



Review

Systems Biology: The elements and principles of Life

Hans V. Westerhoff^{a,b,*}, Catherine Winder^a, Hanan Messiha^a, Evangelos Simeonidis^a, Malgorzata Adamczyk^a, Malkhey Verma^a, Frank J. Bruggeman^b, Warwick Dunn^a

^a Manchester Centre for Integrative Systems Biology, The University of Manchester, United Kingdom

^b Netherlands Institute for Systems Biology, Molecular Cell Biology, VU University Amsterdam, The Netherlands

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ABSTRACT

Systems Biology has a mission that puts it at odds with traditional paradigms of physics and molecular biology, such as the simplicity requested by Occam's razor and minimum energy/maximal efficiency. By referring to biochemical experiments on control and regulation, and on flux balancing in yeast, we show that these paradigms are inapt. Systems Biology does not quite converge with biology either: Although it certainly requires accurate 'stamp collecting', it discovers quantitative laws. Systems Biology is a science of its own, discovering own fundamental principles, some of which we identify here.

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1. Introduction

Even though Systems Biology has had a long and diverse prehistory [1], it has not been quite accepted yet as the new way in which biology should be done, or as the way in which physics should turn to the life sciences. One reason may be that Systems Biology has diverged substantially both from its origins in biochemistry and molecular biology and from its origins in physics and mathematical biology. Systems Biology is heretic in its ambitions and methodologies [2]. Using actual results obtained by Systems Biology, we shall test whether two well-known ambitions of physics that are not shared by molecular biology, are met by Systems Biology: being general and being precise. This discussion will also address Ernest Rutherford's proposition that science is either physics or stamp collecting [3] and examine whether Systems Biology should move biology into the former category.

Two paradigms, i.e. Occam's razor [4] and the prevalence of minimum energy solutions, e.g. [5], are pertinent to much of physics. They are sometimes implemented uncritically in molecular

biology and Systems Biology. We shall examine whether these paradigms make sense in a Systems Biology that aims to address the molecular biology basis of biological function. We shall conclude that Systems Biology is much more physics than biology ever was, and much more biology than physics has been. It is more than an interface between physics and biology.

2. Results

2.1. Is biological chemistry simply physics?

It is generally accepted that living organisms consist of nothing but the same matter that occurs in the dead parts of Nature. In addition the interactions between the various types of matter in biology are again the same as the interactions that occur in 'dead' matter. Although accumulating some elements more than others from their environment, living organisms do not harbor chemical elements that are absent from non-living matter. The components of living organisms are not subject to additional forces (such as the 'vital force') either. At least there is no acceptable evidence for such forces, and for this right reason, scientific explanations do not take them into account. Only one of the four fundamental interactions of physics appears to be directly important for living organisms

* Corresponding author. Address: Manchester Centre for Integrative Systems Biology, The University of Manchester, United Kingdom.

E-mail address: Hans.Westerhoff@manchester.ac.uk (H.V. Westerhoff).

and this is the Coulombic force; the gravitational force, the weak force nor the strong force have much to bear on biology. But of course, there is no doubt they are active also inside living matter.

Herewith, living systems are just a subset of all physical and chemical systems in the universe as we know it. And since fundamental physics is only interested in the most general properties and principles of matter, it is per definition not interested in biology, or it would seem that it should not be. This point of view is held by some physicists and molecular biologists. The former thereby see Life as a non interesting object of study, as just a special case. The latter recognize that they cannot interest physicists in addressing the problems that they, the molecular biologists, find most interesting.

But is biology simply physics, or even simple physics? Below we shall contend that it may be neither and that therefore it should be of great interest to a broad range of scientists, including physicists and biologists who are ready to engage in a new type of science.

2.2. Simple physics: general principles, general laws, Occam's razor and minimum energy

To many, the aim of physics is to understand the universe and everything that is in it, in terms of a limited number of completely general principles. Some of these principles are gauged in highly precise, quantitative laws, using exact equations that can be dealt with analytically. In order to progress towards this aim, physics decomposes the reality that it studies into much smaller parts that consist of very few, well-defined components. It then studies the properties of the components. In its most reductionist form, physics addresses only the components, and suggests that this make the whole understood, since the latter is nothing but the sum of the behaviors of the components.

This paper does not mean to address physics, but the study of living systems. Highly successful disciplines studying these include biochemistry, molecular biology and cell biology. These disciplines have become successful in what they have done, by assimilating some of the strategies of physics, such as the ones described above.

For these strategies to be effective, the number of relevant interactions between the components of the system should be limited, such that indeed the decomposition can be in terms of very few components. The interactions should also be close to linear, close enough for the essential behavior of the whole to be described by a first order Taylor series expansion. Deviations, if at all significant are then treated as minor perturbation terms.

