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Edited by Alexander Dömling

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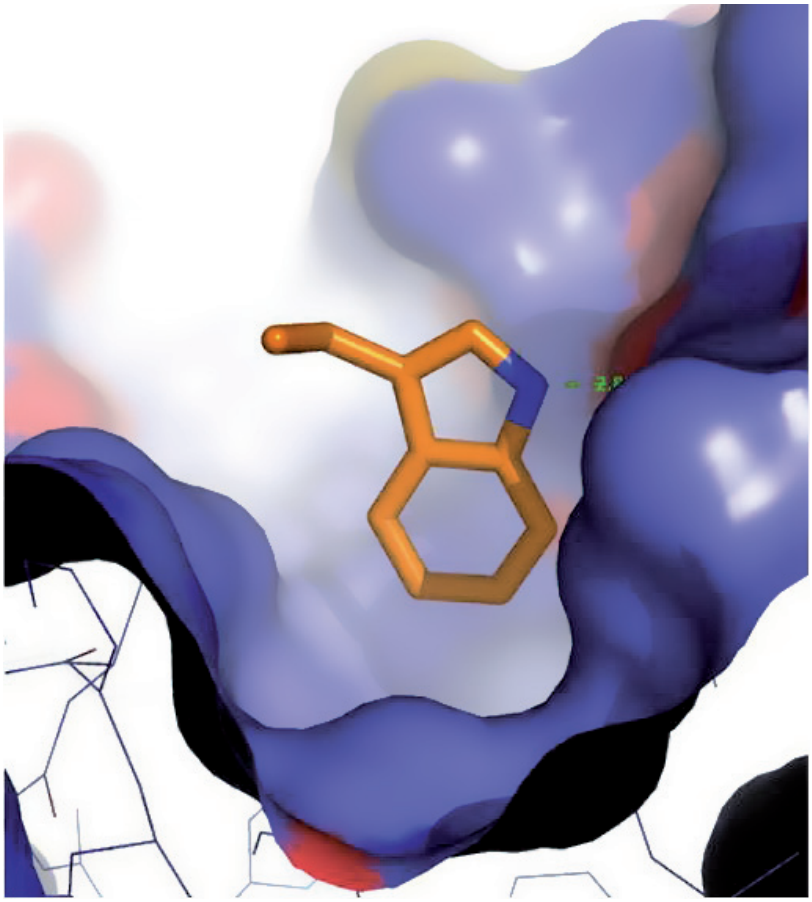

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Protein–Protein Interactions in Drug Discovery

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Cover
Cut-away view of the co-crystal structure of an archetypical protein- protein interaction: the tumor suppressor p53 and the oncogene MDM2 (PDB ID 1YCR). The most deepest buried p53 amino acid Trp23, anchoring the PPI, is shown in sticks presentation, the MDM2 receptor is shown as surface. Also shown the hydrogen bond between the Trp23 indole-NH and the receptor Leu54 carbonyl as green dotted line. The picture was rendered using PYMOL software.

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Preface

In the common definition of the proteome it is considered to be the set of expressed proteins in a given type of cells or an organism at a given time under defined conditions. It is larger than the genome, due to special mechanisms in the biogenesis of proteins, which render them more numerous than the genes at the end. What, however is very special to the proteome is its enriched complexity which arises both from the protein's 3D structure and the functional interaction of the proteins.

Proteins talk to each other. They do it very frequently and in different ways and obviously in large communication networks of sophisticated structure. For yeast, recent research has suggested kind of social life in protein communication:

> *This distinction suggests a model of organized modularity for the yeast proteome, with modules connected through regulators, mediators or adaptors, the date hubs. Party hubs represent integral elements within distinct modules and, although important for the functions mediated by these modules (and therefore likely to be essential proteins), tend to function at a lower level of the organization of the proteome. We propose that date hubs participate in a wide range of integrated connections required for a global organization of biological modules in the whole proteome network (although some date hubs could simply be 'shared' between, and mediate local functions inside, overlapping modules). (. . .) Finally, it is possible that discriminating between date and party hubs might also help to define new therapeutic drug targets.*[1]

Well, there we are in defining ambitious goals for drug development. Interfering with the party gossip and intruding the clandestine dates of proteins for sake of therapeutic benefits is still the freestyle of drug design. The complexity is huge. Large interfaces that resemble flat landscapes, lacking cosy caves or deep pockets, where small molecules might dock made protein-protein interactions clearly undruggable for a long time. With the advent and success of monoclonal therapeutic antibodies (mAbs) however, this view changed. The benefits of mAbs in the cancer

