

Systems biology, connectivity and the future of medicine

J. van der Greef

Abstract: The concept of systems-based strategies in medicine is emerging, with systems pathology guiding an understanding of the multidimensional aspects of disease system fingerprints and systems pharmacology providing insight into dynamic system responses upon (multiple) drug perturbations. Knowledge of the changes of system characteristics during disease progression creates a framework for the design of novel combinatorial treatment strategies. Such a systems-based, combinatorial-therapies approach readdresses the value of the synergistic actions of components of treatments based on natural products and highlights new methodology to study multidimensional intervention via reversed-pharmacology.

1 Introduction

Since the beginning of the last century, the nature of life has increasingly been studied from a systems perspective across different scientific disciplines ranging from quantum physics to cosmology. Numerous excellent overviews and visions have been published related to systems-based science and the necessary integration of sciences with a potentially high impact on research and society. The visionary and authoritative works of Capra [1], Laszlo [2–4] and Sheldrake [5, 6] and the numerous references cited within their books of outstanding systems research are referred to collectively as they form the inspirational basis for this introduction to the impact of a systems approach within life sciences, especially focussed on drug discovery and drug development.

In a recent issue of *Nature Biotechnology* [7], different views were collected, covering a broad range, from vision to opportunities for commercialisation of systems biology in drug discovery, emphasising the need for practical tools to move from vision and future potential towards impacting today's problems and challenges in the biotech and pharmaceutical industries.

The challenges in drug discovery are huge, as highlighted by the fact that target-centred drug discovery, practised by pharmaceutical companies for the past 30 years and recently amplified by the availability of genomic data, has become unproductive to the point where the economic future of the industry is in question. The steady decline of new drug approvals in the USA since 1996 is in sharp contrast with the almost doubling of expenditure on pharmaceutical R&D during the same period.

How can a systems-based approach alter our view of drug discovery and development or, more generally, of human healthcare? What would be the impact of practising a

systems strategy in medicine? Two focus points can be identified: new improved insights in biology and correspondingly novel interventions strategies matching derived from these insights.

First, technology-wise, the evolution of novel -omics tools, biostatistics and bioinformatics over the last decade has enabled (partial) systems fingerprints to be generated efficiently. Quantitative data of different biochemical components in an *in vivo* system, such as transcripts, proteins and metabolites, can be converged to create a correlation network, accomplishing an understanding at a higher level. A correlation network clearly demonstrates the interconnectivity and interdependence of a biological system [8–10]; see Fig. 1 for a typical example of a set of correlated biomarkers at the onset of disease, obtained via comparison of 'normal' versus disease followed by correlation analysis of all the individual biomarkers per object, in this case transgenic mice.

These early studies clearly show that, in this transgenic mouse model, at the onset of atherosclerosis many changes occur, and system changes can only be described by biomarker system fingerprints. The change from single biomarker strategies towards biomarker patterns is a notable change in studying etiology and disease progression. It also underlines the limitation of using a single therapeutic intervention point when multiple pathways are involved. Notably underestimated in systems biology studies so far seems to be the inclusion of (trace) metal homeostasis within system descriptions related to health and disease.

System fingerprinting at the cellular, organ or body fluid level also underpins the concept that, at different levels of complexity, new properties emerge. Systems clearly cannot be understood by studying the fundamental constituents in isolation, because the properties of the parts are not intrinsic, but can be understood within the context of the whole [1]. The so-called system fingerprints at the body fluid level represent the biochemical body language, also reflecting the dynamics of the physiology, providing detailed information on how communication and control mechanisms are functioning in an *in vivo* organism. For multifactorial diseases, studying parts of a system, such as particular cell types, is informative, but is not informative for the organisational level of the larger system [11]. In multifactorial diseases, the system self-organisation is key

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The author is with TNO Systems Biology, Utrechtseweg 48, 3700 AJ Zeist, The Netherlands, Center for Medical Systems Biology, LACDR, Leiden University, P.O. Box 9502, Leiden, The Netherlands and BG Medicine Inc., 610N Lincoln Street, Waltham, MA 02451, USA

