

Nucleobase Peptide Amphiphiles

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GENERAL CONSIDERATIONS

All starting materials were obtained from commercial suppliers and used without further purification unless otherwise noted. All reactions were carried out under argon atmosphere. Dry solvents were obtained commercially and used without further drying, handled via cannula techniques under argon unless stated otherwise. NMR spectra were recorded on a 400 MHz spectrometer. The NMR spectra were recorded in CDCl₃ and DMSO-*d*₆ (¹H at 400 MHz, ¹³C at 100.6 MHz with chloroform-*d*₁ (δ 7.26, ¹H δ 77.0, ¹³C), DMSO-*d*₆ (δ 2.50, ¹H δ 39.43, ¹³C), tetramethylsilane (δ 0.00, ¹H). Data are represented as follows: chemical shifts (ppm), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad), coupling constant *J* (Hz). Routine electrospray ionization mass spectrometry (MS) was performed using a Finnegan LCQ/DUO HPLS/MS system, while high resolution data were obtained on a Kratos MS25RFA high-resolution mass spectrometer. LC/MS was performed on an Agilent Technologies triple quad LC/MS model 6420. Analytical thin-layer chromatography (TLC) was performed on TLC silica gel plates pre-coated with fluorescent indicator. Standard flash chromatography procedures were followed using silica gel 60 (230-400 mesh) with reagent grade eluents. H₂O was de-ionised and microfiltered using a Milli-Q® Millipore machine. 1xTAEMg buffer is composed of 45 mM Tris, 12.5 mM Mg(OAc)₂·6H₂O and 2 mM EDTA. The pH was adjusted to 8 using glacial acetic acid.

Circular Dichroism

CD spectra were measured on an Applied Photophysics Chirascan instrument with a Quantum Northwest Temperature Control TC125 running at 25°C using 2 mM solutions of NPAs in methanol and a 2 mm cell length. Samples were diluted where necessary to achieve extinction arising from absorbance and/or scattering above 200 nm of less than 1 AU (reduced in general by using a thin cell), thus minimising spectral noise. Spectra were collected in triplicate (with the solvent background subtracted) and averaged, followed by smoothing with a pixel window of no more than 5 nm.

Dynamic Light Scattering

Light scattering data were collected on a Brookhaven 90Plus Particle Size Analyser using the same samples as for CD. Data were collected for five minutes at 25°C, and the light scattering intensity averaged over that time, after omissions calculated by the automated dust cut-off filter.

Transmission Electron Microscopy

Samples (3 μL) were deposited on carbon film coated copper EM grids for one minute, followed by blotting off the excess liquid with the edge of a filter paper. The samples were then allowed to dry under air before being imaged using a Tecnai 12 microscope (FEI electron optics) equipped with a Lab6 filament at 120kV. Images were acquired using a Gatan 792 Bioscan 1k x 1k Wide Angle Multiscan CCD Camera (Gatan Inc.). Images for tomography were acquired using a FEI Tecnai G2 F20 operating at 120 kV equipped with a Gatan Ultrascan 4000 4k x 4k CCD Camera System Model 895. A single tilt series of images was collected with the range ± 55°. Tomographic reconstruction was performed using the IMOD (ETomo) programme from Boulder Laboratory for 3-D Electron Microscopy of Cells, Colorado, USA. Publication images were generated in Chimera 1.8 using an inverse-contrast model.

Atomic Force Microscopy

Samples (5 μL) were deposited for one minute on freshly cleaved mica, followed by wicking off excess liquid with the edge of a filter paper. Images were recorded on a Digital Instruments

MultiMode with a NanoScope IIIa controller equipped with a J-Scanner in tapping mode (air), using Olympus AC160 probes (spring constant of 42 Nm^{-1} , nominal resonant frequency of 300 KHz, and tip radius of less than 10 nm). A scan rate of 0.7 – 1.0 Hz was used, and the gains and setpoint were optimised individually for each image. NanoScope Analysis V1.40 was used for image production and analysis.

Cryo-electron microscopy

5 μl of sample was added to Quantifoil (SPI) holey carbon EM grids. Excess fluid was blotted and the sample was flash frozen hydrated by plunging into a bath of liquid ethane using FEI Vitrobot Grid Plunging System (FEI electron optics). The grids were then stored in liquid nitrogen until observation. Images were acquired using FEI Titan Krios 300kv Cryo-S/TEM microscope (FEI, Inc) equipped with Gatan 2k x 2k Digital GIF Camera System at a magnification of 19500 \times corresponding to a pixel size of 0.44 nm (defocus level ranging from – 2.5 to – 4.5 μm) under low dose conditions.

Gelation studies

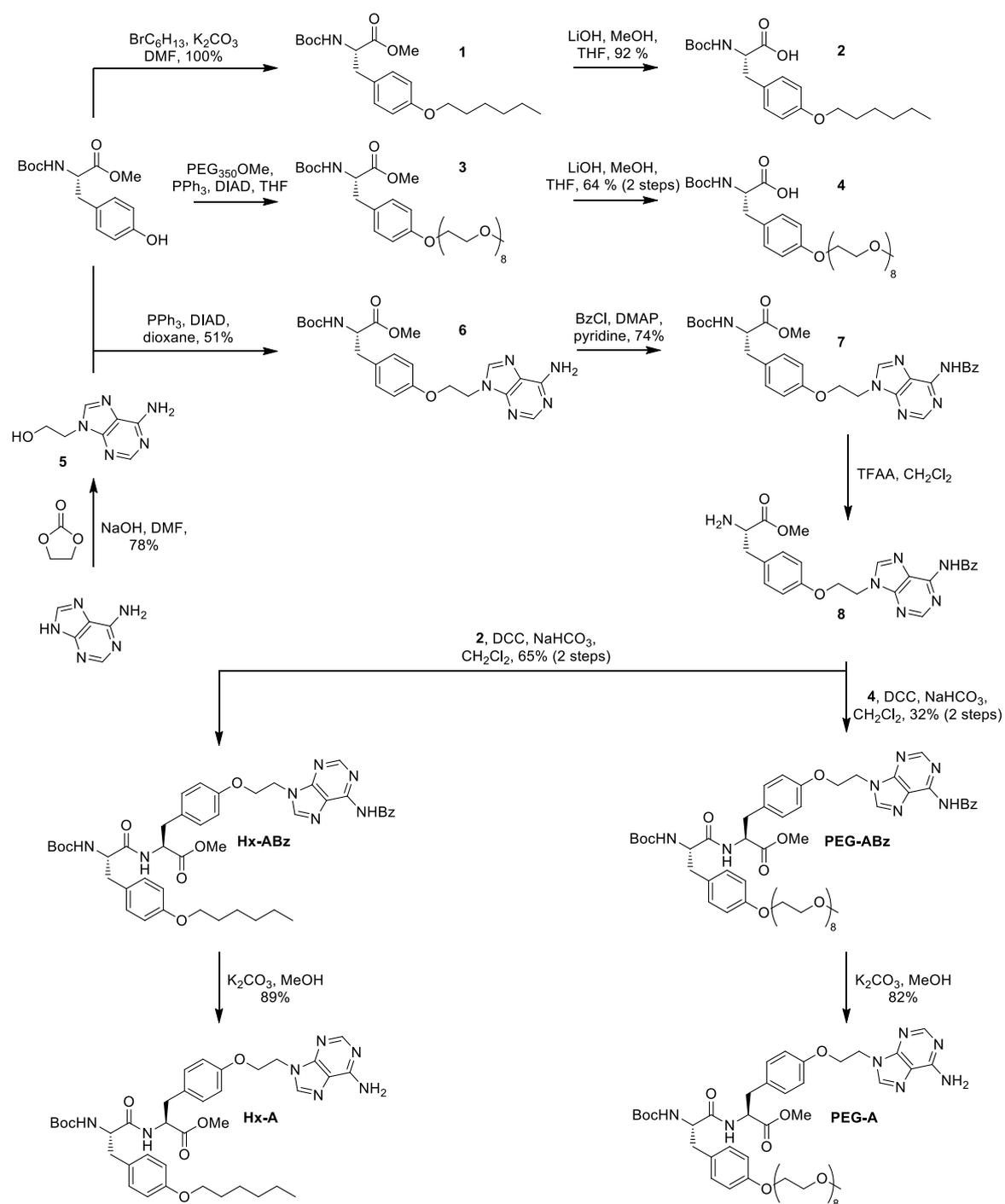
Benzene was gelled by addition of 5 wt% **Hx-ABz** followed by sonication and heating to produce a clear solution. The clear gel was formed upon cooling.

Chloroform and methanol were incorporated into gels by dissolution of 5 wt% **Hx-ABz** followed by slow gaseous diffusion of hexane or diisopropyl ether respectively into the mixture. A cloudy gel was formed from the chloroform mixture, and a clear gel in the presence of some liquid from the methanol mixture.

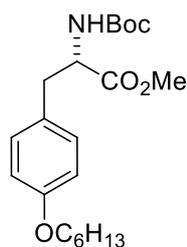
Cytotoxicity Measurement

The cytotoxicity of the **Hx-A/PEG-A** hydrogelator system was assessed using the CellTiter96 kit from Promega according to the manufacturer's instructions. Briefly, HeLa cells (human cervical cancer) were seeded at a density of 10000 cells per well in a 96-well plate in DMEM media (Invitrogen) supplemented with 10% FBS and AB/AM. Hydrogel stock solutions were initially diluted in growth medium to yield final concentrations ranging from 10 to 500 mM (total NPA basis). Cells were incubated for 24, 48, and 72 h in 5% CO_2 at 37°C. After the incubation period, the MTS reagent was added to each well and further incubated for 2 h in 5% CO_2 at 37°C. Subsequently, 96-well plates were allowed to equilibrate at room temperature and the absorbance was read at 490 nm using a BioTek Epoch micro-plate reader. All quantifications were done using GraphPad Prism 5 software.

SYNTHESIS AND CHARACTERISATION



Scheme S1. Synthesis of modified tyrosine-based amino acids as dipeptide precursors.



(S)-methyl-2-(tert-butoxycarbonylamino)-3-(4-(hexyloxy)phenyl)propanoate

(1).¹ 1-Bromohexane (3.8 g, 21.11 mmol) was added to a solution of orthogonally protected (Boc, methoxy) L-tyrosine (4.8 g, 16.24 mmol) in dry DMF (24 mL) over anhydrous K₂CO₃ (4.48 g, 32.5 mmol), under and argon atmosphere. The reaction mixture was stirred at RT until completion (typically 36-48 hr), as monitored by TLC. After consumption of the starting material, the solids were filtrated off and washed with CH₂Cl₂. Combined organic solutions were evaporated and purified by dry flash chromatography (100% CH₂Cl₂ to 3% MeOH/ CH₂Cl₂) to yield the product as pale yellowish dense oil solidifying into white/off white solid 5.8g 5.8g (93%). ¹H NMR (400 MHz CDCl₃) δ 0.90 (t, *J* = 6.9 Hz, 3H), 1.28-1.51 (m, Boc + 3 x CH₂, 15H), 1.72-1.80 (m, 2H), 2.96-3.07 (m, 2H), 3.071 (s, 3H), 3.92 (t, *J* = 6.5 Hz, 2H), 4.53 (br. d, *J* = 7.1 Hz, 1H), 4.95 (br. d, *J* = 7.7 Hz, 1H), 6.81 (d, *J* = 7.9 Hz, 2H), 7.01 (d, *J* = 8.2 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 14.0 (CH₃), 22.6 (CH₂), 25.7 (CH₂), 28.3 (3 x CH₃), 29.2 (CH₂), 31.5 (CH₂), 52.1 (CH₃), 54.5 (CH₂), 67.9 (CH₂), 79.8 (C), 114.5 (2 x CH), 127.6 (C), 130.2 (2 x CH), 155.1 (C), 158.2 (C), 172.4 (C). MS (EI) *m/z* calc. for [M+Na]⁺ 402.2, found 402.1.

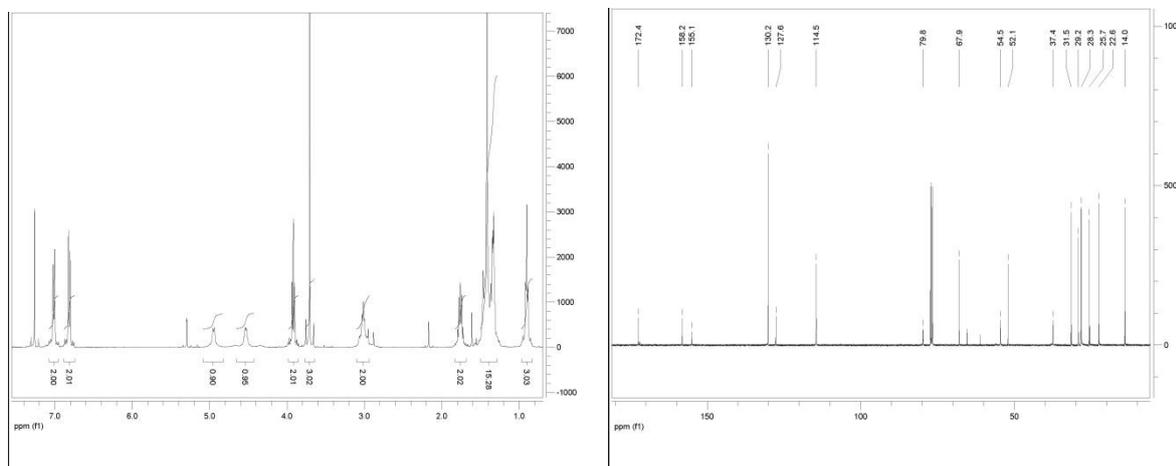
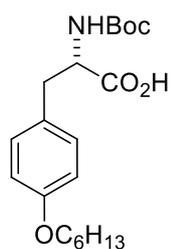


Figure S1. ¹H and ¹³C NMR spectra of **1** (CDCl₃, 298 K).



(S)-2-(tert-butoxycarbonylamino)-3-(4-(hexyloxy)phenyl)propanoic acid

(2).² LiOH (1.1 g, 45.6 mmol) was added to a stirred solution of **1** in MeOH, H₂O and THF (60, 15, 15 mL respectively) at 0°C and the reaction mixture was allowed to warm up to RT stirred for a further hour until completion. Methanol and THF were evaporated and 1M HCl was added to the remaining water solution dropwise to give ca. pH 5. The desired product was extracted with EtOAc (3 x 50 mL), and the combined organic layers dried over Na₂SO₄. Solvent evaporation gave an off white waxy solid. This was subsequently suspended in *n*-hexane (50 mL) and washed with I-hexane (3 x 30 mL), dried under high vac. to give the desired product as white solid 5.1g (92%). ¹H NMR (400 MHz CDCl₃) δ 0.90 (t, *J* = 7.0 Hz, 3H), 1.29-1.46 (m, Boc + 3 x CH₂, 15H), 1.72-1.79(m, 2H), 3.00-3.05 (br. m 1H), 3.11 (dd, *J* = 14, 4.8 Hz, 1H), 3.92 (t, *J* = 6.5 Hz, 2H), 4.53 (br. d, *J* = 4.5 Hz, 1H), 4.93 (br. d, *J* = 7 Hz, 1H), 6.83 (d, *J* = 8.2 Hz, 2 H), 7.08 (d, *J* = 8.1 Hz). 10.10 (br. s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 14.0 (CH₃), 22.6 (CH₂), 25.7 (CH₂), 28.3 (3 x CH₃), 29.2 (CH₂), 31.6 (CH₂), 36.8 (CH₂), 54.5 (CH₂), 68.0 (CH₂), 80.2 (C), 114.6 (2 x CH), 127.5 (C), 130.3 (2 x CH), 155.5 (C), 158.3 (C), 176.3 (C). MS (EI) *m/z* calc. for [M+Na]⁺ 388.2, found 388.0.

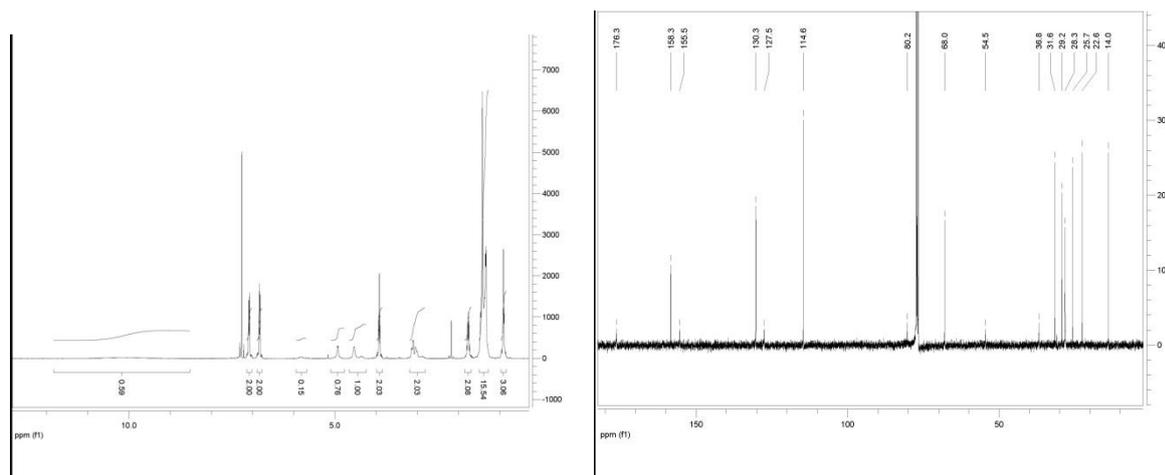
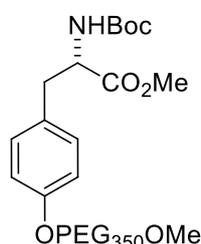
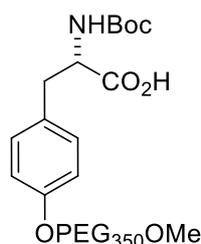


Figure S2. ^1H and ^{13}C NMR spectra of **2** (CDCl_3 , 298 K).



