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Supporting Information

for

Relative Binding Affinities of Molecular Capsules investigated by ESI-MS

By

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Additional ESI-MS data:

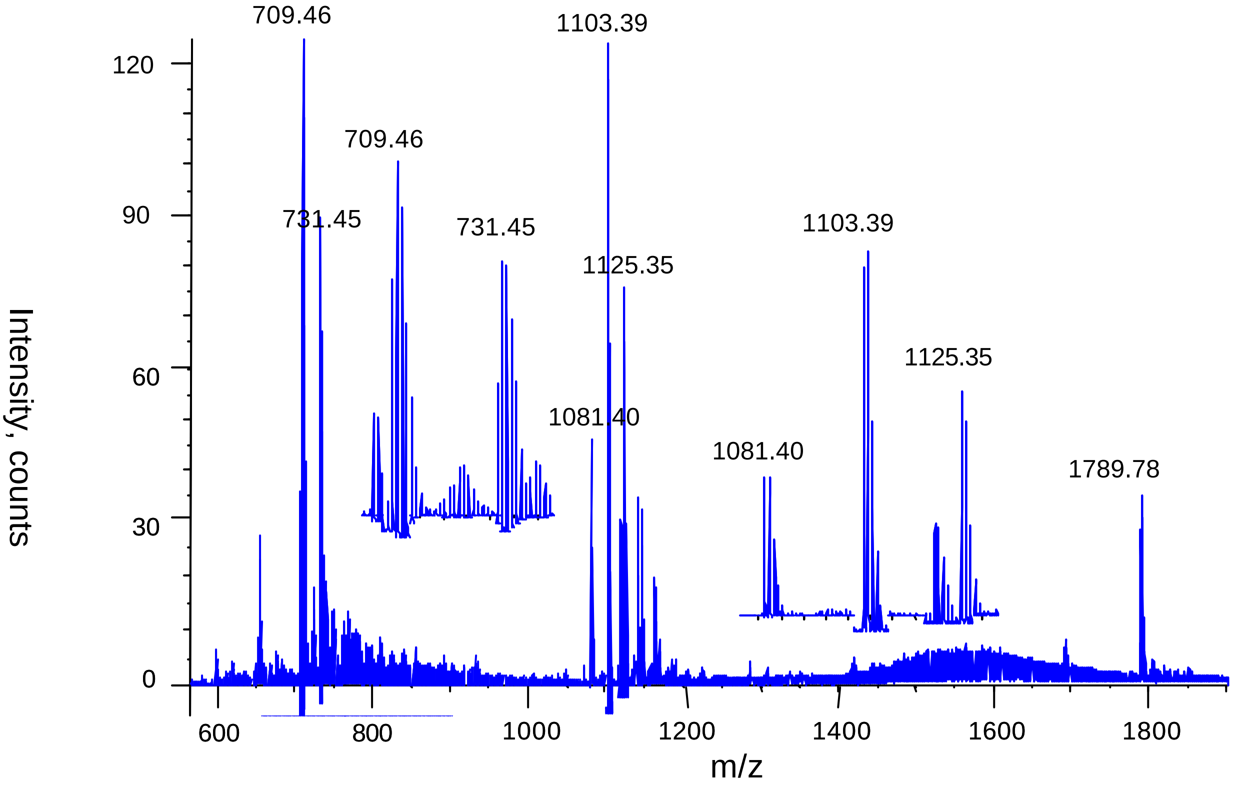


Figure 1: ESI-MS spectrum of the single capsule **1+4.** The spectrum was recorded in the positive ion mode. The calculated masses are: [**4** - 3H$^+$]$^+$: 709.47; [**1** + 5H$^+$]$^+$: 1081.47; [**1+4** + H$^+$]$^+$: 1789.88; all other peaks result from H$^+$/Na$^+$ exchange. No ion peaks were found beyond *m/z* = 1800.

