Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Self-Assembly of the Anti-Fungal Polyene Amphotericin B into Giant Helically-Twisted Nanotapes

Ian William Hamley, *,[a] Steven Kirkham,[a] Radoslaw M. Kovalczyk,[a] Valeria Castelletto,[a] Mehedi Reza,[b] and Janne Ruokolainen[b]

[a]*School of Chemistry, Pharmacy and Food Biosciences, University of Reading, Whiteknights, Reading, RG6 6AD, U.K.*

[b]*Department of Applied Physics, Aalto University School of Science, P.O. Box 15100 FI-00076 Aalto, Finland*

Experimental Methods

Materials

Amphotericin B (AmB) was obtained in the form of powder from Merck Millipore (Nottingham, U.K.). The purity of the compound was determined by analytical HPLC to be 92.5%, and the molecular weight was obtained by electrospray-mass spectrometry to be 924.1 Da. In this work, weighed amount of AmB were dissolved in ultrapure water from a Barnstead Nanopure system to the desired concentration. The pH was then adjusted to 12 through addition of NaOH from Fisher Scientific (USA), dissolved pellets in water to give a 5 M solution.

Pyrene fluorescence

Fluorescence spectra were recorded with a Varian Cary Eclipse Fluorescence Spectrometer with samples in 4 mm inner Quartz cuvettes. The assays were performed using $1.3x10^{-3}$ – 0.13 wt.% AmB, in 2.3 x 10^{-5} wt.% pyrene solution. The samples were excited at λ_{ex}=380 nm, and the fluorescence emission was measured for λ= (400-550nm).

Cryo-TEM

Experiments were carried out using a field emission cryo-electron microscope (JEOL JEM-3200FSC) operating at 200 kV. Images were taken using bright-field mode and zero loss energy filtering (omega type) with a slit with 20 eV. Micrographs were recorded using a Gatan Ultrascan 4000 CCD camera. The specimen temperature was maintained at -187 °C during the imaging. Vitrified specimens were prepared using an automated FEI Vitrobot device using Quantifoil 3.5/1 holey carbon copper grids with 3.5 μm hole sizes. Grids were

cleaned using a Gatan Solarus 9500 plasma cleaner just prior to use and then transferred into an environmental chamber of FEI Vitrobot at room temperature and 100% humidity. Thereafter, 3 μl of sample solution at 1wt.% concentration was applied on the grid, blotted once for 1 second and then vitrified in a 1/1 mixture of liquid ethane and propane at -180 °C. Grids with vitrified sample solutions were maintained in a liquid nitrogen atmosphere and then cryo-transferred into the microscope.

Small-angle X-ray scattering (SAXS)

Experiments were performed on beamline B21 at Diamond Light Source, Harwell, UK. Solutions of Amphotericin B (0.5, 1 and 2 wt%) were loaded into the 96 well plate of an EMBL BioSAXS robot. Solutions were then injected via an automated sample exchanger at a slow and very reproducible flux into a quartz capillary (1.8 mm internal diameter) in the X-ray beam. The quartz capillary was enclosed in a vacuum chamber, in order to avoid parasitic scattering. After the sample was injected in the capillary and reached the X-ray beam, the flow was stopped during the SAXS data acquisition. B21 operated with a fixed camera length (3.9 m) and fixed energy (12.4 keV). The images were captured using a Pilatus 2M detector. Data processing (background subtraction, radial averaging) was performed using the dedicated beamline software Scatter.

Circular Dichroism (CD)

CD spectra were recorded using a Chirascan spectropolarimeter (Applied Photophysics, UK). The sample (0.01 -1 wt.% in water, pH raised to 11 with NaOH) was placed in a cover slip cuvette (0.1 mm thick). Spectra are presented with absorbance $A < 2$ at any measured point with a 0.5 nm step, 1 nm bandwidth, and 1 second collection time per step at 20 °C. In

temperature-dependent experiments (results not shown), the AmB solution was acclimatised at each temperature point for 5 minutes before measurements were taken. The CD signal from the pH 12 alkalised water was subtracted from the CD data of the AmB solution.

Fourier Transform Infrared Spectroscopy (FTIR)

Spectra were recorded using a Thermo Scientific Nicolet IS5 and a Nexus-FTIR spectrometer, both equipped with a DTGS detector. A 20 μL drop of sample (0.1 wt.% in water, pH raised to 12) was analysed using an iD7 ATR accessory, consisting of a surface with a diamond crystal the drop is placed onto, and pressure device, which was lowered onto the drop. Spectra were scanned 128 times over the range of 900-4000 cm^{-1}.

Mass Spectroscopy. Electrospray-ionization mass spectra were recorded using a ThermoFisher Orbitrap XL instrument. Samples, which were presented as 1 mg/mL, were diluted 33 fold (30 μL sample + 970 μL diluent) in water containing 0.1% formic acid.

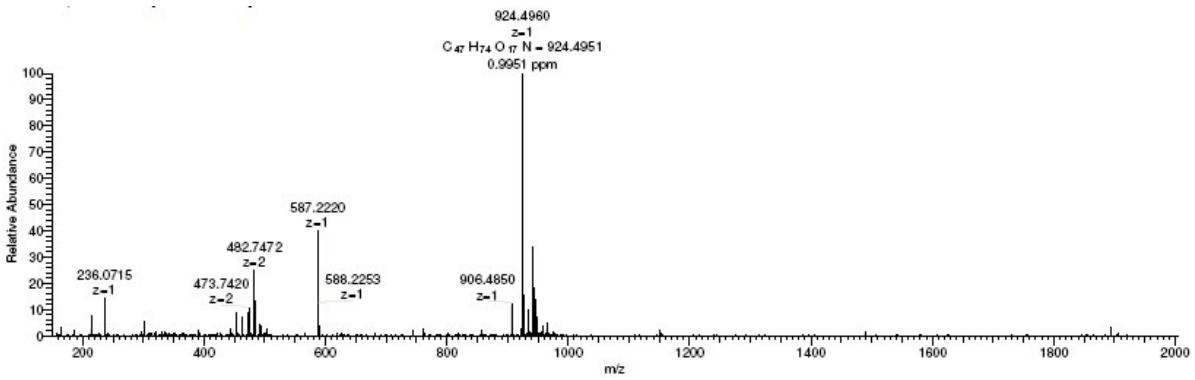
Nuclear Magnetic Resonance (NMR)

The NMR experiments were performed on D_2O solutions of amphotericin B at 0.3 wt% concentration, at room temperature. The proton spectra (^1H) were recorded on a Bruker 500 MHz Avance III spectrometer (11.74 T) equipped with a BBO triple channel probe. The standard Bruker nosypr1d pulse sequence was used to saturate the dominating water signal that originated from the exchange of protons between the Amphotericin and deuterated solvent. No ^{13}C signal was observed on this instrument.

