Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supplementary Information

A theoretical study on Sequence-dependent nanomolar binding of tripeptides containing N-terminal Leucine by Cucurbit[7]uril

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I. Supplementary method

Details of Independent Gradient Method (IGM) analysis

The Independent Gradient Method (IGM)[1] method performed by Multiwfn 3.8 program[2] can estimate the noncovalent interactions between host CB[7] and guest molecules. $\delta g^{inter} = |\nabla\rho^{IGM,\ inter}| - |\nabla\rho|$ is the key descriptor in IGM, which depends on the magnitude of the norm of the electron density gradient calculated by IGM model $\nabla\rho^{IGM,\ inter}$ and the electron density gradient $\nabla\rho$. Besides, δg^{inter} represents the δg function in intermolecular regions which can depict the intermolecular interactions. The strong attractive/repulsive interaction and *v*dW interaction are shown in blue/red and green, severally. The volume cutoff is set as follows: δg^{inter}= 0.01, and other key settings are the program's default settings in all systems.

Reference

1. Lefebvre, C.; Rubez, G.; Khartabil, H.; Boisson, J.-C.; Contreras-García, J.; Hénon, E., Accurately extracting the signature of intermolecular interactions present in the NCI plot of the reduced density gradient versus electron density. *Physical Chemistry Chemical Physics* **2017,** *19* (27), 17928-17936.
2. Lu, T.; Chen, F., Multiwfn: A multifunctional wavefunction analyzer. *Journal of Computational Chemistry* **2012,** *33* (5), 580-592.

Isothermal Titration Calorimetry (ITC)

ITC experiments were carried out with a MicroCal iTC200 at 298.15 K to study the binding force between CB[7] and three different tripeptides (LGG, LGH, and LGK) in aqueous solution. Concentration of CB[7] was calibrated by a titration with 1-adamantanamine of known concentration. Twenty-six injections were performed with 90-second spacing time. The titration traces were integrated by NITPIC, which produced thermograms for further fitting in SEDFHAT. Two individual titrations were carried out for each peptide and a global fitting over the two repeated experiments was performed using the single-site model. The final figures were generated using GUSSI.

