Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Oligopeptide-CB[8] complexation with switchable binding pathways

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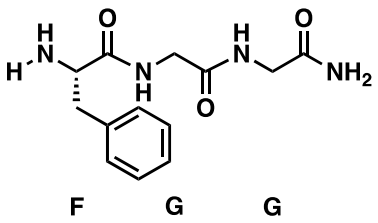
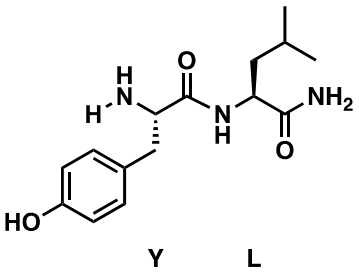
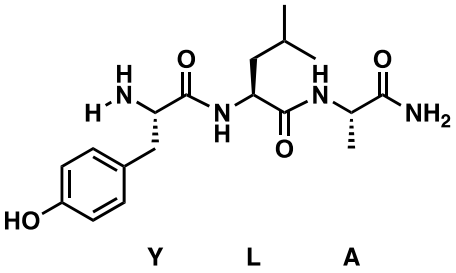
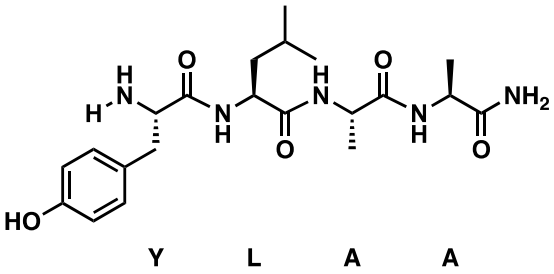
SI-1 Materials and methods

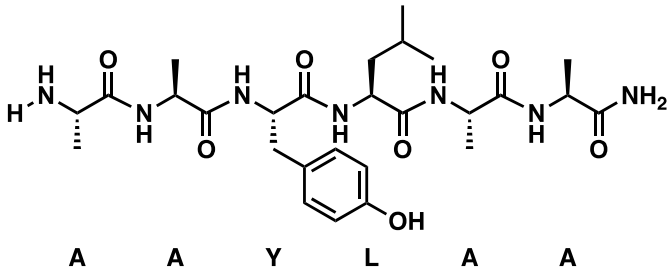
Chemical Structure of Studied Molecules.

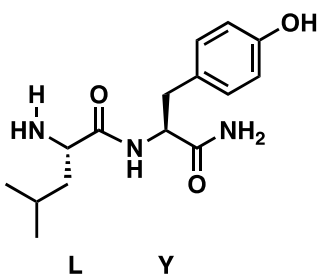


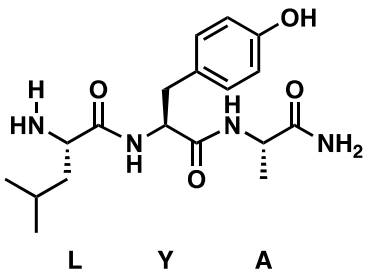


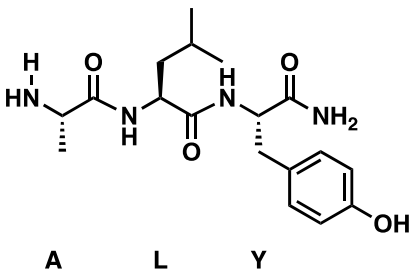


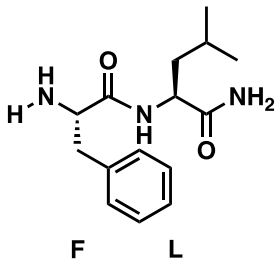


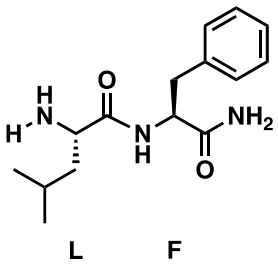


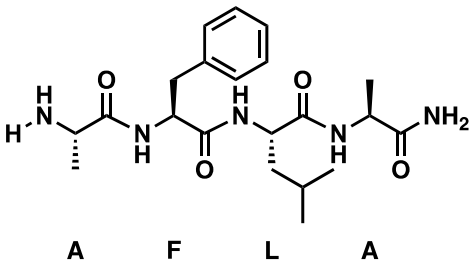


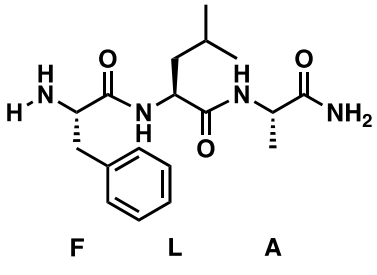


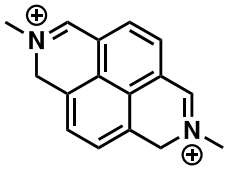


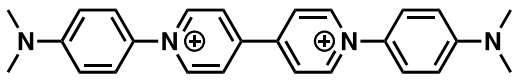












Materials. All Fmoc-protected amino acids, solvents and rink amide 4-methyl-benzhydrylamine (MBHA) resin used for peptide synthesis were purchased from AGTC Bioproducts (UK). All other solvents and reagents were purchased from Sigma-Aldrich (UK) and used as received. Cucurbit[8]uril (CB[8]) was synthesized according to the published procedure [1]. Milli-Q water (18.2 MΩ·cm) was used for preparation of all non-deuterated aqueous solutions. The stock solution of 10 mM sodium phosphate buffer was prepared by mixing sodium phosphate monobasic, sodium phosphate dibasic, and water then adjusting to pH 7.0.

Peptide Synthesis and Characterisation. All peptide sequences were synthesized using solid-phase methodology (FMOC, tBu, MBHA resin) on an automated microwave peptide synthesiser (Liberty, CEM). Crude Peptides were cleaved from the resin with a cleavage cocktail of 95% trifluoroacetic acid (TFA), 2.5% triisopropyl silane and 2.5% DI H_2O and left to shake for 2.5 h. Following cleavage, the crude peptides were precipitated and washed with cold diethyl ether (DEE), then left to dry under vacuum overnight.

The crude peptides were then purified by high pressure liquid chromatography (HPLC) using a Phenomenex C18 Kinetic-Evo column with a 5 micron pore size, a 110 Å particle size and with the dimensions 150 x 21.2 mm. A gradient from 5% acetonitrile 95% water to 100% acetonitrile was run with 0.1% TFA Following purification, peptide identities were verified by analytical HPLC and ^{1}H-NMR.

Isothermal Titration Calorimetry (ITC). All ITC experiments were carried out on a Microcal iTC200 at 298.15 K in 10 mM sodium phosphate buffer (pH = 7.0). In a typical ITC, the host molecule (CB[8]) was in the sample cell, and guest molecule was in the injection syringe with a concentration of about ten times concentration of host. The concentration of CB[8] was calibrated by the titration with a standard solution of 1-adamantanamine. In order to avoid bias or potentially arbitrary offsets caused by manual adjustment of baseline, all raw data (thermograms) of ITC were integrated by NITPIC (v.1.2.0), fitted in Sedphat (v.12.1b), and visualized through GUSSI (v.1.1.0) [2]. For each species, at least two individual titrations were performed for the subsequent global fitting, whose error estimations were carried out by F statistics at the 0.68 confidence level.

Nuclear Magnetic Resonance Spectroscopy (NMR). ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, and ^{1}H DOSY spectra were acquired in heavy water (D_2O) at 298 K and recorded on a Bruker AVANCE 500 with TCI Cryoprobe system (500 MHz) being controlled by TopSpin2.

The 1H DOSY experiments were carried out using a modified version of the Bruker sequence ledbpgp2s involving, typically, 32 scan over 16 steps of gradient variation from 10% to 80% of the maximum gradient. Diffusion coefficients were evaluated in Dynamic Centre (a standard Bruker software) and determined by fitting the intensity decays according to the following equation:

$$I = I_o\, e^{[-D\gamma^2 g^2 \delta^2 (\Delta - \delta/3)]}$$

where I and I_o represent the signal intensities in the presence and absence of gradient pulses respectively, D is the diffusion coefficient, $\boldsymbol{\gamma}$ = 26753 rad/s/Gauss is the ^{1}H gyromagnetic ratio, $\delta = 2.4$ ms is duration

of the gradient pulse, Δ = 100 ms is the total diffusion time and g is the applied gradient strength. Monte Carlo simulation method is used for the error estimation of fitting parameters with a confidence level of 95%.

Ion Mobility Mass Spectrometry (IM-MS). A traveling-wave ion mobility quadrupole time-of-flight mass spectrometer (Vion IMS QTof, Waters) with an electrospray ion source (ESI) was used to detect mass to charge signals and record drift time for each detected signal. All data were acquired and processed by UNIFI system using 'accurate mass screening on IMS data' as the analysis method. The MS settings was summarized as follows: source type: ESI, source temperature: 100 °C, desolvation temperature: 400 °C, cone gas: 20 L/hr, desolvation gas: 600 L/hr, capillary voltage: 2.60 kV, IMS gas: 25 mL/min, IMS wave velocity: 300 m/s, IMS pulse height: 15.0 V, low collision energy: 6.00 eV, high collision energy ramp start: 20.00 eV, high collision energy ramp end: 30.00 eV, collision gas: nitrogen (N_2). Only positive ions were detected in this work. Solution was directly intruded into source without chromatography.

SI-2 Isothermal titration thermograms of peptides with CB[8]

ITC data can supply complexation information including the binding stoichiometry, enthalpy changes (dH), and the binding constant (K_a), which can further deduce Gibbs free energy changes (dG) and entropy changes (dS) through $dG=-RTlnK_a=dH-TdS$, where R is the gas constant and T is the absolute temperature.

Isothermal titration thermograms of the complexation between CB[8] and peptide derivatives of YL, LY and FL were shown in Figure S1-S12. Most titration curves were perfectly fitted by hetero association model (AB model or one-site model). Titration curves of FL and YAL were fitted through stoichiometric model (AB_2 model or sequential binding model). All the data is obtained at 298.15K in 10 mM sodium phosphate buffer pH 7.0 (NaP7). Each thermodynamic data were obtained by the global fitting of at least two repeating experiments.

Table S1. Thermodynamic data for the association of CB[8] with peptide derivatives of YL, LY and FL.

Peptides	Model	Ka AB: M^{-1}, AB2: M^{-2}	dG kcal/mol	dH kcal/mol	TdS kcal/mol	Temp. K	Buffer
YL	AB	8.2×10^6	-9.4±0.1	-13.6±0.1	-4.2±0.2	298.15	NaP7
YLA	AB	8.1×10^6	-9.4±0.1	-12.0±0.1	-2.6±0.2	298.15	NaP7
YLAA	AB	7.1×10^6	-9.3±0.1	-11.0±0.1	-1.7±0.2	298.15	NaP7
AAYLAA	AB	1.8×10^5	-7.2±0.1	-11.6±0.1	-4.4±0.2	298.15	NaP7
LY	AB	1.3×10^7	-9.7±0.2	-13.7±0.2	-4.0±0.4	298.15	NaP7
LYA	AB	1.3×10^7	-9.7±0.2	-12.0±0.2	-2.3±0.3	298.15	NaP7
ALY	AB	1.3×10^6	-8.4±0.1	-11.7±0.1	-3.4±0.1	298.15	NaP7
FLA	AB	1.0×10^7	-9.6±0.1	-12.0±0.1	-2.4±0.1	298.15	NaP7
AFLA	AB	2.1×10^6	-8.6±0.1	-11.4±0.1	-2.8±0.1	298.15	NaP7
LF	AB	6.6×10^6	-9.3±0.2	-12.3±0.2	-3.0±0.4	298.15	NaP7
FL	AB	1.3×10^7	-9.7±0.8	-11.4±0.2	-1.7±1.0	298.15	NaP7
FL	AB2	1.9×10^{11}	-15.4±2.4	-26.7±1.4	-11.3±3.8	298.15	NaP7
YAL	AB	2.7×10^4	-6.0±0.1	-6.2±0.6	-0.2±0.7	298.15	NaP7
YAL	AB2	8.7×10^7	-10.8±0.4	-18.1±2.8	-7.3±3.2	298.15	NaP7

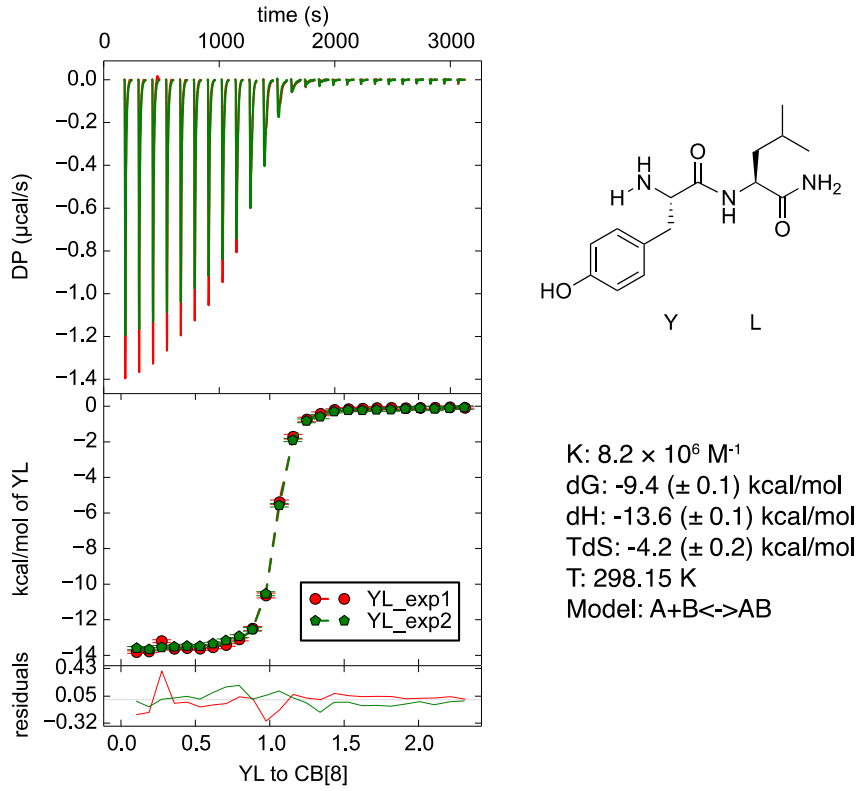


Figure S1. ITC of YL (0.588 mM or 0.592 mM) titrated into CB[8] (0.053 mM). 10 mM sodium phosphate buffer (pH 7.0)., 298.15 K, iTC200, 25 injections of 1.5 µL, 120 s interval.

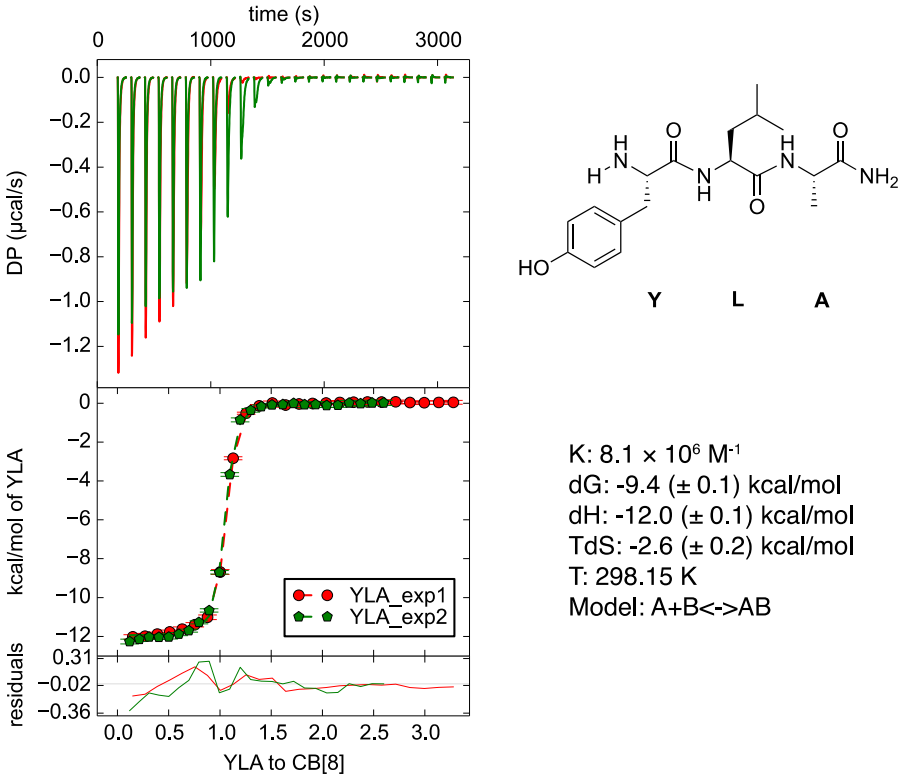


Figure S2. ITC of YLA (0.837 mM or 0.670 mM) titrated into CB[8] (0.053 mM). 10 mM sodium phosphate buffer (pH 7.0)., 298.15 K, iTC200, 25 injections of 1.5 µL, 120 s interval.

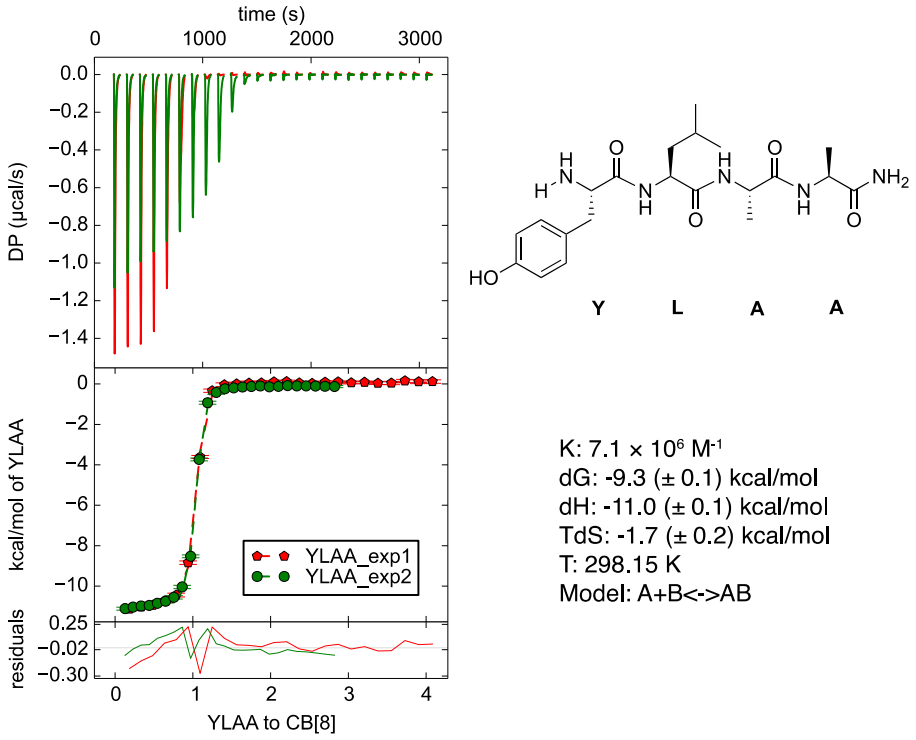


Figure S3. ITC of YLAA (1.05 mM or 0.726 mM) titrated into CB[8] (0.053 mM). 10 mM sodium phosphate buffer (pH 7.0)., 298.15 K, iTC200, 25 injections of 1.5 µL, 120 s interval.

