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Teaser Today, by improving administration, stability and intracellular delivery, the interest in peptides as potential drugs is resurgent, especially for targeting the thousands of intracellular protein–protein interactions implicated in cellular homeostasis and pathological conditions

Interfering peptides targeting protein–protein interactions: the next generation of drugs?

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Protein–protein interactions (PPIs) are well recognized as promising therapeutic targets. Consequently, interfering peptides (IPs) – natural or synthetic peptides capable of interfering with PPIs – are receiving increasing attention. Given their physicochemical characteristics, IPs seem better suited than small molecules to interfere with the large surfaces implicated in PPIs. Progress on peptide administration, stability, biodelivery and safety are also encouraging the interest in peptide drug development. The concept of IPs has been validated for several PPIs, generating great expectations for their therapeutic potential. Here, we describe approaches and methods useful for IPs identification and *in silico*, physicochemical and biological-based strategies for their design and optimization. Selected promising *in-vivo*-validated examples are described and advantages, limitations and potential of IPs as therapeutic tools are discussed.

Introduction

Cell homeostasis depends on a fine-tuned network of protein–protein interactions (PPIs). It has been estimated that the human interactome involves between 130 000 and 600 000 PPIs [1–3]. Because the deregulation of these interactions is often associated with pathology, each of them could be seen as a potential target opportunity, covering a very large range of illnesses [4]. Drugs

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that can specifically modulate these interactions, and thus the downstream signaling pathways they regulate, have become the object of intensive efforts during the past decade [5–7]. Although important therapy successes have been obtained early using peptides [8] and monoclonal antibodies [9] to target membrane or extracellular PPIs, the development of peptides targeting intracellular proteins has been limited by the intrinsic biochemical properties of the peptides [10–12]. In particular, the means of administration, stability and bioavailability of therapeutic peptides have long been of concern. Small-molecule inhibitors were then considered to target intracellular PPIs [7,13–17]; however, the use of small compounds could be limited for target PPIs owing to the specific features of PPI interfaces. In this context, natural or synthetic peptides capable of interfering with PPIs, called interfering peptides (IPs), are receiving increasing attention (Fig. 1) [18–21]. The large contact surfaces involved in PPIs (1500–3000 Å) [3], often missing clear features (such as pockets, grooves or clefts), have been repeatedly noted as an obstacle for their targeting with small molecules and to serve for modeling and design of new small therapeutic molecules [7]. By contrast, peptide molecules designed to interact with large and flat protein surfaces seem to be better adapted. There is increasing evidence for the capacity of IPs to modify several cellular processes and support the notion that they would have a significant potential to become quickly valuable therapeutic tools [22–28]. With a long history of use in therapeutics, peptides are now recognized as being safe and well tolerated [9,12]. The differences in the physicochemical properties between the small molecules and peptides – molecular weight, flexibility and toxicological profiles – raise new challenges for further development. Improvements in the ADME proprieties of the peptides have led to a resurgence of interest in the development of IPs as drugs. For instance, recent reports showed the feasibility of oral and intranasal administration of peptides as well as ways to improve their stability [29–34]. Moreover, the use of cell-penetrating peptides (CPPs) with the capacity of transporting chemically different cargos emerges as a promising option to improve intracellular peptide delivery [35] – another long-term weakness. This opens new perspectives to specifically address target cells and intracellular compartments [36]. For these reasons, we believe that IPs represent a new and exciting class of drug candidates for inhibition of intracellular PPIs. Today, the identification of candidate IPs can be addressed through various means [36]: (i) the characterization of natural occurring peptides; (ii) brute-force strategies relying on the generation of large libraries of peptides that are then screened to identify active candidates; and (iii) the use of peptides corresponding to short fragments located at the protein–protein interface, which are natural candidates to interfere with PPIs. Here, we describe strategies to identify IPs when protein–protein-interacting structures are known (rational design) or when they are not known (empirical approach). Then, we describe methods useful to optimize identified IPs and we describe some promising examples that have been validated in *in vitro* and/or *in vivo* models.

Strategies for IP identification

Fig. 2 summarizes current strategies involved in IP discovery.

Structure-based approaches

Knowledge of the structure of the complex of the interacting proteins is the most useful information. Among biophysical approaches, X-ray crystallography, NMR and cryo-electron microscopy (cryo-EM) remain the techniques that are most widely used to determine the 3D structure of macromolecular complexes and to provide direct access to the identification of the PPIs [37,38]. In addition to X-ray crystallography to determine, in isolation or in interaction, globular domain structures, NMR can also investigate transient and weak PPIs that are essential in cell signaling transduction. Protein interfaces become better understood through the crystal or solution structure of protein complexes. At the time of writing (June 2017), 8096 structures of protein–protein complexes involving 12 936 proteins have been reported in the Protein Data Bank (proteins of at least 30 amino acids, removing protein chain with sequence similarity >90%). In addition, the combination of cryo-EM and small-angle X-ray scattering (SAXS) as low resolution structural techniques with other methods such as X-ray crystallography, NMR, Förster resonance energy transfer (FRET), mass spectroscopy techniques and *in silico* approaches also provides near atomic resolution information to characterize protein interfaces [39,40].

When the structure of the complex can be solved, IPs can be rationally designed based on the direct observation of the natural sequences that mediate PPIs in the proteins. It has been observed

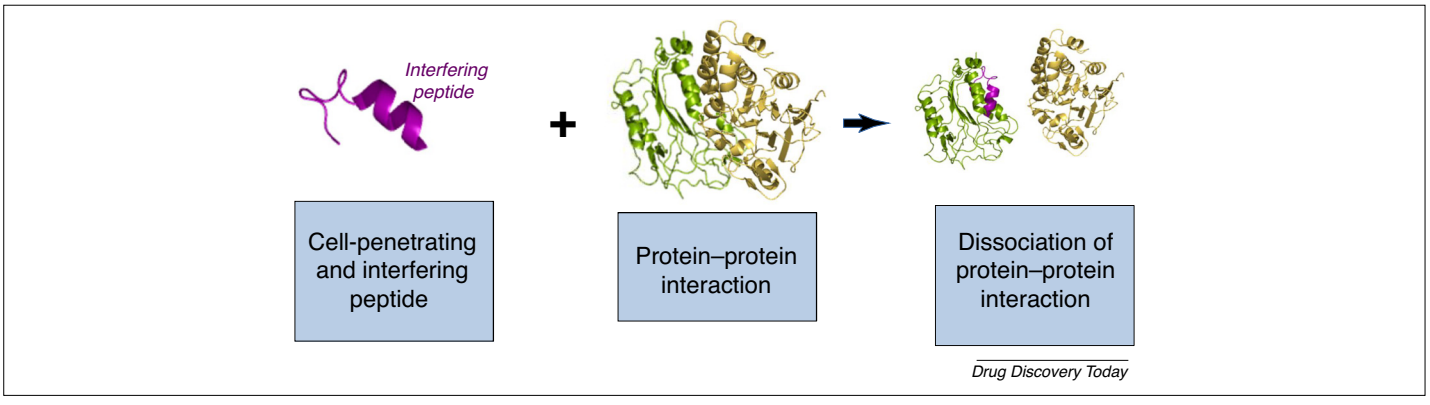

FIGURE 1

Schematic representation of the effect of an interfering peptide (IP) targeting a protein–protein interaction (PPI). An IP is a peptide that can specifically affect the normal interaction between two proteins. In most of cases the use of an IP results in the modulation of a signaling pathway.

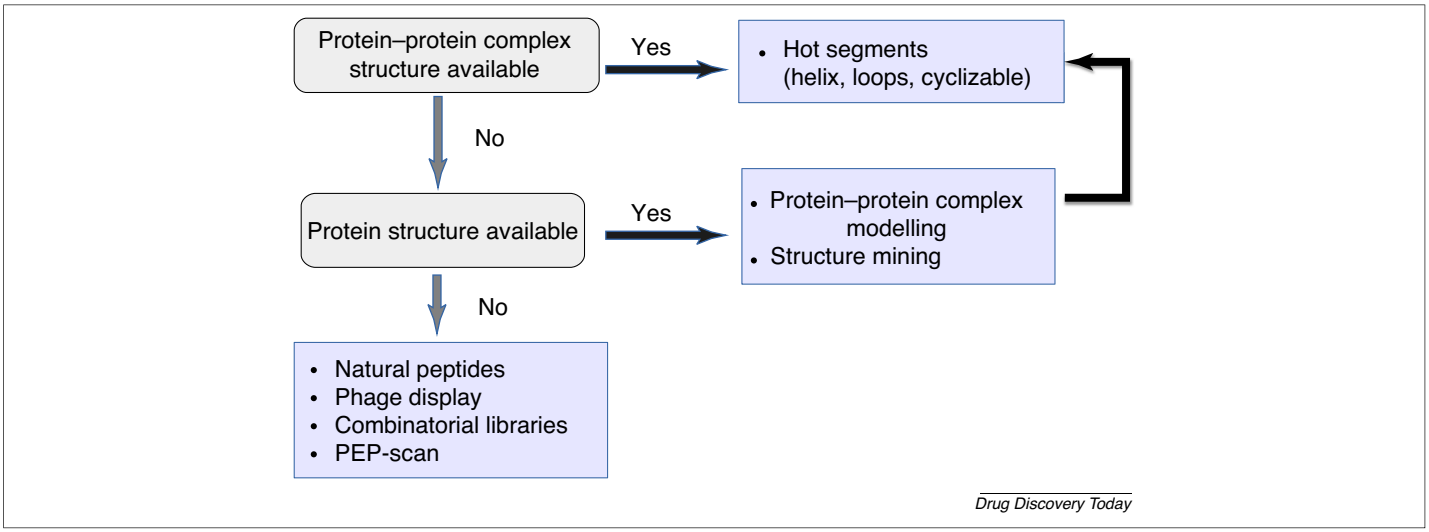

FIGURE 2

Current strategies for IP discovery. Strategies to identify candidate interfering peptides (IPs) vary depending on the availability of knowledge on the 3D structure of the targeted complex. These approaches range from the rational identification of sequences located at the protein–protein interface in the structure of the complex, to the identification of active IP sequences using random or systematic strategies.

that only a few hot segments involving residues located at the PPI seem responsible for the interaction between the partners [41], and the analyses of the 3D structures in interaction using structural bioinformatics methods can help to identify peptides from one partner that could bind to the other partner and thus interfere with the PPI. LoopFinder [42], PeptiDerive [43] or searching for helix interfaces in protein–protein interactions (HIPPs) [44] are examples of *in silico* approaches exploiting this observation. PeptiDerive systematically splits the chains in the interaction as series of fragments of 10 amino acids and identifies those corresponding to hot segments. It has been used successfully to design agonists of the MD2–TLR4 interaction [45]. It is interesting to note that the linear peptide identified originally was not active but inserting a disulfide bond stabilized its variant. Consequently, PeptiDerive now specializes in the identification of hot segments compatible with the use of peptides that can be closed by a disulfide bridge. The HIPP approach specializes in the identification of helical segments that can undergo further modifications such as stapling, whereas LoopFinder focuses on loops that can be cyclized. Submicromolar inhibitors of stonin2 and Eps15, designed using LoopFinder, have recently been reported [46].

