

Electronic Supplementary Information (ESI)

Supramolecular Guest Relay Using Host-Protein Nanocavities: An Application of Host-Induced Guest Protonation

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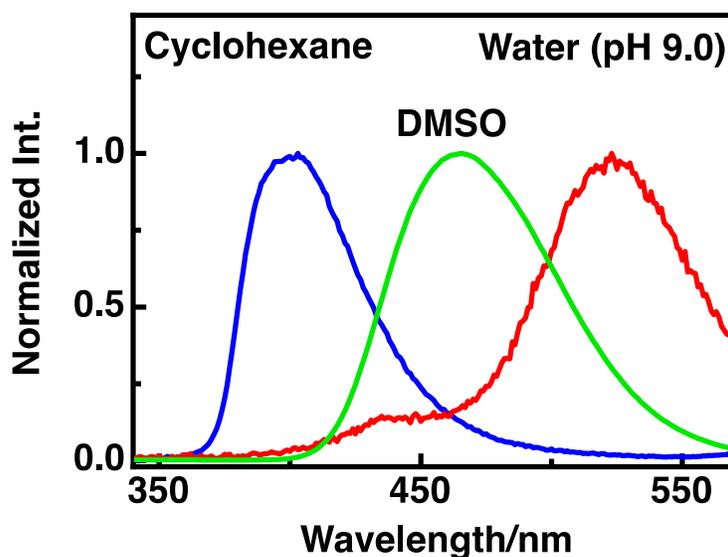


Fig. S1: Normalized fluorescence spectra of PRO in different solvent (Cyclohexane, DMSO and in Water at pH 9.0)

Benesi-Hildebrand analysis:

Equation used for the Benesi-Hildebrand plot:

$$\frac{[PROH]_0}{OD} = \frac{1}{[CB7]} \left(\frac{1}{\epsilon_{PROH} + K_{[PROH \cdot CB7]_b}} \right) + \frac{1}{\epsilon_{PROH}}$$

Where, $[\text{PROH}]_0$ is the concentration of Prodan in its protonated form which was fixed, OD is the optical density which varies with the addition of CB7, $[\text{CB7}]$ is the concentration of CB7, $K_{[\text{PROH}+\text{CB7}]_b}$ is the binding constant between Prodan in its protonated form (measurement was done at pH 3.0) and CB7 and ϵ_{PROH^+} is the molar extinction co-efficient. The binding constant was obtained from the slope of the linear plot between $1/[\text{CB7}]$ vs $[\text{PROH}]_0/\text{OD}$.

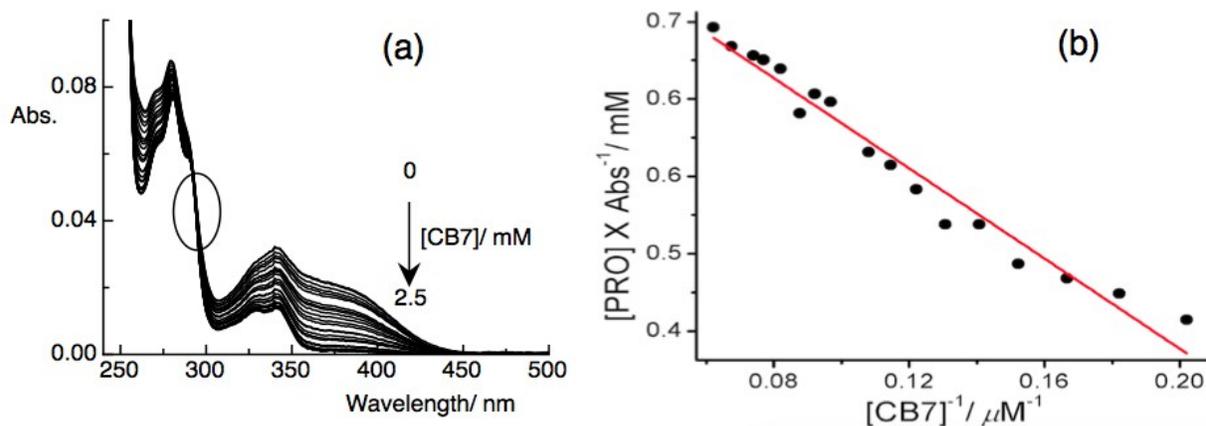


Fig. S2 (a) UV-Vis titration of 10 μM PRO with increasing concentration of CB7 up to 2.5 mM at pH 3.0; (b) show analysis of UV-Vis titration data using modified Benesi–Hildebrand plot. A good correlation is found and thus it reveal a 1:1 association between PRO and CB7.