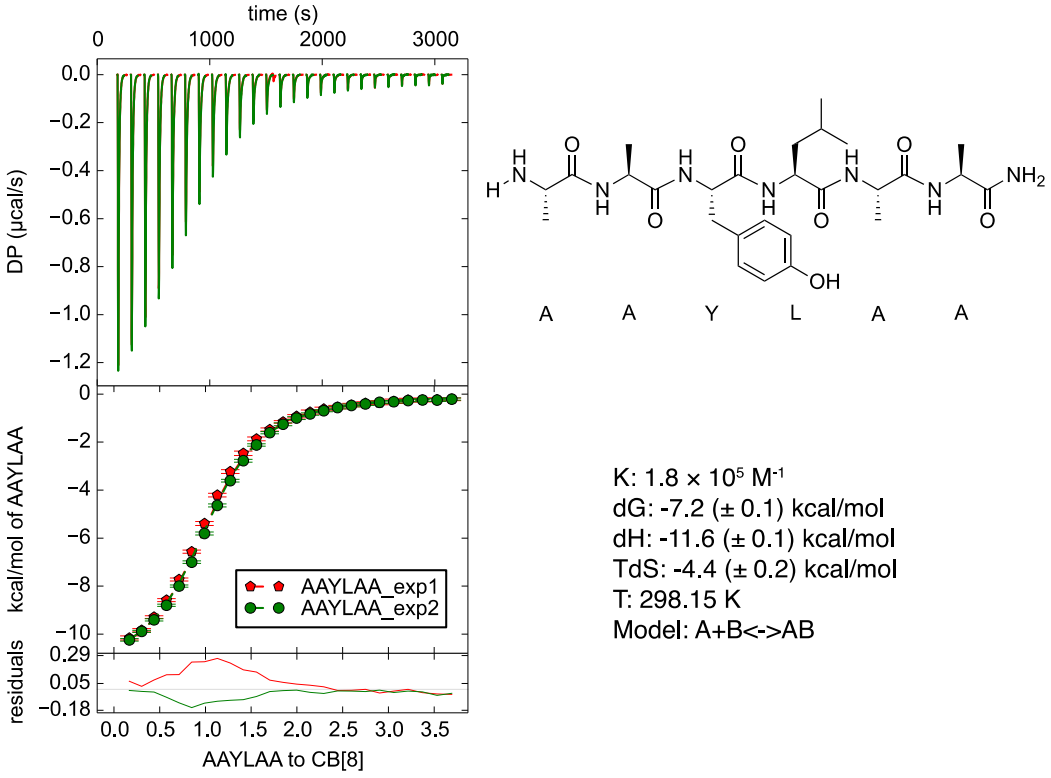


Figure S4. ITC of AAYLAA (0.928 mM or 0.921 mM) titrated into CB[8] (0.053 mM). 10 mM sodium phosphate buffer (pH 7.0)., 298.15 K, iTC200, 25 injections of 1.5 µL, 120 s interval.

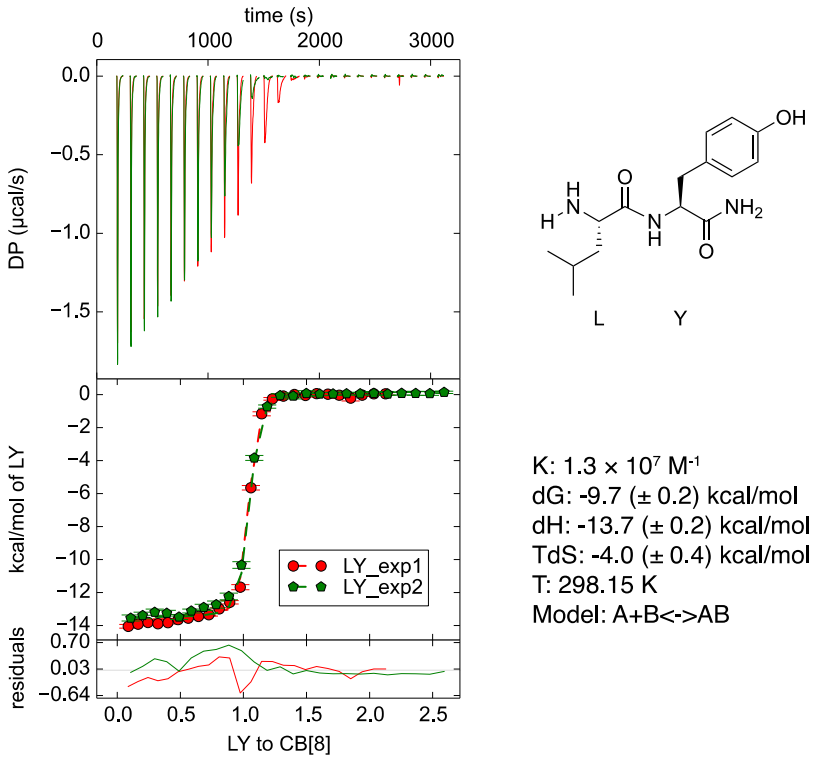


Figure S5. ITC of LY (0.610 mM or 0.742 mM) titrated into CB[8] (0.0587 mM). 10 mM sodium phosphate buffer (pH 7.0)., 298.15 K, iTC200, 25 injections of 1.5 µL, 120 s interval.

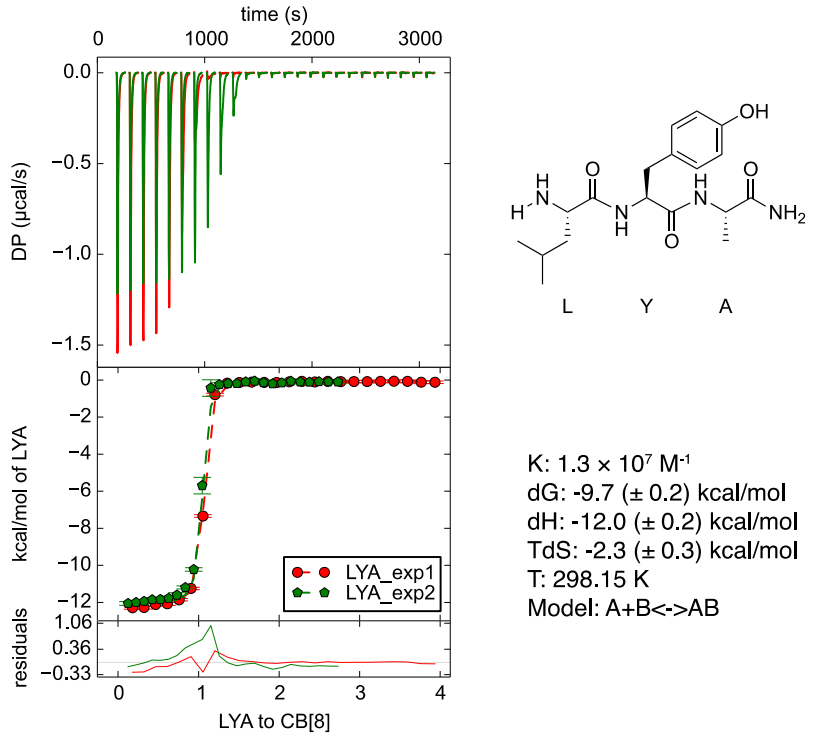


Figure S6. ITC of LYA (1.00 mM or 0.714 mM) titrated into CB[8] (0.053 mM). 10 mM sodium phosphate buffer (pH 7.0)., 298.15 K, iTC200, 25 injections of 1.5 µL, 120 s interval.

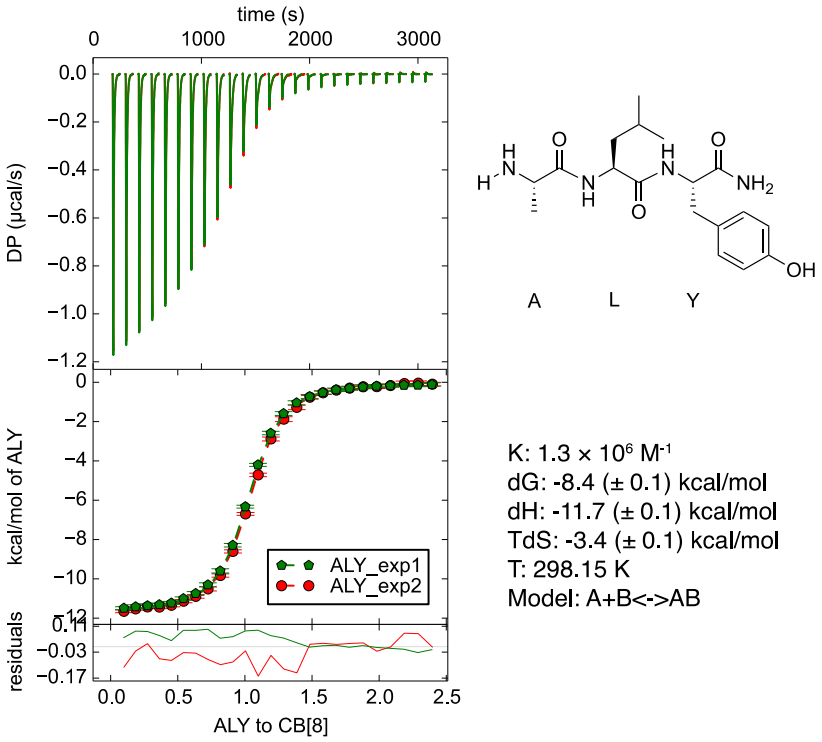


Figure S7. ITC of ALY (0.619 mM or 0.620 mM) titrated into CB[8] (0.053 mM). 10 mM sodium phosphate buffer (pH 7.0)., 298.15 K, iTC200, 25 injections of 1.5 µL, 120 s interval.

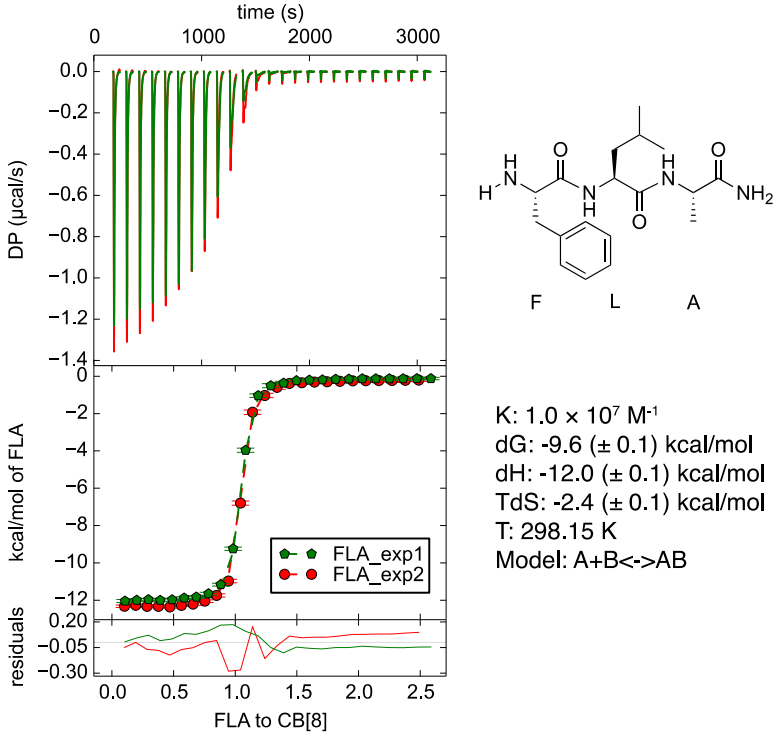


Figure S8. ITC of FLA (0.660 mM or 0.640 mM) titrated into CB[8] (0.053 mM). 10 mM sodium phosphate buffer (pH 7.0)., 298.15 K, iTC200, 25 injections of 1.5 µL, 120 s interval.

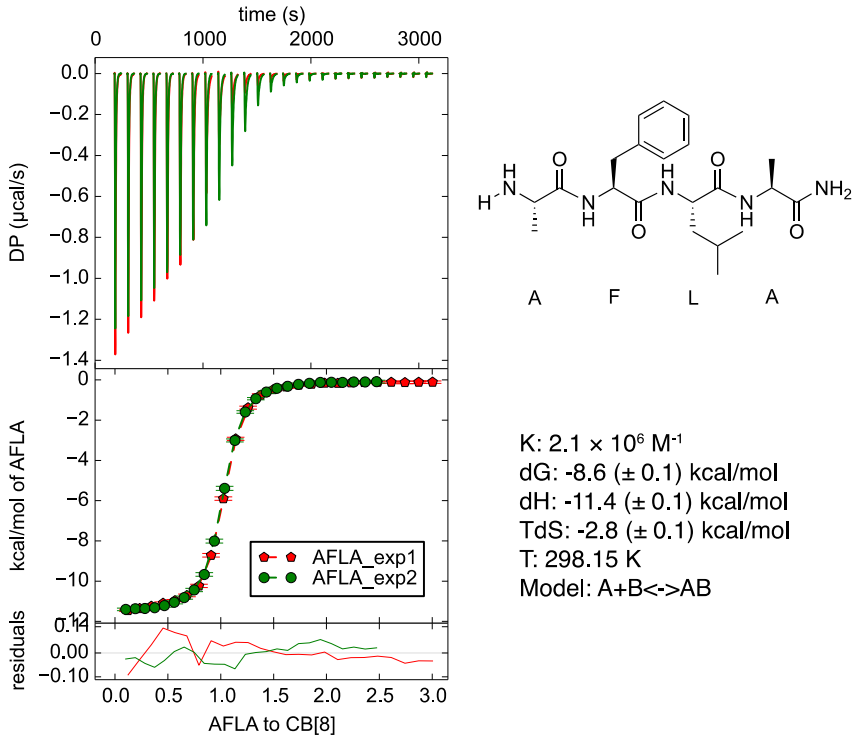


Figure S9. ITC of AFLA (1.05 mM or 0.726 mM) titrated into CB[8] (0.053 mM). 10 mM sodium phosphate buffer (pH 7.0)., 298.15 K, iTC200, 25 injections of 1.5 µL, 120 s interval.

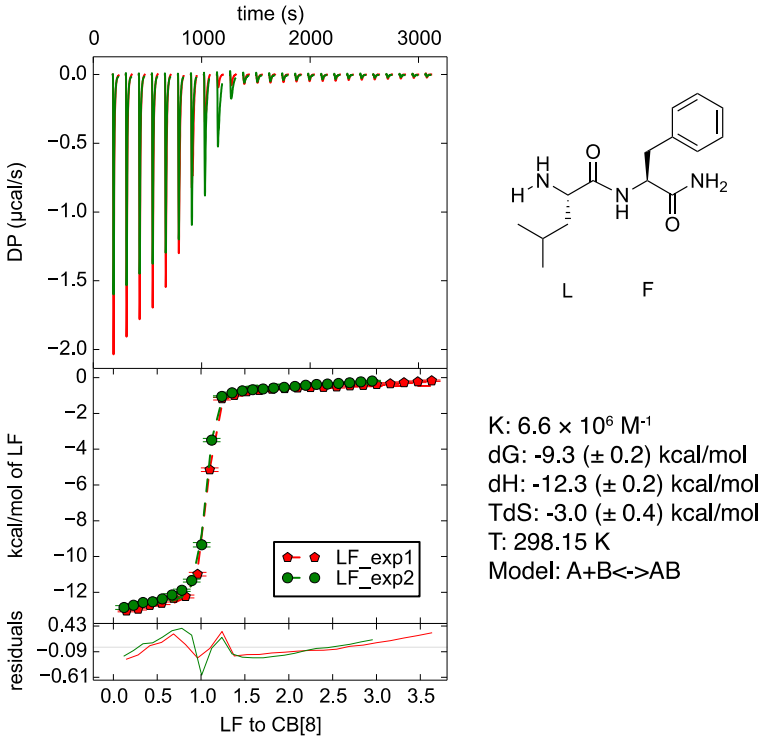


Figure S10. ITC of LF (0.920 mM or 0.790 mM) titrated into CB[8] (0.0587 mM). 10 mM sodium phosphate buffer (pH 7.0)., 298.15 K, iTC200, 25 injections of 1.5 µL, 120 s interval.

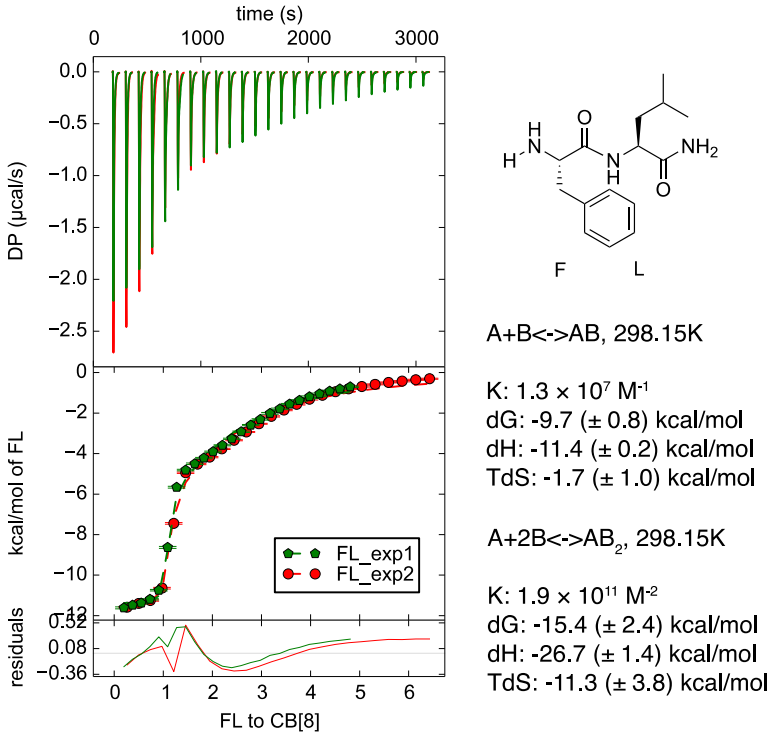


Figure S11. ITC of FL (1.47 mM or 1.76 mM) titrated into CB[8] (0.0587 mM). 10 mM sodium phosphate buffer (pH 7.0)., 298.15 K, iTC200, 25 injections of 1.5 µL, 120 s interval.

