Supplementary Information for
ATP-fuelled self-assembly to regulate chemical reactivity in the time domain

Maria A. Cardona, Leonard J. Prins*
Department of Chemical Sciences, University of Padova, Via Marzolo 1, 35131 Padova, Italy.
# Table of Contents

1. Materials and methods ...................................................................................................................... 3
   1.1 Instrumentation .......................................................................................................................... 3
   1.2 Materials .................................................................................................................................. 4

2. Synthesis and characterisation of the amphiphile C₁₂TACN ................................................................ 5
   2.1 Synthesis and characterisation ............................................................................................... 5
   2.2 Characterization data for C₁₂TACN ......................................................................................... 6

3. ATP-templated self-assembly ............................................................................................................ 7
   3.1 ATP-assisted encapsulation of water-soluble dye Rhodamine 6G ........................................... 7

4. Chemical reactivity in self-assembled assemblies ............................................................................ 8
   4.1 Synthesis and characterisation of hydrazones ........................................................................ 8
   4.2 ¹H NMR and ¹³C NMR spectra .............................................................................................. 10
   4.3 Quantification of the concentrations (UV-Vis and HPLC) ..................................................... 13
       4.3.1 Calibration curves for hydrazone 1A by UV-Vis Spectroscopy .................................... 14
       4.3.2 Calibration curves for hydrazone 1A by HPLC ............................................................ 15
       4.3.3 Calibration curves for hydrazone 2A by HPLC ............................................................ 16
   4.4 UV-Vis spectra of 1 and 1A .................................................................................................... 17
   4.5 TEM and DLS in the presence of reagents ............................................................................. 17
   4.6 Kinetics of formation of 1A by UV-Vis and HPLC ............................................................... 18
   4.7 Reactivity of 1A and 2A in the presence of the enzyme alkaline phosphatase ..................... 21
   4.8 Control of reactivity of hydrazones 1A and 2A by controlling the self-assembly ............... 22

5. Kinetic model ..................................................................................................................................... 24

6. References ......................................................................................................................................... 26
1. Materials and methods

1.1 Instrumentation

**NMR Analysis:** $^1$H were recorded using a Bruker spectrometer operating at 300 MHz for $^1$H. $^{13}$C NMR (proton decoupled) were recorded on a Bruker AV500 operating at 500 MHz for $^1$H and at 126 MHz for $^{13}$C. Chemical shifts, $\delta$ are reported in ppm and were referenced against TMS ($\delta_H = 0.00$ ppm) or the solvent residual peak ($\delta_H = 7.26$ for CDCl$_3$, $\delta_H = 2.49$ ppm for DMSO-$d_6$ residual peak, $\delta_H = 3.31$ for CD$_3$OD residual peak), ($\delta_C = 77.16$ ppm for CDCl$_3$, $\delta_C = 39.52$ ppm for DMSO-$d_6$ residual peak, $\delta_C = 49.00$ for CD$_3$OD residual peak). Abbreviations for multiplicity are as follows: s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, etc.).

**MS Measurements:** ESI-MS measurements were acquired on an Agilent Technologies 1100 Series LC/MSD Trap-SL spectrometer, equipped with an ESI source, hexapole filter and ionic trap.

**pH Measurements:** The pH of buffer solutions was determined at room temperature using a Metrohm-632 pH meter equipped with a Ag/AgCl/KCl reference electrode and calibrated with standard buffer solutions at pH 7.00 and pH 10.00.

**UV-Vis Measurements:** UV-Vis measurements were recorded on a Varian Cary50 spectrophotometer holding a thermostatted multiple cuvette holder or a Varian Cary100 spectrophotometer hosted with a thermostatted multiple cuvette holder. Individual spectra were acquired in Scan Mode while kinetics were followed in Scanning Kinetics Mode. The spectra were then exported and analysed using Excel and plotted using Origin. Analysis were conducted using 1 mL Quartz cuvettes.

**Fluorescence Spectroscopy:** Fluorescence measurements were recorded on a Varian Cary Eclipse fluorescence spectrophotometer equipped with a thermostated cell holder hosting a maximum of 4 cuvettes. Experiments were typically conducted in 1 mL fluorescence quartz cuvettes. Fluorescence spectra were obtained in either scan mode or kinetics mode in which the emission at a particular wavelength was monitored over time. After each addition of the respective component to the sample cuvette, the sample was allowed to equilibrate before measurement.

