European Journal of Medicinal Chemistry xxx (2014) 1–9

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Structure-based inhibition of protein–protein interactions

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ARTICLE INFO

Article history:
Received 6 July 2014
Received in revised form
3 September 2014
Accepted 12 September 2014
Available online xxx

Keywords:
Protein-protein interaction
Computational tools
Inhibitor design
Peptidomimetic
Protein structure

ABSTRACT

Protein–protein interactions (PPIs) are emerging as attractive targets for drug design because of their central role in directing normal and aberrant cellular functions. These interactions were once considered "undruggable" because their large and dynamic interfaces make small molecule inhibitor design challenging. However, landmark advances in computational analysis, fragment screening and molecular design have enabled development of a host of promising strategies to address the fundamental molecular recognition challenge. An attractive approach for targeting PPIs involves mimicry of protein domains that are critical for complex formation. This approach recognizes that protein subdomains or protein secondary structures are often present at interfaces and serve as organized scaffolds for the presentation of side chain groups that engage the partner protein(s). Design of protein domain mimetics is in principle rather straightforward but is enabled by a host of computational strategies that provide predictions of important residues that should be mimicked. Herein we describe a workflow proceeding from interaction network analysis, to modeling a complex structure, to identifying a high-affinity substructure, to developing interaction inhibitors. We apply the design procedure to peptidomimetic inhibitors of Ras-mediated signaling.

1. Introduction

The centrality of protein–protein interaction (PPI) networks in regulating cellular function offers attractive opportunities for drug discovery [1,2]. PPIs are considered fertile yet challenging targets for inhibitor design [3]; however, advances in molecular and structural biology as well as computational chemistry and molecular design have afforded potent inhibitors for previously intractable targets [4]. Mimicry of protein domains that are critical for the formation of native protein–protein complexes offers an attractive approach for the design of PPI inhibitors [5–8]. This protein domain mimicry approach is complementary to small molecule high throughput and fragment based screening approaches, with each method offering distinct advantages [9,10]. While protein domain mimetics employ quaternary structure information to imitate native bound states, small molecule high-throughput screens can reveal binding pockets and molecules that allosterically modulate the binding surface [11–14]. Fragment-based methods identify small molecule binders and employ an iterative approach to recombine them to produce a potent ligand for the target interface [9]. These different yet complementary approaches have yielded orthosteric and allosteric inhibitors while revealing general principles that can be extended to broad classes of PPIs [15]. In this review, we outline steps to the design of protein domain or protein secondary structure mimetics as inhibitors of chosen PPIs. We focus on the design of PPI inhibitors that modulate Ras/Sos and Ras/Raf complexes as model systems, with a focus on description of the *in silico* resources available to guide a project from target selection to compound design. An overview of the process is depicted in Fig. 1.

2. Computational methods to target protein–protein interactions

Commonly, efforts to design novel inhibitors begin with a disease state in mind, rather than a specific protein or a specific protein complex. Disease states can be distinguished from healthy states by comparing the signaling networks present in each; for example, cancers typically exhibit upregulated proliferation signaling circuits. Thus, before arriving at a specific protein complex, one must examine the perturbations native to the disease state and determine what interactions within that signaling network might return it to health.

2.1. From disease state to protein–protein interaction

The majority of inter-species variation owes to differences in the interactions between gene products rather than differences in gene