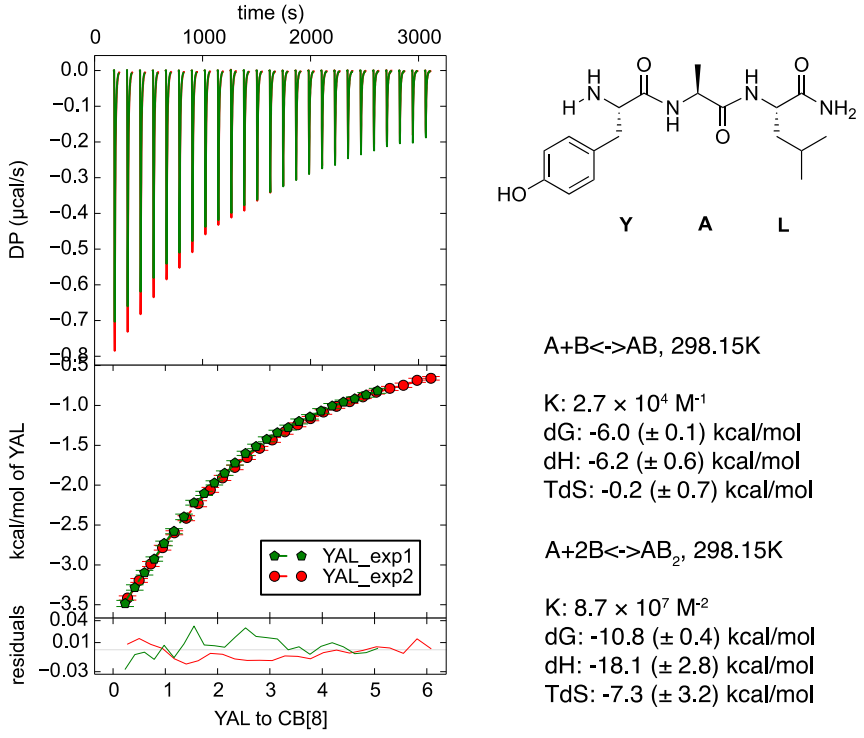


Figure S12. ITC of YAL (1.33 mM or 1.59 mM) titrated into CB[8] (0.053 mM). 10 mM sodium phosphate buffer (pH 7.0)., 298.15 K, iTC200, 25 injections of 1.5 µL, 120 s interval.

SI-3 Ion mobility mass spectra of YLA, YAL and their CB[8] complexes.

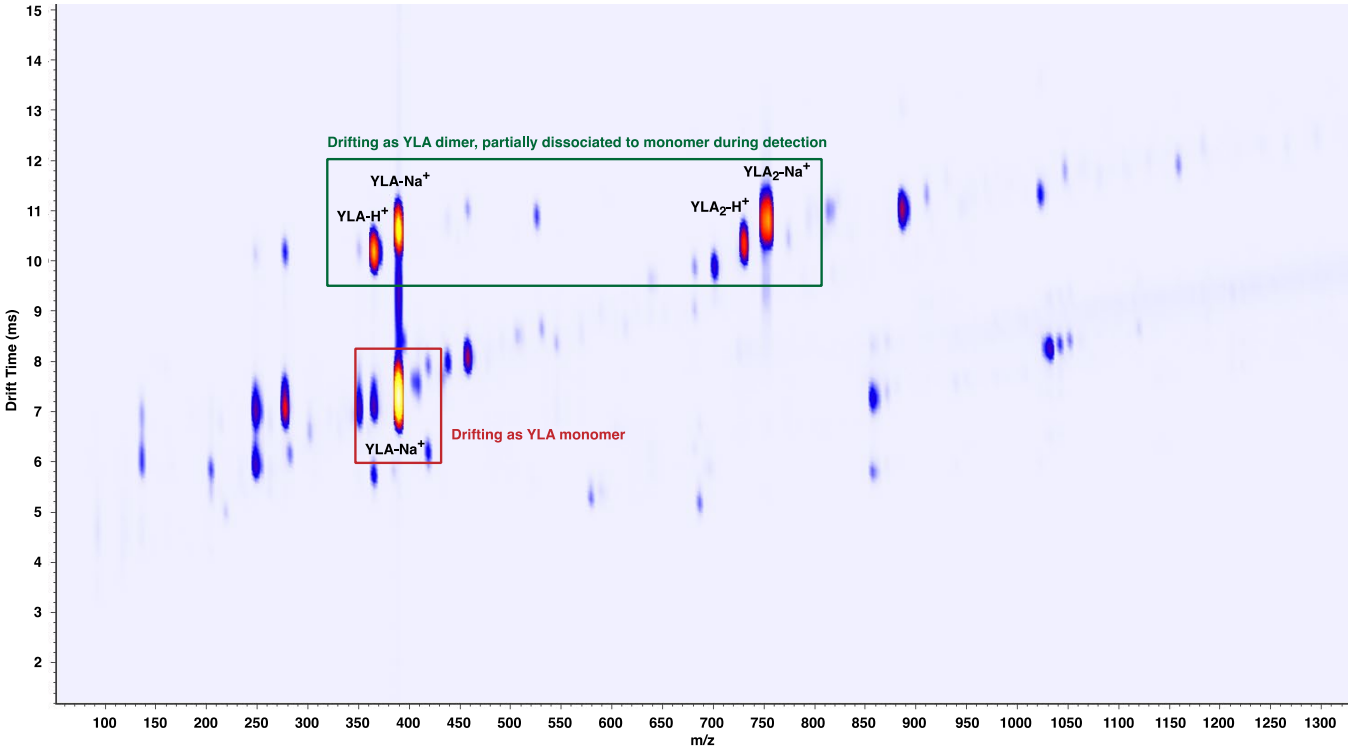


Figure S13. IMMS of the direct injection of YLA aqueous solution showing its tendency towards forming dimeric aggregates.

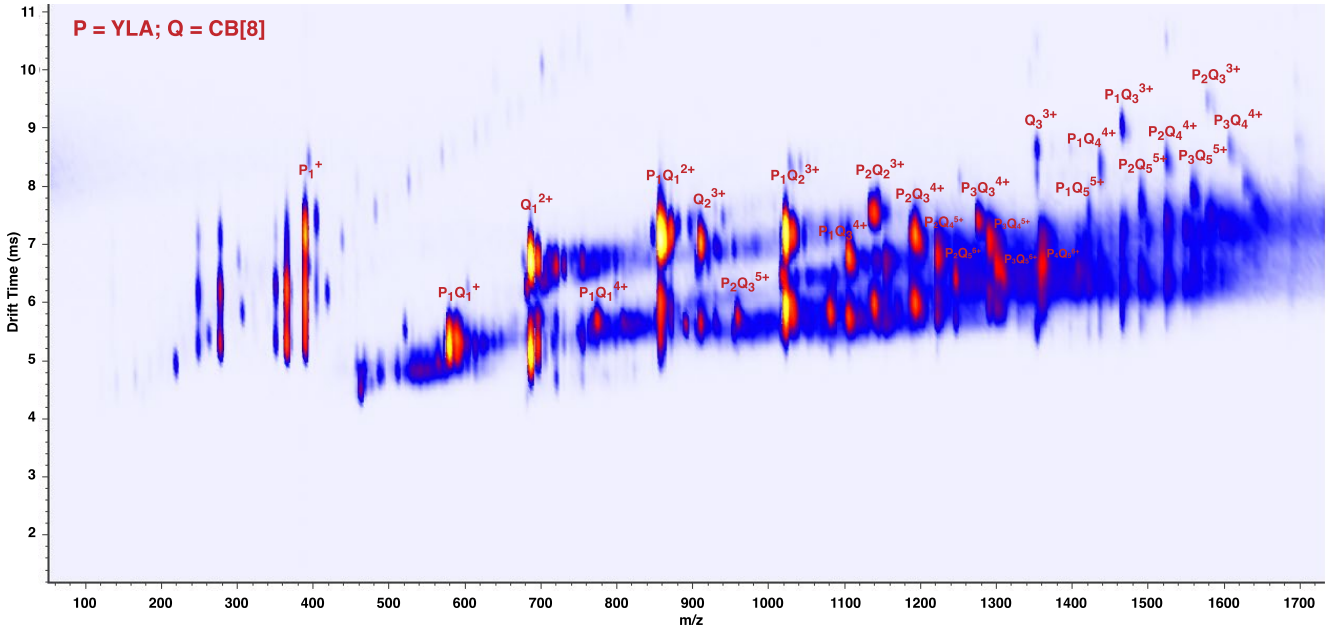


Figure S14. IMMS of the direct injection of YLA-CB[8] (1:1) aqueous solution showing existence of various aggregation species containing multiple CB[8] (Q) and multiple peptide (P).

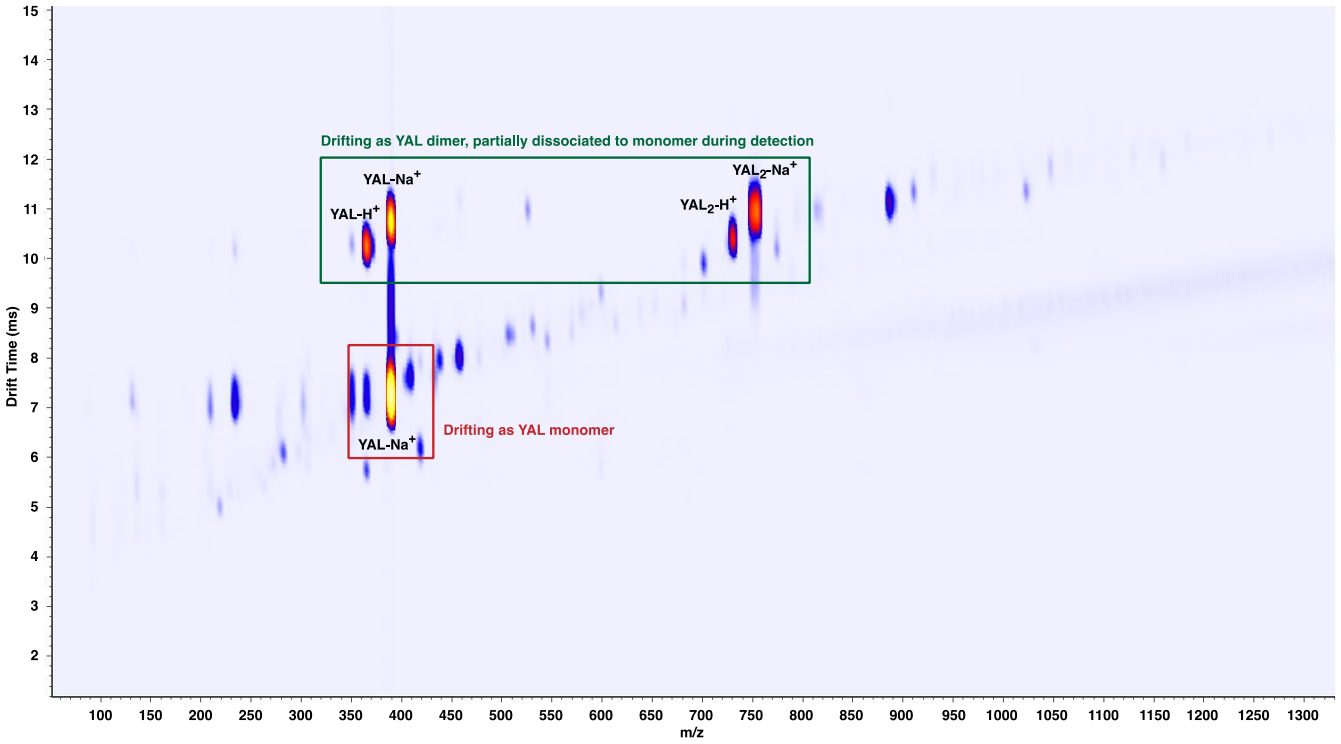


Figure S15. IMMS of the direct injection of YAL aqueous solution showing the same behavior as YLA.

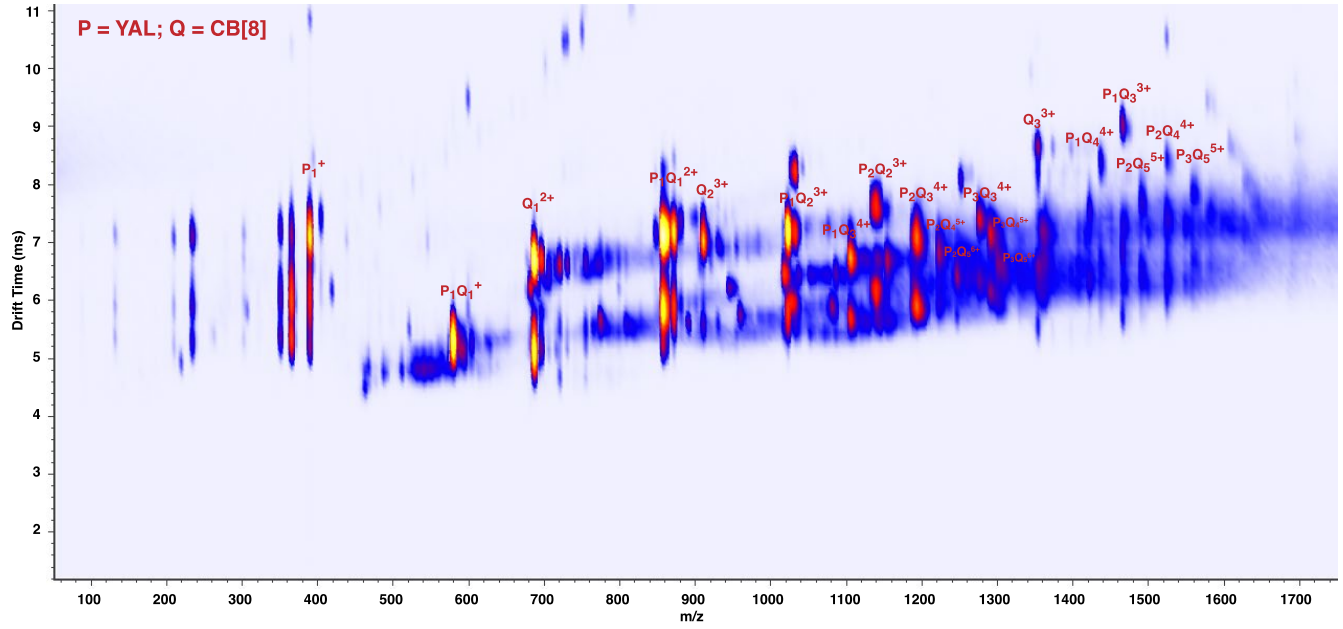


Figure S16. IMMS of the direct injection of YAL-CB[8] aqueous solution showing similar mass pattern as that in YLA-CB[8] system.

SI-4 NMR of peptides and their complexation with CB[8]

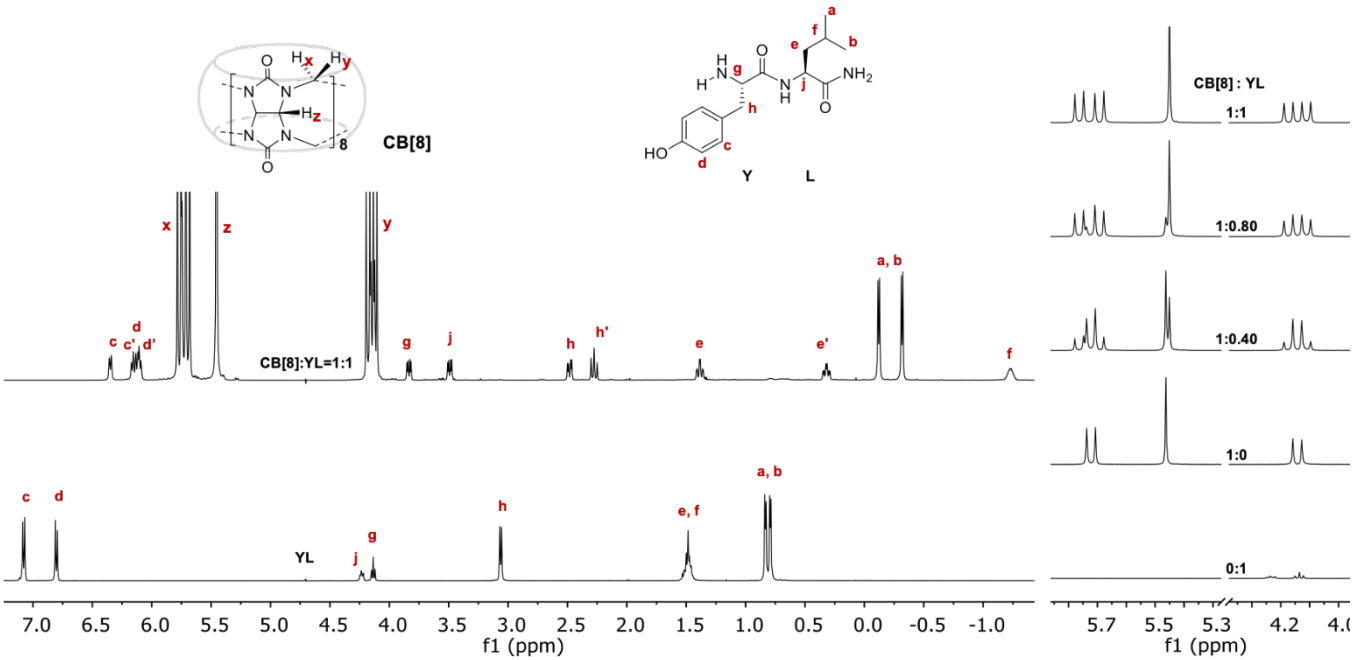


Figure S17. ^{1}H NMR of YL titrated into CB[8] until a ratio of 1:1. (500 MHz, 298 K, D_2O).

