

Supporting Information

A Modular Approach towards Functional Decoration of Peptide-Polymer Nanotapes

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Materials

N,N-Dimethylglycine (DMG, Aldrich, 97%), *N,N*-diisopropylethylamine (DIPEA; Acros, peptide grade), piperidine (Acros, peptide grade), trifluoroacetic acid (TFA; Acros, peptide grade), and 1-benzotriazoloyloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP, NovaBiochem) have been applied as received. *N,N*-dimethylformamide (DMF; Aldrich, 99+%) was distilled prior to use. All other reagents were used as received from Aldrich.

Fmoc-amino acid derivatives (Fmoc-Val-Thr-(psi Me,Me Pro)-OH, Fmoc-Thr(*t*Bu) OH, Fmoc-Asp(*t*Bu) OH, Fmoc-Lys(Boc) OH, Fmoc-Gly OH, Fmoc-Val OH), *N,N*-dimethylglycine (*dmG*) 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), *N*-methyl-2-pyrrolidone (NMP, 99.9+%, peptide synthesis grade) were used as received from IRIS Biotech GmbH, Germany. Dichloromethane (DCM; IRIS Biotech GmbH, peptide grade) was distilled from CaH₂. TentaGel[®] PEG Attached Peptide resin (TentaGel PAP) (loading: 0.24 mmol/g; M_n= 3200, PDI= 1.06 (GPC (THF, calibrated against linear PEO standards, PSS, Germany)) was purchased from Rapp, Polymere GmbH, Tuebingen, Germany.

1 N HCl (Merck; Titripur[®]) and NaOH (Merck, Titripur[®]), NaHCO₃ (99.7%, Acros Organics); Na₂CO₃ (anhydrous, 99.95%, Sigma-Aldrich), CaCl₂·2H₂O (99.5%; Fluka) have been used as received.

The diazotransfer reagent imidazole-1-sulphonyl azide hydrochloride (IS-N₃) has been synthesized according to a reported literature procedure.[E. D. Goddard-Borger and R. V. Stick, *Org. Lett.*, 2007, **9**, 3797]

Instrumentations and methods

Mass spectrometry: Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) measurements were performed on a Voyager-DE STR BioSpectrometry Workstation MALDI-TOF mass spectrometer (Perseptive Biosystems, Inc., Framingham, MA, USA). The samples were dissolved in methanol-acetonitrile (1:1, v/v, with or without TFA) at a concentration of 0.1 mg/mL. One μL of the analyte solution was mixed with 1 μL of alpha-cyano-4-hydroxycinnamic acid matrix solution consisting of 10 mg of matrix dissolved in 1 mL of 0.3% TFA in water-acetonitrile (1:1, v/v, with 0.3% TFA). From the resulting mixture 1 μL was applied to the sample plate. Samples were air-dried at ambient temperature (24°C). Measurements were performed at an acceleration voltage of 20 kV. Each spectrum obtained was the mean of 250 laser shots.

Electrospray-Ionization Mass spectrometry (ESI-MS): Measurements were carried out at an LCMS-ESI-MS from Shimadzu (Duisburg, Germany). The high performance liquid chromatography system has been equipped with a PQ8000 α mass spectrometer (Shimadzu (Duisburg, Germany)). Samples were injected directly without prior chromatographic separation. The samples were dissolved at a concentration of 1 mg/mL either in methanol with 0.1v/v % formic acid or in acetonitrile/water (1:1 v/v). 10 μL of the solution was injected into the MS applying a carrier solvent flow of 0.5 mL/min. The samples were measured in positive mode with a detector voltage of 1.6 kV, an acceleration voltage of 4.5 kV, a temperature of 150 °C and nitrogen carrier gas flow of 4.5 L/min.

Infrared spectroscopy: FT-IR spectroscopy was conducted at a BioRad FTS 6000 equipped with a golden gate ATR crystal. Samples were measured directly as a solid or after lyophilization. (s = strong intensity, m = medium intensity, w = weak intensity).

Nuclear magnetic resonance spectra (NMR): NMR was recorded on a Bruker DPX-400 Spectrometer in D₆-DMSO or D₁-TFA at 400 MHz (¹H-NMR), 100 MHz (¹³C-NMR). The measurements were evaluated by using the Win-NMR Software package from Bruker.

Circular dichroism (CD): Circular dichroism UV spectra were recorded in solution on a Jasco 715 CD spectrometer using a sample concentration of 1 mg/mL and a cuvette with a path length of 1 mm. Measurements were performed at room temperature within the range of 300 nm to 190 nm, a resolution of 0.2 nm, a scan speed of 50 nm/min, a detector response time of 4 sec and a spectral bandwidth of 1 nm. Every measurement was an average of eight single scans, corrected by appropriate background.

Transmission electron microscopy (TEM): TEM was performed on a Zeiss EM 912Ω instrument at an acceleration voltage of 120 kV. Sample preparation was described previously.[Eckhardt, D. *et al.*, *Chem. Commun.*, 2005, 2814] Selected area electron diffraction (SAED) was measured on non-stained samples.

Atomic force microscopy (AFM): AFM was performed on a NanoScope IIIa device (Veeco Instruments, Santa Barbara, CA) in tapping mode. Commercial silicon tips (Type NCR-W) were used with a tip radius between 6 and 10 nm, without tip correction. A constant force of 42 N m⁻¹ and a resonance frequency of 285 kHz was employed. The image was recorded on a 10×10 μm e-scanner. 5 μL of a sample solution of 0.2 mg/mL were spin-coated (2 min; 2500 rpm) on fresh cleaved Mica substrates. Analyses of the aggregate dimension were carried out by averaging over at least 30 values.

Confocal microscopy: Confocal scanning laser fluorescence microscopy images were recorded using a Leica DM IRE2 SP5 system (Leica Microsystems Heidelberg GmbH, Germany), with a 40× HCX Plan APO objective (N.A. = 0.75).

Automated solid phase supported peptide synthesis (SPPS): Peptide sequences were assembled on a PAP resin by following standard FastMoc protocols using a 0.1 mmol scale. The automated synthesis of the conjugate was performed on an ABI 433a peptide synthesizer (Applied Biosystems).

