

## Minireview

# Network medicine

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**Abstract** To more effectively target complex diseases like cancer, diabetes and schizophrenia, we may need to rethink our strategies for drug development and the selection of molecular targets for pharmacological treatments. Here, we discuss the potential use of protein signaling networks as the targets for new therapeutic intervention. We argue that by targeting the architecture of aberrant signaling networks associated with cancer and other diseases new therapeutic strategies can be implemented. Transforming medicine into a network driven endeavour will require quantitative measurements of cell signaling processes; we will describe how this may be performed and combined with new algorithms to predict the trajectories taken by a cellular system either in time or through disease states. We term this approach, *network medicine*.

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### 1. The need for a novel drug development strategy

Drug discovery and development have resulted in many successful therapies over the last century. Current drug development efforts almost uniformly focus on a specific step in a well-described disease pathway and aim to identify highly specific inhibitors for this particular step. However, these strategies have generally been less effective for identifying therapeutically useful approaches for treating complex diseases. This is supported by recent discouraging trends in the areas of complex regulatory diseases including cancer and diabetes. Firstly, the number of submissions for FDA approval declined in the 2000s, and this was coupled with a decline in all phases of drug development. The average price of bringing a drug to market is now approximately 860M USD [1], and there is a general trend towards a rise in expenditure for drug development [2]. Secondly, only 25% of new drugs over the last decade were considered *innovative*, in the sense of a new drug indicated for a previously unmet medical need [3], although this number is likely to increase due to an increasing number of first-in class products [5]. Thirdly, although there was a net rise in small-

molecule, recombinant protein and monoclonal antibody therapeutics approved in the US from 1980 to 2001 this trend has not continued from 1996 to 2002 [4], which indicates a more recent slowdown in the success of drug discovery efforts.

There are other important trends, for example, whereas most current drugs were discovered before their molecular targets were known, a recent trend towards more rational drug design has been observed [8]. Increasingly more drugs are developed in highly specialised biotech companies [6]. Furthermore, it has been suggested that the increase in drug development price is not due to an increase in clinical trial time, as the review process time has actually declined [7]. It has been argued that the current approval and funding systems favour non-risky drug development towards well-studied targets [1]. This trend was also observed in a recent network analysis that found that an overabundance of ‘follow-on’ drugs, that is to say, drugs against already targeted proteins are over-represented in current drug discovery efforts [8].

Recently, more attention has been directed towards molecular drug targets in cellular signalling networks, such as protein kinases and GPCRs. Therefore tools are needed to ‘dig deeper’ in protein networks associated with diseases and it will become increasingly important to address issues such as sensitivity and quantification to focus more research on these frequently low-abundance proteins.

Thus it seems that there are fewer drugs being developed and that creating such compounds is increasingly difficult and expensive. Many papers have been written to discuss the possible economical and political reasons for this, some of which are mentioned above; however few papers [17,19,24,25] have addressed the limitations in the current strategy for selecting drug targets of complex diseases such as cancer, diabetes and mental illness.

Here, we will discuss the possibility that the hunt for a single highly specific compound that targets a single molecular cellular target in many cases will likely fail for complex diseases. Instead we propose that the molecular networks associated with disease and in particular their dynamics as a target for intervention. Thus, we suggest that protein signaling networks are powerful drug targets. Here, we will discuss how quantitative and directional protein interaction networks can be monitored with current proteomics methods, and in combination with recent advances in computational algorithms facilitate the construction of network models to describe transitions between disease states. Having gained insight into these aberrant networks the next step in developing a *network*

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*medicine* is to target the network itself. We suggest two different strategies for this, one involves a synthetic biology approach that aims at rewiring (by adding new interactions) of the network using small molecules or novel synthetic modular proteins, which we previously described [38]. This strategy exploits the modular nature of signalling proteins to change the topology and wiring of the network by adding or depleting interactions. A second path aims at extracting control architectures and hierarchies of kinases to suggest combinations of inhibitors to change the topology and information flow within the network. This strategy relies on the fact that network topology is dynamic and regulated by post-translational modifications such as phosphorylation. Thus, the information flow within the network can be targeted by changing the phosphorylation states of key proteins.

