Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Druggable protein–protein interactions – from hot spots to hot segments

Nir London[1], Barak Raveh[2] and Ora Schueler-Furman[3]

Protein–**P**rotein **I**nteractions (PPIs) mediate numerous biological functions. As such, the inhibition of specific PPIs has tremendous therapeutic value. The notion that these interactions are 'undruggable' has petered out with the emergence of more and more successful examples of PPI inhibitors, expanding considerably the scope of potential drug targets. The accumulated data on successes in the inhibition of PPIs allow us to analyze the features that are required for such inhibition. Whereas it has been suggested and shown that targeting hot spots at PPI interfaces is a good strategy to achieve inhibition, in this review we focus on the notion that the most amenable interactions for inhibition are those that are mediated by a 'hot segment', a continuous epitope that contributes the majority of the binding energy. This criterion is both useful in guiding future target selection efforts, and in suggesting immediate inhibitory candidates – the dominant peptidic segment that mediates the targeted interaction.

Addresses
[1] Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA 94158, USA
[2] Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, CA 94158, USA
[3] Department of Microbiology and Molecular Genetics, Institute for Medical Research Israel-Canada, Hadassah Medical School, The Hebrew University, POB 12272, Jerusalem 91120, Israel

Corresponding author: Schueler-Furman, Ora (oraf@ekmd.huji.ac.il)

Current Opinion in Chemical Biology 2013, **17**:952–959

This review comes from a themed issue **Synthetic biomolecules**

Edited by **Shang-Cheng Hung** and **Derek N Woolfson**

For a complete overview see the Issue and the Editorial

Available online 31st October 2013

1367-5931/$ – see front matter,

http://dx.doi.org/10.1016/j.cbpa.2013.10.011

Introduction

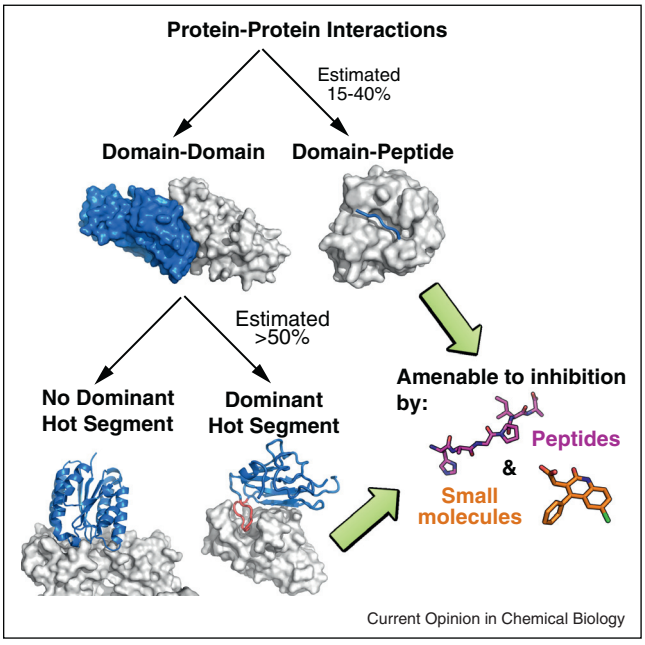
Accurate communication between different proteins is critical to the proper functioning of any living cell. The interaction between protein partners not only establishes macromolecular machineries such as ribosomes, polymerases and proteasomes, but also mediates transient signaling pathways, and timely regulatory processes. Protein–Protein Interactions (PPIs) can be mediated by the classical interaction between two globular protein domains. Alternatively, as much as 40% of the PPIs are estimated to involve peptide–domain interactions, through the binding of a short peptidic linear binding motif to a globular protein domain [1], or even by the co-folding of these linear motifs within two unstructured regions [2].

For some time, protein-protein interfaces were considered relatively flat and featureless (inspired among others by the seminal analysis of protein–protein interfaces by Thornton in 1996 [3]), and therefore hard to 'drug' using small molecules that prefer well-defined binding pockets [4]. However, from early on it was also shown that even seemingly featureless protein–protein interfaces contain 'hot spot' residues [5–7]. These often involve large amino acids such as Tyrosine, Arginine and Tryptophan that bind in small pockets across the interface and contribute the major part of the binding interaction energy. Additional studies that characterized protein–protein interfaces revealed a higher-level organization of these interfaces [8]. The prevalence of hot spot residues in both domain–domain and peptide–domain interfaces [9•] made it theoretically feasible to disrupt protein–protein (and peptide-protein) interactions with small molecules that target their binding sites. In recent years, the number of successful attempts at inhibiting PPIs with small molecules has increased considerably (and also been extensively reviewed by e.g. [10–13,14••] and many others). In this review, we survey distinctive features of protein–protein interfaces that are amenable for inhibition, and posit that these druggable PPIs are often dominated by 'hot segments', an extension of the concept of hot spots to a continuous binding epitope that dominates the interaction [15••]. The prevalence of hot segments in druggable PPIs highlights peptides and their derivatives as a suitable class of PPI inhibitors. We discuss various techniques for further optimization that can propel these starting points into potential drugs.

