

Supplementary Information

Feedback driven autonomous cycles of assembly and disassembly from minimal building blocks

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Supplemental experimental procedures

1. SYNTHESIS

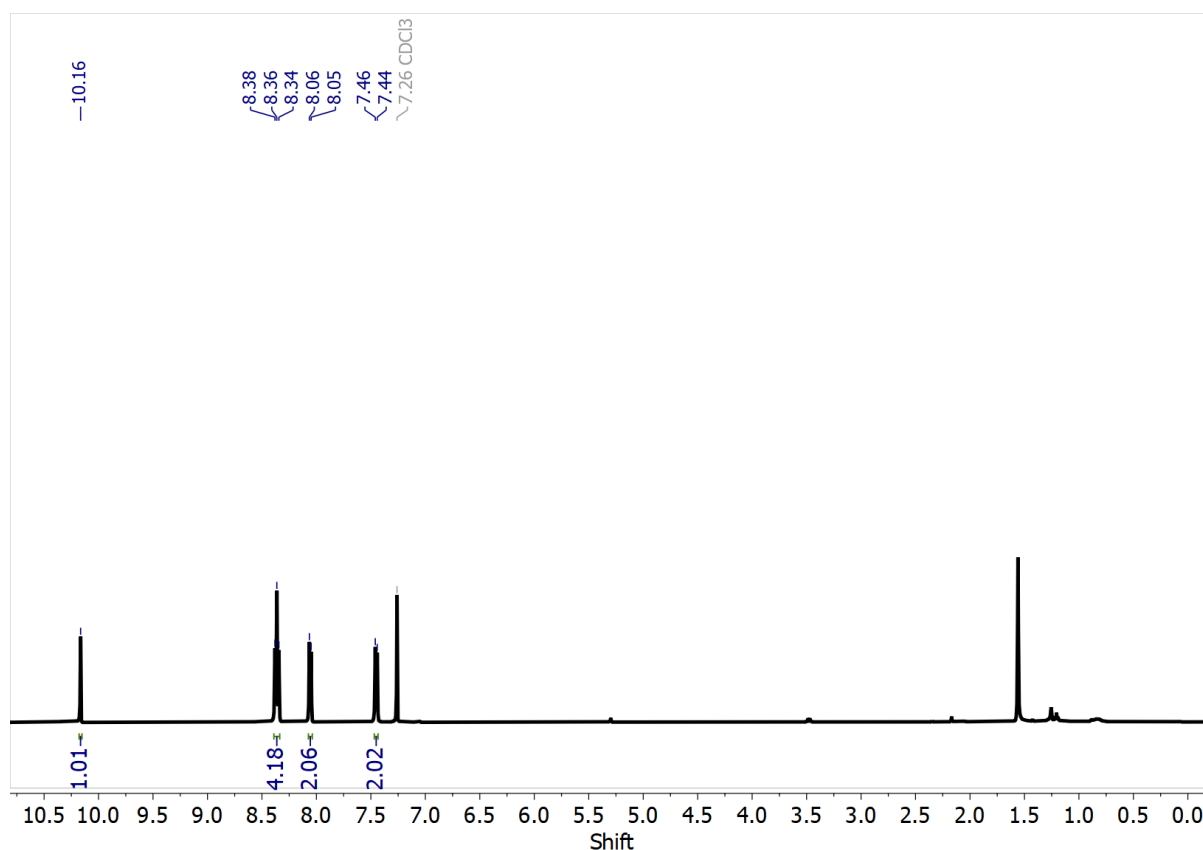
1.1 Preparation and characterization of BC:

BC was synthesized by following previously reported protocol.¹ In an oven dried round bottom flask, freshly prepared 4-formyl benzoyl chloride (2 mmol) was taken in dichloromethane (DCM). To this solution, p-nitrophenol (278 mg, 2 mmol) was added. Then triethyl amine (0.28 ml, 2 mmol) was added dropwise to the solution. Subsequently, the reaction mixture was kept under stirring condition for 2h. The completion of the reaction was monitored by TLC, then solvent was evaporated under vacuo. Then, ethyl acetate was added to the mixture. The organic layer was washed with brine solution, dried on Na₂SO₄, and evaporated under reduced pressure. Then the crude material was purified by silica-gel column chromatography (hexane/ethyl acetate).

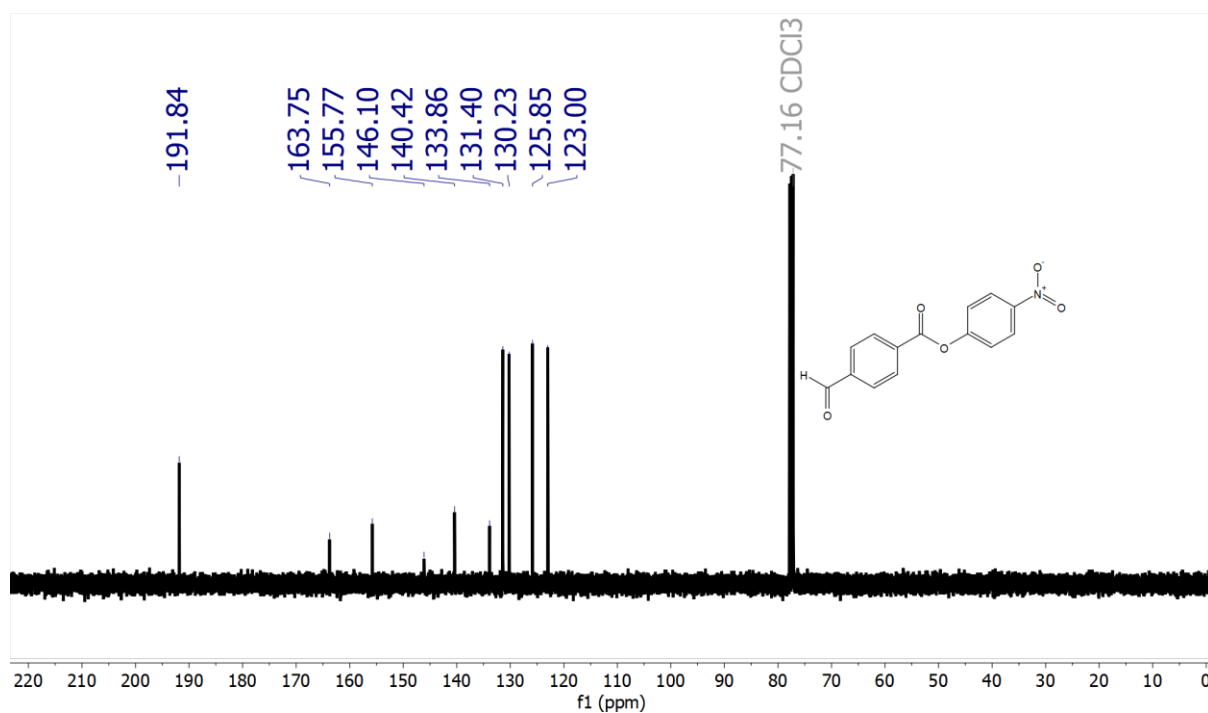
¹H NMR (400 MHz, CHLOROFORM-*D*) δ : 10.16 (s, 1H), 8.36 (m, 4H), 8.06 (d, *J* = 8.1 Hz, 2H), 7.45 (d, *J* = 8.9 Hz, 2H).

¹³C NMR (100 MHz, CHLOROFORM-*D*) δ : 191.84, 163.75, 155.77, 146.10, 140.42, 133.86, 131.40, 130.23, 125.85, 123.00.

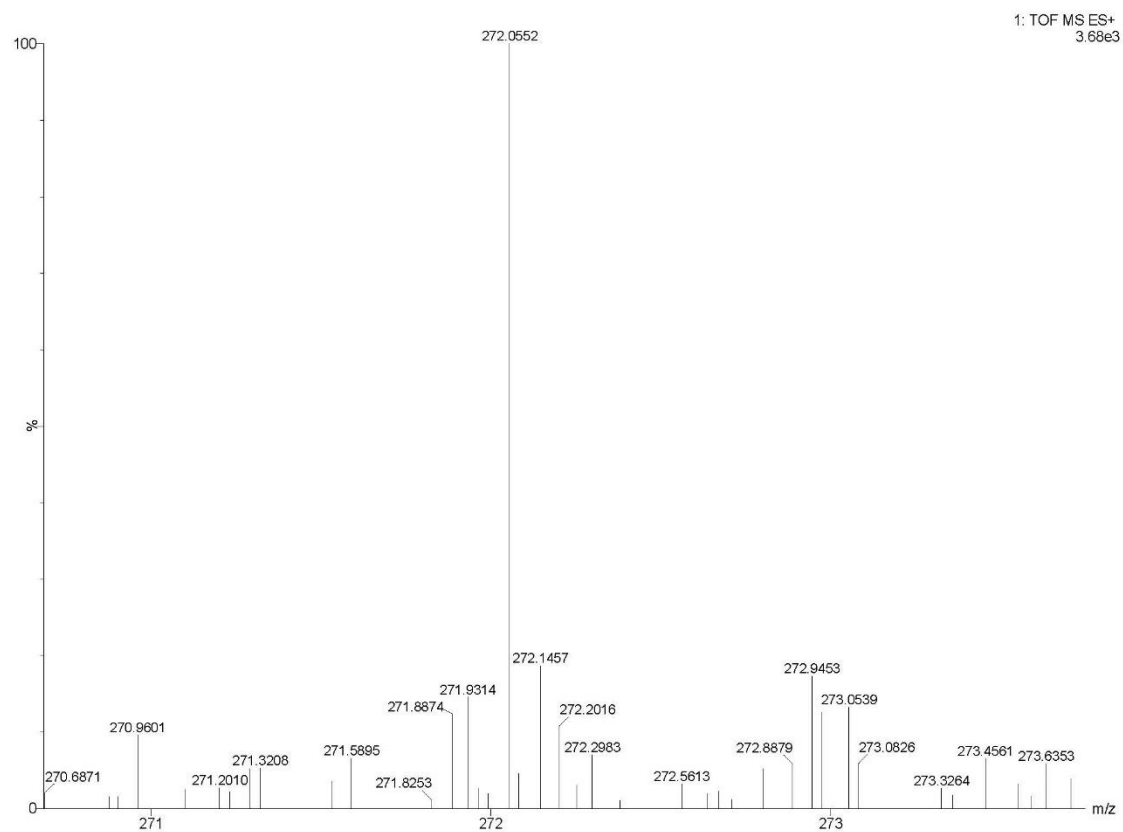
HRMS: calculated *m/z* for [M+H]⁺ = 272.0553, observed *m/z* = 272.0552.