Crystallization experiments

The general sample preparation, setup, calibration procedure as well as the experimental procedure have been described in detail somewhere else. (Gebauer, science, 2008; Gebauer Adv. Mat.; 2009) In crystallization experiments, in brief, CaCl₂ solution (25 mM, preset to pH 9.75) was added to a carbonate buffer solution (pH 9.75; 25 mL; 10 mM; without or with the respective fibre (100 mg/L)) at a constant dose rate (0.01 mL/min). The pH-value was kept constant during the experiments utilizing dilute sodium hydroxide solution (10 mM). The calcium potential was recorded continuously by means of a calcium ion selective electrode. The resulting dilution of the solution was considered in the data evaluation. Beaker, burette tips and electrodes were carefully washed with acetic acid (10%) and water after each experiment.

Note: The small parallel shift of the graph (Figure 2) obtained in the presence of VI b can be attributed to the binding of Ca²⁺ to the fibres. A comparison of the slopes in the pre-nucleation stage with the reference experiment in the absence of any additives (black line) shows that neither of the fibres has a significant influence on the cluster equilibrium.

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Calcium ion binding assay: In calcium binding experiments the respective fibre (about 80 mg/L) was dissolved in degassed water and the solution pH was set to 9.00. Then, CaCl₂ solution (25 mM, set to pH 9.00) was added to 25 mL fibre solution at a constant rate (0.01 mL/min). The pH-value was kept constant during the experiments by titrating sodium hydroxide and hydrochloric acid solution (both 10 mM). The development of the Ca²⁺ potential was recorded simultaneously. Calcium binding can be identified by a parallel shift of the development of the free amount of Ca²⁺ along the x-axis. The binding capacity can be determined by extrapolating the linear stage of the obtained curve to the x-axis. The intercept corresponds to the binding capacity.

Synthesis, aggregation and characterization

Peptide polymer conjugates [dmG-TVTVKV]₂-Template-PEO, I): The synthesis of the carbazole based template and the procedure to obtain **I** via fully automated solid phase supported peptide synthesis (SPPS) followed protocols, which have been described previously.[Eckhardt, D. et al., *Chem. Commun.*, 2005, 2814] All standard amino acids derivatives were coupled by standard Fastmoc-protocols (double coupling, extended coupling times of 45 min). To avoid insufficient couplings within the valine-threonine aggregation domain, a structure breaking pseudoproline Fmoc-Val-Thr(ψ Me,Mepro)OH was introduced at sequence position Thr⁴ (Val³-Thr⁴). Pseudoproline was coupled automatically using double coupling with 5 equiv. excess and coupling times of 55 min. *N,N*-Dimethylglycine (*dmG*) was coupled by hand with bench-top protocols in a self-made glass reactor. 10 equiv. excess and double coupling was applied to achieve complete conversion as confirmed by the colourimetric Kaiser-Test. The product PEO-peptide conjugate was cleaved with 99% TFA (1% TMBS) for 6 hours. TFA was removed under reduced pressure. The crude compound was dissolved in methanol, precipitated twice in Et₂O. The isolated solid was purified by dialysis in MeOH (MWCO 1000), followed by lyophilization in H₂O.

Characterization of **I**

FTIR (ν in cm⁻¹): 3273(s), 3086(w), 2960(w), 2874(s), 1788(w), 1672(m - amine), 1624(s, amide(I)), 1549(m - amide(II)), 1466(m), 1456(w), 1400(w), 1359 (w), 1342(s), 1281(m), 1240 (w), 1201 (m), 1172(w), 1144(m), 1103 (s), 1062 (m), 962 (s), 946 (m), 841 (s), 799 (m), 719(m), 706(m), 667(s)

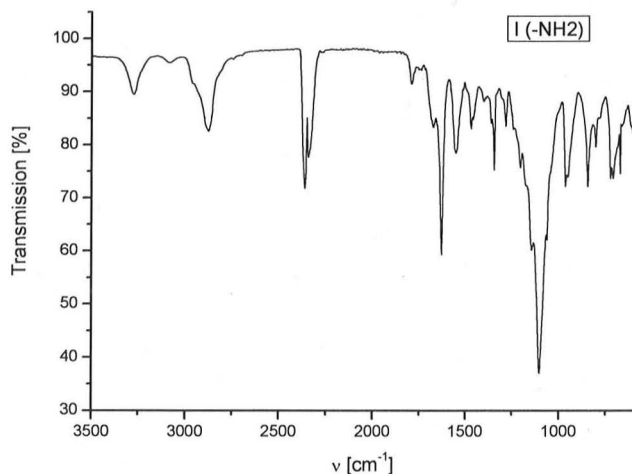


Figure S1. ATR-FT IR of the amine functional conjugate (**I**).

¹H-NMR (400 MHz, d₆-DMSO, RT, δ in ppm): δ = 0.80 (m, 36H, CH₃-Val), 1.03 (br.d, 12 H, CH₃-Thr), 1.27 (m, 4H, γCH₂-Lys), 1.62-1.72 (m, 8H, β,δCH₂-Lys), 1.77 (t, 4H, CH₂-template), 1.9-2.0 (m, 6H, βCH₂-Val), 2.76-2.77 (br. m, 12H, CH₃-dmG), 2.82-2.88 (br.m, 4H, εCH₂-Lys), 2.98-3.04 (m, 6H, CH₂-template), 3.20 (m, 4H CH₂-template), 3.49 (br.s, 290H, PEO), 3.90-4.50 (several m, 16H, αCH-Val, Thr, Lys + βCH-Thr), 4.95 (s, 2, CH₂-carbazole), 7.22 (d, 2H, CH-Ar, template), 7.38 (d, 2H, CH-Ar, template), 7.88 (s, 2H, CH-Ar, template).

