

Metabolic balance sheets

Athel Cornish-Bowden and María Luz Cárdenas

Application of book-keeping principles to metabolic networks provides a powerful technique for understanding the properties of microorganisms and predicting the results of genetic modification.

Stoichiometric analysis has the same function in biochemistry as book-keeping has in business. Both deal with the inputs and outputs in flow systems, and studying the stoichiometric structure of a metabolic network might seem only slightly more exciting than a visit to an accountant's office. Times change, however, and progress in systems biology will involve paying closer attention to stoichiometry than seemed necessary in the past. The benefits should be that, with minimal knowledge of kinetic parameters, we will be able to predict how systems will respond to changes in conditions, and how they can be genetically engineered to produce desirable characteristics.

The growing knowledge of genomes of different organisms has brought new life to the study of metabolic networks, and a striking example appears on page 190 of this issue¹. Stelling and collaborators discuss there the idea that, by breaking a network down into 'elementary flux modes', based on simple accounting for metabolic inputs and outputs, possible properties of the network can be predicted.

A typical biological network such as the central metabolism of the gut bacterium *Escherichia coli* consists of many processes that operate simultaneously and in parallel. For example, many different metabolic products are being synthesized at different rates at the same time as substrates such as glucose are being consumed to supply power for all the activity. Even when all the individual processes have been identified, it is no trivial matter to predict how the properties of the network as a whole will change if the activity of one enzyme is changed.

It used to be widely assumed, for example, that complete elimination of an enzyme activity would have obvious effects, but this expectation has been overturned by observations of the effects of gene knockouts in various organisms: in *E. coli*, fewer than 300 out of 4,000 genes are 'essential' in the sense that deleting one of them prevents growth on a rich medium². Many of the others can be deleted (one at a time) without producing any evident consequences, even for growth on a medium containing restricted nutrients; in other words, they are 'silent'. As well as discovering the functions of these silent genes, it is crucial to know why they are not essential, and elementary flux modes provide a tool for addressing this question.

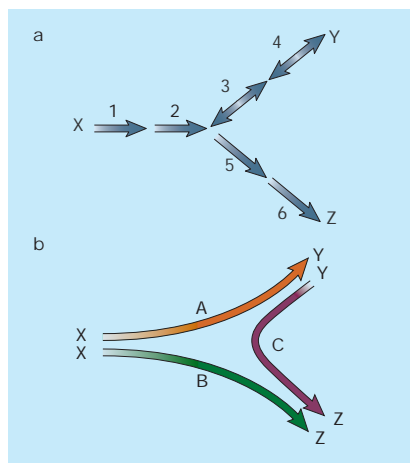


Figure 1 Networks made simple. a, A network of six reactions, of which reactions 3 and 4 are reversible. b, This system can be decomposed into three elementary flux modes, A, B and C. It is evident that transformation of input X into output Y is a function of mode A, and that deleting a reaction that is not part of it (for example number 5) will not prevent this transformation from occurring. This type of analysis has been taken to sophisticated levels by Stelling *et al.*, who in their study of *Escherichia coli* metabolism looked at 43,279 elementary flux modes.

Consider, for example, the simple branched network of six reactions shown in Fig. 1a. There are three subnetworks that account for everything the whole network can do. These are the elementary flux modes, labelled A, B and C in Fig. 1b, of which mode A consists of reactions 1, 2, 3 and 4; mode B of reactions 1, 2, 5 and 6; and mode C of reactions 3, 4, 5 and 6. Analysing the network in this way makes it immediately obvious that transformation of X into Y is a function of flux mode A and that deleting a reaction that is not part of it, for example reaction 5, will not prevent the system from achieving this transformation. Predicting whether a mutant will be able to grow involves identifying elementary flux modes that do not use the missing reaction. In this simple example, the analysis merely confirms what is evident from inspection, but in larger systems this is not the case.