**HPLC Analysis:** The HPLC analysis were performed on an Agilent Technologies 1290 Infinity equipped with a DAD detector and a Quadrupole LC/MS. Conditions: Flow rate: 0.6 ml/min, Gradient: 5-95%B (A: H$_2$O+0.1%HCOOH, B: ACN+ 0.1% HCOOH) from 0.00 to
5.00 min; 95-100 % B in 5 to 5.10 min; 100 % B from 5.10 to 8.10 min; 100-5 % B in 8.10 to 9.10 min; and finally 5% B from 9.10 to 10.00 min. Column: Agilent Zorbax SB-C3 Rapid Resolution HT 3.0 × 100 mm 1.8 micron. Injection volume: 20 µL Column temperature: 40 °C.

**DLS Analysis:** DLS measurements were recorded on a Malvern Zetasizer Nano-S instrument. Samples were analysed in disposable low volume cuvettes.

**TEM Analysis:** TEM images were recorded on a Jeol 300 PX electron microscope. One drop of sample was placed on the sample grid for 1 minute. For staining purposes, it was then placed on a drop of uranyl acetate (2%) for 30 s. The solvent was evaporated before the stained grid was imaged. TEM images were elaborated using the software ImageJ (http://rsb.info.nih.gov/ij/).

**LSCM Analysis:** Confocal images were taken using a laser scanning confocal microscope (BX51WI-FV300-Olympus) coupled to a frequency doubled Ti:Sapphire femtosecond laser at 400 nm, 76 MHz (VerdiV5Mira900-F Coherent). The laser beam was scanned on a 40x40 µm sample area with a 512x512 resolution, using a 60x water immersion objective (UPLSAPO60xW-Olympus).

### 1.2 Materials

All fine chemicals were sourced from commercial suppliers and were used directly without purification unless mentioned. The final solutions for analysis by UV-Vis and HPLC were prepared from stock solutions prepared in the respective solvent and diluted in MilliQ water.

ATP and adenosine were obtained from Sigma Aldrich. Solutions were prepared as an approximately 10 mM solution in MilliQ water by weight and the exact concentration was calculated by UV-Vis spectroscopy using the molar extinction coefficients: ε_{259} (ATP, adenosine) = 15400 M^{-1} cm^{-1}.49 Inorganic phosphate solution was prepared as a 20 mM stock solution by weight in MilliQ water.

The aldehydes *trans*-cinnamaldehyde and 2-pyridinecarboxyaldehyde and the hydrazide 3-hydroxy-2-napthoic hydrazide were purchased from Sigma Aldrich and used without further purification. Solutions were prepared by weight in acetonitrile at the required concentration, typically 20 mM, fresh before use. The concentrations were confirmed by UV-Vis spectroscopy using the molar extinction coefficients: ε (*trans*-cinnamaldehyde) = 25300 M^{-1} cm^{-1}(50), ε (2-pyridinecarboxyaldehyde) = 5290 M^{-1} cm^{-1}.51

The concentration of the Zn(NO₃)₂-stock solution was quantified using EDTA following standard procedures.
The buffer 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was procured from Sigma-Aldrich and used without further purification. The buffer were prepared as 100 mM solutions in MilliQ water and adjusted to a pH of 7.0.

The fluorophores DPH, 1,6-diphenyl-1,3,5-hexatriene and laurdan dye, 6-dodecanoyl-N,N-dimethyl-2-naphthylamine, were procured from Sigma-Aldrich and used without further purification.

The enzyme alkaline phosphatase was obtained from Sigma Aldrich and used without further purification. The solution was diluted in 1 mL water to give a concentration of 1000 U/mL. The solution was further diluted to obtain a concentration of 100 U to be used for lower enzyme concentrations.

2. Synthesis and characterisation of the amphiphile C<sub>12</sub>TACN

2.1 Synthesis and characterisation

Scheme S1. Synthetic scheme for the synthesis of C<sub>12</sub>TACN as the hydrochloride salt.

Di-tert-butyl 1,4,7-triazanonane-1,4-dicarboxylate (b) was synthesized from 1,4,7-triazanonane (a) according to a literature protocol (G. Pieters, A. Cazzolaro, R. Bonomi, L. J. Prins, Chem. Commun. 48, 1916 (2012)).

