LONDON SCHOOL OF ECONOMICS AND POLITICAL SCIENCE

Decomposing Complexity:
The Discovering of Pathway Dynamics

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To Janet and Nathan

I certify that the thesis I have presented for examination for the PhD degree of the London School of Economics and Political Science is solely my own work other than where I have clearly indicated that it is the work of others (in which case the extent of any work carried out jointly by me and any other person is clearly identified in it).

I declare that my thesis consists of 60,984 words.

Adam White

Abstract

Biochemists often adopt what may be called the "Strategy of Decomposition" for the causal discovery of biochemical pathway dynamic behaviours. This involves decomposing a pathway into a set of isolated parts, which are then analysed separately. It is assumed that knowledge gained of the isolated parts can then be used to explain the dynamic behaviours of the whole pathway. My thesis addresses the extent to which use of the Strategy of Decomposition is warranted. I evaluate two challenges contained in Bechtel and Richardson's *Discovering Complexity*. The first challenge is that pathways lack the 'modular' structure assumed in the Strategy of Decomposition. Bechtel and Richardson take biochemists to use a concept of modularity called 'near decomposability'. The second challenge is that pathways have 'Pathway Emergent' behaviours. I reject both challenges. I show that near decomposability is the wrong type of modularity to apply to pathways, and that the occurrence of Pathway Emergence has not been established. I argue that an underlying problem with Bechtel and Richardson's analyses is that they overstate the consequences of feedback and nonlinearity for the Strategy of Decomposition. Instead, the analysis of pathway modularity and emergence needs to be centered on the context-sensitivity of pathways' 'local causal laws'. I identify that the type of modularity assumed in the Strategy of Decomposition is 'causal law modularity', which requires the invariance of local causal laws. I also identify a necessary condition for Pathway Emergence: a pathway must manifest at least one local causal law that is not manifested by its isolated parts. I argue that the use of the Strategy of Decomposition may often be unwarranted. This is because the local causal laws of pathways are highly context-sensitive, and pathways might often not be causal law modular. This context-sensitivity also leaves open the possibility of Pathway Emergence.

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Contents

Chapter 1 - The Causal Discovery of Pathway Dynamic Behaviours

1.1 Introduction

There are formidable challenges to the causal discovery and explanation of biochemical pathway behaviours. Pathways are complex systems, consisting of sequences of interdependent chemical reactions that are nonlinear, context sensitive and often involve multiple feedback loops. Complexity is further increased when pathways are parts of large biochemical networks in which they interact with many other pathways. This complexity means that the causal discovery and explanation of pathway behaviours may sometimes be beyond our cognitive abilities. To make matters worse, there are also considerable barriers to gaining relevant data. For example, the physical structures in which a pathway occurs are often extremely fragile. Measuring pathway operations often appears practically impossible, without first destroying these structures. Yet these structures can play a critical role in determining pathway behaviours.

I shall be focusing on pathway dynamic behaviours. These concern the flows and the changing chemical concentrations that occur within pathways. Pathways are composed of reaction steps, which are themselves composed of the chemicals that bring about that step's reaction. Each reaction step has a rate law that specifies how its rates of reaction depends on the concentrations of its reactants. Pathways can exhibit a wide range of dynamic behaviours including maintaining a single steady state, switching between multiple steady states and oscillating.

Within biochemistry, it is generally agreed that a pathway dynamic behaviour is explained by specifying the mechanism producing that behaviour. This involves specifying the salient parts, operations and organisation of the pathway. 'Causal discovery' of a pathway dynamic behaviour refers to the processes by which biochemists discover these parts, operations and organisation. However, given the complexity of pathways and the lack of data, how should the discipline of biochemistry proceed when attempting to identify these pathways?

Biochemists often respond by adopting what I shall term as the 'Strategy of Decomposition' for the causal discovery of pathway dynamic behaviours. The strategy has three broad stages:

1. An extraction stage; in which the target pathway is separated from its biological context.
2. A decomposition stage; in which the isolated pathway is decomposed into a set of isolated parts, that are then separately analysed.
3. A reconstruction stage; involving using a simulation model to deduce the target behaviour.

My thesis addresses the extent to which the Strategy of Decomposition is warranted. I will evaluate two related challenges to the strategy that are contained in Bechtel and Richardson's book *Discovering Complexity* (2010) and in related papers.[1]

The first challenge is that pathways lack the 'modular' structure assumed in the Strategy of Decomposition. Bechtel and Richardson take biochemists to be applying a concept of modularity called 'near decomposability' that was originally formulated by Herbert Simon (1962, 1973, 1977, 1999, 2002). Systems are modular, in this sense, when the intensity of intra-subsystem interactions is significantly greater than the intensity of inter-subsystem interactions. Bechtel and Richardson argue that pathways are often *not* nearly decomposable because of the effects of feedback loops. Nevertheless, the assumption of near decomposability has been heuristically useful in producing 'false models as a means to truer theories' (Bechtel and Richardson, 2010, p. xxvi).

The second challenge is that pathways sometimes have a type of emergence, that I shall term 'Pathway Emergence'. A key requirement for Pathway Emergence is that a pathway's dynamics cannot be deduced from a 'Deductive Base' that contains statements of: the properties of the pathway's isolated parts, the pathway's organisation, and laws manifested in systems simpler than the whole pathway. But if this is correct, then it appears to undermine the Strategy of Decomposition, which is based on precisely being able to make these types of deductions.

[1] These are papers that are endorsed in the second edition of Discovering Complexity and are authored or co-authored by either Bechtel or Richardson. **In particular, the account of pathway emergence analysed in my thesis was originally proposed in Boogerd et al.'s (2005) paper, which was co-authored by Richardson.**

The aim of this first chapter is to provide the biochemical and philosophical background to my thesis. In section 2, I provide an overview of pathways and pathway dynamic behaviours. In section 3, I review the philosophical literature on mechanisms and on scientific explanation in biochemistry. I argue that pathway dynamic behaviours are explained by 'dynamic mechanistic explanations' that reference local causal laws. In section 4, I explain how the complexity of pathways and the lack of data are formidable challenges to the causal discovery of pathway dynamic behaviours. In section 5, I describe the Strategy of Decomposition that has been employed in the causal discovery of pathway dynamic behaviours. I explain that biochemists often assume that pathways are 'causal law modular'. In section 6, I provide an overview of the remaining chapters of my thesis.

1.2 Pathways

The operations of a cell are accomplished through series of biochemical reactions called pathways. Pathways are a basic analytic construct of biochemistry. It is by understanding pathways that we can understand much of how cells function. There is not a generally agreed definition of what a pathway is, but the following characterisation will suffice for our purposes: a pathway from a *source* chemical X to a *target* chemical Y is a sequence of chemical reactions in which:

(i) X is a reactant of the first reaction,

(ii) Y is a product of the last reaction,

(iii) at least one product[2] of each reaction is a reactant in the next reaction (with the exception of the last reaction in the pathway).

There are three main types of pathway:

- Metabolic pathways. These are either catabolic or anabolic. Catabolic pathways break down molecules into progressively smaller products. The products provide chemically available energy to the cell and metabolic

[2] The product linking each step cannot be a 'currency molecule'. Currency molecules are chemical species that appear in a large number of biological reactions and whose function is primarily to donate or receive electrons e.g. H_2O, ATP, NAD^+ (Holme (2008)). Currency molecules are excluded from providing the links between pathway steps, as otherwise the number of pathways within a biological network becomes too large for the concept of a pathway to be useful. Currency molecules are further discussed in section 3.5.

intermediates for anabolic pathways. Anabolic pathways start from these metabolic intermediates (and other small precursor molecules) and convert them progressively into larger molecules such as nucleic acids, lipids and polysaccharides, which enable the growth, maintenance and duplication of a cell.

- Gene regulatory pathways. These activate or inhibit the production of specific gene products e.g. RNA and proteins.

- Signal transduction pathways. These are triggered by cellular receptors responding to extra-cellular signals. Receptors can begin a pathway within their cell which will eventually elicit a response to the signal such as changing the cell's metabolism, shape or gene expression.

Each of the reactions in a pathway can be represented by a chemical reaction equation, for example:

$$B + C \rightarrow D + E$$

states that a molecule of species B combines with a molecule of species C and is transformed into a molecule of D and a molecule of E. The rate of a reaction is a measure of the rate of change in the concentrations of the reactants and products due to that reaction. A 'rate law' specifies this relationship between the rate of a reaction and the concentration of its reactants. In the above example the rate law is:

$$v = k[B][C] \quad \left(v = -\frac{d[B]}{dt} = -\frac{d[C]}{dt} = +\frac{d[D]}{dt} = +\frac{d[E]}{dt} \right)$$

where v is the rate of reaction, a square bracket [ ] denotes concentration and a lower case k denotes a proportionality constant called the rate constant.

The chemical reaction equations below are for the glycolytic pathway and illustrate how the reactions of a pathway are linked together. For example, glucose-6-phosphate is a product of the first reaction and a reactant in the second reaction step.

Step		Enzyme
1	glucose + ATP → glucose-6-phosphate + ADP + H^+	hexokinase
2	glucose-6-phosphate ↔ fructose-6-phosphate	phosphoglucose isomerase
3	fructose-6-phosphate + ATP → fractose-1,6-bisphosphate + ADP + H^+	phosphofructokinase
4	fructose-1,6-bisphosphate ↔ dihydroxyacetone phosphate + glyceraldehyde-3-phosphate	aldolase
5	dihydroxyacetone phosphate ↔ glyceraldehyde-3-phosphate	triose phosphate isomerase
	(steps 6 to 10 are carried out twice)	
6	glyceraldehyde-3-phosphate + Pi + NAD^+ ↔ 1,3-bisphosphoglycerate + NADH + H^+	glyceraldehyde 3-phosphate dehydrogenase
7	1,3-bisphosphoglycerate + ADP ↔ 3-phosphoglycerate + ATP	phosphoglycerate kinase
8	3-phosphoglycerate ↔ phosphoglycerate	phosphoglycerate mutase
9	2-phosphoglycerate ↔ phosphoenolpyruvate + H_2O	einolse
10	phosphoenolpyruvate + ADP + H^+ → pyruvate + ATP	pyruvate kinase

Fig 1.1 The glycolytic pathway. Similar representations can be found, for example, in Nelson and Cox (2113, p .545) or Alberts (2010, p. 430), the only significant difference being that they provide diagrams of the molecular structures of the substrates. Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; NAD^+, nicotinamide adenine dinucleotide (oxidised form); NADH, nicotinamide adenine dinucleotide (reduced form).

The glycolytic pathway will be used as the main case study in my thesis. This metabolic pathway produces adenosine triphosphate (henceforth: ATP), which provides the energy needed for many of the cell's chemical reactions. For every two molecules of ATP that are initially consumed in the pathway, four molecules of ATP are later created. The chemical reactions in metabolic pathways are nearly always catalysed by enzymes.

A 'pathway dynamic behaviour' is a trajectory in that pathway's phase space; where the phase space has a separate dimension for the concentration values of each of the pathway's chemicals. For example, a dynamic behaviour of the glycolytic pathway would be a trajectory specifying how the concentrations of glucose, glucose-6-phosphate, ATP and so forth changed over time.

My thesis is concerned with the causal discovery and explanation of pathway dynamic behaviours that occur within living organisms. Such pathways are referred to as *in vivo* pathways; *in vivo* being Latin for 'within the living'. As I shall be explaining, the causal discovery of pathways often proceeds by analysing pathways that are not located within a living organism, but instead are located in a laboratory apparatus such as a petri dish or a test tube. Such pathways are referred to as *in vitro* pathways; *in vitro* being Latin for 'within the glass'.

Two attributes of pathways that are critical to their dynamic behaviours are (i) pathways are often regulated by chemical feedback (henceforth: feedback) (ii) pathways are nonlinear. A detailed analysis of both pathway nonlinearity and feedback will be provided in my chapter 2. For now, the following brief overview will suffice.

Chemical feedback occurs when the concentration of a reactant affects the rate of that reactants own production (Epstein and Pojman, 1998, p. 23). Positive feedback increases the rate of production and negative feedback decreases the rate of production. Pathways are often regulated by multiple chemical feedback loops. For example, the diagram below illustrates three of the feedback loops in the glycolytic pathway, that directly affect the third reaction step, in which fructose-6-phosphate (F-6-P) is transformed into fructose-1,6-biphosphate (FDP). These loops help to regulate the pathway, controlling the amount of ATP available in a cell.

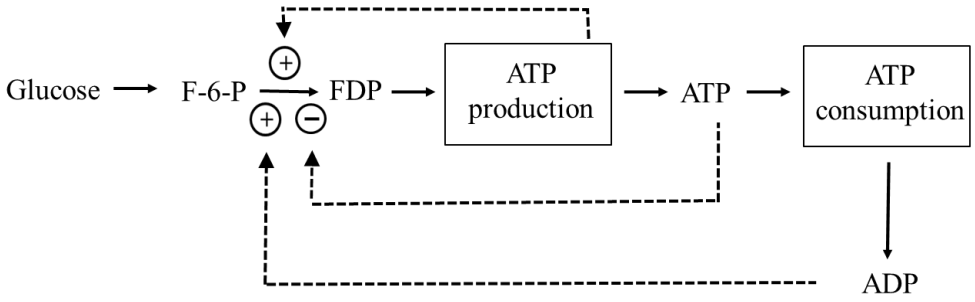


Fig 1.2 Feedback affecting reaction step 3 of the glycolytic pathway. The dashed arrows represent feedback, with (+) being positive feedback and (-) being negative feedback. The undashed lines represent sets of reactions of the glycolytic pathway or of the subsequent transformation of ATP into adenosine diphosphate (ADP). The enzyme for reaction step 3 is phosphofructokinase. Each of the three feedback loops involves a set of reactions that change the conformation of phosphofructokinase, leading to changes the rate of reaction step 3. These reactions between enzymes and reactants of 'later steps' are not usually shown in summary representations of pathways such as Fig 1.1, and hence the presence of feedback loops cannot usually be simply read off from such summary representations. A detailed account of feedback on phosphofructokinase is provided in my chapter 2.

In practice, all pathways are nonlinear. A physical system is nonlinear if it induces a system of equations that do *not* satisfy the 'superposition principle'. The superposition principle is satisfied when (i) any two solutions to the equations can be added together to obtain another solution, and (ii) any solution can be multiplied by any numerical factor to obtain another solution. Nonlinearity has important implications for the explanation of pathway behaviours. Because of nonlinearity, the contributions of each of a system's parts cannot be simply combined to calculate that pathway's dynamic behaviour. Instead, pathway dynamic behaviours are usually calculated by the use of simulation.

Pathways can exhibit a wide range of dynamic behaviours, including such 'exotic dynamics' as oscillating and switching between different steady states. Furthermore, small physical changes (such as changes to the crowdedness of the solution containing the pathway) can lead to sudden dramatic shifts in the dynamic behaviour of a pathway (e.g. via a bifurcation). I shall now illustrate some of these behaviours using case studies referenced in Bechtel and Abrahamsen's (2010) account of the causal discovery of circadian rhythms.

Circadian rhythms are endogenous, physiological cycles of living beings that have a duration close to 24 hours and are entrainable by environmental cues such as daylight and temperature. The multiple pathways for circadian rhythms involve large numbers of reactions, however it will be sufficient for our purposes to consider the relatively simple pathway that was proposed by Goldbeter (1995) for Drosophila (the common fruit fly). The mechanism for maintaining circadian rhythms is located in a small number of neurons of the Drosophila. The proposed mechanism involves the transcription of the *per* gene in the nucleus to produce m-RNA *per*, which is then transported to the cytoplasm and transcribed into the protein PER (in general the symbols for genes are in lower case and italicised, whilst the corresponding proteins are in capitals). PER then undergoes a set of reactions in which it is phosphorylated first to protein PER_1 and then to protein PER_2. The PER_2 is then transported back to the nucleus where it inhibits the further transcription of the *per* gene (this is an example of negative feedback). PER2 gradually degrades and the *per* gene becomes active again and starts to be transcribed. The following diagram represents Goldbeter's model:

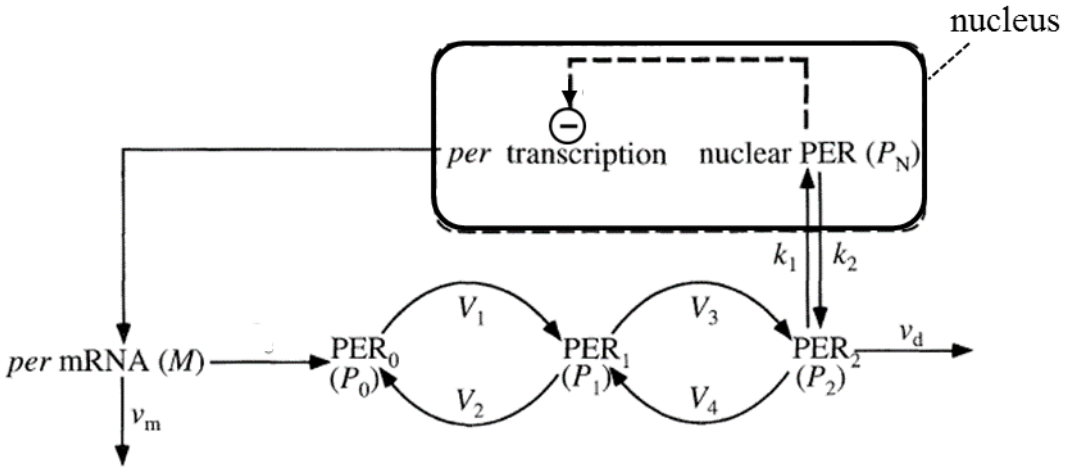


Fig. 1.3 Goldbeter's model of the circadian rhythm of Drosophila. The dashed arrows represent feedback, with (-) being negative feedback. The undashed arrows represent sets of reactions. Abbreviations: M, *per* mRNA in the cytoplasm; P_N, PER in the nucleus; P_0, PER in the cytoplasm; P_1, PER with one phosphoryl group; P_1, P_2, PER with two phosphoryl groups. (Goldbeter, 1995, p. 320).

Goldbeter's model shows how small changes to the rates of a pathway's reactions can result in the pathway changing its behaviour from being in a steady state to oscillating. The pattern of oscillations generated by the model are illustrated below.

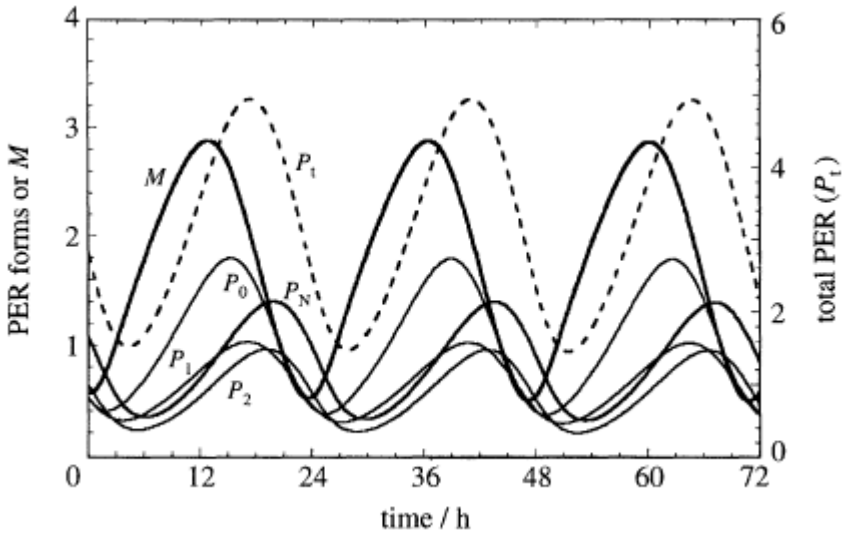


Fig. 1.4. Sample output from Goldbeter's model of the circadian rhythm of Drosophila. Abbreviations: M, *per* mRNA in the cytoplasm; P_N, PER in the nucleus; P_0, PER in the cytoplasm; P_1, PER with one phosphoryl group; P_1, P_2, PER with two phosphoryl groups; P_t = all forms of PER. (Goldbeter, 1995, p. 321).

Subsequent to Goldbeter's model, other genes and proteins were found to play critical roles in the maintenance of circadian rhythms. These were incorporated in the Leloup and Goldbeter (2003) model for the mammalian circadian mechanism. The model was used in generating possible explanations for a variety of circadian

pathologies. These included Advanced Sleep Phase Syndrome in which the natural pattern of falling asleep around midnight is shifted by several hours, so that the subject falls asleep early in the evening and wakes up very early in the morning. Leloup and Goldbeter were able to show that this could be replicated by changing their model's parameter values for the phosphorylation of PER. This is consistent with studies of families with Advanced Sleep Phase Syndrome, which identified the presence of a genetic mutation that affected the production of an enzyme involved in the phosphorylation of PER (Bechtel and Abrahamsen, 2010, p. 327).

The above case studies illustrate the types of dramatic changes in pathway behaviours that can sometimes occur and which will need to be accounted for in the corresponding explanations of pathway dynamic behaviours. I take it that an adequate explanation will need to go beyond just stating the steps by which a pathway's target chemical is produced, it will also need to explain why a pathway is exhibiting one type of dynamic behaviour rather than another. It should provide answers to what Woodward (2003, p. 260) calls 'what-if-things-had-been-different?' questions, such as how would the pathway's dynamic behaviour be different if:

- the initial conditions were different
- there was a perturbation that changed the concentrations in the pathway
- there was a physical change that changed the kinetic parameters of the pathway.

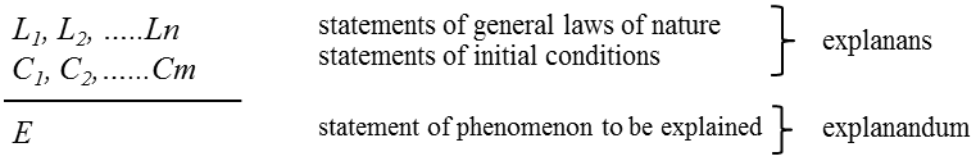
1.3 Explaining Pathway Dynamic Behaviours

How should pathway dynamic behaviours be explained? The deductive-nomological (henceforth: D-N) account of scientific explanation once dominated the philosophy of science. According to this, a phenomenon is explained by showing that it can be deduced from statements of (i) general laws of nature and (ii) initial conditions. But there are significant shortcomings with the D-N account and it does not match the actual explanations provided in biology.

Instead, biologists provide mechanistic explanations, in which a phenomenon is explained by specifying the mechanism responsible for the phenomenon. Before the first publication of *Discovering Complexity* in 1993, little philosophical attention was

paid to this form of explanation. Since then a 'new mechanistic philosophy of science' has flourished. In this section, I will explain what mechanisms are. And that many, if not all the causal regularities found in biological mechanisms are described by 'local' rather than 'general' causal laws. I will then argue that the successful explanation of a pathway dynamic behaviour requires a 'dynamic mechanistic explanation'. This consists of a (i) a qualitative account of a mechanism and (ii) a quantitative account showing that the target dynamic behaviour can be deduced from statements of the pathway's rate laws and initial conditions. However, in contrast to D-N explanations, these laws are 'local causal laws'.

According to the D-N account of scientific explanation, to explain a phenomenon is to subsume it under general laws of nature. For systems with deterministic laws, successful scientific explanations were taken to have the form of the D-N model, as specified by Hempel and Oppenheimer (1948):



The explanans must include statements of general laws of nature that are essential to the derivation of the explanandum. The laws are general, in the sense that statements of the laws make no reference to particulars and are true without exception. The explanandum is explained by showing that it is an instantiation of these laws. A similar account of explanation was held to apply for systems with probabilistic laws, but it is sufficient for our purposes just to focus on D-N explanations.

The D-N Model has been subject to some well-known counterexamples (Salmon, 1984. p. 46-50). Consider, for example, the following deductive argument:

> Every man who regularly takes birth control pills avoids pregnancy
> John Jones regularly takes birth control pills
> John Jones avoids becoming pregnant

This satisfies the criteria for being a D-N explanation but clearly fails to be explanatory. Such counterexamples have highlighted serious shortcomings with the D-N Model, including that: (i) irrelevant premises can be used to deduce and hence 'explain' an explanandum (ii) no temporal priority is required between the explanans

and the explanandum (iii) there is no requirement that the cause of the explanandum be included in the explanans. A further problem for the D-N account is that there are few general laws of nature to be found in biology (e.g. Dupre, 2009, p. 33). Instead the regularities that biologists term as 'laws' are often highly context-sensitive. If the D-N account is correct then few, if any, scientific explanations have been provided within biology.

The D-N account of scientific explanation has been widely rejected by philosophers of biology. With respect to biochemistry, it is recognised that successful scientific explanations are causal explanations, in which a phenomenon is explained by specifying the mechanism that produces it.

But what is a mechanism? The characterisation of a mechanism has been the subject of an intense debate amongst a group of philosophers that I shall refer to as the 'New Mechanists'. The three most prominent characterisations are:

> "Mechanisms are entities and activities organized such that they are productive of regular changes from start or set-up to finish or termination conditions." (Machamer et al., 2000, p .3)
>
> "A mechanism for a behaviour is a complex system that produces that behaviour by the interaction of a number of parts, where the interactions between parts can be characterized by direct, invariant, change-relating generalizations." (Glennan, 2002, p. S344)
>
> "A mechanism is a structure performing a function in virtue of its component parts, component operations, and their organization. The orchestrated functioning of the mechanism, manifested in patterns of change over time in properties of its parts and operations, is responsible for one or more phenomena." (Bechtel and Abrahamsen, 2005, p. 423)

Each of these characterisations, sometimes with minor modifications, has its supporters. There are some differences in vocabulary between the characterisations, for example Machamer et al. use the terms 'entities' and 'activities' rather than 'part' and 'operations'. But there are also some substantial differences, reflecting different views or emphasises on such matters as:

a. the nature of causation
b. the domains of science to which the concept of mechanism applies

c. the importance of cyclical organisation, including feedback within mechanisms.

Nevertheless, there is now considerable agreement on a minimum concept of mechanism that applies across the sciences, and this has been captured by Illari and Williamson:

> "A mechanism for a phenomenon consists of entities and activities organised in such a way that they are responsible for the phenomenon". (Illari and Williamson, 2012, p. 120)

Illari and Williamson's characterisation lays clear the three basic components of a mechanism: its phenomenon, its parts and operations, and its organisation. I shall use this as my base, from which to explain mechanisms.

Craver (2007, p. 7) nicely represents the structure of mechanism:

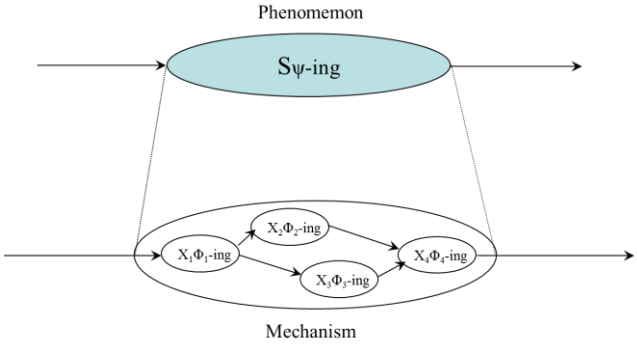


Fig 1.5. Craver's schematic diagram of a mechanism. The mechanism consists of the organised parts (circles) and operations (arrows). The mechanism's phenomenon is S's ψ-ing, its parts are $\{X_1, X_2 \ldots X_m\}$ and its operations are $\{\Phi_1, \Phi_2 \ldots \Phi_n\}$. Mechanisms can have multiple levels, as each node can itself be a mechanism.

A mechanism is a mechanism responsible for a phenomenon. It is functionally individuated by its phenomenon i.e. by the set of inputs and outputs delimiting that phenomenon. There are many types of phenomena. There are mechanisms responsible: for *producing* particular materials (e.g. for producing adenosine triphosphate and pyruvate from glucose); for *exhibiting* particular behaviours (e.g. for neurons exhibiting electrical oscillations); for *maintaining* particular states (e.g. maintaining homeostasis). A mechanism can also be responsible for a system having a capacity; Illari and Williamson (2012, p. 124) give the example of a cell *having a capacity* to metabolise lactose. A cell will only metabolise lactose when glucose is

unavailable. However, the mechanism responsible for having this capacity will exist, even if lactose is never metabolised.

Operations (or 'activities' or 'interactions') are what the parts do within a mechanism. There is considerable disagreement between the New Mechanists as to the nature of causation, and consequently as to how operations should be characterised. Machamer et al. (2000) and Bogen (2005) incorporate a realist, productive view of causation in which "activities are types of causes" (Machamer et al., 2000, p. 7). "An entity acts as a cause when it engages in a productive activity. It is not penicillin that causes pneumonia to disappear, but what the penicillin does" (Machamer et al., 2000, p. 7). They endorse Anscombe's view that the term 'cause' is highly abstract and only becomes meaningful when filled in by more specific causal terms such as "scrape, push, wet, carry, eat, burn, knock over, keep off, squash, make (e.g. noises, paper boats), hurt" (Machamer et al., 2000, p. 7). By contrast, Glennan (2002) refers to a mechanism's parts 'interacting', where an interaction "is an occasion in which a change in a property in one part brings about a change in a property of another part" (Glennan , 2002, S344). This is best understood within the context of Glennan's wider project. Glennan (2009) is proposing an account of (non-fundamental) causation in terms of mechanisms. The proposal is that for two events to be causally related they must be connected by an intervening mechanism. In explaining his notion of interaction, Glennan invokes Woodward's manipulationist theory of causation. Woodward's theory is explained in my chapter 3, but the basic idea is that for X to be a cause of Y:

a) there is an ideal intervention on *X* such that *Y* changes or the probability distribution of *Y* changes.
b) the relationship between *X* and *Y* is invariant i.e. remains unchanged by the intervention.

However, Glennan takes such invariant generalisations to be mechanically explicable i.e. the truth conditions for the generalisations are mechanisms (Glennan, 2009, p. 322). Finally, Bechtel and Abrahamsen (2005, 2010), have been careful to avoid using the word 'cause' or taking any position on the nature of causation, instead simply citing examples of operations such as the adding or removing of hydrogen atoms from a molecule during a chemical reaction. (Bechtel and Abrahamsen, 2005, p. 433). I will return to the subject of causality, below.

There are several types of organisation that may be relevant to the functioning of a mechanism. These include spatio-temporal organisation, such as the locations and conformations of parts, and the temporal orderings, rates and durations of the parts' operations. A second type of organisation, that is critical to the functioning of many complex systems, is the coordinating of the parts' operations by feedback loops. Examples include the maintenance of states such as homeostasis, and 'self-organising systems' in which multiple feedback loops give rise to exotic behaviours such as the synchronised oscillations of neurons (self-organisation is discussed in my chapter 4). It is because of the importance of feedback that Bechtel and Abrahamsen's characterisation of a mechanism refers to to the "orchestrated functioning of a mechanism"; Bechtel (2011, p. 539) elaborates that 'like a player in an orchestra, an individual part may behave differently as a result of operations performed by other parts'. Bechtel criticises Machamer et al. for describing mechanisms as proceeding 'from start or set-up conditions to finish or termination conditions', as this implies a sequential ordering to a mechanism's operations and hence fails to recognize the importance of feedback loops (Bechtel, 2011, p. 536). A similar criticism can be made of Glennan's characterisation. A third type of organisation is near decomposability. As I will explain in my chapter 3, sometimes a mechanism has this type of organisation, in which its parts' behaviours are relatively autonomous of each other.

With respect to my thesis's analyses, Bechtel and Abrahamsen's characterisation provides the best fit for pathway mechanisms. It is consistent with Illari and Williamson's account but has been formulated specifically for the domain of biology, where feedback is ubiquitous. Both Machamer et al. and Glennan's accounts are tied to particular theories of causation, and this does not fit with my thesis which is neutral between these theories.

With respect to causality, my thesis is based on some uncontroversial claims that are, at least implicitly, incorporated into biochemists' analyses of pathway dynamics:

1) a change to the value of a cause will, at least sometimes, lead to a change to the value of its effect.
2) causal relationships relate variables. Variables are properties or magnitudes that can have more than one value; and the values of variables are possessed by particular entities. (Woodward, 2003, p. 39).

3) causal relationships can be expressed as causal equations in which the dependent variable is the effect and independent variables are a complete set of its causes. Causal equations specify functionally correct relationships.
4) A variable X is a direct cause of a variable Y, with respect to a set of variables **V**, if changing X will, at least sometimes, change Y when all other variables in **V** are held constant. Hence changing X can bring about a change in Y without having to change the value of an intermediate variable. The definition of a direct cause is relative to a set of variables **V.** X may be a direct cause of Y relative to the set **V** but an indirect cause relative to a different set **V*** (c.f. Woodward, 2003, p. 55).

The causal equations describing the causal regularities of a system, are statements of that system's 'causal laws' (see for example (Cartwright, 2007, p. 152 – 155)). Causal laws need not be general laws, applying without exception. Instead, they can be local laws, applying in a limited number of contexts. Small changes in context can 'break' a local causal law. Many, if not all, the causal laws that apply within biological mechanisms are local causal laws. For example, when biochemists refer to the rate *laws* of a pathway's chemical reactions, they are not supposing that these rate laws are general laws. They know that rate laws are highly context sensitive; and this is reflected in the experimental procedures they use when discovering these laws (as I will explain in my chapter 3). The regularities that they call 'rate laws' are local causal laws; instantiated when that reaction's reactants are located in the right sort of context.

Biological mechanisms may be viewed as being examples of what Cartwright calls 'nomological machines':

> A nomological machine is "a fixed (enough) arrangement of components, or factors, with stable (enough) capacities that in the right sort of stable (enough) environment will, with repeated operation, give rise to the right kind of regular behaviour that we represent in our scientific laws" (Cartwright, 1999, p. 50).

Consider a toy example of an *in vitro* pathway *S* that is situated in a test tube in a laboratory. Let us assume that the rate laws for *S*'s reaction steps continues to apply over the full range of reactant concentrations occurring within the test-tube (this assumption is routinely made in biochemistry). However if, say, a particular catalyst is added into the test-tube or a powerful electromagnetic force is applied to the test-

tube, then some of the original rate laws of *S* will be broken (i.e. they will no longer describe the causal regularities of *S*). *S* is an example of a nomological machine. If the nomological machine is changed, in this case by adding a new catalyst or applying an electro-magnetic force, then different local causal laws may arise. As I shall now explain, local causal laws play a key role in the explanation of pathway dynamic behaviours.

In mechanistic explanations, a phenomenon is explained by specifying the mechanism responsible for the phenomenon. These specifications often reference multiple compositional levels and bottom out in parts and operations that are taken as relatively fundamental to scientists in that field of science (see Fig. 1.6).

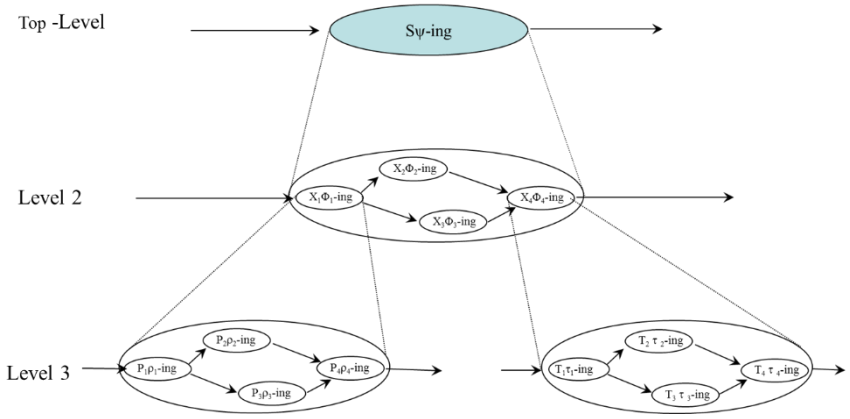


Fig. 1.6. **The multiple levels that may be referenced in a mechanistic explanation**. Craver (2007, p. 190).

Some New Mechanist accounts, most prominently Machamer et al. (2000) have portrayed mechanistic explanations as being qualitative descriptions of a mechanism. According to Machamer et al., a satisfactory explanatory text consists of a description of the producing mechanism in terms of the field or scientist's bottom out activities and entities. For example, in molecular neuroscience the bottom out entities would typically include different types of neurons, ions, neural transmitters and so forth. The bottom-out activities for molecular biology and neuroscience fall into four categories (Machamer et al, 2000, p. 14):

Activities	
geometrico-mechanical	(e.g. turning, pushing)
electro-chemical	(e.g. attracting, bonding)
energetic	(e.g. diffusing)
electro-magnetic	(e.g. conducting).

Machamer et al. (2000, p. 8-13) provide an example of the mechanistic explanation of an action potential in a neuron. The explanation consists of a qualitative account of the bottom-out entities engaging in the types of bottom-out activities listed above.

Both Bechtel (2011, p. 537) and (Boogerd et al. p. 154) criticise Machamer et al. for portraying mechanistic explanations as being qualitative explanations. A qualitative account of a mechanism cannot, by itself, explain why a pathway is exhibiting a particular dynamic behavior, or why a small perturbation may result in an extreme change in behavior. It cannot, for example, explain why a pathway has sustained oscillations rather than damped oscillations (recall section 1.2). An answer to such questions requires an account of how the pathway's dynamic behaviours arise from the multiple non-linear interactions between the pathway's reactants (including feedback loops) and how small differences in concentrations or kinetic parameters can lead to substantially different behaviours. In practice, this requires the use of a simulation model. Such models consist of a system of ordinary differential equations (henceforth: ODEs) whose:

> "variables [and parameters] in the model reflect salient properties of the parts and operations in the mechanism and the equations capture how values of these variables change over time." (Bechtel and Abrahamsen, 2010, p. 19)

A successful explanation of a dynamic behaviour has two components: (i) a qualitative account of the mechanism (ii) a quantitative account provided with the aid of a simulation model. The two accounts must be integrated, with the simulation model's ODEs being 'explicitly anchored' to the qualitative mechanistic account. Bechtel and Abrahamsen call such explanations 'dynamic mechanistic explanations' (Bechtel and Abrahamsen, 2010, p. 323). By 'anchoring' Bechtel and Abrahamsen mean that there is a mapping from the variables and parameters in the equations to the parts and operations specified in the mechanistic account.

The two parts of a dynamic mechanistic explanation complement each other. The qualitative mechanistic account will contain salient details of the mechanism that are not explicitly stated in the ODEs. These should include the context in which the behaviour is occurring, how the parts are organised, how conformational changes of catalysts affect the rates of particular reactions and so forth. Much of this detail is needed for understanding a mechanism and identifying how it can be intervened on. In contrast, the explanation's ODEs are often at a higher level, in effect quantifying aggregate effects of the factors detailed in the qualitative mechanistic account.

I support Bechtel and Abrahamsen's analysis of the need for dynamic mechanistic explanations, but with one addition. The equations in the simulation model must be causal equations. Kaplan and Craver's (2011) make a similar point in their analysis of explanatory models in cognitive and systems neuroscience, when they formulate a 'model-to-mechanism-mapping' (3M) constraint:

> '(3M) In successful explanatory models in cognitive and systems neuroscience (a) the variables in the model correspond to components, activities, properties, and organizational features of the target mechanism that produces, maintains, or underlies the phenomenon, and (b) the (perhaps mathematical) dependencies posited among these variables in the model correspond to the (perhaps quantifiable) causal relations among the components of the target mechanism.' (Kaplan and Craver, 2011, p. 611).

I take the anchoring requirement for successful dynamic mechanistic explanations to be stronger than this: that the dependencies between the variables *must* be mathematically specified (i.e. in the model's ODEs). A dynamic mechanistic explanation is incomplete, to the extent that its causal relationships are not specified in its ODEs, or there are variables or parameters in these ODEs that are not mapped to the qualitative mechanistic account.

The structure of pathway ODE models for dynamic mechanistic explanations is discussed in my chapter 2. For now, it is sufficient to make the following points:

- there will be one ODE for each of the pathway reactants being modelled.
- the left-hand side of the ODE (the dependent variable) is the rate of change of a reactant's chemical concentration and the right-hand side variables are direct causes of the quantity on the left-hand side.

- the other terms on the right-hand side of the ODE are parameters that are constants. In chemically homogenous solutions, these are rate constants (or functions of rate constants and fixed reactant concentrations) whose values depend on properties of the reactants and the context in which the reactants are situated.

Goldbeter's model illustrates the required structure:

$$\frac{dM}{dt} = v_s \frac{K_I^n}{K_I^n + P_N^n} - v_m \frac{M}{K_m + M}$$

$$\frac{dP_0}{dt} = k_s M - V_1 \frac{P_0}{K_1 + P_0} + V_2 \frac{P_1}{K_2 + P_1}$$

$$\frac{dP_1}{dt} = V_1 \frac{P_0}{K_1 + P_0} - V_2 \frac{P_1}{K_2 + P_1} - V_3 \frac{P_1}{K_3 + P_1} + V_4 \frac{P_2}{K_4 + P_2}$$

$$\frac{dP_2}{dt} = V_3 \frac{P_1}{K_3 + P_1} - V_4 \frac{P_2}{K_4 + P_2} - k_1 P_2 + k_2 P_N - v_d \frac{P_2}{K_d + P_2}$$

$$\frac{dP_N}{dt} = k_1 P_2 - k_2 P_N$$

Fig 1.7 The ODEs used in Goldbeter's model of the circadian rhythm of Drosophila (Goldbeter, 1995, p. 320). There are five variables, corresponding to the five modelled reactants. As before, M = *per* mRNA in the cytoplasm, P_N = PER in the nucleus, P_0 = PER in the cytoplasm, (P_1 , P_2) = phosphorylated forms of PER and P_t = all forms of PER. All other terms are constants.

In Goldbeter's model, the independent variables are parts in the mechanism, and there is a causal relationship between these parts and the part referred to in the dependent variable. For example, in the first equation, the independent variables M and P_N (i.e. *per* mRNA and PER in the nucleus) are direct causes of changes in the rate of consumption of *per* mRNA. This corresponds to Goldbeter's putative mechanism where (i) increasing the concentration of P_N decreases the production of *per* mRNA, and (ii) increasing the concentration of *per* mRNA increases the consumption of *per* mRNA (*per* mRNA degrading in the cytoplasm).

But have we now gone full circle and returned to D-N explanations? After all, both D-N explanations and dynamic mechanistic explanations involve explaining a phenomenon by showing that it can be deduced from laws of nature and initial conditions. But there are two important differences. First, the qualitative part of a dynamic mechanistic explanation plays a key role in describing the causal structure that produces the phenomenon. Second, as Bechtel notes, whilst D-N explanations use general laws of nature, the equations in dynamic mechanistic explanations' are 'descriptions of the operations of specific parts' (Bechtel 2011, p. 535). As I have explained, I take these 'descriptions' to be statements of local causal laws.

In closing, pathways can exhibit a variety of 'exotic' dynamic behaviours. A pathway dynamic behaviour is explained by specifying the mechanism that produces it. Bechtel and Abrahamsen correctly emphasise the central role that feedback loops play in pathway mechanisms. An adequate explanation goes beyond just stating the steps by which a pathway's target chemical is produced, it also explains why a pathway is exhibiting one type of dynamic behaviour rather than another. This requires an account of how pathway dynamic behaviours arise from the multiple non-linear interactions between the pathway's reactants (including feedback loops) and how small differences in concentrations or kinetic parameters can lead to substantially different behaviours. This, in turn, requires explanations to have two complementary parts, one qualitative and one quantitative. In such dynamic mechanistic explanations, the quantitative part includes statements of the local causal laws for the mechanism. These are combined with statements of initial conditions to deduce the target dynamic behaviour. In this context, I take 'causal discovery' to refer to the processes by which biochemists discover these parts, operations and organisation referenced by the corresponding dynamic mechanistic explanation. This then includes the discovery of these local causal laws. My thesis will be focusing on the putative modularity and emergent behaviour of pathways; I shall argue in later chapters that it is the invariance of a pathway's local causal laws that is key to these subjects.

1.4 Barriers to Causal Discovery

In vivo pathways are complex systems and we currently have only sparse data on their operations. In this section, I will review four key factors that contribute to the complexity of *in vivo* pathways:

(i) The large number of reactants that are shared between pathways

(ii) The impact of non-reactive interactions between a pathway and other cellular components.

(iii) The presence of multiple feedback loops

(iv) Pathway non-linearity

I will then review the reasons for the sparsity of *in vivo* data. If biochemists are to succeed in explaining *in vivo* pathway dynamic behaviours, their methodologies will need to overcome the formidable challenges posed by pathway complexity and by the sparsity of data.

Pathways often share many of their reactants with multiple other pathways. The chemical reactions occurring with a cell can be represented by a set of biological networks, with separate networks for metabolic interactions, transcriptional regulation interactions, protein-protein interactions and so forth. These networks illustrate the extent to which pathways share reactants and hence have interdependent dynamic behaviours. I will use the metabolic networks of *E. coli* and *streptococcus pneumoniae* to illustrate this interdependency.

Metabolites are the chemical substances that are either intermediates or products of metabolism; they do not include enzymes. In the metabolic networks considered in my thesis, vertices are used to represent specific metabolites and edges connect pairs of vertices that are related as reactant and product. For example, Zhao et al.'s (2006) metabolic network of E. coli depicts 924 metabolites engaged in 1437 reactions. Enzymes are not explicitly represented but it is implicitly understood that there will generally be a unique enzyme associated with each edge. The following graph by Silva, M. et al. (2008) represents the metabolic network for the streptococcus pneumoniae cell.

