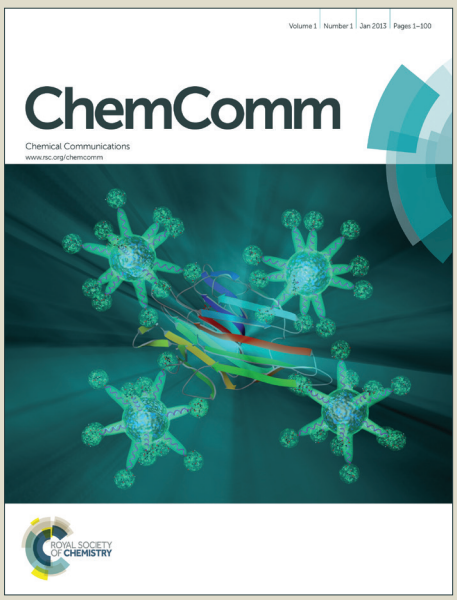
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

ChemComm

RSCPublishing

FEATURE ARTICLE

Modulating protein-protein interactions: the potential of peptides

Cite this: DOI: 10.1039/x0xx00000x

L.Nevola[a]* and E. Giralt[a,b]*

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Protein-protein interactions (PPIs) have emerged as important and challenging targets in chemical biology and medicinal chemistry. The main difficulty encountered in the discovery of small molecule modulators derives from the large contact surfaces involved in PPIs when compared with those that participate in protein-small molecule interactions. Because of their intrinsic features, peptides can explore larger surfaces and therefore represent a useful alternative to modulate PPIs. The use of peptides as therapeutics has been held back by their instability in vivo and poor cell internalization. However, more than 200 peptide drugs and homologous compounds (proteins or antibodies) containing peptide bonds are (or have been) on the market, and many alternatives are now available to tackle these limitations. This review will focus on the latest progress in the field, spanning from "lead" identification methods to binding evaluation techniques, through an update of the most successful examples described in the literature.

1. Introduction

Communication between proteins is involved in almost every biological function. This enormous network system corresponds to a comparably huge map of interactions between protein partners that determines cellular behavior. It is not surprising therefore that protein-protein interactions (PPIs) have emerged as important and challenging targets in chemical biology and medicinal chemistry.[1] PPI modulation would contribute to improving current knowledge of PPI networks, facilitating the understanding of the pathogenic mechanisms involved in diseases and thus paving the way towards the development of novel diagnostic and therapeutic strategies.[2] In general, PPIs are classified as mediated either by the interaction between two protein domains (domain-domain) or by the interaction between a linear sequence of residues of one of the partners and a domain of the other one (peptide-domain) (Figure 1). In the latter, which accounts for almost 40% of known PPIs, the interacting peptide often adopts a specific secondary structure, typically an α-helix, although it can also be completely disordered.[3] However, as a great proportion of domain-domain interactions also involve these "hot segments",

Laura Nevola

After receiving the degree in Medicinal Chemistry, Laura Nevola obtained the Ph.D in Pharmaceutical Science from the University of Rome "Sapienza" (Italy), with a thesis on organic and supramolecular chemistry. In 2007 she joined Prof. Hamilton group as visitant assistant in research at Yale University, where she focused on peptidomimetics for the inhibition of protein-protein interactions. In 2009 she moved to the Institute for Research in Biomedicine (IRB Barcelona) to start as post-doctoral fellow in the group of Proteins and Peptides of Prof. Giralt. From 2013 she is Research Associate in the same group.

Ernest Giralt

Ernest Giralt is Professor of Organic Chemistry at the University of Barcelona and Group Leader at the Institute for Research in Biomedicine (IRB Barcelona) where is the Head of the Chemistry and Molecular Pharmacology Programme. He has published around 420 papers, 16 patents and several books that have become a reference in his field. He is one of the founding members of the European Peptide Society. He has received several awards, among others the Dimitrios Theodoropoulos Award, the National Research Award, and recently the Josef Rudinger Memorial Lecture Award and the Max Bergmann Medal. His major interests lie in the field of peptide synthesis and structure determination, in particular using NMR to the study of complex molecular recognition processes, with emphasis on the design of specific ligands for interaction with protein surfaces, related to possible therapeutic uses. This includes studies concerning new drug delivery systems, amyloid protein aggregation inhibitors and antitumor peptides of marine origin.

they can be characterized by a dominant hot segment-mediated interaction. This is the case of PDZ domain-mediated interactions (PDMIs)[4] and of Src homology 2 (SH2) domain-mediated interactions,[5] both types often targeted by peptides. The modulation of PPIs has been pursued through many approaches, in particular those based on the use of small organic molecules, either derived from natural compounds or from pure organic synthesis.[6] More than 12 small molecule PPI modulators are currently in clinical development, and it is predicted that the market value of these molecules will exceed 600 million EUR in the next 5 years.[7] However, the main difficulty encountered in the discovery of small molecule modulators derives from the large contact surfaces involved in PPIs (~1,500-3,000 Å^2) when compared with those that participate in protein-small molecule interactions (~300-1,000 Å^2). In addition, these protein-protein interfaces are generally flat and often lack the grooves and pockets present on the surfaces of proteins that bind to small molecules.[8] Researchers have thus turned their attention to the possibility of using "more natural" alternative PPI modulators, such as recombinant proteins/antibodies, and peptides, which can explore larger surfaces. Peptides in particular present several advantages: (i) flexibility, which is translated into adaptability to large surfaces; (ii) easy modularity, which increases structural diversity and consequently allows higher selectivity and potency; (iii) size, which limits accumulation in tissue; and (iv) complete biocompatibility, which means low toxicity in humans.[9] The last two features are highly desirable given the growing interest in PPIs as therapeutic targets. However, progress towards the development of therapeutic peptide PPI modulators is hindered by the following drawbacks of these molecules: low stability against degradation by proteolytic enzymes of the digestive system and blood plasma; rapid removal from the circulation (hepatic and renal clearance); poor ability to cross physiological barriers; and potential immunogenicity.[10] In spite of these limitations, the large number of successful peptide PPI modulators reported so far and the great effort to tackle the bottlenecks that impair their use as pharmaceutics are impressive. In addition to features that allow cell and tissue permeability, many chemical modifications and smart linker conjugations[11] have been introduced into PPI modulators in order to reduce proteolytic degradation and improve bioavailability. An entire class of synthetic molecules that mimics the secondary structure of peptides, the so-called peptidomimetics, has been developed and applied to the modulation of PPIs, as extensively described elsewhere.[12, 13]The present review seeks to give an overview of the recent scenario in this field, following the scheme of the common pipeline corresponding to PPI modulator discovery (Figure 2). We will focus first on the approaches used to identify lead compounds (Section 2) and then on the techniques applied to evaluate peptides as PPI modulators in vitro and in living cells (Section 3). Our main goal is to provide a complete and updated description of the successful applications of peptides in PPI modulation and their consequent use in biochemistry, chemical biology, and pharmacology.

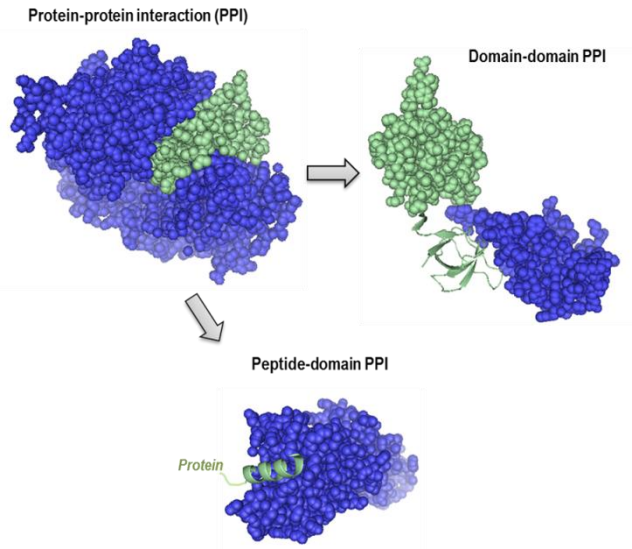


Figure 1. **Protein-protein interactions (PPIs) can be domain-mediated or peptide-mediated**. The domain-peptide type is defined by the interaction of linear peptide regions, in particular the α-helix. However, the presence of a continuous epitope (the peptide) can also be found in a great number of domain-domain interactions.

2. Peptides and PPIs: identifying the lead.

PPIs are extremely difficult to target because of the high surface area, normally flat and featureless, that characterizes the interaction. Peptides are the perfect candidates to tackle this limitation as they are able to closely mimic the principle features of a protein but can be easily synthesized and modified to improve properties such as stability, binding strength, and bioavailability. For applications in medicinal chemistry and chemical biology, peptide research tends to approach lead compound discovery following the same pathways as those used for small molecules. In this section we will briefly address all the techniques used in the discovery of peptide PPI modulators, focusing on the latest approaches published. The first part will describe "Structure-based design", showing the classic and modern approaches for "hot spot" identification (Section 2.1) and the advances made in the synthesis of modified peptides (Section 2.2). The second part will focus on high-throughput screening (HTS), starting from biological techniques such as phage display (Section 2.3), ribosome display (Section 2.4) and mRNA display (Section 2.5) through to *in silico* screening (Section 2.6) and fragment-based selection (Section 2.7).

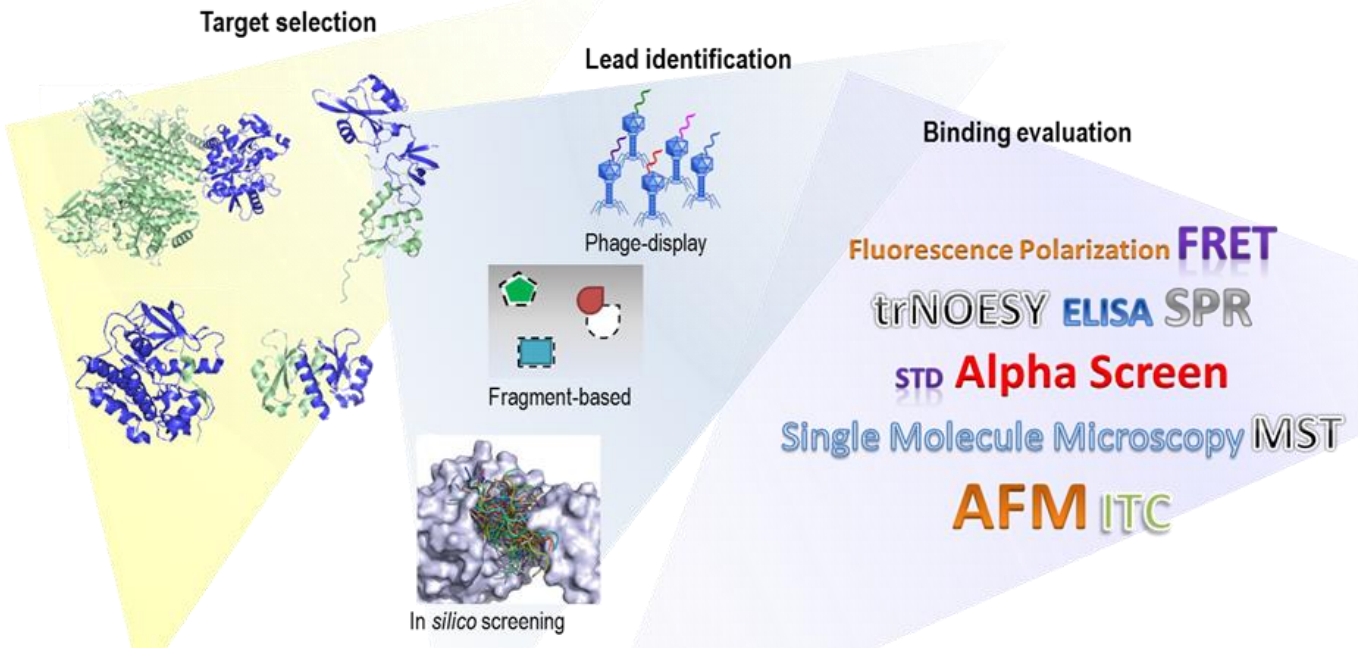


Figure 2. **PPI modulator pipeline**. The discovery of PPI modulators starts from the target selection among the PPI interactome. Several methods, such as phage display, fragment-based discovery, and in silico screening (adapted with the permission from Proteins 2010, 78, 3140-3149), contribute to "lead" peptide identification. The last step in the pipeline is the evaluation of binding proprieties in vitro or in cells, which involves many techniques.