(S)-methyl 3-(4-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yloxy)phenyl)-2-(tert-butoxycarbonylamino)propanoate (3). A mixture of protected tyrosine (3.0 g, 10 mmol), PEG₃₅₀OMe (4.3 g, 12 mmol) and PPh₃ (3.41g, 13 mmol.) in dry THF (30 mL) was stirred at room temperature under argon, then cooled to 0°C, at which point DIAD (2.63g, 13 mmol) was added slowly (over 1 hour) rendering the mixture yellow. The mixture was warmed to room temperature and stirred for 16 h, then concentrated *in vacuo*. The resulting residue was dissolved in CH₂Cl₂

(50 mL) and washed with H₂O (3 x 100 mL), then dried over Na₂SO₄ and concentrated *in vacuo*. The crude product was purified by flash chromatography in 50% EtOAc/hexane followed by 0-15% MeOH/CH₂Cl₂ to afford 6.85g of the product as yellow dense oil containing PPh₃O impurities. The absence of PEG impurities could be confirmed by TLC stained with KMnO₄. The product was then submitted directly for methyl ester deprotection. MS (EI) *m/z* calc. for [M+Na+MeCN]⁺ 725.4, found 725.3.



(S)-3-(4-(2,5,8,11,14,17,20,23-octaoxapentacosan-25-yloxy)phenyl)-2-(tert-butoxycarbonylamino)propanoic acid (4). LiOH (580 mg, 24.1 mmol) was added to the crude residue of **3** dissolved in solution in H₂O and THF (20 and 50 mL respectively) at 0°C and the reaction mixture was allowed to warm up to RT and stirred for 3 more hours until completion. The reaction mixture was neutralized with 1M HCl, evaporated dry and purified by chromatography with 50% EA/CH₂Cl₂ followed by 10% MeOH/CH₂Cl₂ to afford (4.1g, 64% over 2

steps) the desired product as dense colorless oil. ^1H NMR (400 MHz CDCl_3) δ 1.39 (s, 9H), 2.93-3.03(br. m, 2H), 3.34 (s, 3H), 3.53-3.71 (m, 35H), 3.81-3.83 (m, 2H), 4.08-4.10 (m, 2H), 4.46 (br. s, 1H), 5.16 (br. s, 1H), 6.82 (d, $J = 8.4$ Hz), 7.07 (d, $J = 8.4$ Hz, 2H), ^{13}C NMR (100 MHz, CDCl_3) δ 28.2 (3 x CH₃), 37.0 (CH₂), 58.9 (CH₃), 67.2-71.7 (multiple PEG CH₂), 79.4 (C), 114.4 (2 x CH), 129.1 (C), 130.4 (2 x CH), 155.3 (C), 157.4 (C), 175.6 (C). MS (EI) *m/z* calc. for [M-H]⁻ 646.3, found 646.1.

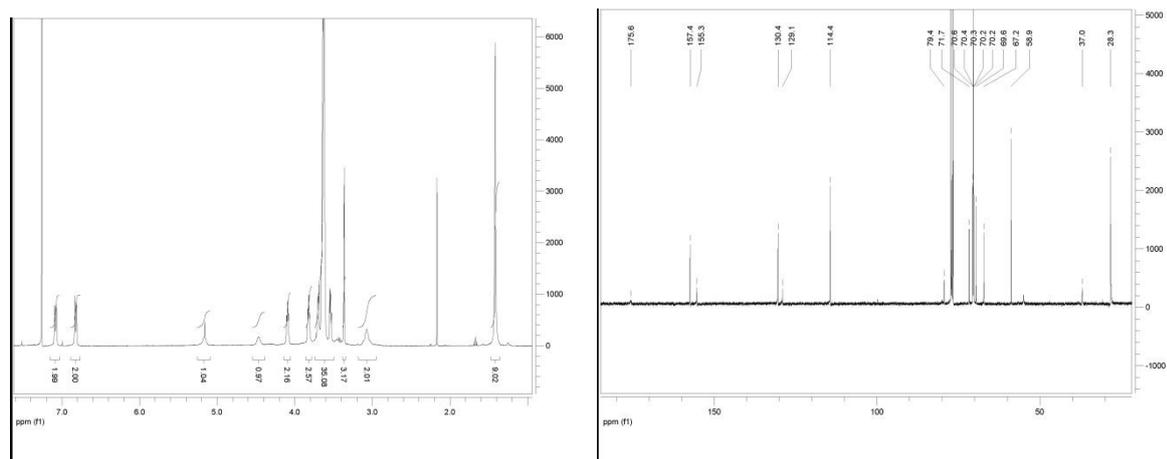
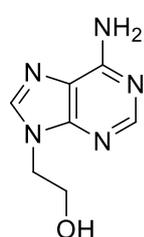


Figure S3. ^1H and ^{13}C NMR spectra of **4** (CHCl_3 , 298 K).



2-(6-amino-9H-purin-9-yl)ethanol (5).³ NaOH (80 mg, 2 mmol) was added to a refluxing (180°C) suspension of adenine (2.7 g, 20 mmol) in dry DMF (30 mL), followed by ethylene carbonate (1.8 g, 22 mmol) and stirred at this temperature until the solution became clear orange/brown (1-1.5h) and CO_2 evolution stopped. The solution was cooled down and DMF evaporated, leaving a yellow solid, which was washed with MeOH (20 mL) and dried under high vac to give 2.85 g (78%) of **5** as a white solid with a minor DMF impurity. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 3.74 (t, $J = 5.3$ Hz, 2H), 4.19 (t, $J = 5.4$ Hz, 2H), 5.03 (s, 1H), 7.21 (s, 2H), 8.08 (s, 1H), 8.14 (s, 1H); ^{13}C NMR (100 MHz, $\text{DMSO-}d$) δ 45.7 (CH_2), 59.2 (CH_2), 118.6 (C), 141.3 (CH), 149.5 (C), 152.2 (CH), 155.8 (C). MS (EI) m/z calc. for $[\text{M}+\text{H}]^+$ 180.1, found 180.2.

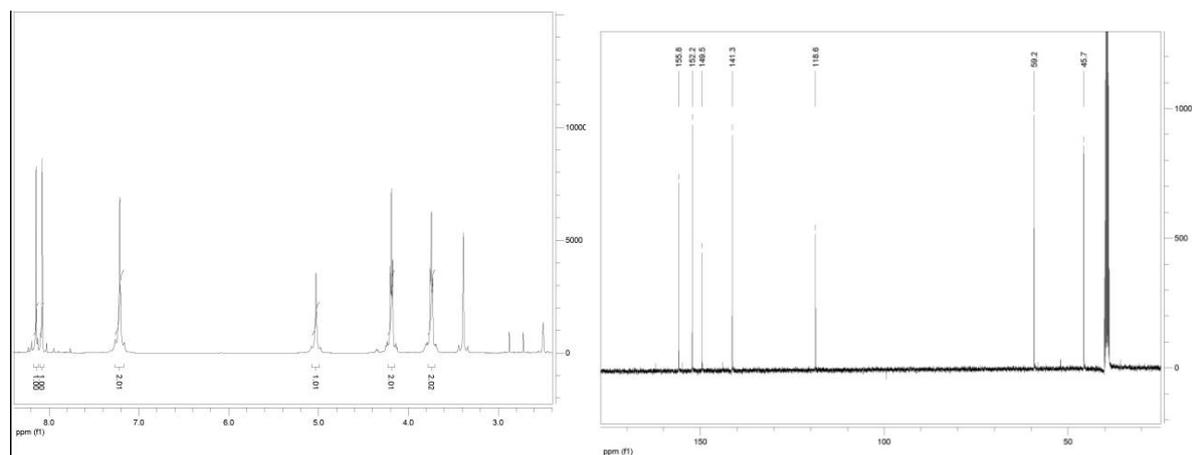
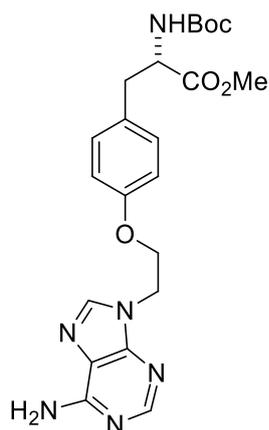


Figure S4. ^1H and ^{13}C NMR spectra of **5** ($\text{DMSO-}d_6$, 298 K).



(S)-methyl-3-(4-(2-(6-amino-9H-purin-9-yl)ethoxy)phenyl)-2-(tert-butoxycarbonylamino) propanoate (6). DIAD (8.5 g, 42.08 mmol) was added slowly (over 2 hours), under argon to a suspension of PPh₃ (12.72 g, 48.55 mmol), **5** (7.0 g, 38.85 mmol) and Boc-, methoxy- protected tyrosine (9.56 g, 32.37 mmol) in dry dioxane (250 mL) at 0°C. The reaction mixture was then allowed to warm to RT and stirred overnight to result a clear yellow/brown solution. The solvent was removed *in vacuo* and the resulting brown oil was purified by chromatography with slow gradient of solvents: 100% CH₂Cl₂ to 6% MeOH/CH₂Cl₂. *n*-Hexane was added to the fractions containing the desired product and left overnight. 7.57 g (51%) of white semi crystalline solid was filtrated and dried under high vac. Remaining solution and fractions containing the desired product were combined and purified again. ¹H NMR (400 MHz CDCl₃) δ 1.39 (s, 9H), 2.93-3.05 (m, 2H), 3.69 (s, 3H), 4.28 (t, *J* = 4.9 Hz, 2H), 4.52 (br. d, *J* = 5.6 Hz, 1H), 4.57 (t, *J* = 4.8 Hz, 1H), 5.07 (br. d, *J* = 7.7 Hz, 1H), 5.85 (s, 2H), 6.76 (d, *J* = 8.2 Hz, 2H), 7.00 (d, *J* = 8.3 Hz, 2H), 7.96 (s, 1H), 8.35 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 28.3 (3 x CH₃), 37.4 (CH₂), 43.2 (CH₂), 52.2 (CH₃), 54.5 (CH), 65.9 (CH₂), 79.9 (C), 114.5 (2 x CH), 119.5 (C), 129.0 (C), 130.4 (2 x CH), 141.3 (CH), 149.9 (C), 153.0 (CH), 155.0 (C), 155.1 (C), 156.9 (C), 172.3 (C). MS (EI) *m/z* calc. for [M-H]⁻ 455.2, found 454.8.

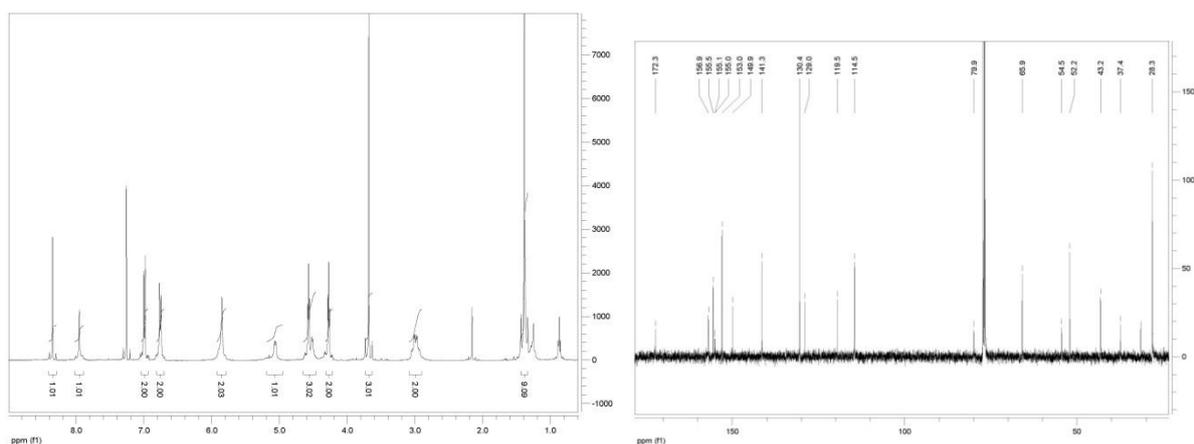


Figure S5. ¹H and ¹³C NMR spectra of **6** (CHCl₃, 298 K).

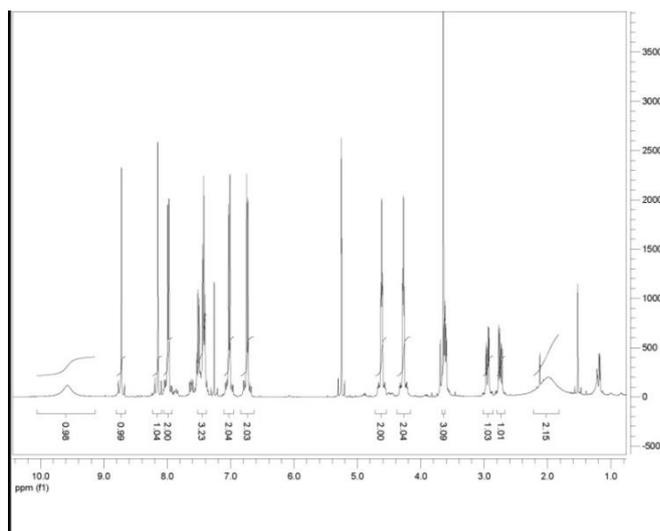
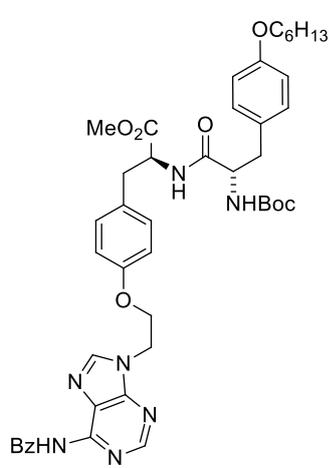


Figure S7. ^1H NMR spectrum of **8** (CDCl_3 , 298 K).

General procedure for synthesis of the Tyr-Tyr dipeptides.⁶

Boc-protected amino acid **2** or **4** (5.0 mmol) and amino acid methyl ester **8** (5.0 mmol) were suspended in dry dichloromethane (40 mL) in an ice bath. After 10 min, dicyclohexylcarbodiimide (6.0 mmol) and sodium bicarbonate (5.0 mmol) were added and the resultant mixture was stirred at room temperature for 24 hr. The precipitate was filtered off and washed with dichloromethane (2 x 20 mL), the filtrate was concentrated and charged on silica column for purification.



Hx-ABz dipeptide, yield 65% (over 2 steps). ^1H NMR (400 MHz CDCl_3) δ 0.87-0.90 (m, 3H), 1.29-1.43 (m, Boc + 3 x CH_2 , 15H), 1.69-1.77 (m, 2H), 2.87-3.03 (m, 4H), 3.63 (s, 3H), 3.89 (t, $J = 6.5$ Hz, 2H), 4.19-4.29 (br. m, 1H), 4.32 (t, $J = 4.8$ Hz, 2H), 4.66 (t, $J = 4.7$ Hz, 2H), 4.67-4.75 (br. m, 1H), 5.09 (br. d, $J = 7.4$ Hz, 1H), 6.3 (br. d, $J = 7.0$ Hz, 1H), 6.71 (d, $J = 8.2$ Hz, 2H), 6.78 (d, $J = 8.1$ Hz, 2H), 6.88 (d, $J = 8.1$ Hz, 2H), 7.05 (d, $J = 8.2$ Hz, 2H), 7.48-7.61 (m, 3H), 8.02 (d, $J = 7.9$ Hz, 2H), 8.16 (s, 1H), 8.90 (s, 1H), 9.18 (br. s, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 14.0 (CH_3), 22.6 (CH_2), 25.7 (CH_2), 28.2 (3 x CH_3), 29.2 (CH_2), 31.5 (CH_2), 37.4 (CH_2), 43.4 (CH_2), 52.2 (CH_3), 53.3 (CH), 55.9 (CH), 65.6 (CH_2), 68.0 (CH_2), 80.1 (C), 114.5 (2 x CH), 114.6 (2 x CH), 122.8 (C), 127.9 (2 x CH), 128.1 (C), 128.79 (2 x CH), 128.85 (C), 130.3 (2 x CH), 130.4 (2 x CH), 132.7 (CH), 133.6 (C), 143.8 (CH), 149.5 (C), 151.9 (C), 152.6 (CH), 155.3 (C), 156.8 (C), 158.2 (C), 164.6 (C), 170.9 (C), 171.3 (C). HRMS (EI) m/z calc. for $[\text{M}+\text{H}]^+$ 808.40284, found 808.40217.

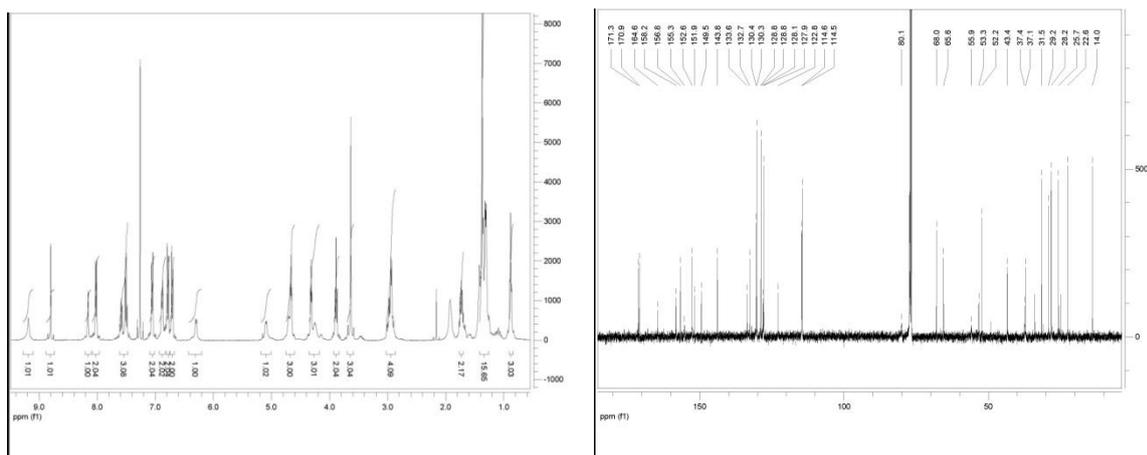
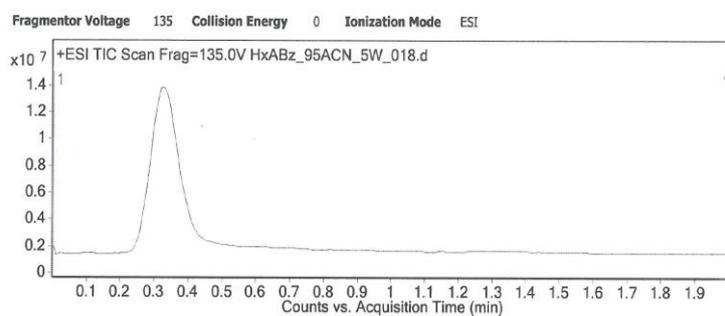


Figure S8. ^1H and ^{13}C NMR spectra of **Hx-ABz** (CDCl_3 , 298 K).