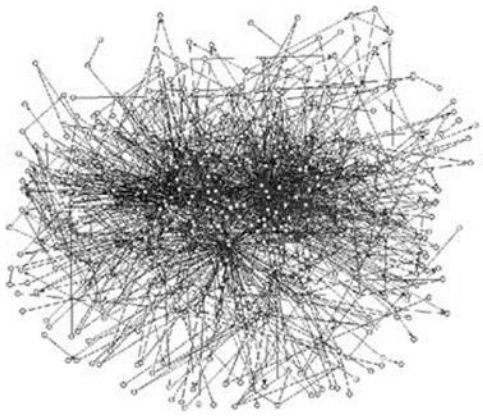


Fig. 1.8 Metabolic network for streptococcus pneumoniae. (Silva, M. et al, 2008, p. 238)

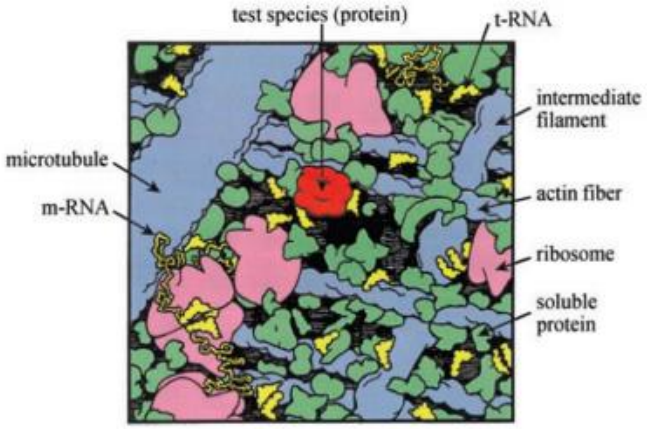
This diagram illustrates that the metabolic operations with a cell are not organised into nicely delimited pathways. *In vivo* pathways are *not* almost closed systems; with each pathway being undisturbed by other pathways, apart from receiving reactants at some beginning stage and releasing products at some end stage. Instead *in vivo* pathways are, what I shall term as, 'pervasively open systems' with the concentrations of the reactants for any particular reaction often being directly determined both by (i) the outputs of its adjacent reactions steps and (ii) the reactions of other pathways. For example, glyceralderhyde-3-phosphate is a reactant in reaction step 6 of the glycolytic pathway:

$$\text{glyceraldehyde-3-phosphate} + \text{Pi} + \text{NAD}^+ \leftrightarrow \text{1,3-bisphosphoglycerate} + \text{NADH} + \text{H}^+$$

The concentration of glyceralderhyde-3-phosphate available for reaction step 6, depends both on (i) the outputs of reaction steps 4, 5 and 7 of the glycolytic pathway and (ii) the reactions of other pathways in the cytoplasm which consume or produce glyceralderhyde-3-phosphate (e.g. glyceraldehyde-3-phosphate is a reactant in the gluconeogenesis pathway, the pentose phosphate pathway, the pathway for thiamine and so forth - from the KEGG Pathway Database; accessed 16/07/2016). The 'pervasive openness' of *in vivo* pathways may greatly increase the number of interactions that need to be determined in the causal discovery of a pathway dynamic behaviour.

A pathway's dynamics are also often highly dependent on non-reactive interactions between the pathway and other cellular components. In practice, biochemistry has historically assumed that pathway reactions occur in chemically homogenous solutions. But this ignores the cellular architecture that compartmentalises a cell and

the ‘crowded’ cellular solution which contains high concentrations of large macromolecules. Minter provides the following illustration of a eukaryotic cytoplasm.



Cartoon of eukaryotic cytoplasm at a magnification of 1,000,000 ×. The test protein molecule (*red*) is in a fluid medium that is crowded by soluble proteins (*green*), RNA species (*yellow*), and ribosomes (*pink*) and confined by cytoskeletal fibers (*blue*).

Fig 1.9. **Cartoon of eukaryotic cytoplasm.** (Minter, 2001, p. 10578)

Trevors et al. (2012, p. 3) illustrates the heterogeneous solution in which *in vivo* pathways are located by listing some of the features of the bacterial cytoplasm. These include:

> “ - Structured and organized gel, not a watery sac enclosed by a cytoplasmic membrane.
> - Contains salts, ions, sugars, amino acids, macromolecules, vitamins, coenzymes and about 2000 different proteins… all nucleic acids… tens of thousands of ribosomes.
> - Spatially varied composition with some compartmentation.
> - Electrostatics is a dominant force.
> - Diffusion is also dominant (e.g. rotational and translational).
> - Hydrophobic effects.”

Such non-reactive interactions can have large scale effects on reaction rates (van Eunen et al., 2012) and these will need to be determined as part of the causal discovery of a pathway dynamic behaviour.

Feedback further adds to the complexity of pathways. To see this, let us first consider the case of an isolated *in vitro* pathway, where there are no inter-pathway interactions. The presence of feedback means that the behaviours of a particular reaction step cannot be explained just in terms of the intrinsic properties of that reaction step, plus the concentrations of reactants resulting from earlier stages in the

pathway. Instead account also has to be taken of later stages in the pathway whose products may also affect the reaction step. In the case of *in vivo* pathways, feedback can result in an additional layer of complexity, as there are often feedback loops involving multiple pathways. For example, many pathways are thermodynamically powered by converting ATP to ADP. There are positive feedback loops between these pathways and the glycolytic pathway, whereby increasing the concentration of ADP increases the rate of at which the glycolytic pathway increases production of ATP, which then increases the rate of production of ADP (see my Fig 1.2.). In my chapter 3, I will review Bechtel and Richardson's account of how the failure to take account of feedback, delayed the causal discovery of the glycolytic pathway.

A fourth factor that contributes to complexity is nonlinearity. Many of the interactions both within a pathway and with other cellular components are nonlinear (including the feedback interactions). Nonlinearity means that the cumulative effects of these interactions cannot be simply calculated, but instead will usually require the construction of a simulation model.

A further challenge to causal discovery is that very little i*n vivo* reaction rate data is currently available. For example, Davidi et al. note that k_{cat} rate constants[3] are a fundamental measure of the dynamic properties of enzymes, these constants are referenced in many models of cellular metabolism, and yet data on these constants 'is scarce and measured *in vitro*, thus may not faithfully represent the *in vivo* situation" (Davidi et al., 2016, p. 3401).

One reason for the lack of relevant *in vivo* data is the lack of technology that can measure chemical concentrations within intact cells. For example, Phillip and Schreiber (2013, p. 1050) note that it is "for this reason, quantitative *in vivo* measures of proteins is still rare". Current technologies that are being pioneered include cross-correlation spectroscopy and in-cell NMR. However, as Zhou et al. (2008, p. 12-13) highlight, there are concerns that such techniques significantly impact on the operations within the target cell, inducing artificial interactions.

Another reason for the sparsity of relevant *in vivo* data, is that the same reactants are often involved in multiple reactions. For example, dynamic mechanistic explanations will require data on the rate constants of that pathway's individual reaction steps.

[3] k_{cat} rate constants specify the maximal turnover rates of enzymes ($V_{max} = k_{cat}$ * concentration of enzyme)

Determining the values of these rate constants, in turn requires gaining data on the separate contribution that each reaction step makes to changes in its reactants' concentrations. But, as I have explained, a pathway's reactant concentrations are often affected both by multiple reactions within that pathway and by multiple reactions of their biochemical networks.

The complexity of *in vivo* pathways and the lack of data are formidable challenges to the causal discovery of pathway dynamic behaviours. Biochemists have responded by adopting the Strategy of Decomposition. This assumes that a pathway can be analysed in isolation of its biological context and decomposed into subsystems called modules, that can be separately studied and used to infer the pathway's mechanisms and explain its behaviours.

1.5 Causal Discovery via the Strategy of Decomposition

In this section, I will provide an overview of the Strategy of Decomposition and highlight some of its key assumptions.

The Strategy of Decomposition consists of multiple steps and the details of the steps will vary from case to case. The process steps described below have been elicited from: Bechtel and Richardson's account of the causal discovery of the glycolytic pathway (Bechtel and Richardson, 2010, p. 153-172), Bechtel and Abrahamsen's account of dynamic mechanistic explanations (Bechtel and Abrahamsen, 2005) and van Eunen et al.'s analysis of differences between *in vivo* and *in vitro* conditions (van Eunen et al. (2014)). I shall be providing further analysis of these process steps in my chapter 3. The steps include:

(i) Identifying the *in vivo* pathway dynamic behaviour to be explained.

(ii) Extracting the *in vivo* pathway from its biological context, creating a corresponding *in vitro* pathway.

(iii) Proposing a functional decomposition of the *in vitro* pathway dynamic behaviour. This will consist in proposing the reactants and products of each of the pathway's reaction steps.

(iv) Creating a separate chemical solution for each putative reaction step. Starting from the functional specification, a test solution is created for each putative reaction step. These will contain the proposed reactants and

products. It will also include any other putative parts of the step that were not specified in the functional decomposition (e.g. the reaction step's enzyme). The test solutions can also be formulated so as to try and replicate salient features of the corresponding *in vivo* reaction steps by, for example, adding crowding agents, using physiological acid/alkaline levels and so forth.

(v) Analysing each of the isolated putative reaction steps. Experiments are carried out to discover the salient properties of each reaction step, including their operations and rate laws.

(vi) Confirming that the putative reaction steps combine to form the *in vitro* pathway and produce the dynamic behaviour to be explained. This will include using the rate laws determined in step (v) to construct a simulation model of the pathway (an account of how this is done is provided in section 2.2.2). The simulation model's output is then validated against observations of the target pathway dynamic behaviour.

The causal discovery of a pathway will normally involve many iterations of these process steps, with evidence from later process steps leading to changes in the parts, operations, organisation and rate laws proposed in earlier process steps. Ideally, there would also be an additional step:

(vii) coupling the simulation model to models of other pathways, so as to construct a single model of the entire biochemical network that the pathway belongs to. The aim would be to calculate the effects of the network on the pathway's dynamics. This would then resolve the 'complexity problem' of discovering the effects of pathways sharing their reactants with multiple other pathways. This corresponds to part of the 'Silicon Cell philosophy' that is advocated by Snoep et al. (2006). However, currently the reaction rate data has not been collected to enable such a model.

Overall, the above process steps correspond to a physical decomposition followed by an 'in silico' reconstruction. This is illustrated below:

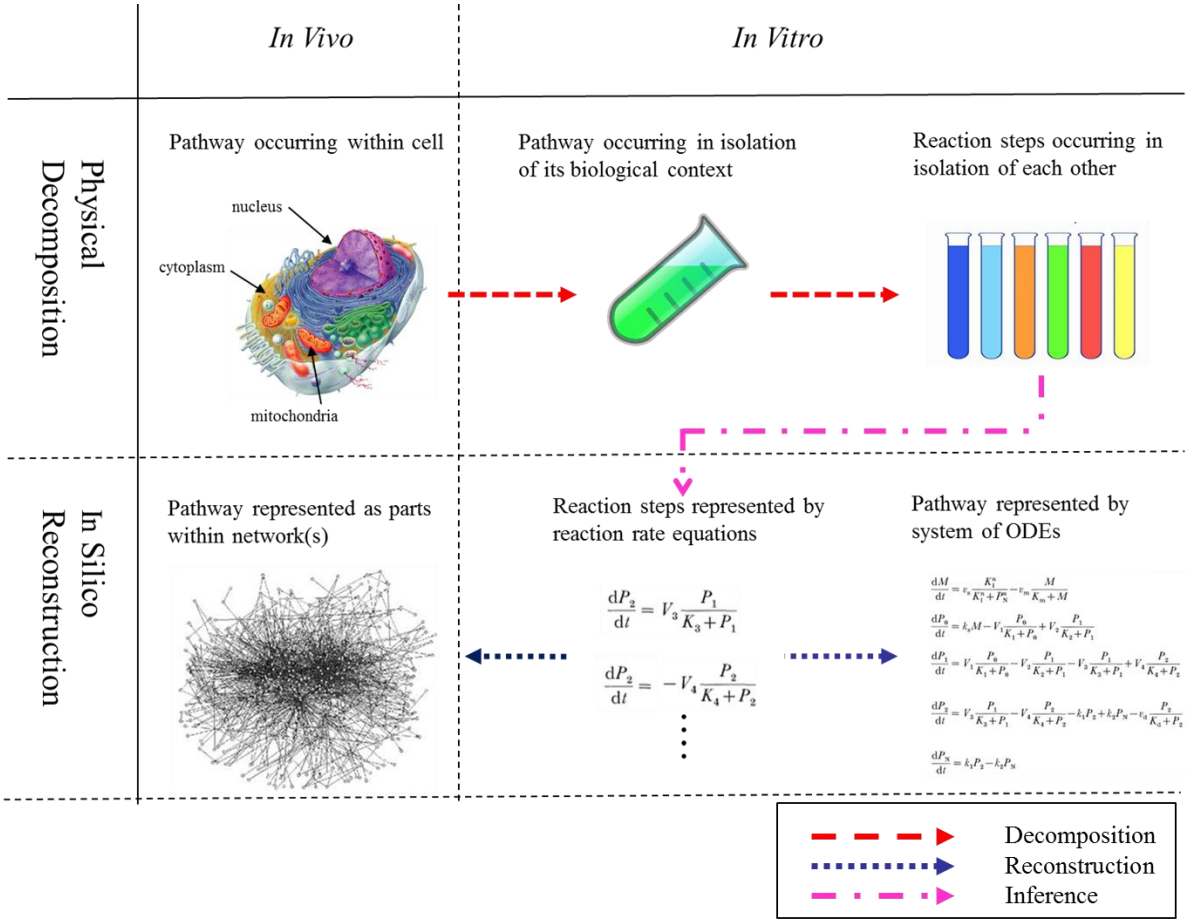


Fig 1.10 The Strategy of Decomposition for the causal discovery of pathway dynamic behaviours.

The process steps above illustrate a general strategy of decomposition, that is widely used in both biology and in other sciences. The general strategy has three broad stages:

1. An extraction stage; in which the target system is separated from its context.
2. A decomposition stage; decomposing the isolated system into a set of isolated parts that can then be separately analysed.
3. A reconstruction stage; involving using a simulation model to derive the target behaviour from statements of the properties of its isolated parts, their arrangement in the system and a general law for combining the rate laws of the isolated parts.

The strategy needs to be applied differently, on a case-by-case basis, based on such factors as the accessibility of data, the degrees to which subsystems interact with each other, and so forth. Sometimes, the strategy will involve a 'physical decomposition' of *S,* such that subsystems are physically isolated from each other. Other times, the strategy may only need to involve a 'conceptual decomposition' in which the subsystems remain in *S* but are each separately analysed, largely ignoring the interactions between *S*'s subsystems. In the case of *in vivo* pathways, their complexity and the lack of data means that physical decompositions will be required. Henceforth, unless otherwise stated, I shall use the term 'Strategy of Decomposition' just to refer to the strategy employed in the causal discovery of pathway dynamic behaviours.

When applying the Strategy of Decomposition, biochemists are, at least implicitly, making an assumption about the invariance of reaction step rate laws. The assumption is that the *in vitro* isolated reaction steps manifest the same rate laws as the corresponding *in vivo* reaction steps. This corresponds to assuming that pathways have a type of modularity that I shall term as 'causal law modularity' (see section 3.6). Consider a system *S* composed of subsystems $C_1 \ldots C_n$. *S* is 'causal law modular' if $C_1 \ldots C_n$ manifest the same local causal laws that are manifested by objects of the same kind as $C_1 \ldots C_n$ that are situated in 'isolation'. In the case of pathways, $C_1 \ldots C_n$ are reaction steps and the local causal laws are their rate laws.

If a pathway is causal law modular, then knowledge gained of its *in vitro* rate laws can be 'exported' and used to provide dynamic mechanistic explanations of its *in vivo* behaviours. Given the complexity of pathways and the lack of data, it is epistemically convenient to believe that pathways are causal law modular. But reaction step rate laws are often very context-sensitive. And the *in vivo* cytoplasm provides a very different context compared to an *in vitro* solution containing just the constituents of a single reaction step. Epistemic convenience is no warrant of truth. Biochemists do attempt to replicate *in vivo* conditions in their *in vitro* experiments. But given the context-sensitivity of rate laws, evidence is required as to why the assumption of causal law modularity is warranted in any particular study. Often no such evidence is provided and the risk exists that the analyses lack adequate epistemic foundations. This is an example of a far more general problem that applies not only to biochemistry but across the natural and social sciences. Namely, how to justify the use of causal knowledge, in cases where there is a difference between the context in

which the explanandum phenomenon occurs and the context in which we can gain causal knowledge about its parts (e.g. Cartwright's (2007)).

1.6 The Road Ahead

I will be focussing on two related challenges to the Strategy of Decomposition that are contained in Bechtel and Richardson's *Discovering Complexity* and in related papers. The first challenge is that pathways lack the 'modular' structure assumed in the Strategy of Decomposition. The second challenge is that pathways are sometimes 'Pathway Emergent'. Both challenges center on the putative consequences of pathways being nonlinear and having feedback.

My Chapter 2 is on nonlinearity and feedback. I begin by defining nonlinearity, and then explain that in practice, all pathways are nonlinear. Nonlinearity is a necessary but insufficient condition for such exotic behaviours as multiple steady states and stable limit cycles (both of which will be explained). I then provide an analysis of feedback. I define feedback and explain the mathematical criteria that systems biologists use to identify the feedback loops of a pathway. I argue that feedback loops are circular causal chains, that can be identified from the system of causal equations referenced in a dynamic mechanistic explanation. I also identify a necessary condition for a pathway to have a feedback loop. My chapter provides the conceptual groundwork that will be used in my chapter 3 and 4 to argue that Bechtel and Richardson's analyses overstate the consequences to the Strategy of Decomposition of the effects of feedback and nonlinearity.

My Chapter 3 is on modularity. Bechtel and Richardson take biochemists to be applying a concept of modularity called 'near decomposability' that was originally formulated by Simon. I shall analyse Simon's concept of near decomposability and explain how Bechtel and Richardson significantly modify this concept, and apply it to pathways. Bechtel and Richardson argue that pathways are often *not* nearly decomposable because of the effects of feedback loops. Nevertheless, the assumption of near decomposability has been heuristically useful in producing 'false models as a means to truer theories'. I shall argue that the concept of near decomposability does *not* apply to pathways, as it is inconsistent with the substantial sharing of parts that occurs between a pathway's reaction steps. I further argue that there is a significant shortcoming in both Simon's and in Bechtel and Richardson's analyses: neither

recognise that there is a plurality of types of modularity. Starting from their analyses of near decomposability, I identify five distinct types of modularity that are important either to the analysis of pathways or to biology more generally. One of these types is 'causal law modularity'. It is causal law modularity that plays a key heuristic role in the causal discovery of pathway dynamic behaviours. I show that feedback does not affect this type of modularity.

My Chapter 4 is on Pathway Emergence. The concept of Pathway Emergence was originally proposed by Boogerd et al. (2005). It is based on their interpretation of C.D. Broad's theory of emergence. Boogerd et al. claim that their concept is relevant to the discovery practices of biochemistry. The concept was subsequently developed in *Discovering Complexity*. A key requirement for Pathway Emergence is that a pathway's dynamics cannot be deduced from a 'Deductive Base' that contains statements of the properties of the pathway's isolated parts, the pathway's organisation, and laws manifested in simpler systems than the whole pathway. Boogerd et al. appear to be proposing a type of emergence that is incompatible with the successful application of the Strategy of Decomposition. The main argument presented for the occurrence of Pathway Emergence takes the form of a simulation case study. Pathway Emergence is linked to pathway nonlinearity and the presence of feedback. I show that their case study does not illustrate Pathway Emergence, and that the claims for existence of this type of emergence are unjustified. I then suggest that perhaps the concept of Pathway Emergence was meant to be based on a far more restrictive notion of non-deducibility than is stated in either Boogerd et al. or in Bechtel and Richardson's writings.

My Chapter 5 draws together the conclusions of my thesis. These can be grouped into two sets. The first set are deflationary about the consequences of feedback and nonlinearity. I take it that Strategy of Decomposition has been successfully developed by biochemists to fully incorporate the effects of nonlinearity and feedback. I reject Bechtel and Richardson's claims about the challenges posed to pathway modularity and to the deducibility of pathway dynamic behaviours. My second set of conclusions emphasise the need to focus on the context sensitivity of pathways' 'local causal laws' (i.e. rate laws). The analysis of pathway modularity and emergence should be centered on the invariance of these causal laws. With respect to modularity, it is causal law modularity that is assumed within the Strategy of Decomposition. With respect to emergence, my analysis identifies a necessary condition for Pathway

Emergence: a pathway must manifest at least one causal law for one of its reactions, which is not manifested by its isolated parts. I finish, by noting that even though Bechtel and Richardson's arguments against the Strategy of Decomposition fail, it does not follow that biochemists' use of the Strategy of Decomposition is thereby warranted. There is a significant risk that the context sensitivity of pathways' local causal laws means that the assumption of causal law modularity is often incorrect. This context-sensitivity also leaves open the possibility of Pathway Emergence.

Chapter 2 - Two Types of Pathway Nonlinearity

2.1 Introduction

In *Discovering Complexity*, Bechtel and Richardson claim that nonlinearity contributes both to non-modularity and to the emergent behavior of biochemical pathways (henceforth: pathways). If they are correct, then nonlinearity results in significant challenges to the Strategy of Decomposition used by biochemists to discover pathway dynamic behaviours (recall section 1.5). In this chapter, I explain that Bechtel and Richardson's analysis uses the term nonlinearity in two senses. Sometimes 'nonlinearity' is used to refer to feedback between chemical reactants, at other times 'nonlinearity' is used to refer to a system of ordinary differential equations (i.e. 'ODEs') that do not satisfy the 'superposition principle' (see below). Bechtel and Richardson do not provide definitions for either type of nonlinearity. This chapter provides the conceptual groundwork for how the two types of nonlinearity apply to pathways, which will then be referenced throughout my thesis. The chapter also introduces an important part of the methodology used throughout my thesis: expressing pathways as sequences of 'elementary reactions'. The rate laws for elementary reactions are very simple and focusing on these equations enables a concise analyses of pathway nonlinearity, modularity and emergence.

The first type of nonlinearity shall be referred to as 'equation nonlinearity'. A pathway is equation nonlinear if its dynamics induce an ODE system that does *not* satisfy the superposition principle; in which case the system of equations cannot be solved by taking each of its variables to be the sum of independent contributions. Equation nonlinearity is a necessary condition for such exotic dynamic properties as limit cycles and multiple steady states. Bechtel and Abrahamsen's arguments for the need for 'dynamic mechanistic explanations' are based on the pathways having both types of nonlinearity (recall section 1.3). Bechtel and Richardson are sometimes referring to equation nonlinearity when they claim that 'nonlinearity' contributes to pathways having emergent behaviors. In chapter 4, I will use this chapter's analysis of equation nonlinearity to dispute Bechtel and Richardson's claim.

The second type of nonlinearity is chemical feedback (henceforth: feedback). Feedback occurs when the concentration of a reactant affects the rate of that

reactants own production. Positive feedback increases the rate of production and negative feedback decreases the rate of production. In my thesis, I shall be focussing on two key claims that Bechtel and Richardson make about the effects of feedback on pathways. Their first claim is that feedback significantly reduces the modularity of pathways. Their second claim is that feedback also contributes to pathways having emergent behaviours. In chapters 3 and 4, I will use this chapter's analysis to dispute both of their claims about feedback.

My analysis of the two types of pathway nonlinearity includes defining each type, analysing its prevalence and explaining how it occurs. I shall also explain how the two types of nonlinearity are related to each other and how they each affect pathway behaviors. Section 2.2 is on equation nonlinearity. I begin by explaining the superposition principle. I then show that a pathway will be equation nonlinear, unless it has a 'possible but highly improbable' structure. I explain that equation nonlinearity is a necessary but insufficient condition for a set of exotic dynamic properties, the most salient to biochemistry being multiple steady states and stable limit cycles. Section 2.3 is on feedback. I provide a definition of feedback between chemical reactants and I explain that systems biology has an accompanying mathematical criterion for identifying feedback in pathways. This criterion will be analysed, in order to gain further insights into the concept of feedback. I elicit what I take to be the criterion's underlying rationale: that feedback requires circular causal chains that can be identified by their pathway 'concentration ODEs'. My analysis also identifies a necessary condition for feedback between elementary reactions. Section 2.4 considers the relationship between the two types of nonlinearity. I explain that it is possible for a pathway to have feedback and *not* be equation nonlinear, but that this would require a 'possible but highly improbable' structure. Section 2.5 reviews the current consensus view on the necessary conditions for a pathway to have either a stable limit cycle or multiple steady states. I explain that the conditions include not only equation nonlinearity and feedback but also that a pathway's reaction rate curves have a 'sigmoidal shape'.

In the later chapters of my thesis, I will be arguing that Bechtel and Richardson overstate the implications of the two types of nonlinearity for the Strategy of Decomposition. But first it is necessary to be clear as to what these types of nonlinearity are, and to understand how they affect pathway dynamic behaviours.

2.2 The First Type of Nonlinearity: Equation Nonlinearity

2.2.1 The Superposition Principle

A mathematical function $f(y)$ is linear with respect to y if it satisfies the following requirements:

- additivity: $f(y_1 + y_2) = f(y_1) + f(y_2)$
- homogeneity: $f(\alpha y_1) = \alpha f(y_1)$

These requirements are combined in the 'superposition principle':

$$f(\alpha y_1 + \beta y_2) = \alpha f(y_1) + \beta f(y_2)$$

where α and β are constants and y is a number or vector. If f(y) does not satisfy the superposition principle then it is nonlinear[4] . This definition of linearity also applies to operators. An operator **L** is linear with respect to x (t) if:

$$\mathbf{L}(\alpha\, x_1(t) + \beta\, x_2(t)) = \alpha\, \mathbf{L}\, x_1(t) + \beta\, \mathbf{L}\, x_2(t)$$

where $x_1(t)$ and $x_2(t)$ are functions.

An equation that does not satisfy the superposition principle is nonlinear.

If a first order ODE is linear with respect to x(t), then it can be expressed in the *standard form* [5]*:*

$$\frac{dx}{dt} + p(t)x(t) = q(t)$$

This equation would be nonlinear if it contained an x(t) term raised to a power other than one (e.g. $x(t)^2$) or if the p (t) term was also a function of x (t). It is simple to show that the *standard form* satisfies the supposition principle:

Let $\mathbf{S} = \left[\frac{d}{dt} + p(t)\right]$ then the *standard form* can be expressed as $\mathbf{S}\, x(t) = q(t)$

$$\mathbf{S}\left(\alpha x_1(t) + \beta\, x_2(t)\right) = \frac{d(\alpha x_1 + \beta\, x_2)}{dt} + p(t)\left(\alpha x_1(t) + \beta\, x_2(t)\right)$$

$$= \alpha\left(\frac{dx_1}{dt} + p(t)x_1(t)\right) + \beta\left(\frac{dx_2}{dt} + p(t)x_2(t)\right)$$

[4] For the sake of readability, I do not always state the variable or function that linearity (nonlinearity) is with respect to. When referring to f(y) I am considering linearity (nonlinearity) with respect to y, for **L** x(t) with respect to x(t) and so forth.

[5] Ordinary differential equation means that there is a single independent variable. In the metabolic pathway models considered in this PhD the independent variable is time (t). These models assume that other possible independent variables such as temperature and pressure remain constant.

$$= \alpha \, \mathbf{S} \, x_1(t) + \beta \, \boldsymbol{S} x_2(t)$$

A system of equations is 'linked' if each of its equations contains at least one variable that is referenced in at least one of the system's other equations. A system of linked ODEs is nonlinear if any of its ODEs is nonlinear.

Throughout the remainder of this chapter, unless otherwise stated, the term 'equation nonlinearity' will only be used to refer to either an ODE that does not satisfy the superposition principle or an entity (such as a pathway) whose behaviour induces a system of linked ODEs one or more of which do not satisfy the superposition principle. My thesis will ignore systems whose dynamics do not correspond to a system of ODEs.

Examples

$\frac{dx}{dt} + 3x(t) = q(t)$ is a linear ODE. This is illustrated by:

$\frac{dx}{dt} + 3x(t) = 1$ has a solution $x(t) = \frac{1}{3}$ *and*

$\frac{dx}{dt} + 3x(t) = e^{-3t}$ has a solution $x(t) = te^{-3t}$ *therefore*

$\frac{dx}{dt} + 3x(t) = 1 + e^{-3t}$ has a solution $x(t) = \frac{1}{3} + te^{-3t}$

The above can be expressed more compactly using an operator **T**.

Let $\mathbf{T} = \left[\frac{d}{dt} + 3\right]$

$\mathbf{T}\, x\,(t) = q\,(t)$

$\mathbf{T}\left(\frac{1}{3}\right) + \mathbf{T}\,(te^{-3t}) = \mathbf{T}\left(\frac{1}{3} + te^{-3t}\right)$

An example of a nonlinear ODE is:

$$\frac{dx}{dt} + x^2(t) = q(t)$$

This does not satisfy the superposition principle:

Let $\mathbf{U} = \left[\frac{d}{dt} + (\,)^2\right]$

Then $\mathbf{U}\, x_1(t) = q_1(t)$ and $\mathbf{U}\, x_2(t) = q_2(t)$

Attempting to apply the superposition principle gives:

$$\mathbf{U}(x_1(t) + x_2(t)\,) = \frac{d(x_1 + x_2)}{dt} + \left(x_1(t) + x_2(t)\right)^2$$

$$= \left(\frac{dx_1}{dt} + {x_1}^2(t)\right) + \left(\frac{dx_2}{dt} + {x_2}^2(t)\right) + 2x_1(t)x_2(t)$$

$$= \mathbf{U}\, x_1(t) + \mathbf{U}\, x_2(t) + 2x_1(t)x_2(t)$$

Therefore $\mathbf{U}(x_1(t) + x_2(t)\,) \neq \mathbf{U}\, x_1(t) + \mathbf{U}\, x_2(t)$ because of the $2x_1(t)x_2(t)$ term.

2.2.2 Pathway Nonlinearity

I shall present an argument showing that, in practice, all pathways are equation nonlinear. Before doing so, I first need to explain the distinction between 'elementary' and 'stepwise' chemical reactions.

Biological networks are composed of intersecting pathways, each pathway is composed of a sequence of stepwise reactions and each stepwise reaction is composed of a sequence of elementary reactions.

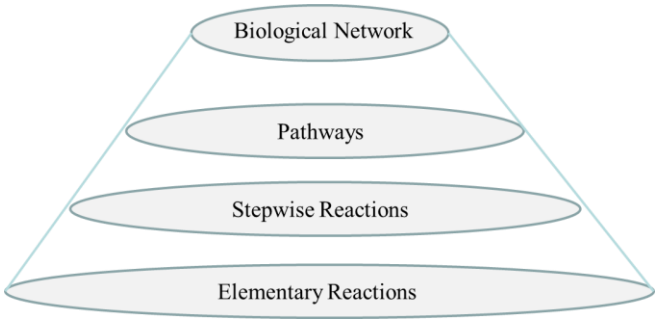


Fig 2.1 The Hierarchical Structure of a Biochemical Network.

Elementary reactions are the most basic type of chemical reaction; they cannot be decomposed into more basic chemical reactions. They are the building blocks for all other types of chemical reaction (Marin, Gregory, Yablonsky, 2011, p. 19). In biochemistry, there are two types of elementary reaction: unimolecular and bimolecular. In unimolecular reactions, single molecules rearrange into one or more product molecules. Bimolecular reactions involve two molecular entities combining and being transformed into product molecules. The 'Law of Mass Action' states that the rates of elementary reactions are proportional to the product of the concentrations of their reactants (Murray, 2002, p. 176)[6], for example for the bimolecular reaction {B + C → D + E} the rate law is:

[6] The accuracy of the Law of Mass Action for crowded intercellular solutions has been challenged. Bajzer et al. (2008) identify two modified Laws of Mass Action that have been proposed by system biologists. The first is based on 'fractal kinetics'. In this the rate coefficient is not constant but instead is a function of time; the law for the reaction A → B + C is then $v = k(t)[A][B]$. The second uses a power law approximation where $v = k(t)[A]^a[B]^b$ where a and b are constants. Throughout my thesis I will assume that the unmodified Law of Mass Action applies. However, mutatis mutandis, my thesis's arguments will also apply with either of these modified laws, as the arguments only rely on the rates of elementary reactions being a function of their reactants' concentrations.

$$v = \ k[B][C] \qquad (v = -\frac{d[B]}{dt} = -\frac{d[C]}{dt} = +\frac{d[D]}{dt} = +\frac{d[E]}{dt})$$

Getting accurate measurements of the rates of elementary reactions is often very problematic; for example their products often have transient existences before being consumed in other elementary reactions. The rate equations used in modelling pathway dynamics therefore usually only refer to stepwise reactions. Stepwise reactions consist of a sequence of several elementary reactions, and their rate laws correspond to the aggregation of their elementary reaction's rate laws. The rate laws for stepwise reactions can be very complex, often referencing several reactants and including several types of parameter (e.g. dissociation constants, Michaelis Menten constants etc.). However stepwise reactions are composed of elementary reactions and true stepwise rate laws can be deduced, at least in principle, from their elementary rate laws. Many of the most commonly used stepwise rate laws have been deduced by aggregating the rate equations of their elementary reactions (see section 2.2.3).

Throughout my thesis, I will explain pathway dynamics by explaining the dynamics of sequences of elementary reactions. I have chosen to do this solely for expository reasons. Whilst the rate laws of stepwise reactions are complex (see for example appendix 1), the rate laws for biochemical elementary reactions are much simpler having only one parameter, a rate constant, which is multiplied by the concentrations of either one or two reactants. Focussing on elementary reactions enables concise analyses, that can pick out the philosophically salient points about pathway dynamics, without being distracted by the complex rate laws that results from the aggregation of multiple elementary rate laws.

I will be focussing on metabolic pathways, however my analysis only makes use of general characteristics of biochemical pathways and should apply equally to other biological domains such as genetics. In considering pathway dynamics, I will use the same assumptions that are commonly used in systems biology: (i) that a pathway takes place within a spatially homogenous chemical solution (ii) those substrates[7] that are not produced in 'earlier' reactions of the pathway flow into the solution at fixed rates (iii) those products that are not involved in 'later' reactions diffuse from the solution at rates proportional to their concentrations. For the sake of readability,

[7] In biochemistry, the term 'substrate' refers to a chemical species that is acted upon by an enzyme.

I will omit these inflows and diffusions when specifying a pathway, as they do not affect this section's conclusions.

In the systems biology literature, the equation nonlinearity of pathways is simply taken to be self-evident:

> "Even the briefest consideration of the dynamics which arise from biochemical reactions kinetics underpinning almost all cellular processes reveals the ubiquity of nonlinear phenomena. The fundamental law of mass action states that when two molecules A and B react upon collision with each other to form a product C
>
> $$A + B \rightarrow C.$$
>
> the rate of the reaction is proportional to the number of collisions per unit time between the reactants and the probability that the collision occurs with sufficient energy to overcome the free energy of activation of the reaction. Clearly, the corresponding differential equation $d\frac{C}{dt} = k\,AB$ [...] is nonlinear" (Cosentino and Bates, 2012, p. 67)

I will now present an argument for pathways being equation nonlinear, that is based on the above quote. My argument is in five stages.

Stage 1. Only Elementary Reactions Need be Considered When Evaluating the Equation Nonlinearity of Pathway Dynamic Behaviours.

As I explained above, elementary reactions are the basic chemical reactions that occur, and stepwise reactions are just composed of sequences of these reactions. An analysis of equation nonlinearity can therefore be carried out at the level of elementary reactions.

Stage 2. Bimolecular Elementary Reactions are Equation Nonlinear

The sequences of elementary reaction that biochemistry classifies as pathways comprise of many elementary reactions, and it is uncontroversial to state that a very high proportion of these are likely to be bimolecular. I will now demonstrate that the rate laws for bimolecular reactions are nonlinear.

In rate equations, [X] stands for the concentration of chemical species X at a particular time. When needed, a time index can be added to this notation, in which case $[X]_t$ stands for the concentration of X at time t and $[X]_0$ is a constant equal to the concentration of X at the beginning of the reaction. For the elementary reaction A + B → C:

$$\frac{d\,[C]}{dt} = k_1[A][B]$$

where k_1 is a rate constant. [A] and [B] are both functions of [C] and hence this ODE does not satisfy the superposition principle. The actual relationship between [A], [B] and [C] will depend on experimental or *in vivo* conditions. Let us consider the case where $[C]_0$ is equal to zero and the reaction takes place in a system where there is no exchange of matter with the surroundings then:

$$[A]_t = [A]_0 - [C]_t$$
$$[B]_t = [B]_0 - [C]_t$$

as for every molecule of C produced, one molecule of A and one molecule of B are consumed.

Substituting into the rate law equation gives:

$$\frac{d\,[C]_t}{dt} = k_1\{[C]_t{}^2 - (\,[A]_0 + [B]_0)[C]_t + [A]_0[B]_0\}$$

Given that $[A]_0$ and $[B]_0$ are constants:

$$\frac{d\,[C]_t}{dt} = k_1[C]_t{}^2 - w_2[C]_t + w_3$$

Where $w_2 = k_1(\,[A]_0 + [B]_0)$ and $w_3 = k_1(\,[A]_0[B]_0)$

This does not satisfy the supposition principle because of the $[C]_t{}^2$ term.

By contrast, the rate laws for unimolecular reactions are modelled using linear ODEs. For example, the rate law for the reaction {A → B + C} is:

$$\frac{d\,[A]}{dt} = -\,k_1[A].$$

Stage 3. Pathways Induce a System of 'Concentration ODEs'

Biochemistry takes there to be a law of nature for homogenous chemical solutions, which I will term as the 'Kinetic Law of Composition'.[8] According to this law, the rate of change of the concentration of a chemical species is equal to the sum of the rates of those reactions that create that chemical species minus the rates of those reactions that consume that chemical species (this is for closed systems; for open systems the net flows into the pathway also need to be added). Consider the following toy closed pathway from B to I:

	Chemical Equation	Rate Law
Step 1	**B** + C → D + E	$v_1 = k_1[B][C]$
Step 2	E + F → G	$v_2 = k_2[E][F]$
Step 3	G + H → C + **I**	$v_3 = k_3[G][H]$

where "k_x" is the rate constant of reaction step x. C is consumed in step 1 and produced in step 3. According to the Kinetic Law of Composition:

$$\frac{d[C]}{dt} = rate\ of\ step\ 3 - rate\ of\ step\ 1$$

$$= \quad v_3 \quad - \quad v_1 \quad = \quad k_3[G][H] - k_1[B][C]$$

The above equation is the 'concentration ODE' for C in this pathway. A pathway induces a system of linked ODEs that incorporates the rate laws of its reactions. There is a separate concentration ODE for each of the pathway's chemical species. For example, in the case of the toy pathway, the corresponding linked system consists of eight concentrations ODEs:

$$\frac{d[B]}{dt} = -v_1 \quad , \quad \frac{d[C]}{dt} = v_3 - v_1 \quad , \quad \frac{d[D]}{dt} = v_1 \quad ,$$

$$\frac{d[E]}{dt} = v_1 - v_2 \quad , \quad \frac{d[F]}{dt} = -v_2 \quad , \quad \frac{d[G]}{dt} = v_2 - v_3$$

$$\frac{d[H]}{dt} = -v_3 \quad , \quad \frac{d[I]}{dt} = v_3 \quad .$$

[8] Sauro (2014, p. 50) refers to this law of composition simply as ' Mass Balance'.

A dynamic behaviour of a pathway can be deduced from the pathway's concentration ODEs plus initial conditions.

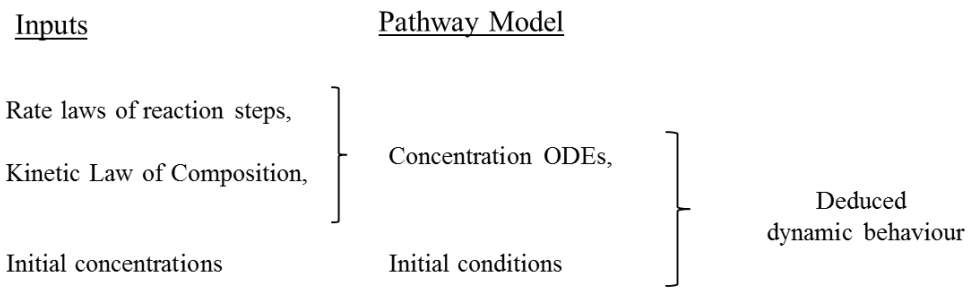


Fig 2.2 Diagram illustrating how concentration ODEs are used to deduce pathway dynamic behaviours for a closed system.

Stage 4. A System of 'Concentration ODEs' That References Bimolecular Rate Laws Will be Equation Nonlinear

(i) The concentration ODEs for a pathway reference that pathway's elementary rate laws. (*from stage 3*)

(ii) It is sufficient for a concentration ODE to be equation nonlinear, that one of the rate laws it references is equation nonlinear. (*this follows from the superposition principle*)

(iii) A concentration ODE that references a bimolecular rate law will be equation nonlinear. (*from (ii) and stage 2*).

(iv) A system of linked ODEs is equation nonlinear if one or more of its ODEs is equation nonlinear. (*this follows from the superposition principle*)

Hence

(v) A system of 'concentration ODEs' that references bimolecular rate laws will be equation nonlinear. (*from (iii) and (iv)*).

Stage 5. There Are 'Possible but Highly Unlikely' Pathway Structures That are Equation Linear

It is theoretically possible for there to be pathways consisting solely of unimolecular reactions i.e. where single molecules unilaterally undergo a sequence of decompositions, e.g.

$$A \rightarrow B + C$$

$$B \rightarrow D + E$$

$$D \rightarrow F$$

Given that the rate laws for unimolecular reactions are equation linear, it follows that the pathway's system of linked concentrations ODEs will also be equation linear. But such a pathway structure is 'possible but highly unlikely'. By this I mean that it appears to be a contingent fact that such structures do not feature in the pathways of biological entities. This can be evidenced by perusing such pathway databases as BioPath, KEGG and Reactome.[9] It may also be the case that other 'possible but highly unlikely' pathway structures can be concocted that would also be equation linear. For example, it appears theoretically possible for the nonlinear terms within each concentration ODE to cancel out, leaving linear ODEs.

This completes my argument for the conclusion that:

Pathways are equation nonlinear (unless they have a possible but highly unlikely structure).

2.2.3 The Nonlinearity of Stepwise Reactions

Given that chemical reactions occur at the level described by elementary reaction equations, it is sufficient in explaining the nonlinear dynamics of pathways to stay at this level. However, it is still useful to consider stepwise rate laws as it is these, for the most part, that are used in actual pathway models. It also provides an opportunity to highlight the importance of 'sigmoid' shaped reaction curves for the occurrence of some exotic dynamics. As will be explained in my section 2.5, systems biologists sometimes use the term 'nonlinear' to refer to these reaction curves.

Before doing this, I need to explain what a reversible reaction is. A 'reversible chemical reaction' consists of two reactions occurring simultaneously. For example, if the following two reactions are both occurring:

$P + Q \rightarrow R + S$ ('forward reaction')

$R + S \rightarrow P + Q$ ('backward reaction')

then these are represented by a chemical reaction equation containing a double headed arrow '↔' i.e.

$P + Q \leftrightarrow R + S$

[9] www.molecular-networks.com/databases/biopath, www.genome.jp/kegg/pathway.html, www.reactome.org

I will now provide an overview of the Michaelis-Menten and Hill rate laws. Many of the rate laws used in modelling metabolic pathways are based either on the Hill or Michaelis-Menten equations. For example, Teusink et al.'s (2000) highly cited model of glycolysis has twelve rate laws of which seven are classified as reversible Michaelis-Menten and one is classified as an irreversible Hill equation (see my appendix 1). The nonlinearity of both types of rate law is clear given that the substrate concentration terms are raised to powers (other than one). Given the high frequency with which rate laws based on Michaelis-Menten and Hill rate laws are used, their nonlinearity provides a good demonstration of the nonlinearity of metabolic pathway models.

The Michaelis-Menten equation is derived by aggregating two elementary reactions and making a simplifying assumption. Consider a reaction in which an enzyme (E) catalyses the transformation of a substrate (S) into a product (P). The stepwise reaction is:

$$S \rightarrow P \quad \text{(enzyme: E)}$$

which is composed of two reactions:

$$E + S \leftrightarrow ES$$
$$ES \rightarrow P + E$$

where ES is an intermediate enzyme-substrate complex. If the assumption is made that the ES complex rapidly reaches a steady state, then it is a simple derivation from elementary rate equations to the Michaelis-Menten equation which has the form:

$$\frac{d\,[P]}{dt} = \frac{a\,[S]}{b + [S]}$$

where a and b are constants (Cornish-Bowden, 2011, p. 29).

There are several more complex equations that are also classified as being 'Michaelis-Menten'. These take account of additional factors such as: multiple substrates, product inhibition and reversible reactions. The derivations for these equations share the key assumption that steady states are rapidly achieved. The forms of these equations resemble that of the basic equation described above.

One source of further complexity can be cooperativity effects, where the binding of one substrate to an enzyme molecule increases the probability of other substrates binding to the same enzyme molecule. This can lead to reaction rates that are

ultrasensitive to changes in substrate concentration. The simplest irreversible stepwise reactions of this type are modelled using the Hill equation which has the form:

$$\frac{d\,[P]}{dt} = \frac{a\,[S]^n}{b + [S]^n}$$

where n is a measure of cooperativity and a and b are constants (Cornish-Bowden, 2011, p. 286). The Hill equation captures the way that cooperation can change the shape of reaction rate curves from hyperbolic to sigmoid.

