ELECTRONIC SUPPORTING INFORMATION

Chemical signal cascading in a supramolecular network

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1. Methods and materials

All chemicals used for the development of the cascade were acquired from Sigma-Aldrich in the highest quality available, except for cucurbit[7]uril (CB7). The latter was prepared by following a published procedure.[1] Its water content was determined to 20% by ^{1}H NMR with malonic acid as internal standard. Memantine and dopamine were used as a hydrochloride salts.

^{1}H-NMR spectra in deuterium oxide (D_2O, >99 atom% D) were recorded with a Varian Mercury 500 MHz or a 400 MHz NMR spectrometer. The chemical shifts (δ/ppm) are referenced to the residual solvent peak D_2O (4.79 ppm). In order to ensure complete equilibration of the multicomponent mixtures, measurements were taken *ca*. 1-3 hours after sample preparation. This timeframe exceeds the previously determined equilibration time period of *ca*. 1-2 minutes, as seen in cuvette experiments that were performed at micromolar concentrations and monitored by optical spectroscopies (see below).

Isothermal calorimetry titrations were performed with a Nano ITC instrument (TGA) and processed by the software NanoAnalyze.

2. Isothermal titration calorimetry

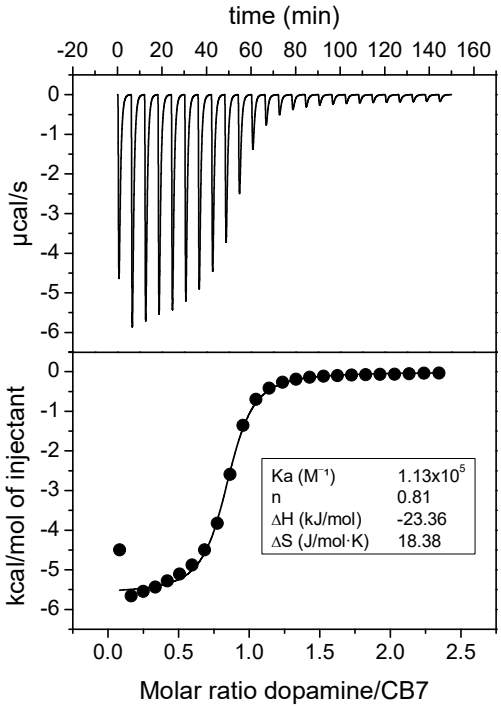

Figure S1. ITC curve for the titration of CB7 (0.87 mM in water) upon consecutive additions of aliquots of a stock solution of dopamine (6.74 mM in water). The experiment was performed at 25 ºC.

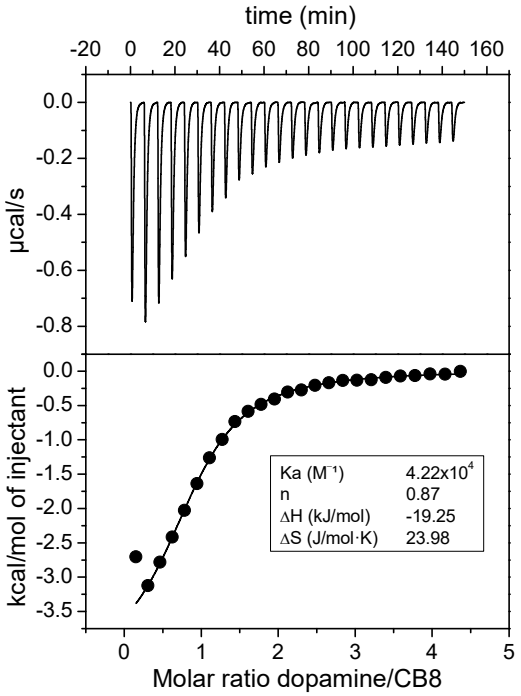

Figure S2. ITC curve for the titration of CB8 (0.10 mM in water) upon consecutive additions of aliquots of a stock solution of dopamine (1.44 mM in water). The experiment was performed at 25 ºC.