[1] Jing-Dong J. Han, Nicolas Bertin, Tong Hao, Debra S. Goldberg, Gabriel F. Berriz, Lan V. Zhang, Denis Dupuy, Albertha J. M. Walhout*, Michael E. Cusick, Frederick P. Roth & Marc Vidal. *Evidence for dynamically organized modularity in the yeast protein–protein interaction network.* Nature, 430, 2004, pp. 88-93

and immune-disease areas showed that interfering with protein-protein interactions (PPIs) is indeed a practicable approach. Unfortunately the proteins parties and dates are taken place inside the cell, with no admittance for mAbs. Hence, the challenge or the "reaching for high-hanging fruit"[2] is to interfere therapeutically by use of small molecules.

In the present volume Alexander Dömling and his co-authors depict a scenario that reveals more than a silver lining on the horizon. There is a variety of new (and old) targets and a plethora of new approaches and insights into molecular mechanisms which raise the hope that in the near future small molecule interference of protein-protein interactions will emerge as a new functional class of therapeutics. Twelve chapters illuminate opportunities, strategies, success stories and pitfalls in the discovery of small molecules targeting protein-protein-interactions.

While this volume grants an encompassing view on the state-of-the-art in the PPI field, it is at the same time structuring the future, since it paves the way for a hopefully greater commitment in drug discovery focusing PPIs.

Not least because of this, the series editors are indebted to the authors and the editor who made this comprehensive issue possible. We are convinced that the book represents an important contribution to the body of knowledge in drug discovery and that it matches the interests of many researches who have adjourned to a promising but rocky field.

In addition, we are very much indebted to Frank Weinreich and Heike Nöthe, both at Wiley-VCH. Their support and ongoing engagement, not only for this book but for the whole series "Methods and Principles in Medicinal Chemistry" adds to the success of this excellent collection of monographs on various topics, all related to drug research.

November 2012

Düsseldorf	Raimund Mannhold
Weisenheim am Sand	Hugo Kubinyi
Zürich	Gerd Folkers

[2] James A. Wells and Christopher L. McClendon. *Reaching for high-hanging fruit in drug discovery at protein–protein interfaces.* Nature 450, 2007, 1001-1009

A Personal Foreword

Therapeutic targeting of protein–protein interactions (PPIs) has been in the past largely the domain of biotech industries. With their exquisite natural selection process, and the resulting high affinity and selectivity towards their targets, monoclonal antibodies (mAbs) and similar biologics have been the only way to target large protein interfaces to effectively compete with the endogenous interaction. Meanwhile, mAbs have overcome their early issues, and the generation of safe, specific, high-affinity, and nonimmunogenic antibodies has become a reality. In fact, a switch of the pharmaceutical industry from focusing traditionally on small molecules towards the biotech drugs has recently been observed based on the therapeutic and business success of the biologics; major takeovers of biotech companies by big pharma excite the industry and shareholders. Severe conditions can meanwhile be treated with mAbs, including some cancers, Crohn's disease, rheumatoid arthritis, transplant rejection, or macular degeneration, just to name a few. However, large biomolecules like mAbs also have intrinsic disadvantages that are hard to overcome, such as lack of oral bioavailability, high cost of goods, and perhaps most importantly mAbs can only target extracellular accessible structures. The majority of PPI drug targets, are, however, localized intracellularly.

The strength of small molecules, in principle, is to overcome exactly the issues of mAbs and similar biologics: they can be made orally bioavailable and decorated with advantageous pharmacokinetics/pharmacodynamics properties, they can be produced orders of magnitude cheaper then biologics, and they can also be designed to penetrate cell membranes to reach intracellular targets. Therefore, small molecules should be ideal to disrupt PPIs. However, PPIs very often consist of large interfaces of greater than 1000 $Å^2$ and do not contain the deep pockets medicinal chemists are used to from other target areas, such as kinases, G-protein-coupled receptors, or proteases. Pocket dimension, form (concave versus convex), and hydrophobicity are, however, key features defining the druggability by small molecules. Analysis of the plethora of available crystal structures of PPIs reveals that only a fraction of PPIs fit the druggability criteria. Nevertheless, while PPIs as a class were considered as undruggable a decade ago, several small molecules advancing into clinical trials recently clearly support the notion that at least a fraction of the PPIs are not only attractive therapeutic targets, but can also be developed to drugs. It can be foreseen

that in the future many more small molecules targeting PPIs will emerge for the treatment of unmet medical needs.