II. Supplementary Figures S1-S5

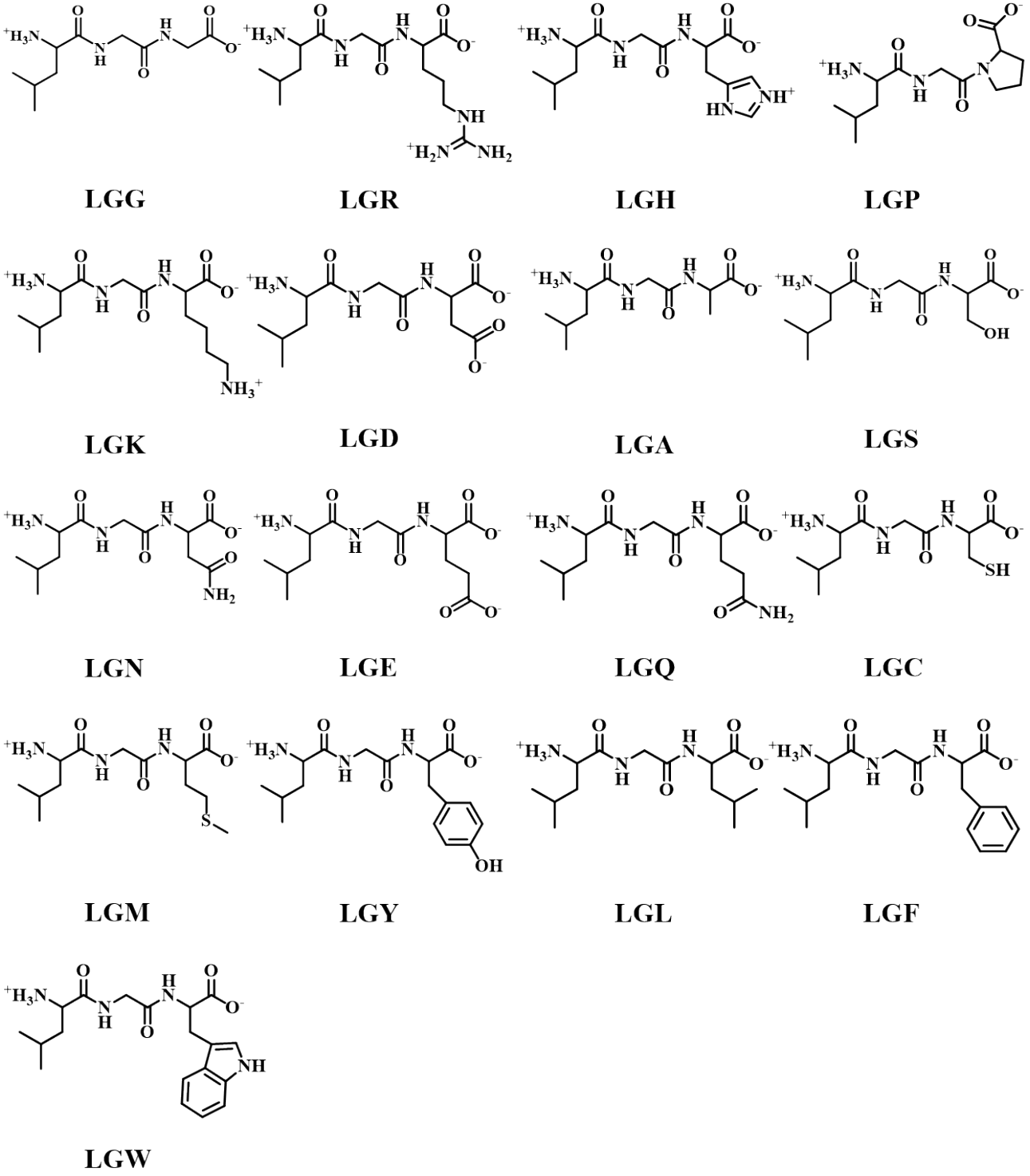
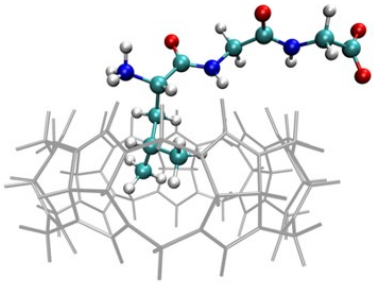
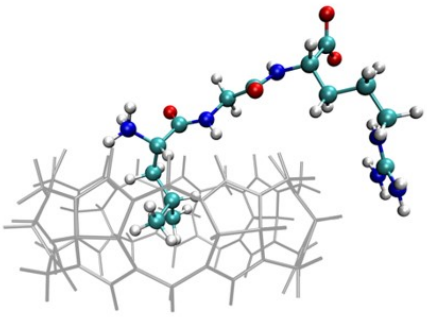


Figure S1 The chemical structural formulas of 17 tripeptides.



CB[7]/LGG



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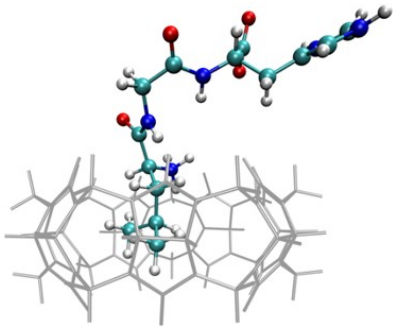
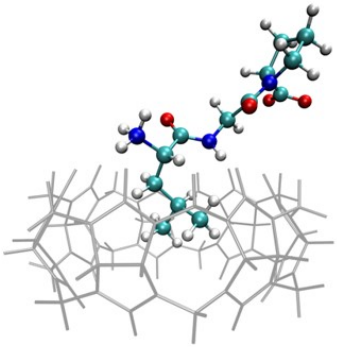
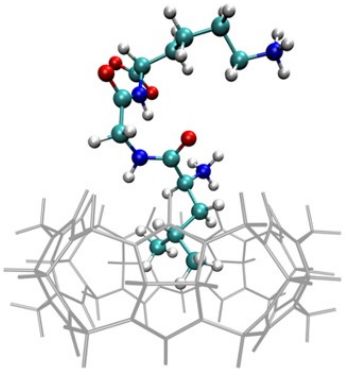


