

Allo-Network Drugs: Extension of the Allosteric Drug Concept to Protein-Protein Interaction and Signaling Networks

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Abstract: Allosteric drugs are usually more specific and have fewer side effects than orthosteric drugs targeting the same protein. Here, we overview the current knowledge on allosteric signal transmission from the network point of view, and show that most intra-protein conformational changes may be dynamically transmitted across protein-protein interaction and signaling networks of the cell. Allo-network drugs influence the pharmacological target protein indirectly using specific inter-protein network pathways. We show that allo-network drugs may have a higher efficiency to change the networks of human cells than those of other organisms, and can be designed to have specific effects on cells in a diseased state. Finally, we summarize possible methods to identify allo-network drug targets and sites, which may develop to a promising new area of systems-based drug design.

Keywords: Allo-network drugs; allosteric drugs; interactome; protein-protein interaction networks; protein structure networks; signaling networks.

1. INTRODUCTION

Fast and affordable drug development is a requirement that contrasts with the current state of drug discovery. Drug development often fails because the development process does not always take into account the vast complexity of the cell and the robustness of its networks. In recent years, systems level and network analyses have become increasingly applied methods in drug design [1-14].

In this review, we first give a network-focused overview of how allosteric changes propagate within proteins, and how this signal transduction process can be extended to protein complexes and larger segments of protein-protein interaction and signaling networks. Further, we describe the benefits and the limitations of network-based methods to analyze allosteric action. Then we introduce the concept of allo-network drugs [15], i.e. drugs acting indirectly, via the inter-protein propagation of changes in cellular networks. We show that allo-network drugs may have special benefits in human cells, and may be designed to have specific effects on cells of diseased organisms. The review is concluded by the suggestion of several methods to identify allo-network drug targets and their binding sites including determination of network centralities, network hierarchy, controllability, assessment of perturbation propagation in networks, the analysis of correlated motions, reverse engineering methods to reveal central and less frequently used pathways, analysis of evolutionary conservation, as well as of biological

disease-related systems data or system-level responses of the cell to drugs. We conclude that the development of allo-network drugs appears as a promising new trend in drug design.

2. THE PROPAGATION OF ALLOSTERIC EFFECTS AND PROTEIN STRUCTURE NETWORKS

2.1. The Concept of Allostery

Several questions of protein dynamics such as the mechanism of allosteric changes gained much attention in the last century [16-22] but have not been completely elucidated yet. The molecular mechanisms of allostery have been typically discussed in terms of two classical models, the Monod-Wyman-Changeux (MWC) [23] and the Koshland-Némethy-Filmer (KNF) [24] models. Although these models were originally developed for describing allostery in homooligomers, their modernized versions also apply to monomeric proteins and other types of allosteric systems [25]. Both models assume that the protein can exist in two major conformations (e.g. tense and relaxed or active and inactive). According to the MWC model, equilibrium exists between the two conformations at all times, and the binding of the allosteric effector only modifies this equilibrium, causing a population shift [26]. In contrast, the KNF model assumes that the new conformation does not pre-exist before the binding of the effector but is instead induced by the binding event through a series of sequential steps. Experimental evidence supports the MWC model in many cases, such that it has been argued that it can claim victory [27], even though it has been acknowledged that local ligand-dependent movements may occur in agreement with the KNF mechanism.

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Others have argued that the MWC and KNF scenarios are just extremes of a range of possible allosteric mechanisms [28].

A modern extension of the MWC mechanism stresses that most proteins do not exist in only two possible states but form a large ensemble of states, and the allosteric change represents a redistribution of population intensities within this ensemble, i.e. a population shift [29]. In addition, allostery may occur without a conformational change [30,31], which led to the new concept of dynamically driven allostery [32] and the notion that all proteins may be capable of being allosterically regulated. A recent classification of allosteric mechanisms considers the extent of conformational change, and whether the allostery is driven by enthalpy, entropy, or both [33].

2.2. Communication Pathways and Intra-Protein Networks

Regardless of the particular mechanism of allostery, it is recognized that information is in some way transmitted from the allosteric site to the substrate site, which implies the existence of communication pathways within the protein. Therefore, considerable experimental and computational effort has been devoted to discovering these pathways. It should be noted, though, that allostery has also been conceptually understood in purely thermodynamic terms, as in the ensemble view of allostery [34], which does not require the existence of specific pathways for the propagation of allosteric information.

The notion of intra-protein communication pathways inspires a network view of proteins. In this view, the protein is represented as a graph where nodes are (usually) amino acid residues and edges are defined based on some relationship between residues [35-45]. Communication from one site to another may thus occur along the edges of the graph.

As detailed in the following examples, there are many ways of defining networks within proteins. The procedure may start with a single structure, several structures (e.g. inactive and active forms), or an ensemble of structures obtained from molecular dynamics (MD) simulations or other approaches. Edges (weighted or unweighted) may be defined based on a simple distance cutoff criterion, interaction strength or energy, the comparison of structures, or an analysis of the ensemble. Experimental results are also used to define various networks within proteins.

2.3. Properties of Protein Structure Networks

The properties of protein structure networks (networks based on static structures) have been studied in detail [35-45]. When the network is defined based on a distance cutoff, interaction cutoff or energy cutoff, it undergoes a percolation transition in a narrow range of the cutoff as the size of the largest connected component increases to eventually cover the whole protein [40,46]. The clustering coefficient tends to be homogeneous in the protein core, and characteristic path lengths of residues have been shown to correlate well with residue fluctuations [39]. Hubs tend to connect secondary structure elements, and have a preference for certain amino acids (mainly aromatic ones and arginine) [40].