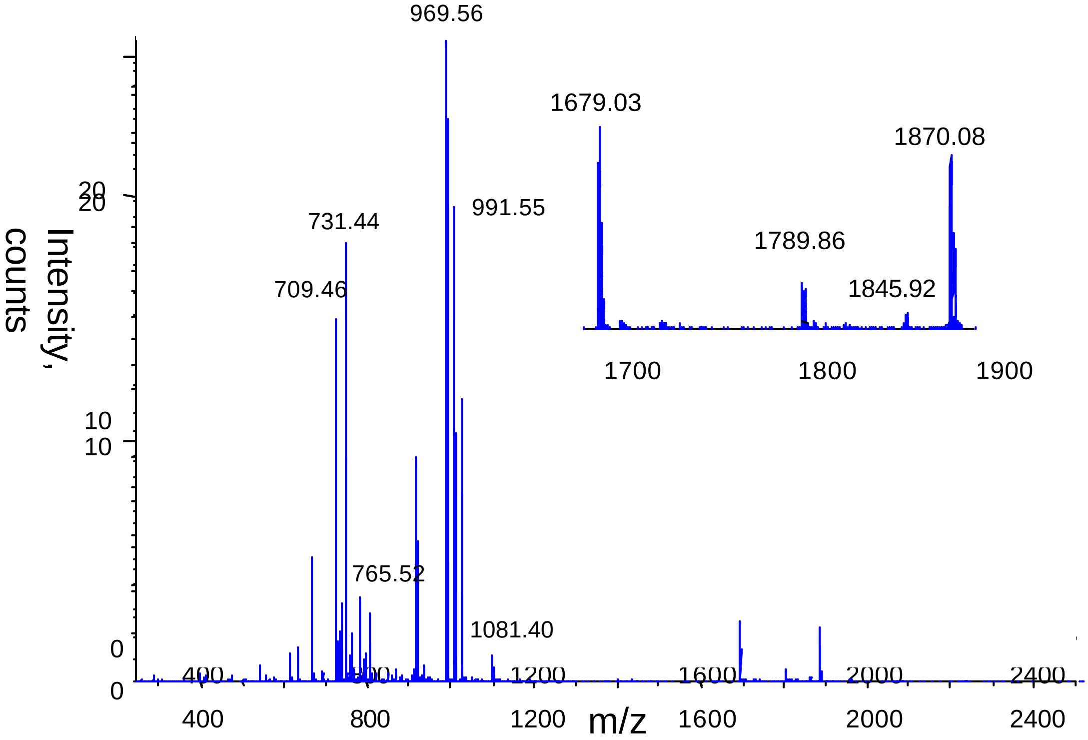


Figure 2. ESI-MS spectrum of the 1:1:1:1 mixture **1+3+4+5**. The spectrum was recorded in the positive ion mode. The calculated masses are: [**4** - $3H^+$]$^+$: 709.47; [**3** - $3H^+$]$^+$: 765.53; [**5** - $3H^+$]$^+$: 969.58; [**1** + $5H^+$]$^+$: 1081.47; [**1+4** + H^+]$^+$: 1789.88; [**1+3** + H^+]$^+$: 1845.94; [**5+4** - $7H^+$]$^+$: 1678.09; [**5+5** - $7H^+$]$^+$ (one molecule pyrazole lost) 1870.11; all other peaks result from H^+/Na^+ exchange. No ion peaks were found beyond *m/z* = 2400.

In the mixtures containing the pyrazolium half-sphere **5** a proton transfer occurred. Therefore new species (neutral half-spheres and additional capsules) were formed and the stoichiometry of the different compounds in the mixtures became less well defined resulting in a certain variation of the relative capsule´s ESI-intensities (I_{ESI}). This was especially pronounced in the mixture **1+3+4+5**, as summarized in Table 1. Interestingly, this variation was not observed for the mixture **2+3+4+5**. We assume, that due to a lower pK_a value the benzylic phosphonate has a markedly lower affinity for the pyrazolium NH^+ proton. For the assignment of relative ESI-MS stabilities we therefore chose the relative intensities obtained with the 1:1:1 mixtures (compare Tables 2 and 6) not containing the pyrazolium halfspheres.