1-Dodecyl-1,4,7-triazacyclononane (C<sub>12</sub>TACN.3HCl): Compound b (0.20 g, 0.61 mmol) and dodecyl bromide (0.18 g, 0.71 mmol) were dissolved in 10 mL acetonitrile in a 50 mL round bottomed flask. K<sub>2</sub>CO<sub>3</sub>,6H<sub>2</sub>O (0.33 g, 2.0 mmol) and NaHCO<sub>3</sub> (0.22 g, 2.57 mmol) were added and the suspension was stirred at 60 °C for 9 hours. The mixture was subsequently filtered on gooch and the solvent evaporated under reduced pressure. The residual solid was then purified by silica flash chromatography using as eluent a solvent mixture of methanol and dichloromethane starting with 2% MeOH, which was gradually increased to 3% MeOH until the product was eluted and then increased to 10% MeOH to elute unreacted b. The protected C12 amphiphile was obtained as a pale, yellow oil (0.22 mg, 71%) and used directly in the next step. The obtained solid was dissolved in 7 mL MeOH in a 25 mL round-bottomed flask. A volume of 6 N HCl (6 mL) was added to the solution and the mixture was stirred for two hours at 60 °C. The solvent was evaporated under reduced pressure and the
solid was collected as a pinkish-white solid (120 mg, 74% yield). The solid was washed with cold chloroform to remove any organic residue.

$^1$H NMR (CD$_3$OD, 300 MHz, 300 K, ppm): $\delta$ 0.86 (m, 3H, CH$_3$), 1.20 (s, 18H, 9 $\times$ CH$_2$), 1.54 (s, 2H, CH$_2$), 2.75 (s, 2H, CH$_2$N), 2.99 (s, 4H, 2 $\times$ CH$_2$N), 3.22 (s, 4H, 2 $\times$ CH$_2$N), 3.45 (s, 4H, 2 $\times$ CH$_2$N).

$^{13}$C NMR (CD$_3$OD, 125 MHz, 300 K): $\delta$ 14.48, 23.78, 25.72, 28.46, 30.52, 30.65, 30.78, 33.12, 43.67, 44.83, 57.25

ESI-MS(ESI+, H$_2$O:CH$_3$CN = 1:1). $m/z$ calculated for C$_{18}$H$_{39}$N$_3$: [M+H]$^+$ 298.3, found 298.3.

N.B. The solid was preserved in the fridge. Prior to experiments, fresh solutions of amphiphile were prepared in small volumes as 10 mM solutions in milliQ water and preserved at room temperature to avoid aggregation and precipitation. The solutions were subsequently diluted to the required experimental concentration. The Zn$^{2+}$ metal complex of C$_{12}$TACN was formed in situ by adding an equimolar quantity of Zn(NO$_3$)$_2$ to a buffered solution of the amphiphile.

### 2.2 Characterization data for C$_{12}$TACN

![Figure S1. $^1$H NMR spectrum of C$_{12}$TACN in MeOD (300 MHz, 300K). (S denotes solvent peaks.)](image-url)
3. ATP-templated self-assembly

3.1 ATP-assisted encapsulation of water-soluble dye Rhodamine 6G

Evidence for the presence of an aqueous interior phase in the assemblies was obtained by encapsulation studies of the cationic water-soluble dye Rhodamine 6G (Rh6G). ATP (25 and 50 μM) was added to a solution of C₁₂TACN·Zn²⁺ (100 μM) and Rhodamine 6G (1 μM). After an incubation period of 5 minutes, the samples were centrifuged for 45 minutes at 14000 rpm, resulting in the precipitation of the assemblies. The absorption spectrum of the supernatant was measured in order to quantify the amount of remaining dye in solution. In all cases, the remaining amount of Rh6G in the supernatant decreased. This indicated an uptake of the dye by the assemblies. The use of a cationic dye excluded that this was simply a result of electrostatic interactions with the (positively charged) amphiphile.

Figure S2. $^{13}$C NMR spectrum of C₁₂TACN in MeOD (125 MHz, 300K).
Figure S3. Decrease in Rh6G concentration in the supernatant as a function of ATP concentration for 100 µM C12TACN•Zn2+. [HEPES] = 5 mM, pH 7.0, [Rh6G] = 1 µM, centrifugation speed = 14000 rpm, centrifugation time = 45 minutes.

4. Chemical reactivity in self-assembled assemblies

4.1 Synthesis and characterisation of hydrazones

Hydrazones were synthesized using general published procedures.³ Generally, the solid hydrazide was dissolved in a minimal amount of ethanol in a round-bottomed flask and the solution was stirred with a magnetic stirrer. An equimolar quantity of the respective aldehyde was added dropwise to the solution whilst stirring the solution. A few drops of TFA were sometimes added as a catalyst. The hydrazones precipitated out of solution as white or yellow solids, which were filtered and if necessary, recrystallised from methanol.