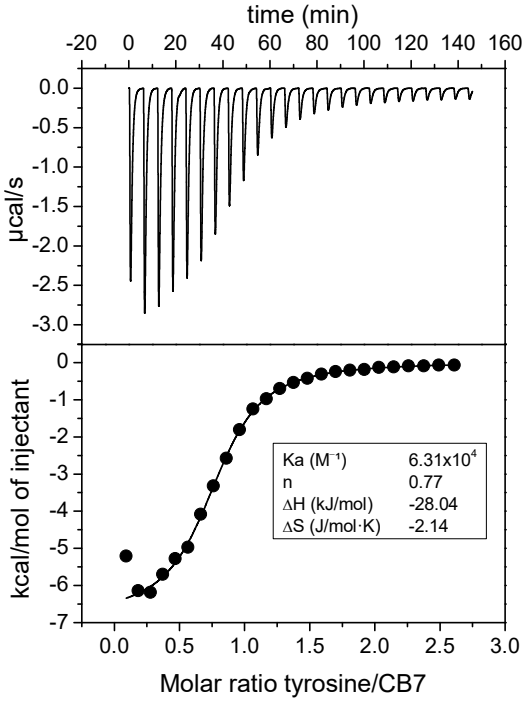

Figure S3. ITC curve for the titration of CB7 (0.35 mM in water) upon consecutive additions of aliquots of a stock solution of tyrosine (3.00 mM in water). The experiment was performed at 25 ºC.

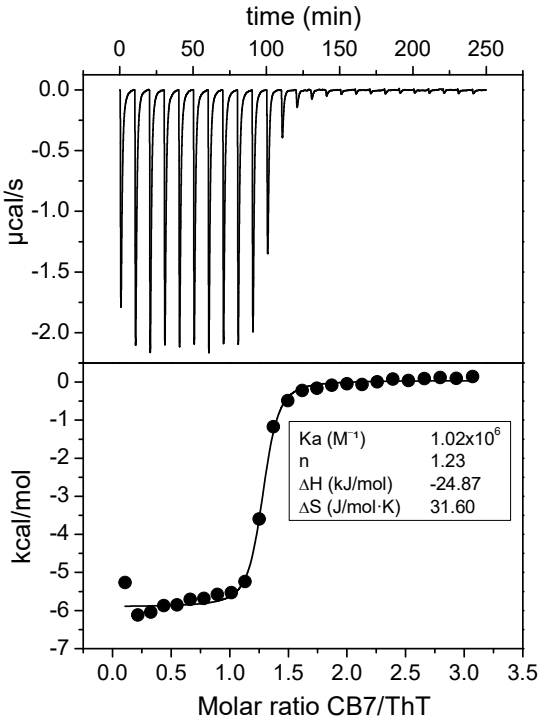

Figure S4. ITC curve for the titration of ThT (0.335 mM in water) upon consecutive additions of aliquots of a stock solution of CB7 (3.40 mM in water). The experiment was performed at 25 ºC.

3. ^{1}H-NMR spectra

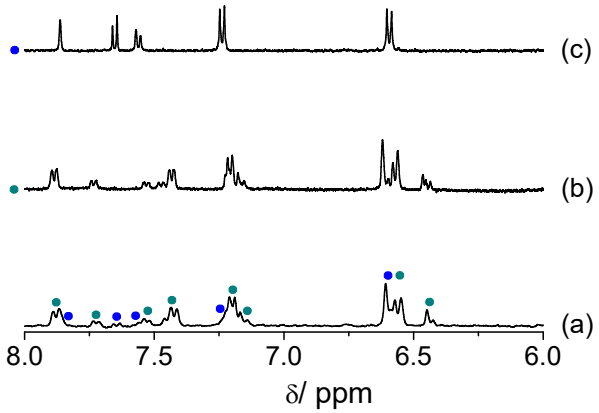

Figure S5. Partial ^{1}H-NMR spectra (in D_2O) of a) mixture of ThT, CB7, and CB8; b) ThT•CB8 (green dots); c) ThT•CB7 (blue dots). [ThT] = 140 μM, [CB7] = [CB8] = 200 μM.

