Supplementary Material (ESI) for Chemical Communications

Supporting information for

Sunlight mediated disruption of peptide-based soft structures decorated with gold nanoparticles

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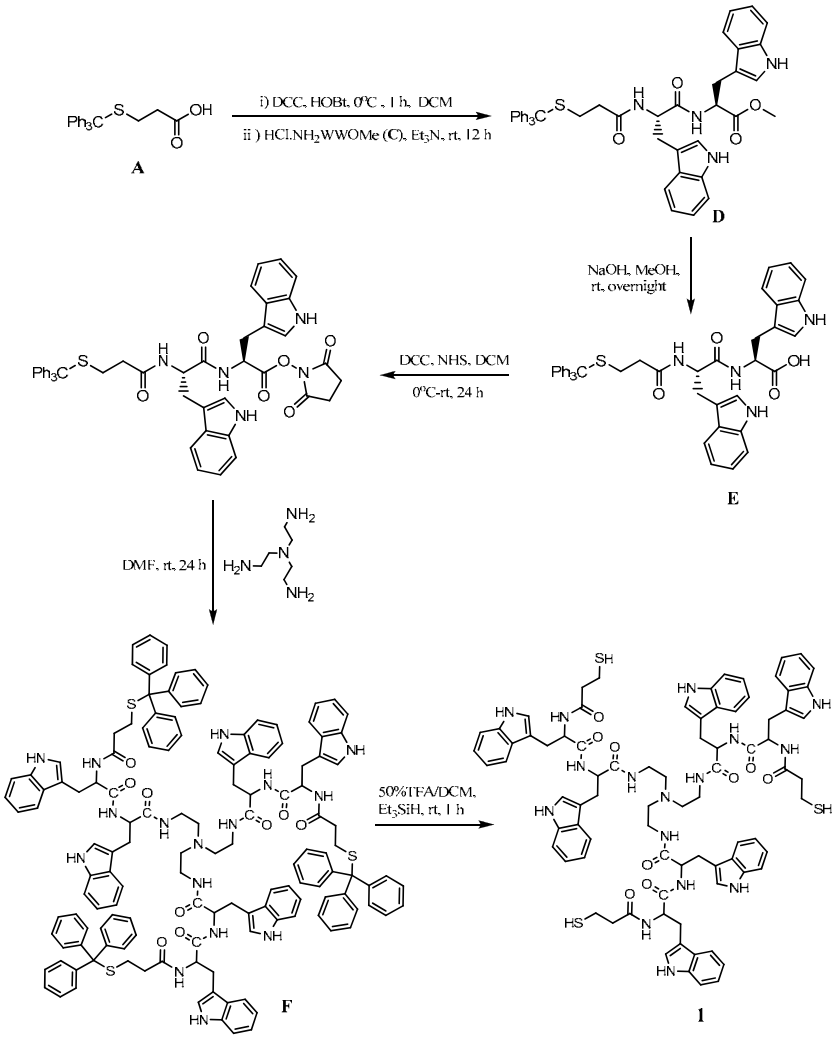
Contents

General Synthetic procedures:

Materials and methods: Dichloromethane, N, N'-dimethylformamide, methanol, were distilled following standard procedures prior to use. N,N'-dicyclohexylcarbodimide, N-hydroxybenzotriazole, amino acids, were purchased from Spectrochem (Mumbai, India) and used without further purification. Triethylsilane, 3-mercaptopropionic acid were purchased from S.D. Fine-chem limited, Mumbai and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on JEOL-JNM LAMBDA 500 model operating at 500 and 125 MHz, respectively. HRMS mass spectra were recorded at IIT Kanpur, India, on Waters, Q-Tof Premier Micromass HAB 213 mass spectrometer using capillary voltage 2.6-3.2 kV. HPLC spectra was recorded (AktaBasic, Amersham Pharmacia) using a µRPC C2/C18 ST 4.6/100 column (Pharmacia Biotech).



Scheme S1: Scheme for synthesis of (Mpa-trp-trp)$_3$tren (**1**)

Synthesis of (*S*-trityl-3-mercaptopropionicacid) (A): 3-mercaptopropionicacid (5 g, 47 mmol) was dissolved in dry dichloromethane (40 mL). Then triethylamine (6.6 mL, 33 mmol) was added and stirred. Solution of trityl chloride (13.2 g, 47 mmol) in dichloromethane (30 mL) was added slowly to this solution. The reaction mixture was stirred for overnight at room temperature. Organic layer was washed with 1N aqueous HCl and brine solution and then dried over anhydrous sodium sulphate. The crude product obtained after evaporation was purified by silica gel column chromatography using dichloromethane and methanol (97:2) solvent system. Yield: 11.5 g (70%) R_f : 0.6 (5% methanol in dichloromethane) . ^{1}H NMR (500 MHz) $CDCl_3$: 1.24 (s, 1H); 2.21-224 (t, 2H, J=7.55Hz); 2.43-2.46 (t, 2H, J=7.25 Hz); 7.20-7.45 (m, 15H). This compound was used without further characterization.

