Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Microstructure manipulation and guest release from cation responsive peptide microspheres

Astha Gupta [c], K. Vijaya Krishna[a] and Sandeep Verma* [a, b]

a Department of Chemistry, Indian Institute of Technology-Kanpur
Kanpur 208016 (UP), India

b DST Unit of Excellence on Soft Nanofabrication, Center for Environment Science and Engineering, Indian Institute of Technology-Kanpur, Kanpur-208016 (UP), India

c Department of Chemical Engineering, Indian Institute of Technology-Kanpur
Kanpur 208016 (UP), India

Materials & Methods

General: Dichloromethane (DCM), N, N'-dimethylformamide and methanol, were distilled following standard procedures prior to use. N, N′-dicyclohexylcarbodimide (DCC), N-hydroxysuccinimide (NHS), L-Histidine, p-Nitrophenol (PNP) were purchased from Spectrochem (Mumbai, India) and used without further purification. Triethylsilane, 3-mercaptopropionic acid, Adenosine 5′-triphosphate disodium salt hydrate (ATP-Na_2), Adenosine 5′-diphosphate disodium salt hydrate (ADP-Na_2), Adenosine 5′-monophosphate disodium salt hydrate (AMP-Na_2), Triethylamine, Dithiothreitol (DTT) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl) were purchased from S. D. Fine-Chem Limited, Mumbai and used without further purification. Tris-(2-aminoethyl) amine (Tren) was purchased from Sigma Aldrich Co., Bangalore and used as obtained. Sodium phosphotungstate (PTA) was acquired from Alfa- Aesar India. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on JEOL-JNM LAMBDA 400 model operating at 400, 100 and 161 MHz, respectively. HRMS were recorded on Waters, Q-Tof Premier Micromass HAB 213 mass spectrometer using capillary voltage 2.6-3.2 kV.

Field Emission Scanning Electron Microscopy (FE-SEM): Field emission scanning electron microscopy images were acquired on FEI QUANTA 200 microscope, equipped with a tungsten filament gun, operating at WD 10.6 mm and 20 kV.

Atomic Force Microscopy: AFM images were recorded with an atomic force microscope (Molecular Imaging, USA) operating under the Acoustic AC Mode, 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 a scan speed of 2.2 lines/s. The data acquisition was done using PicoView 1.8.2® software, while data analysis was done using PicoView.

Transmission Electron Microscopy (TEM): Transmission electron microscopy images were acquired on FEI Tecnai G^2U-Twin 200 kV microscope.

Fluorescence Microscopy: Dye stained structures were examined under fluorescence microscope (Leica DM2500M), provisioned with a Rhodamine filter (absorption 540 nm/emission 625 nm). This filter optimized visualization of rhodamine-treated (positive resolution) structures in comparison to untreated (negative resolution) structures that are virtually invisible to this light.

Focused Ion Beam- Scanning Electron Microscopy (FIB-SEM): Images were acquired with the help of focused-ion-beam/scanning electron microscope dual beam system (NOVA 600 NANOLAB, D97 FEI) using a Gallium ion source (15-30 kV) operating at WD 20 mm and 30 kV. Two ion sources are lying at an angle of 52º with respect to each other. Image of the sample is obtained by tilting the stage by 52º.

UV-Vis Spectroscopy: UV-Vis absorption spectra were recorded on Varian CARY 100 Bio UV-Vis spectrophotometer, with 10 mm quartz cell at 25±0.1 °C.

Circular Dichroism Spectroscopy: The spectra were collected using JASCO J-815 CD spectrometer and a quartz cuvette, with a path length of 1 mm. The experiment was carried out at room temperature. CD spectra were collected between 200 to 270 nm, and each spectrum was the average of three scans. To avoid any instrumental baseline drift between measurements, baseline correction for the samples was done using 50% aq.MeOH.

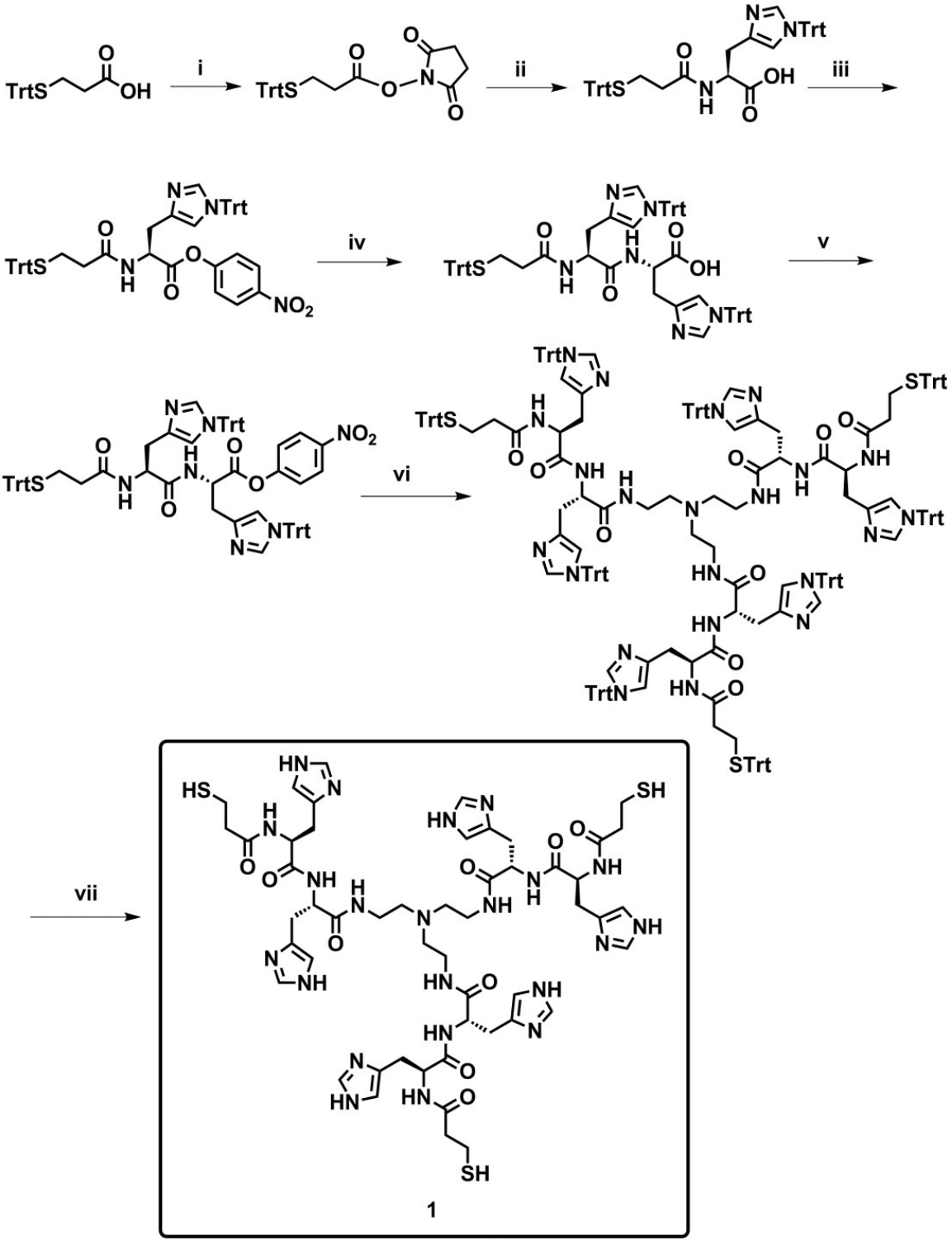
High-performance liquid chromatography (HPLC): The HPLC system (Agilent 1200 Series) consisted of quaternary pump (G1311B), UV Detector (G1365C) and fraction collector (G1364C). Instrumental control, data acquisition, and processing were performed using ChemStation software (Agilent Technologies, Wokingham, UK). An Eclipse XDB C-18 (250×4.6 mm) column with 5 μm particle size, kept at room temperature was used for all separations.

Zeta Potential and Dynamic Light Scattering: The measurement of zeta potential and particle size was done using Delsa Nano from Beckman Coulter India.

Thermogravimetric Analysis: The data was acquired on TGA/DSC 1 Star System from Mettler Toledo International Inc.

Peptide Synthesis

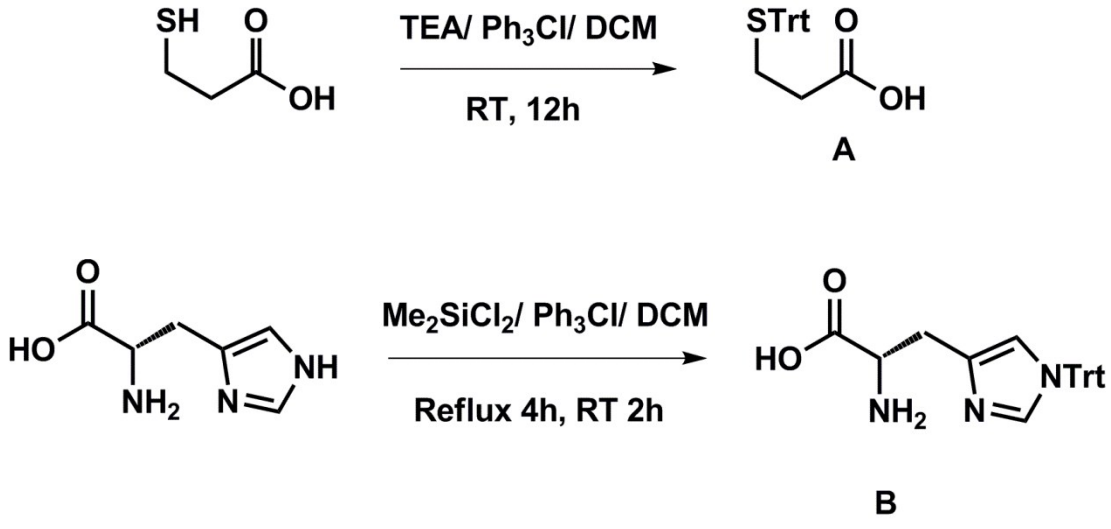
The tripodal peptide was synthesized by conventional solution phase methodology using DCC-NHS coupling (Scheme 1).