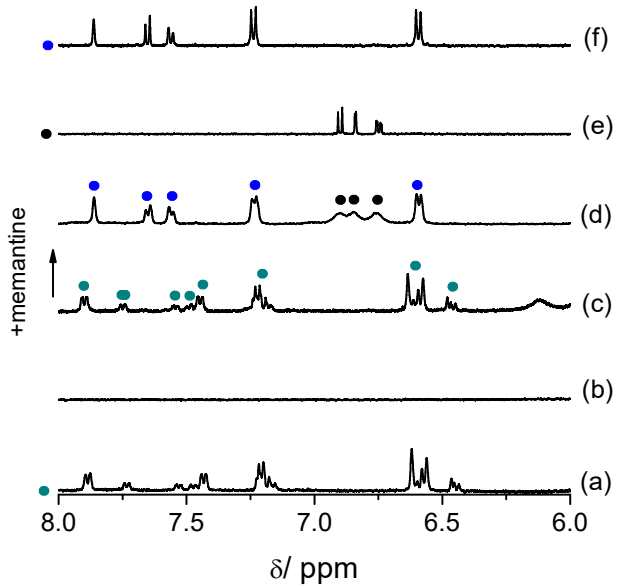

Figure S6. Partial ^{1}H-NMR spectra (in D_2O) of a) ThT•CB8 (green dots); b) dopamine•CB7; c) mixture of ThT, dopamine, CB7, and CB8; d) mixture "c" after addition of memantine; e) free dopamine (black dots); f) ThT•CB7 (blue dots). [ThT] = 140 μM, [dopamine] = [memantine] = [CB7] = [CB8] = 200 μM. Note that the signals of dopamine broaden and join with the baseline on complexation with CB7 (spectrum b).

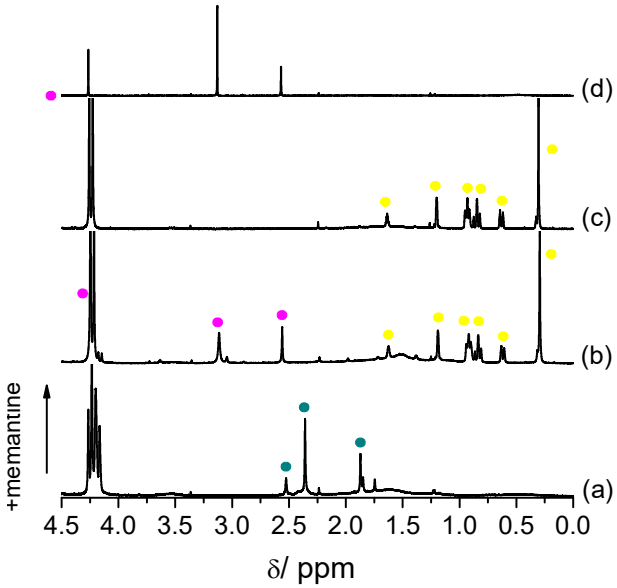

Figure S7. Partial ^{1}H-NMR spectra (in D_2O) of a) ThT•CB8 (green dots); b) solution "a" after addition of memantine; c) memantine•CB8 (yellow dots); d) free ThT (magenta dots). [ThT] = 140 μM, [memantine] = [CB8] = 200 μM.

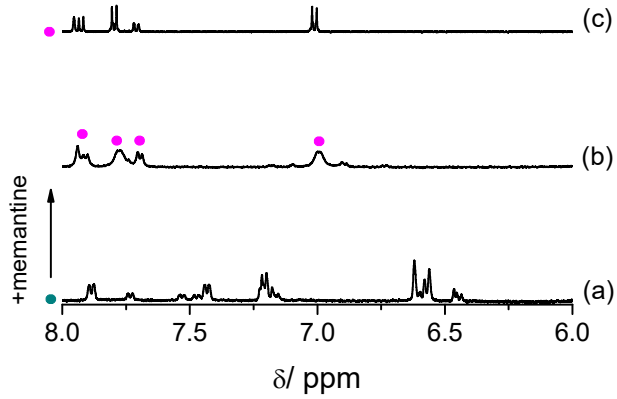

Figure S8. Partial ^{1}H-NMR spectra (in D_2O) of a) ThT•CB8 (green dots); b) solution "a" after addition of memantine; c) free ThT (magenta dots). [ThT] = 140 μM, [memantine] = [CB8] = 200 μM.

