Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Microtubule-Binding R3 Fragment from Tau Self-Assembles into Giant Multistranded Amyloid Ribbons

Jozef Adamcik, Antoni Sánchez-Ferrer, Nadine Ait-Bouziad, Nicholas P. Reynolds, Hilal A. Lashuel, and Raffaele Mezzenga**

anie_201508968_sm_miscellaneous_information.pdf

SUPPORTING INFORMATION

Experimental Section:

R3 fragment: The 26-mer peptide fragment from Tau: $Tau_{306-327}$, VQIVYKPVDLSKVTSKCGSLGNIHHK, was purchased from CS Bio Co. (Menlo Park, CA, USA).

Self-assembly of R3 peptide: 200 μM R3 peptide was dissolved in buffer (10 mM Tris, 50 mM NaF, 0.5 mM dithiothreitol) and pH was adjusted to 7.4. The solution was then vortexed and centrifuged for 20 min at 14000 rpm and the final concentration of the R3 peptide was verified by CD measurements. Such solution was incubated in absence of heparin at 37 °C either with or without constant orbital agitation (1000 rpm, Peqlab, Thriller, Germany). In case of incubation in presence of heparin, the R3 peptide was incubated in presence of 10 μM heparin sodium salt (Applichem GmbH, Germany).

Circular Dichroism (CD) spectroscopy: CD measurements were performed on a Jasco J-815 CD spectrometer operated at 20 °C. All spectra were acquired in the range of 195–250 nm with an increment of 0.2 nm and a scan rate of 50 $nm{\cdot}min^{-1}$. For each sample, five spectra were averaged and smoothed using binomial approximation.

Thioflavin T (ThT) Fluorescence: ThT fluorescence reading was performed in triplicates using a final ThT concentration of 60 μM and a peptide concentration of 60 μM in 50 mM glycine pH 8.5, on a Bucher Analyst AD plate reader (excitation wavelength: 450 nm, emission wavelength: 485 nm). The data are corrected for buffer absorption, performed as triplicates and expressed as mean ± S.D.

X-ray Scattering: Small- and wide-angle X-ray scattering (SAXS and WAXS) experiments were performed using a Rigaku MicroMax-002+ microfocused beam (4 kW, 45 kV, 0.88 mA) with the $\lambda_{CuK\alpha}$ = 0.15418 nm collimated by three pinhole collimators (0.4, 0.3, and 0.8 mm). The scattered SAXS and WAXS intensity was collected in transmission mode by a two-dimensional Triton-200 gas-filled X-ray detector (20 cm diameter, 200 μm resolution) and by a Fujifilm BAS-MS 2025 imaging plate system (15.2 × 15.2 cm^2, 50 μm resolution), respectively. An effective scattering vector range of 0.05 nm^{-1} < q < 25 nm^{-1} was obtained, where q is the scattering wave vector defined as $q = 4\pi \sin \theta/\lambda_{CuK\alpha}$ with a scattering angle of 2θ. Synchrotron SAXS experiments were performed on the SAXS/WAXS beamline at the Australian Synchrotron. Samples were transferred from a 96 well plate to the beamline via a quartz capillary connected to a syringe pump. The experiments used a beam of wavelength of λ= 1.03320Å (12.0 KeV) with dimensions 300 μm x 200 μm and a typical flux of 1.2 x1013 photons per second. 2D diffraction images were recorded on a Pilatus 1M detector in the q-range 0.05-3 nm^{-1}. Spectra were recorded under flow (0.15 $mlmin^{-1}$) in order to prevent X-ray damage from the beam and multiples of 15 spectra were recorded for each time point (exposure time = 1 s) and the averaged spectra are shown after background subtraction against buffer in the same capillary.

Electron microscopy (EM): 3.5 μL of samples were applied onto glow-discharged Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Sciences, Hatfield, PA, USA) for 1 min. The grids were then blotted with filter paper, washed twice with ultrapure water, once with staining solution (Uranylformate 0.7% w/V)), stained for 30 s and blotted off. A Tecnai Spirit BioTWIN electron microscope operated at 80 kV and equipped with a LaB_6 gun and a 4K x 4K FEI Eagle CCD camera was used to observe samples.

