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Protein Domain Mimics as Modulators of Protein–Protein Interactions

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CONSPECTUS

Protein–protein interactions (PPIs) are ubiquitous in biological systems and often misregulated in disease. As such, specific PPI modulators are desirable to unravel complex PPI pathways and expand the number of druggable targets available for therapeutic intervention. However, the large size and relative flatness of PPI interfaces make them challenging molecular targets. This Account describes our systematic approach using secondary and tertiary protein domain mimics (PDMs) to specifically modulate PPIs.

Our strategy focuses on mimicry of regular secondary and tertiary structure elements from one of the PPI partners to inspire rational PDM design. We have compiled three databases (HIPDB, SIPPDB, and DIPDB) of secondary and tertiary structures at PPI interfaces to guide our designs and better understand the energetics of PPI secondary and tertiary structures. Our efforts have focused on three of the most common secondary and tertiary structures: α -helices, β -strands, and helix dimers (e.g., coiled coils).

To mimic α -helices, we designed the hydrogen bond surrogate (HBS) as an isosteric PDM and the oligoioxopiperazine helix mimetic (OHM) as a topographical PDM. The nucleus of the HBS approach is a peptide macrocycle in which the N-terminal i , $i + 4$ main-chain hydrogen bond is replaced with a covalent carbon–carbon bond. In mimicking a main-chain hydrogen bond, the HBS approach stabilizes the α -helical conformation while leaving all helical faces available for functionalization to tune binding affinity and specificity. The OHM approach, in contrast, envisions a tetrapeptide to mimic one face of a two-turn helix. We anticipated that placement of ethylene bridges between adjacent amides constrains the tetrapeptide backbone to mimic the i , $i + 4$, and $i + 7$ side chains on one face of an α -helix.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): P.S.A. is a co-inventor on patent applications on secondary and tertiary structure mimics described in this paper. He is also cofounder of Inthera Bioscience, which is pursuing therapeutic applications of HBS and OHM technologies.

DEDICATION

We dedicate this Account to Neville Kallenbach on the occasion of his 80th Birthday.

For β -strands, we developed triazolamers, a topographical PDM where the peptide bonds are replaced by triazoles. The triazoles simultaneously stabilize the extended, zigzag conformation of β -strands and transform an otherwise ideal protease substrate into a stable molecule by replacement of the peptide bonds.

We turned to a salt bridge surrogate (SBS) approach as a means for stabilizing very short helix dimers. As with the HBS approach, the SBS strategy replaces a noncovalent interaction with a covalent bond. Specifically, we used a bis-triazole linkage that mimics a salt bridge interaction to drive helix association and folding. Using this approach, we were able to stabilize helix dimers that are less than half of the length required to form a coiled coil from two independent strands.

In addition to demonstrating the stabilization of desired structures, we have also shown that our designed PDMs specifically modulate target PPIs in vitro and in vivo. Examples of PPIs successfully targeted include HIF1 α /p300, p53/MDM2, Bcl-xL/Bak, Ras/Sos, and HIV gp41. The PPI databases and designed PDMs created in these studies will aid development of a versatile set of molecules to probe complex PPI functions and, potentially, PPI-based therapeutics.

Graphical abstract

INTRODUCTION

Protein–protein interaction (PPI) modulators have vast potential as specific probes to elucidate complex biological pathways and as therapeutics to correct misregulated PPIs in disease.^{1,2} Developing a systematic strategy to discover high affinity PPI modulators is an area of intensive research.^{3,4} Our efforts have focused on the role and mimicry of secondary

(e.g., α -helix and β -strand) and tertiary (e.g., coiled-coil) structures to inhibit PPI interfaces (Figure 1). Estimates based on peptide backbone dihedral angles suggest that PPI interfaces are composed of roughly equal measures of regular secondary structures (α -helices and β -strands) and nonregular structures.⁵ Regular structural elements display side chain groups in defined orientations, often burying large surface areas and contributing significantly to the overall PPI binding energy.^{6,7} The structural regularity and energetic importance of these secondary and tertiary structures led to the hypothesis that protein domain mimics (PDMs) that imitate interfacial PPI secondary or tertiary structures would act as potent PPI inhibitors.

