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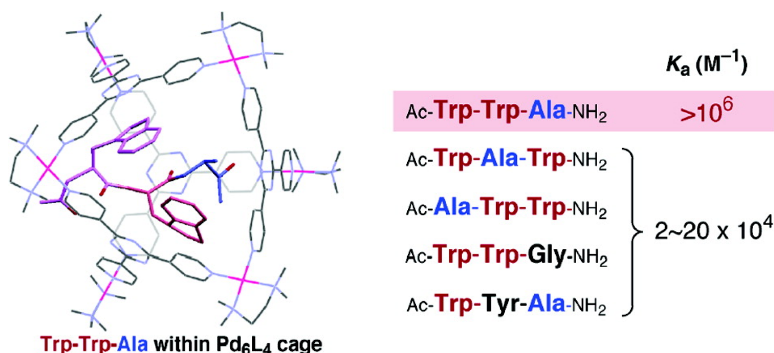

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Sequence-Selective Recognition of Peptides within the Single Binding Pocket of a Self-Assembled Coordination Cage

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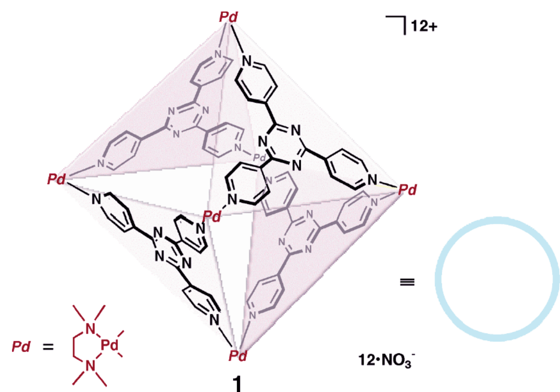
Sequence-selective recognition of peptides is expected to be an essential process for the site-specific recognition of protein surfaces,[1] which leads to the control of protein functions and to the understanding of biological events at protein surfaces such as protein−protein or protein−oligopeptide interactions.[2] Although a few groups have reported artificial receptors for sequence-selective peptide recognition, the highly designed receptors are in their early stages. They are constructed by simply linking small recognition sites such as cyclodextrins,[3] crown ethers,[4] or amide groups,[5] which all bind only a single amino acid residue. Here, we report that the single binding pocket of self-assembled coordination cage **1**[6] can accommodate oligopeptides in a highly sequence-selective fashion. Having a large hydrophobic cavity, cage **1** binds as many as three amino acid residues. NMR and X-ray analyses reveal that the sequence-selective recognition is ascribed to cooperative multiple interactions between the residues and the cavity.



In a first series, the sequence-selective recognition of tripeptides, Ac-X[1]-X[2]-X[3]-NH$_2$ (X^{1-3} = amino acid residues), was examined.[7] We found that cage **1** bound Ac-Trp-Trp-Ala-NH$_2$ (**2**) very strongly (K_a >10^6 M^{-1}).[8] The **1·2** complex was easily prepared by suspending powdered **2** (1 mol equiv) in a D_2O solution of **1** (10 mM) at room temperature for 0.5 h. Strong binding was specific to the Trp-Trp-Ala sequence because the binding of tripeptides possessing those same residues in different sequences, such as Ac-Trp-Ala-Trp-NH$_2$ (**3**) and Ac-Ala-Trp-Trp-NH$_2$ (**4**), was much less effective (K_a = 2.5 × 10^5 and 2.1 × 10^4 M^{-1}, respectively). Even singly mutated tripeptides, such as Ac-Trp-Trp-Gly-NH$_2$ (**5**) and Ac-Trp-Tyr-Ala-NH$_2$ (**6**), showed poorer affinity (K_a = 7.4 × 10^4 and 5.3 × 10^4 M^{-1}, respectively) although they have very similar aromatic−aromatic−aliphatic sequences (Table 1). These results