It has been shown that residues with a high closeness index in the network tend to be functionally important, and are often at active sites [47,48]. Networks spanning protein-protein interfaces have also been analyzed, with the general finding that hot spots at such interfaces tend to occur in clusters and are highly central [49-52]. In addition, protein structure networks have been used in studies of protein folding and unfolding [53-56].

2.4. Methods to Identify Allosteric Pathways in Intra-Protein Networks

Once an intra-protein network has been defined, various techniques can be used to find allosteric communication pathways within the network. One common method is to search for shortest paths connecting the allosteric and substrate sites [57-65]. This method identifies the residues most frequently occurring along these shortest paths. Another concept that is often embraced is to consider the protein as a set of modules (subgraphs with many connections within them but few connections between them). It has been shown that many proteins have a modular architecture, with modules corresponding to (often functionally distinct) subdomains. Residues between modules tend to be conserved and more rigid, and are thought to be involved in allosteric communication [66-69]. Several studies have employed the concept of modules or a related notion [69-71].

2.5. Statistical Coupling Analysis

In 1999, Lockless and Ranganathan [72] published an influential paper that introduced the idea of allosteric communication pathways, which inspired many experimental and computational studies. In this paper, the authors used statistical coupling analysis (SCA), a technique that uses a multiple sequence alignment to identify a coevolving set of residues in a protein family, to identify in the PDZ protein family a sparse network of residues representing what they called evolutionarily conserved pathways of energetic connectivity. Point mutations have been used to experimentally confirm the energetic coupling between the residues in the network. This method was then applied to reveal the communication pathways in a number of other proteins [73-77], and the predictions of the original paper have been supported by NMR measurements [78]. Although these studies showed the success of the SCA method, comparison with thermodynamic coupling data from double mutant cycles showed that while SCA does find some of the physically close, coupled residues, it does not find all of them [79]. Indeed, an exhaustive study using double mutant cycles found that coupling is not a special property of the residues in the coevolving network originally identified for the PDZ family [80]. A more recent lattice model study also noted some apparent discrepancies between SCA and mutational studies [81]. In spite of these apparent limitations, SCA continues to be successfully used in various studies [82-85].

2.6. Networks from Experiments

Although there are many computational techniques to detect allosteric communication pathways, it is difficult to discover those directly using experimental methods. As mentioned, SCA results were validated by mutational studies.

Energetic coupling between residues can be reliably measured by double mutant cycles. However, a full energetic description of an allosteric pathway requires the examination of higher-order couplings, which can only be performed by triple, quadruple, etc. mutants. Clearly, the number of required constructs quickly grows prohibitively high. In an impressive study, 46 different mutants were used to reveal a highly cooperative, sharply delineated allosteric pathway in a voltage-gated potassium channel [86]. Energetic couplings were found to be highly anisotropic, not corresponding to a distance relationship, which suggests that there is an actual pathway of successive physical deformations.

NMR based techniques have been used to discover allosteric communication pathways in a number of proteins, with remarkable results [87,88]. Of special interest is the discovery of dynamic couplings and allosteric behavior in a non-allosteric protein, eglin c [89]. It was demonstrated that energy transmission is unidirectional, i.e. perturbing the protein at one site may elicit a change at another site, but this process does not always occur in the reverse direction. The networks of dynamical couplings were revealed in several different mutants of eglin c, and were found to be different. In one mutant that showed subtle conformational changes from the wild-type protein, the network of dynamical couplings was not physically contiguous. The propagation of allosteric signals was explained by a change in residue rotamer populations [89].

Recently, a highly powerful technique named “chemical shift covariance analysis” (CHESCA) was developed and validated by mutational studies [90,91]. The method provides a detailed picture of both the structurally and the dynamically mediated allosteric changes, quantitatively describing the relative contribution of each residue to binding and allostery.

2.7. Multiple Pre-Existing Pathways

The growing number of experimental and computational studies of allosteric communication pathways led to the concept that there are multiple pathways, which pre-exist before the binding of the allosteric effector, much like all functional conformations pre-exist in the conformational ensemble before an effector is bound [92]. Major and minor pathways may exist as suggested by the different effect of mutations upon the allosteric signal transmission. An MD based computational analysis of communication pathways in Met-tRNA synthase, however, indicated that only fragments of pathways are present in the apo states of the protein, with the full pathway only appearing when both substrates are bound [59]. This indicates the dependence of various pathways on subtle structural changes. A given pathway may only pre-exist in a scarcely populated subset of the native ensemble and thus may not be readily detected in MD simulations.

2.8. Allosteric Communication Pathways from Static Structures

A number of studies simply used a single structure or a small number of structures to define networks, and extract communication pathways. In myosin, this method was used to identify shortest paths between the ATP binding site and the lever arm, defining a communication pathway by con-

served residues along the paths [60]. Another study identified those conserved residues that had the largest contribution to maintaining short paths within the protein structure network; the results showed good agreement with experimental findings for 7 protein families [57]. For proteins undergoing a clear conformational change due to allostery, pairs of structures can be used to obtain an insight into the allosteric mechanism [93]. It was found that a communication pathway is best identified by combining the network of rigid substructures with the network of contact rearrangements [94].