MALDI-TOF-MS

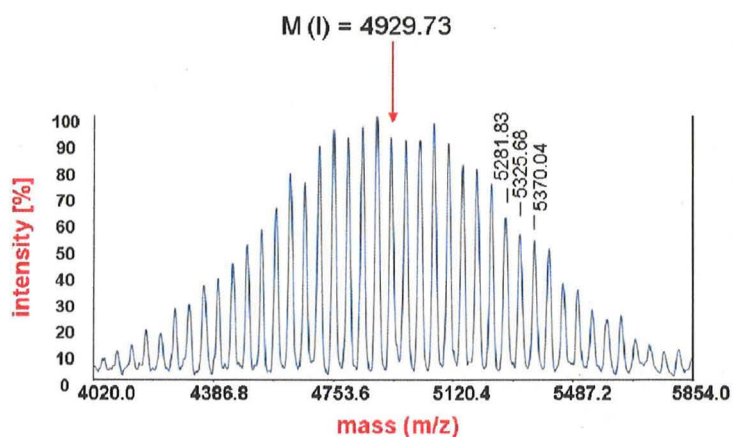


Figure S2. MALDI-TOF-MS of the amine functional conjugate (**I**).

The spectrum shows the typical distribution of a PEO with the characteristic repeat unit of 44 Da. The m/z of each peak can be assigned by: $M^+ = n \times M_{[\text{EO}]} + M_{[\text{template}]} + 2 \cdot M_{[\text{peptide}]} + M_{[\text{endgroupe}]} + M_{[\text{counter ion}]}$.

The experimentally found signal at m/z 4929.73 can be assigned within ± 0.2 Da accuracy to a $[M+K] = 4929.93$ Da by assuming a $M_{[\text{EO}]}$ of 44.04 Da, $n = 71$ units, a mass of the template of $M_{[\text{template}]} = 335.19$ Da, a mass of each peptide chain of $M_{[\text{peptide}]} = 713.9$ Da, a mass of the polymer end groups (1 H = 1 Da) and the mass of a potassium counter ion ($M_{[\text{counter ion}]} = 39$).

Self-assembly of [dmG-TVTVKV]₂-Template-PEO (I): The self-assembly of **I** followed a sequential denaturation-reaggregation strategy as described recently.[Eckhardt, D. et al., *Chem. Commun.*, 2005, 2814] Briefly, compound **I** was dissolved and denatured by a treatment with TFA for 30 min. TFA was removed under reduced pressure, and the residual was diluted with methanol. After concentration of the solution by partially distilling of the methanol, additional methanol was added and removed twice under reduced pressure to minimize the residual TFA level. Subsequently methanol was added to obtain a concentration of ~ 5 mg/mL. Self-assembly of **I** was guided by a solvent exchange procedure via the stepwise dialysis from methanol to water (10% per day; MWCO = 2000 Da). The resulting slow self-assembly process led to the formation of β -sheet tapes that were characterized by AFM, TEM and CD.

CD-Spectroscopy: Cotton effects characteristic for the β -sheet motif 195 nm (positive max, $40.000 \text{ deg} \cdot \text{cm}^2 \cdot \text{dmol}^{-1}$) and 218 nm (negative minimum, $-60.000 \text{ deg} \cdot \text{cm}^2 \cdot \text{dmol}^{-1}$) were observed. Consistent with congener PEO-peptide conjugates, having the same aromatic template, a negative shoulder at 235 nm could be found. Probably this relates to the organization of the aromatic template in the chiral environment as similar shoulders can be observed with phenylalanine rich proteins.

Microscopy:

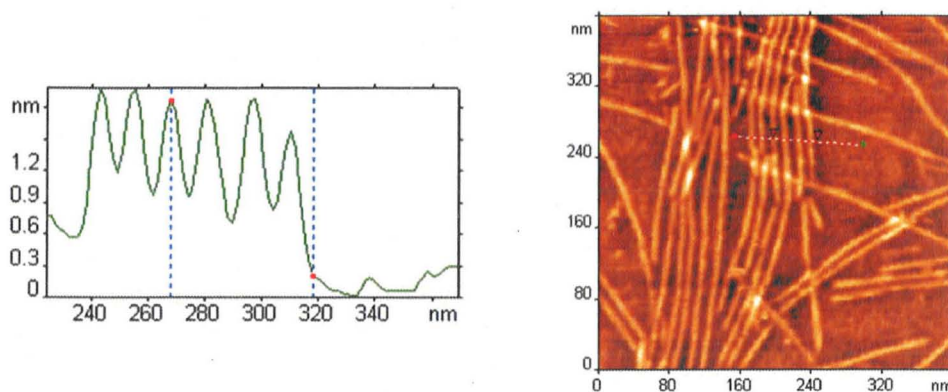


Figure S3. AFM height image and height profile (z range 2 nm, mica) of self-assembled **I**.

Diazo transfer reaction at self-assembled I leading to azido functionalized nanotapes of [dmG-TVTVK^{N₃}V]₂-Template-PEO (II): The amine→azide transformation (diazo transfer) was adapted from a recently described procedure.[E. D. Goddard-Borger and R. V. Stick, *Org. Lett.*, 2007, **9**, 3797] To 5 mL of an aqueous solution of the nanotapes (**I**) with a concentration of ~5 mg/mL (0.005 mmol conjugate, 0.010 mmol amino groups), 3.37 mg potassium carbonate (0.024 mmol, 2.4 equiv.), 0.64 mg copper(II)sulphate (0.004 mmol, 0.4 equiv.) and 5.0 mg imidazole-1-sulphonyl azide hydrochloride (0.024 mmol, 2.4 equiv.) were added. The reaction mixture was shaken 12 h at room temperature. DOWEX™ ion exchange resin was used to remove copper ions and final dialysis (MWCO 2000 Da) against Millipore water led to the solution of azido functionalized nanotapes (**II**). Quantitative conversion was proven by a negative colorimetric Kaiser test, which is highly sensitive for primary amino groups. The accurate concentration was determined by UV-spectroscopy as described previously.[Eckhardt, D. et al., *Chem. Commun.*, 2005, 2814] CD, AFM and TEM (Figure S4) confirmed the preservation of the β -sheet fibres. A small aliquot of the solution was lyophilized, to characterize the product.

Characterization of II

FTIR-ATR (ν in cm^{-1}): 3271(s), 3086(w), 2968(w), 2874(s), 2100(s -azide), 1627(s, amid(I)), 1548(m -amid (II)), 1465(m), 1454(w), 1400(w), 1359 (w), 1342(s), 1278(m), 1240 (w), 1201 (w), 1145 (m), 1101 (s), 1061 (m), 962 (s), 946 (m), 881(w), 841 (s), 800(w), 799 (m), 711(m), 669(m).