WILEY-VCH

Atomic Force Microscopy: Aliquot of 20 μL of R3 peptide solution was deposited on freshly cleaved mica, left to adsorb for 2 min at room temperature, rinsed with MilliQ water, and gently dried with pressurized air. The samples were scanned on Nanoscope VIII Multimode Scanning Force Microscopes (Bruker) covered with an acoustic hood to minimize vibrational noise. The AFM was operated in tapping mode under ambient conditions using commercial silicon nitride cantilevers. All AFM images were flattened to remove background curvature using the Nanoscope Analysis 1.5 software and no further image processing was carried out. To determine the distance between two individual protofilaments the in-house written open source code FiberApp[1] was used.

WILEY-VCH

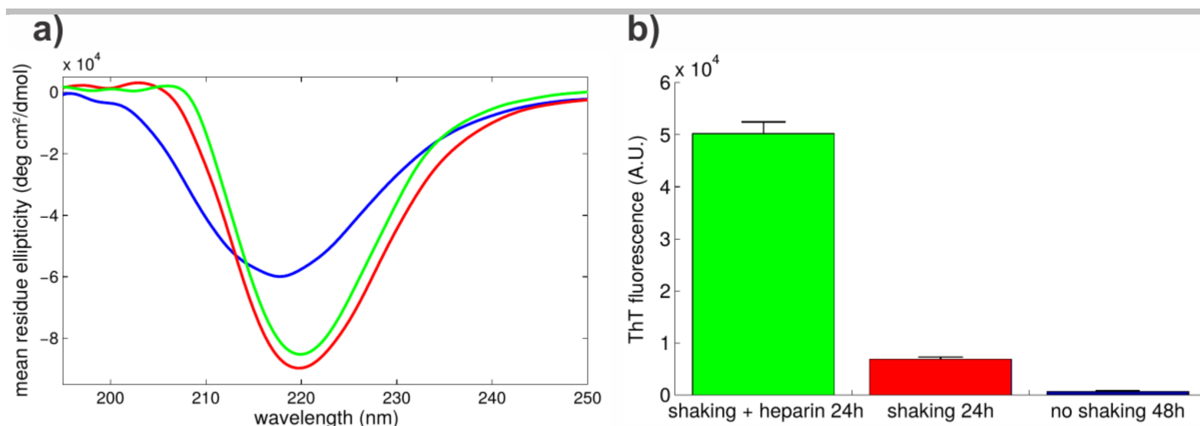

Figure S1. (a) CD spectra of R3 peptide solutions incubated in buffer in absence of heparin at 37 °C with shaking at 1000 rpm for 24 h (red color) and without shaking for 48 h (blue color) and in presence of heparin at 37 °C with shaking at 1000 rpm for 24 h (green color). (b) ThT fluorescence intensity of R3 peptide solutions incubated in buffer in absence of heparin at 37 °C with shaking at 1000 rpm for 24 h (red color) and without shaking for 48 h (blue color) and in presence of heparin at 37 °C with shaking at 1000 rpm for 24 h (green color).