User Chromatograms



User Spectra

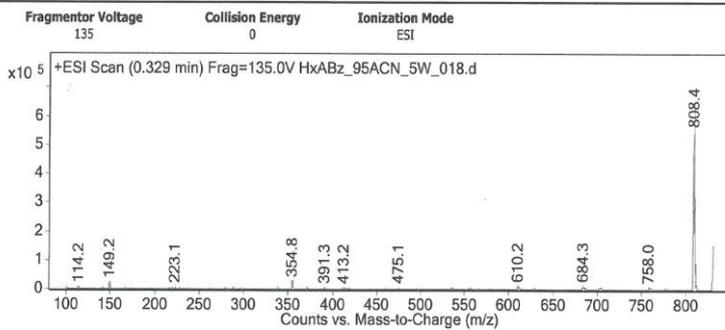
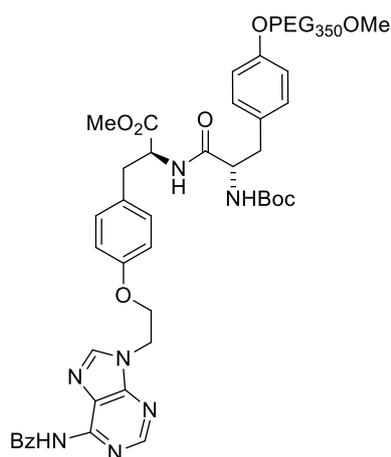


Figure S9. LC-MS trace of **Hx-ABz**. Peak at 808.4 m/z corresponds to $[\text{M}+\text{H}]^+$.



PEG-ABz dipeptide, yield 32% (over 2 steps). ^1H NMR (400 MHz CDCl_3) δ 1.38 (3 x CH_3 , 9H), 2.90-3.02 (m, 4H), 3.37 (s, 3H), 3.53-3.83 (m, PEG + OCH_3 , 33H), 4.06-4.08 (m, 2H), 4.24 (br. d, $J = 6.2$ Hz, 1H), 4.33 (t, $J = 4.9$ Hz, 2H), 4.66-4.72 (m, 3H), 5.05 (br. s, 1H), 6.28 (d, $J = 7.3$ Hz, 1H), 6.71 (d, $J = 8.6$ Hz, 2H), 6.80 (d, $J = 8.7$ Hz, 2H), 6.88 (d, $J = 8.3$ Hz, 2H), 7.06 (d, $J = 8.6$ Hz, 2H), 7.50-7.62 (m, 3H), 8.02 (d, $J = 7.1$ Hz, 2H), 8.16 (br. s, 1H), 8.81 (s, 1H), 9.09 (br. s, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 28.2 (3 x CH_3), 37.1 (CH_2), 37.4 (CH_2), 45.5 (CH_2), 52.3 (CH_3), 53.3 (CH), 59.0 (CH_3), 65.6 (CH_2), 67.4 (CH_2), 69.7-71.9 (multiple PEG- CH_2), 80.2 (C), 114.5 (2 x CH), 114.7 (2 x CH), 122.7 (C), 127.9 (2 x CH), 128.6 (C), 128.8 (2 x CH), 130.3 (2 x CH), 130.4 (2 x CH), 132.7 (CH), 133.6 (C), 143.8 (CH), 149.5 (C), 151.9 (C), 152.6 (CH), 155.3 (C), 156.8 (C), 157.8 (C), 164.6 (C), 170.8 (C), 171.3 (C). HRMS (EI) m/z calc. for $[\text{M}+\text{H}]^+$ 1090.53431, found 1090.53242.

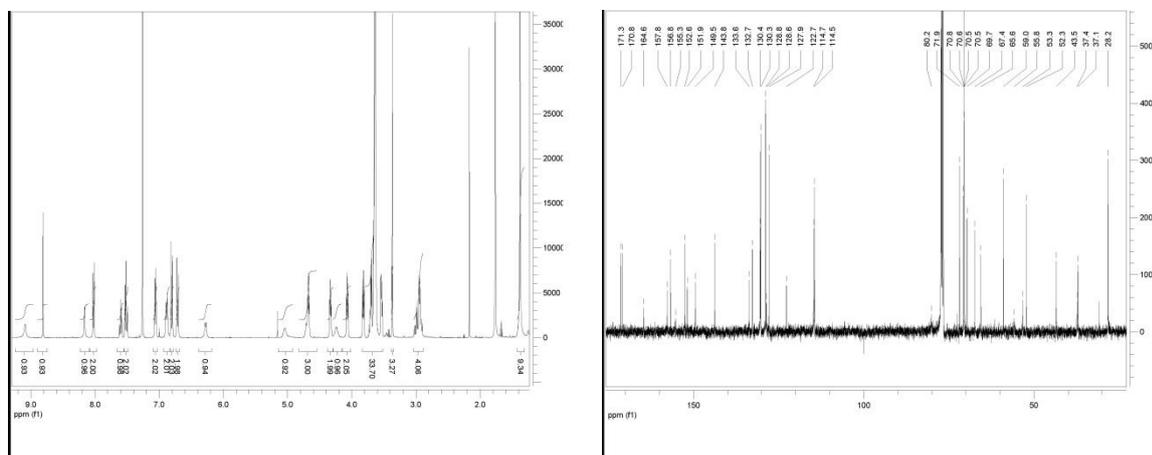
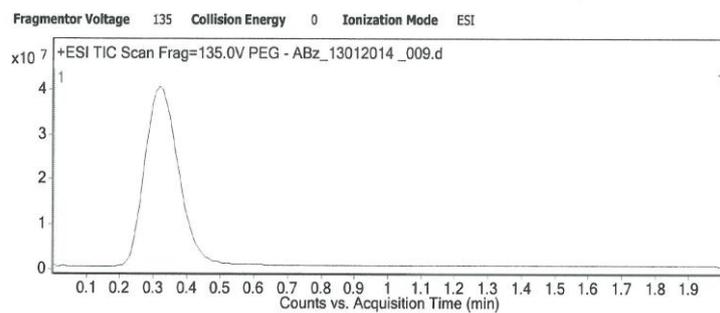


Figure S10. ^1H and ^{13}C NMR spectra of **PEG-ABz** (CDCl_3 , 298 K).

User Chromatograms



User Spectra

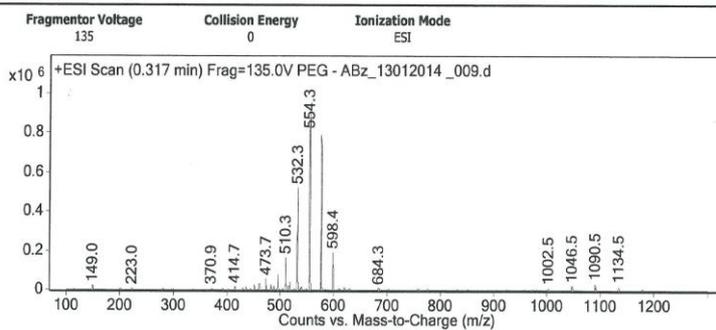


Figure S11. LC-MS trace of **PEG-ABz**. Peak at 1090.5 m/z corresponds to $[M+H]^+$.

General procedure for adenine benzoyl deprotection⁷

To a solution of the Bz protected dipeptide (0.1 mmol) in methanol (3 mL) 5 equivalents of K_2CO_3 was added at RT. Reaction mixture was stirred for 18 hours until completion. Solids were filtered off and washed with methanol (2 x 5 mL). Methanol was evaporated and oily residue purified by column chromatography.

Due to self-assembly, it was impossible to record clear NMR spectra, nevertheless the absence of the benzoyl signals is clear.

For **Hx-A** 0-10% MeOH/ CH_2Cl_2 – 89% isolated yield, white solid. 1H NMR (300 MHz 10:1 $CD_3OD:CDCl_3$) δ 0.91 (q, 3H, $-CH_2CH_3$), 1.14-4.7 (m, 16H, $-CH_2CH_2CH_2-$, iBu), 1.70 (dd, 2H, $-TyrOCH_2CH_2CH_2-$), 2.16 (s, 3H, OCH_3), 2.55-3.08 (br, 4H, $-CH_2-Ar$), 3.89 (dd, 2H, $TyrOCH_2CH_2CH_2-$), 4.20 (1H, br, $-NHCHRCO-$), 4.29 (dd, 2H, $TyrOCH_2CH_2-A$), 4.52 (br, 1H, $-NHCHRCO-$), 4.59, 4.60, 4.52, 4.30, 4.20, 3.88 (dd, 2H, $TyrOCH_2CH_2-A$), 6.76 (m, 4H, ArH), 6.97 (m, 2H, ArH), 7.05 (m, 2H, ArH), 8.13 (d, 1H, AH), 8.21 (s, AH); ^{13}C NMR could not be recorded due to self-assembly at high concentrations. HRMS (EI) m/z calc. for $[M-CH_3+H]^+$ 690.36097, found 690.3638.⁸

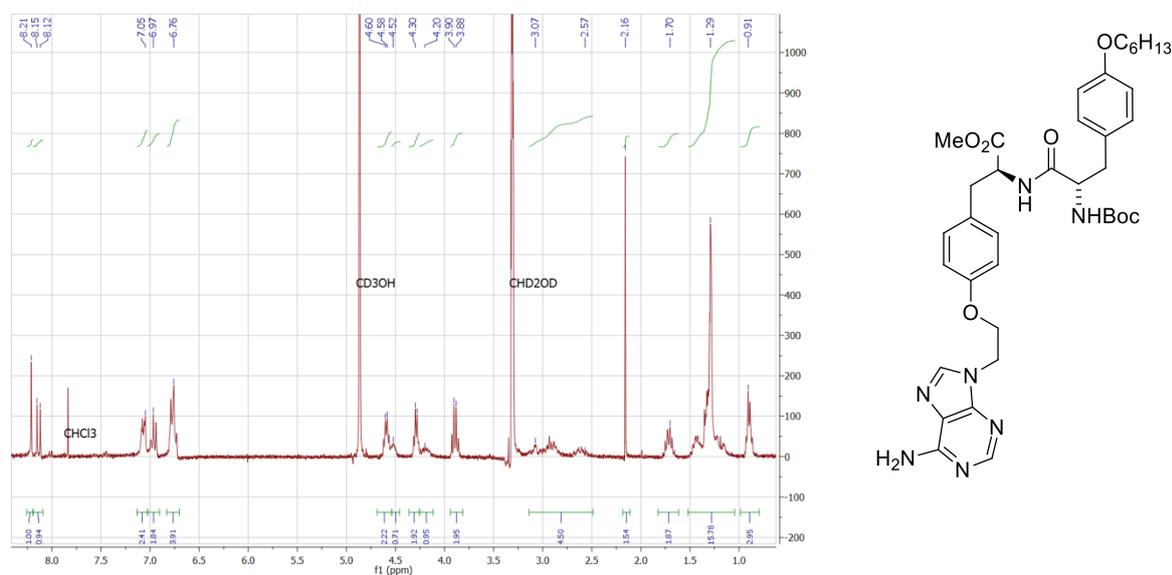
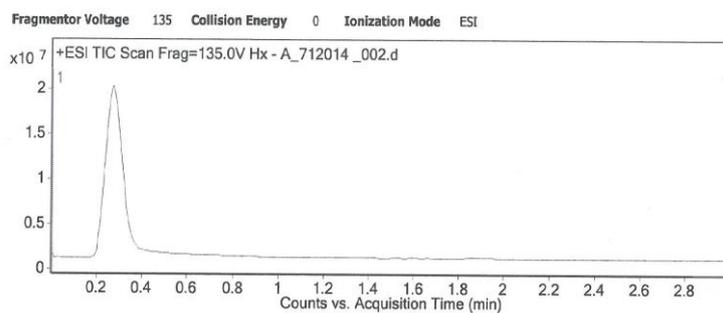


Figure S12. 1H NMR spectrum of **Hx-A** (10:1 $CD_3OD:CDCl_3$, 298 K).

User Chromatograms



User Spectra

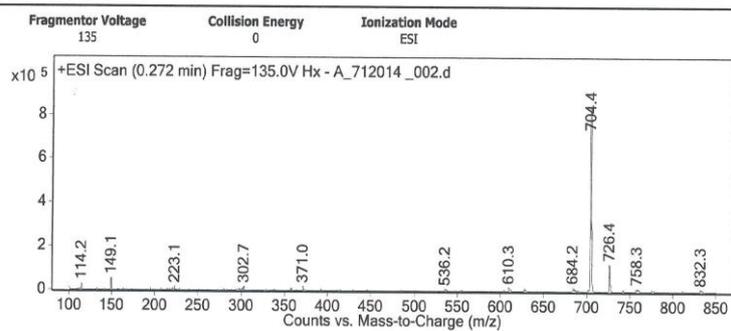
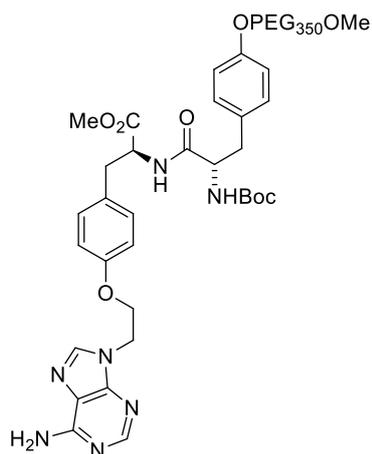
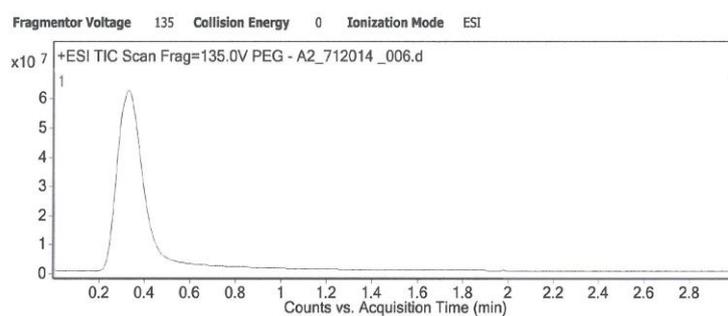


Figure S13. LC-MS trace of **Hx-A**. Peak at 704.4 m/z corresponds to $[M+H]^+$.



For **PEG-A** 10-30% MeOH/CH₂Cl₂ – 82% isolated yield, white solid. Clear ¹H and ¹³C NMR could not be recorded, however the success of transformation could be gauged by TLC, giving a single spot at slightly higher polarity than **PEG-ABz**. HRMS (EI) m/z calc. for [M-CH₃+K]⁺ 1010.44887, found 1010.44783.⁹

User Chromatograms



User Spectra

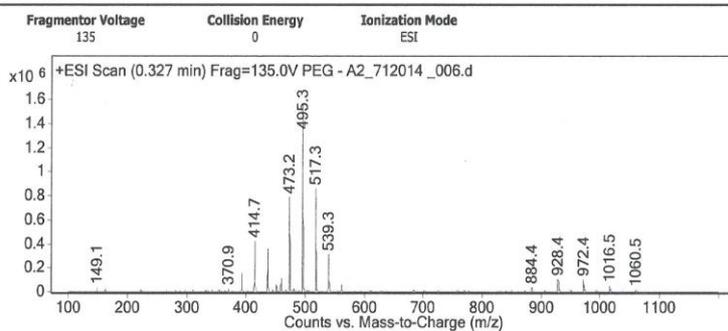


Figure S14. LC-MS trace of **Hx-A**. Peak at 972.4 m/z corresponds to [M-CH₃+H]⁺.