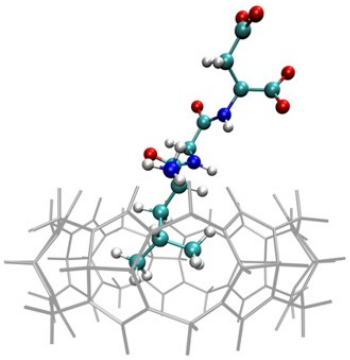
CB[7]/LGH



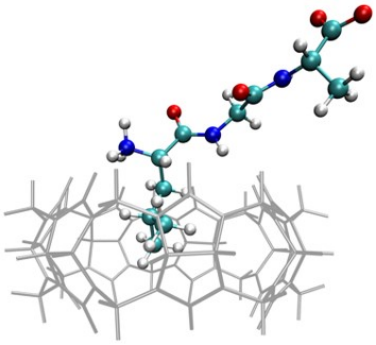
CB[7]/LGP



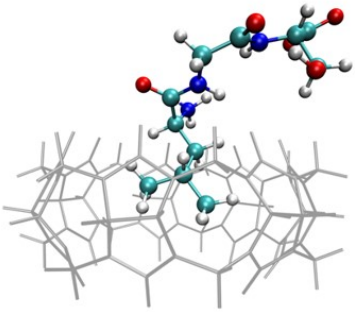
CB[7]/LGK



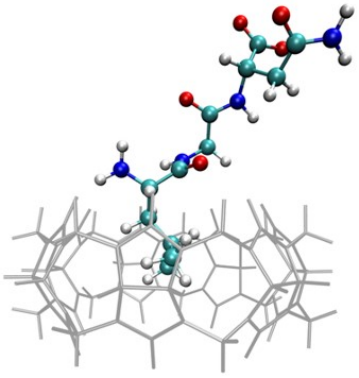
CB[7]/LGD



CB[7]/LGA



CB[7]/LGS



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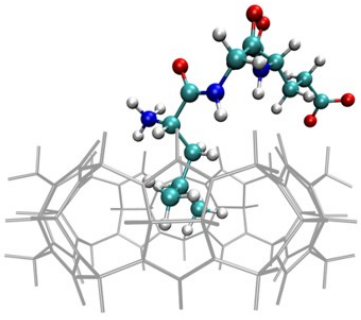
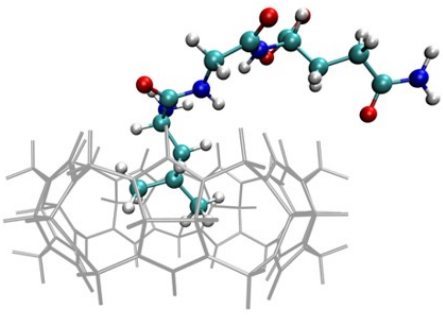
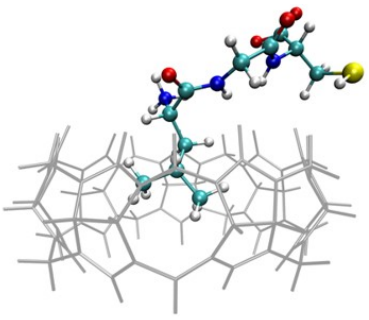