Table 1: Comparison of I_{ESI} (absolute intensities of the M^{+} capsules peaks) in equimolar *1:1:1:1* (three cations, one phosphonate **1**) mixtures obtained in ten independent runs

Run	Mixture	I_{ESI}(**1**+**3**)	I_{ESI}(**1**+**4**)	I_{ESI}(**1**+**5**)	*I*(**1**+**3**)*:I*(**1**+**4**)
1	**1+3+4+5**	0.25	1.3	0	1:5
2	**1+3+4+5**	0.32	0.65	0	1:2
3	**1+3+4+5**	5	9	0	1:1.8
4	**1+3+4+5**	14	22.5	0	1:1.7
5	**1+3+4+5**	8.5	8.7	0	1:1
6	**1+3+4+5**	9.5	12.5	0	1:1.5

[a] calculated error in relative MS intensities: ± 66%.

Table 2: Comparison of I_{ESI} (absolute intensities of the M^{+} capsules peaks) in equimolar *1:1:1* (two cations, one phosphonate **1**) mixtures obtained in two independent runs

run	Mixture	I_{ESI}(**1**+**3**)	I_{ESI}(**1**+**4**)	*I*(**1**+**3**)*:I*(**1**+**4**)
1*	**1+3+4**	144	125.5	1.1:1
2*	**1+3+4**	53.5	63.5	1:1.2

* [1+3]$^{+2}$ and [1+4]$^{+2}$ were observed together with the singly charged capsules.

[a] calculated error in relative MS intensities: ± 13%.

Table 3: Comparison of I_{ESI} (absolute intensities of the M^{+} capsules peaks) in a mixture of 3 eq. phosphonate and 1eq. cations, *3:1:1:1* obtained in two independent runs

run	Mixture	I_{ESI}(**1**+**3**)	I_{ESI}(**1**+**4**)	I_{ESI}(**1**+**5**)	*I*(**1**+**3**)*:I*(**1**+**4**)
1	**1+3+4+5**	4.2	3	0	1.4:1[a]
2	**1+3+4+5**	6.1	10.6	0	1:1.7

[a] calculated error in relative MS intensities: ± 40%.

Furthermore, as shown in experiments where the amount of the cationic half-sphere **4** was increased (compare Table 4), the relative signal intensities of the two capsules changed. Therefore, sample preparation (compound concentrations) was found to be a critical step in determining the relative capsule stabilities by ESI-MS, whereas multiple injections of the same sample always gave identical results.

Table 4: Comparison of I_{ESI} (absolute intensities of the M^{+} capsules peaks) in a mixture of 1eq. phosphonate and 3 eq. **4** and 1eq. of the other cations (*1:1:3:1)* obtained in three independent runs

run	mixture	I_{ESI}(**1**+**3**)	I_{ESI}(**1**+**4**)	*I*(**1**+**5**)	*I*(**1**+**3**)*:I*(**1**+**4**)
1	**1**+**3**+**4**+**5**	0.07	1	0	1:14
2	**1**+**3**+**4**+**5**	3	23	0	1:7.6
3	**1**+**3**+**4**+**5**	10	30	0	1:3

[a] calculated error in relative MS intensities: ± 66%.

Table 5: Comparison of I_{ESI} (absolute intensities of the M^+ capsules peaks) in equimolar *1:1:1:1* (three cations, benzylic tetraphosphonate **2**) mixtures obtained in two independent runs

run	mixture	I_{ESI}(**2**+**3**)	I_{ESI}(**2**+**4**)	I_{ESI}(**2**+**5**)	I(**2**+**3**):I(**2**+**4**)
1	**2+3+4+5**	11.1	2.3	0	4.8:1
2	**2+3+4+5**	4.8	1	0	4.8:1

[a] calculated error in relative MS intensities: < 1%.

