Advancing drug targets for peptide-peptide interactions: Peptide flexibility is key in inhibiting the MDM2/p53 interaction

Review Article

The search for new drug classes has gone beyond the classically druggable genome, which appears to be limited to around 1,500 proteins. One route is the development of molecules that interfere with the Protein-Protein Interactions (PPIs) that are critical to all cellular processes, such as the regulation of cell growth, DNA replication, transcriptional activation, protein folding, and transmembrane signaling.

Compared with the extremely conserved enzyme substrate binding pockets that are targeted by many drugs, PPI interfaces are highly diverse, which should enable the development of selective drugs with low off-target toxicity. The down side is that, in contrast to enzyme substrate binding pockets, PPI interfaces are dynamic and often planar. This puts special demands on the design of drugs that can 'stick' to the flat surfaces of the druggable target protein. Despite this, peptide-based PPI inhibitors with high specificity and affinity have been developed. Examples include LCL-161, an orally available IAP (Inhibitor of Apoptosis Proteins) antagonist that is active against multiple myeloma, glioblastoma and sarcoma, and ABT-199, a potent and selective BCL-2 inhibitor with anti-tumor activity (for a review of PPI inhibitors, see Laraia *et al*, 2015).

One PPI-based drug target that involves a well-defined secondary structure is the interaction between the p53 tumor suppressor, the so-called "guardian of the genome" involved in programmed cell death, and MDM2, an important negative regulator of p53. This interaction involves a "hot spot triad" of three residues, Phe19, Trp23 and Leu26, on one face of the α-helical region of p53. Mimicking this region with a peptide became the focus of a collaborative effort between University of Gothenburg, Sweden and St. Jude Children's Research Hospital in Tennessee, USA and resulted in key insights into the balanced peptide design required to achieve effective PPI inhibition (Danelius *et al*, 2016).

Peptide design and synthesis

The team used a known mimetic of the α-helical region of p53, called Peptide 1 (Figure 1), as the starting point for computer modeling to design peptides that could be expected to form β-hairpins and mimic the three critical side chains of p53 involved in MDM2 binding (Phe19, Trp23, and Leu26). The designs included several β-hairpin inducing features, such as β-branched amino acids, hydrophobic residues at cross-strand positions, and the commonly used D-Pro-Gly β-turn inducers. The team also substituted two glutamic acid residues in positions 4 and 9 with serines to stabilize the structure by interstrand hydrogen bonding. One peptide, 'Peptide 2', (Figure 1) was particularly promising in forming β-hairpins and was used as a template for further modification based on insights into the interaction between MDM2 and p53.

Pro—D-Pro—Phe—Glu—Trp—Asp—Leu—Trp(6-Cl)—Glu—Phe

Peptide 1

Gly—D-Pro—Phe—Ser—Glu—Asp—Leu—Trp(6-Cl)—Ser—Phe

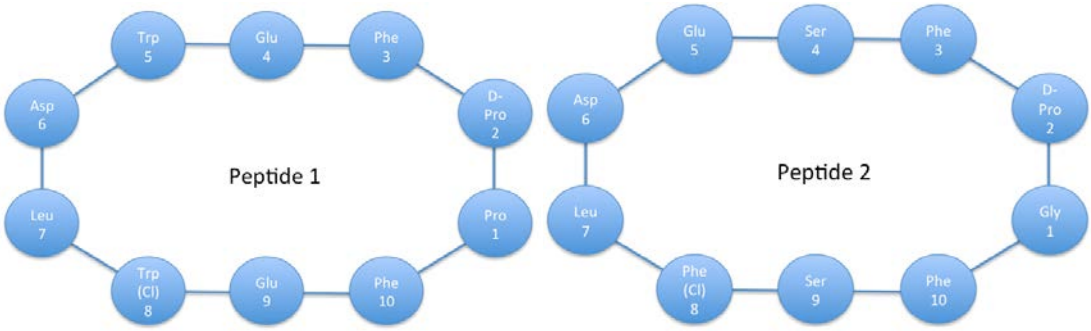

Figure 1 *Schematic representations of two of the four peptides used as mimetics of the α-helical region of p53. Peptide 2 was derived from the original Peptide 1 by substituting Glu4 and Glu9 with serines to stabilize the structure by interstrand hydrogen bonding. A 4-chloro-L-phenylalanine residue was included at position 8 based on the success of MDM2/p53 inhibitors that place a 4-chlorophenyl moiety in the Trp pocket of MDM2. Trp5 was replaced with Glu5 to enable hydrogen bonding and/or ionic interactions with the His96 and Lys94 residues of MDM2 seen in other inhibitors. Based on Figures 2 and 3, Danelius et al, 2016.*

Linear versions of the peptides were synthesized with a PS3 Automated Solid Phase Peptide Synthesizer using Fmoc-*t*-Bu-Trt protecting strategy and the 2-chlorotrityl resin. Peptide couplings were performed with TBTU (N-[(1H-benzotriazol-1-yl) (dimethylamino)methylene]-N-methylmethanaminium hexafluorophosphate N-oxide) as the coupling reagent and N,N-diisopropylethylamine as the base in DMF. After cleavage using 1% TFA in DCM, the peptides were cyclized with a pseudo-high dilution procedure involving HATU as the coupling reagent, followed by global deprotection and purification by reversed phase HPLC. Yields were in the 8–18% range.