Whether or not an effective linear decomposition of a whole system has been found, is in part the art of physics: it may depend on finding a way to redefine its fundamental, or elementary properties (e.g. wave forms rather than particles), as well as new ways of observing the system (and the corresponding definition of operators describing the observations). Focusing on force and acceleration rather than on the more obvious force and velocity, brought Newton success in physics [6].

This reductionist strategy has been highly successful for physics, biochemistry and molecular biology. It has led to many principles that are mostly accepted to be of general validity. Early examples were indeed Newton's 3 laws [6] and the ideal gas law [7]. Acceleration was found to be proportional to force, and was independent of the material out which the object consisted, except for the proportionality constant, mass, which was then an immutable attribute to matter (until the days of Einstein [8]). At a constant temperature, pressure was inversely related to Volume, (almost) independent of the specific gas that was examined [7].

It has been highly important for science in general that with these and other examples, the existence of general and simple principles was demonstrated. Indeed, this was the important victory that science held over approaches that preceded it. And it is

against this backdrop that the principle should be seen that is often attributed to William of Occam, i.e. '*Entia non sunt multiplicanda praeter necessitatem*', or '*hypotheses should not be multiplied beyond necessity*', or rather what he actually wrote [9], i.e. '*pluralitas non est ponenda sine necessitate*' (complexity should not be assumed unnecessarily) [4]. Occam made this statement in the 14th century against a very serious backdrop of domination of scientific undertakings by a Church that offered an explanation for all observations in terms of ill-defined divine forces. Occam may have tried to safeguard rationality by declassifying those other explanations because they involved hypotheses that were not germane to the problem at hand. Newton's (and others') laws demonstrated that Occam's paradigm was of high value for the development of science (and with that for the development of rationalism). Without reference to Occam, Newton insisted on the prevalence of simple explanations: '*We are to admit no more causes of natural things than such as are both true and sufficient to explain their appearances.*' [6]. For understandable, but historical, reasons, 'Occam's razor' (this is what the above principle has been called) has become a paradigm for physics, and for molecular and cell biology.

Physics holds a second paradigm, i.e. that of minimum energy. When studying a ball falling from a roof, one finds that the ball strives towards minimum potential energy, and the steady solution is therefore the one with the lowest free energy. Both the final state and the path towards the final state are expected to be of lowest energy. When solving the Schrödinger equation one also looks for wave functions that have the lowest possible energies as eigenvalues.

For this paper we shall further focus on these four aspects, i.e. the search for fundamental principles, the discovery of quantitative laws, the simplicity paradigm attributed to Occam, and the paradigm of minimum energy.

2.3. Principles of Systems Biology

Biology is sometimes referred to as a 'can of worms'. This refers to the enormous diversity of Biology, with its millions of different species. The statement also suggests absence of general principles from biology, every organism behaving differently. The origin of such ideas may reside in pre-Lamarckian biology, where all species were indeed thought to be different, in origin and hence in nature. But with Lamarck and Darwin, evidence was collected and hypotheses came into existence that contended the opposite, i.e. that species had a common origin and were hence rather similar [10,11]. Of course when the chimpanzee and the human were proposed to have a common origin, this led to disbelief amongst the general public: in human societies chimpanzee and human are worlds apart.

We here refer to this example because actually both contentions are right, i.e. humans and chimpanzees are very different and very similar at the same time; it just depends on the perspective. From the perspective of social, intellectual and economic function, the two species are highly different, but from the point of view of their molecular components, they are virtually identical [12]. The difference may simply be caused by the lack of ability of chimpanzees to engage in social networking and learning through written language: essentially identical components but different abilities for them to network.

When one looks at the biochemical organization of Life on Earth, then the similarities are even stronger: humans are not only very similar to chimpanzees but also to earthworms and to *Escherichia coli*. Twenty amino acids, eight nucleotides, phospholipids, with differences between *Archaea* and the two other kingdoms of Life, but there is high similarity. As already recognized by Kluyver and Donker [13], but substantiated more extensively now when annotating genome sequences [14], there is great unity in biochemistry. Accordingly, biology is definitely *not* a can of worms,

where every new case is unrelated to every previous case. By studying one set of organisms one can learn an awful lot about other organisms.

Also in terms of the organization of their macromolecules there is a substantial similarity between organisms. These can be phrased in terms of fundamental principles that govern interactions between the ‘elementary particles’ of biology, i.e. the genes, the mRNAs and the proteins, and are hence in the domain of Systems Biology. One fundamental principle is that all chemical reactions and transmembrane transport are carried out by proteins, called enzymes and transporter proteins. A second fundamental principle is that every protein is encoded by a string of DNA, the information being transmitted by mRNA. The exceptions to these general principles, are increasingly rare [15] and do not much compromise the stature of these principles as being fundamental. Biology does have general principles.

