Triggering autocatalytic reaction by host-guest interactions

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Electronic Supporting Information

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1. Materials, instrumentation and methods

All chemicals were purchased as reagent grade from commercial suppliers (Sigma-Aldrich, Fluka) and used without further purification. The solvents used (Merck, ChemPur, PoCh) were of analytical grade quality. Solvents and glassware for NMR measurements were purchased from Armar Chemicals.

CB6 was synthesized according to a literature procedure [A. Day et al, J. Org. Chem. 2001, 66, 8094-8100]. Prior to use, CB6 was purified from acid remnants by excessive washing with Millipore water (18.2 MΩ·cm). In brief, acid-contaminated CB6 was mixed with water and sonicated. The obtained slurry was centrifuged and the aqueous solution was discarded. The purification procedure was repeated until an indicator paper showed neutral pH of supernatant. Afterwards, CB6 powder was sonicated and rinsed with copious amounts of water and dried under vacuum (0.1 mbar) at 100°C for 6 hours. Elemental analysis showed no presence of chloride ions in the sample.

NMR spectra were recorded on Bruker (400 and 600 MHz) instruments. The chemical shifts ($\delta$) are given in ppm relative to TMS, coupling constants are (J) in Hz. All experiments were performed in SHP NMR tubes at room temperature in D$_2$O (99.8 atom%). pH was adjusted by addition of diluted solutions of DCl (38 wt% in D$_2$O, 99.5 atom%) and NaOD (30 wt% in D$_2$O, 99.5 atom%). The use of a buffer was discouraged due to: (i) poor substrate solubility; (ii) competition of an external cation for binding to CB6; (iii) termination of autocatalysis. Low-temperature NMR measurements were carried out with D$_2$O pre-saturation. NMR data were analyzed using MestReNova and Origin Software. In view of signal broadening of $S_1$, precipitation of $P_0$ and the high instability (reactivity) of $P_2$, the quantitative information about the process was obtained by integrating NMR signals of aromatic protons of phenolic substrate ($S_2$, $S_3$, $S_1$) and $P_1$. MS spectra were recorded on Maldi SYNAPT G2-S HDMS (Waters) spectrometer. pH was measured using HI 3220 pH Meter equipped with InLab® Micro glass electrode (Mettler Toledo) and converted to pD values by the addition of 0.4 units [P. K. Glasoe et al, J. Phys. Chem. 1960, 64, 188-190]. The X-ray data were collected at 100 K on a SuperNova Agilent diffractometer using CuKα radiation ($\lambda = 1.54184$ Å) and MoKα radiation ($\lambda = 0.71073$ Å). The data were processed with CrysAlisPro (Agilent Technologies, CrysAlisPro, Version 1.171.35.21b). Structures were solved by direct methods and refined using SHELXL-97 (G. M. Sheldrick, Acta Cryst. 2008, 64A, 112-122).
2. Reaction between \( S_1 \) and \( S_2 \)

CB6 powder (3.9 mg, 3.9 µmol) was suspended in 200 µL of \( D_2O \) and then diluted to 600 µL using equimolar (3.9 µmol) aliquots of freshly prepared stock solutions of \( S_1 \) and \( S_2 \) in \( D_2O \). The pD of the suspension was adjusted during the dilution. The final concentration of substrates was 6.5 mM. The experiment was run in parallel with the control experiment. The reaction progress was monitored by \(^1H\) NMR. After the experiment was finished, the suspension was stirred for a few hours with calcium chloride to remove CB6 species. The resulting suspension was centrifuged affording \( P_3 \) as an orange precipitate which is poorly soluble in common organic solvents. The azo product was washed several times with water by centrifugation and dried in vacuo. In order to solubilize the product and to acquire NMR data, DMSO-\( d_6 \)/NH\( _4 \)PF\( _6 \) mixture was used. MS spectrum was recorded using highly diluted solution of the product in MeOH. \(^1H\) NMR (400 MHz, DMSO-\( d_6 \)): \( \delta \) 8.04–7.89 (m, 4H), 7.68 (d, \( J=8.9 \) Hz, 1H), 6.51 (dd, \( J=9.0, 2.4 \) Hz, 1H), 6.35 (d, \( J=2.4 \) Hz, 1H). \(^13C\) NMR (100 MHz, CDCl\( _3 \)): \( \delta \) 215.2, 69.6, 35.9, 31.9, 29.6, 29.6, 29.5, 29.4, 29.3, 28.9, 28.3, 22.7, 14.1, 13.8. HRMS (ESI): \( m/z \) : calcd for \( C_{13}H_{13}N_4O_2 \): 257.1039 [M⁺]; found: 257.1039.