¹H NMR spectrum of **BC**.



¹³C NMR spectrum of BC.



HRMS of BC.

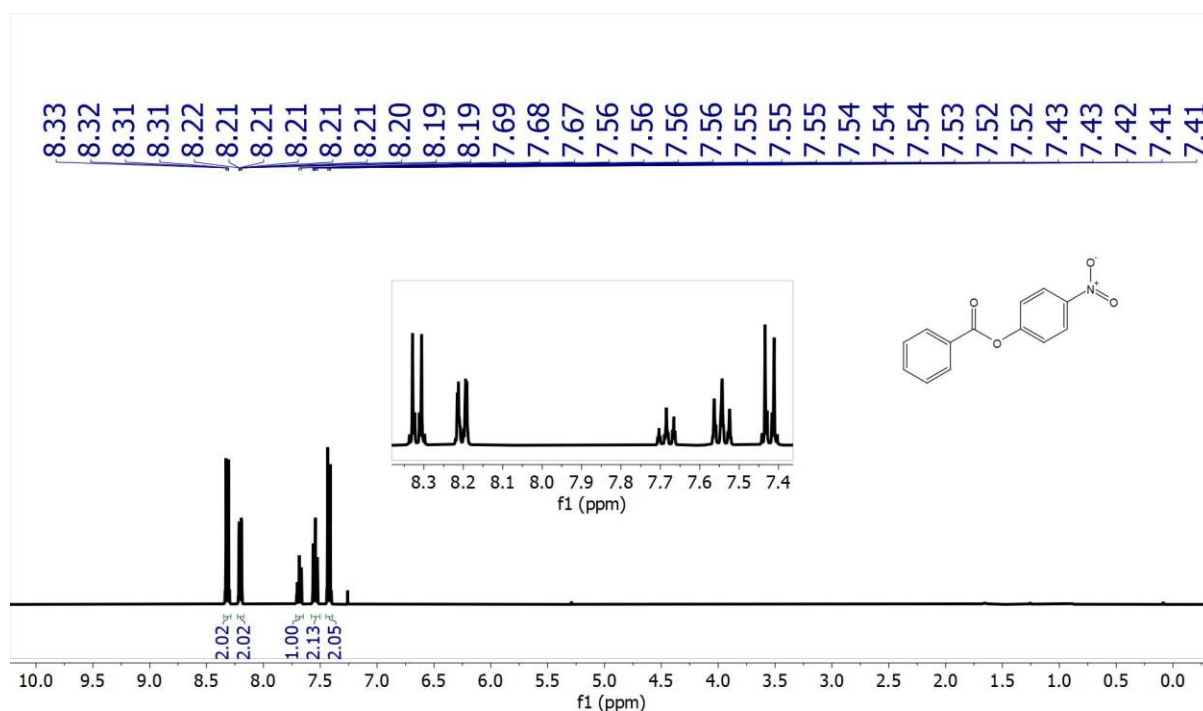
1.2 Preparation and characterization of DC:

DC was also synthesized by using the same protocol which was followed during the synthesis of **BC**. In a round bottom flask, freshly prepared benzoyl chloride (2 mmol) was added to dry dichloromethane (DCM). To this solution, p-nitrophenol (2 mmol) was added followed by the dropwise addition of triethyl amine (2 mmol) to the solution. The progress of the reaction was monitored by TLC, then the solvent was evaporated under vacuum. The reaction mixture was dissolved in ethyl acetate and the organic layer was washed with brine solution, followed by the drying over Na₂SO₄ and evaporated under reduced pressure. Column chromatography was then performed with the crude material and the pure product was obtained (hexane/ethyl acetate).

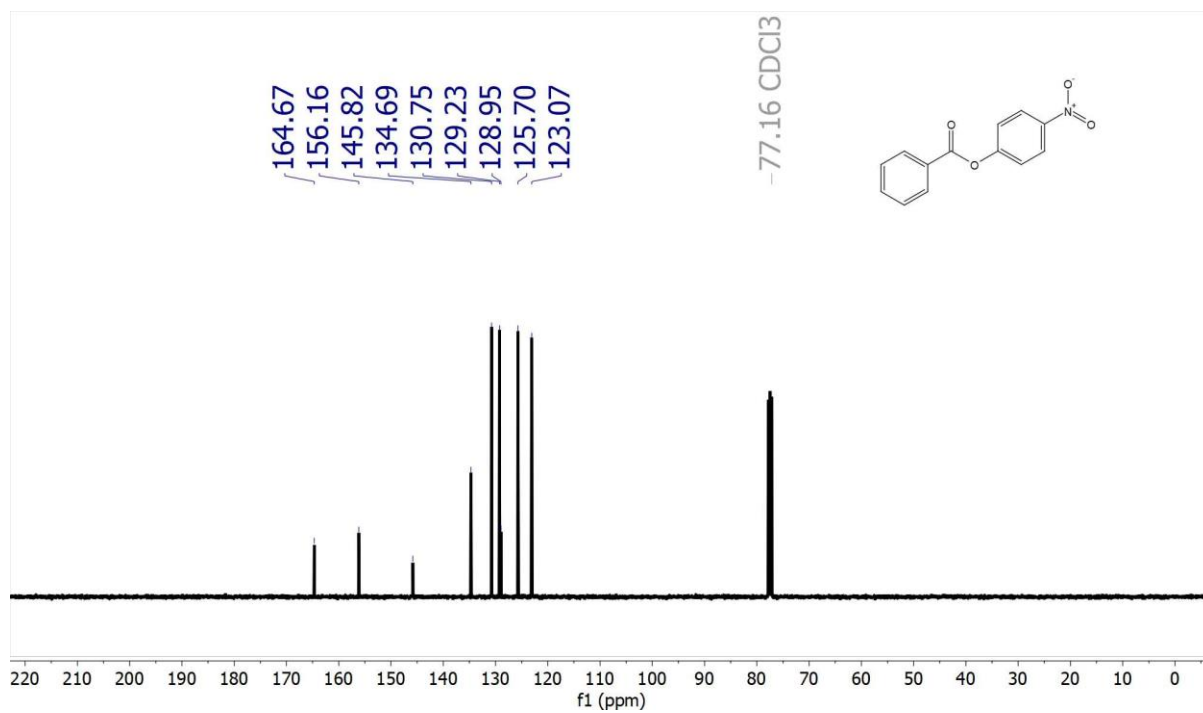
¹H NMR (400 MHz, CHLOROFORM-*D*) δ : 8.34 – 8.29 (m, 2H), 8.22 – 8.18 (m, 2H), 7.71 – 7.66 (m, 1H), 7.57 – 7.51 (m, 2H), 7.45 – 7.40 (m, 2H).

¹³C NMR (100 MHz, CHLOROFORM-*D*) δ : 164.67, 156.16, 145.82, 134.69, 130.75, 129.23, 128.95, 125.70, 123.07.

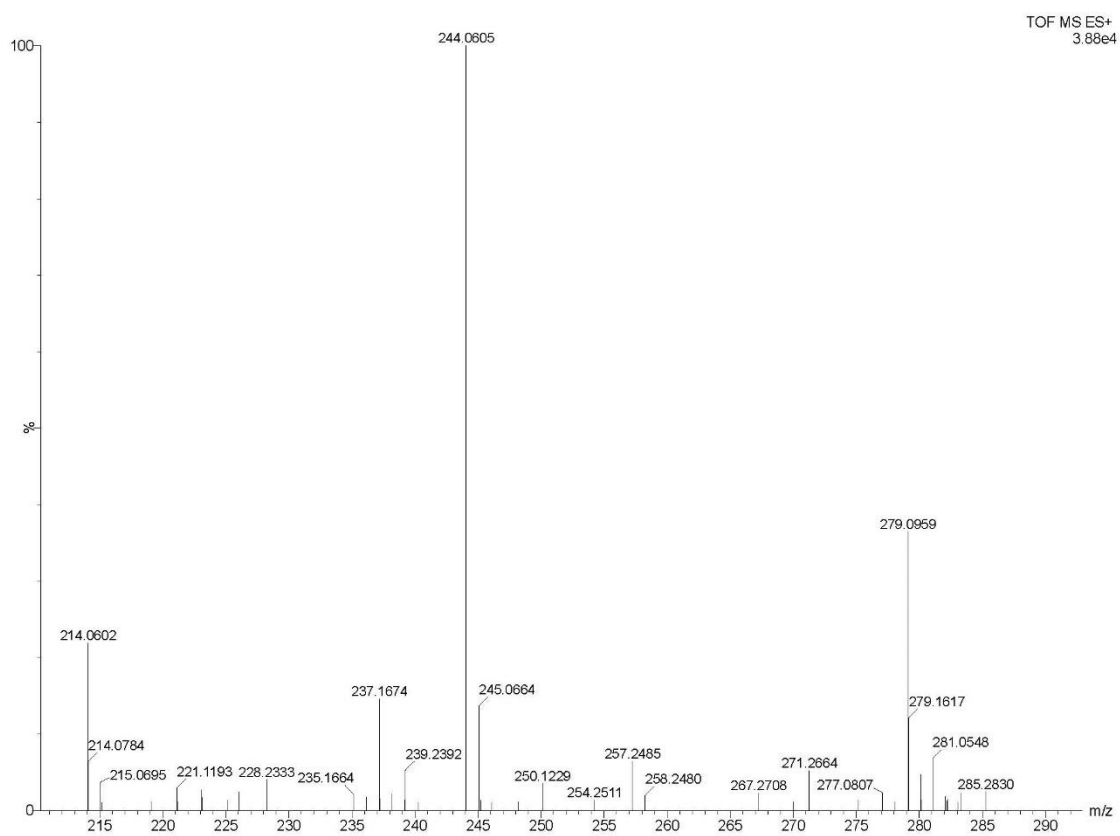
HRMS: calculated m/z for [M+H]⁺ = 244.0604, observed m/z = 244.0605.