Synthesis of *N*-*tert*-butyloxycarbonyl-L-tryptophan-tryptophan methyl ester (B): N-(Boc)-L-Tryptophan (2 g, 6.57 mmol) and 1-hydroxybenzotriazole (1.2 g, 7.88mmol) were dissolved in dry N, N'-dimethylformamide (10 mL) and dichloromethane (10 mL), and reaction mixture was cooled to 0 $^\circ$C under nitrogen atmosphere. Solution of N, N'- dicyclohexylcarbodiimide (1.62 g, 7.85 mmol) in DCM (15 mL) was added into the reaction mixture at 0 $^\circ$C under nitrogen atmosphere. The reaction mixture was stirred at 0 $^\circ$C for one-hour. After one-hour tryptophan methyl ester hydrochloride (1.67 g, 6.57 mmol) was added into the reaction mixture followed by the addition of triethylamine (1.82 mL, 12.94 mmol) and stirring was continued for 24 hours at room temperature. Next, the reaction mixture was filtered to remove N, N'-dicyclohexylurea and filtrate was concentrated in reduced pressure. The residue was dissolved in dichloromethane and organic layer was washed with aqueous 1N HCl and 10% aqueous $NaHCO_3$ solution. The organic layer was then dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude compound was purified through a silica gel column chromatography by using dichloromethane and methanol (97:3) solvent system to give pure compound (1.5g, 45% yield). R_f: 0.8 (10% methanol in dichloromethane). M.P = 170-172°C; $[\alpha]_D^{25}$ = -16° (c 0.5, methanol); ESI-HRMS: $[M+H]^+$, Calculated ($C_{28}H_{33}N_4O_5$) = 505.2451, Found = 505.2454; ^{1}H NMR (400MHz, DMSO-d_6, TMS, δ ppm): 1.28 (s, 9H); 2.83-2.89 (m, 1H); 3.02-3.18 (m, 3H); 3.56 (s, 3H); 4.23-4.24 (m, 1H); 4.53-4.58 (m , 1H); 6.70-6.72 (d, 1H, J = 8.32); 6.94-6.99(m, 2H); 7.02-7.07 (m, 3H,); 7.16(s, 1H); 7.30-7.38(t, 2H, J = 8 Hz, 8 Hz); 7.46-7.48 (d, 1H, J = 7.56 Hz); 7.56-7.58 (d, 1H, J = 7.8 Hz); 8.24-8.26 (d, 1H, J = 7.32 Hz); 10.78 (s, 1H); 10.87 (s, 1H). ^{13}C NMR (125 MHz; DMSO-d_6, δ ppm): 28.63, 33.87, 52.34, 53.56, 55.51, 78.61, 109.70, 110.62, 111.97, 118.98, 121.34, 124.71, 127.61, 127.89, 136.59, 155.64, 172.72.

Synthesis of HCl salt of L-tryptophan-tryptophan methyl ester (C): *N-tert*-butyloxycarbonyl-L-tryptophan-tryptophan methyl ester (1.2 g, 2.37 mmol) was taken in a round bottom flask and 15 mL of 1N HCl in ethyl acetate was added. The reaction mixture was stirred for 2 h under nitrogen atmosphere at room temperature. The solvent was evaporated under reduced pressure and the solid residue was washed with ether thrice to yield compound **C** as white solid (Yield 1.0g, 95%). R_f = 0.4 (10% methanol in dichloromethane). M.P = 178-180 ^{0}C; $[\alpha]_D^{25}$ = -2° (c 0.5, methanol); ESI-HRMS: $[M+H]^+$, Calculated ($C_{23}H_{25}N_4O_3$) = 405.1927, Found = 405.1922; ^{1}H NMR (500 MHz, CD_3OD, TMS, δ (ppm): 3.14-3.18 (m, 2H); 3.35-3.36 (m, 2H); 3.61 (s, 3H); 4.08 (m, 1H); 4.73-4.76 (t, 1H, J = 6.6 Hz, 6.58 Hz); 6.97-7.17 (m, 6H); 7.29-7.36 (dd, 2H, J = 8 Hz, 24.9 Hz); 7.47-7.49 (d, 1H, J= 7.75 Hz); 7.61-7.62 (d, 1H, J = 7.7 Hz).^{13}C (125 MHz; CD_3OD, TMS, δ ppm): 27.27, 37.42, 51.55, 53.46, 53.80, 111.13, 111.31, 118.59, 118.97, 121.20, 121.54, 127.91, 136.91, 168.76, 171.98.

Synthesis of S-Trityl MPA-L-Trp-Trp-OMe (D): S-tritymercaptopropionic acid (1.50g, 4.30 mmol) and 1-hydroxybenzotriazole (0.71 g, 4.57 mmol) were dissolved in dry N, N-dimethylformamide (10 mL) and dichloromethane (10 mL), and reaction mixture was cooled to 0 $^\circ$C under nitrogen atmosphere. Solution of N, N'- dicyclohexylcarbodiimide (0.92 g, 4.45 mmol) in dichloromethane (15 mL) was added into the reaction mixture at 0 $^\circ$C under nitrogen atmosphere. The reaction mixture was stirred at 0 $^\circ$C for one-hour. After one-hour HCl.tryptophan-tryptophan methyl ester (1.9 g, 4.30 mmol) was added into the reaction mixture followed by the addition of triethylamine (1.2 mL, 8.5 mmol) and stirring was continued for 24 hours at room temperature. Next, the reaction mixture was filtered to remove N, N'-dicyclohexylurea and filtrate was concentrated in reduced pressure. The residue was dissolved in dichloromethane and organic layer was washed with aqueous 1N HCl and aqueous 10% $NaHCO_3$ solution. The organic layer was then dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude compound was purified through a silica gel column chromatography by using dichloromethane and methanol (97:3) solvent system to give pure compound **D** as a white solid**.** R_f **:** 0.7 g (10% methanol in dichloromethane) Yield: 2.59g (82%). ESI-HRMS: $[M+H]^+$ Calculated ($C_{45}H_{43}N_4O_4S$) = 735.3005, found = 735.3004; $[M+Na]^+$ Calculated ($C_{45}H_{42}N_4NaO_4S$) =757.2824, found= 757.2833; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.62-1.68 (m, 1H); 1.73-1.77(m, 1H,); 2.43-2.48 (m, 2H); 2.99-3.04 (1H, dd, J=7.45 Hz, 14.3 Hz); 3.10-3.11 (d, 2H, J= 5.15Hz); 3.22-3.26 (dd, 1H J= 5.15Hz, 14.9Hz), 4.65-4.68 (dd, 1H, J=2.3Hz, 7.45 Hz); 4.75-4.76 (1H, dd, J=2.35Hz, 5.75Hz); 5.96-5.97 (d, 1H, J= 6.9Hz), 6.27-6.28(d, 1H, J=6.90Hz); 6.65- 7.43 (m, 25H); 7.76 (s, 1H); 7.99 (s, 1H). ^{13}C (125 MHz; $CDCl_3$, TMS, δ (ppm): 27.22, 27.51,

