ELECTRONIC SUPPLEMENTARY INFORMATION

FOR

A Supramolecular Five-Component Relay Switch that Exposes the Mechanistic Competition of Dissociative versus Associative Binding to Cucurbiturils by Ratiometric Fluorescence Monitoring

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Materials and Methods

All reagents and solvents were commercially available and used as received without further purification. Acridine orange (AO), *trans*-4-[4-(dimethylamino)styryl]-1-methylpyridinium iodide (DSMI), and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma-Aldrich. The putrescine derivative of aminomethyladamantane (AMADA-Put)[1], cucurbit[6]uril (CB6)[2], and cucurbit[7]uril (CB7)[2] were synthesized and purified according to reported literature procedures. For all experiments, Millipore water (<18.2MΩ cm) from an ELGA Labwater Classic water purification system was used. Absorption measurements were performed with a Varian Cary 4000 spectrophotometer and fluorescence spectra were recorded on a Varian Cary Eclipse spectrofluorimeter. ^{1}H NMR spectra were recorded on a JEOL JNM-ECX 400 spectrometer working at 400 MHz. The time-dependent fluorescence data were analyzed using homemade programs written in proFit 6.2.13 (QuantumSoft, Switzerland).

Abbreviations

AMADA-Put: the putrescine derivative of aminomethyladamantane; AO: acridine orange; CB6: cucurbit[6]uril; CB7: cucurbit[7]uril; DSMI: *trans*-4-[4-(dimethylamino)styryl]-1-methylpyridinium iodide.

Derivation of Rate Law for Dissociative Pathway I

The unimolecular dissociation pathway (Fig. 1 in main manuscript, Pathway I) is described by the law of mass action considering binding of CB6 and CB7 with the two respective binding sites of the guest:

$$\mathrm{CB6G} \leftrightharpoons_{k_{\mathrm{outCB6}}}^{k_{\mathrm{inCB6}}} \mathrm{CB6} + \mathrm{G} \tag{EQ1}$$

$$\mathrm{G} + \mathrm{CB7} \leftrightharpoons_{k_{\mathrm{inCB7}}}^{k_{\mathrm{outCB7}}} \mathrm{CB7G} \tag{EQ2}$$

Therein, k_{inCB6}, k_{outCB6}, k_{inCB7}, and k_{outCB7} are the association and dissociation rate constants, which are related to the respective binding constants, K_{CB6} and K_{CB7} by EQ3 and EQ4:

$$K_{\mathrm{CB6}} = \frac{k_{\mathrm{inCB6}}}{k_{\mathrm{outCB6}}} \tag{EQ3}$$

$$K_{\mathrm{CB7}} = \frac{k_{\mathrm{inCB7}}}{k_{\mathrm{outCB7}}} \tag{EQ4}$$

The overall concentration changes can be described by a system of two coupled differential equations, EQ5 and EQ6, in which $[\mathrm{CB6G}]_t$, $[\mathrm{CB6}]_t$, and $[\mathrm{G}]_t$ are the time-dependent concentrations of CB6 host-guest complex, free CB6 host, and free guest, respectively, and $[\mathrm{CB7G}]_t$ and $[\mathrm{CB7}]_t$, the time-dependent concentrations of CB7 host-guest complex and free CB7 host:

$$\frac{\mathrm{d}[\mathrm{CB6}]_\mathrm{t}}{\mathrm{d}t} = k_{\mathrm{outCB6}}[\mathrm{CB6G}]_\mathrm{t} - K_{\mathrm{CB6}}k_{\mathrm{outCB6}}[\mathrm{CB6}]_\mathrm{t}[\mathrm{G}]_\mathrm{t} \tag{EQ5}$$

$$\frac{\mathrm{d}[\mathrm{CB7G}]_\mathrm{t}}{\mathrm{d}t} = K_{\mathrm{CB7}}k_{\mathrm{outCB7}}[\mathrm{CB7}]_\mathrm{t}[\mathrm{G}]_\mathrm{t} - k_{\mathrm{outCB7}}[\mathrm{CB7G}]_\mathrm{t} \tag{EQ6}$$

