

Anionic Amphiphilic Calixarenes for Peptide Assembly and Delivery

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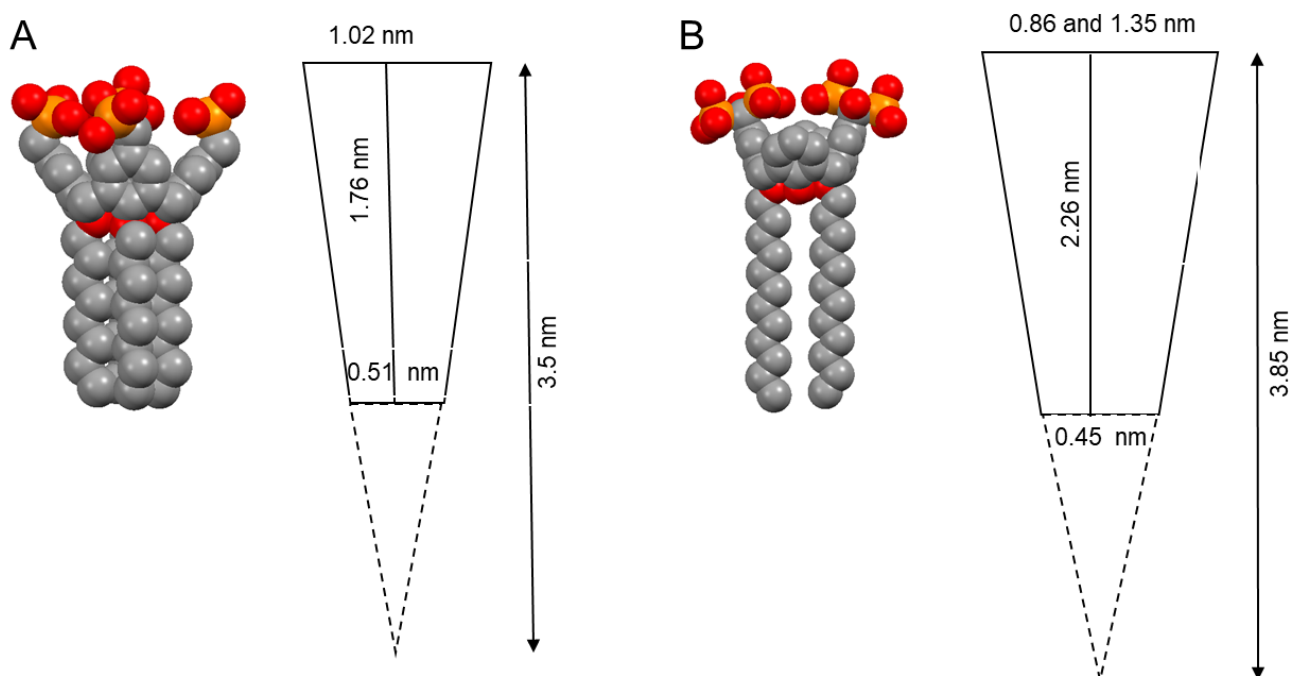


Figure S1. Structure of CAX8 (A) and C780 (B) according to HyperChem 8 (PM3) and corresponding sizes of cone likely formed by these molecules.