27.78, 35.20, 52.34, 52.69,53.47, 66.76, 109.30, 110.04, 111.15, 111.27, 118.32, 118.80, 119.43, 119.73, 122.04, 123.16, 123.55, 126.71, 127.94, 129.55, 135.91, 136.02, 144.59, 170.77, 171.05, 171.63

Synthesis of S-Trityl MPA-L-Trp-Trp-OH (E): S-Trityl MPA-Trp-Trp-OMe (2.4 g, 3.26 mmol) was dissolved in 30 mL of methanol. 1N aqueous NaOH solution (3.9 mL) was added and stirred for 4 hours at room temperature. Reaction mixture was then passed through strong cation exchange resin. Solvent was then evaporated to get the product as white solid. Yield= 2.23 g (94 %). R_f **:** 0.3 (10% methanol in dichloromethane). ESI-HRMS: $[M+Na]^+$ Calculated ($C_{44}H_{40}N_4NaO_4S$) =743.2668, found= 743.2661. ^{1}H NMR (500 MHz, CD_3OD, TMS), δ(ppm) : 1.82-1.89 (m, 2H); 2.22-2.25 (t, 2H, J=7.65Hz); 2.99-3.02 (dd, 1H, J=8.05Hz,); 3.11-3.18 (m, 2H); 3.21-3.24 (dd, 1H, J=5.35Hz); 4.61-4.62 (m, 2H); 6.85-7.49 (m, 25H). ^{13}C (125 MHz; CD_3OD, TMS) δ (ppm): 26.93, 27.46, 31.31, 34.98, 53.71, 53.84, 65.97, 109.31, 109.41, 111.47, 118.42, 118.47, 119.16, 119.59, 121.75, 122.01, 123.55, 123.72, 126.82, 126.98, 127.84, 128.03, 129.61, 135.84, 136.04, 144.66, 171.35, 171.63, 175.14

Synthesis of (S-Trityl MPA-Trp-Trp)$_3$Tren (F): S-Trityl MPA-Trp-Trp-OH (2 g, 2.77 mmol) and N-hydroxysuccinimide (340 mg, 2.95 mmol) was dissolved in dry dichloromethane (40 mL) and cooled to 0^{o}C. A solution of N, N'- dicyclohexylcarbodiimide (610 mg, 2.95 mmol) in dichloromethane was added slowly and stirred for 1hour at 0 $^\circ$C temperature and then 12 hours at room temperature. Dicyclohexylurea was filtered off and organic layer was washed with 10% aqueous $NaHCO_3$ solution and then with brine solution. The organic layer was then dried over anhydrous sodium sulphate and concentrated in reduced pressure. The product obtained was used in the subsequent step without further purification.

This active ester was dissolved in dry N,N'-dimethylformamide (15 mL) at room temperature under nitrogen atmosphere. Solution of tris-(2-aminoethyl)amine in (123 mg, 0.84 mmol) in dry N,N'-dimethylformamide (3 mL) was added into the reaction mixture drop-wise under nitrigen atmosphere at room temperature. The reaction mixture was stirred for additional 24 hours. The reaction mixture was then concentrated under reduced pressure and dissolved in dichloromethane. Organic layer was washed with aqueous 1N HCl and 10% aqueous $NaHCO_3$ solution and then dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude compound was purified through silica gel column chromatography by by using dichloromethane and methanol (97:4) solvent system to give pure compound. Yield= 1.25g (60% for two steps). R_f **:** 0.6 (10% methanol in dichloromethane). ESI-HRMS : $[M+H]^+$ Calculated ($C_{138}H_{133}N_{16}O_9S_3$) = 2254.9637, found = 2254.9680. ^{1}H NMR (500 MHz, DMSO-d_6, TMS),

δ(ppm) : 2.01-2.06 (brd, m, 12H); 2.17 (brd, s, 6H); 2.79-2.84 (m, 6H); 2.89-2.93(m, 6H); 2.99 (brd, s, 6H); 4.42-4.45 (m, 6H); 6.82-7.03 (m, 18H); 7.14-7.24 (m, 51H); 7.45-7.46 (d, 6H, J=4.6Hz); 7.62 (brd, s, 3H); 7.91-7.92 (d, 3H, J=6.45Hz); 7.95-7.96 (d, 3H, J= 6.85Hz); 10.70-10.72 (d, 6H, J=10.75Hz). ^{13}C (125 MHz; $CDCl_3$, TMS) δ (ppm): 27.15, 27.22, 29.52, 34.54, 36.91, 52.12, 54.02, 66.68, 108.96, 109.22, 111.32, 117.87, 118.20, 118.95, 119.03, 121.55, 121.92, 123.31, 123.47, 126.61, 127.23, 127.80, 129.37, 135.91, 136.01, 144.43, 171.27, 171.81, 171.90.

Synthesis of (MPA-Trp-Trp)$_3$Tren (1) : Compound **F** (500 mg, 0.22 mmol) was dissolved in dry dichloromethane (3 mL). Triethylsilane (0.8 mg, 0.67 mmol) was added to the solution and stirred for 5 min. Trifluoroacetic acid (3 mL) was then added and the reaction mixture was stirred for 1 hour. The solution was then concentrated under reduced pressure and diethyl ether was added. The precipitate formed was dried and dissolved in minimum volume of methanol and re-precipitated by adding diethyl ether, this process was repeated three times and the solid was dried in high vacuum pump. Yield=310 mg, (91%). R_f**:** 0.4 streak (10% methanol in dichloromethane). ESI-HRMS : $[M+H]^+$ Calculated ($C_{81}H_{91}N_{16}O_9S_3$) = 1527.6317, found=1527.6317. M.P = 160-162^{0}C; $[\alpha]_D^{25}$ = -11° (c 0.5, methanol). ^1H NMR (500 MHz, DMSO-d_6, TMS), δ(ppm) : 2.03-2.06 (t, 3H, J=7.30Hz); 2.27-2.31 (m, 6H); 2.65-2.70 (m, 6H); 2.81-2.88 (m, 6H) 2.91-3.00 (m, 12H); 3.03-3.10 (m,6H); 4.42-4.46 (m, 3H); 4.50-4.56 (m, 3H); 6.89-6.92 (m, 6H); 6.99-7.01 (d, 6H, J=8.25Hz); 7.06-7.09 (m, 6H); 7.25-7.29 (m, 6H); 7.47-7.48 (d, 3H, J=6.45Hz); 7.51-7.56(m, 3H); 8.05-8.10 (m, 9H); 10.71-10.81(t, 6H); ^{13}C (125 MHz; DMSO-d_6, TMS) δ (ppm): 19.90, 22.50, 23.21, 27.32, 30.55, 51.41, 53.45, 53.76, 109.59, 110.05, 111.35, 118.24, 118.43, 120.90, 121.01, 123.67, 123.74, 127.28, 136.01, 170.72, 171.72, 172.14.