2.4. Laws of Systems Biology

But then, laws of physics are usually more quantitative than the principles we just quoted. Are there any such quantitative laws in biology? Biology has grown to be non-quantitative. In its early days, biochemistry seemed to alter this, with the quantitative formulations of enzyme kinetics. But since then, biochemistry and molecular biology (with the exception of molecular dynamics and X-ray crystallographic structure determinations) have become more and more qualitative, and cell biology has become almost exclusively qualitative. Systems Biology has broken with this restraint of biology from being quantitative and has revealed laws of biology. Examples are summation laws concerning the control and regulation of intracellular fluxes and concentrations [16,17]:

$$\sum_{i=1}^n C_i^J = 1$$

and

$$\sum_{i=1}^n C_i^X = 1$$

C_i^J and C_i^X represent the control of any steady-state flux J and any metabolite X , respectively, by molecular process (often enzyme) i [18]. The so-called connectivity laws are even closer to the fundamental aims of Systems Biology by relating systemic properties to interactive properties of the components. We here reproduce the connectivity theorem referring the elasticities (molecular sensitivities) of all enzymes with respect to metabolite X to their systemic inverses, i.e. the control exerted by those enzymes on the metabolite concentration of X [19]:

$$\sum_{i=1}^n C_i^X \cdot \epsilon_X^i = -1$$

A new such law, which we present here without its proof (which is analogous to the proofs given in [20]), is the summation law with reference to the control of noise by the catalytic processes:

$$\sum_{i=1}^n C_i^{\sigma_X^2} = 0$$

This refers to the variance in any free steady-state variable X in the system. Such laws are not limited to steady state, e.g. [20]:

$$\sum_{i=1}^n C_i^J - C_i^J = 1$$

Time dependence of the flux is quantified by C_i^J and the flux control coefficients are time dependent.

The first two of these laws might be compared with the first law of thermodynamics, which expresses a similar conservation property, except that the sum of a set of energy forms and not of a set of controls is conserved. The third law may be compared with the Gibbs–Duhem law, which similarly addresses the conservation of multiplications of partial energy related forces and changes in exogenous properties. Where most of these Systems Biology laws preceded the onset of mainstream Systems Biology, the two last ones quoted did not. The fourth one has been applied to the control of signal transduction by kinases and phosphatases [21], see also [22]. The fifth awaits application.

Laws on regulation have been discovered in the Systems Biology era, such as the one that distinguishes between metabolic (or direct) and gene-expression (or hierarchical) regulation of an intracellular process [23], the two ρ 's referring to the two types of regulation, respectively:

$$\rho_m + \rho_g = 1$$

All these laws have in common with the second law of thermodynamics and the Schrödinger equation that they cannot be falsified experimentally. They have been derived mathematically on the basis of an assumed or defined structure of the system they are addressing, and of precise definitions [18]. This assumed structure derives from the above two, qualitative, fundamental principles. The utility of these laws can be tested however and it has been, as in explaining the dominance of recessiveness [24], in showing that control can be distributed over many processes in the network [25], in revealing that highly important cellular processes can have very little control on growth rate [26], and in designing experiments proving that regulation was distributed over transcription, translation and metabolic regulation [27].

2.5. No Occam's razor for Systems Biology

2.5.1. Genomes are more complex than simple

With the present understanding of Life, and of the limitations that biochemical processes have, it is possible to estimate the minimum number of processes required to sustain Life. Living systems function essentially at a non-equilibrium steady state. To maintain this steady state they need to import Gibbs free energy, use some of that to drive thermodynamically uphill processes, and dissipate the rest to speed up the process rates [28]. The free energy for the uphill processes is taken from the terminal phosphate bond of ATP. ATP re-synthesis therefore has to be driven by coupling to free energy liberating chemical processes. An important example of such a process is the breakdown of glucose to alcohol and carbon dioxide by yeast. The solution that evolution has generated is a series of steps in a metabolic pathway that are each catalyzed by a protein. This leads to a requirement of at least 10 proteins. The information needed to specify these proteins must be stored in an information molecule, in practice requiring a nucleic acid of at least 3 kbp. The information has to be translated into protein, which requires a nucleic-acid informed protein-synthesizing enzyme system (in practice the ribosome). The nucleotides and amino acids out of which these macromolecules consist, need to be made from what is available outside the cell. The corresponding biosynthetic pathways require at least 88 additional enzymes, assuming that each component requires two enzymes for its synthesis. It is important that all these components of Life are held together. The evolutionary solution for this has been a phospholipid-based membrane, adding a requirement for phospholipid synthesis and transport proteins, requiring another 20 proteins at least. With such an argumentation one readily comes to a minimum requirement for Life of more than 120 proteins, hence more than 120 genes. Genome sequencing has shown that the smallest known genome has some

450 genes [29], subsequent knock-out experimentation suggesting that the minimum number of genes required for Life is slightly in excess of 375 [30]. All these genes are apparently necessary to maintain each other.