The identity and purity of all hydrazones were confirmed by ¹H and ¹³C NMR spectroscopy and ESI-MS. When obtained in DMSO-<sup>d6</sup>, the NMR spectra of the hydrazones generally showed the presence of two isomers.³ On the other hand, only one isomer was observed when the ¹H NMR spectrum of the product was obtained in CD₃OD.

**Hydrazone 1A:** Hydrazone 1A was synthesized from 383 mg (1.89 mmol) 3-hydroxy-2-naphthoic hydrazide and 294 mg (2.22 mmol) trans-cinnamaldehyde in 50 ml absolute ethanol. The solid crystallised out upon concentration of solution and recrystallised from ethanol to give 314 mg of 1A (52.5% yield).

R<sub>f</sub> = 0.5 (hexane:ethyl acetate 80:20)

UV-Vis: λ<sub>max</sub> = 331 nm (methanol)

³¹H NMR (only major isomer: 88%) (DMSO-<sup>d6</sup>, 300 MHz, 300 K, ppm): δ 7.08 (d, J = 4.3 Hz, 2H), 7.25 – 7.41 (m, 6H), 7.48-7.50 (m, 1H), 7.62 (d, J = 7.4 Hz, 2H), 7.73 (d, J = 8.3 Hz, 1H), 7.87 (d, J = 8.3 Hz, 1H), 8.21-8.23 (m, 1H), 8.41 (s, 1H), 11.28 (s, 1H), 11.85 (s, 1H)
$^1$H NMR (MeOD-$d_4$, 300 MHz, 300 K, ppm): δ 7.10 (d, $J = 4.3$ Hz, 2H), 7.29 – 7.40, (m, 6 H), 7.45-7.55 (m, 1H), 7.55-7.64 (m, 2H), 7.70 (d, $J= 8.3$ Hz, 1H), 7.88 (d, $J = 8.3$ Hz, 1H), 8.18 – 8.20 (m, 1H), 8.52 (s, 1H)

$^{13}$C NMR (DMSO-$d_6$, 126 MHz, 300 K, ppm): δ 111.02, 120.77, 124.27, 125.97, 126.32, 127.25, 127.67, 128.70, 129.14, 129.35, 129.46, 130.71, 136.31, 140.13, 150.97, 154.57, 164.17

MS (ESI,+, MeOH:H2O 1:1): m/z calculated for C$_{20}$H$_{16}$N$_2$O$_2$: [M+H]$^+$: 317.3, found 317.2.

HPLC-MS ($\lambda = 331$ nm): $R_t = 4.3$ minutes

Hydrazone 2A: Hydrazone 2A was synthesized from 254 mg (1.25 mmol) 3-hydroxy-2-napthoic hydrazide and 135 mg (1.25 mmol) pyridine carboxyaldehyde in 150 mL absolute ethanol. The orange-yellow solid precipitated out of solution upon evaporation of some of the solution, was filtered, dried and recrystallised from methanol to afford 128 mg of 2A (35% yield).

UV-Vis: $\lambda_{max} = 329$ nm

$^1$H NMR (only major isomer: 86%) (DMSO-$d_6$, 300 MHz, 300 K, ppm): δ 7.21 – 7.55 (m, 4H), 7.74 (d, $J = 8.1$ Hz, 1H), 7.89 (d, $J = 7.2$ Hz, 2H), 8.00 (d, $J = 8.1$ Hz, 1H), 8.40 - 8.44 (m, 2H), 8.62 (s, 1H), 11.10 (s, 1H), 12.11 (s, 1H).

$^{13}$C NMR (DMSO-$d_6$, 126 MHz, 300 K, ppm): δ 110.97, 120.57, 121.34, 124.29, 125.04, 126.34, 127.26, 127.37, 128.71, 129.12, 130.77, 136.31, 137.43, 148.96, 150.07, 153.61, 154.33, 164.49

MS (ESI,+, MeOH:H$_2$O 1:1): m/z calculated for C$_{17}$H$_{13}$N$_3$O$_2$Na: [M+Na]$^+$ 314.2, found 314.1.

HPLC-MS ($\lambda = 331$ nm): $R_t = 3.4$ minutes
4.2 $^1$H NMR and $^{13}$C NMR spectra

Figure S4. $^1$H NMR spectrum of 1A in DMSO-$d_6$ (300 MHz, 300K).