To test this hypothesis, many groups have developed elegant approaches to mimic protein secondary and tertiary structures.^{8–27} One common approach is to covalently stabilize isolated peptides, which are typically unstructured in solution. Preorganization of the peptide in its active conformation may improve upon the affinity of unstructured peptides for their target protein. A second common approach mimics protein surfaces with nonpeptidic scaffolds that can approximate the side chain geometry of binding epitopes. Both approaches have been successful for many protein targets, particularly in the mimicry of α -helices, β -strands, and β -hairpins.²⁸

This Account reviews our efforts in (1) the construction and analysis of an extensive PPI database and (2) the design and evaluation of PPI inhibitors inspired by PPIs identified in the database. We will discuss the database and design of PPI inhibitors in reference to three specific secondary and tertiary structures: α -helices, β -strands, and helix dimers. For each structure, we highlight specific findings from database analysis, scaffolds used for PPI modulator design, and a case study for a biological system of interest. Though many of our PPI modulators were designed prior to database construction, the database continues to inform our target selections and ligand design.

RESULTS AND DISCUSSION

α -Helical PDMs

α -Helices are commonly observed at PPI interfaces, particularly at homodimeric complexes, where approximately 40% of all interface residues are in the α -helical conformation.⁵ Given the prevalence of α -helices at PPI interfaces, we systematically analyzed the Protein Data Bank (PDB) to construct a database (Helical Interfaces in Protein–Protein Interactions Database, or HIPPDDB) of α -helices that contribute significantly to the binding free energy of PPI interfaces.^{29–31} In an attempt to preselect for “targetable” PPIs, all of our databases are constructed using cutoffs for the minimum number of hotspot residues ($G = 1$ Rosetta energy unit) in the structural element. Analysis of HIPPDDB reveals that only a small fraction (14%) of helical PPI interfaces have their hotspots concentrated into binding pockets that might be blocked with traditional small molecules. In the remaining interfaces, hotspots are more widely distributed.

In designing a PPI α -helix mimic, the major decision is which molecular architecture to use as a scaffold for the functional groups that will determine the molecule’s binding affinity and specificity. This selection process is informed by two critical factors: (1) the spacing of

hotspots with respect to each other and (2) alignment of hotspots with respect to the helical faces.

Single-Face Helix Mimics—Analysis of PPI α -helices reveals a hotspot periodicity of 3–4 residues, corresponding to hotspot alignment on a single helical face (Figure 2). Hotspot residues are aligned in this way in approximately 60% of HIPDB helices.³¹ Hotspot alignment on a single helical face led to the hypothesis that a rigid topographical mimic that reproduces the α -helical spacing of functional groups, but not necessarily the peptide backbone, would function as a PPI inhibitor with similar efficacy to a helical peptide. The success of many conformationally rigid scaffolds that mimic α -helix topography solidly supports this hypothesis.^{15,18,20,32}

In our laboratory, we have developed the oligooxopiperazine helix mimetic (OHM) as a topographical helix mimetic (Figure 3A).^{22,32–34} We rationalized that if the goal is to mimic a two-turn α -helix that displays three hotspot residues on the same helical face, i.e. the i , $i + 4$, and $i + 7$ positions, a tetrapeptide may be sufficient. We created a tetrapeptide model that spans the length of a two-turn α -helix and positions its i , $i + 1$, and $i + 3$ residues in positions equivalent to the i , $i + 4$, and $i + 7$ residues of an α -helix. To stabilize the requisite peptide conformation, we used oxopiperazine rings obtained by bridging neighboring amides with ethylene. Though the OHM backbone does not trace that of the α -helix, side chains are presented in i , $i + 4$, and $i + 6/i + 7$ positions. The optimal α -helix side chain mimicry requires the half-chair geometry observed in the piperazine ring. OHMs are efficiently synthesized on solid-phase in successive dipeptide units by alkylating an o -nitrobenzenesulfonyl (o NBS) activated dipeptide under Mitsunobu conditions^{22,35} followed by base-catalyzed cyclization (Figure 3A). In addition to providing conformational stability, the ethylene bridges confer proteolytic stability by protecting the peptide bonds as tertiary amides. The utility of the OHM scaffold for specific, high affinity binding ($K_d = 30$ –500 nM) has been demonstrated with various PPI targets.^{22,32}