The 1 mM solution allowed recording both ^{1}H and ^{13}C spectra on Bruker 700 MHz Avance III spectrometer (16.44T) which was equipped with a four channel cryoprobe with improved sensitivity. The 64 transient were recorded using nosypr1d pulse sequence and average into ^{1}H spectrum. The proton decoupled ^{13}C experiment required 8192 transients, recorded with 4s relaxation delay and averaged into carbon spectrum. COSY, DEPT135 and HSQC spectra were also recorded. All spectra were referenced to the TMS signal at 0 ppm.

Assignment of the resonances is based on the 700 MHz data (both 1D and 2D) with the assistance of ^{1}H 500 MHz spectrum which show better resolution of multiples than corresponding 700 MHz spectrum. The presence of the broad signal and line broadening of other resonances in the 700 MHz spectra is the consequence of the aggregation of amophotericin molecules at higher concentration. The small variation in the chemical shifts between 500 and 700 MHz proton spectra are also attributed to the difference in the sample concentration. The assignment is in agreement with previously published data.[1]

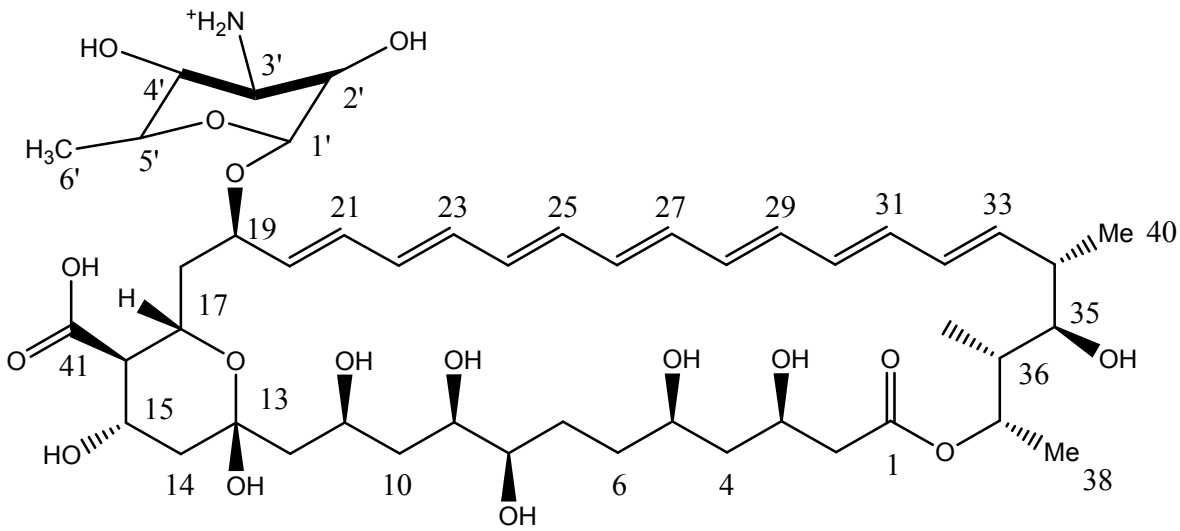
Mass Spectroscopy Data on a Sample at pH 11

