Supporting Information

Template-Dependent (Ir)reversibility of Noncovalent Synthesis Pathways

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1. Materials and methods

Nile Red, nucleotides, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were procured from Sigma-Aldrich. All commercially available reagents were used as received. The enzymes potato apyrase (PA) and alkaline phosphatase (AP) were obtained from Sigma Aldrich and used without further purification. PA was dissolved in 1.0 mL of mQ-water and divided in 20 working aliquots of 50 μL with a concentration of 200 U/mL and preserved at -20 °C. Similarly AP was also dissolved in 1.0 mL of mQ-water and divided into 20 working aliquots of 50 μL with a concentration of 5000 U/mL and preserved at 4°C.

The Zn(NO$_3$)$_2$-stock solution was standardized using EDTA following standard procedures. The stock solutions were prepared both by weight and UV-vis spectroscopy using the molar extinction coefficients: $\varepsilon_{259}$ (AMP and ATP) = 15400 M$^{-1}$cm$^{-1}$.

Fluorescence measurements were performed using a Varian Cary Eclipse fluorescence spectrophotometer equipped with a thermostatted cell holder. UV-visible spectra were measured on a Varian Cary50 spectrophotometer equipped with thermostatted multiple cell holders. Dynamic light scattering (DLS) was performed on a Malvern Zetasizer Nano-S instrument. TEM images were recorded on a Jeol 300 PX electron microscope. First the grid was placed on a drop of sample solution for 1 min and then, for staining, it was placed on a drop of uranyl acetate (2 %) solution for 30 s. The solvent was allowed to evaporate before imaging of the stained grid.
2. Synthesis and characterization of the surfactant - C\textsubscript{16Ph}TACN

\[ \text{4-Hydroxybenzaldehyde 1 (1 g, 8.19 mmol) was added to a solution of KOH (0.46 g) in 30 mL ethanol and stirred in inert atmosphere for 15 minutes. The mixture was then treated with 1-Bromohexadecane (2.49 g, 8.19 mmol)) and refluxed at 78 °C. The reaction was monitored by TLC and ESI-MS. Ethanol was evaporated and the residue was dissolved in diethyl ether and washed with an aqueous solution of 10% KOH followed by water until a} \]
neutral pH was obtained. The organic layer was dried over anhydrous sodium sulphate and then evaporated to dryness. The crude product was purified by flash chromatography (silica gel, eluent: only CHCl₃) yielding product 2 (0.53 g, 19 % yield) as a white solid.

Di-tert-butyl 1, 4, 7-triazanonane-1, 4-dicarboxylate 4 was synthesized according to a literature protocol (G. Pieters, A. Cazzolaro, R. Bonomi, L. J. Prins, Chem. Commun. 48, 1916 (2012)). Compound 4 (0.20 g, 0.58 mmol) was dissolved in 20 mL methanol under inert atmosphere. Glacial acetic acid (0.58 mmol) in a solution of 8 mL methanol was added using a syringe followed by a solution of 2 (0.2 g, 0.58 mmol) in 20 mL methanol. The mixture was cooled to approximately 15 °C and then a solution of sodium cyanoborohydride (0.13 g, 2.08 mmol) in 40 mL methanol was slowly added with a syringe pump (0.25 mL/min) maintaining the temperature at 15 °C. After complete addition, the mixture was stirred at room temperature for 18 hours. Following this, the solution was concentrated under reduced pressure and the product was purified using flash column chromatography (silica gel, eluent: 2% MeOH in CHCl₃) yielding product 5 as a white solid (0.19 g, 49 % yield). Characterization data are conform those reported.

Compound 5 (0.12 g, 0.19 mmol) was solubilized in methanol (about 5mL) and a concentrated HCl-solution (6 N, 5 mL) was added. The mixture was stirred for 6 h at 60 °C after which the solvent was evaporated under reduced pressure with a rotary evaporator. The product 6 was then dried to completeness under high vacuum yielding product 1-(4-(hexadecyloxy)phenyl)-1,4,7-triazonane (0.08 g, 98%) as a pale yellow solid.

**Characterization**

**COMPOUND 2**

**¹H NMR:** (δ ppm, CDCl₃, 298K, 200 MHz): 9.8 (s, 1H), 7.9 (d, J = 8.2 Hz, 2H), 7.0 (d, J = 8.2 Hz, 2H), 4.1 (t, J = 5.9 Hz, 2H), 1.8 (m, 2H), 1.26 (br, 24H), 0.9 (t, J = 6.5Hz, 3H)

**¹³C NMR:** (δ ppm, CDCl₃, 298K, 300 MHz): 191.1, 164.7, 132.4, 130.2, 115.2, 68.9, 32.4, 29.8, 29.5, 26.4, 23.2, 14.6.

Figure S1 $^1$H NMR spectrum of compound 2 in CDCl$_3$, 200 MHz

Figure S2 $^{13}$C NMR spectrum of compound 2 in CDCl$_3$, 300 MHz
Figure S3 ESI-MS (ESI+, H$_2$O:CH$_3$CN = 1:1) spectrum of compound 2