This book aims to address an audience including medicinal chemists, organic chemists, and other interested readers. In 12 chapters written by experts in their fields I try to introduce the reader to the problems and opportunities associated with targeting PPIs by small molecules. Introductory overviews pave the way for specific medicinal chemistry showcases on developmental compounds. Additionally, several chapters introduce technologies relevant for PPI drug discovery, including the interactome and computational chemistry.

Ottmann gives an excellent introductory overview on different PPI drug discovery approaches and compound classes, and the structural features of their interacting proteins.

Next, Schleker *et al*. introduce the reader to different techniques used in systems biology to define the interactome of different species. Scope and limitations of the current experimental and computational identification approaches to obtain interactomes related to human are discussed. Cleary only careful comparison of the large-scale results of orthogonal experimental approaches can lead to meaningful results and a deeper understanding of the interactome's wide regulation of life.

Fry and So stress the importance of three-dimensionality in the modulation of PPIs – a shape feature often under-represented in company screening libraries. From structural analysis based on the Protein Data Bank (PDB) database they deduce simple rules of thumb for when a particular PPI is druggable by small molecules.

Villoutreix *et al*. introduce the reader to computational chemistry approaches towards PPI drug discovery. Detailed discussions on the relationship and complementarity of the shape of the receptor binding site and the scaffold shape clarify why traditional chemical space is relatively unsuited to find PPI antagonists. The specifics of PPI-targeting compound classes are elaborated in a detailed analysis of many descriptors. Rather novel scaffold and privileged structures are needed to address the PPI interfaces.

Web-based techniques to computationally predict small-molecule PPI inhibitors are introduced by Camacho *et al*. The AnchorQuery™ approach makes use of the disproportional high importance of anchoring residues in PPIs, and queries the very large chemical space and one-pot accessibility of multicomponent reactions (MCR). Again, the importance of suitably designed libraries for PPIs is stressed.

Luccarelli *et al*. review the Src homology 3 (SH3) domain as a drug target. SH3 domain proteins comprise a very large class with implications in multiple therapeutic areas. The structural biology is reviewed and, based on the understanding of specific features of the binding pocket, current inhibitor design is discussed.

The PPI of the transcription factor p53 and the oncogene MDM2 is amongst the most intensively studied PPIs with several compounds in late preclinical and early clinical development. Khoury *et al*. review this area with a focus on small-molecule classes supported by structural biology information, their structure–activity relationships (SARs), and the methods of initial hit finding.

Guckian reviews the area of inhibitors of the lymphocyte function-associated antigen-1/intracellular adhesion molecule (LFA-1/ICAM) interaction for the

treatment of autoimmune diseases. LFA-1/ICAM is a case where a recently approved mAb had to be withdrawn from the market due to fatal side-effects. Structural biology as well as different inhibitor classes and their SAR are discussed. Several small molecules are currently being evaluated in clinical trials.

Engel reviews the PIF-binding pocket of the subclass of AGC kinases, which serves as a target site for allosteric modulators and PPI inhibitors. Major advantages of targeting non-ATP sites of kinases would be to obtain more selective compounds and to target kinases previously considered to be nondruggable. The recent discovery of different classes of AGC kinase pocket binders makes this an important area of research.

The discovery and medicinal chemistry evolution of oxytocin receptor antagonists is reviewed by Borthwick and Liddle. Their efforts have led to Retosiban and Epelsiban – two orally bioavailable oxytocin receptor antagonists with superior potency and selectivity for the treatment of preterm birth and related disorders.

Mas-Moruno and Kessler describe peptidic inhibitors targeting cell adhesion receptors (e.g., RGD peptides). Although peptides are not considered as small molecules, much can be learned from the specific modes by which peptides interact with their targets for the development of small-molecule PPI inhibitors.

Last, but not least, McInnes reviews another method to allosterically inhibit the cyclin-dependent kinase (CDK) kinase class by targeting the substrate recognition site. The REPLACE algorithm for the structure-based prediction of PPI binders has been applied to the Polo-box domain of PLK1 (Polo-like kinase 1), resulting in small peptides that are N- and C-capped by organic residues.

The area of small molecules successfully targeting PPIs has recently exploded and therefore only a glimpse of the exciting research in the area can be reflected by the present book. Many interesting works could not be included. To finish, and in the tradition of the Editor's choice, I want to point the reader to several more exciting recent targets.

The small G-protein and oncogene Ras has been recognized for several decades as a major oncology target. A large portion of lung, colon, and pancreas cancer patients bear constitutively active Ras mutations that drive cancer growth. However, until recently no small-molecule drug has been known to directly bind to and effectively modify the downstream pathway. Lately, novel biophysical techniques have shown promise by identifying small molecules inhibiting the Ras effector complex Ras–Sos (Figure 1a) and cocrystal structures solved by two independent groups provide hope that the initial fragment-based hits can be further elaborated towards effective drugs [1,2].