Multi-Face Helix Mimics—The remaining 40% of high affinity PPI α -helices in HIPDB engage their protein partners with two or three helical faces.³⁰ Mimicry of these helices with topographical α -helical mimetics is at best incomplete because those molecules display functional groups equivalent to only a single α -helical face. Isolated α -helical peptides, on the other hand, could effectively mimic their protein counterparts. However, short peptides are conformationally labile, proteolytically unstable and generally bind with weak affinity, in part because of the entropic penalty for folding upon binding.³⁶

In light of these challenges, different strategies have been devised to covalently constrain peptides into the α -helical conformation.^{20,24} We have extensively evaluated a hydrogen bond surrogate (HBS) approach as one such strategy (Figure 3B).^{11,37–43} The crux of this approach is the replacement of a helix-nucleating N-terminal backbone hydrogen bond with an isosteric, covalent carbon–carbon bond.^{44,45} Covalent nucleation of the α -helical conformation leads to helix propagation⁴⁶ and enhances the proteolytic stability of HBS helices compared to their respective unconstrained peptide analogs.⁴⁷ Importantly, by replacing a main-chain hydrogen bond, the number of side chains available for functionalization is maximized while minimizing the size of the covalent constraint.

HBS helices are efficiently synthesized by solid-phase peptide synthesis (SPPS) with three modifications (Figure 3B). The first modification is the incorporation of an *N*-allyl amino acid residue at the *i* + 4 position. *N*-Allyl amino acids can be synthesized in solution or on resin, typically using the *o*NBS protecting group to activate the amine for monoalkylation.³⁵ The second modification for HBS synthesis is the solid-phase incorporation of a 4-pentenoic acid derivative in place of the *i* and *i* + 1 residues.^{11,43} The terminal alkene thus mimics the carbonyl group of the *i* residue. Finally, the third and final modification of SPPS for HBS synthesis is ring-closing metathesis (RCM)⁴⁸ between the alkenes at the *i* and *i* + 4 equivalent positions, forming a covalent macrocyclic α -helix nucleus. The RCM step has been optimized for solid-phase microwave conditions and is compatible with most protecting groups commonly used in Fmoc/tBu-based SPPS.^{11,37} As with OHMs, a variety of HBS helices have been established as inhibitors of specific helix-mediated PPIs.^{38,47,49–52}

Case Study: Inhibition of the HIF1 α -p300/CBP PPI—A pertinent illustration of OHMs and HBS helices as PPI inhibitors both in vitro and in vivo is exemplified by our studies on the inhibition of complex formation between the hypoxia inducible factor 1 α (HIF1 α) transcription factor and the transcriptional coactivator homologues p300 and CBP (Figure 4).^{32,51} The HIF1 α -p300/CBP PPI has been studied extensively because of the central role of HIF1 α in regulating transcription in hypoxic environments, such as solid tumors. HIF1 α engages p300/CBP primarily through its C-terminal transactivation domain (CTAD).^{53,54} While the HIF1 α CTAD is intrinsically disordered in isolation, it folds upon binding to the CH1 domain of p300/CBP, forming two distinct α -helices.^{55,56} Computational and experimental studies have demonstrated an essential role for both helices in high-affinity interaction between HIF1 α and p300/CBP.