2.1 "Hot spot" identification.

Identification of the physical and chemical features of the protein surfaces involved in PPIs has been a challenge for many years. The breakthrough in this field came with the understanding that the "flatness" of the protein-interacting part is decorated by well-defined binding sites, the so called "hot spots", characterized by a high degree of flexibility that confers adaptability to enhance PPI fitting.[7] Although "hot spots" account for less than half of the contact surface, they make a significant contribution to the overall interacting energy.[8] Each "hot" residue is surrounded by an "O-ring" that excludes the solvent. The second level of boundaries is conferred by residues that are partially buried, rendering the spot a unique physico-chemical property.[14] The mapping of "hot spots" is therefore an ideal starting point for the design of modulators—both peptides and small molecules. The main limitation in this regard is the time-consuming and harsh experimental procedures normally required for their identification, which is achieved mainly by systematic cycles of mutagenesis and binding measurements. By means of alanine scanning, "hot spots" have been defined as those sites where mutations cause an increase in the binding free energy of at least 2.0 kcal/mol. [15] The discovery of a rapid and effective method to detect these determinant binding points is highly desirable. From the experimental point of view, several variables of classic alanine scanning have been developed, such as alanine-shaving, shotgun scanning, and in particular computational prediction, through MM-PBSA (molecular mechanics-Poisson–Boltzmann surface area), MM-GBSA (molecular mechanics-generalized Born surface area), and more recently through "post-processing" protocol[16]. Computational and fragment-based approaches have been reported in the literature. In this review we will focus on the most recent cases. An alignment-free method for the prediction of protein interface residues, named iPred, was developed in 2011.[17] It was the first prediction method to include complete structural environment information of a residue in order to determine its interface propensity, introducing the concept of context-dependent scoring. Although designed for small molecules, iPred also proved applicable for recognizing peptide-binding regions on protein surfaces. One of the most challenging issues in the prediction of "hot spots" is the identification—in the absence of knowledge—of the binding site. This question was addressed using a computational method similar to the concept of the multiple solvent crystal structure. The method is based on the combination of the co-solvent mapping method with a double de-coupling calculation of chemical fragments in order to analyze the binding free energy associated with each protein "hot spot"[18] and it has been successfully applied to the hydrophobic interface of Bcl-x_L.[19] Another example of the reliability of computational prediction is given by computational fragment mapping (FTMap) applied to the NEMO/IKK β binding interface, where the use of "fragment-like probes" helps to identify consensus cluster sites that correspond to binding "hot spots".[20] The most recent extension of this concept has led to the definition of "hot loops".[21] The LoopFinder algorithm is a useful tool for searching structure databases for peptide loops at protein-protein interfaces. It has the capacity to show that several of

these interfaces are repeated in many proteins and that they are crucial for their interaction network. This evidence opens up a new scenario for drug targeting and an option for the development of constrained peptide inhibitors.

2.2 Designed Peptides.

Many PPIs are mediated by small peptides, which take on a specific secondary structure to adapt to interacting surfaces. The use of protein segments as PPI modulators has some limitations, due in particular to the high flexibility and to loss of three-dimensional stability when a short sequence is isolated from a global protein. The introduction of chemical modifications helps to solve this problem, providing the constrained peptides with additional features, such as protease resistance, higher selectivity, and greater cellular internalization. This review will focus on recently designed peptides that have been successfully applied to the modulation of PPIs, classifying them on the basis of general chemical modification. We will start with "cyclic peptides", moving from head-to-tail to side-chain to side-chain cyclization, focusing on "stapled peptides" as example of α-helix stabilization. We will then report on other secondary structure stabilizers, such as "other α-helix" and "β-hairpin-constrained" peptides. We will conclude by exploring the field of "bicyclic peptides" and "β-peptides and peptoids". Finally, specific attention will be devoted to "mini-proteins" and "grafted-peptides", and special mention will be given to "photo-switchable peptides" as examples of the remote control of PPIs.

2.2.1 Cyclic peptides. Head-to-tail cyclization was one of the first modifications explored to enhance peptide stability against enzymatic degradation and physical denaturation.[22] Peptides constrained in this way also exhibit remarkable biological activity with extraordinary potency and improved bioavailability, thus attracting the attention of the pharmaceutical industry. Many synthetic methodologies have been developed[22] beyond the classic amide formation, such as the recent imine-induced Ser/Thr ligation[23]; however, finding a highly selective and specific manner to achieve peptide cyclization remains a challenge. Enzymatic reaction could be the answer, as demonstrated by the discovery of butelase I,[24] the first asparagine/aspartate ligase, which performs peptide cyclization with a 95% yield at the fastest rate reported to date. In some cases, head-to-tail cyclic peptides have been successfully used to inhibit PPIs. Cyclic pentapeptides selected from a library of $3.2x10^6$ members were found to inhibit the interaction between HIV Gag protein and the host protein TSG101 (tumor susceptibility gene 101), thus impairing viral budding. [25]More recently, molecules that inhibit the dimerization of the C-terminal binding protein (CtBP) transcriptional repressor were identified from a much larger library of 64 million genetically encoded cyclic peptides. Treatment of human breast cancer cells with these peptides has been reported to reduce their mitotic fidelity, proliferation, and colony-forming potential.[26] The same research group applied the strategy to another PPI of therapeutic relevance, namely hypoxia-inducible factor (HIF) hetero-dimerization, obtaining hexapeptides with high activity in the cell. [27] The other approach most commonly used to produce cyclic peptides is through the reaction between side-chain groups. This class of peptides can feature the oxidative di-sulfide bridge formation of a pair of cysteine residues, as normally occurs in proteins (this issue will be addressed in Section 2.2.7), but also other coupling reactions of distinct nature. Lactam-bridged peptides (amide condensation between the side chain of a Lys and an Asp or a Glu) have been widely used to confer rigidity and define α-helix secondary structure in short peptides[28] and have yielded improved PPI modulators, such as the PDZ domain of the PSD-95/NMDA receptor interaction[29], the HIV-1 Rev/RRE interaction[30], and the HR-N C-terminus domain/HR-C domain of Respiratory Syncitial Virus protein, where a double lactamization was used.[31, 32] Side chain-bridged macrocyclic peptides were prepared using bis-carboxylic acid ring spacers to inhibit PPIs mediated by the third PDZ domain (PDZ3) of PSD-95.33 As already mentioned for the lactam-bridged peptides, the side-chain to side-chain cyclization is devoted mainly to constrain peptide secondary structures and in particular to promote α-helix conformation. In this regard, "stapled" peptides—addressed in the following section—play a major role.

2.2.2 α-Helix-constrained peptides: "stapled" peptides. After the first example of ring-closing metathesis applied to peptides by Grubbs[34], in 2000 Verdine and co-workers reported the synthesis of an all-hydrocarbon cross-linking system through a metathesis reaction to induce α-helix conformation in short peptides.[35] This first article concluded that: "*The major goal of this research program is to improve the pharmacological properties of α-helical peptides through synthetic modification. The present report is an important first step toward that end.*" Since then, Verdine's group and later that headed by Walensky, and many others afterwards, have used the stapling protocol[36] (Figure 3a) to produce α-helix peptide inhibitors of PPIs, consistently obtaining better affinity, higher stability and greater cellular uptake. Successful examples are the Bcl-XL/Bak and the MCL-1/NOXA interactions[37, 38], the p53/MDM2 interaction[39-42], the MAML1/ICN1–CSL complex of NOTCH signaling [43], and β-catenin/T-cell factor (TCF) proteins involved in Wnt signaling[44]. More recently, stapled peptides have been applied to inhibit new anti-cancer targets, such as H3 Lys27 trimethylation by disrupting the EZH2–EED complex and reducing EZH2 protein levels in

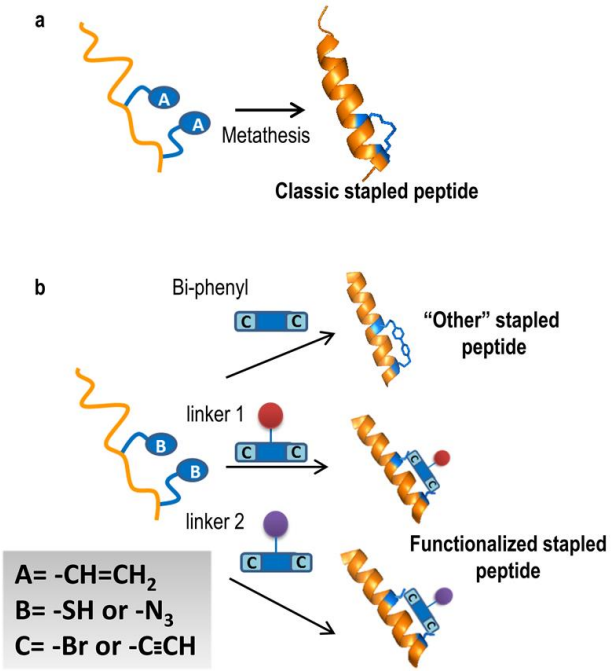


Figure 3. **Classic stapled peptides (a) and their evolution (b).**

leukemia cells.[45]The treatment with the peptide leads to growth arrest and monocyte-macrophage differentiation, thus modulating an epigenetic 'writer' and suppressing cancer cell growth. Stapled peptides have also been used to tackle the problem of PPIs,

considered undruggable because of the extensive and shallow nature of their interfaces. This is the case of GTP-ase signaling, which has been successfully regulated by inhibiting the Rab8a/Rab8a–effector interaction, thus representing a new approach to overcome the limitation of this protein family target. [46] The capacity of stapled peptides to selectively interact with protein surface candidates make them not only direct PPI modulators, but also opens them up to other applications, such as protein probing for drug discovery and structural studies. The latter concept has been applied to photo-reactive stapled BH3 peptides, thus helping to experimentally dissect the interactome of the Bcl-2 family of proteins. [47] Another example is the ATRIP-based sequence stapled peptide that binds tightly to the basic cleft of RPA70N, which is essential for DNA replication, damage response, and repair. By inhibiting the interactions of RPA70N with other peptides/proteins, the stapled peptide was used as probe to find small molecule modulators.[48] The PPI that involves the "juxtamembrane segment" of epidermal growth factor receptor (EGFR) and allows its dimerization has recently been targeted by stapled peptides.[49] Those molecules are allosteric inhibitors and open a third way to inactivate EFGR signaling. The established tight connection between "stapling-α-helix conformation" and "enhanced activity" was discussed and slightly unhinged when the inhibitory capacity of some stapled BimBH3 peptides did not yield the results expected. [50] The same issue, although from a different perspective, has recently been analyzed by the groups of Ottmann and Grossman, opening up the appealing option of using stapling to stabilize peptides, not necessarily in α-helix structure, but also peptides with irregular conformations as PPI modulators.[51] In that paper, constrained peptides with no particular defined secondary structure were shown to inhibit the interaction between the virulence factor exoenzyme S (ExoS) and the human protein 14-3-3.