**COMPOUND 5**

$^1$H NMR: ($\delta$ ppm, CD$_3$OD, 298K, 500 MHz): 7.3 (d, J = 8.4 Hz, 2H), 6.8 (d, J = 8.5 Hz, 2H), 4.0 (t, H = 5.2 Hz, 2H), 3.6 (s, 2H), 3.5 (m, 4H), 3.2 (m, 4H), 2.7 (m, 4H), 1.8 (m, 2H), 1.4 (s, 18H), 1.3 (br, 26H), 0.9 (t, J = 6.9 Hz).

$^{13}$C NMR: ($\delta$ ppm, CD$_3$OD, 298K, 500 MHz): 158.3, 131.6, 129.8, 113.8, 79.8, 67.5, 59.8, 53.8, 52.5, 31.7, 29.4, 29.1, 27.5, 25.8, 22.4, 13.1.

Figure S4 $^1$H NMR spectrum of compound 5 in CD$_3$OD, 500 MHz

Figure S5 $^{13}$C NMR spectrum compound 5 in CD$_3$OD, 500 MHz
Figure S6 ESI-MS (ESI+, H₂O:CH₃CN = 1:1) spectrum of compound 5

**COMPOUND 6: C₁₆PhTACN**

**¹H NMR:** (δ ppm, CD₃OD, 298K, 500 MHz): 7.4 (d, J = 8.5 Hz, 2H), 6.9 (d, J = 8.5 Hz, 2H), 4.0 (t, J = 6.4 Hz, 2H), 3.9 (s, 2H), 3.6 (m, 4H), 3.2 (m, 4H), 3.0 (m, 4H), 1.7 (m, 2H), 1.5 (m, 2H), 1.3 (br, 24H), 0.9 (t, J = 6.9 Hz 3H).

**¹³C NMR:** (δ ppm, CD₃OD, 298K, 300 MHz): 159.5, 131.6, 127.2, 114.3, 68.0, 59.2, 44.3, 42.8, 31.9, 25.9, 22.4, 13.2.

**ESI-MS** (ESI+, H₂O:CH₃CN = 1:1). [M+H]⁺: found: 460.5; calcd: 460.4.

The NMR (¹H, ¹³C) and ESI-MS spectra are shown below (Supplementary Figs. 1-9).
Figure S7 $^1$H NMR spectrum of $\text{C}_{16}\text{PhTACN}$ (compound 6) in $\text{CD}_3\text{OD}$, 500 MHz

Figure S8 $^{13}$C NMR spectrum of $\text{C}_{16}\text{PhTACN}$ (compound 6) in $\text{CD}_3\text{OD}$, 300 MHz
Figure S9 ESI-MS (ESI+, $\text{H}_2\text{O} : \text{CH}_3\text{CN} = 1:1$) spectrum of $\text{C}_{16}\text{Pn}\text{TACN}$ (compound 6)
3. Characterization of aggregates formed by C$_{16}$PhTACN (free ligand)

3.1. Dynamic light scattering (DLS) analysis

**Figure S10** Hydrodynamic diameter of aggregates formed when a 5 mM stock solution of C$_{16}$PhTACN is diluted to 50 µM in aqueous buffer, [HEPES] = 5 mM, pH 7.0, 25 °C.
3.2. Transmission electron microscopy (TEM) analysis

**Figure S11** TEM images of 50 µM solution of $\text{C}_{16}\text{H}_{36}\text{TACN}$. General experimental conditions: [HEPES] = 5 mM, pH 7.0, 25 °C, staining was performed using 2% uranyl acetate.
4. Characterization of aggregates formed by C_{16}PhTACN

4.1. Kinetic study of the formation of the disk like assemblies I

a. TEM analysis

b. DLS Measurements

c. Time dependent Fluorescence

Figure S12 a. TEM images of (1) 50 µM C_{16}PhTACN (free ligand), 50 µM C_{16}PhTACN·Zn^{2+} (2) measurements performed instantly after the addition of 1 equivalent Zn(NO_3)_2, (3) measurements performed 3 hours after the addition of zinc to surfactant, and (4) measurements performed 8 hours after the addition of zinc to surfactant. b. Hydrodynamic diameter of (1), (2), (3) and (4). c. Fluorescent intensity (FI) at 636 nm as a function of time following the process of formation of disk like assemblies I when 1 equivalent of Zn(NO_3)_2 is added to 50 µM C_{16}PhTACN·Zn^{2+} in the presence of Nile Red (2 µM). General experimental conditions: [HEPES] = 5 mM, pH 7.0, 25 °C. Fluorescence measurements- excitation wavelength = 552 nm, slit width (ex/em) = 5/10 nm. TEM analysis - staining was performed using 2% uranyl acetate.
4.2 Additional TEM images of the C\textsubscript{16}PhTACN\textsuperscript{2+} disks I