Physics is accustomed to reduce its systems of study to two or three components, and if that does not work, then perhaps 6. Three hundred and seventy-five is certainly not in the realm of simplicity. The implication of this is substantial. Let us suppose that we study the chemical function of extraction of Gibbs free energy from glucose by a living organism. Chemically this process may be carried out by a series of 14 chemical reactions. Using Occam's razor we might therefore wish to explain biological formation of ATP in terms of the action of 14 proteins [31]. The above implies that this is impossible, as the 14-enzyme pathway cannot be disentangled from the functioning of 361 other gene products.

It gets even worse. For quite some time already, biochemical research has made good use of biological model systems. These model systems had been selected for their simplicity. Two examples are *E. coli* and *Saccharomyces cerevisiae* (baker's yeast). Genome sequencing has revealed that both these organisms are informed by at least 10 times more genes than the minimal organism, i.e. by 4000 [32] and 6000 [33] genes, respectively. Even if one were to extend the concept of 'simple explanations' to explanations in terms of 300 processes, this would not help: for the 'simple' model organisms the explanations might well involve thousands of genes. And for ourselves, we should have to reckon with explanations involving more than 30 000 genes [34]. Because one usually studies an organism under only a subset of the conditions that may have been important during its evolution, the number of gene products required for understanding biological functioning could be smaller than the total number of gene in the genome. However, the number will still be large: For researching living organisms, Occam's razor is not an appropriate paradigm.

2.5.2. Control of biological function is not as simple as possible

One of the areas of biochemistry where the preference for simplicity has had a strong and detrimental influence has been the analysis of what limits or controls metabolic fluxes. Various simple paradigms have found their ways into textbooks without appreciable validation [35]. According to these, any pathway should have a rate-limiting step for its metabolic flux. This step should be (i) the first step in the pathway, (ii) the (most) irreversible step in the pathway, and (iii) the most intensely regulated step in the pathway. If in biological reality, in all metabolic pathways, the first steps were both irreversible and most strongly regulated, then all these three simple paradigms might be operational simultaneously. However, already in the glycolysis of yeast, the first step (glucose transport) is not as far from equilibrium as many other steps, whereas the hexokinase step may well be the most irreversible, and phosphofructokinase the most regulated by allosteric modifiers [31]. The same is true for mitochondrial oxidative phosphorylation, where the first step may be either NADH:UQ oxidoreductase or the ADP import step and where the most irreversible step is catalyzed by the last step, i.e. cytochrome c oxidase. Clearly therefore, the combined triple paradigm would not work. Which of the three simple paradigms would then work? Because there was no effective operational definition of the extent to which a step is rate-limiting, experiments and their discussions remained inconclusive for a long time.

Making the Systems Biology definition of rate-limitation, as introduced by Kacser and Burns [16] and Heinrich and Rapoport [17], operational in terms of inhibitor titration, Groen et al. [25] showed that none of the three simple paradigms was realistic for mitochondrial respiration. The answer to the question what limits oxidative phosphorylation in mitochondria was not even in line with the simplicity of a single rate-limiting step: there was no such

single rate-limiting step; the control was distributed over a number of molecular processes, in a way that was condition and function dependent. Meanwhile it has become clear that this is not an exception [36–40]. Also glycolysis in potato and *Trypanosoma brucei* have distributed flux control [39,41]. In *T. brucei* the strongest controller is the first step, which is not irreversible or strongly regulated. In some cases virtually all control does reside in an initial irreversible step, such as in the control of glycogen synthesis in muscle [42].

2.5.3. Regulation of biological function is not as simple as possible either

Defining regulation in terms of how a living organism adapts its processes to altered external conditions, one may again be inspired to apply Occam's razor and expect that this will be at a single step. Another simple concept was that regulation of the processes in living cells should be confined to the level of transcription. These two simple concepts apply neither to *T. brucei* [23,43], nor to *S. cerevisiae* [27,44], nor to *L. lactis* [45]. Regulation is also distributed.

2.5.4. Occam's razor and the second law of thermodynamics

There is a general reason why Occam's razor should be expected to fail as a paradigm for distinguishing between right and wrong theories: complexity is favored over simplicity by the second law of thermodynamics. The second law of thermodynamics states that if a system has two states of equal energy, say state A and state B, with state B having many substates and A being a single state, then the system will on average move from state A to state B, hence from the simple (because non degenerate and thereby uniform) to the complex state [46,28]. Occam's razor would cut out state B as well as any theory, including the second law of thermodynamics, that would predict state B to be the actual state. Of course, the state degeneracy argument might be claimed to fall within the caveat '*sine Necessitate*', but if we are to admit this escape route in such an *ad hoc* manner, the whole paradigm of Occam loses force.