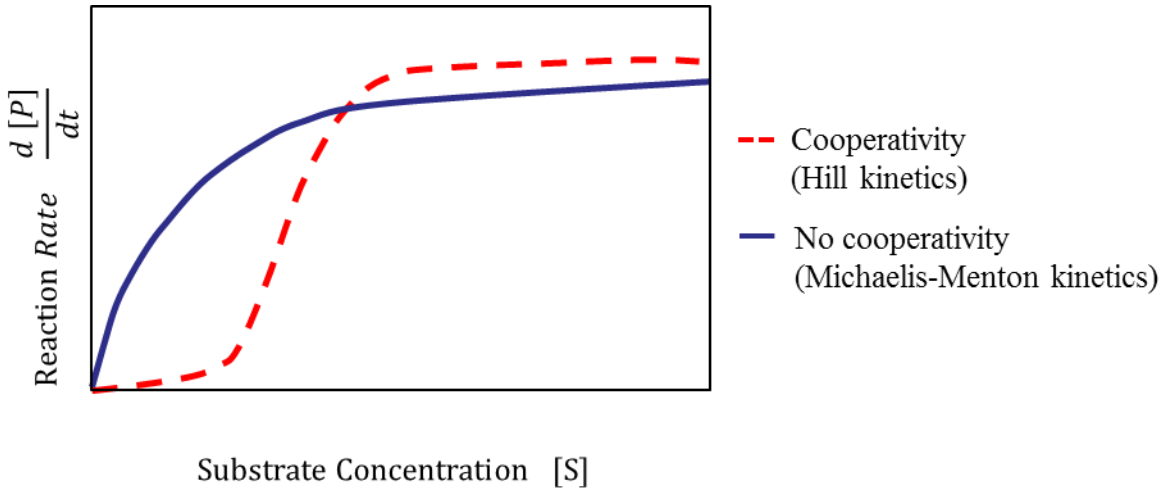


Fig 2.3 Graph comparing Hill kinetics with Michaelis-Menton kinetics. (Sauro, H. 2012, p. 157).

The sigmoid shape is important for the occurrence of exotic behaviour. This is illustrated by an example from Smits et al. (2006) of a simple gene network in which the rates of both the production and deactivation of a protein P are proportional to its concentration [P]. The rate of production is described by a Hill type equation whilst the rate of deactivation is described by a linear equation. When the rates of the production and deactivation are equal, then d [P]/d t = 0 and the system is in a steady state. The graph below illustrates how a sigmoid shape can lead to three steady states (corresponding to the three points where the lines intersect).

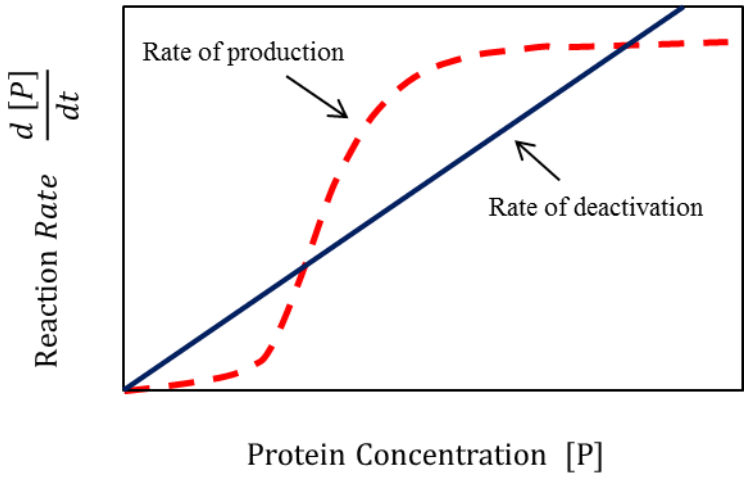


Fig 2.4 The reaction rate curves for a simple gene network (adapted from Smits et al. (2006, p. 261).

The importance of sigmoid rate curves to exotic dynamics will be further discussed in section 2.5. This completes my explanation of how pathways are equation nonlinear. I will now consider some of the consequences of equation nonlinearity for the behaviour of pathways and the explanation of their dynamics.

2.2.4 Exotic Pathway Behaviours

Pathway equation nonlinearity is a necessary but insufficient condition for a set of exotic dynamic properties. The two that appear to be most salient to biochemistry are:

1) multiple steady states
2) stable limit cycles - these correspond to the dynamics of self-sustained oscillations.

I shall provide an overview of these two dynamic properties, explaining their importance to biochemistry and highlighting their dependence on equation nonlinearity.

Multiple Steady States. In vivo systems are open systems, exchanging both matter and energy with surrounding systems. Within their pathways there are net flows of materials and their reactions are therefore not in equilibrium. However, a pathway can be at a 'steady state', where the concentrations of reactants and products remain constant over time. Let us consider a pathway that induces a system of concentration ODEs. The phase space of a pathway will have a dimension for the concentration of each of the pathway's chemicals. A trajectory is then a time ordered set of states, with each state specifying the concentrations of all of the pathway's chemicals. Let **S** be a

column vector of the concentrations of these chemicals. At the steady states of the phase space:

$$\frac{d[S_1]}{dt} = \frac{d[S_2]}{dt} = \frac{d[S_3]}{dt} = \ldots\ldots = 0$$

If a biochemical system's ODEs were all linear then they could be written in matrix form. For example:

$$d[S_1]/dt = a[S_1] + b[S_2]$$
$$d[S_2]/dt = c[S_1] + d[S_2]$$

could be written as: $\dot{\boldsymbol{S}} = \begin{bmatrix} a & b \\ c & d \end{bmatrix} \boldsymbol{S}$. At steady state $\dot{\boldsymbol{S}} = 0$, and the only solutions for such systems are either (i) a single steady state point at the origin of the phase space (i.e. $\boldsymbol{S} = 0$) if the matrix is nonsingular or (ii) a continuum of steady state points if the matrix is singular (see for example Strang (2009, p. 133-144) on the nullspaces of linear systems).

By contrast equation nonlinear systems can have multiple isolated steady states. The graph below illustrates an output from a model by Steuer and Junker (2008) of glycolysis, that identifies three steady states. This model captures key aspects of the dynamics of glycolysis using only two variables: one for the concentration of ATP, the other for the concentration of glyceraldehyde-3-phosphate (henceforth: TP). For two dimensional models, the steady states can be analysed by plotting direction field vectors and the *x* and *y*-nullclines. Direction field vectors indicate the direction that a trajectory would proceed from that point. The *x*-nullcline is the set of points corresponding to $\frac{dx}{dt} = 0$ and the y-nullcline is the set of points corresponding to $\frac{dy}{dt} = 0$. In this graph, the *x*-axis is [ATP] and the y-axis is [TP] and hence the nullclines correspond to $\frac{d[ATP]}{dt} = 0$ and $\frac{d[TP]}{dt} = 0$ respectively.

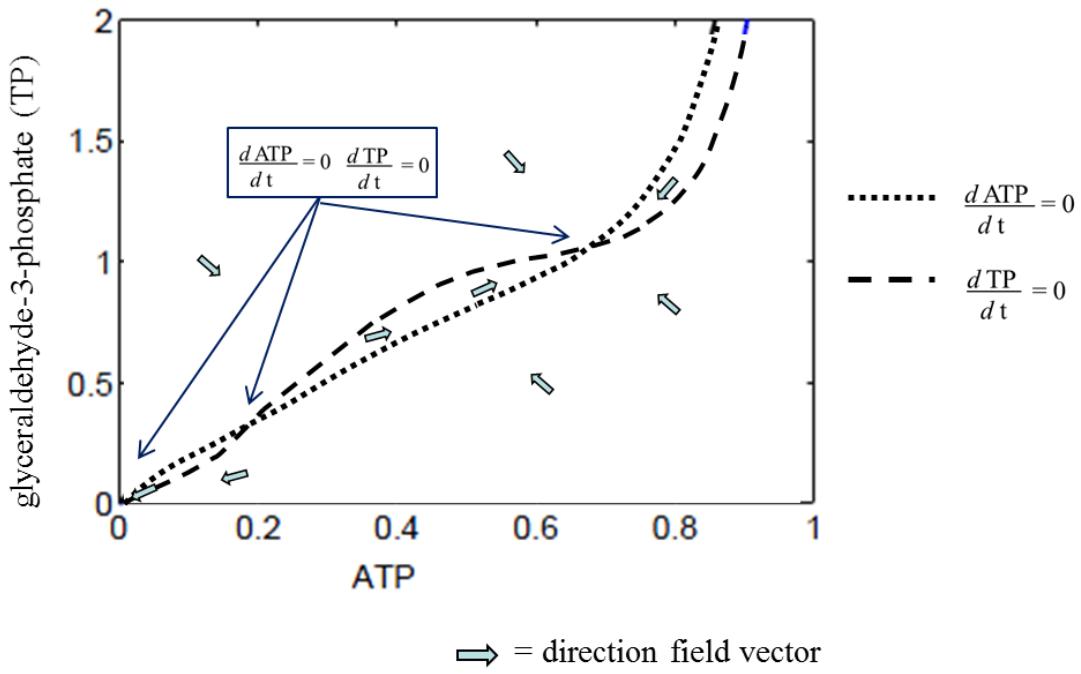


Fig 2.5 The nullclines of a minimal model of glycolysis. (Steuer and Junker, 2008) – adapted from diagrams on pages 173 and 174.

The model uses three reaction rate equations, the first being a Hill type equation, the second a bimolecular equation and the third a Michaelis-Menten equation. Steady states occur at the intersections of the two nullclines. There are three steady states (as indicated by the thin arrows). The two outer steady states are asymptotically stable meaning that they attract nearby points whereas the middle steady state is unstable and repels nearby points. The phase space is divided into two basins of attraction, one for each stable steady state. This is an example of a *bistable* system. If a bistable system is at one steady state, it will only switch to the other steady state if there is a perturbation that moves the system into the other basin of attraction. Bistability is an important system property for cellular signalling networks as it enables switch-like behaviour and 'memories' of perturbations.

Stable Limit Cycles. A stable limit cycle is an isolated closed trajectory that attracts all neighbouring trajectories. This corresponds to the dynamics of self-sustained oscillators, which are systems whose oscillations are internally determined, rather than being determined by an external signal. Their "essential feature" (Pikovsky et al., 2001, p. 29) is that after a small perturbation, they return to their original oscillation. This makes them relatively robust. Biology has many examples of systems whose dynamics correspond to stable limit cycles; including glycolysis and cell division. Only nonlinear systems can have limit cycles.

Linear systems can also have oscillations, for example simple harmonic oscillations. However, such periodic trajectories are neighboured by other closed trajectories. If a state is perturbed it will not be attracted back to its original cycle, instead it will remain perturbed and oscillate at its new amplitude. The oscillations of linear systems are therefore not robust and are unlikely to be viable in noisy 'biological reality'.[10] The diagram below illustrates the difference between limit cycles and linear closed trajectories.

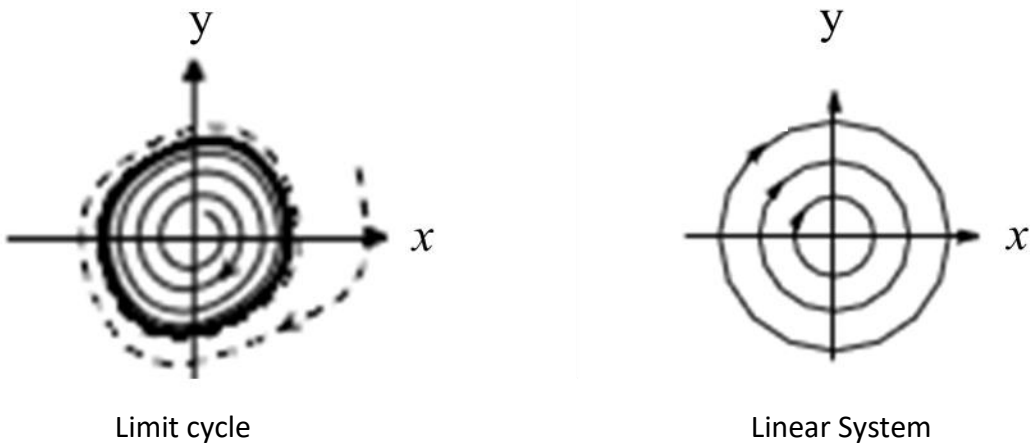


Fig 2.6 Comparison of the trajectories neighbouring a limit cycle and the trajectories for a linear system. (Pikovsky et al., 2001, p. 30 & p. 36)

In the limit cycle diagram, the bold curve is a limit cycle and the other two curves are trajectories being attracted towards the limit cycle. In the linear system, there is a continuum of closed trajectories.

Pathways induce systems of nonlinear ODEs that cannot usually be analytically solved (i.e. there is no set of well-known mathematical operations that will enable exact solutions to be readily calculated). Instead numerical methods or simulation need to be used to find approximate solutions and to identify structures within their phase space such as steady-states and limit cycles. This has implications for providing satisfactory explanations of pathway dynamics. Purely qualitative explanations, such as those proposed by Machamer, Darden and Craver (2000)

[10] Cosentino and Bates (2012, p. 154). A similar argument applies to Lotka-Voterra oscillations which can occur in nonlinear systems: "By reason of their stability or regularity, most biological rhythms correspond to oscillations of the limit cycle type rather than Lokta-Volterra oscillations." (Goldbeter, 1996, p. 6).

(recall section 1.3), will not be able to account for some of the dramatic differences that sometimes occur in pathway behaviours. For example, they cannot account for why some pathways oscillate and others do not. Similarly, they cannot account for how small perturbations can lead to switch-like changes in behaviour. As Bechtel and Abrahamsen (2005) argue 'dynamic mechanistic explanations' are needed in these cases (recall section 1.3). Dynamic mechanistic explanations have two parts:

a) a qualitative explanation of the pathway's grounding mechanism
b) a mathematical explanation of the pathway's dynamics. This includes a statement of that pathway's system of nonlinear ODEs and a description of the solutions to that system (usually determined by simulation). The variables in the ODEs should refer to entities stated in the qualitative explanation.

Equation nonlinearity therefore has important consequences for both pathway behavior and the explanation of pathway dynamics. Equation nonlinearity is a necessary but insufficient condition for a set of exotic behaviours. Equation nonlinearity also means that explanations of pathway behaviours will often need to be dynamic mechanistic explanations.

In conclusion, equation nonlinearity concerns ODEs that do not satisfy the superposition principle. I have explained that bimolecular rate laws are equation nonlinear and that this leads to pathways also being equation nonlinear (unless they have a highly unlikely structure). I have also highlighted that some equation nonlinear systems include reactions with sigmoid shaped rate curves and that these affect exotic dynamics. My analysis has illustrated the methodology of focusing on elementary reactions. As we shall see, treating pathways as sequences of elementary reactions (rather than stepwise reactions) enables concise studies of such subjects as pathway: nonlinearity, modularity, in principle predictability and reducibility. My analysis also helps to illustrate a likely limitation for the conclusions of such studies: that the scope for identifying universal generalisations will be very limited. This is because it may often be the case that a 'possible but highly unlikely' pathway structure can be concocted to provide a counter-example to a putative universal claim. Finally, I have explained that equation nonlinearity has important consequences for both pathway behavior and the explanation of pathway dynamics.

2.3 The Second Type of Nonlinearity: Chemical Feedback

Discovering Complexity helped to bring the importance of feedback to the attention of the philosophy of biology. Bechtel and Richardson do not provide a definition of 'feedback' in pathways but based on how the term is used it refers to 'chemical feedback'. Chemical feedback occurs when the 'concentration of some species affects the rate of its own production.' (Epstein and Pojman, 1998, p. 23).[11] The rate of production of a species is equal to its 'rate of creation' minus its 'rate of being consumed'. Positive chemical feedback increases the rate of production and negative chemical feedback decreases the rate of production. Bechtel and Richardson sometimes use the term 'nonlinear' to refer to chemical feedback. A pathway is 'nonlinear' in this sense, if there is chemical feedback between its reactants, otherwise it is linear. A linear pathway is 'sequentially organised' and a nonlinear pathway is 'nonsequentially organised'.[12] There are other types of feedback that are not 'chemical' but may also be relevant to metabolic pathways, for example 'thermal feedback' in which the heat generated by a reaction increases reaction rates. However, these other types of feedback are not part of Bechtel and Richardson's analysis and do not appear to raise any further substantial philosophical points; hence they will not be addressed in my PhD. This section is *solely* on chemical feedback. The relationship between chemical feedback and equation nonlinearity will be discussed in my section 2.4.

In section 2.3.1, I provide an overview of chemical feedback (henceforth: feedback). I explain that systems biology specifies a mathematical criterion for identifying feedback (henceforth: the 'Systems Biology Criterion for Feedback'). This is a mathematical counterpart to the definition that chemical feedback occurs when the 'concentration of some species affects the rate of its own production.' I examine this criterion, as a means to further analyse the concept of feedback. I elicit what I take to be the rationale underlying this criterion: that feedback loops are circular causal chains that can be identified by their 'causal' concentration ODEs. In section 2.3.2, I argue that the criterion needs to be modified, as it misidentifies individual bimolecular reactions as having positive feedback. In section 2.3.3, I identify a

[11] Epstein and Pojman use the term 'feedback' rather than 'chemical feedback'.
[12] For example, Bechtel and Richardson 2010 –p xxxviii. NB Bechtel and Richardson are discussing mechanisms rather than pathways, but I take it that pathways are mechanisms e.g. the glycolytic pathway is the mechanism for 'the production of pyruvate and ATP from glucose.'

necessary condition for feedback between reactions. In section 2.3.4. I will provide an account of 'allosteric feedback', and explain that this is also a type of chemical feedback.

2.3.1 Feedback Between Chemical Reactions

Feedback occurs by reactions affecting the concentrations of reactants. Feedback can occur within single reactions, for example:

$$Y + X \rightarrow 2X$$

Here there is positive feedback on X, as increasing [X] increases the rate of production of X (Epstein and Pojman, 1998, p. 96). Feedback can also occur between reaction steps within a pathway, with the rate of a 'later' step affecting the rate of an 'earlier' step. An 'earlier' step has a lower step number than a 'later' step. The numerical ordering of a pathway's steps is the temporal order of the reactions that produce the target molecule (e.g. G) starting from the source molecule (e.g. B). For example, consider a simple pathway of elementary reactions from B to G:

Step 1 $A + \mathbf{B} \rightarrow C$

Step 2 $C + D \rightarrow E$

Step 3 $E + A \rightarrow \mathbf{G}$

There is negative feedback on C as increasing [C] increases the rate of production of E (step 2), which increases the rate of consumption of A (step 3) and leads to a reduction in the rate of production of C (step 1). This illustrates how the rate of a 'later' step (step 2) affects the rate of an 'earlier' step (step 1). It is feedback *between* reactions, rather than *within* a reaction, that is relevant to Bechtel and Richardson's analysis of the Strategy of Decomposition.

The 'Systems Biology Criterion for Feedback' uses a Jacobian matrix to identify feedback.[13] When applied to the modelling of pathway kinetics, a Jacobian matrix's elements are partial derivatives of the rate of production of chemical species x_i with

[13] For a set of *m* equations in *m* variables:

$$\begin{aligned} y_1 &= f_1(x_1, \ldots\ldots\ldots x_m) \\ &\vdots \\ y_m &= f_m(x_1, \ldots\ldots\ldots x_m) \end{aligned}$$

A Jacobian matrix is defined as:

$$\begin{bmatrix} \frac{\partial f_1}{\partial x_1} & \cdots & \frac{\partial f_1}{\partial x_m} \\ \vdots & \ddots & \vdots \\ \frac{\partial f_m}{\partial x_1} & \cdots & \frac{\partial f_m}{\partial x_m} \end{bmatrix}$$

respect to the concentration of chemical species x_j . Henceforth the term 'Jacobian matrix' will be used to refer only to matrices of this type. The matrix element a_{ij} is defined as:

$$a_{ij} = \frac{\partial f_i}{\partial [x_j]} \quad \text{where} \quad f_i = \frac{d[x_i]}{dt}$$

f_i is the concentration ODE for x_i. Consider the above pathway from B to G. 'A' is consumed in reaction steps 1 and 3. The Law of Mass Action states that the rate of each step is proportional to the product of the concentrations of their reactants. Hence 'A' is consumed in step 1 at a rate equal to $k_1[A][B]$ and is consumed in step 3 at a rate equal to $k_3[A][E]$. Given the Kinetic Law of Composition (recall section 2.2):

$$f_1 = \frac{d[A]}{dt} = -k_1[A][B] - k_3[A][E] \qquad a_{AE} = \frac{\partial f_1}{\partial [E]} = -k_3[A]$$

Similarly:

$$f_2 = \frac{d[C]}{dt} = \quad k_1[A][B] - k_2[C][D] \qquad a_{CA} = \frac{\partial f_2}{\partial [A]} = k_1[B]$$

$$f_3 = \frac{d[E]}{dt} = k_2[C][D] - k_3[A][E] \qquad a_{EC} = \frac{\partial f_3}{\partial [C]} = k_2[D]$$

The Jacobian matrix for a biochemical system with m substrates is a square matrix of dimension m:[14]

$$\begin{bmatrix} \frac{\partial f_1}{\partial [x_1]} & \cdots & \frac{\partial f_1}{\partial [x_m]} \\ \vdots & \ddots & \vdots \\ \frac{\partial f_m}{\partial [x_1]} & \cdots & \frac{\partial f_m}{\partial [x_m]} \end{bmatrix}_y$$

where y is the point in the phase space at which the matrix is evaluated. If a_{ij} is non-zero then $[x_j]$ is taken to directly affect $[x_i]$. If a_{ij} is positive (negative) then increasing the concentration of x_j increases (decreases) the rate of production of x_i. Feedback

[14] There are no matrix operations involved in identifying feedback loops. As such the elements a_{ij} could also be used if they belonged to an alternative data structure such as a table. However, the matrix form is useful in attempting to define necessary conditions for self-sustained oscillations and multistationarity.

occurs when there is a set of non-zero matrix elements that connect in a loop i.e. a_{ij} a_{jk} a_{kl} ..... $a_{ni} \neq 0$ (Tyson, 2005, p. 234). The sign of the product of a loop's elements indicates whether the feedback is positive or negative. For example, if x_i inhibits the production of x_j, and x_j inhibits the production of x_i, then $a_{ij} < 0$, $a_{ji} < 0$ and $a_{ij}\, a_{ji} > 0$, showing that there is positive feedback. In the above toy pathway example, there is a negative feedback loop as $a_{AE}\, a_{EC}\, a_{CA} = -k_3[A]k_1[B]\, k_1[D] < 0$ (it is less than zero given that rate constants and concentrations are always positive)

I will now argue that the criterion's underlying rationale is that:

(i) feedback loops are circular causal chains

(ii) the direct causal links in these chains are between reactants

(iii) concentration ODEs are 'causal equations' that can be used to identify these circular causal chains.

The chemical equation for a reaction specifies a direct causal relationship between the reactants and the products. For example { A + B → C } specifies that [A] and [B] are both direct causes of [C]. [A] is a *direct cause* of [C], if changing the concentration of A whilst holding fixed the concentrations of all reactants/products other than A and C, leads to a change in [C] (recall section 1.3).

Feedback requires the existence of a feedback loop, which is a circular causal chain. The reaction steps *in* a feedback loop are those with the direct causal relationships that form this chain. Let us consider the following toy pathway:

Step 1 A + B → C

Step 2 C + D → E

Step 3 E → A + F

There is *positive* feedback on A, as increasing [A] increases the rate of production of C (step 1), which increases the rate of production of E (step 2) and leads to an increase in the rate of production of A (step 3). The direct causes in the loop are identified by the 'Systems Biology Criterion for Feedback':

[A] *is a direct cause of* [C] $a_{CA} = \frac{\partial f_2}{\partial [A]} = k_1[B]$

[C] is a direct cause of [E] $a_{EC} = \frac{\partial f_3}{\partial [C]} = k_2[D]$

[E] is a direct cause of [A] $a_{AE} = \frac{\partial f_1}{\partial [E]} = k_3$

There is positive feedback as $a_{AE}\, a_{EC}\, a_{CA} > 0$. The positive feedback loop links A, E, and C. The reaction steps in this loop can be identified by the rate constants of the corresponding matrix elements. For example, the equation for a_{CA} has the rate constant for step 1 i.e. A is a direct cause of C in the reaction {A + B → C}. There is feedback *on* a species x_i, if the concentration of x_i affects the rate of x_i's production. This is the case for all the reactants of the reaction steps *in* a feedback loop.

The 'System's Biology Criterion for Feedback' implicitly requires that a pathway's concentration ODEs are all 'causal equations' i.e. where each equation's right-hand side variables are direct causes of the quantity on the left-hand side. $[x_j]$ is a direct cause of $\frac{d[x_i]}{dt}$, if x_j is a reactant in a reaction that has either x_i as a product (x_i is created) or has x_i as a co-reactant (x_i is consumed). Consider the above toy pathway. Applying the Law of Mass Action and the 'Kinetic Law of Composition':

$$f_2 = \frac{d[C]}{dt} = rate\ of\ reaction\ step\ 1 - rate\ of\ reaction\ step\ 2$$

$$= k_1[A][B] - k_2[C][D]$$

This is a causal equation as $[A]$, $[B]$, $[C]$ and $[D]$ are direct causes of $\frac{d[C]}{dt}$ (as they are reactants in chemical reactions in which C is either produced or consumed). When a Jacobian matrix's f_i terms are all causal ODEs then a non-zero element a_{ij} identifies that $[x_j]$ is a direct cause of $\frac{d[x_i]}{dt}$. Hence direct causes of $\frac{d[x_i]}{dt}$ are 'picked out' by partially differentiating x_i's concentration ODE with respect to each of the concentrations of the pathway's reactants.

I will now demonstrate how using 'non causal' concentration ODEs can lead to the criterion failing to correctly identify feedback loops. Consider again the toy pathway:

Step 1 A + B → C
Step 2 C + D → E
Step 3 E → A + F

Let this pathway take place in a closed system with the starting concentration of D = $[D]_o$ and the starting concentrations of E and F both equal to zero. The concentration of E at time *t* is given by:

$$[E]_t = [D]_o - [D]_t - [F]_t$$

where $[D]_o - [D]_t$ is the amount of [E] that has been created by time *t* and $[F]_t$ is the amount of [E] that has been consumed by time *t*. As explained in my section 2.2.2, the time indexes of concentrations are not normally explicitly stated in reaction rate equations; the default being that all concentrations are at time *t*. Hence:

$$[E] = k_D - [D] - [F]$$

where k_D is a constant equal to D_o.

The causal concentration ODEs for A, C and E are:

$$f_1 = \frac{d[A]}{dt} = -k_1[A][B] + k_3[E] \qquad a_{AE} = \frac{\partial f_1}{\partial [E]} = k_3$$

$$f_2 = \frac{d[C]}{dt} = \quad k_1[A][B] - k_2[C][D] \qquad a_{CA} = \frac{\partial f_2}{\partial [A]} = k_1[B]$$

$$f_3 = \frac{d[E]}{dt} = k_2[C][D] - k_3[E] \qquad a_{EC} = \frac{\partial f_3}{\partial [C]} = k_2[D]$$

The pathway has a positive feedback loop linking A, E and C; with the Jacobian elements $a_{AE} > 0$, $a_{EC} > 0$, $a_{CA} > 0$. However if in the equation for f_1, the [E] term is substituted by $k_D - [D] - [F]$ then:

$$f_1 = \frac{d[A]}{dt} = -k_1[A][B] + k_D - [D] - [F] \qquad a_{AE} = \frac{\partial f_1}{\partial [E]} = 0$$

The equation for f_1 is now a non-causal ODE as it references [F] which is not a direct cause of changes in [C] (F is created in step 3 but is not a reactant in any reaction that creates or consumes C). But now that the equation for f_1 is non-causal, it appears that there is no feedback loop linking A, E and C as $a_{AE}a_{EC}a_{CA} = 0$.

2.3.2 The False Identification of Positive Feedback

In this section, I highlight a limitation of the Systems Biology Criterion for Feedback: it incorrectly identifies that there is positive feedback *within* bimolecular reactions. As such, the criterion needs to be modified, so that it only identifies feedback *between* reactions. In its current form, the criterion is a necessary but insufficient condition for feedback between reactions. The Systems Biology Criterion for Feedback has been used to formally prove that positive feedback is a necessary

condition for the existence of multiple steady states (Soulé, 2003). But given the above problem with bimolecular reactions, such proofs do not reveal whether feedback between reactions is necessary for multiple steady states. Consider a simple system with only two chemical species B and C:

$$B + C \rightarrow D$$

with

$$f_1 = \frac{d[B]}{dt} = -k_1[B][C]$$

$$f_2 = \frac{d[C]}{dt} = -k_1[B][C]$$

This will give the following elements for a Jacobian matrix whose first column elements are partial derivatives with respect to B and whose second column elements are partial derivatives with respect to C.

$$a_{BC} = \frac{\partial f_1}{\partial [C]} = -k_1[B]$$

$$a_{CB} = \frac{\partial f_2}{\partial [B]} = -k_1[C]$$

Therefore $a_{BC}a_{CB} > 0$ and a 'positive feedback loop' is identified connecting B and C.

The only confirmation I have found for this problem is Soliman (2013) entitled 'A stronger necessary condition for the multistationarity of chemical reaction networks'. This does not provide an analysis of the Jacobian criterion for feedback but it does state that:

> "the existence of a positive loop in the Jacobian of the ODE system, is almost always satisfied. Indeed, any binary reaction equipped with Mass-Action kinetics [*i.e. any bimolecular reaction obeying the Law of Mass Action*] will lead to the mutual inhibition of the two substrates, and thus create such a loop." (Soliman, 2013, p. 2290).

Soliman claims that because of this, the ODE modelling community has 'turned to other types of conditions' for multiple steady states other than positive feedback. He cites Craciun et al (2006) and Craciun and Feinburg (2005) as examples though neither of these discuss the ubiquity of positive feedback. Furthermore, there have continued to be papers that discuss the necessity of positive feedback without noting that this necessity is unproven e.g. Novak and Tyson (2008). The Systems Biology Criterion for Feedback needs to be modified to exclude feedback between reactants of the same reaction. For now, it appears to be an open question whether positive feedback between reactions is a necessary condition for pathways having multiple steady states.

2.3.3 A Further Necessary Condition for Feedback

Given that feedback *between* reactions involves a circular causal chain in which a 'later' reaction affects the rate of an 'earlier' reaction, I take it to follow that a necessary condition for a pathway to have such feedback is that it contains one of the following two types of 'later' reaction/ 'earlier' elementary reaction pairs:

Type 1 Feedback. x_i is a reactant in an 'earlier' step and a product in the 'later' step. For example:

Earlier step	$\mathbf{C} + X \rightarrow Y$	+ other products (optional)
Later step	$Y + W \rightarrow \mathbf{C}$	+ other products (optional)

Type 2 Feedback. x_i is a reactant in both an 'earlier' step and the 'later' step. For example:

Earlier step	$X + \mathbf{C} \rightarrow Y$	+ other products (optional)
Later step	$Y + \mathbf{C} \rightarrow W$	+ other products (optional)

This is a necessary condition for feedback, as there is simply no other way in which a 'later' step can affect the rate of an 'earlier' steps via (chemical) feedback. This condition provides a 'visual' means of identifying feedback loops from statements of the elementary reaction steps of a pathway.

2.3.4 Allosteric Feedback

I will finish my section on feedback by considering a possible objection to my analysis: that it does not take account of 'allosteric feedback'. Allosteric enzymes are enzymes that change their conformation as a result of the binding of 'effector' molecules. A chemical species *F* is an 'effector', with respect to enzyme *E,* if *F* can bind to *E,* thereby interconverting *E* between a more-active and a less-active state.[15] This involves the formation of an enzyme-effector complex (Nelson and Cox, 2013, p. 226- 227). This is a type of chemical feedback in which a 'later' reaction step changes the concentration of an effector, which is a reactant in an 'earlier' reaction step. Allosteric feedback occurs when:

(i) the concentration of chemical species *F* affects the rate of production of *F,* and

(ii) at least one reaction within *F*'s feedback loop is catalysed by an enzyme *E* to which *F* is an effector.

Effectors change the rate at which an enzyme catalyses a reaction by changing the conformational states of that enzyme (which is now part of an enzyme-effector complex). Positive effectors promote the transition from less active to more active conformation states. Negative effectors promote the transition from more active to less active conformation states.

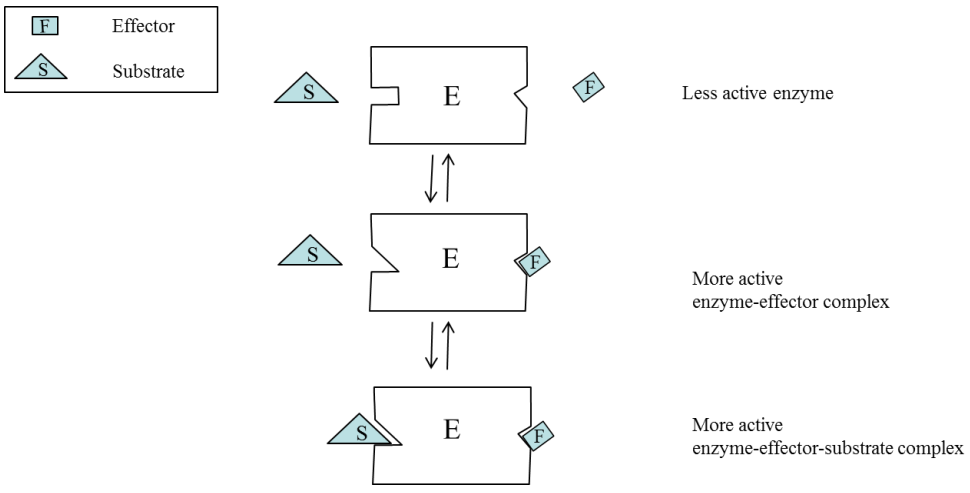


Fig 2.7 Cartoon representation of an allosteric enzyme binding to a positive effector and a substrate. The effector's binding leads to the enzyme having a higher affinity to bind to the substrate; this is illustrated by the shape of E changing as a consequence the effector's binding.

[15] Binding is an attractive interaction between two molecules. The bindings that occur between an allosteric enzyme, its effectors and substrates are non-covalent. There are also other types of feedback where the enzyme becomes covalently bonded to its effectors/substrates. Mutatis mutandis, my analysis of allosteric feedback will also apply in these cases.

Allosteric feedback can be contrasted with 'simple feedback' in which the conformational state of enzymes remains unaltered (in any sense that is relevant to the dynamics of the pathway). I will now explain that, with respect to my analysis of feedback, there is no significant difference between simple and allosteric feedback. This is because both types of feedback:

- have the same necessary condition for 'later reaction' / 'earlier reaction' pairs
- can be modelled using a system of linked concentration ODEs
- are identified by Systems Biology Criterion for Feedback

I will use the enzyme phosphofructokinase (henceforth: PFK) as a case study to illustrate allosteric feedback.

PFK is often taken to be a paradigm example of an allosteric enzyme and plays a key role in the oscillation of the glycoltic pathway (Gustavsson et al., 2012). It catalyses the third reaction step of the pathway:

fructose-6-phosphate + ATP → fractose-1,6-bisphosphate + ADP + H^+

PFK consists of four identical subunits (A, B, C, D):

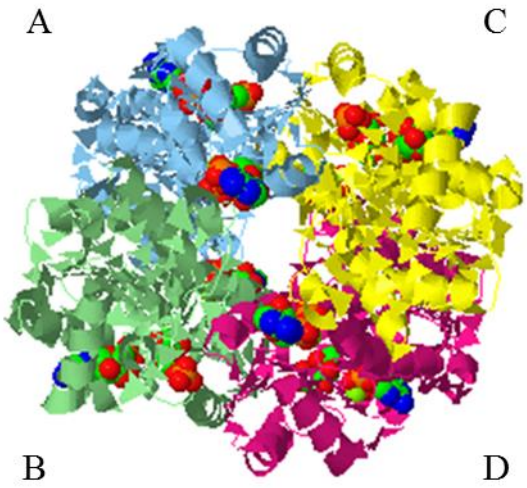


Fig 2.8 Structure of phosphofructokinase.
http://proteopedia.org/wiki/index.php/Phosphofructokinase_(PFK) (accessed 20/07/2016).

Each PFK subunit has two 'binding sites', one for fructose-6-phosphate (henceforth: F6B) and one for ATP. The stepwise reaction proceeds first by F6B and ATP

separately attaching to their binding sites on a subunit. A phosphoryl group (PO_3^{2-}) is then transferred from ATP to F6B.
Each PFK subunit also has an effector site, where an effector molecule can bind. Demin and Goryanin (2009, p. 170 – 186) provide an analysis of PFK in E. Coli, identifying its positive effectors as ADP and guanosine diphosphate and its negative effectors as ATP and phosphoenolpyruvate. In the more active conformational states, PFK has a greater affinity to bind to F6B and this in turn increases the rate at which F6B is transformed into fructose-1,6-bisphosphate (henceforth: F16bP).

A possible misconception is that allosteric feedback does not satisfy the necessary condition for chemical feedback that I identified, i.e. that a pathway has either:

Type 1 Feedback. x_i is a reactant in an 'earlier' step and a product in the 'later' step
or
Type 2 Feedback. x_i is a reactant in both an 'earlier' step and the 'later' step.

Textbook representations of allosteric pathways can sometimes give the false impression that this condition is not met. For example, in the summary representation of the glycolytic pathway below, it appears that phosphoenolpyruvate is not part of a feedback loop involving PFK; as phosphoenolpyruvate only appears to be in two reaction steps, and in its earlier step it is a product.

Step		Enzyme
1	glucose + ATP → glucose-6-phosphate + ADP + H^+	hexokinase
2	glucose-6-phosphate ↔ fructose-6-phosphate	phosphoglucose isomerase
3	**fructose-6-phosphate + ATP → fractose-1,6-bisphosphate + ADP + H^+**	**phosphofructokinase**
4	fructose-1,6-bisphosphate ↔ dihydroxyacetone phosphate + glyceraldehyde-3-phosphate	aldolase
5	dihydroxyacetone phosphate ↔ glyceraldehyde-3-phosphate	triose phosphate isomerase
	(steps 6 to 10 are carried out twice)	
6	glyceraldehyde-3-phosphate + Pi + NAD^+ ↔ 1,3-bisphosphoglycerate + NADH + H^+	glyceraldehyde 3-phosphate dehydrogenase
7	1,3-bisphosphoglycerate + ADP ↔ 3-phosphoglycerate + ATP	phosphoglycerate kinase
8	3-phosphoglycerate ↔ phosphoglycerate	phosphoglycerate mutase
9	2-phosphoglycerate ↔ **phosphoenolpyruvate** + H_2O	einolse
10	**phosphoenolpyruvate** + ADP + H^+ → pyruvate + ATP	pyruvate kinase

However, summary representations such as above often omit many of the elementary reactions that occur between enzymes and effectors.

In the case of phosphoenolpyruvate, there are a set of elemenary reactions that occur within reaction step 3 which involve PFK. These include the reaction:

3a. PFK + **phosphoenolpyruvate** ↔ PFK (phosphoenolpyruvate)

where the bracket indicates that phosphoenolpyruvate is part of a complex in which it is binded to PFK. The PFK (phosphoenolpyruvate) complex then reacts with F6B and ATP to produce fructose-1,6-bisphosphate (F16bP). Reaction step 9 changes the concentration of phosphoenolpyruvate, which is an (unstated) reactant in reaction step 3. Reaction steps 3 and 9 therefore form a Type 1 Feedback pair of steps and hence the necessary condition for feedback is satisfied. Similar accounts can be provided for the other effectors of PFK.[16]

[16] The GDP feedback loop involves reactions in a second pathway. The ATP feedback loops are slightly more complex as ATP is actually in two forms. In reaction step 3 where it is a reactant it is bonded to

A second possible misconception is that the generic structure of a pathway ODE model described in section 2.2 does not capture the effects of the changing conformations of allosteric enzymes. But this is incorrect, the dynamics of any chemical pathway that obeys the Law of Mass Action[17] can, at least in principle, be modelled at the level of elementary reactions with seperate concentration ODE being specified for each chemical species. In the case of the glycolytic pathway, this means there would be a separate concentration ODE for each type of PFK complex. For example there will be a concentration ODE for PFK(phosphoenolpyruvate) which will reference the reactions in which it is created (e.g. reaction step 3a) and the reactions in which it is consumed (e.g. when it reacts with F6B or ATP), In this way the effects of all the reactions involving PFK in its different conformations are captured. The only difference, compared with the glycoltic models found in the literature, would be that there would be many more concentration ODEs. The fact that PFK forms different complexes, with different conformations and different reaction rates does *not* amount to there being some new type of feedback process, that somehow needs to be differently modelled. All that is required is the rate laws for each PFK complex be included within a model.

However *in practice*, the PFK complexes have only transient existences and kinetic data is usually not available for their elementary reactions. Instead biochemists make do with just modelling the entire stepwise reaction. Sometimes the concentrations of PFK (and its complexes) are not explicitly modelled; and instead the reaction rate is simply taken as being a function of F6P, ATP and effectors (e.g. Hynne et al., 2001). By contrast, Tsusink et al. (2000) provide a more detailed model in which PFK is assumed to have just two conformational states: a less active T (tense) conformation or a more active R (relaxed) conformation. The total concentration of PFK is taken to be constant, and the variations in concentrations of PFK in its R and T conformations are calculated from the concentrations of their effectors and the substrates. Tsusink et al.'s rate law equations are provided in my appendix 1.

magnesium i.e. $MgATP2^{-}$, whereas the free form ATP^{4-} acts a negative effector on PFK (Demin and Goryanin, 2009, p. 174).

[17] This includes any modified Law of Mass Action (see this chapter's footnote 3). The only requirement is that rate of the reaction is a function of the concentrations of that reaction's reactants.

In summary, pathways with 'allosteric feedback' can be fully specified in the same way as pathways with 'simple feedback' i.e. by systems of concentration ODEs. It follows that allosteric feedback loops can be identified by the Systems Biology Criterion for Feedback. Indeed, Tyson's (2002) paper specifying the criterion, includes PFK as a paradigm example of biochemical feedback.

In this section, I have provided an overview of feedback. This included using the 'Systems Biology Criterion for Feedback' to elicit that feedback loops are circular causal chains that can be identified by their causal concentration ODEs. I also identified that a necessary condition for a pathway to have feedback between reactions is that it contains one of two types of a 'later' reaction/ 'earlier' reaction pairs. I have suggested that Systems Biology Criterion for Feedback needs to be modified to exclude feedback between reactants of the same reaction. Finally, I have explained that my analysis applies equally to 'simple' and 'allosteric' feedback.

2.4 What is the Relationship Between the Two Nonlinearities?

In this section, I will argue that it is possible for a pathway to have a feedback loop and *not* to be equation nonlinear but that this requires a possible but highly unlikely pathway structure. The structure of my argument is that both premises:

1. feedback between reactions requires there to be at least one bimolecular reaction in a pathway.
2. the existence of a bimolecular reaction within a pathway is sufficient for the pathway to be equation nonlinear.

are true, unless there is a 'possible but highly unlikely' pathway structure. I have already argued that premise two is true unless there is some 'possible but unlikely pathway structure' that leads to the nonlinear terms within a pathway's concentration ODEs cancelling out (recall section 2.2.2). I will now address the correctness of the first premise. My analysis will proceed by separately considering 'Type 1 Feedback' and 'Type 2 Feedback'.

Type 1 Feedback. This involves x_i being in two elementary reaction steps; in its 'earlier' step it is a reactant, in its 'later' step it is a product. For example:

Earlier Step $\quad \mathbf{C} + D \rightarrow E + F$

Later Step F + G → H + **C**

In this example, there is positive feedback as increasing [C] then increases the rate of C's production. Can Type 1 Feedback occur without involving a bimolecular reaction? Not if at least one of the reactions within the feedback loop has two or more products. The following example illustrates why this is so:

Consider a single molecular instantiation of a pathway with the following steps

Step 1 C → D + E

Step 2 ? → C

i.e. a single molecule of C being transformed into a single molecule of D and a single molecule of E and so forth, then:

Given

(i) In an elementary chemical reaction, the number of atoms of each chemical element is conserved. (*The Law of Conserved Atoms)*
e.g. there must be the same number of carbon atoms in the reactants as there are in the products etc.

(ii) Elementary chemical reactions are either unimolecular or bimolecular.

(iii) At least one product of each reaction step is a reactant in the next reaction step *(from the definition of a pathway)*

(iv) The pathway steps include:
step 1 = {C → D +E}
step 2's product is C
where C, D and E are chemical species and therefore are each composed of at least one atom.

Then

From (i), (iv) A molecule of C has more atoms than either a molecule of D or a molecule of E.

From (i), (iv) Steps 2's reactants have the same number of atoms as one molecule of C.

From (iii), (iv) Step 2 must have at least one of D and E as a reactant.

From (i)-(iv) Step 2 must have at least one reactant molecule in addition to either a molecule of D or E. i.e. **step 2 must be a bimolecular elementary reaction**.

This argument will generalise to longer Type 1 feedback loops where at least one unimolecular reaction has at least two products.

The only way a pathway can have a Type 1 Feedback 'later' reaction/'earlier' reaction pair and not include a bimolecular structure is for it to consist solely of unimolecular reactions and for the reactions in its feedback loops to each have only one product (otherwise a bimolecular reaction will be required to provide the atoms needed for reproducing the reactant that starts the loop). This requires a 'possible but highly unlikely' pathway structure, for example:

Step 1 $A \rightarrow B + C$

Step 2 $\mathbf{C} \rightarrow D$

Step 3 $D \rightarrow E$

Step 4 $E \rightarrow \mathbf{C}$

Step 5 $C \rightarrow F + G$

Although the loop from C (step 2) to C (step 4) appears to be logically redundant, it is perfectly feasible for such loops to occur in open biochemical systems, with C, D and E being isomers (i.e. same atomic composition but different arrangements of atoms).