\textbf{Figure S13} TEM images of a 50 µM solution of C\textsubscript{16}PhTACN\textsuperscript{2+}. Experimental conditions: [HEPES] = 5 mM, pH 7.0, 25 °C, staining was performed using 2% uranyl acetate.
5. TEM analysis of aggregates formed by $\text{C}_{16\text{Ph}}\text{TACN} \cdot \text{Zn}^{2+}$ in the presence of ATP

**Figure S14** TEM images of the transformation of the disk like assemblies I instantly after the addition of 15 µM ATP to a solution of 50 µM $\text{C}_{16\text{Ph}}\text{TACN} \cdot \text{Zn}^{2+}$ disks. General experimental conditions: [HEPES] = 5 mM, pH 7.0, 25 °C, staining was performed using 2% uranyl acetate
Figure S15 TEM images taken 6 hours after the addition of 15 µM ATP to a solution of 50 µM C_{16}PhTACN\(\text{Zn}^{2+}\) disks I. General experimental conditions: [HEPES] = 5 mM, pH 7.0, 25 °C, staining was performed using 2% uranyl acetate.
Figure S16 TEM images of spherical aggregates formed when 15 µM ATP is added to a fresh solution of 50 µM C$_{16}$PhTACN$^+$. General experimental conditions: [HEPES] = 5 mM, pH 7.0, 25 °C, staining was performed using 2% uranyl acetate.
6. Determination of the kinetic stability of the different ATP templated structures

**Figure S17.** a. Fluorescent Intensity (FI) at 636 nm as a function of time following the addition of ATP (15 μM) to 50 μM C_{16Ph}TACN·Zn^{2+} disks I (1) to form elongated fibres II (2) and then the addition of potato apyrase (3 U mL^{-1}) (3). b. Fluorescent Intensity (FI) at 636 nm as a function of time when potato apyrase (3 U mL^{-1}) is added to ATP templated spherical assemblies III (15 μM ATP is added to a fresh solution of 50 μM C_{16Ph}TACN·Zn^{2+}) (4). General experimental conditions: [Nile Red] = 2 μM, [CaCl$_2$] = 0.5 mM, [HEPES] = 5 mM, pH 7.0, 25 °C, excitation wavelength = 552 nm, slit width (ex/em) = 5/10 nm.
7. TEM analysis of aggregates formed by C\textsubscript{16}Ph-TACN\textsuperscript{-}Zn\textsuperscript{2+} in the presence of AMP

**Figure S18** TEM images of the aggregates formed when 50 µM AMP is added to a fresh solution of 50 µM C\textsubscript{16}phenylTACN\textsuperscript{-}Zn\textsuperscript{2+}. Experimental conditions: [HEPES] = 5 mM, pH 7.0, 25 °C, staining was performed using 2% uranyl acetate.
Figure S19 TEM images of the assemblies formed 30 mins after the addition of 50 µM AMP to 50 µM C$_{16}$phenylTACN-Zn$^{2+}$ after the formation of the disc like assemblies I. Experimental conditions: [HEPES] = 5 mM, pH 7.0, 25 °C, staining was performed using 2% uranyl acetate
Figure S20 TEM images of the aggregates formed 10 hours after the addition of 50 µM AMP to a solution of 50 µM C16phenylTACN-Zn^{2+} after the formation of the disc like assemblies I. Experimental conditions: [HEPES] = 5 mM, pH 7.0, 25 °C, staining was performed using 2% uranyl acetate.
8. TEM analysis of the AMP-templated system after the addition of enzyme (alkaline phosphatase (AP))

**Figure S21** Additional TEM images taken 12 hours after the addition of alkaline phosphatase to the AMP templated assemblies IV (assemblies IV were obtained by adding AMP to the C$_{16}$TACN-Zn$^{2+}$ disks I). Experimental conditions: [HEPES] = 5 mM, pH 7.0, 25 °C, staining was performed using 2% uranyl acetate.
**Figure S22** Additional TEM images taken 12 hours after the addition of alkaline phosphatase to the AMP templated assemblies IV (assemblies IV were obtained by adding AMP to a fresh solution of C$_{16}$TACN$^2$Zn$^{2+}$). Experimental conditions: [HEPES] = 5 mM, pH 7.0, 25 °C, staining was performed using 2% uranyl acetate.
Figure S23 Additional TEM images taken 24 hours after the addition of alkaline phosphatase to the AMP templated assemblies IV. (Experimental conditions: [HEPES] = 5 mM, pH 7.0, 25 °C, staining was performed using 2% uranyl acetate.