

Supporting Information

Salt-induced Guest Relocation from a Macrocyclic Cavity into a Biomolecular Pocket: Interplay between Cucurbit[7]uril and Albumin

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Experimental Section:

Neutral red hydrochloride (NRH⁺•Cl⁻) was obtained from Fluka, Switzerland, and purified following the reported procedure.¹ CB7 was synthesized according to the reported modified procedure.² BSA was obtained from Sigma-Aldrich and used as received. Nanopure water (Barnstead System; resistivity 0.1 $\mu\text{S cm}^{-1}$) was used throughout for solution preparation. The pH of the solution was adjusted by adding dilute perchloric acid or sodium hydroxide solution and an ORION ionalyzer/901 was used for pH measurements. In the calculation of the CB7 concentration a correction for water content (15 wt %) was considered. Absorption spectra were recorded with a Jasco V530 UV-Vis spectrophotometer (Tokyo, Japan). Steady-state fluorescence spectra were recorded using a Hitachi F-4010 spectrofluorometer (Tokyo, Japan). The samples were excited at the apparent isosbestic point (~520 nm) observed in the absorption spectra of the dye.

NMR measurements

All experiments were performed at ambient temperature in D₂O (99.8%). The pD values of the solutions were adjusted by addition of D₂SO₄ or NaOD. pH readings were taken with a WTW 330i pH meter equipped with a combined pH glass electrode (SenTix Mic) and converted to pD (+0.40 units).³ ¹H NMR spectra (400 MHz) were recorded by using the chemical shift of HOD in D₂O preset at 4.67 ppm as reference. Concentrations of NRH⁺ (1 mM) and CB7 (1 mM) were chosen to obtain sufficient signal strength.

Effect of sodium chloride concentration on the binding constant of NRH⁺•CB7

The experimentally observed equilibrium constant [K_{eq} (NRH⁺)] of NRH⁺•CB7 diminishes by a factor of about 80 with an increase in the NaCl concentration (Fig S1). The simplified equation for the change of the observed reciprocal of the binding constant of NRH⁺•CB7 complex with Na⁺ concentration can be written as^{4,5}

$$\frac{1}{K_{\text{eq}}(\text{NRH}^+)} = \frac{1 + K_2[\text{Na}^+] + K_2K_3[\text{Na}^+]^2}{K_1}$$

where K_1 and K_2 are the equilibrium constants for NRH⁺•CB7 and Na⁺•CB7 complex formation, K_3 stands for the equilibrium constant of Na⁺•CB7 with a second Na⁺. Using this relationship and the result of the nonlinear least-squares fit (Fig. S2), $K_2 = 80$

M^{-1} and $K_3 = 20 \text{ M}^{-1}$ are calculated for the complexation of Na⁺ with CB7 and Na⁺•CB7, respectively.

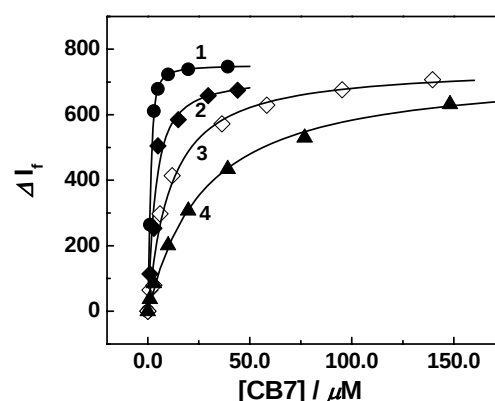


Fig. S1: Binding isotherm of the NRH⁺•CB7 complex at various concentrations of NaCl. [NaCl]/M: 1) 0.0, 2) 0.01, 3) 0.1 and 4) 0.2.

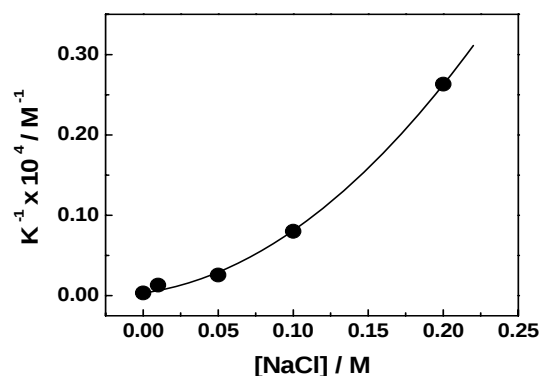
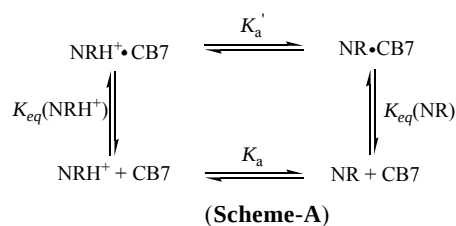


Fig. S2: The reciprocal of the observed equilibrium constant versus NaCl concentration; the line shows the fitted function.