CB[7]/LGE



CB[7]/LGQ



CB[7]/LGC

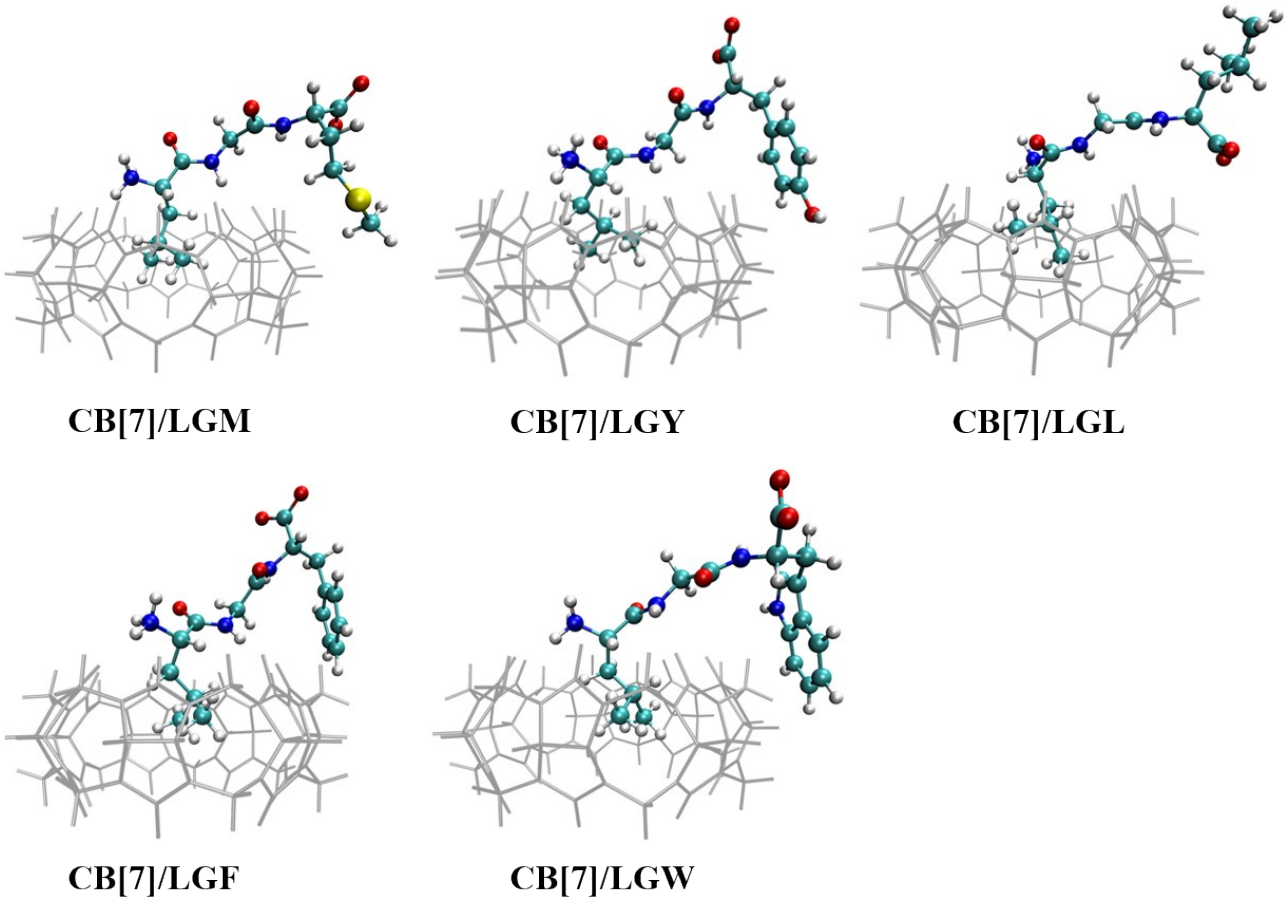


Figure S2 The equilibrated structures of CB[7]/tripeptide complexes after MD simulation. Color codes: cyan refers to C; white refers to H; red refers to O; blue refers to N.

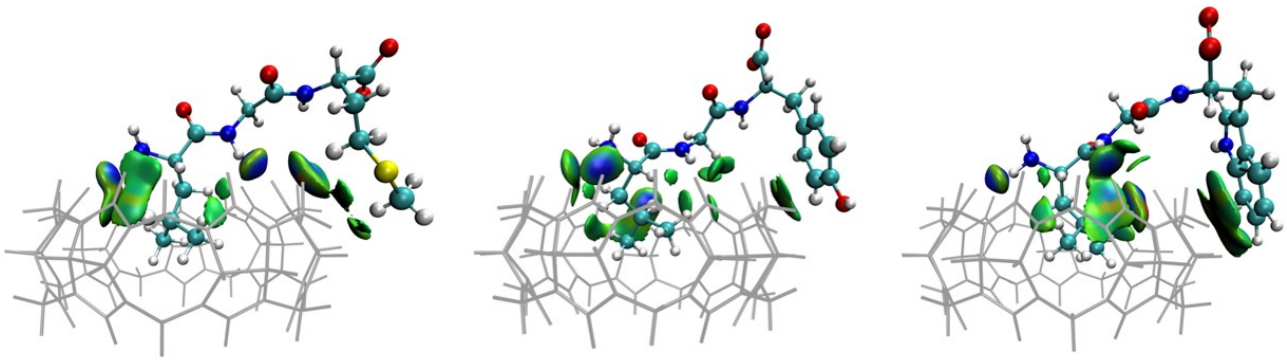


Figure S3 IGM isosurface analysis of LGM, LGY and CB[7]/LGW.