Table 6: Comparison of I_{ESI} (absolute intensities of the M^+ capsules peaks) in equimolar *1:1:1* (two cations, one benzylic tetraphosphonate **2**) mixtures obtained in three independent runs

run	mixture	I_{ESI}(**2**+**3**)	I_{ESI}(**2**+**4**)	I_{ESI}(**2**+**5**)	I(**2**+**3**):I(**2**+**4**)
1	**2+3+4**	90.5	22	0	4:1
2	**2+3+4**	86	21	0	4:1

[a] calculated error in relative MS intensities: < 1%.

ESI-MS/MS experiments (gas-phase experiments)

The ESI-MS/MS data of capsule **1**+**3** are shown in the main manuscript.

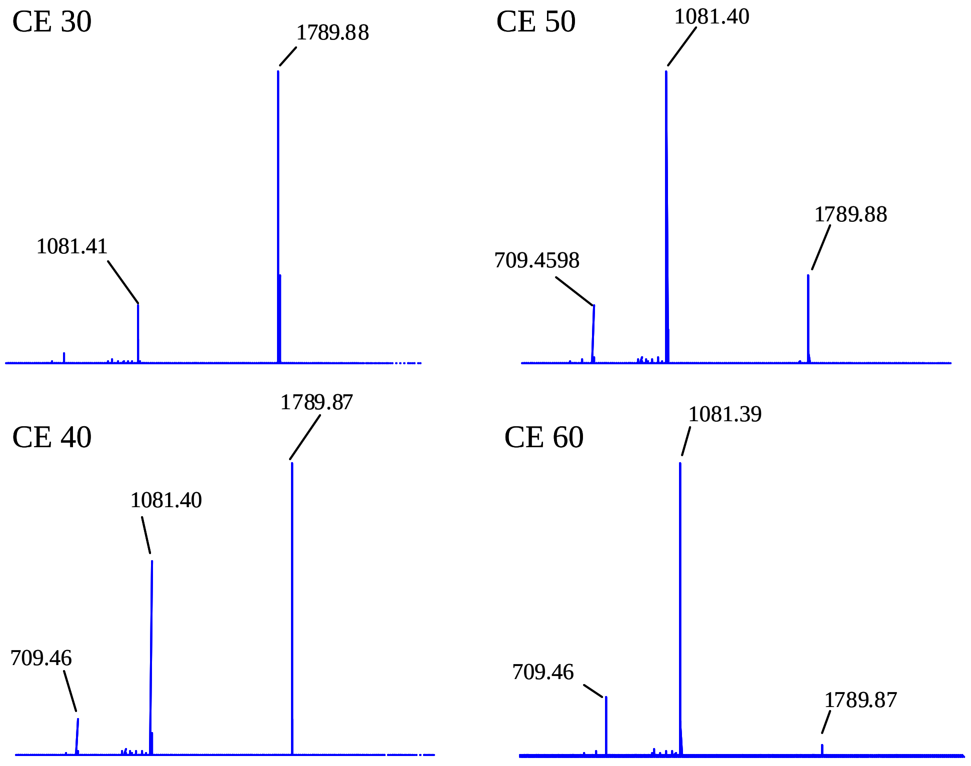


Figure 3: MS/MS data of capsule **1**+**4** at CE values of 30, 40, 50 and 60 Volts.