¹H NMR spectrum of **DC**.



¹³C NMR spectrum of DC.



HRMS of DC.

2. GELATION

To check the gelation at pH 7.5, initially **A'** was dissolved in HEPES buffer (100 mM, pH 7.5) by heating at around 80°C followed by the addition of DMSO solution of **BC** to drive the assembly and the final concentration of **A':BC** was maintained at 50:50 mM.

Similarly, gelation was also checked at pH 6.5 by using MES buffer (100 mM) and in water (Milli Q water) individually. The final composition of the solvent was maintained at 60% DMSO/buffer or DMSO/Milli-Q water. Gel formation was monitored by the vial inversion technique, and the lifetime of the gels was noted down.

Attempts to synthesize **A'BC/A*BC** to check gelation properties

A number of attempts were made to synthesize **A'BC** or **A*BC** but none of the methods could yield the desired product due to solubility issues of the two starting materials or the purification challenges. It must be noted here that in the case of assemblies, the formation of **A'BC** or **A*BC** is driven by the self-assembly process and the challenges of purification need not be dealt. Some of the attempted protocols are described below.

Firstly, a solution of **BC** in anhydrous toluene was added to the flask containing histidine or carnosine in anhydrous toluene under continuous stirring in nitrogen atmosphere. A catalytic amount of PTSA was added to the mixture and the contents were kept for stirring at 90°C for 12 hours.

Replacing toluene with methanol, ethanol or water in the above procedure did not yield the considerable amount of desired product and further purification was proved to be challenging.

3. KINETIC MODELLING

The kinetic model comprises three interlocked events that depict the regulatory interactions occurring within the system. These include (i) assembled state (**AS**) mediated degradation of **ABC** ($k_{abc,as}^-$, **A** = **A*** or **A'**) (ii) the pH variation following the formation of the acid **B** and its effect on **ABC** formation (α_b and K_b) and (iii) the self-assembled state promoting the formation of **ABC** (α_{abc}). Here, (ii) and (iii) enhance **ABC** formation and thus form the two positive feedback loops in the model. Simultaneously, the assembled state (**AS**)-mediated cleavage of the ester acts as a negative feedback mechanism, hindering the formation of **AS** due to the degradation of **ABC**. In the proposed kinetic model, it was assumed that the formation of **ABC** led to a linear increase in the net rate of **ABC** formation. Since the pH change observed experimentally resulted from the formation of the acid **B**, the amount of **B** was used as a proxy for the proton concentration. A significant influence of **B** on the product formation was designed to appear only when a certain amount of **B** was accumulated in the system (mathematically expressed as a Hill function). Experimental findings indicate that the assembly occurred only when precursor **ABC** reached the required critical concentration. Consequently, the rate of assembly was described as a Hill function of the **ABC** concentration (k_{as}^+ and K_{as}). As seen experimentally, the degradation of **ABC** occurred solely in the presence of **AS**. Therefore, **AS**-independent degradation of **ABC** was not included in the model. Based on these assumptions, the rate of concentration change for each chemical species in the system was described as follows.

$$\frac{d[A]}{dt} = -k_{abc}^+ [A][BC] \left(1 + \frac{\alpha_b [B]^n}{K_b^n + [B]^n} + \alpha_{abc} [ABC] \right) + k_{abc}^- [ABC] + k_{abc,as}^- [AS][ABC] \quad (1)$$

$$\frac{d[BC]}{dt} = -k_{abc}^+ [A][BC] \left(1 + \frac{\alpha_b [B]^n}{K_b^n + [B]^n} + \alpha_{abc} [ABC] \right) + k_{abc}^- [ABC] \quad (2)$$

$$\frac{d[B]}{dt} = \frac{d[C]}{dt} = k_{abc,as}^- [AS][ABC] \quad (3)$$

$$\frac{d[AS]}{dt} = \frac{k_{as}^+ [ABC]^n}{K_{as}^n + [ABC]^n} - k_{as}^- [AS] \quad (4)$$

$$\frac{d[ABC]}{dt} = -\frac{d[A]}{dt} - m \cdot \frac{d[AS]}{dt} \quad (5)$$

Here, the Hill coefficients are denoted by n , and m represents a stoichiometric coefficient such that $AS = (ABC)_m$. The stability analysis of the system of ordinary differential equations (ODEs) was conducted by computing the eigenvalues of the Jacobian matrix at the steady state. For this, the steady state of the system was determined analytically.

3.1 Steady state

The total amount of each chemical is conserved since the system is closed. That is,

$$[A] + [ABC] + m \cdot [AS] = A_{\text{tot}} \quad (6)$$

$$[B] + [BC] + [ABC] + m \cdot [AS] = BC_{\text{tot}} \quad (7)$$

where A_{tot} and BC_{tot} denote the initial amount (concentration) of **A** and **BC** in the system. At steady state, the concentration of each chemical species remains constant over time. This implies that the rates of change of **[B]** and **[C]** are both zero at steady state, and hence $d[B]/dt = d[C]/dt = k_{abc,as}^- \cdot [ABC] \cdot [AS] = 0$ (From Eq. (3)). Since the rate constant $k_{abc,as}^- > 0$, either **[ABC]** or **[AS]** must equal zero. However, if only one of them is zero, the condition $d[AS]/dt = 0$ at steady state will not be met. Therefore, both **[ABC]** and **[AS]** must be zero at steady state. Substituting this condition into the equation $d[BC]/dt = 0$ (Eq. (2)), we find that

$$-k_{abc}^+ \cdot A_{\text{tot}} \cdot [BC] \cdot \left(1 + \frac{\alpha_b [B]^n}{K_b^n + [B]^n} \right) = 0 \quad (8)$$

The above condition can only be satisfied when $[BC] = 0$. Therefore, the steady state of the chemical system is given by.

$$[BC] = [ABC] = [AS] = 0, [A] = A_{\text{tot}}, \text{ and } [B] = [C] = BC_{\text{tot}} \quad (9)$$

Analytically, the system attains a unique steady state where the initially supplied hydrophobic ester completely degrades to form its constituents **B** and **C**, halting the formation of **ABC** and **AS**. However, it is important to acknowledge that these theoretical steady state conditions may not precisely represent the conditions within the actual system. The generation of **AS**, triggered by the buildup of **ABC** within the system, is simulated through a Hill function instead of a sudden, discrete transition that signifies a sharp rise in the rate once the concentration of **ABC** surpasses a critical threshold. Due

to the continuous nature of the Hill function, the rate of **AS** formation remains nonzero whenever **ABC** is present in the system. Consequently, even in insignificant quantities, **ABC** drives its own dissociation through the formation of **AS** over a long period, attaining the derived theoretical steady state.

3.2 Stability analysis reveals absence of sustained oscillations

Upon substituting the algebraic expressions for **[A]** and **[B]** obtained from the above conservation laws (Eq. (6) and (7)), the kinetic model is simplified to the following system of three ODEs.

$$\begin{aligned} \frac{d[\mathbf{BC}]}{dt} = & -k_{abc}^+ \cdot (\mathbf{A}_{\text{tot}} - [\mathbf{ABC}] - m \cdot [\mathbf{AS}]) \cdot [\mathbf{BC}] \\ & \cdot \left(1 + \frac{\alpha_b \cdot (\mathbf{BC}_{\text{tot}} - [\mathbf{BC}] - [\mathbf{ABC}] - m \cdot [\mathbf{AS}])^n}{K_b^n + (\mathbf{BC}_{\text{tot}} - [\mathbf{BC}] - [\mathbf{ABC}] - m \cdot [\mathbf{AS}])^n} + \alpha_{abc} \cdot [\mathbf{ABC}] \right) + k_{abc}^- \cdot [\mathbf{ABC}] \end{aligned} \quad (10)$$

$$\frac{d[\mathbf{ABC}]}{dt} = -\frac{d[\mathbf{BC}]}{dt} - k_{abc,as}^- \cdot [\mathbf{AS}] \cdot [\mathbf{ABC}] - m \cdot \frac{d[\mathbf{AS}]}{dt} \quad (11)$$

$$\frac{d[\mathbf{AS}]}{dt} = \frac{k_{as}^+ \cdot [\mathbf{ABC}]^n}{K_{as}^n + [\mathbf{ABC}]^n} - k_{as}^- \cdot [\mathbf{AS}] \quad (12)$$

At steady state, the Jacobian of the above system of ODEs simplifies to the following form.

$$J_{ss} = \begin{pmatrix} -k_{abc}^+ \cdot \mathbf{A}_{\text{tot}} \cdot \left(1 + \frac{\alpha_b \cdot \mathbf{BC}_{\text{tot}}^n}{K_b^n + \mathbf{BC}_{\text{tot}}^n} \right) & k_{abc}^- & 0 \\ k_{abc}^+ \cdot \mathbf{A}_{\text{tot}} \cdot \left(1 + \frac{\alpha_b \cdot \mathbf{BC}_{\text{tot}}^n}{K_b^n + \mathbf{BC}_{\text{tot}}^n} \right) & -k_{abc}^- & m \cdot k_{as}^- \\ 0 & 0 & -k_{as}^- \end{pmatrix} \quad (13)$$

The stability of the fixed point is determined by the eigenvalues of the Jacobian matrix at that fixed point. Oscillatory behavior is observed only when the Jacobian has complex eigenvalues. Here, the three eigenvalues of the Jacobian matrix (Eq. (13)) are given by

$$e_1 = 0 \quad (14)$$

$$e_2 = -k_{as}^- < 0 \quad (15)$$

$$e_3 = -k_{abc}^- - k_{abc}^+ \cdot \mathbf{A}_{\text{tot}} \cdot (1 + \alpha_b \cdot \mathbf{BC}_{\text{tot}}^n / (K_b^n + \mathbf{BC}_{\text{tot}}^n)) < 0 \quad (16)$$

Through the computation of the eigenvalues of the Jacobian matrix for the kinetic differential equations, we have assessed the stability of the chemical system at its theoretical steady state. Our analysis revealed that the nonzero eigenvalues of the system of differential equations are real and negative. The absence of imaginary components in the three eigenvalues signifies the non-existence of oscillatory dynamics in achieving the fixed point. Consequently, the system is deemed stable in its steady state, suggesting a convergence towards the equilibrium without any oscillations or divergent behaviors. Nonetheless, the absence of sustained oscillations does not imply the absence of transient oscillatory dynamics within the system away from the theoretical equilibrium, as we demonstrate through numerical simulations.

