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## Nature and geometry of aromatic reactions in peptides: peptide models to protein engineering

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### Abstract

A thorough knowledge of noncovalent interactions is crucial to the understanding of biological complexity. One of the less well understood but significant weak interactions in nature is the aromatic interaction. Recent studies have provided new insight into the driving force, stability and selectivity of these interactions. The contribution of solvophobic and electrostatic interactions have been shown to be inextricably linked. Moreover, the influence of electrostatic and solvophobic components on the selectivity of aromatic interactions has been demonstrated.

**Keywords:** aromatic interactions; peptide model; protein engineering; model systems; Nature and geometry of aromatic

### Introduction

A thorough knowledge of noncovalent interactions is crucial to the understanding of biological complexity. One of the less well understood but significant weak interactions in nature is the aromatic interaction. Recent studies have provided new insight into the driving force, stability and selectivity of these interactions. The contribution of solvophobic and electrostatic interactions have been shown to be inextricably linked. Moreover, the influence of electrostatic and solvophobic components on the selectivity of aromatic interactions has been demonstrated.

### The nature and geometry of aromatic interactions

Aromatic interactions have been proposed to consist of van der Waals, hydrophobic and electrostatic forces. The relative contribution and magnitude of each of these components is still under investigation. This is complicated by the fact that aromatic groups interact in one of several geometries, depending on the nature of the rings involved. Nonetheless, aromatic interactions are intriguing molecular recognition elements because they are expected to be strong in water because of the hydrophobic component of the interaction yet, at the same time, the interaction should be selective if the electrostatic component is significant, thus providing the best features of both hydrophobic interactions and hydrogen bonding.

Several geometries are attractive, and have been proposed on the basis of the electrostatic component of the interaction (Figure 1). The electrostatic component has been proposed to arise from interactions of the quadrupole moments of the aromatic rings. Although benzene has no net dipole, it has an uneven distribution of charge, with greater electron-density on the face of the ring and reduced electron-density on the edge, which gives rise to the quadrupole moment. The edge-face geometry (Figure 1a), which can be considered a CH- $\pi$  interaction, is found in benzene in the solid state, and is commonly observed between aromatic residues in proteins. The offset stacked orientation (Figure 1b) is also commonly found in proteins and is the geometry of base stacking in DNA. In this geometry, more surface area is buried, and the van der Waals and hydrophobic interactions are increased. This orientation appears to be more common when the electron density on the face of one or both rings is reduced. A third possible geometry is the face-to-face stacked orientation (Figure 1c). This is commonly observed with donor-acceptor pairs and compounds that have opposite quadrupole moments,

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such that the interaction between the faces of the rings is attractive. The benzene–perfluorobenzene interaction is an excellent example of this type of aromatic interaction, and has been calculated to provide  $-15.5 \text{ kJ mol}^{-1}$  in stability. In an elegant study, Cozzi and Siegel have demonstrated the electrostatic contribution to aromatic interactions in the

face-to-face stacked conformation. However, questions still exist regarding the importance of electrostatics relative to dispersion and hydrophobic forces in the edge–face and offset–stacked geometries, which are the geometries commonly found in nature.

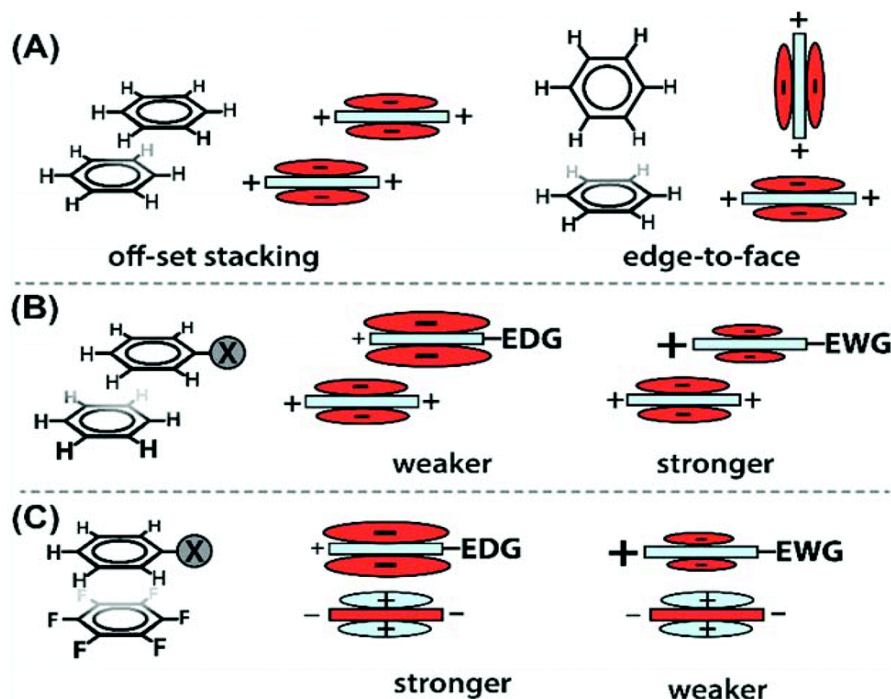


Fig. 1: Geometries of aromatic interactions. (a) edge-face; (b) offset stacked; (c) face-to-face stacked.