Scheme 1: Synthetic Scheme of C_3 symmetric peptide (MPA-H-H)$_3$Tren: **i** = NHS, DCC, dry DCM, 0 °C-1 h, RT-4 h; **ii, iv** = 50% 1,4-dioxane- H_2O, $NaHCO_3$, N^{im}Trt-His, RT-4 h; **iii**, **v** = PNP, DCC, dry DCM, 0 °C-1 h, RT- 4 h; **vi** = Tren, DMF, 12 h; **vii** = TFA, DCM, Triethylsilane, RT-1 h.

Synthesis of 3-S-trityl-mercaptopropionicacid (A) (Scheme 2): MPA (5 g, 47 mmol, 1 eq.) was dissolved in dry DCM (40 mL). Then triethylamine (3.34 g, 4.6 mL, 33 mmol, 0.7 eq.) was added to it and stirred. Solution of trityl chloride (13.13 g, 47 mmol, 1 eq.) in DCM (30 mL) was added slowly to this solution. The reaction mixture was stirred at room temperature overnight. The reaction mixture was then washed with 1 N aq. HCl and brine solution and dried over anhydrous Na_2SO_4. The crude product obtained after evaporation was purified by silica gel column chromatography using 2% MeOH in DCM solvent system. Yield: 11.5 g (70%), R_f = 0.6 (5% MeOH in DCM). ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C, TMS), δ (ppm): 2.21-2.24 (t, 2H), 2.43-2.46 (t, 2H), 7.20-7.45 (m, 15H).

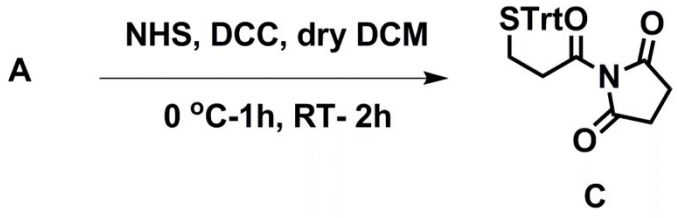
Synthesis of N^{im}-trityl Histidine (B) (Scheme 2): A suspension of histidine (5 g, 32 mmol, 1 eq.) was made in DCM (50 mL). Dimethyldichlorosilane (4.15 g, 3.9 mL, 32 mmol, 1 eq.) was added to the suspension and the mixture was refluxed for 4 h at 45 °C. After 4 h, triethylamine (6.48 g, 8.93 mL, 64 mmol, 2 eq.) was added and it was further refluxed for 15 mins. Triethylamine (3.23 g, 4.46 mL, 32 mmol, 1 eq.) was again added to it, followed by a solution of trityl chloride (8.92 g, 32 mmol, 1 eq.) in DCM while stirring at room temperature. After 2 h, an excess of methanol was added and the solvent was evaporated *in vacuo*. Water was then added to the residue and pH was adjusted to 8-8.5 by drop wise addition of triethylamine. The suspension was shaken well with DCM and the precipitate was filtered and washed with water and diethyl ether to afford the final product. The crude product was used without further purification. Yield: 11 g (85.8%), R_f = 0.3 (10% MeOH in DCM).^{1}H NMR (400 MHz, DMSO-d_6, 25 °C, TMS), δ (ppm): 2.98-3.02 (dd, 2H), 5.72 (s, 1H), 6.55 (brs, 2H), 6.73 (s, 1H), 7.02-7.37 (m, 15H), 7.56 (s, 1H).^{13}C NMR (100 MHz; DMSO-d_6, 25 °C, TMS), δ (ppm): 29.74, 55.01, 75.07, 119.37, 126.79, 128.26, 129.83, 137.60, 138.43, 142.81, 169.79.



Scheme 2: Synthesis of protected S-trityl MPA and N^{im}-trityl Histidine

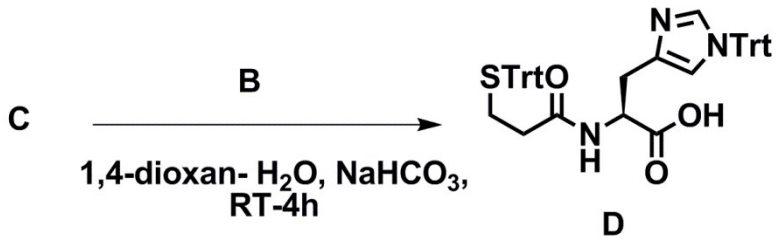
Synthesis of 3-S-trityl MPA-N-hydroxysuccinimide active ester (C) (Scheme 3): A (5 g, 14 mmol, 1 eq.) and NHS (1.98 g, 17 mmol, 1.2 eq.) were dissolved in dry DCM (30 mL) and

the solution was cooled to 0 °C. A solution of DCC (3.54 g, 17 mmol, 1.2 eq.) in DCM was added to it slowly and the mixture was stirred for 1 h at 0 °C and then for 4 h at room temperature. After that, the insoluble dicyclohexylurea was filtered off and the remaining organic layer was washed with 10% $NaHCO_3$ and brine solution and subsequently dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to obtain the final product (**C**). Yield: 4.8 g (78%), R_f = 0.8 (10% MeOH in DCM). The crude product was used without further purification.



Scheme 3: Synthesis of 3-S-trityl MPA-NHS active ester

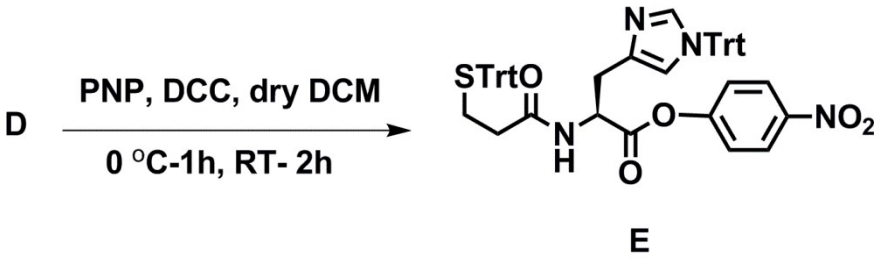
Synthesis of 3-S-trityl mercaptopropanoic acid- N^{im}trityl histidine (D) (Scheme 4): B (4.8 g, 11 mmol, 1 eq.) was dissolved in a 1:1 mixture of water and 1,4-dioxane to which a solution of $NaHCO_3$ (1.84 g, 22 mmol, 2 eq.) in water was added and stirred for 5-10 mins. After that a solution of **C** (4.43 g, 11 mmol) in 1,4-dioxane was added to it and the reaction mixture was stirred for 4 h at room temperature. Then, the solvent was evaporated under reduced pressure and the residue was redissolved in DCM and washed with citric acid. The organic layer was dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The crude product obtained after evaporation was purified by silica gel column chromatography using 3% MeOH in DCM solvent system to obtain the final product (**D**). Yield: 4.5 g (55.5%), R_f = 0.6 (10% MeOH in DCM). ^{1}H NMR (400MHz, DMSO-d_6, 25 °C, TMS), δ (ppm): 2.10-2.13 (t, 2H), 2.21-2.24 (t, 2H), 2.92-2.97 (dd, 2H), 5.31 (s, 1H), 7.00 (s, 1H), 7.13-7.34 (m, 30H). ^{13}C NMR (100 MHz; DMSO-d_6, 25 °C, TMS), δ (ppm): 27.14, 28.15, 33.20, 62.92, 66.67, 86.57, 118.29, 127.10, 128.27, 129.25,136.80, 137.41, 142.83, 144.51, 173.06, 180.10. HRMS $[M+H]^+$ for ($C_{47}H_{42}N_3O_3S$): 728.2947(calculated), 728.2831 (observed).



Scheme 4: Synthesis of 3-S-trityl mercaptopropanoic acid- N^{im}trityl histidine

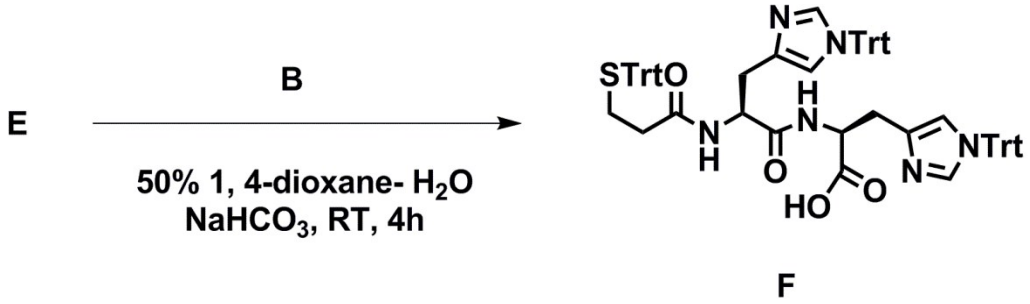
Synthesis of 3-S-trityl mercaptopropanoic acid- N^{im}trityl histidine-4-nitrophenyl active ester (E) (Scheme 5): D (4.5 g, 6 mmol, 1 eq.) and PNP (1.032 g, 7 mmol, 1.2 eq.) were dissolved in dry DCM (30 mL) and the solution was cooled to 0 °C. A solution of DCC (1.44

g, 7 mmol, 1.2 eq.) in DCM was added to it slowly and the mixture was stirred for 1 h at 0 °C and for 4 h at room temperature. After that, the insoluble dicyclohexylurea was filtered off and the remaining organic filtrate was washed with 10% $NaHCO_3$ and then brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated at reduced pressure to obtain the final product (**E**). Yield: 4.8 g (92%). R_f =0.7 (10% MeOH in DCM). The crude product was used without further purification.