For a better description of information flow in the network, some studies use an information theoretical approach, converting the protein structure network into a Markovian model of information propagation [71,95-97]. By defining soft clusters in the Markovian network and identifying hub and ‘messenger’ residues, two main communication pathways were discovered in the GroEL-GroES system [71]. In a later study, it was shown that the commute time between two residues (as calculated from the Markov propagation model) is proportional to the fluctuations in the distance between these residues (as calculated from an elastic network model), thereby bridging the gap between information theory and physics based models [98].

2.9. Communication Pathways from Elastic Network Models

Elastic network models (ENMs) are various coarse-grained models of proteins that are typically defined from a single protein structure based on a residue-residue distance criterion, and studied by normal mode analysis [99]. Such models have proven successful for describing functional motions (including allosteric motions) in proteins, especially when the protein consists of large, rigid regions connected by more flexible linker regions [28,100]. ENMs have been used in various ways to define communication pathways. In the “structural perturbation method”, the response of the network to a local structural perturbation is mapped out to define an allostery wiring diagram [101,102]. The similar “perturbation response scanning” method examines the displacement of residues when a force is applied on a selected residue [103]. A recent “electro-elastic network model” attempts to account for hydration, and is used to study the effect of solvation upon allosteric changes [104].

2.10. Allosteric Communication from an Ensemble of Structures

Recognizing the fact that proteins are dynamic entities, many techniques have been developed to define communication pathways in proteins from ensembles of structures. Molecular dynamics simulation (MD) is the most common method to generate ensembles of protein conformations. Once an MD trajectory is generated, the edges of a residue network are defined based on various quantities obtained by analyzing the trajectory. Edges between residues may be defined based on the correlation of displacements of the atoms (usually the Ca atom) [59,61,64,105], the fluctuation of Ca-Ca distances [106,107], generalized correlations [63], interaction energy correlations [108,109], mutual information between torsion angles [110], and discretized backbone

conformations of segments [65]. In several cases, this network was further filtered or combined with distance based networks to ensure physical contact between correlated residues. The resulting network was then analyzed by the same methods as those used for static structures, i.e. finding shortest paths, hubs, clusters, modules, etc.

A different ensemble-based approach has been adopted by Hilser and colleagues [111,112]. Instead of generating an ensemble of actual conformations, a statistical mechanics approach is used. The COREX algorithm generates states with all possible combinations of locally unfolded or folded segments, and the energies of these states are estimated. In this approach, the energetic couplings between residues can be determined. Using energetic perturbations, the method reveals the cooperative network of the protein, and it can also determine the global allosteric response to mutations [113]. A remarkable conclusion arising from this approach is that cooperative coupling between sites does not require the existence of a specific energetic pathway. Instead, such coupling can be explained by the hierarchy of energetically excited states in the native ensemble. The validity of the approach was experimentally confirmed by creating a new allosteric effect by increasing the probability of some locally unfolded states in adenylate kinase, without changing the native structure [114].

2.11. Energy Propagation in Proteins

An allosteric effect can be regarded as a local energetic perturbation of a protein, which then propagates within the protein matrix to eventually reach the substrate site [115]. Several studies have been devoted to the investigation of energy transmission processes within proteins. The investigation of vibrational energy flow on all-atom models [116] found that the flow is anisotropic and the protein can be approximated as a percolation cluster [117] with a fractal dimension ~ 2.5 . Energy is transferred between vibrational modes via anharmonic coupling. Based on a similar normal mode analysis, frequency-resolved communication maps were constructed for myoglobin [118]. At low frequencies, modes are global, and some channels are observed that could transfer energy away from the heme group. The communication map resembles the contact map. At higher frequencies, however, the map is very frequency-dependent. It should be noted that energy transfer by these processes is typically much faster (on the picosecond time scale) than most allosteric processes, therefore the relevance of these energy flow channels to allostery is uncertain.

Energy flow was also studied by elastic network models. Using an anharmonic model, it was shown that “discrete breathers”, localized vibrational modes easily form in proteins at the stiffest regions, and are able to pump and store energy from neighboring sites. Thus, energy can ‘jump’ from site to site via this mechanism, often covering long distances [119,120]. In a Gaussian network model, the correlations between the energy fluctuations of residues were calculated, and were found to define an interaction pathway which was similar to the pathway defined by the co-evolving network of residues in the PDZ domain [121].

Energy flow within proteins was also investigated by a number of MD techniques. In the method of anisotropic

thermal diffusion, a single amino acid is suddenly heated to 300 K while the rest of the protein is at 10 K, and the diffusion of heat within the protein is monitored [122-124]. In pump-probe MD, selected atoms are excited by oscillating forces, and the transmission of the oscillations to other parts of the protein is monitored [125]. Similar methods are based on measuring the times energy is transmitted from the site of perturbation to other residues [126,127], and the time correlations of the energy flow between residues [128]. Although the perturbations used in these methods may be unphysically large, all these methods reveal the anisotropy and pathways of intra-protein energy transmission. Often, the communication pathways found by these methods are similar to those identified by other methods such as SCA.

A method has been developed to quantitate the local energetic frustration in proteins [129]. ‘Frustratograms’, networks of minimally and highly frustrated residue pair interactions, were calculated for a number of proteins, and it was shown that the regions that reconfigure during an allosteric conformational change tend to be enriched in patches of highly frustrated interactions [130]. This and similar findings have led to the idea that intra-protein communication may occur via a front of highly frustrated residues, and ligand binding may induce a flipping of residues within a ‘frustration tube’ [28].