Type 2 Feedback. This involves x_i being a reactant in both an 'earlier' step and the 'later' step. For example:

Step 1 $\mathbf{A} + B \rightarrow C$

Step 2 $C + D \rightarrow E$

Step 3 $E + \mathbf{A} \rightarrow G$

In this example, there is negative feedback as increasing [A] increases the rate of creation of C (step 1), which increases the rate of creation of E (step 2), increasing the rate of consumption of A (step 3); i.e. increasing [A] *decreases* the rate of production of A.

For Type 2 Feedback to occur without Type 1 Feedback also occurring, the 'later' step must be a bimolecular reaction. Consider the above example, chemical A needs to be a reactant in step 3 but it cannot be a product of the preceding step, otherwise steps 1 and 2 are a Type 1 'later' reaction /'earlier reaction' pair (and my previous argument for equation nonlinearity applies). The 'later' step therefore needs to include two reactants (i) chemical A to close the feedback loop and (ii) a product from the

preceding step (e.g. E) so that the 'later' step is linked to its preceding pathway step. The 'later' step must therefore be a bimolecular reaction.

Hence, a feedback loop will include bimolecular reactions, unless it has a highly unlikely structure (a chain of unimolecular reactions). And as I argued in section 2.2.2, a pathway that includes bimolecular reactions is highly likely to be equation nonlinear.

In conclusion, in theory there could be pathways with feedback that are also equation linear. For example: (i) a pathway consisting solely of unimolecular reactions, with each of the reactions within its feedback loop having only one product. (ii) a pathway where the nonlinear terms in each concentration ODE cancel out. But such pathway structures are 'possible but highly unlikely'.

2.5 Exotic Dynamics and the Two Nonlinearities

I will finish my analysis by summarising the current consensus view on the relationship between the two types of pathway nonlinearity and the occurrence of the dynamic properties of biochemical oscillations and multiple steady states.

Biochemical Oscillations. Novak and Tyson (2008) summarise the consensus view amongst systems biologists on the requirements for biochemical oscillations. They claim to have surveyed all known cases of biochemical oscillators and have identified three general factors that appear to be empirically necessary:

1. delayed negative feedback
2. 'sufficient nonlinearity'
3. balanced times scales of opposing processes.

The opposing processes are (i) a set of reactions that produce the chemical species whose concentration is oscillating and (ii) another set of reactions that reduce the concentration of that chemical species. 'Balanced time scales' refers to the need for these processes to occur with the right timings and strengths so as to produce a limit cycle. If a given biochemical system is capable of oscillating at all, then it will usually only occur for a limited range of parameter values. The role of negative feedback is to bring the system back to its starting position. The role of the delay in this feedback is to make a system continually "overshoot and undershoot the steady state" (Novak and Tyson, 2008, p. 3).

Novak and Tyson use the term *nonlinearity* in 'sufficient nonlinearity' to refer to a requirement for at least one of the reaction rate curves to have a sigmoid-type shape (Novak and Tyson, 2008, p. 7). This 'nonlinearity' can be the consequence of cooperativity or some other types of activity involving the formation of multiple bonds on a substrate. The term *sufficient* in 'sufficient nonlinearity' is used to indicate that the value of a nonlinearity factor (e.g. the n in the earlier formulation of the Hill equation) needs to be within a particular range for oscillations to be feasible.

Some ODE systems with delayed negative feedback and sigmoidal-type reaction curves are capable of producing oscillations for some range of parameter values. Novak and Tyson (2008, p. 7) say that negative feedback 'seems necessary' for self-sustained oscillations. This necessity has been partially proven using Jacobian matrices, for example Snoussi (1998), however these proofs all involve making some simplifying mathematical assumptions; for example, that the Jacobian matrix elements are quasi-monotonous over phase space. A literature search failed to find any published attempts to formally prove the necessity of sigmoidal-type reaction curves. Defining the necessary conditions for self-sustained oscillations is an on-going research program. Based on what has so far been empirically established, it remains a conjecture that negative feedback and sigmoidal-type reaction curves are necessary conditions for self-sustained oscillations.

Multiple Steady States. It remains an open question whether positive feedback is a necessary condition for the existence of multiple steady states (recall section 2.3.3). Again, a literature search failed to find any published attempts to formally prove the necessity of sigmoidal-type reaction curves. The same search also failed to find any cases of multiple steady states in biochemistry that did not involve a sigmoidal-type rate curve. It remains a conjecture whether a sigmoidal-type rate curve is a necessary condition for multiple steady states.

2.6 Conclusion

In *Discovering Complexity*, Bechtel and Richardson use the term 'nonlinearity' in two senses. A pathway can be equation nonlinear but not include feedback, and vice versa. My chapter has introduced the method that will be used throughout my thesis of expressing pathways as sequences of elementary reactions. This has helped to

illustrate that the scope for identifying universal generalisations about the two types of pathway nonlinearity is very limited. This is because it seems often to be the case that a 'possible but highly unlikely' pathway structure can be concocted to provide a counter-example to a putative universal claim.

A pathway is equation nonlinear if its dynamics induce an ODE system that does *not* satisfy the superposition principle. I have shown that a pathway will have this nonlinearity, unless it has a 'possible but highly unlikely' structure. Equation nonlinearity is a necessary but insufficient condition for multiple steady states and for stable limit cycles. Bistability is an important system property for explaining the functioning of cell signaling networks. Stable limit cycles describe the dynamics of self-sustained oscillators, which are commonplace in biochemistry.

Feedback occurs when the concentration of a reactant affects the rate of that reactants own production. Feedback loops are circular causal chains that can be identified from their causal concentration ODEs. Feedback between reactions occurs by a 'later' reaction changing the concentrations of reactants in 'earlier' reactions. I identified that a necessary condition for a pathway to have feedback between its reactions is that it contains one of two types of a 'later' reaction/ 'earlier' reaction pairs. The 'Systems Biology Criterion for Feedback' has been used to prove that positive feedback is a necessary requirement for multiple steady states. However, the criterion incorrectly identifies there being positive feedback *within* bimolecular reactions, and it remains an open question whether feedback *between* reaction is necessary for multiple steady states. The necessity of negative feedback for stable limit cycles is currently only conjectured; a general proof is yet to be provided.

Bechtel and Richardson claim that nonlinearity contributes both to the non-modularity and to the emergent behavior of biochemical pathways. This chapter has clarified the meanings of the two types of nonlinearity that they are referring to; and it has explained how each affects pathway dynamic behaviours. We are now ready to evaluate Bechtel and Richardson's claims. In the remainder of my thesis, I shall avoid the confusion caused by using the term 'nonlinearity' to refer to two distinct concepts; henceforth 'nonlinearity' will only refer to 'equation nonlinearity'.

Chapter 3 - Pathway Modularity and the Effects of Feedback: From Near Decomposability to Invariance of Causal Laws

3.1 Introduction

Biological systems are often assumed to be 'modular'. Werner Callebaut believes that modularity is ubiquitous (Callebaut, 2005, p. 4). Denis Noble talks of 'nature using modular systems' (Noble, 2006, p. 62). Modularity is routinely assumed in systems biology. Herbert Simon pioneered the study of modularity, and took there to be a single type of modularity, with different systems having different degrees of modularity. But now there are considerable differences in how the term is used within biology (Wagner and Altenberg (1996), Newman and Girven (2004), Porcar et al. (2013)). A shared idea is that a system can be decomposed into 'subsystems' that are 'relatively autonomous' from each other. Yet what is meant by 'subsystems' and by 'relatively autonomous' is open to several interpretations and this has led to the variety of proposals as to how 'modularity' should be defined.

This chapter is on the modularity of biochemical pathways, and how it is assumed within the Strategy of Decomposition. The Strategy of Decomposition is a strategy that biochemists often use for the causal discovery of pathway dynamics (recall section 1.5). The Strategy of Decomposition has three broad stages:

1. An extraction stage; in which the target *in vivo* pathway is separated from its biological context, creating an *in vitro* pathway.
2. A decomposition stage; involving decomposing the *in vitro* pathway into a set of isolated parts that can then be separately analysed.
3. A reconstruction stage; involving using a simulation model to deduce the target behaviour from statements of the properties of its isolated parts, their arrangement, plus the Kinetic Law of Composition.

There are two dominant accounts of modularity found within the philosophy of science literature. The first is based on Herbert Simon's concept of 'near decomposability'. Systems are modular, in this sense, when the intensity of intra-subsystem interactions is significantly greater than the intensity of inter-subsystem interactions. This is the type of modularity that Bechtel and Richardson claim is

assumed within the Strategy of Decomposition. My chapter focuses on Bechtel and Richardson's analysis. The second dominant account comes from Woodward, and is part of his conceptual analysis of causality. Woodward's concept has limited relevance to the Strategy of Decomposition; and I will delay considering it until after completing my analysis of near decomposability. At that point, I will be better placed to explain how Woodward's concept would need to be adjusted, so as to be of greater relevance to the Strategy of Decomposition.

Simon states that the complex systems found in biology are very often nearly decomposable (henceforth: ND). He claims that given human beings' limited cognitive abilities, it is only because complex systems are ND that we are able to understand them. In *Discovering Complexity*, Bechtel and Richardson advance Simon's work by analysing how the assumption of near decomposability has been used for causal discovery within biology. Contrary to Simon, they contend that biological systems are often only 'minimally decomposable'. In the case of pathways, they claim that it is feedback that leads to this minimal decomposability. Nevertheless, the assumption of near decomposability is still meant to have been heuristically useful for the causal discovery of pathways, by producing 'false models as a means to truer theories' (Bechtel and Richardson, 2010, xxx).

In contrast both to Simon and to Bechtel and Richardson, I shall argue that there is a plurality of types of modularity and that near decomposability is the wrong type to apply to the causal discovery of pathways. Instead, a different type of modularity, based on the invariance of causal laws is assumed within the Strategy of Decomposition. I shall call this type of modularity 'causal law modularity'. It is the assumption of causal law modularity that plays a key heuristic role in pathway discovery. I shall further argue that Bechtel and Richardson overstate the importance of feedback in reducing pathway modularity.

This chapter proceeds as follows. In section 3.2, I analyse Simon's concept of near decomposability. In section 3.3, I explain how Bechtel and Richardson significantly modify this concept and apply it to pathways. I summarise their account of the causal discovery process for pathways (which I have incorporated into my specification of the Strategy of Decomposition in section 1.5). In section 3.4, I argue that the concept of near decomposability does not apply to pathways, as it is inconsistent with the substantial overlaps that exist between a pathway's reaction steps. I also show that,

contrary to what Bechtel and Richardson claim, significant levels of feedback would not necessarily reduce near decomposability. In section 3.5, I argue that there is a significant shortcoming in both Simon's and in Bechtel and Richardson's analyses: neither recognise that there is a plurality of types of modularity. Starting from their analyses of near decomposability, I identify five distinct types of modularity that could be important either to the analysis of pathways or to biology more generally. One of these types is causal law modularity. In section 3.6, I explain how the assumption of causal law modularity is made within the Strategy of Decomposition. I explain that if a pathway is causal law modular then *in vivo* reaction step rate laws can be discovered by examining *in vitro* reaction steps. In section 3.7, I complete my analysis of modularity by reviewing Woodward's concept and explaining how it might map onto causal law modularity. I then conclude my chapter on a cautionary note; the context-sensitivity of pathway rate laws means that there is a significant risk that pathways will often *not* be causal law modular. In such cases, the Strategy of Decomposition will fail to discover a pathway's *in vivo* rate laws.

3.2 Simon's Analysis of Hierarchy and Near Decomposability

Simon (1962, 1973, 1998, 2002) claims that many natural systems can be considered as hierarchical and ND. Simon bases his claims on both empirical observations and on evolutionary arguments. The aims of this section are: (i) to provide an overview of Simon's theory (ii) to highlight those parts of his theory that are most salient to evaluating pathway near decomposability (iii) to show that Simon's concept of near decomposability entails two distinct types of autonomy regarding the behaviours of subsystems (in section 3.5, these will be used to help identify different types of modularity that are useful to biology).

I will begin this section by describing the hierarchical structure of ND systems. The criteria for a system to be ND will then be specified. I will then explain the two types of autonomy, which shall be termed as: 'dynamic autonomy' and 'structural autonomy'. Dynamic autonomy concerns the mutual independence of subsystems' dynamic behaviours and structural autonomy concerns subsystems continuing to function when the structure of their containing system is changed. I will then provide a summary of Simon's claim that it is only because complex systems are ND that we are able to understand them. Finally, I will explain why Simon's evolutionary

arguments for the ubiquity of near decomposability in biology leave open the possibility that pathways are *not* ND.

Simon uses the image of Chinese boxes to explain hierarchy:

> "In application to the architecture of complex systems, "hierarchy" simply means a set of Chinese boxes of a particular kind [...] Opening any given box in a hierarchy discloses not just one new box within, but a whole small set of boxes; and opening any one of these component boxes discloses a new set in turn [....] a hierarchy is a partial ordering- specifically a tree." (Simon, 1973, p. 5).

The wholes at one level are the parts of the next level up. Simon claims that biological systems have this hierarchical structure. Cells are organised into tissues, tissues into organs and organs into systems; whilst a cell is composed of several subsystems including the nucleus, the cell membrane and the mitochondria (Simon, 1962, p. 469).

A parable about two watchmakers is used to support the claim that biological systems are often organised as a hierarchy of stable subsystems (Simon, 1962, p. 470). Hora and Tempus both make watches consisting of a thousand parts. For each watchmaker, there is a probability p that they will be interrupted whilst a part is being added to an incomplete assembly of the watch's parts. Tempus has holistically designed his watches. This has the consequence that if he is interrupted whilst assembling a watch, the partly assembled watch will fall apart. This means that the probability of Tempus completing a watch is $(1-p)^{1000}$. By contrast Hora has hierarchically designed his watches. Assembly starts by putting together stable subsystems of ten parts each. Ten of these subsystems are then put together to form a larger stable subsystem and finally ten of these subsystems are put together to complete a watch. If Hora is interrupted whilst assembling, he only loses a small amount of work and this will occur with a probability of $(1-p)^{10}$. Simon shows that if p equals 0.01 then Hora will make about four thousand watches in the time it takes Tempus to complete one. This parable is meant to illustrate that:

1. If a process of assembly of many parts is subject to interruption, it will be more successful when there is a hierarchy of stable subsystems.
2. Complex systems will evolve much more rapidly from simple systems if there are stable subsystems.
3. Hierarchical systems are the ones that have time to evolve.

The dynamic properties of hierarchically structured systems can be decomposed into subsystems in order to analyse their behaviours (Simon, 1962, p. 468). A distinction is made between 'fully decomposable'[18] and 'nearly decomposable' systems. A system is 'fully decomposable' if its subsystems can be considered as mutually independent. Simon's only example is of a noble gas, which is fully decomposable because its inter-atomic forces are negligible (Simon, 1962, p. 474). Simon does not provide a tight specification of the necessary and sufficient conditions for near decomposability, however the following criteria can be elicited. A hierarchical system is ND if:

(i) the intensity of intra-subsystem interactions is significantly greater than the intensity of inter-subsystem interactions (for a given level).

(ii) the intensity of interactions (both inter- and intra- subsystem) significantly increases with the lowering of levels.

(iii) satisfaction of (i) and (ii) results in same-level subsystems having a high degree of 'dynamic' and 'structural' autonomy from each other:

(a) 'dynamic autonomy' entails that the dynamic behaviours of subsystems are approximately independent of each other.

(b) 'structural autonomy' entails that each subsystem can continue to function when the structure of its containing system is changed.

I shall refer to the above criteria as The Criteria for Near Decomposability and these will be further explained below. Simon does not distinguish between what I am terming as 'dynamic' and 'structural' autonomy and seamlessly switches between the two. Both are taken to be consequences of (i) and (ii).

Simon does not formally state what is meant by 'intensity of interaction'. However, his concept of near decomposability is based on interactions of greater intensity occurring at faster rates and with greater strength. He provides two examples from the natural sciences in which the intensities of interactions are identified. The first

[18] The term 'fully decomposable' is used by Bechtel's (2010 –pxx). Simon refers to such systems as being 'decomposable systems'.

example concerns the Mellon Institute building reaching thermal equilibrium. In this case the 'interactions' are the transferring of heat between office cubicles, and 'intensity of interaction' refers to the rate of flow of heat. The second example concerns vibrations within a molecular system. In this case the 'interactions' are the bondings, and 'intensity of interaction' is the frequency of vibration; with greater frequencies of vibration corresponding to greater bond strengths (i.e. bond energies) (Simon, 1962, p. 475-76). I will take intensity to be a function of the number and strength of interactions, per unit time.

Simon provides the following explanation for the variabilities of intensities of interactions found within a system:

> "Most interactions that occur in nature, between systems of all kinds, decrease in strength with distance. Hence any given 'particle' has most of its strong interactions with nearby particles." (Simon, 1977, p. 9).

The importance of physical barriers for biological systems is not discussed. Yet this will often be a far more salient factor in explaining sharp changes in intensities of interactions than distance. For example, membranes are a critical factor in explaining the weak interactions between the parts of adjacent cells, or between organelles such as mitochondria, nuclei and the Golgi apparatus. Another example of an important physical barrier is epithelial tissue which covers the surfaces of organs.

The bottom-level of an ND system is characterised relative to a particular analysis. It contains those parts that have been chosen as basic for that analysis for that system. In the Mellon Institute example, the bottom-level parts are office cubicles. In the molecular system example, the bottom-level parts are neutrons, protons and electrons. The following matrix illustrates how the intensity of pairwise interactions at the bottom level determines its subsystems and levels.

	1	2	3	4	5	6	7	8	9	10	11	12
1	1	a	a	ε1	ε1	ε1	ε2	ε2	ε2	ε2	ε2	ε2
2	a	1	a	ε1	ε1	ε1	ε2	ε2	ε2	ε2	ε2	ε2
3	a	a	1	ε1	ε1	ε1	ε2	ε2	ε2	ε2	ε2	ε2
4	ε1	ε1	ε1	1	a	a	ε2	ε2	ε2	ε2	ε2	ε2
5	ε1	ε1	ε1	a	1	a	ε2	ε2	ε2	ε2	ε2	ε2
6	ε1	ε1	ε1	a	a	1	ε2	ε2	ε2	ε2	ε2	ε2
7	ε2	ε2	ε2	ε2	ε2	ε2	1	a	a	ε1	ε1	ε1
8	ε2	ε2	ε2	ε2	ε2	ε2	a	1	a	ε1	ε1	ε1
9	ε2	ε2	ε2	ε2	ε2	ε2	a	a	1	ε1	ε1	ε1
10	ε2	ε2	ε2	ε2	ε2	ε2	ε1	ε1	ε1	1	a	a
11	ε2	ε2	ε2	ε2	ε2	ε2	ε1	ε1	ε1	a	1	a
12	ε2	ε2	ε2	ε2	ε2	ε2	ε1	ε1	ε1	a	a	1

Fig 3.1 A matrix representing the intensity of interactions occurring in a nearly decomposable system (Simon, 2002, p. 589).

In the above matrix a >>ε1>> ε2. The intensity of the interactions of a part with other parts can be read horizontally, so for example part 1 has an intensity of interaction of magnitude '*a*' with parts 2 and 3, 'ε1' with parts 4, 5 and 6, and 'ε2' with parts 7 through 12.

The matrix represents an ND system with three levels:

	Subsystems
3rd level	components 1-6 , 7-12
2nd level	components 1-3, 4-6, 7-9, 10-12
Bottom level	the individual components

2nd level subsystems are determined by choosing an intensity threshold, in this example ε1, and then selecting those parts with pairwise interactions greater than this. In the example matrix, parts 1, 2 and 3 belong to the same subsystem because the intensities of their pairwise interactions with each other are all greater than ε1.

3rd level subsystems can be determined by choosing a second intensity threshold and then selecting those parts with mutual interactions greater than this. The second threshold must be less than the first threshold. In the example matrix the second threshold is ε2; the parts 1, 2, 3, 4, 5, 6 will then belong to the same 3rd subsystem, because the intensity of interactions between them are all greater than ε2.

Subsystems are defined in terms of intensity of interactions, rather than by their functions. As such a subsystem may be involved in a plurality of functions.

This form of matrix will represent a ND system if (a) all the large elements form a diagonal block sequence (b) the other elements have small values (possibly including some zeros). Such a matrix is called a 'nearly decomposable matrix'. If all the other elements are zero then the system is decomposable (Ando and Simon, 1961, p. 114). A dynamic system that can be described by a nearly decomposable matrix has the properties of a nearly decomposable system (Simon, 1962, p. 475).

The example of a molecular complex is used to illustrate how the partitioning of intensities of interactions can reveal a system's ND structure (Simon, 1962, p. 475). The strengths of molecular interactions are: intra-atomic forces >> covalent bonds>> hydrogen bonds >> van der Waal forces. Let us consider a system consisting of just 12 atoms. The subsystems are then:

	Entities	Strongest External Interactions	Subsystems
3rd level	(2) Pairs of molecules	van der Waal forces	cells 1-6 , 7-12
2nd level	(4) Molecules	Hydrogen bonds	cells 1-3, 4-6, 7-9, 10-12
Bottom level	(12) Atoms	Covalent bonds	individual cells

It might be objected that Simon's method for determining subsystems fails to take account of the context sensitivity of interactions. For instance, the interactions between two atoms may well be different if they are in an isolated system rather than being parts of a molecular complex. But this objection is wrong, as an ND matrix references only the interactions of entities fully situated within the system being analysed.

I will use the Mellon Institute example (Simon and Ando (1961)) to illustrate the use of the ND matrix and to explain dynamic autonomy. The example concerns heat flows within the building. The building is divided into rooms and each room is divided into cubicles. At a start time, all cubicles are set to different temperatures.

The dynamics of the building reaching temperature equilibrium are modelled using an ND matrix whose elements are the coefficients of heat transfer between cubicles. For example, the first row will contain the coefficients between the first cubicle and each of the other cubicles in the building. The diagonal block elements of the matrix will correspond to the rooms. Let $Fi_I(t)$ equal the temperature of the i^{th} cubicle of the I^{th} room at time t, let F(t) be a row vector whose parts are $Fi_I(t)$ and let R be the ND matrix. Then:

$$F(t+1) = F(t)R$$

For such dynamical systems (Simon and Ando,1961, p. 116-118):

1. In the short run the values of each $Fi_I(t)$ are dominated by the coefficients of their I^{th} subset. In this example, this corresponds to changes in the temperatures of cubicles being dominated by interactions with other cubicles in the same room.
2. In the long run the $Fi_I(t)$ within each 'I' will be approximately equal i.e. $Fj_I(t) \approx Fk_I(t)$ for any pair <j, k> . However, there can be significant differences between aggregate values of the $Fi_I(t)$ of each 'I' i.e. it can be the case that $Fi_J(t) >> Fi_K(t)$ if $J \neq K$. The changes that occur to these aggregates can be successfully modelled using aggregations of each I subset.
   In the example, this corresponds to each cubicle now being almost at equilibrium with the other cubicles in its room (ie these are local approximate equilibria) but there still being significant differences in the temperatures of different rooms. The changes in each room's average temperature can now be successfully modelled without reference to the temperatures of individual cubicles, which will remain in approximate equilibrium.
3. In the end the values for all $Fi_I(t)$ will be approximately equal. In this example this corresponds to the temperature being almost equal throughout the whole building.

The Mellon Institute building is "the archetype of an ND system [....] Its special characteristic is that equilibrating interactions within boxes at any level take place much more rapidly than the interactions between boxes at the same-level" (Simon, 2002, p. 589).[19]

[19] Although the Mellon Institute analysis is based on representing behaviours by linear ordinary differential equations, Simon claims that 'the qualitative results can be extended to more general cases' (Simon, 2012, p.589). However, his claim needs to further qualified, for example to allow that there may be non-linear systems whose intensity of interactions satisfies the form of an ND matrix but do not have the consequent autonomous behaviours because of sensitive dependence to initial conditions.

Simon and Ando's mathematical analysis helps to unpack Simon's well known 'two propositions':

> (a) in a nearly decomposable system, the short-run behaviour of each of the component subsystems is approximately independent of the short-run behaviour of the other components; (b) in the long run, the behaviour of any one of the components depends in only an aggregate way on the behaviour of the other components." (Simon, 1962, p. 474)

In the Mellon Institute example 'behaviour' refers to the 'rates of change in temperature' and the component subsystems are the rooms. 'Short- run' corresponds to the initial period when each room is in disequilibrium. At this point interactions between cubicles in different rooms have only a small effect. 'Long-run' corresponds to the period when approximate equilibrium has been established within each room. Only the aggregate of each room's internal interactions now needs to be taken into account when modelling rooms moving to temperature equilibrium.

Simon's two propositions capture what I am terming as the 'dynamic autonomy' within ND systems. However, his wording is slightly unclear as to whether the 'components' being referred to are meant to be on the same-level and what their relationship is meant to be to 'component subsystems'. I will take dynamic autonomy to refer to:

a) the short-run dynamic behaviours of same-level subsystems being approximately independent of each other,
b) the long-run dynamic behaviours of each subsystem being approximately independent of the details of other same-level subsystems' internal behaviours.

It is on the basis of dynamic autonomy that Simon classifies molecular systems as ND:

> "Molecular systems are nearly decomposable, the short-run dynamics relating to the internal structures of the subsystems; the long-run dynamics to the interactions of these subsystems" (Simon, 1962, p. 476).

The long-run independence from the details of other subsystems, follows from the internal interactions being of significantly greater intensity than their external interactions. The greater intensities of internal interactions means that "motions will be so rapid that the corresponding subsystems will appear to always be at

equilibrium" (Simon, 1971, p. 10). Only aggregate properties of each subsystem's internal interactions will be relevant to the behaviours of other same-level subsystems.

Events at different levels of a ND system occur with different intensities of interaction, with higher level interactions having significantly lower intensities of interactions. This can have important implications both for the behaviour of a system and for our understanding of that system. When considering the dynamic behaviours at a given level, the slow interactions at the next level up might not be important and can then be ignored (in the short-run). Similarly, the details of the interactions at the next level down might not be important and can then be aggregated. Explanations for the behaviour at a given level will then only need to refer to two levels, the given level and aggregations of interactions from the next lower level. For example, when explaining the seasonal temperature changes for a region, the hourly changes in the temperatures of sub-regions can be aggregated over, whilst the global temperature changes over geological eras can be ignored (Simon, 1998, p. 15).

Summary accounts of near decomposability sometimes fail to adequately refer to dynamic autonomy. For example, in the glossary of his book 'Re-engineering Philosophy for Limited Beings', Wimsatt defines near decomposability as: "The ability to break structures into parts and then reassemble them to solve or engineer problems"[20] (Wimsatt, 2007, p. 358). This fits with ND's being 'structurally autonomous' (see below) but ignores dynamic autonomy. I will be arguing in my section 3.4 that Bechtel and Richardson also fail to adequately take account of the dynamic autonomy required for systems to be ND. Yet dynamic autonomy is a consequence of the structure of ND systems and is extensively analysed by Simon. The following quote illustrates both the importance of dynamic autonomy to Simon's concept of near decomposability and also its relevance to the practice of science:

> "ND systems have very special dynamic behaviour. When disturbed from equilibrium, the subsets at the lowest level of the system return to equilibrium while the sets at the next level above are still changing dynamically (relatively slowly) and the same is true (and even more decisively) for still higher levels,

[20] However it should also be noted that Wimsatt's book also contains an extensive treatment of near decomposability analysed in terms of intensities of interactions (see for example Wimsatt, 2007, p. 181-185)

> which are essentially stationary (but not at equilibrium) on this time scale. In fact-and this property is used continually by the sciences- we can study the equilibrium at any given level without much concern for the slow dynamics at the levels above, and we can study the dynamics at any level without much concern for the rapid return to equilibrium at the levels below" (Simon, 2002, p. 590-591).

Simon focuses on subsystems relaxing towards local equilibria (or in the case of open biological systems: local steady states) following a perturbation to their state. However the concept of dynamic autonomy is more general than relaxation dynamics, concerning the different time scales that apply between:

1. the internal and external interactions of subsystems
2. the behaviours of subsystems at different levels of any ND system.

ND subsystems are also 'structurally autonomous'. This aspect of Simon's theory is not directly relevant to Bechtel and Richardson's analysis of pathway near decomposability. However structural autonomy forms the basis for two of the distinct concepts of modularity that I shall be discussing in my section 3.5.

Structural autonomy concerns each subsystem being able to continue to function when the causal structure of its containing system is changed. Simon does not provide a definition of this type of autonomy but two types of structural change are considered:

a. changes to the number and arrangement of the containing system's subsystems[21],
b. changes to the internal interactions within a subsystem.

I will refer to the accompanying autonomies as 'external' and 'internal' structural autonomy.

Internal structural autonomy is discussed in terms of 'functional equivalence'. A subsystem is 'functionally equivalent' to another if it maps the same inputs to the same outputs. "Functional equivalence permits mutation and natural selection to go on in particular subsystems without requiring synchronous changes in all the other systems that make up the total organism." (Simon, 1977, p. 17). For example, as long

[21] This would be consistent with Wimsatt's (2007, p. 358) definition of near decomposability.

as functional equivalence is maintained, the metabolic efficiency of a subsystem could improve without disrupting other same-level subsystems (Simon, 1977, p.17). Another example given is the circulatory system being loosely coupled to the respiratory and digestive systems (Simon, 1977, p. 17). The circulatory system's inputs include: oxygen from the respiratory subsystem, nutrients from the digestive subsystem and carbon dioxide from muscle subsystems. Its outputs include nutrients to muscle subsystems and so forth. How the circulatory system achieves its tasks is irrelevant to these other subsystems, all that matters are that they are achieved. Evolutionary changes may occur in the circulatory system without necessarily changing the respiratory and digestive systems.

Internal structural autonomy is taken to follow from the 'loose horizontal coupling' between same-level subsystems. This loose coupling is due to the same factors that explain dynamic autonomy: (i) the intensity of subsystems' internal interactions being significantly greater than the intensity of their external interactions, and hence (ii) only the aggregate properties of each subsystem being relevant to other same-level subsystems' behaviours:

> "The loose horizontal coupling permits each subassembly to operate dynamically in independence of the detail of the others; only the inputs it requires and the outputs it produces are relevant for the larger aspects of system behaviour. In programming terms, it is permissible to improve the system by modifying any one of the subroutines, provided that the subroutine's inputs and outputs are not altered." (Simon, 1977, p. 17).

It is in this way Simon links what I am term 'dynamic autonomy' to 'internal structural autonomy'.

Simon seems to be claiming that: *if* changes occur within a subsystem which do not change its inputs and outputs *then* other same-level subsystems will not be affected. This can appear to be an empty claim. Consider any group of causally connected entities within a system. Any interaction it has on any other part of its containing system is an input or an output. It seems vacuous to say that: *'only the inputs it requires and the outputs it produces are relevant for the larger aspects of system behaviour"* (Simon, 1977, p. 16*).* But internal structural autonomy is not an empty concept. The quote in this paragraph gains meaning if it is interpreted as emphasising the relatively large amount of activity that occurs within a subsystem that is irrelevant to other subsystems.

External structural autonomy is implicit in the Hora and Tempus parable. When this type of autonomy occurs, the parts of a system can be rearranged to form new systems. This is the case, for example, with electrical circuits, where parts such as resistors, capacitors, and inductors can be taken from one circuit and used to construct a new circuit. This is the least developed part of Simon's account of near decomposability. It is not clear why this type of autonomy follows from the relatively strong intensities of subsystems' internal interactions. There is no obvious link between external structural autonomy and the other two types of autonomy. Instead external structural autonomy appears to belong to an unrelated concept of modularity. Simon also fails to explain why biological systems would be expected to have external structural autonomy. For example, why would biological subsystems continue to function in different contexts? Further details are also required as what the constraints would be on the combining such subsystems. This type of autonomy could be very important to biology, for example, the discipline of synthetic biology assumes this type of autonomy (as will be discussed in my section 3.5). However, developing a fuller account of external structural autonomy is outside the scope of this chapter. The disconnection between external structural autonomy and the other two types of autonomy points to the need for a plurality of types of modularity.

Two evolutionary arguments are provided for the ubiquity of nearly decomposable biological systems: the Hora and Tempus parable and an argument for the need for internal structural autonomy. The Hora and Tempus parable has been shown to have significant shortcomings. These include:

(i) It assumes that generating new kinds of higher level entities is simply an exercise in combinatorics but evolving multicellular individuals from unicellular ones requires solving many control problems (Lane, 2005, p.90).

(ii) Many evolutionary processes involve the reorganisation rather than addition of genetic material (Zawidski. 1998, p.545).

By contrast, there is widespread support within the discipline of biology for Simon's second argument that evolution requires biological systems to have some degree of internal structural autonomy. This is illustrated by Wagner and Altenberg's (1996, p.971) proposed structure for evolutionary modules (see section 3.5).

Even if biological systems are often ND into evolutionary modules, it does not follow that this provides support for the claims of near decomposability in such fields as

physiology, developmental biology and biochemistry.[22] This is because a biological system can be decomposed in different ways, depending on which interactions are relevant to a particular analysis. This leads to what Wimsatt (1972) has called 'descriptive complexity'. Different biology sub-disciplines select different types of interactions and basic parts when decomposing a system and there is not a one-to-one mapping between the subsystems of these different decompositions. Descriptive complexity means that evidence of one kind of near decomposability (e.g. evolutionary) might not be evidence for another kind of near decomposability (e.g. biochemical). This undermines Simon's strategy of using an evolutionary argument to claim that near decomposability is ubiquitous in biology.

Simon claims that given human beings' limited cognitive abilities, it is only because many complex systems are ND that we are able to understand them. If there are complex systems that are not ND, then analysing their behaviour would involve such detailed knowledge and computation of their elementary interactions as to be beyond us (Simon, 1962, p. 477). Because of near decomposability, we can divide a system into subsystems, aggregate some interactions and, if appropriate, ignore others. The example is given of a system made of five components, each in turn consisting of five parts and each of these having five sub-parts. The system can be examined by analysing just thirty-one subsystems: one subsystem of five components, five subsystems of five parts and twenty-five subsystems of five sub-parts. This is likely to be a much simpler task than analysing a single system of one hundred and twenty-five interacting parts (Simon, 1977, p. 179). Furthermore, we will often only be interested in the behaviours of parts corresponding to a particular level, in which case only the interactions of that level, plus the aggregation of interactions at the next level down might need to be considered. Simon claims that if complex systems are not ND, they may well escape our observation or understanding.

In summary, Simon takes there to be a single type of modularity in which different systems have different degrees of decomposability. Near decomposability entails a conjunction of dynamic and structural autonomies. However external structural autonomy does not appear to be adequately related to other types of autonomy. Simon's evolutionary argument for the ubiquity of near decomposability fails and this leaves open the possibility that pathways are not ND. Simon claims that it is only because many complex systems are ND that we are able to understand them. In

[22] See for example Callebaut, W. et al. (2005).

section 3.4, I shall be arguing that pathways are not dynamically autonomous and therefore are not ND. In section 3.5, I shall explain that complex systems do not need to be ND to be understandable, as there are other types of modularity that can assist in this task. I will then use Simon's analysis to help identify some of the different types of modularity that are important to biology. My claim that pathways are not ND is in contrast to Bechtel and Richardson, who take pathways that do not have feedback to be ND. I shall now consider their analysis.

3.3 Bechtel and Richardson on the Minimal Decomposability of Pathways

In contrast to Simon, Bechtel and Richardson argue that biological systems are often only 'minimally decomposable'. Nevertheless, they claim that the assumption of near decomposability has been a key heuristic for causal discovery in biology. Bechtel and Richardson stay with Simon's single concept of modularity, though they implicitly add three further criteria. These criteria concern subsystem: functionality, behaviour when isolated and self-control. Bechtel and Richardson explain that in minimally decomposable systems, the interactions between subsystems play an important role in determining the internal operations within subsystems. These are integrated systems in which the distinction between subsystems is blurred and systemic organisation provides significant constraints on subsystem functioning (Bechtel and Richardson, 2010, p. 27). Bechtel and Richardson provide case studies to illustrate their claim that complex systems do not need to be ND in order to be understood.

I will focus on Bechtel and Richardson's analysis of biochemical pathways. They describe how the strategies of functional / structural decomposition, and localisation have been used in discovering pathway mechanisms. These twin strategies are claimed to be based on the assumption of near decomposability. They state that the failures of the twin strategies reveal that pathways are often only minimally decomposable. This minimal decomposability is attributed to the presence of feedback. Bechtel and Richardson use the discovery of the metabolic pathway for glycolysis to illustrate their claims.

The aims of this section are to (i) summarise Bechtel and Richardson's concept of near decomposability and explain how it is applied to pathways (ii) explain why

feedback is meant to reduce near decomposability (iii) highlight the key role that Bechtel and Richardson's three additional criteria make to their analysis of pathway near decomposability and the effects of feedback.

Bechtel and Richardson do not provide a concise definition of what a biological mechanism is. However, their analysis is consistent with the later definition provided by Bechtel and Abrahamsen (recall section 1.3):

> "A mechanism is a structure performing a function in virtue of its component parts, component operations, and their organization. The orchestrated functioning of the mechanism, manifested in patterns of change over time in properties of its parts and operations, is responsible for one or more phenomena." (Bechtel and Abrahamsen, 2005, p. 423).

Bechtel and Richardson claim that two types of decomposition are used in discovering pathway mechanisms: functional and structural. In a functional decomposition, a mechanism's function is decomposed into constituent sub-functions. Each sub-function corresponds to a component operation of the mechanism. For example, in metabolic pathways:

> "component operations are then characterised in terms of individual chemical reactions on a series of substrates (e.g. oxidizing or reducing them, adding or removing H_2O, etc.). A successful functional decomposition will identify each operation with its passive parts (the substrate and its resulting product). What it lacks is specification of its active parts – that is the enzyme that initiates and guides each reaction." (Bechtel and Abrahamsen, 2005, p. 433).

In a structural decomposition, a system is decomposed into its physical parts (i.e. subsystems). There are many ways in which a system can be partitioned. What matters for causal discovery is identifying the 'working parts' i.e. the subsystems that perform the operations corresponding to the functional decomposition. In practice, researchers will often identify some of a mechanism's working parts, prior to having completed the mechanism's functional decomposition. For example, they might identify that a particular reactant is critical to a pathway without fully appreciating its functional role.

In a localisation, the proposed sub-functions are identified with the operations of specific working parts. Bechtel and Richardson state that:

> "The notion of decomposability stems from Simon and constitutes the descriptive counterpart of localisation" (Bechtel and Richardson, 2010, p. 25).

Bechtel and Richardson describe a concept of near decomposability that implicitly goes beyond Simon's original account. Their initial account does cover much of Simon's specification, including his quote on his 'two propositions', the example of the intensities of interactions within molecular systems and the Hora and Tempus parable. They also state that:

> 'in relatively simple hierarchies, there will be a relatively high strength of interaction within subsystems as compared with the interactions among subsystems. These are systems that are nearly decomposable' (Bechtel and Richardson, 2010, p. xxix).

But three new criteria are also incorporated in their account of near decomposability, although they are not identified as being new. It is these three criteria, rather than Simon's concept, that do most of the work in Bechtel and Richardson's analysis of pathway causal discovery. This points towards the claim that I shall be making in my section 3.5, that rather than near decomposability, there is a different type of modularity ('causal law modularity') that plays a key heuristic role in pathway discovery.

The first new criterion is that each ND subsystem has a discrete functional role. In the case of a pathway, each subsystem is assumed to perform a separate sub-function of the pathway's overall function; with only minimal interaction with the pathway's other subsystems. This goes beyond Simon's stated concept, in which subsystems are selected only by the intensities of interactions of the elementary parts. Bechtel and Richardson appear to be providing an interpretation of near decomposability that better fits Simon's evolutionary arguments. Although Simon does not discuss it, there may well be evolutionary advantages to 'internally structurally autonomous' subsystems having discrete functional roles that can alter in relative independence of each other (see section 3.5). Nevertheless, the requirement for discrete functional roles` goes beyond what Simon states. Bechtel and Richardson's are adding an extra constraint on what will count as an ND system.

The second new criterion is for ND systems to possess an additional type of autonomy, in that the properties of each part can be determined in isolation of the system's other parts:

> "the behaviour of parts is intrinsically determined. In these cases, it is feasible to determine component properties in isolation of other components, despite the fact that they interact." (Bechtel and Richardson, 2010, p. 26).

The third new criterion concerns the control of parts:

> "For near decomposability, individual components must be controlled by intrinsic factors." (Bechtel and Richardson, 2010, p. 26).

By 'control' is meant the turning on/off or changing the rates of component operations. This entails that the mechanism does not involve significant feedback, as the presence of feedback involves a subsystem having its rates of operation extrinsically controlled by another subsystem or subsystems. When subsystems are:

> "less governed by intrinsic factors, we [then] enter the domain of integrated composite systems, which are *minimally decomposable*." (Bechtel and Richardson, 2010, p. 27).

Bechtel and Richardson use the glycolytic pathway as a case study to illustrate how the twin strategies have been applied. The pathway's reaction steps are:

Step		Enzyme
1	glucose + ATP → glucose-6-phosphate + ADP + H^+	hexokinase
2	glucose-6-phosphate ↔ fructose-6-phosphate	phosphoglucose isomerase
3	**fructose-6-phosphate + ATP → fractose-1,6-bisphosphate + ADP + H^+**	phosphofructokinase
4	fructose-1,6-bisphosphate ↔ dihydroxyacetone phosphate + glyceraldehyde-3-phosphate	aldolase
5	dihydroxyacetone phosphate ↔ glyceraldehyde-3-phosphate	triose phosphate isomerase
	(steps 6 to 10 are carried out twice)	
6	glyceraldehyde-3-phosphate + Pi + NAD^+ ↔ 1,3-bisphosphoglycerate + NADH + H^+	glyceraldehyde 3-phosphate dehydrogenase
7	1,3-bisphosphoglycerate + **ADP** ↔ 3-phosphoglycerate + **ATP**	phosphoglycerate kinase
8	3-phosphoglycerate ↔ phosphoglycerate	phosphoglycerate mutase
9	2-phosphoglycerate ↔ **phosphoenolpyruvate** + H_2O	einolse
10	phosphoenolpyruvate + **ADP** + H^+ → pyruvate + **ATP**	pyruvate kinase

Fig 3.2 The glycolytic pathway - highlighting those chemicals that are parts of the feedback loop involving phosphofructokinase. Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; NAD^+, nicotinamide adenine dinucleotide (oxidised form); NADH, nicotinamide adenine dinucleotide (reduced form); Pi, phosphate group.

There are multiple feedback cycles occurring within the glycolytic pathway. In particular, there are feedback cycles that regulate the activities of some of the enzymes i.e. allosteric feedback (recall section 2.3.4). For example, phosphofructokinase (step 3) is inhibited by ATP and phosphoenolpyruvate but activated by both ADP and guanosine diphosphate (the latter is not part of the pathway but is affected by the concentrations of the metabolites in the pathway). Another example is pyruvate kinase (step 10) which is activated by fructose-1,6-bisphosphate (steps 3 and 4) but inhibited by ATP. These feedback cycles tightly regulate the production not only of ATP and NADH but also the intermediate products in the pathway which are used as building blocks in other pathways, for example fructose-6-phosphate is used in the production of amino sugars.

The glycolytic pathway was discovered in the early decades of the twentieth century. Bechtel and Richardson describe how this process involved (i) conjecturing as to what the mechanism's operations were (i.e. by proposing a functional decomposition) (ii) postulating the existence of substrates, products, enzymes and effectors within

the pathway that could perform these operations (i.e. by proposing a structural decomposition and localisation) (iii) attempting to isolate the reaction steps of the pathway and then seeking experimental evidence of these substrates, products, enzymes and effectors. Bechtel and Richardson identify a key constraint that the discipline of biochemistry placed on an acceptable account of the glycolytic pathway: that the mechanism would involve a linear sequence of reactions i.e. there were no feedback loops. This constraint reflected the lack of importance attached to feedback at the time; consequently it was assumed that pathways were linear.

> "The assumption was tantamount to near decomposability because, if it held, each reaction necessarily occurred in relative independence and could be studied in isolation, provided the appropriate inputs are available." (Bechtel and Richardson, 2010, p. 157).

This assumption hindered the discovery of glycolytic pathway. It was used to infer that any substrate that was an intermediary in the pathway should be able to produce pyruvate (step 10) at least as quickly as glucose. Consequently, the proposal that dihydroxyacetone phosphate (step 5) was an intermediary was initially rejected; as a pathway starting with dihydroxyacetone phosphate produced pyruvate at a slower rate than the glycolytic pathway. Eventually it was recognised that dihydroxyacetone phosphate was an intermediary, but that feedback loops increase the rate of production of pyruvate. But recognising that the glycolytic pathway involved feedback:

> "violated the assumption of linearity and which therefore had the effect of undermining near decomposability" (Bechtel and Richardson, 2010, p. 163).

Bechtel and Richardson's analysis of pathway near decomposability is dependent on their three additional criteria. The first criterion requiring discrete functional roles is needed for their claim that using the 'twin strategies' amounts to assuming near decomposability. The second criterion on isolated subsystems is needed to support the claim that the discovery methods used by biochemists are centered on near decomposability. And, as I will in argue in the next section, the third criterion on self-control is needed for their claim that feedback undermines near decomposability.

Bechtel and Richardson's description of the causal discovery of pathways is incorporated into my account of the Strategy of Decomposition in my section 1.5. My

account also incorporates some of the conclusions from Bechtel and Abrahamsen's (2005) analysis on using simulation to recompose a target dynamic behaviour.