Protein lysine methyltransferases are important regulators of epigenetic signaling. The oncogenic protein SMYD2 represses the tumor suppressors p53 and Rb by a PPI. The use of structural biology information cannot be overestimated, and the structure of apo-SMYD2, SMYD2 bound to a methylated p53 peptide, and in complex with a small molecule competitively inhibiting p53 binding was published recently (Figure 1b) [3].

The von Hippel–Lindau (VHL) syndrome is a rare autosomal dominant genetic orphan disease characterized by abnormal angiogenesis in certain parts of the body.

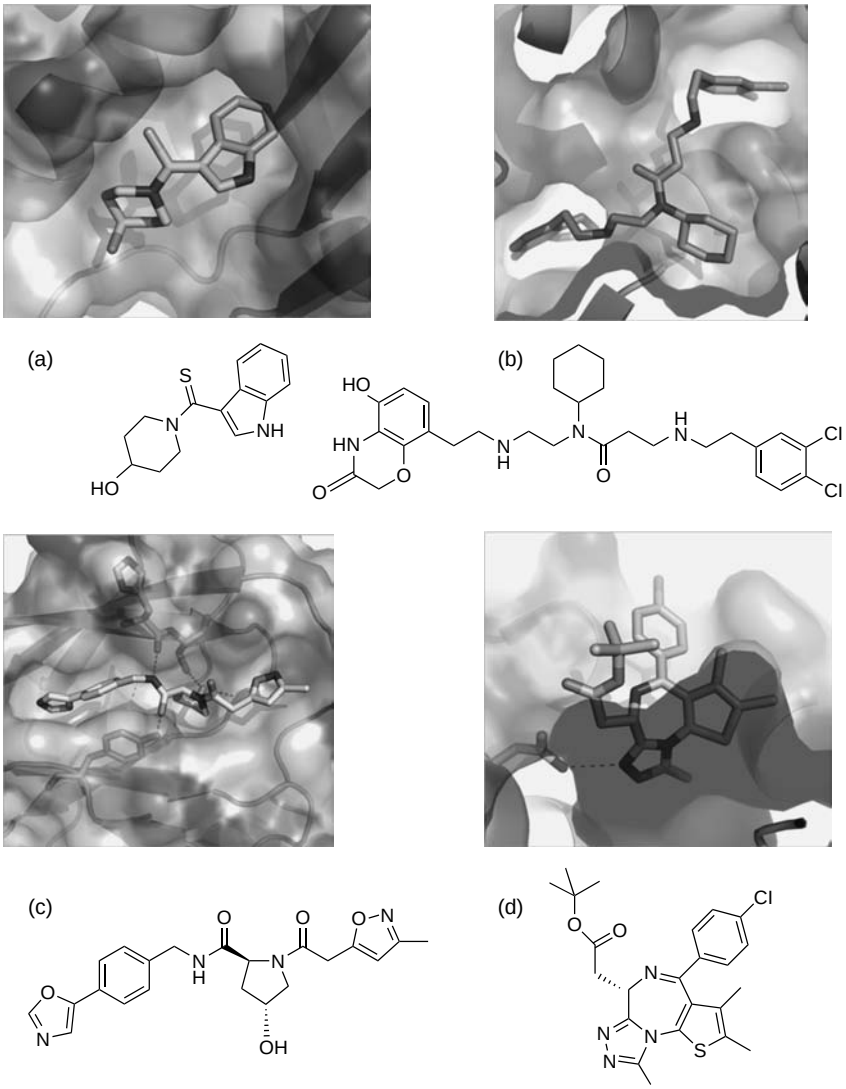

Figure 1 (a) Indole-3-thiocarbonylpiperidine fragment bound to Ras on the Sos interacting interface (PDB ID: 4EPV). (b) Small molecule mimicking the p53 Lys-Me binding to SMYD2 and extending with the dichlorophenyl moiety into a nearby hydrophobic pocket not occupied by the peptide (PDB ID: 3S7B). (c) Small molecule anchoring via the central hydroxyproline ring to the VHL complex and thus inhibiting the contact to HIF-1α (PDB ID: 3ZRC). (d) Cut-away view of the Gewald thiophenodiazepine JQ1 bound into the bromodomain of BRD4 into the Ac-Lys binding site (PDB ID: 3MXF).