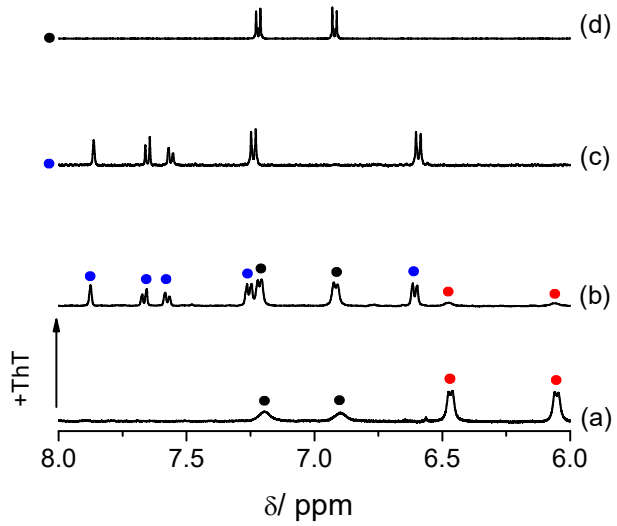

Figure S9. Partial ^{1}H-NMR spectra (in D_2O) of a) tyrosine•CB7 (red dots); b) solution "a" after addition of ThT; c) ThT•CB7 (blue dots); d) free tyrosine (black dots). [ThT] = 140 μM, [tyrosine] = [CB7] = 200 μM.

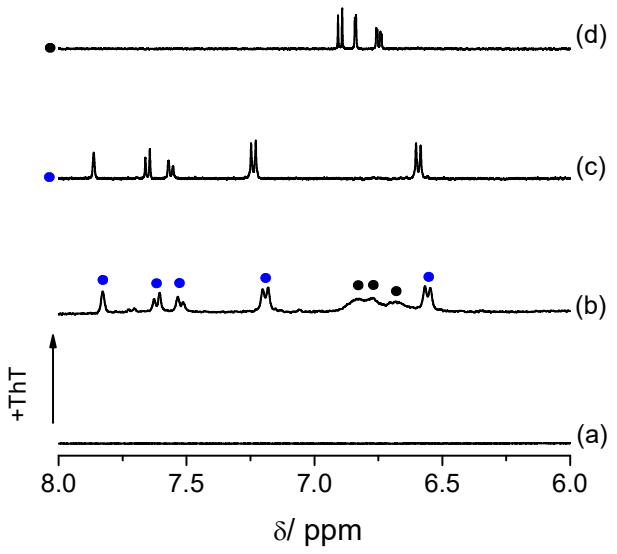

Figure S10. Partial ^{1}H-NMR spectra (in D_2O) of a) dopamine•CB7; b) solution "a" after addition of ThT; c) ThT•CB7 (blue dots); d) free dopamine (black dots). [ThT] = 140 μM, [dopamine] = [CB7] = 200 μM.