Systems of moderate size have previously been decomposed into elementary flux modes^{3,4}. But there has been no analysis of a system that encompasses most of an organ-

ism's metabolic activity, something that is necessary if unintuitive properties are to be deduced. The approach has now come of age with the analysis by Stelling and colleagues¹ of a network of 110 reactions, linking 89 different metabolites, that encapsulates our knowledge of the central metabolism of *E. coli*. This system is much larger than any analysed previously, and its complexity is illustrated by the huge number — 43,279 — of elementary flux modes, far beyond the range of analysis possible by inspection.

The results of Stelling *et al.* allow us to understand some of the experimental properties of *E. coli* in terms of its stoichiometric structure. For example, glucose is involved in 27,099 elementary modes, more than twice as many as glycerol, in agreement with biochemical experience that glucose is the more important energy source. Moreover, around 7% of the elementary modes for glucose allow energy production and growth in the absence of oxygen. But none of the elementary modes for glycerol, succinate or acetate allows this, in agreement with the observation that *E. coli* can grow anaerobically on glucose but not on these other energy sources.

The agreement between theory and experiment provides a basis for believing that this method can produce valid conclusions even without knowing the answer in advance, and so can be used for organisms that are less well known than *E. coli*. Specifically, we can now predict which mutations, in which combinations, a bacterial or yeast culture can tolerate, and what genetic modifications (addition or suppression of catalytic activities) will permit new properties to be created. Moreover, as flux modes may differ in the amount of product they generate per unit of input, they allow insight into how yields may be improved. Organisms such as *E. coli* that can survive in widely varying conditions owe their metabolic robustness, or resistance to mutation, to a high degree of redundancy — in other words, they have a much larger number of elementary flux modes than are absolutely necessary for growth in any particular conditions. More specialized organisms will certainly have less redundancy in their design and fewer elementary flux modes.

Until now, biochemical progress has been driven mainly by new observations rather than theories — in contrast, say, to modern

physics. However, knowledge of elementary flux modes allows predictions of what should happen after a particular genetic manipulation or change in culture conditions, and these predictions can, of course, be tested. Stoichiometric analysis will thus give a boost to hypothesis-driven experimental research in biology.

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1. Stelling, J., Klamt, S., Bettenbrock, K., Schuster, S. & Gilles, E. D. *Nature* **420**, 190–193 (2002).
2. Cséte, M. E. & Doyle, J. C. *Science* **295**, 1664–1669 (2002).
3. Schuster, S. et al. *Bioinformatics* **18**, 351–361 (2002).
4. Van Dien, S. J. & Lidstrom, M. E. *Biotechnol. Bioeng.* **78**, 296–312 (2002).

Earth science

Is it all in the crust?

Simon Lamb

New observations suggest that earthquakes on land are only ‘skin-deep’, confined to the Earth’s outermost layer of crust. This has prompted a rethink of what gives the tectonic plates their strength.

Writing in *GSA Today*, James Jackson¹ is seeking to overturn more than two decades of geological thinking about the movement of the Earth’s surface. He proposes that where these motions occur on land, we need look no deeper than the crust for their driving forces. If he is right, then it is going to be difficult to predict the long-term effect of these forces — and the occurrence of earthquakes.

In the late 1960s, with the advent of the theory of plate tectonics, geophysicists finally seemed to have made sense of earthquakes. The surface of the Earth is covered in a mosaic of rigid plates, and earthquakes, in this theory, occur only in narrow zones where the plates rub against each other. This was good news, because it provided a basic understanding of why there were earthquakes in the first place. But in the 1980s, there were second thoughts.

The plate theory worked well in the oceans, but across the continental land masses, earthquakes seemed to occur all over the place. Gradually a new view emerged that wasn’t plate tectonics at all: in fact, large tracts of the continents seemed to behave more like a fluid².

The idea was that the movements along fault lines in the continental crust were driven by a deeper, fluid-like flow in the underlying mantle³ (Fig. 1a). This opened up new possibilities for earthquake prediction, because the well-known physics of fluid flow, in combination with the flow properties of the mantle, could be used to understand those movements. But if mantle rocks do have such a dominant influence, they must be generally stronger or stiffer than those in the crust.

Jackson, who was himself involved in developing this fluid theory of continents, has now changed his mind. He believes that

we must go back to trying to understand the motions of small plate-like blocks in the crust — bad news for those studying the active fault lines, because, as yet, there is no simple way of predicting how these blocks might behave.