The E3 ubiquitin ligase VHL complex primarily targets the hypoxia-inducible factor (HIF)-1α – a transcription factor involved in the regulation of numerous genes (i.e., involved in angiogenesis and cancer). Using *in silico* methods and structure guided medicinal chemistry the first small molecule ligand for VHL was recently described. The authors used the hydroxyproline motif of HIF-1α as an "anchoring" starting point for their medicinal chemistry efforts (Figure 1c) [4]. Derivatives of the first-generation compounds might evolve into cell-penetrating chemical probes to test the involvement of the VHL complex in disease conditions such as chronic anemia, acute ischemia, and stroke.

Bromodomains are reader elements in the framework of epigenetic control and recognize sequence-specific acetylated lysine side-chains of histones and other proteins. Two small-molecule inhibitors of the LysAc-bromodomain PPI are currently undergoing clinical evaluation for the treatment of artherosclerotic cardiovascular disease, and testis midline and other cancers (Figure 1d) [5,6]. As for many areas in PPIs, the availability of X-ray structures of Apo, Ac-Lys, and ligand-bound protein proved to be invaluable in the early discovery process. A major challenge of the area will be the design of selectivity into the small molecules targeting one of the 61 very similar bromodomains found in the human genome.

I have enjoyed editing this book, and I hope that readers will benefit from the expert reviews on cutting-edge small-molecule PPI research and development.

October 2012
Groningen

Alexander Dömling

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1
Protein–Protein Interactions: An Overview

Christian Ottmann

1.1
Introduction

Protein–protein interactions (PPIs) are implicated in almost all biological processes for any given protein engaged in complexes with other proteins for the majority of its lifetime. In this regard, proteins function not merely as single, isolated entities, but display their roles by interacting with other cellular components. The different interaction patterns are at least as important as the intrinsic biochemical activity status (e.g., of a protein kinase) of the protein itself. Therefore, to understand the biological role of a protein it is of the utmost importance to know the underlying PPI network. This holds especially true in the case of diseases where, for example, mutations in oncogene or tumor suppressor proteins are recognized as the cause for malignancies. An impressive recent example for the relevance of the PPI interplay is the finding that active-site inhibitors targeting the oncogenic kinase B-Raf can under certain circumstances activate the underlying signal transduction pathway (mitogen-activated protein kinase (MAPK) pathway) instead of inhibiting it [1–3]. This finding is a strong reminder that nature in the majority of cases ultimately relies on regulating protein function by PPIs. In addition to taking into account this important concept for the drug development process, targeting PPIs significantly enlarges the "druggable genome" that was initially estimated to comprise around 1500 single protein targets [4]. While this number is still several times higher than the 266 human protein targets actually addressed by currently approved drugs [5], there are diseases that lack a good "conventional" target like an enzyme, receptor, or ion channel. By adding the number of PPIs occurring in the human body, the so-called protein–protein "interactome," this situation will definitely be improved. As the size of the interactome has been estimated to lie between 130 000 [6] and 650 000 [7], successfully addressing PPIs will vastly expand our opportunities for pharmacological intervention.

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1.2
Role of PPIs in Human Physiology

Direct physical interactions of proteins are intricately implicated in the majority of processes in living organisms (Figure 1.1). For example, reception and propagation of growth signals can start with the binding of a proteinaceous signaling molecule like the epidermal growth factor (EGF) to its cell surface receptor (EGFR). This binding triggers the intracellular assembly and activation of signaling complexes comprised, for example, of adapter proteins like Grb2 and Sos and small G-proteins like Ras that – again by physically interacting – activate protein kinases like Raf. Activated Raf then stimulates a phosphorylation cascade via the kinases MEK (mitogen-activated protein kinase/extracellular signal-related kinase) and ERK (extracellular signal-related kinase) that ultimately leads to gene activation via transcription factors like Sp1 and Elk [8]. As each of these steps necessitates direct binding of the components of this signal transduction chain, small molecules inhibiting these interactions could disrupt this proproliferative signaling. Furthermore, stabilization of the inhibitory binding of regulatory proteins like the Raf kinase inhibitory protein (RKIP) [9] and 14-3-3 to components of the pathway (e.g., Raf) might also produce a therapeutic benefit.

Many cellular functions like motility are related to functional changes in the cytoskeleton. For example, dynamic assembly and disassembly of actin filaments are based on the interaction of actin with itself and with protein partners like ADF/cofilin and profilin [10]. Biological (surface) recognition, like in the immune system, is also mediated by PPIs as in the case of binding of lymphocyte function associated antigen (LFA)-1 presented on the surface of immune cells to intracellular adhesion molecule (ICAM)-1 found on the surface of endothelial cells [11]. This interaction enables immune cells to attach to the walls of blood vessels and to migrate into neighboring tissue to initiate inflammation.