Scheme A

As reported,⁶ the protolytic thermodynamic equilibrium for the present Dye•CB7 system is expected to follow a four-state model as in Scheme A, involving the complexed and uncomplexed dye both in the protonated and unprotonated forms.



According to the thermodynamic cycle in Scheme A, the acidity constant of the encapsulated dye, $\text{p}K_a'$, can be directly determined from the measured binding constants for the neutral and protonated forms, $K_{eq}(\text{NR})$ and $K_{eq}(\text{NRH}^+)$, in combination with the $\text{p}K_a$ value of the uncomplexed dye using the following relation.⁶

$$K_a' = K_a K_{eq}(\text{NR}) / K_{eq}(\text{NRH}^+)$$

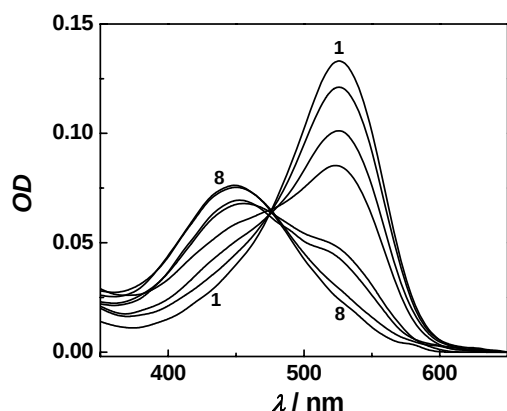


Fig. S3: Absorption spectra of neutral red ($2.9 \mu\text{M}$) in water with $150 \mu\text{M}$ CB7 at 0.01 M of NaCl at different pH values: (1) 4.8, (2) 7.1, (3) 7.7, (4) 8.1, (5) 8.7, (6) 8.9, (7) 9.8 and (8) 10.8.

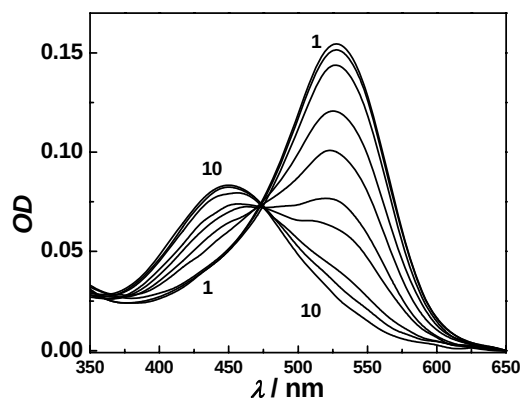


Fig. S4: Absorption spectra of neutral red ($2.9 \times 10^{-6} \text{ M}$) in water with $150 \mu\text{M}$ CB7 at 0.5 M of NaCl at different pH values: (1) 3.6, (2) 5.0, (3) 5.9, (4) 6.6, (5) 7.0, (6) 7.4, (7) 7.6, (8) 8.1, (9) 8.5 and (10) 10.0.

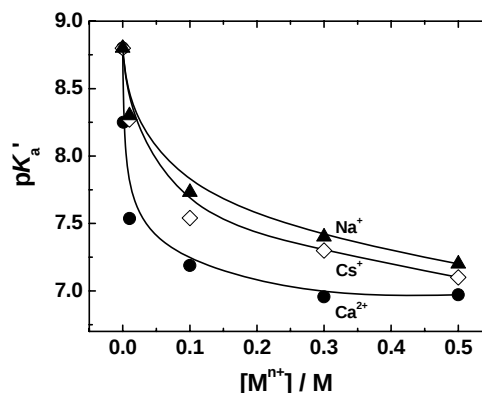


Fig. S5: Plot of the apparent $\text{p}K_a'$ values of the Dye•CB7 complex at different concentrations of metal ions $[\text{M}^{n+}]$. The symbols $\bullet$, $\diamond$, and $\blacktriangle$ represent Ca^{2+} , Cs^+ and Na^+ ions, respectively.

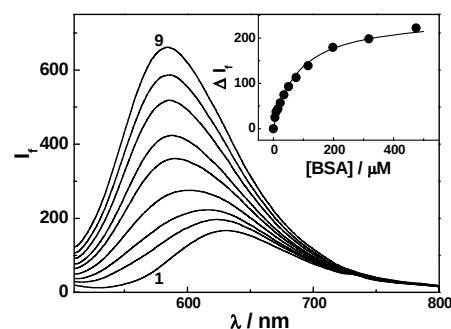


Fig. S6: Fluorescence spectra of the NR form of neutral red ($6.5 \mu\text{M}$) in aqueous solution at pH 8 with increasing concentration of BSA. [BSA]/ μM : (1) 0, (2) 5, (3) 15, (4) 35, (5) 76, (6) 115, (7) 198, (8) 318 and (9) 475. Inset: Fluorescence titration curve of NR in the presence of BSA, $\lambda_{\text{mon}} = 630 \text{ nm}$.