Considering that $[\mathrm{CB6}]_{\mathrm{tot}}$, $[\mathrm{CB7}]_{\mathrm{tot}}$, and $[\mathrm{G}]_{\mathrm{tot}}$ are the total concentrations of CB6, CB7, and guest, respectively, law of mass conservation requires that:

$$[\mathrm{CB6}]_\mathrm{tot} = [\mathrm{CB6}]_\mathrm{t} + [\mathrm{CB6G}]_\mathrm{t} \tag{EQ7}$$

$$[\mathrm{CB7}]_\mathrm{tot} = [\mathrm{CB7}]_\mathrm{t} + [\mathrm{CB7G}]_\mathrm{t} \tag{EQ8}$$

$$[\mathrm{G}]_\mathrm{tot} = [\mathrm{CB6G}]_\mathrm{t} + [\mathrm{CB7G}]_\mathrm{t} + [\mathrm{G}]_\mathrm{t} \tag{EQ9}$$

Combining EQ7-9 with EQ 5-6 gives a set of two coupled differential equations, EQ10 and EQ11, containing two unknowns, $[\mathrm{CB6}]_t$ and $[\mathrm{CB7G}]_t$:

$$\frac{\mathrm{d}[\mathrm{CB6}]_\mathrm{t}}{\mathrm{d}t} = k_{\mathrm{outCB6}}([\mathrm{CB6}]_\mathrm{tot} - [\mathrm{CB6}]_\mathrm{t}) - K_{\mathrm{CB6}}k_{\mathrm{outCB6}}[\mathrm{CB6}]_\mathrm{t}([\mathrm{G}]_\mathrm{tot} - [\mathrm{CB6}]_\mathrm{tot} + [\mathrm{CB6}]_\mathrm{t} - [\mathrm{CB7G}]_\mathrm{t}) \tag{EQ10}$$

$$\frac{\mathrm{d}[\mathrm{CB7G}]_\mathrm{t}}{\mathrm{d}t} = K_{\mathrm{CB7}}k_{\mathrm{outCB7}}([\mathrm{CB7}]_\mathrm{tot} - [\mathrm{CB7G}]_\mathrm{t})([\mathrm{G}]_\mathrm{tot} - [\mathrm{CB6}]_\mathrm{tot} + [\mathrm{CB6}]_\mathrm{t} - [\mathrm{CB7G}]_\mathrm{t}) - k_{\mathrm{outCB7}}[\mathrm{CB7G}]_\mathrm{t} \tag{EQ11}$$

The boundary conditions required to solve the differential equations are found in the table below. Therein, $[\mathrm{CB6G}]_0$, $[\mathrm{CB6}]_0$, $[\mathrm{G}]_0$, $[\mathrm{CB7G}]_0$, and $[\mathrm{CB7}]_0$ are the initial concentrations of CB6 host-guest complex, free CB6 host, free guest, CB7 host-guest complex and free CB7 host, respectively, at time $t = 0$ s.

Time	[CB6G]	[CB7]	[G]	[CB6]	[CB7G]
0	$[CB6]_{tot}-[CB6]_0$	$[CB7]_{tot}$	$[G]_0$	$[CB6]_0$	0
t	$[CB6]_{tot}-[CB6]_t$	$[CB7]_{tot}-[CB7G]_t$	$[G]_{tot}-[CB6]_{tot}+[CB6]_t-[CB7G]_t$	$[CB6]_t$	$[CB7G]_t$

Since CB7 has just been added at t = 0 s, $[CB7]_0 = [CB7]_{tot}$ and $[CB7G]_0$ = 0 M, whereas the initial concentrations $[CB6]_0$, $[G]_0$, and $[CB6G]_0$ can be obtained from the equilibrium:

$$K_{CB6} = \frac{[CB6G]_0}{[CB6]_0 \times [G]_0} \quad \text{(EQ12)}$$

$$[CB6]_0 + [CB6G]_0 = [CB6]_{tot} \quad \text{(EQ13)}$$

$$[G]_0 + [CB6G]_0 = [G]_{tot} \quad \text{(EQ14)}$$

Combining EQ12-14 gives:

$$[CB6G]_0 = \frac{([G]_{tot}+[CB6]_{tot}+1/K_{CB6})}{2} + \sqrt{\frac{([G]_{tot}+[CB6]_{tot}+1/K_{CB6})^2}{4} - [CB6]_{tot}[G]_{tot}} \quad \text{(EQ15)}$$

$$[CB6]_0 = [CB6]_{tot} - \frac{([G]_{tot}+[CB6]_{tot}+1/K_{CB6})}{2} - \sqrt{\frac{([G]_{tot}+[CB6]_{tot}+1/K_{CB6})^2}{4} - [CB6]_{tot}[G]_{tot}} \quad \text{(EQ16)}$$