Given the importance of the HIF1 α helices in p300/CBP binding, we hypothesized that mimics of either α -helix would be sufficient to inhibit the HIF1 α -p300/CBP PPI. HBS helices based on the HIF1 α CTAD sequence were designed and found to form stable α -helices in solution. Each HBS helix binds to the cognate p300/CBP CH1 domain with submicromolar affinity.^{49,51}

Motivated by the success of the HBS helices, a series of HIF1 α -based OHMs were also designed to mimic the hotspot residues of one of the HIF1 α CTAD helices whose hotspots lie on a single helical face.³² Hotspot mimicry using the OHM scaffold results in nanomolar binding affinity for the CH1 domain, suggesting that these OHMs capture the majority of the high-affinity elements of the HIF1 α helix. HBS and OHM analogues lacking computationally predicted hotspot residues display negligible binding to the p300/CBP CH1 domain, suggesting that both types of helix mimetics recognize their target domain at the intended binding site in a sequence-specific manner.

In addition to successfully binding to p300/CBP, the designed HBS helices and OHMs also efficiently downregulate HIF1 α -mediated transcription activation in cell culture.^{32,49,51} Transcription of specific HIF target genes, such as *VEGFA*, *LOX*, and *GLUT1*,⁵⁷ is downregulated upon treatment with designed HBS helices or OHMs. Gene expression profiling of treated A549 cells under hypoxic conditions revealed 2-fold up- or

downregulation of up to 600 transcripts, including transcripts from many genes involved in the “hallmarks of cancer.”⁵⁸

The HBS and OHM mimics of the HIF1 α helix also proved to be effective in reducing tumor growth in vivo in mouse xenograft models (Figure 4).^{32,51} For HBS helix treatment, 786-O human renal cell carcinoma xenografts in BALB/c mice showed greater than 50% volume reduction over a 4-week treatment course. Similar reduction in tumor volume was observed in BALB/c mice with MDA-MB-231 human breast adenocarcinoma xenografts upon similar OHM treatment. Daily weight measurements suggested no toxicity for either compound. Post-treatment tumor imaging with the near-infrared tumor contrast agent IR-783 revealed reduced tumor volume and no evidence of tumor metastasis.

The success of HBS helices in reducing the HIF1 α -p300/CBP interaction in vitro and reducing tumor volume in vivo studies prompted further investigation of the potential of HIF1 α helix mimetics to reduce other cancers associated with p300/CBP PPIs. The CH1 domain of p300/CBP is known to interact with numerous transcription factors, including p53.⁵⁹ In human papillomavirus-positive head and neck squamous cell carcinoma (HPV-positive HNSCC), the p300/CBP-p53 PPI is disrupted by the viral protein E6, reducing p53 stability and transcriptional activity.⁶⁰ It was thus hypothesized that HIF1 α helix mimetics might block the p300-E6 PPI and reactivate p53.⁶¹ Indeed, a HBS helix derived from one of the HIF1 α CTAD helices reactivates p53 in HPV-positive HNSCC but not HPV-negative HNSCC. The HBS helix also potentiates the effects of cisplatin, suggesting that combination therapy with the HIF1 α HBS helix and reduced cisplatin dosing may improve the toxicity profile of cisplatin treatment. Interestingly, a HBS helix derived from the other HIF1 α CTAD helix does not reactivate p53, suggesting partial but incomplete overlap of the E6 and HIF1 α binding sites on p300/CBP.

Overall, these studies highlight the utility of HBS helices and OHMs as modulators of p300/CBP PPIs in vitro and in vivo. Further studies are expected to provide insight into the specificity of HBS helix/OHM PPI targeting.

β -Strand PDMs

The similar frequency of α -helices and β -strands at heterodimeric interfaces (26% and 24%, respectively) underscores the essential role of β -strands in PPIs.⁵ Thus, to complement HIPDB, we constructed SIPPDB (Strand Interfaces in Protein–Protein Interactions Database), a database of β -strands that contribute significantly to the binding free energy of their respective PPI interfaces.⁶²

With the exception of position $i + 2$, the expected periodicity of β -strand hotspots on a single face is not as evident as the hotspot periodicity of 3–4 observed for α -helices (Figure 5). PPI β -strands also tend to be shorter than PPI α -helices. Our survey of PPI β -strands revealed that only one-quarter of these strands engage their protein partners with hotspots on a single face. In contrast, almost one-third of PPI β -strands have binding energy contributions distributed equally across their 2 faces.