Figure S5. $^1$H NMR spectrum of 1A in MeOD (300 MHz, 300K).
Figure S6. $^{13}$C NMR spectrum of 1A in DMSO-$d_6$ (126 MHz, 300K).

Figure S7. HPLC chromatogram of 1A (20 µM) monitored at 331 nm. Retention time: 4.3 min. A volume of 2 µL of the stock solution of the compound (in DMSO-$d_6$, 10 mM) was added to an aqueous buffered solution (HEPES, 5 mM, pH 7) of 1 mL. Conditions: Flow rate: 0.6 ml/min, Gradient: 5-95 %B to 95-5%B (A: H$_2$O+0.1%HCOOH, B: ACN+0.1% HCOOH) in 0.00 to 5.00 min; 95-100 % B in 5 to 5.10 min; 100 % B from 5.10 to 8.10 min; 100-5 % B in 8.10 to 9.10 min; and finally 5% B from 9.10 to 10.00 min. Column: Agilent Zorbax SB-C3 Rapid Resolution HT 3.0 × 100 mm 1.8 micron. Injection volume: 20 µL. Column temperature: 40 °C.
Figure S8. $^1$H NMR spectrum of 2A in DMSO-d$_6$ (300 MHz, 300K).

Figure S9. $^{13}$C NMR spectrum of 2A in DMSO-d$_6$ (126 MHz, 300K).
Figure S10. HPLC chromatogram of 2A (20 µM) monitored at 331 nm. Retention time: 3.4 min. 2 µL of the stock solution of the compound (in DMSO-d₆, 10 mM) was added to an aqueous buffered solution (HEPES, 5 mM, pH 7) of 1 mL. Conditions: Flow rate: 0.6 ml/min, Gradient: 5-95 %B to 95-5%B (A: H₂O+0.1%HCOOH, B: ACN+0.1% HCOOH) in 0.00 to 5.00 min; 95-100 % B in 5 to 5.10 min; 100 % B from 5.10 to 8.10 min; 100-5 % B in 8.10 to 9.10 min; and finally 5% B from 9.10 to 10.00 min. Column: Agilent Zorbax SB-C3 Rapid Resolution HT 3.0 × 100 mm 1.8 micron. Injection volume: 20 µL. Column temperature: 40 °C.

4.3 Quantification of the concentrations (UV-Vis and HPLC)

The stock solutions of reactants were prepared in acetonitrile to ensure adequate solubility. Mixing the reactants in buffer, amphiphile and vesicles resulted in the formation of a tiny fraction of product at the starting time-point. This effect was probably due to the small percentage of organic solvent in the solution. In cases where reactions needed to be compared, the values were normalised for the initial quantity of product formed upon mixing. The exact concentration of the aldehydes was determined by UV-Vis spectroscopy. The reagent solutions were prepared fresh prior to each experiment.

The corrected absorbance obtained by UV-Vis spectroscopy and the integrated area obtained by HPLC at the appropriate wavelength were converted to concentration using the appropriate calibration curves in the appropriate solvent. Solutions were prepared with the reagents at different concentration and the absorbance at the \( \lambda \) of analysis or the area under the peaks at the appropriate wavelength was plotted against the concentration. The slope of the linear curve thus obtained was used to determine the \( \varepsilon \) in the case of UV-Vis measurements and the conversion factor in the case of the HPLC measurements.
4.3.1 Calibration curves for hydrazone 1A by UV-Vis Spectroscopy

**Figure S11.** Calibration curve for hydrazone 1A in (a) HEPES buffer pH 7.0 monitoring the $\lambda_{\text{max}}$ peak at 329 nm and (b) 200 µM C$_{12}$TACN·Zn$^{2+}$ monitoring the $\lambda_{\text{max}}$ peak at 408 nm. [HEPES] = 5 mM, pH 7.0.

**Figure S12.** Calibration curve for hydrazone 1A in 200 µM amphiphile10, 200 µM ATP at pH 7.0 monitoring the $\lambda_{\text{max}}$ peak at 408 nm. The two graphs show two different concentration ranges. [HEPES] = 5 mM, pH 7.0.
4.3.2 Calibration curves for hydrazone 1A by HPLC

**Figure S13.** Calibration curve for hydrazone 1A in HEPES buffer pH 7.0 (a) between 0-1 µM and (b) between 0-25 µM. [HEPES] = 5 mM, pH 7.0.

**Figure S14.** Calibration curves for hydrazone 1A in acetonitrile, monitoring the area of the hydrazone peak at 331 nm. [HEPES] = 5 mM, pH 7.0.