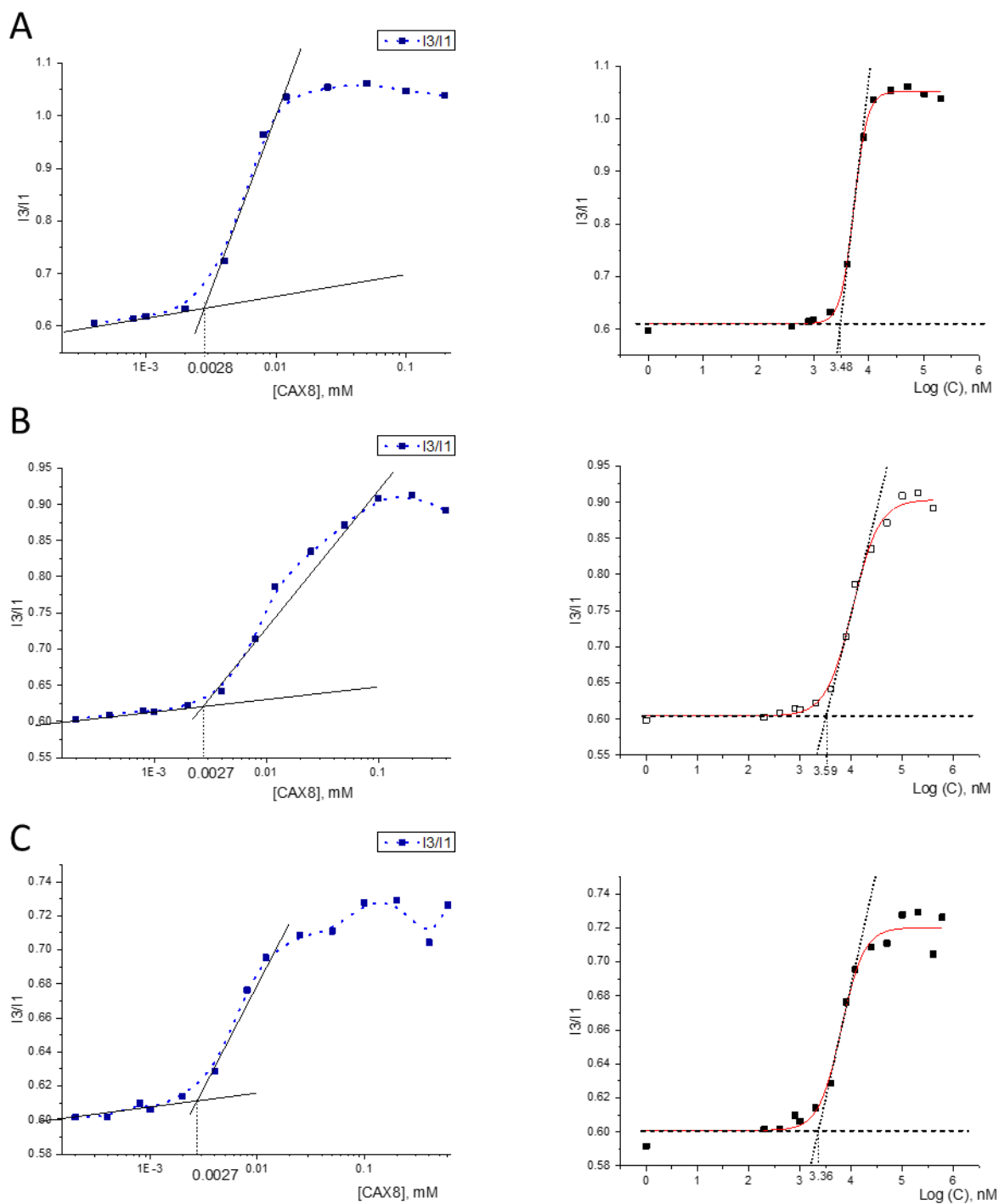


Figure S2. Fluorescence intensity ratio $I_{3/1}$ of pyrene as a function of CAX8 concentration in deionized water (A), 20 mM Tris buffer, pH 7.4 (B) and 0.2 mM NaOH (C). Determination of CMC manually (left) and using a Boltzmann sigmoidal fitting. The numbers in the X-scale indicated the estimated CMC.