One significant challenge with β -strand PPI modulators is that β -strands are ideal protease substrates.⁶³ To overcome this challenge and the general conformational instability of isolated peptides, many approaches have been investigated to simultaneously stabilize the β -strand conformation, protect or replace the peptide backbone, and retain critical functional groups.⁶⁴ These approaches can be roughly classified into two categories: use of nonpeptidic amino acid analogs to stabilize individual β -strands^{9,13,65–68} or protection of individual strands in the context of a β -hairpin stabilized by noncovalent interactions or macrocyclization (Figure 6A).^{12,23,69} Both approaches have been reviewed recently,^{64,70} so we will limit our discussion here to the triazolamer scaffold developed in our laboratory.

Triazolamers—To design conformationally and proteolytically stable, single-strand β -strand mimics, we developed the triazolamer scaffold. In this scaffold, the peptide backbone is replaced with 1,3-substituted 1,2,3-triazoles.^{13,67,71} This scaffold recapitulates peptide side chain interactions but cannot hydrogen bond in the same way as a peptide backbone. The triazole moiety is synthesized from amino acid-derived alkynes and azides.⁷² Peptide backbone replacement protects these molecules from protease degradation. Stabilization of the β -strand conformation is driven by dipole–dipole interactions between triazoles, which have large dipole moments. Both solution NMR and X-ray diffraction studies have confirmed zigzag triazolamer conformations that mimic the pleats observed in β -strands.^{13,73} (Figure 6A, B). The $i, i + 2$ distance between triazolamer side chain C β s (7.9 Å) is similar to the $i, i + 2$ distance in β -strands (7.2 Å), making this an appealing scaffold for the design of not only PPI modulators but also protease inhibitors.

Case Study: Inhibition of HIV-1 Protease—Buoyed by the promising biophysical properties of triazolamers, we conducted initial studies to evaluate triazolamers as PPI inhibitors. Preliminary investigations focused on HIV-1 protease (HIVPR), as protease–substrate interactions can be thought of as transient PPIs (Figure 6C, D).⁶⁷ Initial designs were based on two tetrapeptide inhibitors that adopt a strand-like conformation in the protease active site.⁷⁴ FRET-based activity assays⁷⁵ revealed robust inhibition of HIV-1 protease by the designed triazolamers and demonstrate the potential of this scaffold for the rational design of inhibitors involving a β -strand. Further evaluation of triazolamer-based PPI inhibitors are in progress.

Helix Dimer PDMs

The results above demonstrate the advantages of secondary structure mimics as PPI inhibitors. Nonetheless, many PPI interfaces do not possess a single, energetically dominant α -helix or β -strand. Instead, these interfaces often rely on tertiary structures to drive complex formation, where hotspots are distributed over multiple secondary structure motifs.³

One of the simplest and best studied examples of tertiary structure is the coiled coil.^{76–78} Coiled coils form a helical dimer and can occur in either parallel or antiparallel orientation. We constructed DIPDDB (Dimeric Interfaces in Protein–Protein Interactions Database) to systematically examine helix dimers at PPI interfaces.⁷⁹ To construct the database, we applied two separate filters to HIPDDB. The first filter examined the geometry between pairs

of interface helices, retaining only helix pairs with (1) an antiparallel or parallel orientation and (2) an interhelical distance compatible with typical coiled coil hydrophobic cores. The second filter examined the energetics of the PPI interface helices and retained only those helix pairs where both helices contributed significantly to the calculated binding energy. The overlap between these two filtered sets was refined to yield DIPPDDB.