Notice: IR shows the occurrence of a vibration at 2100 cm^{-1} , which is the asymmetric N-N-N stretching frequency and characteristic of $-\text{N}_3$.

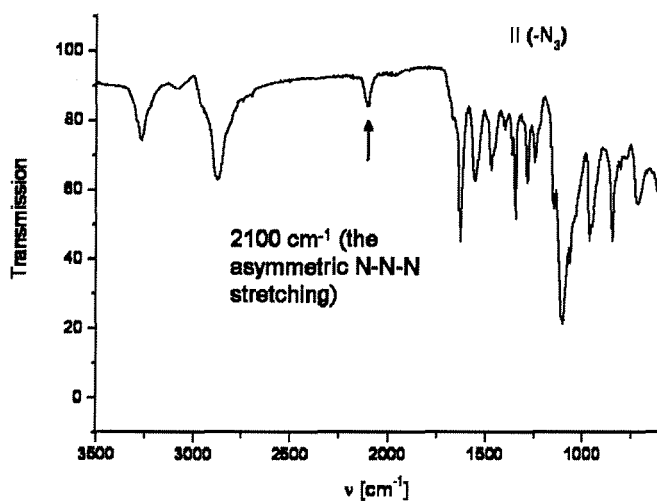


Figure S4. ATR-FT IR of the azide functional conjugate (II).

MALDI-TOF-MS

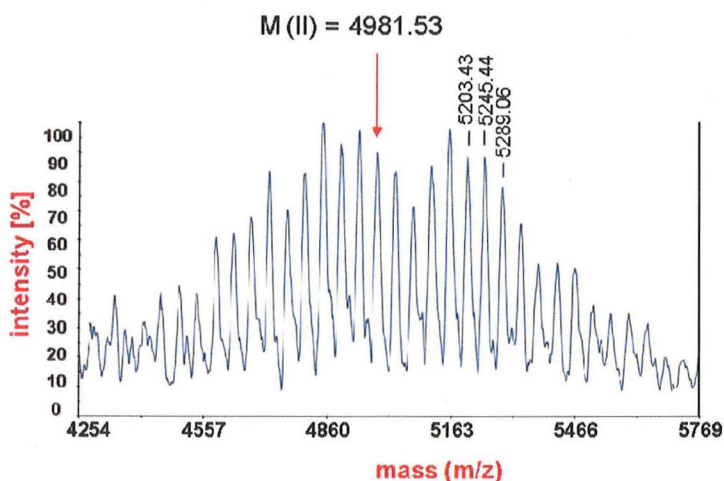


Figure S5. MALDI-TOF-MS of the azide functional nanotapes II.

The mass spectrogram shows the typical distribution of PEO with the characteristic repeat unit of 44 Da. The m/z of each peak can be assigned to II within ± 1 Da accuracy. The experimentally found signal at m/z 4981.53 can be assigned to $[M+K] = 4981.93$ Da by assuming a $M_{[EO]}$ of 44.04 Da, $n = 71$ units, the mass of the template of $M_{[template]} = 335.19$ Da, a mass of each peptide chain of $M_{[peptide]} = 739.9$ Da, a mass of the polymer end groups ($1 H = 1$ Da) and the mass of a potassium counter ion ($M_{[counter\ ion]} = 39$).

Mass signals in between the main signals could be assigned to the loss of N_2 , which is known to occur in the presence of azides during the MALDI-process during in source fragmentation. Remaining starting material, at which azide transfer was not quantitative could be excluded due to a negative Kaiser test, which is a highly sensitive and selective test for primary amino groups.

¹H-NMR (400 MHz, TFA, RT, δ in ppm): δ = 0.48-0.53 (m, 36H, CH₃-Val), 0.82-0.86 (m, 12H, CH₃-Thr), 1.00, 1.12, 1.32, 1.39, 1.52, 1.62* (multiple resonances m, ~20H, γCH₂-Lys, δCH₂-Lys, βCH₂-Lys, CH₂-template, CH₂-template), 2.29-2.41 (m, 3H, βCH-Val), 2.62 (br. s, 12H, CH₃-dmG), 2.74 -2.81 (m, 3H, βCH-Val), 2.98-3.06 (m, 4H CH₂-template), 3.39 (br. s, 288 H, O-CH₂-CH₂-O), 3.79, 3.84, 3, 92, 4.04, 4.29, 4.35** (multiple resonances m, 22H, αCH-Val, αCH-Lys, αCH-Thr, βCH-Thr, εCH₂-Lys, PEO-O-CH₂-CH₂-NH-Gly-template), 4.66 (s, 2H, N-CH₂-carbazole), 6.76-6.82 (m, 4H, 2×CH-Ar template), 7.39 (s, 2H, CH-Ar template).

Note: The resonances δ = 0.48-0.53 ppm (Val) and 6.76-6.82 ppm (template) and 3.39 ppm (PEO) were utilized to confirm the ratio of peptide/template and PEO (36.0 :4.0:295).

* Resonances are not baseline separated and can only be integrated cumulatively.