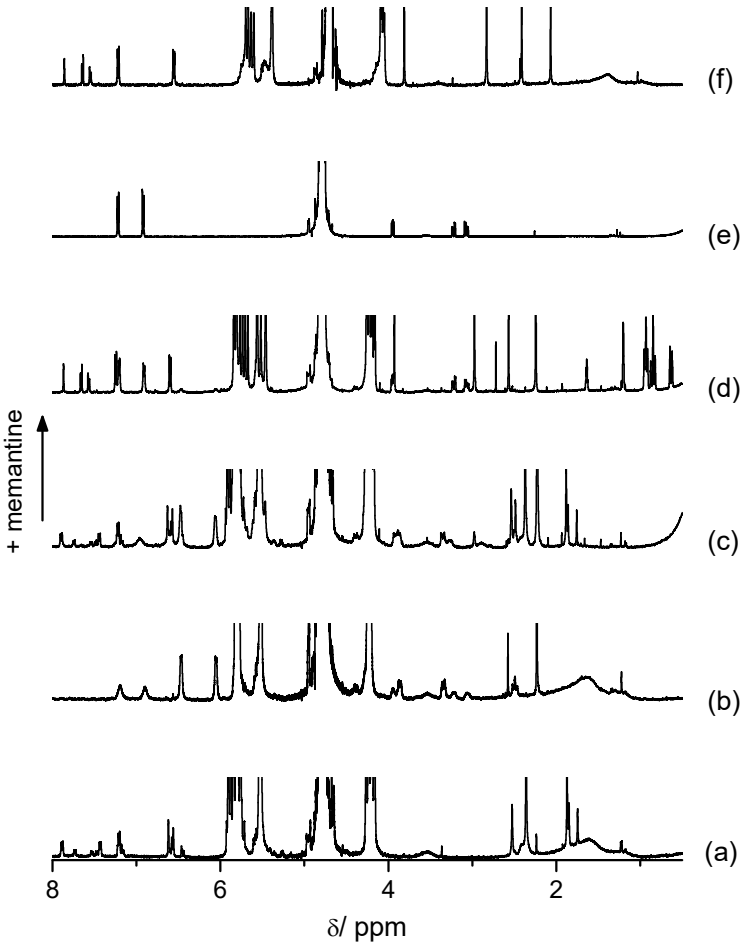

Figure S11. ^{1}H-NMR spectra (D_2O) of a) ThT•CB8; b) tyrosine•CB7; c) the four-component mixture of ThT, tyrosine, CB7, and CB8; d) the four-component mixture after addition of memantine; e) free tyrosine; f) ThT•CB7. [ThT] = 140 μM, [tyrosine] = [memantine] = [CB7] = [CB8] = 200 μM. The spectra correspond to the partial spectra shown in Figure 3 of the main text.

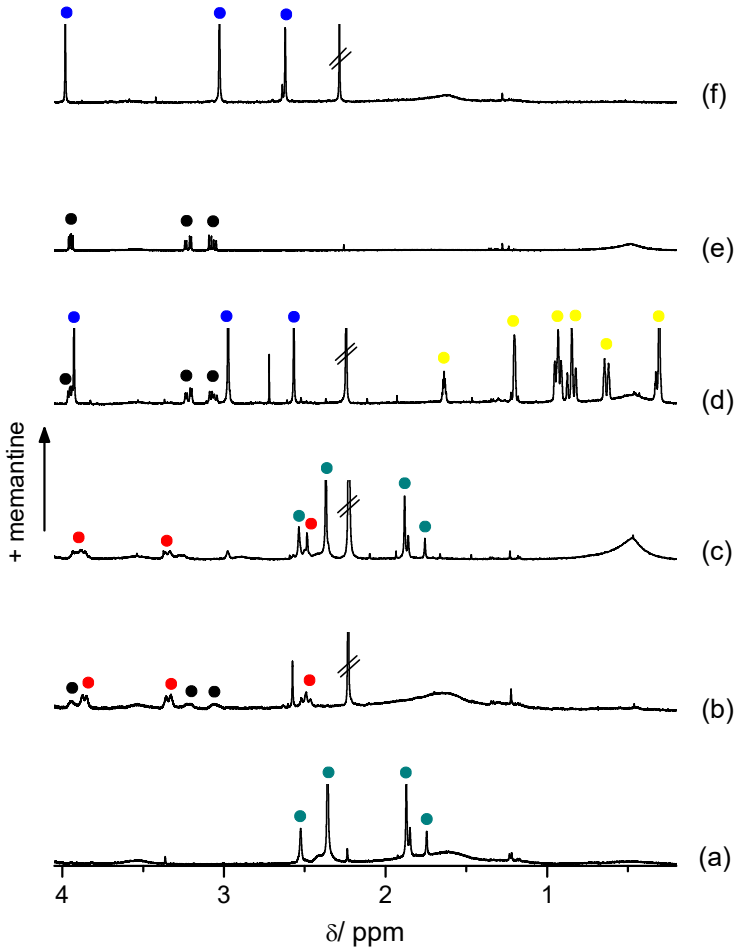

Figure S12. Partial ^{1}H-NMR spectra (D_2O) of a) ThT•CB8 (green dots); b) tyrosine•CB7 (red dots – complex; black dots – free tyrosine); c) the four-component mixture of ThT, tyrosine, CB7, and CB8; d) the four-component mixture after addition of memantine (yellow dots correspond to the signals of CB8-bound memantine) ; e) free tyrosine (black dots); f) ThT•CB7 (blue dots). [ThT] = 140 μM, [tyrosine] = [memantine] = [CB7] = [CB8] = 200 μM.

4. Simulation of the titration of the four-component mixtures with memantine

The algorithm used to simulate the speciation of the multicomponent system containing two hosts (CB7 and CB8) and three guests (memantine, thioflavin T, and dopamine/tyrosine) was based on a system of five equations constructed from the mass balance and equilibrium expressions. This system of equations was then numerically solved using the Newton-Raphson algorithm implemented in a conventional spreadsheet software to calculate the equilibrium concentrations of all species from the binding constants and initial concentrations.