When the structures of the partners are available but not that of the complex, *in silico* approaches can to some extent still prove to be useful. First, modeling techniques, exploiting either brute-force, co-evolutionary or homology information, can provide low- or medium-resolution models of protein–protein complexes that could be used to locate the interface and beyond, and assist the identification of candidate peptides. Several such approaches have been reported recently [47,48]. Whether the structural approximations of the models are compatible or not with the identification of hot segments with an accuracy similar to that reached from experimentally resolved structures is still a matter for investigation.

Finally, not attempting to build the complex structure, information about the interface region on the structure, either predicted or using *in vitro* techniques such as mutation analysis, can also be valuable. It has been observed that, for a significant number of protein–peptide complexes, the binding pose adopts structural patterns similar to those found in a single-chain fold [49]. Mining the increasing collection of protein structures can help to obtain information about protein segments likely to interact with a protein patch. PepComposer [50] is an example of such an approach. Although it was shown able to identify segments mimicking those of protein–peptide complexes, this approach has not yet led to the effective discovery of new interactive peptides.

Off-structure approaches: phenotype- and target-oriented approaches

When no structural information of the partners is available, several *in vitro* approaches have been used with success to identify bioactive peptides. Among the phenotype-oriented approaches, screening of natural peptides [51] and of artificial libraries containing peptides synthesized on a solid support [52] have often been employed. Libraries enable simultaneous testing of a high number of variants in a single screen. Libraries can be designed using mixed codons at protein interface positions [53]. Randomization can be tailored by the mixture of nucleotides as well as their ratio. This has been applied with success to study the NOX-CFTR-CK2 interactions during lung development [54].

Other approaches include phage display, where filamentous bacteriophages expressing peptides and exposing them on their surface are analyzed, and the production of IPs by recombinant adeno-associated virus [54]. Phage display was described by Smith in 1985 [55] and has been one of the most used techniques for *in vitro* screening of small peptides targeting proteins [56–61]. With these strategies, the peptides produced can be tested for their affinity to bind a selected target and for their capacity to modify a cellular process (development, proliferation, differentiation apoptosis, senescence, etc.). For example, phage display has been used to identify IPs targeting the vascular endothelial growth factor (VEGF)–neuropilin (NRP)-1 interaction [62].

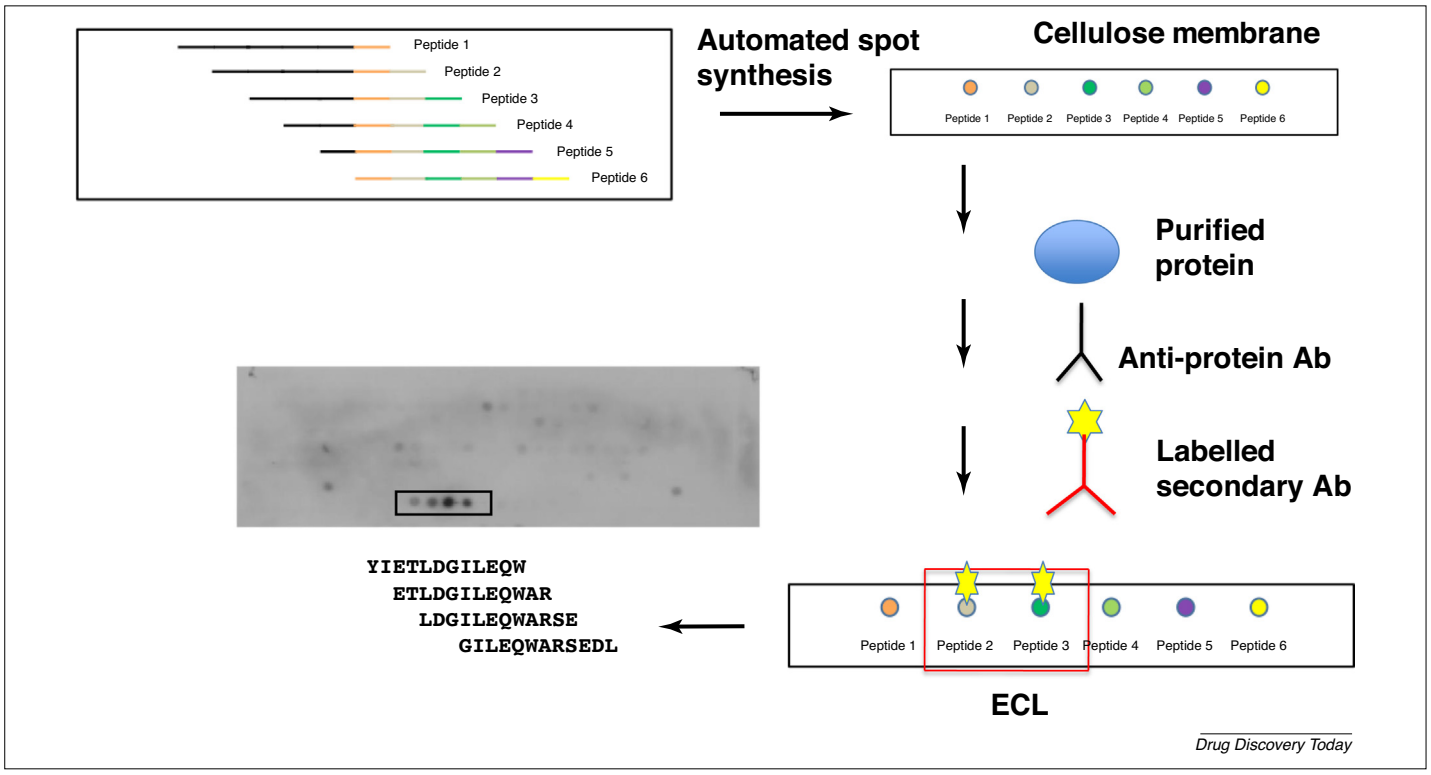

FIGURE 3

Schematic representation of the PEPscan approach. Overlapping dodecapeptides (with a shift of two amino acids) spanning the complete sequence of one of the proteins of the complex are prepared and spotted in a solid support. The membrane is hybridized with the other purified interacting protein of the complex. The presence of a complex is detected using an antibody against the purified protein, followed by a labeled secondary antibody.

The most powerful target-oriented approach to identify IPs is PEPscan technology. The principle of PEPscan is to scan the sequence of one of the partners split as a series of overlapping peptides, systematically testing the ability of each peptide to compete with the formation of PPIs (Fig. 3). The overlap between consecutive segments offers the possibility of precise identification of the binding site(s) between two proteins of interest and improves confidence in the results by introducing some redundancy in the tests. The sizes of peptides as well as the step of the sliding window along the sequence are of course crucial. Common values are 12 and two, respectively [63,64]. Complex formation is usually monitored by western blot. To deal with the large number of experiments required, PEPscan technology is based on the synthesis of peptide arrays bound to a solid support such as cellulose. Peptide arrays provide a rapid approach to screen PPIs. They were used for the identification of T and B epitopes [65,66]. Peptide arrays offer almost an unlimited choice of size and format. The peptide lengths usually range from 5 to 30 amino acids. SPOT synthesis is the technique used to generate this type of peptide array [63,67], based on solid-phase fluorenylmethyloxycarbonyl (Fmoc)-based chemistry [64].

SPOT synthesis is particularly flexible with respect to the numbers and scales that can be accomplished: the arrays can be adapted in terms of length of the peptide, spot size, number of spots per membrane and spotted volume [63,67]. Any reported system can be used that results in a signal that is trapped at the peptide site (e.g., ELISA, western blot, etc.). Rebollo and co-workers adapted peptide microarrays and generated overlapping dodecapeptides scanning the complete sequence of several proteins involved in tumoral transformation and apoptosis. The peptide arrays were prepared by automated spot synthesis (Abimed) into an amino-derived cellulose membrane as described [64,67]. Using this approach, the binding sites of several PPIs have been identified: the oncoprotein Ras and the kinase Raf [68]; the cysteine protease caspase 9 and the serine/threonine phosphatase PP2A [69]; the serine/threonine phosphatase PP2A and its physiological inhibitor the oncoprotein SET [70]; the cysteine protease caspase 9 and the oncoprotein SET [71]; the interaction between the transcription factors of the hippo signaling pathway TEAD, Yap and TAZ [72]; and the interaction Ras–Raf [73]. This approach was also used to identify the binding sites between proteins from the *Plasmodium falciparum* parasite [74].

Candidate peptide optimization

As illustrated in Fig. 4, candidate peptide optimization benefits from contributions from different fields.

Structural characterization of the target–IP interaction

Knowledge of the mode-of-interaction between the peptide and the target is crucial for optimization. On the experimental side, a combination of biophysical methods is needed to fully describe PPIs and optimize the design of a IPs. Thermodynamic and kinetic description of complexes can be achieved using calorimetry and surface plasmon resonance (SPR) methods. Microcalorimetry enables the thermodynamic characterization of molecular interactions in solution. Isothermal titration calorimetry (ITC) enables the direct determination of binding stoichiometry, equilibrium constant and variation of enthalpy (ΔH) and entropy (ΔS). SPR

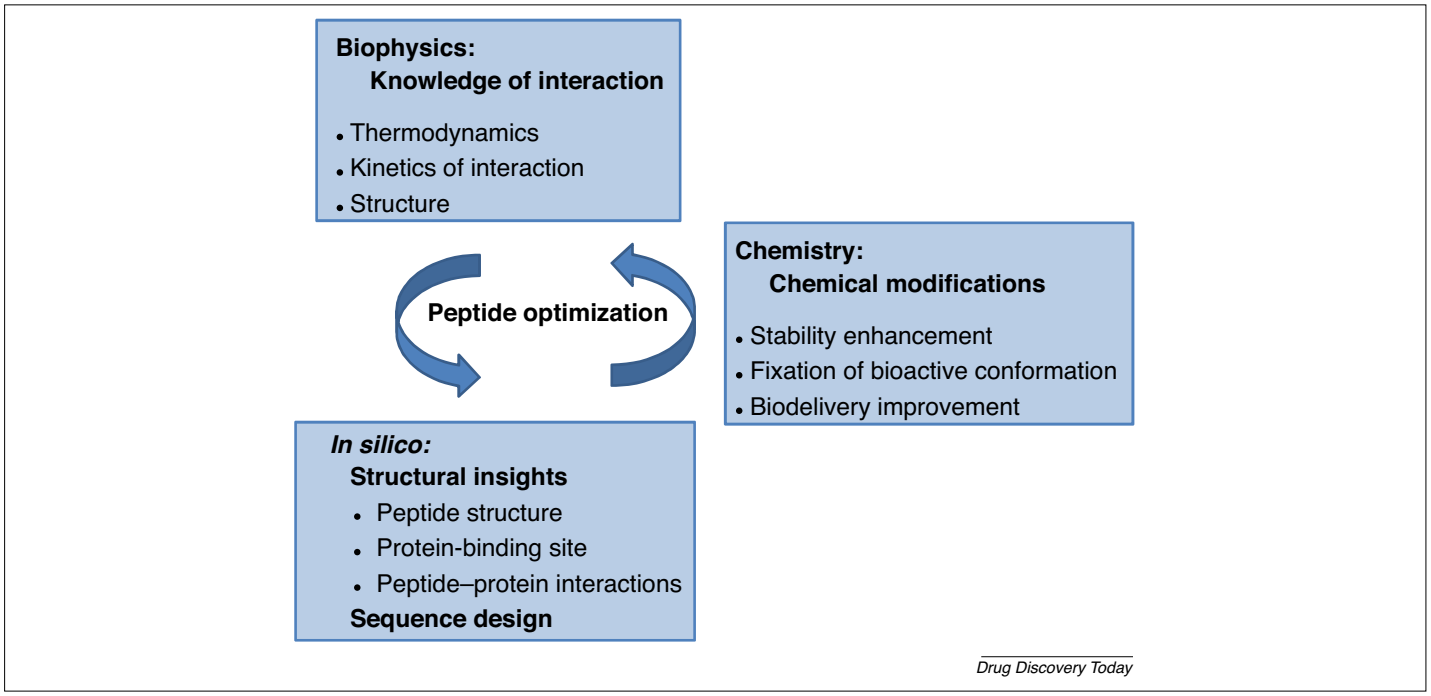

FIGURE 4

Flowchart of peptide optimization: biophysical, chemical and *in silico* contributions are iteratively required. The characterization of peptide–protein interaction at the molecular level, using biophysical and *in silico* approaches, guides the choice of chemical modifications likely to improve peptide activity as well as ADME properties.

