

# Probing the Stability of Multicomponent Self-Assembled Architectures based on Cucurbit[8]uril in the Gas Phase

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## S.1 Synthesis

**General:** All solvents and reagents were used as supplied. O-(benzotriazol-1-yl)-N,N,N,N-tetramethyluronium-hexafluoro-phosphate (HBTU) was purchased from CEM. Fmoc protected amino acids, DMF and NMP were purchased from AGTC Bioproducts. Rink Amide ChemMatrix resin was purchased from NovaBiochem. All other chemicals were purchased from Sigma-Aldrich.

### Synthesis of trimer 1

**Synthesis of 1-(8-bromooctyl)-[4,4'-bipyridin]-1-ium bromide.** 4,4'-bipyridine (5 g, 32 mmol) and 1,8-dibromooctane (17.4 g, 64 mmol) were dissolved in EA and stirred at 50 °C for one week. Fractions of precipitated product were collected every 24 h, washed with EA and dried in vacuum. The resulting off-white powder (9.25g, 68% yield) was used in the next step without any further purification. <sup>1</sup>H NMR (d<sub>6</sub>-DMSO): δ 9.30 (d, 2H), 8.90 (d, 2H), 8.68 (d, 2H), 8.08 (d, 2H), 4.68 (t, 2H), 3.55 (t, 2H), 2.00 (m, 2H), 1.82 (m, 2H), 1.43 (m, 8H). <sup>13</sup>C NMR (d<sub>6</sub>-DMSO): δ 152.2, 150.9, 145.3, 140.8, 125.4, 121.9, 60.3, 35.2, 32.1, 30.6, 28.2, 27.8, 27.3, 25.3; HRMS (*m/z*): [M]<sup>+</sup> (without counterions) calculated 347.1123; found 347.1118.

**Synthesis of 1-(8-azidoctyl)-[4,4'-bipyridin]-1-ium bromide.** A mixture of 3.2 g (7.5 mmol) 1-(8-bromooctyl)-[4,4'-bipyridin]-1-ium bromide and 1.3 g (20 mmol) NaN<sub>3</sub> in water was stirred for 2 h at 80°C. The solvent was subsequently removed and the remaining oil dissolved in acetonitrile and filtered to remove any solids. The product (2.3 g, 79% yield) was used without further purification. <sup>1</sup>H NMR

(d<sub>6</sub>-DMSO):  $\delta$  9.30 (d, 2H), 8.93 (d, 2H), 8.70 (d, 2H), 8.11 (d, 2H), 4.70 (t, 2H), 3.37 (t, 2H), 2.02 (t, 2H), 1.58 (t, 2H), 1.37 (s, 8H). <sup>13</sup>C NMR (d<sub>6</sub>-DMSO):  $\delta$  152.2, 150.9, 145.3, 140.8, 125.4, 121.9, 60.3, 50.6, 30.6, 28.2, 28.1, 26.0, 25.3; HRMS (*m/z*): [M]<sup>+</sup> (without counterions) calculated 310.2032; found 310.2012.

#### Synthesis of tris-[[4-(8-(4,4'-bipyridinium-1-yl)-octan-1-yl)-1H[1,2,3]triazol-1-yl]methyl]amine tribromide.

A mixture of 1-(8-azidoctyl)-[4,4'-bipyridin]-1-ium bromide (2.3g, 5.9 mmol), trispropargylamine (242 mg, 1.8 mmol) and CuI (113 mg, 0.6 mmol) in 18 ml DMF was stirred at 60°C for 2 days. After removal of the solvent the reaction mixture was titrated with water. The water phase was filtered and evaporated. The remaining solid was re-dissolved in DMF and reacted with 3 g azide modified Merrifield resin<sup>1</sup> and 60 mg CuI at at 60°C for 24 h. The DMF solution was then filtered and evaporated, the remaining oil titrated with water. After filtration and evaporation, the remaining solid was dissolved in MeCN/MeOH and filtered through a plug of neutral aluminum oxide. The product was obtained as light brown solid. (1.5 g, 64% yield). <sup>1</sup>H NMR (d<sub>6</sub>-DMSO):  $\delta$  9.28 (d, 6H), 8.92 (br, 6H), 8.68 (d, 6H), 8.09 (s, 6H), 8.08 (s, 3H), 4.66 (t, 6H), 4.37 (t, 6H), 3.64 (s, 6H), 1.98 (t, 6H), 1.85 (s, 6H), 1.32 (s, 24H). <sup>13</sup>C NMR (d<sub>6</sub>-DMSO):  $\delta$  152.6, 151.2, 145.7, 125.8, 60.7, 30.6, 29.6, 28.2, 28.1, 25.7, 25.3; HRMS (*m/z*): [M]<sup>3+</sup> (without counterions) calculated 353.8943; found 353.8912.