In a similar vein, just as much as there is a subliminal sentiment that solutions should be as simple as possible, there is a sentiment that if nature has a great many solutions to any particular problem, then it will try them all. Thereby multiplicity and diversity, which is one form of complexity and almost the opposite of Occam's simplicity, is the rule [47]. One of the principles of microbial ecology, proposed by Beyerinck and Baas-Becking (see [48]), is that whenever a habitat fulfills the conditions necessary for Life, one will find a microorganism in it.

2.5.5. The status of Occam's razor

There may indeed be cases that the simplest solution is realistic, such as in the sigma orbital between two hydrogen atoms, which has the simplest possible structure. This may be due to the fact that the simplest symmetry around an object (H_2^+) that is itself simple, has the lowest energy (but see below), and therefore not just due to a universal law that prefers simplicity. After all, Occam's razor has not been *proven* to be valid, neither theoretically nor experimentally. Where the evidence has been analyzed in the literature, the more complex hypothesis turned out to be correct, or the simpler hypothesis was correct but for other reasons than simplicity [47].

2.6. No minimum energy or maximum efficiency criterion for Systems Biology

2.6.1. The minimum energy principle is inappropriate for living systems

For closed systems, thermodynamic equilibrium is the ultimate steady state. At equilibrium there are no processes, i.e. this corre-

sponds to death. Consequently, all living organisms are partly-open, ‘metabolic’ [28] systems, importing substances of high Gibbs energy and excreting substances of lower Gibbs energy, whilst being closed for many other substances such as enzymes and ATP [28]. The substances out of which living organisms consist have been optimized for good performance of their catalytic (enzymes), information storage (DNA) and impermeability function (lipids), rather than for being low in energy. Biomass has approximately the same Gibbs energy content per carbon atom as glucose [28].

2.6.2. Maximum thermodynamic efficiency is not a good paradigm

One might transpose the paradigm of physics that systems tend to be in the lowest energy state, to a paradigm of maximum thermodynamic efficiency. Thermodynamic efficiency has been defined as the total output power in terms of useful work including the synthesis of new biomass, divided by the total input power [28,49]. The notion that living organisms have close to maximum thermodynamic efficiencies, has attracted biologists and systems biologists alike. Most recently the notion has affected genome-wide flux balance analysis (FBA), e.g. [50,51]. Genome-wide FBA has become possible after the annotation of large numbers of genome sequences in terms of biochemical function. The corresponding genes have subsequently been projected into genome-wide metabolic maps. After some upgrading, these can also function as road maps in the sense that they can indicate (i) which products can be made from any given set of substrates and (ii) at which rates and efficiencies and (iii) which routes are then followed. The fluxes through the networks specified by the maps need to fulfill the requirement that for no metabolite inside the network there be a net production or removal rate. In practice, this steady state requirement still admits a great many flux patterns, differing in the net yield of ATP.

Flux balance analysis goes one step further and assumes that the organism’s evolutionary history has selected the pathways with the highest biomass yield. This has had a similar persuasive strength as the minimum energy principle had in physics, and indeed FBA is being used by many research groups as a standard methodology to calculate the fluxes through pathways in living organisms, with some success.

There are three caveats with this FBA approach. First, even if maximum efficiency were the criterion to dictate where the fluxes should go, it is not clear whether indeed the organism would have the ability to attain that optimum. When calculated from published growth yields, the thermodynamic efficiency of microbial growth was very low, much lower than what was predicted on the basis of optimal efficiency. Growth yields and efficiencies were more consistent with a combined optimization for efficiency and growth rate [49,52]. Second, it is unclear whether the circumstances under which the actual flux patterns are important for the researcher are identical to the circumstances under which the organism has evolved. The optimum flux pattern would depend on such external conditions, including the types of substrates available for its energy metabolism. And thirdly, it is unclear whether the criterion of maximum efficiency is important at all for the organisms under consideration [53]. Many organisms live in habitats where there is ample Gibbs free energy available in their carbon substrates, but where other conditions are challenging, such as high temperatures, high amounts of toxic substances such as ethanol, or competition with other organisms.

2.6.3. Maximum growth yield or ATP is not a good paradigm

Thermodynamic efficiencies can be limited by the available mechanisms. If glycolysis can only make two ATP’s per glucose and not three, then that limits the realistically possible thermodynamic efficiency. FBA chooses between pathways that are mecha-

nistically realistic but differ in ATP yield. The issue therefore may not be thermodynamic efficiency but whether microorganisms have growth yields that are close to what is theoretically possible. By calculating what was needed in terms of ATP to make biomass, Stouthamer predicted growth yields for a variety of microorganisms. Comparing this to growth yields that he had corrected for growth rate independent maintenance, he found that the experimental growth yields were far below the theoretical ones [54]. Perhaps not surprisingly therefore, cases have already been reported where predictions by FBA concerning the flux pattern were not realistic [53,55].