Our current understanding indicates that allosteric changes may have two major molecular mechanisms. In allosteric proteins where signaling involves only a small number of amino acids, a switch-type conformational change is typical. When allosteric signaling involves a large number of amino acids signals usually propagate at multiple, seemingly fuzzy trajectories which tend to converge at inter-domain boundaries. While protein segments involved in switch-type allosteric changes may be more rigid, protein segments harboring multiple trajectories may be more flexible. Some proteins may have a mixture of the above two mechanisms for the propagation of conformational changes. If the binding of an allosteric effector makes certain protein segments more rigid, the signal may propagate by a mechanism closer to the first, switch-type mechanism, resulting in an efficient, saltatory signal transduction. This can be conceptualized as the propagation of a ‘rigidity-front’ [1,45,131]. The ‘rigidization’ of a protein segment may accelerate the propagation of the allosteric change within the segment and induce similar changes in the neighboring segment. Rigidity front propagation may use sequential energy transfers [22,120], and thus may significantly increase the speed of the allosteric change [1,45]. The rigidity front propagation model combines elements of ‘rigidity propagation’ [132-134] with the ‘frustration front’ concept of Zhuravlev and Papoian [28], and it is in agreement with the recent proposal of Dixit and Verkhivker [135] suggesting an interaction network of minimally frustrated (rigid) anchor sites and locally frustrated (flexible) proximal recognition sites to play a key role in allosteric signaling.

2.12. Finding Allosteric Sites by Network-Based Methods

Discovering new allosteric sites in proteins opens up a new way of designing more specific drugs [87]. Thus, it is of interest to develop methods that can identify allosteric hot

spots on protein surfaces [136,137]. Communication pathways predicted by SCA were used to find allosteric hot spots in dihydrofolate reductase (DHFR), and a chimeric protein was created by inserting a light-sensitive domain into DHFR at a predicted allosteric site, thereby obtaining an enzyme that can be regulated by light [84]. As a continuation of this experiment, it was shown by the same method that the set of all allosteric hot spots is almost identical to the set of surface sites connected to network defined by SCA, and a similar conclusion was made about a PDZ domain [85]. These findings also give an insight into how new sites for allosteric regulation may naturally evolve.

2.13. Limitations of Protein Structure Networks for the Study of Allosteric Communication

Studying allosteric communication by network analysis of protein structure graphs is a powerful approach based on an intuitive concept, and has yielded plausible and occasionally, experimentally validated results. However, it is not without limitations.

As we have seen, there are many ways of defining intra-protein networks, and not all methods give the same result. An MD based study using restraints from NMR experiments showed that the network obtained by mapping structural changes differs from that obtained from dynamical changes, and both are different from the co-evolving network obtained by SCA (although the overlap between the SCA and the dynamical network is larger) [138]. The network obtained by mapping torsion angle correlations is also different from that obtained from position correlations [110]. The hubs in a network obtained from Lennard-Jones energies tend to be located in the protein core, while those defined in the network based on full interaction energies tend to be at the surface [46].

A network constructed from a single, static structure cannot account for conformational changes. Communication pathways may not always pre-exist or may only be present in a scarcely populated subset of the native ensemble, thus easily evading detection even via ensemble-based methods. It was shown that the full communication path only gets assembled in Met-tRNA synthase when both substrates are bound [59]. Methods based on MD simulations yield networks reflecting different time scales of events, from the picosecond [126] to the tens of nanoseconds [110] range. In an MD study based on torsion angles, correlations between distant side chains were seen without a correlated chain of residues between them [110]. In this case, the correlation is probably mediated by events on a faster time scale that is not captured by the method.

Communication pathways built from residue-residue contact-based protein structure networks often lack a true physical basis (with the exception of elastic networks). Different types of perturbations may activate different communication pathways within a protein; e.g. a targeted, strong perturbation elicited by ligand binding may get propagated differently than thermal noise from the solvent [58]. Protein structure networks often do not take into account the differences in these perturbation types.

Most network-based methods to reveal communication pathways do not take side chain rotations into account, even

though these have been shown to be involved in allosteric communication via rotamer population shifts [89,110,138,139]. Side-chain rotations generate their own network, which is impossible to map out from a static structure [140]. Many methods only use C α coordinates, although it has been shown that side chains should be included for best results [70].

All intra-protein network construction methods consider residues as nodes in the network. Although this is an obvious choice, residues have widely different sizes and physico-chemical properties, which may bias the results of the analysis. For example, hubs are very often aromatic residues [57], which is at least in part due to their bulkiness [40]. In fact, residue types have different network properties due to their physico-chemical differences [141,142]. It was shown that a graph with chemical groups (groups of chemically similar atoms) as nodes is more suitable for the analysis of MD simulations than residue-based graphs [143].

Allostery itself may occur by a number of mechanisms, involving large or small or no conformational change, driven by enthalpy (structure), entropy (dynamics) or both [33], and the time scale of the allosteric change may also vary over a wide range. The protein may be highly modular or may consist of only a single, rigid domain. Ideally, the choice of method to describe communication pathways should depend on the particular protein, as well as the type and time scale of the allosteric change under study.