2.2.3 Other α-helix-stabilized peptides. As already mentioned, many PPIs are mediated by α-helix peptides. This observation justifies the intense research performed in the field of helix inducers or helix stabilizers. In addition to the use of stapled peptides (see 2.2.2), many methodologies addressing this issue have been reported in the literature (Figure 3b). In many cases helix induction is achieved by reacting a rigid linker with a pair of cysteine residues located in the desired position (generally i, i+4; i, i+7; i, i+11). Bis-aryl methylene bromide, in particular 4,4-bis-bromomethyl-biphenyl (Bph) and 6,6-bis-bromomethyl-bipyridine (Bpy), provided satisfactory inhibition in the p53/MDM2 PPI and improved cell permeability. [52] The same group obtained similar results applying cross-linking to a peptide directed at the Noxa/MCL1 interaction. [53] Perfluoroarylation of cysteine residues was used for mild functionalization of unprotected peptides [54], obtaining a new class of α-helix-induced peptides characterized by the rigidity and lipophilicity of the perfluroaromatic linkers. The copper-catalyzed alkyne-azide cycloaddition ("click" reaction) is another strategy used to induce helix conformation in short peptides.[55] It has been successfully applied to find strong inhibitors of the interaction between β-catenin and B-cell CLL/lymphoma 9 (BCL9). [56] One of the most recent examples is the use of the double-click method applied to peptide inhibitors of the p53/MDM2 interaction[57], where linear diazidopeptides are reacted in solution with dialkynyl linkers to create bis-triazole-stapled peptides under Cu (I) catalysis. An extra modification was also introduced, adding a functionalized group to the staple linkage itself. [58] Upon examination of all these protocols, the best way to induce the α-helix is still undecided. Fairlie and co-workers established a rank order, comparing first a model penta-peptide modified in various ways (Figure 4), and then confirming the conclusion in a 13-er.[59] In their hands, when the peptide achieved self-helicity in water, almost all the stapled peptides worked fine, while in very short and flexible ones the best result was obtained in the lactam cycle. Another unusual approach is the introduction of a coordination motif (HCM) into the peptide sequence. HCMs consist of a natural metal-coordinating residue,

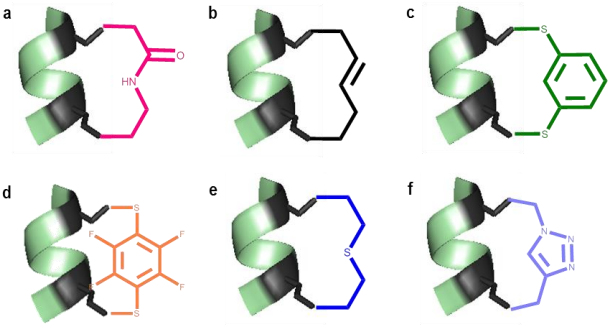


Figure 4. **Comparison of stapling techniques.** Ranking of various "stapling" classes according to Fairlie and al.[51]: lactam (a), hydrocarbon (b), aryl (c), perfluoroaryl (d), thioether (e), and "click" triazole (f).

such histidine (His), at position *i* and an unnatural bi-dentate chelating group, such as 8-hydroxyquinoline (Quin), attached to the side chain at position *i* + *7*.[60]Upon metal (Zn) coordination, a peptide that mimics the BH3 domain of the pro-apoptotic protein Bax has been shown to enhance α-helix content. As a final example, mention is given to the hydrogen bond surrogates (HBS) approach, which involves simple substitution of an intramolecular i→i + 4 hydrogen bond by a covalent linkage. This reaction has been optimized by Arora and co-workers using microwave-assisted ring-closing metathesis[61] and used to develop inhibitors of the interaction between the CH1 region of co-activator protein p300 and the C-TAD domain of hypoxia-inducible factor 1 R (HIF-1 R), involved in the expression of vascular endothelial growth factor (VEGF) or VEGF receptor[62]. The same research group has mimicked a fragment of Sos factor, stabilizing the helix with the HBS method to inhibit the Ras-Sos interaction, involved in the cancer-related aberrant receptor tyrosine kinase (RTK) signaling. [63]An alternative is provided by the use of restricted templates positioned at the N-terminus of a peptide that can induce helicity. This was first proved through a restricted di-proline as N-capping[64] and confirmed recently through scaffold ProM-5, a Pro-Pro analogue made rigid by means of an ethylidene bridge.[65]

2.2.4 β-Hairpin-stabilized peptides. Protein recognition mediated by peptides also involves another diffuse secondary structure, namely β-hairpins. This structure normally comprises two antiparallel β-strands connected by a turn or a loop sequence. Many strategies have been devoted to enhancing the stability of these hairpins in order to modulate PPIs. The main aim was to develop peptide epitope mimetics by introducing a constrained template, by head-to-tail cyclizing or by connecting the strands with di-sulfide bridges. [66] In addition to targeting cell membrane receptors and protein-RNA interactions, and mimicking vaccine, β-hairpin-stabilized peptides have shown potential to modulate PPIs by mimicking an α-helical epitope in the N-terminal segment of the p53 protein and binding with nanomolar affinity to MDM2 (Figure 5).[67] Stabilized β-hairpins have been used to construct a new class of highly efficient binding peptides, aptides (Figure 6), inspired on the structure of antibodies and that have shown affinity in the nanomolar range with slow dissociation rates against several targets.[68]An unusual PPI has been targeted by the aptide (APT), which involves the unfolding of the fibronectin extradomain B (EDB) and the displacement of the intramolecular β-sheet by an intermolecular one.[69] This modification perturbs the interaction of EDB with the

FN8 domain of fibronectin, which is involved in cell adhesion and movement.

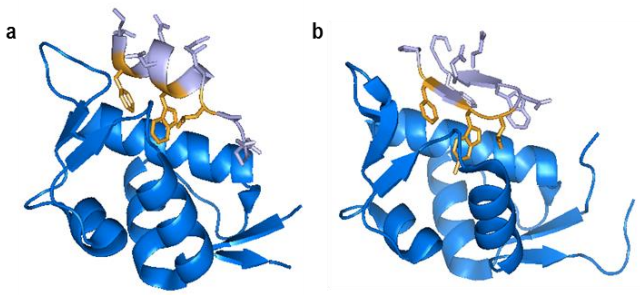


Figure 5. **β-Hairpins as mimetics of α-helix.** Interaction between MDM2 and an α-helical epitope in the N-terminal segment of p53 [PDB 3DAC] (a), and interaction between MDM2 and the β-hairpin-stabilized peptidomimetic [PDB 2AXI] (b).

2.2.5 Bicyclic peptides. Rigidifying the structure of PPI modulators has been shown to improve their binding affinity/specificity and metabolic stability, as is the case of bicyclic peptides. Generally, bicyclic peptide inhibitors have been discovered by HTS of libraries generated by phage or mRNA display, as will be covered in detail in Sections 2.3 and 2.4. To guarantee chemical diversity, many synthetic strategies following a rational design approach have been developed, sometimes adding an extra-rigid element to the construct,

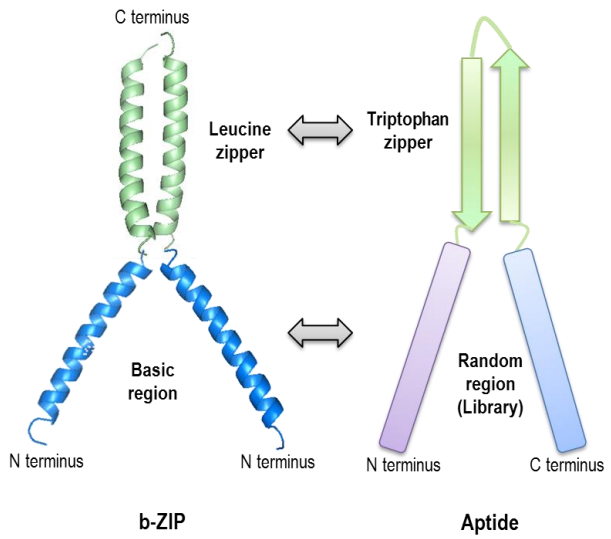


Figure 6. **Aptides are bio-inspired designed high affinity peptides.** In this prototype, the Trp-Trp-induced β-sheet scaffold confers secondary structure stability, while the N- and C-terminal moieties guarantee diversity for affinity optimization.

like in the case of triazole-bicyclic peptoids.[70] Recent examples are the synthesis of an 11-residue peptide macrocycle that binds the Src homology 2 (SH2) domain of growth factor receptor-bound protein 2 (Grb2)[71] and also the synthesis and screening of large combinatorial libraries based on the use of planar trimesic acid as a scaffold for the inhibition of tumor necrosis factor-α (TNFα) dimerization. [72] The same group has published a novel approach to produce bicyclic peptides containing a cyclic segment of a cell-penetrating peptide (CPP) and a cyclic inhibitor of protein-tyrosine phosphatase 1B (PTP1B).[73] This bicyclic system renders the impermeable PPI inhibitor permeable and retains the same uptake- enhancing feature when applied to other cases.[74]

2.2.6 β-Peptides and peptoids. The introduction of chemical modifications has been applied to peptides especially to improve their stability to proteolysis, but retaining the binding propriety, mainly connected to the folding of the peptide-mimetic. A successful example is given by β-peptides, oligomers of β-amino acids, a class of foldamers that can adopt well-characterized secondary structures, spreading from 8-helix, 10-helix, 12-helix and 14-helix. Seebach[75], Schepartz[76, 77] and Gellman[78, 79] groups have focused their research on the understanding of β-peptide and α and β-peptide mixture structure, and have applied them to target PPI, such as MDM2-p53. Peptoids are oligomers of N-alkyl glycine where the side chain is attached to the backbone nitrogen instead of the α-carbon. They adopt predictable conformations and are resistant to proteolytic degradation. As linear or crosslinked[80] sequence, they have been used to target PPI, such as again MDM2/p53[81], but also protein receptors. More recently, peptoids mixed with α-peptides (peptomers) where used to bing polo-box domain of polo-like kinase 1.[82]

2.2.7 Mini-protein and "grafted" peptides. One method by which to enhance the selectivity and affinity for PPI is to explore larger surfaces, mimicking the regular recognition feature of a protein receptor or an antibody. In this context, DARPins (Designed Ankyrin Repeat Proteins) play an important role by mediating several PPIs in nature.[83] In fact, these genetically engineered antibody mimetics exhibit highly specificity and high affinity in protein binding and they have already been used in cancer therapy, thus showing great potential for further applications. The example of small proteins able to perform the same action as large ones has prompted their applicability also in the field of protein recognition and PPI modulation, leading to the expression or synthesis of modified miniature proteins or mini-proteins. Nature provides a specific structural model in this regard, represented by cyclotides, plant-derived disulfide-rich mini-proteins. Because of their size and stability, cyclotides find particular applications in the modulation of

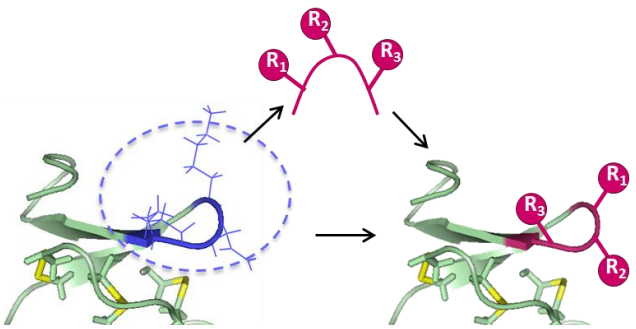


Figure 7. **"Grafted" peptides are a strategy to display diverse binding epitopes.** Some cyclotides, such as kalata B1 [PDB 2F2J], have been used as scaffolds for incorporating a range of epitopes, obtaining grafted peptides with novel biological activities.[84]

PPIs. [84] In addition to their intrinsic activities, cyclotides have been used as scaffolds for the introduction of epitopes with biological activities, thus obtaining a new class of molecule called "grafted" peptides (Figure 7). This approach led to the discovery of the engineered grafted cyclotide MCo-PMI to antagonize intracellular p53 degradation. This peptide shows high stability in human serum and is cytotoxic both in vitro and in vivo.[85] In this case, the great advantage is the high permeability of some of these cysteine-rich scaffolds, as recently reported.[86] Other mini-protein scaffolds have been used for the same purpose, such as the Avian Pancreatic Polypeptide (aPP), which was grafted to an active epitope for MDM2 recognition.[87] In that paper, Schepartz and co-workers coupled the approach to phage-display selection, thus demonstrating

the huge potential in the PPI field. Re-engineering and mutation studies were used to discover novel mini-proteins to inhibit the interaction between androgen/estrogen receptor and their co-activators.[88] Finally, a combination of phage-display and molecular grafting led to the discovery of a highly specific mini-protein, targeting the membrane protein D114, that interacts with Notch1 receptor to promote the vascularization of cancer cells. [89]