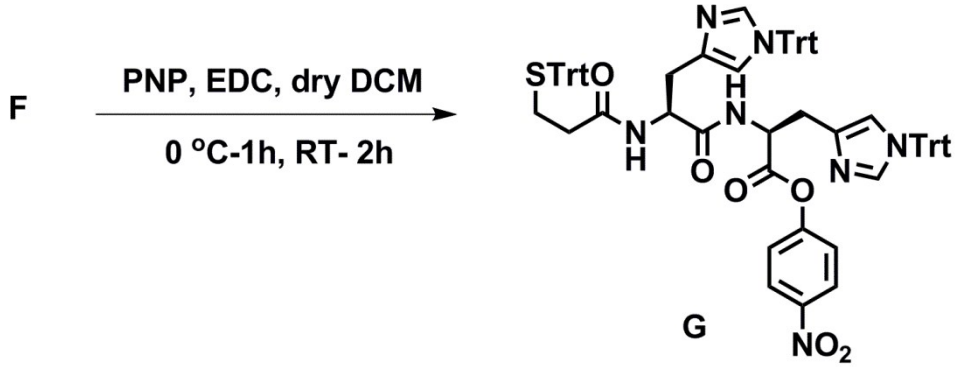
Scheme 5: Synthesis of 3-S-trityl mercaptopropanoic acid- N^{im}trityl histidine - PNP active ester

Synthesis of 3-S-trityl-mercaptopropanoic acid- N^{im}trityl histidine- N^{im}trityl histidine (F) (Scheme 6): **B** (2.17 g, 5 mmol, 1 eq.) was dissolved in a 1:1 mixture of water and 1,4-dioxane and a solution of $NaHCO_3$ (0.94 g, 11 mmol, 2 eq.) in water was added to it. It was stirred for 5-10 mins. After that a solution of **E** (4.8 g, 5 mmol, 1 eq.) in 1,4-dioxane was added to it and the reaction mixture was stirred for 4 h at room temperature. Then, the solvent was evaporated under reduced pressure and the residue was dissolved in DCM and washed with citric acid. The organic layer was dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The crude product obtained after evaporation was purified by silica gel column chromatography using 4% MeOH in DCM solvent system to obtain the final product (**F**). Yield: 3 g (48%). R_f= 0.5 (10% MeOH in DCM).^{1}H NMR (400 MHz, DMSO-d_6, 25 °C, TMS), δ (ppm): 2.11-2.15 (t, 2H), 2.21-2.25 (t, 2H), 2.96-3.02 (dd, 4H), 5.3 (s, 2H), 6.96 (s, brd, 2H), 7.19-7.31 (m, 45H),7.97(s, 1H), 7.99 (s, 1H),8.12 (brs, 2H), 10.6 (s, 1H). ^{13}C NMR (100 MHz; DMSO-d_6, 25 °C, TMS), δ (ppm):27.14, 28.15, 31.20, 33.30, 56.51, 62.92, 86.59, 118.35, 127.20, 128.26, 129.35, 136.75, 137.25, 142.81, 144.20, 170.71, 173.4, 180.25. HRMS $[M+H]^+$ for ($C_{72}H_{63}N_6O_4S$): 1107.4632 (calculated), 1107.4631 (observed).



Scheme 6: Synthesis of 3-S-trityl-mercaptopropanoic acid- N^{im}trityl histidine- N^{im}tritylhistidine

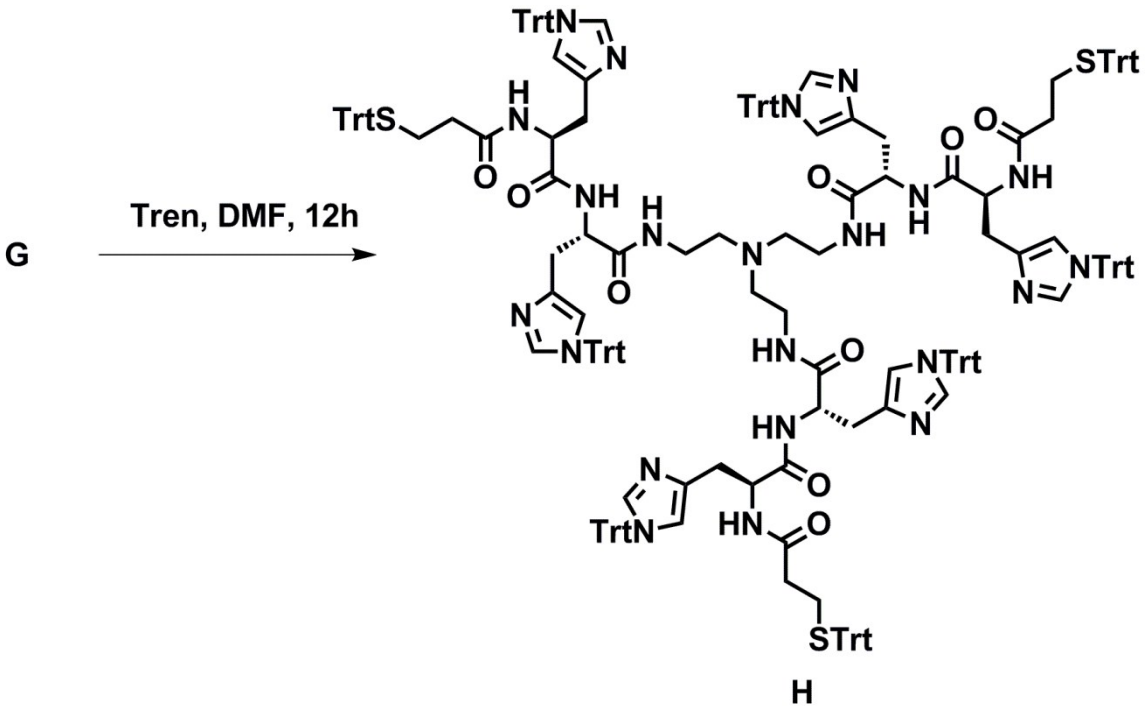
Synthesis of 3-S-trityl-mercaptopropanoic acid- N^{im}trityl histidine- N^{im}trityl histidine PNP active ester (G) (Scheme 7): F (3 g, 2 mmol, 1 eq.) and PNP (0.45 g, 3 mmol, 1.2 eq.) were dissolved in dry DCM (30 mL) and the solution was cooled to 0 °C. A solution of EDC.HCl (0.46 g, 3 mmol, 1.2 eq.) in DCM was added to it slowly and the mixture was stirred for 1 h at 0 °C and for 4 h at room temperature. After that, the organic layer was washed with 10% $NaHCO_3$ and then brine solution and subsequently dried over anhydrous Na_2SO_4 and concentrated at reduced pressure to obtain the final product (**G**). Yield: 2.9 g (87%). R_f = 0.6 (10% MeOH in DCM). The crude product was used without further purification.



Scheme 7: Synthesis of 3-S-trityl-mercaptopropanoic acid- N^{im}trityl histidine- N^{im}trityl histidine PNP active ester

Synthesis of (3-S-trityl-mercaptopropanoic acid-N^{im}trityl histidine- N^{im}trityl histidine)$_3$Tren (H) (Scheme 8): G (2.9 g, 2 mmol, 1 eq.) was dissolved in N,N'-dimethylformamide (25 mL) and Tris-(2-aminoethyl) amine (0.12 g, 117 µL, 0.7 mmol, 0.3 eq.) was added to it slowly while stirring. It was left to stir overnight at room temperature. Then, the solvent was evaporated under reduced pressure and the residue was redissolved in DCM and washed with citric acid. The organic layer was dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The crude product obtained after evaporation was purified

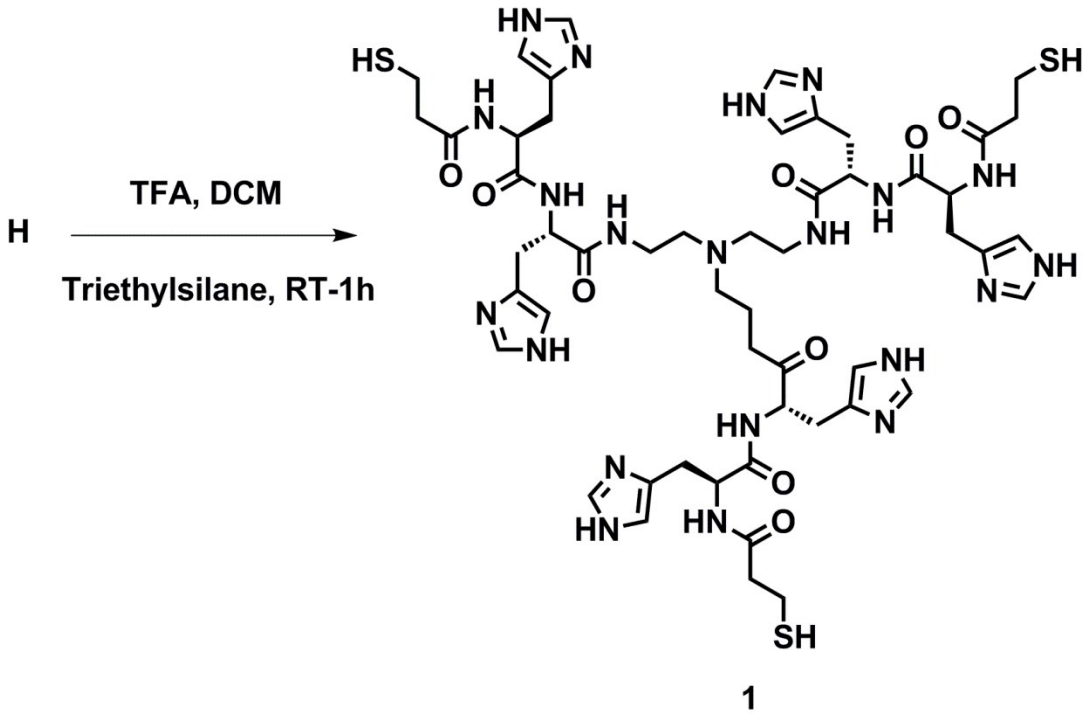
by silica gel column chromatography using 6% MeOH in DCM solvent system to obtain the final product (**H**). Yield: 1.9 g (23%). R_f= 0.4 (10% MeOH in DCM). ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C, TMS), δ (ppm): 1.94-2.22 (m, 12H), 2.59-2.63 (m, 12H), 2.76-2.84 (m, 12H), 4.31 (brs, 6H), 6.94 (brs, 6H), 7.07-7.23 (m, 135H), 7.71-7.94 (m, 6H), 8.12-8.16 (m, 9H).^{13}C NMR (100 MHz; DMSO-d_6, 25 °C, TMS), δ (ppm), δ (ppm): 27.51, 29.53, 34.22, 53.58, 56.51, 61.40, 66.46, 67.74, 74.88, 74.97, 121.14, 127.14, 128.45, 128.60, 129.54, 129.71, 137.42, 137.87, 137.98, 138.06, 138.18, 142.66, 142.76, 144.91, 171.04,171.19, 172.27, 173.35, 174.54, 174.99. HRMS {$[M+2H]^{2+}$}/2 for ($C_{222}H_{198}N_{22}O_9S_3$)/2: 1707.7549 (calculated), 1707.7399 (observed).