3.3 Estimation of parameter values for numerical simulations

Hill coefficients (n) in the model were assumed to be equal to 5, and the stoichiometric coefficient m was assumed to be equal to 100. Degradation rate constant of the assembled state (k_{as}^-) was approximated from the experimentally measured lifetime of the assemblies (Fig. 2g). Assuming an average lifetime of 4h, the degradation rate constant is given by

$$k_{as}^- \approx \frac{1}{\text{Lifetime}} = 0.25 \text{ h}^{-1} \quad (17)$$

In the unbuffered system, the experimentally measured concentration of **C** at 5h (following one assembly cycle) was approximately 10 mM (Fig. 4e). Hence, the average rate of **C** formation can be approximated as $\frac{10 \text{ mM}}{5 \text{ h}} = 2 \text{ mM/h}$. Therefore,

$$k_{abc,as}^- \cdot [\text{AS}] \cdot [\text{ABC}] \approx 2 \text{ mM/h} \quad (18)$$

and

$$k_{abc,as}^- \approx \frac{2 \text{ mM/h}}{[\text{AS}] \cdot [\text{ABC}]} \quad (19)$$

Since this is a closed system and all reactions were assumed to occur in a single compartment,

$$[\text{C}] + [\text{BC}] + [\text{ABC}] + 100 \cdot [\text{AS}] = 50 \text{ mM} \quad (20)$$

Thus, at $t = 5 \text{ h}$

$$[\text{BC}] + [\text{ABC}] + 100 \cdot [\text{AS}] = 40 \text{ mM} \quad (21)$$

From Eq. (19), it can be observed that the value of $k_{abc,as}^-$ reaches its minimum when the denominator is maximized. Combining with Eq. (21), the lowest possible value for $k_{abc,as}^-$ was determined to be $0.5 \text{ mM}^{-1} \text{ h}^{-1}$, and this minimum value is attained when the concentration of **ABC** is 20 mM. Conversely, the maximum value of $k_{abc,as}^-$ lacks a clear definition, as either **AS** or **ABC** can potentially approach zero. Nevertheless, for numerical simulations, an upper bound of 10^4 was assumed for $k_{abc,as}^-$, based on the calculations assuming minimum values of **ABC** and **AS** at $t = 5 \text{ h}$ were 1 mM and 0.001 mM, respectively.

Experiments show that the assembly process takes around 1.5-2h to gradually realize the assembled state (Fig. 3e). Hence, from 0 to 1.5h, we assume that only the **ABC** formation and dissociation reactions are active. Hence, the rate of formation of **ABC** is given by

$$\frac{d[\text{ABC}]}{dt} = k_{abc}^+ \cdot [\text{A}] \cdot [\text{BC}] - k_{abc}^- \cdot [\text{ABC}] \quad (22)$$

Let K_{crit} be the concentration of **ABC** (in mM) at $t = 1.5 \text{ h}$, after which **AS** starts to form. Hence, the average rate of **ABC** formation till $t = 1.5 \text{ h}$ is given by $\frac{K_{\text{crit}}}{1.5} \text{ mM/h}$. That is,

$$k_{abc}^+ \cdot [\text{A}] \cdot [\text{BC}] - k_{abc}^- \cdot [\text{ABC}] \approx \frac{K_{\text{crit}}}{1.5} \text{ mM/h} \quad (23)$$

or

$$k_{abc}^+ \cdot (50 - K_{\text{crit}}) \cdot (50 - K_{\text{crit}}) - k_{abc}^- \cdot K_{\text{crit}} \approx \frac{K_{\text{crit}}}{1.5} \text{ mM/h} \quad (24)$$

Hence,

$$k_{abc}^+ = \frac{K_{\text{crit}} \cdot \left(\frac{2}{3} + k_{abc}^-\right)}{(50 - K_{\text{crit}})^2} \text{ mM}^{-1} \text{ h}^{-1} \quad (25)$$

Here K_{crit} is bounded between 0 and 50 mM. For simplicity, we assumed that the time scale of **ABC** dissociation is similar to the time scale of **AS** degradation ($k_{abc}^- \sim k_{as}^-$), and let $k_{abc}^- = 0.1 \text{ h}^{-1}$. Then

$$k_{abc}^+ \approx \begin{cases} 3 \times 10^{-4} \text{ mM}^{-1} \text{ h}^{-1} & \text{If } K_{\text{crit}} = 1 \text{ mM} \\ 3 \times 10^{+2} \text{ mM}^{-1} \text{ h}^{-1} & \text{If } K_{\text{crit}} = 49 \text{ mM} \end{cases} \quad (26)$$

However, these computations were performed assuming the absence of the **ABC**-driven generation of **ABC**. Consequently, the actual range of k_{abc}^+ is likely to be even lower than the above values. Since α_{abc} was arbitrarily sampled from 10^{-4} to 10^4 mM^{-1} , k_{abc}^+ was proportionally sampled from 10^{-8} to $10^{-2} \text{ mM}^{-1} \text{ h}^{-1}$.

Assembled state was observed to form rapidly from 1.5h to 2h. If AS_{max} denotes the maximum **AS** at $t = 2\text{h}$, the average rate of **AS** formation is given by $\frac{AS_{\text{max}}}{0.5} = 2 \cdot AS_{\text{max}} \text{ mM/h}$. Hence at $t = 1.5\text{h}$

$$2 \cdot AS_{\text{max}} \approx \frac{k_{as}^+ \cdot K_{\text{crit}}^n}{K_{as}^n + K_{\text{crit}}^n} = \frac{k_{as}^+ \cdot K_{\text{crit}}^n}{2 \cdot K_{\text{crit}}^n + K_{\text{crit}}^n} = \frac{k_{as}^+}{3} \text{ mM/h} \quad (27)$$

Here, we have used a simplifying assumption that $K_{as}^n = 2 \cdot K_{\text{crit}}^n$. Then k_{as}^+ is given by $\sim 6 \cdot AS_{\text{max}}$. For parameter estimation, we assumed that the average [**AS**] is 10^3 times lower than K_{crit} . This also agrees with the previous assumption that **AS** = (**ABC**)₁₀₀. Consequently, the values for k_{as}^+ was sampled around 10^{-2} mM/h , assuming that K_{crit} is approximately 10 mM. Due to the absence of information regarding α_{abc} , the sampling range for α_{abc} was arbitrarily defined. Considering that the initial concentrations were set at 50 mM, the upper bounds for K_b and K_{as} were also set to 50 mM.

3.4 Numerical simulations

The system of six ODEs was examined through numerical simulations to identify parameter regimes that could generate multiple (≥ 2) (dis)assembly cycles. Parameter values for the kinetic model were estimated using experimentally observed data (see Section 3.3). Nevertheless, precise estimation of some parameter values was not feasible due to limited experimental data. To overcome this, we generated multiple parameter sets by sampling around the identified values. The Latin Hypercube method in logarithmic scale was employed for parameter sampling, generating one million parameter sets for the numerical simulations. Supplementary Table 1 summarizes the sampling ranges used for each kinetic parameter in the model.

Initial conditions for simulations were set identical to the experimental setup. Therefore, both **A** and **BC** concentrations at time $t = 0\text{h}$ were set as 50 mM, maintaining a 1:1 ratio. All other concentrations were set to zero. Numerical simulations with the one million parameter sets were carried out using *solve_ivp* from the SciPy Python package (version 1.10.0), based on the LSODA and BDF methods. The absolute and relative tolerance values for the solvers were set to 10^{-12} and 10^{-10} , respectively. With each parameter set, the system of ODEs was numerically integrated till 1000 hours (arbitrarily set). The numerical integration was also halted prematurely when the **BC** concentration fell below 0.01 mM, as reassembly would not be possible in such cases.

Parameter	Unit	Approximated value/range	Sampling range for numerical simulations	Remarks
k_{abc}^+	$\text{mM}^{-1} \text{ h}^{-1}$	10^{-4} to 10^2	10^{-8} to 10^{-2}	See section 2.4.3
k_{abc}^-	h^{-1}	0.1	10^{-3} to 10^{+1}	

$k_{abc,as}^-$	$\text{mM}^{-1} \text{h}^{-1}$	0.5 to 10^4	10^{-1} to 10^{+4}	
α_b	---	---	10^{-2} to 10^{+4}	Arbitrarily set
K_b	mM	---	10^{-2} to 50	See section 2.4.3
α_{abc}	mM^{-1}	---	10^{-4} to 10^{+4}	Arbitrarily set
K_{crit}	mM	10	---	See section 2.4.3
k_{as}^+	mM h^{-1}	10^{-2}	10^{-4} to 1	
k_{as}^-	h^{-1}	0.25	0.25	
K_{as}	mM	20	10^{-2} to 50	

Supplementary Table 1. Parameter values and Latin Hypercube sampling ranges for the numerical simulations.

Parameter values used for generating Figs. 6c, d and e:

$k_{abc}^+ = 2.89 \times 10^{-7} \text{ mM}^{-1} \text{ h}^{-1}$, $k_{abc}^- = 3.72 \times 10^{-1} \text{ h}^{-1}$, $k_{abc,as}^- = 5.98 \times 10^3 \text{ mM}^{-1} \text{ h}^{-1}$, $\alpha_b = 1.29$, $K_b = 1.81 \times 10^{-1} \text{ mM}$, $\alpha_{abc} = 4.18 \times 10^3 \text{ mM}^{-1}$, $k_{as}^+ = 6.20 \times 10^{-4} \text{ mM h}^{-1}$, $k_{as}^- = 2.50 \times 10^{-1} \text{ h}^{-1}$, and $K_{as} = 3.68 \times 10^{-1} \text{ mM}$.

3.5 Numerical simulations predict transient oscillation

The analysis revealed transient (damped) oscillatory behavior of **AS** for several parameter sets ($n = 5139$). This finding highlights the presence of non-sustained oscillations within the system, underscoring the intricate nature of the catalyzed assembly-disassembly phenomenon. An example of such a case is shown in Figure 6c, where multiple (dis)assembly cycles can be observed as transient peaks. For several parameter sets, the time scale of the simulated assembly agrees with that of the experimental observations. The stepwise decrease of **BC** concentration can be an indication that its hydrolysis is enhanced when **ABC** assembly is populated in the system. However, since the changes in concentrations are depicted as continuous processes in the model, the steep changes can be observed to become smoother after the first reassembly event ($\sim 10\text{h}$). These simulations demonstrate the consistency of the proposed model with the observed kinetics. Nevertheless, these findings do not exclude the possibility of an alternative mechanism that could produce similar kinetics.

