Controlling the conformational constraint of peptides to modulate their target affinity

Müller, C.

2019

document version
Publisher's PDF, also known as Version of record

Chapter 1

Introduction

Introduction

Every cell contains a plethora of different proteins, acting as molecular tools for the most diverse processes and procedures. Those proteins consist of amino acids, which are connected via peptide bonds and form long chains with certain properties, depending on their amino acid sequence. These chains exist in different shapes and orientations depending on their specific amino acid composition and sequence. Amino acid chains can fold into so-called secondary structures for example α-helices, β-sheets and different turn motifs. These individual secondary structure elements interact to form a higher ordered tertiary structure, giving shape to a protein. If proteins form higher organized complexes, their structure is spoken of as quaternary.[1] Peptides are per definition small chains of amino acids. Therefore, every protein can also be considered a polypeptide.[2] Peptides, as well as proteins, fulfill certain tasks in the cell, for example as signaling peptides included in proteins, steering protein localization. This also means that peptides can be included in bigger protein macromolecules. Like proteins, peptides are also capable of forming secondary, tertiary and quaternary structures.[3] In nature, the connection of amino acids towards peptide chains is an enzymatic process (referred to as translation). It is also possible to assemble peptides chemically, for instance via solid-phase peptide synthesis.[4] The opportunities of this technique exceed the simple peptide-translation, since it is possible to incorporate unnatural building blocks. This provides a variety of opportunities for the design and use of peptides in diverse applications.

Peptides as protein-protein interaction inhibitors

Protein-protein interactions (PPIs) play a central role in a multitude of biological processes, such as subcellular localization and enzymatic activities. Such interactions between protein surfaces are mediated by electrostatic attraction, hydrogen bonding and the hydrophobic effect.[5,6] The formed complexes harbor different physiological functions: e.g. permanent complexes are involved in the formation of the cytoskeleton, while reversible and transient interactions often play a role in signaling pathways.[7,8] Macromolecular complexes are formed by at least partially structured protein domains. One example involves Fts proteins, which form the divisome, thereby being responsible for bacterial cell division, and will play an important role in this thesis.[9] PPIs can occur between secondary structures like helical peptides ((e.g. coiled-coil)), but also flexible peptides or more complex tertiary and quaternary structures like proteins. Because of their important role in the highly organized network of cell regulation, malfunctioning is associated with numerous diseases.[10–12]

Modulators of PPIs are therefore considered of great potential for the development of novel drug candidates in particular when aiming for so far difficult targets.[11,13,14] However, targeting PPIs is a difficult endeavor, as many of these interactions involve rather flat and large interfaces (about 800 - 2000 Å) which are very hard to address using small molecular scaffolds.[5,15,16] Until the recent years, most therapeutic entities were either large molecular biologics or small molecules.[11] Biologics, such as antibodies, harbor binding motifs suitable to interact with interfaces involved in PPIs.[10,15,17] Two major drawbacks of biologics is their limited oral availability and cell permeability.[17] The other class, classical small molecules, in principle provides oral availability and has been successfully used to obtain drugs inhibiting PPIs when harboring a suitable binding pocket.[10,15] However, they are often too small to effectively interact with flat protein surfaces and thereby inhibit the interactions of a protein complex.[15] Large macrocycles establish a third class of therapeutics, addressing difficult target structures and lying between small molecules and big biologics.[17–19] In contrast, such macrocycles have a bigger structure allowing them to interact with widespread interaction patches on protein surfaces. One source of macrocyclic inhibitors are peptide binding epitopes. Typically, these binding epitopes are identified in the interaction area of one of the protein complex partner and are defined by their secondary structure (e.g. α-helix, β-sheet and turn) which they exhibit in their bound state (bioactive conformation). After extraction of the binding epitope, these short peptides however, lose their secondary structure and exist in an ensemble of conformational states in solution (bio-inactive conformation). Linear, peptide fragments such as these maintain low cell permeability and due to their highly flexible nature in solution, possess high protease instability.[20] However, by stabilizing such peptides into their bioactive conformation, it is possible to derive bioavailable and stable inhibitors for PPI inhibition. [21,22] The stabilized peptidic molecules mimicking binding epitopes of the natural precursor are generally referred to as peptidomimetics.[24–29]

