Supporting Information

for

Sequence Recognition and Self-Sorting of Dipeptide by Cucurbit[6]uril and Cucurbit[7]uril

Mikhail V. Rekharsky,[a] Hatsuo Yamamura,[b] Young Ho Ko,[c] N. Selvapalam,[c] Kimoon Kim,*[c] and Yoshihisa Inoue*[a,d]

[a] ICORP Entropy Control Project (JST), Department of Applied Chemistry, Osaka University, 2-1 Yamada-oka, Suita 565-0871, Japan, [b] Graduate School of Technology, Nagoya Institute of Technology, Gokiso-cho, Showa-ku Nagoya 466-8555, Japan, [c] National Creative Research Initiative Center for Smart Supramolecules and Department of Chemistry, Division of Molecular and Life Sciences, Pohang University of Science and Technology, San 31 Hyojadong, Pohang 790-784, Korea Department of Applied Chemistry and [d] Osaka University, 2-1 Yamada-oka, Suita 565-0871, Japan

e-mail: inoue@chem.eng.osaka-u.ac.jp

Table S1. Stability constant (K), standard enthalpy ($\Delta H°$) and entropy changes ($T\Delta S°$) for complexation reactions of cucurbit[7]uril (CB[7]) with zwitterionic Ala-Phe and Phe-Ala in pure water and selected dipeptide amides in 0.1 M NaCl[a)] at T = 298.15 K

Complexation reaction	Solvent	K/M^{-1}	$K_{\text{Phe-X}}$ / $K_{\text{X-Phe}}$	$\Delta H°$ /kJ mol^{-1}	$T\Delta S°$ /kJ mol^{-1}
CB[7]^0 + Ala-Phe^{+-} = [CB[7]•Ala-Phe]$^{(+-)}$	H$_2$O	$(1.3 \pm 0.1) \times 10^3$		-34.6 ± 0.3	-16.8 ± 0.4
CB[7]^0 + Phe-Ala^{+-} = [CB[7]•Phe-Ala]$^{(+-)}$	H$_2$O	$(1.9 \pm 0.3) \times 10^7$	1.5×10^4	-49.4 ± 0.5	-7.9 ± 0.5
[CB[7]•2Na]$^{2+}$ + $^+$Gly-Phe-NH$_2$ = [CB[7]•Gly-Phe-NH$_2$•Na]$^{2+}$ + Na$^+$	0.1M NaCl	$(4.4 \pm 0.3) \times 10^4$		-30.7 ± 0.3	-4.2 ± 0.3
[CB[7]•2Na]$^{2+}$ + $^+$Phe-Gly-NH$_2$ = [CB[7]•Phe-Gly-NH$_2$•Na]$^{2+}$ + Na$^+$	0.1M NaCl	$(3.7 \pm 0.3) \times 10^6$	84	-30.9 ± 0.3	6.5 ± 0.3
[CB[7]•2Na]$^{2+}$ + $^+$Ala-Phe-NH$_2$ = [CB[7]•Ala-Phe-NH$_2$•Na]$^{2+}$ + Na$^+$	0.1M NaCl	$(4.5 \pm 0.3) \times 10^4$		-32.3 ± 0.3	-5.7 ± 0.4
[CB[7]•2Na]$^{2+}$ + $^+$Phe-Ala-NH$_2$ = [CB[7]•Phe-Ala-NH$_2$•Na]$^{2+}$ + Na$^+$	0.1M NaCl	$(8.1 \pm 0.4) \times 10^6$	180	-41.4 ± 0.4	-2.0 ± 0.4
[CB[7]•2Na]$^{2+}$ + $^+$Gly-Tyr-NH$_2$ = [CB[7]•Gly-Tyr-NH2•Na]$^{2+}$ + Na$^+$	0.1M NaCl	$(7.5 \pm 0.4) \times 10^4$		-39.2 ± 0.4	-11.4 ± 0.4
[CB[7]•2Na]$^{2+}$ + $^+$Tyr-Gly-NH$_2$ = [CB[7]•Tyr-Gly-NH$_2$•Na]$^{2+}$ + Na$^+$	0.1M NaCl	$(1.1 \pm 0.2) \times 10^7$	147	-42.7 ± 0.4	-2.6 ± 0.4

a) It is widely recognized that addition of hydrophobic residues and/or positive charges to a guest leads in general to a large enhancement of the affinity towards CB[7] and other CB homologues (see references below). Thus, we may expect much higher affinities for the selected dipeptide amides compared to zwitterionic analogs. Taking into account the instrumental upper limit of ITC in affinity determination of about 10^7–10^8 M^{-1}, we used aqueous 0.1 M NaCl solution rather than pure water. Sodium cations serve as competitive guest for CB[7] portal and thus reduce complex stability in about 20 times as it is demonstrated by the data presented Table S2.