Considering only the formation of 1:1 complexes, the following binding equilibria apply in a multicomponent mixture with two hosts (H_1 and H_2) and three guests (G_1, G_2, and G_3):

$$H_1 + G_1 \overset{K_{11}}{\rightleftharpoons} H_1G_1 \qquad H_2 + G_1 \overset{K_{21}}{\rightleftharpoons} H_2G_1$$

$$H_1 + G_2 \overset{K_{12}}{\rightleftharpoons} H_1G_2 \qquad H_2 + G_2 \overset{K_{22}}{\rightleftharpoons} H_2G_2$$

$$H_1 + G_3 \overset{K_{13}}{\rightleftharpoons} H_1G_3 \qquad H_2 + G_3 \overset{K_{23}}{\rightleftharpoons} H_2G_3$$

Scheme S1. Binding equilibria established in a multicomponent mixture containing two hosts and three guests.

$$[H_1]_0 = [H_1] + [H_1G_1] + [H_1G_2] + [H_1G_3] \qquad (S1)$$

$$[H_2]_0 = [H_2] + [H_2G_1] + [H_2G_2] + [H_2G_3] \qquad (S2)$$

$$[G_1]_0 = [G_1] + [H_1G_1] + [H_2G_1] \qquad (S3)$$

$$[G_2]_0 = [G_2] + [H_1G_2] + [H_2G_2] \qquad (S4)$$

$$[G_3]_0 = [G_3] + [H_1G_3] + [H_2G_3] \qquad (S5)$$

Substituting the concentrations of the complexes by the product of the respective binding constant with the equilibrium concentrations of free host and guest (i.e., $[H_iG_j] = K_{ij}[H_i][G_j]$) leads to a system of five equations and five unknown variables:

$$[H_1] + K_{11}[H_1][G_1] + K_{12}[H_1][G_2] + K_{13}[H_1][G_3] - [H_1]_0 = 0 \qquad (S6)$$

$$[H_2] + K_{21}[H_2][G_1] + K_{22}[H_2][G_2] + K_{23}[H_2][G_3] - [H_2]_0 = 0 \qquad (S7)$$

$$[G_1] + K_{11}[H_1][G_1] + K_{21}[H_2][G_1] - [G_1]_0 = 0 \qquad (S8)$$

$$[G_2] + K_{12}[H_1][G_2] + K_{22}[H_2][G_2] - [G_2]_0 = 0 \qquad (S9)$$

$$[G_3] + K_{13}[H_1][G_3] + K_{23}[H_2][G_3] - [G_3]_0 = 0 \qquad (S10)$$

The solutions of this system of equations provides the equilibrium concentrations of all free species that can be inserted in the equilibrium equations (i.e. $[H_iG_j] = K_{ij}[H_i][G_j]$) to calculate the concentration of the complexes and construct the speciation plots (see main text for tyrosine and Figure S13 for dopamine).

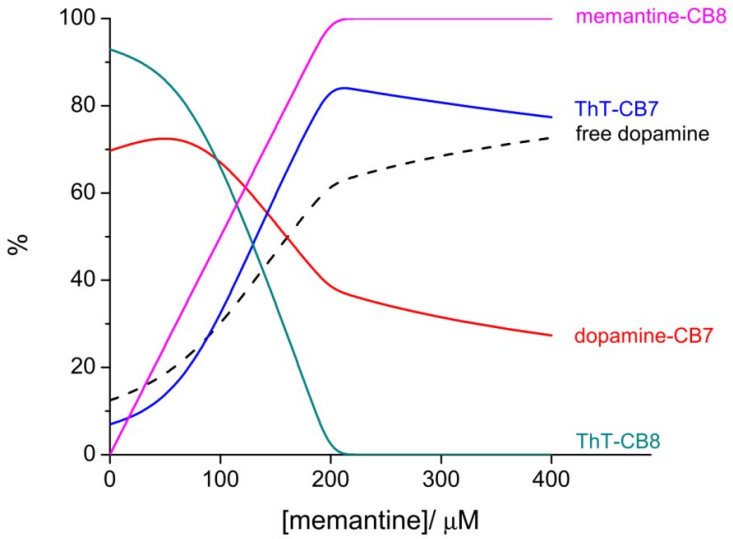

Figure S13. Distribution of the main species upon titration of the four-component mixture of ThT (140 μM), dopamine (200 μM), CB7 (200 μM), and CB8 (200 μM) with memantine. The percentage of each species is expressed relative to the maximum possible concentration. Sub-stoichiometric amounts of ThT were chosen to maximize the complexation degree of this dye with CB8. ThT-CB8 stands for the mixture of the different possible complexes with varying stoichiometric compositions.