Scheme 8: Synthesis of (3-S-trityl-mercaptopropanoic acid-N^{im}trityl histidine- N^{im}trityl histidine)$_3$Tren

Deprotection of (S-tritylmercaptopropanoic acid-N^{im}trityl histidine- N^{im}trityl histidine)$_3$Tren (1) (Scheme 9): H (1.9 g, 0.5 mmol, 1 eq.) was dissolved in DCM (5 mL) and triethylsilane(0.58 g, 799 µL, 5 mmol, 9 eq.) was added to it. After 2-3 minutes, a 2 % solution of trifluoroacetic acid in DCM (10 mL) was added to it and the mixture was stirred for 1 h in nitrogen atmosphere. The solvent was evaporated *in vacuo* and the residue was washed with diethyl ether followed by anion exchange chromatography to obtain the final product (**1**). Yield: 0.5 g (73.5%). R_f = 0.2 (35% MeOH in DCM). ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C, TMS), δ (ppm): 1.03 (s, 1H), 1.04 (s, 1H), 1.06 (s, 1H), 2.09-2.21 (m, 6H), 2.36-2.40 (m, 6H), 2.77-2.89 (m, 6H), 2.93-3.15 (m, 6H); 3.21-3.37 (m, 6H), 4.45-4.53 (m, 6H); 7.23-7.28 (m, 6H); 8.2-8.4 (m, 9H); 8.8-8.9 (m, 6H). ^{13}C NMR (100 MHz; DMSO-d_6, 25 °C, TMS), δ (ppm): 15.69, 27.53, 34.31, 42.8 (merged with DMSO peak), 52.41, 65.44,

66.54, 127.27, 128.55, 129.56, 170.59, 170.69. HRMS $[M+H]^+$ for $C_{51}H_{72}N_{22}O_9S_3$: 1233.5093 (calculated), 1233.5092 (observed).



Scheme 9: Deprotection of (S-tritylmercaptopropanoic acid-N^{im}trityl histidine- N^{im}trityl histidine)$_3$Tren

Microscopic evaluation of 1:

Sample preparation for FE-SEM: 100 µL aliquots of **1** (1 mM in 9:1 water: methanol) were prepared by adding water (pH 6) and incubated in an eppendorf for 45 mins after which a 10 µL aliquot of the mixture was deposited on a copper stub and allowed to air-dry. The images were acquired after gold coating of the samples for 45 s.

DTT Treatment: 100 µL aliquots of **1** (1 mM in 9:1 water: methanol) were prepared by adding water (pH 6) and incubated in an eppendorf for 45 mins after which 0.5 eq. of DTT and 0.5 eq. treithylamine were added to it and incubated for 1 h. A 10 µL aliquot of the mixture was deposited on a copper stub and allowed to air-dry. The FESEM images were acquired after gold coating of the samples for 45 s.

Sample preparation for fluorescence microscopy: For dye encapsulation study, 1 μL Rhodamine B dye solution (0.1 mg/mL in water) was added to **1** (1mM in 9:1 water: methanol) and incubated for 45 mins. 10 μL of the solution was spread on a glass slide, dried at room temperature, and imaged under fluorescence microscope using 100 X lens. For studying response to Mg^{+2} ions, 2 μL of $MgCl_2$ (2 eq. w.r.t. **1**) was added after the 45 mins incubation with dye and further incubated for 6 h before spreading 10 μL of the solution on a glass slide, drying at room temperature, and imaging under fluorescence microscope using 100 X lens.

Microscopic evaluation of 1-ATP Complex:

Sample preparation for FE-SEM: 50 μL aliquots of **1** (1mM in 9:1 water: methanol) and ATP-Na_2 (0.1 mM in water) were co-incubated in an eppendorf for 45 mins after which a 10 μL aliquot of the mixture was deposited on a copper stub and allowed to air-dry. The images were acquired after gold coating of the samples for 45 s.

DTT Treatment: 50 μL aliquots of **1** (1mM in 9:1 water: methanol) and ATP-Na_2 (0.1 mM in water) were co-incubated in an eppendorf for 45 mins after which 0.5 eq. DTT and 0.5 eq. triethylamine were added to it and incubated further for 1 h. A 10 μL aliquot of the mixture was deposited on a copper stub and allowed to air-dry. The FESEM images were acquired after gold coating of the samples for 45 s.

Sample preparation for AFM: 50 μL aliquots of **1** (1mM in 9:1 water: methanol) and ATP-Na_2 (0.1 mM in water) were co-incubated for 45 mins in a eppendorf after which 10 μL of the mixture was deposited on a glass slide and allowed to air-dry prior to imaging.

Sample preparation for fluorescence microscopy: For dye encapsulation study, 5 μL Rhodamine B dye solution (1 μM/mL in water) was added to a mixture of **1** (1mM in 9:1 water: methanol) and ATP-Na_2 (0.1 mM in water) and incubated for 45 mins. 10 μL of the solution was spread on a glass slide, dried at room temperature, and imaged under fluorescence microscope using 100 X lens. For studying response to metal ions, 2 μL of 2.5 mM metal chloride solution ($MgCl_2$, $CaCl_2$ and KCl) was added after the 45 mins incubation with dye and further incubated for 6 h before spreading 10 μL of the solution on a glass slide, drying at room temperature, and imaging under fluorescence microscope.

Sample preparation for TEM: 50 μL aliquots of **1** (1mM in 9:1 water: methanol) and ATP-Na_2 (0.1 mM in water) were co-incubated in an eppendorf for 45 mins after which a 10 μL aliquot of the mixture was deposited on a 200 mesh copper grid and allowed to air-dry, followed by negative staining with 2% uranyl acetate solution. The excess stain was removed with a tissue paper and the grid was allowed to air-dry before acquisition of images.

Sample preparation for FIB-SEM: 50 μL aliquots of **1** (1mM in 9:1 water: methanol) and ATP-Na_2 (0.1 mM in water) were co-incubated in an eppendorf for 45 mins after which a 10 μL aliquot of the mixture was deposited on a copper stub and allowed to air-dry. The images were acquired after gold coating of the samples for 45 s.

Microscopic evaluation of 1-ATP-PTA Complex

Sample preparation for FE-SEM: 1 μL of PTA (0.67 mM in water, 0.1 eq. w.r.t. **1**) was added to these co-incubated samples and gently mixed for 1 min before deposition of a 10 μL aliquot on a copper stub and allowed to air dry. The images were acquired after gold coating of the samples for 45 s.

Sample preparation for AFM: 1 μL of PTA (0.67 mM in water, 0.1 eq. w.r.t. **1**) was added to these co-incubated samples and gently mixed for 1 min before deposition of a 10 μL aliquot on a glass slide and allowed to air-dry prior to imaging.

Sample preparation for TEM:1 μL of PTA (0.67 mM in water, 0.1 eq. w.r.t. **1**) was added to these co-incubated samples and gently mixed for 1 min before deposition of a 10 μL aliquot on a 200 mesh copper grid and allowed to air-dry, after which it was negatively stained with 2% uranyl acetate solution. The excess stain was removed with a tissue paper and the grid was allowed to air dry before acquisition of images.

Sample preparation for FIB-SEM: 1 μL of PTA (0.67 mM in water, 0.1 eq. w.r.t. **1**) was added to these co-incubated samples and gently mixed for 1 min before deposition of a 10 μL aliquot on a copper stub and allowed to air dry. The images were acquired after gold coating of the samples for 45 s.

Sample preparation for UV-Vis Spectroscopy: Absorbance spectra were recorded for fresh solutions of both **1** and ATP-Na_2 (10 μM in 50% aq. MeOH) and for a 45 min co-incubated solution of **1**- ATP-Na_2 complex.

Sample preparation for Circular Dichroism Spectroscopy: Spectra were collected for **1** (0.5 mM) and **1**- ATP-Na_2 complex (0.5 mM) upon incubation for 45 mins.

HPLC: The preparation of mobile phase was adapted from a reference.[1] All solvents used were HPLC grade and further passed through 0.2 μm membrane filter prior to their use. The flow rate was 1 mL/min and the detection wavelength was set at 260 nm for all the runs.

Buffer A: 39 mM K_2HPO_4, 26 mM KH_2PO_4, and 10 mM TBAHS in water adjusted to pH ~ 6.0 with H_3PO_4.

Buffer B: 39 mM K_2HPO_4, 26 mM KH_2PO_4 and 25% acetonitrile in water, also adjusted to pH ~ 6.0 with H_3PO_4.