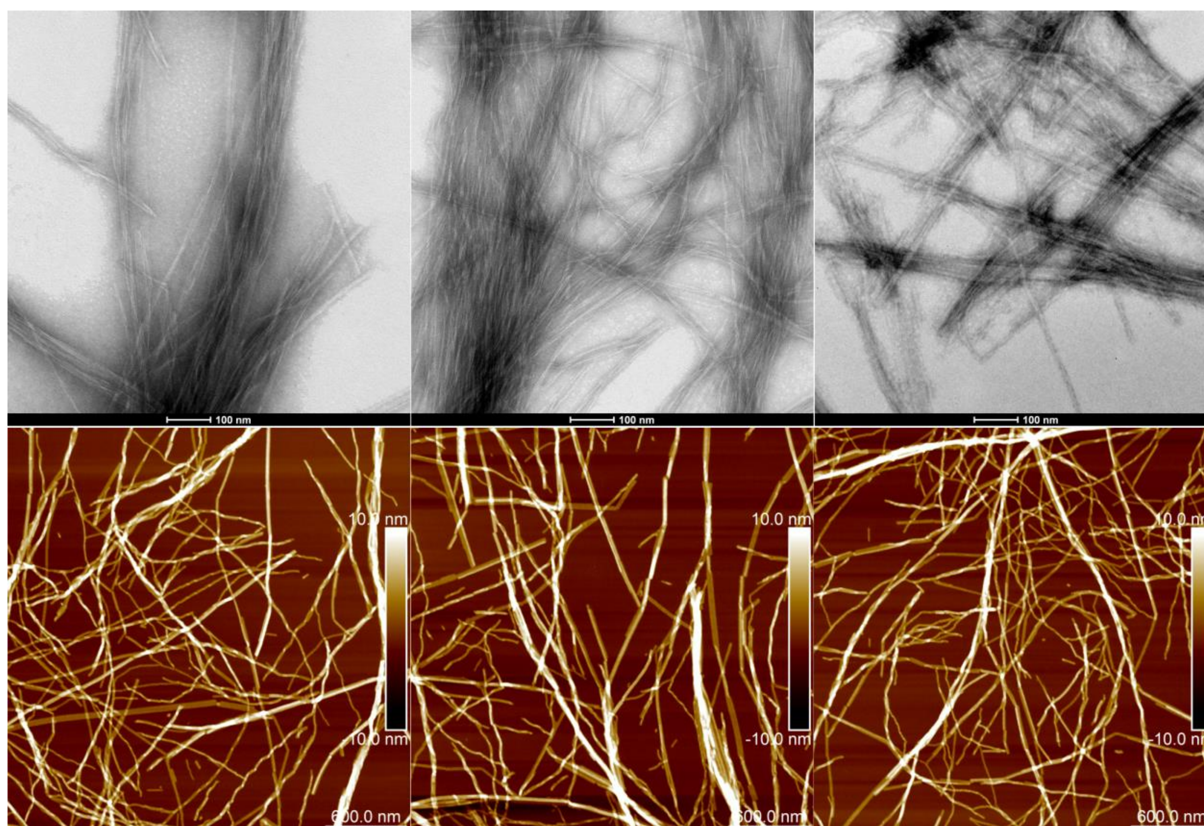

Figure S2. TEM (upper row) and AFM (lower row) images of self-assembled R3 peptide after incubation in buffer in presence of heparin.

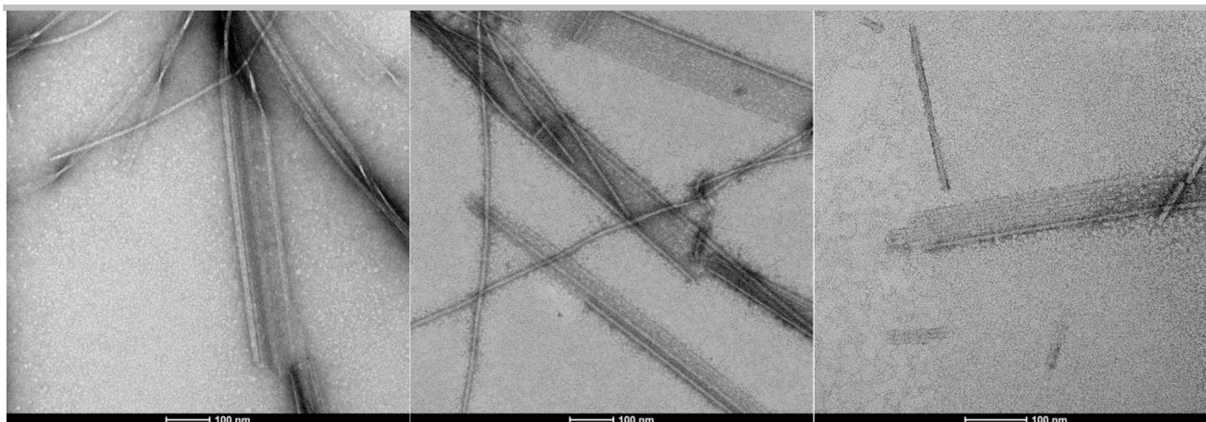

Figure S3. TEM images of self-assembled R3 peptide after incubation in buffer in absence of heparin without shaking.