### Aromatic interactions in peptides

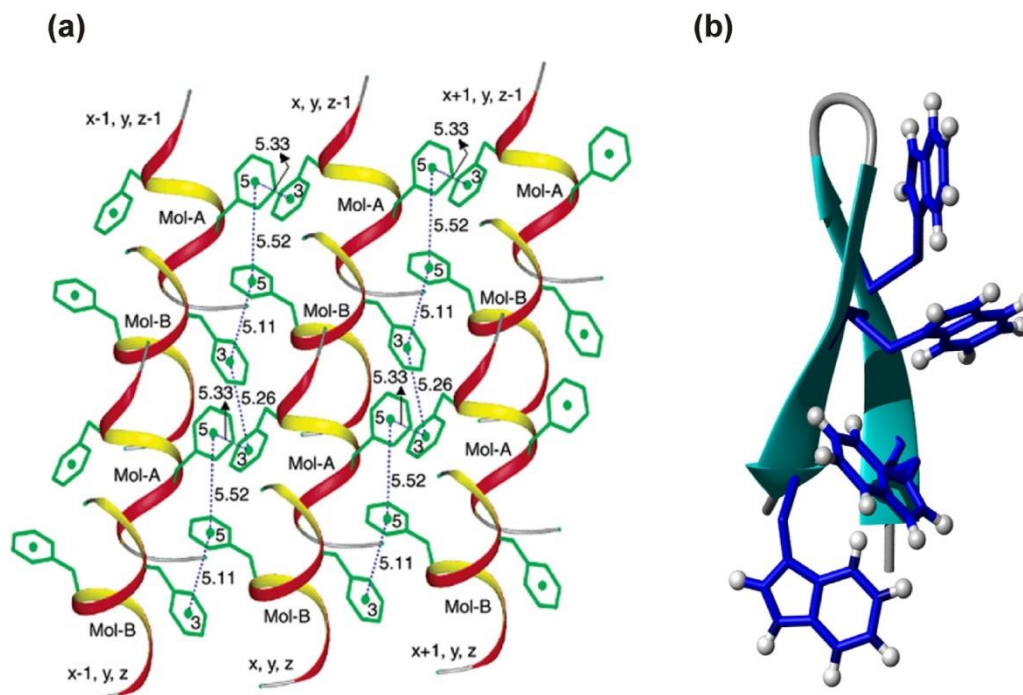
Aromatic interactions have recently been studied in the context of peptide secondary structure. Butterfield and Waters have found that aromatic interactions between two phenylalanines in the  $i$  and  $i+4$  residues in a  $\alpha$ -helix can provide up to  $-3.3 \text{ kJ mol}^{-1}$  to the stability of a  $\alpha$ -helix in water. The geometry is believed to be an edge–face interaction. In a comparison of cross-strand interactions between phenylalanines and cyclohexylalanines (Cha) in a  $\beta$ -hairpin peptide in water, Tatko and Waters found that Phe residues show a preference for self-association. The Phe residues were found to interact in an edge–face geometry despite being solvent exposed. Moreover, the Phe–Phe cross-strand pair was found to be enthalpically more favorable and entropically less favorable than the Cha–Cha interaction, suggesting that a classical hydrophobic interaction is not the driving force for the Phe–Phe association.

### Aromatic Interactions: Peptide Models to Protein Engineering

In the past few decades, numerous groups have investigated aromatic-aromatic interactions using peptide model systems. These investigations have led to a better insight in the context of fundamental forces driving protein folding, stability and in biomolecular recognition. On the basis of secondary structure, the interaction between the aromatic rings has been thoroughly investigated in both isolated  $\alpha$ -helix and  $\beta$ -hairpin model peptide systems. Waters and co-workers investigated the incorporation of Phe residues at  $i$  and  $i+14$  positions of designed helical peptides and provided experimental evidence for the stabilizing role of aromatic interactions. They also showed that this