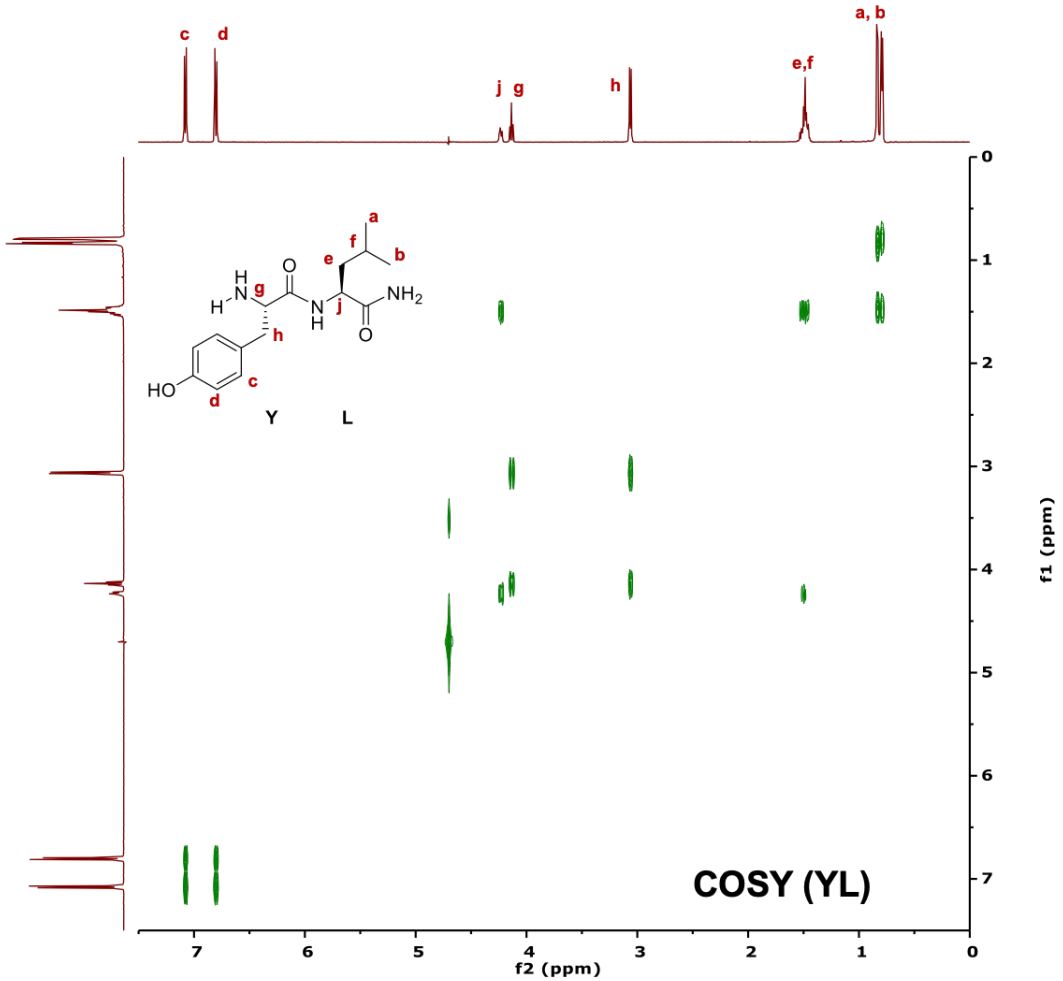


Figure S18. COSY of YL (500 MHz, 298 K, D_2O).

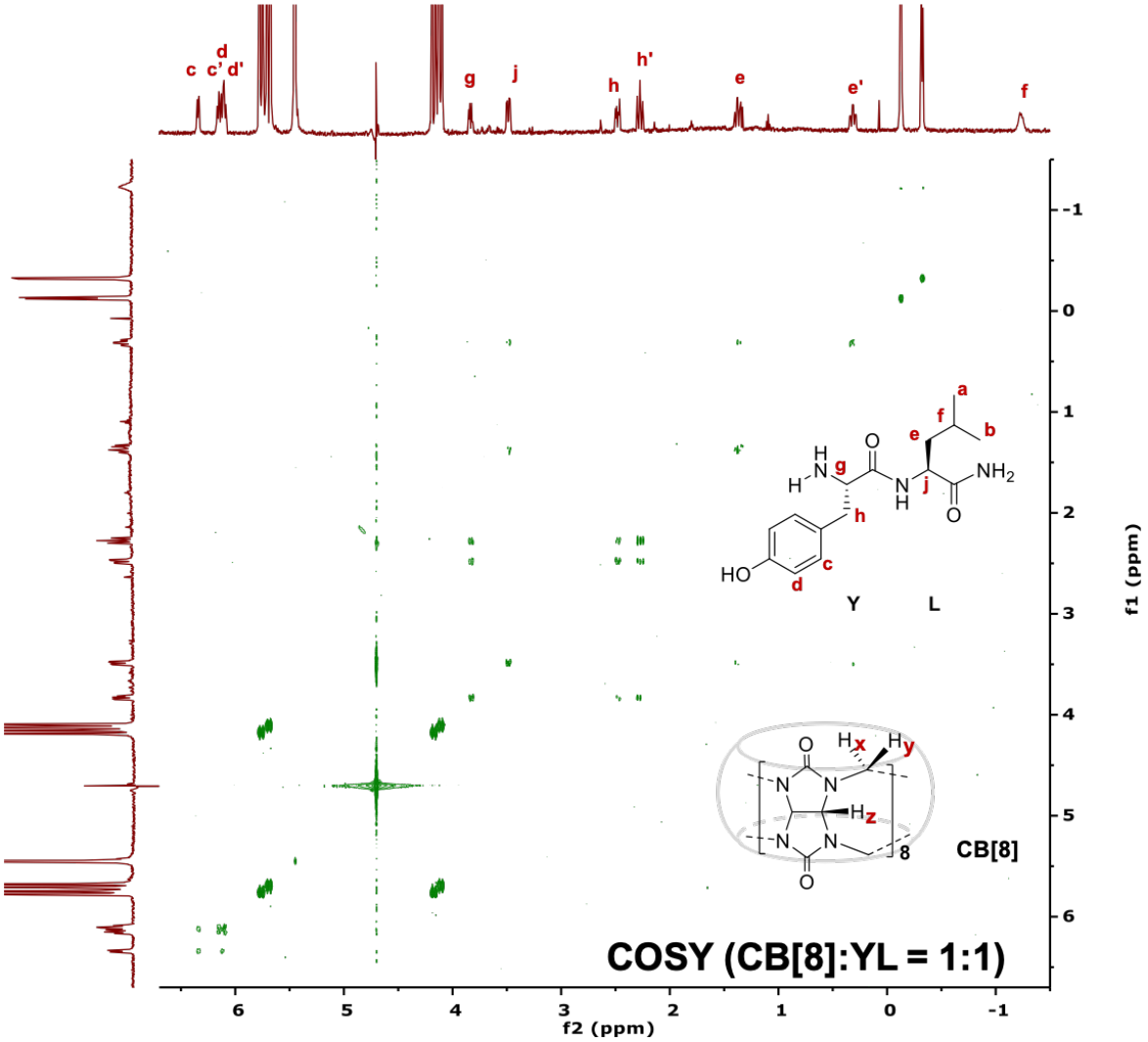


Figure S19. COSY of YL:CB[8]=1:1 (500 MHz, 298 K, D_2O).

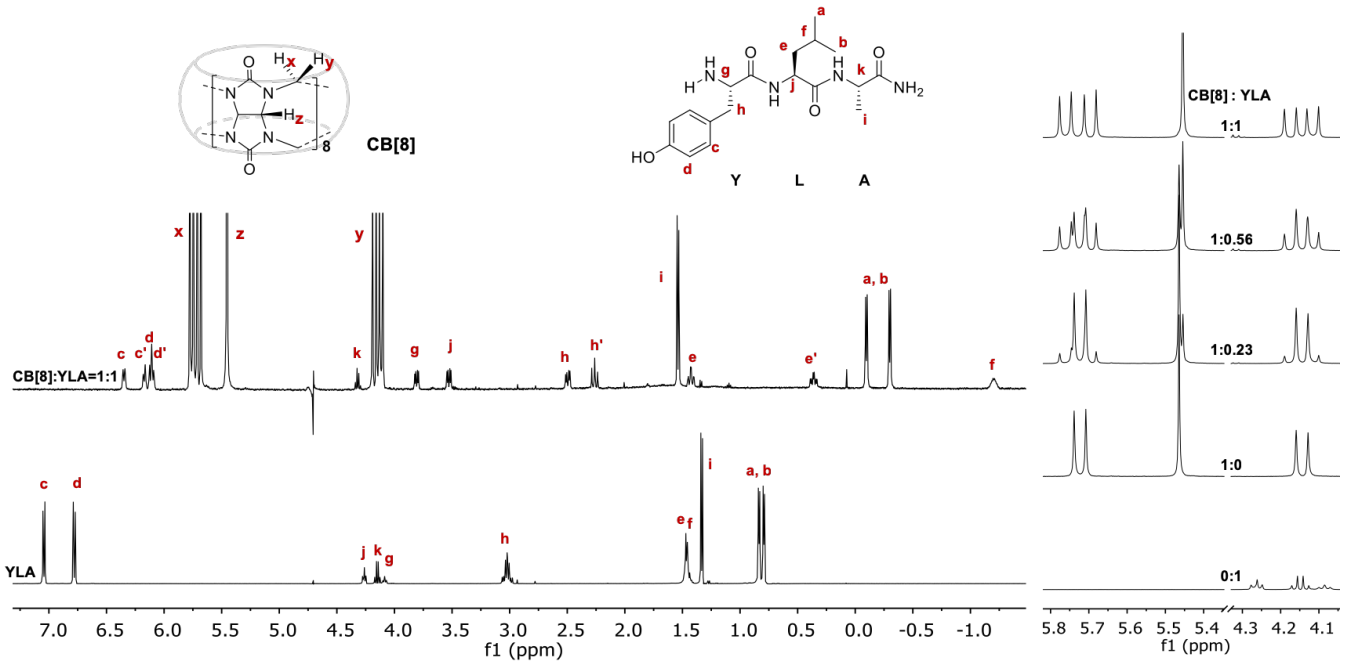


Figure S20. ^{1}H NMR of YLA titrated into CB[8] until a ratio of 1:1. (500 MHz, 298 K, D_2O).

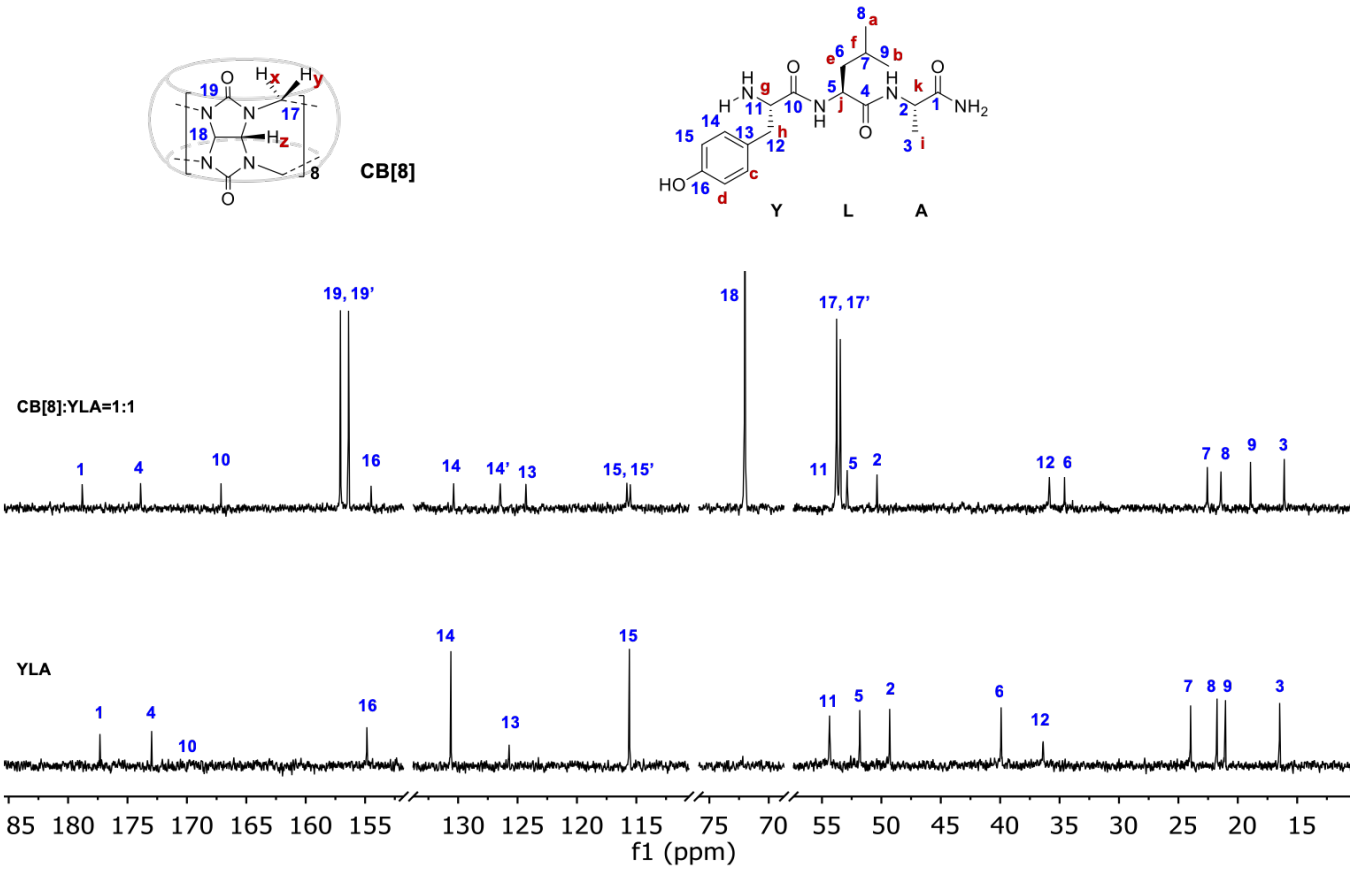
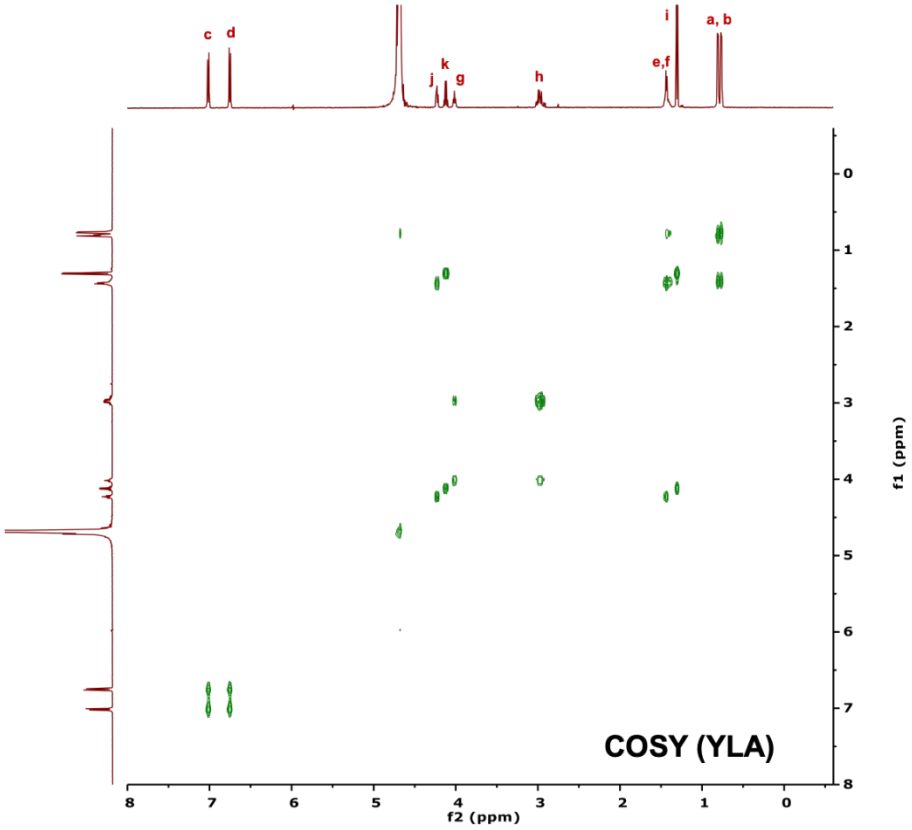


Figure S21. ^{13}C NMR of YLA titrated into CB[8] until a ratio of 1:1. (125 MHz, 298.15 K, D_2O).



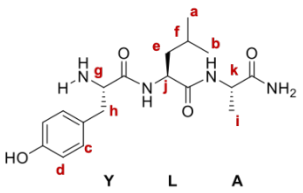
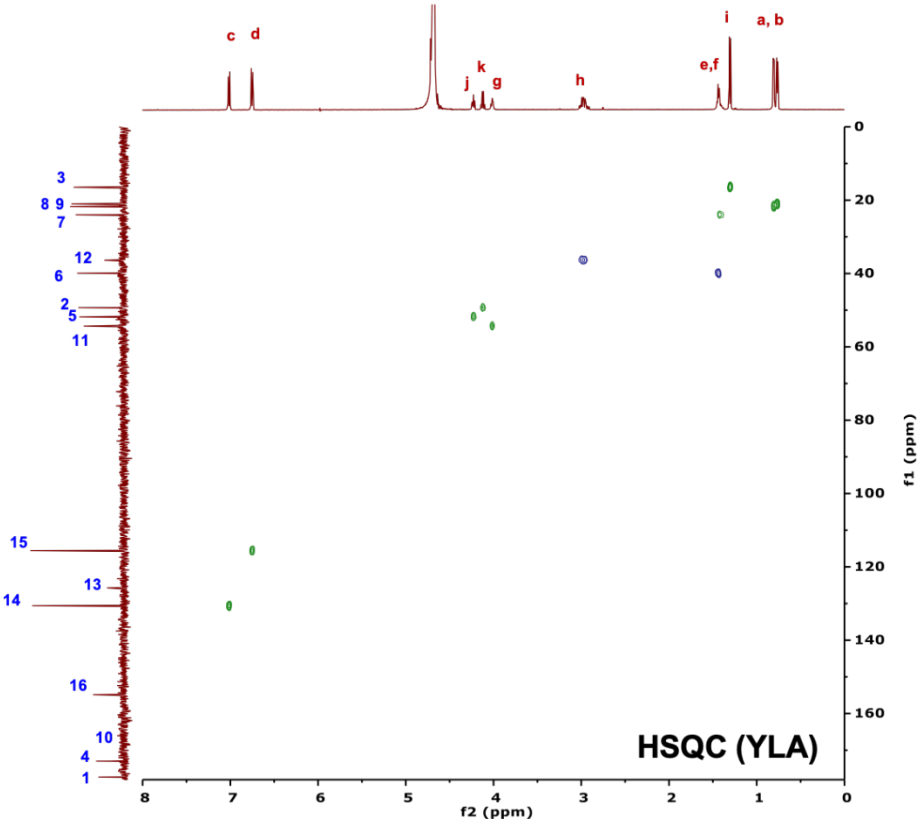


Figure S22. COSY of YLA. (500 MHz, 298 K, D_2O).



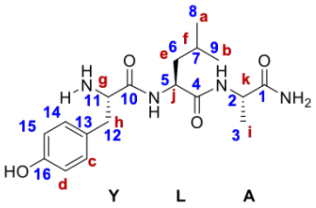


Figure S23. HSQC of YLA. (500 MHz, 298 K, D_2O).