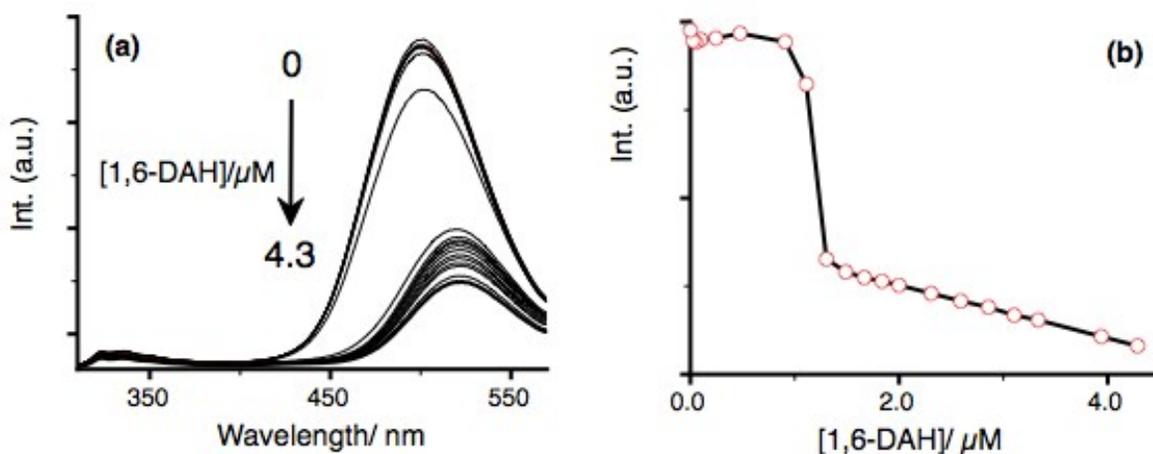


Fig. S3: (a) Fluorescence displacement titration of PRO•CB7 complex with addition 1,6- diaminohexane, (b) a plot of fluorescence intensity at 500 nm of PRO•CB7 complex with increasing concentration of 1,6- diaminohexane

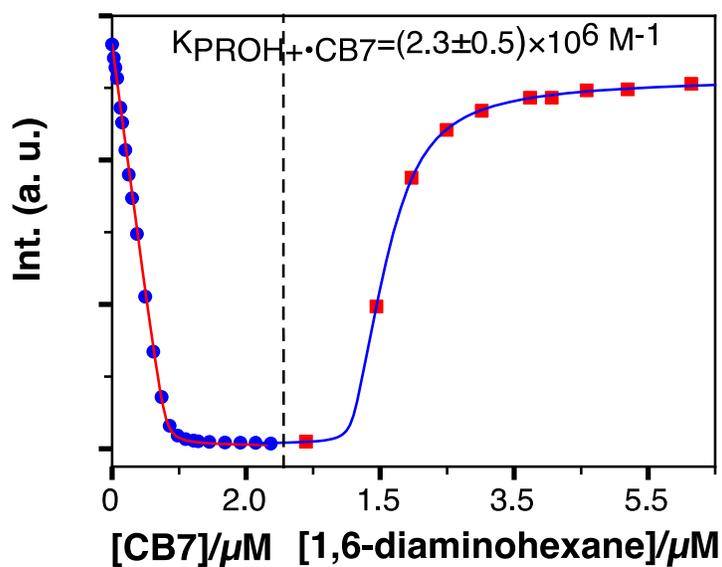


Fig. S4: Left part of the dotted line shows the plot of fluorescence intensity at 500 nm of 10 μ M PRO with increasing concentration of CB7 at pH 3.0, solid line shows fitting of the data using 1:1 binding equation yielding a binding constant $2.3 \pm 0.5) \times 10^6 M^{-1}$. Right side of the graph shows increase in fluorescence intensity due to displacement of PRO from CB7 cavity upon addition of 1,6- diaminohexane.