interaction is stronger when placed near the C-terminus than in the center of a helix. Balaram and co-workers also reported several short helical peptides containing Trp or Phe residues involved in intra- and interhelix aromatic interactions. They observed that peptides in which Phe side chains were on the same face of the helix showed both intrahelix and interhelix aromatic interactions [Fig. 2(a) the peptides in which Phe side chains were placed on opposite faces of the helix resulted in only interhelix aromatic interactions. Their studies using peptide models showed that “the energy landscape for a pair of interacting phenyl rings consists of a broad, relatively flat minimum, which appears to be somewhat rugged, with several local minima separated by small energy barriers” Supramolecular assembly is also possible in peptide structures. One such example from the DeGrado group of a peptide dimer of  $\alpha$ 2D has already been discussed earlier. Since the first report of Trpzip  $\beta$ -hairpin peptides, shown in Figure 2(b), by the Cochran group, Trp-Trp pairs at the non-hydrogen bonding position has proved to be excellent hairpin stabilizing elements. Numerous hairpin scaffolds incorporating Trp-Trp, Trp-non-Trp and other aromatic pairs have since been designed by us, and groups of Andersen, Jimenez, Keiderling, Kelly, Waters and others. These extensive investigations using peptide models have led us to a thorough understanding of aromatic-aromatic interactions.

Aromatic interactions have also proved as useful structure stabilizing elements in protein engineering. The Kelly group has shown that the incorporation of a single cross-strand Trp-Trp pair at the nonhydrogen bonding position in an autonomously folded protein hPin1 WW domain significantly increased its thermodynamic stability.



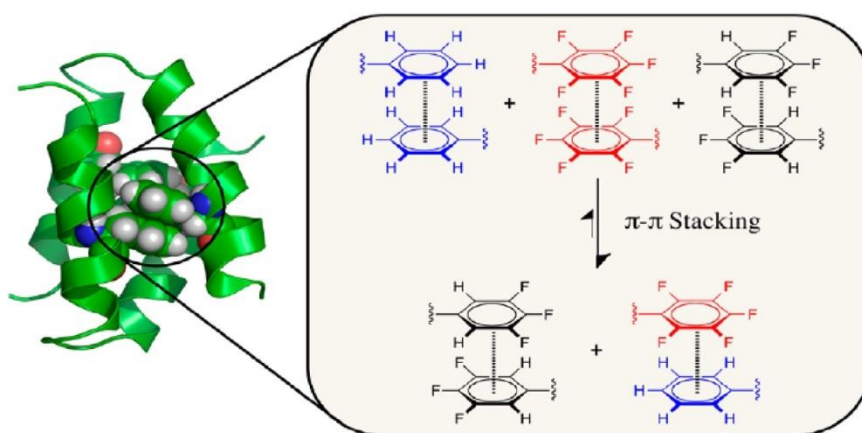
**Fig. 2:** Aromatic interactions in peptide models. (a) A short helical synthetic peptide (Boc-Aib-Ala-Phe-Aib-Phe-Ala-Val-Aib-OMe) displays strong inter-helix aromatic interactions in the crystal

The protein lost its function due to restricted backbone motion caused by highly stabilizing Trp-Trp interaction, suggesting that proteins have evolved to balance stability against functional demands in various cases.

In a designed three-stranded peptide  $\beta$ -sheet nucleated by  $^D$ Pro-Gly segment, Balaram and coworkers incorporated  $\beta$ -phenylalanine at positions facing each other. The structural fold of the  $\beta$ -sheet promoted the N-terminal and C-terminal  $\beta$ -phenylalanines to participate in long-range aromatic-aromatic interactions. Such strategies incorporating backbone modified  $\beta$ - and  $\gamma$ -aromatic amino acid residues has gathered substantial interest in the de novo design of proteolytically resistant bioactive peptides and proteins. Aromatic residues further stabilize such scaffolds through cross-strand interactions. Fluorination of aromatic amino acids is also now widely exploited in protein engineering; excellent work and review in this area has been reported by

the GAO group. In general terms, fluorination increases the hydrophobicity of the molecule, thus favoring a “hydrophobic effect” in protein folding and stability. The Gellman group probed the effect of substituting Phe-Phe interactions in a small protein villin headpiece subdomain with perfluoro phenylalanine. They found that the substitution of aryl side chains with fluoro-aryl side chains could result in stabilizing the folded conformation of proteins; however the effect cannot be generalized. Figure 3 illustrates one such successful example using  $\alpha$ 2D. Tatko and Waters also reported that the halogen substituent in aromatic amino acid side chains can enhance edge-to-face aromatic interactions, resulting in increased strand stability.

The incorporation of aromatic pairs is therefore increasingly attracting attention in protein engineering and the design of peptide-based bio-nanomaterials.



**Fig. 3:** Schematic representation of  $\alpha$ 2D, a de novo designed model system that forms a four helix bundle. This system has been widely exploited to study aryl-perfluoroaryl interaction as an attractive strategy in protein engineering.

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