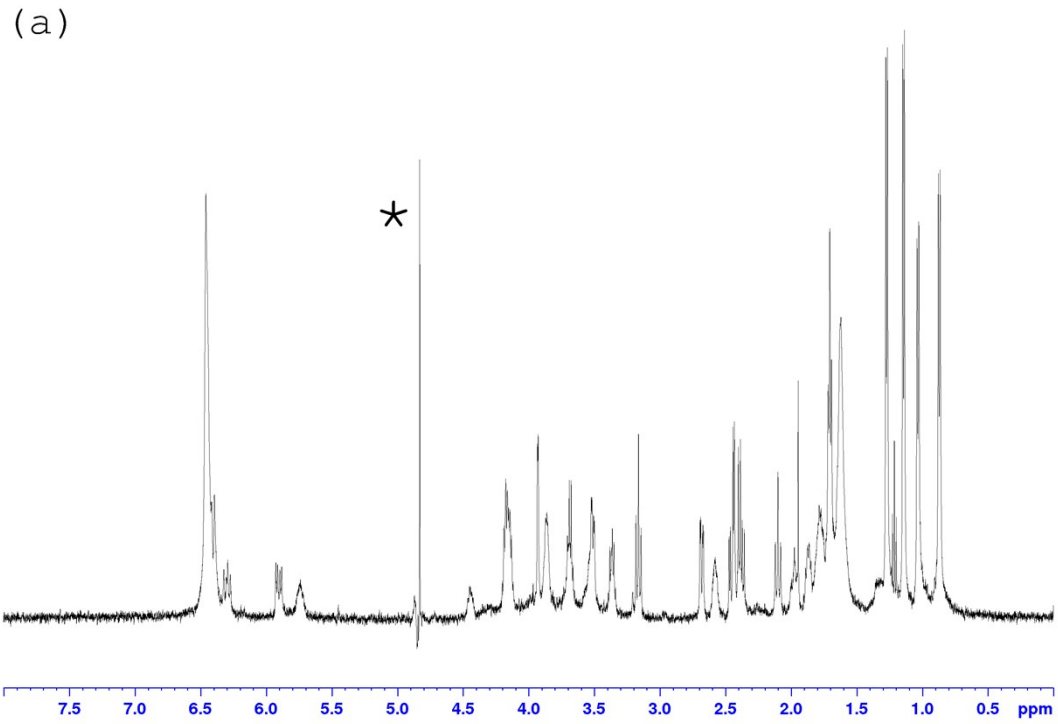


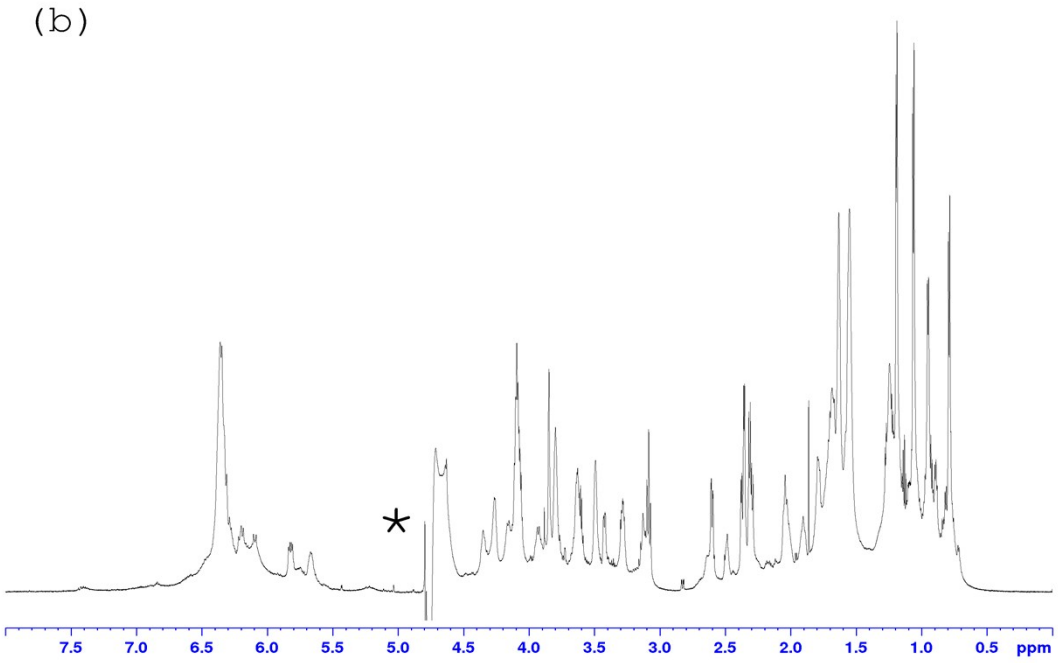
SI Fig.1 ES-MS spectra for a sample at pH 11, measured one week after sample preparation.

The expected molar mass is 924.1 g mol^{-1}.

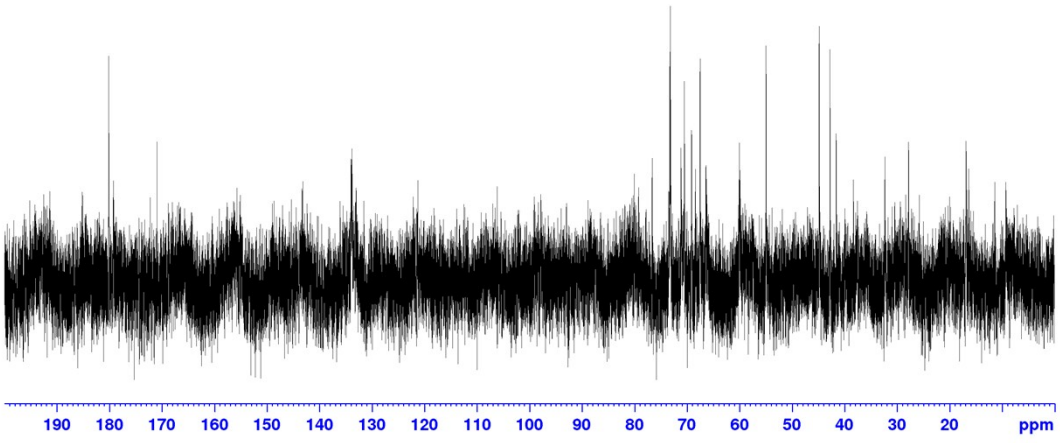
NMR Spectrum







SI Fig.2. ^{1}H NMR spectra recorded on 500 MHz (a) and 700 MHz (b) spectrometers at room temperature. Asterisk denotes the residue of the saturated water signal.



SI Fig.3. ^{13}C NMR spectrum recorded on 700 MHz spectrometer at room temperature.

SI Table.1. Assignment of the resonances in the 700 MHz NMR spectra.

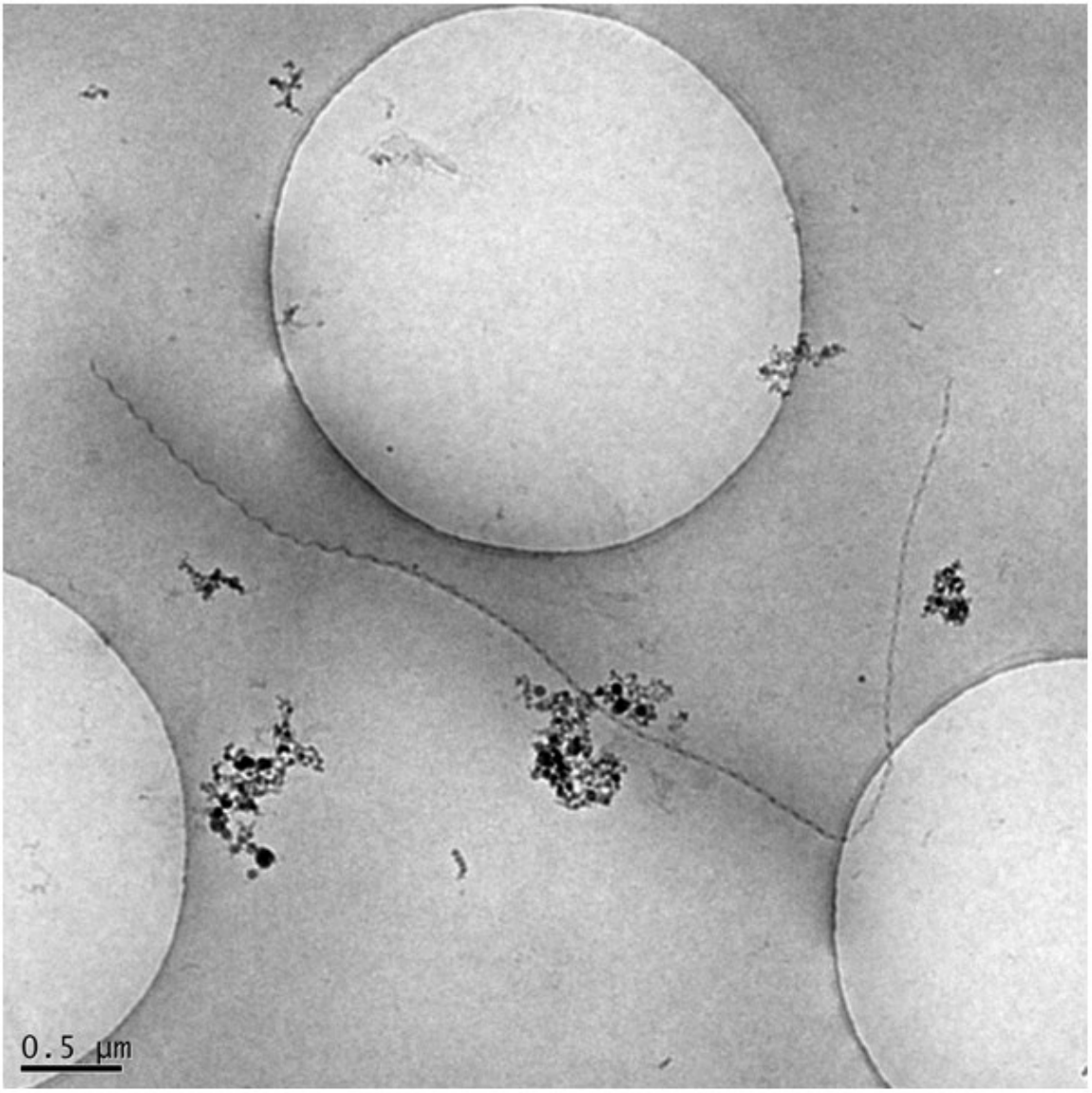
Carbon atom position in the molecule	^{13}C chemical shift / ppm	^{1}H chemical shift of the corresponding proton(s) / ppm
C1	179.3	-
C2	42.8	1.63
C3*	66.3	ca. 4.10
C4	44.8	2.36
C5	71.2	3.60
C6	32.3	1.56
C7	27.9	1.55
C8*	73.4	ca. 3.29
C9*	73.4	ca. 3.29
C10	40.1	1.68
C11*	67.5	ca. 4.10
C12	37.8	1.73
C13	171.0	-
C14	44.8	2.31
C15	67.6	4.12
C16		
C17		
C18*	ca. 40	1.73
C19	77.9	4.38
C20	132.6	5.67
Chain C21-C31*	ca. 133.5	6.4 - 6.0
C32	130.4	6.20
C33	139.6	5.82
C34	38.3	2.50
C35	76.7	3.45
C36	41.6	1.81