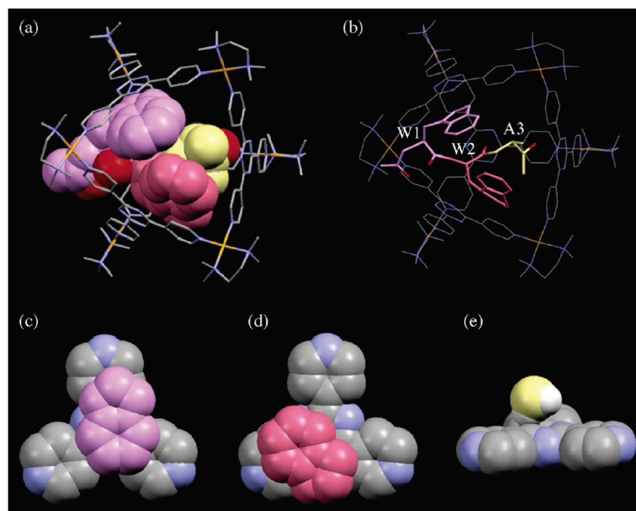


Figure 1. Crystal structure of **1·2**. Peptide **2** in the cavity is represented by (a) space-filling and (b) cylindrical model. The π−π interactions of **1** with indole rings of (c) W1 and (d) W2. (e) The CH−π interaction between **1** and methyl group of A3.

Table 1. Association Constants of **1** with Peptides in Water

Peptides	K_a (M^{-1})[a]
Ac-Trp-Trp-Ala-NH$_2$ (**2**)	>10^6
Ac-Trp-Ala-Trp-NH$_2$ (**3**)	2.5 x 10^5
Ac-Ala-Trp-Trp-NH$_2$ (**4**)	2.1 x 10^4
Ac-Trp-Trp-Gly-NH$_2$ (**5**)	7.4 x 10^4
Ac-Trp-Tyr-Ala-NH$_2$ (**6**)	5.3 x 10^4
Ac-Gly-Gly-Ala-NH$_2$ (**7**)	no binding
Ac-Trp-His-Ala-NH$_2$ (**8**)	no binding
Ac-Tyr-Tyr-Ala-NH$_2$ (**9**)	4.7 x 10^3
Ac-Ala-Trp-Trp-Ala-NH$_2$ (**10**)	>10^6
Ac-Ser-Gly-Ala-Trp-Trp-Ala-NH$_2$ (**11**)	>10^6

[a] Measured by UV−vis titration at 20 °C.

suggest that the two indole rings and the Ala methyl group in **2** should cooperatively interact with the cage in the **1·2** complex.

In fact, the multiple interactions of the methyl and indole groups with the cage were revealed by X-ray crystallographic analysis.[9] Single crystals were obtained after an aqueous solution of **1·2** complex stood at room temperature for 4 d. The diffraction data were collected by synchrotron X-ray irradiation. The crystallographic analysis showed that tripeptide **2** is fully encapsulated in the cavity of **1** (Figure 1a,b). As predicted, all residues interact very efficiently with cage **1**. Namely, two indole rings are stacked on the triazine ligand by π−π interaction (3.4−3.5 Å), while the Ala methyl group interacts with another ligand by CH−π contact

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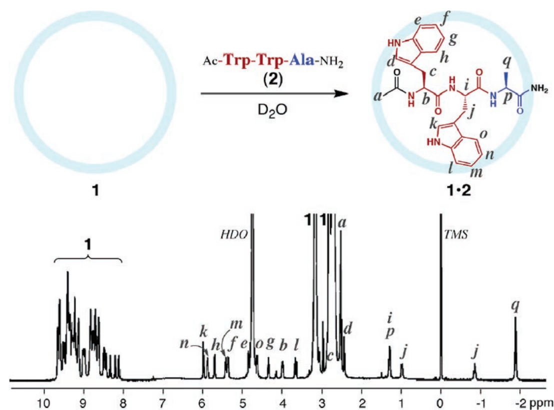

Figure 2. ^{1}H NMR of **1·2** in D_2O (500 MHz, 10 mM, 27 °C, TMS as external standard).

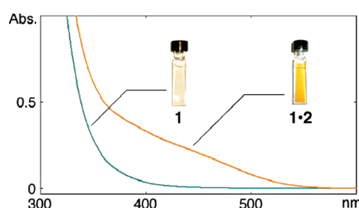

Figure 3. UV−vis spectra of free cage **1** and complex **1·2** (H_2O, 0.2 mM, rt).

(2.5 Å) (Figure 1c−e). Despite the enclathration within the restricted cavity, the peptide backbone is fixed in an extended conformation.