instruments enable the analysis of molecular interactions in real time. This technique is notably suited for the determination of the rates of association (k_{on}) and dissociation (k_{off}) between biological macromolecules. Fluorescence spectroscopy, associated with stopped-flow-equipment, can also be applied to study the molecular interactions in real time to determine the properties of fast reactions with a time resolution of milliseconds. Altogether, these biophysical properties of peptide–protein complexes could be used to decrease the entropic cost upon binding for peptides that explore multi-conformational states in the free-form in solution. Peptides have a tendency to lose their stable conformation out of the 3D protein context. Thus, minimizing the entropic cost and increasing the enthalpic energy of binding and the residence time could be achieved by constraining the backbone in hairpins, stapled peptides, scaffolds mimicking the structural and/or functional properties of natural peptides (see below) [75]. Such thermodynamics and kinetics approaches have been recently used to optimize affined and stable peptides inhibiting the Keap1–Nrf2 PPI. Nrf2 is a key transcriptional factor and, along with Keaps, its primary regulator, is a member of a signaling pathway involved in oxidative stress and inflammation [76].

On the *in silico* side, enormous progress has been recently achieved to fill the gap resulting from decades of protocol optimization to assist small-compound design compared with that of peptide-specific protocols. Indeed, in contrast to small compounds, computer-assisted peptide design usually starts from only a few peptide sequences and key issues specific to peptide design are first in terms of conformational sampling owing to the larger number of degrees of freedom, and second in terms of scoring. Specific approaches addressing the *de novo* prediction of peptide structure [77–79], peptide-binding site [80–83], peptide–protein complex conformation [84–87], among others, are now accumulating.

Given the target structure and the peptide sequence, *in silico* approaches start providing useful information on candidate patches of interaction on the protein surface, with a sensitivity and specificity close to 37% [83], meaning that the approximate location of the interaction region can usually be identified. Fig. 5 illustrates how the pepATTRACT server [83] can mimic a PEPscan experiment applied to the identification of peptides interfering with the PP2A–caspase 9 and Ras–Raf complexes. One clearly sees that the interactions of the experimentally identified IPs target the interface region, whereas the peptides corresponding to other parts of the sequence tend, for this PPI, to target a different patch. Moving one step further, the search for the exact binding mode of interaction between the protein and the peptide – predicting the complex structure – turns out to be more challenging. Whereas it has been demonstrated that starting from an approximate complex (RMSD 5 A) [84] can result in the generation of high-quality poses, the general question of the blind docking (i.e., complex conformation generation for the complete exploration of protein surface) or local docking (specifying a candidate binding site) is still open. Briefly, most current approaches can generate high-quality poses, but often fail to identify them among the large number of decoys that are usually generated. Whereas most approaches consider full peptide flexibility, the accounting for the small protein conformation adaptation upon peptide binding would be necessary to make it possible to discriminate between correct and incorrect poses. As a consequence, the estimation of binding affinities given the peptide sequence and protein structure remains approximate [88]. In addition, conformational heterogeneity in protein–peptide interactions might be more frequent than

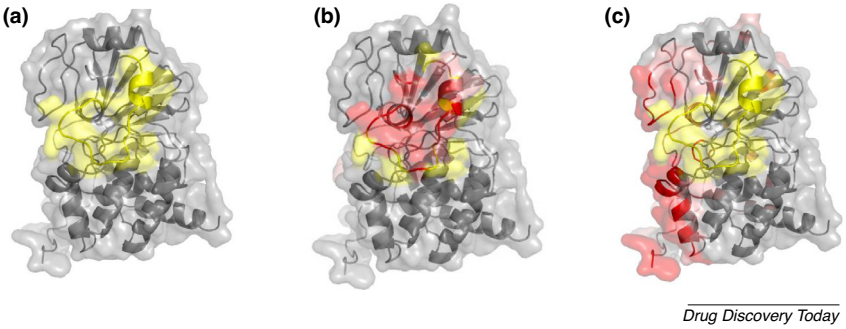

FIGURE 5

In vitro and *in silico* analysis of protein–protein (PPI) interaction. The example of phosphatase PP2A and caspase 9 interaction is shown. **(a)** PP2A catalytic chain is shown in gray and in yellow is the identified patch using *in vitro* PEPscan screening. **(b)** The interaction patch (red) predicted *in silico* using the caspase 9 interfering peptide identified *in vitro*. **(c)** The cumulated propensities of interaction for the PEPscan-negative caspase 9 peptides target a different surface area of PP2A.

initially thought, increasing the complexity of affinity and specificity estimation [89].

Turning to the more difficult question of proposing sequence variants, some stochastic approaches to infer knowledge about the sequence preferences likely to have some affinity with a specified protein have been reported [90,91]. Considering the even more difficult question of how specific are interactions among a collection of protein partners, some interesting results have been obtained for the PDZ family. The results by Ollikainen [92] highlight the importance of backbone flexibility and sequence tolerance to this respect, and Zheng *et al*. [93] have been able to design peptides with differential binding affinities for N2P2 compared with M3P6. Among the recent advances, it is noteworthy that the latest developments are beginning to result in fully integrated pipelines. When the structure of a PPI complex is available, PinaColada [94] provides an evolutionary approach designed for the blind design of a peptide binding to a specified patch, which will first identify candidate hot segments and then iteratively modify the peptide sequence to identify the best candidates to interact with the protein.

Chemical optimization of IPs

Issues in peptide chemistry to address poor resistance to protease degradation or problems of cell permeability have recently resulted in a diversification of the approaches to optimize peptides [95,96]. For instance, peptide-scanning approaches have diversified and can now not only contribute to structure–activity analyses but also to the optimization of peptide pharmacological properties using, among others, *N*-substitution, lactam cyclization or aza-amino scanning procedures [97]. Retro-inverso sequences [98] have for instance been very successful when applied to restore the tissue homeostasis of senescent cells in response to chemotoxicity and aging using a retro-inverso peptide of FOXO4 to perturb its interaction with p53 [27]. Chemical modifications are also useful to establish covalent links between the peptide and its target: for instance Michael acceptors have been successfully introduced in IPs (see below) [99]. Some diproline mimetics, locking the PPI conformation, have been synthesized and peptides containing them showed no loss of affinity for the protein [100]. Peptides with modulation of conformation have also been described: introduction of a photoswitchable group led to *cis–trans* isomerization of a double bond and thus modification of PPI inhibition [101]. Another recent approach lies in peptidomimetic foldamers [102]. Grison *et al*. described α/β/γ-hexapeptides mimicking the primary sequence of $p53_{19\text{-}26}$, and possessing three key substituents at the same position [103]. Their resulting peptides adopt a well-defined helical conformation (mimicking the α-helix) and the three side-chains were appropriately oriented. Owing to the unnatural character of these foldamers, they showed increased proteolytic stability and one efficiently inhibited the p53–hDM2 interaction. There are however as yet no data on potential toxicity of these species.

For IPs, excluding coupling with CPPs [104], current cyclization techniques seem to emerge as the most promising approach. Indeed, cyclization is primarily a mean to fix peptide structure closer to the bioactive conformation, hence to reduce the entropic cost upon binding, thereby increasing its binding efficiency and biological activity. Having fixed the conformation, a better basis for rational design is provided. Second, cyclization is also a way to improve resistance to degradation and improve cell penetrability [105]. Clearly, most techniques for the chemical optimization of peptides are not specific to IPs but some recent advances seem to fit their development well.

α-Helical peptide stapling

Stapling (i.e., the introduction of cross-linking between two side-chains) is probably one of today's most attractive strategies to stabilize an α-helix conformation [106]. To perform stapling, the ring-closing metathesis (RCM) reaction is the most widely used. It implies the design of a peptide containing two non-natural quaternary α-amino acids, with a terminal alkene on the side-chain. The metathesis reaction on the peptide induces cyclization and consequently blocks its α-helix conformation. Applying this strategy, Rezaei Araghi *et al*. improved the affinity and selectivity of a potent lead peptide (selected from a library using yeast-surface display) for myeloid cell leukemia 1 (Mcl-1) – an antiapoptotic protein [107]. The resulting stapled peptide, also containing non-natural α-amino acids, is highly helical and possesses enhanced protease resistance. Starting from a RCM monostapled peptide, Cromm *et al*. introduced a second stapling, increasing further its proteolytic stability, cellular uptake and binding affinity for Rab8a GTPase [108].

Click chemistry, which is compatible with biological conditions [109], is another powerful approach to form stapled peptides. Non-natural α-amino acids are necessary, with side-chains bearing for example azides. Lau *et al*. performed the *in situ* stapling by double-strain-promoted azide–alkyne cycloaddition (SPPAC) simultaneously with screening processes to select an optimal candidate with high binding affinity for MDM2 (targeting thus the oncogenic p53–MDM2 interaction) [110]. The crystal structure of the MDM2-stapled peptide complex showed that the bis(triazolyl) staple forms hydrophobic interactions with the protein. Targeting the same PPI, Serrano *et al*. recently used both chemical reactions [RCM and copper-catalyzed azide–alkyne cycloaddition (CuAAC)] with a chiral linker to improve helicity, proteolytic stability and binding of the resulting peptide [111].

Finally, stapling can also result from using natural amino acids. For instance, Diderich *et al*. built phage display libraries of peptides with cysteine residues in position i and $i+4$ [112]. Chemical cyclization occurred easily on the phage, increasing the helicity of the peptides. These cyclized peptides were then selected according to their affinity to β-catenin and ultimately two compounds showed better affinity than the parent peptides. In general, it has become accepted that building a cyclic dimer of an active peptide through two disulfide bonds connecting two pairs of cysteines can improve its cell-penetration ability and therefore its inhibitor activity, knowing that the disulfide bonds are cleaved under reductive cytosolic conditions (applications of disulfide bonds for therapeutic peptides have been recently reviewed [113]). This strategy has been successfully validated against HIV-1 transcription [114]. In another direction, Sanchez-Murcia *et al*. demonstrated a better capacity of lactam-bridging involving the side-chains of a glutamic acid and a lysine as opposed to hydrocarbon-bridged cyclic peptides to inhibit the dimerization of a trypanothione reductase involved in Leishmaniasis [115].