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http://dx.doi.org/10.1016/j.ejmech.2014.09.047
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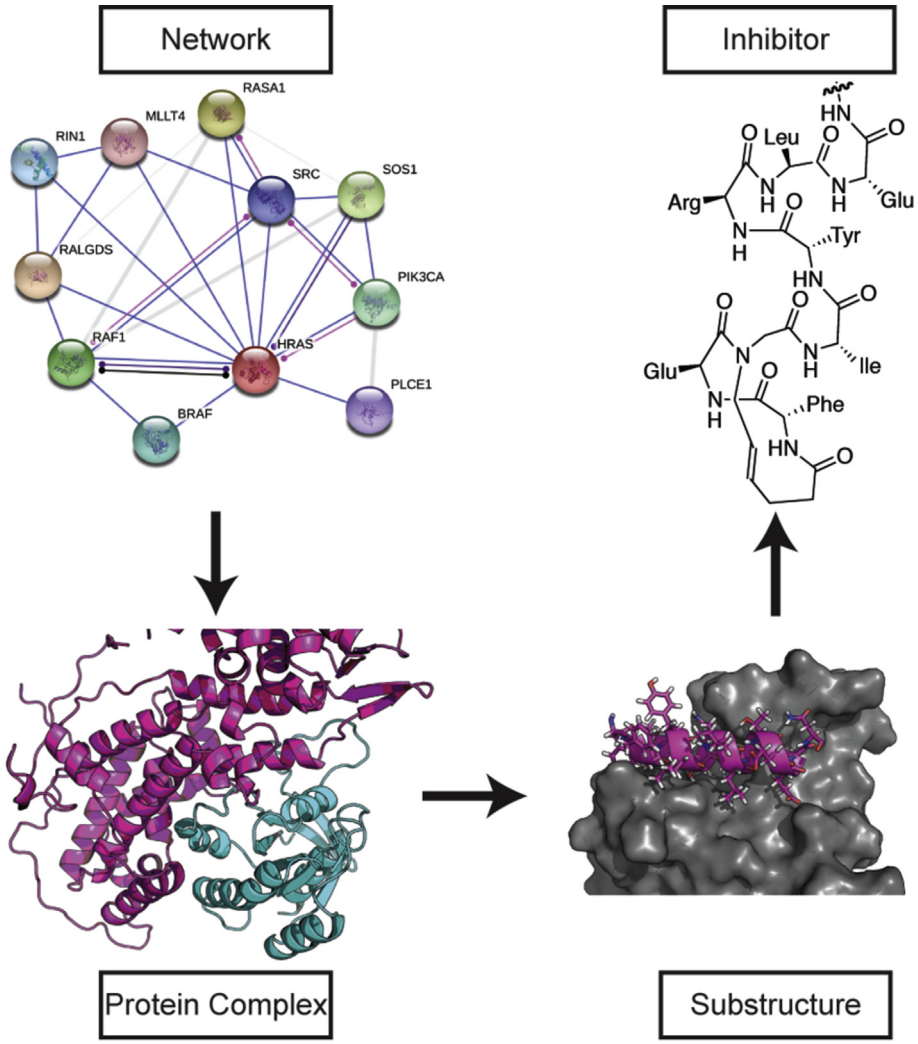

Fig. 1. Analysis of a diseased signaling network gives rise to specific protein complexes of interest, after which that complex is analyzed to identify minimal units of structure relevant for mimicry. Finally, a specific inhibitor molecule is designed based on that sub-structure.

sequences [16]. The connectivity of nodes in PPI networks is often employed to distinguish types of targets for prospective modulation [17]. High connectivity nodes likely have more off-target effects, which can potentially produce toxicity; on the other hand, low-connectivity nodes may be unlikely to have a meaningful effect on the disease phenotype. Synthetic inhibitors may be designed to be "frequent hitters" that are intrinsically nonselective or to specifically engage more than one target [18,19]. As an example of the latter case, tumors with wild-type p53 frequently over express two negative regulators, Mdm2 and Mdmx; drug molecules that promiscuously bind both negative regulators are highly desirable [20].

PPI networks are typically evaluated using gene knockdown strategies, such as RNAi, which result in total and irreversible abrogation of a protein's effects. Under such conditions, high-connectivity nodes are likely to produce a strong toxic effect. A distinguishing feature of molecular interaction inhibitors is that they are uniquely capable of specifically disrupting one edge of a network where the impact of modulating high-connectivity nodes can be titrated in a concentration dependent manner [21–24]. Thus, synthetic inhibitors afford dose-dependent controlled inhibition of specific sets of interactions for a particular protein [24–26].

Given a network believed to describe the interactions relevant to a certain disease state, the identification and analysis of the most important and inhibition–amenable interaction nodes is critical to develop useful PPI inhibitors. Several network analysis tools have been described. Network metrics beyond node connectivity can aid in target selection; for example, the pairwise disconnectivity index measures how essential a given protein is for sustaining the connection between two others [27]. Networks can even be used as inferential tools to support the existence of protein–protein interactions for which there exists no direct experimental evidence [28]. Johnson's interface interaction network, or IIN, describes which protein interfaces are commonly bound by multiple proteins and thus permits the early identification of potential off-target effects [29].

2.2. From protein–protein interaction to a structural model

Though there are hundreds of thousands of protein–protein interactions predicted in humans, there are fewer than twenty thousand non-redundant multiprotein complexes in the Protein

Please cite this article in press as: A.M. Watkins, P.S. Arora, Structure-based inhibition of protein–protein interactions, European Journal of Medicinal Chemistry (2014), http://dx.doi.org/10.1016/j.ejmech.2014.09.047