Bechtel and Richardson's case study successfully illustrates the impact that feedback can have on pathway dynamics. However, it makes two claims that I shall now argue against:

- that pathways without feedback are ND.
- that feedback undermines near decomposability.

3.4 Pathways are not Nearly Decomposable

The aims of this section are (i) to provide a set of arguments explaining why pathways are *not* ND, irrespective of whether they have feedback (ii) to show that even if pathways were ND, significant levels of feedback would not necessarily reduce that near decomposability (iii) to explain that Bechtel and Richardson's three additional criteria do not affect the arguments for pathways not being ND. Showing that pathways are not ND will prepare the ground for my section 3.5, in which I consider alternative types of modularity.

I will begin by reviewing the parts, interactions and intensities of interactions that would be relevant for a pathway near decomposition. Two reasons will then be provided as to why the concept of near decomposability does not apply to pathways. The first is that key interactions cannot be classified as being either intra-subsystem or inter-subsystem. The second is that pathways are not dynamically autonomous. I will also explain that for *in vivo* systems, a reaction step's inter-subsystem interactions are likely to be of a greater intensity than their intra-subsystem interactions. I will then explain why the impact of feedback would not necessarily be to reduce near decomposability. Finally, I will consider Bechtel and Richardson's three additional criteria.

Bechtel and Richardson's analysis concerns the near decomposability of a pathway into its reaction steps (henceforth: RSs). A pathway is taken to have two levels:

	Subsystems
2nd level	Reaction steps
Bottom level	Reactants (including enzymes and effectors) and products

The bottom level interactions are chemical reactions.

Pathway near decomposability and the effects of feedback will be evaluated relative to The Criteria for Near Decomposability that were elicited from Simon's writings:

(i) the intensity of intra-subsystem interactions is significantly greater than the intensity of inter-subsystem interactions (for a given level).

(ii) the intensity of interactions (both inter- and intra- subsystem) significantly increases with the lowering of levels.

(iii) satisfaction of (i) and (ii) results in same-level subsystems having a high degree of dynamic and structural autonomy.

Bechtel and Richardson's three additional criteria will then be considered and it will be explained why they are (a) not relevant to the arguments showing that pathways are not ND (b) are only trivially relevant to the claim that feedback would impact on near decomposability.

In Simon's two examples of ND systems, the interactions are between components that persist during their interactions. The office cubicles continue to exist whilst heat is transferred between them. Similarly, neutrons and protons continue to exist whilst bonding with each other. This is in contrast to the transformations that occur in chemical reactions where reactants are chemically changed into products. This transformational relationship will now be explained.

Consider an elementary reaction between chemical species B and C. The chemical reaction equation:

$$B + C \rightarrow D + E$$

states that a molecule of species B combines with a molecule of species C and is transformed into a molecule of D and a molecule of E. In biochemistry, there are two types of elementary reaction: unimolecular reactions which involve only one molecular entity and bimolecular reactions which involve two molecular entities. Elementary reactions occur via the formation of an activated complex. For the above reaction to occur a molecule of B needs to collide with a molecule of C to form an activated complex which is then transformed into the products. Not all collisions will be 'effective' and lead to the formation of the products. For a collision to be effective a variety of conditions will need to be satisfied, such as the molecules having sufficient kinetic energy and having the correct relative orientations.

Bartholomay (1960) has formulated a 'molecular set theory' that mathematically represents the chemical equations of elementary reactions. This further clarifies the relationship between reactants and products. Bartholomay's analysis can be illustrated for the same chemical reaction as above. A chemical reaction is represented in terms of events between elements of the sets: **B**, **C**, **D**, **E**. These elements are individual molecules, for example the initial number of molecules of B is n_B and its elements are $b^{(i)}$, i.e. $b^{(i)} \in \mathbf{B}$ (i= 1, 2,..., n_B). A reaction occurs when an element of **B** 'effectively collides' with an element of **C** and the two elements are transformed into elements of **D** and **E**. The chemical equation is represented as the molecular set transformation:

$$T: \mathbf{B} \times \mathbf{C} \rightarrow \mathbf{D} \times \mathbf{E}$$

Where 'x' stands for the Cartesian product. The domain **B** x **C** is the set of all possible (b,c) pairs and the co-domain **D** x **E** is the set of all possible (d, e) pairs. Each individual pair $(d^{(ij)}, e^{(ij)})$ is traceable to an individual pair $(b^{(i)}, c^{(j)})$. The individual values of the transformation:

$$T(b, c) = (d, e)$$

will depend on which random collisions between molecules of **B** and **C** are 'effective'. In cases where $b^{(i)}$ 'effectively collides' with $c^{(j)}$ the above equation can be read as 'transformation T applied to molecule $b^{(i)} \in \mathbf{B}$ and molecule $c^{(j)} \in \mathbf{C}$ produces molecules $d^{(ij)} \in \mathbf{D}$ and $e^{(ij)} \in \mathbf{E}$.

Applying the concept of near decomposability to chemical reactions might appear problematic, as there are no direct interactions between a specific reactant molecule and its product molecules. In each molecular level reaction, specific reactant molecules (e.g. $b^{(9)}$, $c^{(3)}$) are transformed into specific product molecules (e.g. $d^{(9,3)}$,$e^{(9,3)}$). These product molecules therefore only come into existence after their corresponding reactant molecules no longer exist. The relationship between reactants and products is causal but not directly interactive. However, reactant molecules are connected to their product molecules by a chain of interactions that start with the 'effective' collisions of reactants, include the relevant internal interactions of the structures in the activated complex and end with the formation of the product. It is these chains of interactions which would have to be considered in attempting to nearly decompose a pathway.

Bechtel and Richardson do not explain what the intensities of interactions are for reaction steps. As previously explained, Simon does not formally state what is meant by 'intensity of interaction'. However, he provides two examples from the natural sciences. In these 'intensity of interaction' refers to (1) the rate of flow of heat and (2) the strength and frequency of bonds. I am taking intensity to be the product of the number and strength of interactions, per unit time.

In order to help analyse what the intensities of interaction are for RSs, consider the following elementary reaction:

$$A + B \rightarrow C + D$$

Let us first consider the internal interactions. For step 1 these include molecules of A and B colliding, and the breaking and forming of various bonds leading to the formation of C and D. One possible measure is the 'rate of a reaction'. This is the rate of change in the concentrations of reactants and products. For step 1:

$$\text{Rate of reaction } = -\frac{d\,[A]}{dt} = -\frac{d\,[B]}{dt} = +\frac{d\,[C]}{dt} = +\frac{d\,[D]}{dt}$$

Where [x] equals the concentration of substance x, a negative sign means that the concentration is decreasing and a positive sign means that the concentration is increasing. But the rate of a reaction does not measure the number of bonds being broken and formed, or the strengths of these bonds. This suggests that some thermodynamic factor should also be part of the measurement of the internal intensity. Further research would be needed to establish how the internal intensity of

reactions should be measured for near decompositions. This would need to ensure that the same measure is used for both internal and external intensities so that their relative magnitudes could be compared. However, I will now argue that such research is unnecessary, as the concept of near decomposability does not apply to pathways.

I identify two main reasons for this non-applicability, both center on the overlap of the constituents of RSs. I will use the following toy pathway:

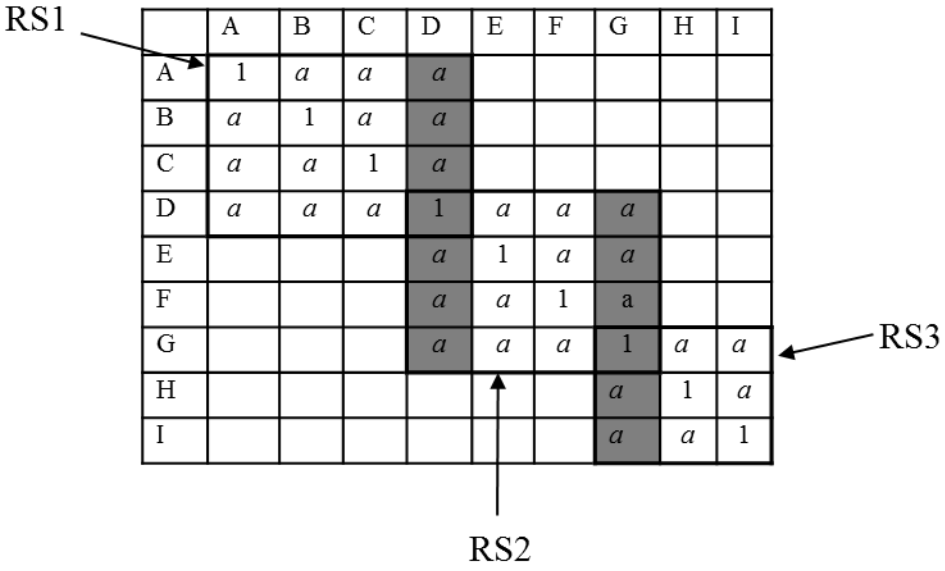
Step 1 $A + B \leftrightarrow C + D$

Step 2 $D + E \leftrightarrow F + G$

Step 3 $G + H \leftrightarrow I$

where the double headed arrows indicate that the reactions are reversible. As I explained in section 2.2.2, a reversible chemical reaction actually consists of two reactions occurring simultaneously, a 'forward reaction' (e.g. $A + B \rightarrow C + D$) and a backwards reaction (e.g. $C + D \rightarrow A + B$).

A substantial number of molecules in a pathway will be in more than one RS. For example, in the toy pathway RS2 is composed of molecules of D, E, F and G. However, the molecules of D are also constituents of RS1 and the molecules of G are also constituents of RS3. The extent of the overlaps can be illustrated using an ND matrix, where the shaded cells highlight that D is in both RS1 and RS2, and that G is in both RS2 and RS3:



	A	B	C	D	E	F	G	H	I
A	1	*a*	*a*	*a*					
B	*a*	1	*a*	*a*					
C	*a*	*a*	1	*a*					
D	*a*	*a*	*a*	1	*a*	*a*	*a*		
E				*a*	1	*a*	*a*		
F				*a*	*a*	1	a		
G				*a*	*a*	*a*	1	*a*	*a*
H							*a*	1	*a*
I							*a*	*a*	1

Fig 3.3 A matrix representing the intensities of interactions within a toy pathway with three reaction steps.

The first reason for pathways not being ND is that key interactions cannot be classified as being either 'inter-subsystem' or 'intra sub-system'. Let us consider RS2:

Step 2 $D + E \leftrightarrow F + G$

Perhaps its inter-subsystem interactions are with steps 1 and 3? After all D is produced in step 1 and G is consumed in step 3. But what is the interaction between RS1 and RS2? It is not the transferring of D molecules, given that all the existing molecules of D are in both RSs. Perhaps it is the interactions within RS1 that produce D? However, these are intra-subsystem transactions of RS1 and if these are also counted as inter-system interactions for RS2, then there is double counting, which will invalidate the near decomposition of the pathway. We appear to be left in the absurd position of not being able to assign an inter-subsystem interaction between the two RSs. The Criteria for Near Decomposability cannot therefore be applied.

Even if there was a satisfactory way of assigning inter-subsystem interactions, there might still be a problem insofar as they might not be of a significantly weaker intensity than intra-subsystem interactions. Simon's stated rationale for why inter-subsystem interactions are weaker is based on the intensity of interactions decreasing with distance (though undoubtedly he would have allowed for other reasons). But his stated reason, at least, does not apply to pathways where many reactants will often be in the same chemical solution and are effectively co-present.

The second reason for non-applicability is that RS's are not dynamically autonomous. This is illustrated by pathways not having the relaxation dynamics of ND systems. RSs usually cannot achieve approximate equilibrium in near independence of each other. For a pathway without feedback (or a portion of a pathway) to be in chemical equilibrium each of its reaction steps needs to be a reversible reaction that is itself in equilibrium.[23] Consider again the reversible chemical reaction:

$$A + B \leftrightarrow C + D$$

At equilibrium, the reaction rate of the forward reaction v_1 is equal to the reaction rate of the backward reaction v_2 and there is no net change in the concentrations of A, B, C and D. In order to show that the RSs usually cannot achieve approximate equilibrium in near independence of each other, we will consider a simple pathway

[23] (i) Open biological systems have steady states rather than equilibrium. (ii) All chemical reactions are reversible. However, reactions are often classified as irreversible when their forward rate greatly exceeds their backward rate.

without feedback consisting of two reversible reactions. The same reasoning will also apply for longer pathways that do not have feedback.

Step 1 $A + B \leftrightarrow C + D$

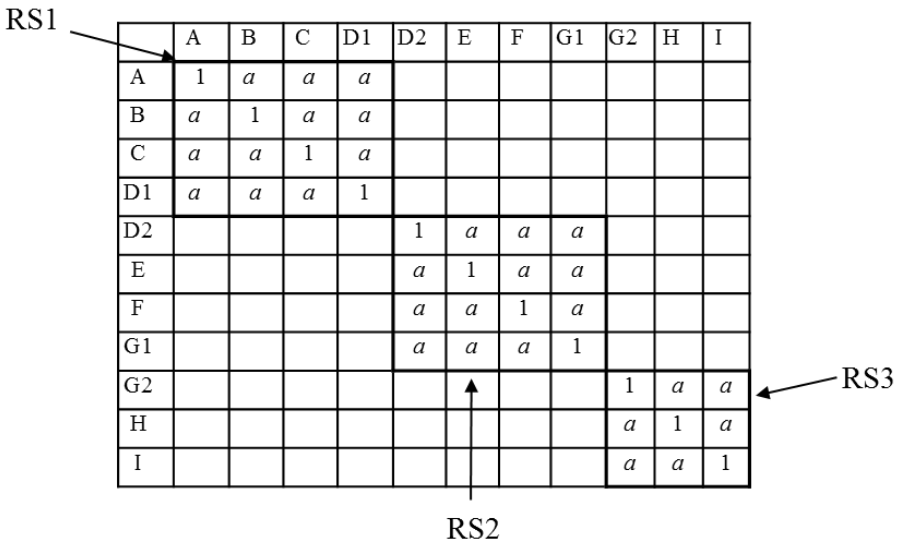
Step 2 $D + E \leftrightarrow F + G$

For RS1 to be at equilibrium, its forward and backward rates of reaction must be equal and the concentration of D must be constant. The same requirements apply to the RS2. This means that the two RSs have to come to equilibrium simultaneously. This is because if an RS is not at equilibrium then it is either producing or consuming a net quantity of D. The other RS will then not be able to satisfy both the requirements of (i) its forward and backward reaction rates being equal and (ii) maintaining a constant concentration of D. The problem is that D is a constituent of both RSs. Hence when starting from disequilibrium, the RSs' short-term behaviours are not 'approximately independent'.[24]

The toy example understates the extent to which RSs overlap within actual pathways. This is because some chemicals are involved in several RSs. For example, in the glycolytic pathway ATP is a reactant or product in four of the ten RSs. The same is true for both ADP and H^+.

A possible counter-argument to my two reasons would be to claim that a near decomposition of a system should only use 'component parts', which are defined by their performing a specific function and their mapping onto a specific physical part. As such there can be several component parts corresponding to a single physical part. For example, the bottom level of the toy pathway would not include reactant D, but instead 'D qua a reactant in RS1' and 'D qua a reactant in RS2'. I will refer to these as 'D1' and 'D2' respectively. This approach would seem to be consistent with Bechtel and Richardson requiring that subsystems have discrete functional roles; it simply extends the same requirement to the bottom level of the ND system. If this approach is accepted, then there are no problems with RS's having overlapping constituents. The corresponding ND matrix would then have the desired diagonal block sequence:

[24] There will be some pathways that have an RS (or a few RSs) that can achieve approximate equilibrium independently of its pathway's other RSs. This could be the case if an RS's rates of reaction are much faster than its adjacent RSs. In such cases the RS can achieve equilibrium while its adjacent RSs remain effectively stationary. However, it seems highly unlikely that such a pattern of adjacent fast and slow RSs exists across a significant proportion of any pathway that is recognised by the discipline of biochemistry.



	A	B	C	D1	D2	E	F	G1	G2	H	I
A	1	*a*	*a*	*a*							
B	*a*	1	*a*	*a*							
C	*a*	*a*	1	*a*							
D1	*a*	*a*	*a*	1							
D2					1	*a*	*a*	*a*			
E					*a*	1	*a*	*a*			
F					*a*	*a*	1	*a*			
G1					*a*	*a*	*a*	1			
G2						*a*			1	*a*	*a*
H									*a*	1	*a*
I									*a*	*a*	1

Fig 3.4 A matrix representing the intensities of interactions within a toy pathway with three reaction steps, in which reactant D is separated into 'component parts' D1 and D2.

But this is an unacceptable representation of the pathway. By failing to identify that the same physical parts are constituents in two RSs, it is falsely implied that the RSs are dynamically autonomous and can independently come to separate equilibria. Using 'component parts' in this way leads to intra-subsystem interactions being omitted, for example D in RS2 is now independent of reactants A and B by being reclassified as D2. But this then leads to minimally decomposable systems being misclassified as being ND. Simon's concept of ND is based on bottom-level entities being physical parts. This is demonstrated by his claim that if a dynamic system can be described by an ND matrix, then it has the properties of an ND system (Simon, 1962, p. 475). If component properties are used, then this claim is false. It should also be noted that Bechtel and Richardson are focused solely on a concept of modularity that can play a useful role in the actual practice of biology. It would therefore be unacceptable to 'save' a putative application of near decomposability by concocting transient component parts whose identity depends on whether they have just been created or are about to be transformed. Such a concoction would be practically useless given that the component parts are co-present in the same chemical solution.

Even if the concept of near decomposability did apply to pathways, it is likely that it would have very limited applicability to *in vivo* pathways. This is because *in vivo* pathways exist within large networks of biochemical reactions. This leads to many reactions being highly interdependent, with the same chemical substance often being involved in multiple reactions. I will illustrate this using a metabolic network of E.

coli. A recently constructed metabolic network of E. coli depicts 924 metabolites engaged in 1437 reactions (Zhoa et al., 2006, p. 2). Metabolites are the chemical substances that are either intermediates or products of metabolism; they do not include enzymes. A small subset of this network is the glycolytic, pentose phosphate and Entner-Doudoroff pathways which all convert glucose to pyruvate. The following diagram shows their interconnections.

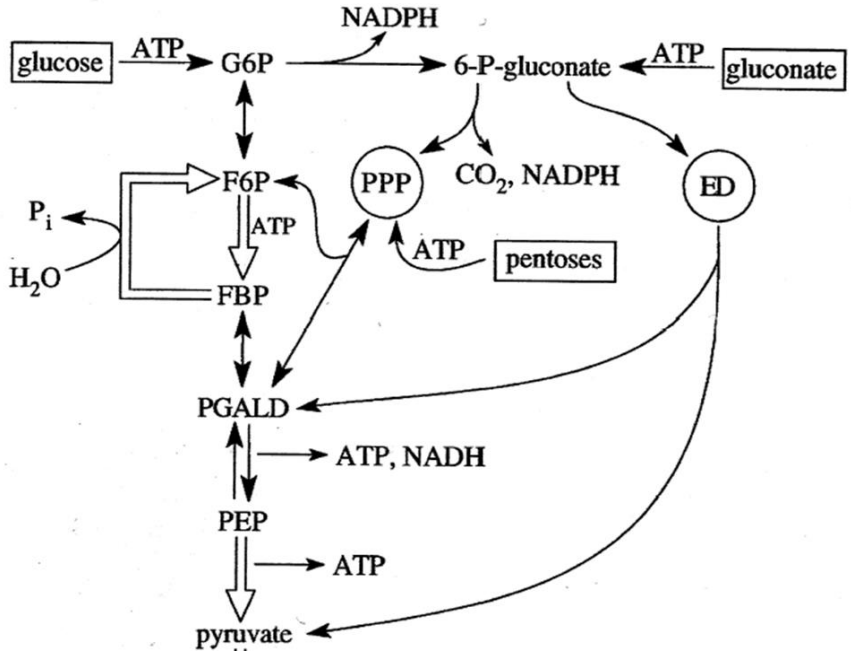


Fig 3.5 Diagram of the central metabolic pathways of E. coli. The left hand pathway is the glycolytic pathway. Some of the reaction steps have been omitted. Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; NADPH, nicotinamide adenine dinucleotide phosphate; NADH, nicotinamide adenine dinucleotide (reduced form); F6B, fructose-6-phosphate; PGALD, glyceraldehyde-3-phosphate. PPP is the pentose phosphate pathway and ED is the Entner-Doudoroff pathway (White, D., 2007, p. 197).

Diagrams such as the above can give a misleading impression of the extent of interaction between pathways. This is because some of the metabolites are also components of many other pathways and reactions. For example, Wagner and Fell (2001) carried out their analysis on a subset of E. coli's metabolic network, consisting of only 367 reactions and found that 3-phosphoglycerate (steps 7 and 8 of the glycolytic pathway) directly interacted with thirteen other metabolites, whilst pyruvate directly interacted with thirty-nine metabolites.[25]

[25] Wagner, A. and Fell, D. (2001) –Details of the pathways included in their network are on p. 1804. Two metabolites 'directly interact' if they occur in the same chemical reaction (either as substrates or products), this is referred to as 'connectivity' on p. 1806.

The strong interconnectivity between pathways means that *in vivo* pathways often would not be ND (even if the applicability problems did not apply). Many of the metabolites in individual RSs will be interacting (i.e. reacting) with the metabolites of other pathways; when attempting a decomposition these interactions between RSs will count as inter-subsystem interactions for the RS. Given the extent of these interactions, it seems highly likely that for many RSs, the intensity of their inter-subsystem interactions will *not* be significantly smaller than the intensity of their intra-subsystem interactions.

I have shown that the concept of near decomposability does not apply to pathways and, furthermore, that the RSs of *in vivo* pathways are subject to strong inter-subsystem interactions. I will now evaluate the impact that feedback would have on pathways if, contrary to my arguments, pathways without feedback were ND.

Bechtel and Richardson claim that feedback undermines near decomposability because it means that subsystems are almost continuously impinging on each other. They state:

> "What is important about mechanism with positive and negative feedback for our purposes is the tension they place on the assumption of decomposability or near decomposability. The more the various operations in the mechanism affect each other, the less successful is a sequential account of the mechanism in which each operation is treated as independent of the others." (Bechtel and Richardson, 2010, p. xxxv)

I will use a toy (non-chemical) example to illustrate that even significant levels of feedback do not necessarily reduce near decomposability.

Let us consider a toy example which consists of two non-overlapping subsystems X and Y. X receives feedback from subsystem Y. If it was not for the feedback the system would be ND. However, the feedback significantly increases the rate at which X completes its internal activities and produces its output. In turn, the increasing rate of output from X increases the rate at which Y operates.

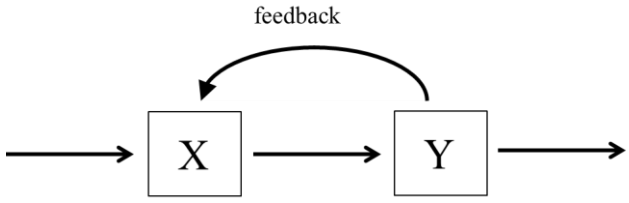


Fig 3.6 Toy example of pathway in which subsystem X receives feedback from subsystem Y

Does the feedback then mean that the system is not ND? It depends on whether the feedback sufficiently lowers the *intra-subsystem intensity / inter-subsystem intensity* ratios of the subsystems. In this example, the feedback increases both the intensities of the intra and inter-sub-system interactions of X. The extent to which the ratios are increased or decreased by the feedback will depend on further details about the feedback and the internal rate laws of X Without these details the effect of the feedback on near decomposability cannot be determined.

The general point is that the impact of feedback is not necessarily to reduce near decomposability. It will depend on a variety of factors that are specific to each particular system. Bechtel and Richardson claim that feedback results in the glycolytic pathway not being ND. Even if pathways without feedback were ND, the correctness of their claim could not be established without an analysis of the effects of the feedback on the *intra-subsystem intensity / inter-subsystem intensity* ratios of the RSs. Such an analysis is not provided.

Bechtel and Richardson's three additional criteria for near decomposability do not affect my arguments that pathways are not ND. The additional criteria are:

a) subsystems have discrete intrinsic functions.
b) the properties of each subsystem can be determined in isolation of the system's other subsystems

c) the subsystems of a mechanism are considered to be controlled by intrinsic factors.

Given that these criteria are supplementary to Simon's concept, the fact that Simon's concept does not apply to pathways is sufficient for Bechtel and Richardson's modified concept also to be inapplicable. In Bechtel and Richardson's account of pathway causal discovery, most of the work is done by their three new criteria. At no point in their analysis of pathways are either intensities of interaction, dynamic autonomy or structural autonomy considered. Relative to just their new criteria, it is plausible that pathways without feedback are modular. Criterion (a) is satisfied with a reaction step's functional role being to convert its substrate into products (recall section 3.3), criterion (b) is often assumed by the discipline of biochemistry to be correct, though I shall question the correctness of this assumption in my section 3.6, criterion (c) is satisfied as there is no feedback. It also trivially follows from criterion (c) that a pathway with significant levels of feedback would not be modular. Perhaps a different type of modularity, defined solely in terms of these additional criteria would better capture their claimed relationships between pathway causal discovery, modularity and feedback? But this would then be capturing a type of 'modularity' that was, perhaps, being assumed by biochemists in the early decades of the twentieth century; and is no longer relevant. The ubiquity of feedback in biochemistry is now well understood.

3.5 A Plurality of Types of Modularity

Simon claims that given our limited cognitive abilities it is only because complex systems are ND that we are able to understand them. But pathways are not ND and yet we have often gained a good understanding of their mechanisms. This points to a significant shortcoming in Simon's analysis: that it does not recognise that there is a plurality of types of modularity that can be usefully applied both in biology and in science more generally. The same criticism applies to Bechtel and Richardson's analysis. I propose a modified version of Simon's claim:

> *given our limited cognitive abilities, it is only to the extent that biological systems are nearly modular in some relevant sense, that we are able to understand them.*

Furthermore, there are types of modularity whose primary benefit is not in enabling understanding, but in enabling the manipulation, partial replacing or the reengineering of biological systems.

Starting from Simon's and Bechtel/Richardson's analyses of near decomposability, five types of modularity will be identified. I have already shown that their concepts of near decomposability entail a conjunction of distinct types of autonomy. I will demonstrate that each of these types of autonomy can be used to formulate a distinct type of modularity that is important either to the analysis of pathways or to biology more generally. And that these types capture some of the different ways in which biologists apply the notion of modularity. Some of these types of modularity apply to systems that are not ND. One of these is 'causal law modularity' and in my section 3.6, I will argue that it is this type of modularity, rather than near decomposability, that is central to the pathway discovery (and is assumed within the Strategy of Decomposition). I take the identification of these five types of modularity to be an original contribution to the philosophy of biology.

I shall only be concerned with types of modularity where a system *S* being modular is relative to an analysis of a target phenomenon of *S*. A satisfactory specification of a type of modularity needs to state both the criteria for identifying what constitutes a subsystem and the sense in which these subsystems are required to be autonomous. I take it that for any type of modularity to be generally useful in biology, its modules must correspond to a functional decomposition of the target phenomenon; for example, it is unlikely that a decomposition into non-functional modules would contribute to an explanation of a target phenomenon. For the five types of modularity that I identify, each module of a decomposition consists of the organised parts and operations that collectively achieve that module's function. The different types of modularity, differ in their type of autonomy.

Different types of modularity might be useful for different types of analysis. For example, a different type of modularity might be useful to an analysis concerning re-engineering compared to an analysis concerning causal discovery. A system *S* may have only one of these types of modularity or it may have several. I will use the following notation: a system *S* is composed of subsystems $C_1 \ldots C_n$. The first two types of modularity require invariance between properties manifested by $C_1 \ldots C_n$ and properties manifested by objects of the same kind as $C_1 \ldots C_n$ that exist in a different

context to *S*. The other three types of modularity solely concern the properties manifested by $C_1...C_n$ whilst situated within *S*.

Causal law modularity. The first type of modularity relates to Bechtel and Richardson's requirement that:

> "it is feasible to determine component properties in isolation of other components, despite the fact that they interact." (Bechtel and Richardson, 2010, p. 26).

Bechtel and Richardson do not specify which types of properties this is meant to apply to. I take there to be two requirements. First, the properties need to be invariant in the sense of being manifested both by subsystems $C_1...C_n$ and by objects of the same kind as $C_1...C_n$ that are situated in isolation. Second, these properties need to be useful in helping to understand the target phenomenon. For biological systems, the required properties cannot be subsystem behaviours, as the interactions between subsystems mean their behaviours are not invariant relative to being in isolation. Instead, if we look to the practices of contemporary biochemistry, we see that the required properties are taken to be local causal laws. Local causal laws are the laws that apply in a particular context. They can be highly context sensitive, applying to a limited number of contexts and small changes in their context can 'break' a law. Biochemistry assumes 'causal law modularity' in which subsystems $C_1...C_n$ are autonomous in the sense that they manifest local causal laws that are also manifested by objects of the same kind as $C_1...C_n$ that are situated in 'isolation'. When analysing the dynamics of pathways, the relevant local causal laws are the RS's rate laws. Biochemistry takes it that these have the required invariance, that these can be combined to produce laws that apply to the whole system *S,* and can then be used to explain *S*'s behaviours (recall 2.2.2). In section 3.6, I will explain how biochemistry justifies its assumption that pathways are often causal law modular.

External structural modularity. This requires subsystems $C_1...C_n$ to be autonomous in the sense that they have the same functionality (i.e. input-output relations), as objects of the same kind as $C_1...C_n$ that are parts of other target systems. The choice of target systems will be relative to a particular analysis. This is consistent with Simon's account of 'external structural autonomy' but adds that this type of modularity is relative to a specific set of target systems. This added qualification is necessary for the concept of external structural modularity to have any general applicability in biology; given the context-sensitivity of biological sub-systems (as explained below).

When systems have external structural modularity, a part-based engineering approach can be used to construct new systems. Systems such as electrical circuits have this type of modularity.

A central assumption of the discipline of synthetic biology is that genetic systems have external structural modularity. Synthetic biology aims to build novel biological functions and systems.

> "synthetic biology is perhaps best defined by some of its hallmark characteristics: predictable, off-the-shelf parts and devices with standard connections, robust biological chassis (such as yeast and E. coli) that readily accept those parts and devices, standards for assembling components into increasingly sophisticated and functional systems and open-source availability and development of parts, devices, and chassis." (www.synbioproject.org)[26]

Subsystems ('parts and devices' in the above quote) can be either molecules or functional portions of molecules, for example promoters, ribosome binding sites and transcriptional repressors which are then joined together. One of the aims of synthetic biology is the construction of novel artificial pathways.

However, it should be noted that the success of synthetic biology has so far been limited. For example, a recent editorial in Nature Methods noted that 'the field has yet to reach the point where genetic parts can be predictably combined to a desired outcome' (Nature Methods, Vol 11, No 5, 2014). This is because context sensitivity is a key characteristic of biological systems. The problem that context sensitivity poses to external structural modularity, can be illustrated by considering two toy molecules S_1 and S_2. S_1 consists of four subsystems A, B, C, D with the structure A-B-C-D, where the hyphens represent chemical bonds. Hence A is bonded to B, B is bonded to both A and C, and so forth. Now consider S_2 which has the structure B-C-D-A. The functionality of A might be very different when it is a part of S_1 compared with being a part of S_2. Amongst the reasons for this are that the functionality of a chemical subsystem can vary significantly depending on what it is directly bonded to. So, if B is electrophilic ('electron loving') and D is electrophobic ('electron hating'), it might be the case that A has a positive electrical charge in S_1 but a negative electrical charge in S_2. Such differences can result in different functionalities. It should also be noted that the functionality of A may also be significantly affected by the context in which S is located. For example, A's functionality may vary with the acidity of its surrounding

[26] This is from the definition used by Synberc that is cited on www.synbioproject.org accessed 3/12/15

solution. Because of context sensitivity, the parts-based engineering vision of synthetic biology may simply not be realisable (Guttinger, 2013). Synthetic biology has been able to develop some interesting novel systems, but the methods it has used are closer to 'kludging' than 'rational design methods' of a parts-based approach (O'Malley, 2011). Kludging (klumsy, lame, ugly, dumb but good-enough) refers to an iterative process of trial and error, workarounds and tweaking.

Synthetic biology is still in its infancy and the extent to which genetic systems are 'external structural modular' is unclear. Before this can be properly evaluated, the concept of external structural modularity needs to be more fully developed. Simon's account only provides the barest sketch of the required autonomy. Although synthetic biology frequently refers to the 'modularity' of biological systems, a rigorous conceptual analysis of the term seems lacking. Such an analysis is needed to address such factors as how the set of target systems should be selected and in what respective physical contexts are *S* and the target systems required to be modular. Providing such an analysis is beyond the scope of this PhD.

Internal structural modularity. This requires that *S's* subsystems are autonomous in the sense that changes to the internal causal relationships of a subsystem do not change the functionality of *S*'s other subsystems i.e. the input / output relationships of *S*'s other subsystems remain unaltered. I identify three ways in which this is important within biology. First, in enabling us to manipulate systems and hence learn about their causal structures. If *S* has this type of autonomy then, at least in principle, interventions can be carried out on one subsystem C_i in order to evaluate the causal relationships between C_i and *S*'s other subsystems. Such interventions could correspond to Woodward's notion of ideal interventions, as described later in my section 3.7. Second, in providing opportunities for replacing subsystems within *S*. For example, it is possible to replace one subsystem with another that is functionally equivalent e.g. replacing a damaged cochlear with a cochlear implant. Third, in enhancing the evolutionary capabilities of organisms (recall section 3.2).

Wagner and Altenberg (1996) provide a paradigm account of what the structure of evolutionary modules might be. They argue that adaptation requires that organisms can produce stepwise improvements in fitness, and that stepwise improvements are more likely if the effects of genetic mutations are restricted so as not to compromise past improvements. This, in turn, requires that the effects of pleiotropy are limited

(Wagner and Altenberg, 1996, p. 971); pleiotropy being the condition in which a single gene influences more than one trait, an 'array of phenotypic effects is typical for most genes and results from the interconnections between the biochemical and cellular pathways that the genes control" (Snustad and Simmons, 2015, p. 80).

Wagner and Altenberg illustrate their proposed concept of evolutionary modularity:

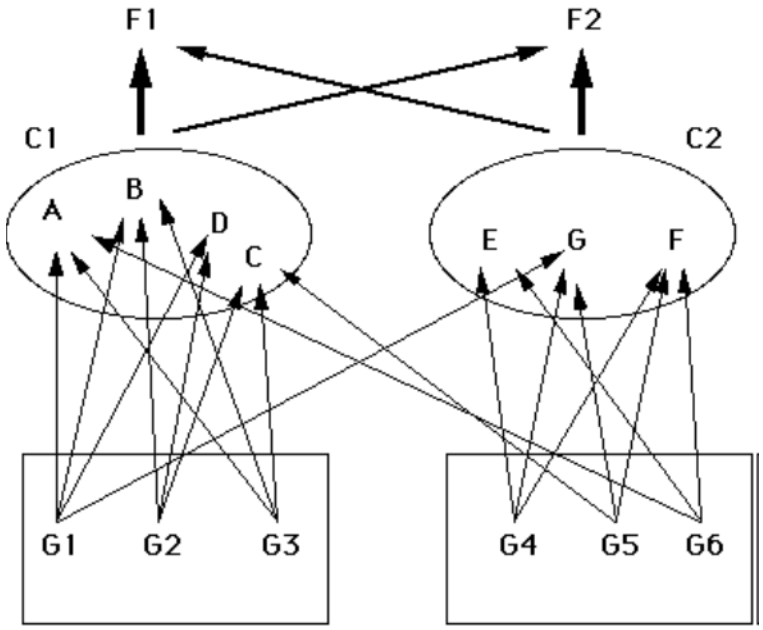


Fig 3.7 Example of two evolutionary modules (Wagner and Altenberg, 1996, p. 970). Wagner and Altenberg do not state what their arrows represent, but presumably the arrows between genes and character complexes represent causal relationships and the arrows between character complexes and functions represent constitutive relationships.

A module includes a grouping of genes and their phenotypic characters. In the above diagram there are two modules:

	Module One	Module Two
Genes	{G1, G2, G3}	{G4, G5, G6}
Character complex	{A, B, C, D}	{E, F, G}
Primary function	F1	F2

Wagner and Altenberg say that the groupings are modular because there are more pleiotropic effects within each group than between them. The phenotype characters are grouped in a 'character complex' which has a primary function. These primary functions are largely independent of each other. Nature can then select for genetic mutations that improve one function without strongly impacting on other functions.

Examples of such low polygeny, low pleiotropy functions include hair colour, immunoglobin antigen binding and enzyme activity (Wagner and Altenberg, 1996. p. 972).

Few strong interactions modularity. S's subsystems are autonomous in this sense when each part only has strong interactions with a small proportion of the system's other parts. When this is the case then there can be a significant reduction in the number of interactions that need to be taken account of in order to understand the whole system. This type of autonomy is possessed by ND systems, as is illustrated by Simon's example of the system of one hundred and twenty-five interacting parts being understandable in terms of the interactions between thirty-one parts. This type of modularity can also be possessed by non-ND systems. For example, when analysing pathways, it may often be the case that the chemical species of an RS will only have strong interactions with a limited number of the pathway's other RSs.

This appears to be the type of modularity that some systems biologists take to be present in biological networks, including metabolic networks. Biological networks are analysed using graph theory. Graphs are composed of vertices and edges, for example $G = (V, E)$ where V is a set of vertices and E is a set of edges. An edge e_{ij} connects vertices v_i to v_j. In directed graphs edges are orientated so that $e_{ij} \neq e_{ji}$, whilst in undirected graphs edges have no orientation so that $e_{ij} = e_{ji}$. A vertex's 'degree' is the number of edges attached to it. In the case of metabolic networks, vertices are used to represent specific metabolites and edges connect pairs of vertices that are related as reactant and product. Enzymes are not explicitly represented but it is implicitly understood that there will generally be a unique enzyme associated with each edge. Directed edges are used to represent irreversible reactions and undirected edges to represent reversible reactions.

Systems biologists have attempted to decompose graphs representing biological networks into sets of sub-graphs such that the vertices in each sub-graph are densely connected but the connections between the sub-graphs are sparse. In these cases, the sub-graphs are called 'community structures' or 'modules'. The modularity of a

decomposition can be measured using Newman and Girvan's (2004) 'modularity coefficient'. Consider a network that is decomposed into k sub-graphs. A k x k symmetric matrix m can then be defined where m_{ij} is the fraction of the network's edges that connect vertices in sub-graph i to vertices in sub-graph j. The trace of the matrix m is the sum of the elements on its main diagonal i.e. Tr $m = \sum_i m_{ii}$. This equals the fraction of the network's edges that connect vertices within the same sub-graph. Here is an example for a network of 150 vertices that has been decomposed into three sub-graphs:

Number of edges connecting sub-graphs

	1	2	3
1	25	6	10
2	6	35	4
3	10	4	50

$\Rightarrow \quad \boldsymbol{m} =$

	1	2	3
1	$\frac{25}{150}$	$\frac{6}{150}$	$\frac{10}{150}$
2	$\frac{6}{150}$	$\frac{35}{150}$	$\frac{4}{150}$
3	$\frac{10}{150}$	$\frac{4}{150}$	$\frac{50}{150}$

$$\text{Tr } \boldsymbol{m} = \frac{25}{150} + \frac{35}{150} + \frac{50}{150} = \frac{11}{15}$$

Fig 3.7 Example of the first steps in calculating the Newman Girvan modularity coefficient.

The matrices show that there are twenty-five edges connecting vertices in sub-graph 1 to other vertices in sub-graph 1, six edges connecting vertices in sub-graph 1 to vertices in sub-graph 2 and so forth. The row sum $a_i = \sum_j m_{ij}$ equals the fraction of edges that connect vertices in sub-graph i to other vertices in the network (including other vertices in sub-graph i). If the edges in a network were randomly distributed then the expected value of m_{ij} would equal $a_i\, a_j$. Hence if there is no clustering into modules then E(m_{ii}) = a_i^2. The modularity coefficient Q is defined as:

$$Q = \text{Tr } \boldsymbol{m} - \sum_i (a_i^2) \qquad \text{(Newman and Girvan, 2004, p. 026113 –7)}$$

If the number of edges in each putative module is randomly distributed then Q is likely to have a value near zero, whilst values of Q approaching one indicate strong modularity. Guimerá et al. (2004. p. 2) define the modularity of a network as being the largest modularity coefficient of all possible partitions of the network.

Graphs of metabolic networks have been generated with the aid of genome sequencing. Sequencing data enables the identification of an organism's enzymes. Reaction databases (e.g. the Kyoto Encyclopaedia of Genes and Genomes Database (henceforth: KEGG)) can then be used to match these enzymes to their chemical reactions and hence create a list of all the metabolic reactions occurring within an organism.

In such graphs the highest degree vertices correspond to 'currency metabolites'. These substances include ATP, ADP, NADH, NAD^+ and Pi. They often function as electron carriers or carriers of functional groups such as phosphates or methyl groups. Other substances such as H_2O and CO_2 are also classified as currency metabolites; as such there does not appear to be a general functional definition for current metabolites, rather they just are substances that appear in a multitude of reactions. When graphs have been constructed that include currency metabolites, they have been found not to be modular. However, if the currency metabolites are then excluded the graphs are found to be 'modular'. The effect of excluding currency metabolites is illustrated with the following graphs for *Streptococcus pneumoniae*, the left hand graph includes currency metabolites, the right hand does not.

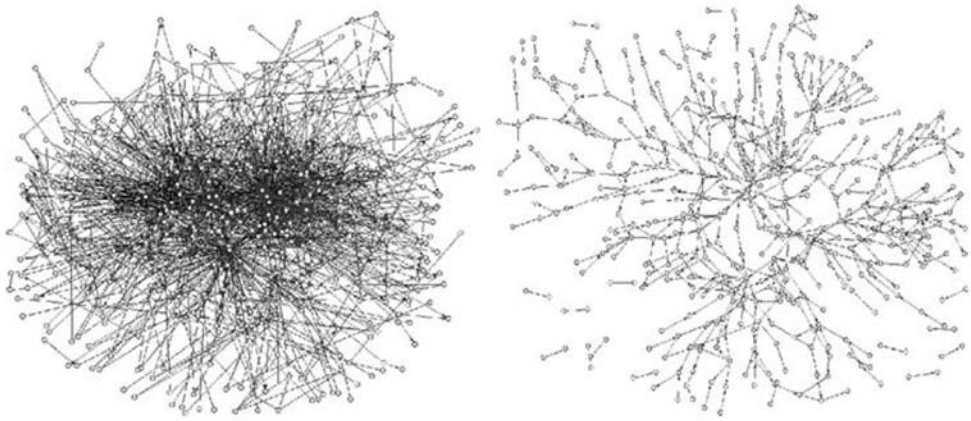


Fig 3.8 Diagrams of the metabolic network for *Streptococcus pneumoniae* - with and without currency molecules. (Silva, M. et al., 2008, p. 238)

Holme (2009, p. 1) justifies the exclusion of currency metabolites on the view that the 'higher functionality, and thus the most interesting information' is contained in the organisation of the non-currency metabolites. To illustrate this, Holme uses as an analogy a graph representing a science conference. Each vertex represents a person and two vertices are connected if the two people had a conversation with each other. Most of the scientists would have conversed only with the reception staff and with

some of the small cluster of scientists working in their particular field. The functional output of the conference was the advancement of science and what most mattered for this were the conversations within these clusters of scientists. The reception staff would have been important for the conference to work but they would have had a secondary, background role. If the reception staff were included in the graph their vertices would have the highest degrees and this would hide the modular structure of the scientists' interactions. Ma and Zeng (2003) illustrate the currency metabolite interactions in the glycolysis pathway:

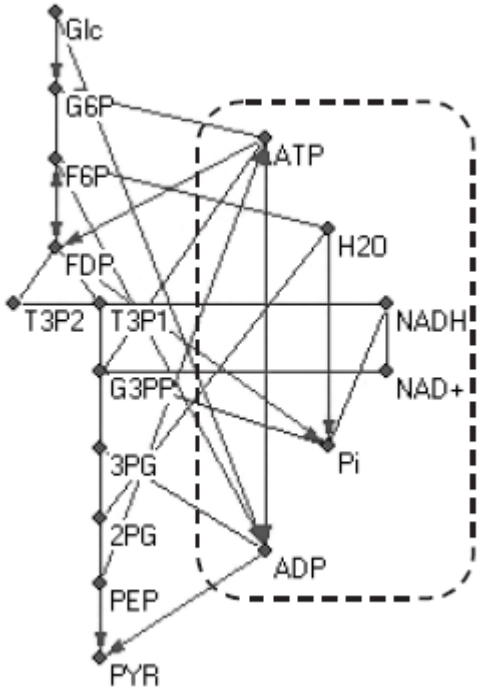


Fig 3.9 Diagram of the glycolytic pathway highlighting the currency metabolites. Abbreviations of currency metabolites: ADP, adenosine diphosphate; ATP, adenosine triphosphate; NAD^+, nicotinamide adenine dinucleotide (oxidised form); NADH, nicotinamide adenine dinucleotide (reduced form); Pi, phosphate group (Ma, H. and Zeng, A., 2003, p. 271)

Zhoa et al. (2006) provide a modularity analysis of the E.coli's metabolic network. By excluding currency metabolites and using the Newman and Girvan metric, twelve 'modules' are identified.