2.6.4. Yeast

In the Manchester Centre for Integrative Systems Biology, we develop, test, adapt and implement Systems Biology methodologies in the context of carbon and energy metabolism of *S. cerevisiae*. We therefore decided to test the paradigms of maximum energetic efficiency or maximum ATP yield for this organism growing under conditions of excess growth substrates. We first performed a flux balance analysis for the genome-wide metabolic network of *S. cerevisiae* growing on glucose only, requiring steady state and maximum growth yield. This led to the predictions that all carbon flux should run to carbon dioxide only, that there should be much respiration, and that there should be no flux to ethanol (Table 1).

Experimentally the case that is relevant here is often examined by growing the organism in batch cultures. However, the organism then adapts in the beginning to the conditions of affluence and re-adapts when it approaches stationary phase. Such a cultivation method may bring the organism in a metabolic quasi steady state only. Gene expression may never relax to a steady state, as this could take more than four cell cycles.

Long lasting steady states are most often effected experimentally by growing the organism in a chemostat. However, chemostats are unstable when all substrates are present in excess. We therefore engaged a permittostat, i.e. a variant of the turbidostat, in which the rate at which new medium is added to the culture vessel is controlled by fixing the dielectric permittivity, which monitors the concentration of living cells [56]. We grew *S. cerevisiae* until they reached maximum permittivity (because of reaching stationary phase), switched on the medium pump, set the permittostat to 75% of the maximum permittivity, and sampled eight dilution times later. Carbon was analyzed in the exometabolome and the off-gas. As shown in Table 2, growth was not respiratory but largely fermentative, indicating that under these high-glucose steady-state conditions, yeast cells do not behave according to the FBA predictions (Table 1, column 3).

We then added an extra constraint to FBA: we limited the fluxes through the mitochondria. This led to a better correspondence with experimental results (Table 1, column 5). This ad hoc fitting suggests that limitations of mitochondrial activity might play a role. At any rate, the sole paradigm of maximum ATP yield of catabolism does not apply to *S. cerevisiae*, and neither does standard FBA.

2.7. If not just physics or just piology, then what?

2.7.1. Physics and stamp collecting?

Both medicine and biology have started much by categorizing their field. For medicine this was in anatomy and for biology in botany. The classification of species in terms of the similarities in, and differences between, their appearances was triggered by the sheer multitude of species on the one hand and the apparent discontinuity between species on the other hand. Similarity seemed to be due to common functional requirements [10], or to common origin [11]. The act of ‘stamp collecting’ led to hypotheses, the most famous and productive perhaps being the theory of evolution through mutation and natural selection [11]. In an important sense

Table 1

Predictions of flux balance analysis for the *S. cerevisiae* genome-wide metabolic network. The third column uses the standard criteria of steady state and maximum ATP yield, the fifth does the same but with restrictions on mitochondrial activity, set so as to come closer to the experimental observations. The sixth column gives results expected if there were only fermentation to ethanol.

Flux type	Reaction equation	Flux (relative to glucose import)			
		ATP maximization	Expected for exclusive respiration	Mitochondrial constraint	Expected for fermentation
Ethanol import	$M_{\text{etoh_e}} \rightleftharpoons M_{\text{etoh_c}}$	0.0	0	-1.8	-2
ATP maintenance	$M_{\text{atp_c}} + M_{\text{h2o_c}} \rightleftharpoons M_{\text{adp_c}} + M_{\text{h_c}} + M_{\text{pi_c}}$	17.5	18	1.5	1
Glucose import	$M_{\text{glc_D_e}} \rightleftharpoons M_{\text{glc_D_c}}$	1	1	1	1
Hexokinase	$M_{\text{atp_c}} + M_{\text{glc_D_c}} \rightleftharpoons M_{\text{adp_c}} + M_{\text{g6p_c}} + M_{\text{h_c}}$	1.0	1	1.0	1
bc1 Complex	$2 M_{\text{ficytc_m}} + 2 M_{\text{h_m}} + M_{\text{q6h2_m}} \rightleftharpoons 2 M_{\text{h_c}} + 2 M_{\text{focytc_m}} + M_{\text{q6_m}}$	12.0	12	0.3	0
Oxygen diffusion	$M_{\text{o2_e}} \rightleftharpoons M_{\text{o2_c}}$	6.0	6	0.2	0

Table 2

Fluxes into the exometabolome in a yeast permissive experiment in well-defined medium with 4% glucose as the only carbon source. Set point was 75% of the maximum attained during the preceding batch growth phase. Carbon balance was 105%. Flux inaccuracies are on the order of 10%.