#### Synthesis of Methyl-tris-[4-(8-(1'-methyl-4,4'-bipyridinium-1-yl)octan-1-yl)-1H[1,2,3]triazol-1-yl]- methylammonium tribromide tetraiodide (1)

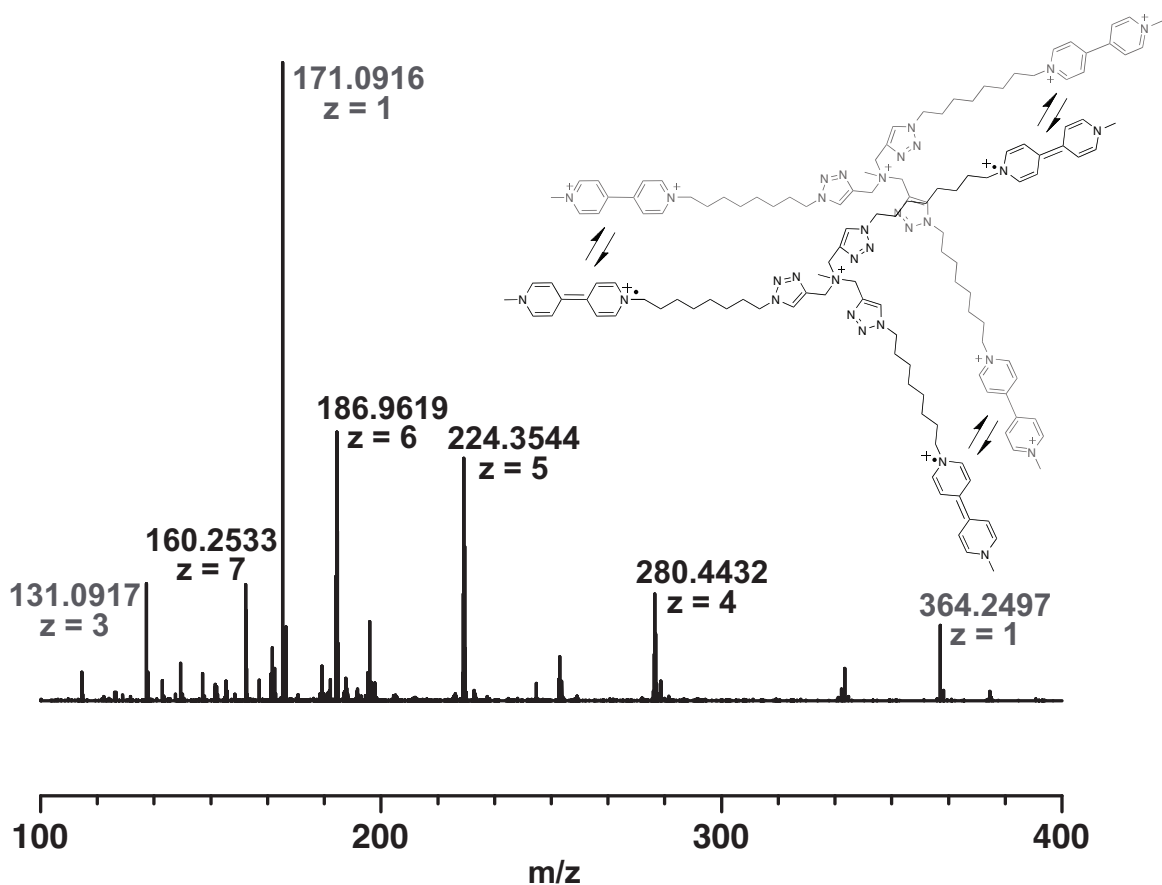
. 1g of tris-[[4-(8-(4,4'-bipyridinium-1-yl)-octan-1-yl)-1H[1,2,3]triazol-1-yl]methyl]amine tribromide (0.77 mmol) was stirred with excess methyl iodide (1.1g, 7.7mmol) in EtOH at 43°C for 48 h. The red precipitate was filtered and washed several times with hot EtOH to yield the product (1 g, 69% yield). <sup>1</sup>H NMR (d<sub>6</sub>-DMSO):  $\delta$  9.45 (d, 6H), 9.33 (d, 6H), 8.83 (m, 12H), 8.62 (s, 3H), 4.75 (t, 6H), 4.64 (s, 6H), 4.50 (m, 18H), 2.0 (b, 6H), 1.91 (t, 6H), 1.32 (s, 24H); <sup>13</sup>C NMR (d<sub>6</sub>-DMSO):  $\delta$  148.4, 148.1, 146.5, 145.6, 134.7, 128.7, 126.4, 126.1, 60.7, 50.1, 48.1 30.7, 29.6, 28.2, 25.8, 25.4; HRMS (*m/z*): [M]<sup>7+</sup> (without counterions) calculated 160.2533; found 160.2533 (see FigureS1).