Under our experimental conditions, the time-dependent fluorescence intensity, I_t, and the concentration of the time-dependent concentration of CB6 not bound with the guest, $[CB6]_t$, are linearly related (see also Figure S2b and note that we neglect the interaction with the much weaker binding dye DSMI) and the fluorescence intensity of the fully dye-complexed host, I_{dh}, refers to CB6 not bound with the ditopic guest, whereas the intensity of the free dye, I_d, refers to a host fully complexed with the ditopic guest. The conversion of the fluorescence intensity changes into concentration changes is then achieved using EQ 17, which can be rearranged to give EQ18:

$$\frac{[CB6]_t}{[CB6]_{tot}} = \frac{I_t - I_d}{I_{dh} - I_d} \quad \text{(EQ17)}$$

$$I_t = I_d + \frac{(I_{dh} - I_d) \times [CB6]_t}{[CB6]_{tot}} \quad \text{(EQ18)}$$

Equations EQ 11, EQ12, EQ 16, and EQ 18 are then implemented into proFit V6.2.13 (QuantumSoft, Switzerland) and numerically solved using the Runge-Kutta method.

Derivation of Rate Law for Associative Pathway II

The associative pathway II involves the formation of a ternary complex, CB6GCB7, with the respective association and dissociation rate constants k_{inCB6t}, $k_{outCB6t}$, k_{inCB7t}, and $k_{outCB7t}$:

$$\mathrm{CB6G} + \mathrm{CB7} \leftrightharpoons_{k_{inCB7t}}^{k_{outCB7t}} \mathrm{CB6GCB7} \leftrightharpoons_{k_{outCB6t}}^{k_{inCB6t}} \mathrm{CB7G} + \mathrm{CB6} \quad \text{(EQ19)}$$

Since no stable ternary complex could be detected by ^{1}H NMR, we can assume a constant low concentration of the ternary complex during the relay process, which allows to apply the steady-state approximation ($d[CB6GCB7]_t/dt=0$). Furthermore, the relay process is essentially irreversible such that we set $k_{inCB6t} = 0\ M^{-1}\ s^{-1}$.

According to steady-state approximation: $\frac{d[CB6GCB7]_t}{dt} = 0$, we have:

$$k_{inCB7t}[CB6G]_t[CB7]_t - k_{outCB7t}[CB6GCB7]_t - k_{outCB6t}[CB6GCB7]_t + k_{inCB6t}[CB7G]_t[CB6]_t = 0 \quad \text{(EQ20)}$$

Assume $k_{inCB6t} = 0$,

$$k_{inCB7t}[CB6G]_t[CB7]_t - k_{outCB7t}[CB6GCB7]_t - k_{outCB6t}[CB6GCB7]_t = 0 \quad \text{(EQ21)}$$

$$[CB6GCB7]_t = \frac{k_{inCB7t}[CB6G]_t[CB7]_t}{k_{outCB7t}+k_{outCB6t}} \quad \text{(EQ22)}$$

From EQ19 we get:

$$\frac{d[CB6]_t}{dt} = k_{outCB6t}[CB6GCB7]_t - k_{inCB6t}[CB7G]_t[CB6]_t \quad \text{(EQ23)}$$

Combining EQ22 with EQ23 and applying the assumption of $k_{inCB6t} = 0$ give us EQ24:

$$\frac{d[CB6]_t}{dt} = \frac{k_{outCB6t}\times k_{inCB7t}}{k_{outCB7t}+k_{outCB6t}}[CB6G]_t[CB7]_t \quad \text{(EQ24)}$$

Then we define the apparent rate constant $k_{app} = \frac{k_{outCB6t}\times k_{inCB7t}}{k_{outCB7t}+k_{outCB6t}}$, which affords

$$\frac{d[CB6]_t}{dt} = k_{app}[CB6G]_t[CB7]_t \quad \text{(EQ25)}$$

Similar as Pathway I, we consider that $[CB6]_{tot}$, $[CB7]_{tot}$, and $[G]_{tot}$ are the total concentrations of CB6, CB7, and guest, respectively, law of mass conservation requires EQ7 and EQ8. And in Pathway II, we ignore the small concentration of CB6GCB7 and G, so we have:

$$[G]_{tot} = [CB6G]_t + [CB7G]_t \quad \text{(EQ26)}$$

Combining EQ7, EQ8, EQ25, and EQ26 gives a differential equation, EQ27, containing one unknown, $[CB6]_t$.