C37*	66.2 (or 67.5)	4.27 (or 4.05)
C38	16.4	1.06
C39	9.3	0.79
C40	11.4	0.95
C41	180.1	-
C1’		
C2’	70.5	3.85
C3’	55.0	2.60
C4’	73.1	3.10
C5’	73.2	3.30
C6’	16.9	1.18

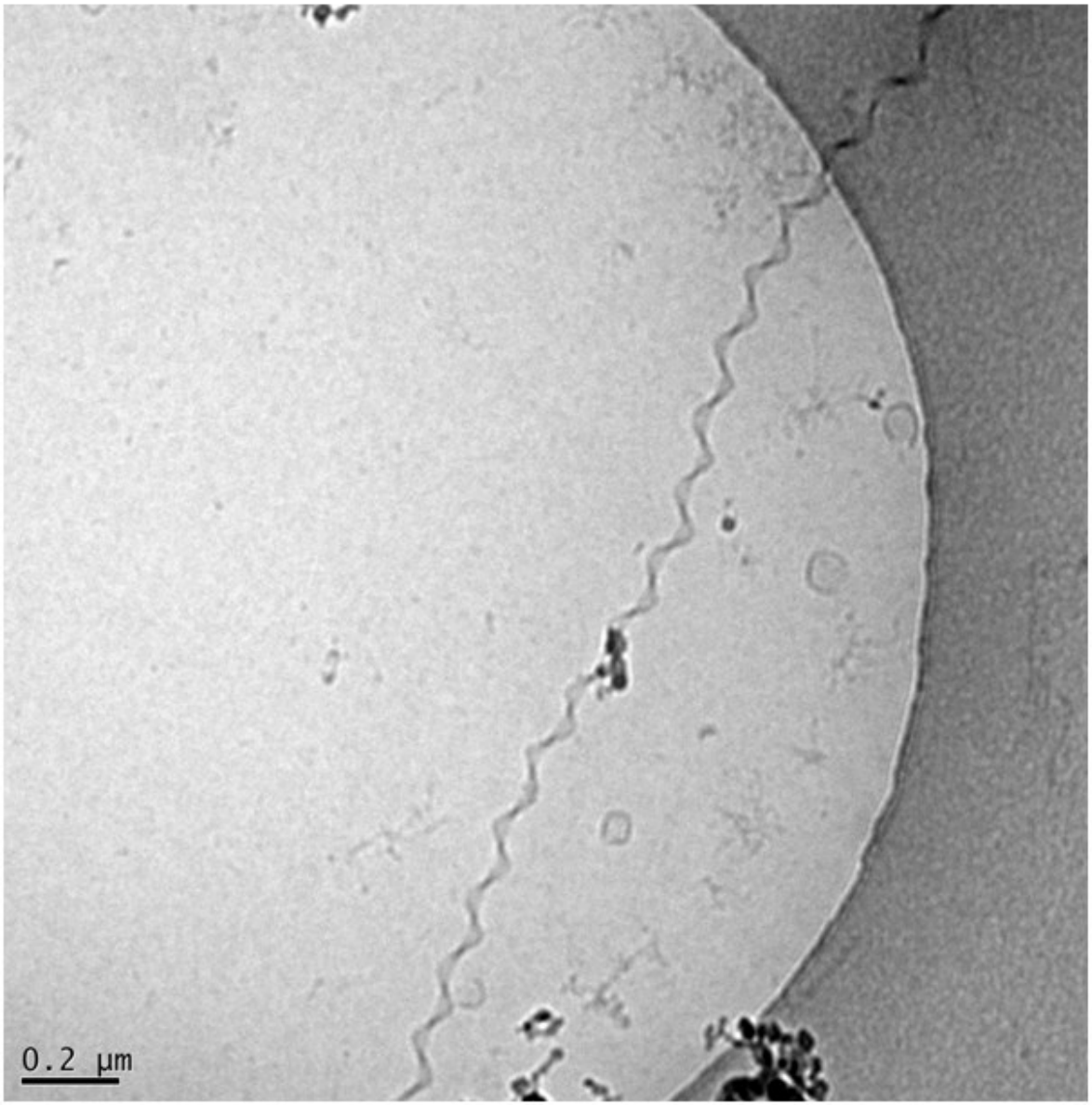
***** Asterisk indicates the tentative assignment because of the low resolution due to broadening and strong overlap, or resonances falling into region of the saturated water signal. The assignment of the carbons C16, C17 and C1’ was not possible on the basis of recorded spectra.