Macrocyclization of irregular peptides

Nature offers many examples of macrocycles as therapeutic agents such as erythromycin (antibiotic), cyclosporin (immunosuppressant) and somatostatin (hormone). Typically, they are 500–2000 Da in size, have 12-membered, or more, ring architecture and they do not obey Lipinski's rules [116]. Stabilizing an irregular peptide structure by macrocyclization [117] without disrupting the biological properties of the peptide turns out of be more challenging but some promising results are emerging. Interestingly, Glas *et al*. synthesized a peptide involved in the binding interaction between the virulence factor exoenzyme *S* and human adaptor protein 14-3-3 [118]. By crystallization of the protein 14-3-3ζ in a complex with the peptide, they found that using a carbon chain increases the hydrophobic interactions. Thus, they synthesized, by RCM and subsequent hydrogenation, a stapled peptide that significantly enhanced the binding affinities for 14-3-3ζ (by stabilization of the irregular peptide and enhancement of the target binding). This approach offers an interesting access to irregular peptide-derived PPI inhibitors. The same group later demonstrated that similar results can be obtained if the irregular peptide secondary structure is constrained through ring-closing alkyne metathesis [119].

Other ways of cyclization under investigation for IPs are known to increase cell permeability [120]. For instance, using head-to-tail cyclization, Upadhyaya *et al*. reported cell-permeable cyclic peptides that are selective and potent inhibitors of Ras-GTP, preventing its interaction with effector proteins [121]. These peptides have been identified using a library screening approach. Inspired by a previously described long linear peptide inhibiting the Hsp90–Cyp40 interaction, Buckton *et al*. generated small cyclic peptides that can inhibit the same interaction as well as the Hsp90-mediated protein folding [122].

Qian and co-workers have proposed another kind of macrocycle [123]. A bicyclic peptide associates a cyclic CPP and part of a known inhibitor of PPIs between NEMO and IKK. The CPP is cyclized through a disulfide bridge and the cargo which is also cyclic, linked to the CPP by the same disulfide link. It induced better cellular uptake and proteolytic stability. After entering the cell, the disulfide bonds are both cleaved by the intracellular glutathione, leading to high activity of the cargo.

Finally, cyclic peptoid libraries have been designed. For instance, an inhibitor of the Skp2–p300 interaction, inducing p53-mediated apoptosis and cell growth inhibition on cancer cells, has been identified from a library of triazine-bridged cyclic peptoids [124]. In another study, a novel cyclized peptoid–peptide chimera was selected for its capacity to inhibit the β-catenin–α-catenin interaction [125]. It should be noted that, in some cases, where the chemical modifications are most important, the properties of the macrocycles obtained could become closer to those of the small compounds.

β-turn mimetics

Although the appearance frequency of β-strands at heterodimeric interfaces is similar to that of α-helices [126], the use of peptides mimicking β-strands is much less frequently documented because β-stranded peptides are ideal substrates of peptidases. Two major directions have been investigated: the use of nonpeptidic amino acid analogs to stabilize individual strands and the introduction of chemical modifications to promote β-sheet formation [127]. For the former approach, an imidazole-based minimalist peptidomimetic has been described disrupting the PCSK9/LDLR PPI with a micromolar IC_{50} [128]. For the latter, a β-sheet-inducing (D)-pro-pro sequence or a dibenzofuran (DBF) turn mimetic has been used to build a CD2-based modulator of PPIs between CD2 and CD58 [129]. In general, a peptide backbone rigidifying into the bioactive conformations provides an entropic advantage, thereby improving bioactivity. However, a molecular flexibility in certain circumstances was shown to be important for the bioactivity of the modified peptides, as shown recently for the cyclic β-hairpin-based MDM2 inhibitors targeting MDM2–p53 PPIs [130].

Some promising examples of IPs

At present, some tens of validated IPs are under investigation, as described in Table 1. Most of the targeted interactions are implicated in cell death, proliferation and angiogenesis, with diverse fields from developmental studies to cardiovascular, neurological or infectious diseases.

IPs in cancer

Several IPs have been validated *in vitro* and *in vivo* (preclinical validation) and some are already in clinical development. For the

TABLE 1

Some interesting examples of interacting peptides (IPs).

Targeted interaction	Process concerned	Peptide sequence	Derived from	Development Route of admin Stability/ solubility	Refs
FOXO4–p53	Senescence	HITLRKEPASEIAQSIL-EAYSQNGWAN RRSGGKRPPPRRRQRRKKRGOH	FOXO4 sequence Dretro inverso (DRI) isoform	Preclinical *in vivo* IP 4 h/high	[27]
NOX-CFTR-CK2	Lung development	Sequences <20 aa of length	Recombinant adeno-associated virus (rAAV)	Preclinical *in vivo* IA	[54]
CaMKIIα–MUPP1	Human fertilization	SIAPNV and SIVMNV	MUPP1 sequence	Preclinical *in vitro*	[158]
D1–D2 dopamine receptors	Antidepressant effect	$D2_{IL329}$ GNCTHPEDMKL-CTVIMKSNGSF PVNRRRV $D2L_{IL3292}$IMKSNGSFPVNRRRV	D2 sequence D2 sequence	Preclinical *in vivo* IN	[142]
EphB2–ADDLs	Alzheimer's disease	Pep21 VQYAPRQLGL Pep25 PRIYISDLLA Pep32 NGVTDQSPFS Pep63 VFQVRARTVA	Peptide array assay	Preclinical *in vivo* ICe	[24]
GluA2–GAPDH	Neuron and cortical dendrite development	YYQWDKFAYLYDSDRGLSTLQ-QVLDSAAEK	GluA2 sequence	Preclinical *in vitro*	[141]
GluN2A–Rph3A	Stabilization at post-synaptic membranes	EDSKRSKSLLPDHASDNPFLHT-YQDDQRLVIGRCDSDPYKH	Rph3A sequence	Preclinical *in vivo* IV/IP	[159]
JNK–c-Jun	Cerebral ischemia, neuronal degeneration, inflammatory diseases	D-JNKI-1 (syn. XG-102)	Jun N-ter sequence (DRI)	Clinical Phase III SCo/IV/IT/SCu	[160,161]
p53–GAPDH	Prevention ischemic stroke damage	IPELNGKLTGMAFRVPTANV	GAPDH sequence	Preclinical *in vivo* ICV	[23]
δPKC–PDK	Cardiac injury	ALSTE (cyclizated) ALSTD (cyclizated)	δPKC sequence PDK sequence	Preclinical *in vivo* SCu	[162]
BAX–HN	Antioxidative, antiapoptosis, neuroprotective and cardioprotective effects	Humanin (HN): MAPRGFSCLLLLTSEIDLPVKRRA	Mammalian mitochondrially derived peptide	Preclinical *in vivo* IV	[163]
Casp9–PP2A	Cell death/proliferation	YVETLDDIFEQWAHSEDL	CASP9 sequence	Preclinical *in vivo* IP 2 h/high	[69]
Ras–Raf	Cell death/proliferation	KMSKDGKKKKKKSRTRCTVM	KRas sequence	Preclinical *in vivo* IP	[68]
ACC11 oligomerization	Cell death/migration/ invasion	AKLNAEKLKDFKIRLQYFAR-GLQVYIRQLRLALQGKT	ACC11 sequence	Preclinical *in vivo* IP–SCu 24 h/high	[134]
Mic–Max	Cell death/proliferation	MRRKNHYHQQDIDDLK-RQNALLEQQVRAL	MAX sequence and genetic library	Preclinical *in vitro*	[164]
PML–RARα or PLZF–RARα	Cell death/proliferation (leukemia)	Oligomerization regions of PML (aa 221–361) and PLZF (aa 1–125)	Genetic library	Preclinical *in vitro*	[135]
p53–p73	Cell death/proliferation	Pool of 13 peptides	DNA binding domain of human p73 (131–310 aa)	Preclinical *in vitro*	[165]
eIF4E–Angel1	Cell death	RRKYGRDFLLRF	Angel1 sequence	Preclinical *in vitro*	[166]
FZD7–DVL	Cell death	GKTLQSWRRFYH	FZD7 sequence	Preclinical *in vivo* IV	[167]
p53–HDM2	Cell death/proliferation	$AcLTFXEYWAQLXSNH_2$	*In situ* stapling	Preclinical *in vitro*	[110]
CXCR4–CXCL12	Cell death/proliferation (chemotherapy in acute myeloid)	E5: GGRSFFLLRRIQGC-RFRNTVDD	CXCR4 sequence	Preclinical *in vivo* SCu	[168]
MTP–NRP1	Angiogenesis/ proliferation/migration	ILITIIAMSALGVLLGAV-CGVVLYRKR	The TM sequence of NRP1	Preclinical *in vivo* IP	[169]
Siah–SIP Siah–PHYL	Cell death/cell proliferation	BI-107G3: Ac-RQIKIWFQ-NRRMKWKK PPPPPPPPPP KLRPVA-MVRPβVR-NH2 (covalent binding)	Consensus binding sequence SIP and $PHYL_{108–130}$	Preclinical *in vitro*	[99]
uPA–uPAR	Angiogenesis/migration/ invasion	AcetylKPSSPPEENH2	uPA sequence	Preclinical *in vivo* IP	[170]
CXCL12–CXCR4	Angiogenesis/mitosis	KGVSLSYRK KGVSLSYR (CTCE-9908)	CXCL12 sequence	Clinical Phase I/II–IV	[171]
α5β1integrin–fibronectin	Angiogenesis/migration/ invasion	AcPHSCNNH2	Fibronectin sequence	Preclinical *in vitro*	[172]

TABLE 1 (*Continued*)

Targeted interaction	Process concerned	Peptide sequence	Derived from	Development Route of admin Stability/ solubility	Refs
αvβ3 and αvβ5 integrins with RGD motif of ECM components	Angiogenesis/cell death/ migration	Cilengitide: cyclo(RGNDFNMV)	RGD binding site	Clinical Phase I–IV	[173]
VEGFR1–VEGF, VEGFR1–PlGF and VEGF–PlGF	Angiogenesis/migration	GNQWFI	Synthetic peptide library	Preclinical *in vivo* SCu	[174]
VEGF–NRP1	Angiogenesis/proliferation	ATWLPPR	Phage library	Preclinical *in vivo* IP	[62]
p53–HDM2	p53 ubiquitination	p28 (NSC745104) (aa 5077 of Zurin)	Cupredoxin azurin sequence (DRI) iso I	Clinical Phase I–IV	[154]
TLR4–MD2	Inflammation/tumor immunity	DDDYSFCRAL CAA-DDDYSFCRAL-AAC	$MD2_{299-108}$ sequence + linkers to macrocyclization	Preclinical *in vitro*	[45]
NEMO–IKK	Inflammation/cell death/ proliferation/immunity/	TALDWSWLQTE BMBRRRRΦFCALDWSWLQC I IS SI I IS SI	IKKb sequence	Preclinical *in vitro*	[123]
Keap1–Nrf2	Inflammation	Acc[CLDPETGEYLC]OH	Nrf2 sequence	Preclinical *in vitro*	[75]
TLR4–SPA	Inflammation	GDFRYSDGTPVNYTNWYRGE	The SPA sequence	Preclinical *in vivo* IP	[175]
TLR4MyD88	Cytokine production	RDFIPGV RDVLPGT	TLR4 sequence MyD88 sequence	Preclinical *in vivo* IP	[176]
Hsp90–nuc	Inhibition ATPase of Hsp90	RELWDD	ATP binding site of the N-terminal domain of Hsp90	Preclinical *in vitro*	[177]
3A–3A (FMD)	FMD virus replication	FFEGMVHDS FFEGMVHDSIKEELRPLIQQ	A3 sequence	Preclinical *in vitro*	[149]
PB1–PB2	Influenza virus replication	$PB1c_{676-757}$ $PB1_{731-757}$	PB1 sequence (aa 676–757) PB1 sequence (aa 731–757)	Preclinical *in vitro*	[150]
ZIKV E	Zika other flavivirus replication	Z2: MAVLGDTAWDFGSV-GGALNSLG KGIHQIFGAAF	ZIKV E protein (residues 421–453)	Preclinical *in vivo* IV/IP	[151]

Abbreviations: Route of Admin, route of administration; DRI, D-retro-inverso isoform; IA, intraamniotic injection; Ice, intracerebral injection; ICV, intracerebroventricular injection; IN, intranasal administration; i.p., intraperitoneal injection; IT, intratympanic injection; IV, intravenous injection; IVit, intravitreal injection; SCo, subconjunctival injection; SCu, subcutaneous injection.