E-mail: vandergreef@pharma.tno.nl

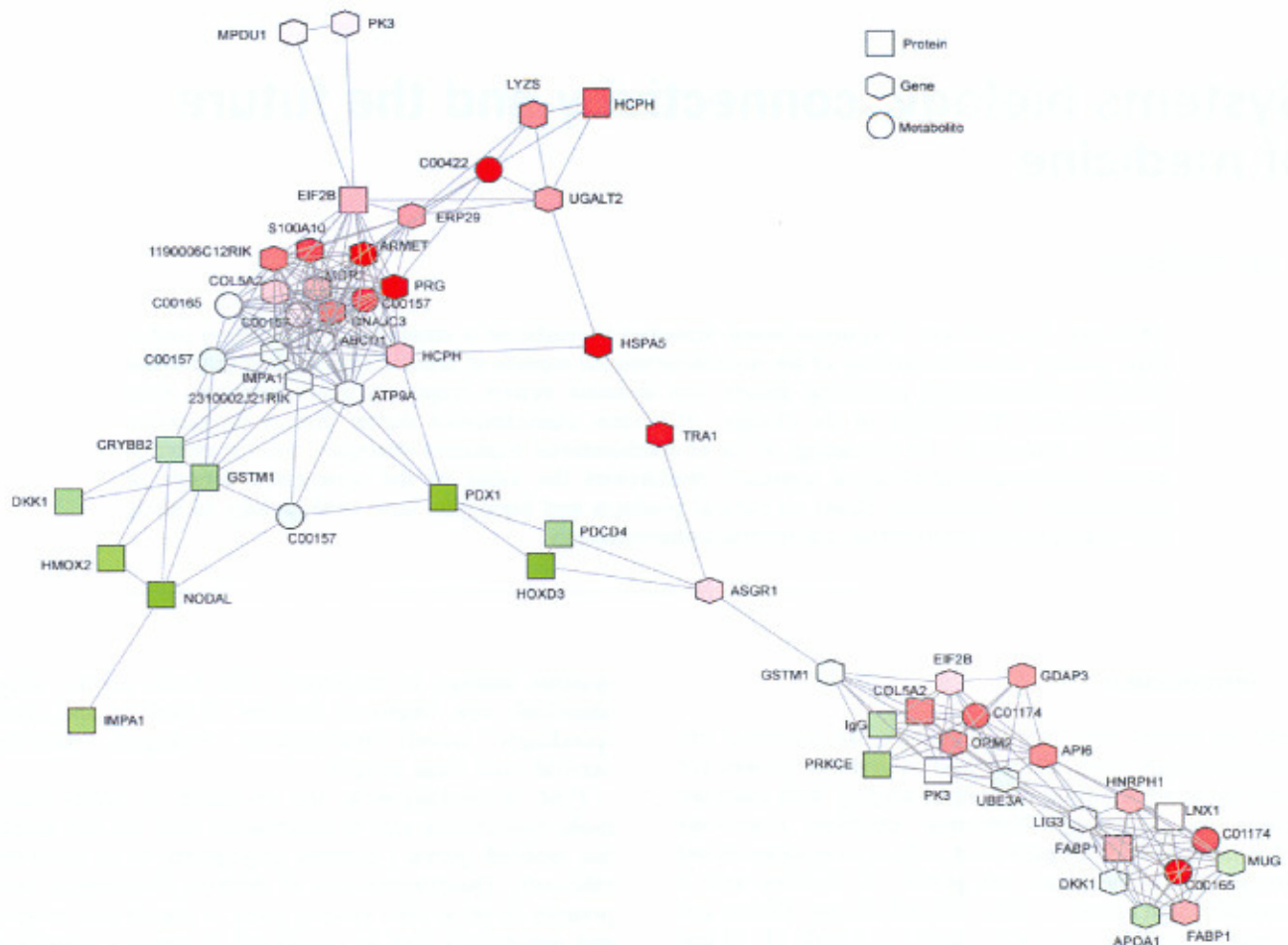


Fig. 1 Typical correlation network of disease showing the connectivity and interdependence

to understanding the onset of a disease or the loss of homeostasis [12]. In addition, the above brings into perspective the importance of moving from symptom-related research in drug discovery – in a late stage of the disease – towards the onset of the phenomena, bridging the gap between nutritional and pharmaceutical research [13]. In other words, understanding the homeostasis of the human body and the homeostatic regulatory capacity upon perturbations of the biology is mandatory for a step towards the detection of early markers of disease. It is established that, in the earliest state of disease progression, perturbation of the system followed by studying the system dynamics is very informative and of high diagnostic value. A typical example is the oral glucose tolerance test (OGTT), but the level of evaluation is typically limited to glucose as biomarker, which could be strongly improved by a dynamic system response using system fingerprints instead.