On average, the first and last hotspot residues for DIPPDDB helix dimers are separated by approximately 13 residues (or two helical turns, see Figure 7).⁷⁹ This average distance is larger than the analogous hotspot separation observed in isolated PPI α -helices (approximately 7 residues or one helical turn). Nonetheless, this spacing represents a relatively compact hotspot distribution compared with the minimum length of approximately 21 residues required to form a stable coiled coil in solution from two separate peptide chains.^{80,81}

We also analyzed the chemistry of the dimerization interface between PPI helix dimers. Interestingly, we observed that these interfaces often deviate from idealized hydrophobic coiled coil packing. This deviation from idealized packing may represent functional constraints imposed by the PPI interface, which can involve helix dimerization interface residues.⁷⁹

Minimal Coiled Coil Mimetics Using the Salt Bridge Surrogate (SBS) Approach

—In light of these findings, we embarked upon a series of studies to identify chemical strategies for stabilizing minimal coiled coil mimetics as PPI inhibitors (Figure 8A).²⁶ Ultimately, we obtained the desired stability for an 18-residue helix dimer (9 residues per helix) by employing azide–alkyne click chemistry to covalently link positions that typically form salt bridge interactions. This salt bridge surrogate (SBS) approach is analogous to the HBS approach, in that it replaces a stabilizing noncovalent interaction with a covalent interaction. To synthesize SBS helix dimers, an azidolysine residue is incorporated at a salt bridge position in one peptide by SPPS. Propargyl ether is then coupled under copper(I)-catalyzed alkyne–azide cycloaddition (CuAAC) conditions.^{72,82} The remaining free alkyne is then coupled to a Fmoc-protected azidolysine amide, which is used for further SPPS.

Case Study: The NHR2/N2B Interaction—Having established the SBS approach for stabilizing an idealized 18-residue helix dimer, we examined the use of this method to constrain a biological helix dimer, namely a minimal coiled coil derived from the Nerve homology 2 (NHR2) protein (Figure 8B). NHR2 binds to the NHR2-binding (N2B) motif of E-proteins through an antiparallel coiled coil and plays an essential role in leukemogenesis.⁸³ Adding the SBS cross-link to an otherwise native NHR2 sequence did not result in stable helix formation or detectable binding to the isolated N2B motif. Optimization of the helix dimerization interface was required to obtain a stable helix dimer that displayed a native-like binding affinity to the N2B motif. Further stabilization of the dimer interface by a cysteine-mediated disulfide bridge further improved both helicity and binding affinity for the N2B target. Taken together, these experiments highlight the combination of the SBS approach with hydrophobic core optimization to create potent helix dimer PPI inhibitors. Importantly, the SBS approach is sequence independent and provides

access to helix dimer mimetics that are less than half the size of the smallest stable coiled coil,⁸¹ improving the prospects of these compounds for intracellular applications.

CONCLUSIONS

In summary, we have described a systematic strategy that combines computational and experimental studies to generate PDMs that target specific PPIs with high affinity and specificity. Our computational databases (HIPDB, SIPPDB, and DIPDB) provide a foundation for understanding the energetics of secondary and tertiary structures at PPI interfaces. The overarching theme of our experimental approach is the introduction of new covalent bonds to drive peptides into specific bioactive conformations. In the HBS and SBS approaches, the new covalent bonds stabilize helical structure by replacing native hydrogen bonding or ionic interactions. For triazolamers and OHMs, new covalent bonds drive the formation of topographical β -strand and α -helix scaffolds, respectively. We have successfully translated information from several of our database entries into effective PPI inhibitors that target specific PPIs in vitro. While some of the PDMs described herein have shown in vivo potential, potent engagement of intracellular targets remains a key challenge. Interplay between computational PPI analysis and evaluation of synthetic PPI inhibitors will allow for the development of a versatile set of molecules to probe complex PPI functions and to inspire the design of new PPI-based therapeutics.

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ABBREVIATIONS

HBS	hydrogen bond surrogate
OHM	oligooxopiperazine helix mimetic
PPI	protein-protein interaction
PDM	protein domain mimic
PDB	Protein Data Bank
SBS	salt bridge surrogate
SPPS	solid phase peptide synthesis

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Figure 1. Examples of (A) α -helices, (B) β -strands, and (C) helix dimers at protein-protein interaction interfaces. PDB codes: (A) 1BXL (Bcl-xL/Bak), 1YCR (p53/MDM2); (B) 1OY3 (NF- κ B homodimer), 1F3U (Rap30/Rap74); (C) 2IW5 (LSD1/CoREST), 3CL3 (vFLIP/IKK γ).