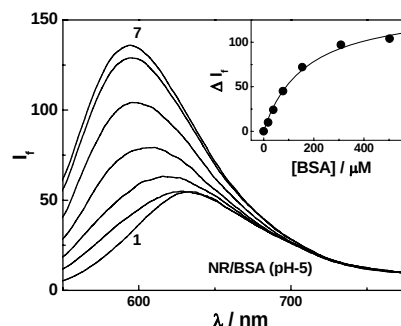


Fig. S7: Fluorescence spectra of the NRH^+ form of neutral red ($3.0 \mu\text{M}$) in aqueous solution at pH 5 with increasing concentration of BSA. [BSA]/ μM : (1) 0, (2) 18, (3) 38, (4) 77, (5) 154, (6) 308 and (7) 502. Inset: Fluorescence titration curve of NRH^+ in the presence of BSA, $\lambda_{\text{mon}} = 595 \text{ nm}$.

NMR investigation of the salt-induced dye displacement at pD 6.4

The salt-induced displacement of the dye from the CB7 cavity was also demonstrated by ^1H NMR chemical shift changes (Fig. S8). We monitored the complexation-induced shifts of the alkyl

($-\text{CH}_3$) and alkyl amino protons ($-\text{N}(\text{CH}_3)_2$), which showed more distinct signals than the phenazine protons; the latter became very broad in the complex. The spectrum of neutral red (1 mM) at pD 6.4 is shown in Fig. S8a (higher pH values were less suitable, because precipitation occurred in the presence of BSA). Addition of CB7 (1 mM) to the solution of NRH^+ resulted in a broadening of the signals and, most characteristic, the separation between the two methyl group signals ($\Delta\delta$, Table S1) decreased and changed from -0.74 ppm to -0.17 ppm (Fig. S8b), with a downfield shift for the alkyl protons ($-\text{CH}_3$) and an upfield shift for the alkyl amino protons ($-\text{N}(\text{CH}_3)_2$); moreover, the CB7 methylene resonances split into an apparent triplet as a consequence of the symmetry breaking in the complex. The addition of NaCl (500 mM) was expected to release the dye from the CB7 cavity and resulted indeed in a “recovery” of the NMR signals (Fig S8c, compare $\Delta\delta = -0.66$ ppm). These shift variations upon addition of salt were qualitatively identical in the presence of BSA (Fig S8d-f), but due to precipitation problems, the concentration of BSA was limited to $10\text{ }\mu\text{M}$, and also the concentration of CB7 needed to be reduced for these control measurements (0.5 mM).

Table S1. Environment-induced ^1H NMR Shifts of the resonances of the dye neutral red (1 mM) in D_2O at pD 6.

additive	δ/ppm		$\Delta\delta/\text{ppm}$
	$-\text{CH}_3$	$-\text{N}(\text{CH}_3)_2$	
none	1.98	2.72	-0.74
CB7 (1 mM)	2.30	2.47	-0.17
CB7 (1 mM) + NaCl (500 mM)	2.06	2.72	-0.66
BSA (10 μM)	2.01	2.77	-0.76
BSA (10 μM) + CB7 (0.5 mM)	2.29	2.47	-0.18
BSA (10 μM) + CB7 (0.5 mM) + NaCl (500 mM)	2.06	2.72	-0.66

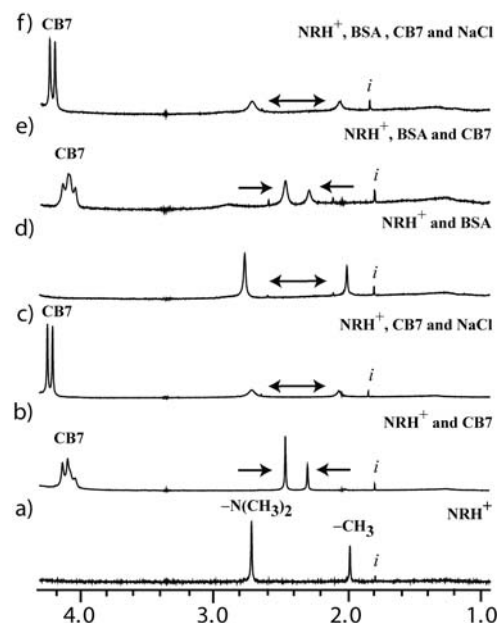


Fig. S8: ^1H NMR spectra (400 MHz) in D_2O at pD 6.4 of neutral red in different environments: a) NRH^+ (1 mM), b) NRH^+ (1 mM) and CB7 (1 mM), c) NRH^+ (1 mM), CB7 (1 mM) and NaCl (500 mM) d) NRH^+ (1 mM) and BSA (10 μM), e) NRH^+ (1 mM), BSA (10 μM) and CB7 (0.5 mM), f) NRH^+ (1 mM), BSA (10 μM), CB7 (0.5 mM) and NaCl (500 mM). CB7 labels the methylene resonances of the CB7 macrocycle. “i” an minor impurity in the commercial dye sample. The diagnostic complexation-induced shifts are represented by arrows.

References

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