#### Synthesis of trimer 2

**1,3,5-Benzenetricarbonyl triazide** was prepared according to literature procedures as a 0.1 M solution in toluene.<sup>2</sup>

#### Synthesis of Benzene-1,3,5-tricarbamic acid, tris[(1'-methyl-4,4'-bipyridinium)-1-ethyl] ester hexachloride (2) in analogy to.

<sup>3</sup> 25 mL of the 0.1 mM 1,3,5-Benzenetricarbonyl triazide (2.5 mmol) was refluxed for 2 hours (the conversion can be followed by FTIR) under nitrogen. Hereafter, the solvent was removed under reduced pressure. Anhydrous acetonitrile (50 mL) was added and the solid was redissolved in a N<sub>2</sub> atmosphere. 1-(2-hydroxyethyl)-1methyl-[4,4bipyridine]-1,1dium di(hexafluorophosphate) (4.0 g, 7.9 mmol) was added as a solid at once. To the clear solution, a drop of dibutyltindilaurate was added and the solution was stirred at room temperature for 24 hours. The conversion can be followed by FTIR (disappearance of the CO stretch). Upon full conversion, tetrabutyl ammonium chloride (5.6 g, 20 mmol) dissolved in a minimum amount of acetonitrile was added to the solution and a precipitate formed. The



**Figure S1:** Trimer 1: signals appear for species in charge states 4+ to 7+, presumably due to rearrangement. Numbers in grey indicate fragments.

precipitate was collected by suction filtration, washed with 50 mL acetonitrile and 50 mL of acetone and then dried in a vacuum oven. Recrystallization from ethanol/water mixtures yielded the title compound: 1.5 g (50%).  $^1\text{H}$  NMR ( $d_6$ - $\text{D}_2\text{O}$ ):  $\delta$  9.23 (d, 6H), 9.10 (d, 6H), 8.62 (d, 6H), 8.54 (d, 6H), 7.11 (s, 3H), 5.13 (d, 6H), 4.78 (d, 2H), 4.55 (s, 3H).  $^{13}\text{C}$  NMR ( $d_6$ - $\text{D}_2\text{O}$ ):  $\delta$  154.49, 151.15, 149.94, 146.73, 146.56, 139.02, 127.42, 127.10, 63.52, 61.15, 48.82. HRMS of the pure substance was not possible due to immediate fragmentation on account of charge repulsion. HRMS of trimer **2**·CB[8]<sub>3</sub> ( $m/z$ ):  $[\text{M}]^{6+}$  (without counterions) calculated 805.7618; found 805.7591.