The inclusion geometry shown by the X-ray analysis is in good agreement with the NMR observations. In the ^{1}H NMR, the Ala methyl signal at δ −2.0 and the indole aromatic protons around δ 6.0−2.5 are considerably upfield shifted due to the shielding effect of the cage (Figure 2). A clear NOE correlation between the Ala methyl group (signal *q* in Figure 2) and one indole ring (signal *e*) was observed, which was explained by the tight contact of these proton pairs (2.6 Å) as revealed by X-ray analysis. Similarly, two indole rings (signals *g* and *n*) are correlated by NOE and shown to be in close contact (2.8 Å). In the δ 10.0−8.0 region, the pyridyl protons of the cage were observed in a very complex pattern indicating the desymmetrization of the cage. The motion of **2** is restricted by enclathration, and therefore all pyridine protons of the cage become inequivalent. We note that the clear desymmetrization of the cage in NMR is only observed for **2**. It seems that the motions of other tripeptides in the cavity are not strictly restricted, and therefore, the ^{1}H NMR cage signals are simply broadened.

The efficient $\pi-\pi$ stacking observed by X-ray and NMR is ascribed to charge transfer from the indole rings to the electron-deficient triazine ligand.[10] The color of the solution turned yellow upon formation of the **1·2** complex. In the UV−vis spectrum, broad absorption around 350−550 nm was observed (Figure 3).

The sequence selectivity changes significantly when electronically and sterically different tripeptides are used. For instance, Ac-Gly-Gly-Ala-NH_2 (**7**) that possesses no aromatic residue was not bound at all. Ac-Trp-His-Ala-NH_2 (**8**) that involves a cationic residue (His) did not show any interactions with the cage due probably to cationic repulsion with the cage. Ac-Tyr-Tyr-Ala-NH_2 (**9**) was bound $>10^2$ times more weakly than **2**, indicating the importance of electron donation from electron-rich indole rings to the electron-deficient ligand[10] (Table 1).

Accordingly, cage **1** recognizes specifically the Trp-Trp-Ala sequence in oligopeptides consisting of more than three amino acid residues. For example, tetrapeptide **10** and hexapepetide **11**, both possessing the Trp-Trp-Ala sequence, were strongly bound to **1** ($K_a > 10^6$ M^{-1}, respectively).[8] This specificity is confirmed by NMR where only the Trp-Trp-Ala part of these oligopeptides was dramatically upfield shifted.[11]

In summary, we have shown the sequence-selective recognition of peptides by the single binding pocket of cage **1**. As organic modification of the cage is easy and related large, hollow structures with different shapes and sizes have been previously prepared,[12] the design of single-pocket receptors for specific sequences of oligopeptides is our next challenge. Ultimately, the pinpoint recognition of protein surfaces by these self-assembled hollow receptors is the main goal of the present study.

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Supporting Information Available: Experimental procedures, physical properties of **1·2**, NMR spectra, UV−visible titrations (PDF) and crystallographic data of **1·2** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) Association constants were measured by the UV titration at a charge-transfer band in water. Spectra were analyzed by a nonlinear curve-fitting procedure. See the Supporting Information.
(8) Since UV titrations of **1·2**, **1·10**, **1·11** showed the saturation at nearly 1 equiv of **1**, exact values of K_a could not be calculated by this measurement. Thus, their K_a's were estimated to be 10^6 M^{-1}.
(9) Crystal data for **1·2**: $C_{135}H_{202}N_{54}O_{54}Pd_6$, $M = 4083.90$, Monoclinic, space group $C2/c$, cell parameters $a = 70.38(1)$ Å, $b = 17.181(3)$ Å, $c = 42.159(8)$ Å, $\beta = 99.90(3)°$, $V = 50217(2)$ Å^3, $T = 15(2)$ K, $Z = 8$, $D_c = 1.068$ g cm^{-3}, λ (synchrotron) = 0.6890 Å, 142086 reflections measured, 47250 unique ($R_{int} = 0.0714$) which were used in all calculations. The structure was solved by direct method (SHELXL-97) and refined by full-matrix least-squares methods on F^2 with 2114 parameters. R1 = 0.1518 ($I > 2\sigma(I)$) and wR2 = 0.4521, GOF = 1.378; max/min residual density 1.532/−1.824 eÅ^{-3}. CCDC reference number 247331. It is noteworthy that in the crystal of **1·2**, the 12 nitrate anions as well as the water molecules were highly disordered, giving rise to a large R1 value. However, the guest molecule was found from Fourier difference maps, and only minor disorder over the peptide backbone chain was found.
(10) Four triazine ligands composing cage **1** are highly electron deficient due to multiple electron withdrawing from three palladium cations.
(11) For the NMR assignments of a site-specific recognition of **1·10** and **1·11**, see the Supporting Information.
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