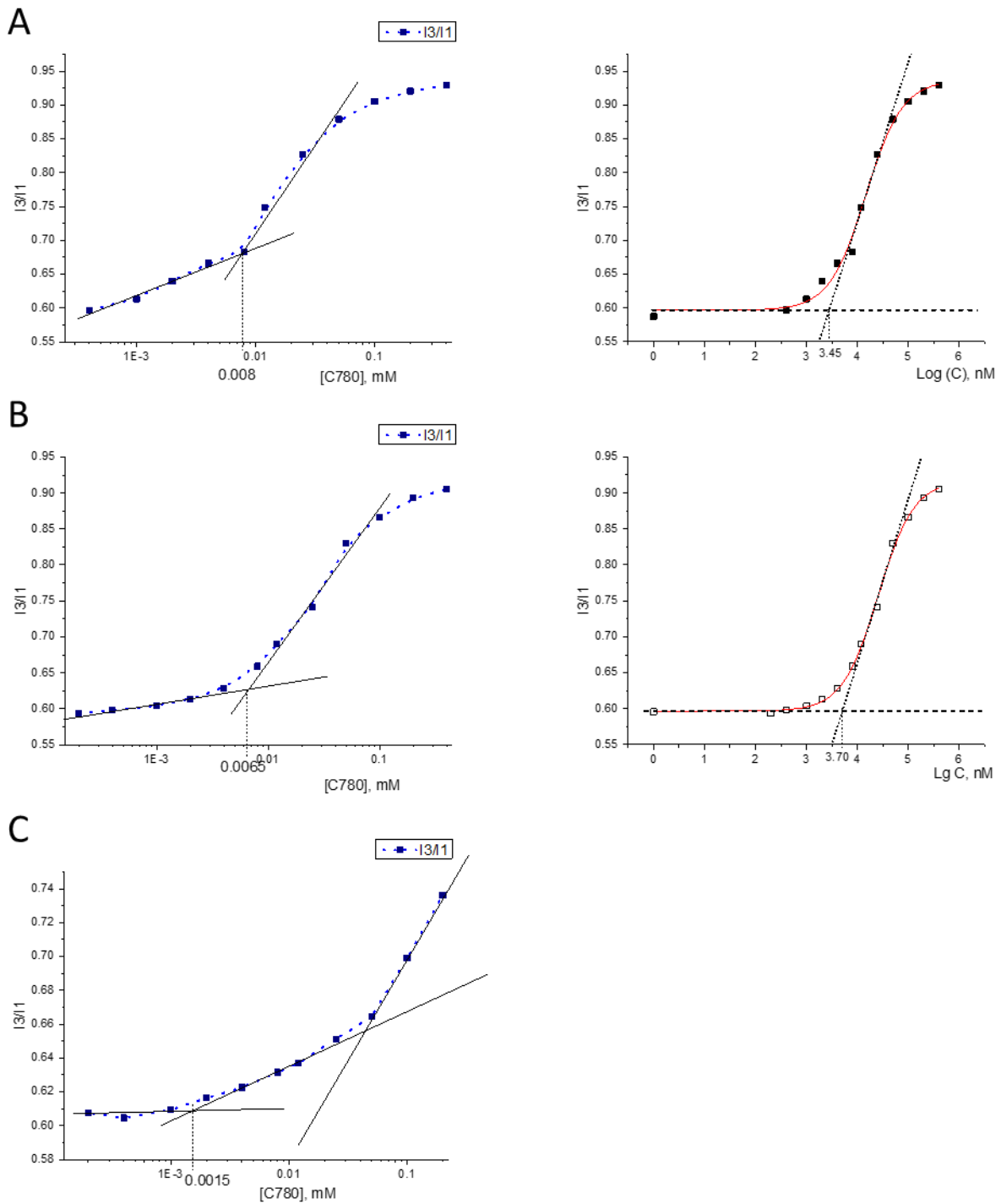


Figure S3. Fluorescence intensity ratio I3/I1 of pyrene as a function of C780 concentration in deionized water (A), 20 mM Tris buffer, pH 7.4 (B), and 0.2 mM NaOH (C). Determination of CMC manually (left) and using a Boltzmann sigmoidal fitting. In case of (C) Boltzmann sigmoidal fitting was not successful and, therefore, not used. The numbers in the X-scale indicated the estimated CMC.

Table S1. Estimated CMC values of CAX8 and C780 based on manual approach, Boltzmann sigmoidal fitting (BSF) and average (av.) of the two methods.

	water			Tris			NaOH		
	manual	BSF	av.	manual	BSF	av.	manual	BSF	av.
CMC of CAX8 (μM)	2.8	3.1	3.0 \pm 0.2	2.7	3.3	3.0 \pm 0.4	2.7	2.3	2.5 \pm 0.3
CMC of C780 (μM)	8	2.8	5.4 \pm 3.7	6.5	5.1	5.8 \pm 1.0	1.5	-	-