Names of genes are designed following their official symbol as is the common usage (https://www.ncbi.nlm.nih.gov/gene).

preclinical validation of antitumor peptides, patient-derived xenograft models (PDX) are normally used. In PDX models, a piece of original tumor is implanted (in most cases subcutaneously) in an animal. The tumor growth is in an environment that, although non-human, mimics the native environment. Such models promise to capture the genetic complexity of human cancers better that cell line culture or genetically engineered mice. However, PDXs have shortcomings because they are developed in mice lacking a normal immune response (to prevent human tumors from being rejected). Efforts are underway to engineer mice that mimic aspects of the human immune system [131,132].

Caspase 9–PP2A

Taking advantage of the PDX models, Rebollo and co-workers validated two different cell-penetrating IPs. The first, DPT C9 h, blocks the interaction between the cysteine protease caspase 9 and the serine/threonine phosphatase PP2A. The antiproliferative effect of this peptide on a triple-negative breast cancer PDX and on a luminal B model was well established [69]. This peptide was also active on primary B cells from chronic lymphocytic leukemia (CLL) patients but not on B cells of healthy donors [133]. They have also validated a cell-penetrating IP blocking the interaction between the oncogene Ras and the kinase Raf, Mut3DPT Ras, on two CLL and two lymphoma xenograft models [68]. The first peptide will be soon tested in a clinical trial.

AAC-11 oligomerization

ACC-11 (antiapoptotic clone 11 or API5) activity is mediated through several PPIs, mediated by its binding domain (BD). Increased expression of AAC-11 confers a survival advantage to cancer cells and contributes to tumor invasion and metastases, whereas its deregulation reduces resistance to chemotherapeutic drugs. Thus, inactivation of AAC-11 might constitute an attractive approach for cancer therapy. IPs derived from the AAC-11 BD prevent its oligomerization and inhibit its interaction with partners, blocking its antiapoptotic properties. This IP selectively kills cancer cells while sparing normal cells. It can also inhibit the growth of BRAF wild-type and V600E mutant melanoma xenograft tumors, through induction of apoptosis and necrosis, without evidence of toxicity in mice [134].

PML–RARα oligomerization

The PML protein present in nuclear bodies is involved in multiple genome maintenance pathways including the DNA damage response, DNA repair, telomere homeostasis and p53-associated

apoptosis. The dominant-negative PML–RARα fusion protein expressed in acute promyelocytic leukemia (APL) disrupts PML function by blocking hematopoietic differentiation. An interfering peptide targeting the oligomerization domain of PML–RARα or PLZF–RARα represents a promising tool to further elucidate the biology of this leukemia and its treatment [135].

FOXO4–p53

A peptide derived from FOXO4, which perturbs its interaction with p53, has been recently described [27]. In senescent cells, the selective interaction between FOXO4 and p53 causes p53 nuclear exclusion and cell-intrinsic apoptosis. FOXO4 IP was well-tolerated *in vivo* and neutralized doxorubicin-induced chemotoxicity. Moreover, this IP restored fitness, fur density and renal function in fast-aging XpdTTD/TTD and naturally aged mice. This shows that therapeutic targeting of senescent cells is feasible under conditions where loss of health has already occurred and in doing so tissue homeostasis can effectively be restored.

SIAH–SIP, SIAH–PHYL

Subsequent to several successful cases of effective therapeutics, drugs that bind their target by means of covalent attachment have recently come back into favor following initial fears by the pharmaceutical industry about possible off-target effects [136]. Consequently, the irreversible inhibition of the PPIs by covalent binding of IPs has also been tried. This approach, first designed to improve the binding affinity of small-molecule drugs for their receptor, was originally proposed by Shokat and colleagues to obtain selective inhibitors of protein kinases [137]. It involves the introduction of a mildly reactive group (usually a Michael acceptor such as an acrylamide) designed to react selectively with a cysteine thiol group present in the target-binding site. This approach was applied to obtain more-potent and −selective SIAH-IPs. Through its interactions, the E3 ligase SIAH1 is involved in different cellular processes such as cell death and mitosis [23,138,139]. Starting from a peptide derived from the Siah-interacting protein Phyllopod, which was modified with a suitably reactive 'warhead', several modified peptides were obtained that can form a covalent bond with a cysteine residue present in the P1 pocket of SIAH [99]. This work shows the feasibility of use of covalent inhibition of PPI with IPs that are potentially orders of magnitude more effective than the parent peptides.

IPs in neurobiology

p53–GAPDH. A recent study showed that disruption of the nuclear p53–GAPDH complex by an IP derived from the GADPH sequence protects against ischemia-induced neuronal damage (cell death in rats subjected to glutamate) [23]. GADPH is a cytosolic metabolic enzyme that, under different stimuli (for example glutamate treatment), translocates to the nucleus through its interaction with the E3 ligase SIAH1 [138,140]. In the nucleus, GADPH binds p53, upregulating its expression and phosphorylation, inducing cell death. *In vitro* disruption of the p53–GAPDH complex prevents cell death upon glutamate stimulation. GAPDH nuclear translocation plays a crucial part in cell death. Disruption of the nuclear p53–GAPDH complex *in vivo* protected from ischemia-induced cell death in rats subjected to temporary middle cerebral artery occlusion [23]. These observations open the way to consider this IP as a potential therapeutic option for ischemic stroke treatment.

GluA–GAPDH

Targeting other interactions of GAPDH was also shown to have a therapeutic interest. Using an IP derived from GluA2 protein and targeting the GluA2–GAPDH interaction, it was shown that this PPI is necessary for neuron and cortical development. This result demonstrates the interest of the IPs in studies of physiological tissue development and differentiation [141].

EphB2–ADDL

Shi *et al*. used a peptide to disrupt the interaction between EphB2 and ADDL proteins [24] and showed that blocking this PPI rescues impaired synaptic plasticity and memory deficits in a mouse model of Alzheimer's disease. This result suggests that inhibition of the EphB2–ADDL interaction with the IP Pep63 could be a promising strategy for the treatment of Alzheimer's disease.

D1–D2

Interestingly, it was shown that an IP designed to disrupt the interaction between the D1 and D2 dopamine receptors has an antidepressant effect. It was tested using the forced swimming test (FST) and the learned helplessness (LH) task, two preclinical tests for antidepressant efficacy [142]. Moreover, it was shown that this IP can be efficiently delivered to relevant brain areas using a pressurized intranasal device [30].

Concluding remarks and future perspectives

IPs seem today to be a credible source of novel drugs for a large number of pathologies. Based in their long-term of utilization, peptides have confirmed their good profile of tolerability and safety with few off-target effects [9]. Biologically active peptides are expressed in virtually all the living species and they have constituted the first source of potential druggable peptides. Peptides binding physiological membrane receptors (like oxytocin and insulin) were the first to be used as medicines [143] and are still being improved today [144]. At present, peptides used as antigens represent the majority of those tested in clinical trials [145]. There are >7000 naturally occurring peptides that have been identified, some with a proven (and others with a promising) potential as therapeutic tools. For instance, they are a rich source of antimicrobial agents [12,146,147]. In a similar way, animal venoms are also an important source of bioactive peptides with miscellaneous activities [148]. Moreover, thousands of synthetic peptides derived from synthetic libraries or techniques of genetic expression in viruses have been biologically characterized. At the same time, peptides interfering with protein–protein viral assembly have also been identified [149–151].

The design of synthetic IPs implies a rational approach for drug development, where the choice of the PPI to be targeted, to obtain a desirable biological effect, is the crucial step. Small molecules have already been used to target intracellular PPIs [13–17]. *A priori,* IPs directly derived from PPI sequences and with their capacity to target large surfaces are better placed than small molecules to target a PPI. A strengths, weaknesses, opportunities and threats (SWOT) analysis of IPs is shown in Table 2.

Owing to their biological importance, p53 and MDM2 proteins and their interactions are two of the best studied. Different classes of small inhibitors of this interaction have been designed and developed, and currently seven such compounds are being evaluated in clinical trials as anticancer drugs (among them nutlin, spirooxindole, isoquilinone and piperidinone) [152]. Similarly,

TABLE 2

A strengths, opportunities, weaknesses, threats (SOWT) analysis of interfering peptides (IPs) as potential drugs.

Strengths
• Combined selective and specific targeting
• Good efficacy and potency for intracellular targeting
• Better adapted than small molecules to target large surface of PPI
• Intracellular concentration not dependent of flow pumps as for small molecules
• Less prone to generate mechanisms of drugs resistance
• Predictable metabolism and low toxicity[a]
• Less immunogenic than recombinant proteins or monoclonal antibodies[a]
• Costs have been steadily declining[a]
Weaknesses
• Most IPs need to add a CPP sequence to improve membrane permeability
• Prone to aggregation[a]
• Short half-life and fast elimination of most IPs if not stabilized[a]
• Less experience in clinical development than for small molecules
Opportunities
• The potential number of IPs is almost unlimited
• Good specific intracellular penetration when fused with CPP
• New alternative delivery routes of administration[a]
• Specific chemical modification to improve ADME[a]
• Increased number of PPIs known to be used for rational structure-based design
• Possibility of design multifunctional IPs
• Availability of natural peptides evolutionarily selected for specificity and stability
• Present penury in the discovery of new effective drugs
• Present resurged interest in peptides as drugs
Threats
• Present lower investment of pharmaceutical industry than for small compounds
• Competition with of small molecules targeting PPIs
• No approved IPs as drugs yet

[a] Not specific for IPs.

this p53 interaction (and others) has been targeted by IPs [153,154] sometimes associated with improvements such as peptide stability [155]. Because most IPs do not have an intrinsic ability to enter cells, major advances in their delivery have been made possible through their modification [120] or their fusion with CPP sequences [35]. These fused CPP–IPs can cross biological membranes and, adding specific sequences, they can be addressed to specific intracellular compartments [36]. Associated to others improvements in administration and stability, IPs are now well positioned for development as drugs [3,8,12,31,156]. The concept of IPs has been validated for ~40 PPIs and it is very likely that this number will increase rapidly.