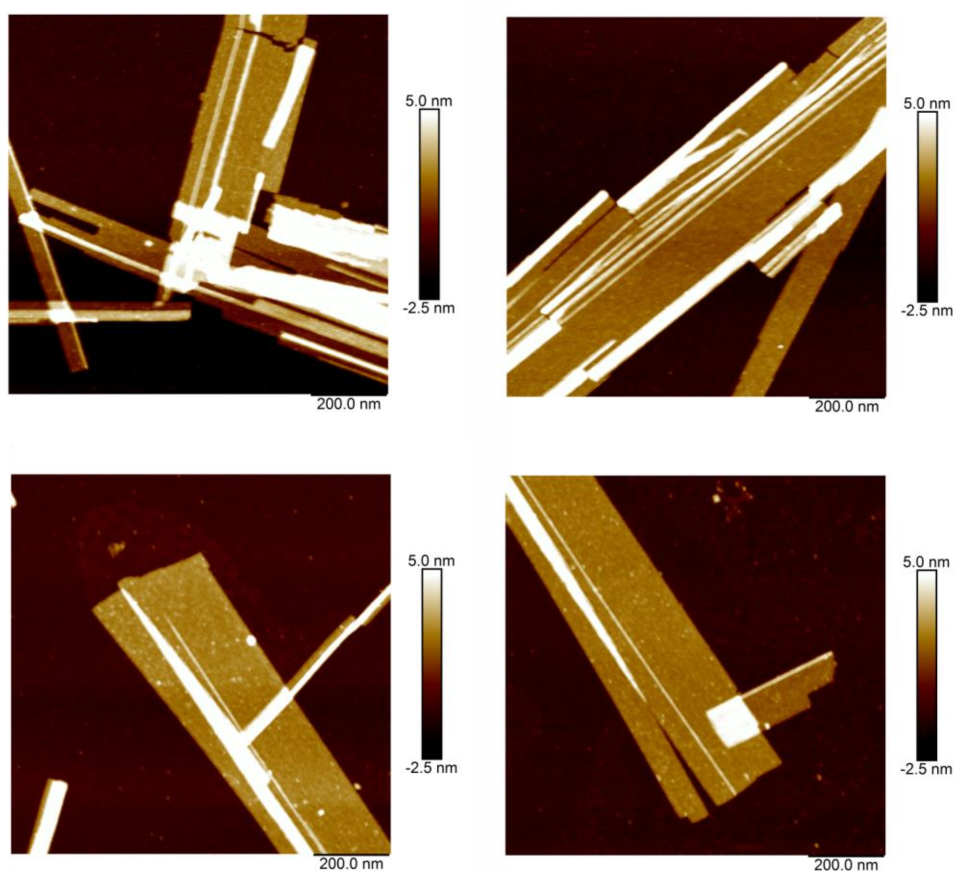

Figure S4. High-resolution AFM height images of flat multistranded ribbons self-assembled from R3 peptide after incubation in buffer in absence of heparin without shaking for 1 week.

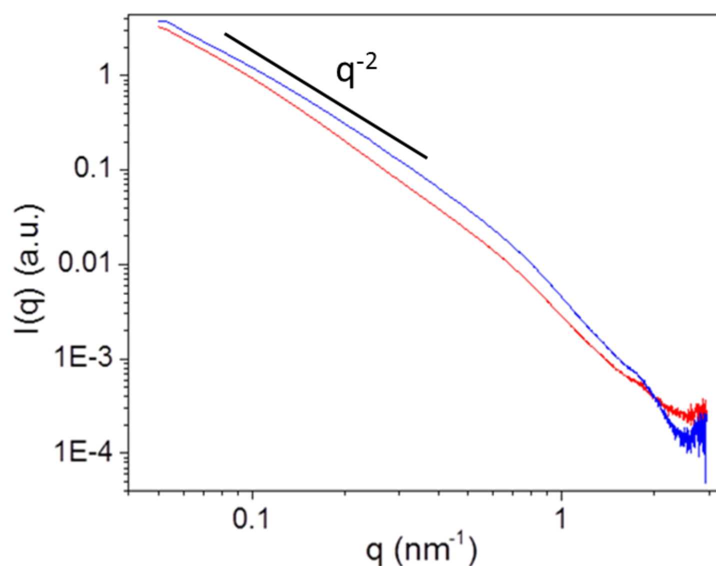

Figure S5: Synchrotron SAXS spectra of 1 wt% solutions of the assembled R3 peptide in the absence of heparin in buffer for 1 week (blue color) and 24 hours (red color), confirming the development of q^{-2} dependence over time. Fitting showed that in the q range 0.005-0.4 nm^{-1} the spectra after 24 h corresponded to $q^{-2.28}$ ($R^2 = 0.99$) whereas after 1 week the spectra corresponded to $q^{-2.05}$ ($R^2 = 0.99$).

REFERENCES:

[1] I. Usov, R. Mezzenga, *Macromolecules* **2015**, 48, 1269-1280.

WILEY-VCH