Figure 2.

Distribution of α -helix hotspots at PPI interfaces. Hotspot frequency is plotted as a bar graph (A) and on an idealized α -helix (B). The data show increases in hotspot frequency every 3–4 residues, corresponding to alignment of hotspots on a single α -helical face (helical faces indicated with black arcs). The color legend depicts fractional occurrence of hot spot residues (G for alanine mutation > 1 kcal/mol) at each position.

Figure 3.

Design rationale for hydrogen bond surrogate (HBS) helices and oligooxopiperazine helix mimetics (OHMs). (A) (Left) An α -helix projects its i , $i+4$, and $i+7$ side chains on a single helical face. A tetrapeptide can be modeled to mimic these side chain projections (center left) and synthetically realized using half-chair oxopiperazine rings (center). Overlay of the OHM model with an idealized α -helix (center right) shows excellent mimicry of a single helical face. The general synthetic scheme for OHMs (right): (a) *o*NBS-Cl, 2,4,6-collidine; (b) 2-bromoethanol, triphenylphosphine (PPh₃), diisopropylazodicarboxylate (DIAD); (c) base (e.g., 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU)); (d) 2-mercaptoethanol, DBU. (B) (Left) The helix-nucleating N-terminal hydrogen bond can be transformed into a covalent bond (center left), specifically a carbon–carbon bond (center). Overlay of the HBS helix crystal structure with an α -helix shows excellent mimicry. The general synthetic scheme for HBS helices (right): (a) SPPS for secondary amine, (b) SPPS, (c) 4-pentenoic acid, *N,N'*-diisopropylcarbodiimide (DIC); (d) ring-closing metathesis (RCM).

Figure 5.

Distribution of β -strand hotspots at PPI interfaces. Hotspot frequency is plotted as a bar graph (A) and on an idealized β -strand (B). With the exception of position $i+2$, the data show a minimal increase in hotspot frequency every 2 residues, corresponding to alignment of hotspots on a single β -strand face. The color legend depicts fractional occurrence of hot spot residues (ΔG for alanine mutation > 1 kcal/mol) at each position.

Figure 6.

Triazolamers as β -strand mimetics. NMR (A) and X-ray crystallographic (B) data support a zigzag geometry for triazolamers that mimics β -strands. The solid and dashed arrows in panel (A) indicate strong and weak ROESY cross peaks, respectively, in the NMR spectrum. (C) The interaction of the tetrapeptide inhibitor L-400,417 (stick representation) with HIV-1 protease (HIVPR) dimer (green cartoon) was used to guide triazolamer HIVPR inhibitor design (overlay on right with L-400,417 in green and a triazolamer design in purple). (D) Examples of a triazolamer inhibitor (left) and negative control (right), showing IC_{50} 's for HIVPR protease inhibition.

Figure 7. Distribution of helix dimer hotspots at PPI interfaces. The frequency of particular spacings between the first and last hotspots is plotted as a bar graph (A) and on an idealized coiled coil (B). The spacing between first and last hotspots is usually shorter than 3 heptads. The color legend depicts fractional occurrence of hot spot residues (G for alanine mutation > 1 kcal/mol) at each spacing.

Figure 8. Salt bridge surrogate (SBS) helix dimers as PPI inhibitors. (A) Different bis-triazole SBS linkages (middle left) were synthesized as part of an idealized coiled coil (top left) and monitored by circular dichroism (CD, bottom left), demonstrating that a propargyl ether-azidolysine linkage (green) favored helix formation. A helical wheel diagram and structural model based on NMR restraints are shown on the right. (B) The SBS approach was applied to the NHR2 coiled coil (top left). Though the SBS alone did not stabilize the helix dimer as monitored by CD, combining the SBS with optimization of the helix dimer interface yields a minimal helix dimer with significant helicity and native-like binding affinity for N2B (NHR2-N2B $K_d = 356 \mu\text{M}$). Binding affinity was further enhanced with a disulfide bridge.