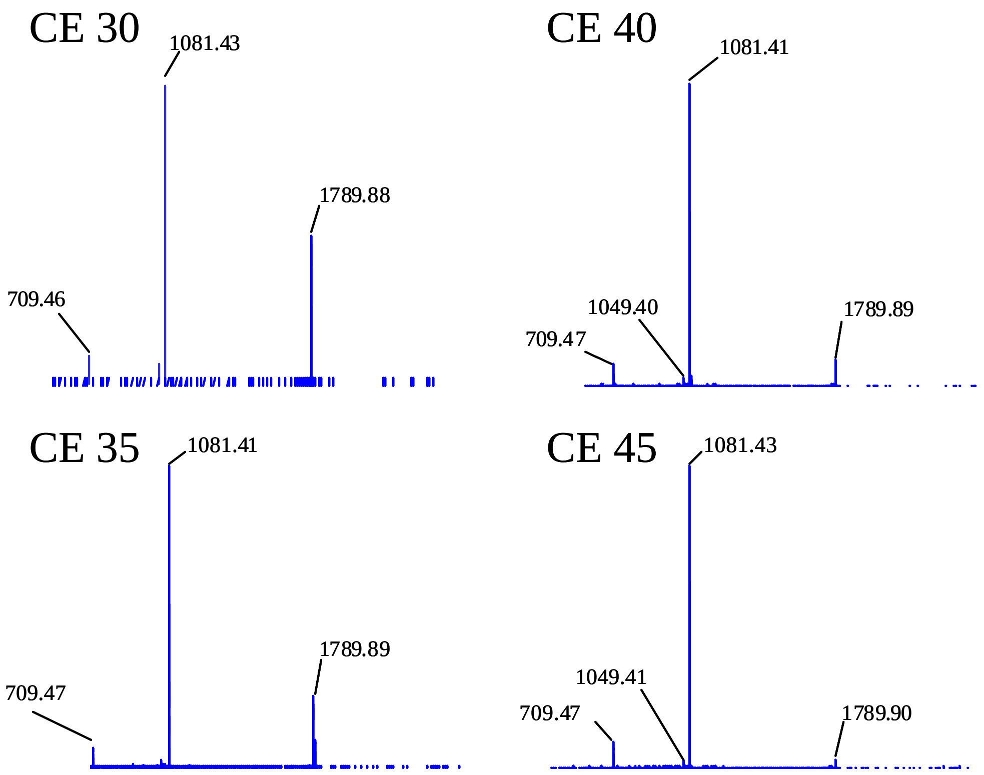


Figure 4: MS/MS data of capsule **2**+**4** at CE values of 30, 35, 40 and 45 Volts.

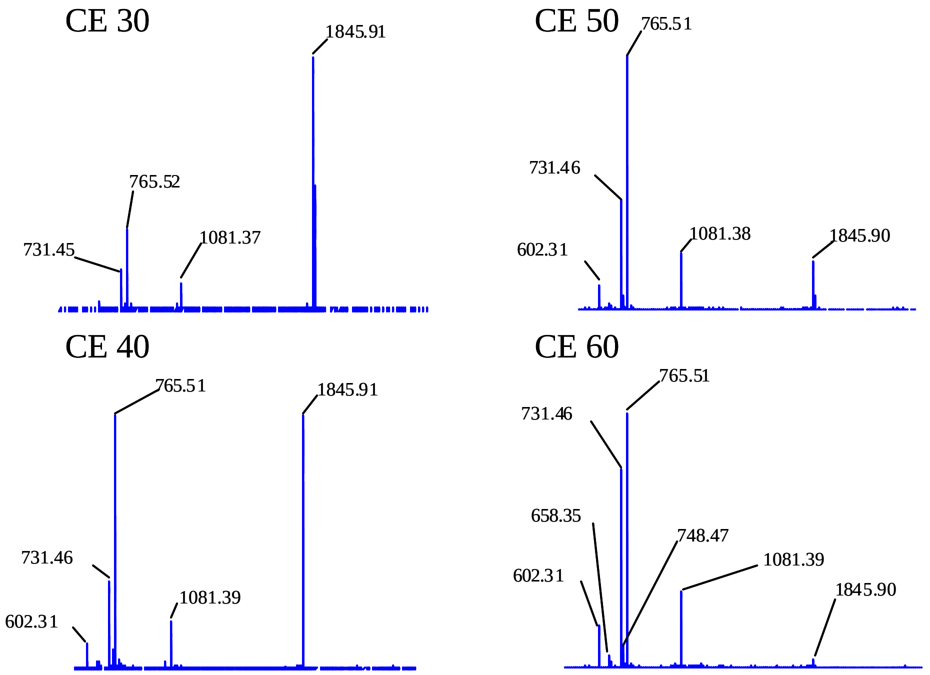


Figure 5: MS/MS data of capsule **2**+**3** at CE values of 30, 40, 50 and 60 Volts.