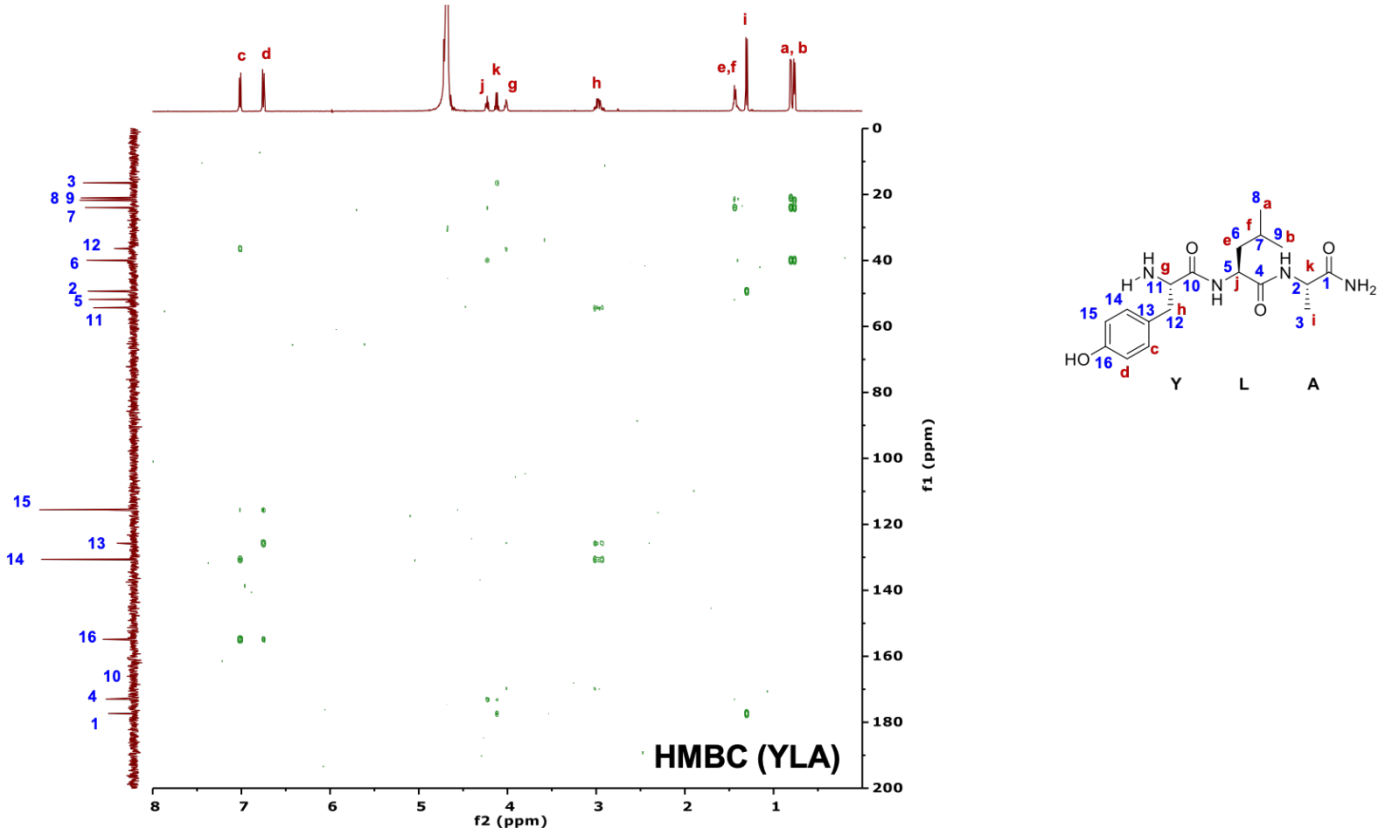


Figure S24. HMBC of YLA. (500 MHz, 298 K, D_2O).

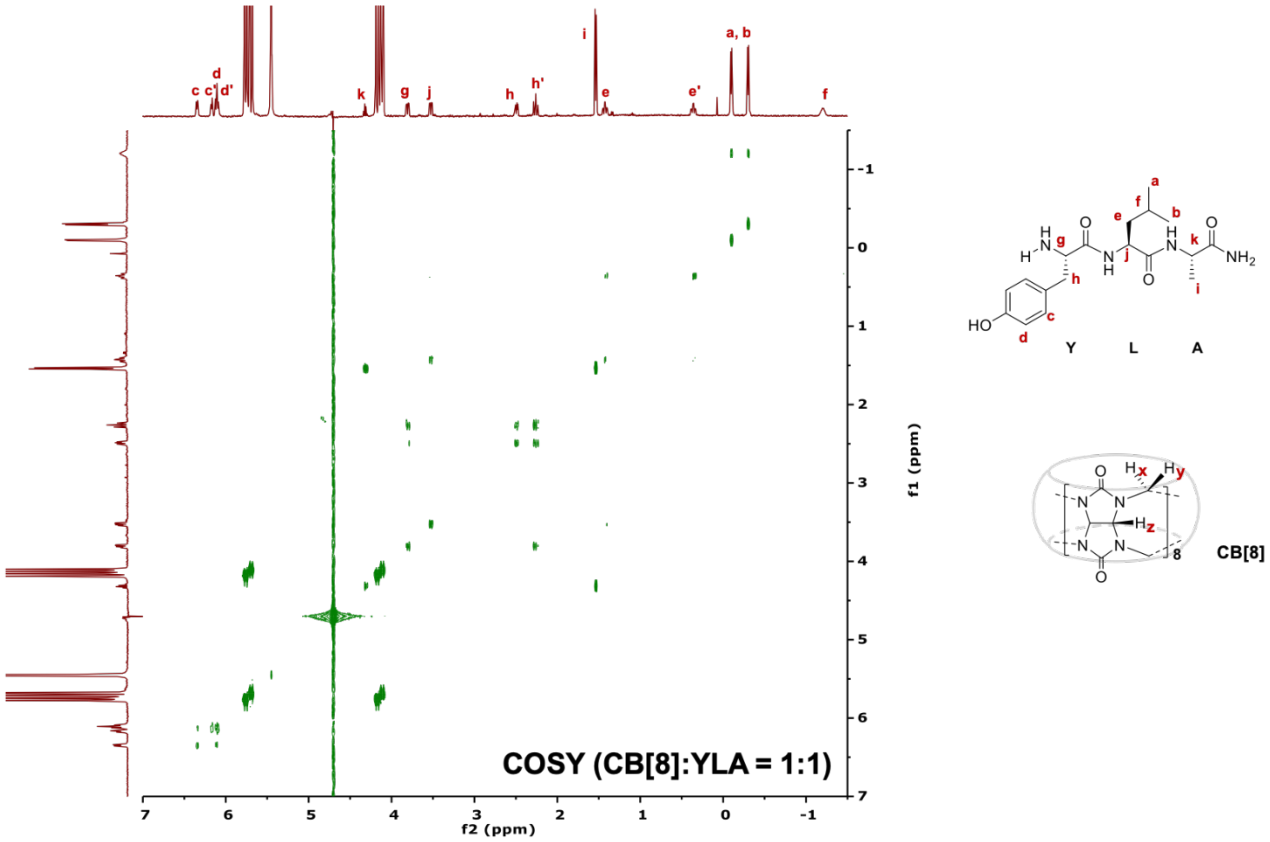


Figure S25. COSY of YLA:CB[8]=1:1. (500 MHz, 298 K, D_2O).

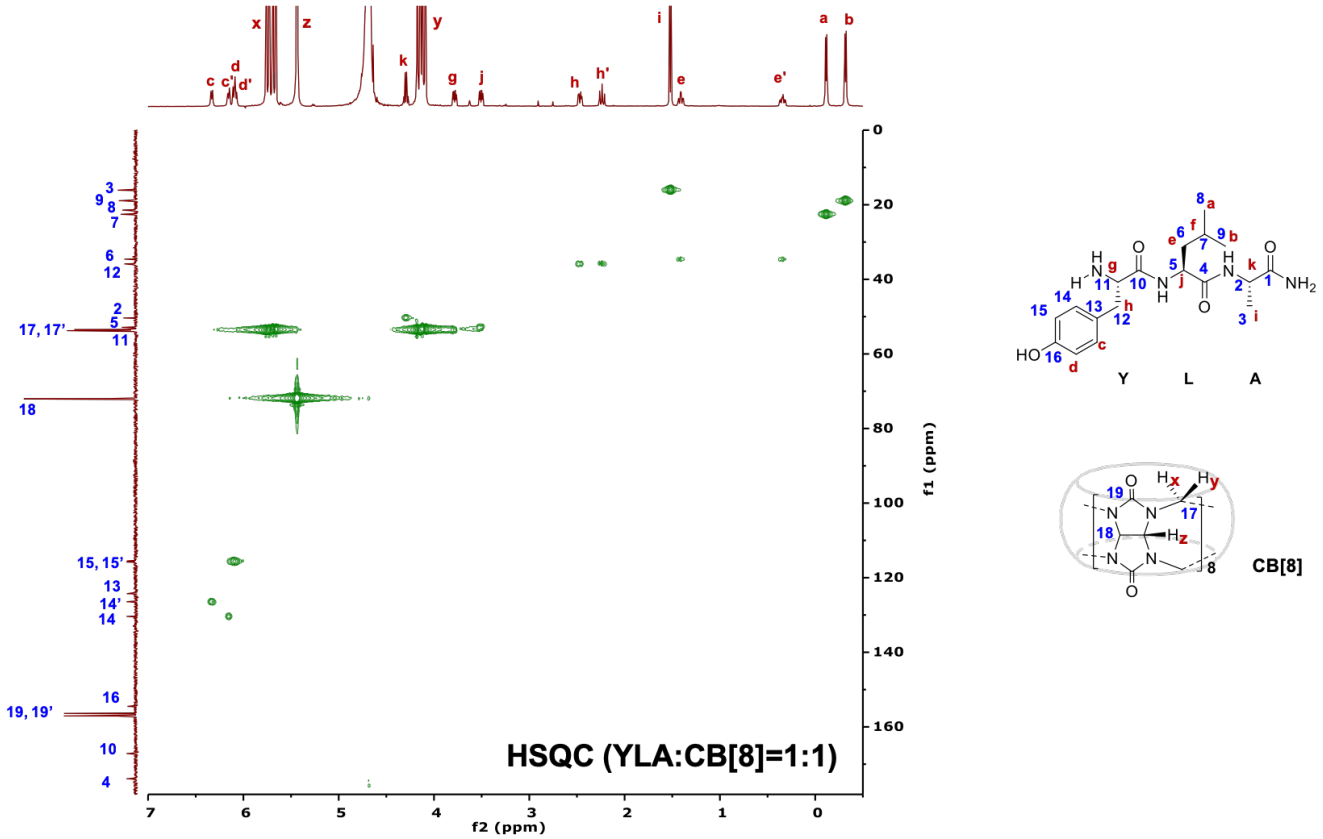


Figure S26. HSQC of YLA:CB[8]=1:1. (500 MHz, 298 K, D_2O).

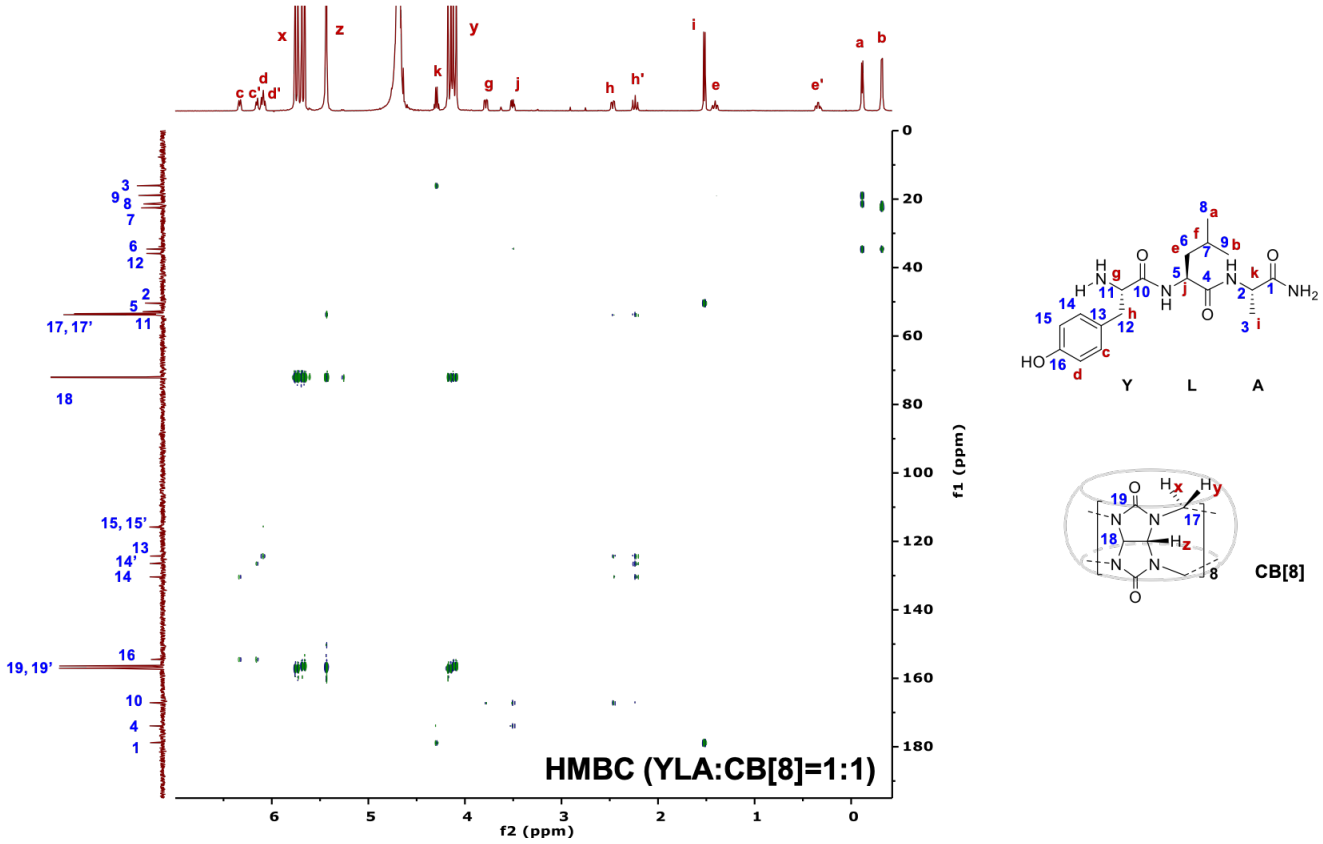


Figure S27. HMBC of YLA:CB[8]=1:1. (500 MHz, 298 K, D_2O).

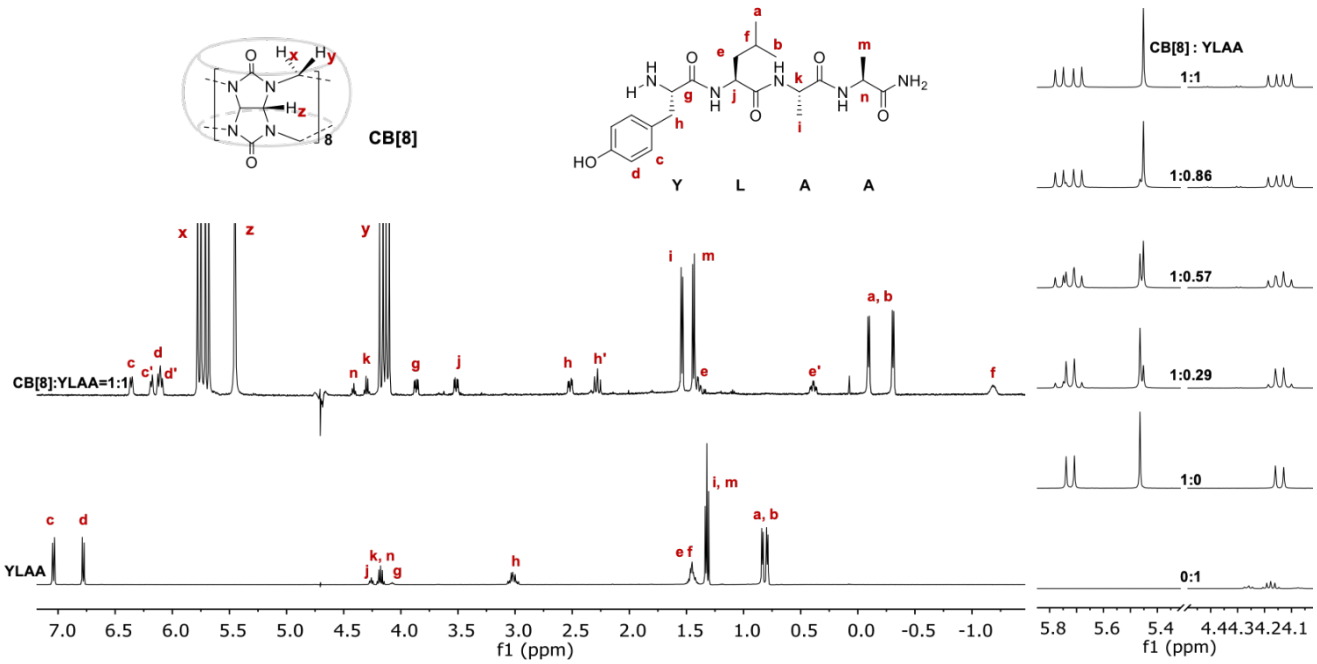


Figure S28. ^{1}H NMR of YLAA titrated into CB[8] until a ratio of 1:1. (500 MHz, 298 K, D_2O).

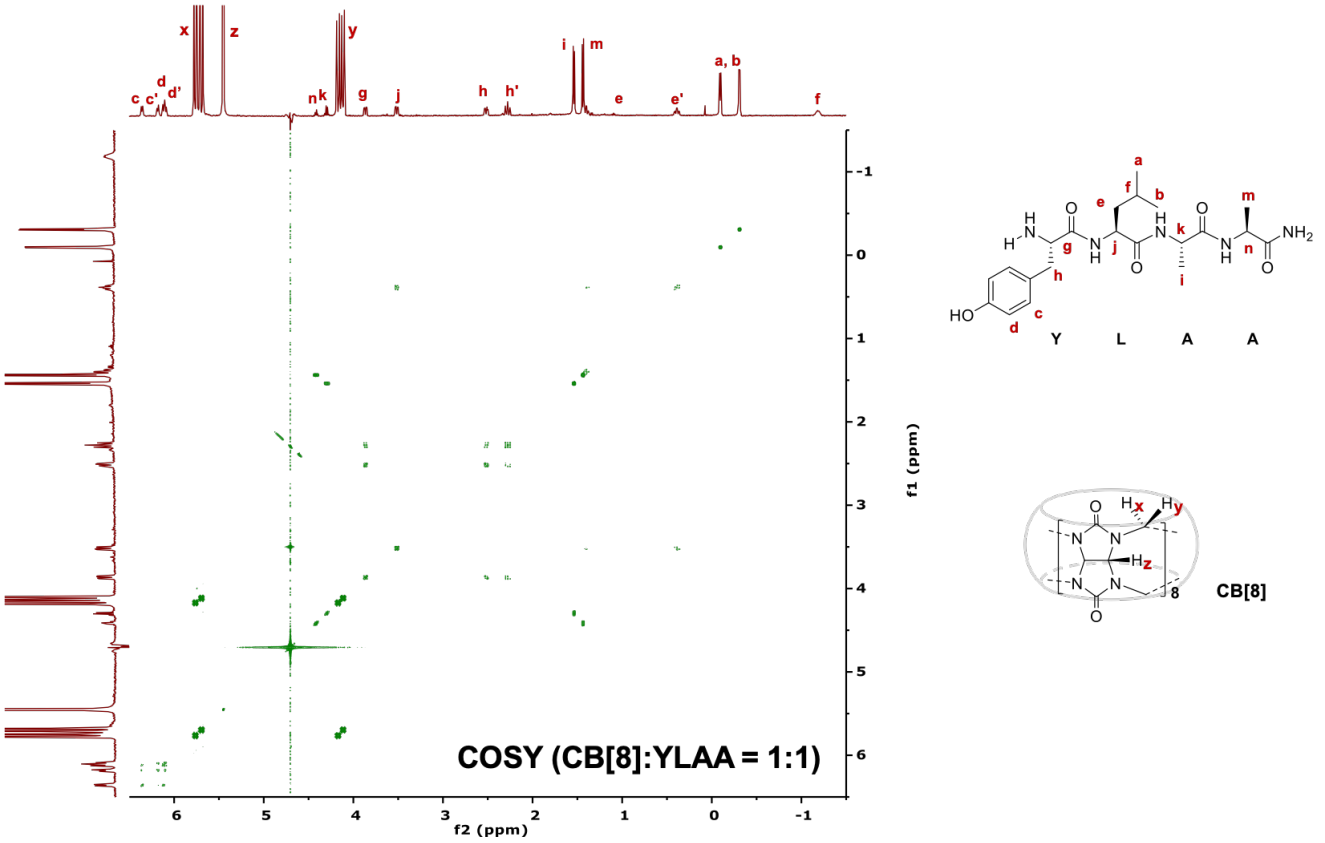


Figure S29. COSY of YLAA:CB[8]=1:1. (500 MHz, 298 K, D_2O).