** Complex resonances could not be assigned individually.

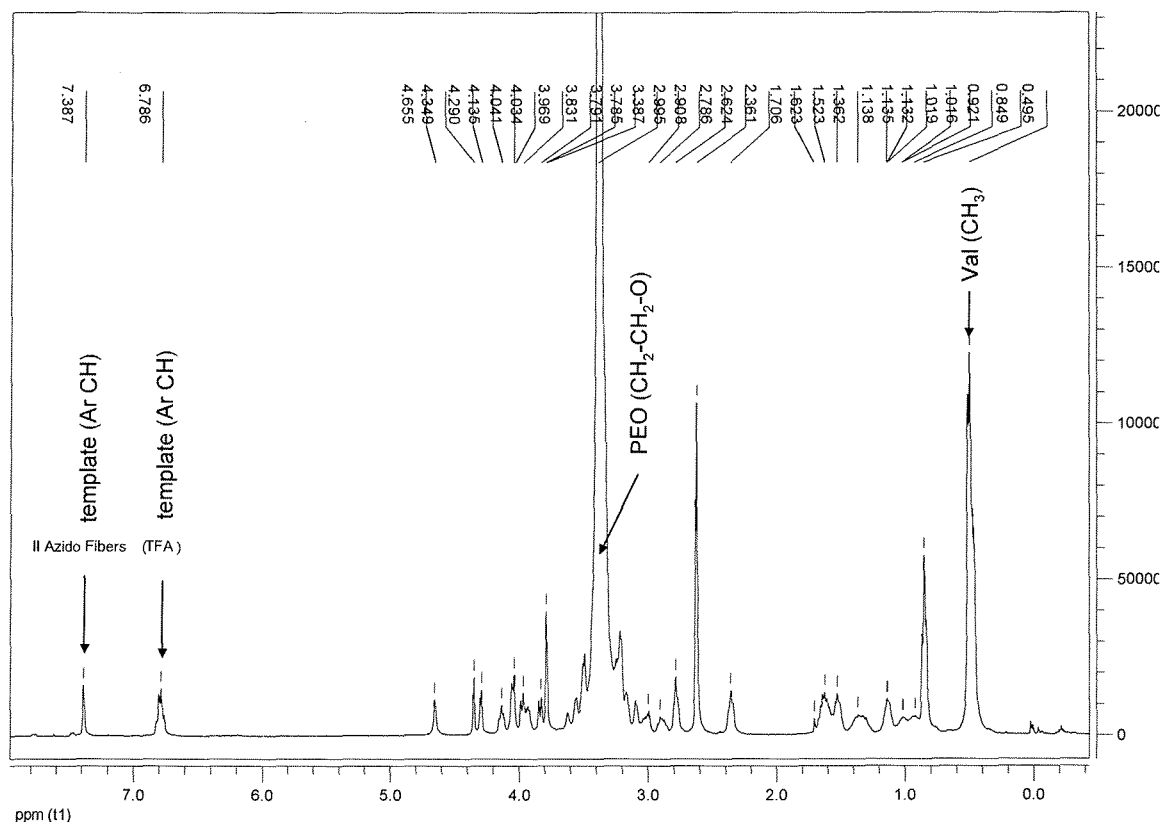


Figure S6. ATR-FT IR of the azide functional conjugate in TFA (II).

CD-spectroscopy shows cotton effects characteristic for the β -sheet motif (196 nm (positive max, $+41.000 \text{ deg} \cdot \text{cm}^2 \cdot \text{dmol}^{-1}$) and 217 nm (negative minimum, $-40.000 \text{ deg} \cdot \text{cm}^2 \cdot \text{dmol}^{-1}$)). A negative shoulder at 235 nm could be found which is consistent with congener PEO-peptide conjugates, having the same aromatic template.

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Microscopy

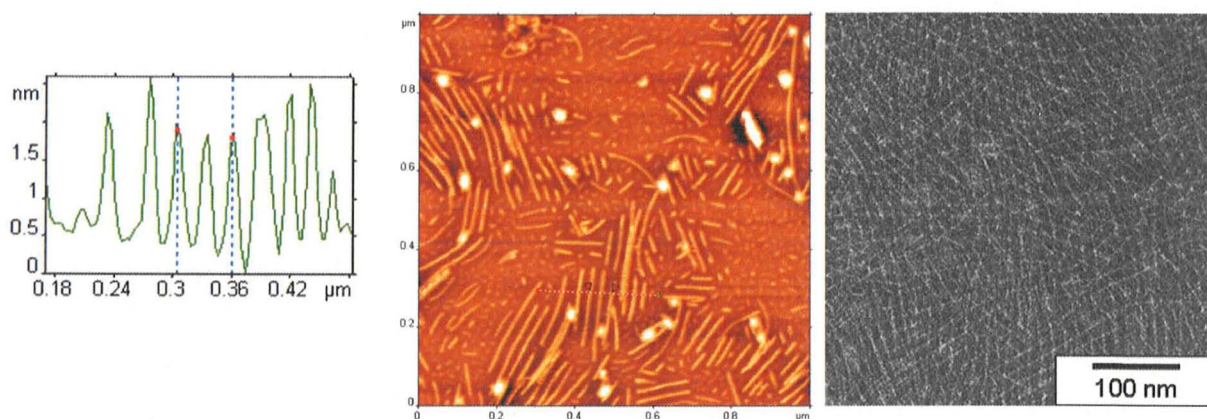


Figure S7. Self-assembled nanotapes of **II**. AFM height image and height profile (middle and left, z range 3 nm, mica) and TEM micrograph on carbon coated copper grid (right, negative stained with ¹⁰ Uranyl acetate).

Modification of the azido-functional nanotapes via click ligation (standard protocol)

20 mL of an aqueous solution of azide functionalized nanotapes (**II**, 1 mg/mL, 0.004 mmol **II**, 0.008 mmol N₃), 2.4 mg copper(II)sulphate (0.016 mmol, 2 equiv.) and 2.2 equiv. of a functional alkyne were added. The reaction mixture was degassed with nitrogen. Under inert gas atmosphere 8.00 mg sodium ascorbate (0.04 mmol, 5equiv.) was added in small portions to generate the catalytic copper(I) catalyst *in situ*. The mixture was agitated 12 h at room temperature. The copper catalyst was removed with DOWEXTM and the solution was purified by means of dialysis against Millipore water (MWCO 2000 Da). The accurate concentration was determined by UV-spectroscopy and the solution was investigated with CD, AFM and TEM. A small aliquot was lyophilized to analyse the product by NMR, IR and MALDI TOF.

1. Introducing alkyne functionalized fluorescein onto the azide-functional nanotapes (**II** → **III b**)

Alkyne functionalized fluorescein (**III a**) was synthesized by the slow addition of a solution of 0.29 g propargylamine (5.3 mmol, 1.05 equiv.) in MeOH to a solution of 2 g fluorescein thioisocyanate (spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one-3',6'-dihydroxy-6-isothiocyanato, 5 mmol, 1 equiv.) in MeOH. After stirring the reaction mixture overnight, the solvent was distilled off under vacuum and the product was lyophilized three times from water under high vacuum to remove the excesses of the propargylamine.