2.2.8 Photo-switchable inhibitors of PPIs (PIPPIs). Since PPIs are precisely orchestrated in cells, the advantage of complementing the pharmacological selectivity of peptide inhibitors with a means of controlling their kinetics and site of action has recently been underlined. This possibility has been explored by regulating the activity of PPI inhibitors with light, thus controlling their effects with spatiotemporal patterns of illumination. Photo-sensitive cross-linkers were introduced in peptides to regulate their structure[90] and to modulate their binding properties.[91, 92] Recently, we have

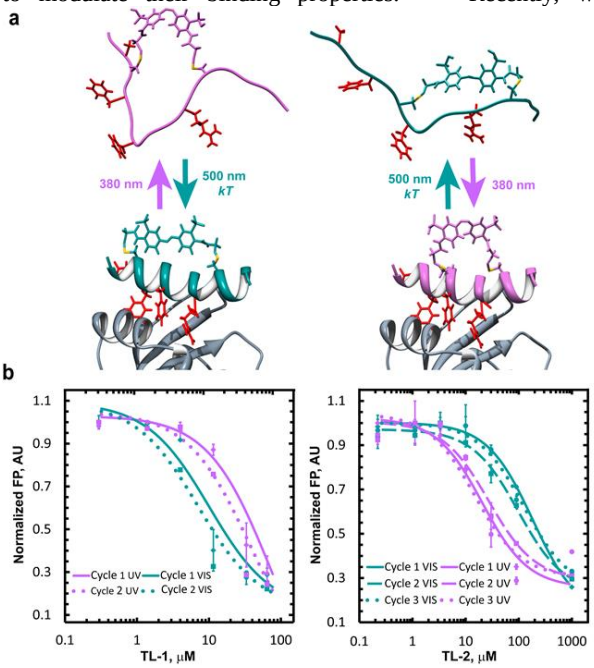


Figure 8. **Traffic light peptides (TLs) to modulate PPIs.** TLs (a) are cell-permeable photo-regulators of PPIs involved in CME, as shown by FP competition assays (b). This propriety confers 'stop' and 'go' signals to control membrane endocytosis, thus allowing the spatiotemporal patterning of membrane receptor internalization in living cells.[93]

developed photo-switchable peptides to control clathrin-mediated endocytosis (CME).[93] These inhibitors, which we call Traffic Light (TL) peptides, are cell-permeable photo-regulators of CME that enable 'stop' and 'go' signals to control membrane endocytosis, thus allowing the spatiotemporal patterning of membrane receptor internalization in living cells (Figure 8). Lately, interest in the photo-control of PPIs is growing, as classical HTS techniques have recently been applied to design a photo-switchable peptide library by phage display and ribosome display (see related Section 2.3 and 2.4).

2.3 Phage Display

The use of biological techniques for drug discovery is already a reality. Phage display in particular has been widely used to build diverse peptide libraries for HTS, and it is a common tool in the field of PPI modulation. The features and advantages of this procedure, such as the high mutability rate, complemented by the "affinity selection" are known[94]. This review aims to focus on the latest modification that has amplified the potential of phage display. In particular, the introduction of small linkers on phage displayed peptides—thereby adding chemical diversity with the aim to mimic the epitopes of folded

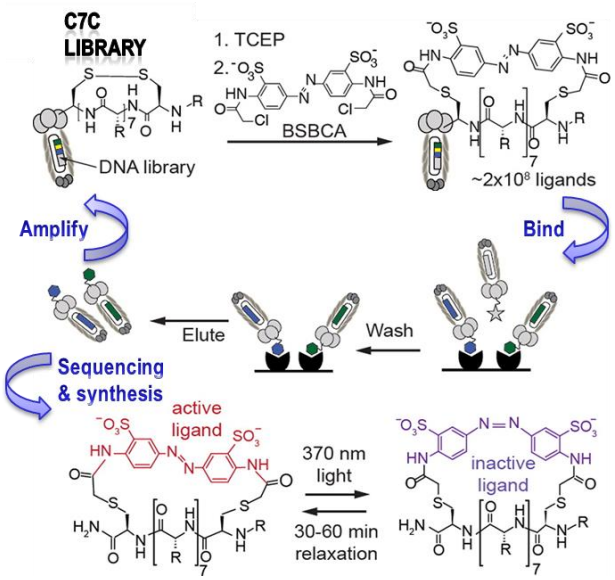


Figure 9. **Genetically encoded light-responsive library.** Light-sensitive cross-linkers have been introduced on a phage display library to select light-responsive protein binders. Adapted with permission from (ACS Chem. Biol. 2014, 9, 443-450). Copyright (2014) American Chemical Society.

proteins—has led to a new generation of encoded combinatorial libraries.[95] Chemical modifications were further explored by means of introducing light-sensitive cross-linkers on the phage[96] and adding light-driven phage selection[97] (Figure 9). Another amplification of this technique is the proteomic-peptide phage display (ProP-PD), which couples bioinformatics, oligonucleotide arrays, and peptide phage display to explore the interactome of human PDZ domain, applied in particular to study cellular and pathogen-host PPIs.[98] Phage display has also been used to find the perfect mimetic of natural proteins in PPIs. This is the case of the C-terminal of Nedd8, whose high affinity mimetic was found by selective selection over NAE (Nedd8-activating enzyme), providing an inhibitor of this post-translational modification.[99]

2.4 Ribosome Display

The limitations derived from creating a random peptide library in living cell have been addressed by the development of in vitro techniques, such as ribosome display and mRNA display, which guarantee the same high diversity of phage display but improved efficiency.[100] This technology relies on non-covalent ternary polypeptide-ribosome-mRNA complexes, which ensure the coupling of genotypes and phenotypes and does not require transformation. Using this approach, a complex library of $1.2x10^{14}$ independent members based on the scaffold of amino acids T20–V109 of protein D has been developed.[101] Similarly to what we have described in Section 2.3, the possibility of incorporating non-canonical amino acids in ribosome display

has led to the preparation of a photo-responsive peptide aptamer. t-RNA carrying azobenzene-coupled lysine was used to prepare an azobenzene-containing peptide library, which recognizes a protein target. [102] More recently, ribosome display has been used to screen the estrogen receptor surface, leading to the discovery of novel proline-rich peptide binders.[103]

2.5 mRNA display.

One of the most important advances in peptide library preparation through bio-techniques is the synthetic reprogramming of the genetic code, which allows the simultaneous introduction of multiple non-proteinogenic amino acids in the peptide sequence. The techniques involves the use mRNA display combined with mutant aminoacyl t-RNA synthetase enzymes, which catalyze the aminoacylation of the RNA strand containing the anticodon sequence for a given amino acid.[104] Suga and coworkers have channeled great effort into developing a more versatile aminoacylation catalyst, obtaining types of "flexible tRNA acylation ribozymes" (flexizymes) that are active in a specific combination with leaving groups for the preparation of acyl tRNAs (Figure 10).

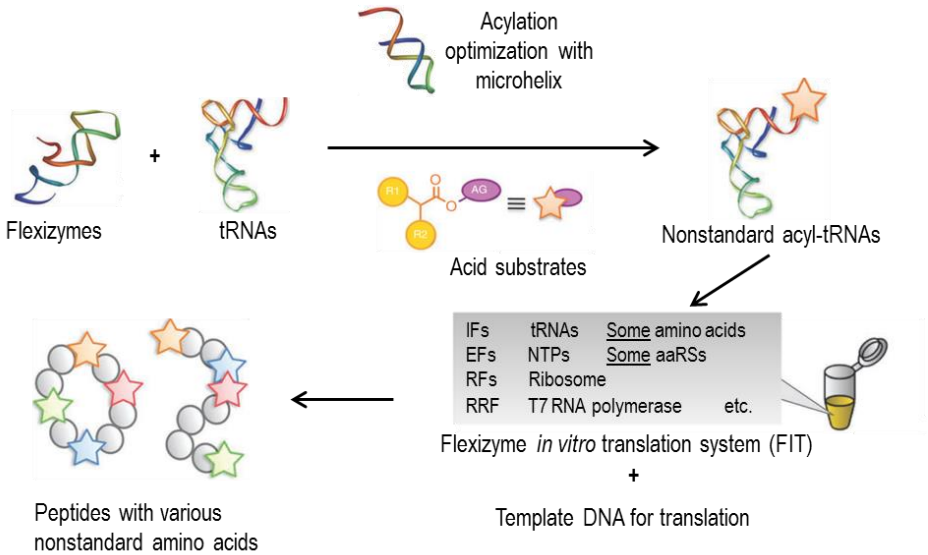


Figure 10. **Flexizymes allow the expression of non-standard peptides.** Code reprogramming is achieved by the acylation of tRNA by flexizymes in combination with specific leaving groups. The expression of peptides with non-proteinogenic amino acids is performed with a FIT system. Adapted with permission from (Nat. Prot. 2011, 6, 779-790).

These entities are used with an appropriate translational system, where some codons are emptied by excluding the chosen proteinogenic amino acid ("vacant codon") and reassigned by supplying the desired Xaa-tRNAs capable of reading the vacant codons, thus allowing the synthesis of proteins and peptides incorporating several unnatural amino acids (Figure 10).[104, 105] Recent examples of the great potential of mRNA display in the discovery of high affinity protein binders are the isolation of unnatural cyclic peptides that bind the protease thrombin with low nanomolar affinity[106] and the identification of macrocyclic peptides that antagonize VEGFR2 activity in living cells.[107]

2.6 In silico Screening

The advances in protein structure characterization by crystallography or NMR have allowed the application of computational methods for the design and virtual screening of drug candidates against desired PPIs.[108] In silico selection is a common procedure in small semi-rigid molecule screening, while the high level of flexibility in protein-peptide interactions limits their use in this field. However, reliable computational approaches have been developed to model this kind of interaction. [109] An elaborate framework for *in silico* selection of candidate inhibitory peptides for protein interactions has been recently reported.[110] It combines the screening of linear segments on interfaces of globular proteins (using the Rosetta modeling framework), peptide docking experiments (using a specific protocol for flexible peptides), and energy funnel analysis, finally applied to the EphB4–EphrinB2 interaction. Molecular dynamic simulations have also been used to predict the inhibition of short peptides (β-sheet breakers) that mimic the 17–21 region of the Aβ1–40 over the Aβ1– 40 peptide interaction in water.[111] A computational approach has also led to the discovery of inhibitors of the Ubiquitin E3 Ligase-SCFFbx4 to control TRF1 degradation, a process involved in telomerase activity. [112]

2.7 Fragment-based Discovery

The fragment-based approach is now widely used in the pharmaceutical industry, since it allows novel areas of chemical space to be explored more efficiently, even when the initial hits have low affinity. [113] A single fragment, in fact, will not normally disrupt PPIs, but can be tethered to other fragments and optimized into potent lead molecules. Fragment screening is divided into two stages: a preliminary phase by SPR or other fast-response techniques (such as virtual screening), and a second one in which a more focused validation of the hits is performed by X-ray crystallography, NMR, or ITC to obtain more specific information on the binding. One limitation is the difficulty in growing the fragment into a mature ligand directly at the protein interface. This approach is followed mainly to find small molecule inhibitors of PPIs, but it can also be useful to identify peptide inhibitors, pursuing the construction of hybrid peptides characterized by the presence of unnatural elements. This is the case of a novel stapled helix peptide inhibitor of 70N protein, which was discovered by identifying the amino acids that bind to the "hot spots" though a fragment-based approach and by selecting unnatural amino acids to substitute them.[48] The application of this method is highlighted in other two examples recently reported: the first is the discovery of peptides to target the interaction

between the tumor suppressor BRCA2 and the recombination enzyme RAD51, the latter involved in DNA repair[114, 115]; and the second is the finding of metallo-peptides to inhibit the interaction of CAL PDZ domain (CALP) with the cystic fibrosis transmembrane conductance regulator (CFTR).[115]

3. Peptides and PPI: evaluation of binding in vitro/in living cells.

Another bottleneck regarding PPI inhibition, as in medicinal chemistry research in general, is the evaluation of the effective binding of selected candidates to the target protein, and therefore the measurement of their inhibitory capacity. Many techniques have been applied to evaluate PPIs in vitro, especially when addressing the development of a reliable HTS methodology. Of these, Fluorescence/Chemiluminescence (Section 3.1) is probably one of the most useful; Nuclear Magnetic Resonance (NMR), Surface Plasmon Resonance (SPR) and Isothermal Calorimetry (ITC) are also commonly reported in literature, as described respectively in Sections 3.2, 3.3 and 3.4. Because of the extensive literature on these subjects, this review will focus specifically on their most recent applications in peptide discovery, exploring newer technical alternatives such as Surface Acoustic Wave (SAW) in Section 3.3 and Microscale Thermophoresis (MST) in Section 3.4. Finally, the urgent need for and effort channeled into "visualizing" PPIs and their inhibition by small molecule and/or peptides in living cells, prompted us to dedicate a special paragraph to Microscopy (Section 3.5).