SUPPLEMENTARY MICROSCOPY IMAGES AND ANALYSIS

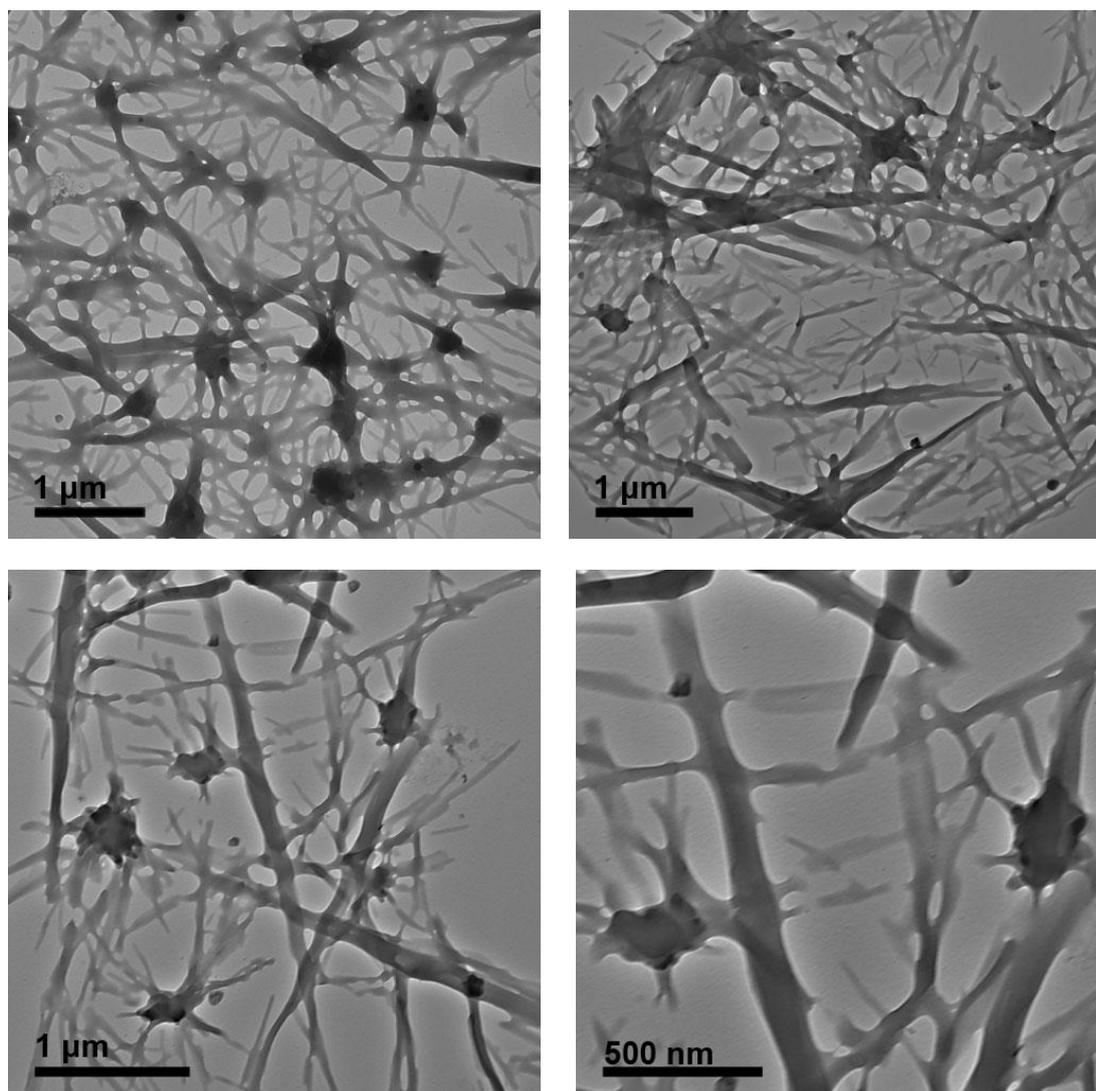


Figure S15. TEM images of **Hx-ABz** precipitate microcrystals (deposited from 1:9 MeOH:H₂O).

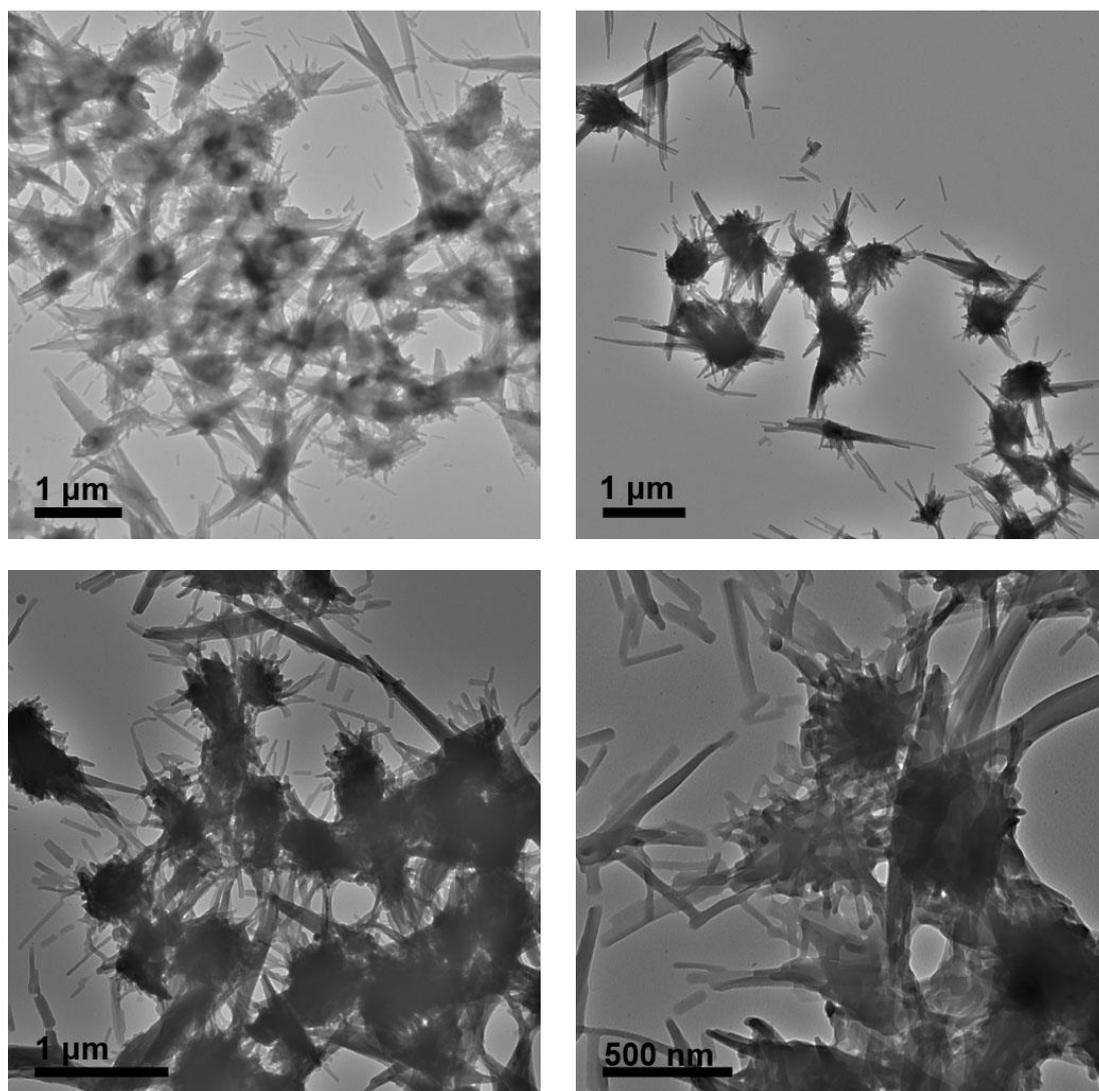


Figure S16. TEM images of **Hx-ABz** precipitate microcrystals (deposited from 1:9 MeOH:TAEMg buffer).

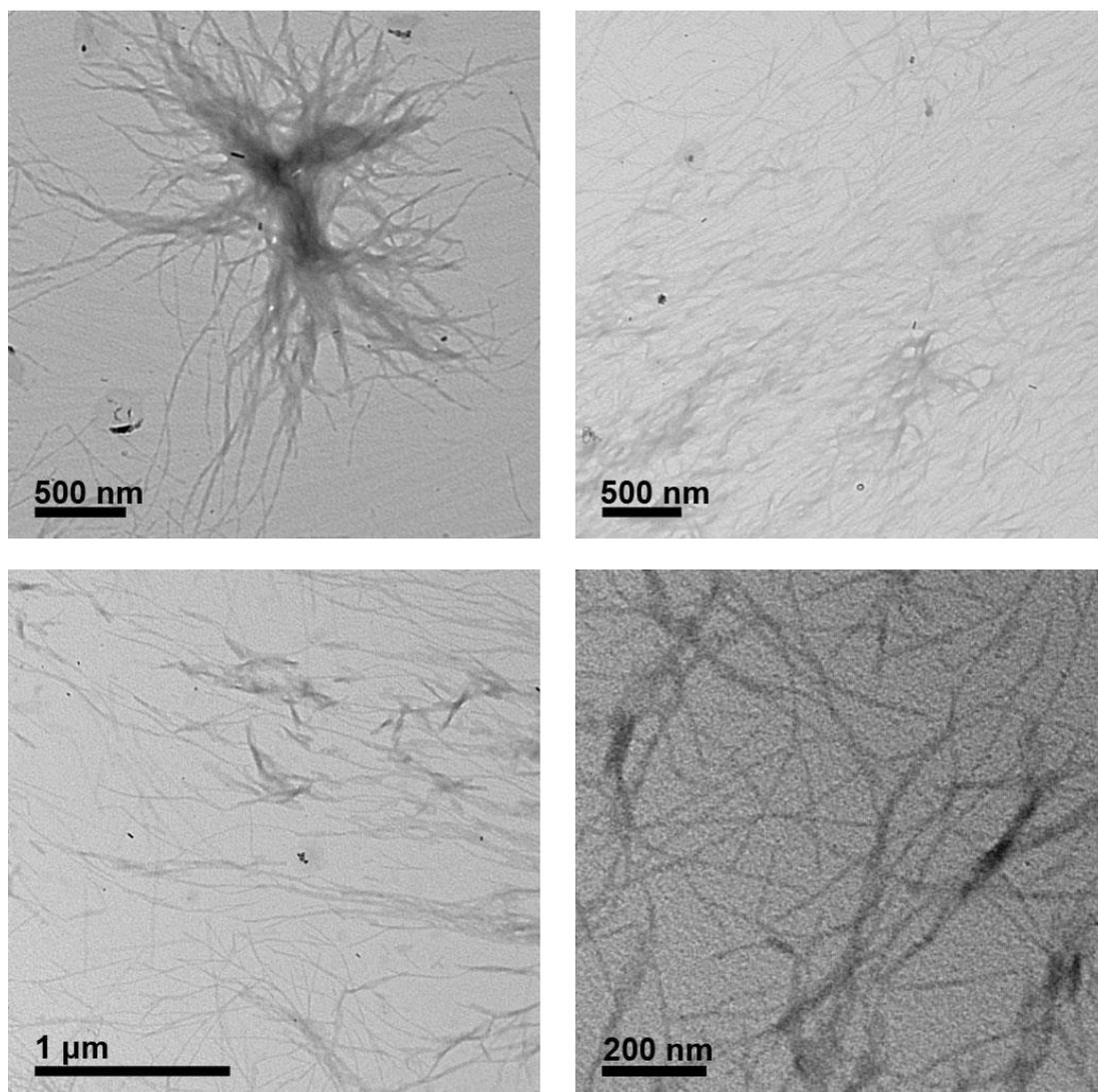


Figure S17. TEM images of **Hx-ABz** benzene gel. Fibre width = 19 ± 3 nm.

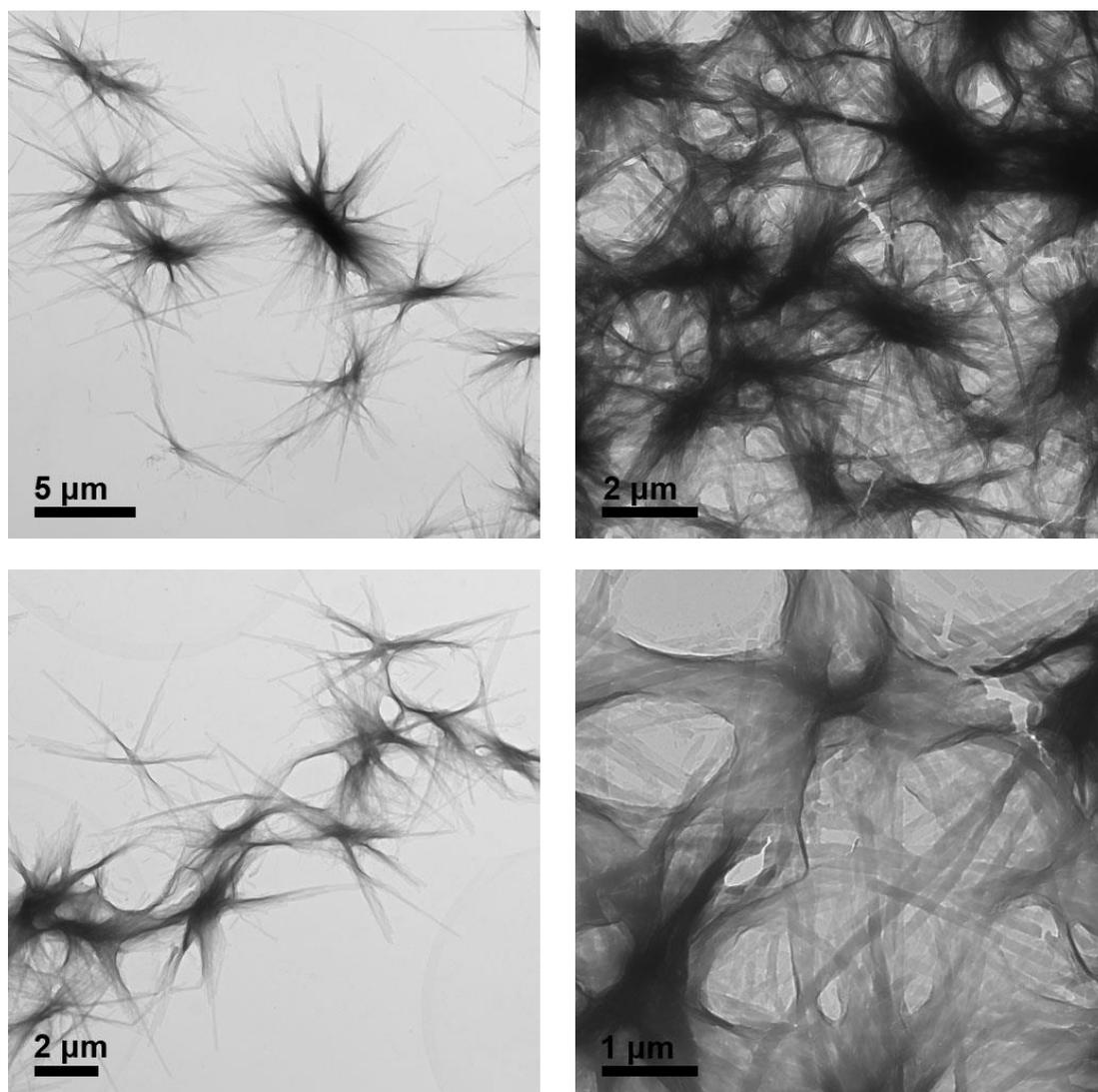


Figure S18. TEM images of **Hx-ABz** chloroform/hexane gel. Constituent fibre width = 122 ± 21 nm.

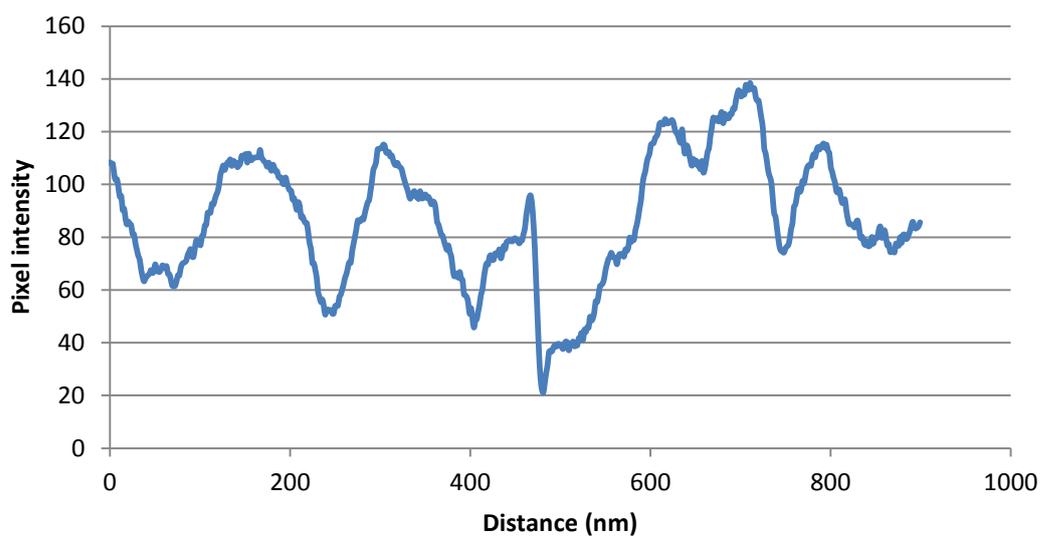
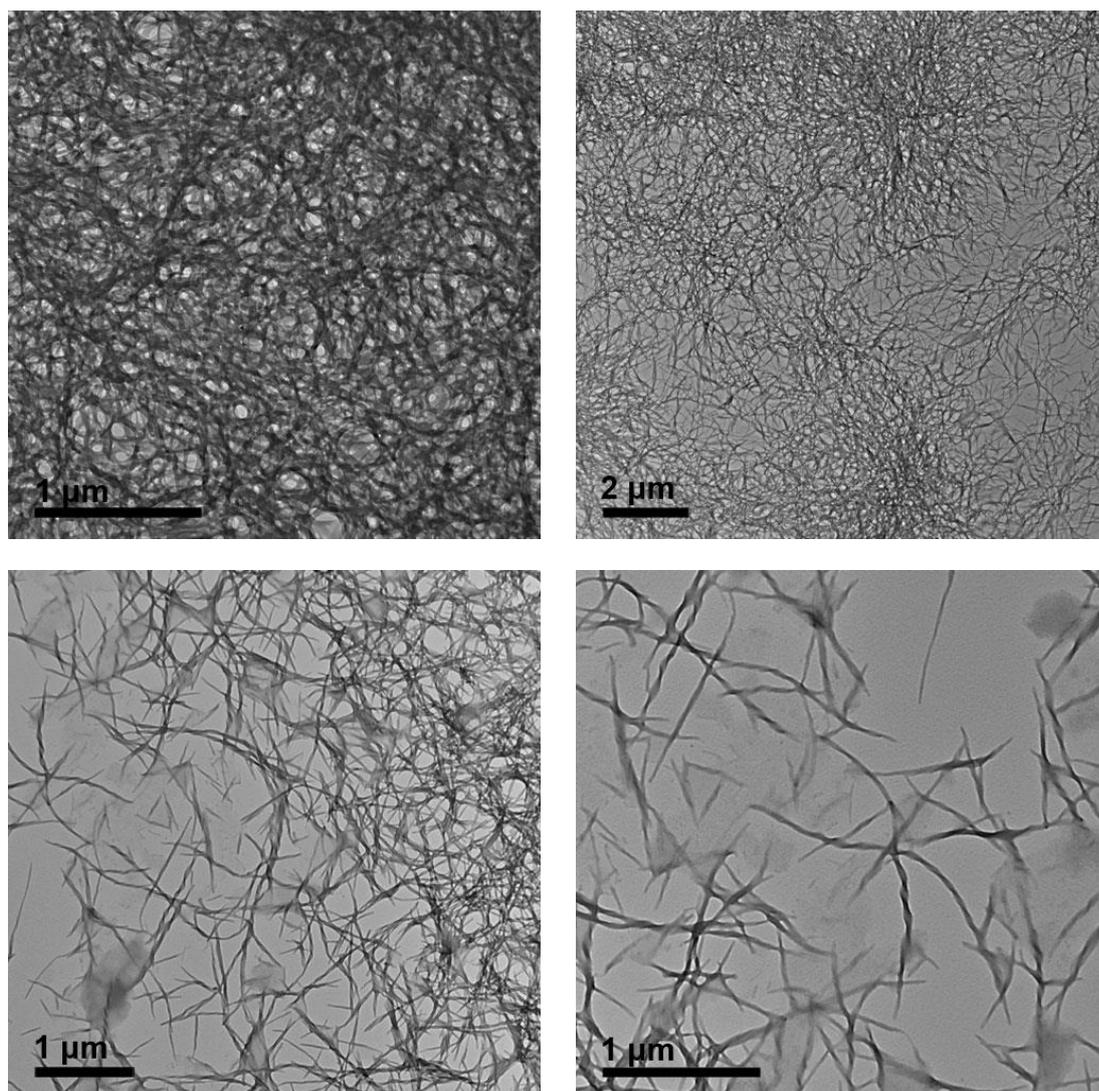


Figure S19. TEM images of **Hx-ABz** methanol/diisopropyl ether gel (fibre width = 41 ± 8 nm) and graph showing variation in pixel intensity along a fibre. Helical pitch is irregular, but averages to approximately 140 nm.