¹H-NMR (**III a**, 400 MHz, MeOH, δ in ppm): δ = 2.27 (s, 1H, CCH), 4.02 (s, 1H, -CH₂-CCH), 6.40-6.58 (m 4H, CH-Ar), 7.00 (s, 2H, CH-Ar), 7.28-7.41 (m, 2H, CH-Ar), 7.69 (s, 1H, CH-Ar).

Modification of the azido-functional nanotapes with alkyne functionalized fluorescein has been performed according to the standard protocol with alkyne functionalized fluorescein (**III a**) as alkyne.

Characterization of **IIIb**

FTIR-ATR (ν in cm^{-1}): 3269(s), 3086(w), 2961(w), 2872(s), 2100 (vw)*, 1749(m), 1670(m), 1627(s, amide(I)), 1612(w), 1572(m), 1548(w – amide(II)), 1506(w), 1460(m), 1386 (m), 1321(s), 1250 (w), 1203 (m), 1168(w), 1103 (s), 1062 (w), 1040(m), 995(w), 950 (m), 914(w), 846 (s), 829(w), 819(w), 800(w), 717(m), 690(w), 671(w), 663(w), 646(w), 624(w), 617(w).

Note: The intensity of the N_3 vibration at 2100cm^{-1} was significantly reduced, indicating not quantitative but high conversion of the click coupling reaction. A rough estimation of the conversion was feasible by comparing the signal integral intensity of the azide vibration band of **III b** with that of the starting material **II**. This reveals a conversion of <75%.

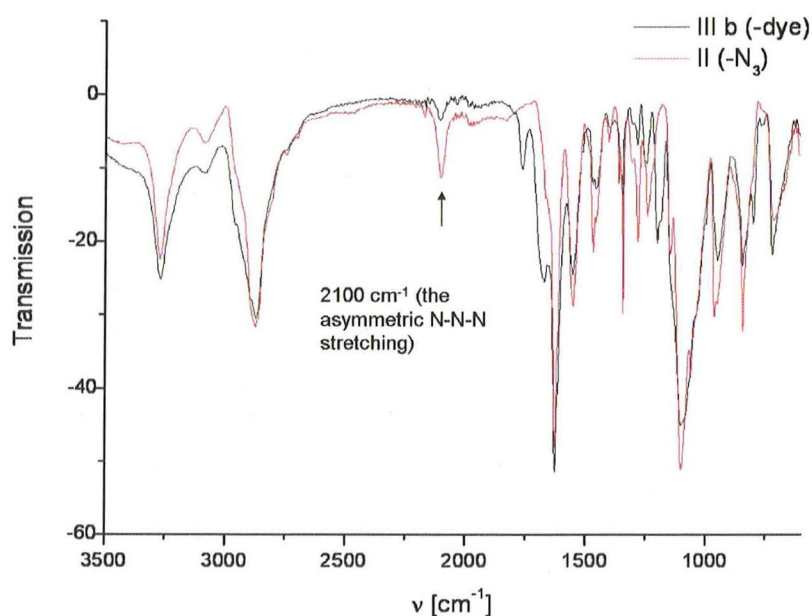


Figure S8. ATR-FT IR of the dye functional conjugate (**III b**).

¹H-NMR (400 MHz, D₁-TFA, δ in ppm): δ = 0.47-0.56 (m, 36H, CH₃-Val), 0.83-0.89 (m, 12H, CH₃-Thr), 1.08, 1.19, 1.54, 1.64* (multiple resonances, m, ~20H, ^γCH₂-Lys, ^δCH₂-Lys, ^βCH₂-Lys, CH₂-template, CH₂-template), 1.71 & 1.96 (not assigned), 2.34-2.42 (m, 6H, ^βCH-Val), 2.64 (br. s, 12H, CH₃-dmG), 3.39 (br. s, 300 H, O-CH₂-CH₂-O), 3.54-4.55** (multiple resonances m, ^αCH-Val, ^αCH-Lys, ^αCH-Thr, ^βCH-Thr, ^εCH₂-Lys, PEO-O-CH₂-CH₂-NH-Gly-template), 5.06 (br. s, 2H, N-CH₂-carbazole), 5.18 (br. s, 4H, CH₂-dye), 6.78-6.86 (resonances overlaid, m, ~12H, CH-Ar template (4Hs), CH-Ar dye (8Hs)), 7.00 (not assigned), 7.10-7.14 (m, ~4H, CH-Ar dye), 7.37 (m, ~2H, CH-Ar template), 7.48-7.50 (m, ~4H, CH-Ar dye), 8.06 (s, 2H, CH-triazole).

Note: The resonances δ = 0.47-0.56 ppm (Val) and 8.06 (1,2,3-triazole) were utilized to confirm the ratio of peptide/dye (36.0 : 1.7), that corresponds to about 85% conversion.

* Resonances are not baseline separated and can only be integrated cumulatively.