5. Following of ThT displacement and re-complexation by optical spectroscopies

The cascade can be also followed by drawing on the photophysical properties (UV/vis absorption and fluorescence) of ThT. ThT has typical spectral fingerprints when present as free dye or when bound either by CB7 or CB8 (see Figure S14 and S15). The free dye shows are relatively broad absorption band with a maximum at 412 nm. On addition of CB7 this band suffers a significant bathochromic shift (by 10 nm) and hypochromic effect.[2] In the presence of CB8 the dye maintains almost the same band position (λ_{max} = 414 nm) but is subjected to a rather pronounced hyperchromic effect.[3] In accordance with the dominance of the ThT•CB8 species in the initial four-component mixture, the

UV/vis absorption spectrum shows the corresponding spectral pattern (see Figure S16). As concerns the fluorescence, the 2:2 and 2:1 complex stoichiometries of ThT•CB8 promote the observation of excimer emission with a typically broad band and maximum at *ca*. 565 nm, while the dye alone or in presence of CB7 shows only weak emission at a maximum of *ca*. 485 nm.[3] Accordingly, in the four-component mixture exclusively the excimer band is seen.

The titration of the four-component mixture with memantine and monitoring the spectral absorbance and emission patterns of ThT leads to conclusion that ThT is competitively displaced from CB8 and re-located in the CB7 cavity. The initially present spectral features of ThT•CB8 disappear and at the endpoint of the titration the typical spectra of the ThT•CB7 have emerged (see Figure S17).

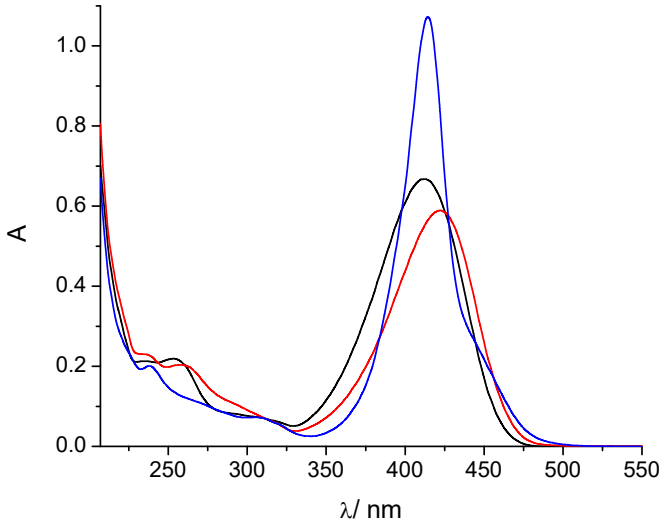

Figure S14. UV/vis absorption spectra of ThT (18.5 μM) alone (black line) and in the presence of CB7 (30 μM, red line) or CB8 (30 μM, blue line); in water.

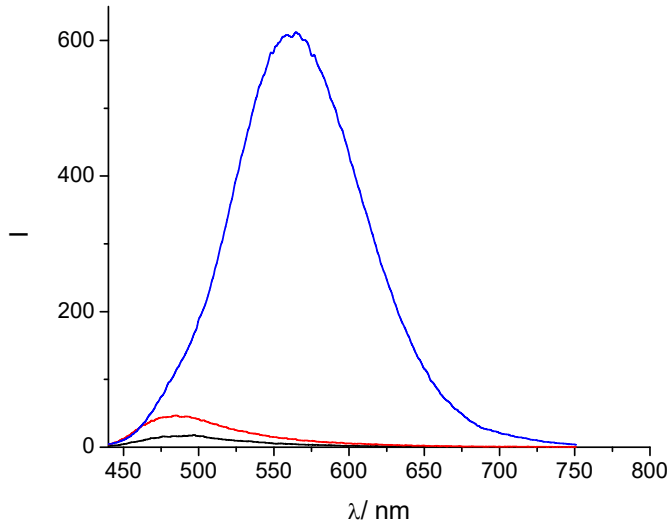

Figure S15. Fluorescence spectra (λ_{exc} = 427 nm) of ThT (18.5 μM) alone (black line) and in the presence of CB7 (30 μM, red line) or CB8 (30 μM, blue line); in water.