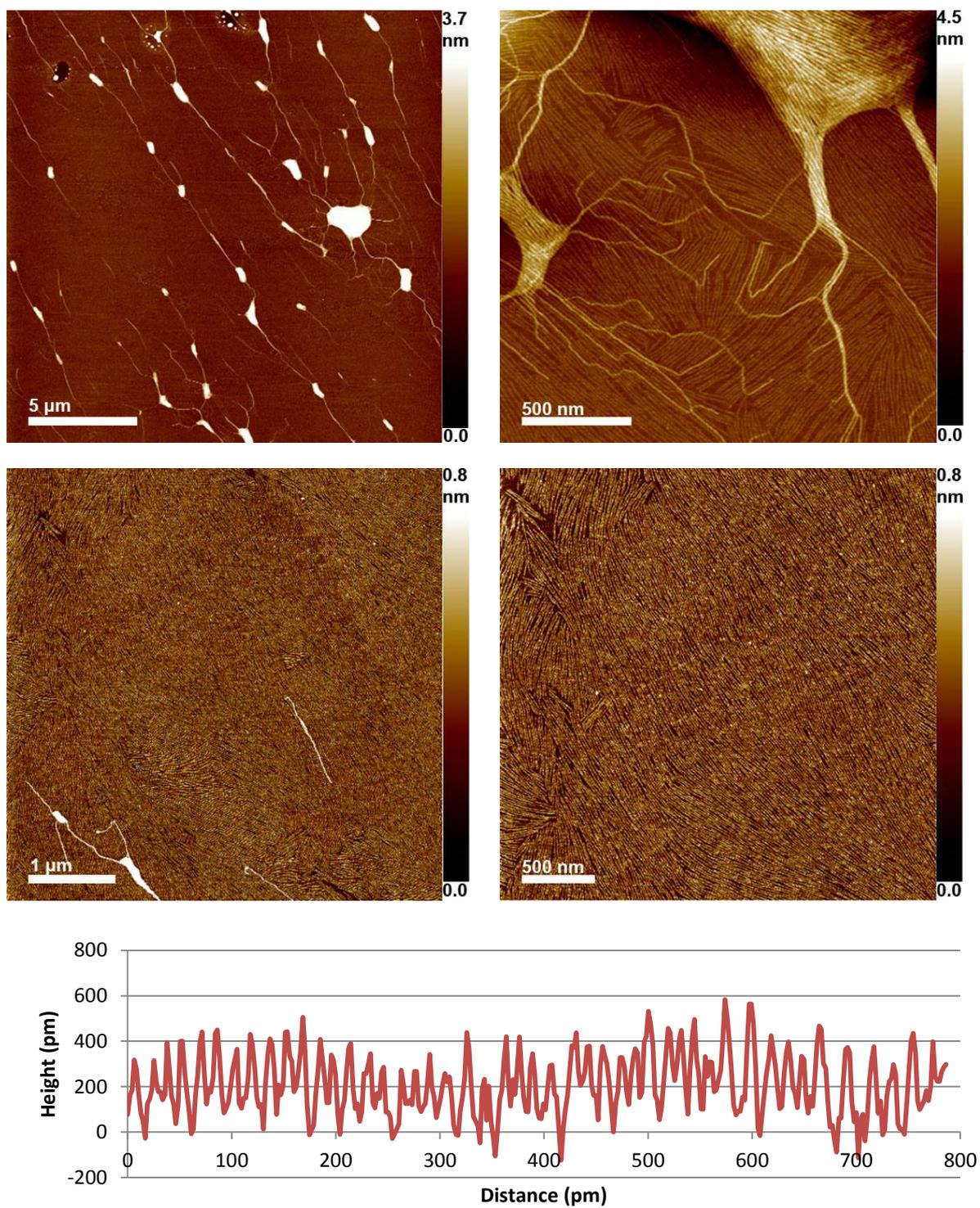


Figure S20. Additional AFM images of **Hx-A** (deposited from 1:9 MeOH:TAEMg), and representative height cross section illustrating regularity of height (400 pm) and spacing (17 nm)

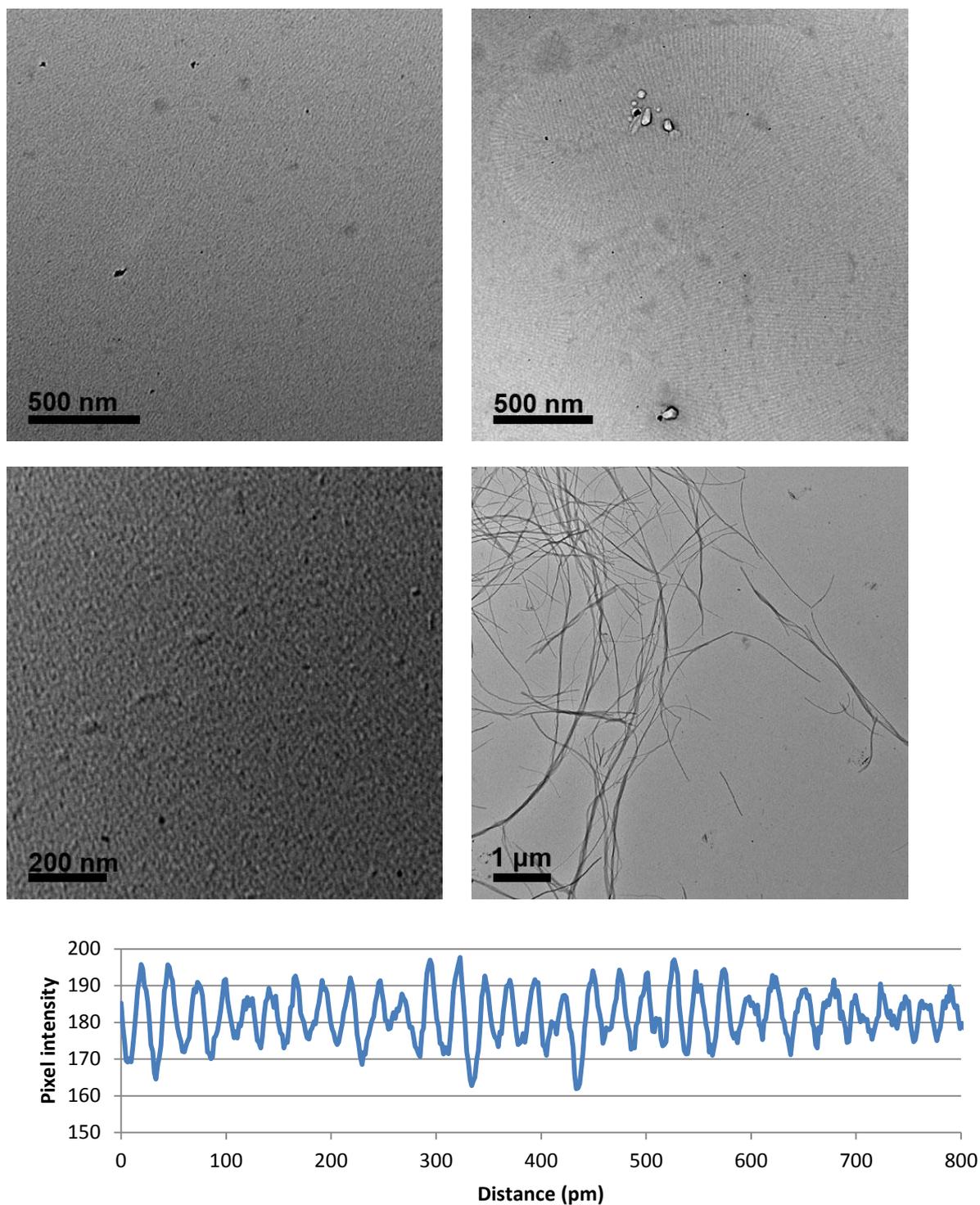


Figure S21. Additional TEM images of **Hx-A** (deposited from 1:9 MeOH:TAEMg, contrast digitally enhanced) including the single observed area with larger rod-like species (bottom right), and representative graph illustrating periodic change in pixel intensity across the ridged portions making up the majority of the sample (mean 24 nm distance between peaks).

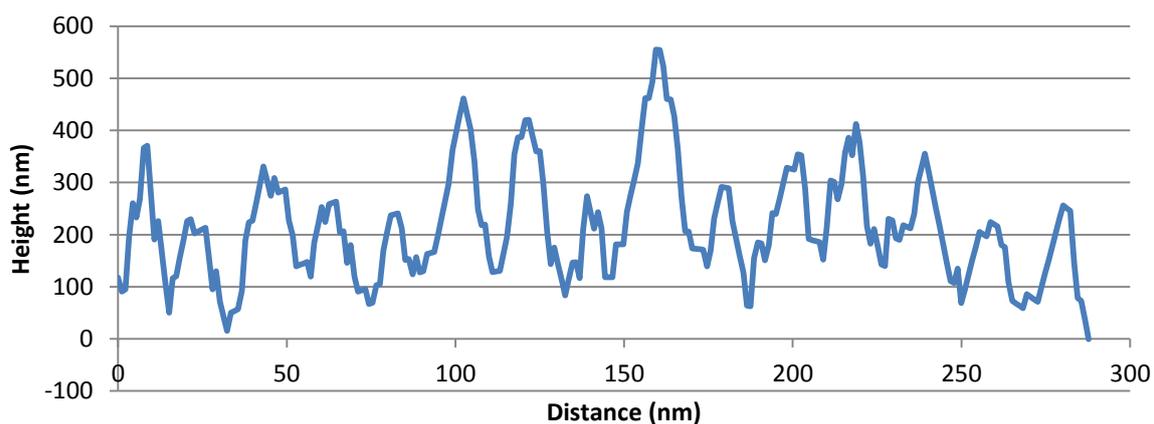
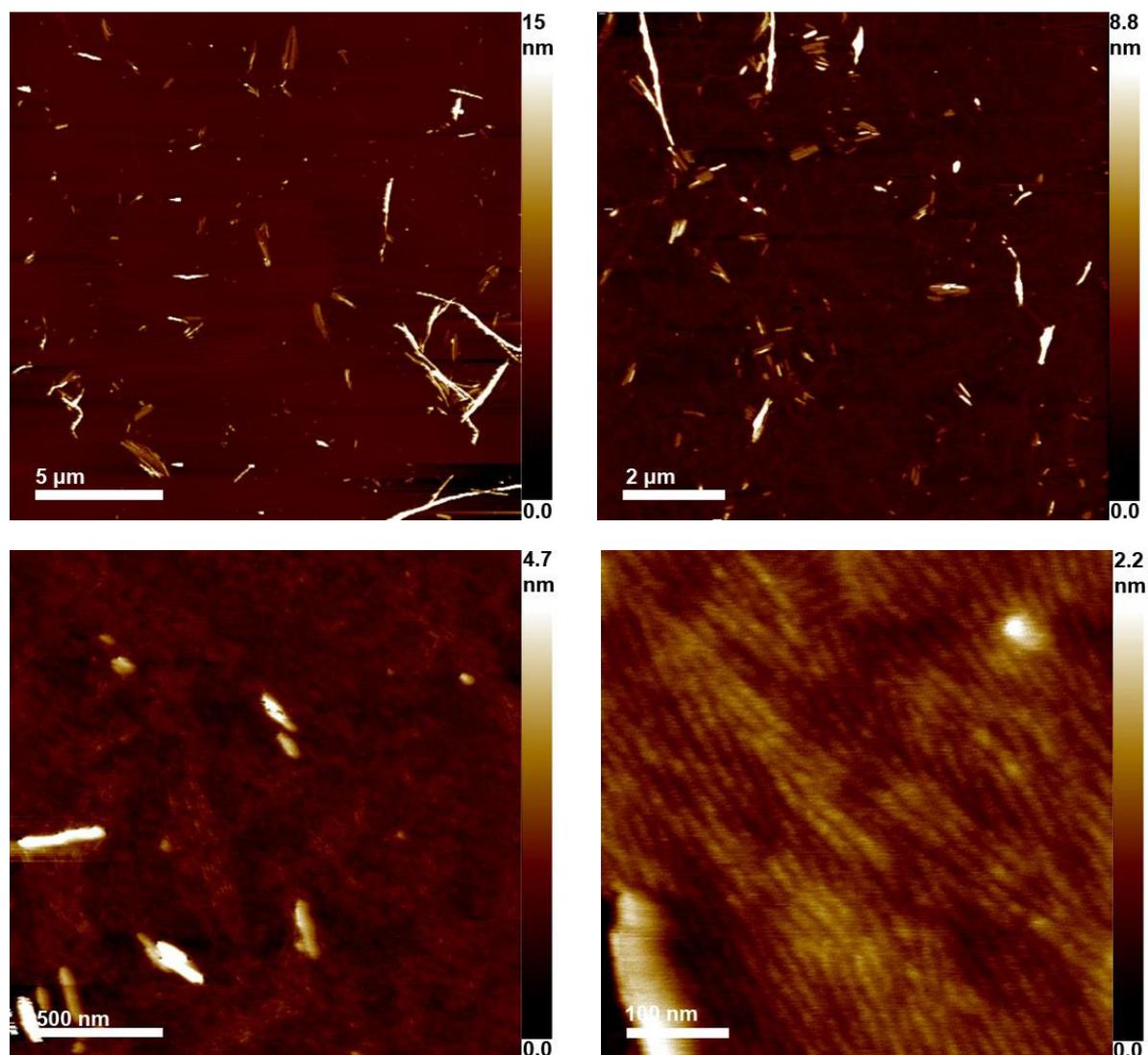


Figure S22. Additional AFM images of Hx-A/T₁₀(deposited from 1:9 MeOH:TAEMg) and representative height cross section of ridged background illustrating regularity of height (200 - 300 pm) and spacing (19 nm) of peaks. Only rods, and no large globular structure decorated with ridges (as in Fig. S3) were seen.