**Figure S15.** Calibration curves for hydrazone 1A in 100 µM C$_{12}$TACN·Zn$^{2+}$, monitoring the area of the hydrazone peak at 331 nm. [HEPES] = 5 mM, pH 7.0.
Figure S16. Calibration curves for hydrazone 1A in 100 µM C12TACN·Zn2+, 200 µM ATP (assemblies), monitoring the area of the hydrazone peak at 331 nm. [HEPES] = 5 mM, pH 7.0.

4.3.3 Calibration curves for hydrazone 2A by HPLC

Calibration curves were prepared separately for the hydrazone 2A in buffer, in 100 µM C12TACN·Zn2+ and for assemblies obtained from mixing 200 µM ATP with 100 µM C12TACN·Zn2+ at five different concentrations. They were injected in the HPLC and the area of 2A was plotted against the concentration. Linear regression of the plots gave an equation which was used to change the area into concentration.

Figure S17. Calibration curves showing plots of the integrated area against the concentration of 2A for standard solutions in (a) aqueous buffer, (b) 100 µM C12TACN·Zn2+ and (c) 100 µM C12TACN·Zn2+, 200 µM ATP. [HEPES] = 5 mM, pH 7.0.
4.4 UV-Vis spectra of 1 and 1A

Figure S18. UV-Vis spectra of 1 (20 µM, pink) and 1A (20 µM) in aqueous buffer at pH 7.0 (blue) or in the presence of C₁₂TACN·Zn²⁺ (100 µM) and ATP (200 µM) (red - * denotes the absorption band from ATP).

4.5 TEM and DLS in the presence of reagents

The figure below shows the TEM of the aggregates formed from the surfactant and ATP in the presence of 1 (a) and 1 and A after 8 hours (i.e. in the presence of product). The figure shows that the structure is preserved in all three cases.

Figure S19. (a) TEM image of aggregates with [C₁₂TACN·Zn²⁺] = 100 µM, [ATP] = 200 µM and [I] = 20 µM. (b) TEM image of aggregates with [C₁₂TACN·Zn²⁺] = 100 µM, [ATP] = 200 µM and [I] = [A] = 20 µM after 8 hours (i.e. containing 1A). The scale bar corresponds to 50 nm.
4.6 Kinetics of formation of 1A by UV-Vis and HPLC

Figure S20a shows the kinetics of formation of 1A as determined by HPLC when 20 µM 1 and A were reacted in the presence of 100 µM C_{12}TACN•Zn^{2+} and increasing concentrations of ATP. The linear part of the curve (typically the first three points) was used to calculate the initial rate of reaction at each ATP concentration. The values obtained were plotted against ATP concentration as shown in Figure S20b. Plotting ln v_0 against ln [ATP] (Figure S21) gave an order of reaction of 0.9.

Figure S20. (a) Graph showing the increase in the concentration of 1A when carrying out the reaction between 1 and A in the presence of 100 µM C_{12}TACN•Zn^{2+} and increasing concentrations of ATP. Graphs are plotted from the same experiment. (b) Initial rate of formation of 1A as a function of concentration of ATP determined from graph (a). [HEPES] = 5 mM, pH 7.0, [C_{12}TACN•Zn^{2+}] = 100 µM, [1] = [A] = 20 µM.

Figure S21. A plot of ln initial rate against ln [ATP]. The gradient of the straight line graph gave the order of reaction (0.9).
Figure S22 compares the kinetics of formation of 1A in HEPES buffer pH 7.0, 100 µM C\textsubscript{12}TACN-Zn\textsuperscript{2+} and in assemblies formed from 100 µM C\textsubscript{12}TACN-Zn\textsuperscript{2+}, 200 µM ATP. The top graph shows the kinetic traces while the graphs below shows the UV-Vis spectra over time under the different conditions. Figure S23 shows the same experiment followed with HPLC-MS. Figure S24 shows the MS of the product peak with a retention time of 3.9 minutes, with an m/z value of 317 g/mol [M+H].

![Figure S22](image)

**Figure S22.** Concentration of hydrazone 1A as a function of time (top) and UV-Vis spectra at regular intervals (bottom) of the reaction of 20 µM reagents in (a) aqueous buffer (b) C\textsubscript{12}TACN-Zn\textsuperscript{2+} and (c) assemblies. [HEPES] = 5 mM, pH 7.0, [C\textsubscript{12}TACN-Zn\textsuperscript{2+}] = 100 µM, [ATP] = 200 µM, [I] = [A] = 20 µM.
**Figure S23.** Concentration of hydrazone 1A as a function of time (top) and HPLC chromatograms (initial and final) (bottom) of the reaction of 20 µM reagents in (a) aqueous buffer (b) C_{12}TACN·Zn^{2+} and (c) assemblies. [HEPES] = 5 mM, pH 7.0, [C_{12}TACN·Zn^{2+}] = 100 µM, [ATP] = 200 µM, [1] = [A] = 20 µM.

