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Review

Flexibility and small pockets at protein–protein interfaces: New insights into druggability

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ABSTRACT

The transient assembly of multiprotein complexes mediates many aspects of cell regulation and signalling in living organisms. Modulation of the formation of these complexes through targeting protein–protein interfaces can offer greater selectivity than the inhibition of protein kinases, proteases or other post-translational regulatory enzymes using substrate, co-factor or transition state mimetics. However, capitalising on protein–protein interaction interfaces as drug targets has been hindered by the nature of interfaces that tend to offer binding sites lacking the well-defined large cavities of classical drug targets. In this review we posit that interfaces formed by concerted folding and binding (disorder-to-order transitions on binding) of one partner and other examples of interfaces where a protein partner is bound through a continuous epitope from a surface-exposed helix, flexible loop or chain extension may be more tractable for the development of "orthosteric", competitive chemical modulators; these interfaces tend to offer small-volume but deep pockets and/or larger grooves that may be bound tightly by small chemical entities. We discuss examples of such protein–protein interaction interfaces for which successful chemical modulators are being developed.

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Contents

1. Introduction

Multiprotein assemblies mediate the majority of cellular processes, including receptor activation, signal transduction, DNA replication, recombination and repair, and other regulatory events that require high signal-to-noise in cell regulation. Multiprotein assemblies often arise from initial weak binary interactions followed by cooperative, higher-order complex formation, giving high selectivity while at the same time being transient as required for termination of regulatory signals (Higueruelo et al., 2013a).

Multiprotein regulatory systems are assembled mainly through protein–protein interactions (PPIs). Whereas enzyme superfamilies that mediate many signalling events may number hundreds of homologues in the human genome – more than 500 protein kinases and over 600 putative E3 ubiquitin (Ub) ligases (Li et al., 2008) – multiprotein regulatory systems differ widely across each superfamily. The specificity of PPIs offers potential for the development of chemical and biological modulators that target specific pathways, with advantages of selectivity that tend to be

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difficult to achieve through inhibitors of members of enzyme superfamilies, which tend to be mechanism based, targeting transition/intermediate states or co-factor-binding sites that are similar across the superfamily (Bolanos-Garcia et al., 2012).

Using criteria derived from retrospective analyses of successful drugs, protein–protein interaction sites have historically been described as undruggable (Hopkins and Groom, 2002). Indeed, many protein–protein interfaces, especially those in obligate complexes such as homo-oligomers for the presence of which usually improves stability, have been viewed as large, flat and featureless, and thus difficult targets for the development of small molecule antagonists (Blundell et al., 2000, 2006; Jones and Thornton, 1996). With the wealth of information available from structural biology programmes, and advances in experimental and computational assessment of druggability, this traditional view of protein–protein interaction interfaces is being reassessed (Kastritis and Bonvin, 2013; Loving et al., 2014; Villoutreix et al., 2014), presenting new insights for the development of "orthosteric" PPI modulators that compete for the binding-site surface of a PPI interface, typically with the objective of sterically inhibiting the association of a multiprotein complex.

In this review we highlight the importance of relatively small pockets that can lead to very selective binding at PPI interfaces (Blundell et al., 2006; Jubb et al., 2012; Koes and Camacho, 2012a, 2012b). We show that small, single-residue sub-pockets and regions of surface depth bound by continuously interacting peptide segments extend the concept of druggability in ways peculiar to protein–protein interactions (Ben-Shimon and Eisenstein, 2010; Fuller et al., 2009; Guo et al., 2014; Koes et al., 2012; Kozakov et al., 2011; Li et al., 2004b; London et al., 2010, 2013; Rajamani et al., 2004; Winter et al., 2012) and provide tractable sites for the development of chemical modulators (Arkin et al., 2014). We posit that interactions involving short peptides, linear binding motifs within larger intrinsically disordered regions or within loops or loop-termini of globular proteins, and possibly linear epitopes arising from surface exposed helices, can provide promising binding sites. The loss of entropy on binding a flexible peptide is likely countered by binding larger sidechains, such as those of tryptophan, tyrosine, phenylalanine or arginine, in distinct preformed pockets (Blundell et al., 2006), or even smaller hydrophobic residues such as alanine in pockets where they may relieve energetically "unhappy" surface waters (Huggins et al., 2011).