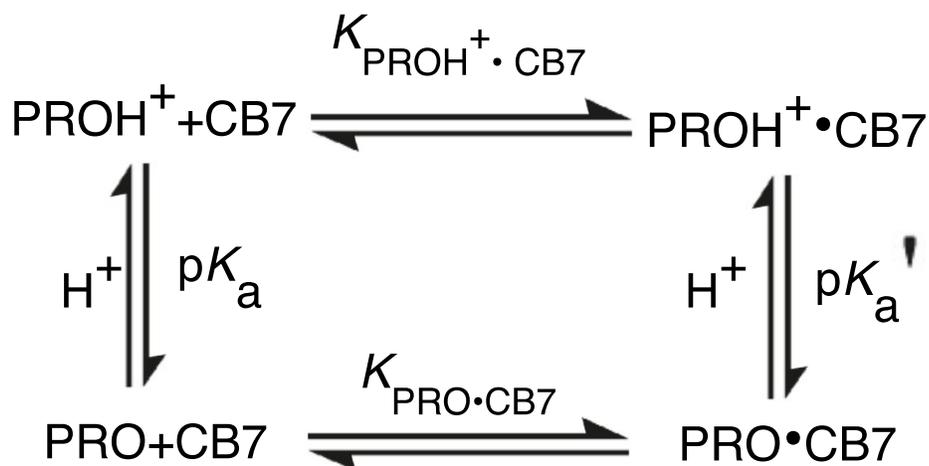


Fig. S5: Thermodynamic cycle showing protonation, deprotonation and binding equilibrium between free PRO and complexes between PRO and CB7.

pH dependent binding and fitting formula for getting pK_a values:

The pH dependent of PRO absorbance in absence and in presence of CB7 was fitted using a two state and four-state model respectively as described by Nau and coworkers^{1, 2} by considering a single protonated and unprotonated complex-see equation (1 and 2).

$$pH = pK_a + \log_{10} \frac{[\text{PRO}]}{[\text{PROH}^+]} \quad \text{-----(1)}$$

$$\begin{aligned}
 A/d = & P\{\varepsilon_{\text{CB7.PRO}} K_a' + \varepsilon_{\text{CB7.PROH}^+} [H^+] + \frac{K_a'}{([\text{CB7}]_o - P\{K_a' + [H^+]\})K_{\text{CB7}} (\varepsilon_{\text{PRO}} + \varepsilon_{\text{PROH}^+} [H^+]/K_a)}\} \\
 \text{With } P = & \frac{[G]_o + [CD]_o}{2(K_a' + [H^+]) + 2K_a K_{\text{PRO}}(K_a' + [H^+])^2} - \frac{K_a'([H^+] + K_a)}{\sqrt{(K_a K_{\text{PRO}}([PRO]_o + [\text{CB7}]_o)(K_a' + [H^+]) + K_a'([H^+] + K_a))^2 - 4K_a^2 K_{\text{PRO}}^2 [\text{CB7}]_o [PRO]_o (K_a' + [H^+])^2}} \\
 & \frac{2K_a K_{\text{PRO}}(K_a' + [H^+])^2}{\text{-----}} \\
 - (2)
 \end{aligned}$$

Where, A is the experimental UV absorbance normalized for the selected path length (d), $\epsilon_{\text{CB7}\cdot\text{PRO}}$, $\epsilon_{\text{CB7}\cdot\text{PROH}^+}$, ϵ_{PRO} , and ϵ_{PROH^+} are the extinction coefficients of the unprotonated and protonated complexed and uncomplexed PRO at the particular wavelength, respectively, K_a and K'_a (to be fitted) are the acidity constants of the uncomplexed and complexed PRO, K_{PRO} is the binding constant of the unprotonated complex (see main text), and $[\text{PRO}]_0$ and $[\square\square 7]_0$ are the total concentrations of guest and host.

