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Teaser The present review proposes a classification of the successfully stabilized protein–protein interactions (PPIs) using small molecules because it represents a new era for PPI modulation that needs to be addressed.

Stabilization of protein–protein interaction complexes through small molecules

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Most of the small molecules that have been identified thus far to modulate protein–protein interactions (PPIs) are inhibitors. Another promising way to interfere with PPI-associated biological processes is to promote PPI stabilization. Even though PPI stabilizers are still scarce, stabilization of PPIs by small molecules is gaining momentum and offers new pharmacological options. Therefore, we have performed a literature survey of PPI stabilization using small molecules. From this, we propose a classification of PPI stabilizers based on their binding mode and the architecture of the complex to facilitate the structure-based design of stabilizers.

Introduction

Protein–protein interactions (PPIs) play a key part in the vast majority of biological processes. The human interactome has been estimated to contain up to 650 000 [1] interactions and therefore represents an unprecedented opportunity for pharmacological innovation. Modulation of PPI complexes can be achieved in several ways. They can be inhibited or stabilized through allosteric or orthosteric binding. Allosteric and orthosteric binding can lead to complex stabilization or inhibition. Allosteric modulators bind at a distant location from the interface and remotely act on protein complex association by triggering a conformational change. Orthosteric modulators bind near or at the interface and directly promote complex stabilization (by acting as a glue) or inhibition (by competing with one of the protein partners). Whereas the identification of allosteric and orthosteric PPI inhibitors has witnessed an impressive number of success stories [2–4], the strategy of stabilizing PPIs remains underexploited.

Similarly to the inhibition of PPIs, stabilization of dimers or oligomers can lead to either activation or inhibition of a biological function. But stabilizing PPI complexes with small compounds (i.e. targeting regions at or near the interfaces of two or multiple proteins) can

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benefit from a significantly higher specificity [5]. The fact that such small molecules do not compete with any of the canonical partners (or substrate when dealing with enzymes), as with most current drugs, prevents the necessity for having a binding affinity in the low nanomolar range to initiate a physiological effect [5]. Jasmonate compounds, for example, elicit a biological response despite their binding affinity being only in the micromolar range [6] (Table 1). PPIs display greater sequence diversity than enzyme active sites, and therefore it should be relatively easy to obtain higher specificity for PPI modulators than for enzyme ligands [5]. A study of rim-exposed (i.e. periphery of the binding interface) PPI cavities revealed that they are physicochemically highly similar to cavities observed in classical drug targets [7]. Moreover, as opposed to a significant number of PPIs targeted by inhibitors, the binding pockets formed by the association of two or several partners show a more favorable druggable profile [5,7]. Fig. 1 shows the state function for the hypothetical formation of a protein trimer in the presence and absence of a PPI stabilizer.

Given the advantages of therapeutic intervention by PPI modulation, it is not surprising that we observe a growing interest in PPI stabilization by natural and synthetic compounds. Such compounds are identified through various techniques including virtual ligand screening (VLS) and HTS. In this review, we describe several targets for which such compounds have been identified. We will discuss their modulation mechanisms, the associated pathologies, techniques used for their discovery and the architecture of the resulting PPI–modulator complexes. We use this information to suggest a conceptual scheme for the classification of stabilized PPI complexes to assist bioscientists interested in the structure-based design of PPI stabilizers.