Synthesis of gold nanoparticles:

~15 nm sized gold nanoparticles were prepared by reduction of $HAuCl_4$ with trisodium citrate in aqueous solution[1].

1. *Journal of Chemical Education*, 2004, **81,** 544a.

UV-Vis studies:

UV-Vis absorption spectra were recorded on Varian CARY 100 Bio UV-Vis spectrophotometer respectively, with 10 mm quartz cell at 25±0.1 $^{\circ}$C. For spectra of **1,** 1 mM solution was prepared in 9:1 methanol:water. Spectra for AuNP was recorded diluting 10 times as synthesized by adding water. For 1-AuNP spectra **1** (1mM) was first incubated for 1 h in methanol to have preformed spherical structures. 100 µL of AuNP solution (as synthesized) in water was added to 900 µL of this solution ,mixed gently and further incubated for another hour and subsequently spectra was recorded.

Microscopic studies:

Atomic Force Microscopy (AFM) – Samples were imaged with an atomic force microscope (Molecular Imaging, USA) operating under the Acoustic AC mode (AAC), with the aid of a cantilever (NSC 12(c) from MikroMasch). The force constant was 0.6 N/m, while the resonant frequency was 150 kHz. The images were taken in air at room temperature, with the scan speed of 2.2 lines/sec. The data acquisition and analysis were done using PicoView 5® software. 10 µL aliquot of fresh solutions of **1** (1 mM; 9:1 methanol: water) was deposited on onto freshly cleaved mica surface at room temperature and uniformly spread using a spin-coater operating at 200-500 rpm (PRS-4000). The sample-coated mica was dried in air for 30 minutes at room temperature, followed by AFM imaging.

Scanning Electron Microscopy (SEM) – Field emission scanning electron microscopy images acquired on FEI QUANTA 200 microscope, equipped with a tungsten filament gun, operating at WD 10.6 mm and 20 kV. A 10 µL aliquot of the fresh and aged sample solution of **1** (1 mM; 9:1 methanol: water) or **1** coincubated with gold nanoparticles were placed on copper stubs. The samples were dried at room temperature for 30 minutes and then vacuum dried for another 30 minutes and subsequently imaged without gold coating. 0.5 eq. DTT and 0.5 eq of triethylamine were added to 10 days aged aged sample solution of **1** (1 mM; 9:1 methanol: water) and further incubated for 12 hours followed by imaging in SEM.

Fluorescence microscopy (FM) – Dye stained structures were examined under a fluorescence microscope (Leica DM2500M), provisioned with a Rhodamine filter (absorption 540 nm/emission 625 nm). This filter optimized visualization of rhodamine-treated (positive resolution) compared with

untreated (negative resolution) vesicles that are virtually invisible to this light. For dye encapsulation study **1** was dissolved in 1 μM Rhodamine B dye solution (9:1 methanol:water) such that final concentration of **1** be 1mM. 10 μL of each solution was spread on a glass slide, dried at room temperature, and imaged under fluorescence microscope using 100 X lens.

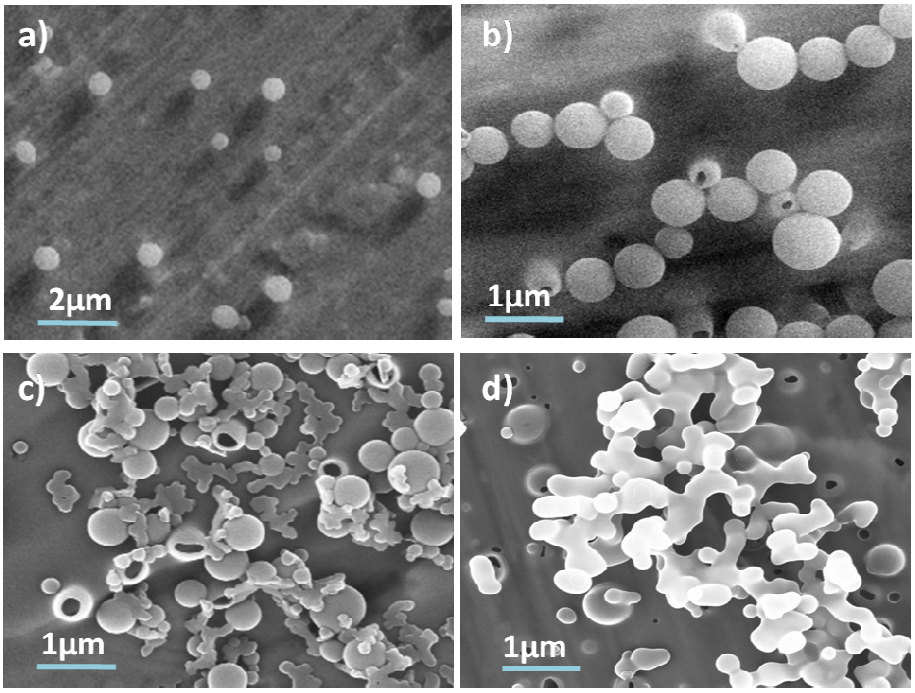


Figure S1: SEM micrographs of 1mM (Mpa-trp-trp)$_3$tren (**1**) in methanol alone (on Cu stubbs) a) Fresh b) 3days aged c) 6 days aged d) after 18 days.