Method: The method used consisted of a gradient for Buffer B varying from 2 to 70% during the course of a run. The method used for separation is as follows:

Time (Mins)	Buffer A (%)	Buffer B (%)
0	98	2
10	92	8
30	30	70

Before use, the column was preconditioned using 10 column volumes of buffer B and 30 column volumes of buffer A. The samples were prepared by co-incubating equal volumes of 1 mM solution of **1** (9:1 water: methanol) with 0.1 mM solution of ATP-Na_2 in water. 20 µL of **1**-ATP-Na_2 complex was then injected into the column after appropriate time period. After each run, the column was washed with acetonitrile (2-50%) in phosphate buffer for 30 mins to elute any remaining compounds. The standards of pure ATP-Na_2, ADP-Na_2 and AMP-Na_2 were used to identify them in mixture.

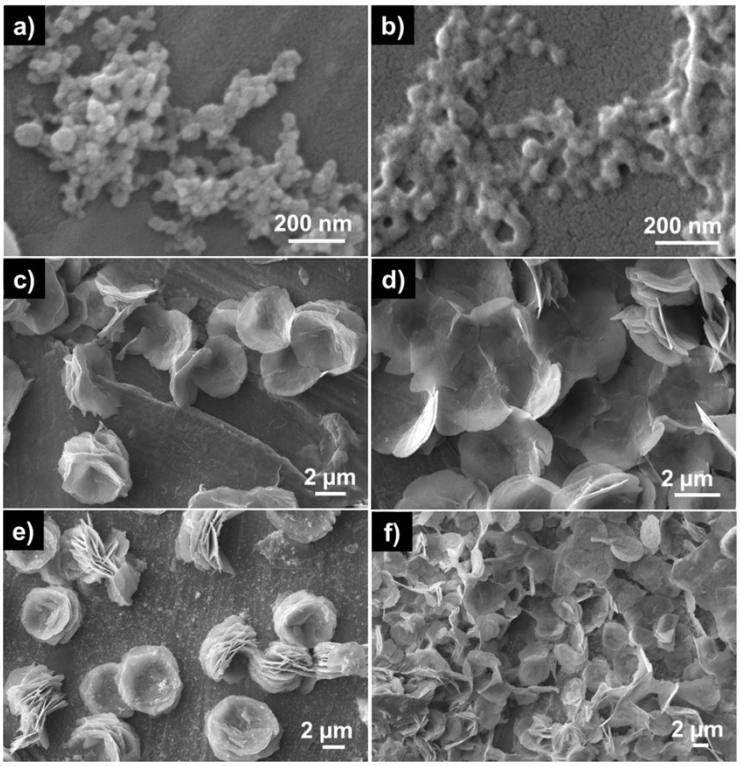


Figure S1. Stability of different self- assembled soft structures after treatment with a), c), e): heat (65 °C for 15 mins); b), d) and f): ultrasound (15 mins). a), b) Microspheres of **1** remain

unaffected; c), d) **1**-ATP coacervates degrade; e), f) PTA coated **1**-ATP coacervates are resistant to thermal treatment but break upon exposure to ultrasound.

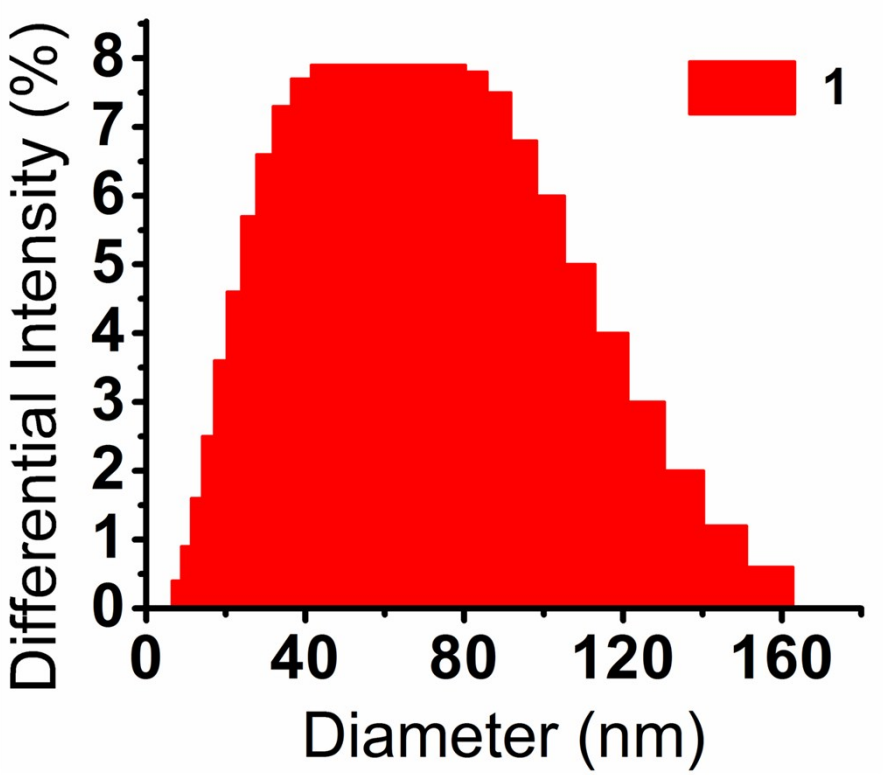


Figure S2. Size distribution of peptide nanospheres obtained by Dynamic Light Scattering.

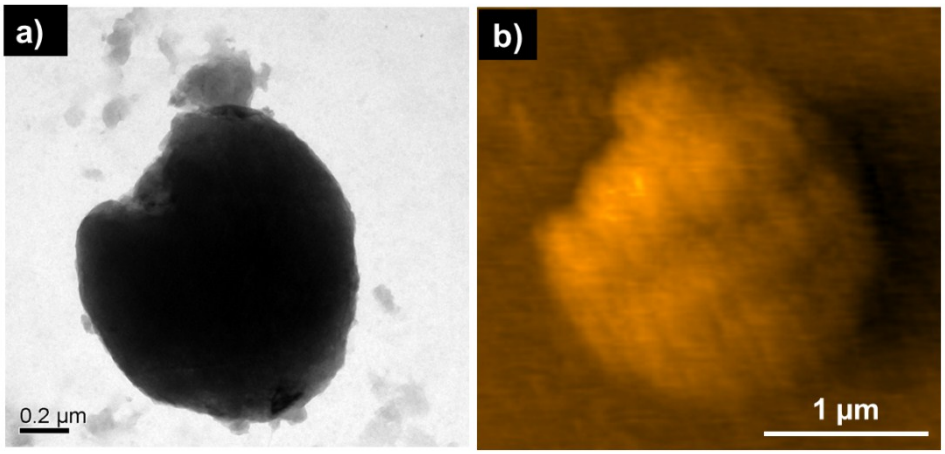


Figure S3. Microscopic images for **1**-ATP complex a) TEM image of doughnut shaped soft structure; b) AFM micrograph.

The interaction of the peptide with ATP was confirmed by UV-Vis absorbance studies. It was observed that the specific absorbance of ATP at 260 nm was quenched upon its incubation with the peptide for 45 mins. The spectrum obtained upon co-incubation of **1** and ATP (Fig S5 (a), green) had two absorbance maxima, one at 205nm, corresponding to peptide bond which is shifted by 2 nm from the characteristic absorbance of the peptide at 203 nm (Fig S4 (a), black) and the other at 260 nm. Both the molecules show a synergistic behaviour upon interaction with each other.

Subsequently, circular dichroism studies were also performed to study the effect of interaction with ATP on secondary structure of **1** (Fig. S5 (b)). It had a native conformation of anti- parallel β sheet as shown by the peak at 208nm. Upon incubation with ATP, a new peak at 218nm was observed which is indicative of a random coil/ irregular conformation. This shows that non covalent interactions other than hydrogen bonding are involved in formation of the microstructures.

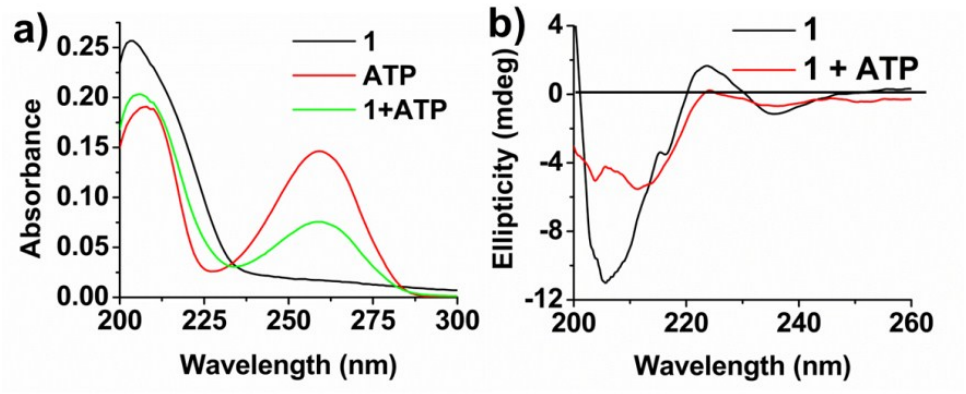


Figure S4. a) UV Absorbance curves for **1** (black), ATP (red) and **1**-ATP complex (green) showing quenching of absorbance at 260 nm; b) CD Spectra for **1** (black) and **1**-ATP complex (red) showing change in secondary structure.