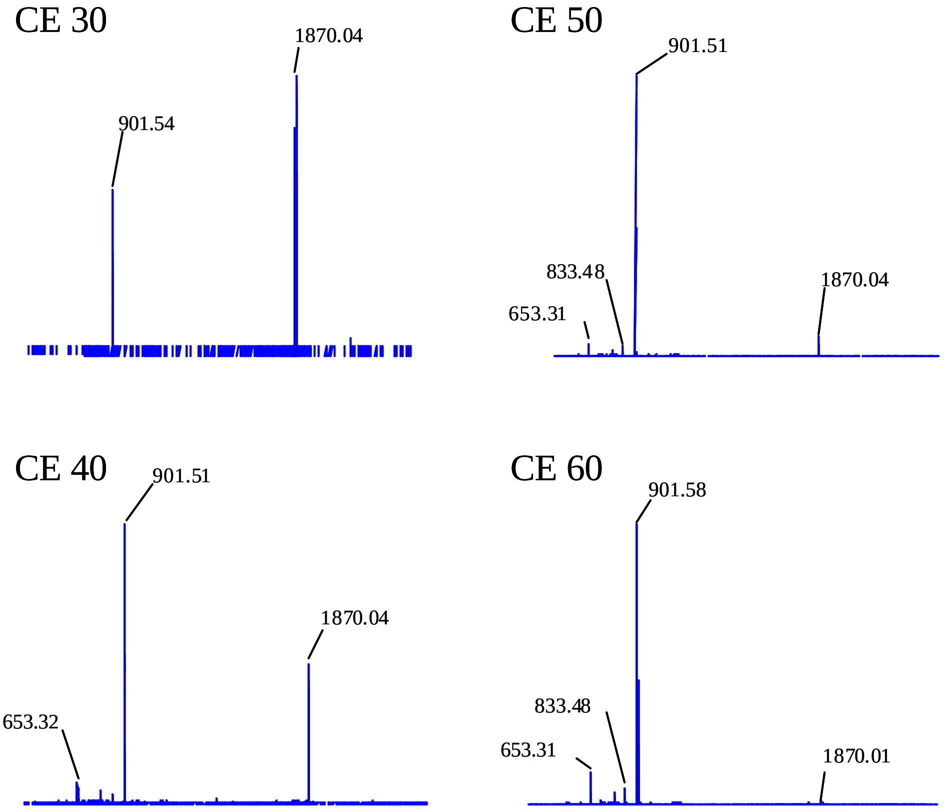


Figure 6: MS/MS data of the newly discovered tetrapyrazole (**5`**)/tetrapyrazolium capsule **5`**+**5**. This capsule was found to be as stable in the gas-phase as capsule **2**+**3**.

Computational Methods

Molecular mechanics calculations: Cerius^2, Molecular Simulations Inc. Force-field: Dreiding 2.21. Monte-Carlo-Simulations and Molecular Dynamics: The program MacroModel 7.0 was used for model building procedures and as graphical interface. Force-field parameters were taken from the built-in force fields, which were in some cases modified versions of the classical published versions. OPLS-AA was chosen for all minimizations and Monte Carlo as well as MD calculations, because Amber* was shown by various other groups to produce misleading conformational preferences with calixarenes. Minimizations were initially carried out in the gas phase, then in aqueous solution. Most complex structures were virtually identical under both conditions, indicating the strong enthalpic preference and hence stability of these arrangements. Energy minimizations were conducted over 2000 iterations on a Silicon Graphics O2 workstation. The best structures were subjected to conformational searches with at least 2000 step Monte Carlo simulations. For some optimized conformations molecular dynamics calculations were subsequently carried out at room temperature for 100 ps and without any external restraints (such as hydrogen bonds etc.).