Above mentioned macrocyclization of the derived peptides can lead to preorganization and stabilization of the bioactive conformation. Different strategies to pre-organize secondary structures have been evolved.[30] Starting with the most common structure of all protein-protein interfaces of PPIs, the α-helix, the most prominent methods are side chain to side chain crosslinks and the incorporation of N-terminal caps.[30] Development of α-helical stabilization approaches started in the early 1980s.[31] In general, α-helices are stabilized by intramolecular hydrogen bonds formed by the peptide's backbone and salt bridges formed by amino acid residues. Ways to stabilize a succession of turns within the helix generally involve bridging one residue to another with a crosslink. For this purpose, amide bonds between lysine and glutamic acid and disulfides have been used.[32,33] Despite these, thiol- and triazole-crosslinks have also been examined (figure 1).[30] Thiol-based crosslinks circumvent the drawback of disulfides. Even though many disulfide approaches are successful, thioether-bridged peptides bear the advantage of being stable under reducing

conditions as they are found inside of most eukaryotic cells. Cysteines react selectively with electrophiles. Different bis- and triselectrophiles and different configured cysteine (-derivatives) have been applied to create mono- and bicyclic peptides with increased cellular uptake.[34–39] Another well characterized and common way to stabilize α-helices is the hydrocarbon peptide stapling technique.[40] The incorporation of α-methyl-α-alkenyl amino acids during solid-phase peptide synthesis and their side chain to side chain crosslinking by ring closing metathesis can lead to increased α-helicity, improved protease resistance and increased cellular uptake compared with their natural precursors.[31,41–43] Further developments of this well-described method are the use of two isolated hydrocarbon crosslinks or the synthesis of stitched peptides harboring a spiro ring junction.[44,45] All described methods for α-helical stabilization have also been used in context of designing PPI inhibitors.

Stabilization of β-strands and β-sheets is also of great interest, as they are highly involved in the proteins' tertiary and quaternary structure, as well as in protein aggregation and PPIs. Several methods, such as turn-mimetics that nucleate β-sheet formation, covalent and non-covalent macrocyclization and β-strand enforcing residues, have been used and also combined to stabilize modified β-strands, β-hairpins and β-sheets.[46–48] Only few approaches of PPI inhibitors based on β-sheets are described.[30] Also the stabilization of turn structures has been addressed already, showing the same effects: cyclization leads to increased protease stability and in most cases, increased cellular uptake.[21,30,49] All these macrocyclization approaches described above, are aiming for pre-organized high affinity peptide-based inhibitors.

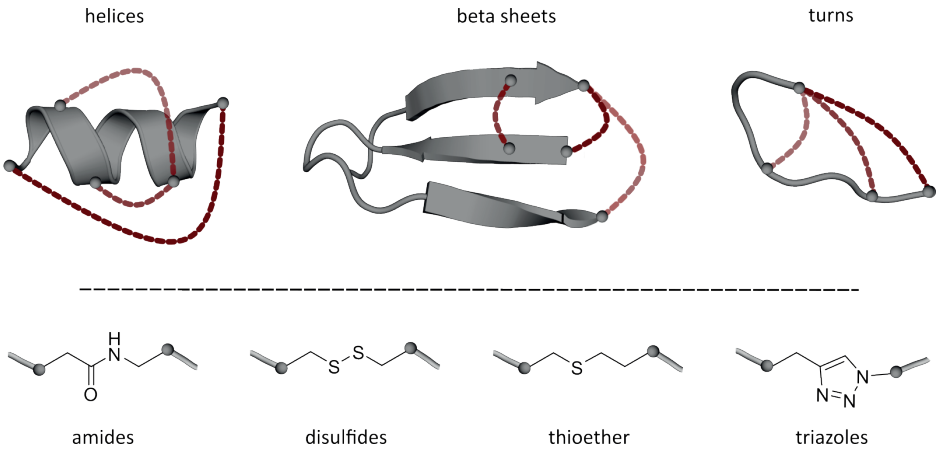

Figure 1. Exemplary depiction of different possibilities to pre-organize and stabilize secondary structures, such as helices, beta-sheets and turns with differently modified crosslinks.

In summary, the stabilization of the epitope-based peptides' bioactive conformation prior to the binding event often leads to increased protease stability, higher cell permeability and to reduced entropic penalty upon binding and thus increased affinity.[23] Thus, chemical modifications of naturally-derived peptides can improve the development of relevant drugs targeting PPIs.