**Synthesis of Trp-Gly-Gly-Gly-Gly (WG5)** . Solid phase peptide synthesis was performed on a Liberty Microwave Peptide Synthesizer (CEM Corporation) using Fmoc-strategy. The Fmoc group was removed in each step with 20% v/v piperidine in DMF for 3 min using a microwave power of 45 W. The maximum temperature was set to 75°C. The coupling step was performed with 5 equivalents of Fmoc protected amino acid in DMF (0.2 M), 4.5 equivalents of HBTU in DMF (0.45 M) and 10 equivalents of DIPEA in NMP (2 M). All couplings were performed for 10 min at 25 W at a maximum temperature of 75°C. Following completion of the sequence the peptide was released from the resin with concomitant removal of side chain protecting groups by treatment with TFA/thioanisole/phenol/ $\text{H}_2\text{O}$ /3,6-dioxo-1,8-octane-dithiol (82.5/5/5/5/2.5, v/v/v/v/v) at room temperature for 3 h. The crude peptide was precipitated

with cold (-20°C) diethyl ether, isolated by centrifugation and washed with cold diethyl ether. The crude product was purified on a Varian 940-LC using a preparative Varian Polaris C8 column applying a linear gradient of 5% to 95% B in 30 min. The mobile phases were 0.1% TFA in H<sub>2</sub>O (eluent A) and 0.1% TFA in MeCN (eluent B). <sup>1</sup>H NMR (D<sub>2</sub>O): δ 7.67 (d, 1H), 7.56 (d, 1H), 7.38 (s, 1H), 7.31 (t, 1H), 7.23 (t, 1H), 4.39 (t, 1H), 3.85-4.01 (m, 10H), 3.45 (d, 2H). HRMS [M+H]<sup>+</sup> calculated 489.2205; found 489.2210.

PEGylated second guests were synthesized according to a previous report.<sup>3</sup>

## S.2 mass spectrometry

**General:** Mass Spectrometry was performed on an LTQ Velos Orbitrap (Thermo Scientific Fisher), equipped with HESI probe and an infusion pump. Spectra were recorded with the highest possible resolution (100000). Signals in the spectra in the main text and below are shown without decimal places for clarity. The HESI temperature was set to 40°C, a spray voltage of 3.8kV was used for an infusion rate of 5 μl/min. Samples were prepared from aqueous solutions of all components to yield a final concentration of 50 μM of the ternary complex under investigation.

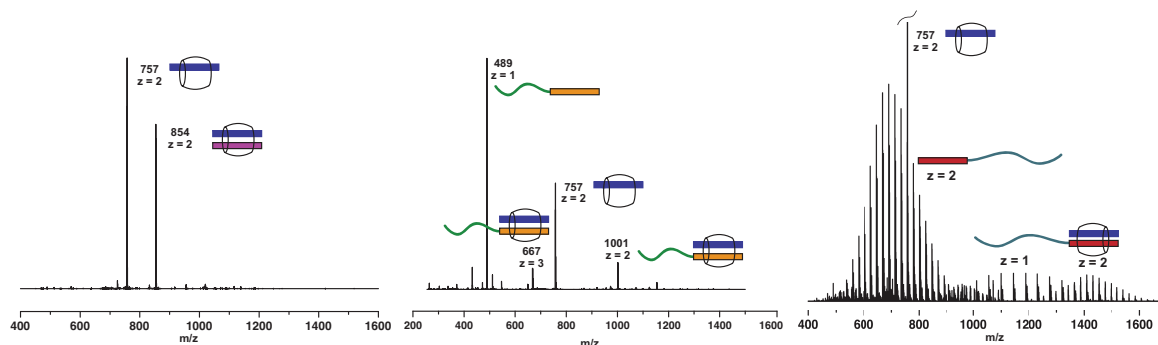


Figure S2: Full spectrum of MV·CB[8] with Ant-NH<sub>2</sub>, WG5 and Np-PEG.