To justify such a paradigm shift, good evidence is needed. It has come in the form of new observations about the nature of the Earth’s outer shell, comprising the crust and uppermost mantle and called the lithosphere. Jackson’s argument rests on the idea that a portion of the continental lithosphere is elastic and can be bent or flexed, supporting substantial stresses, rather like a springy beam. This beam must hold a substantial part of the strength or stiffness of the lithosphere. A critical question is where in the lithosphere this elastic beam lies.

It now seems that all earthquakes in the continents occur only in the crust^{4,5}, not in the mantle. This important discovery has emerged from an increased number of seismic studies, combined with improved computer techniques to model the data. As earthquakes can only occur where the Earth shows springy or elastic behaviour, Jackson argues that the brittle crust is itself the springy beam. And he has more evidence up his sleeve.

McKenzie and Fairhead’s study⁶ of the strength of gravity in northern India shows that this region has bent under the weight of the Himalayas as though it is an elastic beam about 40 km thick — a thickness that nearly matches that of the crust (40–45 km). If you bend an elastic beam, the outside of the curve stretches, but the inside is compressed. The earthquakes in northern India follow exactly this behaviour, with extensional motion at shallow depths and compression deeper down (Fig. 1b). In the simplest case, the point of crossover between these two different motions — called the neutral surface — marks the middle of the beam. In India, the neutral surface turns out to be almost exactly at the middle of the crust.

The conclusion, says Jackson¹, is obvious: the strength of the Indian lithosphere must lie in the crust. And the relation between beam thickness, crustal thickness and earthquakes in other continents studied by McKenzie and Fairhead⁶ seems to point the same way. Jackson is not surprised because he thinks that the underlying mantle contains water, but that the lower crust above it is dry. In this case, given the likely temperatures in the lithosphere, laboratory experiments on rocks suggest that the mantle is weaker than the crust.

But there are some chinks in the armour of Jackson’s argument. For example, the measured depth of the neutral surface in India could be misleading because the lithosphere may be supporting not just the stresses induced by bending, but also the sideways push of the Himalayas and Tibet. These extra stresses will raise the level of the neutral surface in the effective elastic beam. If the neutral surface really is at the middle of

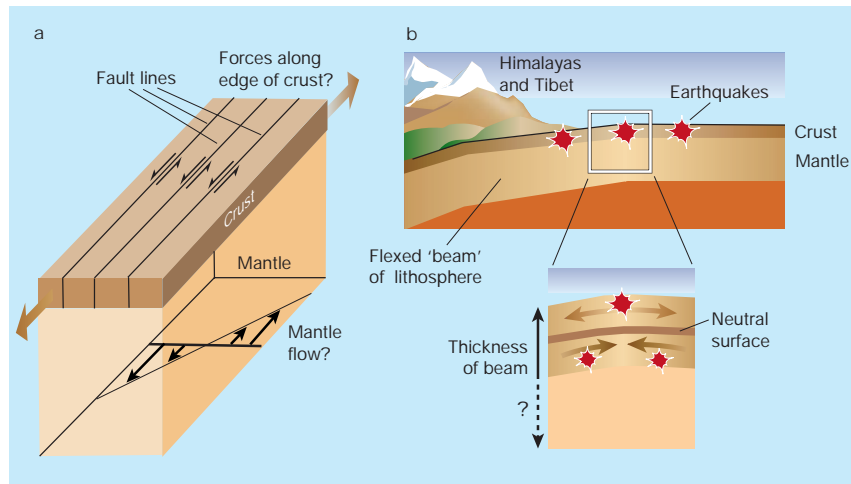


Figure 1 Continental conundrum. a. Are the forces that move fault lines on land in the Earth’s crust, as one block rubs against another, or do they lie much deeper in the underlying mantle? b. Jackson¹ argues that the beam-like behaviour of northern India, flexed under the weight of the Himalayas and Tibet, may provide the answer. The top of the bent beam is stretched, whereas deeper down it is compressed: earthquakes are confined to the crust and show exactly this pattern of deformation, so at least part of the beam must lie there too.