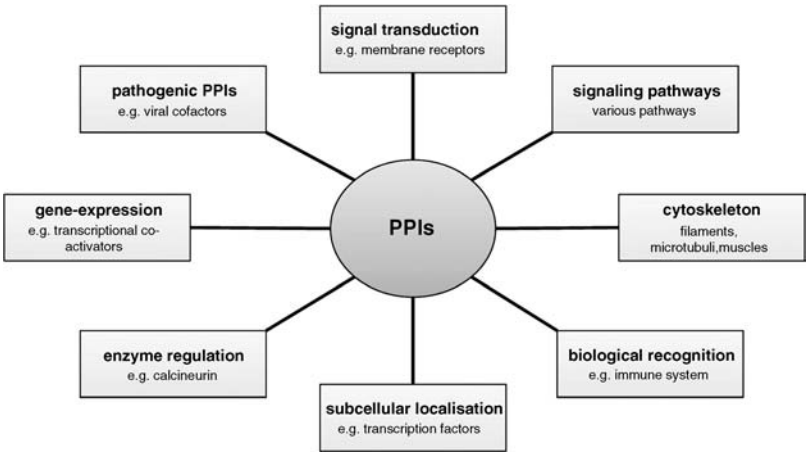

Figure 1.1 Examples of the role of PPIs in human physiology.

The control of subcellular localization is another important aspect of protein regulation performed by PPIs. For example, the transcription factor NFκB is prevented from nuclear import upon complexation with its negative regulator IκB [12]. The 14-3-3 adapter proteins play a similar role in the case of the FoxO transcription factor family [13]. Also, direct regulation of biochemical activity by PPIs is performed many times by PPIs. The phosphatase calcineurin is activated upon complexation with Ca^{2+}-activated calmodulin and repressed upon binding to cabin (calcineurin binding protein) or calcipressin [14]. Another important process involving PPIs is the functional constitution of transcriptional complexes. While transcription factors of the Tcf (T cell factor) LEF (lymphoid enhancer factor) family can directly bind to DNA, transcription starts only when coactivators like β-catenin additionally interact with Tcf/LEF [15]. Many proteins of disease-causing organisms need host proteins as cofactors for their pathogenic activity. For example, exoenzyme S from *Pseudomonas aeruginosa*, an opportunistic, pneumonia-causing bacterium, has to interact with host 14-3-3 proteins to be able to transfer an ADP-ribose moiety from NAD^+ to small G-proteins like Ras [16], thereby inhibiting its target proteins [17].

1.3 Regulation of PPIs

Given the importance and number of PPIs in the living cell it is no surprise that they have to be tightly orchestrated at any moment in time. The occurrence and perseverance of PPIs is governed by the two principal variables local concentration and intrinsic binding energy of the binary interaction [18]. The first is regulated by transcriptional and translational mechanisms, subcellular (co-)localization, degradation rates, and temporary storage. The second can be influenced by covalent modifications like phosphorylation, and by changes in pH, ionic strength, and temperature (Figure 1.2). Furthermore, additional PPIs can modulate binary interactions. They can be inhibitory when, for example, the interaction interface of one partner is masked by binding to the same interface or by simple sterical obstruction. They can also be stabilized, for example, when the third interacting protein binds simultaneously to both protein partners. Such a "bridging" or "assembly platform" function has been described for the A-kinase anchoring proteins (AKAPs) [19] and the kinase suppressor of Ras (KSR) [20]. It is now clear that the local architecture of such signaling complexes is one of the keys to understand regulation and specificity of signaling events.

1.4 Structural Features of PPI Interfaces

PPIs can be established between identical and nonidentical protomers leading to homo- or heterodimeric complexes, respectively. In the following, a number of examples are discussed in more detail. Small-molecule inhibitors have been identified for these PPIs (Table 1.1), strongly validating the general approach to pharmacologically interfere with the interaction of proteins.