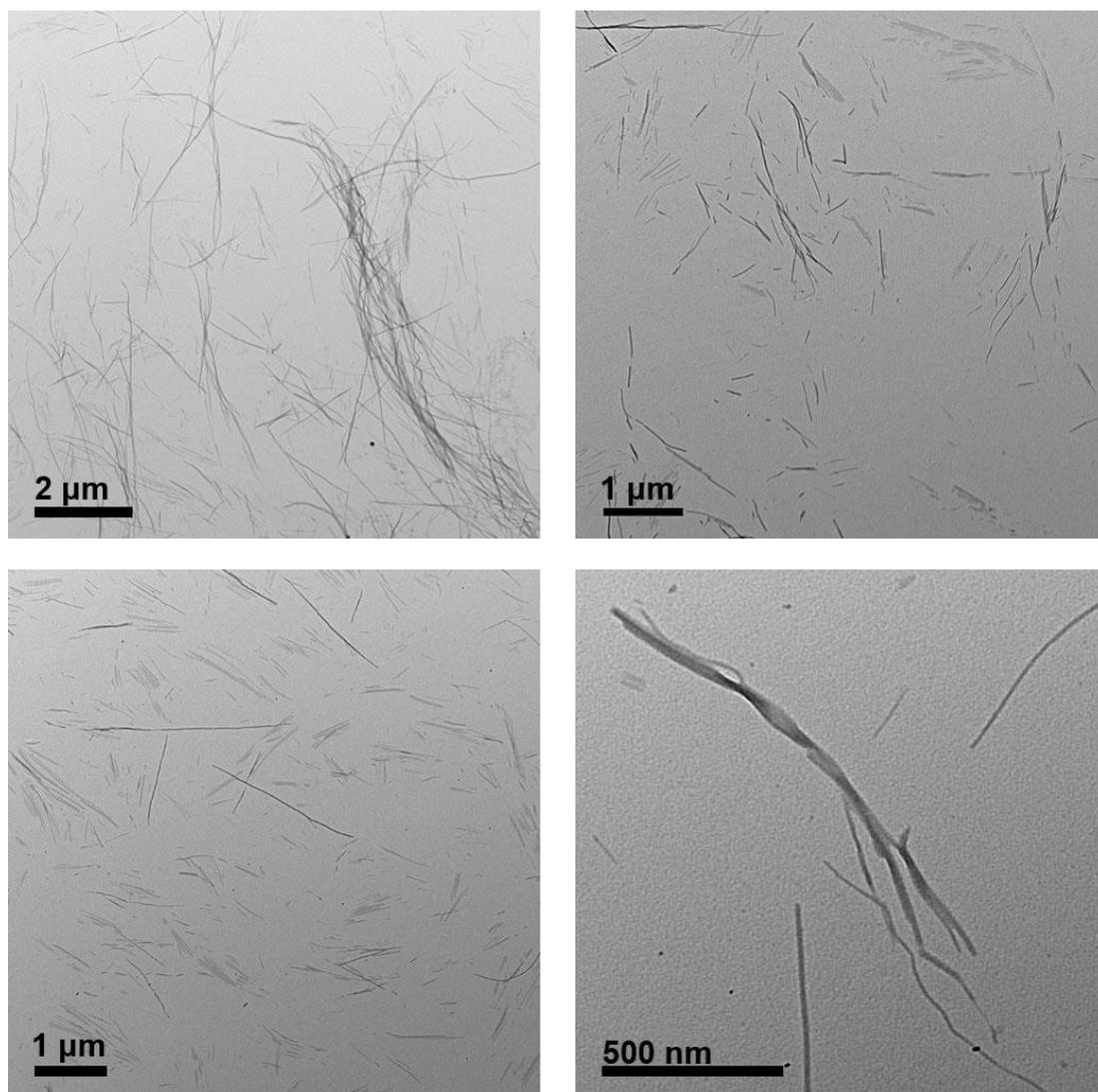


Figure S23. Additional TEM images of **Hx-A/T₁₀** (deposited from 1:9 MeOH:TAEMg).

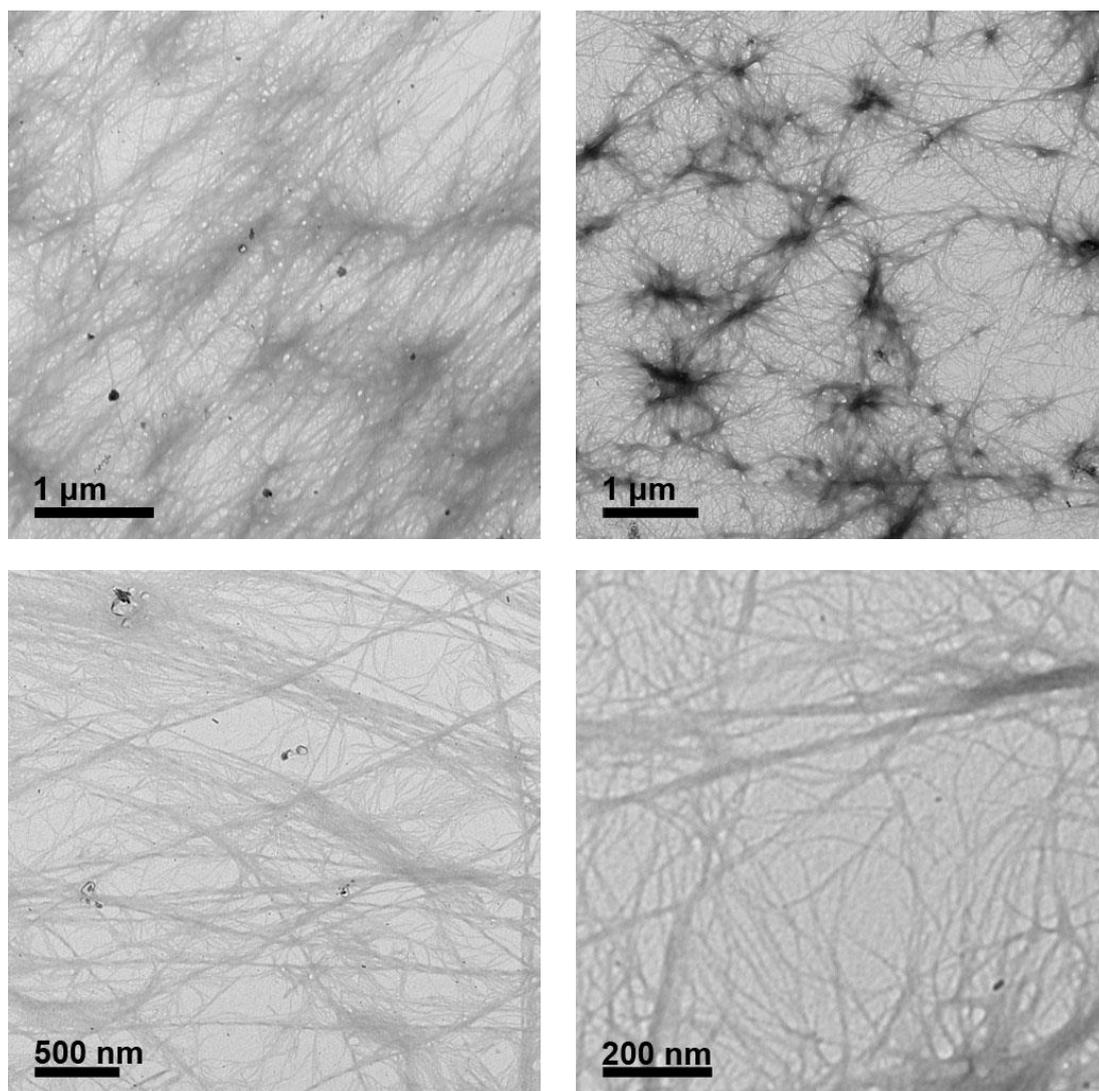


Figure S24. Additional TEM images of **Hx-A/DMSO** hydrogel. Fibre width varies greatly, between 8 and 40 nm.

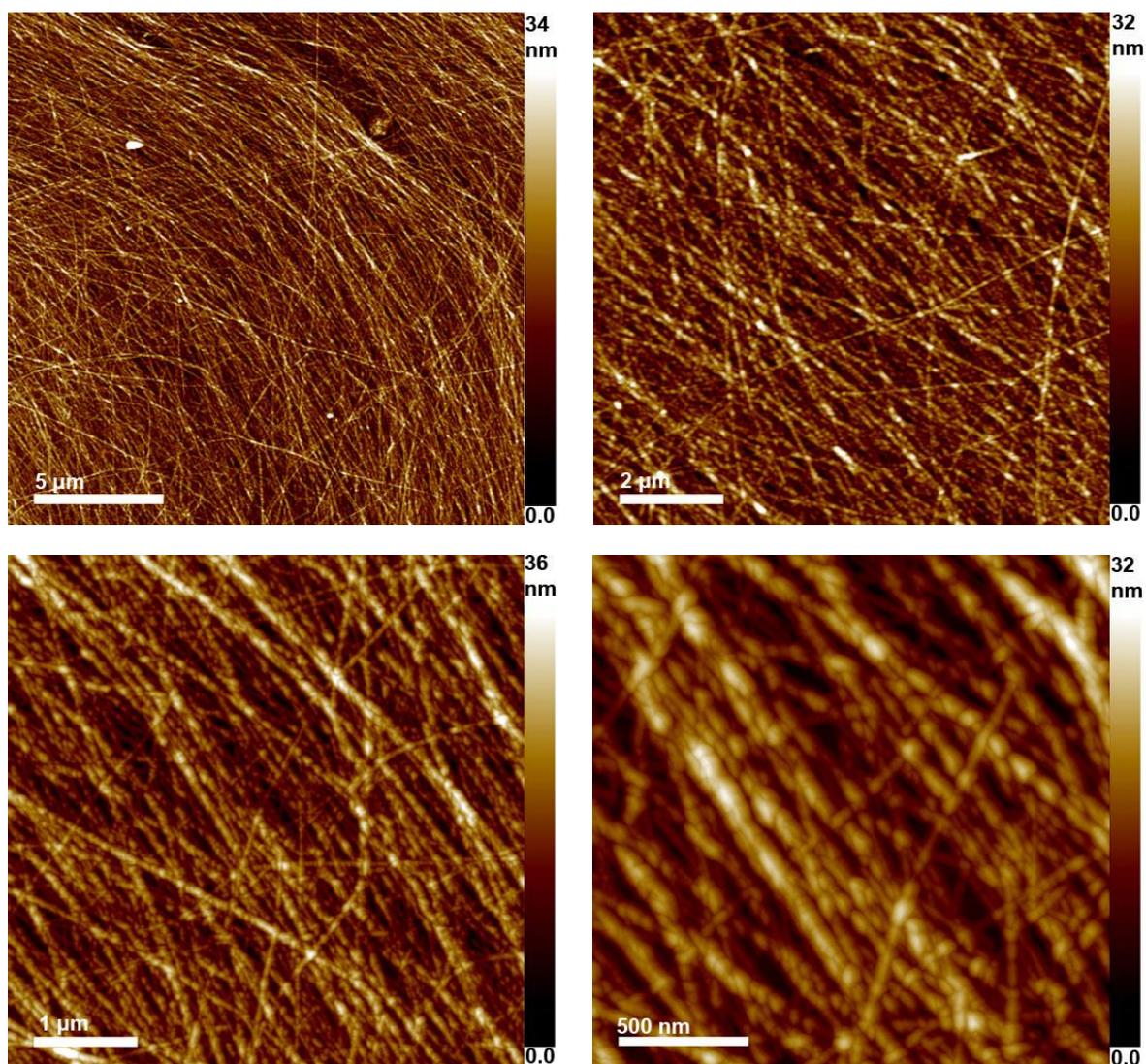


Figure S25. AFM images of **Hx-A/DMSO** hydrogel. Absolute measurement of fibre height is impeded by background of overlapping strands, but is estimated at 10-15 nm.

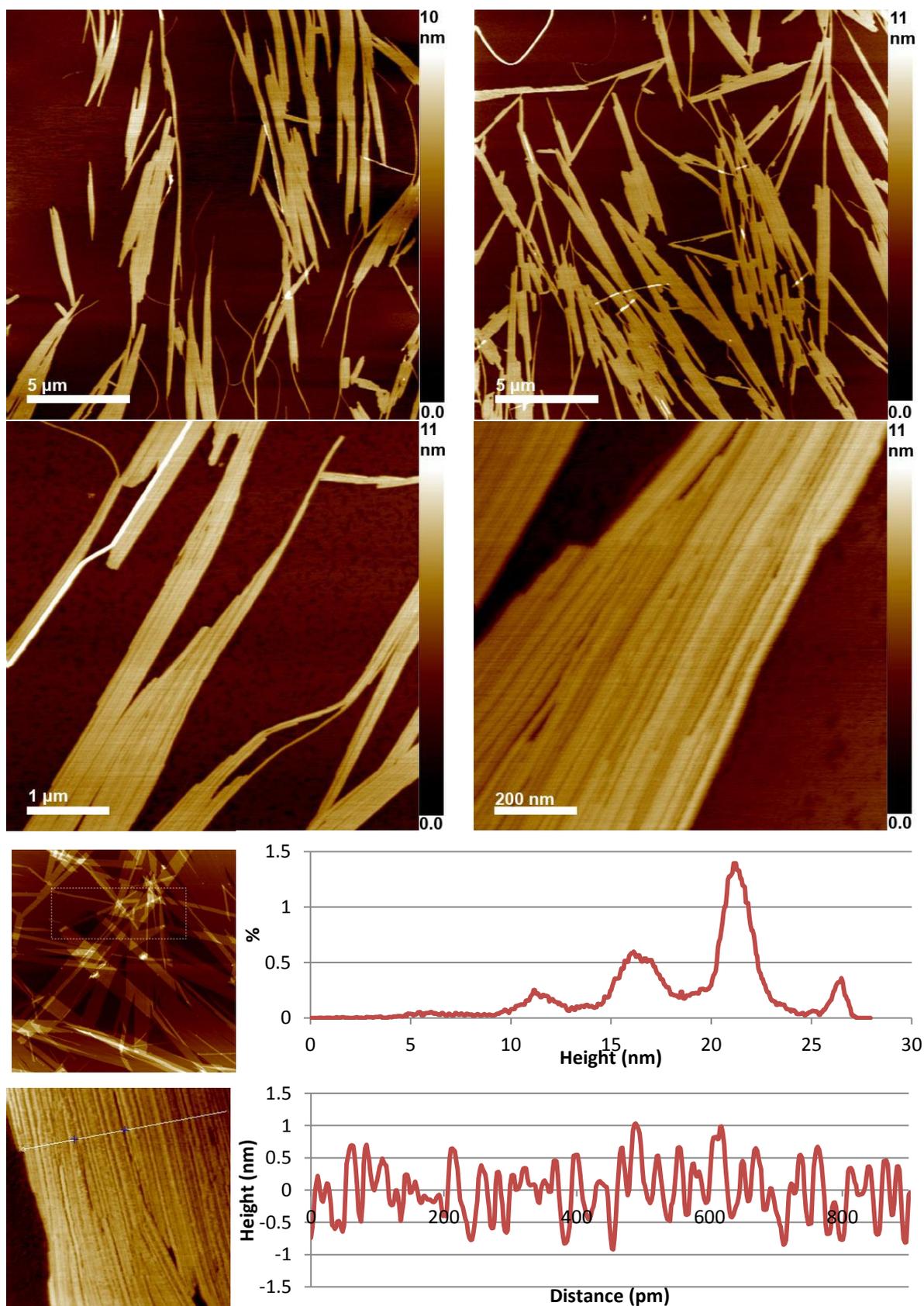


Figure S26. Additional AFM images of **PEG-ABz** (deposited from 1:9 MeOH:H₂O), and height analysis showing peaks for 1, 2, 3, and 4 layers of lamellae (4.9 ± 0.4 nm per layer), and variation of height (ca. 1 nm) across a lamella (mean 26 nm distance between peaks).

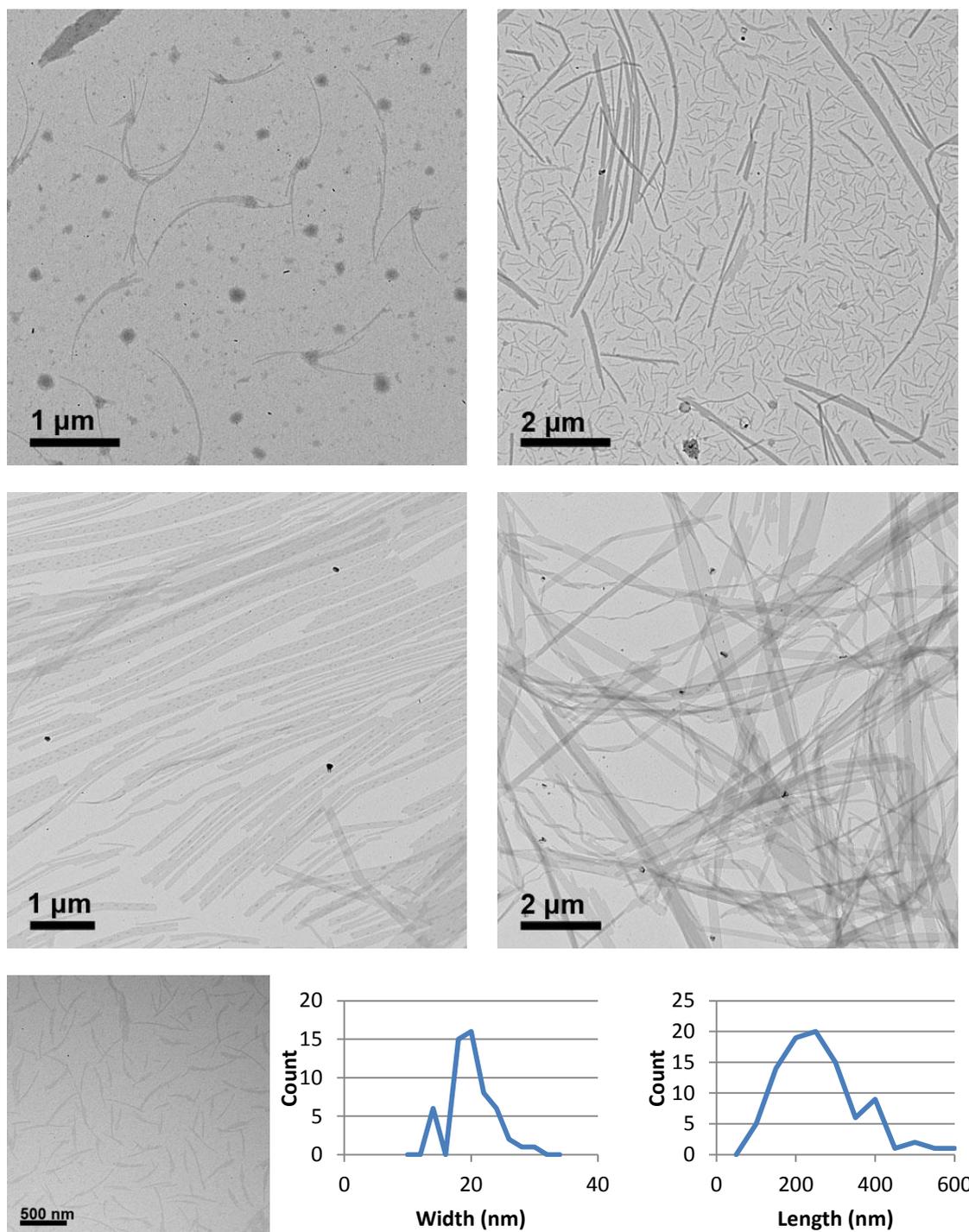


Figure S27. Additional TEM images of **PEG-ABz** (deposited from 1:9 MeOH:H₂O), and size analysis of the curved rod-like micelles (width = 20.1 ± 3.6 nm, length = 287 ± 99 nm).