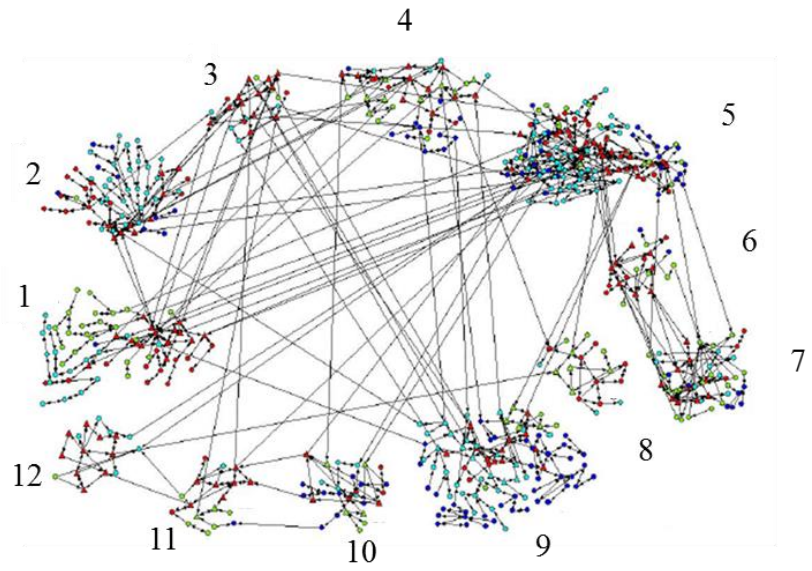


Fig 3.10 Modularity analysis of the E.coli's metabolic network (Zhao et al., 2006, p. 5)

However, their analysis highlights a significant shortcoming in the systems biologists' approach to decomposing biological networks. There is no requirement for each of the 'modules' to have a recognised function. In the above decomposition, each module contributes to several functions[27] and each function has contributions from several modules. For example, module 5 is involved in: amino acid metabolism, carbohydrate metabolism, energy metabolism, lipid metabolism, nucleotide metabolism and the metabolism of cofactors and vitamins. Modules 1,2,3,5,6,7 and 9 contribute to carbohydrate metabolism. 'Textbook' pathways such as those for glycolysis, pentose phosphate and tricarboxylic acid are each spread over several modules. The following table lists some of the metabolites and modules of the glycolysis pathway:[28]

Substance	Reaction Steps (as specified in section 2.2)	Module
glucose-6-phosphate	1,2	6
fructose-6-phosphate	3,4	6
glycerone phosphate	4,5	5
3-phosphoglycerate	7,8	5
Phosphoenolpyruvate	9,10	1
Pyruvate	10,11	1

Fig 3.11 Table listing some of the metabolites shared by 'modules' of Zhao et al.'s (2006) decomposition of E.coli's metabolic network.

[27] Zhoa uses the KEGG pathway classification of functions.
[28] Zhoa et al (2006) –p. 8

Module five also contains some of the metabolites from the pentose phosphate pathway.[29]

Zhoa et al.'s findings are broadly representative of the (few) analyses that have been carried out on the modularity of metabolic pathways. Once currency metabolites are excluded, there is modularity but the modules do not correspond to pathways or have a recognisable function. Zhoa et al. are optimistic "Hopefully, more research will clarify the biological significance of the underlying difference between topological modules and traditional pathways" (Zhoa et al., 2006, p. 4). But it seems unclear whether the modules identified are 'biologically meaningful' or merely artefacts. I take a fundamental problem with such analyses is that they are based on either the Newman- Girvan metric, or some variation of it, which count all edges as being equal. No account is taken of the vastly different rates of reactions or of the effects of feedback.

Dynamic modularity. This requires *S* to be dynamically autonomous, which as previously specified requires that:

i) the short-run dynamic behaviours of same-level subsystems are approximately independent of each other,
ii) the long-run dynamic behaviours of each subsystem are approximately independent of the details of other same-level subsystems' internal behaviours.

Dynamic autonomy is a consequence of the relative intensities of a system's intra-subsystem and inter-subsystem interactions. This applies to ND systems. When a subsystem's behaviour is dynamically modular then it may be possible to study and understand its behaviour *in situ*, whilst ignoring its interactions with other subsystems (at least in the short term). As such, dynamic modularity can be epistemically important when either (i) the assumption of causal law modularity fails i.e. when subsystems $C_1 \ldots C_n$ manifest local causal laws that are different to those manifested by objects of the same kind as $C_1 \ldots C_n$ that are situated in isolation or (ii) it is impracticable to study subsystems of *S* in isolation of each other. Arguably the

[29] Ribose 5-phosphate and erythrose 4- phosphate from the pentose phosphate pathway

latter applies to much of cell biology, where subsystems are often too fragile to maintain their structures when separated from *S*. For example, i*n vitro* analyses often require destroying much of the structure of a subsystem (e.g. an organelle) and analysing the behaviour of its constituents in chemically homogenous solutions.

In summary, the analyses of both Simon and of Bechtel and Richardson miss that there is a plurality of types of modularity. Contrary to Simon, the complex systems found in biology do not need to be ND to be understood. Other types of modularity can assist in this task. Modularity can also be important for manipulating, replacing and reengineering of biological systems. I will now focus on causal law modularity, as it is this type of modularity that is central to the causal discovery of pathways.

3.6 It's Causal Law Modularity That Matters for Pathway Discovery

The Strategy of Decomposition is based on the assumption that pathways are causal law modular. This is done by assuming that RS rate laws are invariant in the sense that:

> each of the rate laws manifested within a pathway will be manifested in its 'appropriately formulated' isolated reaction step.

I will term this the 'rate law invariance' assumption. The rate law for an RS specifies how its rate varies with the concentrations of its reactants (recall section 1.2). An 'appropriately formulated' isolated RS consists of that step's substrates, products, enzymes and effectors, plus any non-reactive constituents of its pathway that impact on the dynamics of that step. If the 'rate law invariance' assumption is correct then a pathway's RS rate laws can be studied in isolation of each other.

There are three broad stages to the Strategy of Decomposition (recall section 1.5). In the 'extraction stage' the target *in vivo* pathway is separated from its biological context, creating an *in vitro* pathway. In the 'decomposition stage', a functional decomposition is proposed for the target pathway dynamic behaviour. A separate *in vitro* chemical solution is then created for each of the pathway's putative RSs. Each chemical solution will contain the reactants, effectors, enzyme and products that are thought to constitute that RS. These are the putative 'appropriately formulated' isolated reaction steps for the pathway. Experiments are carried out to discover the salient properties of each isolated reaction step, including their operations and rate laws. In the 'reconstruction stage', simulation modelling is used to confirm that the

putative rate laws combine to produce the target pathway dynamic behaviour. The decomposition and recomposition stages are based on the 'rate law invariance' assumption being correct for the target pathway; i.e. it is assumed that knowledge gained of isolated RS rate laws can be used to model the *in vivo* RSs of the target pathway.

Prima facie, the 'rate law invariance' assumption is problematic. This is because the rate constants referenced in a RS rate law are highly context sensitive and small changes in context can lead to changes in their values and therefore to changes in the rate law. This is explained by the context sensitivity of rate constants of the elementary reactions that constitute an RS.[30] The values of elementary rate constants are proportional to the fraction of reactant molecules that have 'effective collisions' per unit time. Effective collisions are those collisions that lead to a chemical reaction. Elementary rate constants vary by context because the number of 'effective' collisions varies by context (for given concentrations of reactants). Many factors have been identified that contribute to this; for example, the reactivity of an enzyme can vary with the acidity of the solution or with the concentrations of other solutes that change that enzyme's conformation. Also, there can be crowding and confinement effects that can have a large impact on the number of effective collisions.

The requirement that the isolated RS be 'appropriately formulated' is meant to take account of the context sensitivity of their rate laws. van Eunen et al. (2012) is an example of an analysis which attempts this by using isolated reaction steps that are as "crowded" as the whole pathway and which have the same "effectors". [31] However, the 'rate law invariance' assumption may still sometimes be false. Consider a toy pathway consisting of the following steps:

Step 1 $B + C \leftrightarrow D$

Step 2 $D + E \leftrightarrow F$

Step 3 $F + G \leftrightarrow H$

Step 1's 'appropriately formulated' reaction step would include B, C, D but exclude E, F, G and H. The 'rate law invariance' assumption misses the importance of non-

[30] The rate constants for step-wise reactions will be functions of the rate constants of elementary reactions (plus some reactant concentrations).

[31] Crowders reduce the space available in which reactions can occur and also reduce rates of diffusion. The interactions between crowders and reactants are non-reactive and the interactions do not require a particular crowder to be present. The effects of crowding are particularly important when considering the differences between *in vitro* and *in vivo* dynamics. See for example García-Contreras et al. (2012).

reactive interactions between the constituents of a RS and the pathway's other reactants and products. For example, the presence of E, F, G or H might be necessary before Step 1's reactants acquire the same conformations they have in the pathway; and conformations can affect rates of reaction. I will term this as the 'Other Reactants Objection'.

It is epistemically desirable that *in vivo* pathways are causal law modular. Directly measuring the rate constants of pathway reactions is often difficult, if not infeasible (recall section 1.4). One of the reasons for this is that the same reactants will often be simultaneously involved in several reaction steps. Another reason is that it is often the case that the structures being investigated are very fragile, and we have very limited direct access without significantly disturbing their processes. Biochemists use the 'invariance of rate laws' assumption to warrant using the rate laws of *in vitro* preparations to model the corresponding *in vivo* mechanisms. But the *in vitro* preparations provide a very different context for the RSs they wish to analyse. There seem strong reasons to doubt that the correctness of the 'invariance of rate laws' assumption. In addition to the 'Other Reactants Objection', biochemists may simply not be able to adequately replicate the crowded, heterogenous environment found within a living cell. Evidence is therefore required as to why the 'rate law invariance' assumption is warranted in a particular study. Normally no such evidence is provided.

How well does causal law modularity reconcile with Bechtel and Richardson's analysis of pathway causal discovery? (recall section 3.3). Let us switch the type of modularity from near decomposability to causal law modularity. The focus is now on identifying the RS's rate laws and assuming 'rate law invariance'. This provides a good fit with Bechtel and Richardson's methodology of using the twin strategies of decomposition and localisation to: (i) identify the functionality of each of a pathway's RSs (ii) identify each RS's working parts and (iii) 'to determine component properties in isolation of each other' (Bechtel and Richardson, 2010, p.26). However, as I will now explain, there is not a fit with the claim that feedback undermines modularity.

For feedback to undermine a pathway's causal law modularity, feedback would have to result in the 'rate law invariance' assumption being wrong for at least one of that pathway's RSs. i.e. one of the rate laws manifested within the pathway would need to be *not* manifested by the corresponding appropriately formulated isolated RS. The

type of feedback that is being considered is chemical feedback, which occurs when the concentration of a chemical species X affects the rate of production of X (recall section 2.2). This feedback affects a RS by changing the concentrations of the constituents of that RS. Let us consider a pathway *S* with a step RS_e consisting of a single elementary reaction:

$$A + B \rightarrow C$$

Let us assume that if there is no feedback then:

the rate law for 'RS_e in isolation' = the rate law for 'RS_e in *S*'

What difference might feedback make to the rate law for 'RS_e in *S*' ? Feedback from another of *S*'s RSs only affects the rate of RS_e by changing the concentrations of A or B. Now it is an uncontroversial assumption of biochemistry that changes in the concentrations of reactants of an RS do not change its rate law.[32] As such, feedback does not result in a different rate law being manifested in *S* than in the appropriately formulated isolated RS_e. Mutatis mutandis, the same arguments apply for stepwise RSs which are aggregations of elementary RSs (recall section 2.2).

But what if somehow the 'uncontroversial assumption' was wrong, and feedback led to some concentrations, say $[A]_{new}$ and $[B]_{new}$ at which a new rate law is manifested. Even this, in itself, would not undermine causal law modularity. As long as the new rate law is manifested by both the isolated RS_e and by the RS_e when it is part of *S* (at the concentrations $[A]_{new}$ and $[B]_{new}$) then causal modularity is maintained.

In my section 3.1, I stated that there are two dominant accounts of modularity found within the philosophy of science literature. The first is on 'near decomposability'. My chapter's starting point was Simon's and Bechtel/Richardson's analyses of near decomposability. From this I identified five types of modularity; one of these being causal law modularity. I have explained that the Strategy of Decomposition is based on the assumption that pathways are causal law modular; and that this is done by assuming that RSs are 'rate law invariant'. I will now consider the second dominant account, which is provided by Woodward (1999, 2003, 2008, 2013). Prima facie, this might appear to be similar to causal law modularity, as it is also based on the

[32] This is illustrated by the pathway ODE models referenced in this thesis having one-to-one relationships between their rate laws and reaction steps (see for example appendix 1), i.e. each reaction step is taken to have exactly one rate law. It is theoretically possible that feedback could result in a reactant having an extremely low concentration that would result in a different type of reaction kinetics occurring. However, (i) I am unaware of any pathways for which this is the case (ii) this possibility is covered by my next paragraph.

invariance of causal laws. However, it is a very different concept and has little relevance to the Strategy of Decomposition.

3. 7 Woodward's Concept of Modularity

Woodward provides an alternative concept of modularity that is part of his counterfactual theory of causation. For Woodward, modularity concerns the invariance of causal generalisations relative to 'ideal' interventions (NB. Woodward prefers to use the term 'causal generalisations' rather than 'causal laws'). The key idea is that in modular systems, causal generalisations are distinct and can, at least in principle, be changed independently of other causal generalisations. However, Woodward's concept is significantly different to my 'causal law modularity' and is not assumed within the Strategy of Decomposition.

For Woodward, causal relationships relate variables. Variables are properties or magnitudes that can have more than one value; and the values of variables are possessed by particular entities. (Woodward, 2003, p. 39). In summary, a variable *X* is a cause of variable *Y* if:

a) there is an ideal intervention on *X* such that *Y* changes or the probability distribution of *Y* changes.
b) the relationship between *X* and *Y* is invariant i.e. remains unchanged by the intervention.

This involves the key notions of an ideal intervention and invariance, which I will now explain.

An 'ideal intervention' on *X* with respect to *Y* exogenously changes the value of *X*, such that any change that occurs to the value of Y occurs only because of the change in the value of *X*. Woodward's specification of an ideal intervention involves an 'intervention variable' *I* which acts like a 'switch'. When *I* is 'switched on':

I1. *I* causes *X*. *X*'s value is solely a function of *I*.
I2. This means that all connections between *X* and its pre-intervention causes are 'broken'.
I3. *I* changes the value of *Y*, if at all, only by changing *X*.
I4. *I* does not alter the relationship between *Y* and any of its causes Z that are not on a directed path from *X* to *Y*. (Woodward, 2008, p.202-203)

An ideal intervention on *X* with respect to *Y* consists in *I* being 'switched on'.

The relationship between X and Y is 'invariant' if it holds for at least one 'testing intervention'. Let the relationship between X and Y be represented by the generalisation $Y = G(X)$. A testing intervention is an ideal intervention that changes the value of X from, say, x_0 to x_1 and establishes that:

$$G(x_0) = y_0 \neq G(x_1) = y_1.$$

A necessary and sufficient condition for a generalisation between variables X and Y to represent a causal relationship is that it is invariant (Woodward, 2003, p.250).

An invariant generalisation is specified by a causal equation in which in which the dependent variable is the effect and the independent variables are a complete set of its causes. For example, consider a system S with three variables X, Y, Z, in which Y has a single direct cause X, and Z has two direct causes Y and X (see Fig 3.12). The corresponding causal equations are:

equation (3.1) $Z = bX + cY$

equation (3.2) $Y = aX$

where a,b,c are coefficients.

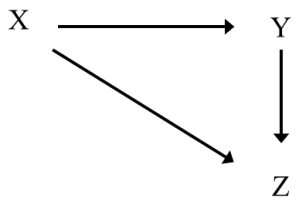


Fig 3.12 The causal system corresponding to equations (3.1 - 3.2). The arrows represent direct causal relationships.

Woodward's concept of modularity applies to systems of causal equations. Woodward provides the following definition:

> "Modularity. A system of equations is modular iff (i) each equation is invariant under some range of interventions on its independent variables and (ii) for each equation, it is possible to intervene on the dependent variable in that equation in such a way that only the equation in which that dependent variable occurs is disrupted while the other equations in the system are left unchanged." (Woodward, 2008, p. 221)

A causal structure S (for example a mechanism) is modular if the system of equations specifying S's causal generalisations is modular. It is important to note that Woodward is not claiming that modularity is relative to any set of interventions on

the dependent variables, only that there is at least one ideal intervention for each dependent variable which will not affect the correctness of the system's other equations.

In some of his earlier papers (e.g. Woodward, 1999) it is claimed that modularity is a universal characteristic of correctly specified systems of causal equations. This was based on the view that causal generalisations are always distinct and can, at least in principle, be independently changed. It follows that an ideal intervention on a dependent variable (an effect) should not alter the correctness of a system's other causal equations. For example, if system *S* is correctly specified by equations:

equation (3.1) $Y = aX$

equation (3.2) $Z = bX + cY$

then an intervention that fixes the value of *Y* to *y* (i.e. replaces equation (3.1) with *Y*= y) should not affect the correctness of equation (3.2) If an intervention on *Y* did lead to equation (3.2) being functionally incorrect, then this would mean that there was a causal connection between *Y* and *Z*, and *S*'s causal structure that had not been fully and accurately specified (Woodward, 2003, p.327). Woodward demonstrates how the causal relationships within *S* can be misrepresented by functionally correct equations, by substituting equation (3.1) into equation (3.2) giving:

equation (3.1) $Y = aX$

equation (3.3) $Z = dX$ (where $d = b + ac$)

Equations {3.1, 3.3} is 'observationally equivalent' to equations {3.1, 3.2} in the sense that both imply the same patterns of correlations between *X*, *Y* and *Z* (Woodward, 2003, p.330). However, equations {3.1, 3.3} misrepresents *S,* missing that Y is a direct cause of *Z* (see Fig 3.13). An ideal intervention that fixes the value of *Y* to *y* (i.e. replaces equation (3.1) with *Y*= y) would lead to equation 3.3 being incorrect i.e. $Z \neq dX$ .Woodward's general point being that of all the 'observationally equivalent' representations for a particular system, it is only the modular representation that correctly specifies that system's causal structure.

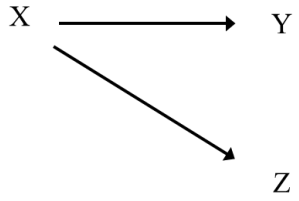


Fig 3.13 The causal system implied by equations {3.1, 3.3}. The arrows represent direct causal relationships.

However, Woodward's claim about the universality of modularity has been subject to 'structure changing' counter-examples. A prominent counter-example is Mitchell's (2012, p. 70-84) analysis of a hypothetic gene network, in which a gene is made inoperative by a 'knock out' intervention. The network is robust, and the intervention leads to a global reorganisation of the entire genetic network such that it still produces the same output Z (see Fig 3.14). The robustness of the network means that it is not possible, even in principle, to carry out an intervention on the target gene without changing the causal generalisations that apply to the other network genes. As such, it appears that Woodward's condition of modularity fails for this case.

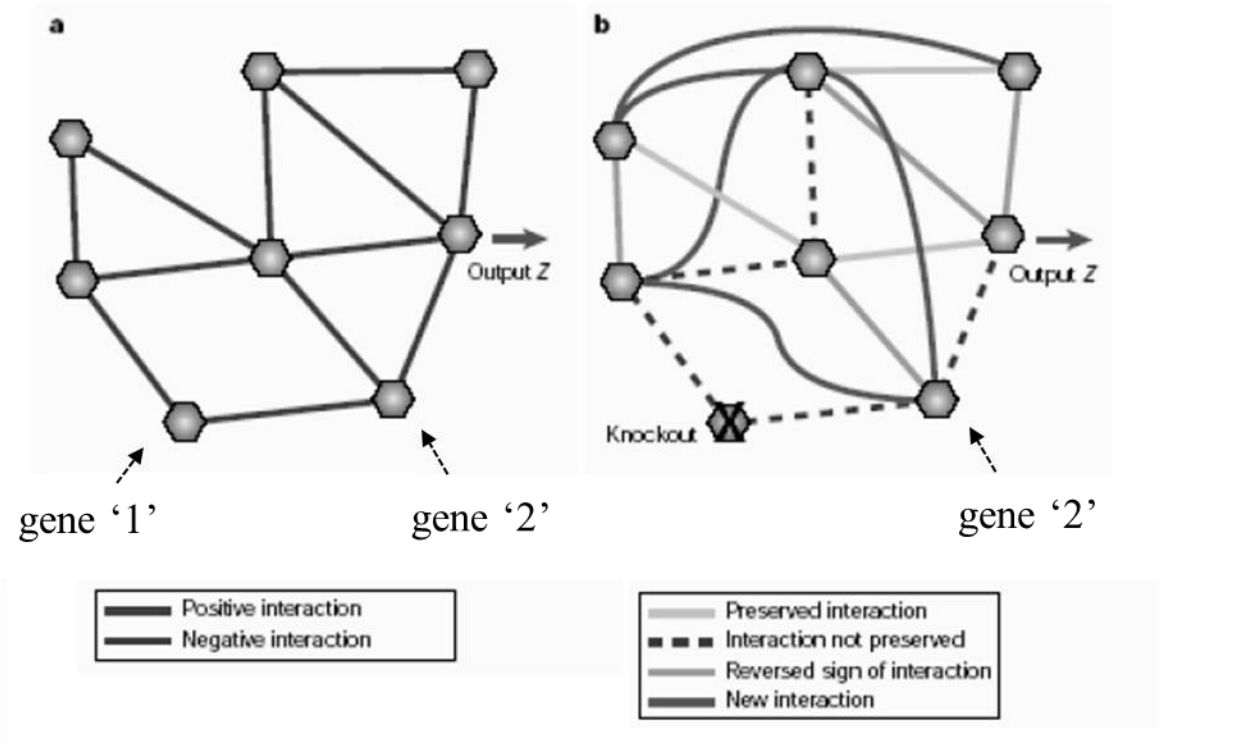


Fig. 3.14 The effects of knock-out experiment on the causal structure of a genetic network. (a) is before intervention (b) is after intervention. (adapted from Mitchell, 2012, p. 72)

One option for Woodward would be simply to claim that the above representation of the gene network is incomplete. For example, the knocking out of the gene would presumably trigger a series of chemical reactions that result in the re-organisation of the network; yet the causal relationships corresponding to these chemical reactions are not represented. Prima facie, it seems a plausible claim that if the full causal structure of the network were specified, then the network would have a modular structure.

Instead, Woodward reviews Mitchell's counter-example (Woodward, 2013, p. 54-55) and concedes that it is plausible that some biological systems may not be modular. But there seems to be a considerable tension between Woodward's theory of causation and his relaxing of his universality of modularity claim. This can be illustrated using the above gene network example. Let *gene 1* be the gene that is to be knocked out. If *gene 1* is a cause of the state of *gene 2* then on Woodward's account there must be an ideal intervention on *gene 1* with respect to *gene 2*. Criterion *I4* for an ideal intervention requires that the corresponding intervention variable *I* does not alter the relationship between *gene 2* and any of its causes that are not on a directed path from *gene 1* to *gene 2*. But in the example, *gene 2*'s causal relationships within the network are completely altered. Woodward does not address how his notion of ideal interventions is consistent with causal systems not being modular. However, further analysing the coherence of Woodward's theory falls outside the scope of my PhD and will not be further considered.

Woodward (2013) also makes clear that modularity is relative to a set of interventions. For a system of equations, even if there was an intervention for each dependent variable that does not disrupt the other equations, there may be other interventions on the dependent variables for which the system of equations breaks down. Woodward states:

> "modularity comes in degrees and is relative to a class of changes or interventions. Generalisation G_k characterising the causal relationships among one set of components might be stable under certain sorts of changes C_i in the causal relationships characterising other subsets of components but not under changes $C_j \neq C_i$ in these relationships. Or G_k might be stable under changes in some causal relationships elsewhere in the system but not under changes in other causal relationships. Or perhaps changes elsewhere change G_k but only in small or minor ways." (Woodward, 2013, p. 52)

How does Woodward's concept of modularity fit into my analysis of the five types of modularity? One difference is that Woodward does not relate his criteria for modularity to the functional decomposition of a target system. Another difference is that Woodward does not seem to allow for modules consisting of multiple parts acting in accordance with multiple causal generalisations. Instead modules are characterised by their single causal generalisation. But these are both minor differences, and Woodward's concept could be easily amended to incorporate these

points. Let us suppose they are incorporated, and let us also make the (highly contested) assumption that Woodward's theory of causation is correct. Given that "modularity comes in degrees and is relative to a class of changes or interventions", two of the types of modularity that I identify correspond to ways in which a system *S* can also be modular in Woodward's sense:

- *Internal structural modularity*, which requires that *S's* subsystems are autonomous in the sense that changes to the internal causal relationships of a subsystem do not change the functionality of *S*'s other subsystems. Systems that are internal structural modular would also be modular in Woodward's sense, relative to ideal interventions on the internal causal laws of single subsystems.
- *Causal law modularity,* which requires that *S*'s subsystems $C_1...C_n$ are autonomous in the sense that they manifest local causal laws that are also manifested by objects of the same kind as $C_1...C_n$ that are situated in 'isolation'. Systems that are causal law modular would also be modular in Woodward's sense, relative to a set of ideal interventions on multiple variables that would, in effect, replicate a physical decomposition of *S* into isolated subsystems. Hence causal law modularity can imply 'Woodward modularity' but only if we accept Woodward's theory of causality (and make minor amendments to his concept of modularity).

The other three types of modularity are distinct from Woodward's concept. *External structural modularity* compares *S*'s subsystems to other target systems which may have very different compositions. *Few strong interactions modularity* and *dynamic modularity* both concern the properties manifested by $C_1...C_n$ whilst situated within an un-intervened *S*.

Woodward's concept is not directly relevant to the causal discovery of pathways, and need not be further considered in my thesis. Although his concept concerns the invariance of causal laws, it is not specifically about the invariance between the properties manifested by $C_1...C_n$ and by objects of the same kind as $C_1...C_n$ that are situated in 'isolation'. A system can be modular in Woodward's sense but fail to be 'causal law modular'. It is only the latter that are relevant to the causal discovery of pathways.

3.8. Conclusion

Simon takes there to be a single concept of modularity, in which decomposability comes in degrees. Near decomposability is determined by the relative intensities of subsystem interactions and entails a conjunction of dynamic and structural autonomies. Simon states that the complex systems found in biology are often ND and it is only because they are ND that we can understand them. Bechtel and Richardson disagree. They think that biological systems are often only minimally decomposable but that the assumption of near decomposability has been heuristically useful in causal discovery.

Bechtel and Richardson take the twin strategies of functional /structural decomposition and localisation to be based on the assumption of near decomposability. They implicitly add three criteria to Simon's account and it is these criteria that do most of the work in their analysis of pathways. Bechtel and Richardson take pathways without feedback to be ND but state that feedback undermines near decomposability.

I have shown that the concept of near decomposability does not apply to pathways because of the substantial overlap between their RSs. Furthermore, the assumption of near decomposability would have little relevance to *in vivo* pathways where there are substantial inter-pathway interactions. I have also shown that the claimed effects of feedback on near decomposability have not been established.

A significant shortcoming in both Simon and in Bechtel and Richardson's accounts is that neither recognizes that there is a plurality of types of modularity. Starting from their analyses of near decomposability I have identified five types of modularity that are important to the analysis of pathways or to biology more generally. These demonstrate that, contrary to Simon, the complex systems found in biology do not need to be ND to be understood. Other types of modularity can assist in this task. The different types of modularity can also be important for manipulating, replacing and reengineering of biological systems.

The Strategy of Decomposition is based on an assumption of pathways being modular. But, contrary to Bechtel and Richardson, the assumed type of modularity is causal law modularity. Feedback does not undermine causal law modularity. It is highly desirable that pathways are causal law modular as otherwise our abilities to

determine pathway rate laws and explain pathway dynamics seem very limited. But rate laws are highly context-sensitive and some pathways may not be causal law modular. In such cases, the Strategy of Decomposition will fail to discover a pathway's *in vivo* rate laws.

Chapter 4 - Emergence in Biochemical Pathways

4. 1 Introduction

The term 'emergent' has frequently been used by scientists to describe some properties of non-linear biological systems. Yet they rarely define what they mean by emergence and when a definition is offered it is often vague. Several attempts have been made by philosophers to elicit an interesting concept of emergence from scientists' usage of the term. These have included: Wimsatt (1997), Bedau (2003, 2008), Mitchell (2009, 2012) and Boogerd et al. (2005). A shared idea is that emergent properties are systemic properties which are in some way 'novel' relative to the properties of their system's parts. But the notion of 'novelty' is itself open to many interpretations and this has contributed to the variety in the concepts of emergence being proposed.

In what follows, I will focus on Boogerd et al.'s concept, which is based on their interpretation of C.D. Broad's concept of emergence. Boogerd et al.'s concept applies to the dynamics behaviours of pathways and I will use the term 'Pathway Emergence' to refer to their concept. They claim that their concept is relevant to the discovery practices of biochemistry. There are two criteria for a pathway dynamic behaviour to be Pathway Emergent. The first is a 'non-deducibility' criterion requiring that the behaviour cannot *in principle* be deduced from a 'Deductive Base' that contains statements of the properties manifested by the pathway's isolated parts, the pathway's organisation, and laws manifested in simpler systems. The second is a 'qualitative difference' criterion requiring that emergent behaviours must be qualitatively different to those manifested by the pathway's isolated parts. Bechtel and Richardson (2010) further develop the concept of Pathway Emergence, linking it to nonlinearity and feedback. Boogerd et al.'s paper has been frequently cited in a number of prominent publications. Despite this considerable influence, it has been subject to very little critical evaluation. [33]

[33] Boogerd et al.'s paper has been cited 159 times, according to Google Scholar (18/09/2016). The only paper that appears to critique their concept is Theurer (2014), which focusses on problems with specifying the complexity of systems.

If pathways are Pathway Emergent, then this would seem to have significant consequences for the applicability of the Strategy of Decomposition. To recap, the strategy has three broad stages:

1. An extraction stage; in which the target *in vivo* pathway is separated from its biological context, creating an *in vitro* pathway.
2. A decomposition stage; involving decomposing the *in vitro* pathway into a set of isolated parts that can then be separately analysed.
3. A reconstruction stage; involving using a simulation model to deduce the target behaviour from statements of the properties of its isolated parts, their arrangement, plus the Kinetic Law of Composition.

If a pathway dynamic behaviour is Pathway Emergent, then it seems to follow that the deduction in the reconstruction stage will fail. Boogerd et al. appear to be proposing a type of emergence that is incompatible with the successful application of the Strategy of Decomposition.

In this chapter, I argue that the claims for the existence of Pathway Emergence are unsuccessful. In section 4.2, I provide a literature review on emergence. In section 4.3, I provide an analysis of Pathway Emergence. In doing so, I explain that there are significant gaps in the specifications provided by both Boogerd et al. and by Bechtel and Richardson. I then provide the best interpretation that I can find, that is consistent with Broad's concept. In section 4.4, I review the steps by which pathway dynamic behaviours are deduced in biochemistry. In section 4.5, I explain Boogerd et al.'s argument for Pathway Emergence, which takes the form of a case study. I identify a necessary condition for it to satisfy the non-deducibility criterion: the manifestation of at least one rate law in a pathway that is not manifested by its isolated parts. I then show that the case study does not satisfy this necessary condition. Hence, as it stands, the claims for Pathway Emergence are unjustified.

Two options for advancing the analysis of Pathway Emergence are then considered. In section 4.6, I consider whether, even though it was not provided, there still is a plausible argument for the existence of Pathway Emergence. This is because the rate laws within a pathway are highly context sensitive; and these laws might sometimes be non-deducible from the pathway's Deductive Base. But this argument is speculative, and hence the existence of Pathway Emergence has not been established. In section 4.7, I consider whether the concept of Pathway Emergence was meant to be based on a far more restrictive notion of non-deducibility than is stated in the

writings of either Boogerd et al. or in Bechtel and Richardson. I provide evidence that the 'deductions' referred to were intended to be restricted to only a particular type of law of composition that aggregates dynamic behaviours- thereby excluding simulations of a whole pathway. This yields an alternative concept of emergence that I call 'Weak Pathway Emergence'. The case study does succeed in demonstrating Weak Pathway Emergence, but this is very different to Broad's concept of emergence. Furthermore, the concept provides little insight into the challenges that biochemists face in the causal discovery of pathway behaviours. In section 4.8, I conclude that the claims that pathways manifest a type of emergence that challenges the Strategy of Decomposition are unsubstantiated.

4. 2 Concepts of Emergence

In this section, I review five concepts of emergence, that I take to be representative of the contemporary literature on emergence and which, prima facie, apply to pathway dynamics. These are the concepts of: Kim (1999), Wimsatt (1997), Bechtel and Richardson (1993)[34], Bedau (2003, 2008), and Mitchell (2009, 2012). My review will not include the concept of Pathway Emergence, the analysis of which will begin in the next section. My aims are:

i. to situate the concept of Pathway Emergence within the wider literature on emergence.
ii. to illustrate that none of the other contemporary concepts challenges the applicability of the Strategy of Decomposition.

First, let me explain some key terms. 'Properties' will be used to refer only to manifested properties, and I will include 'behaviours' as a subset of manifest properties. 'Systemic properties' are properties that belong to a system as a whole, including those that can also be possessed by its parts, such as weight. It is only systemic properties that can be emergent. 'Physical monism' is the ontological thesis that the only kind of substance is physical matter. All the concepts that I shall consider are physically monist.

[34] I will be referring to Bechtel and Richardson's 1993 concept of emergence as 'Uniform Components Emergence'. It is conceptually distinct from the concept of Pathway Emergence. Identical accounts of Uniform Components Emergence are provided in both editions of *Discovering Complexity* i.e. in both the 1993 and 2010 editions. When discussing Uniform Components Emergence, I have chosen only to reference the 1993 edition; this is to avoid any confusion with Bechtel and Richardson's later analysis of Pathway Emergence, which is entirely in the 2010 edition of *Discovering Complexity*.

I will use the following notation, let:

(i) S be any system composed of parts $C_1 \ldots C_n$ in arrangement R

(ii) P_s be a systemic property of S

(iii) $\boldsymbol{P_1 \ldots P_n}$ denote the sets of properties that $C_1 \ldots C_n$ manifest within S

(iv) $\boldsymbol{I_1 \ldots I_n}$ denote the sets of properties that objects of the same kind as $C_1 \ldots C_n$ manifest when in isolation.

A 'law of composition' equates a systemic property to an aggregate function of its parts' properties. I will take laws of composition to have the following form:

If parts $C_1 \ldots C_n$ have properties $\boldsymbol{P_1 \ldots P_n}$, and S consists of these parts in arrangement R, then S has property $P_S = f_R(\boldsymbol{P_1, \ldots, P_n})$, for some f_R.

where f_R may be either a linear or a nonlinear function. An example of a law of composition is that the mass of S is the sum of the masses of its parts. Another example is the 'Kinetic Law of Composition' (recall section 2.2.2).

Concepts of emergence can be classified into two general types: 'strong emergence' and 'weak emergence'. Strongly emergent properties are 'ontologically irreducible' to more fundamental properties and laws from which they emerge (Vintiadis, 2016). They have novel causal powers that are not present in their parts. A necessary condition for a systemic property P_s to be strongly emergent is that it must be *in principle* non-deducible, relative to a 'Deductive Base' that contains statements of S's part properties, laws that apply to the parts, and the arrangement of the parts .[35] But note, this *in principle* non-deducibility is an epistemological criterion for identifying what is a metaphysical type of emergence (Vintiadis, 2016; O'Connor and Wong, 2015; McLaughlin, 2008, p. 55). Non-deducibility is a consequence of an emergent property being a novel addition to the ontology of the world. Hence, a P_s is non-deducible because it is strongly emergent, it is not strongly emergent because it is non-deducible. Strong emergence is the type of emergence that was originally proposed by the British Emergentists, including Broad. For example, Broad thought that the behaviour of sodium chloride could not be deduced from the properties of sodium and chlorine in isolation (Broad, 1925 , p. 59). Today there is considerable

[35]Whenever my thesis refers to a property being deducible from a Deductive Base, it is to be understood that this is shorthand for there being a valid argument whose premises are *statements* of there being the cited properties, laws and organisational relationships and whose conclusion is a *statement* of there being the target systemic property.

scepticism as to whether there are any genuine cases of strong emergence. For example in his analysis of British Emergentism, McLaughlin states that there is 'not a scintilla of evidence that there are emergent causal laws or powers' (McLaughlin, 2008, p. 23). By contrast, Chalmers (2006, p. 247) thinks there is exactly one clear case of strong emergence, and that is the phenomenon of consciousness.

Different specifications for *S*'s Deductive Base lead to different concepts of strong emergence. For example, Pathway Emergence and Kim's concept are both examples of strong emergence, using the following respective Deductive Bases:

Deductive Base For Pathway Emergence	**Deductive Base For Coexisting Systemic / Part Properties**
Statements of:	Statements of:
i Properties $\boldsymbol{I_1}\ldots\boldsymbol{I_n}$ that objects of the same kind as $C_1\ldots C_n$ manifest when in isolation from S	i Properties $\boldsymbol{P_1}\ldots\boldsymbol{P_n}$ that $C_1\ldots C_n$ manifest in S
ii Arrangement R	ii Arrangement R
iii Laws of composition*	iii Laws of composition*
iv Other laws of nature*	
Used by Boogerd et al. (2005)	Used by Kim (1999)

* manifested in simpler systems

Fig 4.1 Example of Deductive Bases of strong concepts of emergence

Weak emergent concepts do not require in principle non-deducibility. Instead, a necessary condition for a *Ps* to be emergent is that it is 'unexpected' in some specified sense, relative to a specified Deductive Base for *S*. Different specifications of 'unexpected' and of *S*'s Deductive Base lead to different concepts of weak emergence. Unexpectedness is often cashed out, at least in part, by highlighting some aspect of the deduction of P_s that is taken to be philosophically interesting. For example, in Bedau's concept a necessary condition for P_s to be weakly emergent is that it is deducible, but only by the use of simulation.[36]

[36] Simulation is a form of deduction using μ-recursive functions. According to the Church-Turing thesis, the class of μ-recursive functions (a subset of the class of partial recursive functions) is exactly the class of functions which can be computed by Turing machines. For a discussion of this see Boolos, Burgess and Jefferey (2007). This came from a personal communication with my supervisor J. Alexander.

A further distinction is made between 'diachronic' and 'synchronic' concepts of emergence. Diachronic concepts are concerned with how an emergent property is produced i.e. with the dynamic history that leads to the emergent property. A diachronic emergent P_s temporally evolves from S's earlier non-emergent state. Diachronic concepts have been proposed to try to specify the sense in which the term 'emergence' is used in complexity science. For example, traffic jams (Bedau, 2008, p. 448) and the patterns formed by raiding army ants (Camazine et al., 2001, p. 257) are putative examples of diachronic emergence. Diachronic concepts are usually also weak concepts of emergence (Vintiadis, 2016). By contrast, synchronic concepts are 'timeless' (Stephan, 1999) in that P_s is *not* compared with the properties that its parts $C_1 \ldots C_n$ manifest at some earlier (or later) time. There are two types of synchronic concept found in the emergence literature. First, there are concepts that compare P_s with its coexisting part properties $\boldsymbol{P_1 \ldots P_n}$; for example consciousness is claimed to be emergent relative to the part properties of its co-existing neurons. Second, there are concepts that compare P_s with properties manifested by objects of the same kind as $C_1 \ldots C_n$ that are situated within other systems that are simpler than S; for example, Broad's comparison of sodium chloride with properties manifested by chorine and sodium in isolation.

Of the concepts that I shall now consider: Kim (1999) is a synchronic concept of strong emergence; Wimsatt (1997) and Bechtel and Richardson (1993) are both synchronic concepts of weak emergence, and Bedau (2003, 2008) and Mitchell (2012) are both diachronic concepts of weak emergence. Each of the concepts will be relevant to my later analysis of Pathway Emergence.

Kim (1999) considers a paradigm formulation of strong synchronic emergence. A P_s is emergent iff it is:

- supervenient. A systemic property P_s supervenes on properties $\boldsymbol{P_1 \ldots P_n}$ iff whenever anything has $\boldsymbol{P_1 \ldots P_n}$ it necessarily has P_s.
- non-deducible from a Deductive Base containing statements of $\boldsymbol{P_1 \ldots P_n}$, arrangement R and laws of composition manifested in simpler systems than S (henceforth: the Coexisting Systemic / Part Properties Deductive Base). Only laws of composition manifested in simpler systems are allowed, so as to prevent the concept of emergence from being vacuous. For example, if

statements of the laws of composition that are actually manifested by *S* are included, then any P_s would be deducible.

- causally efficacious. P_s should have novel causal powers that are functionally irreducible to the causal powers of its parts $C_1 \ldots C_n$ in *S*. This involves 'downward causation' whereby P_s can cause the instantiation of part properties.

An emergent property P_s is novel because of its novel causal powers. This is a synchronic concept of emergence as $\boldsymbol{P_1 \ldots P_n}$ and P_s, are simultaneously manifested by *S*. Having specified his concept of strong emergence, Kim then provides several convincing arguments against its occurrence. I will focus on just two of these arguments. The first claims that the concept of downward causation is incoherent. The second claims that if a systemic property P_s can be functionally defined, then it will be deducible from its Deductive Base.

Kim's Causal Exclusion Argument can be roughly summarised as follows: let there be two systemic properties M and M* which supervene on ***P*** and ***P**** respectively. Let there be a putative causal relationship where an instantiation of M is the cause of an instantiation of M*, which I will denote by M=> M*. Given that M=> M* and M supervenes on ***P***, Kim claims that it follows that ***P***=> ***P****. The situation can be represented as:

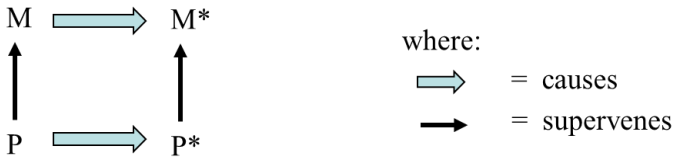


Fig. 4.2 Kim's causal exclusion argument.

Kim (1999, p. 24) stipulates his 'principle of downward causation': to cause the instantiation of any property (other than those at the bottom level) you must cause the constituent base from which it arises. Hence M can only be the cause of M* by causing ***P****. But ***P*** alone is sufficient to cause ***P**** which realises M*; Kim concludes that ***P*** does all the causal work, and that M is epiphenomenal and irrelevant to causing M*. Kim (2006, p. 548) states that "Downward causation is the raison d'être of emergence, but it might well turn out to be what in the end undermines it".

Kim's second argument is based on the functional reduction of systemic properties, such that any causally efficacious P_s is identified with its realisers in S i.e. with parts $C_1 \ldots C_n$ and their properties $\boldsymbol{P_1} \ldots \boldsymbol{P_n}$. There are three steps: (Kim, 1999, p. 10-11)

1. Define (or redefine) P_s in terms of its causal role i.e. in terms of the properties that cause P_s to be instantiated and the properties that P_s instantiates.
2. Find the realisers of this causal role at the level of S's parts.
3. Find a theory at the level of S's parts that explains how they fulfil the causal role constitutive of P_s.

Kim claims that if a P_s is functionalisable then it can be reduced to the functions performed by S's parts and is therefore not emergent. Kim concludes that emergentism may be an empty concept, but that the most promising candidate for being emergent are the phenomenal qualities of consciousness which he thinks may be 'non-functionalisable' (Kim, 1999, p. 18).

Mitchell (2009, 2012) rejects Kim's claim of the extent to which systemic properties are functionally reducible. She notes that Kim is making the assumption that there is always a unique and complete description of a P_s in terms of its lower level. However descriptions are idealisations or abstractions, they are always incomplete:

> "Any representation – be it linguistic, logical, mathematical, visual or physical - stands for something else. To be useful, it cannot include every feature in all the glorious detail of the original, or it is just another full blown instance of the item it represents. Something must be left out, and what is left out is a joint product of the nature of the representing medium (Perini, 2005) and the pragmatic purposes the representation serves……What now of the mapping between two different descriptions of a phenomenon, one higher level, one microstructural?" (Mitchell, 2009, p. 31)

Hence Kim's functional reduction strategy is claimed to fail; there is no reason to think that a functional specification of a P_s will always be identical with a corresponding functional specification of S's parts' operations (Mitchell, 2012, p. 178).

Mitchell's argument seems compelling when considering reductions between different domains of science; for example, reducing biochemical phenomena to the entities, properties and laws described by physics. However, I do not take it to apply

to functional reductions of pathways. This is because the functional descriptions of both the higher and lower levels will be made using the same theoretical vocabulary and referring to the same types of entities. For example, a functional specification of the glycolytic pathway will include terms for the quantities of reactants into the pathway (e.g. glucose, ATP, ….), the quantities of the products out of the pathway (e.g. pyruvate, ATP, …..) and so forth. Likewise, the functional specifications of each of *S*'s reaction steps will include terms for the quantities of reactants into that step (e.g. glucose, ATP) and products out of that step (e.g. glucose-6-phosphate, ADP, H^+) and so forth. The mapping between the levels is clearly unproblematic. Indeed, Bechtel and Richardson's account of causal discovery of pathways centers on the functional decompositions of pathway behaviours that biochemists actually carry out (recall section 3.3).

I take Kim's arguments to succeed in the case of pathways. Pathway do not have P_s with novel causal powers that are functionally irreducible to the causal powers of its parts $C_1 \ldots C_n$ in *S*. As we shall see, Boogerd et al. also support Kim's conclusions, and incorporate the assumption that pathway dynamic behaviours are deducible from their Coexisting Systemic / Part Properties Deductive Base into their analysis of Pathway Emergence.