Peptides as probes

As described before, PPIs rank among the most promising targets in current drug discovery efforts.[50] Since the human interactome contains about 650 000 interactions, it is of the utmost importance to provide researchers with tools to identify interaction partners, biophysically characterize the interactions and detect the potency of potential inhibitors. Accordingly, peptides can be deployed not only as inhibiting molecules themselves, but also as molecular probes.[51,52] Since direct tagging of interaction partners is challenging, those additionally added probes can provide specificity and fluorescent functions that are needed. Advantages of peptides compared to other classes of molecules lie in their ability to be designed as highly specific and affine molecules, but also to be equipped with fluorophores or other detectable compartments. Applications of such probes are e.g. *i*) the readout of cell-based PPI inhibition assays via fluorescence, *ii*) the identification of new PPIs via fluorescent-based pull down assays and *iii*) the identification of potential inhibitors in a fluorescent-based high-throughput screening.[53,54] Overall, simply designed experiments with comparably high signal-to-noise ratios define the gold-standard in PPI characterization.

Outline of this thesis

Chapter 2 (*Synthesis of a Peptidomimetic - Inhibition of the protein-protein interaction between FtsB and FtsQ in bacterial cell division*) highlights the interaction between FtsB and FtsQ as an interesting target for a bacterial cell division inhibitor, based on the involvement of these proteins in early stages of cell division. Since a crystal structure of the FtsB-FtsQ protein complex is available, crucial interacting residues and their spatial arrangement are known. This is an excellent starting point for the development of high affinity peptidomimetics. During its development it is necessary to enhance affinity compared to the wildtype peptide via crosslinking strategies leading to secondary structure stabilization. **Chapter 3** (*Coiled-coil Peptide Beacon: A Tuneable Conformational Switch for Protein Detection*) describes a new approach towards a peptidic probe for protein detection and sensing of protein-protein interactions. The design, synthesis and biophysical characterization of these so-called coiled-coiled peptide beacons are described. Synthetic peptides can also be used to as probes to understand the role of protein modifications in cells, as shown in **Chapter 4** (*Plant cysteine oxidases are dioxygenases that directly enable arginyl transferase-catalysed arginylation of N-end rule targets*). Here, the use of peptides as substrates of arginyl transfer RNA transferase in plants is described. Next, because of PTEN's crucial role in a wide range of protein-protein interactions, **Chapter 5** (*The Therapeutical Potential of PTEN Modulation Targeting strategies from Gene to Protein*) summarized the development of PTEN modulation as a therapeutic target.

Literature

1. Alberts, B. *et al. Molecular Biology of the cell*. (Garland Science, 2002).
2. Eaborn, C. Compendium of chemical Terminology: IUPAC Recommendations. *J. Organomet. Chem.* **356,** C76–C77 (1988).
3. Berg JM, Tymoczko JL, S. L. *Quaternary Structure: Polypeptide Chains Can Assemble Into Multisubunit Structures*. *Biochemistry.* (W H Freeman, 2002).
4. Merrifield, R. B. Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. *J. Am. Chem. Soc.* **85,** 2149–2154 (1963).
5. Valdar, W. S. J. & Thornton, J. M. Protein-protein interfaces: Analysis of amino acid conservation in homodimers. *Proteins: Struct. Funct. Genet.* **42,** 108–124 (2001).
6. Keskin, O., Tsai, C., Wolfson, H. & Nussinov, R. A new, structurally nonredundant, diverse data set of protein-protein interfaces and its implications. *Protein Sci.* **13,** 1043–1055 (2004).
7. Jansen, R., Greenbaum, D. & Gerstein, M. Relating whole-genome expression data with protein-protein interactions. *Genome Res.* **12,** 37–46 (2002).
8. M.A.Nooren, I. & Thornton, J. M. Diversity of protein-protein interactions. *EMBO J.* **22,** 3486–3492 (2003).
9. Natale, P., Pazos, M. & Vicente, M. The Escherichia coli divisome: Born to divide. *Environ. Microbiol.* **15,** 3169–3182 (2013).
10. Villoutreix, B. O. *et al.* Drug-like protein-protein interaction modulators: Challenges and opportunities for drug discovery and chemical biology. *Mol. Inform.* **33,** 414–437 (2014).
11. Fry, D. C. & Vassilev, L. T. Targeting protein-protein interactions for cancer therapy. *J. Mol. Med.* **83,** 955–963 (2005).
12. Kar, G., Gursoy, A. & Keskin, O. Human cancer protein-protein interaction network: A structural perspective. *PLoS Comput. Biol.* **5,** (2009).
13. Milroy, L.-G., Grossmann, T. N., Hennig, S., Brunsveld, L. & Ottmann, C. Modulators of Protein–Protein Interactions. *Chem. Rev.* **114,** 4695–4748 (2014).
14. Ivanov, A. A., Khuri, F. R. & Fu, H. Targeting protein – protein interactions as an anticancer strategy. *Trends Pharmacol. Sci.* **34,** 393–400 (2013).
15. Laraia, L., McKenzie, G., Spring, D. R., Venkitaraman, A. R. & Huggins, D. J. Overcoming Chemical, Biological, and Computational Challenges in the Development of Inhibitors Targeting Protein-Protein Interactions. *Chem. Biol.* **22,** 689–703 (2015).