Characteristics of successfully inhibited PPIs

Following the accumulation of examples of small molecules that inhibit PPIs, databases such as TIMBAL [16], 2P2I [17] and iPPI-DB [18] have been dedicated to the collection of small molecule PPI inhibitors and relevant structural data on these PPIs. The 2P2I database organizes inhibited PPIs in two classes: peptide–domain interactions and domain–domain interactions [19•] (see Figure 1 and Table 1). A structural comparison of these complexes to other heterodimeric complexes suggested that the druggable 2P2I interfaces involve a much smaller buried surface area, are more hydrophobic, and do not show major conformational changes upon binding. Interestingly, we previously found that these features, in

Figure 1



Protein–protein interactions that are peptide-mediated or contain an interface hot-segment are suitable targets for inhibition. A substantial fraction of interactions (estimated up to 40% [1]) are mediated by a linear peptide stretch (domain–peptide interactions). Among the remaining domain–domain interactions, about half of the protein–protein interactions with solved structures contain one contiguous interface hot segment that contributes the dominant part of binding energy (as estimated using the PeptiDerive protocol [15••], see text).

particular the latter, are also characteristic of peptide–protein interactions [9•]. Another structural analysis of inhibited PPIs divided the interactions into four classes, based on whether they are narrow/wide and tight/loose [14••]. Among these, the narrow (surface area <2500 Å^2) and tight ($K_d < 200$ nM) PPIs are more amenable to inhibition. In turn, the inhibition of narrow and loose interactions often involves conformational changes and allosteric effects. Since these are more difficult to model and design, they are usually identified in *a posteriori* structural analyses of inhibitors detected by high throughput screening (HTS) approaches [14••].

A large share of PPIs are dominated by a hot segment

As evident from the large proportion of peptide-mediated interactions that are targeted by small molecule inhibitors (see Table 1), this prevalent form of interaction seems particularly suitable for inhibition. Peptide-mediated interactions are obviously dominated by a continuous binding epitope (namely, the peptide). More surprisingly however, this holds also for domain–domain interactions (e.g. interactions between two globular protein partners): these too are often dominated by one hot segment.

We have previously developed a protocol that, based on a solved complex structure, identifies the contiguous peptide epitope within a protein that contributes most to binding (Rosetta PeptiDerive [15••]). In a systematic analysis of standard benchmarks of domain–domain interactions using PeptiDerive, we estimated that more than 50% of those interactions are mediated by one hot segment that contributes the majority of the binding interaction energy [15••] (Figure 1). Application of the same methodology to the entire Protein Data Bank (PDB; [20]) has led Teyra *et al.* [21•] to estimate that almost 700 protein families are dominated by a hot segment at the interface of the protein–protein interaction. In a similar study that was restricted to α-helix mediated interactions, Jochim and Arora [22•] highlighted a set of ~400 complexes that contain a dominant α-helix at the interface.

Hot segments are good predictors of PPI druggability

To examine the relation between druggability and the existence of hot segments, we apply here the aforementioned PeptiDerive protocol to the druggable protein–protein complexes enlisted in the 2P2I database. The results suggest that indeed, one contiguous peptide dominates the interaction in practically all of these reported cases (Table 1). Trivially, for all 7 peptide-protein interactions, one short dominant epitope contributes 64–99% of the estimated total interaction energy. Less trivially so, however, PPIs of the second domain–domain class are also dominated by a single short continuous epitope that contributes 43–71% of the interaction energy. Remarkably, in all cases, we found that this epitope binds to the same site that is targeted by a known small molecule inhibitor (Figure 2). We conclude that it is the presence of a dominant hot segment at a protein–protein interface that often renders this PPI druggable.

Additional computational approaches for the prediction of PPI druggability

Accumulating knowledge about druggable PPIs has spurred the development of computational approaches to predict the 'druggability' of a query PPI. The most straightforward approach is to detect hot spot residues, either by experiment [5] or by computation [23]. This usually requires a solved structure of the PPI, or a very accurate model. Such models may be built using protein docking protocols of PPIs [13] or peptide-docking protocols [24].

The structure of a protein complex is not always available, but luckily it is also not always necessary in order to determine the druggability of an interaction. Using 'pocket biased' conformational sampling of unbound proteins on a subset of 2P2I, Johnson and Karanicolas find that druggable protein interaction sites display low-energy pocket-containing conformations that are not found elsewhere on the protein surface [25••].