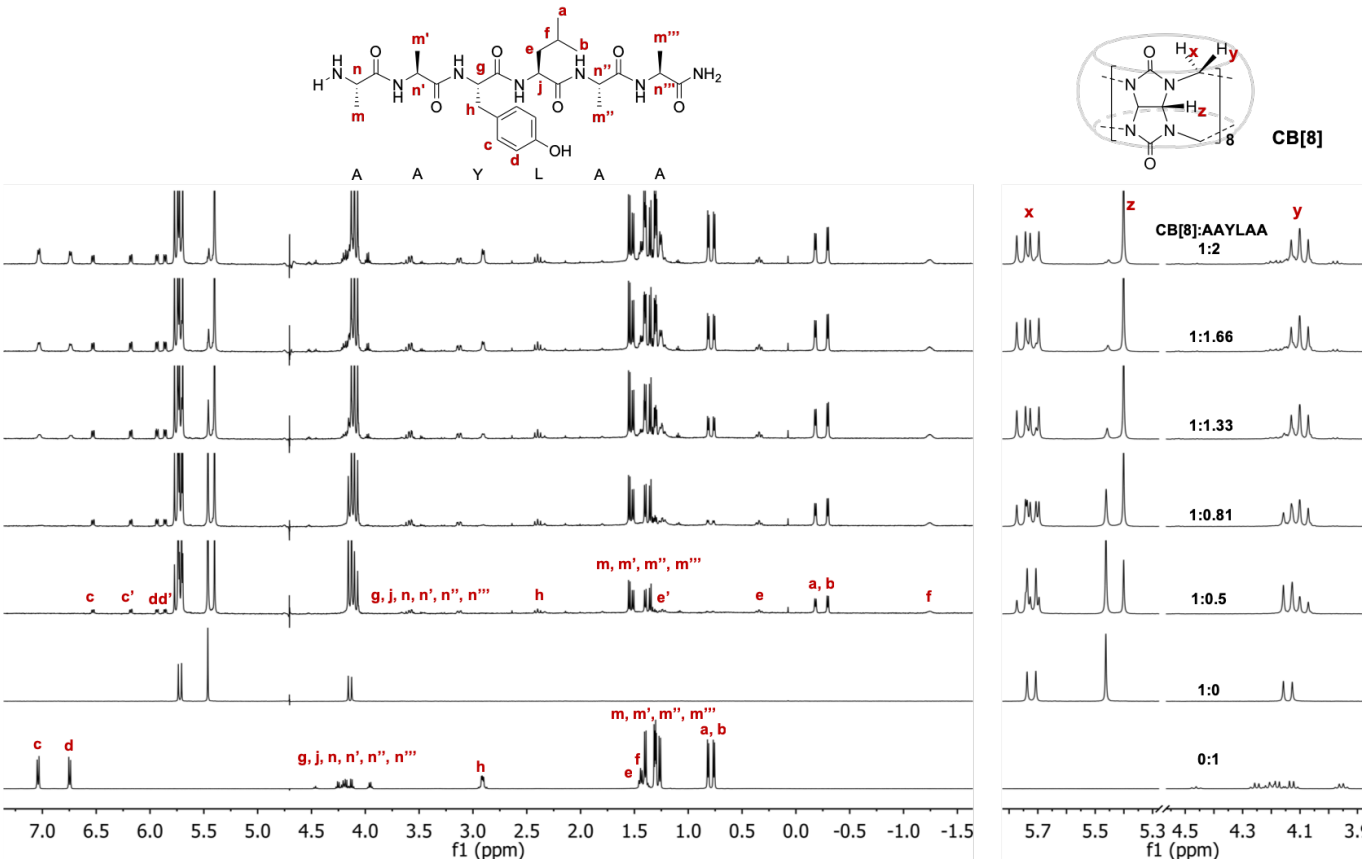


Figure S30. ^{1}H NMR of AAYLAA titrated into CB[8] until a ratio of 2:1. (500 MHz, 298 K, D_2O). The weaker binding requires excess of AAYLAA to consume all free CB[8].

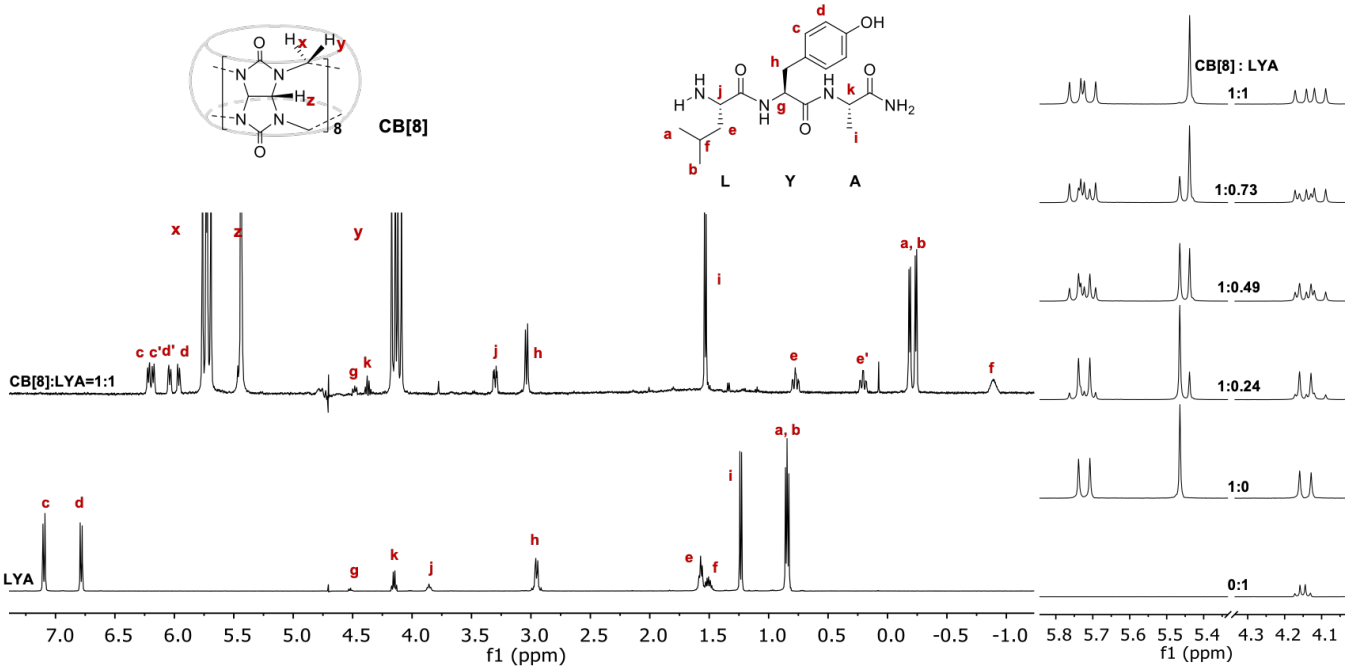


Figure S31. ^{1}H NMR of LYA titrated into CB[8] until a ratio of 1:1. (500 MHz, 298 K, D_2O).

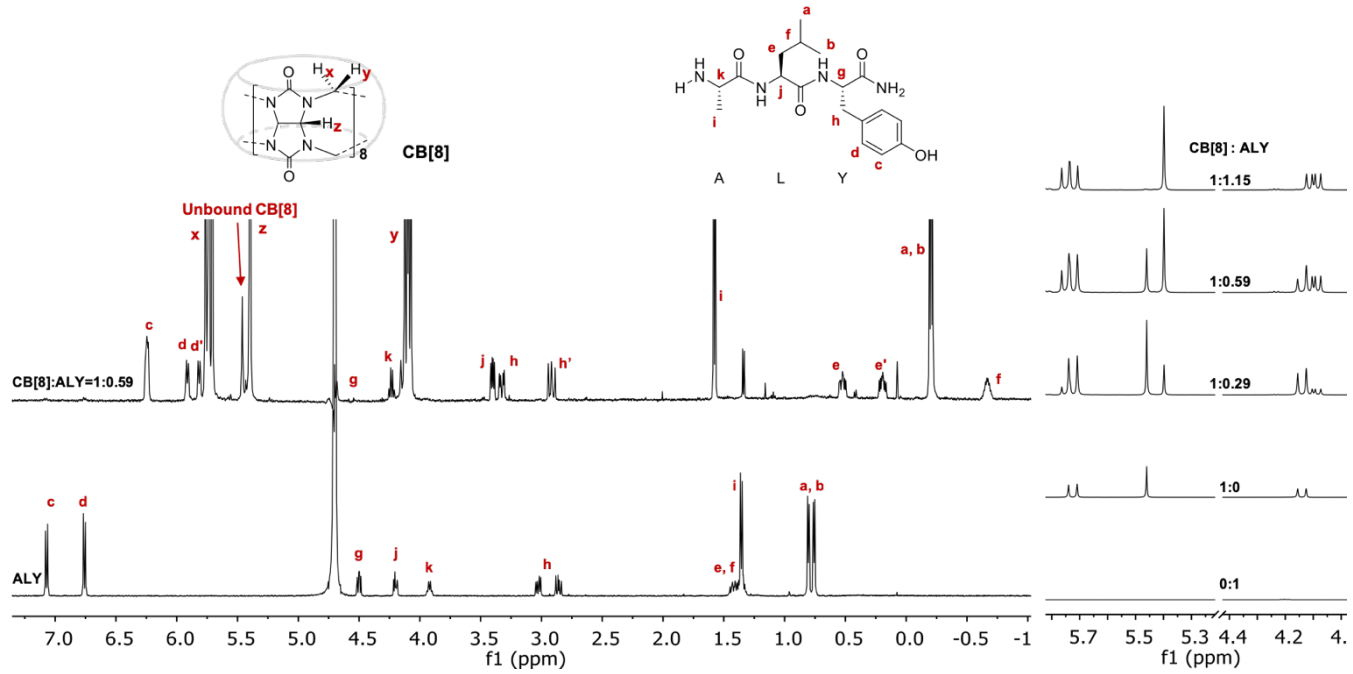


Figure S32. ^{1}H NMR of ALY titrated into CB[8]. (500 MHz, 298 K, D_2O).
The weaker binding requires excess of ALY to consume all free CB[8].

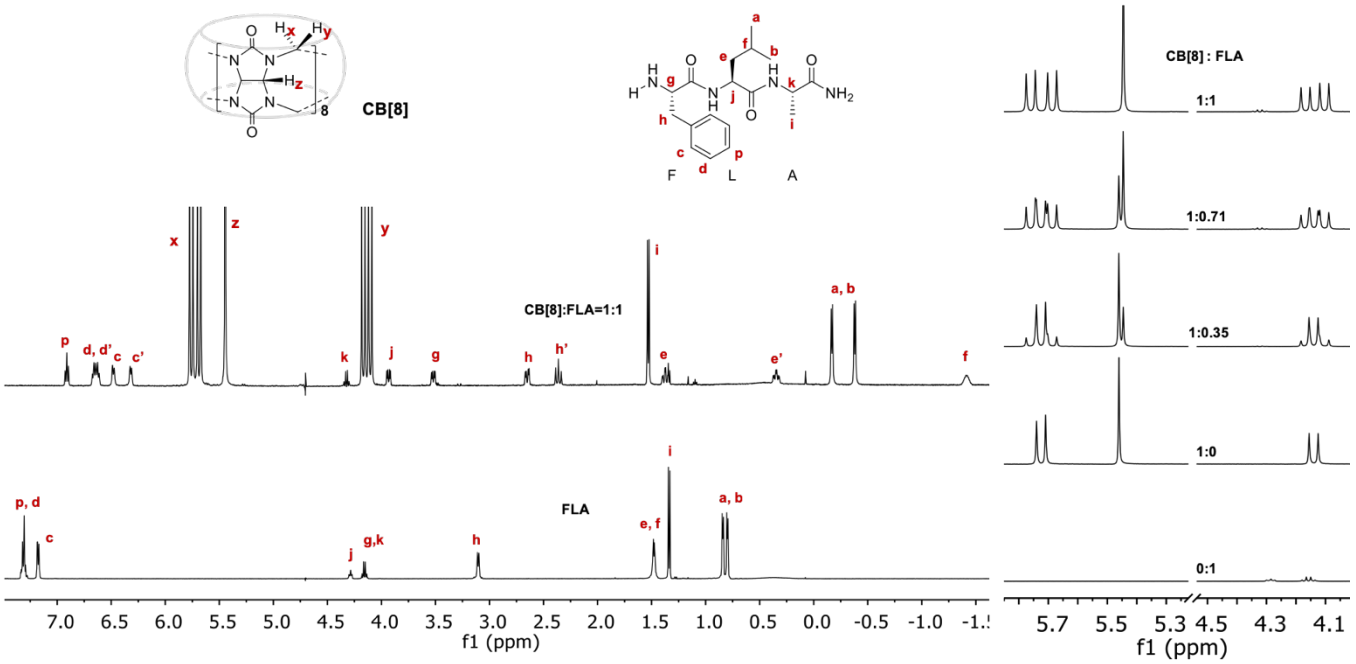


Figure S33. ^{1}H NMR of FLA titrated into CB[8] until a ratio of 1:1. (500 MHz, 298 K, D_2O).

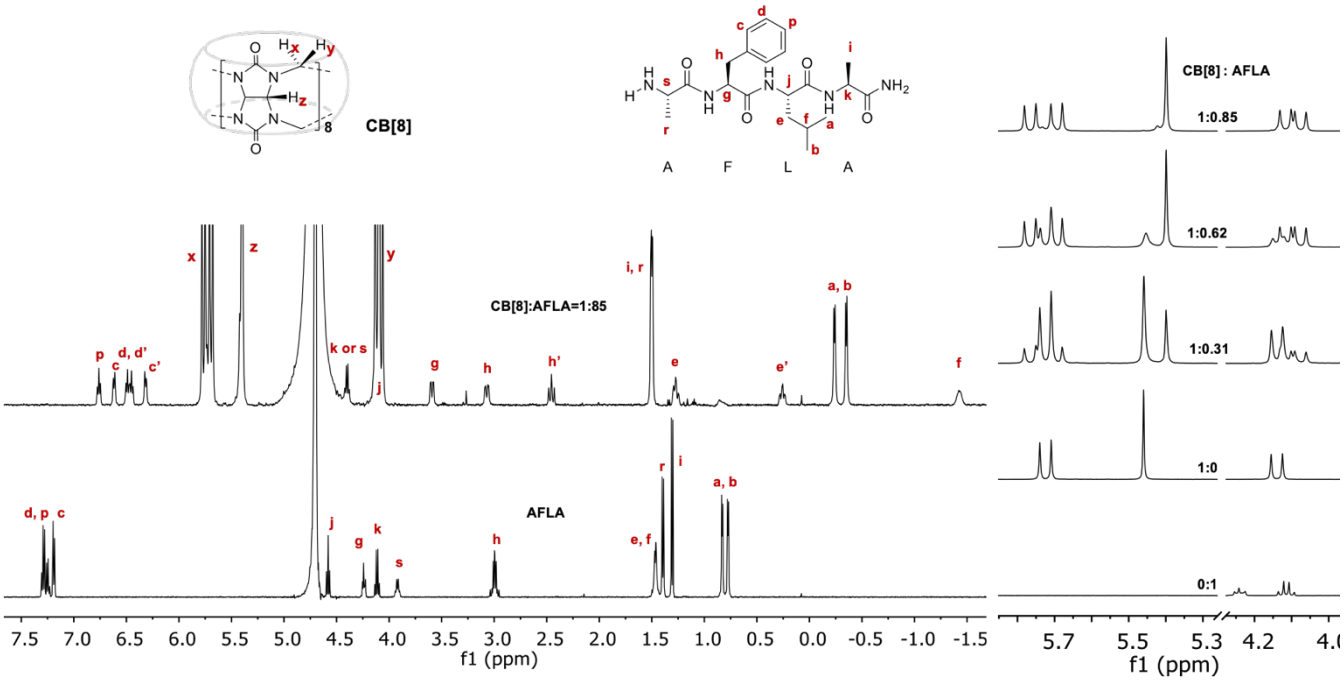


Figure S34. ^{1}H NMR of AFLA titrated into CB[8]. (500 MHz, 298 K, D_2O).