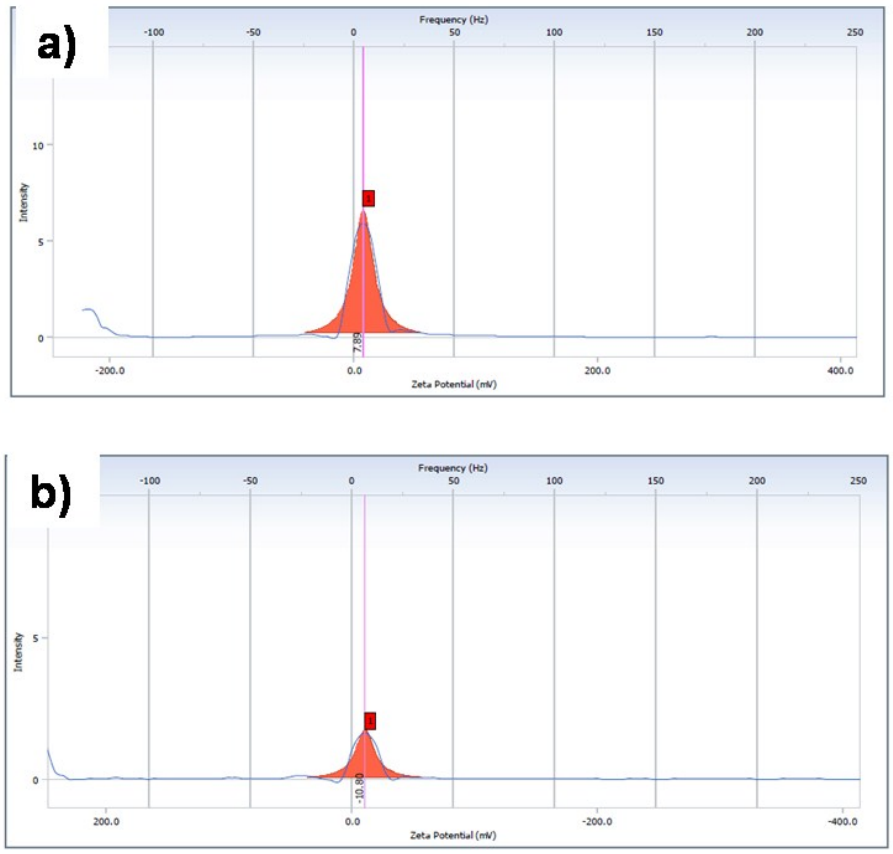


Figure S5. Zeta potential measurement a) **1**-ATP complex, b) **1**-ATP-PTA complex.

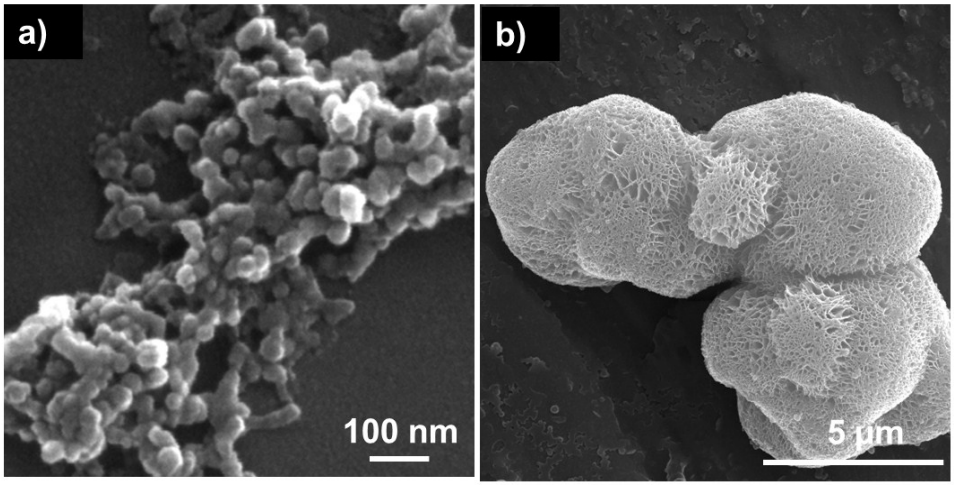


Figure S6. a) 1 after incubation with PTA shows no change in morphology; b) Zoomed view of 1-ATP hybrid microsphere upon treatment with DTT exhibiting the internal porous structure upon erosion of top layer

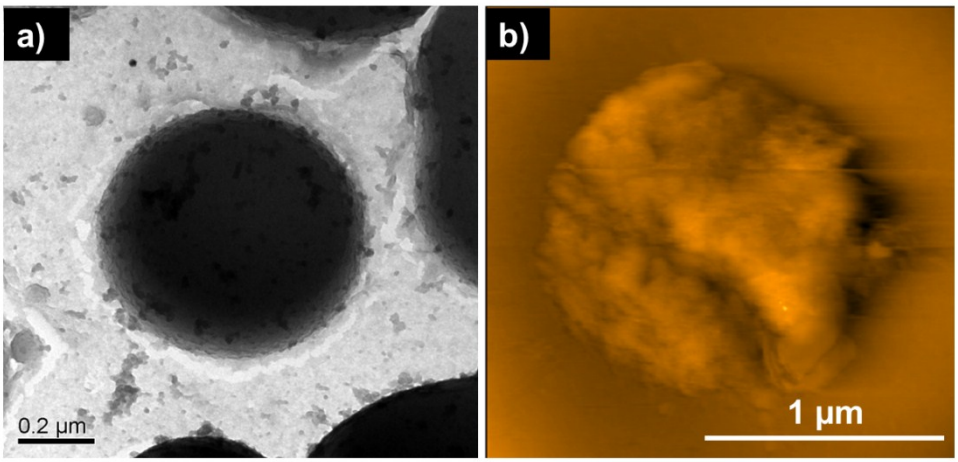


Figure S7. Microscopic images for **1**-ATP-PTA complex a) TEM image of the PTA coated doughnut; b) AFM micrograph.

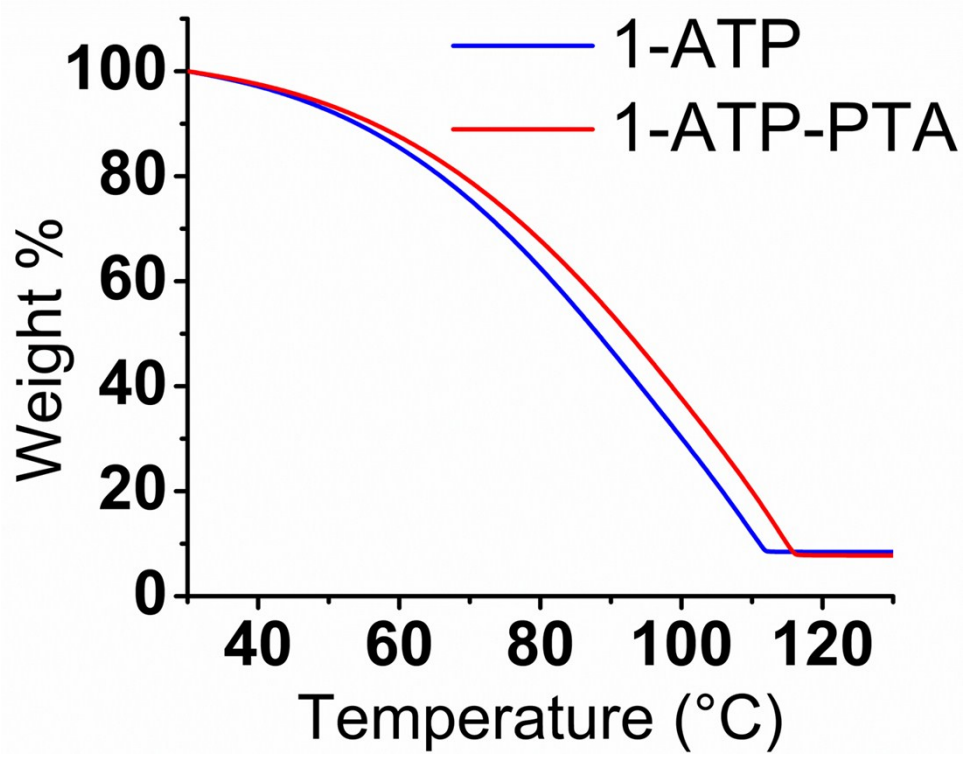


Figure S8. TGA plots for **1**-ATP (blue) and **1**-ATP-PTA (red)

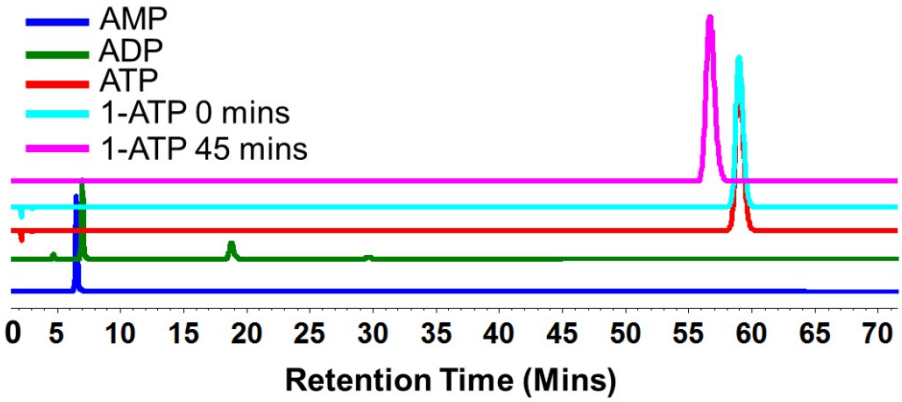


Figure S9. HPLC retention time curves for different molecules.