Even though network construction methods will always yield some communication network, allosteric 'information transmission' may in some cases not involve any particular pathway at all, as suggested by the ensemble view of allostery [144]. This may typically be the case for proteins where disordered regions or domains optimize allosteric coupling [45,144], or where significant segments of the protein structure are extremely rigid [1,45]. Network-based methods to reveal allosteric pathways may have difficulties with handling significant disorder (i.e. extreme flexibility, plasticity) or extreme rigidity.

Finally, intra-protein networks usually do not include solvent molecules, even though they may be part of the allosteric pathway. It has been shown that water molecules at the interface between the subunits of hemoglobin carry a large part of the energy and are probably involved in allosteric communication [145].

Future research will probably yield more advanced methods that will surmount some of the weaknesses of existing methods. Despite the limitations, the findings of many network-based analyses of allostery have been supported by experiments, and the network view of proteins offers a useful framework for the discussion of allosteric processes.

3. PROPAGATION OF SIGNALS IN CELLULAR NETWORKS

The propagation of allosteric signals is not limited to single protein chains. The classical models of allostery describe allosteric effects in oligomeric proteins [23,24]. Network models of allosteric signal propagation have been applied to multimeric protein complexes [49-52]. Many of the examples of the preceding sections were also protein complexes.

Cellular function can be described in terms of cellular networks such as protein-protein interaction networks, signal transduction networks, gene transcription networks, metabolic networks, etc. [1,146,147]. Evolution has optimized the cellular machinery to rapidly respond to changing conditions by the use of modular functional organization and large multimolecular assemblies. The binding of an allosteric effector to one protein may induce conformational or dynamic changes in adjacent proteins. The propagation of conformational changes is usually anisotropic and traverses across a pathway, thereby facilitating signal transduction over large distances (hundreds or thousands of angstroms). Thus, binding to a protein may elicit a limited, specific, and distant functional change in the network [15,33,115,148].

A prime example of such functioning is the gigantic Mediator complex, which relays signals from specific DNA regulatory elements to initiate gene-specific transcription hundreds of angstroms away [149,150]. However, long-range signaling does not necessarily require a giant complex, since the signal can also propagate in a stepwise manner, where only two nodes are involved in each signal transmission step. As an example of specific propagation of long-range changes, Maslov and Ispolatov [151] used the law of mass action to calculate the effect of a two-fold increase in the expression of a single protein on the free concentration of other proteins in the yeast interactome. Despite of an exponential decay of changes, there were a few highly selective pathways where concentration changes propagated to a larger distance [151]. The perturbation dynamics of signaling networks was extensively analyzed including ~10,000 phosphorylation events in an experimental study of yeast cells [152]. The size of the human interactome has been estimated to have 650,000 interactions [153]. Though several datasets have been published in the last decade [154-157], we are still a long way from deciphering the full complexity of the human interactome. Human signaling networks extend this complexity by the inclusion of directed and conditional interactions, as well as microRNAs [1].

The number of possible regulatory combinations for a given gene increases dramatically with an increase in input complexity and network size. For example, with 100 genes and 3 inputs per gene, there are about a million input combinations for each gene in the network, resulting in 10^{600} different network wiring diagrams [158]. Systems-level molecular networks have a 100-fold larger size. Estimates of the number of possible states of the yeast interactome range from $10^{7,200}$ to $10^{79,000,000,000}$ [159], which are all unimaginably high numbers. The number of possible states of the human interactome, which is an order of magnitude larger, must be even higher. At this level of system complexity, current descriptions of network dynamics contain several simplifications, e.g. they neglect node-specific delays, differences in individual dissipation patterns, effects of water, or molecular crowding. Most importantly, most current network-level cellular signal transduction models do not take into account the highly anisotropic nature of the perturbation propagation inside protein structures [115]. Construction of atomic-level resolution protein-protein interaction and signaling networks, with interacting 3D protein structures bound or docked at their experimentally shown or predicted binding sites, will be a major step towards high-resolution

signal transduction models to understand long-range allosteric action. This step will add a new dimension of information especially to protein-protein interaction networks, which at present mostly contain probability-type interactions lacking structural and signal propagation details. A few protein-protein interaction networks containing 3D protein structures have already been assembled [160-163], providing initial steps towards this goal.

4. ALLO-NETWORK DRUGS: EXTENSION OF THE CONCEPT OF ALLOSTERIC DRUGS TO CELLULAR NETWORKS

Traditional, orthosteric drugs bind to active sites of enzymes. However, their selectivity is hard to ensure as proteins acting on the same substrates (e.g. protein kinases which all use ATP) have similar active sites. To circumvent this problem, a number of allosteric drugs have been developed, mainly for seven transmembrane receptors, GPCRs [11,14,164-167] (see also other papers of this issue). Because allosteric drugs bind to sites different than the active site, they can be more specific, and thus usually have fewer side effects. Furthermore, allosteric drugs allow fine modulation of function instead of the inhibition of the protein achieved by most orthosteric drugs.

The combination of the concepts that intra-protein allosteric communication can be described *via* protein structure networks and that cellular function can be understood in terms of protein-protein interaction and signaling networks inspired a novel suggestion for drug target discovery: allo-network drugs (Fig. (1), [15]). In this concept, instead of targeting a malfunctioning protein directly, a different protein in the cellular network neighborhood is targeted by the allo-network drug. The concept urges that drug discovery should harness the long-distance allosteric signal transduction pathways created by evolution to extend the scope of drug discovery targets. In allo-network drug action, the binding of the drug triggers a pathway of conformational (or dynamic) changes over a segment of the protein-protein interaction or signaling network, finally reaching a target protein, where it may then, for example, enhance or inhibit another pathway of propagating conformational changes. Because pathways evolved by nature are limited and specific, allo-network drugs have the potential to be highly selective and disease-specific. This approach may dramatically increase the number of target proteins that can be considered as drug targets.