16. Moreira, I. S., Fernandes, P. A. & Ramos, M. J. Hot spots-A review of the protein-protein interface determinant amino-acid residues. *Proteins: Struct. Funct. Bioinforma.* **68,** 803–812 (2007).
17. Bakail, M. & Ochsenbein, F. Targeting protein-protein interactions, a wide open field for drug design. *Comptes Rendus Chim.* **19,** 19–27 (2016).
18. Mallinson, J. & Collins, I. Macrocycles in new drug discovery. *Future Med. Chem.* **4,** 1409–1438 (2012).
19. Dougherty, P. G., Qian, Z. & Pei, D. Macrocycles as protein–protein interaction inhibitors. *Biochem. J.* **474,** 1109–1125 (2017).
20. Zhang, X. X., Eden, H. S. & Chen, X. Peptides in cancer nanomedicine: Drug carriers, targeting ligands and protease substrates. *J. Control. Release* **159,** 2–13 (2012).
21. Glas, A. *et al.* Constrained peptides with target-adapted cross-links as inhibitors of a pathogenic protein-protein interaction. *Angew. Chem., Int. Ed.* **53,** 2489–2493 (2014).
22. Azzarito, V., Long, K., Murphy, N. S. & Wilson, A. J. Inhibition of α-helix-mediated protein-protein interactions using designed molecules. *Nat. Chem.* **5,** 161–173 (2013).
23. Giannis, A. & Kolter, T. Peptidomimetics for Receptor Ligands—Discovery, Development, and Medical Perspectives. *Angew. Chem., Int. Ed.* **32,** 1244–1267 (1993).
24. Ripka, A. S. & Rich, D. H. Peptidomimetic design. *Curr. Opin. Chem. Biol.* **2,** 441–452 (1998).
25. Azzarito, V., Long, K., Murphy, N. S. & Wilson, A. J. Inhibition of α-helix-mediated protein-protein interactions using designed molecules. *Nat. Chem.* **5,** 161–173 (2013).
26. Akram, O. N. *et al.* Tailoring Peptidomimetics for Targeting Protein-Protein Interactions. *Mol. Cancer Res.* **12,** 967–978 (2014).
27. Cummings, M. D. *et al.* Substituted 1,4-benzodiazepine-2,5-diones as α-helix mimetic antagonists of the HDM2-p53 protein-protein interaction. *Chem. Biol. Drug Des.* **67,** 201–205 (2006).
28. Kharb, R., Rana, M., Chander Sharma, P. & Shahar Yar, M. Therapeutic importance of peptidomimetics in medicinal chemistry. *J. Chem. Pharm. Res.* **3,** 173–186 (2011).
29. Pelay-Gimeno, M., Glas, A., Koch, O. & Grossmann, T. N. Structure-Based Design of Inhibitors of Protein-Protein Interactions: Mimicking Peptide Binding Epitopes. *Angew. Chem., Int. Ed.* **54,** 8896–8927 (2015).

30. Toniolo, C. *et al.* Structural versatility of peptides from Cα,α-dialkylated glycines. II. An IR absorption and1H-nmr study of homo-oligopeptides from Cα,α-diethylglycine. *Biopolymers* **27,** 373–379 (1988).

31. Chorev, M. *et al.* Cyclic Parathyroid Hormone Related Protein Antagonists: Lysine 13 to Aspartic Acid 17 [i to (ì + 4)] Side Chain to Side Chain Lactamization. *Biochemistry* **30,** 5968–5974 (1991).

32. Jackson, D. Y., Schultz, P. G., King, D. S., Chmielewski, J. & Singh, S. General Approach to the Synthesis of Short α-Helical Peptides. *J. Am. Chem. Soc.* **113,** 9391–9392 (1991).

33. Spokoyny, A. M. *et al.* A perfluoroaryl-cysteine SNAr chemistry approach to unprotected peptide stapling. *J. Am. Chem. Soc.* **135,** 5946–5949 (2013).