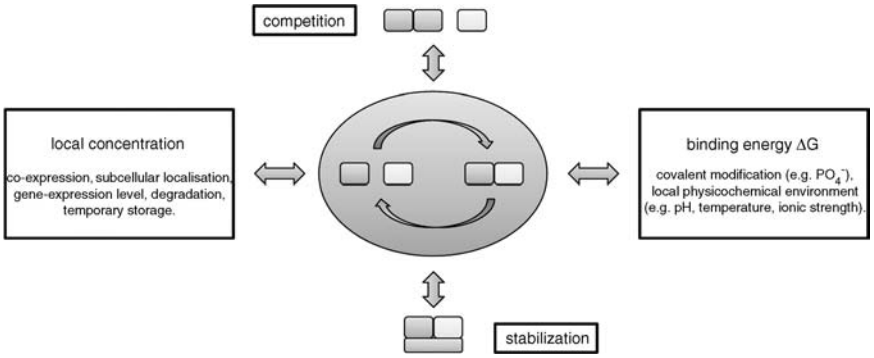

Figure 1.2 Factors governing the occurrence and perseverance of PPIs. Important control mechanisms for the oligomerization state of interacting proteins. The association–dissociation equilibrium between monomeric and multimeric states is regulated by the partners' local concentration and their mutual binding affinity. Additional cellular or pharmaceutical factors can compete for one partner or stabilize the dimeric complex. (Adapted from Nooren and Thornton [18].)

Table 1.1 Small-molecule PPI inhibitors from the pharmaceutical industry.

Target	Compound	Identification	Affinity (μM)	Reference	Company
Bcl-2	ABT-737	SAR by NMR	$K_i = 0.001$	[21]	Abbott
HDM2	Nutlin-2	HTS (biochemical)	$IC_{50} = 0.14$	[22]	Roche
TNF-α	SP307	combinatorial fragment assembly	$IC_{50} = 22$	[23]	Sunesis
RSV F_1	BMS-433771	HTS (cellular)	$EC_{50} = 0.012$	[24]	BMS
RSV F_1	JNJ 2408068	HTS (cellular)	$EC_{50} = 0.00016$	[25]	Johnson & Johnson
iNOS	PPA250	HTS	$IC_{50} = 0.082$	[26]	SSP
iNOS	compound **21b**	HTS	$K_d = 0.00029$	[27]	Berlex Biosciences
iNOS	compound **6**	HTS	$IC_{50} = 0.012$	[28]	Adolor
B7.1	compound **2**	HTS	$IC_{50} = 0.030$	[29]	Wyeth
HPV E1/E2	compound **10**	HTS	$IC_{50} = 0.35$	[30]	Boehringer Ingelheim
ZipA	pyridylpyrimidine 1	HTS	$K_i = 12$	[31]	Wyeth
IL-2	SP4206	tethering	$K_d = 0.07$	[32]	Sunesis
HDM2	TDP665759	HTS	$IC_{50} = 0.7$	[33]	Johnson & Johnson
LFA-1	LFA878	HTS	$IC_{50} = 0.05$	[34]	Novartis
LFA-1	compound **4**	epitope transfer	$IC_{50} = 0.0014$	[35]	Genentech/ Roche

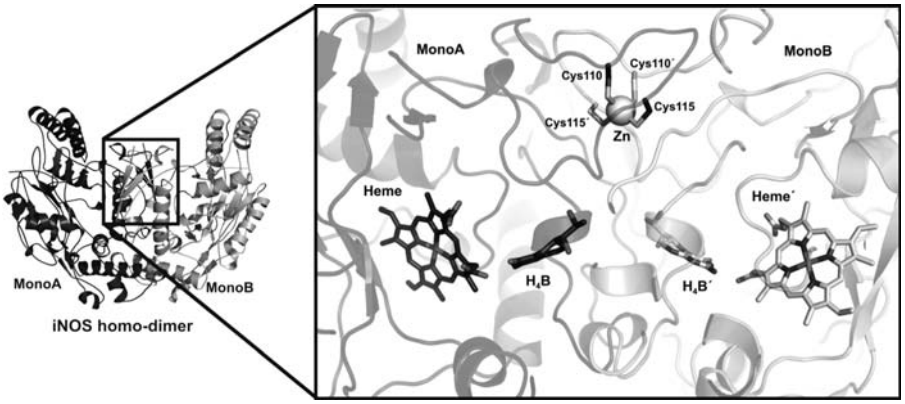

Figure 1.3 Structure of the iNOS homodimer. General topology of the iNOS dimer, and expanded, detailed view of the dimer interface with the cysteine-coordinated zinc ion and the cofactors H_4B and heme.

1.4.1
iNOS Homodimer

An example for a homodimeric protein complex is the inducible nitric oxide synthase (iNOS) that produces the signaling molecule NO from L-arginine [36–38]. To perform its catalytic activity NOS depends on the tightly bound cofactors tetrahydopterin (H_4B), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and iron protoporphyrin IX (heme). This enzyme is only active as a homodimer, and the crystal structures of the (dimeric) oxygenase domain [38] explained this fact by showing that the dimerization interface shapes the functional binding sites for the cofactors H_4B and heme (Figure 1.3). It also displays a large intersubunit cavity of about 750 $Å^3$ that is separated from the surrounding bulk solvent when a zinc ion is coordinated by two cysteines from protomer A and two cysteines from protomer B. With 69% nonpolar and 31% polar amino acids, the interface of the iNOS dimer shows a distribution that is typical for the majority of known homodimers. The contact surface of roughly 2900 $Å^2$ is rather flat. Nonetheless, mainly due to the special situation characterized by interface-bound cofactors, inhibitors of dimer formation could be identified successfully.