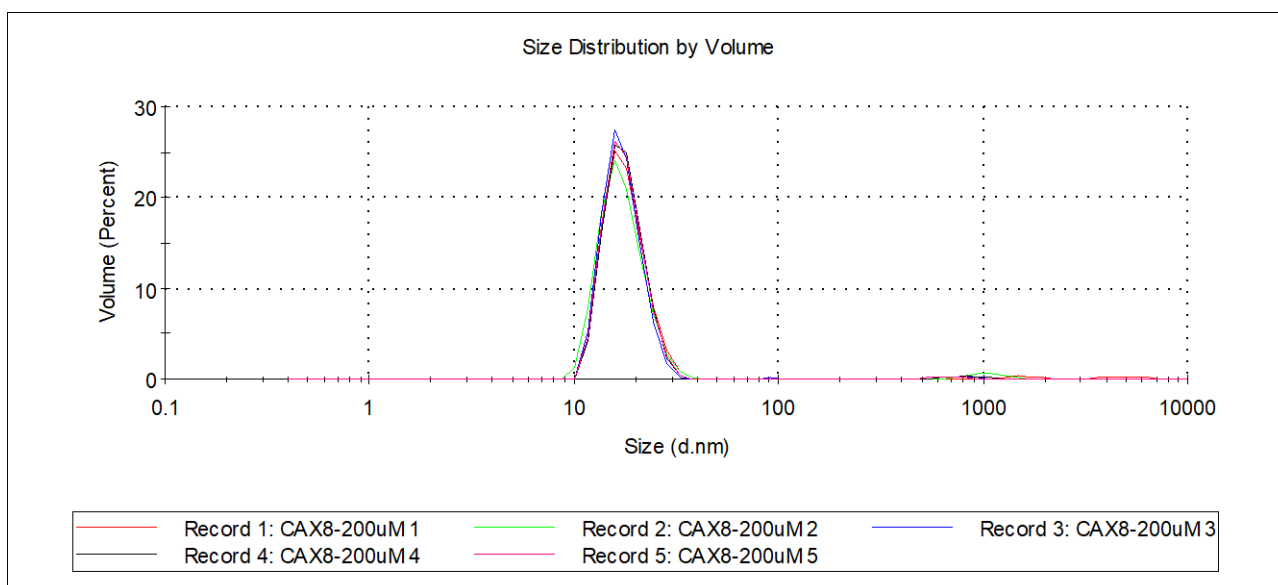


Figure S4. Size distribution (by volume) for CAX8 in water. Concentration 200 μ M, mean size: 18 ± 0.5 nm (by volume); 20 ± 0.5 nm (by intensity); 16 ± 0.4 nm (by number); PDI 0.640.