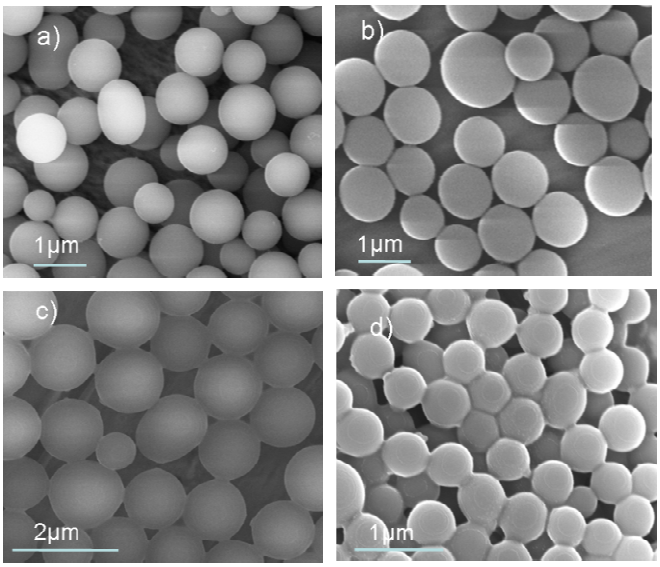


Figure S2: SEM micrographs of 1mM (Mpa-trp-trp)$_3$tren (**1**) in 9:1 methanol: water solvent system a) fresh b) 3 days aged c) 7 days aged and d) after 16 days.

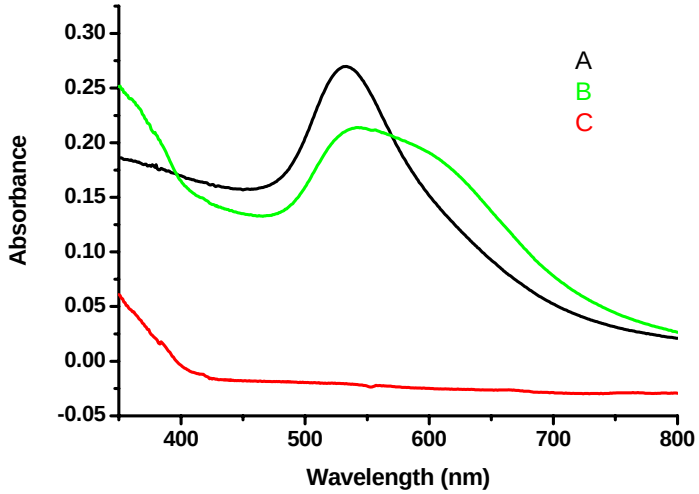


Figure S3: UV-Visible spectra A) AuNP solution in water, citrate ion as stabilizing agent, B) AuNP solution in presence of (Mpa-trp-trp)$_3$tren (**1**) in 9:1 methanol: water C) (Mpa-trp-trp)$_3$tren (**1**) alone in methanol

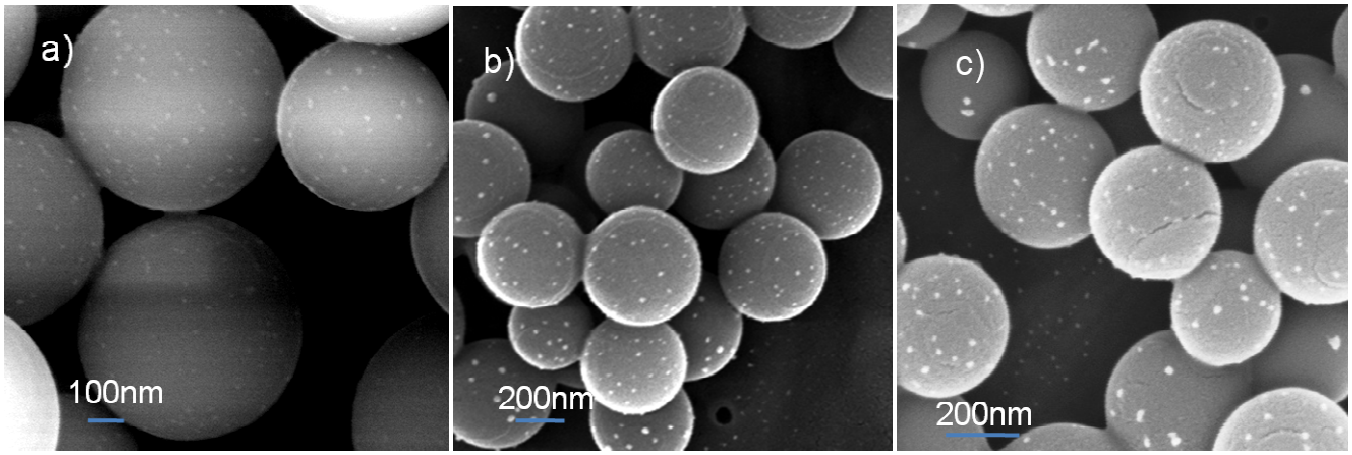
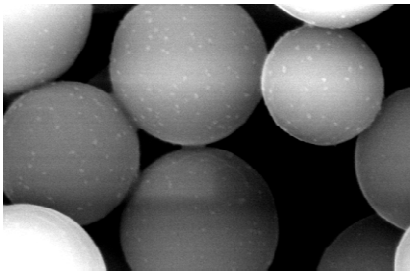


Figure S4: SEM micrographs of 1mM (Mpa-trp-trp)$_3$tren (**1**) in 9:1 methanol: water with 0.1mM AuNP a) fresh b) 3 days aged c) after 16 days. (Images were taken on Cu stubs without gold coating).