Earlier works already pointed towards an allo-network type drug action, like the suggestion of inter-protein propagation of allosteric effects [10,168] and the possible use of such an effect in drug design [7]. In fact, drugs that can be considered allo-network drugs already exist. Combination therapies and multi-target drugs combine multiple effects, often at places distant from the malfunctioning protein in the cellular network [2]. Examples from cancer drugs include B-RAF and MEK combination therapy [169,170] and rapamycin/FKBP12, an inhibitor of the mTORC1 complex [171,172]. Moreover, drug-target network studies revealed that in more than half of the established 922 drug-disease pairs, drugs do not target the actual disease-associated proteins, but bind to their 3rd or 4th neighbors. However, the

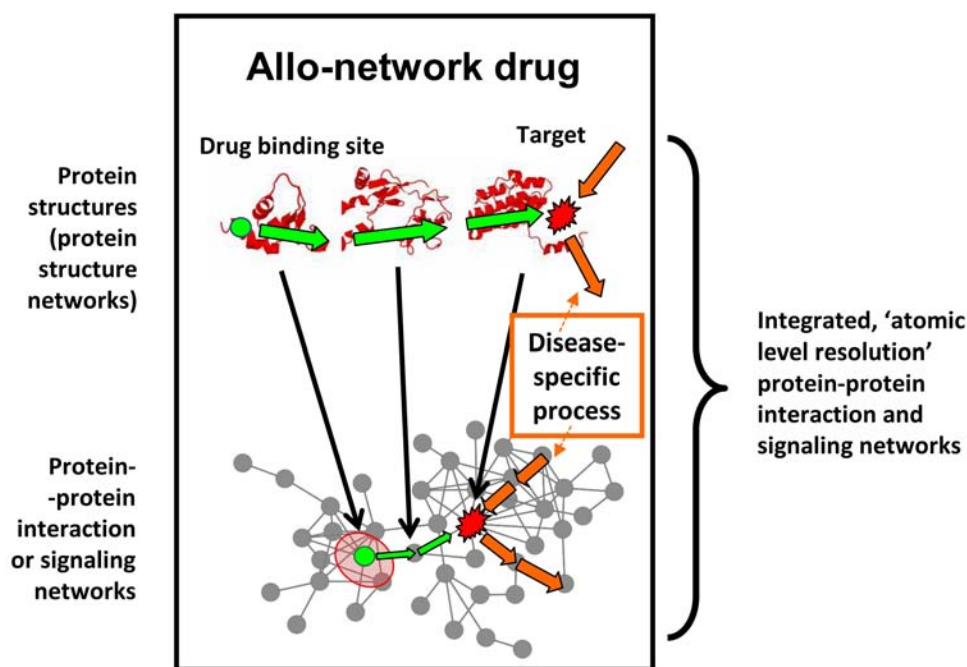


Fig. (1). Allo-network drugs. The top part of the figure illustrates the protein structures of the allo-network drug target containing the primary binding site (green circle), a 'transmission' protein, and the final target. The bottom part of figure illustrates the positions of the same 3 proteins in the human protein-protein interaction or signaling network. The red ellipse illustrates the 'action radius', i.e. the network perturbation induced by the primary target. In the top part, signal propagation (illustrated by the light green arrows) extends beyond the original drug binding protein and affects two neighboring proteins in the network *via* specific interactions. The pharmacologically active final target is marked by a red asterisk. Orange arrows illustrate an intracellular pathway of propagating conformational changes, which is disease-specific in case of successful allo-network drugs. Allo-network drugs allow indirect and specific targeting of key proteins by a primary attack on a 'silent' protein which is not involved in major cellular pathways. Targeting 'silent', 'by-stander' proteins, which specifically influence pharmacological targets, not only expands the current list of drug targets, but also causes much less side-effects and toxicity. (*The color version of the figure is available in the electronic copy of the article.*)

distance between drug targets and disease-associated proteins was regarded as a sign of palliative drug action [3,13], and the expansion of the concept of allosteric drug action to the interactome level has been formulated only recently [15].

Thinking in network terms, the action of drugs can be perceived as a network perturbation. The drug-induced attack either destroys the network of infectious or cancer cells, or shifts the pathophysiological network status back to normal [115,173-175]. Perturbation mediators in anti-infectious or anti-cancer therapies are often at cross-roads of cellular pathways. On the contrary, in other diseases such as diabetes or neurodegenerative diseases, efficient drug targets are not directly involved in major cellular pathways but indirectly influence them in a highly efficient manner [1]. Thus, the indirect but highly directed action of allo-network drugs can achieve specific, limited changes at the systems level, with fewer side-effects and lower toxicity than those of conventional drugs [15]. In agreement with this assumption, drugs with targets less than 3 steps (or more than 4 steps) from a disease-associated protein were suggested to have more side-effects and to fail more often [176]. However, rational drug design proceeded in the opposite direction, identifying drug targets closer to disease-associated proteins than earlier [3]. The recently observed reversal of this trend may be more productive. Allo-network drugs point exactly to this direction.