Considering the number of existing PPIs and their implications in pathology, this approach could be used eventually to treat any disease where responsible PPIs are identified and targetable, and models to test the biological effects of IPs are available. Although in theory PPIs targeted by peptides can be obtained in two ways: (i) direct binding to the PPI surfaces (orthosteric modulation); (ii) binding other regions of the interacting proteins (allosteric regulation), the former strategy has been to date the most investigated, but progress in understanding allostery could open promising perspectives. The direct targeting of sequences on the interaction surface has almost always yielded peptides disrupting the interaction. It is expected that stabilization of PPIs (when desirable) or a more specific disruption of the PPIs can also be obtained through finer allosteric modulation.

We can summarize a general strategy to be used to design IPs, consisting of a succession of alternate steps of rational and experimental approaches. The first step, based on the available biological knowledge, is the choice of PPI to be targeted. The second is the identification of sequence to be mimicked by the IP based on structural, *in silico* and off-structure approaches. Finally, the biological effects of these candidate IPs are to be tested using *in vitro* and *in vivo* models. This step is normally followed by a rational IP optimization, including the modeling of the interacting peptide protein for the design of new IPs as well as the use of chemically modified sequences for improving stability, solubility and cell permeability. Again, a candidate peptide must be biologically validated. This strategy relies on exploiting various complementary disciplines: chemistry, biology, biophysics and modeling.

Given the increasing number of reported IPs and their biological potential, some are likely to advance to clinical testing in the near future. Faced with the present penury in the discovery of new effective small-molecule drugs [157], it will be highly desirable that the renewed interest in peptides as drugs could be consolidated by using IPs targeting intracellular PPIs. Such an evolution could have beneficial consequences in the treatment of different pathologies ranging from degenerative diseases to cancer.

Conflicts of interest

Mrs Rebollo is a cofounder of PEP-therapy. The other authors have no conflicts of interest to declare.

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References

1 Stumpf, M.P.H. *et al.* (2008) Estimating the size of the human interactome. *Proc. Natl. Acad. Sci. U. S. A.* 105, 6959–6964
2 Venkatesan, K. *et al.* (2009) An empirical framework for binary interactome mapping. *Nat. Methods* 6, 83–90
3 Cunningham, A.D. *et al.* (2017) Peptides and peptidomimetics as regulators of protein–protein interactions. *Curr. Opin. Struct. Biol.* 44, 59–66
4 Modell, A.E. *et al.* (2016) Systematic targeting of protein–protein interactions. *Trends Pharmacol. Sci.* 37, 702–713
5 Berg, T. (2003) Modulation of protein–protein interactions with small organic molecules. *Angew. Chem. Int. Ed.* 42, 2462–2481
6 Uversky, V.N. (2013) Intrinsic disorder-based protein interactions and their modulators. *Curr. Pharm. Des.* 19, 4191–4213
7 Petta, I. *et al.* (2016) Modulation of protein–protein interactions for the development of novel therapeutics. *Mol. Ther.* 24, 707–718
8 Kaspar, A.A. and Reichert, J.M. (2013) Future directions for peptide therapeutics development. *Drug Discov. Today* 18, 807–817
9 Kinch, M.S. (2015) An overview of FDA-approved biologics medicines. *Drug Discov. Today* 20, 393–398
10 Fletcher, S. and Hamilton, A.D. (2006) Targeting protein-protein interactions by rational design: mimicry of protein surfaces. *J. R. Soc. Interface* 3, 215–233

11 Vlieghe, P. *et al.* (2010) Synthetic therapeutic peptides: science and market. *Drug Discov. Today* 15, 40–56
12 Fosgerau, K. and Hoffmann, T. (2015) Peptide therapeutics: current status and future directions. *Drug Discov. Today* 20, 122–128
13 Wells, J.A. and McClendon, C.L. (2007) Reaching for high-hanging fruit in drug discovery at protein–protein interfaces. *Nature* 450, 1001–1009
14 Nero, T.L. *et al.* (2014) Oncogenic protein interfaces: small molecules, big challenges. *Nat. Rev. Cancer* 14, 248–262
15 Arkin, M.R. *et al.* (2014) Small-molecule inhibitors of protein–protein interactions: progressing toward the reality. *Chem. Biol.* 21, 1102–1114
16 Bakail, M. and Ochsenbein, F. (2016) Targeting protein–protein interactions, a wide open field for drug design. *C.R. Chim.* 19, 19–27
17 Zhuang, C. *et al.* (2017) Small molecules inhibiting Keap1-Nrf2 protein–protein interactions: a novel approach to activate Nrf2 function. *MedChemComm* 8, 286–294
18 Mason, J.M. *et al.* (2008) iPEP: peptides designed and selected for interfering with protein interaction and function. *Biochem. Soc. Trans.* 36, 1442–1447
19 Gaida, A. *et al.* (2009) Selection of peptides interfering with protein–protein interaction. *Methods Mol. Biol.* 535, 263–291
20 Villoutreix, B.O. *et al.* (2014) Drug-like protein–protein interaction modulators: challenges and opportunities for drug discovery and chemical biology. *Mol. Inf.* 33, 414–437
21 Recio, C. *et al.* (2017) The potential therapeutic application of peptides and peptidomimetics in cardiovascular disease. *Front. Pharmacol.* 7, 526http://dx.doi.org/10.3389/fphar.2016.00526
22 Jouaux, E.M. *et al.* (2008) Targeting the c-Myc coiled coil with interfering peptides. *J. Pept. Sci.* 14, 1022–1031
23 Zhai, D. *et al.* (2014) Disruption of the nuclear p53-GAPDH complex protects against ischemia-induced neuronal damage. *Mol. Brain* 7, 20
24 Shi, X.-D. *et al.* (2016) Blocking the interaction between EphB2 and ADDLs by a small peptide rescues impaired synaptic plasticity and memory deficits in a mouse model of Alzheimer's Disease. *J. Neurosci.* 36, 11959–11973
25 Mine, Y. *et al.* (2016) Biomimetic peptides for the treatment of cancer. *Anticancer Res.* 36, 3565–3570
26 Pleasant-Jenkins, D. *et al.* (2017) Reversal of maladaptive fibrosis and compromised ventricular function in the pressure overloaded heart by a caveolin-1 surrogate peptide. *Lab. Invest.* 97, 370–382
27 Baar, M.P. *et al.* (2017) Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging. *Cell* 169, 132–147
28 Ellert-Miklaszewska, A. *et al.* (2017) Short peptides interfering with signaling pathways as new therapeutic tools for cancer treatment. *Future Med. Chem.* 9, 199–221
29 Fitches, E.C. *et al.* (2012) Fusion to snowdrop lectin magnifies the oral activity of insecticidal ω-hexatoxin-Hv1a peptide by enabling its delivery to the central nervous system. *PLoS One* 7, e39389
30 Brown, V. and Liu, F. (2014) Intranasal delivery of a peptide with antidepressant-like effect. *Neuropsychopharmacology* 39, 2131–2141
31 Karsdal, M.A. *et al.* (2015) Lessons learned from the clinical development of oral peptides. *Br. J. Clin. Pharmacol.* 79, 720–732
32 Nevola, L. and Giralt, E. (2015) Modulating protein–protein interactions: the potential of peptides. *Chem. Commun.* 51, 3302–3315
33 Zorzi, A. *et al.* (2017) Acylated heptapeptide binds albumin with high affinity and application as tag furnishes long-acting peptides. *Nat. Commun.* 8, 16092
34 Naylor, M.R. *et al.* (2017) Cyclic peptide natural products chart the frontier of oral bioavailability in the pursuit of undruggable targets. *Curr. Opin. Chem. Biol.* 38, 141–147
35 Guidotti, G. *et al.* (2017) Cell-penetrating peptides: from basic research to clinics. *Trends Pharmacol. Sci.* 38, 406–424
36 Cerrato, C.P. *et al.* (2017) Cell-penetrating peptides with intracellular organelle targeting. *Expert Opin. Drug Deliv.* 14, 245–255
37 Lengyel, J. *et al.* (2014) Towards an integrative structural biology approach: combining Cryo-TEM, X-ray crystallography, and NMR. *J. Struct. Funct. Genom.* 15, 117–124
38 Cuniasse, P. *et al.* (2017) Structures of biomolecular complexes by combination of NMR and cryoEM methods. *Curr. Opin. Struct. Biol.* 43, 104–113
39 Schindler, C.E.M. *et al.* (2016) SAXS data alone can generate high-quality models of protein–protein complexes. *Structure* 24, 1387–1397
40 Schlundt, A. *et al.* (2017) Integrated structural biology to unravel molecular mechanisms of protein–RNA recognition. *Methods* 118–119, 119–136
41 London, N. *et al.* (2013) Druggable protein–protein interactions–from hot spots to hot segments. *Curr. Opin. Chem. Biol.* 17, 952–959
42 Gavenonis, J. *et al.* (2014) Comprehensive analysis of loops at protein-protein interfaces for macrocycle design. *Nat. Chem. Biol.* 10, 716–722
43 Sedan, Y. *et al.* (2016) Peptiderive server: derive peptide inhibitors from protein–protein interactions. *Nucleic Acids Res.* 44, W536–541
44 Bullock, B.N. *et al.* (2011) Assessing helical protein interfaces for inhibitor design. *J. Am. Chem. Soc.* 133, 14220–14223
45 Gao, M. *et al.* (2014) Rationally designed macrocyclic peptides as synergistic agonists of LPS-induced inflammatory response. *Tetrahedron* 70, 7664–7668
46 Siegert, T.R. *et al.* (2016) Analysis of loops that mediate protein–protein interactions and translation into submicromolar inhibitors. *J. Am. Chem. Soc.* 138, 12876–12884
47 Szilagyi, A. and Zhang, Y. (2014) Template-based structure modeling of protein–protein interactions. *Curr. Opin. Struct. Biol.* 24, 10–23
48 Yu, J. *et al.* (2016) InterEvDock: a docking server to predict the structure of protein–protein interactions using evolutionary information. *Nucleic Acids Res.* 44, W542–549
49 Vanhee, P. *et al.* (2009) Protein–peptide interactions adopt the same structural motifs as monomeric protein folds. *Structure* 17, 1128–1136
50 Obarska-Kosinska, A. *et al.* (2016) PepComposer: computational design of peptides binding to a given protein surface. *Nucleic Acids Res.* 44, W522–528
51 Chassagnon, I.R. *et al.* (2017) Potent neuroprotection after stroke afforded by a double-knot spider-venom peptide that inhibits acid-sensing ion channel 1a. *PNAS* 114, 3750–3755
52 Guixer, B. *et al.* (2016) Chemically synthesized peptide libraries as a new source of BBB shuttles: Use of mass spectrometry for peptide identification. *J. Pept. Sci.* 22, 577–591
53 Eisenberg, M. *et al.* (2013) Dynamic combinatorial libraries of artificial repeat proteins. *Bioorg. Med. Chem.* 21, 3450–3457
54 Cohen, J.C. *et al.* (2009) Small interfering peptide (siP) for *in vivo* examination of the developing lung interactome. *Dev. Dyn.* 238, 386–393
55 Smith, G.P. (1985) Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science* 228, 1315–1317
56 Hagemann, U.B. *et al.* (2008) Selectional and mutational scope of peptides sequestering the Jun-Fos coiled-coil domain. *J. Mol. Biol.* 381, 73–88
57 Heinis, C. *et al.* (2009) Phage-encoded combinatorial chemical libraries based on bicyclic peptides. *Nat. Chem. Biol.* 5, 502–507
58 Bertoldo, D. *et al.* (2016) Phage selection of peptide macrocycles against β-catenin to interfere with Wnt signaling. *ChemMedChem* 11, 834–839
59 Zade, H.M. *et al.* (2017) Biased selection of propagation-related TUPs from phage display peptide libraries. *Amino Acids* 49, 1293–1308
60 Kuzmicheva, G.A. and Belyavskaya, V.A. (2016) [Peptide phage display in biotechnology and biomedicine. *Biomeditsinskaia Khimiia* 62, 481–495
61 Bakhshinejad, B. *et al.* (2016) Phage display biopanning and isolation of target-unrelated peptides: in search of nonspecific binders hidden in a combinatorial library. *Amino Acids* 48, 2699–2716
62 Starzec, A. *et al.* (2006) Antiangiogenic and antitumor activities of peptide inhibiting the vascular endothelial growth factor binding to neuropilin-1. *Life Sci.* 79, 2370–2381
63 Frank, R. (2002) The SPOT-synthesis technique. Synthetic peptide arrays on membrane supports–principles and applications. *J. Immunol. Methods* 267, 13–26
64 Frank, R. and Overwin, H. (1996) SPOT synthesis: Epitope analysis with arrays of synthetic peptides prepared on cellulose membranes. *Methods Mol. Biol.* 66, 149–169
65 Luu, T. *et al.* (1996) Automated multiple peptide synthesis: improvements in obtaining quality peptides. *Int. J. Pept. Protein Res.* 47, 91–97
66 Adler, S. *et al.* (1994) T cell epitope analysis with peptides simultaneously synthesized on cellulose membranes: fine mapping of two DQ dependent epitopes. *FEBS Lett.* 352, 167–170
67 Gausepohl, H. *et al.* (1992) Automated multiple peptide synthesis. *Pept. Res.* 5, 315–320
68 Tian, L. *et al.* (2016) Identification of Ras/Raf binding site and design of interfering peptide with potential clinical application. *Integr. Mol. Med.* 3, 1–9http://dx.doi.org/10.15761/IMM.1000259
69 Arrouss, I. *et al.* (2013) Specific targeting of caspase-9/PP2A interaction as potential new anti-cancer therapy. *PLoS One* 8, e60816
70 Rebollo, G. *et al.* (2016) Pro-Apoptotic Set and Pp2a Peptides. WO2016156536 (A1)
71 Rebollo, G. *et al.* (2016) Peptides That Inhibit Binding Between Set and Caspase-9. WO2016156538 (A1)
72 Rebollo, G.A. *et al.* (2016) Peptide Inhibitors of Tead/Yap-Taz Interaction. US2016264636 (A1)
73 Rebollo, G.A. *et al.* (2017) Pro-Apoptotic Ras and Raf Peptides. US2017015718 (A1)
74 Rebollo, A. Interfering peptides interfering PPI into the Plasmodium falciparum parasite. Patent granted