## 2. Directional and quantitative phosphorylation networks

Any given cell in a physiological environment receives numerous simultaneous input cues that must be processed and integrated to determine changes in cellular behaviour such as migration, proliferation, apoptosis and differentiation. Reversible protein modifications are one of the underlying mechanisms that govern such cellular information processing. In particular, protein phosphorylation has proven to be a primary driving force behind cellular signal propagation. Through its ability to control protein–protein interactions, complex formation, enzyme activity and protein degradation and translocation, phosphorylation impacts every aspect of cellular biology [9]. All these modes of regulation are essential for proper cellular organisation and responses to input cues and we will refer to the set of these events as a *phosphorylation network*. Recent technical developments in mass spectrometry (MS) have permitted the identification and quantitation of thousands of *in vivo* protein phosphorylation sites. We recently introduced computational algorithms, NetworKIN and NetPhorest [10,11] to further enhance the modelling of such networks based on experimentally validated phosphorylation sites.

In combination with methods such as single-cell monitoring by phospho-flow [42,43], kinase-activity assays and conventional immuno-blotting, mass-spectrometry is steadily enhancing our capability to sample the states and dynamics of cellular signalling networks: The phospho-flow technology is based on the detection of a phosphorylation event by a specific antibody within a single cell. Firstly, cells are labelled by antibodies recognizing specific phosphorylated proteins (for example p-ERK or p-PKB) and cell markers (for instance CD20 or CD33). Secondly, they are sorted and measured by FACS thereby providing quantitative measurement of single-cell signalling events. High-throughput kinase assays provide important information for systems modelling [23,24]. Various forms of kinase assays have been developed that use either kinase specific immunopurification or specific kinase chemosensors [44,45]. The advantage of these activation-based assays is that they provide a direct measurement of enzyme activity, rather than an inferred indirect activity based on alterations in phosphorylation state.

However, integration of these data are key for their interpretation and utility in modelling of cellular phenotypes. Therefore, computational tools have been developed to combine these heterogeneous data sets and construct predictive models. These approaches have provided insights into the complex cel-

lular biology of signalling systems. We argue that these data-driven models are important for the understanding of cellular function under a variety of conditions such as disease, differentiation, migration and apoptosis.

Mass-spectrometry is a powerful approach to the analysis of protein networks [12–16], which can be effectively used for protein quantitation, as well as the analysis of post-translational modifications (PTM) and protein dynamics. Stable isotopes can be introduced into proteins in cell culture (SILAC), for quantitative or comparative purposes [14]. These reagents provide information of the relative amounts of proteins between the network states sampled, and identify state-specific binding partners. Another reagent type (iTRAQ, [17,18]) allows 4–8 channel multiplexed experiments in which several (currently 4–8) different samples can be combined; the relative amounts of a protein can therefore be simultaneously quantified across multiple experimental conditions. In particular, iTRAQ allows for the relative quantitative analysis of tissue samples, which is important for the analysis of networks in different cancer states and during tumour progression. Furthermore, it allows the dynamic reorganization of protein networks to be monitored over time. Perhaps the most challenging aspect of proteomics involves the analysis of PTMs [14–16]. PTMs are especially important for the dynamical changes to protein network topology, since interactions are frequently dependent on modifications such as phosphorylation [17,18].

Thus our ability to monitor cell signalling networks under conditions relevant for disease states and their transitions, such as gain of metastatic potential within a tumour, is rapidly growing [19].

## 3. Cue, signal, response – from networks to cell behaviour

Constructing network models from protein–protein interaction methods like LUMIER [20], yeast 2-hybrid [21] and MS [12,13] is essential for systems level understanding of the cellular machinery. However, these networks are even more powerful when integrated with cellular outcome and quantitative phenotypic data [22], which makes it possible to describe the information processing within the system. A series of papers from the Lauffenburger, Yaffe and White labs have combined cue, signal and response measurements to construct models of cellular decision processes [23–28]. The state of nodes within a signaling network was interrogated (“signal”) in a systematic manner using phospho-flow, kinase assays, antibody-arrays and mass-spectrometry, and correlated to cellular phenotypes (“response”) such as apoptosis, proliferation and migration. The signals used to perturb the system (“input cues”) were varied and combined to obtain measurements of cellular responses to conflicting (“orthogonal”) signals. The resulting data were subsequently subjected to data-driven modeling by applying a combination of partial least square regression (PLSR) and principal component analysis (PCA), for numerical modelling and data condensation, respectively. Using this approach on large phosphorylation data sets have proven a powerful way of deriving knowledge about critical sites and establishing predictive models of cellular systems. As an example, the White and Lauffenburger labs applied PLSR to phospho-proteomic data and phenotypic measurements (migration and proliferation) from cells expressing EGFR

alone or in combination with HER2, in order to produce a coefficient vector indicating the importance of the identified phosphorylation sites with respect to either migration or proliferation [26,27]. By constructing a model to recapitulate the measured data, the authors identified 9 phosphorylation events that alone could be used to predict a cellular decision (proliferation versus migration) [26,27]. PLSR has also been applied to generate systems models for cytokine- or sepsis-induced apoptosis and to predict a common effector hypothesis for signal integration [24,25]. These models have proven useful in predicting cellular decision processes; for example the kinase MK2 was identified by the Yaffe lab to play a pro-survival role based on its activation profile in the PLSR model [28].