3.1 Fluorescence/Chemiluminescence

Fluorescence labelling of proteins and peptides has been extensively used in the detection, identification, and evaluation of PPIs. "Direct" modification in wavelength and/or fluorescence intensity can be used; however, "indirect" fluorescence phenomena can also be detected, such as the Fluorescence Polarization (FP) or the Fluorescence Resonance Energy Transfer (FRET). In the first case, an interesting approach is the application of PDZ domain-mediated interactions of probes based on natural peptide ligands connected to an environment-sensitive flPDZ domain.[116] FP has been extensively applied to many PPIs, in particular when used in competitive assays, which avoid the time-consuming synthesis of fluorescence-labelled libraries to be screened. FP is based on the modulation of the anisotropy value when the sample is treated with polarized light. The increment of molecular weight caused by the interaction with a protein of a small fluorescent-labeled compound corresponds to high values, while the interaction disruption corresponds to low ones (Figure 11a). FP has recently been coupled to "tethering" to identify peptide fragments that disrupt the PPI between the KIX domain the transcriptional activator peptide pKID.[117] FRET is based on the overlap of the donor fluorescence emission spectrum with the acceptor excitation spectrum of the two labels coupled to the interaction partners (Figure 11b). The expression of fused fluorescence-labeled proteins guarantees the detection of PPIs with standard fluorescence spectrometers, but also their visualization in cell compartments through microscopy (see Section 3.5). A recent example of the potential of FRET in PPI evaluation is the optimization of a HTS steady-state assay for the Nrf2-Keap1 interaction using a 16-mer

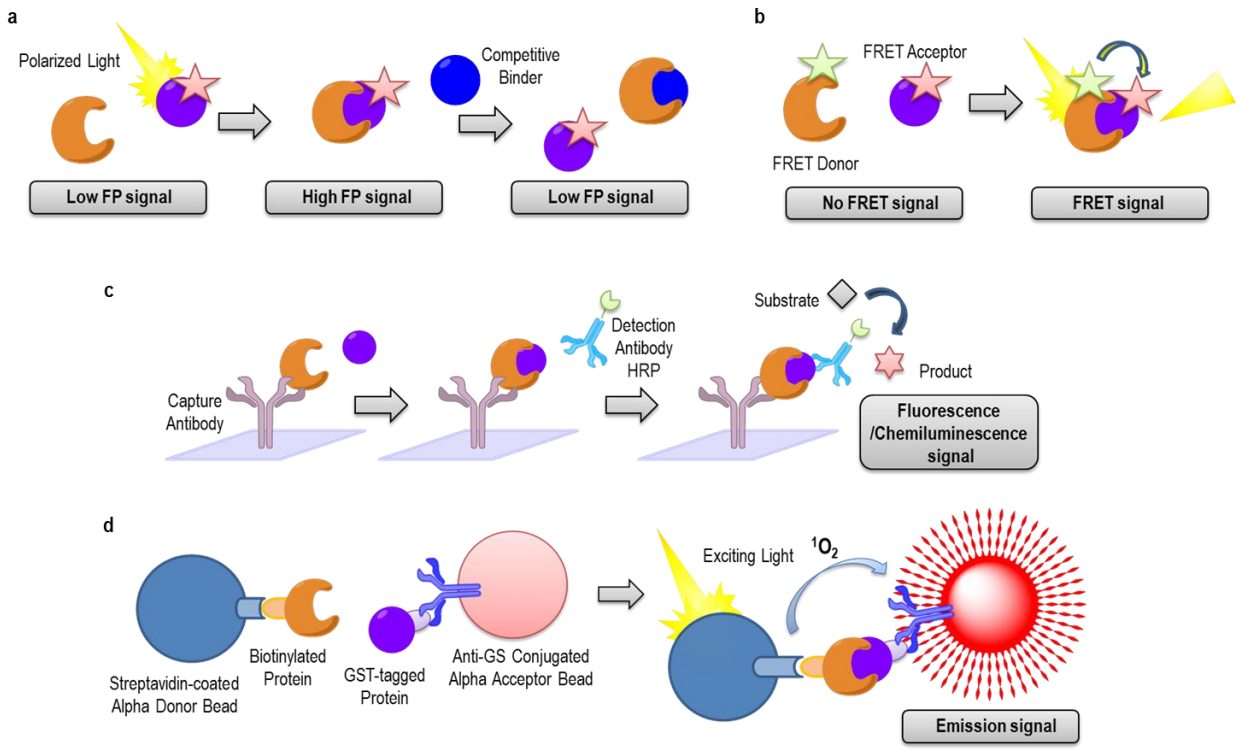


Figure 11. **Fluorescence/Chemiluminescence are the methodologies most commonly used for the validation of PPI modulation**. Fluorescence polarization [FP] as a direct and competitive binding assay (a). Fluorescence resonance energy transfer (FRET) technique (b). Enzyme-linked immunosorbent assay (ELISA) technique (c). Amplified luminescent proximity homogeneous assay screen (ALPHA Screen) technique (d).

peptide fused with CFP (Cyan Fluorescent Protein) as probe for the screening.[118] Enzyme-linked immunosorbent assays (ELISAs) works by immobilizing one of the partner proteins to a surface and by detecting the amount of the second partner protein after incubation. The detection requires the presence of a specific antibody, recognized by an enzyme-coupled secondary antibody responsible for the detection signal, from chromogenic, fluorescent to chemiluminescent read outs (Figure 11c). Amplified luminescent proximity homogeneous assay screen (ALPHA Screen) and the ELISA analogue ALPHAlisa represent the latest advances in proximity-fluorescence-based assay. Both protein partners are connected to beads. The donor bead brings a photosensitizing phthalocyanine, which releases singlet oxygen after excitation. The singlet oxygen is able to diffuse in solution within a radius of 200 nm (broader windows when compared with FRET) to the acceptor bead, which emits the detection signal (Figure 11d). Although the technology is still relatively expensive (protein modification and specific reader machine), it is highly reliable and useful in HTS and has been successfully applied to peptides evaluation for STAT family proteins[119] and integrase-LEDGF/p75 interactions.[120] A curious aspect we wish to underline is the recent use of peptides as a functional tool to improve the performance of fluorescent-based assay technology. Two examples are the WW and SH3 peptides used as "helper-interaction module" when added to FRET fluorophore pairs to improve the narrow range of FRET-permitting distances (<10 nm), and applied to the Ras-Raf1 interaction.[121] In another case, octreotide (a somatostatin-like cyclic peptide) affinity for graphene, and consequent quenching of the fluorescein signal when coupled to it has been used to develop a sensor for the detection of somatostatin receptor in cancer cells.[122]

3.2 NMR

Among the reported techniques, NMR is the only one that gives specific information at atomic resolution on the structural nature of PPIs or on protein-peptide interactions. However, NMR is limited by the use of isotopic enriched complexes, by the time required for bi-or tri-dimensional experiments, and by the high amount or concentration of protein needed.[123] Nevertheless, some NMR-based methods have great potential to identify PPI modulators, even in HTS. Chemical Shift Perturbation (CSP), transferred Nuclear Overhauser Effect (trNOESY), interligand NOEs, Target Immobilized NMR Screening (TINS), 19F-NMR screening and Saturation Transfer Difference (STD) have been extensively reviewed elsewhere and applied to a large number of systems.[124-127] A recent application of STD led to the evaluation of a peptide library for the modulation of p53-Mdm2 and VEGF-VEGFR1.[128] "Grafted proteins" offer the possibility to tackle the high protein concentration problem in NMR sample and have been successfully explored for PDZ-domain and its C-terminal peptide protein partners.[129] The use of 13C-Methyl SOFAST HMQC experiment allowed important gains in terms of signal-to-noise and the evaluation of the interaction of cognate peptides using less than 500 µg of protein.

3.3 Label-free on-surface assays: SPR and SAW

SPR is a label-free method commonly used in drug discovery. However, one of the interaction partners, often the protein over the peptide, needs to be anchored to a gold chip. When excited with polarized light, the coated chip absorbs part of the energy, reflecting a beam at a specific angle (SPR angle). The interaction with the protein partner or binding peptides alters this angle, providing the kinetics and the binding affinity of the interaction (Figure 12a). In addition to the direct binding assay, surface competition assays or inhibition in solution assays have been performed, mainly to eliminate the possible alteration of affinity resulting from the immobilization.[130] In some cases, the best results come about from immobilizing the smaller partner, which means high costs and a time-consuming procedure. These limitations have been partially solved by the development of a bi-modal imprinting chip, which transfers the peptide from the screening bead to the SPR chip, leading to the peptide binders for the hemagglutinin A-antibody AHA interaction.[131]An alternative is the expression of His-tag or biotinylated peptides that have been screened by SPR for the interaction between Plasmodium falciparum aldolase and thrombospondin-related anonymous proteins (TRAPs).[132] The same principle is used in SAW, where the mass change on the gold chip surface is translated into shifts of phase and amplitude of the exciting acoustic wave (Figure 12b). The main advantage of this technique is the combination of the SAW biosensor with mass spectrometry, which amplifies the potential for PPI evaluation.[133] SAW has not been applied

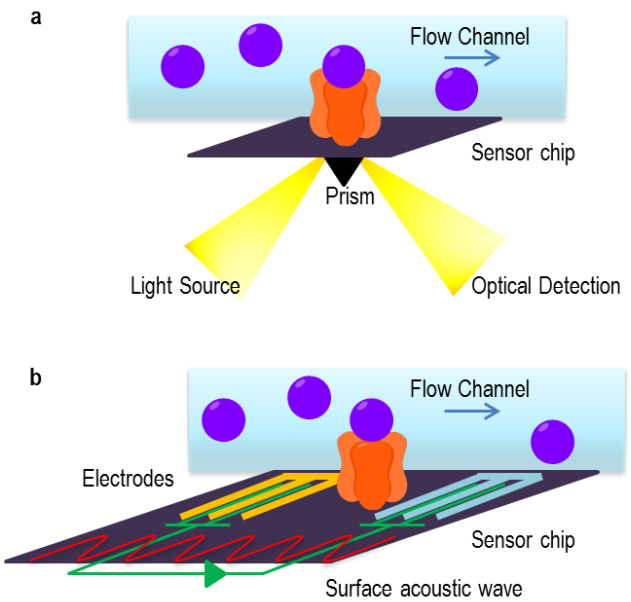


Figure 12. **Label-free on-surface methodologies are often used in the first step of binder validation.** Surface Plasmon Resonance (SPR) technique (a). Surface acoustic wave (SAW) technique (b).