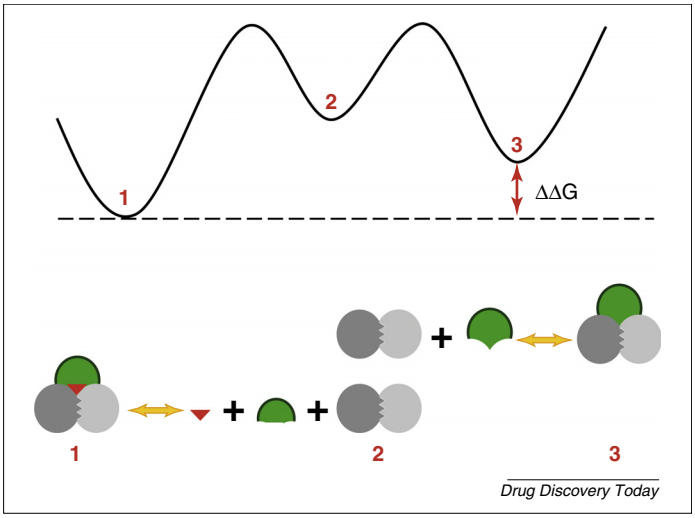

FIGURE 1

State diagram for a trimeric complex in the presence and absence of a protein–protein interaction (PPI) stabilizer. The grey protein dimer (2) can bind to the green protein partner in the presence (1) or absence (3) of the small red PPI stabilizer. The $\Delta\Delta G$ of binding the small red stabilizer is indicated. This figure illustrates the thermodynamic concept that binding always leads to a state with a lower energy but does not always need to lead to stabilization. For this hypothetical case this means that in the presence of the small red modulator there will be more trimer present than in its absence.

Description of PPI modulator complexes

PPI complexes can be heteromers, homomers or quasi homomers. Quasi homomers are multimers consisting of homologous monomers in which the individual proteins perform identical or highly similar functions, like murine double minute 2/x (MDM2 and MDMX) [8] or c-Myc and Max [8]. So far, all heteromers and quasi homomers with solved structures have been dimers, whereas homomers also exhibit higher protein copy numbers and various structural symmetry. As described by Monod *et al.* [9], oligomers of identical units or homologous protein units can be categorized as isologous or heterologous with structural symmetry [10]. Isologous homomer association is characterized by the same interaction patch from both partners around a twofold axis of symmetry. Conversely, heterologous homomer association uses distinct interaction patches from the different partners that, without a closed (cyclic) symmetry, can lead to infinite aggregation [11]. Our literature survey of a series of diverse PPI targets that were successfully stabilized through the binding of small molecules revealed several strong trends in the mode of binding of those small molecules and in the global architecture of the resulting complexes. Indeed, the majority of stabilized PPI complexes can be described using simple structural features. The main feature is the nature of the partners and their symmetry in the complex. Homomers (homodimers and oligomers) are mostly *n*-fold symmetric where *n* is the copy number of the monomer in the complex. The second feature is the location of the cavity in which stabilizers bind. Two types of locations were observed: peripheral and enclosed. Peripheral cavities are located at or in the direct vicinity of the interface. They are solvent exposed whereas enclosed cavities are completely buried in the core of the PPI complex. The stoichiometry of the stabilizer within PPI complexes is not used as a feature because of its unpredictability. However for a significant number of complexes more than one copy of a given stabilizer was found to bind to the protein complex. This was observed mostly in homomers and quasi homomers. The two structural features for stabilized PPI complexes combined with structural data (X-ray crystallography, NMR) naturally lead to a classification of all PPI stabilizers that exert their stabilizing effect through binding at or very near to the PPI interface.

Fig. 2 summarizes the six PPI–stabilizer classes and subclasses that result from the analysis of all cases of PPI with known stabilizers (at the time of the survey) and a three-dimensional structure using the aforementioned structural features. Class A stabilizers are associated with heteromer complexes. Class B stabilizers are associated with homomers and quasi homomers that are associated in an isologous way. Class M stabilizers are homodimers or oligomers of more than two monomers that associate in a heterologous manner. Subclasses 1 and 2 relate to the cavity type being either peripheral or enclosed.

Class A: heterodimers

At the time of this study, four complexes were classified as class A1 (the 14-3-3 proteins with four different partners, ubiquitin–E2–ubiquitin-conjugating-enzyme, ARF1–Sec7 and HDAC3–SMRT-DAD complexes) and two as class A2 (the plant growth hormones and SK2–CaM). With four complexes, the 14-3-3 family was the largest of all families studied.

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