**Figure S24.** MS-spectrum of the product peak formed when 1 and A were reacted in assemblies [HEPES] = 5 mM, pH 7.0, [C_{12}TACN·Zn^{2+}] = 200 µM, [1] = 5 µM, [A] = 25 µM. MS (ESI,+, MeOH:H_{2}O 1:1): m/z calculated for C_{20}H_{16}N_{2}O_{2} [M+H]^{+} 317.3; found 317.2.
Figure S25 shows the control experiment in which ATP was added to the buffer in the presence of C$_{12}$TACN Zn$^{2+}$ by UV-Vis. There was no evidence of product formation over time.

Figure S25. (a) UV-Vis spectra at regular intervals of the reaction of 20 µM reagents in 200 µM ATP. [HEPES] = 5 mM, pH 7.0 [I] = [A] = 20 µM.

4.7 Reactivity of 1A and 2A in the presence of the enzyme alkaline phosphatase

Figure S26 shows the kinetic traces obtained when hydrazones 1A and 2A were formed from solutions of their respective reactants (20 µM) in the presence of C$_{12}$TACN Zn$^{2+}$ (100 µM) and the enzyme alkaline phosphatase (0.5 U) upon repeated addition of 50 µM aliquots of ATP. The formation reactions were carried out in separate vials for the two reactants. A separate experiment was carried out in the absence of reactants but in the presence of laurdan dye (2 µM) in order to monitor the formation and degradation of the assemblies by fluorescence spectroscopy.
Figure S26. (a) Figure showing the F.I. of laurdan dye in 100 µM surfactant and 0.5 U alkaline phosphatase and upon repetitive addition of 50 µM ATP. (b) Figure showing the concentration of 1A in 100 µM surfactant and 0.5 U alkaline phosphatase and upon repetitive addition of 50 µM ATP. (c) Figure showing the concentration of hydrazone 2A in 100 µM surfactant and 0.5 U alkaline phosphatase and upon repetitive addition of 50 µM ATP. λ<sub>ex</sub> = 370 nm, Slits = 5/10 nm (ex/em), 25 °C, [HEPES] = 5 mM, pH 7.0, [Laurdan] = 2 µM, [Surfactant] = 100 µM, [ATP] = 50 µM, [alkaline phosphatase] = 2 U, [1] = [2] = 20 µM, [A] = 40 µM.

4.8 Control of reactivity of hydrazones 1A and 2A by controlling the self-assembly

The different response of the two reactions to the absence or presence of templated assemblies were also assessed by first carrying out the reactions in HEPES buffer pH 7.0 and ATP. ATP on its own did not effect product formation and under these conditions, the formation of 2A was much more favoured than that of 1A. Addition of C<sub>12</sub>TACN·Zn<sup>2+</sup> (100
µM) at around 450 minutes, however, led to a strong enhancement in the rate of formation of 1A but did not significantly affect the formation of 2A. Consequently the formation of 1A became the preferred reaction after some time (Figure S27).

**Figure S27.** Graph showing the increase in 1A and 2A in aqueous buffer and ATP and upon addition of C12TACN:Zn²⁺ to form assemblies. [C12TACN·Zn²⁺] = 100 µM, [ATP] = 200 µM, [1] = [2] = [A] = 20 µM.

The following is a duplicate experiment for the final experiment in which ATP is added to a solution containing reagents 1, 2 and A, C12TACN·Zn²⁺ and the enzyme alkaline phosphatase (AP) under optimized conditions in terms of concentration.

**Figure S28.** Duplicate experiment showing the increase in concentration of 1A and 2A over time when a mixture of 1 (20 µM), 2 (20 µM) and A (40 µM) were added to a solution of C12TACN·Zn²⁺ (100 µM) and alkaline phosphatase (0.5 U) and to which ATP (50 µM) was added at different times. [HEPES] = 5 mM, pH 7.0, [C12TACN·Zn²⁺] = 100 µM, [ATP] = 50 µM, 100 µM, [alkaline phosphatase] = 0.5 U, [1] = [2] = 20 µM, [A] = 40 µM.
5. Kinetic model

A kinetic model was developed to simulate ATP-regulated product formation. The ATP-induced transition from an unassembled to an assembled state is treated as 1:1 complex formation between surfactant and ATP under saturation conditions. This implies that the addition of ATP quantitatively converts surfactant in vesicles. Evidently, this does not correspond to reality, but from a modelling point of view it is the most effective way to describe a transition between two states.