When focussing on body fluid system fingerprints, it needs to be realised how powerful and complex they are. Human physiology is strongly influenced by lifestyle, but especially by body–mind interactions such as stress or thinking patterns. Dynamics of the patterns or change thereof are mandatory to starting to understand systems, as was pointed out in the concept of dynamical disease [14]. Indeed, for various diseases, it is known that the moment of drug administration is critical for optimal function. Studying chronobiology at the systems level can shed light on many rhythm-related diseases. In this sense, the awareness that changes in one part of the systems show up with considerable time delays in other parts is essential for understanding the biofeedback mechanisms involving multiple compartments as elegantly described and analysed

for metabolic syndrome [15]. Linking a top–down approach starting from system fingerprinting at the body fluid level with bottom–up oriented pathway and network levels in specific cell types is an enormous challenge, but our preliminary work indicates that using cross-compartment correlation networks can be used to facilitate this process. Moreover, in patient studies based on body fluid profiling in premenstrual syndrome [16], it has been pointed out that time warping for differences in menstrual cycle rhythms is essential in studying disease patterns.

Following the above path of thinking, the definition of system science as the science of organised complexity evolves, and it becomes understandable that in contemporary systems science, typically, a number of different things and interactions are studied and their behaviour is noted as a whole under different influences [1]. The biological system, as visualised [17] as the life complexity pyramid, illustrates nature's holarchy with relatively 'simple' systems at the bottom and a few complex ones at the top. In a much wider context, given the connectivity between systems, it has been argued, building on the non-locality as observed in quantum physics at the micro-atomic scale, that each system is likely to be nested within a hierarchy of non-locally connected coherent systems [1–4]. Revealing and studying the element of coherence in biology is an important and challenging concept to be investigated in this century, referred to in literature as quantum biology. This raises the important question of whether we can comprehensively measure the organisational and communication details when limited to measuring biochemical molecules only. Understanding the connectivity throughout the universe reminds us further that studying the human

body is still a reductionistic approach and will have limitations in the ability to understand its complexity [4]. Beautiful examples and inspiring insights are obtained from nature in this respect; for instance, the notion that groups of animals such as insects, birds and fish behave like newly formed organisms. A nice illustration is given in Fig. 2, showing a photograph of such, a flock of birds. The group behaviour of these flocks when a raptor attacks [18] is spectacular. An amazing demonstration of group behaviour can also be seen at sunset, when the members of the flock suddenly take their positions in an astonishing flow.

It has been pointed out correctly [5, 6] that 'boids'-models, which simulate emergent behaviour based on neighbour-neighbour interactions, cannot explain this group behaviour, especially because no recognisable leader can be identified. In addition, an advanced model [19], which explains how a small informed fraction of a large group can be effective with great accuracy in decision-making and behaviour of animal groups on the move, provides a possible explanation for information flow, but does not account for the enormous speed of the communication process that is observed in certain cases. Videotaping [5, 6] has revealed that, in certain flocks, the response of the group is faster than the reaction speed of the individuals. Such observations highlight the coverage issue in medical systems biology of biocommunication and control mechanisms. Possibly, we are missing extremely relevant signalling.

However, despite the complexity and limited ability of the most advanced technology platforms today to produce system fingerprints and to record dynamics, carefully chosen strategies can make it possible to zoom in on the aspects of interest and design the correct experiments [13]. Several advanced strategies focus on the cellular level, both for intervention studies [20–22] and for modelling, but a cellular-level focus alone is too limited to drive a paradigm shift in drug discovery. Observing the connectivity in biology reveals how regulation in normal circumstances takes place via multiple interactions of multiple (sometimes low-affinity) bioactive components or components with multiple functions (pleiotropic) arising, for instance, from the neuroendocrine system or via nutrition involving multiple compartments. Metabolic syndrome is a good example of multiple interactions, involving the CNS, adipose and muscle tissues, pancreas, liver, HPA and HPT axis, and so on.

The discovery of the second brain [23], being our enteric nervous system (ENS), and the related complex pattern of systems communication among others with the CNS illustrates this even more. In addition, it has been pointed out

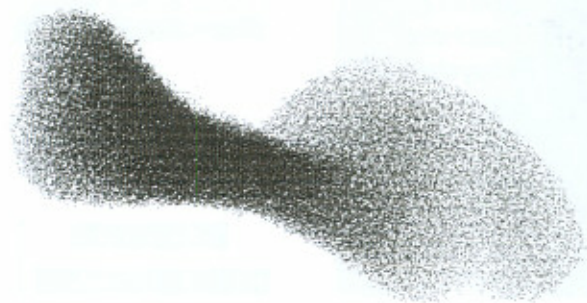


Fig. 2 A flock of starlings, demonstrating the connectivity and organism behaviour (photo with kind permission of Manuel Presti)

how the complexity of human systems is increased by symbiotic microorganisms and parasites [24] in our gut system.