$$\frac{d[CB6]_t}{dt} = k_{app}([CB6]_{tot} - [CB6]_t)([CB7]_{tot} + [CB6]_{tot} - [CB6]_t - [G]_{tot}) \quad \text{(EQ27)}$$

The boundary conditions required to solve the differential equations are as same as Pathway I, i.e., EQ16. The conversion of the fluorescence intensity changes into concentration changes is also achieved using EQ18. Equations EQ27, EQ 16, and EQ 18 are then implemented into proFit V6.2.13 (QuantumSoft, Switzerland) and numerically solved using the Runge-Kutta method.

Supporting Results

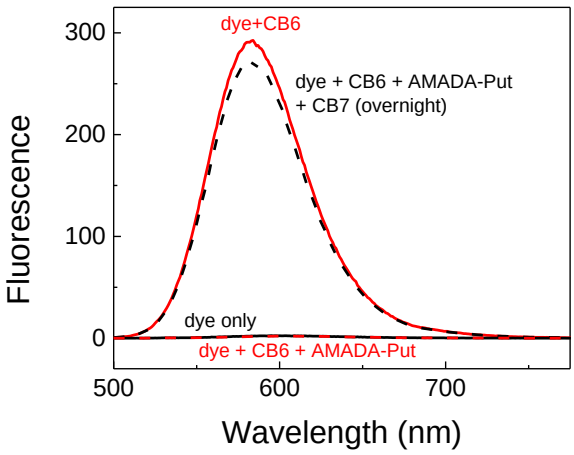

Figure S1. Fluorescence spectra of 4 μM DSMI (black solid line), after addition of 4 μM CB6 (red solid line), 5 μM ADAMA-Put (red dashed line) and 5 μM CB7 overnight (black dashed line) in 10 mM Tris, pH 7.5, λ_{exc} = 450 nm.

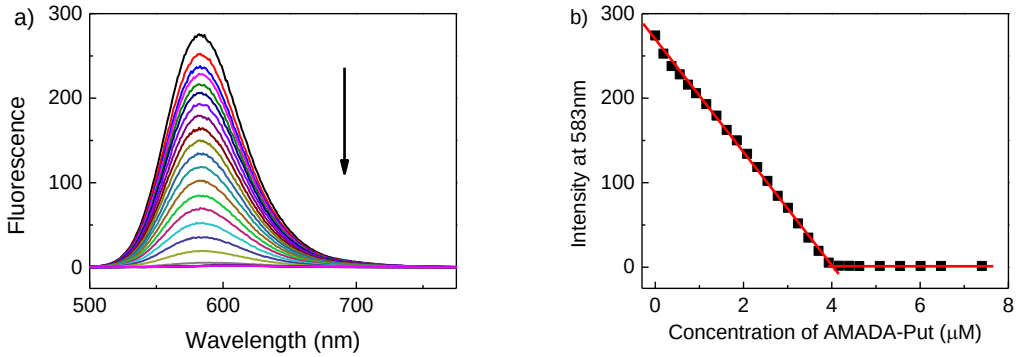

Figure S2. a) Fluorescence spectral changes upon addition of AMADA-Put (0 to 7.4 μM) to 4 μM CB6 and 4 μM DSMI in 10 mM Tris, pH 7.5, λ_{exc} = 450 nm. b) Corresponding fluorescence changes at 583 nm.

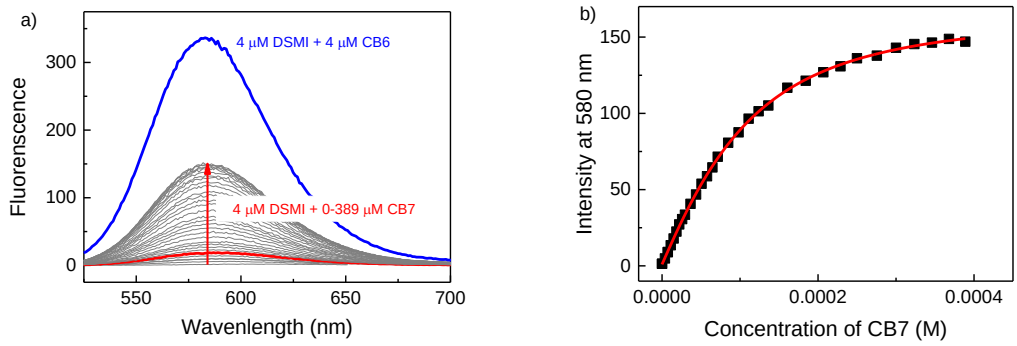

Figure S3. a) Fluorescence spectral changes (λ_{exc} = 450 nm) of 4 μM DSMI upon addition of 0 to 389 μM CB7 (gray thin lines, the thick red line is 15 μM) compared to 4 μM CB6 (blue) in 10 mM Tris, pH 7.5. b) Corresponding fluorescence changes at 580 nm of the CB7 titration.