References
1. Mock, W. L. In *Comprehensive Supramolecular Chemistry*; Vögtle, Ed.; Pergamon: Oxford, 1996; Vol. 2, p 447.
2. Lee, J.W.; Samal, S.; Selvapalam, N.; Kim, H.-J.; Kim, K. *Acc. Chem. Res.* **2003**, *36*, 621.
3. Mock, W.L.; Shih, N.-Y. *J. Org. Chem.* **1986**, *51*, 4440.

Table S2. Comparison of stability constant (K), standard enthalpy ($\Delta H°$) and entropy changes ($T\Delta S°$) for complexation reactions of cucurbit[7]uril (CB[7]) with zwitterionic Phe-Gly and Phe-Ala in pure water and in 0.1 M NaCl at T = 298.15 K

complexation reaction	solvent	K/M^{-1}	$\Delta H°$ /kJ mol^{-1}	$T\Delta S°$ /kJ mol^{-1}
CB[7]^0 + Phe-Gly^{+-} = [CB[7]•Phe-Gly]$^{(+-)}$	H$_2$O	$(3.0 \pm 0.4) \times 10^7$	-47.4 ± 0.5	-4.7 ± 0.5
[CB[7]•2Na]$^{2+}$ + Phe-Gly^{+-} = [CB[7]•Phe-Gly•Na]$^{+(+-)}$ + Na$^+$	0.1 M NaCl	$(1.7 \pm 0.2) \times 10^6$	-32.0 ± 0.3	3.7 ± 0.4
CB[7]^0 + Phe-Ala^{+-} = [CB[7]•Phe-Ala]$^{(+-)}$	H$_2$O	$(1.9 \pm 0.3) \times 10^7$	-49.4 ± 0.5	-7.9 ± 0.5
[CB[7]•2Na]$^{2+}$ + Phe-Ala^{+-} = [CB[7]•Phe-Ala•Na]$^{+(+-)}$ + Na$^+$	0.1 M NaCl	$(7.9 \pm 0.3) \times 10^5$	-30.6 ± 0.3	3.1 ± 0.4

Table S3. Stability constant (K), standard enthalpy ($\Delta H°$) and entropy changes ($T\Delta S°$) for complexation reactions of cucurbit[6]uril (CB[6]) with selected zwitterionic dipeptides and dipeptide amides in 0.1 M NaCl at T = 298.15 K