** Complex resonances could not be assigned individually.

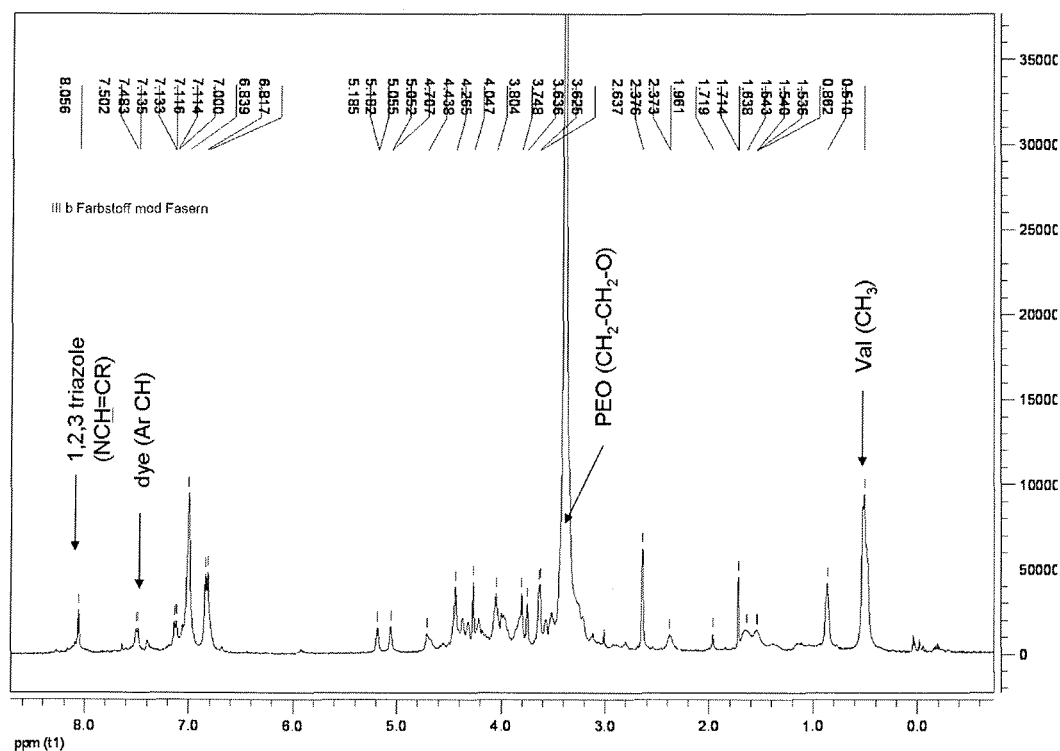


Figure S9. ¹H-NMR of the dye functional conjugate in TFA (**III b**).

MALDI TOF-MS: **III b** could not be desorbed properly. This might be explained by the presents of a dye that absorbs the laser light, leading to an unfavourable energy transfer to the macromolecule and preventing desorption without fragmentation.

CD-Spectroscopy: Cotton effects characteristic for the β -sheet motif (195 nm (positive max, 37.000 deg \cdot cm² \cdot dmol⁻¹) and 217 nm (negative minimum, -32.000 deg \cdot cm² \cdot dmol⁻¹)). The additional negative shoulder at 238 nm could be found, which is consistent with previous observations and probably due to aromatic entities positioned in a chiral environment of the peptide.

Microscopy:

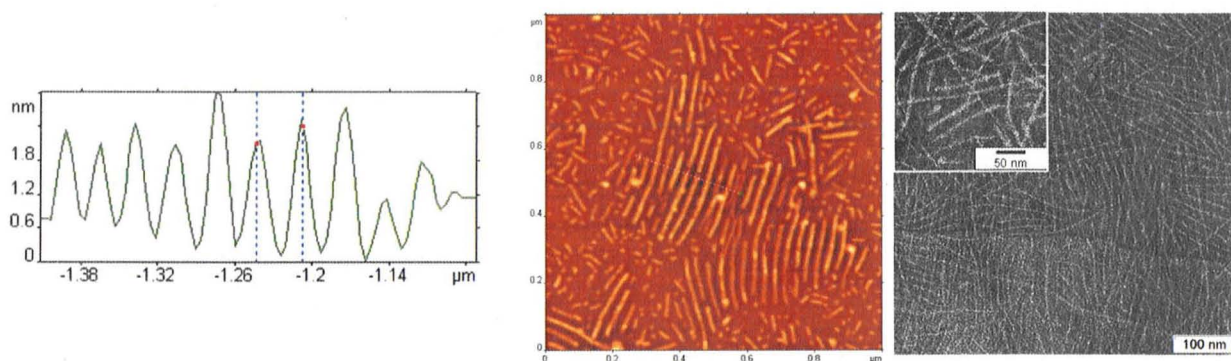


Figure S10. Self-assembled nanotapes of **III b**. AFM height image (middle) and corresponding height profile (left) (z range 3 nm, mica) and TEM micrograph (right) on carbon coated copper grid (negative stained with Uranyl acetate).

Table S1. Dimensions of nanofibres as determined from evaluating AFM and TEM micrographs

functional PEO-peptide tapes	distances (AFM) [nm] ^{a)}	height (AFM) [nm] ^{b)}	width peptide (TEM) [nm] ^{c)}	width (TEM) [nm] ^{d)}
amine (I)	15.5 ± 1.5	1.69 ± 0.2	4.4 ± 1.4	15.0 ± 1.5
azide (II)	(27.0 ± 3) ^{e)}	1.62 ± 0.2	4.0 ± 0.4	16.0 ± 2.0
dye (III b)	(28.0 ± 3) ^{e)}	2.2 ± 0.3	4.8 ± 0.9	14.6 ± 1.8

^{a)} distances of tape structures in parallel packing; ^{b)} heights from isolated single tapes with respect to the substrate; ^{c)} structural region in negatively stained structures with reduced contrast (less stained inner region); ^{d)} distance between core structures in close parallel packing; ^{e)} number of tapes packed parallel have been low, leading to more loose packings and unreliably larger apparent widths (evaluation of TEM micrographs are more valid).

Confocal scanning laser fluorescence microscopy allows for the visualization of fluoresceine functionalized assemblies. Figure S6 shows on glass surfaces the web-like aggregate (not shown). Under higher dilution more separated bundles occur (cf. Figure S6). The lengths of these bundles reach up to 5 μm which indicates aggregation on the glass substrate. Imaging of single nanotapes or the resolution of a fine structure within the observed bundles is clearly beyond the resolution limit of light microscopy.