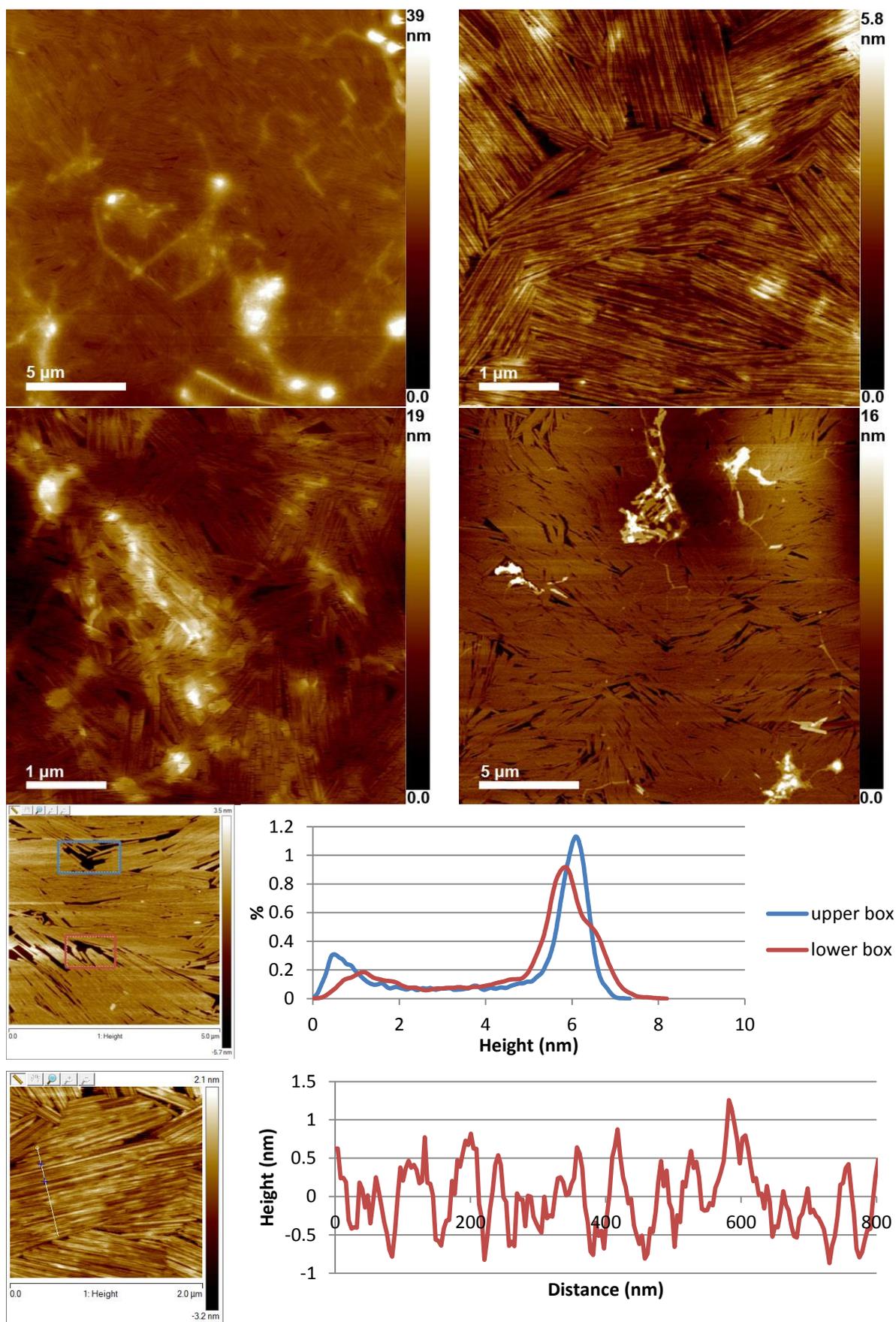


Figure S28. AFM images of PEG-ABz (deposited from 1:9 MeOH:TAEMg) and height analysis (5.1 ± 0.6 nm), and variation of height (ca. 1 nm) across a lamella (mean 38 nm distance between peaks).

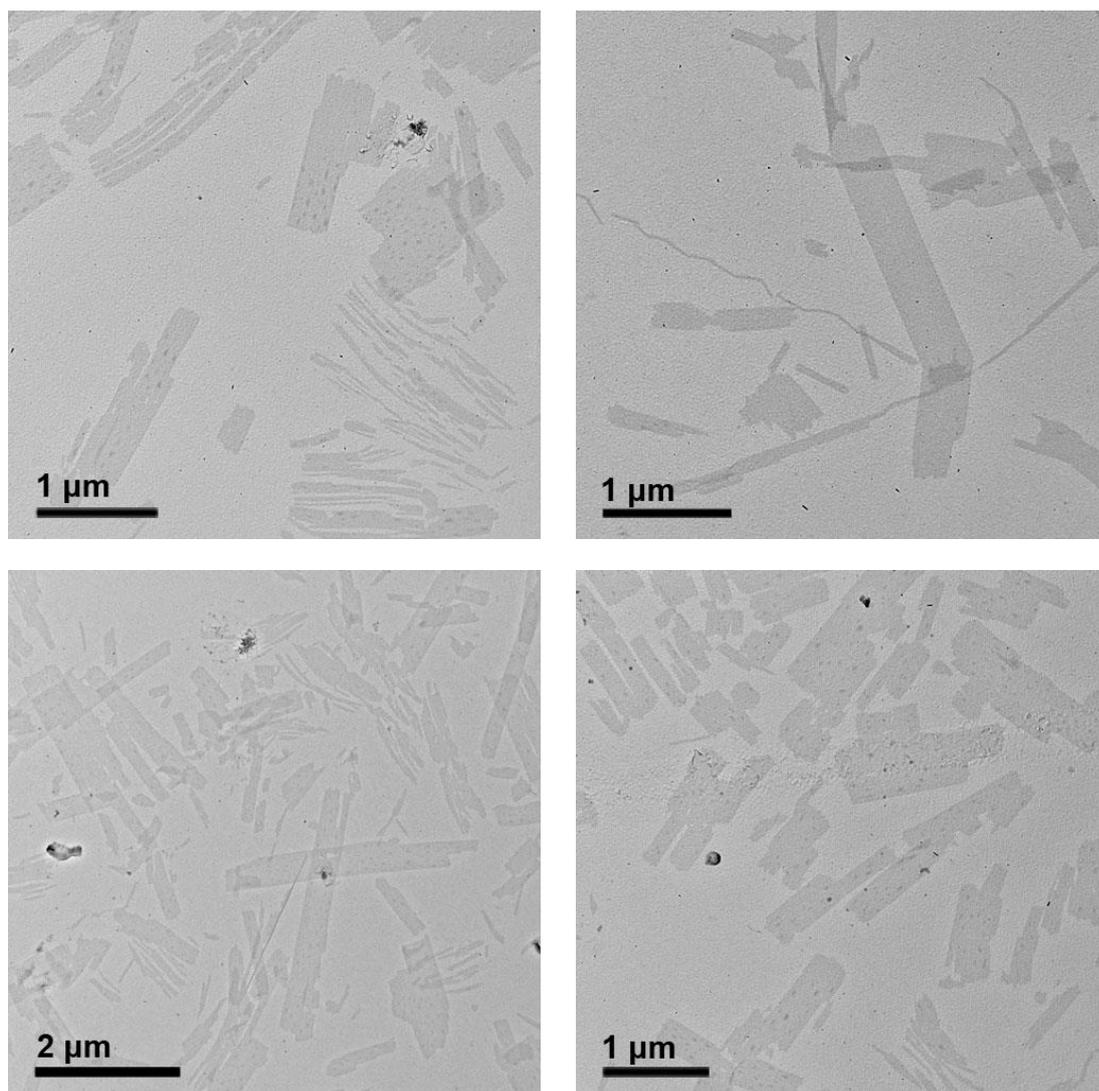


Figure S29. TEM images of **PEG-ABz** (deposited from 1:9 MeOH:TAEMg).

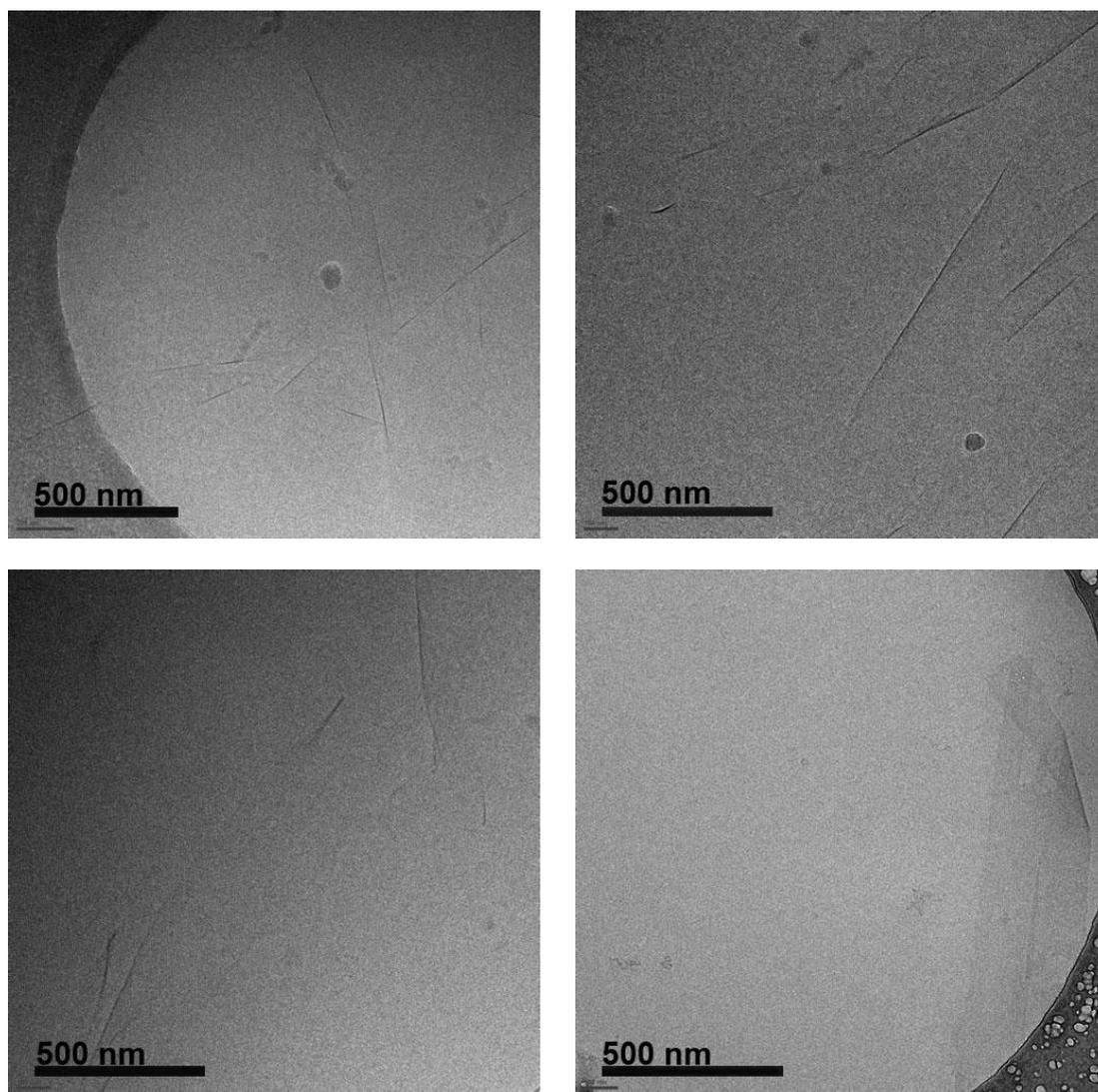


Figure S30. Cryo-EM TEM images of self-assembled **PEG-ABz** in water. In order to locate the nanostructures, the solution was concentrated tenfold compared those used in other studies of **PEG-ABz**.

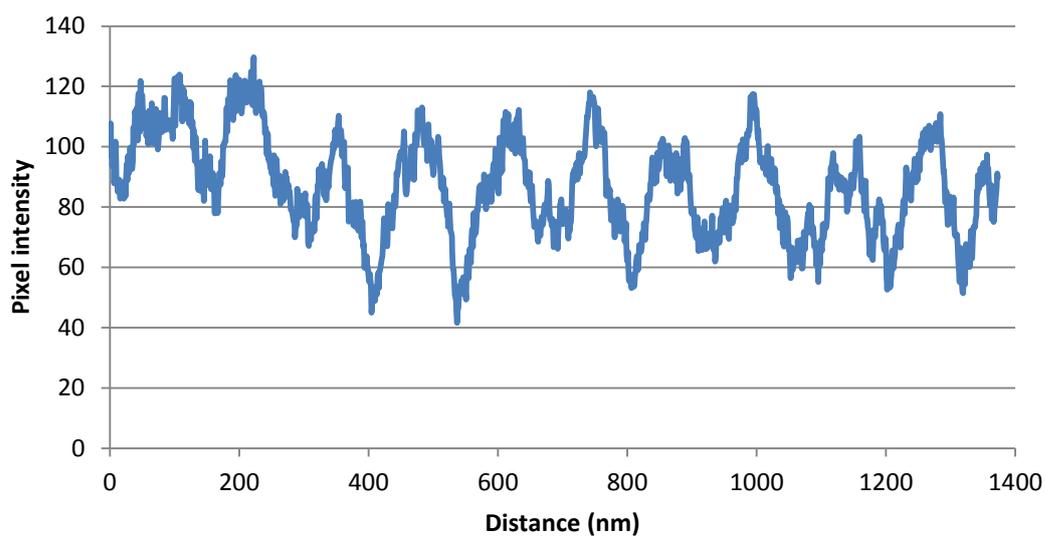
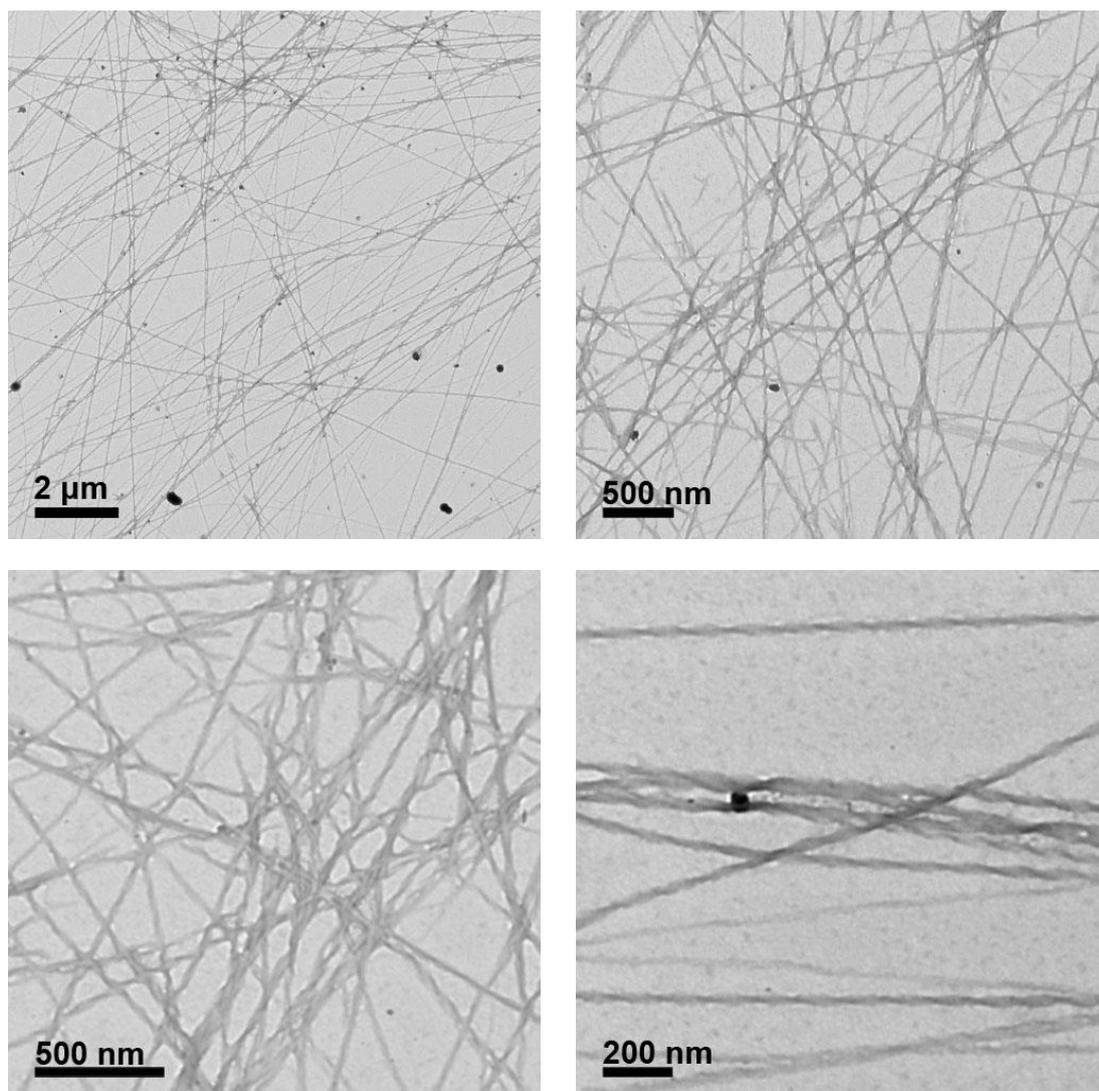


Figure S31. TEM images of **Hx-A/PEG-A** hydrogel (fibre width = 27 ± 5 nm), and variation in pixel intensity along a fibre, indicating twist frequency (average 127 nm/turn).