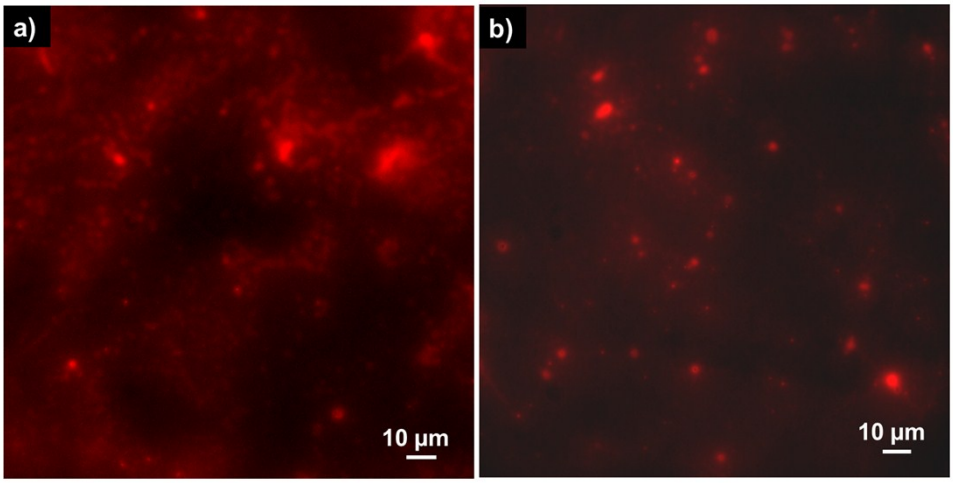


Figure S10. Release of dye from **1**-ATP hybrid soft structures upon incubation with different metal ions a) $Ca^{2+;}$ b) K^{+}

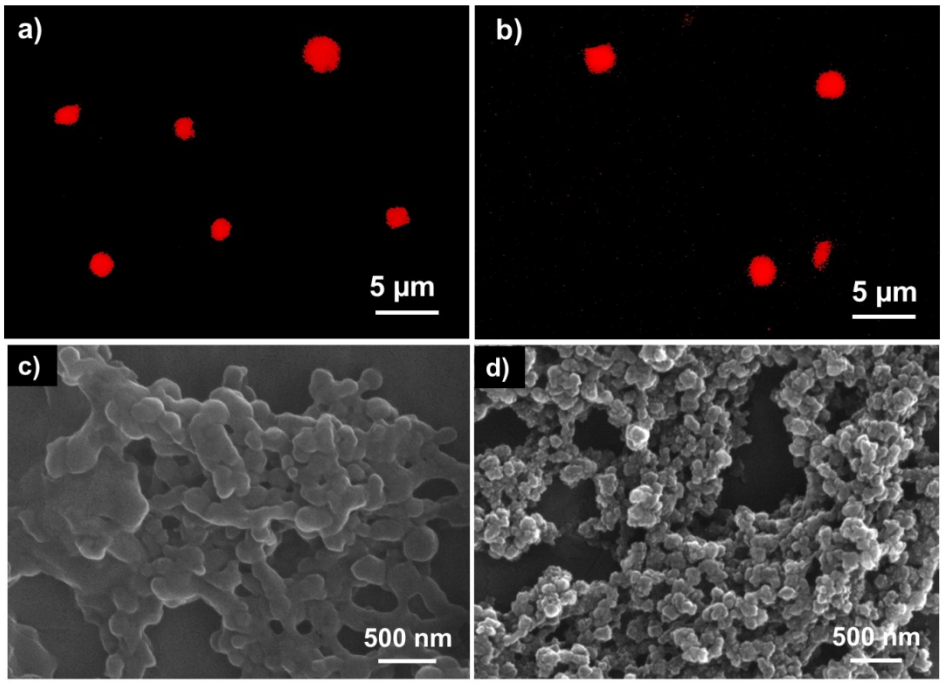
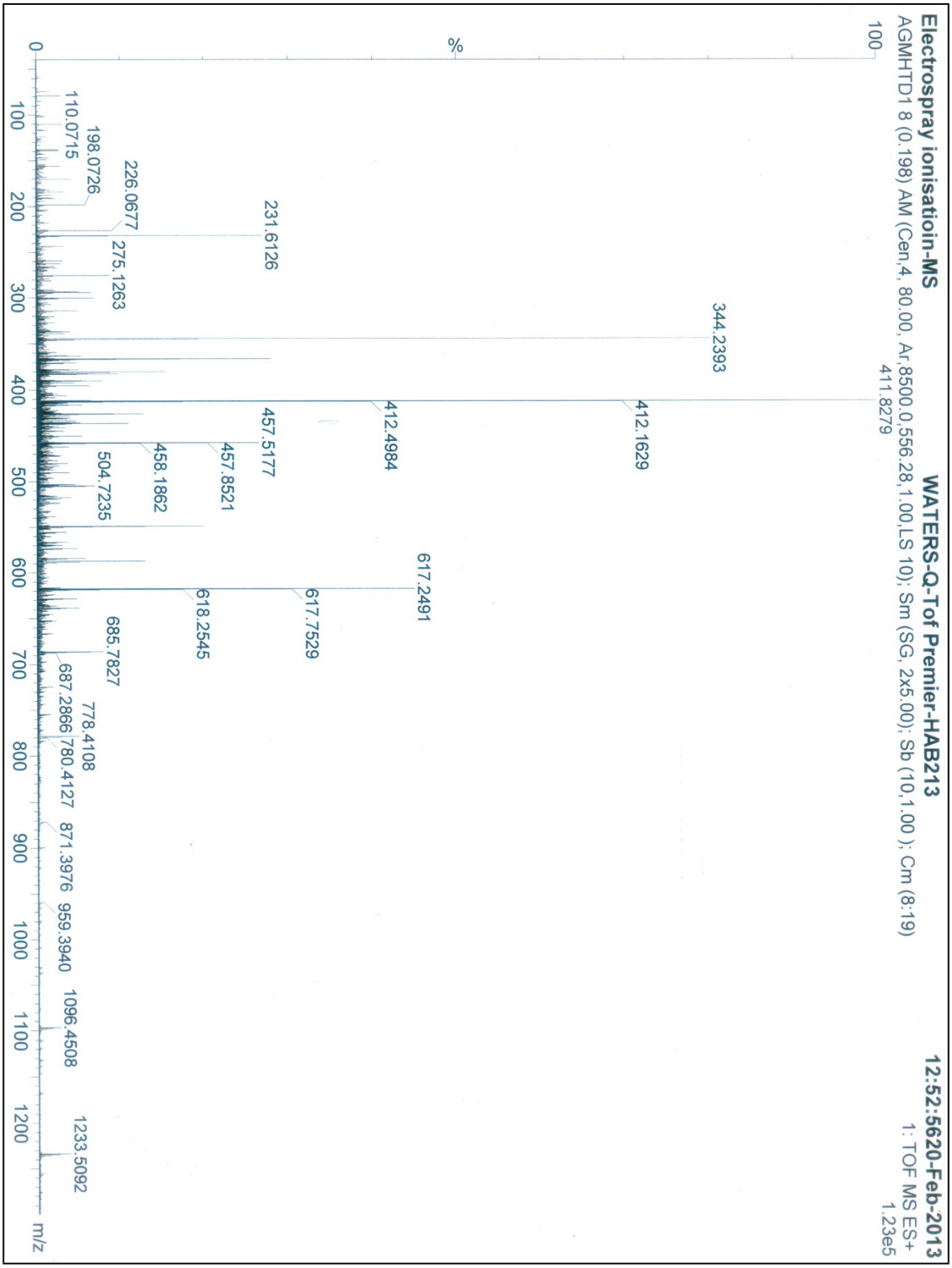


Figure S11. Fluorescent microscopy of dye stained soft structures a) Dye stained nanospheres of 1; b) Release of dye from them after incubation with Mg^{+2} ions for 6 h; c) FESEM micrograph of (a); d) FESEM micrograph of (b).