3.6 Identification of (dis)assembly cycles

Parameter sets that yielded multiple (dis)assembly cycles were identified by locating the peaks in **[AS]** obtained from numerical simulations. This was achieved using the *find_peaks* function in the SciPy package. Each peak in **[AS]**, indicating a successful assembly event, had to meet specific criteria: a minimum concentration of 1 nM and a minimum prominence value of $0.1 \times \text{AS}_{\text{max}}$ mM. Note that, though represented as concentration, **[AS]** denotes only the extent of the assembled state in the system. Here AS_{max} , for each parameter set, denotes the maximum **[AS]** observed during the simulation, and the prominence of a peak measures how much a peak stands out from the surrounding baseline. Additionally, it was assumed that two successive assembly events should be separated by a

minimum time interval of 1 hour, and the duration of each assembly-disassembly cycle should remain below 50 hours. These arbitrary constraints were set to filter out parameter sets that give insignificant short-term fluctuations in the **[AS]**, which could otherwise be misconstrued as assembly events.

3.7 Effect of feedback regulations on reassembly

By systematically eliminating each regulatory interaction, we conducted simulations of the kinetic model using the selected parameter sets. This allowed us to investigate the influence of individual regulatory interactions on the reassembly process. For parameter sets that exhibited reassembly, the model was simulated with the values of α_b , α_{abc} set to zero and $k_{abc,as}^- \cdot [\mathbf{AS}] = k_d$ set to a constant, one at a time. By setting k_d as a constant, the effect of changes in **[AS]** on the degradation of **ABC** is eliminated, effectively transforming **ABC** degradation into a first-order process. For each parameter set, k_d was assumed to be $k_{abc,as}^- \times (0.01 \cdot \text{AS}_{\max})$. Here, $\text{AS}_{\max}$ denotes the maximum assembly concentration observed during the initial simulation without any restrictions on the feedback regulations.

A total of 5139 parameter sets that showed multiple (dis)assembly cycles were used to study the effect of different regulatory interactions. When the **AS**-mediated degradation of **ABC** was removed from the model ($k_d = \text{constant}$), only a single selected parameter set demonstrated reassembly. Similarly, in the absence of **ABC**-mediated enhancement of **ABC** formation ($\alpha_{abc} = 0$), only a small fraction of the parameter sets ($n = 266$) exhibited two assembly cycles, with no instances of more than two cycles observed. However, even after eliminating the enhancement in **ABC** formation due to **B** ($\alpha_b = 0$), the majority of parameter sets ($n = 4461$) showed reassembly, characterized by two or more assembly cycles. No common parameter set exhibited reassembly when each of the three feedback regulations was removed from the model (Supplementary Fig. 16).

3.8 Robustness of the kinetic model to parameter variations

To examine the effect of changes in individual parameter values and initial concentrations on reassembly, we conducted numerical simulations with the parameter sets that demonstrated reassembly. During these simulations, we varied each parameter value individually while keeping all others constant. This process was iteratively repeated for each of the selected parameter sets, with the parameter values systematically incremented and decremented by a specific factor relative to their base value. Concretely, if k_i denotes any kinetic parameter from parameter set i , then the new perturbed parameter value was defined as

$$k_i^{\text{perturbed}} = k_i \cdot \delta^{\pm 1} \quad (28)$$

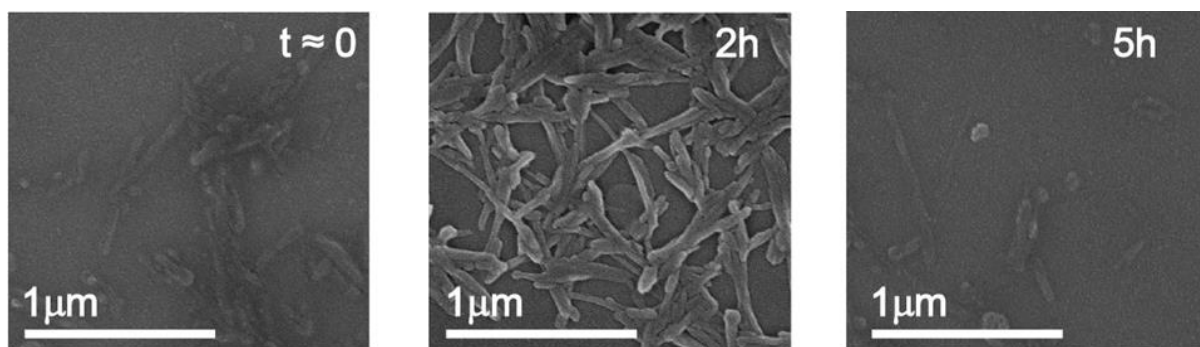
In this analysis, the perturbation factor δ was arbitrarily varied up to 2. Following a similar approach to the kinetic parameters, the initial conditions of the model (concentrations of **A** and **BC**) were also subjected to a two-fold variation to investigate their impact on the reassembly process. In both cases, we evaluated the capability of the system to display two or more (dis)assembly events after each perturbation, thereby gaining insights into the robustness of the kinetic model.

This process was repeated for each of the selected parameter sets, where the perturbation factor δ was varied as 1.1, 1.5, and 2. Supplementary Figures. 17a-c provide an overview of the number of parameter sets that continue to exhibit reassembly when each parameter value is individually varied. Notably, alterations of individual parameters by up to a 2-fold change did not significantly impact the model's capacity to exhibit multiple assembly cycles. Even when subjected to these perturbations, a

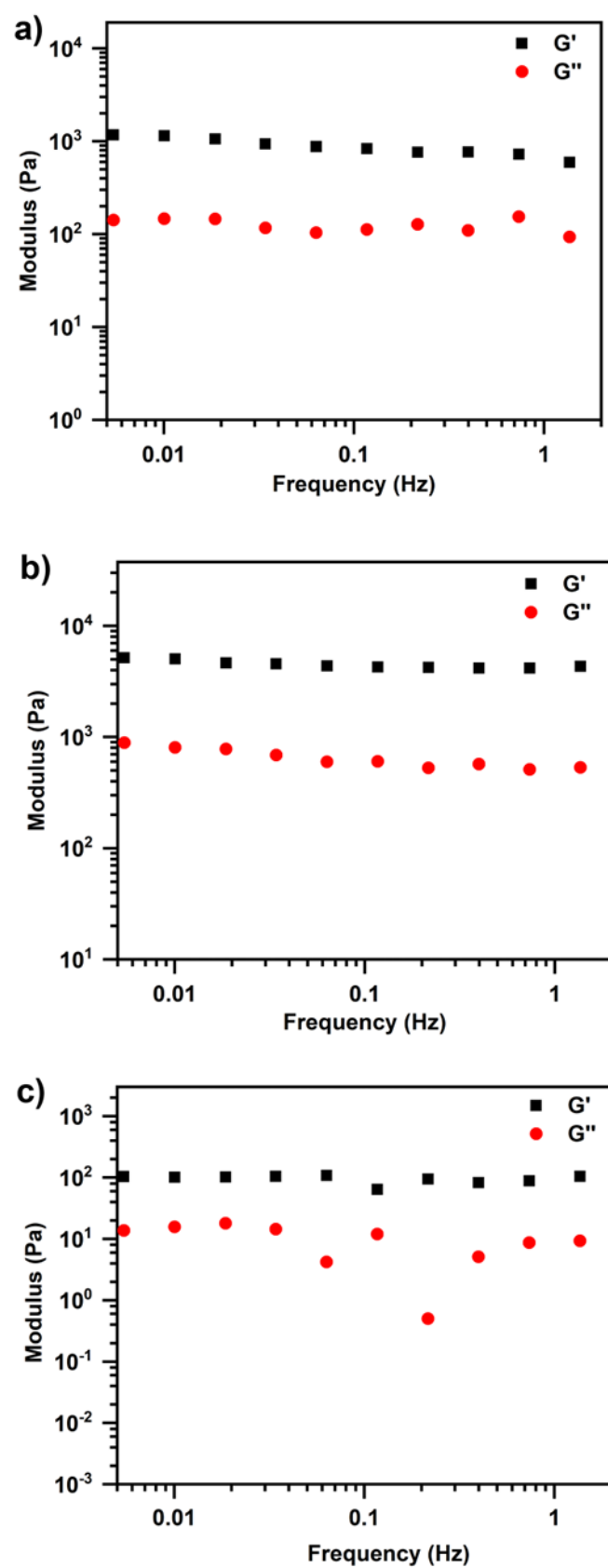
substantial proportion of parameter sets that initially demonstrated reassembly behavior continued to yield comparable kinetics. Similarly, the initial conditions (**A** and **BC** concentrations) of the model were also subjected to a two-fold variation for each of the selected parameter sets. The results of the analysis, investigating their impact on the reassembly process, are summarized in Supplementary Figure 17d. This reveals the robustness of the model, as it consistently exhibits multiple (≥ 2) assembly cycles even when the parameters and initial conditions are perturbed. In this analysis, the value of k_{as}^- (kept at 0.25 h^{-1}) was not subjected to any variation.