Additional Cryo-TEM Images

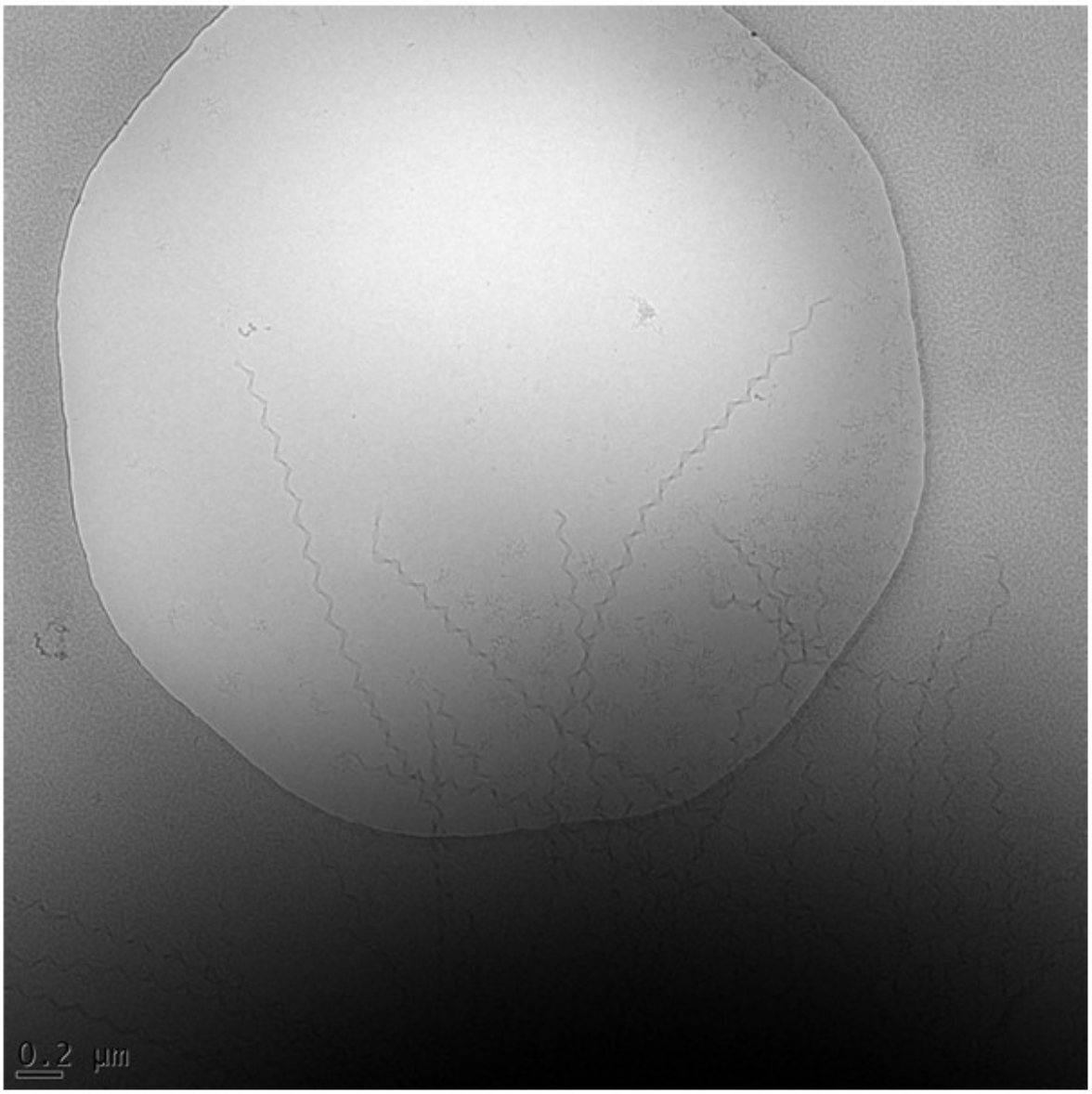
(a)



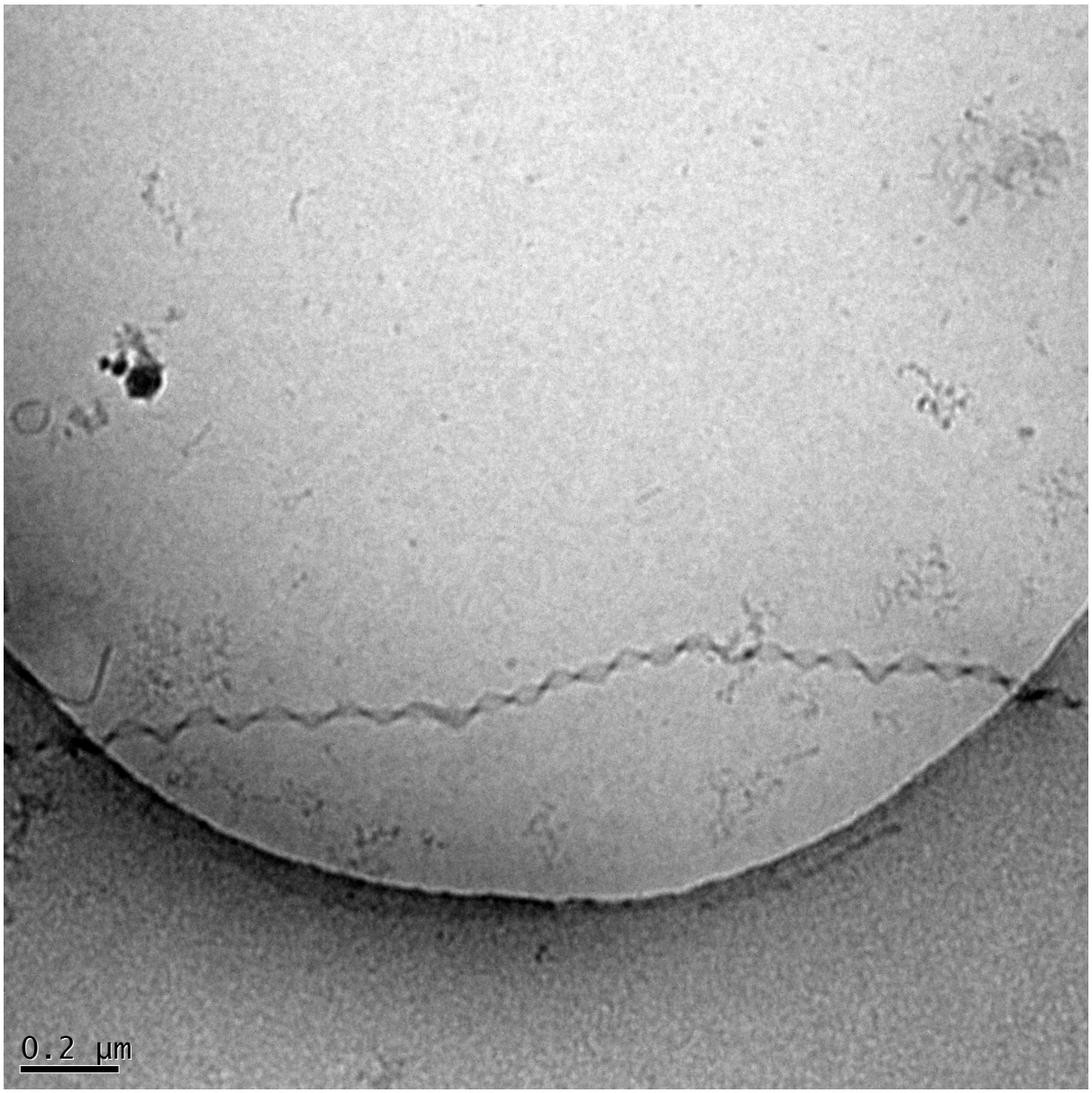
(b)