Element	Weight %	Weight % σ	Atomic %
Carbon	36.334	1.278	71.284
Nitrogen	2.580	3.040	4.306
Oxygen	1.525	0.238	2.688
Sulfur	0.494	0.063	0.369
Copper	33.665	1.135	12.485
Zinc	24.095	0.849	8.686
Tin	0.813	0.123	0.161
Gold	0.213	0.225	0.025

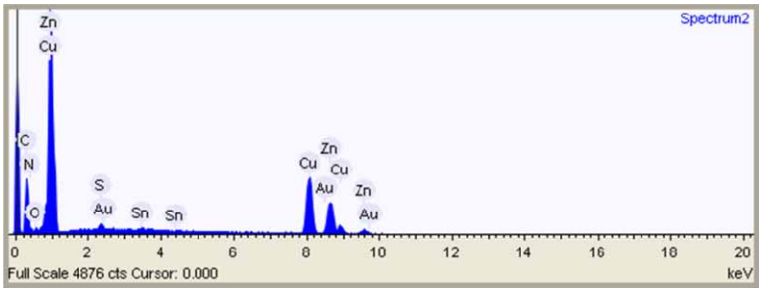


Figure S5: SEM EDAX spectrum of hybrid **1**-AuNP spheres.

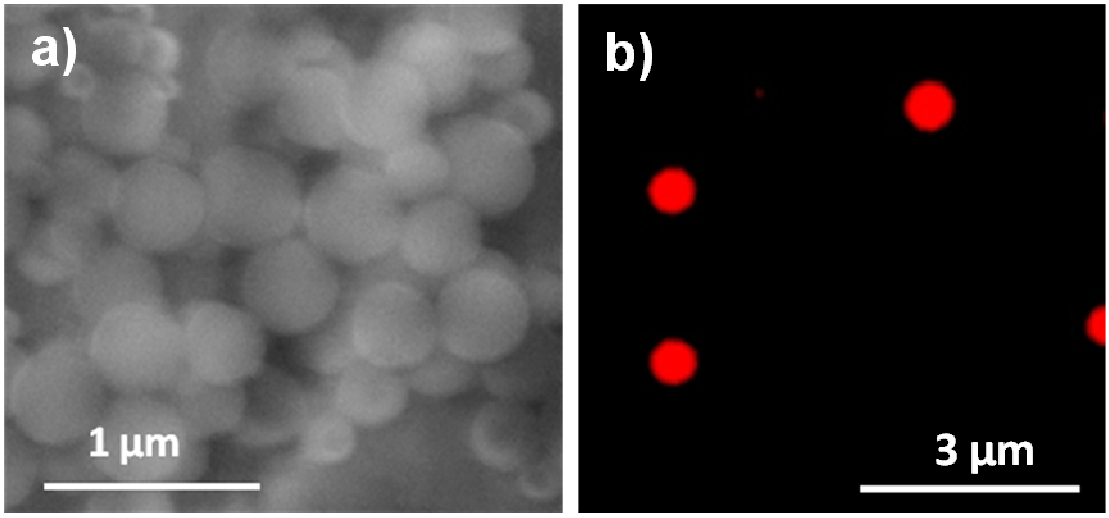


Figure S6: a) SEM micrographs of 1mM $(Mpa\text{-}trp\text{-}trp)_3$tren (**1**) in 9:1 methanol: water exposed to sunlight for 6 h b) Fluorescence microscopy image of Rhodamine B dye encapsulated spheres (without AuNP) after 6 h sunlight exposure.