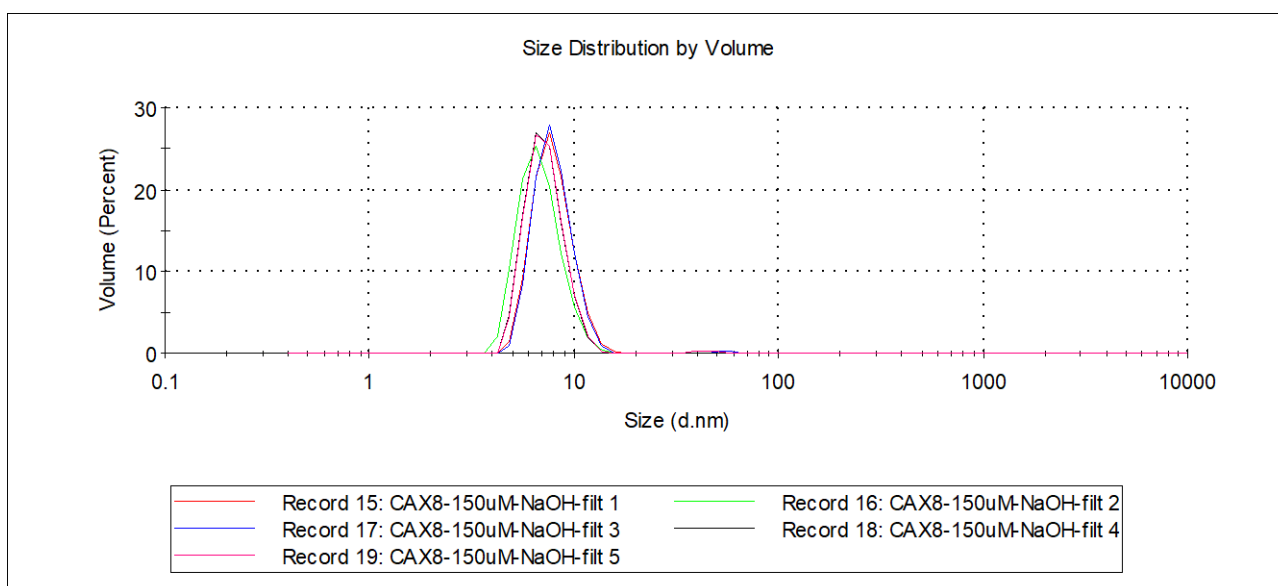


Figure S5. Size distribution (by volume) for CAX8 (μ M) in NaOH solution 100 mM. Mean size: 7.5 ± 0.5 nm (by volume); 8.3 ± 0.5 nm (by intensity); 6.8 ± 0.5 nm (by number); PDI 0.600.

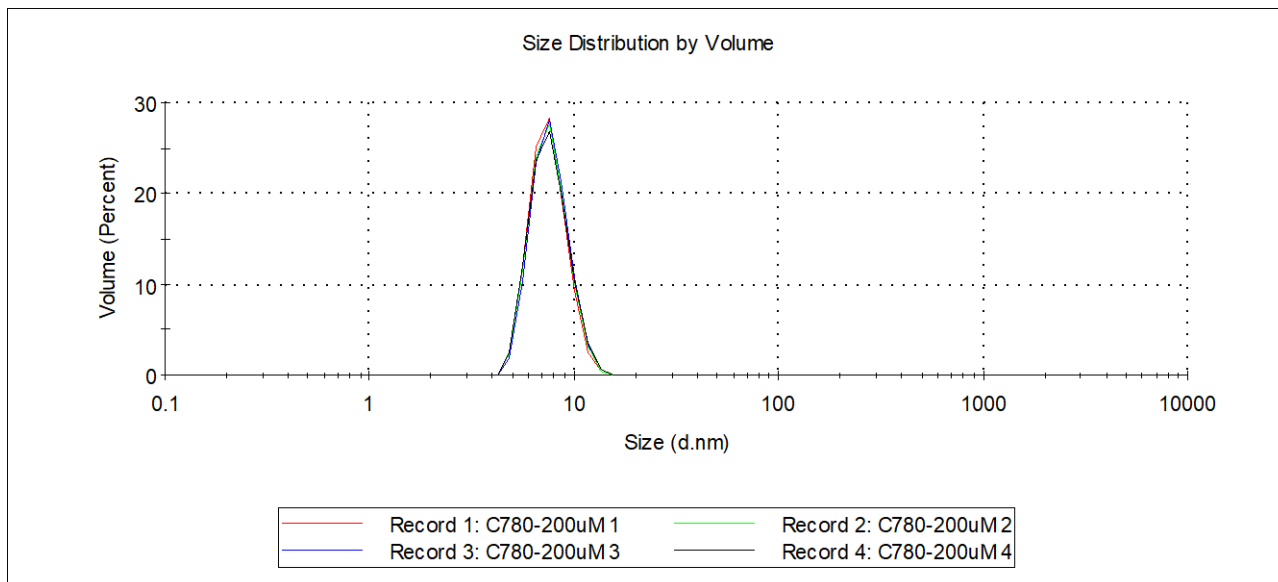


Figure S6. Size distribution (by volume) for C780 in water. Concentration 200 μ M, mean size 7.7 ± 0.1 nm (by volume); 8.4 ± 0.1 nm (by intensity); 7.1 ± 0.1 nm (by number); PDI 0.58.