(c)

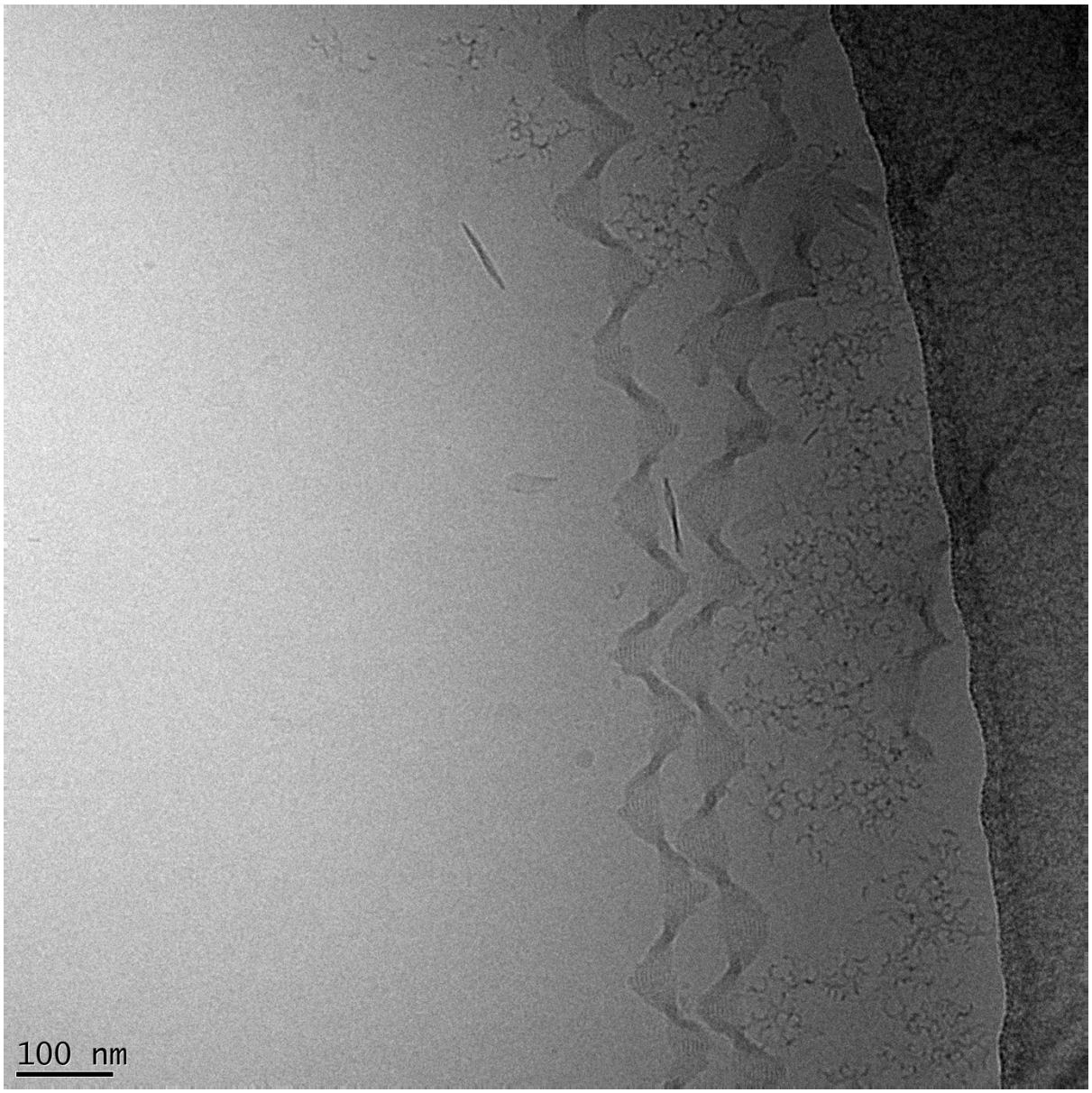


SI Fig.3. Low magnification cryo-TEM images showing extended helical nanotape structures. (a, b) 1 wt% solution. (b) 0.1 wt% solution.