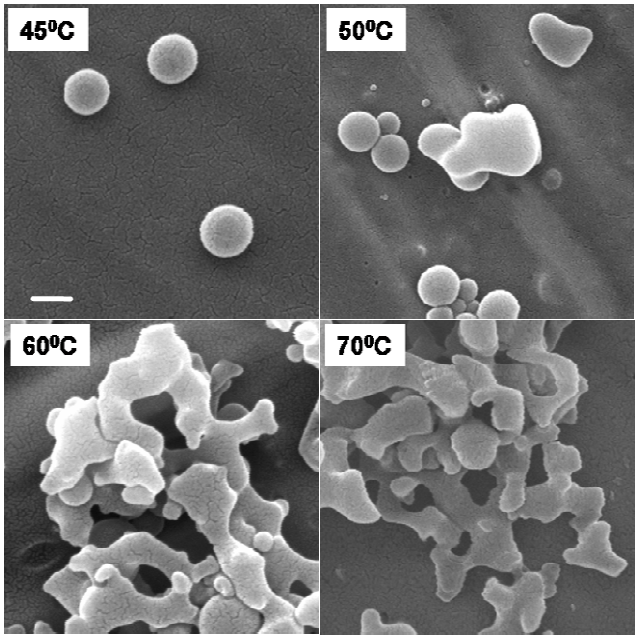
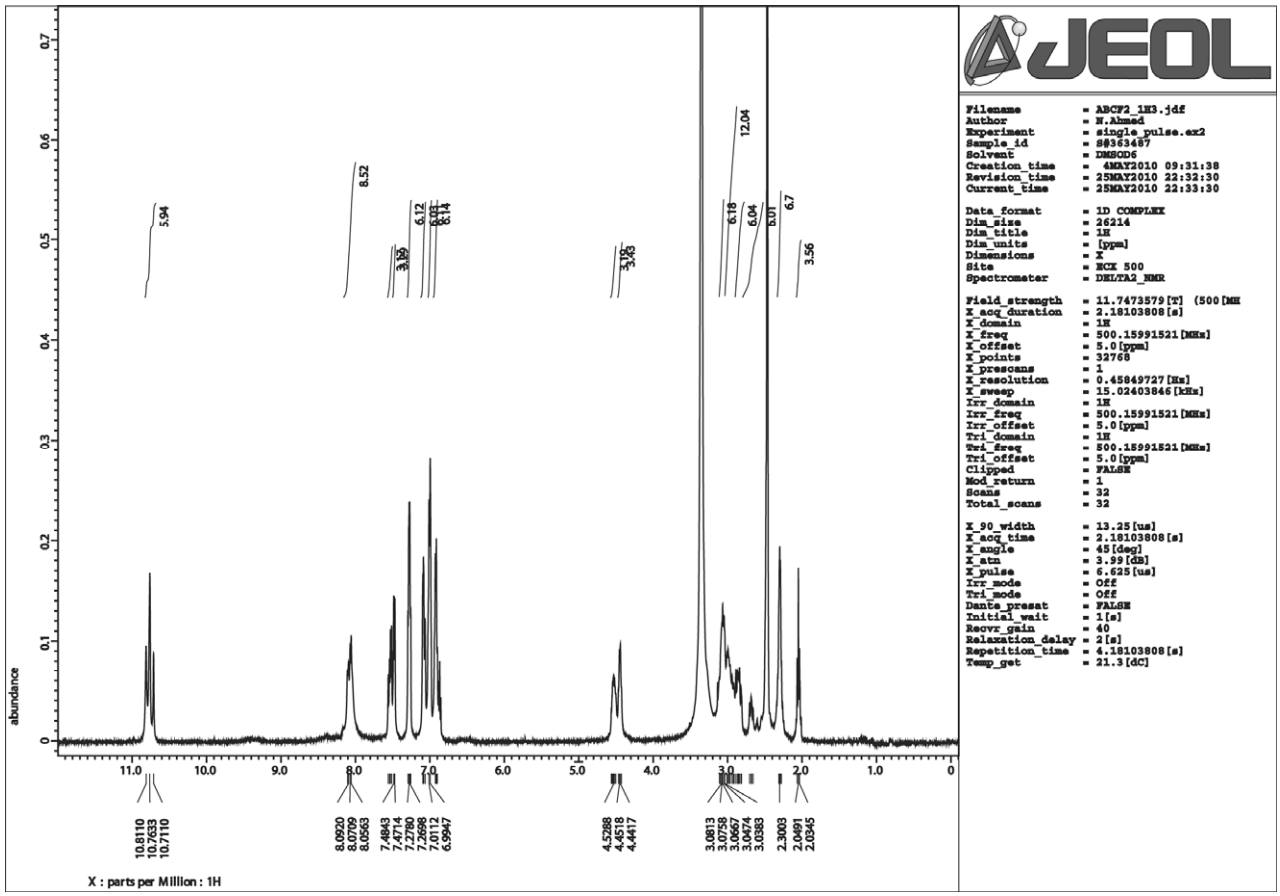
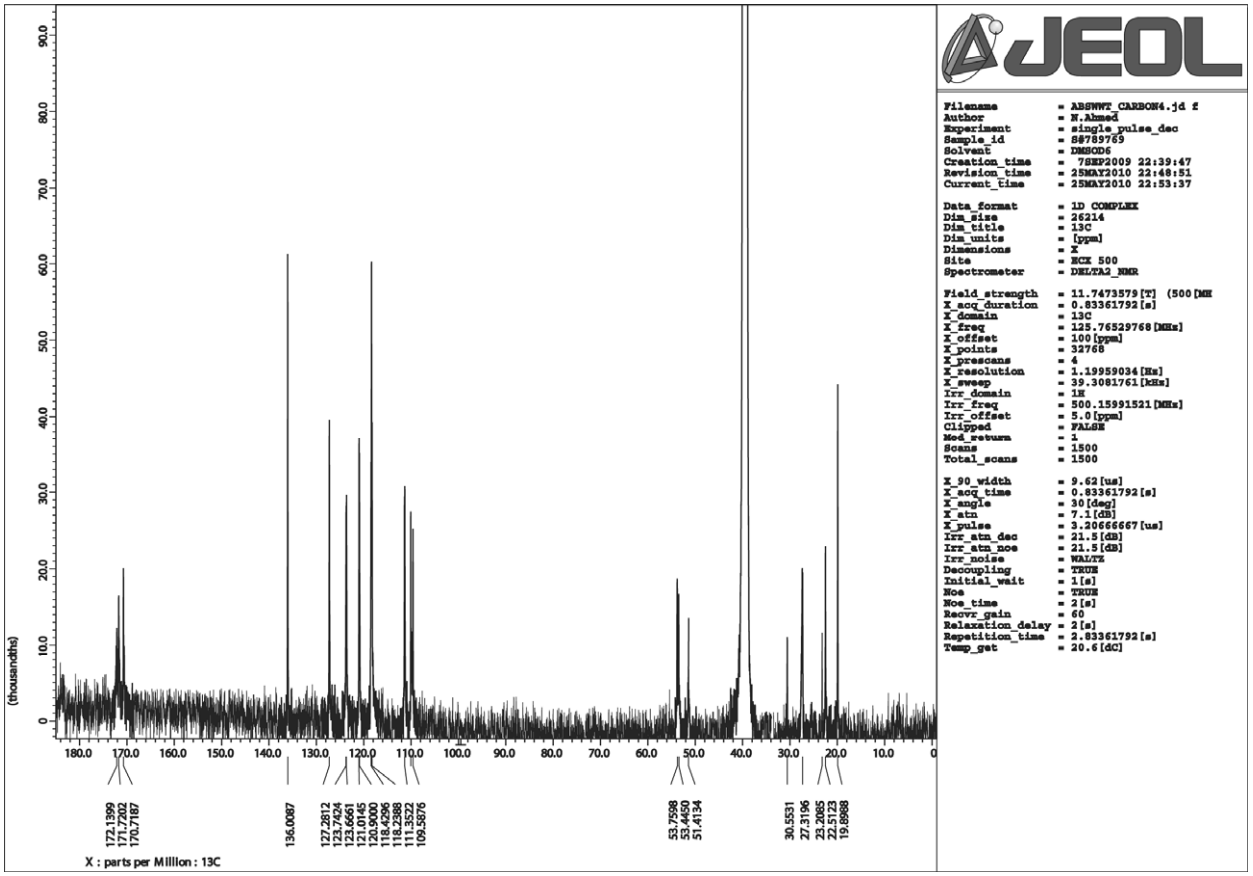


Figure S7: Self-assembly (Mpa-trp-trp)$_3$tren (**1**) in 9:1 methanol: water at different temperatures. Scale bar 200 nm in all images.