Electrospray ionisatioin-MS
WATERS-Q-Tof Premier-HAB213
12:52:5620-Feb-2013
AGMHTD1 8 (0.198) AM (Cen,4, 80.00, Ar,8500.0,556.28,1.00,LS 10); Sm (SG, 2x5.00); Sb (10,1.00); Cm (8:19)
1: TOF MS ES+
1.23e5
%
m/z
411.8279
344.2393
412.1629
412.4984
617.2491
231.6126
457.5177
457.8521
458.1862
504.7235
617.7529
618.2545
685.7827
687.2866
778.4108
780.4127
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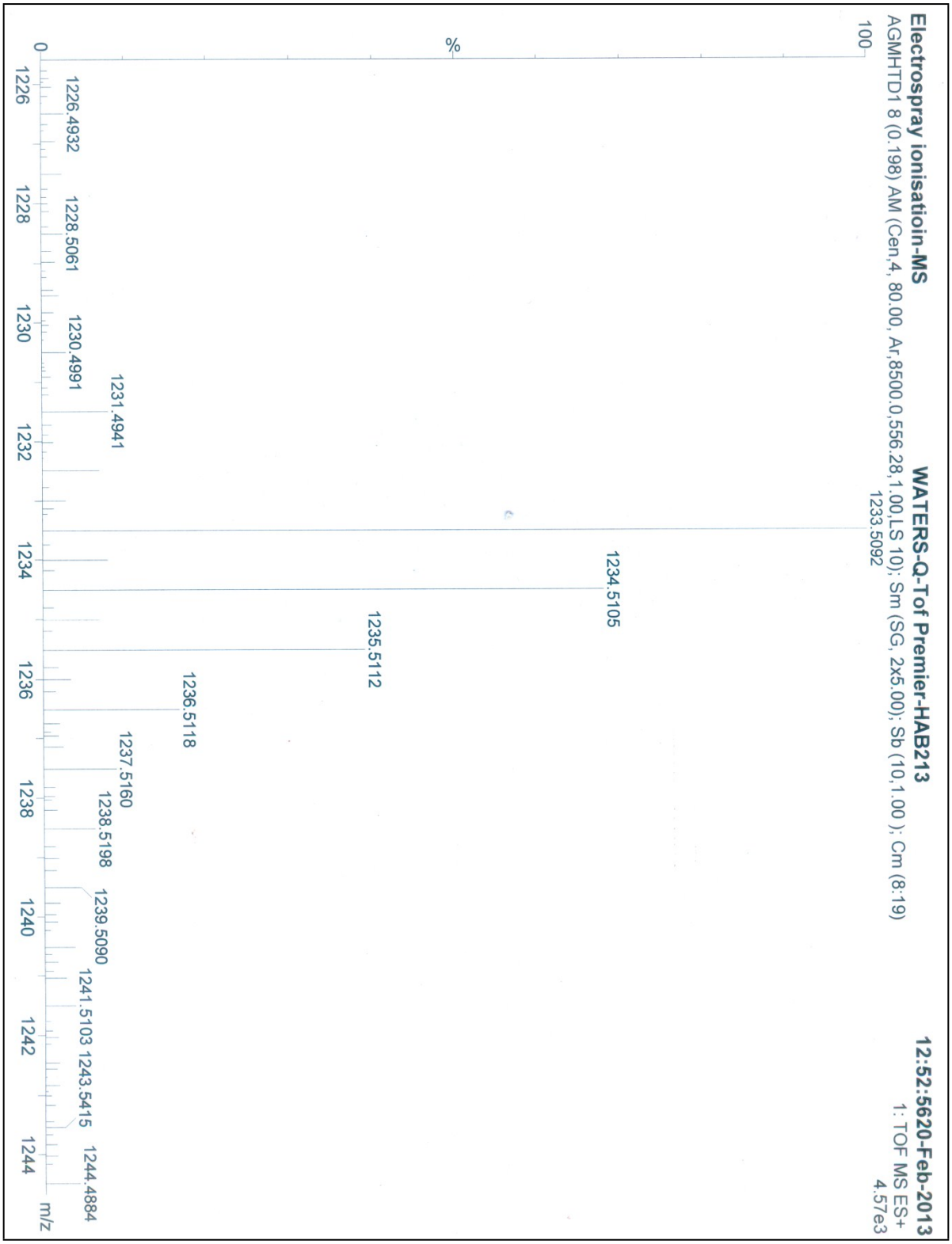
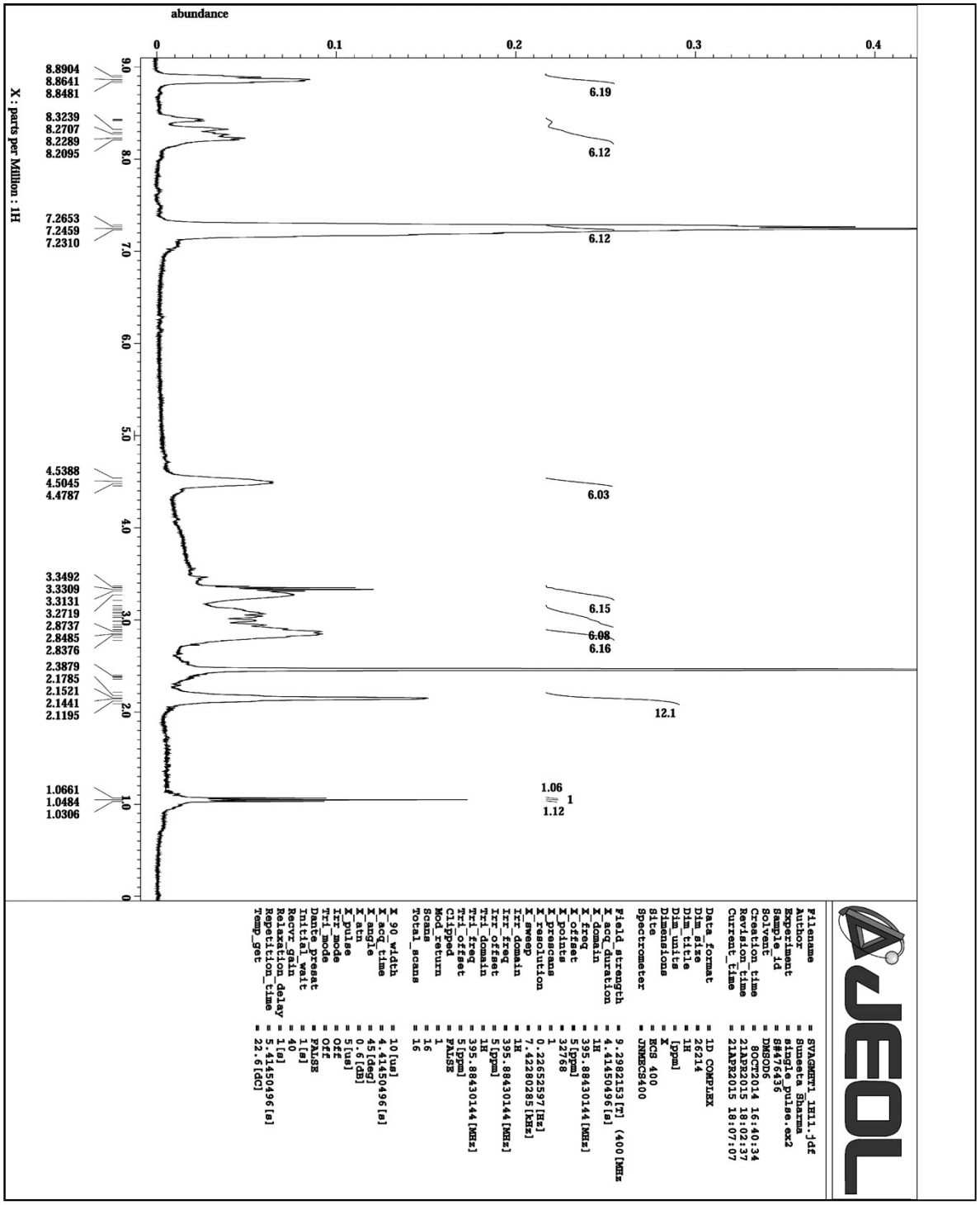
1233.5092
226.0677
275.1263
198.0726
110.0715

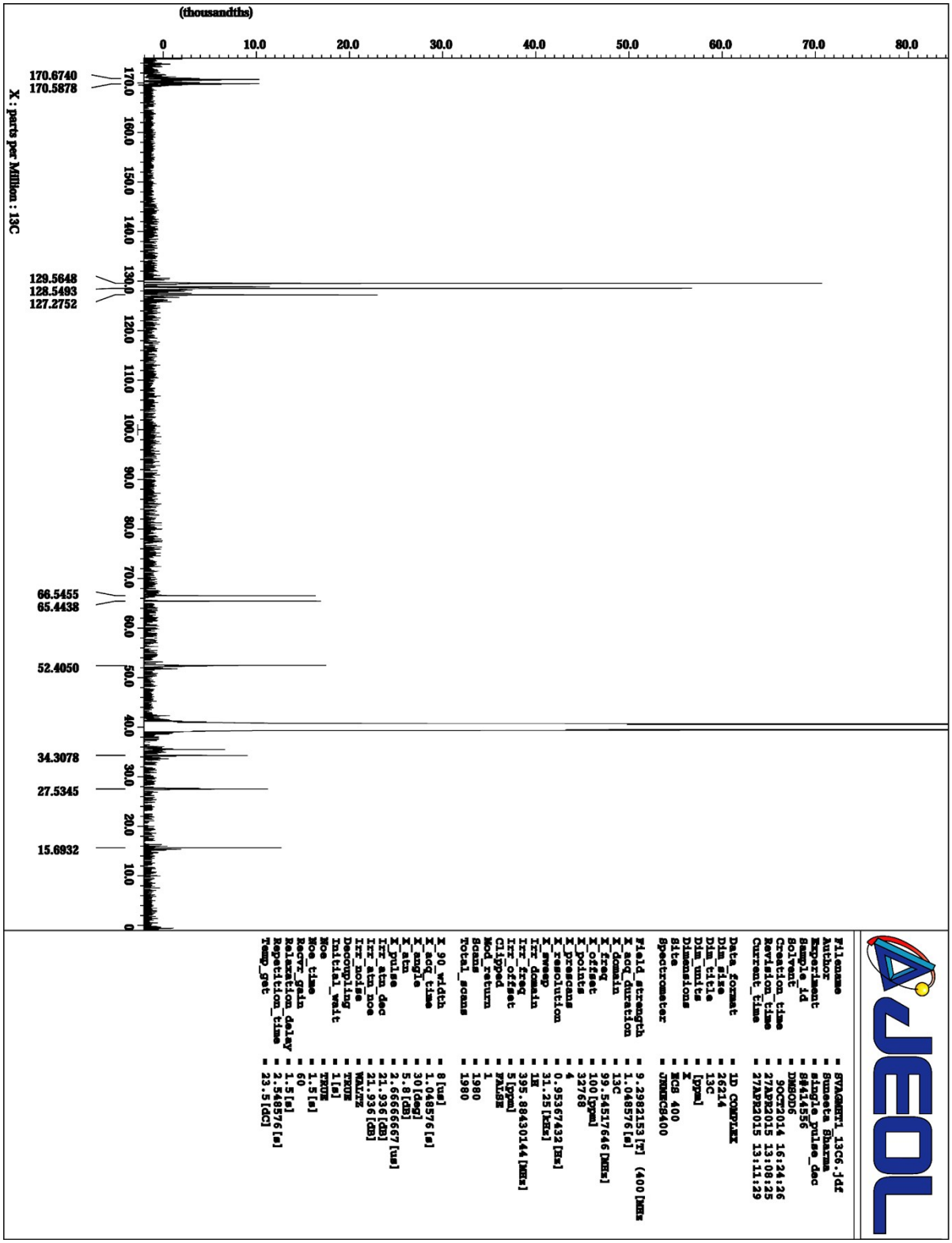
HRMS of **1**



Zoomed HRMS of **1**



^{1}H NMR Spectrum of **1**



^{13}C NMR Spectrum of **1**

References

1. A. Contreras-Sanz, T.S. Scott-Ward, H.S. Gill, J.C. Jacoby, R.E. Birch, J. Malone-Lee, K.M.G. Taylor, C.M. Peppiatt-Wildman and S.S.P. Wildman, *Purinergic Signalling*, 2012, **8**, 741.
2. (a) Z. Song, K. J. Parker, I. Enoh, H. Zhao and O. Olubajo, *Magn. Reson. Chem*, 2010, **48**, 123; (b) T.-Y. D. Tang, M. Antognozzi, J. A. Vicary, A. W. Perriman and S. Mann, *Soft Matter*, 2013, **9**, 7647.