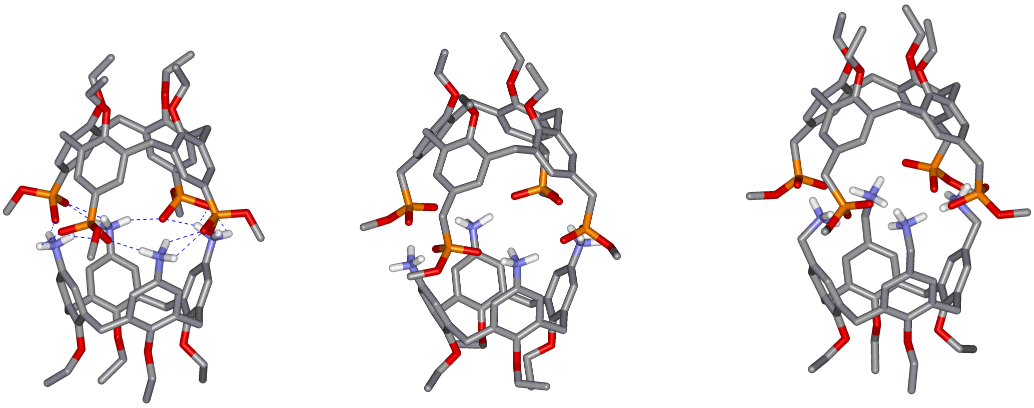
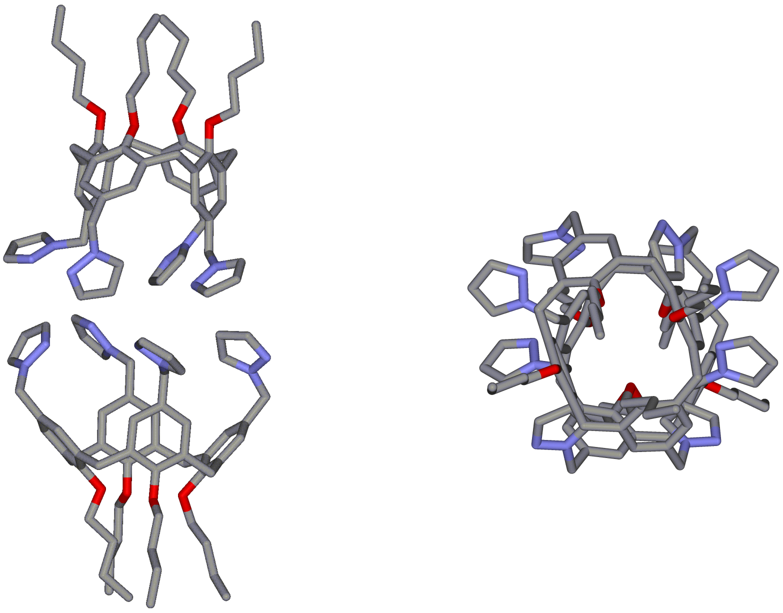


Figure 7: Three optimized structures: (a) tetraphosphonate–tetraanilinium assembly; (b) tetrabenzylphosphonate–tetraanilinium asssembly; (c) tetrabenzylphosphonate–tetrabenzylammonium assembly. Monte-Carlo simulations: MacroModel 7.0, OPLS-AA, 3000 steps, water.



iscovered pyrazolium / pyrazole capsule from the proton transfer side
ty. Energy-minimzation: MacroModel 7.0, OPLS-AA, 1000 steps,
water.