In modern drug research '1 drug–1 target' has been a successful strategy, especially for the late stage of a disease. The concept of having a system fingerprint available with the new technology platforms opens up the opportunity not only to monitor the systems pharmacology via the effect of a (pleiotropic) single component, but also allows studying effects of complex mixtures using the systems pathology fingerprint as a basis for combinatorial intervention development [25]. Furthermore, the whole discovery and development process is improved as the approach enables efficacy and safety monitoring in all stages, but is especially efficient in translational studies in comparing systems fingerprints cross species.

2 Combinatorial interventions

Combination therapy has undergone several stages in the past decennia, from undesired via acceptable from the compliance perspective, to a new phase of pharmaceutical invention. The commercial drive is now well recognised because combining a blockbuster drug going off-patent into a new combo-product extends the patent position and the highly desirable business position. On the other hand, the better understanding of biology from the connectivity perspective and the better insight into how homeostasis is regulated via multitarget and multicompartiment interactions at the system level opens up a totally new perspective for future medicine. Knowing that ~50% of the quoted costs of ~1 billion USD for taking a new drug to the market is attributable to failure costs, the view on failure and desired effects can be re-examined from a systems perspective. In biology, the homeostasis is apparently orchestrated via multiple bioactive compounds interacting in multiple mutually interdependent pathways or compartments, such as inflammation, and the bioactives do not necessarily need to be high affinity for a certain receptor as the total pattern of activity can be multiplied by synergetic effects. In other words, all the drugs that have failed on the basis of efficacy might still be very valuable in combination. The same holds true for those compounds that have failed because at the required dosing, safety issues were discovered, because in combination they could be dosed at levels below the toxicity threshold.

In reality, the combination concept is very old, and has been the basis for herbal medicine for several thousand years, but scientific support for combinations has been very limited due to several issues, in particular, complexity. It is, however, clear that from the isolation of single active components synergetic effects could never have been discovered. Purification and isolation often reduce the efficacy of such mixtures, which has been overcome by either higher dosing, with the risk of side effects, or via chemical modification to obtain high-affinity ligands. Combinatorial, synergetic and opposing effects can be expected in herbal mixtures. A beautiful example of how strong synergetic effects can be has been shown in a study on the growth inhibition effects on *Staphylococcus aureus* of berberine [26]. Berberine, in combination with 5'-methoxyhydrocarpin, a multidrug pump inhibitor and a component that in itself has no significant effect at all on growth inhibition, experiences a very strong potentiation of its effect. In a recent investigation of ginseng [27], it was convincingly demonstrated how opposing principles in the extracts function and how this can lead to new insights into the mechanisms of action and control in combinatorial intervention.

Of course, the tight control of complex mixtures such as herbal medicine is a challenge in itself, but, in principle, this can be handled from a systems biology perspective [28]. A randomised controlled double-blind non-inferiority clinical trial for a hypericum extract versus paroxetine for the indication of depression is a good illustration of the performance of such complex mixtures [29]. The complexity of multitargeted, multifaceted or multidimensional pharmacology rapidly increases when research on intervention moves from a single target and a single component to multiple targets and components (Fig. 3).

Research, directed towards the modernisation of herbal medicine, based on systems biology, demonstrates [30] that not only can changes in systems be monitored using body fluid fingerprinting, but that optimisation of complex compositions is possible both at the efficacy and safety level. Moreover, the approach of reversed-pharmacology [13] can be extended from a single component to a multi-component mixture [28]. The experimental design of varying herbal mixtures or using batches with variable composition and biological effects allows:

- the discovery of groups of bioactive components in relation to a biological endpoint;
- the discovery of groups of components in relation to a single biomarker or biomarker fingerprint; and
- quality control based on knowledge of seasonal variation and knowledge of bioactive component profiles, such as its correlation with *in vivo* system biomarkers.

Today, successful combinations of synthesised new chemical entities (NCEs) such as glucovance for type 2 diabetes, caduet for cardiovascular, advicor for hypercholesterolaemia, advair for asthma and many combinations in anti-HIV, chemotherapy, and so on, are on the market or in development. The combination is based on clinical experience, but in drug discovery based on chemical mixtures, the challenge is now to design a preclinical strategy to achieve this goal and impressive results have been published for the cell level [20]. The step from the cellular to the systems level is the challenge for future medicine and such a strategy has been outlined based on a systems approach [25].