5. ALLO-NETWORK DRUGS AS SPECIFIC EFFECTORS OF DISEASE-AFFECTED HUMAN CELLS

Human cells seem to have developed a larger complexity of their networks than other organisms. This is especially true for signaling networks, where major signaling pathways have all possible crosstalks in human cells, while the same is not at all true in *C. elegans* or in *Drosophila* [177]. The complexity of regulation also increased through additional binding factors, as, for example, apparent in the structure of the transcriptional Mediator mega-complex [149,178].

Not only the static network structure but network dynamics also increased in human cells. The variability of human protein-protein interactions is much larger than that seen in other interactomes. Besides single-nucleotide polymorphisms, alternative splicing, addition of N- or C-terminal tags, partial proteolysis and other post-translational modifications (such as phosphorylation), as well as changes in protein expression patterns may dramatically re-configure protein complexes [179]. Moreover, the human interactome seems to have a higher proportion of 'sticky' proteins leading to more promiscuous interactions than the *E. coli* or *S. cerevisiae* interactomes [180]. As an additional factor of network dynamics, the human interactome is enriched with disordered proteins, causing dynamically fluctuating, 'fuzzy' interaction patterns [45,181].

The increased inter-pathway signaling, as well as the coupled large assemblies, all extend the propagation pathway repertoire of inter-molecular allosteric changes. Complex organization is helped by increased modularity, but increased variability and disordered proteins help to cross module boundaries and amplify intra- and inter-protein allosteric relays [148]. Thus, allo-network drug-like action occurs most where it is needed most: in human cells.

Promising allo-network drugs should interfere with disease-specific pathways [1,15]. Targeting disease-induced dynamical changes in molecular networks may be focused on transient interactions specific to disease. Thus, allo-network drugs may also provide a novel solution to uncompetitive, 'interfacial' drug action [173,182,183].

Finally, current drugs usually inhibit protein-protein interactions [184]. Allo-network drugs may also trap a protein in its inactive state [87,185]. This way, the inter-molecular, long-range conformational change is prevented by the allo-network drug. However, importantly, besides inhibition, allo-network drugs may stabilize, restore and/or activate a protein, its function, or one (or more) of its interactions [186].

6. POSSIBLE METHODS TO IDENTIFY ALLO-NETWORK DRUG TARGETS

As it is conceivable from Fig. (1), at the molecular network level, allo-network drug design needs to solve three closely related problems. 1.) An appropriate pharmacological target protein playing a crucial role in shifting the disease state of the cell back to normal should be found. 2.) An appropriate allo-network drug target protein should be found which is in the network neighborhood of the pharmacological target protein but plays no major role in cellular processes and therefore its direct manipulation has no major side-effects. 3.) A communication channel should exist between the allo-network target of point 2 and the pharmacological target of point 1, i.e. the changes of the allo-network target should selectively propagate towards the pharmacological target in the atomic-resolution network structure.

Since the above points 2 and 3 are related and set rather stringent requirements for the allo-network drug target, search procedures can be greatly facilitated by increasing the resolution of data on cellular networks (the interactome and/or the signaling network) to atomic or amino acid level. To obtain a map of a protein complex at atomic resolution, docking of 3D protein structures and the consequent connection of their protein structure networks are needed. A few protein-protein interaction networks containing 3D protein structures have already been assembled [160-163], providing initial steps towards this goal. These efforts may be extended by mapping the protein structures into low-resolution EM density maps [187]. Thus, allo-network drug targeting requires the integration of our knowledge on protein structures, molecular networks, and their dynamics, focusing particularly on disease-induced changes. The identification of promising drug targets requires a deep understanding of how the cellular networks (interactomes and signaling networks) work, along with detailed structural knowledge of the proteins and complexes involved. To map the cellular networks at the atomic resolution level, experimental structural and

functional data need to be combined with modeling tools to predict which proteins interact and how [188]. Construction of these atomic level cellular networks should also take into account that proteins in different conformations may interact with different partners and different drug molecules; therefore, dynamic protein-protein interaction [161] and signaling networks, as well as conformation-dependent drug-target networks [189] have to be used.

We list a few possible methods to define allo-network drug target sites and allo-network pathways to the pharmacological targets in cellular networks at atomic level resolution (Fig. (2)).

- Central residues play a key role in the transmission of allosteric changes [65,71,95,97,101,102,127,135,190-193]. We may use a number of centrality measures [194], including perturbation-based or game-theoretical assumptions [115,195], to find the level of importance of proteins and pathways in interactomes and signaling networks, and important amino acids in their extensions to atomic level resolution [69].
- At both the molecular network level and its extension to atomic level resolution, we may extract network hierarchy [69,196-201] to assess the importance of various nodes (proteins and/or amino acids).
- We may find nodes or edges controlling the network by the application of recently published methods [201-207].
- Network-based analysis of perturbation propagation is a fruitful method to identify intra-protein allosteric pathways [21,44,45,59-61,71,93,97,102,111,127,208]. A successful candidate for the inter-protein allosteric pathways involved in allo-network drug action disturbs network perturbations specific to a disease state of the cell at a site distant from the original drug-binding site. Perturbation analysis [115,195] applied to the atomic-level-resolution interactome in combination with disease-specific protein expression patterns may help the identification of such allo-network drug targets.
- A general strategy for the identification of allosteric sites may involve finding large correlated motions between binding sites. This can reveal which residue-residue correlated motions change upon ligand binding, and thus can suggest new allosteric sites [209] even in integrated networks of protein mega-complexes.
- Reverse engineering methods [158] allow us to discriminate between 'high-intensity' and 'low-intensity' communication pathways both in molecular and atomic level networks, and thus may provide a larger safety margin for allo-network drugs.
- Combination of evolutionary conservation data has proven to be an efficient predictor of intra-protein signaling pathways [60,85,210-212]. Similar approaches may be extended to protein neighborhoods helping to find starting sites for allo-network drug action.
- Disease-associated single-nucleotide polymorphisms (SNPs; [213]) and/or mutations [214] may often be part of the propagation pathways of allosteric effects. In-frame mutations are enriched in interaction interfaces [214], and provide an interesting dataset that could be