Table 7: Calculated gas phase stabilities for calix[4]arene half-spheres and related molecular capsules (MacroModel 7.0, OPLSS-AA, 1000 steps)

capsule	$H_{A.H.}$ [kcal/mol]	$H_{C.H.}$ [kcal/mol]	$H_{Cap.}$ [kcal/mol]	? H [kcal/mol]
1+3	229	154	-479	-862
1+4	229	314	-285	-828
1+5	229	53	199	-83
2+3	115	154	-548	-817
2+4	115	314	-357	-786
2+5	115	53	143	-25

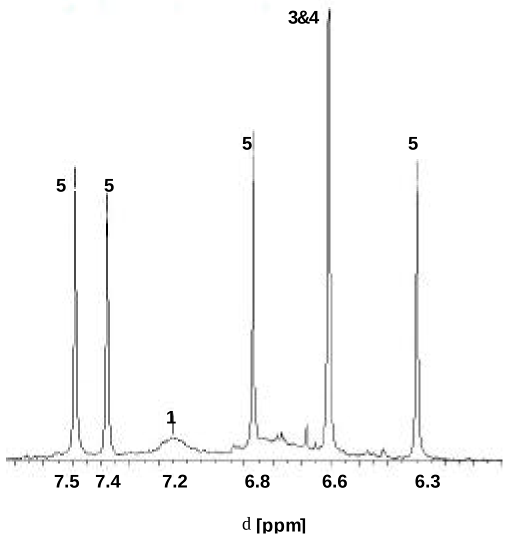


Figure 9. NMR of the 1:1:1:1-mixture (expansion of the interesting region). Note the broad averaged NMR signal of tetraphosphonate **1** for all three capsules as well as the simple set of NMR signals for all cationic half-spheres. **3** and **4** are even shift-isochronous.

RRK calculations:

In comparing relative peak intensities from CAD experiments, one has to adress the issue of unimolecular dissociation rates, which may vary between capsules of a different number of internal degrees of freedom. Especially in the case of capsules **1**+**3** and **1**+**4** the higher CE_{50} value of the former (54V vs. 43V) may simply reflect its extra methylene groups in the cationic benzylic half-sphere. In order to examine if kinetic shifts may be significantly influence our varying CAD results, one could perform Rice-Ramsperger-Kassel-Marcus calculations (RRKM). This is indeed the method of choice, if all the vibrational functions of a given molecule are known. However, in our case, we deal with molecular capsules of more than 200 atoms and we do not know their vibrational functions. Therefore, we carried out RRK calculations for according to the following formula:

$$k_{uni} = f\,[E - E_0 / E]^{S-1}$$

with E = collisional energy, E_0 = binding energy and S = vibrational degrees of freedom = 3N-6.

Assuming, that collisional and binding energies are the same for both capsules, the ratio of their unimolecular dissociation constants would be:

$$k_{1+3} : k_{1+4} = [E - E_0 / E]^{(S1+3 - 1) - (S1+4 - 1)} = [E - E_0 / E]^{36}$$

Capsule **1**+**3** has 273 atoms, while capsule **1**+**4** lacks four methylene groups, i.e., 12 atoms; thus, it has only 261 atoms. This gives 813 vs. 777 vibrational degrees of freedom with a difference of 36, so that the ratio of $k_{1+3} : k_{1+4} = [E - E_0 / E]^{36}$. We conclude, that indeed, the potential difference in rates of unimolecular dissociation may be substantial. This is especially true, if E approaches E_0, so that the CE_{50} values may in part be determined by kinetic shifts. However, although we cannot exclude significant contributions of varying internal degrees of freedom, there are also results indicating that this is not the case: the CE_{50} value for **3**+**2** is lower than that for **1**+**4**, although the former (doubly benzylic half-spheres) has much more internal degrees of freedom than the latter.

Table 8. Exact masses of all calixarene halfspheres and their assemblies.

Halfsphere	Exact mass
[1+5H^{+}]$^{+}$	1081.42
[2+5H^{+}]$^{+}$	1081.42
[3-3 H^{+}]$^{+}$	765.53
[4-3 H^{+}]$^{+}$	709.47
[5-3 H^{+}]$^{+}$	969.58
[1+3+H^{+}]$^{+}$	1845.94
[1+4+H^{+}]$^{+}$	1879.88
[1+5+H^{+}]$^{+}$	2049.98