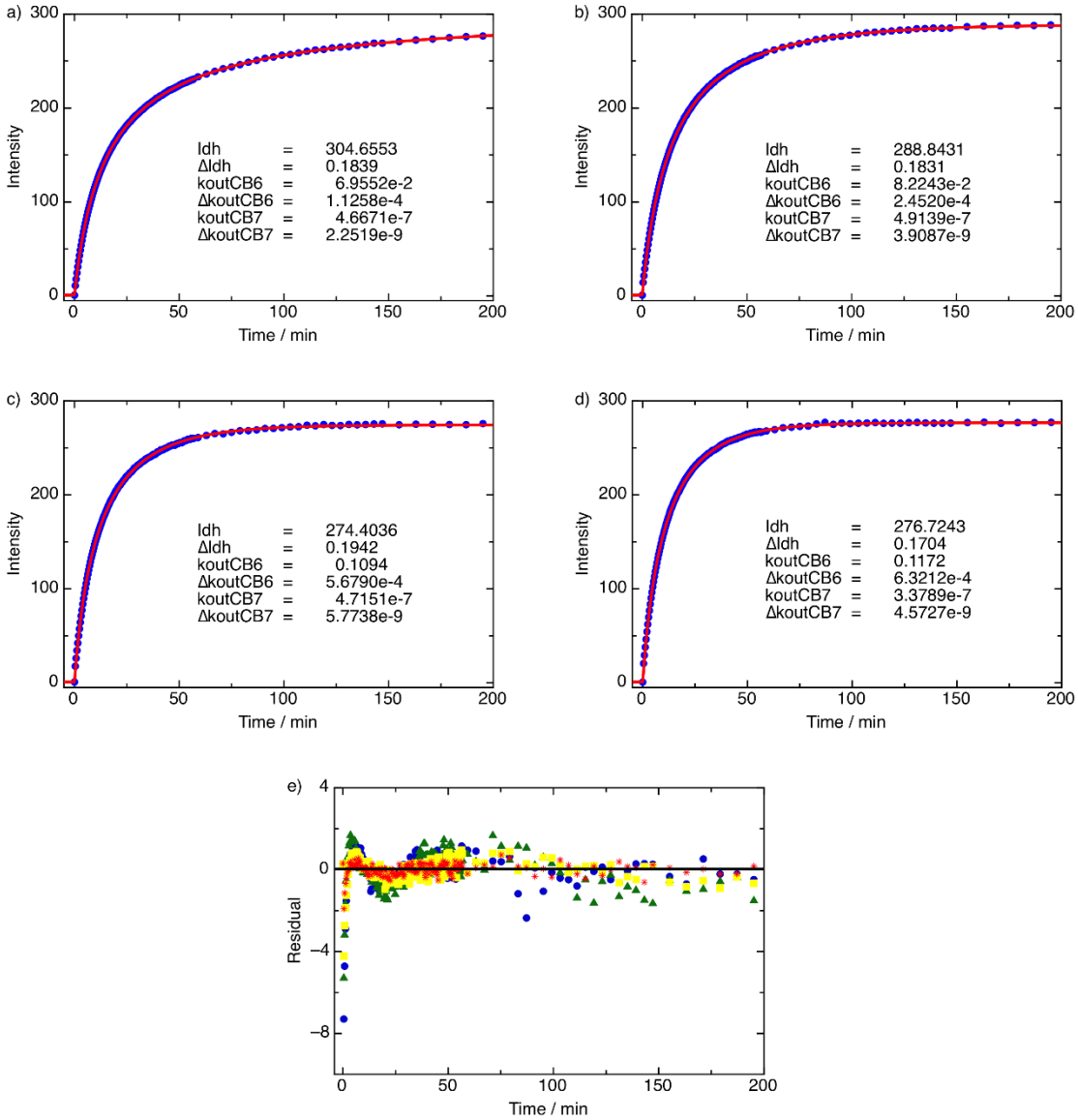

Figure S4. Fitting results of time-dependent fluorescence data with different CB7 concentrations using the unimolecular dissociation pathway model: a) [CB7] = 5 μM, b) 7.5 μM, c) 10 μM, and d) 15 μM. e) Residuals plots of corresponding fitting results: [CB7] = 5 μM (red star), 7.5 μM (yellow square), 10 μM (green triangle), and 15 μM (blue circle).