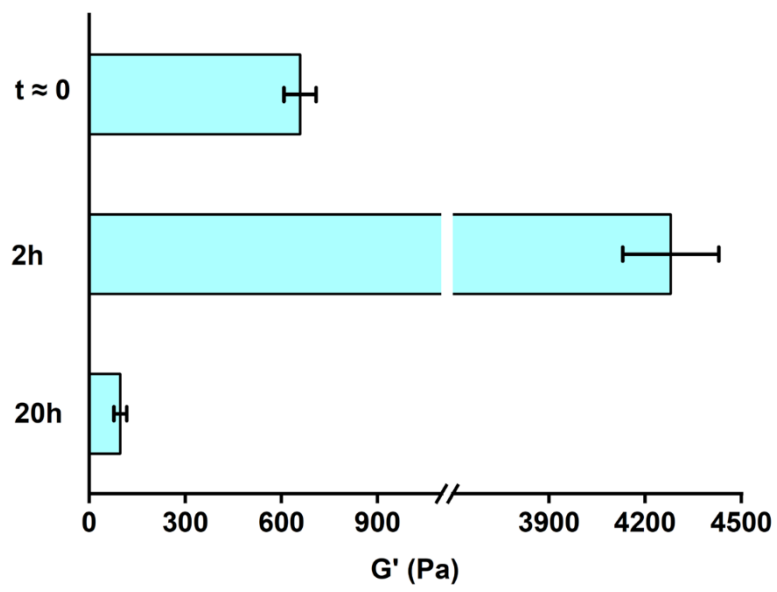
Supplementary Figure 1. Vial image of the system containing 50 mM **B** + 50 mM **A'** in 60% v/v DMSO/HEPES buffer (pH 7.5) solution at 2h.



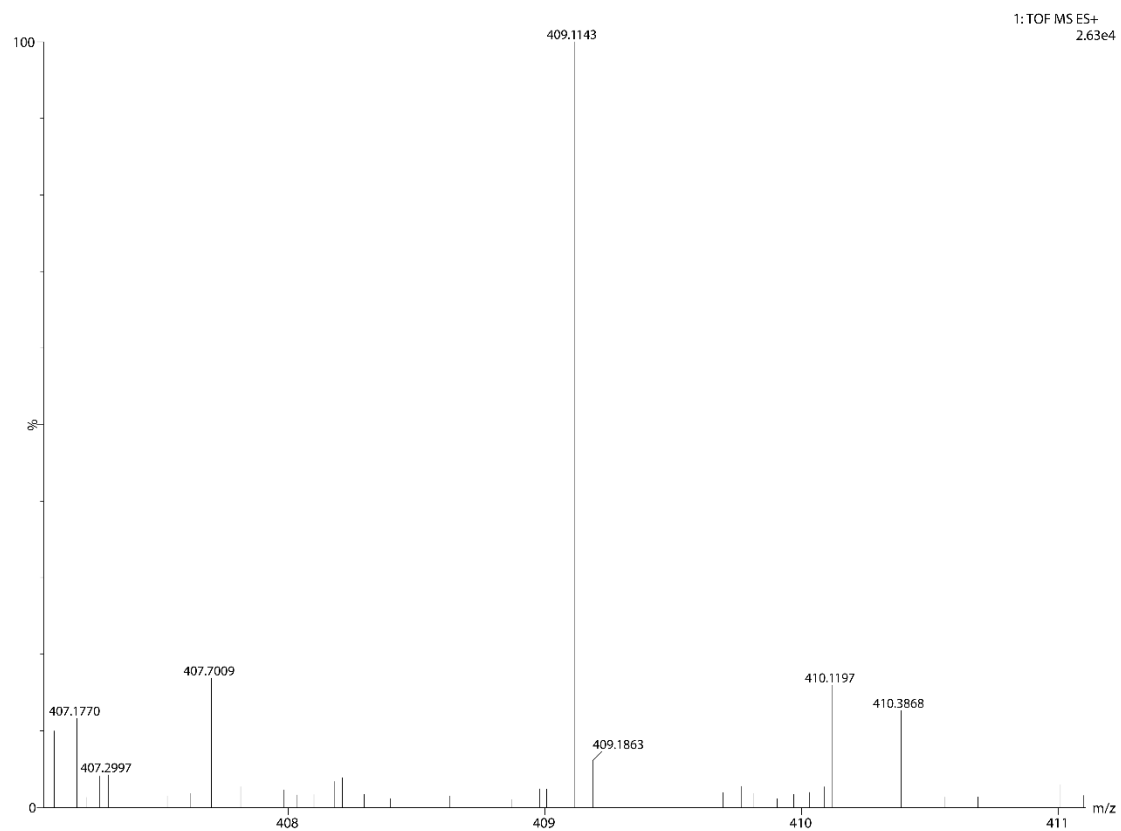
Supplementary Figure 2. SEM images of the system containing 50 mM **BC** + 50 mM **A'** in 60% v/v DMSO/HEPES buffer (pH 7.5) solution at different time intervals.



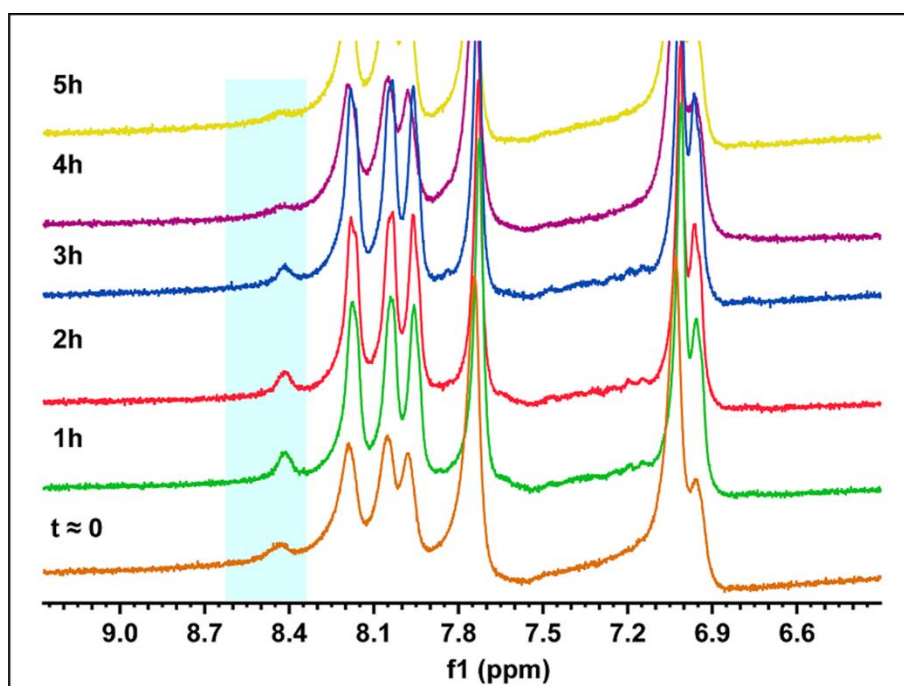
Supplementary Figure 3. Frequency sweep of pH 7.5 system (50 mM BC + 50 mM A') at (a) ~0h, (b) 2h, and (c) 20h.



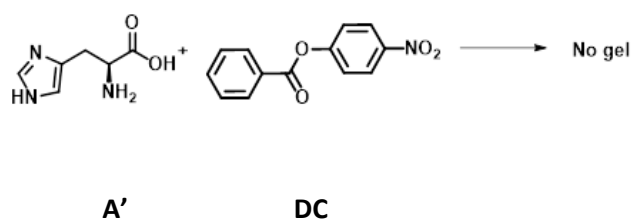
Supplementary Figure 4. Temporal change in storage modulus (G') at 1Hz of the system (50 mM BC + 50 mM A') at pH 7.5 (60% DMSO/HEPES buffer).



Supplementary Figure 5. HRMS data of gel, calculated for $[A'BC+H]^+$: 409.1148; found: 409.1143.



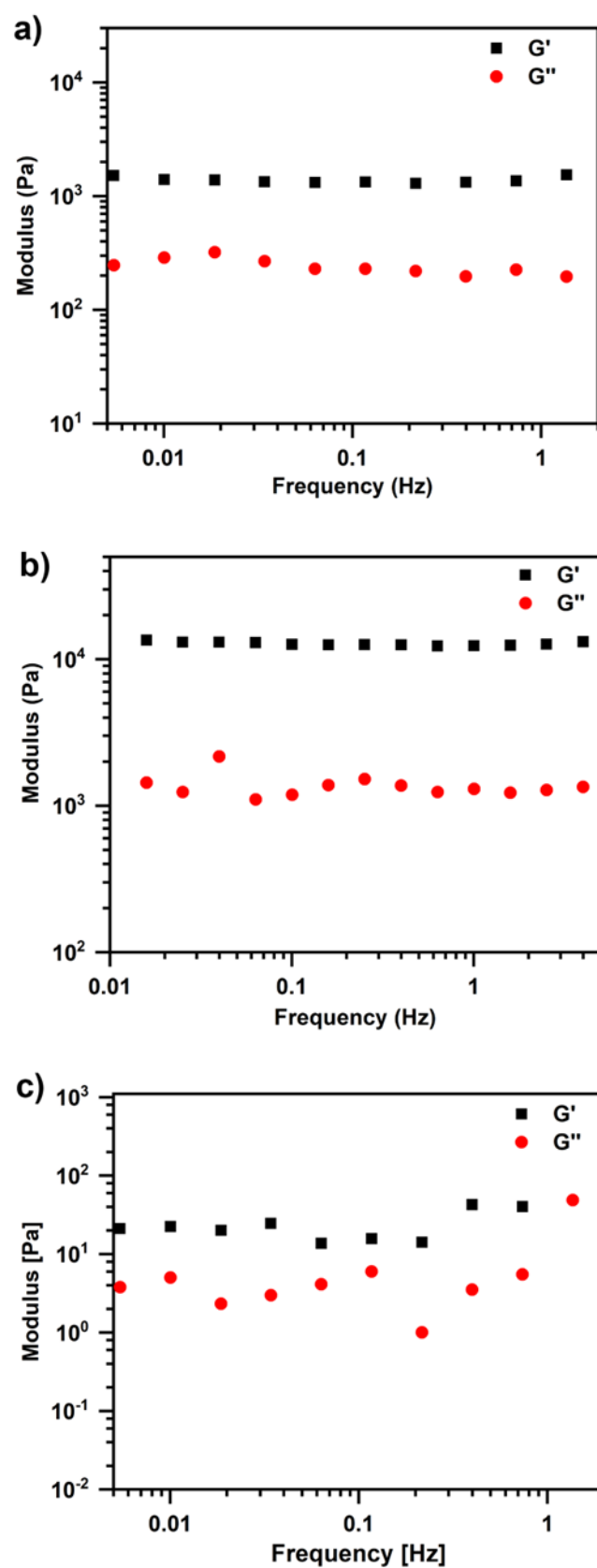
Supplementary Figure 6. Time-resolved ¹H NMR spectrum of the system (50 mM **BC** + 50 mM **A'**) at pH 7.5 (60% DMSO/HEPES buffer).