2. Flexibility in partner interactions

Binary PPIs, which have been targeted in drug discovery and in which different degrees of conformational change and loss of entropy occur on binding, can be described by three models: those where both partners have preformed, relatively rigid structures; those where one or both of the preformed structures undergo significant conformational changes on interaction; and those where one of the structures folds as it binds (Fig. 1) (Blundell and Wood, 1982; Blundell et al., 2006; Pawson and Nash, 2003). There are also some cases where both partners may fold on interaction, but these are relatively uncommon and may less likely provide targets, at least for binding to one of the partners in isolation; for example where homodimers that are expressed simultaneously fold together permanently in an intertwined or interdigitated structure (Bonvin et al., 1994; Kishan et al., 1997). Numerous databases including the 3D Interaction Domains (3DID (Stein et al., 2011); http://3did.irbbarcelona.org/), Domain Annotated Protein–protein Interaction Database (DAPID (Chen et al., 2006); http://gemdock.life.nctu.edu.tw/dapid) and PICCOLO (Bickerton et al., 2011) (http://www-cryst.bioc.cam.ac.uk/piccolo), have documented structural aspects of PPIs and shown that each of these models is quite common; for reviews of structures, lists of databases and tools for studying protein–protein interactions see (Tuncbag et al., 2009; Villoutreix et al., 2013; Winter et al., 2012).

The first two models involve interactions between globular proteins (see Fig. 1). These represent the "traditional" PPI interface, often described as large (~1500–3000 Å^2), flat and relatively featureless interfacial surfaces (Blundell et al., 2000; Jones and Thornton, 1996). The view that these interfaces are featureless has been challenged by the discovery that a few amino acids – so-called hotspots (Clackson and Wells, 1995) – may contribute the majority of interaction free energy in many PPI systems, giving reason for some optimism with respect to targeting specific "hot regions" with chemical modulators (Bogan and Thorn, 1998; Clackson and Wells, 1995; Cukuroglu et al., 2014; Wells and McClendon, 2007). It has been proposed that continuously interacting interface "segments" (Jones and Thornton, 1996; London et al., 2013; Pal et al., 2007) may also play a major role in the architecture of globular protein interfaces, for example the interfaces in TEM1-BLIP and EphB4-EphrinB2 (London et al., 2010).

The third model of protein interaction involves a natively unstructured protein that folds upon interaction with another partner. This was proposed for peptide hormones in the 1970s by Robert Schwyzer (Schwyzer et al., 1979) and experimentally exemplified by X-ray analysis and NMR studies of glucagon in the Blundell and Wüttrich labs (Braun et al., 1983; Sasaki et al., 1975) suggesting a disorder-to-order transition on receptor binding from glucagon with a single turn of helix in solution by NMR (Braun et al., 1983) to one with a much longer region defined by X-ray analysis in the trimer (Sasaki et al., 1975) and at lipid interfaces (Braun et al., 1983) and proposed at the receptor (Blundell, 1979; Blundell and Wood, 1982). Subsequently, Wright & Dyson (Wright and Dyson, 1999, 2009) showed that such concerted folding and binding involving peptides or disordered regions of polypeptide chains is actually widespread in intracellular regulatory systems. To obtain a high-affinity interaction, it would be expected that the smaller surface area provided by peptides and small continuous epitopes requires surface pockets to anchor the peptide in order to maximise intermolecular interactions and to benefit entropically from surface water release into bulk solvent.

An example of a protein–protein interface involving concerted folding and binding of a flexible peptide is the binding of human recombinase Rad51 to BRCA2 in an interaction that is essential for DNA double-strand-break repair through homologous recombination (Pellegrini et al., 2002). The BRC4 peptide found in BRCA2 folds into a defined 3-dimensional structure only upon interacting with Rad51, a disorder-to-order transition (Fig. 2) (Pellegrini et al., 2002). BRCA2 binding disrupts self-association of RAD51 by mimicking RAD51's conserved self-association motif, FxxA (Pellegrini et al., 2002). The conserved phenylalanine of the FxxA motif of BRC4 binds in a deep "anchor" pocket of Rad51, while the conserved alanine binds in a small hydrophobic pocket. Binding to both pockets probably contributes to favourable entropic changes in the system through the release of energetically "unhappy" waters (Huggins et al., 2011).

3. The landscapes of pairwise protein–protein interfaces

Drug-like molecules typically exert their actions through binding to high-affinity sites of the right shape and chemical composition. These were traditionally viewed to not be present in the relatively flat and featureless PPI interfaces. Analyses of PPI interfaces using new computational tools can identify key residues in interfaces mediating the protein–protein interaction (Pires et al., 2014) and potential binding sites (Hendlich et al., 1997; Kalidas and Chandra, 2008; Laurie and Jackson, 2005; Morita et al.,

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