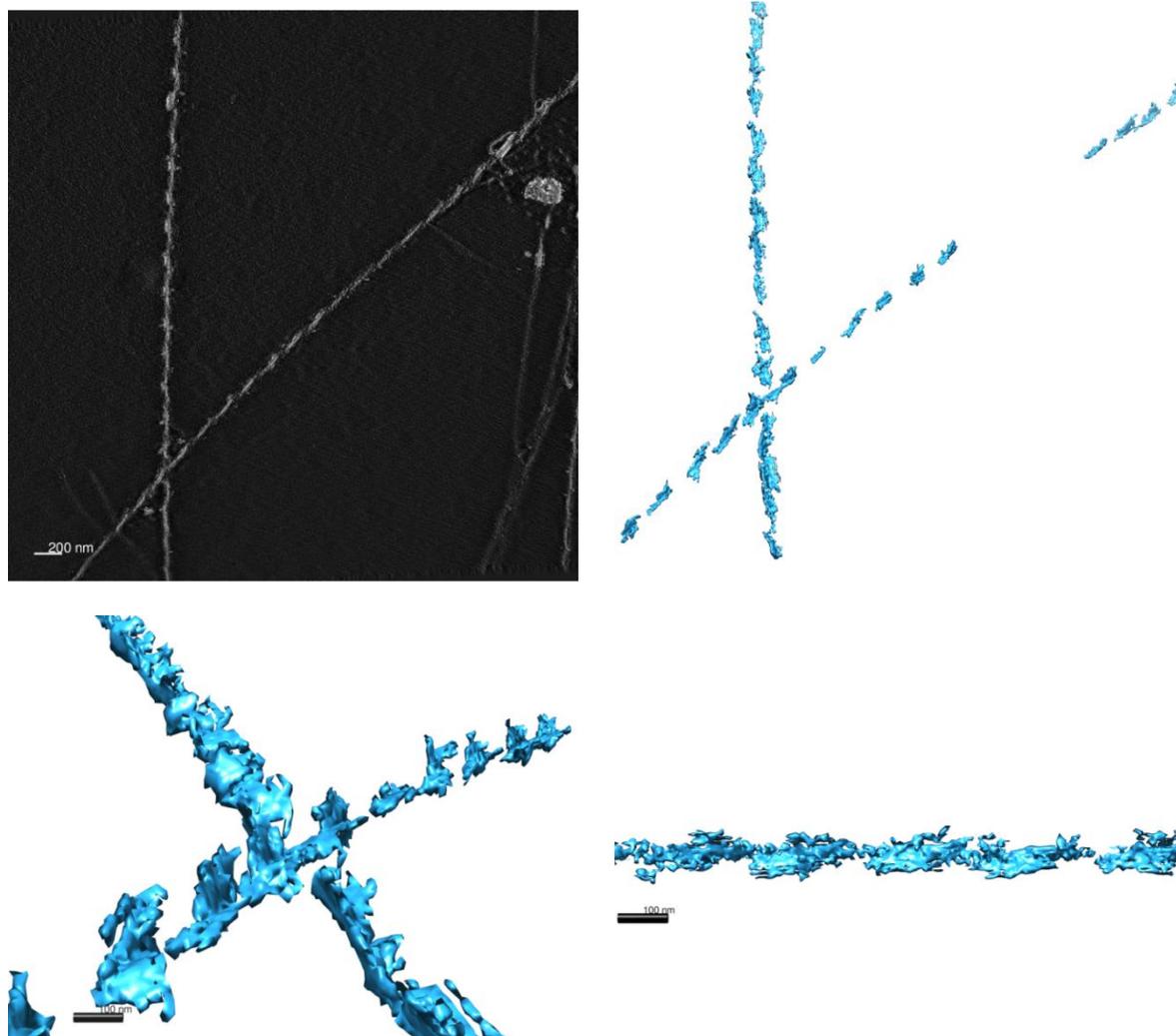


Figure S32. TEM tomographic 3D reconstruction of **Hx-A/PEG-A** hydrogel illustrating left-handed helicity and regularity of period.

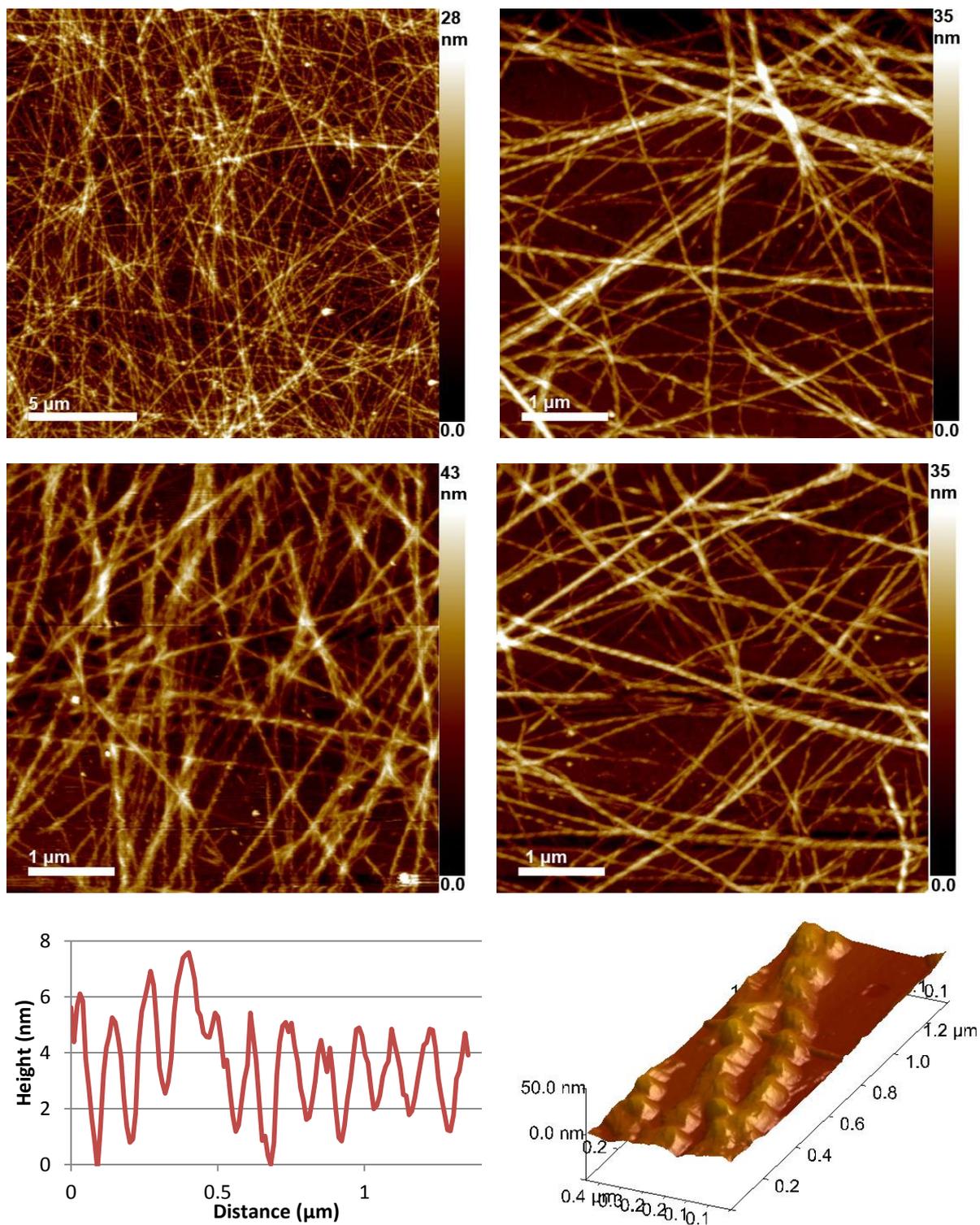


Figure S33. AFM images of **Hx-A/PEG-A** hydrogel, variation in height along a fibre (ca. 4 nm amplitude), indicating average twist frequency of 113 nm/turn, and 3D plot illustrating left-handed helical twist of the fibres.

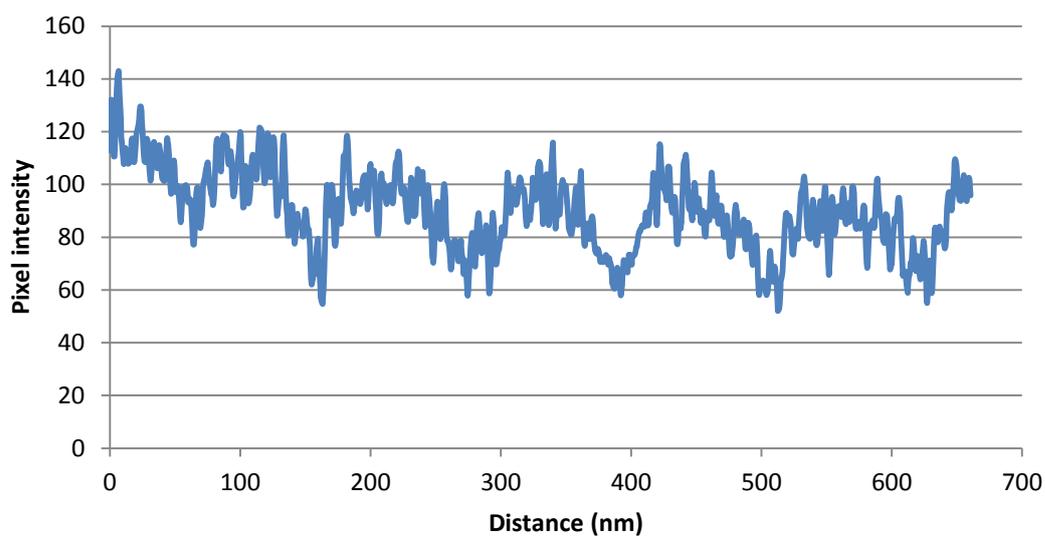
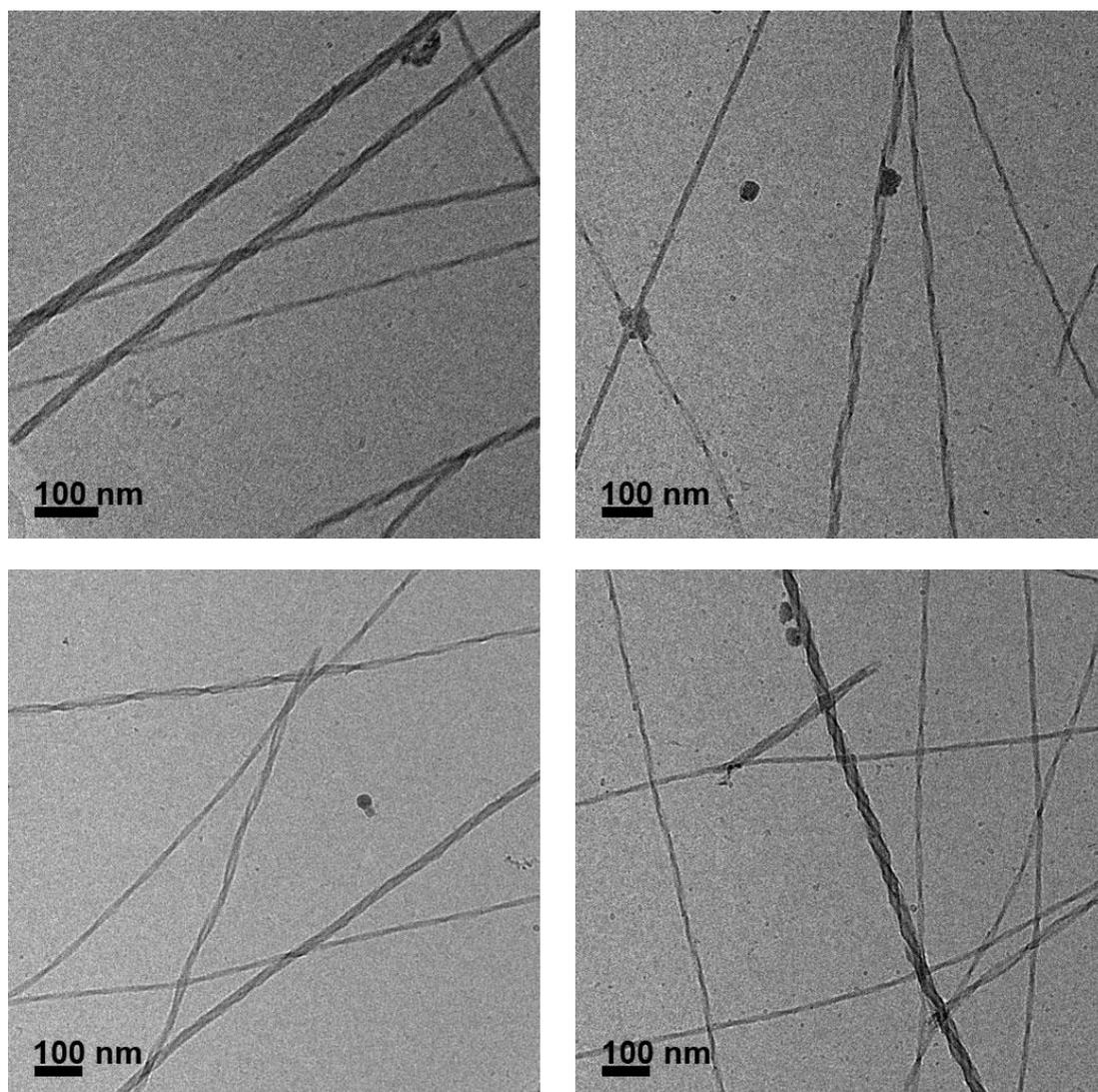


Figure S34. Further cryo-EM images of Hx-A/PEG-A (1:1) frozen from 2 mg/mL solution, and variation in pixel intensity along a fibre, indicating twist frequency (average 110 nm/turn).

CIRCULAR DICHROISM

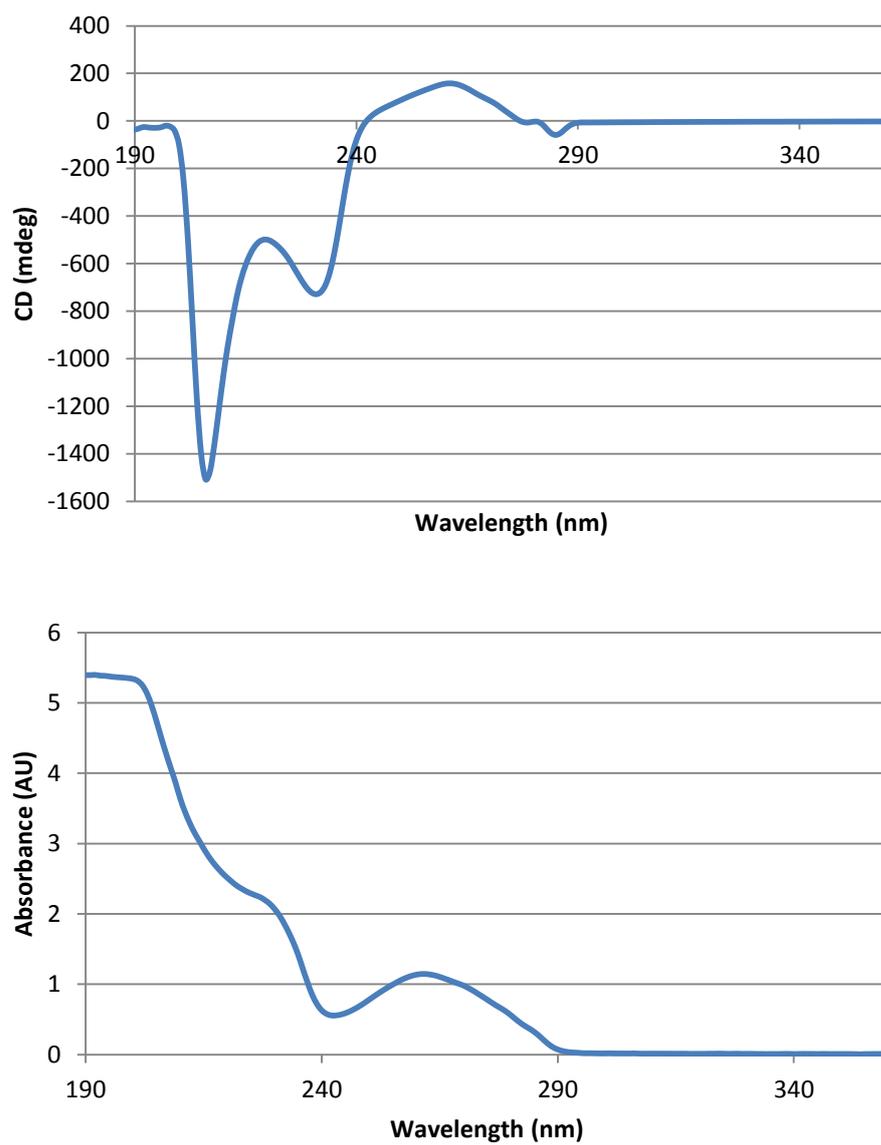


Figure S35. CD (top) and absorbance (bottom) spectra of **Hx-A/PEG-A (1:1)** at 1 mg/mL in water.

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- (7) Zhong, M.; Strobel, S. A. *J. Org. Chem.* **2007**, *73*, 603-611.
- (8) The HR-MS suggests methyl ester deprotection in addition to benzoyl deprotection. However, the methanolysis conditions are well established for N-benzoyl protection in the presence of a methyl ester, and the moderately reduced polarity of the product, as measured by TLC, is not consistent with a carboxylic acid. Furthermore, as a control, deprotection of both the exocyclic amine and the carboxylic acid could be achieved using hydroxide bases, giving a much more polar product (by TLC). Finally, using a different instrument, the $[M+H]^+$ peak for the intact product (704.4 m/z) was observed by LC-MS, instead of the free acid. We therefore regard the apparent loss of the methyl group as an artifact arising from the ionisation conditions.
- (9) As with Hx-A, we regard the apparent loss of the methyl protecting group as an artifact arising from ionisation conditions.