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Stabilization of Protein–Protein Interactions in chemical biology and drug discovery

David Bier [a, b, 1], Philipp Thiel [c, 1], Jeroen Briels [a, b], Christian Ottmann [a, b, *]

[a] Laboratory of Chemical Biology, Department of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, Den Dolech 2, 5612 AZ Eindhoven, The Netherlands
[b] Department of Chemistry, University of Duisburg-Essen, Universitätsstrasse 7, 45117 Essen, Germany
[c] Applied Bioinformatics, Center for Bioinformatics, and Dept. of Computer Science, University of Tübingen, Sand 14, 72076 Tübingen, Germany

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ABSTRACT

More than 300,000 Protein–Protein Interactions (PPIs) can be found in human cells. This number is significantly larger than the number of single proteins, which are the classical targets for pharmacological intervention. Hence, specific and potent modulation of PPIs by small, drug-like molecules would tremendously enlarge the "druggable genome" enabling novel ways of drug discovery for essentially every human disease. This strategy is especially promising in diseases with difficult targets like intrinsically disordered proteins or transcription factors, for example neurodegeneration or metabolic diseases. Whereas the potential of PPI modulation has been recognized in terms of the development of inhibitors that disrupt or prevent a binary protein complex, the opposite (or complementary) strategy to stabilize PPIs has not yet been realized in a systematic manner. This fact is rather surprising given the number of impressive natural product examples that confer their activity by stabilizing specific PPIs. In addition, in recent years more and more examples of synthetic molecules are being published that work as PPI stabilizers, despite the fact that in the majority they initially have not been designed as such. Here, we describe examples from both the natural products as well as the synthetic molecules advocating for a stronger consideration of the PPI stabilization approach in chemical biology and drug discovery.

1. Introduction

Modulation of Protein–Protein Interactions (PPIs) with small molecules is a promising strategy for the development of basic chemical biology tools as well as drug discovery. While 10 years ago this field was still somewhat "exotic", an impressing number of success stories have in the meantime established small molecule PPI modulation as an integral part of chemical biology. Furthermore, there is most probably no pharmaceutical company that has not at least a few PPIs among their actively followed target portfolio. However, this increase of activities and enthusiasm seems to apply mostly on targeted **inhibition** of PPIs with the opposite strategy, targeted **stabilization** of protein complexes, being widely overlooked (Thiel et al., 2012). This certainly is astonishing given the number of excellent examples from nature, where small molecule natural products like rapamycin, cyclosporine A, FK506, brefeldin A, forskolin, fusicoccin A, auxin, jasmonate, brassinolide, and inositol tetraphosphate convey their physiological activity by stabilizing PPIs. Structural elucidation of their target protein complexes showed that these natural products bind mostly to ***rim-of-the-interface*** pockets and thus directly stabilize the proteins' interaction by simultaneously establishing contacts to both partners. This direct mode-of-action is different from also known ***allosteric*** stabilizers of PPIs like paclitaxel, which stabilizes microtubule without contacting more than one protein. Here, we will exclusively discuss the direct, "molecular glue"-like enhancement of PPIs.

In addition to the still growing number of natural-product examples of PPI stabilizers we also review recent examples of synthetic small molecules originating from chemical biology/drug discovery programs that have been found to inhibit their target protein function by acting as stabilizers of an inactive, hetero- or homo-oligomeric state of their target proteins. These compounds

* Corresponding author. Laboratory of Chemical Biology, Department of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, Den Dolech 2, 5612 AZ Eindhoven, The Netherlands.
E-mail addresses: c.ottmann@tue.nl, christian.ottmann@uni-due.de (C. Ottmann).
[1] Contributed equally.

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have thus been found in screens for inhibitors of protein functions with the only later – and sometimes surprising – structural biology finding that they are also PPI interface binders that in principle work in the same way as the aforementioned natural products.

2. Natural-product PPI stabilizers

2.1. Rapamycine, cyclosporine A and FK506

Maybe the best-known and most-widely applied PPI stabilizers are the immunosuppressants rapamycin (Rp), cyclosporine A (CsA), and FK506. For some time it was a puzzling question how these quite different molecules could convey their similar physiological activity, especially given the fact that binding to their direct cellular receptor proteins – Cyclophilin A for CsA (Handschumacher et al., 1984), FKBP12 for Rp and FK506 (Bierer et al., 1990) – is not sufficient to explain their immunosuppressive effect. This obscurity was elucidated by the finding that the complexes of Cyclophilin/CsA and FKBP12/FK506 bound to and inhibited the phosphatase calcineurin (Liu et al., 1991), whereas the FKBP12/Rp complex associated with FRAP (later mTOR, mammalian Target of Rapamycin) and inhibited its protein kinase activity (Brown et al., 1994). The structural details of how these molecules convey their PPI-stabilizing action were reported shortly later by the publication of the ternary complex structures of FKBP12/FK506/Calcineurin (Griffith et al., 1995), FKBP12/Rp/mTOR (Choi et al., 1996), and Cyclophilin/CsA/Calcineurin (Huai et al., 2002). All three molecules are deeply buried in the interface of their respective target complexes (Fig. 1). This binding mode is insofar different from the following examples since CsA, FK506 and Rp are not binding to a preformed pocket of a binary complex but rather induce the interaction of proteins that do not interact under physiological conditions.