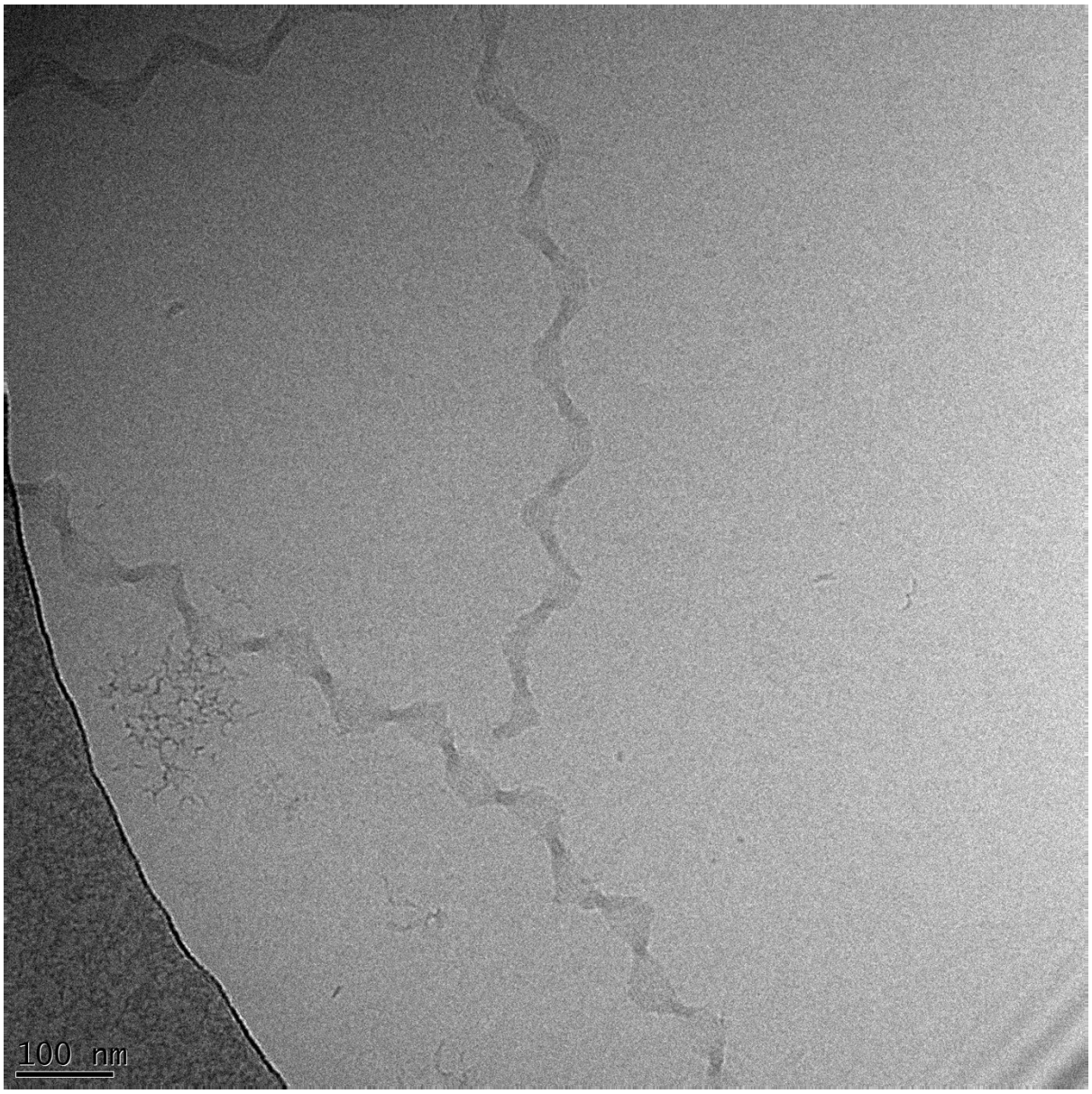


SI Fig.4. Example cryo-TEM image (for a 0.1 wt% AmB sample) showing variation of helical pitch within a single twisted tape.

(a)



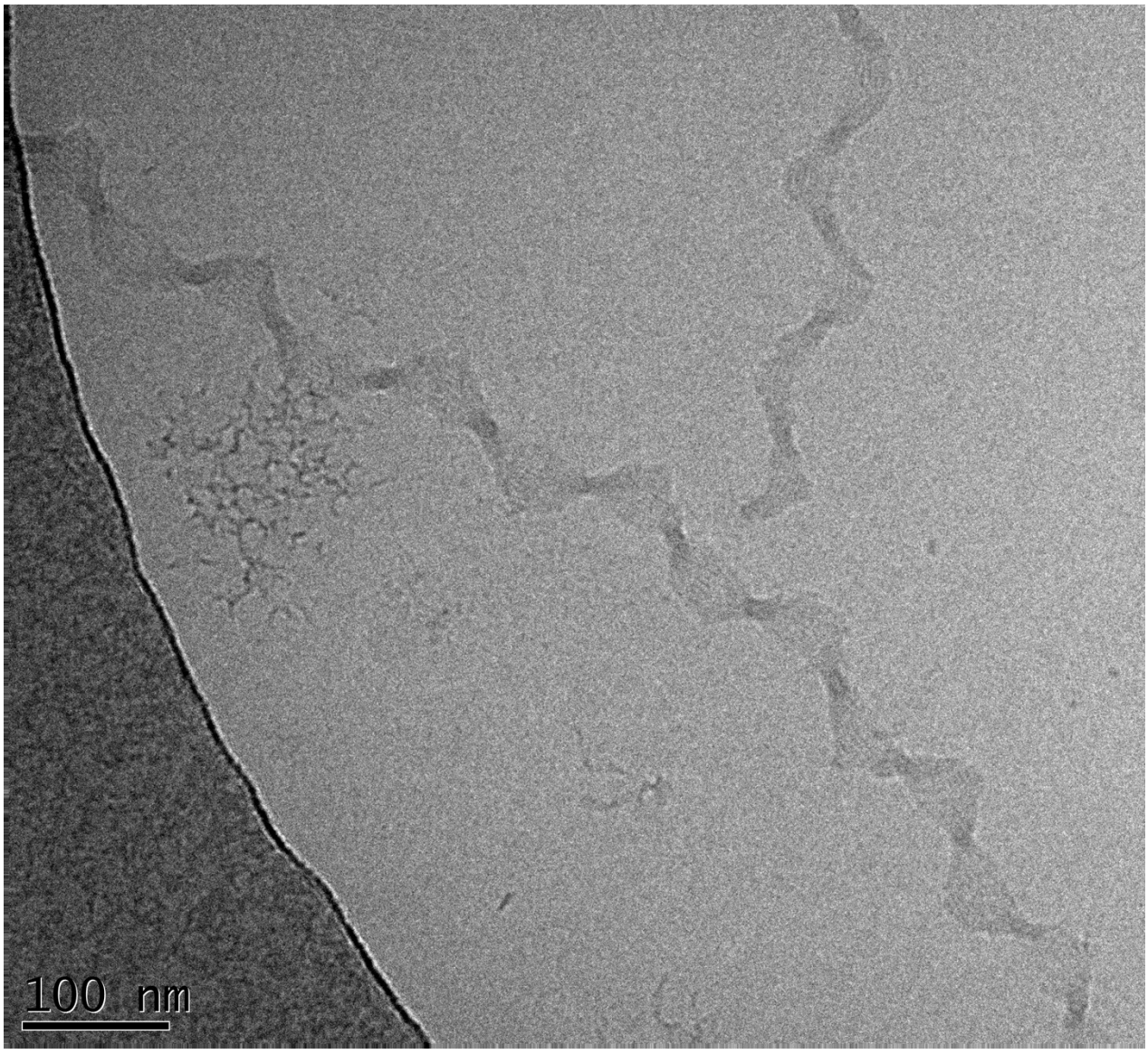
(b)