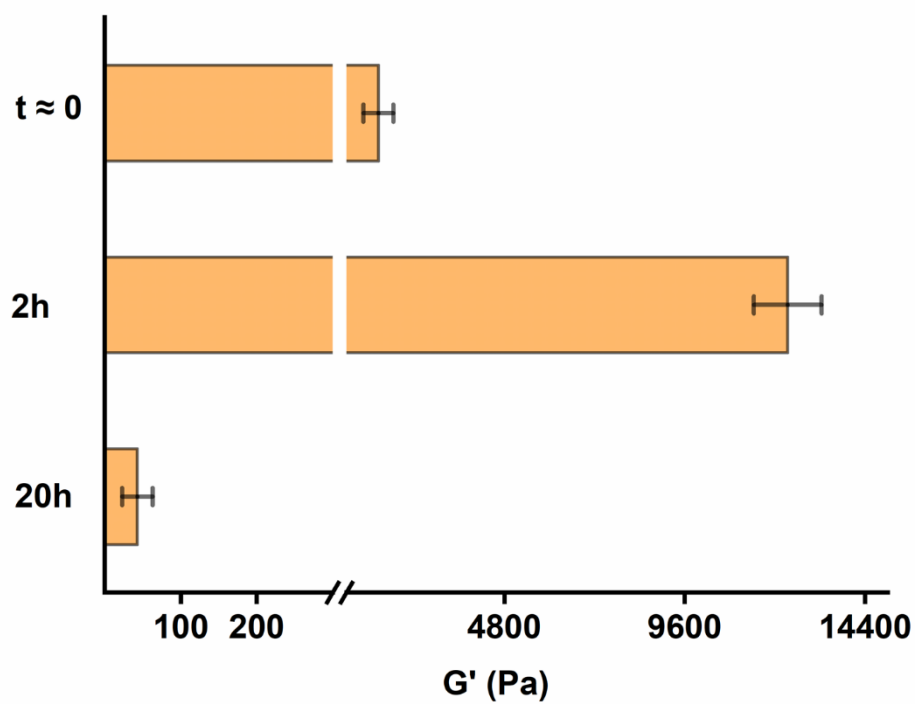
Supplementary Figure 7. Representative vial images of the system containing 50 mM **A'** and 50 mM **DC** at pH 7.5 (60% DMSO/HEPES buffer).



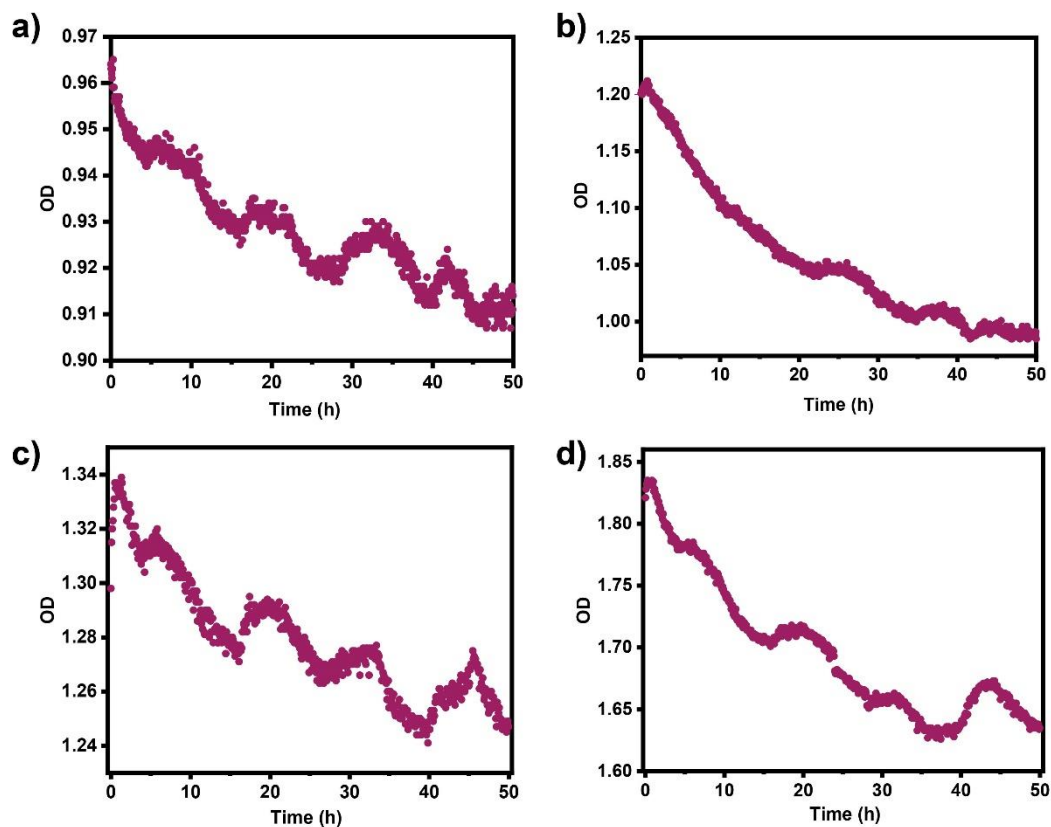
Supplementary Figure 8. Representative vial image of the system containing 50 mM **A'** and 50 mM **B** at pH 6.5 (60% DMSO/HEPES buffer) at 2h.



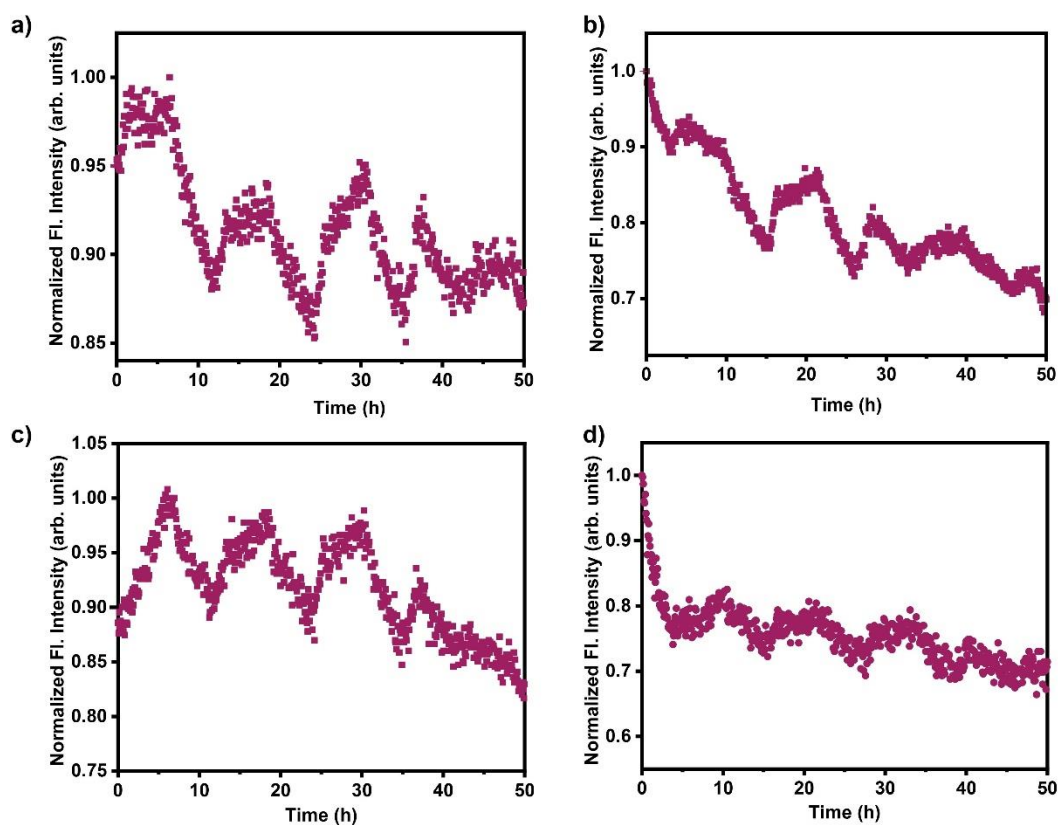
Supplementary Figure 9. Frequency sweep of the pH 6.5 system (50mM BC + 50mM A') at (a) ~0h, (b) 2h and (c) 20h.



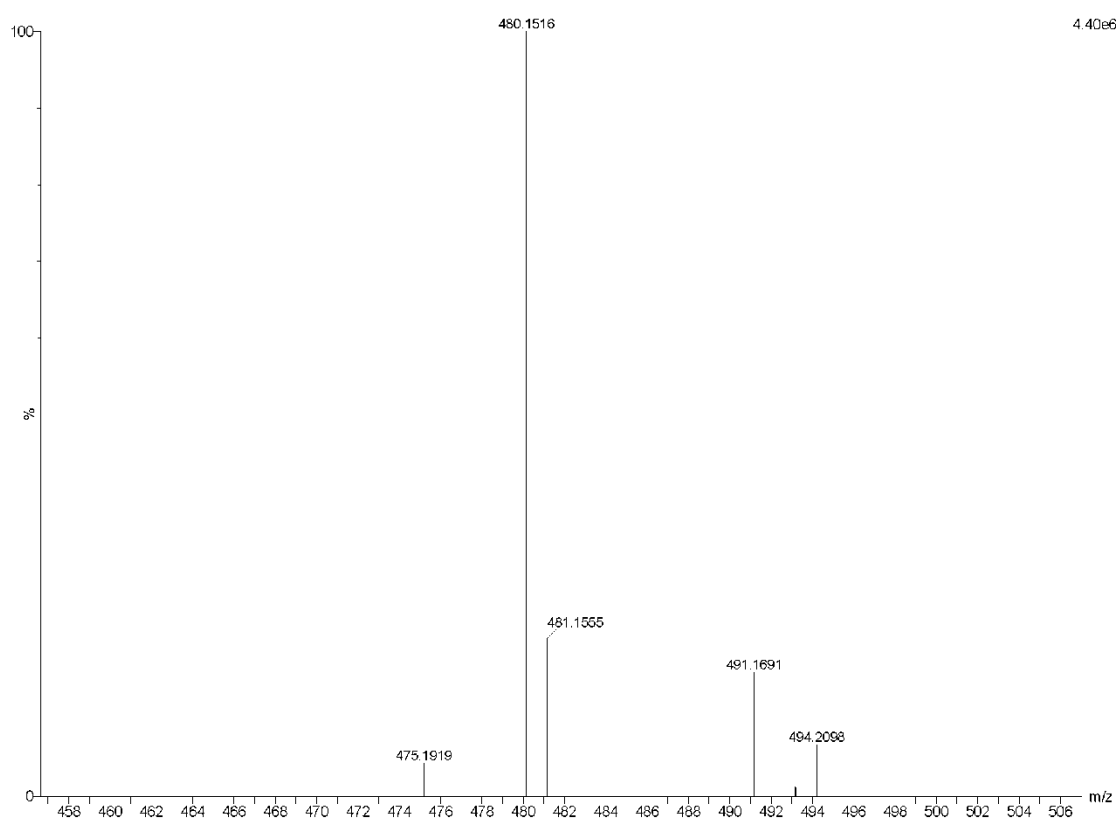
Supplementary Figure 10. Temporal change in storage modulus (G') at 1Hz of the system (50mM BC + 50mM A') at pH 6.5 (60% DMSO/buffer).