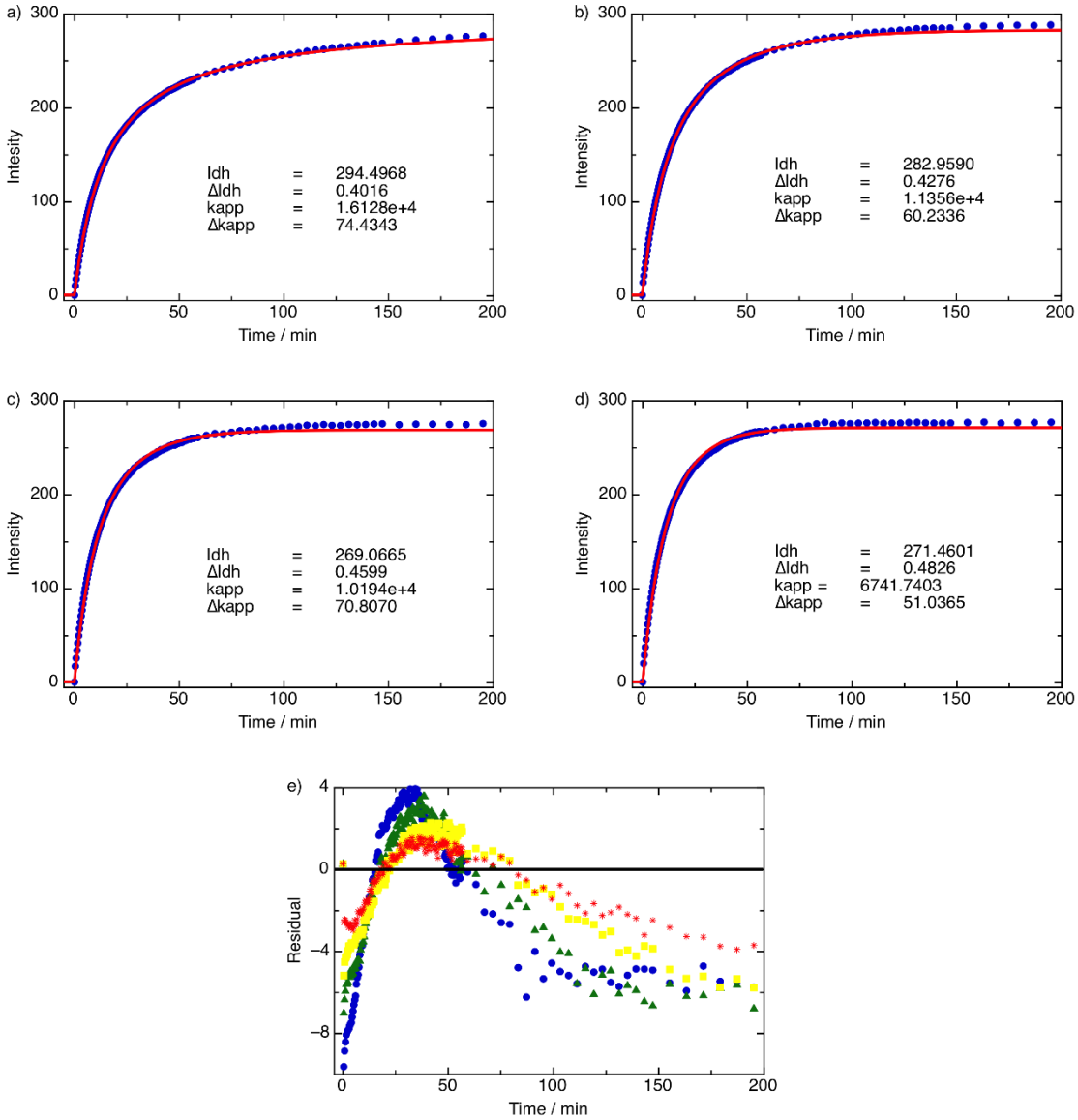

Figure S5. Fitting results of time-dependent fluorescence data with different CB7 concentrations using the associative pathway model: a) [CB7] = 5 μM, b) 7.5 μM, c) 10 μM, and d) 15 μM. e) Residuals plots of corresponding fitting results: [CB7] = 5 μM (red star), 7.5 μM (yellow square), 10 μM (green triangle), and 15 μM (blue circle).