Flux	Flux (mmoles C/h/g dryweight)	Flux percentage of (glucose) input
J_{Biomass}	3.2	6
J_{CO_2} (offgas)	9.8	20
J_{Ethanol} (exometabolome plus off-gas)	36.1	74
J_{Acetate} (exometabolome)	0.2	0
J_{Glycerol} (exometabolome)	1.0	2
$J_{\text{Acetaldehyde}}$ (exometabolome)	0.1	0
$J_{\text{Trehalose}}$ (exometabolome)	0.7	1

therefore, biology has a long history of data-driven hypothesis generation, or empiricism. The considerable extent to which the original classification of living species has more recently been confirmed by the much more direct techniques of molecular biology is amazing. It is safe to conclude therefore that ‘stamp collecting’ is not one of the lesser aspects of Science. Consequently, a statement such as ‘Science is either physics or stamp collecting’ should not be meant to devalue the stamp collecting sciences. Arguably (because both laws of thermodynamics are empirical in origin), Rutherford’s assertion that stamp collection be outside the realm of physics, might be something to uphold, but then with the explicit understanding that there are high quality sciences that do not copy the paradigms of physics. Chemistry may be one example [3], and biology another one.

However, biology has not kept to its initial focus on classification. Especially with the onsets of biochemistry and biophysics, it has made inroads into hypothesis-driven science, where phenomena were explained in terms of underlying principles of physics and chemistry only. Early examples were the explanation of saturation of enzyme catalyzed reactions, through the development of enzyme kinetics by Henri, Michaelis and Menten, and the explanation of the induction of gene expression by lactose, by Jacob and Monod. And with the development of molecular biology enabling the cloning and expression of genes, many more such examples followed, even though mainstream research may have moved away from the precise testing of quantitatively formulated hypotheses. Bottom-up Systems Biology is championing this hypothesis-driven part of biology, whereas top-down Systems Biology is engaging more in the empirical aspects. Is Systems Biology then a simple sum of physics and biology?

2.7.2. A new type of science, next to physics and biology, with new paradigms

The aim of Systems Biology is to combine the two aspects in non-linear, synergistic ways [1,2]. A spiral, inducing hypotheses

from top-down Systems Biology, and testing them in a bottom-up fashion, followed by broader validation of more precise forms of the hypotheses in the top down arena, etc., should be an immensely powerful strategy.

With this, we are proposing a new set of paradigms, replacing the ones favoring minimum potential energy, maximal thermodynamic efficiency and maximal simplicity. The minimum potential energy paradigm should be replaced by some paradigm that recognizes that living organisms are ‘metabolic systems’ [28], i.e. strategically open systems. They should therefore not have minimal free energy because they require storage and shuttle compounds that contain substantial free energy. To the extent that maximum thermodynamic efficiency does not conflict with performance in other functions that are or have been important in evolution, it may be a secondary criterion. With respect to Occam’s razor, we propose a new paradigm, i.e. that an explanation in terms of fewer than 300 gene products is less likely to be true and complete than an explanation making a provision for the possible influence of more than 300.

We at the Manchester Centre for Integrative Systems Biology devise and test strategies for Systems Biologies, as others do. We assimilate the strategies that have been shown to work in the existing scientific disciplines, if they are suitable for Systems Biology. The above reflects that our strategy is not going to be just stamp collecting, nor reduction to a very small number of components, nor assuming minimum energy or maximum simplicity for Systems Biology.

This may all be right, but is it practical as well? Occam’s razor may not be quite appropriate, but in the history of science it has led to important breakthroughs, and starting hypothesis-driven analyses with more than 300 components is impracticable both in terms of experiments and in terms of modeling them dynamically. Likewise, the maximum yield assumed by flux balance analysis, may be wrong, but the procedure has produced useful results [50]. And, stamp collecting may not be enough, but certainly we want to base our Systems Biology on reality, and reality is a multitude of processes all with their own parameter values.

One of our Systems Biology aims is the creation of a genome-wide, experiment based mathematical model of yeast metabolism, inclusive of its control by gene expression and signal transduction. A strategy to reach this aim would be to overexpress all *S. cerevisiae* genes, purify the corresponding proteins, determine their kinetic and affinity properties and rate equations, and then put all those properties, together with measured protein concentrations, into a mathematical model. This stamp collecting based, bottom-up strategy is not the one we shall quite follow, although we shall follow it partly.

Because a consensus map of the genome-wide metabolic network has become available for yeast [14], we start with the most important on that map, neglecting what is less important. This neglecting is paradoxical, as whatever we select, will be connected to the remaining network with virtually all other gene products of

the organism (see above). And the selection of what is most important could be subjective.