75 Pelay-Gimeno, M. *et al.* (2015) Structure-based design of inhibitors of protein–protein interactions: mimicking peptide binding epitopes. *Angew. Chem. Int. Ed.* 54, 8896–8927
76 Lu, M.-C. *et al.* (2015) Binding thermodynamics and kinetics guided optimization of potent Keap1–Nrf2 peptide inhibitors. *RSC Adv.* 5, 85983–85987
77 Das, R. and Baker, D. (2008) Macromolecular modeling with rosetta. *Annu. Rev. Biochem.* 77, 363–382
78 Singh, S. *et al.* (2015) PEPstrMOD: structure prediction of peptides containing natural, non-natural and modified residues. *Biol. Direct* 10, 73
79 Lamiable, A. *et al.* (2016) PEP-FOLD3: faster *de novo* structure prediction for linear peptides in solution and in complex. *Nucleic Acids Res.* 44, W449–454
80 Saladin, A. *et al.* (2014) PEP-SiteFinder: a tool for the blind identification of peptide binding sites on protein surfaces. *Nucleic Acids Res.* 42, W221–226
81 Yan, C. and Zou, X. (2015) Predicting peptide binding sites on protein surfaces by clustering chemical interactions. *J. Comput. Chem.* 36, 49–61
82 Bohnuud, T. *et al.* (2017) Detection of peptide-binding sites on protein surfaces using the Peptimap server. *Methods Mol. Biol.* 1561, 11–20
83 de Vries, S.J. *et al.* (2017) The pepATTRACT web server for blind, large-scale peptide–protein docking. *Nucleic Acids Res.* http://dx.doi.org/10.1093/nar/gkx335 [Epub ahead of print]
84 London, N. *et al.* (2011) Rosetta FlexPepDock web server–high resolution modeling of peptide–protein interactions. *Nucleic Acids Res.* 39, W249–253
85 Trellet, M. *et al.* (2013) A unified conformational selection and induced fit approach to protein–peptide docking. *PLoS One* 8, e58769
86 Schindler, C.E.M. *et al.* (2015) Fully blind peptide–protein docking with pepATTRACT. *Structure* 23, 1507–1515
87 Kurcinski, M. *et al.* (2015) CABS-dock web server for the flexible docking of peptides to proteins without prior knowledge of the binding site. *Nucleic Acids Res.* 43, W419–424
88 Spiliotopoulos, D. *et al.* (2016) dMM-PBSA: a new HADDOCK scoring function for protein–peptide docking. *Front. Mol. Biosci.* 3, 46
89 Sharma, R. *et al.* (2015) Fuzzy complexes: specific binding without complete folding. *FEBS Lett.* 589, 2533–2542
90 Unal, E.B. *et al.* (2010) VitAL: Viterbi algorithm for *de novo* peptide design. *PLoS One* 5, e10926
91 Bhattacherjee, A. and Wallin, S. (2013) Exploring protein–peptide binding specificity through computational peptide screening. *PLoS Comput. Biol.* 9, e1003277
92 Ollikainen, N. (2017) Flexible backbone methods for predicting and designing peptide specificity. *Methods Mol. Biol.* 1561, 173–187
93 Zheng, F. *et al.* (2015) Computational design of selective peptides to discriminate between similar PDZ domains in an oncogenic pathway. *J. Mol. Biol.* 427, 491–510
94 Zaidman, D. and Wolfson, H.J. (2016) PinaColada: peptide–inhibitor ant colony *ad-hoc* design algorithm. *Bioinforma. Oxf. Engl.* 32, 2289–2296
95 Nevola, L. and Giralt, E. (2015) Modulating protein–protein interactions: the potential of peptides. *Chem. Commun.* 51, 3302–3315
96 Rezaei Araghi, R. and Keating, A.E. (2016) Designing helical peptide inhibitors of protein–protein interactions. *Curr. Opin. Struct. Biol.* 39, 27–38
97 Eustache, S. *et al.* (2016) Progress with peptide scanning to study structure–activity relationships: the implications for drug discovery. *Expert Opin. Drug Discov.* 11, 771–784
98 Brugidou, J. *et al.* (1995) The retro-inverso form of a homeobox-derived short peptide is rapidly internalized by cultured neurons: a new basis for an efficient intracellular delivery system. *Biochem. Biophys. Res. Commun.* 214, 685–693
99 Stebbins, J.L. *et al.* (2013) Structure-based design of covalent Siah inhibitors. *Chem. Biol.* 20, 973–982
100 Reuter, C. *et al.* (2015) Design and stereoselective synthesis of ProM-2: a spirocyclic diproline mimetic with polyproline type II (PPII) helix conformation. *Chem. Eur. J.* 21, 8464–8470
101 Albert, L. *et al.* (2017) Controlled inhibition of methyltransferases using photoswitchable peptidomimetics: towards an epigenetic regulation of leukemia. *Chem. Sci.* 8 (6), 4612–4618http://dx.doi.org/10.1039/C7SC00137A
102 Mathieu, L. *et al.* (2013) Helical oligomers of thiazole-based γ-amino acids: synthesis and structural studies. *Angew. Chem. Int. Ed.* 52, 6006–6010
103 Grison, C.M. *et al.* (2016) An α-helix-mimicking 12, 13-helix: designed α/β/γ-foldamers as selective inhibitors of protein–protein interactions. *Angew. Chem. Int. Ed.* 55, 11096–11100
104 Kurrikoff, K. *et al.* (2016) Recent *in vivo* advances in cell-penetrating peptide-assisted drug delivery. *Expert Opin. Drug Deliv.* 13, 373–387
105 García-Pindado, J. *et al.* (2017) Bike peptides: a ride through the membrane. *J. Pept. Sci.* 23, 294–302
106 Cromm, P.M. *et al.* (2015) Hydrocarbon stapled peptides as modulators of biological function. *ACS Chem. Biol.* 10, 1362–1375
107 Rezaei Araghi, R. *et al.* (2016) Rapid optimization of Mcl-1 inhibitors using stapled peptide libraries including non-natural side chains. *ACS Chem. Biol.* 11, 1238–1244
108 Cromm, P.M. *et al.* (2016) Protease-resistant and cell-permeable double-stapled peptides targeting the Rab8a GTPase. *ACS Chem. Biol.* 11, 2375–2382
109 King, M. and Wagner, A. (2014) Developments in the field of bioorthogonal bond forming reactions–past and present trends. *Bioconjug. Chem.* 25, 825–839
110 Lau, Y.H. *et al.* (2015) Double strain-promoted macrocyclization for the rapid selection of cell-active stapled peptides. *Angew. Chem. Int. Ed.* 54, 15410–15413
111 Serrano, J.C. *et al.* (2017) A new methodology for incorporating chiral linkers into stapled peptides. *ChemBioChem* 18, 1066–1071
112 Diderich, P. *et al.* (2016) Phage selection of chemically stabilized α-helical peptide ligands. *ACS Chem. Biol.* 11, 1422–1427
113 Góngora-Benítez, M. *et al.* (2014) Multifaceted roles of disulfide bonds. Peptides as therapeutics. *Chem. Rev.* 114, 901–926
114 Jang, S. *et al.* (2014) Cell-penetrating, dimeric α-helical peptides: nanomolar inhibitors of HIV-1 transcription. *Angew. Chem. Int. Ed.* 53, 10086–10089
115 Sánchez-Murcia, P.A. *et al.* (2015) Comparison of hydrocarbon-and lactam-bridged cyclic peptides as dimerization inhibitors of *Leishmania infantum trypanothione* reductase. *RSC Adv.* 5, 55784–55794
116 Russo, A. *et al.* (2016) Targeting undruggable proteins: design of synthetic cyclopeptides. *Curr. Med. Chem.* 23, 748–762
117 Gao, M. *et al.* (2015) Targeting protein-protein interfaces using macrocyclic peptides. *Pept. Sci.* 104, 310–316
118 Glas, A. *et al.* (2014) Constrained peptides with target-adapted cross-links as inhibitors of a pathogenic protein–protein interaction. *Angew. Chem. Int. Ed.* 53, 2489–2493
119 Cromm, P.M. *et al.* (2016) Constraining an irregular peptide secondary structure through ring-closing alkyne metathesis. *ChemBioChem* 17, 1915–1919
120 Qian, Z. *et al.* (2017) Targeting intracellular protein–protein interactions with cell-permeable cyclic peptides. *Curr. Opin. Chem. Biol.* 38, 80–86
121 Upadhyaya, P. *et al.* (2015) Inhibition of Ras signaling by blocking Ras–effector interactions with cyclic peptides. *Angew. Chem. Int. Ed.* 54, 7602–7606
122 Buckton, L.K. *et al.* (2016) The first report of direct inhibitors that target the C-terminal MEEVD region on heat shock protein 90. *Chem. Commun.* 52, 501–504
123 Qian, Z. *et al.* (2017) Enhancing the cell permeability and metabolic stability of peptidyl drugs by reversible bicyclization. *Angew. Chem. Int. Ed.* 56, 1525–1529
124 Oh, M. *et al.* (2016) A chemical inhibitor of the Skp2/p300 interaction that promotes p53-mediated apoptosis. *Angew. Chem. Int. Ed.* 55, 602–606
125 Kawakami, T. *et al.* (2016) Directed evolution of a cyclized peptoid–peptide chimera against a cell-free expressed protein and proteomic profiling of the interacting proteins to create a protein–protein interaction inhibitor. *ACS Chem. Biol.* 11, 1569–1577
126 Guharoy, M. and Chakrabarti, P. (2007) Secondary structure based analysis and classification of biological interfaces: identification of binding motifs in protein–protein interactions. *Bioinformatics* 23, 1909–1918
127 Guitot, K. *et al.* (2011) The combination of prolinoamino acids and cyclopropylamino acids leads to fully functionalized, stable β-turns in water. *ChemBioChem* 12, 1039–1042
128 Stucchi, M. *et al.* (2016) Disrupting the PCSK9/LDLR protein–protein interaction by an imidazole-based minimalist peptidomimetic. *Org. Biomol. Chem.* 14, 9736–9740
129 Gokhale, A. *et al.* (2011) Conformationally constrained peptides from CD2 to modulate protein–protein interactions between CD2 and CD58. *J. Med. Chem.* 54, 5307–5319
130 Danelius, E. *et al.* (2016) Flexibility is important for inhibition of the MDM2/p53 protein–protein interaction by cyclic β-hairpins. *Org. Biomol. Chem.* 14, 10386–10393
131 Roberts, R.B. *et al.* (2004) Modeling the cancer patient with genetically engineered mice: prediction of toxicity from molecule-targeted therapies. *Cancer Cell* 5, 115–120
132 Reinisch, A. *et al.* (2016) A humanized bone marrow ossicle xenotransplantation model enables improved engraftment of healthy and leukemic human hematopoietic cells. *Nat. Med.* 22, 812–821
133 Arrouss, I. *et al.* (2015) Cell penetrating peptides as a therapeutic strategy in chronic lymphocytic leukemia. *Protein Pept. Lett.* 22, 539–546
134 Jagot-Lacoussiere, L. *et al.* (2016) A cell-penetrating peptide targeting AAC-11 specifically induces cancer cells death. *Cancer Res.* 76, 5479–5490
135 Beez, S. *et al.* (2012) Targeting the acute promyelocytic leukemia-associated fusion proteins PML/RARα and PLZF/RARα with interfering peptides. *PLoS One* 7, e48636
136 Singh, J. *et al.* (2011) The resurgence of covalent drugs. *Nat. Rev. Drug Discov.* 10, 307–317
137 Maly, D.J. *et al.* (2004) A mechanism-based cross-linker for the identification of kinase-substrate pairs. *J. Am. Chem. Soc.* 126, 9160–9161

