beam line ($\lambda = 0.978$ Å) of the DND-CAT at the Advanced Photon Source, Argonne, IL, using a MARCCD detector. Data were integrated and merged with DENZO/SCALEPACK.[15] Space group $P2_12_12_1$; cell dimensions $a = 24.52$, $b = 43.02$, $c = 46.68$ Å. The overall $R_{merge}$ for all reflections between 20 and 1 Å was 4.7%.

Structure Determination and Refinement: The structure was solved by molecular replacement using the DNA decamer described in ref. [16] as the initial model and refined with the programs CNS[17] and SHELX-97.[18] After monitoring the $R_{free}$ using 10% of the reflections, all reflections were included in the final rounds of isotropic refinement. Hydrogen atoms were added in SHELX-97 and all atoms including the spermine molecule and solvent water molecules were treated anisotropically. Coordinates and structure factors have been deposited in the Protein Data Bank (accession code 1KGK).

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# De Novo Protein Surface Design: Use of Cation–π Interactions to Enhance Binding between an α-Helical Peptide and a Cationic Molecule in 50% Aqueous Solution**

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We have previously shown that synthetic guanidinium-based receptors (such as **1**; Scheme 1) can recognize aspartate- and glutamate-containing peptides in 10% aqueous methanol.[1–5] The principal driving force for binding was ion pairing and hydrogen bonding between the carboxylate and guanidinium groups that leads, with optimal matching of the sequence, to peptide stabilization in a helical conformation. In order to extend this approach to targets of more biological relevance we sought to identify peptide sequences that could recognize the receptor with increased affinity and in a more aqueous solvent system. One strategy to increase the strength of the interaction would be to provide additional binding contacts between the receptor and the helical peptide. Herein we report the combination of ion pairing and cation–π interactions projected from a helical surface to enhance the affinity of the synthetic receptor for the peptide. This approach represents an example of de novo design of a protein surface where the properties of the surface of a protein (in this case the helical peptide) are modified to optimize binding to a target molecule.

The design of the peptide sequences exploited a "molecular hinge" strategy that we had used earlier in synthetic receptors for nucleotide bases.[6, 7] In this approach acylaminopyridine and naphthalene groups were linked to provide binding through hydrogen-bonding and π-stacking interactions (Scheme 1A). In this current research our target peptides incorporate four aspartate groups, each flanked above or below by aromatic residues on the helix surface. In this way the tetraguanidinium receptor can potentially make both hydrogen-bonding and cation–π contacts[8] with side-chain groups on the peptide (Scheme 1B).[9, 10]

Three peptides were synthesized with four aspartic acid residues in the *i*, *i+3* relationship that has been shown to be

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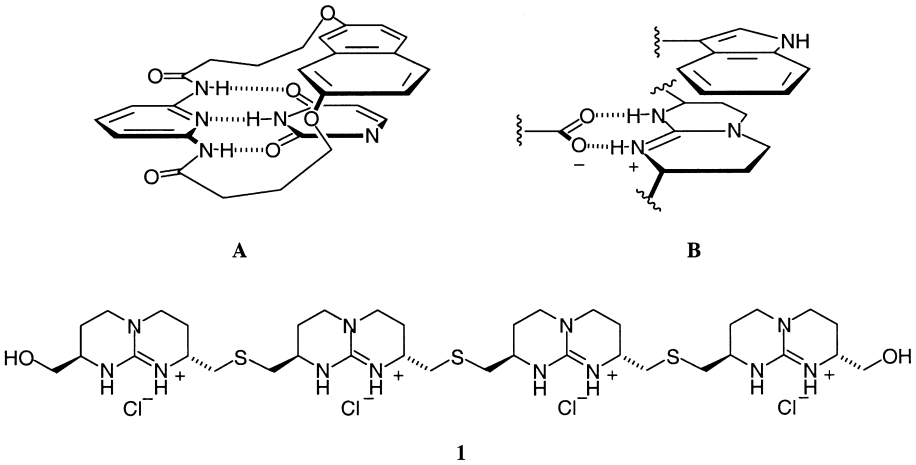


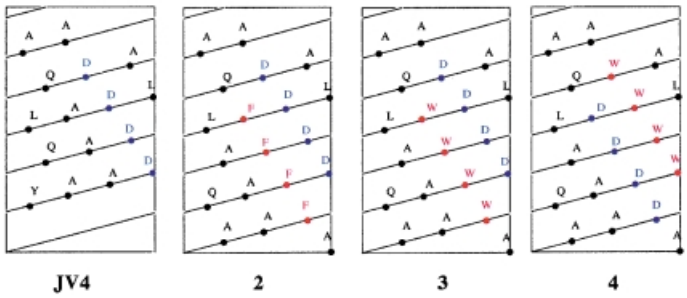
A B

1

Scheme 1. A) Molecular hinge design for the recognition of a nucleotide base.[6] B) Postulated binding geometry of the guanidinium binding site formed from a proximal aspartate and tryptophan group on an $\alpha$-helical surface.

required for optimal binding to receptor **1**.[4, 5] In peptides **2** and **3**, Phe and Trp residues, respectively, were placed on the C-terminus side of each Asp residue in the sequence, while in peptide **4** the placement of the Asp and Trp residues was switched (Figure 1). Binding comparisons were made to a control peptide, **JV4**, which contains four aspartate residues but no flanking aromatic residues.