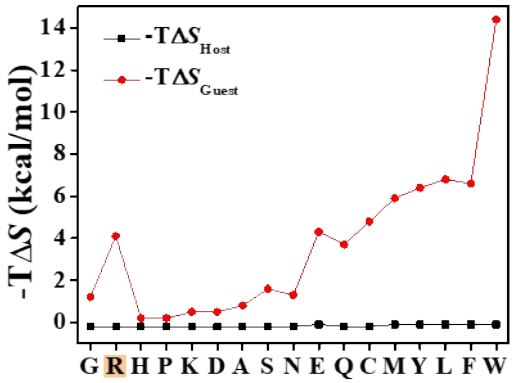


Figure S4 The entropy variations of the host ($-T\Delta S_{Host}$) and guest ($-T\Delta S_{Guest}$) molecules.

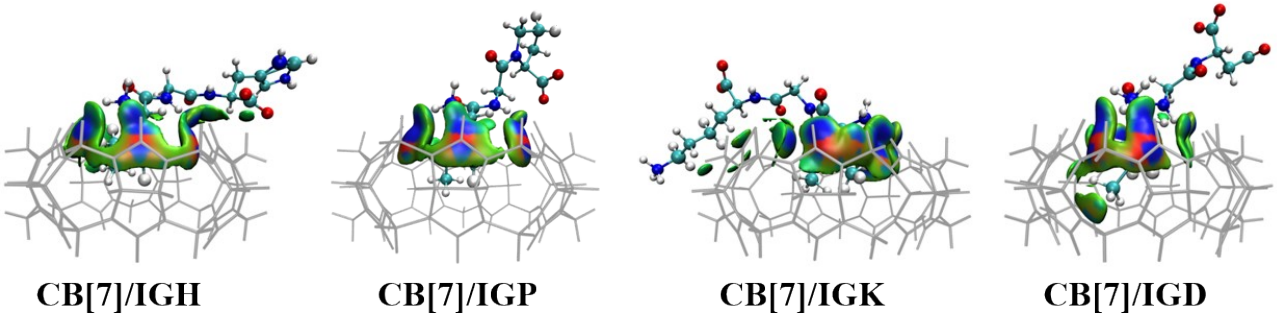


Figure S5 IGM isosurface analysis of CB[7]/IGH, CB[7]/IGP, CB[7]/IGK and CB[7]/IGD.

Thermodynamic parameters for binding between tripeptides and CB[7]

From ITC experiments, a series of thermodynamic parameters, including Gibbs free energy changes (ΔG), binding constant (K_a), enthalpy changes (ΔH) and entropy changes ($T\Delta S$), are obtained for the binding of each tripeptide with CB[7], as shown below in Figure S6-S8.