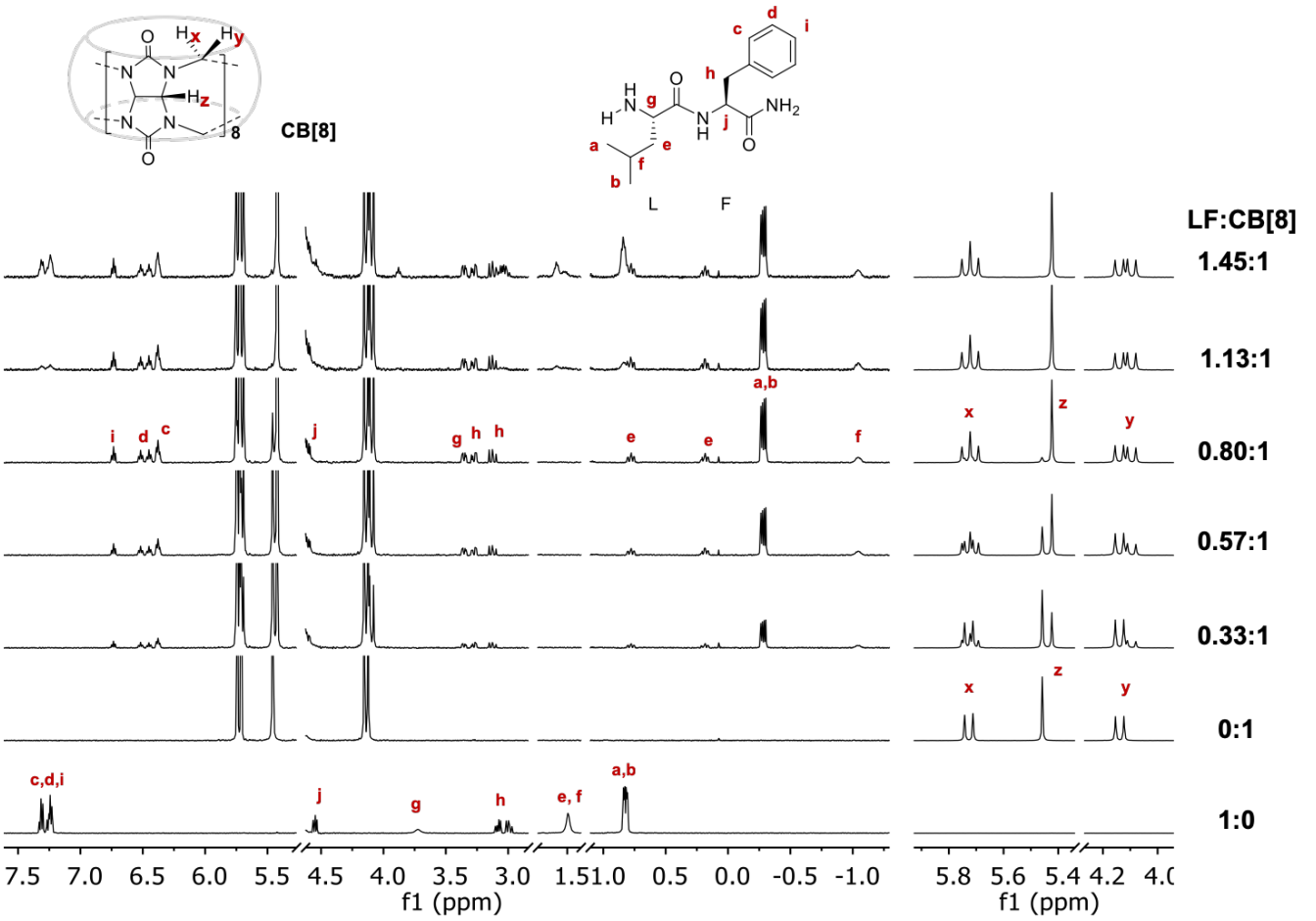


Figure S35. ^{1}H NMR of LF titrated into CB[8]. (500 MHz, 298 K, D_2O).

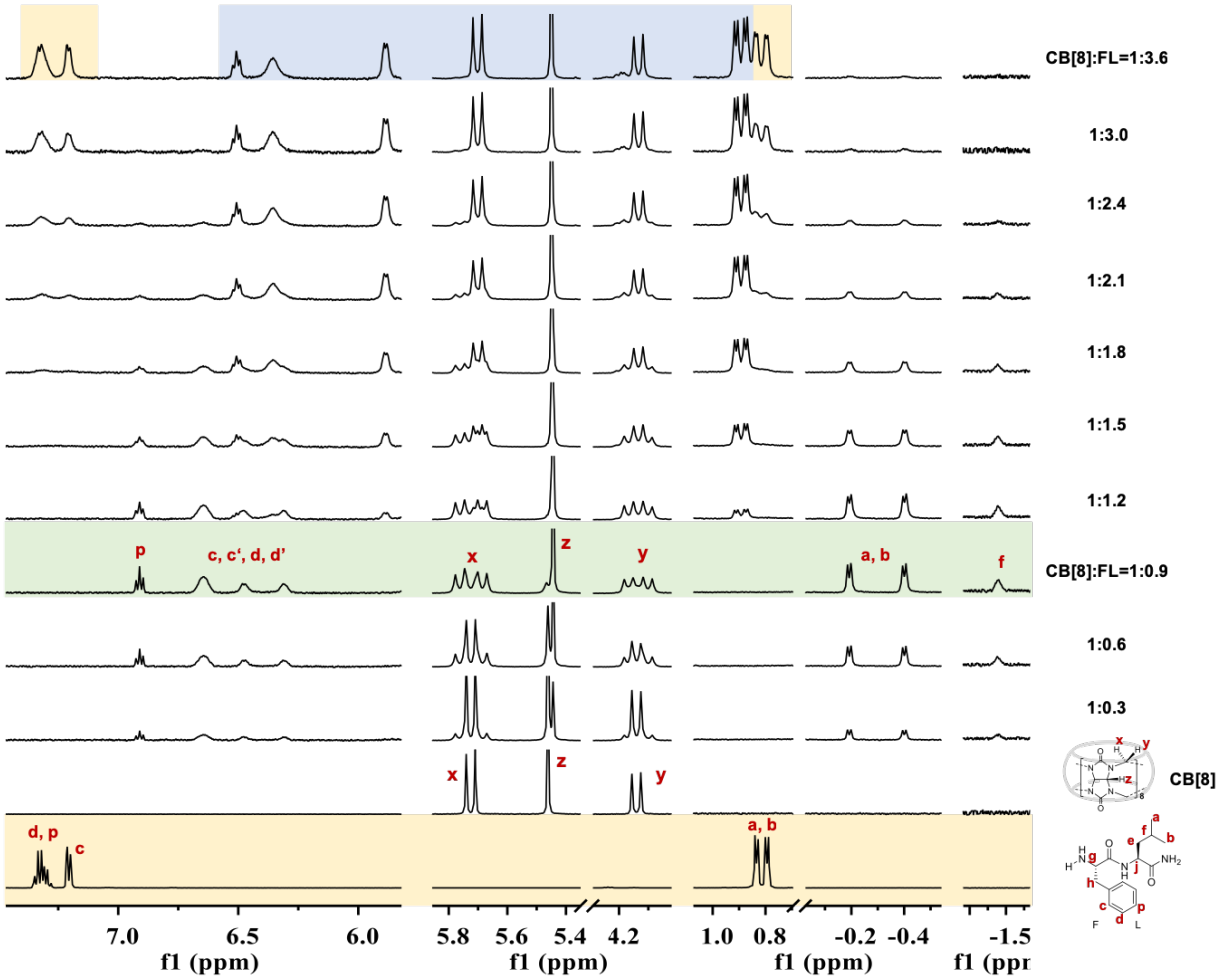


Figure S36. ^{1}H NMR of FL titrated into CB[8]. (500 MHz, 298 K, D_2O).

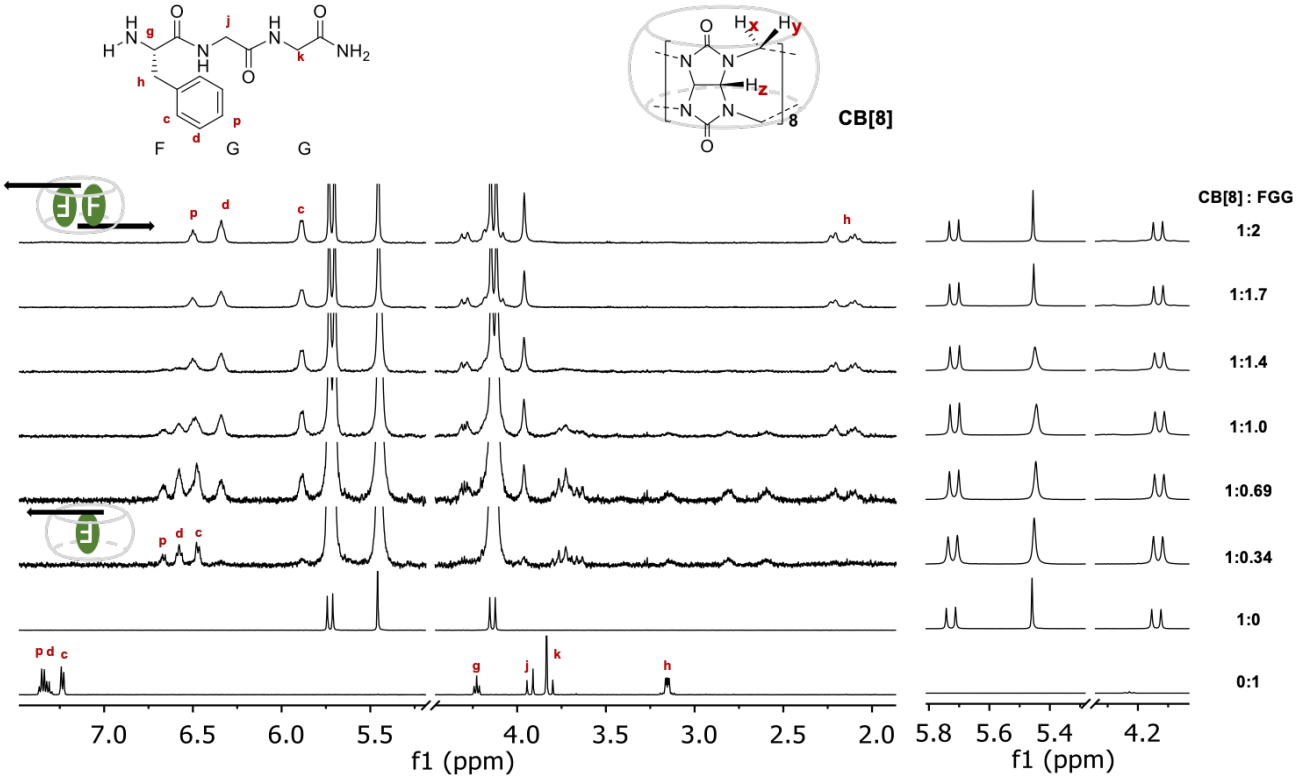


Figure S37. ^{1}H NMR of FGG titrated into CB[8]. (500 MHz, 298 K, D_2O).

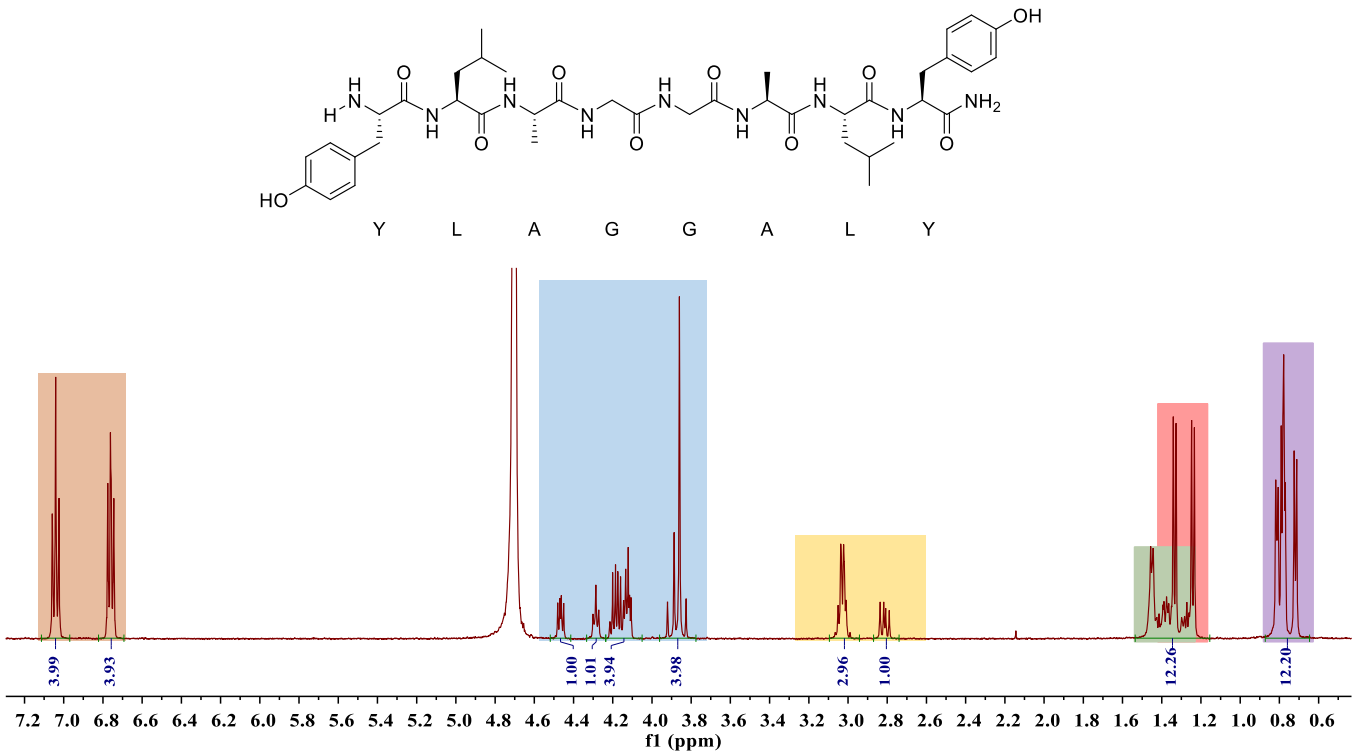


Figure S38. ^{1}H NMR of YLAGGALY. (500 MHz, 298 K, D_2O).

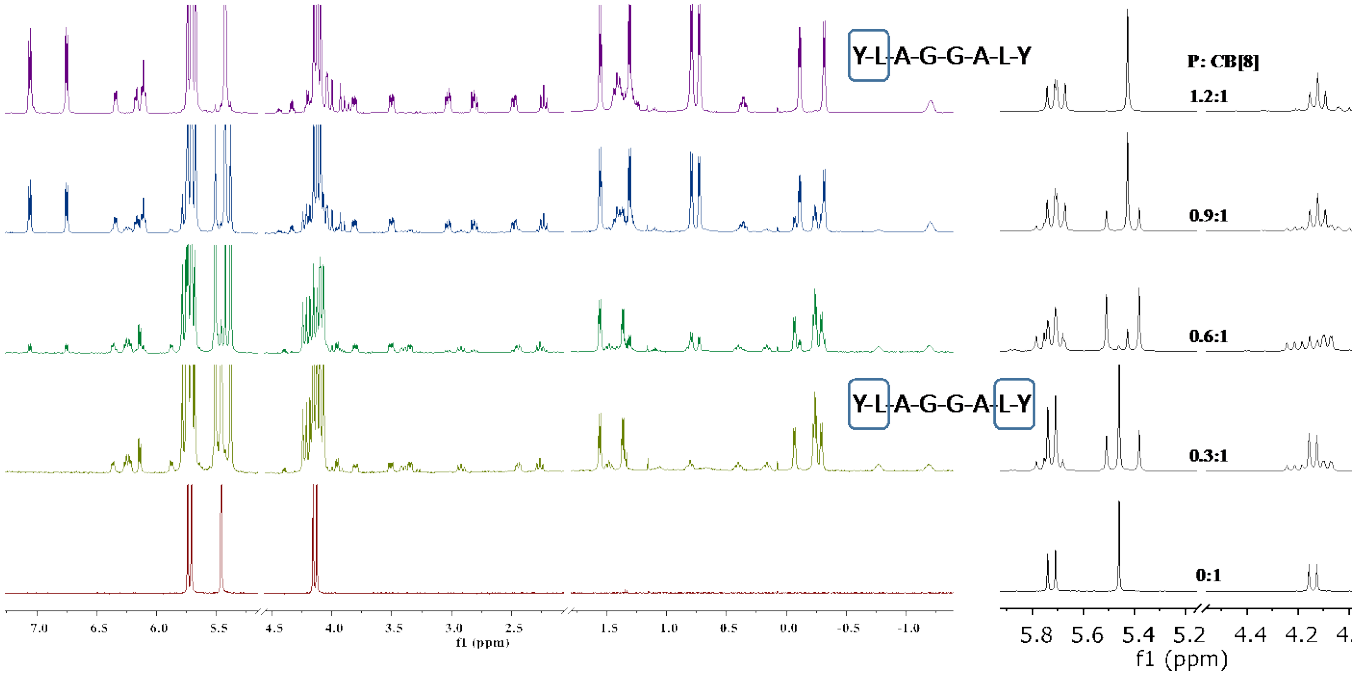


Figure S39. ^{1}H NMR of YLAGGALY titrated into CB[8]. (500 MHz, 298 K, D_2O).

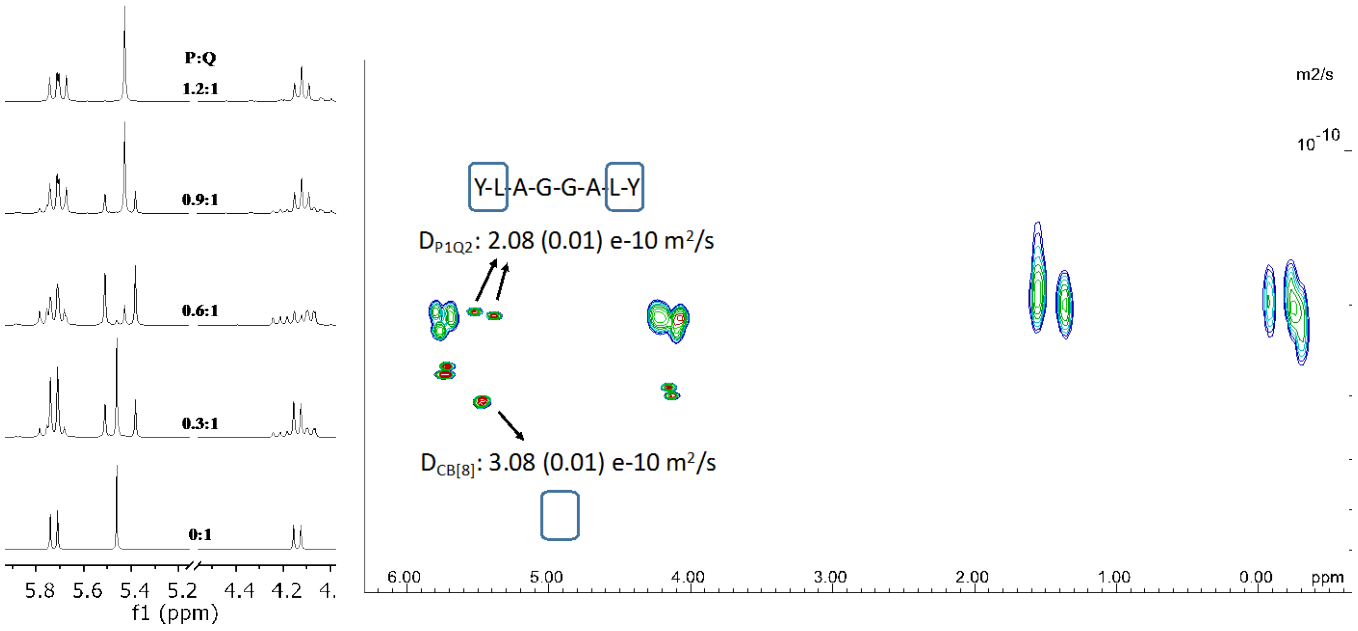


Figure S40. DOSY of YLAGGALY: CB[8] = 0.3:1. (500 MHz, 298 K, D_2O).