2.2. Cell biology tool compounds brefeldin A and forskolin

Brefeldin A (BFA) is an important cell biology tool compound that is used for the analysis of membrane trafficking. BFA inhibits golgi functions by stabilizing the dead-end complex of the small G protein ARF1 with its guanine exchange factor ARF-GEF (Peyroche et al., 1999). Although BFA shows impressive cellular effects its actual IC_{50} of about 10–15 μM (Table 1) and a ten-fold stabilization of the dead-end complex (Peyroche et al., 1999; Zeeh et al., 2006) is surprisingly low. The X-ray structure of BFA in complex with ARF1 and the Sec7 domain of the human ARF-GEF ARNO (Fig. 1) shows how BFA is accommodated in a pocket at the ARF1/Sec7 interface with one third of its surface contacting Sec7 and two thirds contacting ARF1 (Renault et al., 2003). A similar binding mode can be observed with a further cell-biology tool compound, forskolin (FSK), which activates adenylyl cyclase and hence increases the intracellular cAMP level (Sunahara et al., 1997). The X-ray structure of the complex between BFA, the two adenylyl subunits C1 and C2 and the stimulatory G protein subunit $G_{s\alpha}$ showed how FSK promotes the formation of the active, cAMP-producing complex (Tesmer, 1997). In this structure, FSK binds to a composite, strongly hydrophobic pocket that is formed at the rim of the interface of C1/C2 heterodimer (Fig. 1). Comparable to BFA, also with FSK a relatively low EC_{50} of about 10 μM (Table 1) for the activation of adenylyl cyclase is observed (Seamon et al., 1981; Sunahara et al., 1997), hinting at the possibility that the uncompetitive nature of these compounds allow for high physiological activity despite relatively low binding affinities.

2.3. Fusicoccines, stabilizers of 14-3-3 PPIs

A further example of a rim-of-the-interface binding small molecule PPI stabilizer is the diterpene glycoside fusicoccin A (FCA), a wilt-inducing fungal toxin (Ballio et al., 1964) that is stabilizing a complex of the adapter protein 14-3-3 and the plasma membrane H^+-ATPase PMA of plants (Chung et al., 1999; Svennelid et al., 1999). Elucidation of the crystal structure of FCA bound to 14-3-3 and the C-terminal regulatory domain (CT52) of PMA revealed that the natural product fills a gap in the interface of the binary 14-3-3/CT52 complex thereby stabilizes the interaction, mainly by decreasing the dissociation rate (Ottmann et al., 2007). The K_D of FCA binding to the complex of 14-3-3 and an 8-amino acid peptide derived from the phosphorylated C-terminus of PMA has been measured by ITC to be ~0.7 μM (Table 1) and the complex is stabilized in the presence of FCA by a factor of 90 (Würtele et al., 2003).

FCA can also nicely be used as chemical biology tool compound in a human physiology context. We have for example shown that FCA stabilizes the interaction of 14-3-3 proteins with the C-terminal recognition motif of estrogen receptor alpha (ERα). Here, FCA-stabilized binding of 14-3-3 proteins to ERα inhibits the transcriptional activity of the nuclear receptor by interfering with its dimerization and presumably the nuclear import (De Vries-van Leeuwen et al., 2013).

The fact that FCA is well-tolerated in human cells makes this molecule a nice candidate for a chemical inducer of dimerization (CID) of any protein of interest. To use FCA in such a way we fused 14-3-3 and the PMA peptide CT52 with different proteins of interest (POIs) to change their subcellular localization. As an application example we used FCA to induce the secretion of the cytokine IL-8 from cells transfected with NFκB/CT52 fusions (Skwarczynska et al., 2013).

An FCA-related natural product is the anti-cancer agent cotylenin A (CNA), which has been shown to be active against leukemia (Honma et al., 2003), breast cancer (Kasukabe et al., 2005), and ovary carcinoma (Honma et al., 2005). A possible explanation of these anti-cancer activities might be stabilization of 14-3-3 PPIs with partner proteins like the protein kinases of the Raf family (Dumaz and Marais, 2003; Molzan and Ottmann, 2012; Molzan et al., 2010; Ottmann et al., 2009). Recently, we have shown how CNA binds to the interface of a complex between 14-3-3 and the N-terminal 14-3-3 recognition motif of C-Raf and increases the apparent K_D of the complex from 350 nM down to 20 nM by biophysical measurements and protein crystallography (Molzan et al., 2013). The family of fusicoccins is also amenable to semi-synthetic derivatization in order to enhance specificity towards a given 14-3-3 PPI. Starting from the natural product fusicoccin J the group of Kato synthesized a derivative (FC-THF) harboring an additional ring system that stabilized the binding of 14-3-3 to the potassium channel TASK3 leading to an increase of plasma membrane insertion of this channel in *Xenopus* oocytes (Anders et al., 2013). The EC_{50} of the stabilizing activity of FC-THF towards the 14-3-3/TASK3 complex was measured to be ~12 μM (Table 1) (Anders et al., 2013), a value very similar to the activities of BFA and FSK.

2.4. Microtubule stabilizers

A number of microtubule-targeting molecules like vinblastine, colchicine, phomopsin A, and ustiloxin D are well-known modulators of microtubule stability. Protein crystallography studies revealed that vinblastine and phomopsin A act as a wedge between at the β1-α2 interface and restrict its flexibility (Wang et al., 2007). Here, both molecules stabilize a helical, curved assembly of tubulin subunits thereby preventing the buildup of straight protofilaments (Cormier et al., 2008). Although the net effect of these natural

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