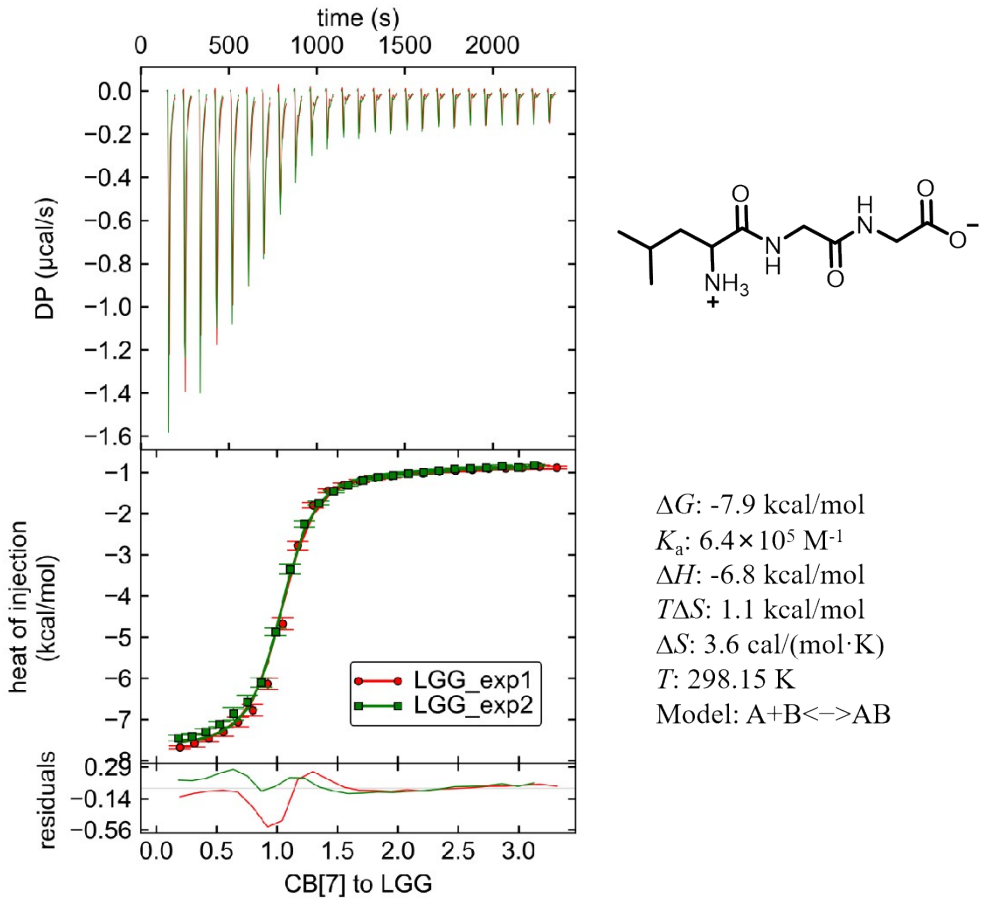
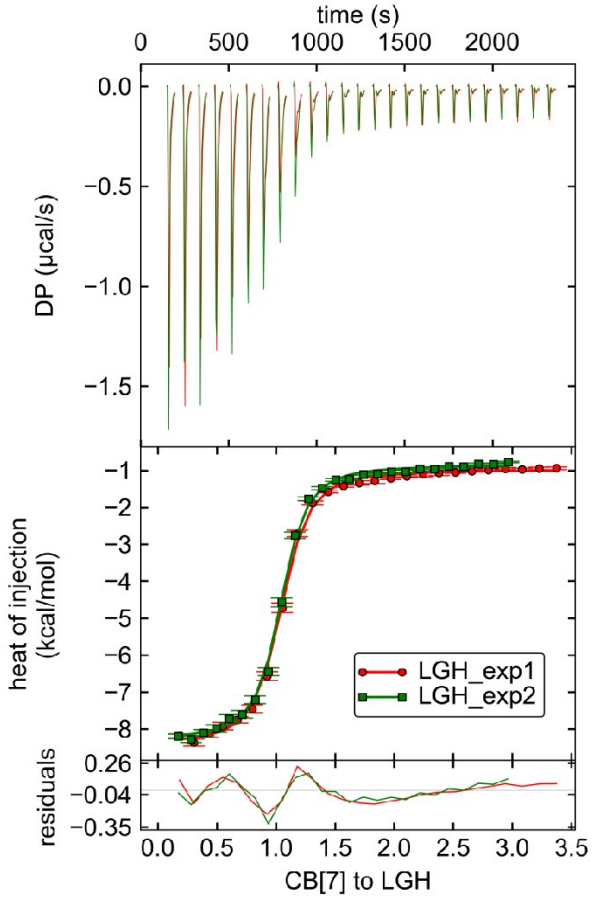
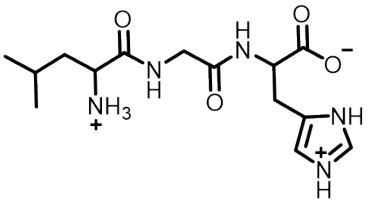


Figure S6 Thermogram at 298.15 K for the titration of CB[7] (1.3 mM) into LGG (0.08 mM or 0.09 mM) aqueous solutions.





ΔG: -8.1 kcal/mol
K_a: 9.1×10^5 M^{-1}
ΔH: -7.4 kcal/mol
$T\Delta S$: 0.7 kcal/mol
ΔS: 2.4 cal/(mol·K)
T: 298.15 K
Model: A+B<−>AB

Figure S7. Thermogram at 298.15 K for the titration of CB[7] (1.3 mM) into LGH (0.08 mM or 0.09 mM) aqueous solutions.