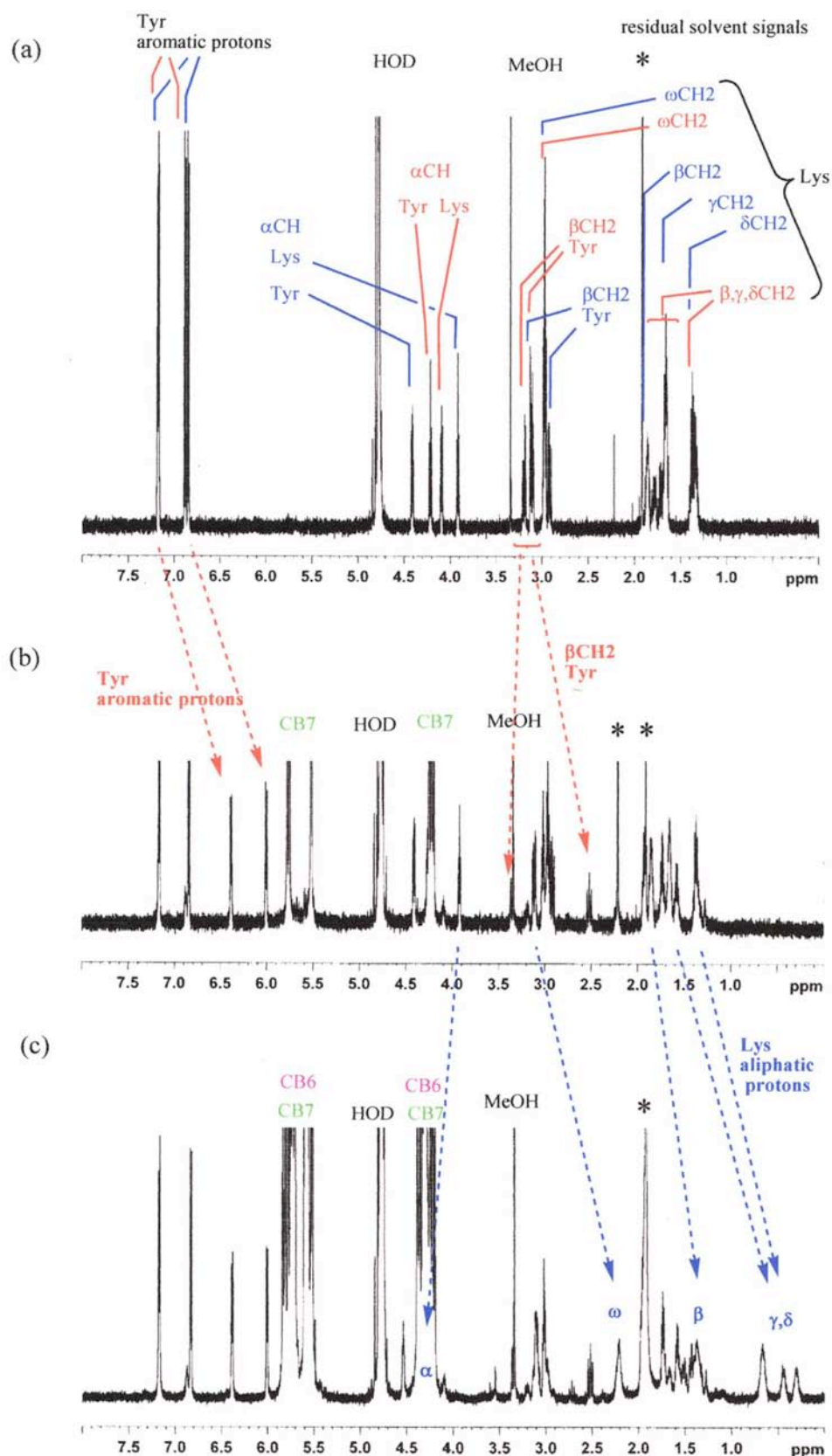| complexation reaction	solvent	K/M^{-1}	$\Delta H°$ /kJ mol^{-1}	$T\Delta S°$ /kJ mol^{-1}
[CB[6]•2Na]$^{2+}$ + $^+$Leu-Ala-NH$_2$ = [CB[6]•Leu-Ala-NH$_2$•Na]$^{2+}$ + Na$^+$	0.1M NaCl	650 ± 10	-12.0 ± 0.2	4.1 ± 0.3
[CB[6]•2Na]$^{2+}$ + $^+$Leu-Phe-NH$_2$ = [CB[6]•Leu-Phe-NH$_2$•Na]$^{2+}$ + Na$^+$	0.1M NaCl	400 ± 10	-11.3 ± 0.2	3.6 ± 0.3
[CB[6]•2Na]$^{2+}$ + $^+$Phe-Leu- NH$_2$ = [CB[6]•Phe-Leu-NH$_2$•Na]$^{2+}$ + Na$^+$	0.1M NaCl	50 ± 20	-6 ± 1	3 ± 1
[CB[6]•2Na]$^{2+}$ + Lys-Ala-NH$_2$$^{2+}$ = [CB[6]•Lys-Ala-NH$_2$•Na]$^{3+}$ + Na$^+$	0.1M NaCl	16000 ± 1000	-11.9 ± 0.2	12.1 ± 0.3

Fig. S1. NMR spectra of (a) an aqueous solution of Tyr-Lys (1 mM) and Lys-Tyr (1 mM), (b) solution (a) + CB[7] (1 mM), (c) solution (b) + CB[6] (3 mM). Protons of Tyr-Lys and Lys-Tyr were marked in red and blue, respectively.

Detailed description and explanation of NMR spectra presented in Fig. S1

We first prepared a solution containing the same concentration (1 mM) of Tyr-Lys and Lys-Tyr (Fig. S1a). Addition of an equimolar amount of CB[7] results in large differences in NMR spectrum indicating specific inclusion of Tyr-Lys into CB[7] (Fig. S1b). Indeed, the aromatic protons of Tyr-Lys were upfield shifted (up to 1 ppm) and β-CH$_2$ (next to the phenol moiety) of Tyr also revealed a significant shift, while small shifts of aliphatic protons of the Lys residue were also observed. At the same time, the positions of all Lys-Tyr protons are intact, indicating that Lys-Tyr remains in the bulk solution. Subsequent addition of an excess amount of CB[6] (1 mM Tyr-Lys + 1 mM Lys-Tyr + 1 mM CB[7] + 3 mM CB[6]) caused significant chemical shifts of protons of Lys-Tyr. In contrast, its aromatic protons remain mostly intact since it is expected that the large phenol ring remains in the bulk water after complex formation, all aliphatic protons of Lys moiety are shifted (Fig. S1c), in particular at the γ-, δ- and ω-protons. This clearly demonstrates that CB[6] interacts with Lys-Tyr and an aminobutyl moiety of the Lys was specifically included by CB[6] cavity.