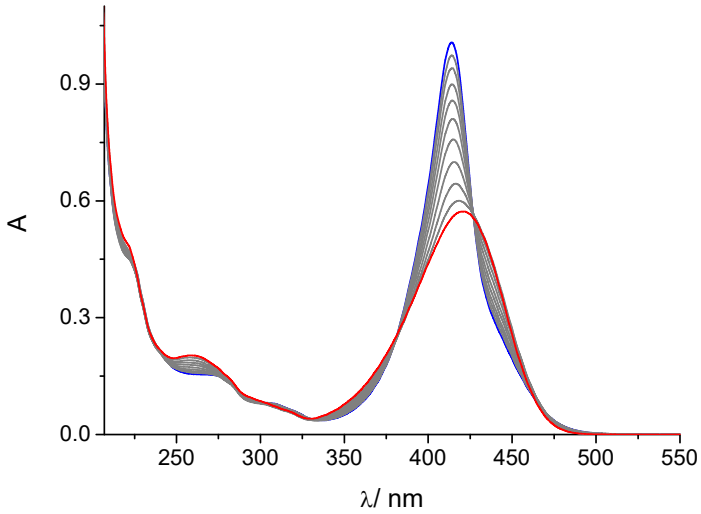

Figure S16. UV/vis absorption titration of the four-component mixture of ThT (18.5 μM), CB7 (30 μM), CB8 (30 μM), and tyrosine (30 μM) with increasing amounts of memantine (0-30 μM); in water. The blue spectrum represents the initial spectrum and the red one corresponds to the final spectrum.

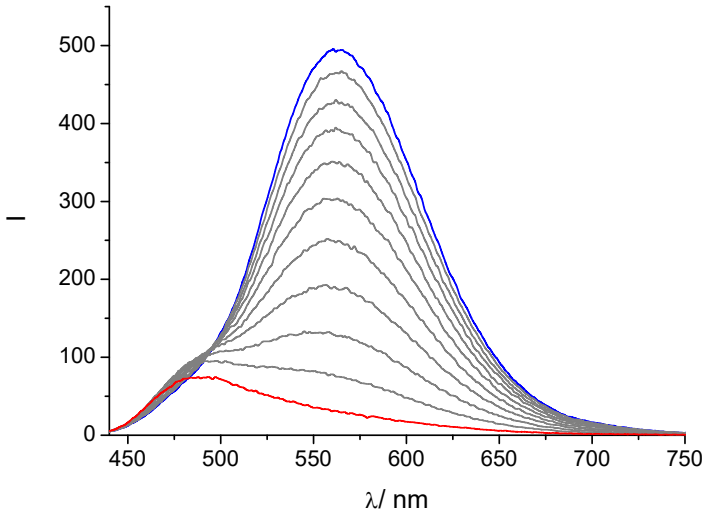

Figure S17. Fluorescence titration (λ_{exc} = 427 nm, isosbestic point in UV/vis absorption titration; see Figure S16) of the four-component mixture of ThT (18.5 μM), CB7 (30 μM), CB8 (30 μM), and tyrosine (30 μM) with increasing amounts of memantine (0-30 μM); in water. The blue spectrum represents the initial spectrum and the red one corresponds to the final spectrum.

References

1. C. Márquez, F. Huang and W. M. Nau, *IEEE Trans. Nanobiosci.*, 2004, **3**, 39-45.
2. S. D. Choudhury, J. Mohanty, H. P. Upadhyaya, A. C. Bhasikuttan and H. Pal, *J. Phys. Chem. B*, 2009, **113**, 1891-1898.
3. J. Mohanty, S. D. Choudhury, H. P. Upadhyaya, A. C. Bhasikuttan and H. Pal, *Chem. Eur. J.*, 2009, **15**, 5215-5219.