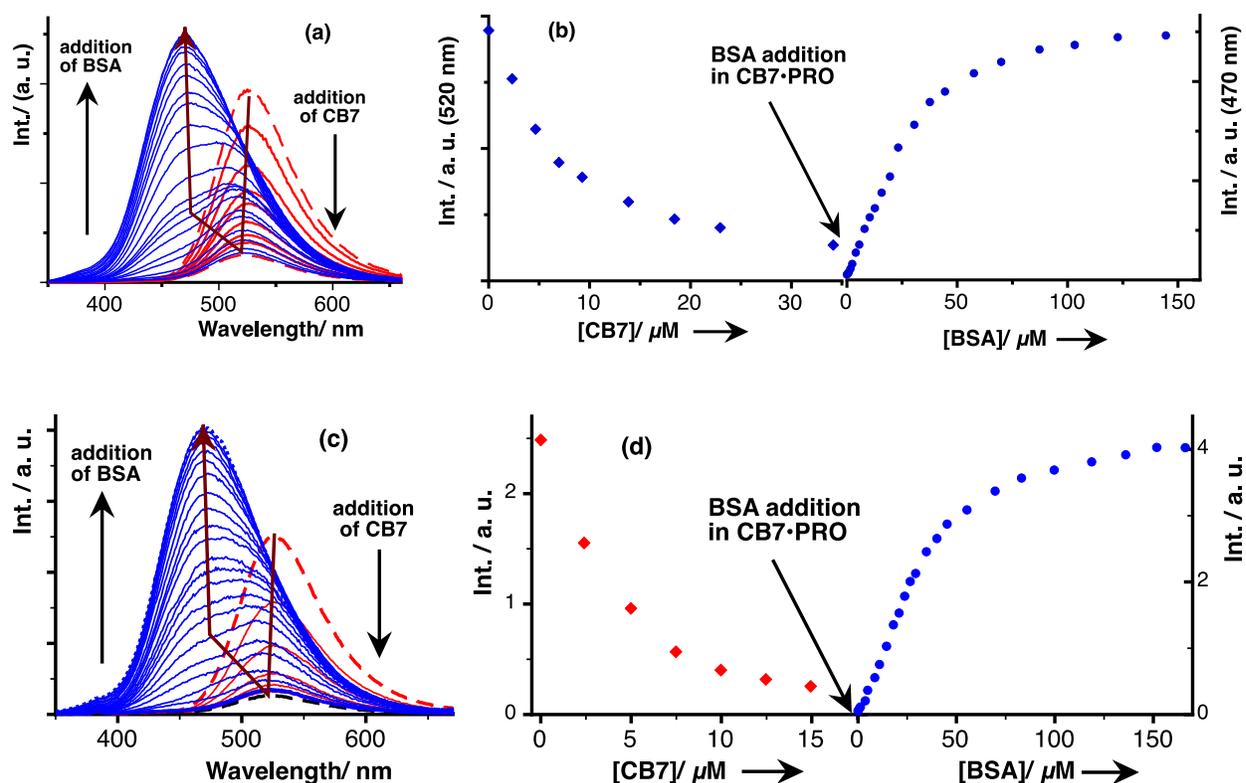


Fig. S6 Switch on relocation process of PRO from CB7 cavity to hydrophobic cavity of BSA at pH 7.3 (a, b) and pH 6.0 (c, d). Fluorescence titration spectra of PRO with incremental addition of CB7 and subsequent addition of BSA in the preformed PRO•CB7 complex gradual increase in fluorescence intensity reaching to a plateau, $\lambda_{\text{ex}}=340$ nm (a, c). Plot of fluorescence intensity against concentration of CB7 or BSA at 520 nm in case of CB7 and 470 nm for BSA respectively (b, d).

References:

1. H. Bakirci, A. L. Koner, T. Schwarzlose and W. M. Nau, *Chem. -A Eur. J.*, 2006, **12**, 4799-4807.
2. A. L. Koner, I. Ghosh, N. Saleh and W. M. Nau, *Can. J. Chem.*, 2011, **89**, 139-147.