**JV4** Ac-AAADQLDALDAQDAAY-$CONH_2$
**2** Ac-AAADQLDFLDFADFAQFAAA-$CONH_2$
**3** Ac-AAADQLDWLDWADWAQWAAA-$CONH_2$
**4** Ac-AAAWQLWDLWDAWDAQDAAA-$CONH_2$



JV4 2 3 4

Figure 1. Sequences and helix net diagrams of synthesized peptides.

Binding studies on receptor **1** with peptide **4** in 1:1 trifluoroethanol (TFE):water by CD spectroscopy showed a large increase in the helical character of the peptide (from 13 to 51 %) with one equivalent of **1**. Since the internal aromatic residues[11, 12] contributed to the CD signal, a more accurate analysis of helicity was attempted using NMR spectroscopy (see below).[13] The binding isotherm obtained by CD analysis pointed to an interaction too strong to measure accurately.[14] Titrations monitored by the fluorescence of the Trp residue, however, allowed the use of lower concentrations and an affinity constant of $1.1 \times 10^8\,\text{M}^{-1}$ was measured (Table 1). Dilution experiments and Job analysis confirmed that the peptide does not aggregate at relevant concentrations and the stoichiometry of the complex is 1:1. The relative position of the Asp and Trp residues is clearly important since the binding

Table 1. Binding constants of **1** with various peptides in 1:1 TFE:water at 25 °C.

| Peptide | Binding constant [$\text{M}^{-1}$] |
|---|---|
| **JV4** | $3.2 \times 10^6$ |
| **2** | $3.8 \times 10^6$ |
| **3** | $4.2 \times 10^6$ |
| **4** | $1.1 \times 10^8$ |

of **1** to peptide **3** was 100-fold weaker (association constant of about $10^6\,\text{M}^{-1}$) than to **4**. This $K_a$ value is comparable to that of **1** and **JV4** ($3.2 \times 10^6\,\text{M}^{-1}$) where no aromatic residues are available for cation–$\pi$ interactions and suggests that the Trp residues in **3** do not participate in binding. Similarly, the Phe residues in peptide **2** do not enhance binding ($K_a = 3.8 \times 10^6\,\text{M}^{-1}$) in 1:1 TFE:water.

A comparison of the chemical shifts of the side-chain protons in the free peptide and in the complex helped to determine which side chains are in contact with the receptor (Figure 2). The diastereotopic $\beta$ protons of the Asp and Trp side chains in the free peptide have degenerate chemical shifts as a consequence of rapid rotation around the $\chi^1$ and $\chi^2$ dihedral angles of the side chain. However, these resonances are strongly anisochronous in the complex and are indicative of restricted motion of the side chains through the establishment of noncovalent interactions with **1**.[4]

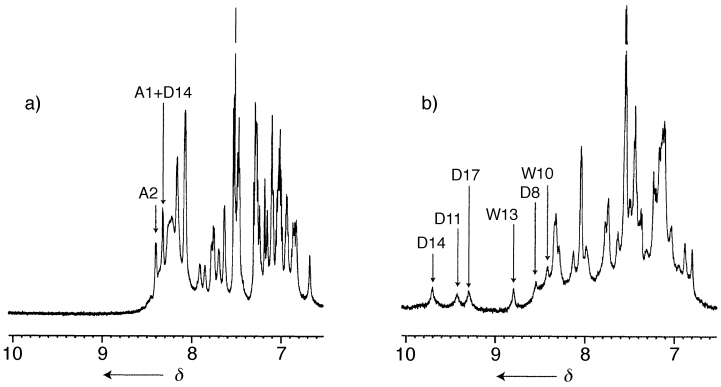



Figure 2. 1D $^1$H NMR spectra (Bruker DMX, 600 MHz, $CD_3OH$:$H_2O$ (9:1), 278 K) of **4** (a) and complex **4**:**1** (b). The labels correspond to those residues with downfield-shifted NH resonances.