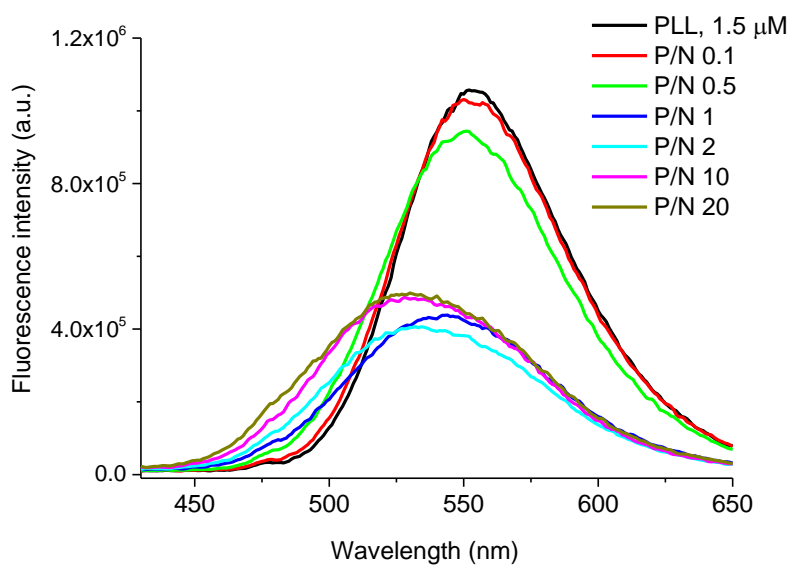
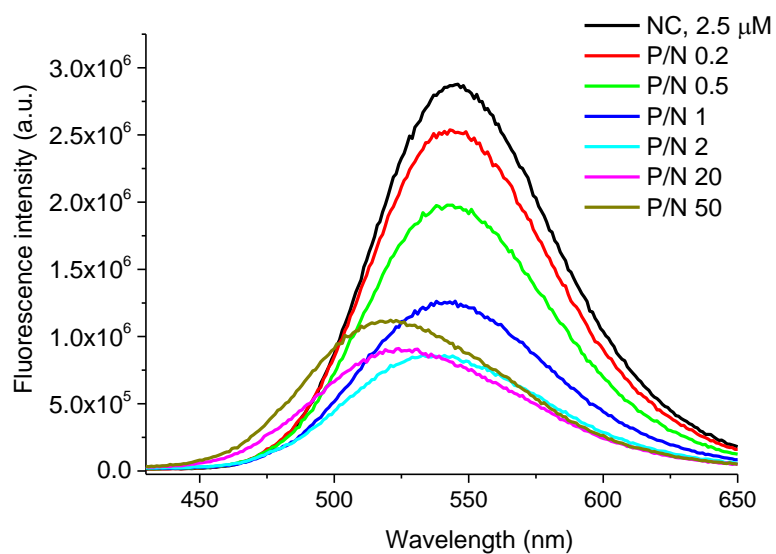
A**B**

Figure S7. Fluorescence spectra of poly-L-lysine 20-mer labelled at its N-terminus by the 3-hydroxyflavone probe (a) and NC(11-55) peptide labelled at its N-terminus by the 3-hydroxyflavone probe (b) in the presence of increasing concentrations of the non-amphiphilic anionic calixarene C-781.

Table S2. Hydrodynamic diameters of labelled NC(11-55)-LRh and its complexes with calixarenes according to DLS and FCS measurements.^a

Sample		P/N	DLS (nm)	FCS (nm)
NC(11-55)-LRh		0	-	2.2±0.2
C780	NC(11-55)-LRh	2	26.6±5.5	22±2
		5	23.5±4.9	10±1
CAX8	NC(11-55)-LRh	2	27.9±2.5	21±1
		5	25.8±2.2	24±1

^a The values are average of at least 3 measurements; the error is standard deviation. P/N is the ratio of the negatively charged phosphonates of calixarene to the positively charged ammonium groups of peptides.

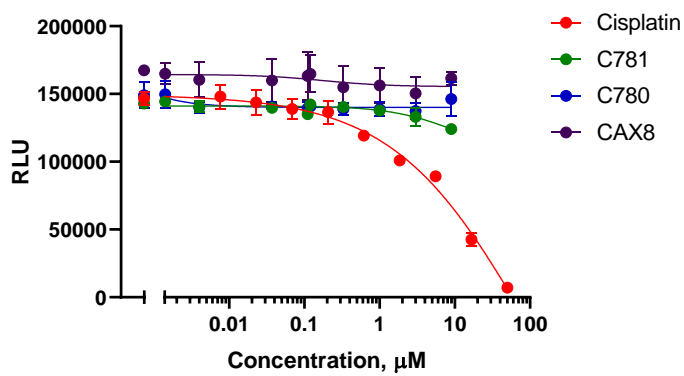


Figure S8. Cytotoxicity of anionic calixarenes, determined with CellTiterGlo assay. The cells were incubated for 72 h at different concentrations of calixarene and cisplatin (positive control) at 37°C. Error is standard deviation (n = 6).