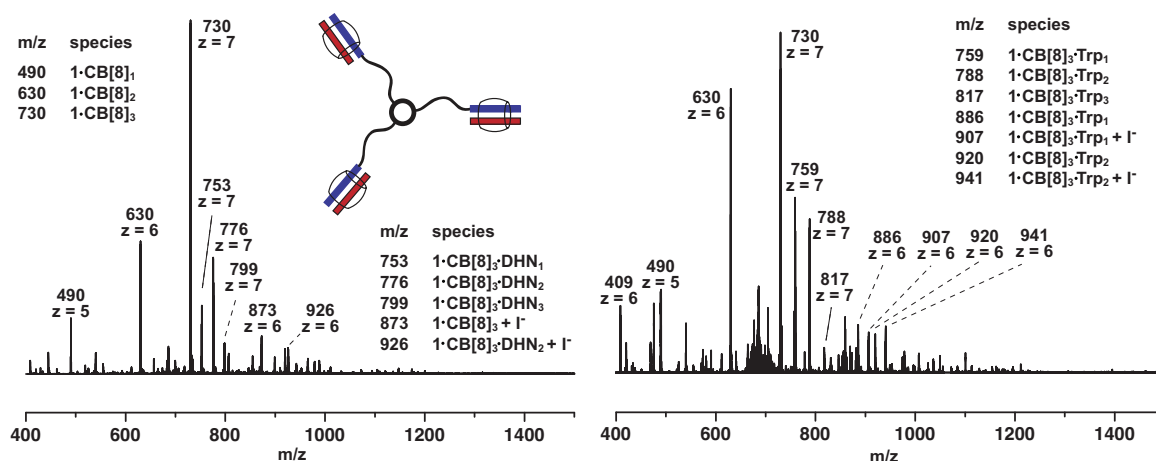
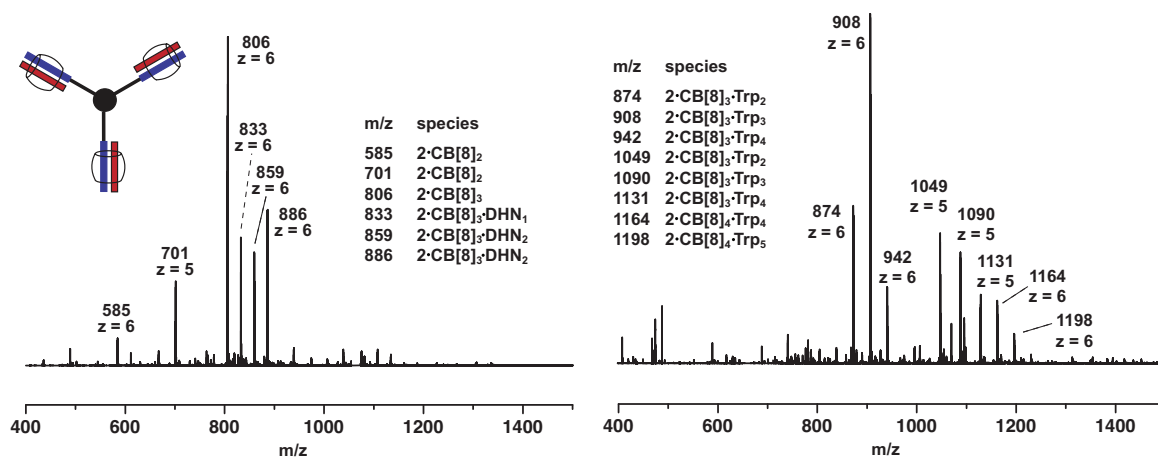
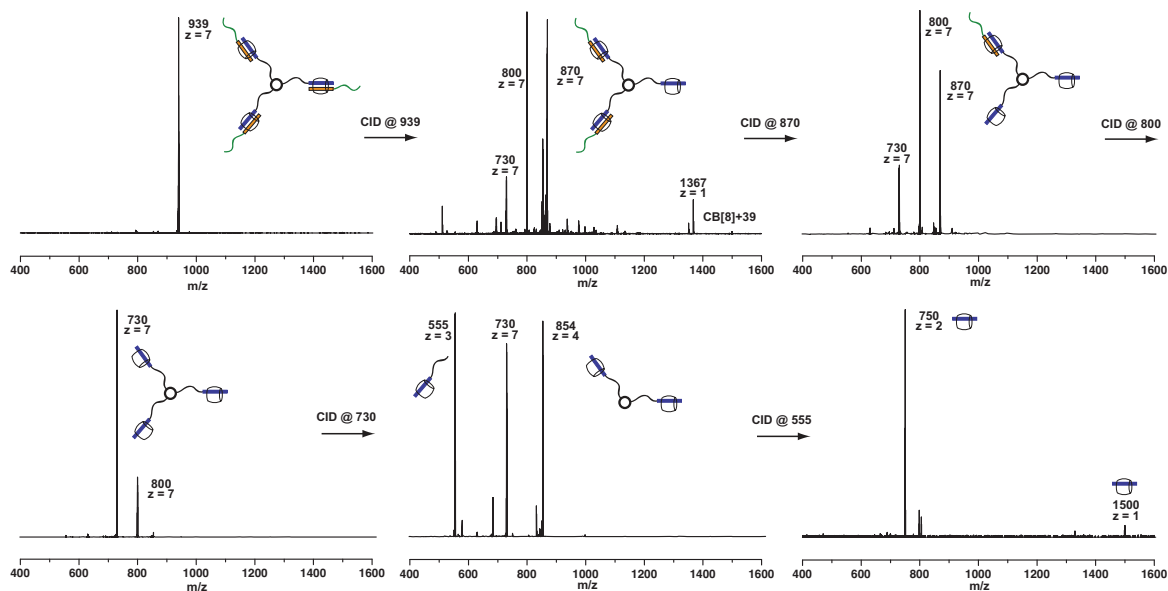