specifically to peptide screening, but a starting point is given by the study of protecting factor humanin and β-amyloid peptides involved in Alzheimer disease[134] and the epitope identification of tyrosine-nitrated peptides derived from prostacyclin synthase.[135]

3.4 Label-free off-surface assays: ITC and MST

ITC is commonly used as a secondary screening technique since it has the capacity to provide thermodynamic information of the PPI under study.[136] It is, in fact, based on the measurement of temperature changes during the protein/peptide titration, and therefore during an exothermal or endothermal interaction. The values are correlated to the enthalpy and entropy contribution to binding affinity. The time-consuming experiments and the large amount of protein needed for the titration have been tackled with the development of NanoITC, which is characterized by higher sensitivity in a shorter time and with lower sample concentration. In contrast, MST is a novel and highly sensitive technique that provides affinity and thermodynamic information of the interaction using a small amount of protein partners. MST is based on thermophoresis, the directed movement of molecules in a temperature gradient, which strongly depends on a variety of molecular properties

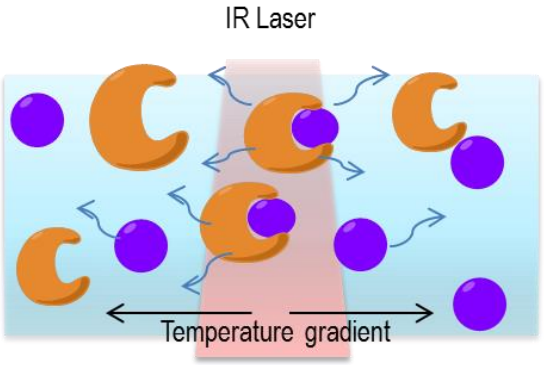


Figure 13. **Microscale thermophoresis (MST) is one of the latest methods to be applied to PPI evaluation.** The use of label-free off-surface techniques reduces the risk of inadequate evaluation caused by chemical modifications of the protein.

such as size, charge, hydration shell, and conformation (Figure 13). It is a immobilization-free method, which distinguishes it from SPR; however, in several applications non-specific fluorescent-labeling is needed, especially in cell lysate analysis.[137] However, label-free MST is possible using highly sensitive instrumentation to detect the intrinsic protein fluorescence and it has been shown to allow reliable binding evaluation.[138] MST has recently been used to evaluate the inhibitory capacity of peptides in the N-type Ca^{2+} channels (CaV2.2) and axonal collapsin response mediator protein 2 (CRMP2) interaction, involved in pain hypersensitivity.[139] Another case reported is peptides derived from the voltage-dependent anion channel (VDAC1), which interacts with Bcl-x_L and is implicated in the control of apoptosis.[140]

3.5 Microscopy

In this last section we would like to mention the current uses of microscopic techniques to detect and evaluate PPIs, and also peptide-peptide interactions, and peptide-protein interactions. The development of Single Molecule Force Spectroscopy using Atomic Force Microscope (AFM-SMFS), reviewed elsewhere[141], has led to the visualization of protein ensembles, DNA-protein complexes, and also peptide-protein interactions, as for domain I of integrin with synthetic collagen-related triple helix peptides.[142] One of the main applications has been the study of amyloid peptide assemblies on membrane surfaces.[143] This technique can be also extended to living cell membrane systems, as recently reported for the effect of anti-EGF antibody on the HER2-modulated EGF-EGFR interaction.[144] Some drawbacks are also encountered in this method. In addition to the sample−surface interaction, the tip-sample interaction may limit analysis and lead to artifacts. Moreover, AFM can be used only in a few cellular studies. However, single molecules can be extended to the imaging when coupled to other spectroscopic techniques, such as in the case of single molecule-FRET or single molecule-TIRF (total internal reflection microscopy). The feasibility of improving the visualization of PPIs in cells, thus studying their dynamics and reaction in response to drug treatments, is unquestionable. The same objective has recently been pursued in bacteria, where the use of a far-red/near-infrared (FR/NIR) fluorescence light-up probe allows the detection and visualization of specific protein/polyprotein-peptide interactions using confocal microscopy.

Conclusions

PPI modulation is one of the hot topics in diverse fields of recent research, ranging from biochemistry to chemical biology and pharmacology. For many years the focus has been on finding strong inhibitors against intracellular PPIs through the screening of small molecules. More recently, the development of new techniques for the identification and evaluation of these molecules has hugely extended the field, highlighting the potential of peptides as competitive alternatives. The introduction of remotely controlled PPI inhibitors, as in the case of PIPPIs, offers a very interesting portfolio of applications, such as in the dissection of the role of PPIs in cell biology. A less explored approach that can lead to effective results is the possibility of stabilizing PPIs rather than inhibiting them, as occurs in several examples in nature. To date, this option has been addressed only with templating ligands[145] or with small molecules[146]; however, it offers potential applications to peptides.

In addition to the advantages already described, peptide PPI modulators still present several drawbacks as drug candidates—these related in particular to pharmacodynamic and pharmacokinetic issues. In spite of these limitations, more than 200 peptide drugs and homologous compounds (proteins or antibodies) containing peptide bonds are (or have been) on the market10, and many companies are now devoted to peptide discovery. Addressing issues such as proteolytic degradation and poor cell internalization are currently the main objectives pursued by several research groups, and a number of solutions have been provided. Improved stability in vivo has been demonstrated by modifying the peptide backbone, introducing for instance β-amino acid residues.[147] Another example is given by the now consolidated class of CPPs[148, 149], which have been used as drug delivery systems[150]. The use of peptides as "shuttles" across cellular barriers has been also applied specifically to the blood-brain barrier (BBB) [151-156] as an option to tackle the difficult delivery of drugs to the central nervous system. While many issues are yet to be solved, the growing interest in peptide PPI modulators, which is reflected in the increasing number of publications, attests the relevance of this research field and the feasibility of accomplishing challenging milestones in the next decade.

Acknowledgements

This work has been supported by MINECO-FEDER (BIO2013-40716-R) and the Generalitat de Catalunya (XRB and 2014-SGR-521).

Notes and references

* Corresponding Author

[a] Institute for Research in Biomedicine (IRB Barcelona), C/ Baldiri Reixac 10, 08028 Barcelona – Spain

E-mail: laura.nevola@irbbarcelona.org

E-mail: ernest.giralt@irbbarcelona.org

Fax: (+)34 93 403 71 26 Phone: (+)34 934037127

[b] University of Barcelona (UB)

1. M. R. Arkin and J. A. Wells, Nature reviews. Drug discovery, 2004, 3, 301-317.
2. S. Jaeger and P. Aloy, IUBMB life, 2012, 64, 529-537.
3. N. London, B. Raveh and O. Schueler-Furman, Current opinion in chemical biology, 2013, 17, 952-959.
4. D. Grillo-Bosch, D. Choquet and M. Sainlos, Drug discovery today. Technologies, 2013, 10, e531-540.
5. D. Kraskouskaya, E. Duodu, C. C. Arpin and P. T. Gunning, Chemical Society reviews, 2013, 42, 3337-3370.
6. L. G. Milroy, T. N. Grossmann, S. Hennig, L. Brunsveld and C. Ottmann, Chemical reviews, 2014, 114, 4695-4748.
7. T. L. Nero, C. J. Morton, J. K. Holien, J. Wielens and M. W. Parker, Nature reviews. Cancer, 2014, 14, 248-262.
8. J. A. Wells and C. L. McClendon, Nature, 2007, 450, 1001-1009.
9. A. P. Higueruelo, H. Jubb and T. L. Blundell, Current opinion in pharmacology, 2013, 13, 791-796.
10. P. Vlieghe, V. Lisowski, J. Martinez and M. Khrestchatisky, Drug discovery today, 2010, 15, 40-56.
11. V. M. Ahrens, K. Bellmann-Sickert and A. G. Beck-Sickinger, Future medicinal chemistry, 2012, 4, 1567-1586.
12. N. T. Ross, W. P. Katt and A. D. Hamilton, Philosophical transactions. Series A, Mathematical, physical, and engineering sciences, 2010, 368, 989-1008.
13. V. Azzarito, K. Long, N. S. Murphy and A. J. Wilson, Nature chemistry, 2013, 5, 161-173.
14. I. S. Moreira, P. A. Fernandes and M. J. Ramos, Proteins, 2007, 68, 803-812.
15. A. A. Bogan and K. S. Thorn, Journal of molecular biology, 1998, 280, 1-9.
16. R. T. Bradshaw, B. H. Patel, E. W. Tate, R. J. Leatherbarrow and I. R. Gould, Protein engineering, design & selection : PEDS, 2011, 24, 197-207.
17. T. Geppert, B. Hoy, S. Wessler and G. Schneider, Chemistry & biology, 2011, 18, 344-353.
18. C. Y. Yang and S. Wang, ACS medicinal chemistry letters, 2010, 1, 125-129.
19. C. Y. Yang and S. Wang, ACS medicinal chemistry letters, 2011, 2, 280-284.
20. M. S. Golden, S. M. Cote, M. Sayeg, B. S. Zerbe, E. A. Villar, D. Beglov, S. L. Sazinsky, R. M. Georgiadis, S. Vajda, D. Kozakov and A. Whitty, Journal of the American Chemical Society, 2013, 135, 6242-6256.
21. J. Gavenonis, B. A. Sheneman, T. R. Siegert, M. R. Eshelman and J. A. Kritzer, Nature chemical biology, 2014.
22. C. J. White and A. K. Yudin, Nature chemistry, 2011, 3, 509-524.
23. C. T. Wong, H. Y. Lam, T. Song, G. Chen and X. Li, Angew Chem Int Ed Engl, 2013, 52, 10212-10215.
24. G. K. Nguyen, S. Wang, Y. Qiu, X. Hemu, Y. Lian and J. P. Tam, Nature chemical biology, 2014.
25. A. Tavassoli, Q. Lu, J. Gam, H. Pan, S. J. Benkovic and S. N. Cohen, ACS chemical biology, 2008, 3, 757-764.
26. S. K. N. Charles N. Birts, Charlotte A. Mardle, Franciane Hoakwie, Patrick J. Duriez, Jeremy P. Blaydes* and a. A. Tavassoli*, Chemical Science, 2013, 4, 3046-3057.
27. E. Miranda, I. K. Nordgren, A. L. Male, C. E. Lawrence, F. Hoakwie, F. Cuda, W. Court, K. R. Fox, P. A. Townsend, G. K. Packham, S. A. Eccles and A. Tavassoli, Journal of the American Chemical Society, 2013, 135, 10418-10425.
28. A. M. Felix, E. P. Heimer, C. T. Wang, T. J. Lambros, A. Fournier, T. F. Mowles, S. Maines, R. M. Campbell, B. B. Wegrzynski, V. Toome and et al., International journal of peptide and protein research, 1988, 32, 441-454.
29. T. Li, D. Saro and M. R. Spaller, Bioorganic & medicinal chemistry letters, 2004, 14, 1385-1388.
30. N. L. Mills, M. D. Daugherty, A. D. Frankel and R. K. Guy, Journal of the American Chemical Society, 2006, 128, 3496-3497.
31. N. E. Shepherd, H. N. Hoang, V. S. Desai, E. Letouze, P. R. Young and D. P. Fairlie, Journal of the American Chemical Society, 2006, 128, 13284-13289.
32. R. S. Harrison, N. E. Shepherd, H. N. Hoang, G. Ruiz-Gomez, T. A. Hill, R. W. Driver, V. S. Desai, P. R. Young, G. Abbenante and D. P. Fairlie, Proceedings of the National Academy of Sciences of the United States of America, 2010, 107, 11686-11691.
33. G. Udugamasooriya, D. Saro and M. R. Spaller, Organic letters, 2005, 7, 1203-1206.
34. H. Blackwell and R. H. Grubbs, Angew Chem Int Ed Engl, 1998, 37, 3281-3284.
35. P. J. Schafmeister C. E., and Verdine G. L., Journal of American Chemical Society, 2000, 122, 5891-5892.
36. Y. W. Kim, T. N. Grossmann and G. L. Verdine, Nature protocols, 2011, 6, 761-771.
37. L. D. Walensky, A. L. Kung, I. Escher, T. J. Malia, S. Barbuto, R. D. Wright, G. Wagner, G. L. Verdine and S. J. Korsmeyer, Science, 2004, 305, 1466-1470.
38. M. L. Stewart, E. Fire, A. E. Keating and L. D. Walensky, Nature chemical biology, 2010, 6, 595-601.
39. F. Bernal, A. F. Tyler, S. J. Korsmeyer, L. D. Walensky and G. L. Verdine, Journal of the American Chemical Society, 2007, 129, 2456-2457.
40. F. Bernal, M. Wade, M. Godes, T. N. Davis, D. G. Whitehead, A. L. Kung, G. M. Wahl and L. D. Walensky, Cancer cell, 2010, 18, 411-422.
41. Y. S. Chang, B. Graves, V. Guerlavais, C. Tovar, K. Packman, K. H. To, K. A. Olson, K. Kesavan, P. Gangurde, A. Mukherjee, T. Baker,