I will now consider some weak concepts of emergence. In weak concepts, an emergent property *Ps* is novel in the sense of being 'unexpected'. I will start with Wimsatt (1997) and Bechtel and Richardson (1993)'s synchronic concepts of emergence. Both are intended to draw attention to the dependence that a P_s may have on (i) the organisation of a system's parts and (ii) the interactions between the system's parts.

In Wimsatt's concept, emergence is defined negatively, as corresponding to a failure of aggregation. Emergent properties are systemic properties that are not 'mere aggregates' of their part properties. For a P_s to be aggregative with respect to a decomposition of *S,* it must satisfy the following equation and meet four conditions (Wimsatt, 1997, p. S376):

$$P_S = f_R(\boldsymbol{P_1}, \ldots, \boldsymbol{P_n})$$

1. Intersubstitution. – P_s is invariant to rearranging of its parts or their inter-substitution.

2. Size scaling. – P_s remains qualitatively similar (identity or changing only in value) upon the adding or subtracting of component parts.
3. Decomposition and reaggregation – P_s is invariant under operations of decomposition and recomposition of its parts. Wimsatt states that this 'suggests an associative function' (Wimsatt, 1997, p. S376) i.e. that the grouping of variables does not matter, for example for variables a,b,c the following multiplications are equivalent: $(a \times b) \times c = a \times (b \times c)$
4. Linearity – f_R must be a linear function. This requirement means that there are no cooperative or inhibitory interactions between the parts (Wimsatt, 1997, p. S376).

To be 'truly aggregative' P_s must satisfy these conditions for all possible decompositions of S.

Wimsatt recognises that the conditions for aggregativity are very demanding and will rarely be met. They require that P_s depends on $\boldsymbol{P_1,\ldots,P_n}$ in a highly atomistic way. Consequently, emergence is ubiquitous. Amongst the examples of emergence that Wimsatt cites are traffic jams, the cooperative binding of oxygen by haemoglobin and even a heap of stones (as its shape and stability will depend on how the stones are organised). Different forms of emergence can then be classified by identifying the different ways in which the four conditions for aggregation fail.

Relative to Wimsatt's concept of emergence, the dynamic behaviours of pathways are emergent. The behaviours of a pathway's parts (i.e. its reaction steps) are highly inter-dependent. Pathways are nonlinear systems, whose dynamic behaviours cannot be deduced by merely aggregating the properties of its parts. However, this type of emergence does not challenge the applicability of the Strategy of Decomposition to pathways. In the decomposition stage, each part (i.e. reaction step) is analysed in isolation, but the behaviours of the whole pathway are not then assumed to be some linear aggregation of the behaviours of the reaction steps. Instead in the reconstruction stage, a simulation model is used that is meant to fully captured the nonlinear interactions between the reaction steps.

Bechtel and Richardson (1993) propose a concept of weak emergence that I will term as 'Uniform Components Emergence'. Some biological networks may be emergent in the sense that they have a systemic property whose values are determined solely by the organisation of the network, rather than by distinctive properties of their parts

(Bechtel and Richardson, 1993, p. 202-229). They cite Kaufmann's (1993) model of how genetic regulatory systems might function as a result of the patterns of connections between genes, with each gene acting as a Boolean operator. In such cases the isolated parts would not perform tasks that would feature in a functional decomposition of the whole system. According to Bechtel and Richardson, the Strategy of Decomposition would fail in such cases:

> "analysing the components in isolation throws no light on the phenomenon under investigation.....[such] systems thus defy some of our traditional tools for studying natural systems, for these tools rely on being able to decompose the system, work on components singly, and then build up again to understand the whole." (Bechtel and Richardson, 1993, p. 228)

For Wimsatt, emergence corresponds to there being *any* dependence between a P_s and *S*'s organisation *R*. For Bechtel and Richardson, Uniform Components Emergence corresponds to P_s being *solely* dependent on *S*'s organisation *R* (given that its components have certain uniform properties). Clearly pathways are not Uniform Components emergent, given they are not networks and have parts with very different properties. However, I have included this concept in my review as it illustrates how Bechtel and Richardson link emergence to the failure of the Strategy of Decomposition. This idea is carried forward, and incorporated into the concept of Pathway Emergence (Richardson is one of the co-authors of Boogerd et al. (2005)).

Bedau (2003, 2008) and Mitchell (2009, 2012) both propose diachronic concepts of weak emergence. They both cite paradigm examples of complex behaviours that are taken from the complexity science literature. For example, a flock of birds might be flying without any apparent formation but over time a vee shaped pattern arises. The vee shaped pattern is taken to be a diachronic emergent property.

Bedau's key proposal is that emergent systems are 'complex' in the sense that their dynamic behaviours are 'computationally incompressible' and can only be deduced by the use of simulation. There can be no 'short-cut derivation' (Bedau, 2003, p. 15) of a target behaviour, it 'cannot be determined by any computation that is essentially simpler than the intrinsic natural computational process by which the system's behaviour is generated' (Bedau, 2003, p.10).

According to Bedau, a P_s manifesting at time *t,* is weakly emergent iff it is:

1. nominally emergent. A nominally emergent property is a systemic property that cannot be possessed by its parts. For example, 'being a circle' is a nominal emergent property as none of a circle's parts are a circle; a circle being composed of individual points that do not have shape.
2. locally reducible. This means that the value of P_s at any particular time *t* is required to be deducible from statements of the part properties manifested at that time *t,* the arrangement *R* of $C_1...C_n$ at that time *t* and the laws of composition manifested in simpler systems than *S* (i.e. P_s is deducible relative to *S*'s Coexisting Systemic / Part Properties Deductive Base). The reason why this type of reducibility is termed as 'local' is because Bedau wants to emphasis the context sensitivity of the properties manifested by $C_1...C_n$. For example *Ps* might not be deducible using statements of the properties manifested by objects of the same kind as $C_1...C_n$ which are not located in *S* (Bedau, 2003, p. 14).
3. deducible, but only by simulation. This deducibility is relative to a Deductive Base stating:

   – the properties that $C_1...C_n$ manifest at an earlier time t_1

   – the arrangement of $C_1...C_n$ at an earlier time t_1

   – the laws of nature manifested in *S*

   – initial conditions

   – boundary conditions

   where t_1 can be any earlier time in *S*'s temporal evolution. Bedau describes the use of simulation to perform such deductions as "crawling the micro-causal web' of *S* and 'aggregating and iterating the earlier local micro-interactions over time' (Bedau, 2008, p. 446).

Bedau has formulated a concept of emergence that is meant to correspond to one of the key ideas being researched in complexity science: that the complexity of a system can be measured by its 'algorithmic complexity'. For example, Kolmogorov Complexity has been proposed as a possible measure. The Kolmogorov Complexity of an object is the shortest computer program that would generate a description of that object. For example, consider the following string of text:

ACACACACACACACACACAC (string 1)

Only a short program specifying 'Print AC ten times' is needed to describe this string. Now consider the randomly generated string:

A C B C G C B A A G A B G G A A B A C (string 2)

Describing this string would require a longer program: Print "A C B C G C B A A G A B G G A A B A C". String 2 is taken to be more complex because the description of it cannot be compressed.

Bedau's concept can be understood, at least in part, as an attempt to capture the idea of algorithmic complexity and apply it to Nature's nonlinear physical systems. According to Bedau, weak emergence is commonplace; in biology it:

> "seems to characterize a vast number of global properties of complex systems in molecular and cellular biology, including regulatory gene networks, metabolic networks, and the process by which proteins fold into three-dimensional structures." (Bedau, 2008, p. 454)

Relative to Bedau's concept, the dynamic behaviours of pathways seem to be emergent. But as with Wimsatt's concept, this type of emergence does not challenge the applicability of the Strategy of Decomposition, but simply highlights the need for simulation modelling.

Mitchell's account of weak emergence focusses on self-organising systems:

> "Self-organization and feedback make scientific sense of emergent features of complex systems." (Mitchell, 2012, p. 184).

Mitchell does not provide a definition of self-organisation but her paper makes several references to Camazine et al. (2003) who provide the following definition:

> "Self-organisation is a process in which a pattern at the global level of a system emerges solely from numerous interactions among the lower-level components of the system. Moreover, the rules specifying interactions among the system's components are executed using only local information, without reference to the global pattern." (Camazine et al., 2003, p. 8).

Self-organising systems are one of the main types of systems studied in complexity science. In self-organised systems, there is no central controller, instead the components' behaviours are coordinated through multiple nonlinear feedback loops. (Camazine et al., 2003, p.16-26).

Mitchell cites Wimsatt's analysis of emergence and states that "emergence is identified with certain types of non-aggregative compositional structures" (Mitchell,

2012, p. 179). However, in contrast to Wimsatt, Mitchell is proposing a diachronic concept of emergence. Kim's explication of emergence is criticised for being too static and not capturing the dynamic nature of emergence. Mitchell's main examples of emergent behaviour are the self-organising behaviours of flocks of birds and of bees' storage of honey in hives. She also cites Tyson (2003), whose paper is on pathway dynamic behaviours.

According to Mitchell, there are three key requirements for a property to be emergent: downward causation, unpredictability and novelty (Mitchell, 2012, p. 181). Mitchell does not explain her novelty requirement, but it appears to include: (i) the requirement that properties be nominally emergent and (ii) that emergent properties are causally efficacious via downward causation (Mitchell, 2012, p. 179).

Mitchell explains how feedback can result in downward causation:

> "negative and positive feedback both stabilize phenomena at a higher level and constrain the behavior of the components at the lower level. Feedback provides an operational understanding of one type of downward causation where system level properties constrain and direct the behavior of the components." (Mitchell, 2012, p. 182)

So characterised, downward causation does not pose a challenge to the Strategy of Decomposition. For example, feedback can lead to pathways having such topological structures as limit cycles (recall 2.2.4). These structures act as constraints on the behaviours of the parts of their systems, and can be portrayed as 'directing' a pathway's reactants into patterns of rising and falling concentrations. But this is all explained by the regular causal interactions between reactants that are described by the pathway's reaction rate laws (and by the Kinetic Law of Composition that combines these laws). There are no additional higher level laws that coordinate the reactions and orchestrate a pathway's dynamic behaviours. This is illustrated by the specification of Teusink et al.'s (2000) model of the glycolytic pathway; the model consisting solely of the reaction rate laws and the Kinetic Law of Composition (see my appendix 1). In the case of pathways, the type of downward causation that Mitchell is characterising, is fully captured by the rate laws that the Strategy of Decomposition aims to discover.

The only grounds Mitchell cites for unpredictability are that some deterministic non-linear systems have sensitive dependence to initial conditions (henceforth: sensitive dependence), which she states is often associated with chaotic systems

(sensitive dependence is a necessary but insufficient condition for a system to be chaotic (Smith, 1998, p.170)). Although not specified, I take it that this unpredictability is relative to the same Deductive Base that I specified in my account of Bedau's concept. In summarising the emergent behaviours found in self-organising systems, Mitchell states that:

> "Interactions are often chaotic, displaying both positive and negative feedback, which can generate novelty in the overall response which is not predictable from the intrinsic properties of the individual components." (Mitchell, 2012, p. 184)

Before I assess this part of Mitchell's concept, I first need to explain sensitive dependence. The unpredictability due to sensitive dependence is that: "any bundle of initial conditions spreads out more than a specific diameter representing the prediction accuracy of interest" (Werndl, 2009, p. 202). In such cases, a dynamic behaviour cannot be predicted using our *knowledge* of initial conditions and the dynamic laws of that system. This is because immeasurably small differences in initial conditions can lead to significantly different outcomes. Hence Mitchell's claim of unpredictability is a claim about predictability *in practice*. However, Mitchell is incorrect in taking sensitive dependence (or chaos) to be prevalent in biological self-organising systems. In systems with sensitive dependence, small perturbations can lead to significantly different outcomes. This is not biologically viable, given the noisy environments in which biological systems exist. Biological systems would not be able to survive if their pathways were so highly sensitive to small perturbations. Camazine et al. notes that "natural selection tunes the parameters of living systems to avoid chaos". Boogerd et al. (2005, p.143) take pathway dynamic behaviours to only be chaotic in pathological conditions. Relative to Mitchell's diachronic concept, pathway dynamic behaviours do not appear to be emergent, at least in non-pathological cases.

This completes my review of the contemporary literature on emergence. None of the concepts considered challenges the applicability of the Strategy of Decomposition to pathway dynamic behaviours. The concept of Pathway Emergence was formulated against the same background of ideas that motivated the weak concepts of emergence that I have just considered. This was, and remains, a background in which the discipline of complexity science analysed systems that are:

- nonlinear

- have multiple feedback loops
- taken to be computationally incompressible.

And in which complexity scientists term such system's dynamic behaviours as 'emergent'. A further background to the concept of Pathway Emergence is Bechtel and Richardson's concept of Uniform Components Emergence, which links emergence to a failure of the Strategy of Decomposition. As we shall now see, the concept of Pathway Emergence is very different to these weak concepts.

4.3 The Concept of Pathway Emergence

The concept of Pathway Emergence is based on Boogerd et al.'s (2005) interpretation of Broad. It is claimed to be a strong concept of emergence and applies to pathway dynamic behaviours. A key requirement for Pathway Emergence is for a pathway to have a systemic property that is 'non-deducible'. Boogerd et al. state that a systemic property is emergent if it:

> "cannot be 'deduced' from the behavior of parts, together with a 'complete knowledge' of the arrangement of the system's parts and the properties they have in isolation or in other simpler systems." (Boogerd et al., 2005, p. 135).

The concept was further developed by Bechtel and Richardson (2010) who link Pathway Emergence with pathway nonlinearity and the presence of feedback loops. But there are significant gaps and ambiguities in the two accounts provided of Pathway Emergences. This section provides the best interpretation that I can find for the specification of Pathway Emergence. This is primarily based on Boogerd et al. and Bechtel and Richardson's explicit statements on Pathway Emergence but it also: (i) makes use of Broad's writings to help fill in some gaps (ii) includes a criterion for Pathway Emergence that has only implicitly been included in their analyses. The section is in two parts; the first focuses on Boogerd et al.'s interpretation of Broad, the second explains how this is applied to pathway dynamics.

In *Mind and Its Place in Nature* Broad formulates a strong synchronic concept of emergence. Chapter 2 of his book is entitled "Mechanism and its Alternatives" and is devoted to considering the possibility of emergence "before the level of life" (Broad, 1925, p. 44). He states that he cannot give a conclusive example of emergentism but "it is logically possible with a good deal in its favour" (Broad, 1925, p. 59). He also

thinks that chemistry "seems to offer the most plausible example of emergent behaviour" (Broad, 1925, p. 65). Broad explains emergence by contrasting it to what he calls 'mechanistic theory'. Consider any system S composed of parts $C_1...C_n$ in arrangement R. According to mechanistic theory, systemic properties of S can be deduced, at least in principle, from a Deductive Base containing statements of:

1. properties that objects of the same kind as $C_1...C_n$ manifest when in simpler systems than S and
2. arrangement R and
3. laws of composition manifested in simpler systems than S and
4. other laws of nature manifested in simpler systems than S.

Broad cites artificial machines such as clocks as being paradigm examples of where the mechanistic theory successfully applies (Broad, 1925, p. 460). The behaviour of a clock can be deduced from the behaviours and organisation of its springs, wheels etc. together with the appropriate laws of physics and laws of composition.

For Broad, a systemic property P_s of system S is emergent if and only if it cannot be deduced, even in principle, from its Deductive Base. A Deductive Base is restricted to statements of properties and laws manifested in simpler systems than S. This captures the idea that emergent properties arise as the complexity of systems increases. 'Simpler systems' are taken to exclude systems of a similar complexity to S. This non-deducibility is not relative to our cognitive abilities or the current state of science, even a 'mathematical archangel' with 'complete knowledge' could not deduce an emergent property from its system's Deductive Base (Broad, 1925, p. 70, and p. 61).

Broad is proposing a metaphysical concept of emergence (Vintiadis, 2016; O'Connor and Wong, 2015; McLaughlin, 2008, p. 55; Wilson, 2015, p. 36). The requirement for in principle non-deducibility is an epistemological criterion for a metaphysical phenomenon. As Vintiadis (2016) explains:

> "The impossibility of prediction which he [i.e. Broad] cites as a criterion of emergence is a consequence of the metaphysical structure of the world; the mathematical archangel could not have predicted emergent properties not because of complexity or because of the limits of what may be expressed by lower-level concepts, but because emergent facts and laws are brute facts or else are laws that are in principle not reductively explainable."

Boogerd et al.'s interpretation of Broad is inconsistent as to which properties should be stated in *S*'s Deductive Base. Their initial analysis refers to properties that objects of the same kind as $C_1 \ldots C_n$ manifest when situated within other systems that are simpler than *S* (I will use the notation $\boldsymbol{O_1 \ldots O_n}$ to refer to the sets of these properties). However, their paper then switches to referring to properties $\boldsymbol{I_1 \ldots I_n}$, that objects of the same kind as $C_1 \ldots C_n$ manifest when in isolation. Both options lead to viable notions of emergence. I will interpret Boogerd et al. as taking the properties to be $\boldsymbol{I_1 \ldots I_n}$., although mutatis mutandis, my arguments against the occurrence of Pathway Emergence will apply if the properties are taken to be $\boldsymbol{O_1 \ldots O_n}$. I have chosen $\boldsymbol{I_1 \ldots I_n}$. as this is consistent with: (i) the bulk of their paper, which contains multiple references to properties of parts in isolation, (see for example (Boogerd et al, 2005, p. 149, p. 150, p. 156- 159). (ii) their case study which contrasts an 'emergent system' with its 'subsystems studied in isolation' (Boogerd et al., 2005, p. 156) (iii) Bechtel and Richardson's analysis in *Discovering Complexity* of the Strategy of Decomposition; which entails discovering $\boldsymbol{I_1 \ldots I_n}$ in order to explain a target P_S.[37]

Boogerd et al. interpret Broad as having two conditions for emergence, one for each stage of deducing a systemic property P_S. In the first stage *S*'s Deductive Base is used to deduce the properties $\boldsymbol{P_1 \ldots . P_n}$ that $C_1 \ldots C_n$ manifest within *S*. In the second stage properties $\boldsymbol{P_1 \ldots . P_n}$, arrangement *R* and laws of composition are used to deduce P_S. My following diagram illustrates the two stages:

[37] Theurer (2014) interprets Boogerd et al. as selecting $\boldsymbol{O_1 \ldots O_n}$ which is characterised as being the properties of 'less complex systems' than *S*. The paper then focuses on the problems of complexity being 'ill-defined and difficult to measure' and argues that the concept of Pathway Emergence is therefore 'rendered unacceptably relative and epistemic' (Theurer, 2014, p. 283). If Theurer's argument is accepted, I take it to equally apply to Broad's concept and to many other strong concepts of emergence.

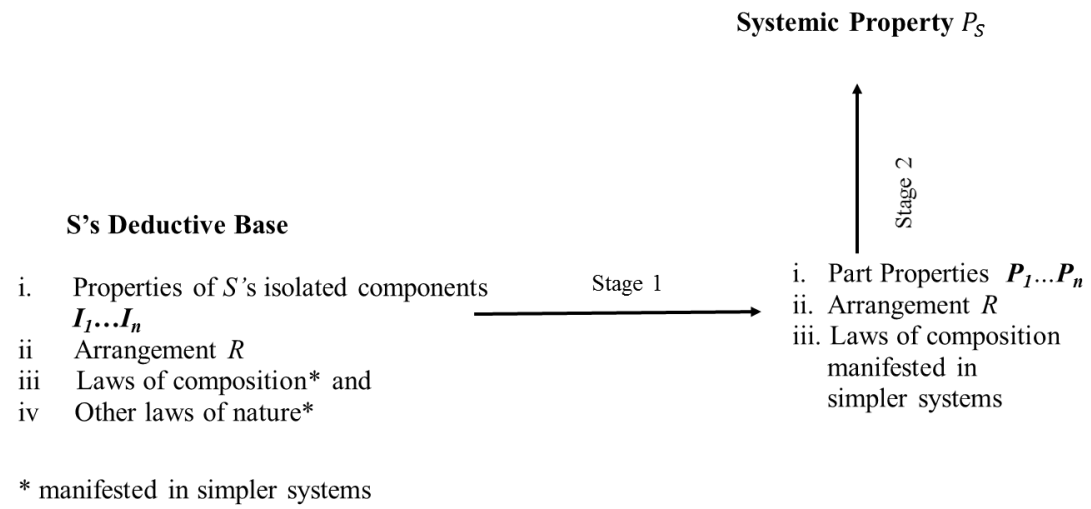


Fig 4.3 Boogerd et al.'s interpretation of Broad's concept of emergence

If there is a part property of *S* that cannot be deduced in stage one, then any systemic property of *S* that is a function of that part property is also taken as being non-deducible. This is the 'horizontal condition' for emergence. If there is a systemic property of *S* that cannot be deduced in stage two, then this is the 'vertical condition' for emergence. The following example illustrates the two conditions.

Consider a system *S* consisting of parts C_1, C_2 which are organised in relation *R*. Let C_1 have a property Q_1 that is manifested when C_1 is situated within *S*, but is not manifested when an object of the same kind as C_1 is in isolation i.e. $Q_1 \in \boldsymbol{P_1}, Q_1 \notin \boldsymbol{I_1}$.

In the first stage, the horizontal stage, the properties $\boldsymbol{P_1}, \boldsymbol{P_2}$ that C_1, C_2 manifest when situated within *S*, are deduced from *S*'s Deductive Base:

1. $\boldsymbol{I_1}, \boldsymbol{I_2}$, *R* and
2. laws of composition manifested in simpler systems than *S* and
3. other laws of nature manifested in simpler systems than *S*.

If Q_1 cannot be deduced from the above, then any systemic property of *S* that is a function of Q_1 is also taken as being non-deducible and hence emergent.

The second stage, the vertical stage, of the deduction starts with $\boldsymbol{P_1}, \boldsymbol{P_2}$ and *R*. *S*'s systemic properties are deduced from:

1. $\boldsymbol{P_1}, \boldsymbol{P_2}$, *R* and
2. laws of composition manifested in simpler systems than *S*

(NB. This is the Coexisting Properties Deductive Base used in Kim's (1999) analysis). Any systemic property of *S* that cannot be deduced from the above is emergent.

It is only the horizontal condition that Boogerd et al. take to be relevant to the occurrence of Pathway Emergence. Boogerd et al. do not believe that vertical emergence occurs in biochemistry. (Boogerd et al., 2005, p. 142). They follow Kim (1999) and take it that the only plausible examples of vertical emergence would be properties that cannot be functionally defined, such as the qualitative character of our phenomenal experiences. For Boogerd et al., biochemical systemic properties can be functionally defined and hence can be 'vertically deduced'. Boogerd et al. make a related point that biochemical systemic properties have mechanistic explanations (e.g. Machamer et al. (2000)) and that this entails rejecting vertical emergence. "Fulfilling the vertical condition means there would be a failure of mechanistic explanation. The properties (and behaviours) of the system would be inexplicable in terms of the properties (and behaviours) of the parts as they function in the system" (Boogerd et al. 2005, p. 136). Instead Boogerd et al. propose a concept of Pathway Emergence that is consistent with emergent properties being "both functionalizable and mechanistically explained" (Boogerd et al. 2005, p. 140).

Boogerd et al. summarise their analysis of Broad:

> "We associate Broad's emergence with a strong notion of emergence..[that includes].. synchronic unpredictability. In the form we have identified and described, synchronic unpredictability means that a systemic property is not predictable, even in principle, from the properties of subsystems in isolation." (Boogerd et al., 2005, p.159)

This concludes my analysis of Boogerd et al.'s interpretation of Broad.

Boogerd et al. now apply their interpretation of Broad's concept to the dynamic behaviours of pathways. A 'pathway dynamic behaviour' is a trajectory in that pathway's phase space; where the phase space has a separate dimension for the concentration values of each of the pathway's chemicals.[38] Dynamic behaviours are the systemic properties that are meant, at least sometimes, to be Pathway Emergent.

Boogerd et al. do not discuss whether emergence is relative to a particular decomposition of *S*. However, their case study shows that they allow for multiple decompositions. Boogerd et al. take the basic parts of a pathway to be its reaction

[38] This comes from a personal communication with Bruggeman, who is one of the co-authors of Boogerd et al.

steps. They also consider sequences of reaction steps from *S* to be parts under alternative possible decompositions. I will take it that a dynamic behaviour will need to be non-deducible relative to any decomposition where:

a) *S* consists of $C_1 \ldots C_n$ in some arrangement *R* and
b) Each C_i is either a reaction step of *S* or a sequence of reaction steps of *S* and
c) No C_i is of similar complexity to *S*.

I will refer to any $C_1 \ldots C_n$ that satisfies these criteria as being an 'admissible decomposition' of *S*. For example, if *S* consists of three reaction steps then there are three admissible decompositions:

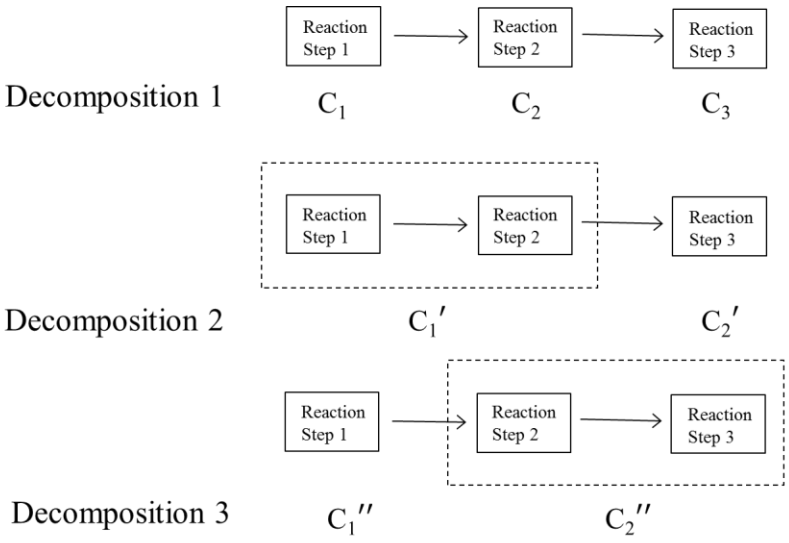


Fig 4.4 Illustration of possible decompositions of a pathway with three reaction steps.

A Deductive Base includes statements of the properties, arrangement and laws corresponding to any such decomposition.

In order to be able to deduce a particular dynamic behaviour, it is necessary to include a statement of the state of that pathway for at least one time point. Boogerd et al. therefore implicitly expand Broad's Deductive Base so that it includes initial conditions. Boogerd et al.'s analysis concerns 'open' pathways, whose behaviours are dependent on net chemical flows entering and exiting that pathway. They therefore further expand Broad's Deductive Base to include boundary conditions.

In the 2nd edition of *Discovering Complexity* Bechtel and Richardson develop the concept of Pathway Emergence. They state that a system can 'reasonably be counted emergent' (Bechtel and Richardson, 2010, p. xlvi) if two conditions are met:

(i) There is feedback. "The component operations will then systematically depend on each other and to the extent that feedback is system-wide, these dependencies will result in operations that are specific to the system.... the behavior of components within a system will not be "predictable" from the behavior in significantly simpler systems." (Bechtel and Richardson, 2010, p. xlvi).

(ii) "the nonlinearities affecting component operations must in turn affect the behavior of the system. For example, such organization can introduce oscillatory states or instabilities that would not be present in significantly simpler systems or linear systems." (Bechtel and Richardson, 2010, p. xlvi).

The rationale for these two conditions is not explained. Although Bechtel and Richardson do not state it, both are necessary conditions both for stable limit cycles and for multiple steady states (recall section 2.3).[39] I take the two conditions to highlight a criterion for Pathway Emergence that has been only implicitly included in Boogerd et al. and is not part of Broad's concept.[40] The criterion restricts emergence to dynamic behaviours that are 'qualitatively different' from those manifested by their isolated parts. The phrase 'qualitatively different' comes from Boogerd et al.'s case study, when they are comparing a pathway with an unstable steady state to pathways with stable steady states (see section 4.5); however they do not define what the phrase means. I take it that two pathways have 'qualitatively different' dynamics if they have a different number of either: stable steady states, stable limit cycles, unstable steady states or unstable limit cycles.[41] In biochemistry, as far as I am aware, pathways have been found only to have either zero or a very low number for any of these steady states or limit cycles. If two pathways differ in the number of any of these states or cycles then their phase spaces can be taken to have significantly different topologies and this can result in significantly different dynamic behaviours.

[39] These are general requirements for pathways in homogenous solutions that are at constant temperature; see for example Novak et al. (2008).

[40] See for example Boogerd et al. (2005, p.156). This requirement has been confirmed in a personal communication with Bruggeman, e.g. see quote in section 4.5.

[41] This definition could be expanded to include other types of attractors / repellors that are not part of Boogerd et al.'s analysis e.g. strange attractors. For explanations of these see Strogatz (1994).

The requirement for qualitatively different dynamics appears to be an ad-hoc addition. But perhaps this is meant to capture the causal novelty required by a strong concept of emergence? It is not immediately clear. Let us first see what Boogerd et al.'s argument for Pathway Emergence is, and then we will return to the role of this 'qualitative difference'.

In summary, I interpret the concept of Pathway Emergence as having both a 'non-deducibility' and a 'qualitative difference' criterion.

Let $C_1 \ldots C_n$ be the parts of a decomposition of pathway S such that (a) S consists of $C_1 \ldots C_n$ in arrangement R and (b) each C_i is either a reaction step of S or a sequence of reaction steps of S and (c) no C_i is of similar complexity to S. A dynamic behaviour of a pathway S is Pathway Emergent if and only if, for all such decompositions it:

(a) Cannot be deduced, even in principle, from:
  (i) properties $\boldsymbol{I_1 \ldots I_n}$ that objects of the same kind as $C_1 \ldots C_n$ manifest when in isolation and
  (ii) the arrangement R and
  (iii) laws of composition manifested in simpler systems than S and
  (iv) other laws of nature manifested in simpler systems than S and
  (v) initial conditions and
  (vi) boundary conditions.

(b) is 'qualitatively different' from the dynamic behaviours manifested by S's isolated parts.

Henceforth these shall be referred to as the 'Criteria for Pathway Emergence'.

4.4 Deducing Pathway Dynamic Behaviours

In this section, I will briefly recap the steps by which pathway dynamic behaviours are deduced in biochemistry (recall section 2.2.2), and I will structure the material in a way that will facilitate my evaluation of Boogerd et al.'s case study.

Elementary reactions are the most basic type of chemical reaction and involve one or more chemical species reacting directly to produce a product. However, the chemical

reaction equations used in modelling pathway dynamics usually only refer to stepwise reactions. A stepwise reaction is an aggregation of a sequence of elementary reactions where at least one product of each elementary reaction is a reactant in the next reaction. Stepwise reactions are composed of elementary reactions and true stepwise rate laws can be deduced, at least in principle, from their elementary rate laws. Therefore*:*

> *only elementary reactions need be considered when evaluating the in principle deducibility of pathway dynamic behaviours.*

The dynamics of pathways are often modelled using ordinary differential equations (i.e. 'ODEs'). For chemically homogenous solutions at constant temperature, the discipline of biochemistry often assumes that the dynamic behaviours of a pathway are fully determined by statements of:

a) the rate laws that its reaction steps have within that pathway and
b) initial concentrations and
c) net flows of chemicals entering/exiting the pathway and
d) a law for combining these net flows with the pathway's rate laws (i.e. the Kinetic Law of Composition).

Henceforth this will be referred to as the 'Biochemistry Base'.

The deduction of a pathway dynamic behaviour can be viewed as having two stages. In the first stage, a separate concentration ODE is deduced for each of *S*'s chemical species from statements of *S*'s: reaction step rate laws plus the Kinetic Law of Composition. The Kinetic Law of Composition states that the rate of change of the concentration of a chemical species is equal to the sum of the rates of those reactions that create that chemical species minus the rates of those reactions that consume that chemical species (this is for closed systems; for open systems the net flows into the pathway also need to be added).

For example, let us return to the toy pathway considered in section 2.2.2:

Chemical Equation	Rate Law
Step 1 B + C → D + E	$v_1 = k_1[B][C]$
Step 2 E + F → G	$v_2 = k_2[E][F]$
Step 3 G + H → C + I	$v_3 = k_3[G][H]$

Consider chemical species E, which is produced in step 1 and consumed in step 3. According to the Kinetic Law of Composition:

$$\frac{d[E]}{dt} = rate\ of\ step\ 1 - rate\ of\ step\ 2$$

$$= \quad v_1 \quad - \quad v_2$$

$$= \quad k_3[B][C] - k_1[E][F]$$

The above equation is the 'concentration ODE' for E in this pathway. A pathway's concentration ODEs are taken by the discipline of biochemistry to fully determine the topography of a pathway's phase space and its pathway's dynamic behaviours.

In the second stage, the target pathway dynamic behaviour is deduced from statements of the pathway's concentration ODEs plus initial conditions and net flows into the pathway. This is usually carried out by the use of simulation.

The following diagram summarises the relationship between the Biochemical Base, concentration ODEs and the deduction of dynamic behaviour (the square brackets group together the factors that are used to: (i) deduce the concentration ODEs and (ii) to deduce a dynamic behaviour).

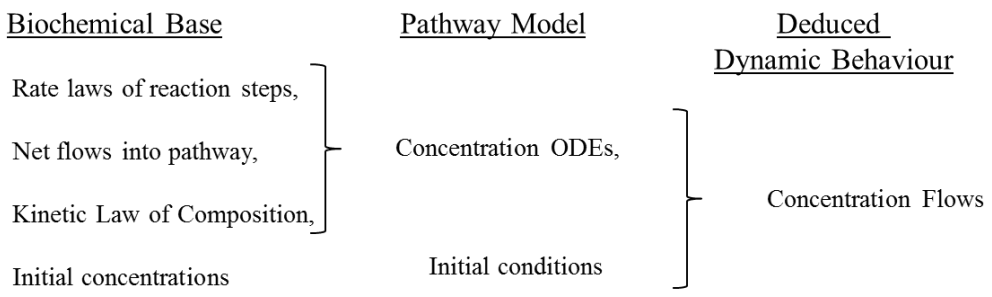


Fig 4.5 Diagram illustrating the relationships between the Biochemical Base, a pathway model and the deduction of a dynamic behaviour

4.5. Boogerd et al.'s Case Study – A Simulation Example of Pathway Emergence?

Boogerd et al. argue for the occurrence of Pathway Emergence by providing 'an explicit case study drawn from molecular cell physiology that [shows] biochemical networks display this kind of emergence" (Boogerd et al., 2005, p. 131). The case study is a simulation of a pathway and its dynamic behaviours are fully mathematically specified. In order to evaluate the case study, this section will first identify a necessary condition for the pathway's dynamic behaviours to be non-deducible from its Deductive Base. It will then be shown that the case study does not satisfy this condition and therefore fails to illustrate Pathway Emergence. Boogerd et al. link non-deducibility to the values of its 'state-dependent component properties'. Bechtel and Richardson link non-deducibility to the presence of feedback. It will be argued that neither state-dependent component properties nor feedback affect the deducibility of dynamic behaviours.

Boogerd et al.'s case study assumes that the Biochemical Base is sufficient for deducing its pathway's dynamic behaviours. The Biochemical Base of a pathway contains statements of that pathway's rate laws, net flows, initial concentrations and the Kinetic Law of Composition. A necessary requirement for pathway *S* to be Pathway Emergent is then: *S*'s Deductive Base does *not* entail its Biochemical Base. Given that statements of the Kinetic Law of Composition, initial concentrations and boundary conditions will be contained in both, it follows that *S*'s Deductive Base must not entail statements of *S*'s rate laws. This is illustrated below (the arrows represent relationships of entailment):

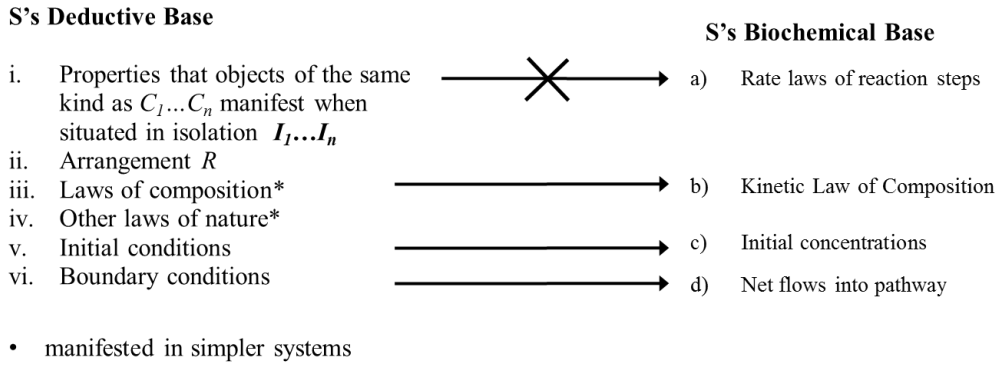


Fig 4.6 Diagram illustrating a necessary requirement for a pathway *S* to be Pathway Emergent.

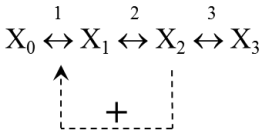
As the only type of reaction that needs to be considered when evaluating in principle deducibility are elementary reactions, it further follows that a necessary requirement for Pathway Emergence is that relative to all the admissible decompositions of a pathway *S*:

> *S must manifest at least one rate law for one of its elementary reactions, which is not manifested by its isolated parts.*

Henceforth this shall be referred to as the 'New Rate Law Requirement'. This requirement is not identified in Boogerd et al.

The title of Boogerd et al.'s paper is 'Emergence and Its Place in Nature: A Case Study of Biological Networks' but their paper concerns emergence in pathways. The case study is a simple pathway with a single feedback loop. Given that biological networks are composed of pathways, it can be argued that if there is emergence at the level of pathways then there is also emergence at the level of biological networks. But it is important to understand that Boogerd et al. are not claiming that biological networks have emergent properties in virtue of being networks, rather the putative emergence is meant to be occurring at the level of pathways. It should also be noted that although Boogerd et al. are using a simulation case study to illustrate Pathway Emergence, the claim is that this kind of emergence occurs in actual biological pathways.

The case study consists of an ODE simulation model of a hypothetical metabolic pathway of three reaction steps:



Model A

X_0 , X_1 , X_2 and X_3 are substrates and there is a positive feedback loop whereby X_2 increases the rate of the first reaction step. Boogerd et al. specify the conditions for model *A* to be in a stable steady state. They then decompose *A* into two isolated models:[42]

Model A_1 Model A_2

The conditions for models A_1 and A_2 to be in a stable steady state are then specified. It is shown that it is possible for A_1 and A_2 to be stable but for *A* to be unstable. This is claimed to illustrate that *A* can have emergent behaviour.

Models *A*, A_1 and A_2 are all constructed using different combinations of just three rate laws. *A* uses all three rate laws, A_1 uses the first two and A_2 uses the last two. The values of each rate constant remain the same across models; for example the rate constants for reaction step one will have the same values in both *A* and A_1. Boogerd et al. use dynamic systems theory to identify some stability conditions. Model *A* has a stable steady state if the following two conditions are met (Boogerd et al, 2005, p. 158):

[42] I take it be a weakness of the case study that A_1 and A_2 compositionally overlap. Allowing such decompositions would unnecessarily complicate the concept of Pathway Emergence. For example, it is unclear how statements of properties of A_1 and A_2 could be advantageous for deducing systemic properties of *A* compared with only using properties from non-overlapping components. Furthermore, their inclusion would then seem to require a rule of aggregation that somehow adjusts for the overlapping. Such a rule would seem too ad-hoc to be regarded as a law of composition.

$$\frac{\partial v_1}{\partial [X_1]} - \frac{\partial v_2}{\partial [X_1]} + \frac{\partial v_2}{\partial [X_2]} - \frac{\partial v_3}{\partial [X_2]} < 0 \qquad \text{(condition 1)}$$

$$\underbrace{\left(\frac{\partial v_1}{\partial [X_1]} - \frac{\partial v_2}{\partial [X_1]}\right)}_{\text{Term 1}} \underbrace{\left(\frac{\partial v_2}{\partial [X_2]} - \frac{\partial v_3}{\partial [X_2]}\right)}_{\text{Term 2}} - \underbrace{\left(\frac{\partial v_1}{\partial [X_2]} - \frac{\partial v_2}{\partial [X_1]}\right)\left(\frac{\partial v_2}{\partial [X_1]}\right)}_{\text{Term 3}} > 0 \qquad \text{(condition 2)}$$

Model A's Stability Condition

Where v_1, v_2 and v_3 are the rates of the reaction steps and $[X_1]$, $[X_2]$, $[X_3]$ are concentrations of X_1, X_2 ,X_3 respectively. Models A_1 and A_2 each have a stable steady state if:

$$\underbrace{\frac{\partial v_1}{\partial [X_1]} - \frac{\partial v_2}{\partial [X_1]}}_{\text{Term 1}} < 0 \quad \text{and} \quad \underbrace{\frac{\partial v_2}{\partial [X_2]} - \frac{\partial v_3}{\partial [X_2]}}_{\text{Term 2}} < 0 \qquad \text{(conditions 3)}$$

Model A_1 and A_2 Stability Conditions

Notice that Term 1 and Term 2 of stability condition 2 are also terms in stability conditions 3. From this it follows that Model A will be unstable, yet Models A_1 and A_2 stable if:

$$\underbrace{\left(\frac{\partial v_1}{\partial [X_1]} - \frac{\partial v_2}{\partial [X_1]}\right)}_{\text{Term 1}} \underbrace{\left(\frac{\partial v_2}{\partial [X_2]} - \frac{\partial v_3}{\partial [X_2]}\right)}_{\text{Term 2}} \leq \underbrace{\left(\frac{\partial v_1}{\partial [X_2]} - \frac{\partial v_2}{\partial [X_1]}\right)\left(\frac{\partial v_2}{\partial [X_1]}\right)}_{\text{Term 3}}$$

Term 1 < 0

Term 2 < 0

This equation makes clear how stability conditions 3 can be true but stability condition 2 false and that hence "it is possible to have an unstable system even under the assumption that subsystems are stable in isolation" (Boogerd et al, 2005, p. 158). It also helps to illustrate that the same rate laws are being used in all three models.

Boogerd et al. conclude that:

> "The behavior of A_1, in isolation is sometimes qualitatively different from the behavior of A_1 in A, and therefore, since the behavior of A is a function of A_1, understood as a component, the behavior of A cannot generally be derived from studies on simpler subsystems of A. In general, the (dynamic) behavior of A is not simply the superposition of the (dynamic) behaviors of its subsystems studied in isolation. Dynamic interactions can bring about qualitatively new behavior in complex systems. This is precisely where prediction of system behavior on the basis of simpler subsystems fails. We cannot predict the behavior of the components within the entire system and so cannot predict systemic behavior. This is emergence, with novel system behavior that cannot be predicted on the basis of the behavior of simpler subsystems." (Boogerd et al, 2005, p. 156).

The case study does help to illustrate how the qualitatively different criterion could be satisfied: A has an unstable steady state whilst A_1 and A_2 have stable steady states. But the case study does not illustrate the type of non-deducibility needed for Pathway Emergence. A Deductive Base includes statements of all the properties that a system's parts manifest in isolation. The manifested properties of A_1 includes the rate laws for reaction step one and reaction step two. The manifested properties of A_2 includes the rate law for reaction step three. The rate laws for A are therefore manifested by its isolated parts and the dynamic behaviours of A can be deduced using these laws, the Kinetic Law of Combination, boundary conditions and initial concentrations. The case study fails to illustrate Pathway Emergence because it fails to satisfy the New Rate Law Requirement.

In their overview of pathway simulation, Boogerd et al. emphasise the importance of 'state-dependent component properties' and these are clearly meant to play a central role in illustrating Pathway Emergence. State-dependent component properties are functions of two types of properties (Boogerd et al, 2005, p. 158):[43]

[43] Boogerd et al. refer to these simply as 'component properties'. However I have termed them as 'state-dependent component properties' in order to make clear how they differ from other properties of components.

(i) state properties of the system. These are systemic properties which vary as the state of the system varies. The relevant properties in the case study are a pathway's concentrations.

(ii) properties intrinsic to the component; these are state-independent, relational properties specifying the relationship between two or more chemicals. The relevant properties in the case study are rate constants.

Boogerd et al. cite rates of reactions and elasticity coefficients amongst their examples of component properties. Both are ultimately functions of elementary rate constants and pathway concentrations. In the case study, the pathway concentrations are deduced by simulation and rate constants are known and do not change; hence the values of the state-dependent component properties are entirely deducible and do not provide any support to claims of non-deducibility relative to a Deductive Base.

Perhaps the putative non-deducibility is meant to be related to Model *A* having a feedback loop? This would be consistent with Bechtel and Richardson's including feedback as one of their two conditions needed for a system to 'reasonably be counted emergent'. To recap, Bechtel and Richardson are referring to chemical feedback which occurs when the 'concentration of some species affects the rate of its own production.' (Epstein and Pojman, 1998, p. 23). Consider a toy pathway of the following elementary reactions:

Step 1 $B + C \rightarrow D + E$

Step 2 $E + F \rightarrow G$

Step 3 $G + H \rightarrow C + I$

There is feedback on C as:

- **increasing concentration [C]** increases the rate of production of E which, (in Step 1)
- increases the rate of production of G which, (in Step 2)
- **increases the rate of production of C**. (in Step 3)

Neither in Bechtel and Richardson's book nor in Boogerd et al.'s paper is the relationship between feedback and non-deducibility explained. As I have explained in section 3.3, Bechtel and Richardson provide an account of how in the early 20th century biologists assumed that the glycolytic pathway did not have feedback loops. This hampered the discovery of the actual reaction steps of the pathway; because the

rate of an isolated reaction step can be very different to the rate of the same reaction step when subject to feedback (Bechtel and Richardson, 2012, p. 149 -172). This is illustrated in the above example, where, ceteris paribus, the rate of step one would be faster when situated within the pathway than when in isolation (as step three produces C, which leads to an increases in the rate of step one). Bechtel and Richardson's analysis illustrates how feedback can complicate the dynamic behaviours of a pathway, but it does not illustrate non-deducibility relative to its Deductive Base.