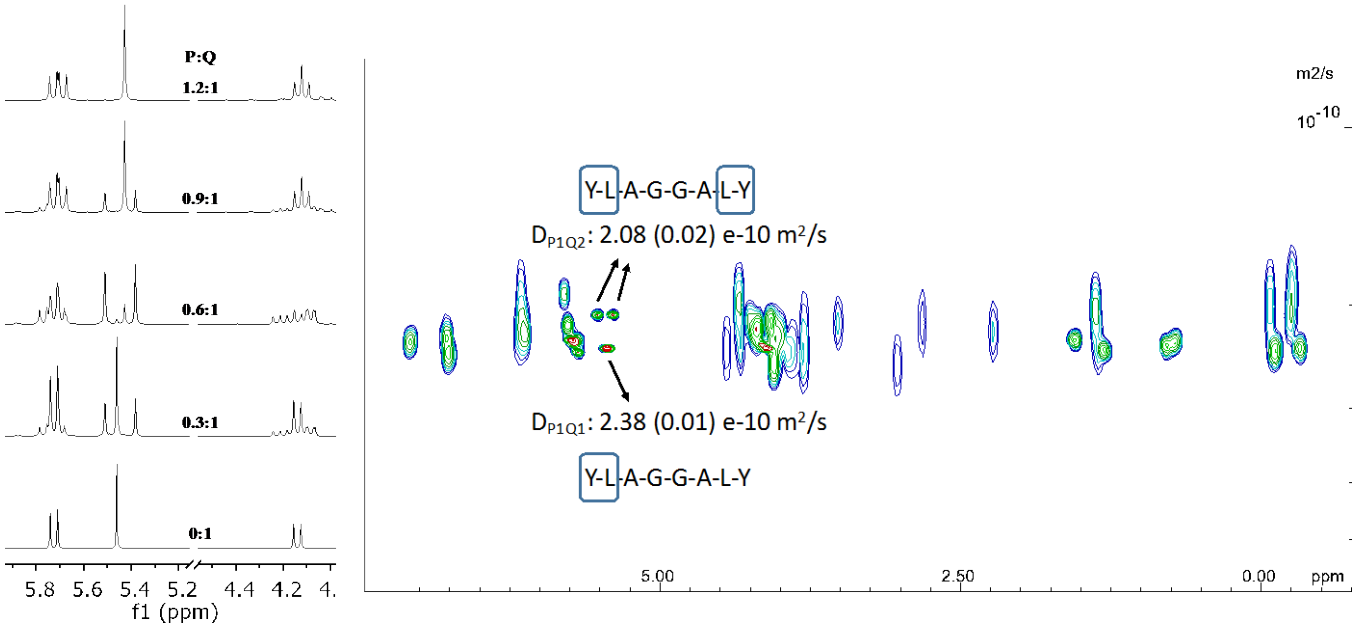


Figure S41. DOSY of YLAGGALY: CB[8] = 0.9:1. (500 MHz, 298 K, D_2O).

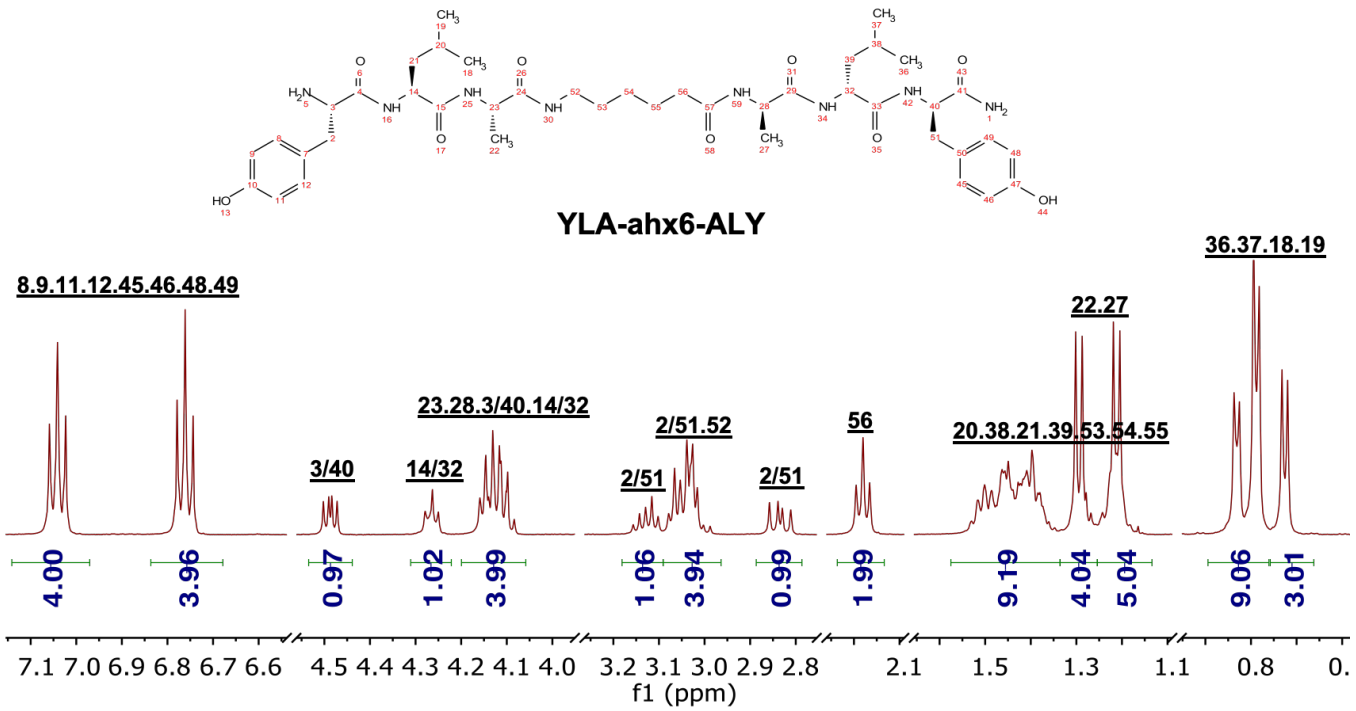


Figure S42. ^{1}H NMR of YLA-ahx6-ALY. (500 MHz, 298 K, D_2O).

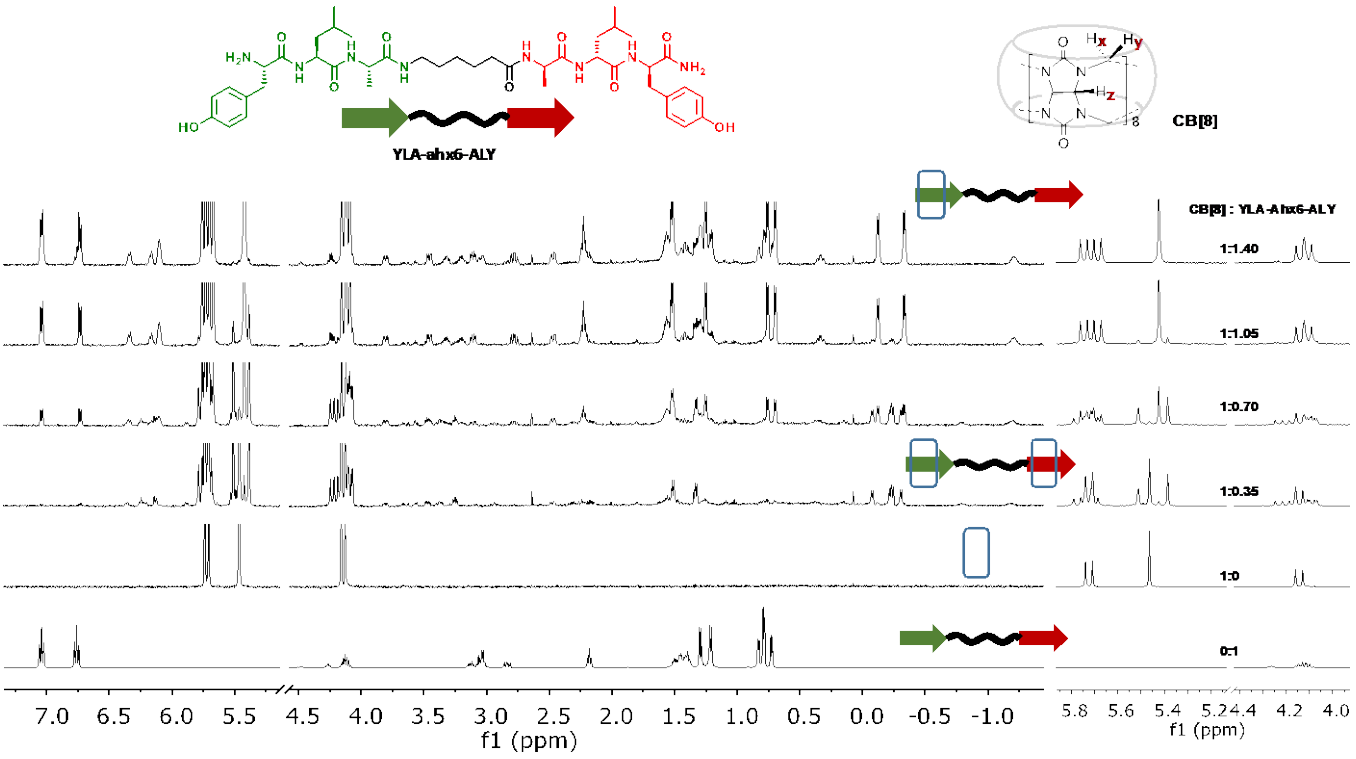


Figure S43. ^{1}H NMR of YLA-ahx6-ALY titrated into CB[8]. (500 MHz, 298 K, D_2O).

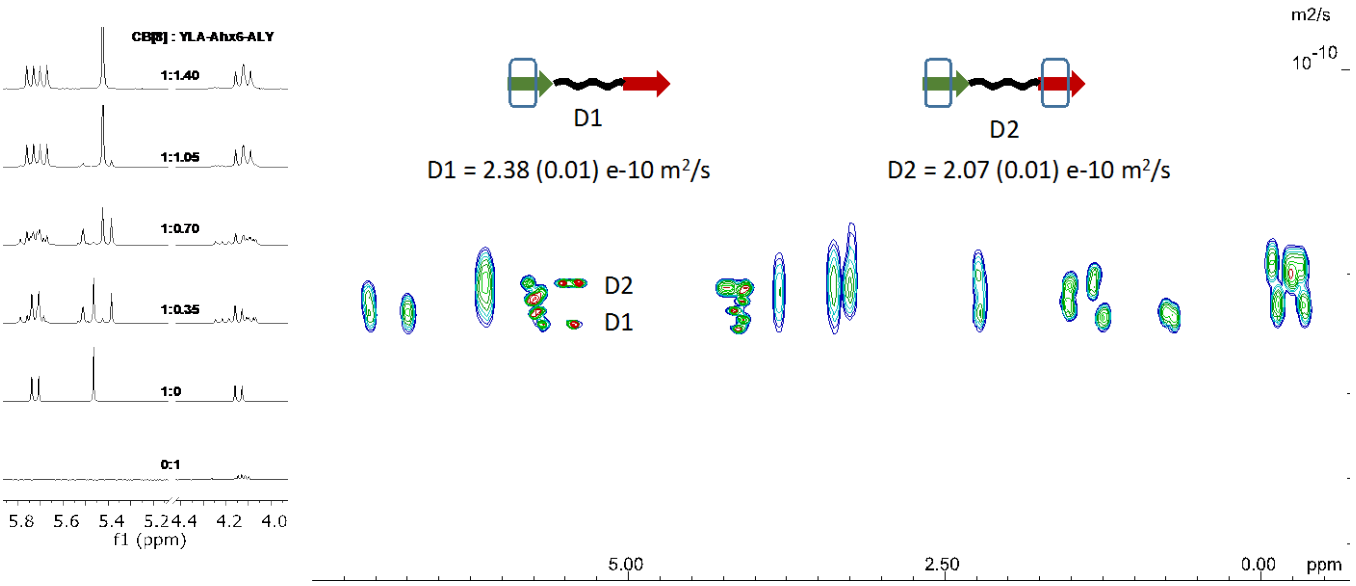


Figure S44. DOSY of YLA-ahx6-ALY: CB[8] = 0.70:1. (500 MHz, 298 K, D_2O).

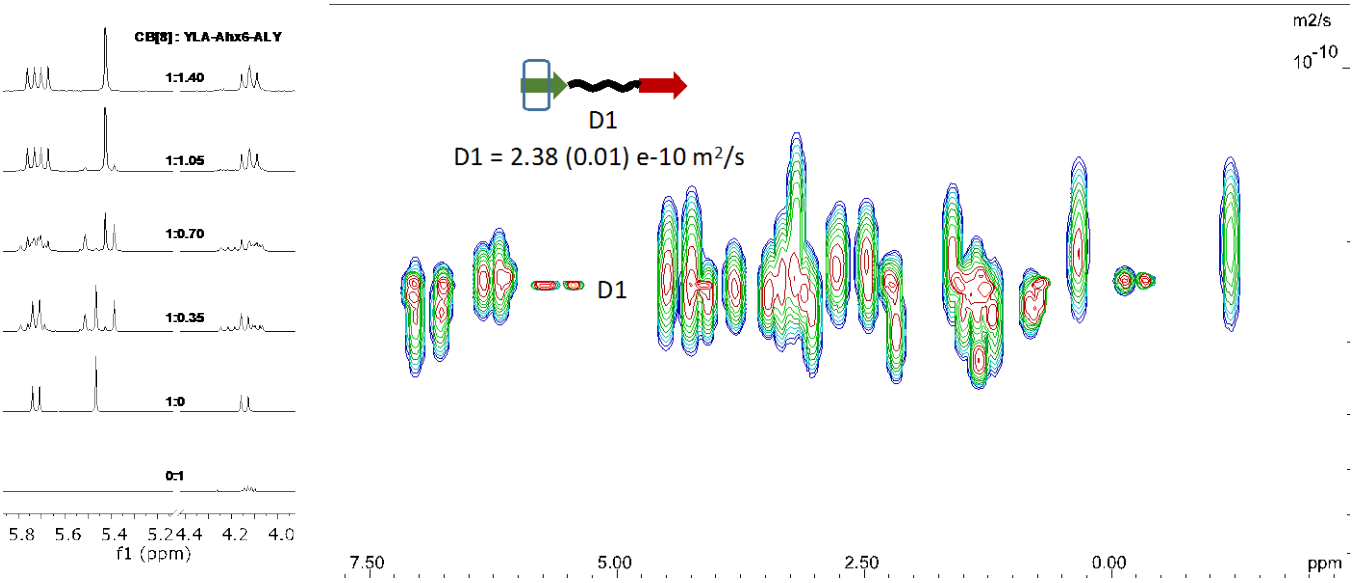


Figure S45. DOSY of YLA-ahx6-ALY: CB[8] = 1.40:1. (500 MHz, 298 K, D_2O).

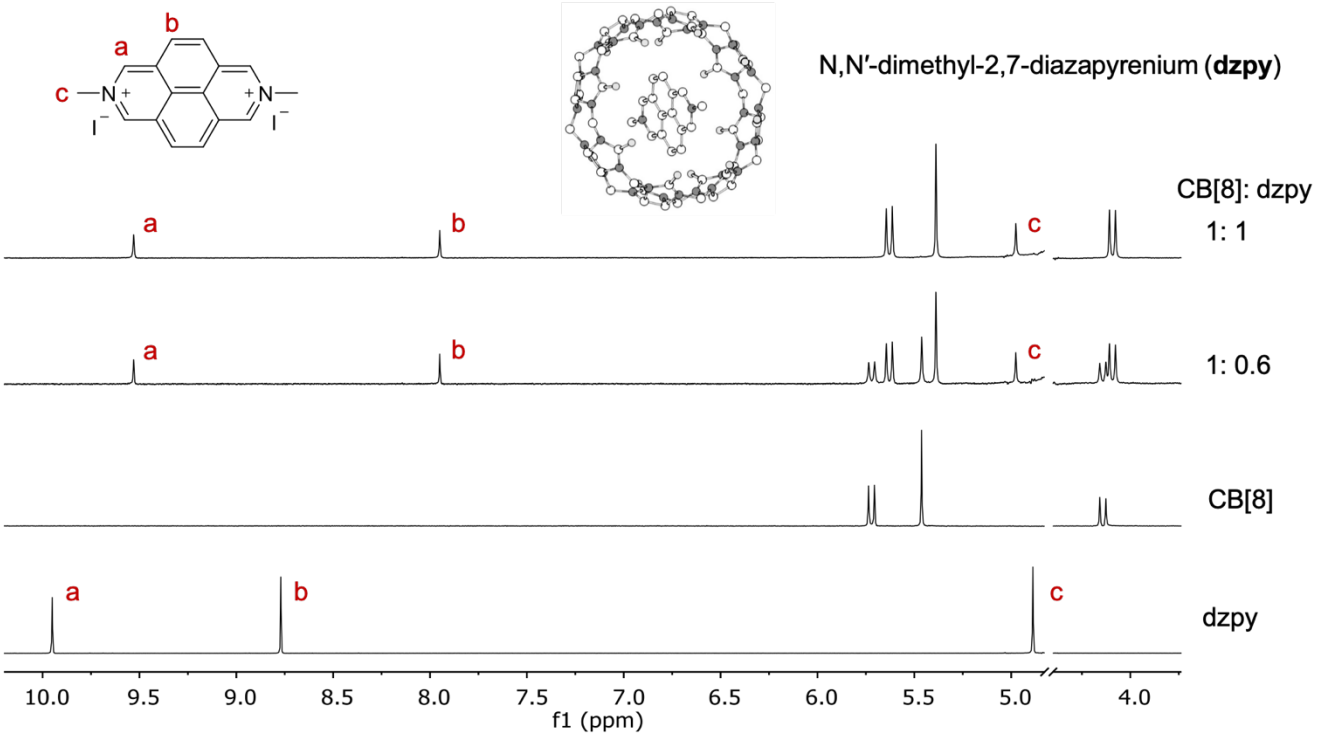


Figure S46. ^{1}H NMR of dzpy titrated into CB[8]. (500 MHz, 298 K, D_2O).

Reference:

[1] Day, A.; Arnold, A. P.; Blanch, R. J.; Snushall, B. *J. Org. Chem.* **2001**, *66* (24), 8094–8100.

[2] Brautigam, C. A.; Zhao, H.; Vargas, C.; Keller, S.; Schuck, P. *Nat. Protocols* **2016**, *11* (5), 882–894.