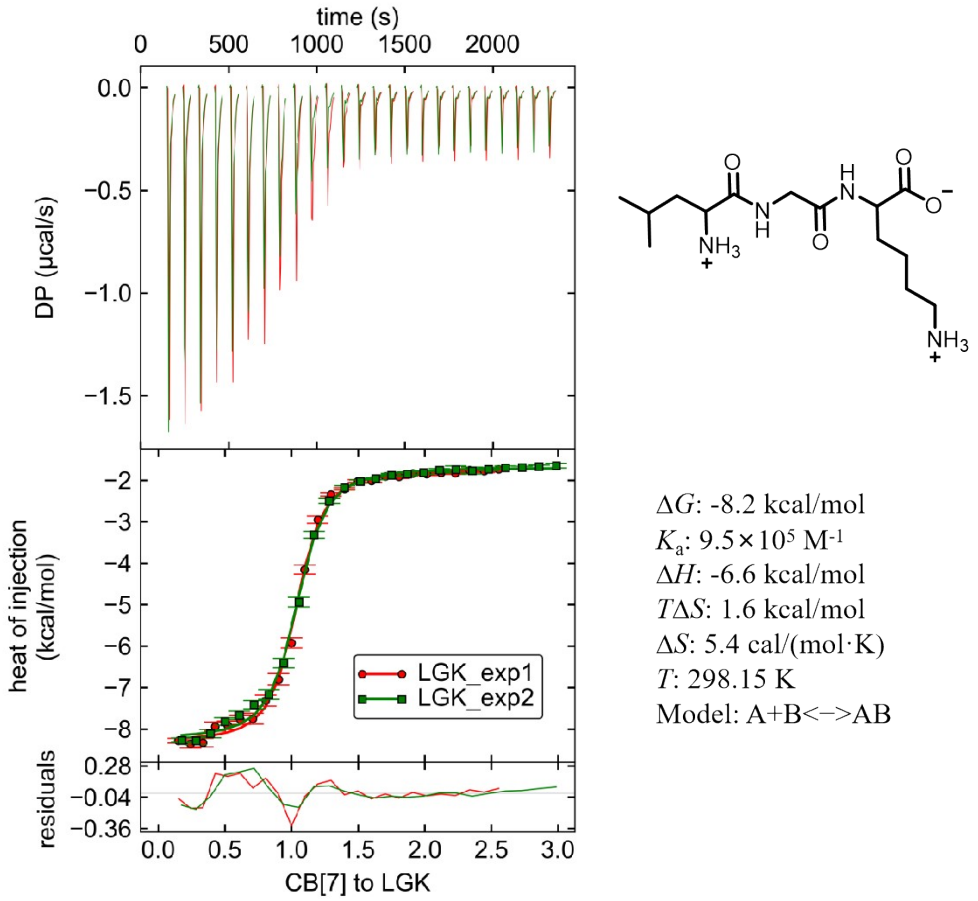


Figure S8. Thermogram at 298.15 K for the titration of CB[7] (1.3 mM) into LGK (0.11 mM or 0.09 mM) aqueous solutions.

III. Supplementary Tables S1-S4

Table S1 Thermodynamic parameters of binding free energy (ΔG), enthalpy (ΔH), and entropy ($-T\Delta S$) in kcal/mol as well as the binding constant K_a in M^{-1} for comparing the binding of CB[7] to LGX with that to LXG (X = R, H, P, K and D).

	ΔG	K_a	ΔH	$-T\Delta S$
CB[7]/LGR	-12.4	**1.1×10^9**	**-16.3**	3.9
CB[7]/LRG	-9.5	**8.3×10^6**	**-9.8**	0.3
CB[7]/LGH	-9.3	6.0×10^6	-9.3	0
CB[7]/LHG	-5.8	1.7×10^4	-9.8	4.0
CB[7]/LGP	-8.8	**2.6×10^6**	-8.8	**0**
CB[7]/LPG	-2.6	**78.4**	-7.9	**5.3**
CB[7]/LGK	-8.5	1.6×10^6	-8.8	0.3
CB[7]/LKG	-7.3	2.1×10^5	-9.8	2.5
CB[7]/LGD	-8.5	**1.6×10^6**	-8.8	**0.3**
CB[7]/LDG	-4.4	**1.6×10^3**	-10.0	**5.6**

Table S2 Thermodynamic parameters of binding free energy (ΔG), enthalpy (ΔH), and entropy ($-T\Delta S$) in kcal/mol as well as the binding constant K_a in M^{-1} for comparing the binding of CB[7] to LGX with that to LGGX (X = R, H, P, K and D).

