Molecular Recognition

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Introducing Quadrupole Interactions into the Peptide Design Toolkit

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Proteins and peptides are often described as the machinery of life. Even the simplest bacteria have hundreds of proteins, and these proteins work in concert with each other to conduct thousands of distinct functions. The fidelity of these functions relies on the specificity of interactions between the units of the machinery, between the proteins.

By studying the forms and functions of proteins, and tracing these back to amino acid sequences the "rules" for their self-assembly can be obtained, thus allowing de novo peptide design and yielding novel forms and functions.[1] The design of linear amino acid sequences that fold into defined secondary structures, or motifs, such as α-helices or β-sheets is relatively well understood. The greater challenge is to engineer specific interactions between these motifs, that is between precisely positioned side chains. An improved ability to direct intermolecular interactions will increase the functionality of the peptide assemblies that we are able to construct, and correspondingly, increase their potential applications.

Until recently, the chemical toolkit for introducing peptide–peptide molecular recognition has consisted of four tools, or noncovalent interactions between amino acid side chains. These are ionic interactions, hydrogen bonding, hydrophobic interactions, and π stacking.[2] Zheng and Gao have recently described a new tool, the quadrupole interaction (Figure 1), which is a refinement of π stacking.[3] The ways in which these tools are utilized to impart specificity to peptide interactions are touched upon in the following paragraphs, with particular reference to coiled coils, which are the best understood peptide machinery.[4,5]

1. Hydrophobic interactions. The hydrophobic effect is generally the strongest component in protein and peptide quaternary interactions, and the degree of steric matching between hydrophobic side chains is important for increasing the stability of the complexes. However the interactions are not as specific as those between hydrophilic side chains. With regards to coiled coils, hydrophobic design principles have been used to greatest effect in determining the oligomerization state or relative orientation of α-helices in complexes rather than specifying binding partners. This strong but non-exclusive form of molecular recognition is complemented with other binding mechanisms in de novo peptides.
2. Ionic interactions. Amino acids with charged side chains are important determinants of specificity in peptide complexes, and this is the most frequently used strategy. Specificity is often achieved by negative design, whereby a unique structure is achieved by destabilizing the other possible complexes.[6] Many heterodimeric coiled coils have charged residues bordering the hydrophobic core such that one helix is positively charged and the other negatively charged, hence preventing homodimeric coiled-coils from forming. Controlling intermolecular electrostatic interactions by using changes in pH values or salt concentrations can also be used to switch peptide binding specificity. This concept has been demonstrated with iterative pH cycles, specifically replacing one, two, or all three initial helices of a coiled-coil trimer.[6]
3. Hydrogen bonds. Hydrogen bonds are also used to impart specificity to coiled coils by the placement of hydrogen-

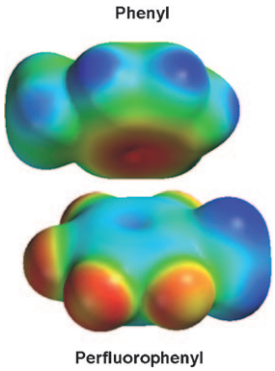

Figure 1. Space-filling model of the aromatic side chains of phenylalanine and perfluorophenylalanine stacked face-to-face. The distribution of electrostatic potential is indicated, with blue being positive, and red negative. Phenyl and perfluorophenyl have opposite quadrupole moments, allowing a net electrostatic attraction between the π faces to occur.[3]

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bonding residues within the hydrophobic core. The correct register of hydrogen-bond-forming polar residues enhances one low-energy structure over the other possible structures. The high degrees of binding specificity that can be designed into the coiled-coil interaction by utilizing hydrogen bonding has been exemplified by the formation of three distinct heterodimers from solutions of six peptides. The selectivity was introduced by substituting a single, natural or urea-derived hydrogen-bond-forming amino acid in a core position of each peptide.[7]

4. π–π Interactions. Aromatic interactions consist of a hydrophobic component and an electrostatic component arising from the quadrupole moment.[8] They are quite prevalent in protein cores, and have been used to stabilize peptide interactions in both α-helices and β-sheets. As with hydrophobic interactions they have predominantly been used to stabilize specific configurations in homocomplexes, rather than specific interactions between different peptides.[8] Because of it's complexity, having ill-determined hydrophobic and electrostatic components, variable packing geometries, and interactions with other moieties, this tool is rather poorly understood.