Flexibility confers activity

Emma Danelius and her colleagues investigated the relationship between the flexibility of the peptides and their ability to inhibit the MDM2/p53 interaction. They used a combination of NMR spectroscopy and computational methods called NAMFIS (NMR Analysis of Molecular Flexibility in Solution) that describes conformations in solution. The inhibition of the interaction was determined by a fluorescence polarization assay that measured the displacement of a fluorescently labeled wild type p53 peptide bound to MDM2. The team also characterized the binding of the peptide using surface plasmon resonance spectroscopy.

The accepted norm for increasing the bioactivity of peptides is to add rigidity to give them an entropic advantage. This is seen, for example, in α-helices and β-turn mimetics. But adding rigidity has been questioned in the field of PPIs, where there seems to be a need for a balance between flexibility and rigidity to achieve the desired activity. And this is exactly what the team discovered. There was an inverse correlation between the inhibitory activity of their peptides and the hairpin stability (Figure 2). Peptide 1 had the lowest β-hairpin stability (24%) and was the most active inhibitor (2.6 μM IC50) compared to peptide 2 with the highest β-hairpin stability (61%) and least active inhibitor (23.9 μM IC50).

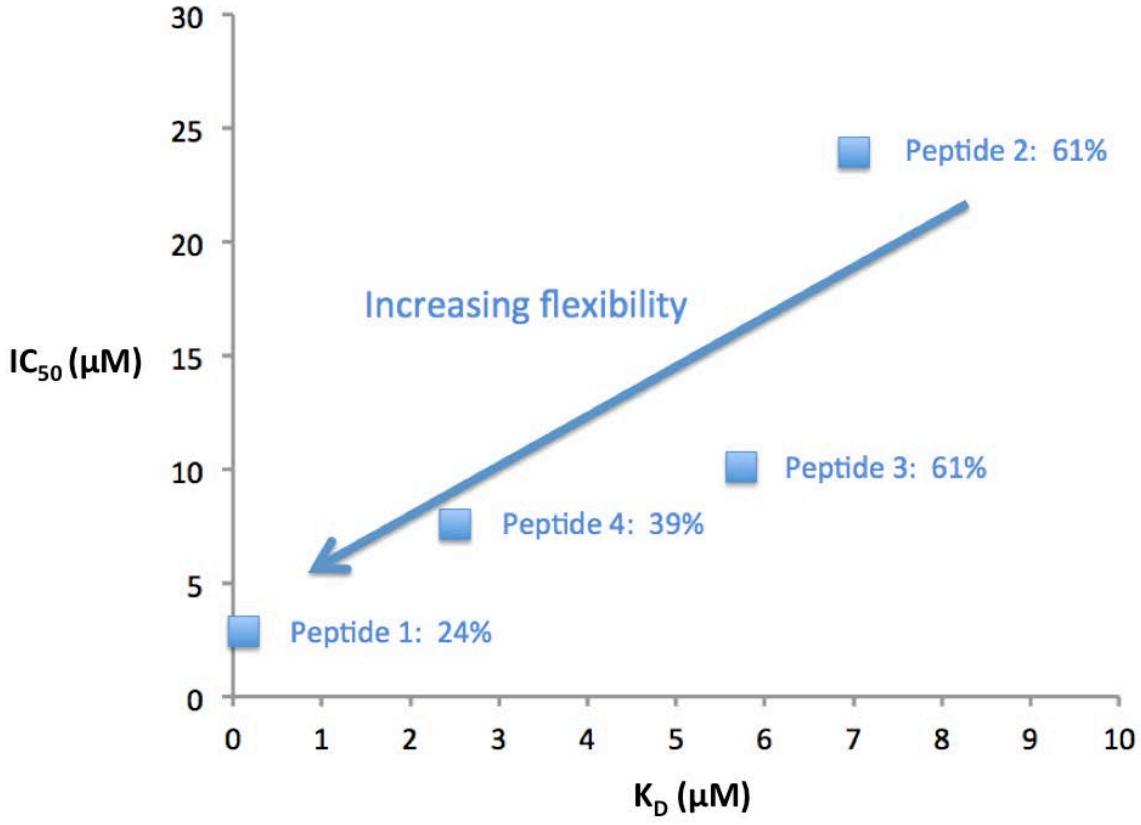

Figure 2 *There was a clear relationship between the peptides' inhibitory activity (IC_{50}) and their binding affinity (K_D), both of which increased with increasing flexibility. The labeling includes the peptide name (1–4) and the percentage of the β-hairpin in solution based on analysis by NAMFIS. The figure was created based on the data presented in Table 2 (Danelius et al, 2016).*

A promising future for peptide-based PPI inhibition

A large proportion of PPIs are mediated by protein secondary structures and α helices are frequently involved. This means that short α-helical peptides based on the key binding hotspot, such as the peptides studied by Emma Danelius and her colleagues, can be potent inhibitors of PPIs. There may even be a "ready-made" lead compound for every α-helix-based PPI, but designing them will demand a lot of modulation to stabilize peptides that accurately mimic protein secondary structure to deliver fine-tuned therapy with high selectivity.

References

Overcoming Chemical, Biological, and Computational Challenges in the Development of Inhibitors Targeting Protein-Protein Interactions. Laraia, L *et al.* Chem Biol. 2015 Jun 18; 22(6): 689–703. doi: 10.1016/j.chembiol.2015.04.019 PMCID: PMC4518475. Pubmed: https://www.ncbi.nlm.nih.gov/pubmed/26091166

Flexibility is important for inhibition of the MDM2/p53 protein-protein interaction by cyclic β-hairpins. Danelius E, *et al*. Org Biomol Chem. 2016 Nov 8;14(44):10386-10393. Pubmed: https://www.ncbi.nlm.nih.gov/pubmed/27731454