K. Darlak, C. Elkin, Z. Filipovic, F. Z. Qureshi, H. Cai, P. Berry, E. Feyfant, X. E. Shi, J. Horstick, D. A. Annis, A. M. Manning, N. Fotouhi, H. Nash, L. T. Vassilev and T. K. Sawyer, Proceedings of the National Academy of Sciences of the United States of America, 2013, 110, E3445-3454.
42. C. J. Brown, S. T. Quah, J. Jong, A. M. Goh, P. C. Chiam, K. H. Khoo, M. L. Choong, M. A. Lee, L. Yurlova, K. Zolghadr, T. L. Joseph, C. S. Verma and D. P. Lane, ACS chemical biology, 2013, 8, 506-512.
43. R. E. Moellering, M. Cornejo, T. N. Davis, C. Del Bianco, J. C. Aster, S. C. Blacklow, A. L. Kung, D. G. Gilliland, G. L. Verdine and J. E. Bradner, Nature, 2009, 462, 182-188.
44. T. N. Grossmann, J. T. Yeh, B. R. Bowman, Q. Chu, R. E. Moellering and G. L. Verdine, Proceedings of the National Academy of Sciences of the United States of America, 2012, 109, 17942-17947.
45. W. Kim, G. H. Bird, T. Neff, G. Guo, M. A. Kerenyi, L. D. Walensky and S. H. Orkin, Nature chemical biology, 2013, 9, 643-650.
46. J. Spiegel, P. M. Cromm, A. Itzen, R. S. Goody, T. N. Grossmann and H. Waldmann, Angew Chem Int Ed Engl, 2014, 53, 2498-2503.
47. C. R. Braun, J. Mintseris, E. Gavathiotis, G. H. Bird, S. P. Gygi and L. D. Walensky, Chemistry & biology, 2010, 17, 1325-1333.
48. A. O. Frank, B. Vangamudi, M. D. Feldkamp, E. M. Souza-Fagundes, J. W. Luzwick, D. Cortez, E. T. Olejniczak, A. G. Waterson, O. W. Rossanese, W. J. Chazin and S. W. Fesik, Journal of medicinal chemistry, 2014, 57, 2455-2461.
49. J. K. Sinclair, E. V. Denton and A. Schepartz, Journal of the American Chemical Society, 2014.
50. T. Okamoto, K. Zobel, A. Fedorova, C. Quan, H. Yang, W. J. Fairbrother, D. C. Huang, B. J. Smith, K. Deshayes and P. E. Czabotar, ACS chemical biology, 2013, 8, 297-302.
51. A. Glas, D. Bier, G. Hahne, C. Rademacher, C. Ottmann and T. N. Grossmann, Angew Chem Int Ed Engl, 2014, 53, 2489-2493.
52. A. Muppidi, Z. Wang, X. Li, J. Chen and Q. Lin, Chem Commun (Camb), 2011, 47, 9396-9398.
53. A. Muppidi, K. Doi, S. Edwardraja, E. J. Drake, A. M. Gulick, H. G. Wang and Q. Lin, Journal of the American Chemical Society, 2012, 134, 14734-14737.
54. A. M. Spokoyny, Y. Zou, J. J. Ling, H. Yu, Y. S. Lin and B. L. Pentelute, Journal of the American Chemical Society, 2013, 135, 5946-5949.
55. S. Cantel, C. Isaad Ale, M. Scrima, J. J. Levy, R. D. DiMarchi, P. Rovero, J. A. Halperin, A. M. D'Ursi, A. M. Papini and M. Chorev, The Journal of organic chemistry, 2008, 73, 5663-5674.
56. S. A. Kawamoto, A. Coleska, X. Ran, H. Yi, C.-Y. Yang and S. Wang, J. Med. Chem., 2012, 55, 1137-1146.
57. Y. H. Lau, P. de Andrade, N. Skold, G. J. McKenzie, A. R. Venkitaraman, C. Verma, D. P. Lane and D. R. Spring, Organic & biomolecular chemistry, 2014, 12, 4074-4077.
58. Y. H. Lau, P. de Andrade, S.-T. Quah, M. Rossmann, L. Laraia, N. Skoeld, T. J. Sum, P. J. E. Rowling, T. L. Joseph, C. Verma, M. Hyvoenen, L. S. Itzhaki, A. R. Venkitaraman, C. J. Brown, D. P. Lane and D. R. Spring, Chem. Sci., 2014, 5, 1804-1809.
59. A. D. de Araujo, H. N. Hoang, W. M. Kok, F. Diness, P. Gupta, T. A. Hill, R. W. Driver, D. A. Price, S. Liras and D. P. Fairlie, Angew Chem Int Ed Engl, 2014, 53, 6965-6969.
60. S. J. Smith, K. Du, R. J. Radford and F. A. Tezcan, Chem Sci, 2013, 4, 3740-3747.
61. A. Patgiri, M. Z. Menzenski, A. B. Mahon and P. S. Arora, Nature protocols, 2010, 5, 1857-1865.
62. L. K. Henchey, S. Kushal, R. Dubey, R. N. Chapman, B. Z. Olenyuk and P. S. Arora, Journal of the American Chemical Society, 2010, 132, 941-943.
63. A. Patgiri, K. K. Yadav, P. S. Arora and D. Bar-Sagi, Nature chemical biology, 2011, 7, 585-587.
64. D. S. Kemp, J. G. Boyd and C. C. Muendel, Nature, 1991, 352, 451-454.
65. V. Hack, C. Reuter, R. Opitz, P. Schmieder, M. Beyermann, J. M. Neudorfl, R. Kuhne and H. G. Schmalz, Angew Chem Int Ed Engl, 2013, 52, 9539-9543.
66. J. A. Robinson, Accounts of chemical research, 2008, 41, 1278-1288.
67. J. A. Robinson, Chimia, 2013, 67, 885-890.
68. S. Kim, D. Kim, H. H. Jung, I. H. Lee, J. I. Kim, J. Y. Suh and S. Jon, Angew Chem Int Ed Engl, 2012, 51, 1890-1894.
69. T. K. Yu, S. A. Shin, E. H. Kim, S. Kim, K. S. Ryu, H. Cheong, H. C. Ahn, S. Jon and J. Y. Suh, Angew Chem Int Ed Engl, 2014.
70. S. B. L. Vollrath, S. Brase and K. Kirshenbaum, Chem. Sci., 2012, 3, 2726-2731.
71. J. S. Quartararo, P. Wu and J. A. Kritzer, Chembiochem : a European journal of chemical biology, 2012, 13, 1490-1496.
72. W. Lian, P. Upadhyaya, C. A. Rhodes, Y. Liu and D. Pei, Journal of the American Chemical Society, 2013, 135, 11990-11995.
73. W. Lian, B. Jiang, Z. Qian and D. Pei, Journal of the American Chemical Society, 2014, 136, 9830-9833.
74. Z. Qian, J. R. LaRochelle, B. Jiang, W. Lian, R. L. Hard, N. G. Selner, R. Luechapanichkul, A. M. Barrios and D. Pei, Biochemistry, 2014.
75. D. Seebach and J. Gardiner, Accounts of chemical research, 2008, 41, 1366-1375.
76. A. D. Bautista, J. S. Appelbaum, C. J. Craig, J. Michel and A. Schepartz, Journal of the American Chemical Society, 2010, 132, 2904-2906.
77. E. V. Denton, C. J. Craig, R. L. Pongratz, J. S. Appelbaum, A. E. Doerner, A. Narayanan, G. I. Shulman, G. W. Cline and A. Schepartz, Organic letters, 2013, 15, 5318-5321.
78. W. S. Horne, J. L. Price and S. H. Gellman, Proceedings of the National Academy of Sciences of the United States of America, 2008, 105, 9151-9156.
79. W. S. Horne, M. D. Boersma, M. A. Windsor and S. H. Gellman, Angew Chem Int Ed Engl, 2008, 47, 2853-2856.
80. S. G. Lee and J. Chmielewski, Chembiochem : a European journal of chemical biology, 2010, 11, 1513-1516.
81. T. Hara, S. R. Durell, M. C. Myers and D. H. Appella, Journal of the American Chemical Society, 2006, 128, 1995-2004.
82. R. N. Murugan, J. E. Park, D. Lim, M. Ahn, C. Cheong, T. Kwon, K. Y. Nam, S. H. Choi, B. Y. Kim, D. Y. Yoon, M. B. Yaffe, D. Y. Yu, K. S. Lee and J. K. Bang, Bioorganic & medicinal chemistry, 2013, 21, 2623-2634.
83. Y. L. Boersma and A. Pluckthun, Current opinion in biotechnology, 2011, 22, 849-857.
84. D. J. Craik, R. J. Clark and N. L. Daly, Expert opinion on investigational drugs, 2007, 16, 595-604.