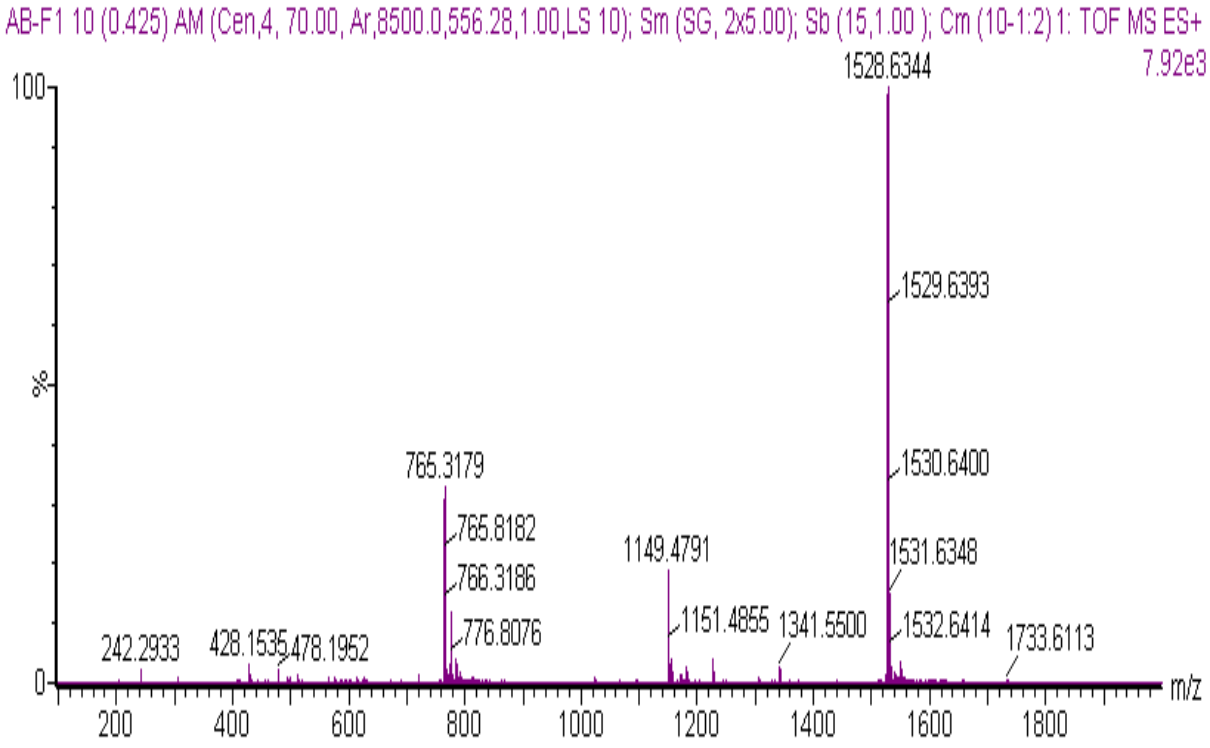
^{1}H spectra of **1:**



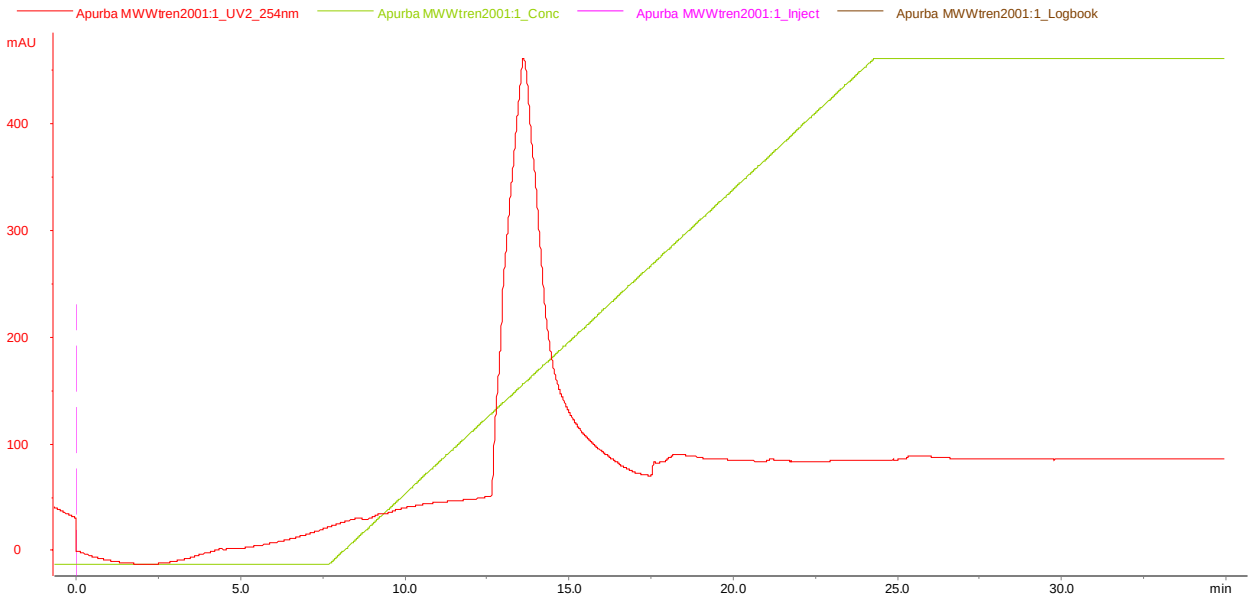
^{13}C spectra of **1:**



HRMS spectra of **1**:



HPLC of **1:**



Solvent System: A. 0.1% TFA inWater B. 0.1% TFA in Acetonitrile. Concentration gradient 0-100% of B in 20 min. Flow rate: 0.5mL/min.