138 Nemani, M. *et al.* (1996) Activation of the human homologue of the *Drosophila sina* gene in apoptosis and tumor suppression. *Proc. Natl. Acad. Sci. U. S. A.* 93, 9039–9042
139 Bruzzoni-Giovanelli, H. *et al.* (1999) SIAH-1 inhibits cell growth by altering the mitotic process. *Oncogene* 18, 7101–7109
140 Hara, M.R. *et al.* (2005) S-Nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. *Nat. Cell Biol.* 7, 665–674
141 Lee, F.H.F. *et al.* (2016) Disrupting GluA2-GAPDH interaction affects axon and dendrite development. *Sci. Rep.* 6, 30458
142 Pei, L. *et al.* (2010) Uncoupling the dopamine D1-D2 receptor complex exerts antidepressant-like effects. *Nat. Med.* 16, 1393–1395
143 THPdb. Available at: http://crdd.osdd.net/raghava/thpdb/index.html
144 Jazayeri, A. *et al.* (2017) Crystal structure of the GLP-1 receptor bound to a peptide agonist. *Nature* 546, 254–258
145 ClinicalTrials.Gov. Available at: https://clinicaltrials.gov/
146 Otvos, L., Jr (2000) Antibacterial peptides isolated from insects. *J. Pept. Sci.* 6, 497–511
147 Li, J. *et al.* (2007) Anti-infection peptidomics of amphibian skin. *Mol. Cell. Proteomics* 6, 882–894
148 Vetter, I. *et al.* (2011) Venomics: a new paradigm for natural products-based drug discovery. *Amino Acids* 40, 15–28
149 González-Magaldi, M. *et al.* (2015) Peptides interfering 3A protein dimerization decrease FMDV multiplication. *PLoS One* 10, e0141415
150 Li, C. *et al.* (2013) A peptide derived from the C-terminus of PB1 inhibits influenza virus replication by interfering with viral polymerase assembly. *FEBS J.* 280, 1139–1149
151 Yu, Y. *et al.* (2017) A peptide-based viral inactivator inhibits Zika virus infection in pregnant mice and fetuses. *Nat. Commun.* 8, 15672http://dx.doi.org/10.1038/ncomms15672
152 Nayak, S.K. *et al.* (2017) p 53-Mdm2 interaction inhibitors as novel nongenotoxic anticancer agents. *Curr. Cancer Drug Targets* 17
153 Fischer, P.M. (2006) Peptide, peptidomimetic, and small-molecule antagonists of the p53–HDM2 protein–protein interaction. *Int. J. Pept. Res. Ther.* 12, 3–19
154 Warso, M.A. *et al.* (2013) A first-in-class, first-in-human, Phase I trial of p28, a non-HDM2-mediated peptide inhibitor of p53 ubiquitination in patients with advanced solid tumours. *Br. J. Cancer* 108, 1061–1070
155 Danelius, E. *et al.* (2016) Flexibility is important for inhibition of the MDM2/p53 protein–protein interaction by cyclic β-hairpins. *Org. Biolmol. Chem.* 14, 10386–10393
156 Mason, J.M. (2010) Design and development of peptides and peptide mimetics as antagonists for therapeutic intervention. *Future Med. Chem.* 2, 1813–1822
157 Fojo, T. *et al.* (2014) Unintended consequences of expensive cancer therapeutics–the pursuit of marginal indications and a me-too mentality that stifles innovation and creativity: the John Conley lecture. *JAMA Otolaryngol. Neck Surg.* 140, 1225
158 Zhang, Y.-L. *et al.* (2016) Structure-based identification of CaMKIIα-interacting MUPP1 PDZ domains and rational design of peptide ligands to target such interaction in human fertilization. *Amino Acids* 48, 1509–1521
159 Stanic, J. *et al.* (2015) Rabphilin 3A retains NMDA receptors at synaptic sites through interaction with GluN2A/PSD-95 complex. *Nat. Commun.* 6, 10181
160 Borsello, T. *et al.* (2003) A peptide inhibitor of c-Jun N-terminal kinase protects against excitotoxicity and cerebral ischemia. *Nat. Med.* 9, 1180–1186
161 Chiquet, C. *et al.* (2017) Postoperative ocular inflammation: a single subconjunctival injection of XG-102 compared to dexamethasone drops in a randomized trial. *Am. J. Ophthalmol.* 174, 76–84
162 Qvit, N. *et al.* (2016) Selective phosphorylation inhibitor of delta protein kinase C–pyruvate dehydrogenase kinase protein–protein interactions: application for myocardial injury *in vivo*. *J. Am. Chem. Soc.* 138, 7626–7635
163 Guo, B. *et al.* (2003) Humanin peptide suppresses apoptosis by interfering with Bax activation. *Nature* 423, 456–461
164 Jouaux, E.M. *et al.* (2009) Improving the interaction of Myc-interfering peptides with Myc using molecular dynamics simulations. *J. Pept. Sci.* 15, 5–15
165 Di Agostino, S. *et al.* (2008) The disruption of the protein complex mutantp53/p73 increases selectively the response of tumor cells to anticancer drugs. *Cell Cycle* 7, 3440–3447
166 Masse, M. *et al.* (2014) An eIF4E-interacting peptide induces cell death in cancer cell lines. *Cell Death Dis.* 5, e1500
167 Nambotin, S.B. *et al.* (2011) Pharmacological inhibition of Frizzled-7 displays anti-tumor properties in hepatocellular carcinoma. *J. Hepatol.* 54, 288–299
168 Li, X. *et al.* (2015) Improving chemotherapeutic efficiency in acute myeloid leukemia treatments by chemically synthesized peptide interfering with CXCR4/CXCL12 axis. *Sci. Rep.* 5, 16228
169 Arpel, A. *et al.* (2016) Inhibition of primary breast tumor growth and metastasis using a neuropilin-1 transmembrane domain interfering peptide. *Oncotarget* 7, 54723–54732
170 Guo, Y. *et al.* (2000) A peptide derived from the nonreceptor binding region of urokinase plasminogen activator (uPA) inhibits tumor progression and angiogenesis and induces tumor cell death *in vivo*. *FASEB J.* 14, 1400–1410
171 Chatterjee, S. *et al.* (2014) The intricate role of CXCR4 in cancer. *Adv. Cancer Res.* 124, 31–82
172 Khalili, P. *et al.* (2006) A non-RGD-based integrin binding peptide (ATN-161) blocks breast cancer growth and metastasis *in vivo*. *Mol. Cancer Ther.* 5, 2271–2280
173 Taga, T. *et al.* (2002) αv-Integrin antagonist EMD 121974 induces apoptosis in brain tumor cells growing on vitronectin and tenascin. *Int. J. Cancer* 98, 690–697
174 Bae, D.-G. *et al.* (2005) Anti-Flt1 peptide, a vascular endothelial growth factor receptor 1–specific hexapeptide, inhibits tumor growth and metastasis. *Clin. Cancer Res.* 11, 2651–2661
175 Awasthi, S. *et al.* (2015) Structure of a TLR4-interacting SPA4 peptide. *RSC Adv.* 5, 27431–27438
176 Hines, D.J. *et al.* (2013) Prevention of LPS-induced microglia activation, cytokine production and sickness behavior with TLR4 receptor interfering peptides. *PLoS One* 8, e60388
177 Raman, S. *et al.* (2015) First structural view of a peptide interacting with the nucleotide binding domain of heat shock protein 90. *Sci. Rep.* 5, 17015