Analysis of NOE data and $\alpha$H secondary chemical shifts showed peptide **4** to have nascent helical secondary structure in only the first ten residues. Interaction with the receptor, however, caused a dramatic amplification of helicity in the rest of the sequence, as seen by the increase in the intensity and number of N-N (*i*, *i+1*) and nonsequential N-N (*i*, *i+2*) NOE interactions, characteristic of helical peptides,[15] which in the complex are present all along the sequence of the peptide.

Conclusive evidence for helix stabilization as a consequence of recognition is provided by changes in the secondary shifts of the NH protons (Figure 2). NH secondary shifts

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($\Delta\delta_{NH} = \delta_{NH}$(experimental) $- \delta_{NH}$(random coil[15])) are related to the length of the hydrogen bond between the NH group at position *i* and the CO group of the residue at *i* – *4* in helical peptides. A periodic trend in $\Delta\delta_{NH}$ values which is observed for the complex (Figure 3) formed between **4** and **1** is typical

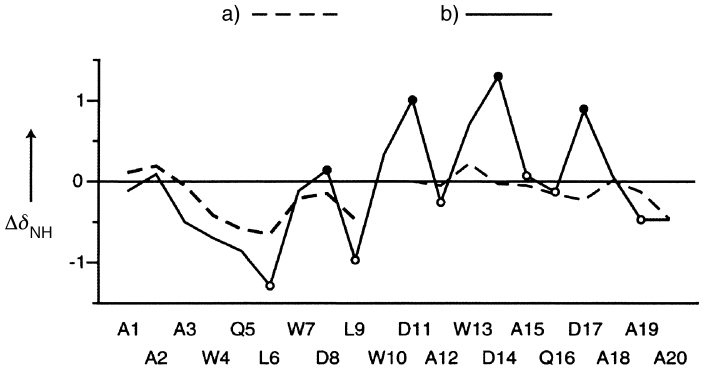


Figure 3. Secondary chemical shifts (NH) as a function of sequence for **4** (a) and complex **4**:**1** (b). Receptor-bound residues on the concave face of the helix are labeled with filled circles and those on the convex face with open circles.

of bent helices,[16] where the hydrogen bonds formed by residues on the convex face of the helix are shorter and cause a downfield shift of the NH protons involved. The residues exhibiting higher values of $\Delta\delta_{NH}$ are those that interact with **1** which suggests that the helix is bent towards the receptor for optimal interaction.

The 30-fold difference in affinity between **3** and **4** for **1** clearly suggests that the participation of the Trp residues in binding is dependent on their position along the helix. Both **3** and **4** have similar initial helicities; therefore, conformational bias cannot be the origin of the differential binding. Changes in the pKa values of the Asp groups[17] as a result of their different positions with respect to the helix dipole are unlikely to be important. The closer proximity of the Asp groups to the C-terminus in **4** would be expected to lead to a decrease in affinity, not the increase in binding strength that is seen. Molecular modeling studies[17] of the relative position of the Asp and Trp side chains along the helix surface provide an explanation. In peptide **3** the indole groups are staggered relative to the aspartate residues and achieve little overlap with the guanidinium binding regions even when the $\chi^2$ bonds of the Trp residues are rotated to a more binding-favorable orientation (Figure 4 a and b).[18] In contrast, the aromatic side chains in peptide **4** can easily adopt a conformation that places the indole groups within stacking distance of the bound cations (Figure 4).

The interaction of **4** and **1** was also investigated in 100% water. An increase in the fluorescence emission of the Trp group in **1** that reached saturation at approximately one equivalent of **4** suggested strong binding. However, at higher concentrations of **4** aggregation effects became more prominent and precluded quantitative analysis of the binding.

In summary, we have designed a short $\alpha$-helix as a model protein surface for the recognition of a small molecule by cation–$\pi$ interactions. The peptide receptor not only binds

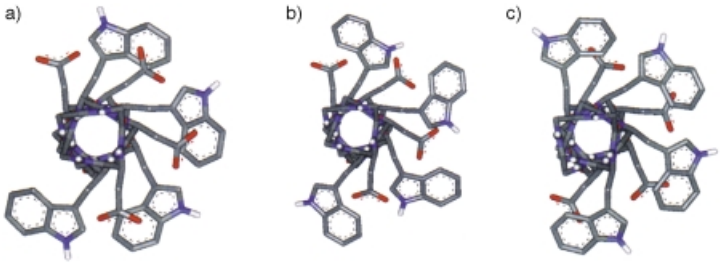


Figure 4. Comparison of the overlap of Trp and Asp side chains in the binding sites of **3** with the preferred side-chain conformations (a), **3** with the bond between the Trp $\beta$ and $\gamma$ carbon atoms rotated 180° (b), and **4** with the preferred side-chain conformation (c). All views are down the helix axis from the N-terminus and nonessential side chains are eliminated for clarity.

with high affinity but also acts to strongly stabilize the helix conformation of the peptide.

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