To determine what is most important for the organism itself, as well as for the use mankind makes of it, we consider yeast leavening dough and yeast making wine. We simplify to an idealized growth medium. Then we anticipate that under these conditions, *S. cerevisiae* only makes use of a small part of its network. To examine which parts of the network it might use theoretically, we implemented standard flux balance analysis for this condition, which indeed led to a greatly reduced number of fluxes in the network (see the same phenomenon in *E. coli*: [57]). However, the flux balance analysis prediction (Table 1) was wrong, as of course yeast is known to ferment glucose to alcohol under those conditions, which we confirmed experimentally (Table 2).

We now restrain mitochondrial activity so as to get close to predicting the right ethanol flux (Table 1, column 5). From this we may calculate the internal fluxes and this should guide us as to which enzymes we wish to characterize to come up with the first version of our kinetic model.

Although this strategy may look at what is most important, it may well miss processes that are almost equally important, not because they carry much of the flux, but because they regulate that flux. Here we use two approximate, additional strategies. The first is based on the phenomenon that if a pathway branches into two further pathways carrying different amounts of flux, then enzymes in the minor branch will tend not to have much control on the fluxes through the major pathway [58]. This rationalizes a strategy that first goes for the pathways carrying most flux. This strategy is risky for living systems, as evolution may have favored pathways carrying very little flux but bringing about a regulatory metabolite (such as cAMP) that then has a strong effect on enzyme activities in the main pathway.

The better strategy is the one that determines the regulatory strengths that run through the network [59], particularly the ones that impinge on the main flux one begins with. Only other pathways that are parts of loops with large regulatory strengths should then be included. In practice the determination of these regulatory strengths is difficult, although it has been accomplished for signaling networks [60]. For all the enzymes in the pathway we now determine whether they have significant elasticity coefficients [18] with respects to metabolites of other pathways. Second, for the pathways that we study we engage in dynamic hierarchical regulation analysis [61], to see if they are regulated significantly by factors outside the pathway.

And then there is the strategy of modularization, where the hope is that intracellular networks are composed of a number of subnetworks that are heavily networked within themselves but have very few connections between them. To the extent that intracellular networks are indeed scale free [62], this strategy seems unlikely to be realistic, but on the other hand the concept of pathways and elementary modes [63] suggests that if one would look at dynamic pathways with the fluxes in them, then this simplification through modularization may work.

3. Discussion

Physics, chemistry and biology are highly dynamic disciplines that cannot be gauged into any uniform or precise definition. Indeed, when we discussed the new science Systems Biology, it will have become clear that there are also new physics, and new chemistry. For arguments' sake we have here adhered to more classical and restrictive definitions of physics and biology. In this classical sense, physics is the science that is after the fundamental principles that govern the universe. Examples are (i) the elementary particles of physics, electrons, protons and neutrons at first, but much

smaller elementary particles such as the Higg's boson, now, and (ii) the four fundamental interactions that physics tries to reduce to four derivatives of a single universal interaction. Again oversimplifying, we have described the scientific world around Systems Biology as consisting of two hemispheres, i.e. physics (plus chemistry and mathematics and engineering) and biology (plus medicine). However, chemistry is not just physics. And neither is medicine equal to biology. All these disciplines are important and challenging in their own right. And again, we quite agree with Rutherford that Science is either stamp collecting or physics rather. However, we are explicit that stamp collecting (top-down science) and physics (bottom-up science) are equally challenging and important, with their synthesis being even more so.

Although we have much referred to classical physics as a static model for the sake of comparison with the developing Systems Biology, we have not really dealt much with physics itself. Notwithstanding the success of physics' paradigms of simplicity and minimum energy, one should perhaps wonder what the scientific basis is of generality, simplicity and minimum energy within physics itself. A brief account already sheds considerable doubt on their validity within physics. First, the acceleration experienced by a snowflake is usually not proportional to its mass, but close to zero, independent of its mass. At steady state it moves at a constant rate proportional to its mass, the standard acceleration of gravity, and inversely proportional to the surface area of the snowflake:

$$v = m \cdot g / \rho$$

Before that steady state is attained the snowflake follows the equation:

$$m \cdot a = m \cdot g - \rho \cdot v$$

which certifies that Newton's equation of motion is not lost or invalid, but that reality is more complex as it does not operate in vacuo. As the name suggests, also the ideal gas law is only valid for idealized gases and low pressures, where the gas molecules are far enough apart not to attract or repel each other. For real gases at atmospheric pressure, virial coefficients correct for first, second and higher order aberrations. Therefore, the fact that the principles of Systems Biology that we reviewed above may not be entirely generally applicable, should not matter; they may well be as general as the principles of physics.

The discussions in this paper of paradigms might seem academic. They are not however: the implicit disagreement about them is a great burden on the younger developers of this new discipline. Their methodologies are not accepted by their peers or professors from physics or biology, neither are their manuscripts finding the complex but real solutions to simple problems, rather than simple apparent solutions to truly complex issues. A clear methodology for Systems Biology is needed [64].

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