The rate of hydrazone bond formation depends on the assembly state of the surfactant. For each hydrazone $1A$ and $1B$ these rates were determined both in the assembled and unassembled state by fitting the data reported in Figure 3b+d of the manuscript. The obtained rates were overall rates including the – possible – background reaction. To permit a smooth transition of the rate from the assembled to the non-assembled state (and back) under dissipative conditions, the surfactant (or vesicle) concentration were always included in the rate equation. ATP hydrolysis was described using first order decay kinetics.

The addition of ATP after a certain time interval was simulated by taking the calculated concentrations of the system prior to ATP-addition as input values for a new simulation in the presence of ATP.

The model was implemented in MicroMath Scientist for Windows (version 2.01).

```
// MicroMath Scientist Model File
IndVars: T
DepVars: H1A, H2A, V, S, ATP, H1, H2, A, H1TOT, H2TOT, ATOT
Params: ka, kd, kcat, k1s, k1v, k2s, k2v, V0, S0, ATP0, H1A0, H2A0, H10, H20, A0
V'=ka*S*ATP-kd*V
S'=-ka*S*ATP+kd*V
ATP'=-ka*S*ATP+kcat*ATP
H1A'=k1s*S*H1*A+k1v*V*H1*A
H1'=k1s*S*H1*A-k1v*V*H1*A
H2A'=k2s*S*H2*A+k2v*V*H2*A
H2'=k2s*S*H2*A-k2v*V*H2*A
A'=-k1s*S*H1*A-k1v*V*H1*A-k2s*S*H2*A-k2v*V*H2*A
//
H1TOT=H1+H1A
H2TOT=H2+H2A
ATOT=A+H1A+H2A
//
T=0
V=V0
S=S0
ATP=ATP0
```
H1A = H1A0
H1 = H10
H2A = H2A0
H2 = H20
A = A0

***

The following abbreviations are used

Dependent variables:  \( H1A = 2A \); \( H2A = 1A \); \( V = \text{vesicle} \); \( S = \text{surfactant} \); \( ATP = ATP \); \( H1 = 2 \); \( H2 = 1 \); \( A = A \);

Parameters:

| indicator | description | value          |
|-----------|-------------|----------------|
| ka        | association rate constant vesicle assembly | 1.00E+10        |
| kd        | dissociation rate constant vesicle disassembly | 1.00E+00        |
| kcat      | rate constant for ATP hydrolysis | 1.50E+04        |
| k1s       | rate of formation for H1A in non-assembled state | 4.99E+04        |
| k1v       | rate of formation for H1A in assembled state | 3.21E+04        |
| k2s       | rate of formation for H2A in non-assembled state | 9.09E+03        |
| k2v       | rate of formation for H2A in assembled state | 7.29E+04        |
| V0        | Initial vesicle concentration | Simulation dependent |
| S0        | Initial surfactant concentration | Simulation dependent |
| ATP0      | Initial ATP concentration | Simulation dependent |
| H1A0      | Initial concentration of H1A | Simulation dependent |
| H2A0      | Initial concentration of H2A | Simulation dependent |
| H10       | Initial concentration of H1 | Simulation dependent |
| H20       | Initial concentration of H2 | Simulation dependent |
| A0        | Initial concentration of A | Simulation dependent |
Figure S29. Fits of the data reported in Figure 3b and d to the kinetic model to obtain the values for $k_{1s}$ (a), $k_{1v}$ (b), $k_{2s}$ (c) and $k_{2v}$ (d) reported above.

6. References

1. Fulmer, G. R.; Miller, A. J. M.; Sherden, N. H.; Gottlieb, H. E.; Nudelman, A.; Stoltz, B. M.; Bercaw, J. E.; Goldberg, K. I. Organometallics 2010, 29, 2176.
2. Maiti, S.; Fortunati, I.; Ferrante, C.; Scrimin, P.; Prins, L. J. Nat. Chem. 2016, 8, 725.
3. Gasparini, G.; Martin, M.; Prins, L. J.; Scrimin, P. Chem. Commun. 2007, 1340.