Ultimately, one needs to accomplish this at the systems level *in vivo* using a strategy and a design that are practically realisable. The systems pathology and systems pharmacology approach in combination offer this opportunity. The approach of random mixing of large compound



Fig. 3 A systems view is a driver towards multiple targets, multiple components systems wide. The complexity is rapidly increasing but is not necessarily a bottleneck and emphasises the need for other ways of carrying out scientific experiments

collections and using partial read-out systems is not feasible for a system strategy.

Drug-induced dynamic system response profiling can, in principle, offer this and the strategy of reversed-pharmacology can be used to reveal unknown mechanisms of action as experimentally have been demonstrated [30] and discussed in this context as a viable approach [13].

Based on the disease system fingerprint and individual response profiles of individual drugs for the same target, drugs designed for other targets, or compounds with unknown pleiotropic effects in an *in vivo* animal model, combination opportunities can be identified. Knowledge of multiple disease system fingerprints and evaluation of the common denominator part also identifies multiple-disease options and an initial drug combination can be suggested, which in a follow-up experiment is monitored at the systems level for new synergetic effects by systems fingerprinting and a biological response for that particular disease when available.

3 Future perspectives

The introduction highlighted the essential concept of connectivity and the recognition of connectivity in the world around us. In Fig. 4 this is illustrated based on detailed discussion by Capra, Laszlo and Sheldrake. The recognition that phenomena at the quantum level such as non-locality can be found at the biology level opens up the possibility to perform novel experiments regarding mind-body interactions (e.g. stress and thought patterns), observer effects (e.g. doctor-patient), synchronicity, rhythms of life, coherence, and so on. The need for additional measurement technologies is high, as they can bridge the different perspectives and can also bridge the different views on diagnosis of patients. The latter is of course key before starting evaluation of different interventions. Many proposals are being made, but from observations such as biophoton emission *in vivo* [31] as an important biocommunication mechanism within systems, we are aware that we are still far away from comprehensively mapping the signalling events in human systems. Quantum biology could become an important subject for future research in medicine and healthcare. Imaging as a non-invasive tool is becoming more and more attractive, especially in combination with biochemical profiling oriented systems approaches. Several issues also become apparent based on the above regarding the development of personalised medicine. Both

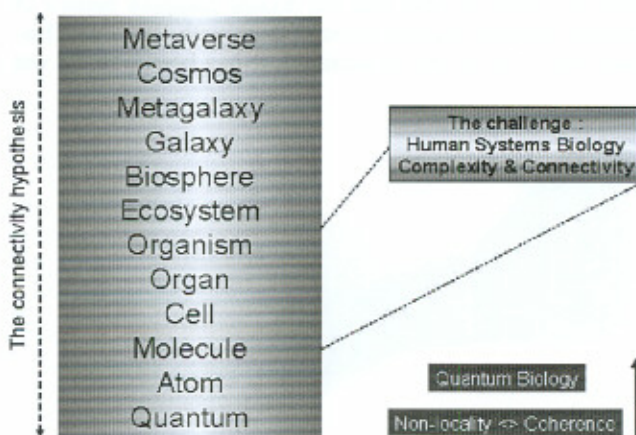


Fig. 4 A systems view on the world; recognition that the connectivity within the human being as well towards the world is key for development of novel approaches and encourages future work on quantum biology

system fingerprinting to stratify patient populations as well as fine-tuning of intervention methods are important concepts for a personalised healthcare approach. If integrative medicine using combinations of drugs or different approaches can be further fine-tuned to a person, but with accepted core concepts, then clinical trials need to be designed differently in the future and not focussed on epidemiology, but as treatment effects as a package. Furthermore, it stimulates the further investigation of placebo effects from different perspectives; for instance, the observation by imaging [32] that some individuals demonstrate similar effects without treatment demonstrates that the mind is powerful and that enhancing of the healing capacity of the mind would be a great development in the improvement in health of a patient. In the immediate future, studying the dynamics of biological systems is key. It is known that abrupt changes in behaviour can occur in far-from-equilibrium conditions within dynamic systems – bifurcation points – and can have long-term implications, prompting development of new strategies related to the study of disease states based on dynamic biomarker patterns. For instance, sudden changes in health condition can occur as is observed in depression, when a combination of a number of factors apparently occurs and patients experience depression as suddenly falling into a deep and endless hole. In episodic disease attacks, such as in migraine, the combination of multiple elements seems to create a trigger, which initiates the development of an attack, but the system's capacity to regulate back to homeostasis is still sufficient. A new insight or change in consciousness can seemingly instantaneously improve the health condition. Opportunities for systems research in this century are enormous and systems research has great potential for a beneficial impact on the quality of life.

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