Resolving the structure of \( P_3 \): The NMR, MS and X-ray analysis confirmed only the structure of cationic part of azo dye while leaving the nature of counterion unresolved. According to NMR (Fig. S1) during the reaction the hydrolysis of aceturate salt of azo product (\( \text{pre-}P_3 \)) takes place that should lead to the formation of corresponding hydroxide (\( P_3 \)). Indeed, the NMR spectrum of crude \( P_3 \) (before treatment with calcium chloride) does not contain aceturate signals (Fig. S2). Theoretically, the product \( P_3 \) could exist also as a zwitterion. However, based on the pK\( _a \) values (pK\( _{a1} \)=7.59, pK\( _{a2} \)=11.67) of a structurally and electronically close 4-(4-nitrophenylazo)resorcinol dye [W.H. Inskeep et al, Proc. Natl. Acad. Sci. U.S.A. 1968, 59, 1027-1029], the hydroxide form of \( P_3 \) seems to be more likely under the reaction conditions (pD=4.2).
Fig. S2 $^1$H NMR spectrum of diluted solution of crude P$_3$ in MeOH-$d_4$. 
Fig. S3 $^1$H and $^{13}$C NMR spectra of P$_3$ in DMSO-$d_6$/NH$_4$PF$_6$ mixture.
3. Reaction between $S_1$ and $S_3$

The experiment was performed similarly as outlined above using the same molar quantities of reagents. After the experiment was finished, the suspension was diluted with ethanol and filtrated. The crystals of product $P_4$ obtained upon evaporation of the solvent were washed with cold water and dried in vacuo. $^1$H NMR (400 MHz, methanol-$d_4$): $\delta$ 8.04–7.91 (m, 4H), 7.91–7.84 (m, 2H), 6.97–6.90 (m, 2H), 3.75 (s, 2H), 1.97 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 175.4, 171.6, 166.6, 162.2, 156.1, 146.1, 129.1, 128.8, 125.3, 122.5, 115.6, 43.2, 21.2. HRMS (ESI): m/z: calcd for $C_{13}H_{13}N_4O$: 241.1089 [M$^+$]; found: 241.1092.

![NMR spectra](image)

**Fig. S4** Time-resolved $^1$H NMR spectra of the reaction mixture at initial pH=7.2. The lack of the downfield shift of aceturate signal indicates that no (or insignificant) product hydrolysis takes place.
Fig. S5 $^1$H and $^{13}$C NMR spectra of $P_4$ in MeOH-$d_4$. 
4. Low-temperature NMR measurements of $S_1$-CB6 mixture

Stoichiometric mixture of $S_1$ (15 mg, 30 µmol) and CB6 (30 mg, 30 µmol) was sonicated in 600 µL of D$_2$O and filtered through a short pad of cotton using glass Pasteur pipette. The NMR spectrum was first recorded at 25°C; then the transparent solution was immediately cooled to 4°C at which all subsequent NMR spectra were taken.

Fig. S6 $^1$H NMR spectra of $S_1$-CB6 mixture in D$_2$O at a) 25°C; b) 4°C.
Fig. S7 $^1$H 2D COSY of $S_1$-CB6 mixture in D$_2$O at 4 °C.