34. Timmerman, P., Beld, J., Puijk, W. C. & Meloen, R. H. Rapid and quantitative cyclization of multiple peptide loops onto synthetic scaffolds for structural mimicry of protein surfaces. *ChemBioChem* **6,** 821–824 (2005).

35. Micewicz, E. D. *et al.* Bridged Analogues for p53-Dependent Cancer Therapy Obtained by S-Alkylation. *Int. J. Pept. Res. Ther.* **22,** 67–81 (2016).

36. Muppidi, A. *et al.* Rational design of proteolytically stable, cell-permeable peptide-based selective Mcl-1 inhibitors. *J. Am. Chem. Soc.* **134,** 14734–14737 (2012).

37. Baeriswyl, V. *et al.* Bicyclic peptides with optimized ring size inhibit human plasma kallikrein and its orthologues while sparing paralogous proteases. *ChemMedChem* **7,** 1173–1176 (2012).

38. Angelini, A. *et al.* Chemical macrocyclization of peptides fused to antibody Fc fragments. *Bioconjug. Chem.* **23,** 1856–1863 (2012).

39. Schafmeister, C. E., Po, J. & Verdine, G. L. An All-Hydrocarbon Cross-Linking System for Enhancing the Helicity and Metabolic Stability of Peptides. *J. Am. Chem. Soc.* **122,** 5891–5892 (2000).

40. Grossmann, T. N. *et al.* Inhibition of oncogenic Wnt signaling through direct targeting of -catenin. *Proc. Natl. Acad. Sci. U. S. A.* **109,** 17942–17947 (2012).

41. Sun, T. L., Sun, Y., Lee, C. C. & Huang, H. W. Membrane permeability of hydrocarbon-cross-linked peptides. *Biophys. J.* **104,** 1923–1932 (2013).

42. Chu, Q. *et al.* Towards understanding cell penetration by stapled peptides. *Medchemcomm* **6,** 111–119 (2015).

43. Bird, G. H. *et al.* Hydrocarbon double-stapling remedies the proteolytic instability of a lengthy peptide therapeutic. *Proc. Natl. Acad. Sci. U. S. A.* **107,** 14093–14098 (2010).

44. Hilinski, G. J. *et al.* Stitched α-helical peptides via bis ring-closing metathesis. *J. Am. Chem. Soc.* **136,** 12314–12322 (2014).

45. Schneider, J. P. & Kelly, J. W. Templates That Induce α-Helical, β-Sheet, and Loop Conformations. *Chem. Rev.* **95,** 2169–2187 (1995).

46. Nowick, J. S., Smith, E. M. & Pairish, M. Artificial β-sheets. *Chem. Soc. Rev.* **25,** 401–415 (1996).

47. Venkatraman, J., Shankaramma, S. C. & Balaram, P. Design of folded peptides. *Chem. Rev.* **101,** 3131–3152 (2001).

48. Chatterjee, J., Rechenmacher, F. & Kessler, H. N-Methylation of peptides and proteins: An important element for modulating biological functions. *Angew. Chem., Int. Ed.* **52,** 254–269 (2013).

49. Houk, K. N., Leach, A. G., Kim, S. P. & Zhang, X. Binding Affinities of Host-Guest, Protein-Ligand, and Protein-Transition-State Complexes. *Angew. Chem., Int. Ed.* **42,** 4872–4897 (2003).

50. Fry, D. C. Targeting protein-protein interactions for drug discovery. *Methods Mol. Biol.* **1278,** 93–106 (2015).

51. Kuner, T., Tokumaru, H. & Augustine, G. J. Peptides as probes of protein-protein interactions involved in neurotransmitter release. *Curr Top Membr.* **52,** 551–570 (2002).

52. Cai, L., Shi, W. & Liu, K. Peptides and Peptidomimetics as Tools to Probe Protein-Protein Interactions–Disruption of HIV-1 gp41 Fusion Core and Fusion Inhibitor Design. *Cdn.Intechopen.Com* (2011).

53. Macarrón, R. & Hertzberg, R. P. *Design and implementation of high-throughput screening assays. Methods Mol. Biol.* **565,** 1-32 (2009).

54. Caraus, I., Alsuwailem, A. A., Nadon, R. & Makarenkov, V. Detecting and overcoming systematic bias in highthroughput screening technologies: A comprehensive review of practical issues and methodological solutions. *Brief. Bioinform.* **16,** 974–986 (2015).