Chemical feedback occurs as a consequence of there being at least one reaction within a pathway that changes the concentration of a chemical species X, where X is also a reactant of an 'earlier' step of the pathway (in the toy pathway this happens with chemical species C). The laws for all the reactions within a pathway are specified by a pathway's concentration ODEs, including the reactions that cause feedback. As I have explained in my section 2.3, the Systems Biology Criterion for Feedback is based solely on a pathway's concentration ODEs. Hence there is nothing about feedback per se that leads to a non-deducibility that would ground Pathway Emergence. The only way that feedback could lead to such non-deducibility would be if it was to lead to changes in the rate laws themselves, and this is something that neither Bechtel and Richardson nor Boogerd et al. claim. Nevertheless, the presence of a feedback loop in the case is not irrelevant, as it is a necessary condition for satisfying the 'qualitatively different' criterion for Pathway Emergence.

In conclusion, a number of problems have been identified with Boogerd et al.'s case study: (i) it does not illustrate non-deducibility relative to its Deductive Base (ii) the emphasis on state-dependent component properties is unjustified (iii) there is no relationship between feedback and non-deducibility (iv) the New Rate Law Requirement has not been recognised. These problems strongly suggest that Boogerd et al. did not fully specify their intended concept of emergence.

4.6 Might There Still be Pathway Emergence?

There is a plausible argument for pathways sometimes being Pathway Emergent. This is because elementary rate constants in biochemistry are highly context sensitive. The New Rate Law Requirement for pathway *S* can be satisfied if one of its elementary reactions:

(i) manifests a rate constant with value k_s, when situated within *S*,

(ii) does *not* manifest a rate constant with value $k_{s.,}$ when in isolation or when situated within any isolated part from an admissible decomposition of *S*.

Prima facie, this often occurs within biochemistry. This is because elementary rate constants are proportional to the fraction of reactant molecules that have 'effective collisions' per unit time (recall section 3.6). Effective collisions are those collisions that lead to a chemical reaction. Elementary rate constants vary by context because the number of 'effective' collisions varies by context (for given concentrations of reactants). Many factors have been identified that contribute to this including: concentrations of solutes changing enzyme conformations, crowding and confinement effects. At present biologists are often not able to accurately deduce how rate constants will change with context. Perhaps this non-deducibility is sometimes indicative of Pathway Emergence?

In Boogerd et al.'s case study, the New Rate Law Requirement is not satisfied. A_1 and A_2 are specified such that their rate laws are also manifested by *A*. This is in line with the assumption that pathways are causal law modular. *S* is 'causal law modular' if $C_1...C_n$ manifest the same local causal laws that are manifested by objects of the same kind as $C_1...C_n$ that are situated in 'isolation'. Biochemists often make this assumption via the Rate Law Invariance Assumption (recall section 3.6):

> each of the rate laws manifested within a pathway will be manifested in its 'appropriately formulated' isolated reaction step.

An 'appropriately formulated' isolated reaction step consists of that step's reactants and products, plus any non-reactive constituents of its pathway that impact on the dynamics of that step.

I will take it that Boogerd et al. meant to specify that the reaction steps referenced in a pathway's Deductive Base are 'appropriately formulated'. This is consistent with (i)

their case study (ii) their claim that the concept of Pathway Emergence is relevant to the practices of biochemistry (see section 4.7) (iii) their claim that "Importantly, all kinetic values can be measured *in vitro*" (Boogerd et al. , 2005, p. 147). The New Rate Law Requirement is then a negative version of the Rate Law Invariance Assumption. When the Rate Law Invariance Assumption is correct, the New Rate Law Requirement is not satisfied and there is no instantiation of Pathway Emergence. Pathway Emergence and causal law modularity are then related concepts. For a pathway *not* to be causal law modular, it is necessary that at least one of its reaction steps manifests a rate law that is not manifested by the corresponding isolated reaction step i.e. the New Rate Law Requirement is a necessary condition for a pathway *not* to be causal law modular. And the New Rate Law Requirement is also a necessary condition for Pathway Emergence.

The New Rate Law Requirement might therefore often be satisfied, for the same reasons that pathways might often not be causal law modular. In section 3.6, I identified two reason why an appropriately formulated reaction step might manifest a different rate law compared to its corresponding pathway reaction step. First there is my 'Other Reactants Objection' i.e. the joint presence of all, or nearly all of the pathway's reactants and products might be necessary before some reactants acquire the same conformations they have in the pathway; and conformations can affect rates of reaction . Second, biochemists may simply not be able to adequately replicate the crowded, heterogenous environment found within a living cell. Hence there are strong grounds for claiming that the New Rate Law Requirement might often be satisfied and this leaves open the possibility of Pathway Emergence.

4.7 Perhaps the Deductive Base was Meant to be Further Restricted?

I will argue that Boogerd et al. intended to specify a weaker criterion for the non-deducibility of dynamic behaviour. The criterion is:

> A dynamic behaviour of a pathway *S* is 'Weakly Non-Deducible' if and only if for all admissible decompositions it cannot be deduced, even in principle, from:
>
> (i) the dynamic behaviour of *S*'s isolated parts and
>
> (ii) a 'law of composition' manifested in simpler systems than *S,* which aggregates these dynamic behaviours.

As earlier explained, 'dynamic behaviour' refers to a pathway's trajectories in a pathway's phase space, where the phase space has a separate dimension for the concentration values of each of the pathway's chemicals. It is these behaviours that are, at least sometimes, meant to be emergent. I will present evidence for Weak Non-Deducibility being Boogerd et al.'s implicit notion of non-deducibility. Adding the qualitatively different criterion (as specified in the Criteria for Pathway Emergence) to this Weak Non-Deducibility criterion results in a concept of emergence that I will term 'Weak Pathway Emergence'. I will argue that Weak Pathway Emergence is a very different notion of emergence to Broad's.

The Weak Non-Deducibility criterion clarifies the earlier quote from the case study that was meant to explain emergence: "In general, the (dynamic) behavior of *A* is not simply the superposition of the (dynamic) behaviors of its subsystems studied in isolation." However, the notion of law of composition that this requires is different to that I specified in section 4.2. A law of composition was taken as specifying a relationship between a system and properties that its parts manifest when they are situated *within that* system. By contrast the type of law of composition that I take Boogerd et al. to imply has the form:

> If objects of the same kind as $C_1 \ldots C_n$ manifest properties $\boldsymbol{I_1 \ldots I_n}$ when they are in isolation and *S* consists of $C_1 \ldots C_n$ in arrangement *R*, then *S* has property $P_S = f_R(\boldsymbol{I_1, \ldots, I_n})$, for some function f_R.

A dynamic behaviour of pathway *S* would then be Weakly Non-Deducible if and only if there is no law of composition of the above form where:

- properties $\boldsymbol{I_1 \ldots I_n}$ are dynamic behaviours
- P_S is equal to the dynamic behaviour of *S*.

I will continue to reserve use of the term 'law of composition' for laws that aggregate the properties $\boldsymbol{P_1 \ldots P_n}$ that $C_1 \ldots C_n$ manifest in *S* and I will use the term 'law of composition (isolated parts)' to refer to laws that aggregate $\boldsymbol{I_1 \ldots I_n}$.

In general, pathways are Weakly Non-Deducible. This is because the concentrations of a pathway's chemicals will often be dependent on all, or nearly all, of the reaction steps in the pathway. This can be illustrated using another toy pathway *T* where all the reactions are reversible (which is typically the case in biochemistry):

Step 1 $B + C \leftrightarrow D$

Step 2 $D + E \leftrightarrow F$

Step 3 $F + G \leftrightarrow H$

Let system T_1 be an isolated system consisting of just reaction step one and reaction step two. Let system T_2 be an isolated system consisting of just reaction step three. Because all the reaction steps are reversible, the concentrations of *T*'s chemical species are dependent on all the reaction steps in the pathway. For example, the values of [D] in *T* depend on the rates of reaction step one, reaction step two and reaction step three. D is both a reactant and a product in reaction step one i.e. it is a product in the forward reaction B + C→ D and a reactant in the backwards reaction D → B + C. Similarly, D is both a reactant and a product in reaction step two. Even though D is neither a reactant nor a product in reaction step three, reaction step three still affects [D] as it affects [F] which in turn affects [D] via the backwards reaction F → D + E . The effects of these pathway-wide dependences means that the concentrations for *T* are Weakly Non-Deducible from the concentrations occurring in T_1 and T_2.

In a personal communication, Bruggeman provides some support for my interpretation that the type of non-deducibility required was Weak Non-Deducibility. Bruggeman describes a pathway with three reaction steps with a different enzyme catalysing each step. He states that:

> "the behaviour of a part in the system depends on all parts; in this case this leads to nonlinear functions and, hence, aggregativity (simple reduction) does not apply. Now, if the system composed out of three enzymes can display a behaviour that is qualitatively different (qualitatively can be strictly defined in mathematics, in fact this is already done in bifurcation theory) from the behaviour of that of any of its subsystems (all single enzyme or pairs of enzymes systems) then one could call the behaviour "new" and hence, weakly emergent as it still reducible in principle if not analytically then by simulation. This is our argument and comes very close to Broad."

My interpretation also helps makes sense of the problems identified with the case study. First, whilst Model *A*'s dynamics are deducible relative to its Deductive Base, they are also Weakly Non-Deducible. Second, the New Rate Law requirement does not need to be satisfied in order for a pathway to be Weak Non-deducible. Third, the focus on state-dependent component properties can be explained. In the case study, state-dependent component properties depend on pathway concentrations; whose

trajectories in phase space are, by definition, dynamic behaviours. As dynamic behaviours are Weakly Non-Deducible, it follows that the state-dependent component properties are also Weakly Non-Deducible. However, this interpretation does not explain the focus on feedback. A pathway that did not have a feedback loop would also be Weakly Non-Deducible.

A recent paper by Kolodkin et al. on Pathway Emergence provides further support for this interpretation. The paper is written by twelve system biologists including Boogerd and two of the other co-authors of the Boogerd et al. paper: Bruggeman and Westerhoff. Kolodkin et al. summarise the account of Pathway Emergence provided by Boogerd et al. They state that:

> "emergence arises when R(A,B,C), e.g. the behavior of A, B and C in organizational state R cannot be predicted from the properties of A,B and C in isolation or in configurations simpler than R."

However they then proceed:

> "… we expect to be able to deduce R(A,B,C) from the complete knowledge of the behavior of A, B, and C in isolation or in other (perhaps crowded) systems such as S_1(A,B), S_2(A,C) and S_3(B,C), if we add also the knowledge of state-dependent properties of A, B and C or S_1(A,B), S_2(A,C) and S_3(B,C). For example, the behavior of A within the system R(A,B,C) may depend not only on relational properties of A (which can be determined in isolation), but also on the state of the system, i.e. on the activity of B and C….These (state-dependent) component properties and the state independent relational properties together constitute R(A,B,C). In fact, while given a mathematical description of a whole network, we are able to integrate both relational and state-dependent component properties of A, B and C. Theoretically, using a comprehensive computer simulation, we can replicate the network that contains all the biological molecules themselves and produce whatever emergent property may arise… even the strongest emergent property should become calculable." (Kolodkin et al, 2012, pp. 192-193).

So emergent properties are taken to be entirely deducible by simulation. Also note that relational properties (which include rate constants) are taken as being determinable from isolated parts.

But what might the motivation be for formulating this concept of Weak Emergence? I suggest that it is the product of a synthesis of several ideas, some of which are present in other concepts of weak emergence (recall section 4.2).

1. Non-aggregativity. Weak Pathway Emergence resembles Wimsatt's concept of emergence. Indeed, Boogerd et al. reference Wimsatt's concept in their discussion of pathway dynamics:

   > "If a system is purely aggregative, all its systemic properties depend only linearly on the properties of the parts (Wimsatt 1976, 1986). In complex biochemical systems, aggregative system properties are a function only of the intrinsic properties of the parts; for example, the mass of a bacterium is simply the sum of the masses of the parts. The flux through a biochemical pathway, in contrast, depends non-linearly on the concentrations of its constituent enzymes. This is not an aggregative property." (Boogerd et al., 2005, p. 151)

   But there are several significant differences with Wimsatt's concept. First, Wimsatt's compositional equation:

   $$P_S = f_R(\boldsymbol{P_1}, \ldots, \boldsymbol{P_n})$$

   is not required to be a law of composition that is manifested in simpler systems than *S*. Second, Weak Pathway Emergence does not include Wimsatt's requirements of: invariance to inter-substitution, size scaling or 'decomposition and reaggregation'. Third, Weak Non-Deducibility aggregate properties $\boldsymbol{I_1 \ldots I_n}$, rather than $\boldsymbol{P_1}, \ldots, \boldsymbol{P_n}$. I will consider why Boogerd et al. might have chosen $\boldsymbol{I_1 \ldots I_n}$, in my point 4 below.
2. Complexity. Pathways are complex systems, that are only deducible by simulation. In Weak Pathway Emergence, P_s is taken to be deducible from *S*'s Biochemical Base, by using simulation. In this respect Weak Pathway Emergence resembles Bedau's concept of emergence. However, Bedau's is a diachronic concept in which a *Ps* at a time *t* is emergent relative to properties manifested at some earlier time than *t*. By contrast, Weak Pathway Emergence is a synchronic concept with deductions using statements of properties, $\boldsymbol{I_1 \ldots I_n}$, and not comparing P_s to properties manifested at some earlier time.
3. Qualitative difference. A motivation for the qualitatively different criterion appears to be that it captures the fact that as the number of reaction steps in a pathway incrementally increases, there can be sudden qualitative changes in the dynamics of that pathway. Such sudden changes can appear 'surprising'. For example, in the case study the pathway comprising reaction step one and

reaction step two has a stable steady state but adding reaction step three leads to the pathway having an unstable steady state.

4. Emergence as a challenge to the causal discovery of pathway dynamic behaviours. The idea of linking emergence with the Strategy of Decomposition is already present in Bechtel and Richardson (1993). Weak Pathway Emergence might be taken as being a useful concept for emphasising the need for the strategy to take account of all the interactions within a pathway. This interpretation is supported by Boogerd et al.'s concluding paragraph:

> "From a methodological point of view, if we attack a biological problem experimentally or theoretically, beginning with the constituents of cells treated in isolation, then the lack of a systemic context can be an impediment to scientific research. With some systemic effects, decomposition may reveal mechanistic explanations, but this depends critically on understanding the behavior of parts as components. Beginning with the behavior of parts in radically different contexts, or in much simpler contexts, will sometimes fail to reveal their contributions to system behavior. Sometimes it will succeed. Sometimes it does not (Boogerd et al. 2002). In these cases, systemic behavior cannot be extrapolated from the behavior of parts in simpler systems, rendering them emergent. We think that this is a general phenomenon for other complex systems." (Boogerd et al. 2005, p.160).

However, it must be emphasised that Boogerd et al. are taking systemic effects to be fully captured by using simulation to deduce pathway dynamic behaviours. As such they are failing to allow for the possibility that the rate laws manifested in a pathway may be different to those manifested by their isolated reaction steps (i.e. that some pathways may *not* be causal law modular).

If Boogerd et al. did intend to propose Weak Pathway Emergence, then this is a concept that is very different to Broad's. Broad's is a metaphysical concept of emergence, in which emergent properties are in principle non-deducible because they are ontologically novel. By contrast, Weak Non-Deducibility simply follows from pathways being nonlinear. Perhaps Weak Non-Deducibility can be described as being in some way 'Broadian', but this would be a highly qualified claim that needs to be

explicitly stated. In practice, virtually all pathways are likely to have Weakly Non-Deducible dynamics. This would lead to the Criteria for Pathway Emergence effectively collapsing to just the qualitative difference criterion that a systemic property is emergent if it is 'qualitatively different' from the systemic properties of simpler systems.

The concept of Weak Pathway Emergence highlights the fairly obvious point that pathway dynamic behaviours are non-aggregative. This provides little insight into the challenges that biochemists face in the causal discovery of pathway behaviours. Perhaps a more interesting concept of weak emergence could be formulated that centered on the *in practice* gap between the causal laws that are manifested in *in vitro* solutions and the causal laws that arise in the more complex environment of living cells. This would be a weak concept as it allows for the possibility of *in principle* deducibility. As I have argued in my chapter 3, the assumption of causal law modularity is the key assumption underpinning the Strategy of Decomposition, and there seem good reasons to think that this assumption will often be false. A weak concept of emergence that centered on this assumption could help to facilitate the debate on the extent to which biochemists are justified in using the Strategy of Decomposition.

4.8 Conclusion

Boogerd et al. claim that pathways can sometimes be Pathway Emergent. They state that it is a strong concept, based on Broad theory of emergence. A key requirement is that a pathway's dynamics are non-deducible relative to their Deductive Base. Prima facie the existence of Pathway Emergence would undermine the Strategy of Decomposition that is used for the causal discovery of pathway dynamic behaviours. This is because the strategy's reconstruction stage involves making exactly the same deduction that Pathway Emergence claims will sometimes not be possible.

There are significant gaps in the specification provided for Pathway Emergence. Boogerd et al.'s argument for Pathway Emergence takes the form of a simulation case study. But the case study does not illustrate Pathway Emergence as it does not satisfy the New Rate Law Requirement.

There is still a plausible argument for pathway dynamics sometimes satisfying the Criteria for Pathway Emergence. This is because of the context sensitivity of rate constants in biochemistry. At present biologists are often not able to accurately deduce how rate constants will change with context. Perhaps this non-deducibility is sometimes indicative of Pathway Emergence. However, this is speculative and the claim that pathway dynamics are sometimes Pathway Emergent has not been established.

Perhaps Boogerd et al. did not correctly specify what they meant by non-deducibility and their intended concept was meant to be Weak Pathway Emergence. But this is a very different notion of emergence to Broad's. Furthermore, the concept provides little insight into the challenges that biochemists face in the causal discovery of pathway behaviours. I conclude that the claims that pathways manifest a type of emergence that challenges the Strategy of Decomposition are unsubstantiated.

Chapter 5 - Conclusions

> "A central challenge of biochemistry is to understand the influences of cellular organisation and macromolecular associations on the function of individual enzymes and other biomolecules – to understand function *in vivo* as well as *in vitro*."
> (Nelson and Cox, 2013, p. 10)

The Strategy of Decomposition is used in biochemistry for the causal discovery of pathway dynamic behaviours. My thesis has addressed the question of whether the use of this strategy is warranted. I have focused on two challenges to the Strategy of Decomposition that are contained in Bechtel and Richardson's *Discovering Complexity* and in related papers. The first challenge is that pathways lack the 'modular' structure assumed in the Strategy of Decomposition, where Bechtel and Richardson interpret modularity as 'near decomposability'. The second challenge is that pathways sometimes have 'Pathway Emergent' behaviours such that their dynamic behaviours cannot be deduced from statements of the properties manifested by the pathway's isolated parts, the pathway's organisation, and laws manifested in simpler systems. I have rejected both challenges. I have argued that near decomposability is the wrong type of modularity to apply to pathways. I have also shown that the occurrence of Pathway Emergence has not been established. An underlying problem with Bechtel and Richardson's analyses is that they overstate the consequences of feedback and nonlinearity for the Strategy of Decomposition.

Instead, the analysis of pathway modularity and emergence needs to be centered on the context-sensitivity of pathways' reaction step rate laws (henceforth: rate laws). These rate laws are local causal laws that are easily broken. I have argued that the key assumption that underpins the Strategy of Decomposition is that pathways are 'causal law modular'. A system is causal law modular if its subsystems $C_1 \ldots C_n$ manifest the same causal laws that are manifested by objects of the same kind as $C_1 \ldots C_n$ that are situated in 'isolation' (recall section 3.5). It is epistemically desirable that this assumption is correct. Knowledge gained of *in vitro* rate laws can then be 'exported' and used to provide dynamic mechanistic explanations of *in vivo*

pathways. But the context-sensitivity of rate laws means that there is a significant risk that pathways will sometimes *not* be causal law modular, and hence the Strategy of Decomposition may fail to discover the *in vivo* rate laws.

In this brief concluding chapter, I will draw together these and the other findings from my preceding chapters. I will also explain how my findings may be applied not just to pathways, but also to other types of self-organising systems found in biology.

Pathway dynamic behaviours are explained by 'dynamic mechanistic explanations' (recall section 1.3). Dynamic mechanistic explanations describe the causal structure (i.e. the mechanism) that produces the explanandum behavior. A dynamic mechanistic explanation consists of two parts: (i) a qualitative account of the mechanism that produces the behaviour (ii) a quantitative account, which is provided with the aid of a simulation model. The qualitative account includes describing the organised parts, operations and context in which the explanandum behaviour is occurring. The quantitative account shows how the explanandum behaviour follows from the dynamic laws of its parts, a law of composition plus initial conditions. I added one requirement to the general account of dynamic mechanistic explanations provided by Bechtel and Abrahamsen: that the equations in the simulation model must be causal equations. I have taken the 'causal discovery' of a pathway dynamic behaviour to refer to the processes by which biochemists discover the parts, operations, organisation and context referenced by the corresponding dynamic mechanistic explanation. This includes discovering a pathway's rate laws.

The complexity of pathways and the lack of *in vivo* data represent considerable barriers to the causal discovery of pathway dynamic behaviours. I identified four key factors that contribute to pathway complexity (recall section 1.4):

(i) The large number of reactants that are shared between pathways. *In vivo* pathways are 'pervasively open' with reactants often interacting with multiple pathways.

(ii) The impact of non-reactive interactions between a pathway and other cellular components. *In vivo* reactions take place in crowded, heterogeneous solutions in which, for example, there may be electrostatic interactions that change reactants' conformations.

(iii) The presence of multiple feedback loops.

(iv) Pathway non-linearity.

I explained that the lack of *in vivo* data is due, in part, to the current lack of technology that can measure chemical concentrations of intact cells. It is also due, in part, to reactants often being involved in multiple reactions, preventing the identification of individual rate constants.

Biochemists have adopted the Strategy of Decomposition as a response to these barriers to causal discovery (recall section 1.5). I characterised the strategy as having three broad stages:

1. An extraction stage; in which the target *in vivo* pathway is separated from its biological context, creating an *in vitro* pathway.
2. A decomposition stage; involving decomposing the *in vitro* pathway into a set of isolated parts that can then be separately analysed.
3. A reconstruction stage; involving using a simulation model to deduce the target behaviour from statements of the properties of its isolated parts, their arrangement, plus the Kinetic Law of Composition.

Critically, the Strategy of Decomposition incorporates the assumption that a target pathway is 'causal law modular' (recall section 1.5). If a pathway is causal law modular, then causal knowledge gained in the decomposition stage can be used to provide dynamic mechanistic explanations of *in vivo* pathway behaviours. I have argued that this is the key discovery heuristic being used in many biochemistry / systems biology analyses (such as those cited in my thesis). There seem strong reasons to think that biochemists use of this heuristic may be unwarranted. After all rate laws are highly context sensitive, and it is far from clear that the *in vitro* chemical solutions used by biochemists can adequately replicate *in vivo* conditions.

Bechtel and Richardson are silent on this key heuristic. Instead, their analyses on the Strategy of Decomposition focus on the consequences of feedback and nonlinearity. Feedback is meant to undermine near decomposability, and both feedback and nonlinearity are meant to contribute to Pathway Emergence. An aim of my Chapter 2 was to provide the conceptual groundwork of how these concepts apply to pathways, in order to later evaluate their claims.

I have explained that a pathway is nonlinear if its dynamics induce an ODE system that does not satisfy the superposition principle. All pathways are nonlinear, unless they have a 'possible but highly improbable' structure. Nonlinearity is a necessary but insufficient condition for various exotic dynamic properties such having multiple steady states or having limit cycles. Pathway nonlinearity has important implications for the explanation of pathway dynamic behaviours. It is because nonlinear ODEs do not usually have analytic solutions, that dynamic mechanistic explanations use simulation modelling to deduce target dynamic behaviours.

Feedback is ubiquitous in biochemistry. I identified that feedback between reactions consists of circular causal chains, such that increasing the concentration of a reactant affects the rate of that reactants own production. Feedback occurs by 'later' reaction steps changing the concentrations of reactants of 'earlier' reaction steps (in the case of allosteric feedback this involves changing the concentrations of effectors). The presence of feedback means that the behaviours of a particular reaction step cannot be explained just in terms of the rate laws of that reaction step, plus the concentrations of reactants resulting from earlier stages in the pathway. Instead account may also need to be taken of later stages in the pathway. Negative feedback is taken to be a necessary condition for stable limit cycles, though a general proof is yet to be provided. Positive feedback appears to be a necessary requirement for multiple steady states, but the proofs for this are flawed, insofar as they fail to exclude 'feedback' within bimolecular reactions.

Bechtel and Richardson brought the importance of feedback and nonlinearity to the attention of the New Mechanists. And they correctly highlighted that these two types of 'nonlinearity' mean that biochemists need to adopt a holistic approach to causal discovery, being aware of how the reactions steps within a pathway can impact on each other's operations. However, their analyses fail to show that feedback and nonlinearity lead to a type of pathway non-modularity or emergence that significantly challenges the Strategy of Decomposition.

Bechtel and Richardson's analysis of modularity is based on Herbert Simon's concept of near decomposability (recall chapter 3). They claim that the assumption of near decomposability has been used in the causal discovery of pathways. They argue that because of feedback, pathways are often only 'minimally decomposable'; but they claim that the assumption of near decomposability has still been heuristically useful

for the causal discovery of pathways. I have explained that, contrary to Bechtel and Richardson, the concept of near decomposability does not apply to pathways, as it is inconsistent with the substantial overlaps that exist between a pathway's reaction steps. Furthermore, there is a significant shortcoming in both Simon's and in Bechtel and Richardson's analyses: neither recognise that there is a plurality of types of modularity. Starting from their analyses of near decomposability, I have identified five distinct types of modularity that could be important either to the analysis of pathways or to biology more generally. One of these types is causal law modularity. Neither feedback nor nonlinearity affects the causal law modularity of pathways.

The theory of Pathway Emergence is based on Boogerd et al.'s interpretation of Broad (recall chapter 4). The theory was further developed by Bechtel and Richardson who link Pathway Emergence both to nonlinearity and to presence of feedback loops. There are two criteria for a pathway dynamic behaviour to be Pathway Emergent. The first is a 'non-deducibility' criterion requiring that the behaviour cannot *in principle* be deduced from a 'Deductive Base' that contains statements of the properties manifested by the pathway's isolated parts, the pathway's organisation, and laws manifested in simpler systems. The second is a 'qualitative difference' criterion requiring that emergent behaviours must be qualitatively different to those manifested by the pathway's isolated components.

As stated in Boogerd et al. (2005), Pathway Emergence is a strong concept of emergence. If a pathway were Pathway Emergent, then this would seem to have significant implications for the Strategy of Decomposition, as deductions of pathway dynamic behaviours would not be possible. As part of my analysis of Pathway Emergence, I identified a necessary condition for a pathway to satisfy the non-deducibility criterion: the pathway must manifest at least one rate law that is not manifested by its isolated reaction steps (i.e. the pathway must *not* be causal law modular). Boogerd et al.'s argument for Pathway Emergence takes the form of a case study, but the case study does not satisfy this necessary condition. Hence Boogerd et al.'s argument for Pathway Emergence fails. Nevertheless, there still is a plausible argument for the existence of Pathway Emergence. This is because the rate laws within a pathway are highly context sensitive; and these laws might sometimes be non-deducible from the pathway's Deductive Base. But this last argument is speculative and I have therefore concluded that the existence of Pathway Emergence has not been established.

I have also suggested that Boogerd et al. may have intended to propose a concept of emergence that is based on a far more restrictive notion of non-deducibility. I have termed this concept as Weak Pathway Emergence. The claim of in principle non-deducibility would then amount to the claim that nonlinear systems have non-aggregative properties. Perhaps Weak Pathway Emergence can be described as in some way 'Broadian', but this would be a highly qualified claim that needed to be explicitly stated. Feedback is not a factor in this 'non-deducibility', and virtually all pathways are likely to have Weakly Non-Deducible dynamics. The concept of Weak Pathway Emergence does highlight the need for the Strategy of Decomposition to use simulation modelling. But the concept provides little insight into the challenges that biochemists face in the causal discovery of pathway behaviours. Perhaps a more relevant concept of weak emergence could be formulated that centered on the *in practice* gap between the causal laws that are manifested in *in vitro* solutions and the causal laws that arise in the more complex environment of living cells

My thesis conclusions can be grouped into two sets. The first are deflationary about the consequences of feedback and nonlinearity. I take it that Strategy of Decomposition has been successfully developed by biochemists to fully incorporate the effects of nonlinearity and feedback. I have rejected Bechtel and Richardson's claims about the challenges posed to pathway modularity and to the deducibility of pathway dynamic behaviours. I now want to briefly note three other possible misconceptions (which are not part of Bechtel and Richardson's analysis). First, it might be thought that pathway feedback involves some higher-level laws that are distinct from the rate laws of reaction steps. But in the case of chemical feedback this is wrong, as is illustrated by my toy examples and by the sample pathway model in appendix 1. A pathway's reaction steps interact with each other by adjusting the concentrations of reactants that are shared by two or more reactions steps. There is no further higher-level feedback law that also needs to be discovered. Second, nonlinearity is not metaphysically interesting. For example, the nonlinearity of bimolecular reactions can be simply explained by collision theory (recall section 2.2.2). Pathway nonlinearity does not imply that a pathway has some novel causal powers 'over and above' those possessed by its parts. Third, pathway nonlinearity does not lead to *in practice* unpredictability due to 'sensitive dependence to initial conditions' (at least in non-pathological cases). This is because biological systems

would not be able to survive in their noisy environments if their pathways were highly sensitive to small perturbations (recall section 4.2).

My second set of conclusions emphasise the need to focus on the context sensitivity of reaction step rate laws. As I have already noted, the key assumption underpinning the Strategy of Decomposition is that pathways are causal law modular. This assumption is made by assuming that the context sensitivity of rate laws can be taken account of by using 'appropriately formulated' *in vitro* reaction steps (recall section 3.6). Given the complexity of pathways and the lack of data, it is epistemically convenient to believe that pathways are causal law modular. But the *in vivo* cytoplasm may provide a very different context compared to an 'appropriately formulated' *in vitro* solution. Epistemic convenience is no warrant of truth and evidence is required as to why the assumption of causal law modularity will be correct in any particular study. Often no such evidence is provided and the risk exists that the analyses lack adequate epistemic foundations. This is an example of a far more general problem that applies not only to biochemistry but across the natural and social sciences (recall section 1.5). Namely, how to justify the use of causal knowledge, in cases where there is a difference between the context in which the explanandum phenomenon occurs and the context in which we can gain causal knowledge about its parts.

I will close by suggesting how my analysis of the Strategy of Decomposition can be extended beyond the domain of pathways. Pathways can be paradigm examples of self-organising systems. In self-organised systems, there is no central controller, instead the components' behaviours are coordinated through multiple nonlinear feedback loops. (recall section 4.2). In general, the dynamic behaviours of self-organising systems also require dynamic mechanistic explanations. Camazine et al. (2003) provide case studies for several systems that they take to be self-organising. These include: pattern formation in slime molds, fish schooling, trial formation in ants, nectar selection by honey bees and feeding aggregations of bark beetles. In each of these, dynamic behaviours of the whole system are explained by determining the causal laws for the interactions between the system's parts, applying a law of composition to combine these laws into a system of linked ODEs and then using a simulation model to derive the explanandum behavior (e.g. Camazine et al., 2003, p. 206). As with pathways, the causal discovery of these behaviours requires determining the causal laws describing the local interactions between the parts. Once these have been discovered, it is taken as unproblematic to use a simulation model to

derive the effects of the parts' nonlinear interactions, including their feedback loops. Nonlinearity and feedback are not, in themselves, significant problems to the causal discovery and explanation of a target behaviour. Problems may arise if a physical decomposition of the system is required to gain knowledge of its parts' causal laws. In such cases, it is epistemically convenient to assume that the target system is causal law modular. But, as in the case of pathways, evidence is then required as to why the assumption of causal law modularity is warranted.

Appendix 1 - Example of a Pathway ODE Model of Glycolysis

Teusink et al. (2000) provide a specification of an ODE model of glycolysis in their paper "Can yeast glycolysis be understood in terms of *in vitro* kinetics of the constituent enzymes? Testing biochemistry". The paper documents how each of the rate laws for the stepwise reactions of glycolysis were experimentally determined from *in vitro* solutions. Statements of the rate laws were then combined using the Kinetic Law of Composition and used to simulate the glycolytic pathway. The model's outputs were compared with aggregate *in vivo* flux data (e.g. the net consumption of glucose). The model was initially very inaccurate and so was modified to include some of the interactions with other metabolic pathways of yeast. This improved the results though there were still some large errors (Teusink et al. p. 5317 – 5319).[44]

The reason for adding a specification of Teusink et al.'s model as an appendix are:

1. To confirm the description of the structure of pathway ODE models (recall section 2.2.2).
2. To illustrate the complex structure of the stepwise rate laws that are often used in biochemistry. These rate laws often reference many types of kinetic parameter. However, true stepwise rate laws can be deduced, at least in principle, from the rate laws of elementary reactions (plus some reactant concentrations), and elementary rate laws have much simpler structures. It is for this reason that my thesis has used elementary rate laws, rather than stepwise rate laws, to explain pathway nonlinearity, feedback, modularity and emergence (recall section 2.2.2).
3. To illustrate that pathway ODE models are fully specified by the rate laws of reaction steps (plus any equations for net flows in/out of the pathway and the Kinetic Law of Composition). There are no other 'higher-level' laws needed to model chemical feedback. The feedback results solely from individual reactions steps changing the concentrations of their own reactants / products, which are also reactants in some earlier reaction steps (recall section 2.3.3).

[44] A later paper my van Eunen (2012) improved on these results by using crowded solutions. In a personal communication one of the co-authors H. Westerhoff characterized the average errors of the latter model as being 'about 30%'.

A.1 The Yeast Glycolytic Pathway

Step		Enzyme
0	Transportation of glucose into the yeast cell	
1	glucose + ATP → glucose-6-phosphate + ADP + H^+	hexokinase
2	glucose-6-phosphate ↔ fructose-6-phosphate	phosphoglucose isomerase
2b	glucose-6-phosphate + ATP → glycogen (branch 1)	
2c	glucose-6-phosphate + ATP → trehalose (branch 2)	
3	fructose-6-phosphate + ATP → fractose-1,6-bisphosphate + ADP + H^+	phosphofructokinase
4	fructose-1,6-bisphosphate ↔ dihydroxyacetone phosphate + glyceraldehyde-3-phosphate	aldolase
5	dihydroxyacetone phosphate ↔ glyceraldehyde-3-phosphate	triose phosphate isomerase
5b	dihydroxyacetone phosphate → glycerol (branch 3)	
	(steps 6 to 10 are carried out twice)	
6	glyceraldehyde-3-phosphate + Pi + NAD^+ ↔ 1,3-bisphosphoglycerate + NADH + H^+	glyceraldehyde 3-phosphate dehydrogenase
7	1,3-bisphosphoglycerate + ADP ↔ 3-phosphoglycerate + ATP	phosphoglycerate kinase
8	3-phosphoglycerate ↔ phosphoglycerate	phosphoglycerate mutase
9	2-phosphoglycerate ↔ phosphoenolpyruvate + H_2O	einolse
10	phosphoenolpyruvate + ADP + H^+ → pyruvate + ATP	pyruvate kinase
11	pyruvate ↔ acetaldehyde + CO_2	pyruvate decarboxylase
12	acetaldehyde + NADH + H^+ ↔ ethanol + NAD^+	alcohol dehydrogenase
12b	acetaldehyde + 3 NAD^+ + 4 ATP → succinate + 3 NADH + 4 ADP (branch 4)	

(Reaction steps 2b, 2c, 5b and 12b are branches to other metabolic pathways within the yeast cell).

A.2 The Concentration ODEs (Teusink et al., 2000, p. 5314)

(1) $\frac{d[Glc_{in}]}{dt} = v_{transport} - v_{HK}$

(2) $\frac{d[G6P]}{dt} = v_{HK} - v_{PGI} - v_{treholse} - v_{glycogen}$

(3) $\frac{d[F6P]}{dt} = v_{PGI} - v_{PFK}$

(4) $\frac{d[F1,6bP_2]}{dt} = v_{PFK} - v_{ALD}$

(5) $\frac{d[Trio-P]}{dt} = 2\,v_{PGI} - v_{GraPDH} - (\,v_{glycerol}\,)$

(6) $\frac{d[BPG]}{dt} = v_{GraPDH} - v_{PGK}$

(7) $\frac{d[3GriP]}{dt} = v_{PGK} - v_{PGM}$

(8) $\frac{d[2GriP]}{dt} = v_{PGM} - v_{ENO}$

(9) $\frac{d[phosphoenolpyruvate]}{dt} = v_{ENO} - v_{PYK}$

(10) $\frac{d[PYR]}{dt} = v_{PYK} - v_{PDC}$

(11) $\frac{d[AcAld]}{dt} = v_{PDC} - v_{ADH}\ (-\,2\,v_{succinate}\,)$

(12) $\frac{d[P]}{dt} = v_{HK} - v_{PFK} - v_{PGK} + v_{PYK} - v_{ATPase}\,(-\,v_{treholse} - v_{glycogen} - v_{succinate}\,)$

(13) $\frac{d[NADH]}{dt} = v_{GraPDH} - v_{ADH}\,(-\,v_{glycogen} - v_{succinate}\,)$

(14) $\frac{d[NAD]}{dt} = \frac{d[NADH]}{dt}$

where: [Trio – P] = [glycerone phosphate] + [GraP] and P = 2[ATP] + [ADP]

Abbreviations: AcAld, acetaldehyde; ADH, alcohol dehydrogenase; ALD, fructose-1,6-bisphosphate aldolase; ENO, phosphopyruvate hydratase; F1,6bP2, fructose-1,6-bisphosphate; F2,6bP2, fructose 2,6-bisphosphate; F6P, fructose 6-phosphate; GraPDH, d-glyceraldehyde-3-phosphate dehydrogenase; G6P, glucose-6-phosphate; 2GriP, 2-phosphoglycerate; 3GriP, 3-phosphoglycerate; HK, hexokinase; PDC, pyruvate decarboxylase; PGI, phosphogluco isomerase; PFK, phosphofructokinase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; PYK, pyruvate kinase; PYR, pyruvate.

A.3 The Rate Laws (Teusink et al., 2000, p. 5326 - 5329)

The rate laws reference numerous parameters such as equilibrium constants, Michaelis-Menten constants, control flux coefficients, mass-action ratios and maximal rates of reactions. I will not provide definitions of each of these; it is sufficient for our purposes to note these are each parameters is ultimately a function of the rate constants of elementary reactions and/or reactant (including enzyme) concentrations.

1. Reaction steps 2, 8, 9. The rate laws for the reactions catalysed by PGI, PGM and ENO have the same form. These are reactions with one substrate, one product and are taken to have reversible Michaelis-Menten kinetics (recall section 2.2.3).

   (R1) $$v = V^{+} \frac{a}{K_a} \left(1 - \frac{T}{K_{eq}}\right) / \left(1 + \frac{a}{K_a} + \frac{p}{K_p}\right)$$

   where a and p are the concentrations of the substrate and product, T is the mass action ratio (this is equal to a / p), K_{eq} is the equilibrium constant , K_i is the Michaelis-Menten constant for chemical i and V^{+} is the maximum rate of the reaction (see Sauro, 2012, p. 328).

2. Reaction steps 1, 6, 7, 10. The rate laws for the reactions catalysed by HK, GraPDH, PGK and PYK all have the same form. This is the form for reversible Michaelis-Menten reactions for two non-competing substrate-product pairs.

   (R2) $$v = V^{+} \frac{ab}{K_a K_b} \left(1 - \frac{T}{K_{eq}}\right) / \left(1 + \frac{a}{K_a} + \frac{p}{K_p}\right)\left(1 + \frac{b}{K_b} + \frac{q}{K_q}\right)$$

   where a and b are the concentrations of the substrates, and p and q are the concentrations of the products.

3. Reaction step 0. The flow of glucose across the membrane and into the cell is modelled by a diffusion equation.

   (R3) $$v_{transport} = V^{+} \frac{[Glc_{out}] - [Glc_{in}]}{K_{Glc}} \Big/ \left(1 + \frac{[Glc_{out}]}{K_{Glc}} + \frac{[Glc_{in}]}{K_{Glc}} + Ki \frac{[Glc_{out}]}{K_{Glc}} \frac{[Glc_{in}]}{K_{Glc}}\right)$$

   GLc_{out} is extracellular glucose, GLc_{in} is intracellular glucose. K_i are equilibrium constants that depend on the relative mobilities of the carriers of glucose.

4. Reaction step 11. The reaction catalyzed by pyruvate decarboxylase (PDC) has cooperative kinetics and is modeled by an irreversible Hill equation, with the

parameter n_H being a measure of cooperativity and $K_{0.5}$ being the half maximal concentration constant (recall section 2.2.3).

(R4) $$\upsilon_{PDC} = V^{+} \left(\frac{[PYR]^{n_H}}{K_{0.5}} \right) / \left(1 + \frac{[PYR]^{n_H}}{K_{0.5}} \right)$$

5. Reaction step 12. The reaction catalysed by alcohol dehydrogenase (ADH) is taken to be 'ordered bi-bi', involving a sequence of two biomolecular reactions (hence 'bi-bi). First NADH binds to ADH forming an enzyme-substrate complex and then the acetaldehyde then binds to that complex.

(R5) $$\upsilon_{ADH} = V^{+} \frac{ab}{K_a K_b} - V^{-} \frac{pq}{K_p K_{iq}} \Big/ \left(1 + \frac{a}{K_a} + \frac{K_a b}{K_{ia} K_b} + \frac{K_q p}{K_p K_{iq}} + \frac{q}{K_{iq}} + \frac{ab}{K_a K_b} \frac{K_q ap}{K_{ia} K_p K_{iq}} \right) + \frac{K_a bq}{K_{ia} K_b K_{iq}} + \frac{pq}{K_p K_{iq}} + \frac{abp}{K_{ia} K_b K_{iq}} + \frac{bpq}{K_{ia} K_b K_{iq}}$$

where a is [ethanol], b is [NAD], p is [acetaldehyde] and q is [NADH], V^{+} is the maximal reaction rate for the forward reaction and v^{-} is the maximal reaction rate for the reverse reaction.

6. Teusink et al. do not explicitly model the reaction processes in which ATP is consumed. Instead a simple linear rate law is assumed.

(R6) $$\upsilon_{ATPase} = k_{ATPase} [ATP]$$

7. Reaction step 4. PFK is an allosteric enzyme (recall 2.3.4). Teusink et al. model three positive effectors: ATP, $F1,6bP_2$, $F2,6bP_2$ and one negative effector AMP. Neither $F1,6bP_2$, nor $F2,6bP_2$ are reactants or products of the glycolytic pathway (except in their capacity of being effectors of PFK) but the concentrations of both are effected by the concentrations of products of the glycolytic pathway. Teusink et al. identify several other effectors of PFK which have not been modelled in order to avoid 'combinatorial explosion (Teusink et al., 2000, p. 5329). The unmodeled effectors are: ammonium, phosphate, H^{+}, fructose 2,6 – biphosphate and ADP. PFK is assumed to have only two conformational states: a relaxed state R and a Tense state T. They simplify their equation by assuming F6P does not bind to PFK in the T state. An adapted form of the Monod, Wymann, Changeux model for allosteric enzymes is used as the rate law. The overall rate law is very complex and is therefore broken down into several components. In effect, the rate law calculates the concentration of PFK that is in the R state from the concentrations of PFK's effectors (and associated kinetic parameters). The rate of reaction step 4 is then determined from the concentrations of PFK in the R state and the concentrations of the reactants F6P and ATP.

(R7) $$\upsilon_{PFK} = V^{+} \frac{g_R \lambda_1 \lambda_2}{R^2 + LT^2}$$

where:

(R 7.1) $$\lambda_1 = [F6P] \ / \ K_{R,\,F6P}$$

(R 7.2) $$\lambda_2 = [ATP] \ / \ K_{R,\,ATP}$$

(R 7.3) $$R = 1 + \lambda_1 + \lambda_2 + g_R \lambda_1 \lambda_2$$

(R 7.4) $$T = 1 + c_{ATP} \lambda_2$$

(R 7.5) $$L = L_0 \cdot \left(\frac{1 + \frac{C_{i,ATP}}{K_{ATP}}}{1 + \frac{[ATP]}{K_{ATP}}} \right)^2 \cdot \left(\frac{1 + \frac{C_{i,AMP}}{K_{AMP}}}{1 + \frac{[AMP]}{K_{AMP}}} \right)^2 \cdot \left(\frac{1 + \frac{C_{i,F2,6bP}}{K_{F2,6bP}} + \frac{C_{i,F1,6bP}}{K_{F1,6bP}}}{1 + \frac{[F2,6bP]}{K_{F2,6bP}} + \frac{[F1,6bP]}{K_{F1,6bP}}} \right)^2$$

c_{ij} are control flux parameters (see Cornish-Bowden, 2014, section 13.4)

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