Fig. S8 $^1$H 2D ROESY of $S_1$-CB6 mixture in D$_2$O at 4 °C showing chemical exchange between $S_1$-CB6 complexes and free $S_1$. 
5. Reaction between $S_1$ and $S_2$ at sub-stoichiometric CB6 loading
The experiment was performed similarly as outlined above using the same molar quantities of reagents except CB6 which was used in a catalytic amount (0.7 mg, 0.7 µmol, 20mol%).

Fig. S9 Plots of the time dependencies of the conversion factor at different CB6 loadings.

6. Monitoring of pD changes in the reaction between $S_1$ and $S_2$
The experiment was performed similarly as outlined above using the same molar quantities of reagents. In order to measure pD, the reaction mixture from NMR tube was periodically transferred to a glass vial.

Fig. S10 Plots of the time dependence of the conversion factor and pD.
7. Competitive binding of $S_3$ to CB6

The experiment was carried out similarly as outlined above using the same molar quantities of components. Before mixing with substrates, CB6 (3.9 mg, 3.9 µmol) was first solubilized in 200 µL of D$_2$O using an excess of calcium chloride (3.3 mg, 30 µmol).

![Fig. S11](image)

**Fig. S11** $^1$H NMR spectra of: a) $S_1$-$S_3$-CB6 mixture; b) $S_1$-$S_3$-CaCl$_2$-CB6 mixture.

8. Competitive binding of 1,5-pentanediamine to CB6 in the reaction between $S_1$ and $S_2$

The experiment was carried out similarly as outlined above using the same molar quantities of components. 1,5-pentanediamine was used in stoichiometric amount.

![Fig. S12](image)

**Fig. S12** Plots of the time dependence of the conversion factor for CB6-catalyzed azo coupling between $S_1$ and $S_2$ in the presence (empty circles) and absence (filled circles) of 1,5-pentanediamine at initial $pD$=7.5.
9. Kinetic model of the reaction

The autocatalytic reaction is modelled by the following system of two equations:

\[ S_1 + D_2O + D^+ \overset{k_1}{\underset{k_1'}{\leftrightarrow}} P_1 + P_2 + D^+ \]  \hspace{1cm} \text{(S1a)}

\[ P_2 + S_2 \overset{k_2}{\underset{k_2'}{\leftrightarrow}} P_3 + D^+ \]  \hspace{1cm} \text{(S1b)}

In our approach we assumed that both reactions, (S1a) and (S1b), are strongly shifted towards the product side. That is,

\[ k_1 \gg k_1' \]  \hspace{1cm} \text{(S2a)}

\[ k_2 \gg k_2' \]  \hspace{1cm} \text{(S2b)}

Under this assumption, reaction equations (S1a) and (S1b) yield the following system of six rate equations:

\[ \frac{d[S_1]}{dt} = -k_1 [S_1][D^+] \]  \hspace{1cm} \text{(S3a)}

\[ \frac{d[P_1]}{dt} = k_1 [S_1][D^+] \]  \hspace{1cm} \text{(S3b)}

\[ \frac{d[P_2]}{dt} = k_1 [S_1][D^+] - k_2 [P_2][S_2] \]  \hspace{1cm} \text{(S3c)}

\[ \frac{d[S_2]}{dt} = -k_2 [P_2][S_2] \]  \hspace{1cm} \text{(S3d)}

\[ \frac{d[P_3]}{dt} = k_2 [P_2][S_2] \]  \hspace{1cm} \text{(S3e)}

\[ \frac{d[D^+]}{dt} = k_2 [P_2][S_2] \]  \hspace{1cm} \text{(S3f)}

To obtain equations (S3a) – (S3c) we made use of the fact that the concentration of deuterium oxide hardly changes during the reaction, [D$_2$O] \approx \text{const}. For each value of pD the above set of rate equations was solved numerically and the concentrations of all species as functions of time were determined. The value of the parameter \( k_2 \) was the same for the catalysed and neutral (control) reaction. Two different values of the parameter \( k_1 \) - \( k_1' \) and \( k_2' \) were used for, respectively, the neutral and catalysed reaction. The quantity \( \gamma(t) \) (the conversion factor) defined as the following ratio:

\[ \gamma(t) = \frac{[P_1](t)}{[S_2](t) + [P_1](t)} \times 100\% \]  \hspace{1cm} \text{(S4)}

was calculated for the neutral and catalysed reactions. The set of the three parameters \((k_1^c, k_1^n, k_2)\) was determined by least-squares fit to the experimental results \((\gamma_{\text{exp}}^c \text{ and } \gamma_{\text{exp}}^n)\). In our approach, for a given value of pD, the values of the three rate constants were determined based on the sum of the squared errors (SSE) calculated as follows:

\[ \text{SSE}(k_{1}^{c}, k_{1}^{n}, k_{2}) = \sum_{i} (\gamma_{\text{exp}, i}^{c} - \gamma_{\text{theor}, i}^{c})^2 + \sum_{i} (\gamma_{\text{exp}, i}^{n} - \gamma_{\text{theor}, i}^{n})^2 \]  \hspace{1cm} \text{(S5)}

The three parameters \((k_{1}^{c}, k_{1}^{n}, k_{2})\) corresponded to the minimum of SSE. The results obtained for three values of pD are summarized in Table S1. In our calculations we employed initial concentrations of the substrates \([S_1](t = 0) = 6.1 \text{ mM and } S_2(t = 0) = 10 \text{ mM}].
The initial concentration of \( S_1 \) was set slightly lower than that of \( S_2 \) to account for the fact that in all experiments the conversion factor \( \gamma \) did not exceed 94%. The theoretical and experimental conversion factors for \( pD = 8.0 \), 7.4, and 6.1 are plotted in Figure S13.

In our approach the hydrolysis rate constant, \( k_{H} \), of \( S_1 \) is given by the product \( k_{H} = k_1[D^+] \). We tested the kinetic model in which the hydrolysis rate constant contained also the uncatalyzed reaction rate term, \( k_{aq} \), that is, \( k_{H} = k_{aq} + k_1[D^+] \). The fit to the experimental data yielded however \( k_{aq} \) equal to zero within the statistical errors.

**Table S1** The parameters obtained by least-square fits of the solutions of the kinetic equations (S1a) – (S1f) to the experimental data. The quantity \( AF \) is the acceleration factor defined as \( AF = \frac{k_2^c}{k_1^c} \). The quantities \( R^2_n \) and \( R^2_c \) denote, respectively, the \( R \)-square goodness of fit measure for the neutral and catalysed reaction.

| pD  | \( k_2 \) [M\(^{-1}\) day\(^{-1}\)] | \( k_1^H \) [M\(^{-1}\) day\(^{-1}\)] | \( k_1^c \) [M\(^{-1}\) day\(^{-1}\)] | \( AF \) | \( R^2_n \) | \( R^2_c \) |
|-----|-----------------|-----------------|-----------------|------|--------|--------|
| 8.0 | (9.70 ± 0.20) × 10\(^3\) | (5.0 ± 0.1) × 10\(^1\) | (16.2 ± 0.2) × 10\(^1\) | 3.24 ± 0.10 | 0.925  | 0.999  |
| 7.4 | (8.50 ± 0.25) × 10\(^2\) | (8.5 ± 0.1) × 10\(^1\) | (46.5 ± 0.5) × 10\(^1\) | 5.47 ± 0.12 | 0.959  | 0.992  |
| 6.1 | (2.40 ± 0.20) × 10\(^2\) | (4.3 ± 0.1) × 10\(^2\) | (22.0 ± 1.0) × 10\(^2\) | 5.12 ± 0.35 | 0.926  | 0.973  |

**Fig. S13** Plots of the time dependencies of the conversion factor for different values of pD. Solid lines represent least-square fits of the solutions of the model to the experimental data.