Methods to find allo-network drugs

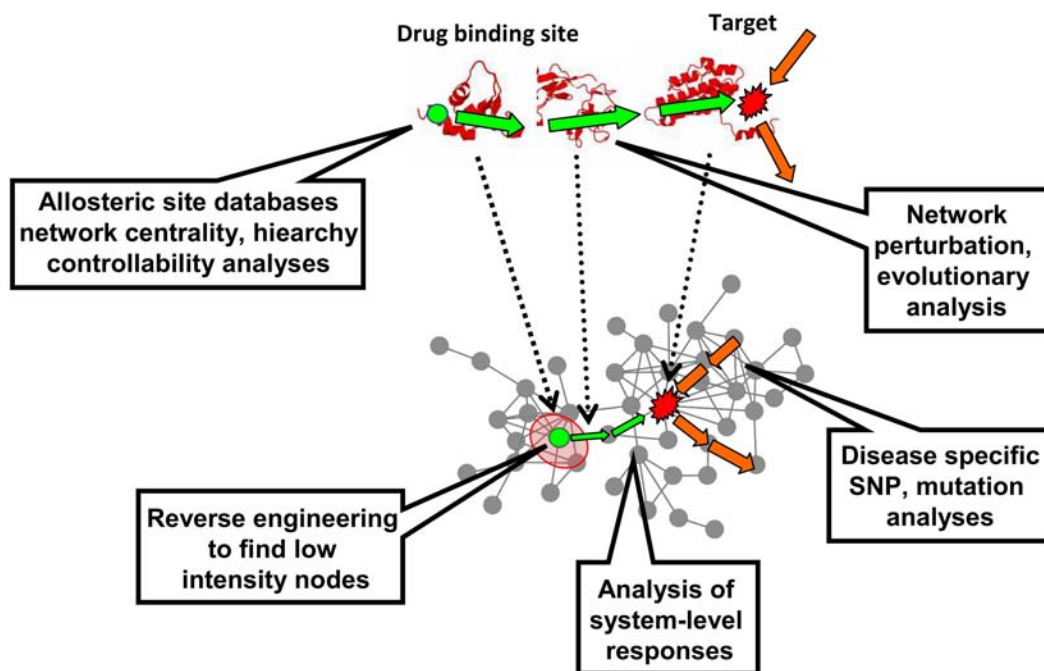


Fig. (2). Methods to find allo-network drugs. All middle segments and notations of the figure are the same as those explained in the legend of Fig. (1). (Top part: protein structures; green circle: allo-network drug binding site; light green arrows: signal propagation; red asterisk: pharmacologically active target; orange arrows: disease-specific pathway; bottom part: protein-protein interaction or signaling networks; red ellipse: 'action radius'.) Boxes highlight the various network- and system-based methods helping the identification of allo-network drugs. (The color version of the figure is available in the electronic copy of the article).

used to predict the existence of allo-network drug binding sites.

We also give a few additional hints and considerations for successful allo-network drug finding.

- Databases of allosteric binding sites (<http://mdl.shsmu.edu.cn/ASD> [215]) help the identification of possible sites of allo-network drug action. However, allo-network drugs may also bind to sites not used by natural ligands, and inter-molecular allosteric effects may use other sites beyond those involved in intramolecular allostery.
- Drugs may fail when the cell bypasses drug action via a parallel pathway. Therefore, it is important to obtain a complete view of the network and to identify possible alternative pathways [216].
- Because allo-network drugs have systems-level effects, cell-based assays can be used to find such compounds, similar to classical allosteric drugs. A number of experimental methods can be used to find and analyze cellular system-level responses. This may involve extensive pathway profiling and high-throughput RNAi screening [217].

Despite the considerable challenges, detailed structural and functional knowledge and understanding of the pathways and information flow in the cellular networks at atomic level resolution will help to circumvent the long trial-and-

error process that was followed by evolution to develop allo-network action.

CONCLUSION AND PERSPECTIVES

We predict that allo-network drug related systems-level and network analysis methods will develop to 'rational allo-network drug design' protocols in the coming years, and hope that these efforts will result in novel drugs with greater selectivity and effectiveness. Allo-network drugs may involve drug targets that are not related at all to usual drug target families.

However, the development of rational allo-network drug design protocols requires 1.) a much more detailed understanding of cellular network structure and dynamics at the atomic resolution level including forward and backward loops; 2.) understanding allosteric signal propagation in networks and finding the key points where this dynamics can be influenced in an indirect but efficient and selective manner from a larger distance [31]; 3.) incorporation of disease-specific systems-level data and data on dynamics and network specificities [216].

Despite the considerable challenges, the rapidity of the increase in systems-level data on healthy and diseased cells and the remarkable progress of network analysis methodology in the last few years gives us strong hope that the rational development of allo-network drugs will be a widely pursued option of the drug industry in a few years.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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