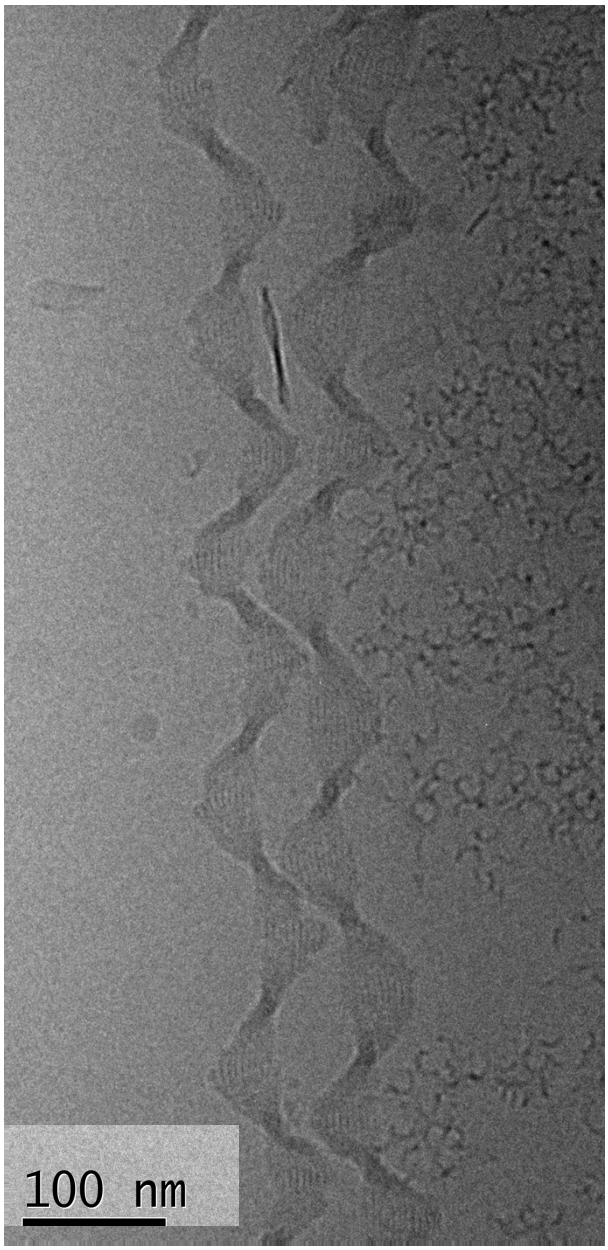
(c)

SI Fig.5. Cryo-TEM images showing (a,b) tapes coexisting with network-like structures for 0.1 wt% AmB, (c) tapes coexisting with short straight fibrils observed in some regions of the TEM grid for 1 wt% AmB.

(a)

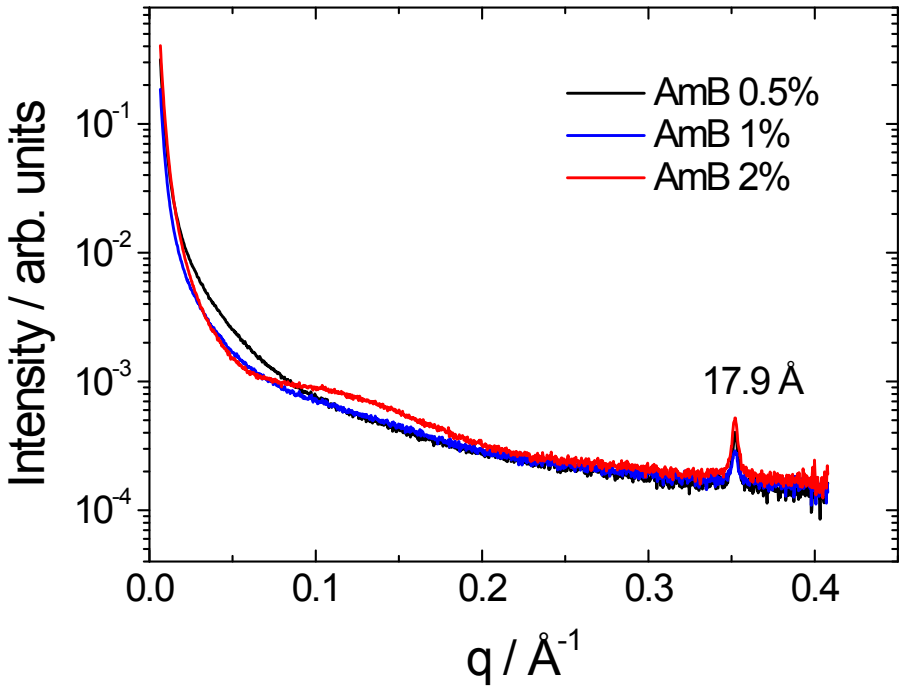


(b)



SI Fig.6. (a,b) Cryo-TEM images showing that twisted tapes comprise individual filaments.

Solution SAXS



SI Fig.7. SAXS intensity profiles from solutions of AmB at pH 11 at the three concentrations indicated.

References

1 (a) C. M. McNamara, S. Box, J. M. Crawforth, B. S. Hickman, T. J. Norwood, and B. J. Rawlings, *J. Chem. Soc.-Perkin Trans. 1*, 1998, 83-87; (b) B. Murphy, K. Anderson, C. Borissow, P. Caffrey, G. Griffith, J. Hearn, O. Ibrahim, N. Khan, N. Lamburn, M. Lee, K. Pugh, and B. Rawlings, *Organic & Biomolecular Chemistry*, 2010, **8**, 3758-3770.