Zheng and Gao have now investigated the quadrupole interaction and shown that this can be used as a new tool to impart specificity to peptide interactions. In a recent paper they demonstrated that the correct placement of aromatic and perfluoroaromatic rings can direct selective protein–protein interactions.[3] The peptide α_2D folds into a homodimer in which each peptide is in a helix-loop-helix conformation having a phenylalanine side chain from each helix stacked face-to-face to form a hydrophobic core. A double mutant in which both phenylalanines are substituted for the fluorinated equivalent also forms a dimer with the same structure. The fluorinated complex has an increased stability (a melting temperature of 80 °C in comparison to 30 °C for the wild type at 25 μM) because of the increased hydrophobicity of the fluorine atoms in comparison to hydrogen atoms. When these two stable dimers are mixed there is a complete transition from homodimers to heterodimers (Figure 2).[3] The specificity of the heterodimer is a result of the quadrupole interaction of the stacked aromatic and perfluoroaromatic rings. This molecular recognition arises because the electrostatic potential of aromatic molecules tends to be negative inside the aromatic ring, whereas for perfluoroaromatic compounds the quadrupole moment is of nearly equal magnitude, but of opposite polarity because of the electron-withdrawing fluorine substituents, thus leading to negative electrostatic potential at the fluorine atoms outside the ring. This arrangement of electronic charge results in a net electrostatic attraction between parallel π-faces (Figure 1).

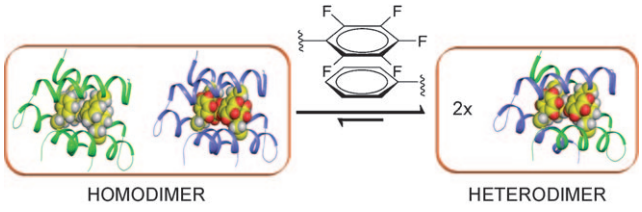

Figure 2. The quadrupole stacking between aromatic and perfluoroaromatic rings is able to direct specific protein–protein interactions, resulting in the exclusive formation of heterodimers upon mixing a pair of homodimers with aromatic and perfluoroaromatic cores.[3]

In this study it was estimated by a double mutation cycle that each phenylanaline perfluorophenylanaline pair contributes $-(1.0\pm0.3)$ kcal mol^{-1} to the binding energy of the dimer.[3] This energetic scale is comparable to that of weak hydrogen bonds, and generally weaker than ionic and hydrophobic interactions. Proteins and peptides typically have a folding stability of approximately 5–10 kcal mol^{-1},[8] which results from a combination of stabilizing and destabilizing interactions. The combined magnitude of these energies is many times greater than the final sum. To more accurately predict the outcome (in terms of both stability and specificity) of this interplay of noncovalent interactions, it would be beneficial to quantify the quadrupole interaction in more detail.

Although the quadrupole interaction of the phenyl/perfluorophenyl pair has been known to lead to molecular recognition of small molecules,[9,10] two previous reports in which the quadrupole interaction was utilized for peptide or peptoid molecular recognition indicated that no stabilization was observed.[11,12] This was probably because the precise stacking geometry of the aromatic rings that is necessary for stabilization could not be achieved. Therefore, despite the easy incorporation of fluoroarenes into peptide sequences, both chemically and recombinantly, it may not be easy to design peptide sequences such that the phenyl and perfluorophenyl side chains have the optimum stacking geometry.

In summary, the quadrupole interaction between aromatic and perfluoroaromatic rings has been shown to be effective in determining binding specificity between peptides. As such it represents a new design tool for specific peptide interactions. For this tool to be widely and confidently used, the quadrupole interaction should be investigated further, both in terms of binding energy and binding geometry. Nevertheless, it has been shown to add to the design rules that allow peptides to be used as sophisticated building blocks for the assembly of nanomaterials, and as such widens the scope of functional peptide assemblies.

Although we are still some way away from being able to consistently program a peptide to achieve the desired function, we are becoming more competent with the available chemical tools for engineering specific interactions between peptides, and as more tools are clarified the realm of possible peptide machines will grow.

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