Figure S3: Full spectra of trimer 1·CB[8]<sub>3</sub>·DHN<sub>3</sub> and 1·CB[8]<sub>3</sub>·Trp<sub>3</sub>.



**Figure S4:** Full spectra of trimer 2-CB[8]<sub>3</sub>·DHN<sub>3</sub> and 2-CB[8]<sub>3</sub>·Trp<sub>3</sub>. Only for the self-assembly mixture containing trimer 2 and Trp aggregates beyond the expected stoichiometry were found.



**Figure S5:** CID experiments of 1-CB[8]<sub>3</sub>·WG<sub>53</sub> illustrating the stepwise dissociation pattern.

1 <sup>st</sup> guest	2 <sup>nd</sup> guest	E <sub>50</sub>
MV	DHN	4.5
MV	Trp	4
MV	Ant-NH <sub>2</sub>	5.5
MV	Flu-NH <sub>2</sub>	4.5
MV	WG5	17
MV	Np-PEG	22.5
MV	Ant-PEG	24
MV	Azo-PEG	23.5
MV	Flu-PEG	24
MV	DBF-PEG	23.5
<b>1</b>	DHN <sub>1-3</sub>	2.5
<b>1</b>	Trp <sub>1-3</sub>	2.5
<b>1</b>	WG5 <sub>1-3</sub>	23-24.5
<b>1</b>	Ant-PEG <sub>1-2</sub>	37-38
<b>1</b>	Np-PEG <sub>1</sub>	37
<b>2</b>	DHN <sub>1-3</sub>	3.5
<b>2</b>	Trp <sub>1-3</sub>	6.5-8
<b>2</b>	WG5 <sub>1-3</sub>	31.5-35

**Table S1:** dissociation energies of ternary complexes.

## References

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- [2] van Gorp, J.; Vekemans, J.; Meijer, E. *J. Am. Chem. Soc.* **2002**, *124*, 14759–14769.
- [3] Biedermann, F.; Appel, E. A.; del Barrio, J.; Gruending, T.; Barner-Kowollik, C.; Scherman, O. A. *Macromolecules* **2011**, *44*, 4828–4835.