Table S1. Fitting results of time-dependent fluorescence data

[CB7] (μM)	k_{outCB6} (10^{-3} s^{-1})	k_{outCB7} (10^{-9} s^{-1})
5	1.16 ± 0.01	7.78±0.04
7.5	1.37 ± 0.01	8.19±0.07
10	1.82 ± 0.01	7.86±0.10
15	1.95 ± 0.01	5.63±0.08

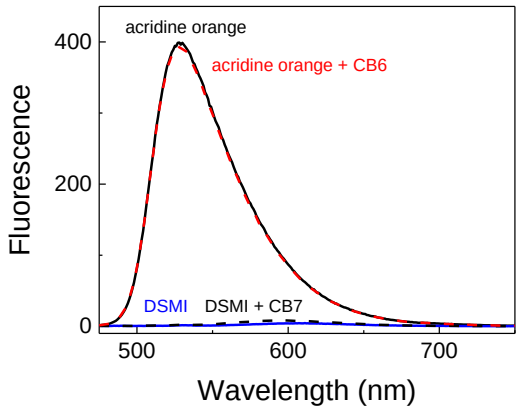

Figure S6. Fluorescence spectra of 1.5 μM AO in absence (black solid line) and presence of 1.5 μM CB7 (red dashed line), and of 1.35 μM DSMI in absence (blue solid line) and presence of 1.5 μM CB7 (blue dashed line) in 10 mM Tris, pH 7.5, λ_{exc} = 450 nm.

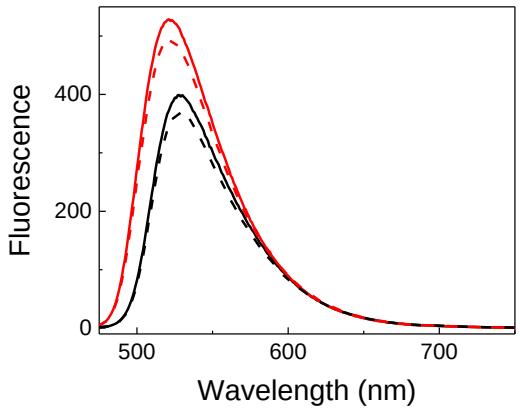

Figure S7. Fluorescence spectra (λ_{exc} = 450 nm) of 1.5 μM AO in absence (black lines) and presence of 1.5 μM CB7 (red lines) in 10 mM Tris, pH 7.5. In presence of additional 1.35 μM DSMI (dashed lines), the fluorescence is slightly reduced regardless of the presence of CB7.

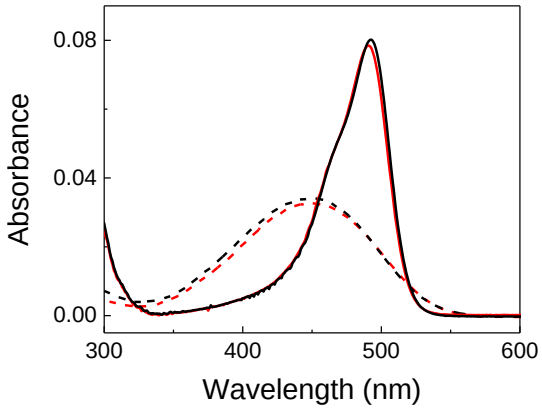

Figure S8. Absorption spectra in 10 mM Tris, pH 7.5 of 1.5 μM AO (solid lines) and 1.35 μM DSMI (dashed lines) in absence (black) and presence (red) of 1.5 μM CB7 or 1.35 μM CB6, respectively.

Reference

1. A. Hennig, A. Hoffmann, H. Borcherding, T. Thiele, U. Schedler and U. Resch-Genger, *Chem. Commun.*, 2011, **47**, 7842-7844.
2. C. Marquez, H. Fang and W. M. Nau, *IEEE Trans. Nanobiosci.*, 2004, **3**, 39-45.