	ΔG	K_a	ΔH	$-T\Delta S$
CB[7]/LGR	-12.4	**1.1×10^9**	**-16.3**	3.9
CB[7]/LGGR	-7.6	**3.4×10^5**	**-9.3**	1.7
CB[7]/LGH	-9.3	6.0×10^6	-9.3	0
CB[7]/LGGH	-6.6	6.4×10^4	-9.6	3.0
CB[7]/LGP	-8.8	**2.6×10^6**	-8.8	**0**
CB[7]/LGGP	-5.2	**6.1×10^3**	-9.6	**4.4**
CB[7]/LGK	-8.5	1.6×10^6	-8.8	0.3
CB[7]/LGGK	-8.1	8.0×10^5	-9.3	1.2
CB[7]/LGD	-8.5	1.6×10^6	-8.8	0.3
CB[7]/LGGD	-6.9	1.1×10^5	-10.0	3.1

Table S3 Thermodynamic parameters of binding free energy (ΔG), enthalpy (ΔH), and entropy ($-T\Delta S$) in kcal/mol as well as the binding constant K_a in M^{-1} for comparing the binding of CB[7] to LGX with that to IGX (X = R, H, P, K and D).

	ΔG	K_a	ΔH	$-T\Delta S$
CB[7]/LGR	-12.4	1.1×10^{9}	-16.3	3.9
CB[7]/IGR	-2.4	**56.0**	-8.4	6.0
CB[7]/LGH	-9.3	6.0×10^{6}	-9.3	0
CB[7]/IGH	-4.4	$\mathbf{1.6\times10^{3}}$	-7.6	3.2
CB[7]/LGP	-8.8	2.6×10^{6}	-8.8	0
CB[7]/IGP	0.2	$\mathbf{7.1\times10^{-1}}$	-7.9	8.1
CB[7]/LGK	-8.5	1.6×10^{6}	-8.8	0.3
CB[7]/IGK	-2.2	**40.1**	-7.2	5.0
CB[7]/LGD	-8.5	1.6×10^{6}	-8.8	0.3
CB[7]/IGD	0.6	$\mathbf{3.7\times10^{-1}}$	-7.9	8.5

Table S4 Thermodynamic parameters of CB[7] binding with tripeptide containing N-terminal aromatic AAs, including ΔG, ΔH, $-T\Delta S$ in kcal/mol as well as the binding constant K_a in M^{-1}.

	ΔG	K_a	ΔH	$-T\Delta S$
CB[7]/FGG	-5.7	1.4×10^4	-12.4	6.7
CB[7]/FGE	-12.8	2.1×10^9	-19.6	6.8
CB[7]/FGK	-11.9	4.7×10^8	-18.2	6.3
CB[7]/FGR	-11.6	2.8×10^8	-18.4	6.8
CB[7]/FGH	-10.2	4.7×10^7	-17.2	7.0
CB[7]/FGS	-3.6	4.2×10^2	-12.5	8.9
CB[7]/FGL	-2.9	1.3×10^2	-11.7	8.8
CB[7]/FGD	-2.3	4.7×10	-12.6	10.3
CB[7]/FGQ	-1.8	2.1×10	-13.1	11.3
CB[7]/FGM	-1.3	8.9	-12.5	11.2
CB[7]/FGY	0.4	0.5	-13.5	13.9
CB[7]/WGG	-9.1	4.5×10^6	-12.4	3.3
CB[7]/WGR	-15.1	9.5×10^{10}	-18.2	3.1
CB[7]/WGK	-3.0	1.4×10^2	-11.7	8.7
CB[7]/WGH	-6.5	5.4×10^4	-12.0	5.5
CB[7]/YGG	-4.9	3.7×10^3	-11.5	6.6
CB[7]/YGR	-14.9	7.0×10^{10}	-17.0	2.1
CB[7]/YGK	-15.4	1.7×10^{11}	-16.7	1.3
CB[7]/YGH	-7.8	4.8×10^5	-10.0	2.2

Table S5 Thermodynamic data of three systems on experiment compared with theoretical values, including ΔG, ΔH, $-T\Delta S$ in kcal/mol as well as the binding constant K_a in M^{-1}.

	ΔG		K_a		ΔH		$-T\Delta S$	
	Cal.	Exp.	Cal.	Exp.	Cal.	Exp.	Cal.	Exp.
LGG	-8.1	-7.9	8.0×10^5	6.4×10^5	-9.1	-6.8	1.0	-1.1
LGH	-9.3	-8.1	6.0×10^6	9.1×10^5	-9.3	-7.4	0	-0.7
LGK	-8.5	-8.2	1.6×10^6	9.5×10^5	-8.8	-6.6	0.3	-1.6