1.4.2
β-Catenin/Tcf4 Complex

The Wnt pathway found to be constitutively activated in many colorectal cancers is dependent on the interaction of β-catenin with transcription factors of the Tcf/LEF family. Normally, the transcriptional coactivator β-catenin can be sequestered in the cytoplasm and the Tcf transcription factor is inhibited by complexation with negative regulators of the Groucho family [39]. Upon Wnt activation, β-catenin is translocated into the nucleus and binds to Tcf to constitute the active transcriptional complex [40].

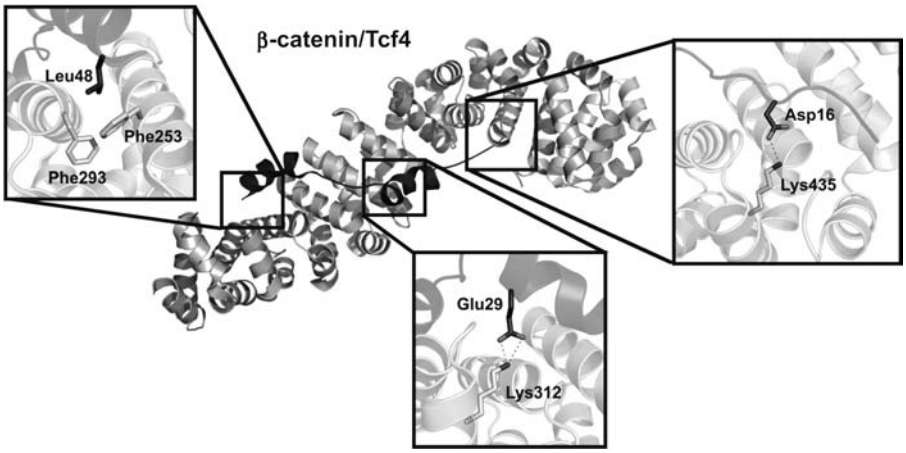

Figure 1.4 Complex of Tcf4 (black ribbon) bound to β-catenin (gray ribbon). Three hotspots of the interaction are presented in structural detail with key residues of Tcf4 (black sticks) and β-catenin (light gray sticks) labeled. Polar contacts are shown as black dotted lines.

The crystal structure of the human β-catenin/Tcf4 complex [41,42] revealed the multisite binding nature of the interaction with three regions of Tcf4 to be important for binding to β-catenin (Figure 1.4): (i) an extended N-terminal sequence, (ii) a kinked α-helix, and (iii) a second extended segment followed by the C-terminal α-helix. The binding module of Tcf4 wraps around the 12-membered armadillo-repeat region of β-catenin. Three essential interaction "hotspots" have been identified in the β-catenin/Tcf4 interface; a salt bridge between Tcf4 Asp16 and β-catenin Lys435, a hydrophobic contact of Tcf4 Leu48 to Phe253 and Phe293 of β-catenin, and a second salt bridge between Glu29 of Tcf4 and β-catenin Lys312. Disruption of one (or several) of these contacts by a small-molecule PPI inhibitor may successfully abolish binding of Tcf4 to β-catenin.

1.4.3
LEDGF/HIV-IN Complex

For a productive infection HIV depends on the viral integrase (IN) that integrates the genetic material of the virus into the host cell's DNA [43]. The human transcriptional coactivator LEDGF (lens epithelium-derived growth factor) is an essential host protein as cofactor for the function of IN that, among others, locates IN to the nucleus [44]. The interaction is mediated between the catalytic core domain (CCD) of IN and the IN-binding domain (IBD) of LEDGF [45]. The IN CCD/LEDGF IBD complex crystallized as an IN CCD dimer with two LEDGF IBD copies attached at opposing sites (Figure 1.5) [46]. An interhelical loop of IBD binds to a pocket at the IN dimer interface burying approximately 1300 $Å^2$ of protein surface. Binding is driven by the hydrophobic contact of LEDGF residue Ile365 to a pocket concomitantly established by IN residues Leu102, Ala128, Ala129, and Trp132 from one chain of the IN dimer (chain B), and Thr174 and Met178 from the other chain of the