85. Y. Ji, S. Majumder, M. Millard, R. Borra, T. Bi, A. Y. Elnagar, N. Neamati, A. Shekhtman and J. A. Camarero, Journal of the American Chemical Society, 2013, 135, 11623-11633.
86. C. D'Souza, S. T. Henriques, C. K. Wang and D. J. Craik, European journal of medicinal chemistry, 2014.
87. J. A. Kritzer, R. Zutshi, M. Cheah, F. A. Ran, R. Webman, T. M. Wongjirad and A. Schepartz, Chembiochem : a European journal of chemical biology, 2006, 7, 29-31.
88. M. D. Seoane, K. Petkau-Milroy, B. Vaz, S. Moecklinghoff, S. Folkertsma, L.-G. Milroy and L. Brunsveld, MedChemComm, 2013, 4, 187-192.
89. F. Zoller, A. Markert, P. Barthe, W. Zhao, W. Weichert, V. Askoxylakis, A. Altmann, W. Mier and U. Haberkorn, Angew Chem Int Ed Engl, 2012, 51, 13136-13139.
90. J. R. Kumita, O. S. Smart and G. A. Woolley, Proceedings of the National Academy of Sciences of the United States of America, 2000, 97, 3803-3808.
91. L. Guerrero, O. S. Smart, C. J. Weston, D. C. Burns, G. A. Woolley and R. K. Allemann, Angew Chem Int Ed Engl, 2005, 44, 7778-7782.
92. B. Schierling, A. J. Noel, W. Wende, T. Hien le, E. Volkov, E. Kubareva, T. Oretskaya, M. Kokkinidis, A. Rompp, B. Spengler and A. Pingoud, Proceedings of the National Academy of Sciences of the United States of America, 2010, 107, 1361-1366.
93. L. Nevola, A. Martin-Quiros, K. Eckelt, N. Camarero, S. Tosi, A. Llobet, E. Giralt and P. Gorostiza, Angew Chem Int Ed Engl, 2013, 52, 7704-7708.
94. G. P. Smith and V. A. Petrenko, Chemical reviews, 1997, 97, 391-410.
95. C. Heinis, T. Rutherford, S. Freund and G. Winter, Nature chemical biology, 2009, 5, 502-507.
96. M. R. Jafari, L. Deng, P. I. Kitov, S. Ng, W. L. Matochko, K. F. Tjhung, A. Zeberoff, A. Elias, J. S. Klassen and R. Derda, ACS chemical biology, 2014, 9, 443-450.
97. S. Bellotto, S. Chen, I. Rentero Rebollo, H. A. Wegner and C. Heinis, Journal of the American Chemical Society, 2014, 136, 5880-5883.
98. Y. Ivarsson, R. Arnold, M. McLaughlin, S. Nim, R. Joshi, D. Ray, B. Liu, J. Teyra, T. Pawson, J. Moffat, S. S. Li, S. S. Sidhu and P. M. Kim, Proceedings of the National Academy of Sciences of the United States of America, 2014, 111, 2542-2547.
99. B. Zhao, K. Zhang, E. B. Villhauer, K. Bhuripanyo, H. Kiyokawa, H. Schindelin and J. Yin, Chembiochem : a European journal of chemical biology, 2013, 14, 1323-1330.
100. M. He and M. J. Taussig, Nature methods, 2007, 4, 281-288.
101. L. M. Yang, J. L. Wang, L. Kang, S. Gao, Y. H. Liu and T. M. Hu, PloS one, 2008, 3, e2092.
102. M. Liu, S. Tada, M. Ito, H. Abe and Y. Ito, Chem Commun (Camb), 2012, 48, 11871-11873.
103. S. Fuchs, H. D. Nguyen, T. T. Phan, M. F. Burton, L. Nieto, I. J. de Vries-van Leeuwen, A. Schmidt, M. Goodarzifard, S. M. Agten, R. Rose, C. Ottmann, L. G. Milroy and L. Brunsveld, Journal of the American Chemical Society, 2013, 135, 4364-4371.
104. J. Morimoto, Y. Hayashi, K. Iwasaki and H. Suga, Accounts of chemical research, 2011, 44, 1359-1368.
105. Y. Goto, T. Katoh and H. Suga, Nature protocols, 2011, 6, 779-790.
106. Y. V. Schlippe, M. C. Hartman, K. Josephson and J. W. Szostak, Journal of the American Chemical Society, 2012, 134, 10469-10477.
107. T. Kawakami, T. Ishizawa, T. Fujino, P. C. Reid, H. Suga and H. Murakami, ACS chemical biology, 2013, 8, 1205-1214.
108. T. L. Blundell, B. L. Sibanda, R. W. Montalvao, S. Brewerton, V. Chelliah, C. L. Worth, N. J. Harmer, O. Davies and D. Burke, Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 2006, 361, 413-423.
109. M. Rubinstein and M. Y. Niv, Biopolymers, 2009, 91, 505-513.
110. N. London, B. Raveh, D. Movshovitz-Attias and O. Schueler-Furman, Proteins, 2010, 78, 3140-3149.
111. V. Minicozzi, R. Chiaraluce, V. Consalvi, C. Giordano, C. Narcisi, P. Punzi, G. C. Rossi and S. Morante, The Journal of biological chemistry, 2014, 289, 11242-11252.
112. J. Lee, D. W. Sammond, Z. Fiorini, J. P. Saludes, M. G. Resch, B. Hao, W. Wang, H. Yin and X. Liu, Chembiochem : a European journal of chemical biology, 2013, 14, 445-451.
113. C. W. Murray and D. C. Rees, Nature chemistry, 2009, 1, 187-192.
114. D. E. Scott, M. T. Ehebauer, T. Pukala, M. Marsh, T. L. Blundell, A. R. Venkitaraman, C. Abell and M. Hyvonen, Chembiochem : a European journal of chemical biology, 2013, 14, 332-342.
115. R. Kundu, P. R. Cushing, B. V. Popp, Y. Zhao, D. R. Madden and Z. T. Ball, Angew Chem Int Ed Engl, 2012, 51, 7217-7220.
116. M. Sainlos, W. S. Iskenderian and B. Imperiali, Journal of the American Chemical Society, 2009, 131, 6680-6682.
117. J. M. Lodge, T. J. Rettenmaier, J. A. Wells, W. C. Pomerantz and A. K. Mapp, MedChemComm, 2014, 5, 370-375.
118. M. Schaap, R. Hancock, A. Wilderspin and G. Wells, Protein science : a publication of the Protein Society, 2013, 22, 1812-1819.
119. K. Takakuma, N. Ogo, Y. Uehara, S. Takahashi, N. Miyoshi and A. Asai, PloS one, 2013, 8, e71646.
120. C. Cavalluzzo, F. Christ, A. Voet, A. Sharma, B. K. Singh, K. Y. Zhang, E. Lescrinier, M. De Maeyer, Z. Debyser and E. Van der Eycken, Journal of peptide science : an official publication of the European Peptide Society, 2013, 19, 651-658.
121. R. Grunberg, J. V. Burnier, T. Ferrar, V. Beltran-Sastre, F. Stricher, A. M. van der Sloot, R. Garcia-Olivas, A. Mallabiabarrena, X. Sanjuan, T. Zimmermann and L. Serrano, Nature methods, 2013, 10, 1021-1027.
122. F. Bianying, G. Linjie, W. Lihua, L. Fan, L. Jianxin, G. Jimin, F. Chunhai and H. Qing, Analytical chemistry, 2013, 85, 7732-7737.
123. E. Giralt, M. Peczuh and X. Salvatella, Protein surface recognition : approaches for the inhibition of protein-protein interactions for drug discovery, John Wiley & Sons, Chichester, West Sussex.
124. X. Salvatella and E. Giralt, Chemical Society reviews, 2003, 32, 365-372.
125. A. Bhunia, S. Bhattacharjya and S. Chatterjee, Drug discovery today, 2012, 17, 505-513.
126. M. Pellecchia, I. Bertini, D. Cowburn, C. Dalvit, E. Giralt, W. Jahnke, T. L. James, S. W. Homans, H. Kessler, C. Luchinat, B.

Meyer, H. Oschkinat, J. Peng, H. Schwalbe and G. Siegal, Nature reviews. Drug discovery, 2008, 7, 738-745.
127. M. Goldflam, T. Tarrago, M. Gairi and E. Giralt, Methods Mol Biol, 2012, 831, 233-259.
128. M. A. Bonache, B. Balsera, B. Lopez-Mendez, O. Millet, D. Brancaccio, I. Gomez-Monterrey, A. Carotenuto, L. M. Pavone, M. Reille-Seroussi, N. Gagey-Eilstein, M. Vidal, R. de la Torre-Martinez, A. Fernandez-Carvajal, A. Ferrer-Montiel, M. T. Garcia-Lopez, M. Martin-Martinez, M. J. de Vega and R. Gonzalez-Muniz, ACS combinatorial science, 2014.
129. J. M. Vieville, S. Charbonnier, P. Eberling, J. P. Starck and M. A. Delsuc, Journal of pharmaceutical and biomedical analysis, 2014, 89, 18-23.
130. L. Choulier, Y. Nomine, G. Zeder-Lutz, S. Charbonnier, B. Didier, M. L. Jung and D. Altschuh, Analytical chemistry, 2013, 85, 8787-8795.
131. W. Wang, M. Li, Z. Wei, Z. Wang, X. Bu, W. Lai, S. Yang, H. Gong, H. Zheng, Y. Wang, Y. Liu, Q. Li, Q. Fang and Z. Hu, Analytical chemistry, 2014, 86, 3703-3707.
132. L. E. Boucher and J. Bosch, Journal of molecular recognition : JMR, 2013, 26, 496-500.
133. G. Treitz, T. M. Gronewold, E. Quandt and M. Zabe-Kuhn, Biosensors & bioelectronics, 2008, 23, 1496-1502.
134. M. Maftei, X. Tian, M. Manea, T. E. Exner, D. Schwanzar, C. A. von Arnim and M. Przybylski, Journal of peptide science : an official publication of the European Peptide Society, 2012, 18, 373-382.
135. M. Dr Gusanu, B. A. Petre and M. Przybylski, Journal of peptide science : an official publication of the European Peptide Society, 2011, 17, 184-191.
136. M. M. Pierce, C. S. Raman and B. T. Nall, Methods, 1999, 19, 213-221.
137. C. J. Wienken, P. Baaske, U. Rothbauer, D. Braun and S. Duhr, Nature communications, 2010, 1, 100.
138. S. A. Seidel, C. J. Wienken, S. Geissler, M. Jerabek-Willemsen, S. Duhr, A. Reiter, D. Trauner, D. Braun and P. Baaske, Angew Chem Int Ed Engl, 2012, 51, 10656-10659.
139. S. M. Wilson, B. S. Schmutzler, J. M. Brittain, E. T. Dustrude, M. S. Ripsch, J. J. Pellman, T. S. Yeum, J. H. Hurley, C. M. Hingtgen, F. A. White and R. Khanna, The Journal of biological chemistry, 2012, 287, 35065-35077.
140. N. Arbel, D. Ben-Hail and V. Shoshan-Barmatz, The Journal of biological chemistry, 2012, 287, 23152-23161.
141. A. Rajendran, M. Endo and H. Sugiyama, Chemical reviews, 2014, 114, 1493-1520.
142. S. J. Attwood, A. M. Simpson, S. W. Hamaia, D. Bihan, D. Roy, R. W. Farndale and M. E. Welland, International journal of molecular sciences, 2013, 14, 2832-2845.
143. C. C. Chang, J. C. Althaus, C. J. Carruthers, M. A. Sutton, D. G. Steel and A. Gafni, PloS one, 2013, 8, e82139.
144. X. Zhang, X. Shi, L. Xu, J. Yuan and X. Fang, Nanomedicine : nanotechnology, biology, and medicine, 2013, 9, 627-635.
145. S. Gordo, V. Martos, E. Santos, M. Menendez, C. Bo, E. Giralt and J. de Mendoza, Proceedings of the National Academy of Sciences of the United States of America, 2008, 105, 16426-16431.
146. P. Thiel, M. Kaiser and C. Ottmann, Angew Chem Int Ed Engl, 2012, 51, 2012-2018.
147. R. W. Cheloha, A. Maeda, T. Dean, T. J. Gardella and S. H. Gellman, Nature biotechnology, 2014, 32, 653-655.
148. C. Bechara and S. Sagan, FEBS letters, 2013, 587, 1693-1702.
149. I. Martin, M. Teixido and E. Giralt, Chembiochem : a European journal of chemical biology, 2011, 12, 896-903.
150. M. Moreno, E. Zurita and E. Giralt, Journal of controlled release : official journal of the Controlled Release Society, 2014, 182, 13-21.
151. M. Teixido, E. Zurita, M. Malakoutikhah, T. Tarrago and E. Giralt, Journal of the American Chemical Society, 2007, 129, 11802-11813.
152. M. Malakoutikhah, M. Teixido and E. Giralt, Angew Chem Int Ed Engl, 2011, 50, 7998-8014.
153. R. Prades, S. Guerrero, E. Araya, C. Molina, E. Salas, E. Zurita, J. Selva, G. Egea, C. Lopez-Iglesias, M. Teixido, M. J. Kogan and E. Giralt, Biomaterials, 2012, 33, 7194-7205.
154. B. Oller-Salvia, M. Teixido and E. Giralt, Biopolymers, 2013, 100, 675-686.
155. M. Malakoutikhah, R. Prades, M. Teixido and E. Giralt, Journal of medicinal chemistry, 2010, 53, 2354-2363.
156. M. Malakoutikhah, B. Guixer, P. Arranz-Gibert, M. Teixido and E. Giralt, ChemMedChem, 2014, 9, 1594-1601.2. S. Jaeger and P. Aloy, IUBMB life, 2012, 64, 529-537.