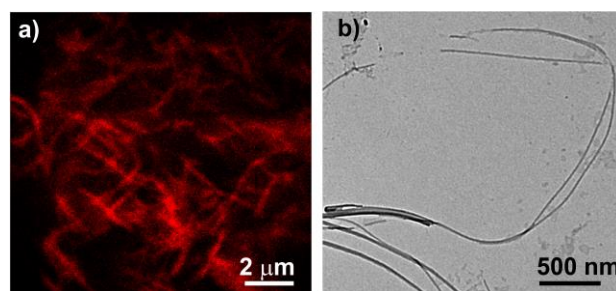
Supplementary Figure 11. Time-dependent change in OD of A^*+BC system. The given data (a-d) are taken from 4 independent experiments to confirm the periodic changes. 4 out of 8 individual experiments showed multiple cycles.



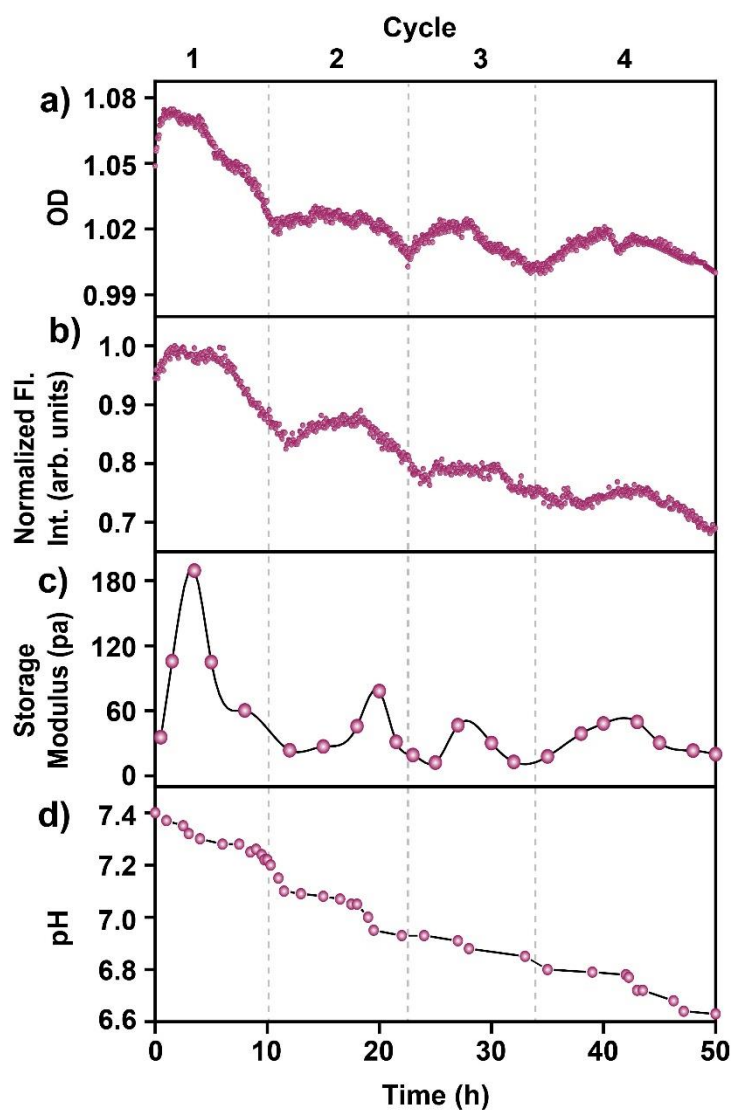
Supplementary Figure 12. Time-dependent change in fluorescence intensity of DPH incubated in A^*+BC system. The given data (a-d) are taken from 4 independent experiments to confirm the periodic changes. 4 out of 8 individual experiments showed multiple cycles.



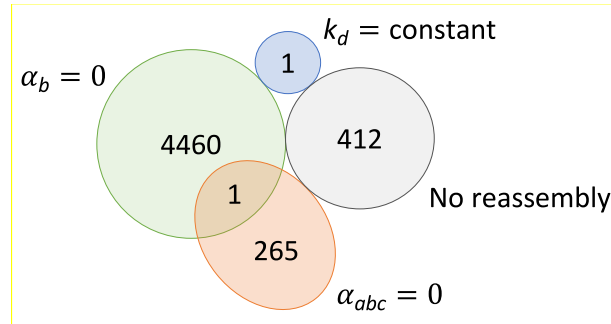
Supplementary Figure 13. HRMS data of turbid solution, calculated for $[A^*BC+H]^+$: 480.1519; found: 480.1516.



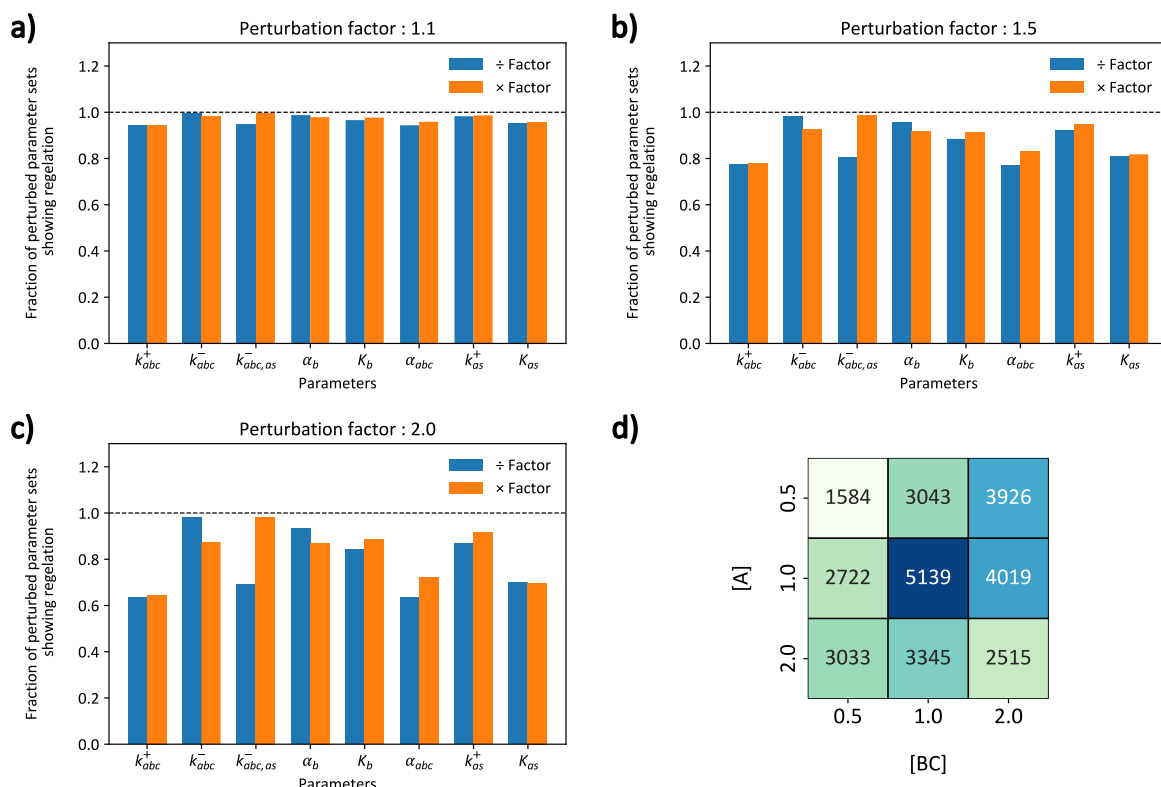
Supplementary Figure 14. (a) Confocal and (b) TEM images of the system containing A^*+BC .



Supplementary Figure 15. The time-dependent changes in **(a)** optical density, **(b)** fluorescence intensity, **(c)** storage modulus (spline fitted), and **(d)** pH with samples taken from a single batch (one system containing A^*+BC). Dotted lines have been added to dictate the number of cycles.



Supplementary Figure 16. Importance of feedback loop. The figure illustrates the number of parameter sets (out of 5139) that continue to exhibit reassembly after removing each feedback from the model individually. The intersection in the figure represents only the number of common parameter sets rather than the number of parameter sets that display reassembly when multiple feedback loops are removed simultaneously.



Supplementary Figure 17. a), b), c) Each subfigure represents the proportion of parameter sets that continue to exhibit reassembly after perturbing each parameter value by factors of 1.1, 1.5, and 2, respectively. Here, y-axis was scaled by the total number of parameter sets that showed reassembly. **d)** Number of parameter sets (out of 5139) that show multiple assembly cycles after varying initial concentrations of **A** and **BC** up to two-folds from the base value. The middle cell ($[A] = [BC] = 1.0$) represents the base case where both **A** and **BC** initial concentrations are 50 mM.

Supplementary Reference

1. Tecilla, P., Tonellato, U., Veronese, A., Felluga, F. & Scrimin, P. Acceleration of p-nitrophenyl ester cleavage by Zn(II)-organized molecular receptors. *J. Org. Chem.* **62**, 7621-7628 (1997).