In addition direct sampling of a signaling network can yield new information. For example, by performing MS quantification of a signaling network Huang et al. [16] found that amplification of c-MET, a receptor tyrosine kinase (RTK), can lead to resistance to an inhibitor of the EGFR RTK in tumours expressing ErbB3/EGFRvIII. Further by using phospho-specific antibody arrays or phospho-proteomics, U87 cells expressing EGFRvIII and a lung carcinoma cell line resistant to Gefitinib (Iressa) were shown to exhibit increased c-MET activation. Combinatorial treatment with both an EGFR inhibitor and a c-MET inhibitor had synergistic effects on growth inhibition, survival and anchorage-independent growth [16,29]. This work shows the power of molecular networks in deriving new drug strategies.

Technologies other than MS can be used to reveal how therapeutic compounds influence cellular signaling. For instance, to study how the signalling networks in acute myelogenous leukemia (AML) cancer cells are correlated with clinical outcome, the lab of Gary Nolan utilised unsupervised clustering of phospho-flow data to group samples from patients according to their differential network profiles (or signatures). The authors observed that patient response to chemotherapy was highly correlated with the initial clustering based on the signalling network state, thus indicating that the behaviour of the individual network can be used to determine and predict patient responsiveness and choice of treatment [30].

#### 4. Network medicine – targeting networks

Above we have discussed how networks and in particular their dynamical properties can be used to link cellular information processing to cellular outcome and phenotype. Therefore, we propose to exploit the knowledge of networks to investigate mechanisms for targeting the network itself.

In a network-disease survey Barabasi and co-workers showed that the so-called essential genes are more likely to be hub proteins which are components of anchored and widely expressed multi-protein complexes [31]. Furthermore, their study showed that most disease genes are more varied in expression patterns and with no tendency to encode such hub proteins [31]. Similarly a network analysis identified markers not as individual genes but as subnetworks of interacting proteins that could classify breast cancer metastasis [32]. It was further shown that genes with known breast cancer mutations reside between such subnetworks interconnecting many differentially expressed genes.

Another issue to consider is that of “cell specificity”, Miller-Jensen et al. [24] showed that common effector processing net-

works mediate cell-specific responses to external cues. The importance of this work and that of White and colleagues [17,19,26] is that it is the *network utilisation* that seems to be changed in different disease states. Thus, by identifying these subnetworks that are common across cell types but which are utilised to different extents, for example through changes in phosphorylation, one can envisage they can be targeted.

These studies point to the importance of network topology in finding markers of disease. Other studies focus on the utility of network information in unravelling new disease genes [46]. Thus, several papers have addressed the idea of using network information to unravel new oncogenic components, for example by integrating various systematic datasets to construct network models that identify new oncogenes in breast cancer [33,34]. By integrating complementary genomic approaches, Boehm and colleagues showed that the IKK-epsilon kinase is a new breast cancer oncogene. The authors presented an approach that predicted a new mechanism for NF-kB activation in breast cancer downstream of PI3K kinase. These studies underline the utility of data integration and integrative databases like STRING, STICH and Phospho.ELM that incorporate the majority of large-scale datasets and cover most current methodologies [35–37].

We envisage two types of approaches to target networks: firstly, the network could be re-wired with small molecules or synthetic modular proteins that introduce novel protein interactions or inhibit existing ones [38–40]. Secondly, the information flow within the network can be targeted by applying small-molecules against several nodes (for example kinases) simultaneously. To develop this strategy, one would analyse the topology of the measured network and correlate it to phenotypic markers (either molecular or macroscopic). This will result in a better understanding of how the aberrant network dynamics contribute to disease and will help to define multiple protein nodes that can be targeted to rewire the network, or induce the specific collapse or topology change of aberrant networks. We previously argued how this could be done by utilising the modular nature of signalling networks, through the generation of new modular synthetic proteins [38].

Given such a potential network drug (or combination of drugs) one would want to carry out extensive sensitivity analyses [41] in various cancer cell lines and with orthogonal input cues [23] in order to get as realistic as possible a picture of the response space for the drug candidate. It is essential to point out that structural and molecular based drug development will continue to play a central role in determining the best leads for a given node within the network. Thus, transforming medicine into a network driven endeavour goes hand in hand with a systems level understanding of cellular information processing.

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