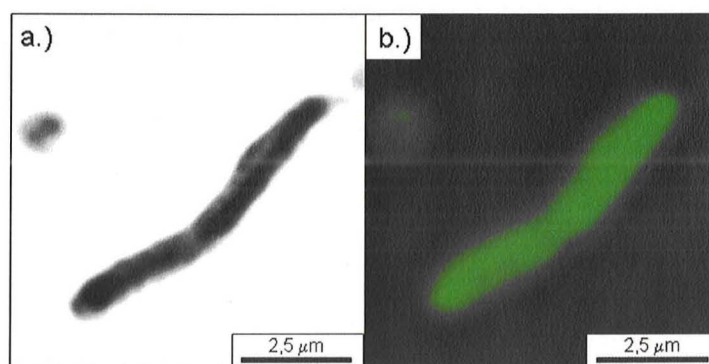


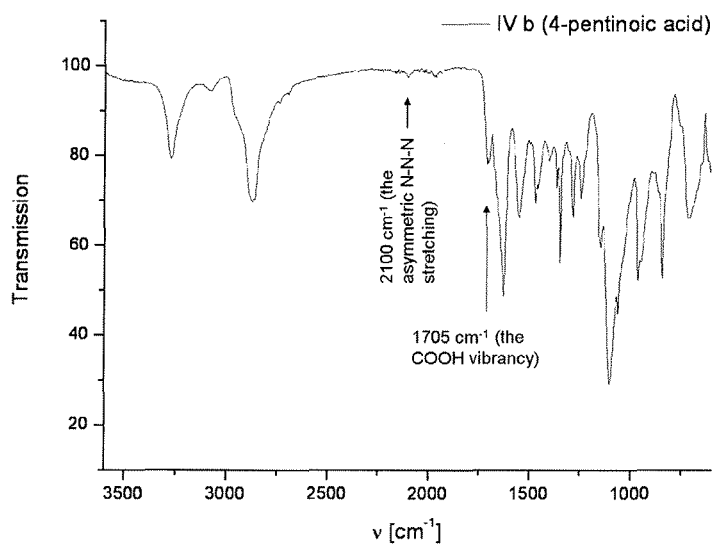
Figure S11. Confocal scanning laser fluorescence microscopy image of bundles of fluoresceine functional nanofibres (III b) (light microscopy image (a), and confocal fluorescence image (b)).

2. Synthesis of 4-pentinoic functional fibres (IV b)

Synthesis was proceeded analogue to the standard protocol with 4-pentinoic acid (IV a) as alkyne. After purification the product was characterized with IR, MALDI-TOF and NMR:

FTIR-ATR (ν in cm^{-1}): 3269(s), 3086(w), 2961(w), 2872(s), 2100 (vw)*, 1705 (m), 1622(s – amide(I)), 1625 (s), 1549(s – amide(II)), 1466(s), 1454(s), 1398(m), 1357(m), 1342(s), 1303(w), 1278(m), 1240 (s), 1143 (w), 1097(s - PEO), 1058(m), 960(s), 840(s), 711(m).

The intensity of the N_3 vibration at 2100cm^{-1} was significantly reduced, indicating nearly quantitative conversion of the click coupling reaction.



10 Figure S12. ATR-FT IR of the pentinoic acid functional conjugate (IV b).

MALDI-TOF

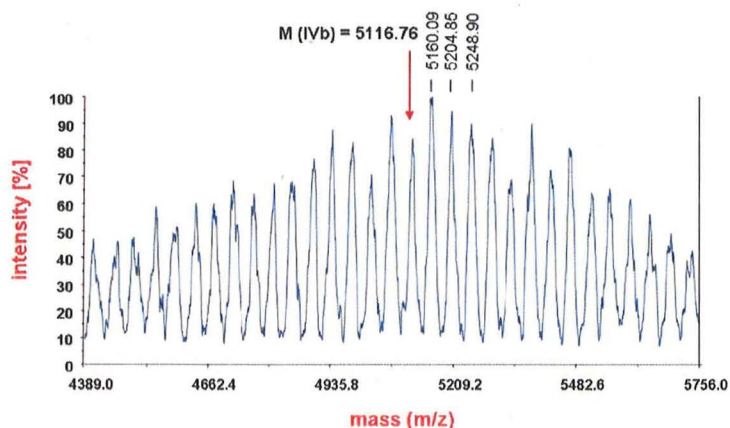


Figure S13. MALDI-TOF-MS of **IV b** (ACHC as matrix).

The spectrum shows the typical distribution of PEO with the characteristic repeat unit of 44 Da. The m/z of each peak can be assigned to **IV b** within ± 1 Da accuracy. For instance the experimentally found signal at m/z 5116.76 can be assigned to $[M+Na]^+ = 5117.99$ Da by assuming a $M_{[EO]}$ of 44.04 Da, $n = 70$ units, a mass of the template of $M_{[template]} = 335.19$ Da, a mass of each peptide chain of $M_{[peptide]} = 838.0$ Da, a mass of polymer the end groups (1 H = 1 Da) and the mass of a sodium counter ion ($M_{[counter\ ion]} = 23$ Da).

1H -NMR (400 MHz, DMSO, RT, in ppm): $\delta = 0.78$ - 0.86 (m, 36H, CH_3 -Val), 1.00 (d, 6H, CH_3 -Thr, $^3J = 5.99$ Hz), 1.05 (d, 6H, CH_3 -Thr, $^3J = 5.77$ Hz), 1.22 (m, 4H, $^{\gamma}CH_2$ -Lys), 1.49 (m, 4H, $^{\beta}CH_2$ -Lys), 1.62 (m, 1 H,), 1.75 (t, 4H, $^{\delta}CH_2$ -Lys, $^3J = 7.3$ Hz), 1.89-1.99 (m, 6H, $^{\beta}CH$ -Val), 2.70-2.80 (multiple resonances* 4+12H, $^{\alpha}CH$ pentinoic acid CH_3 -dmG), 3.00-3.15 (m, 4H, $^{\beta}CH$ pentinoic acid), 3.39 (d, 4H CH_2 -template), 3.47 (br. s, 290H, PEO), 3.93-3.97 (m, 8H, $^{\beta}CH$ Thr + 4H, $^{\epsilon}CH$ Lys)*, 4.09 (dt, 2H, $^{\alpha}CH$ Lys), 4.19-4.40 (multiple m*, 10H, $^{\alpha}CH$ Val & Thr), 4.93 (s, 2, CH_2 -carbazole), 7.22 (d, 2H, $^3J = 8.04$ Hz, CH-Ar template), 7.37 (d, 2H, CH-Ar template), 7.75 (s, 2H, triazole), 7.86 (s, ~2H, CH-Ar template). * Resonances are not baseline separated and can only be integrated cumulatively.

CD-Spectroscopy: Cotton effects characteristic for the β -sheet motif could be found (195 nm (positive max, $30.000 \text{ deg} \cdot \text{cm}^2 \cdot \text{dmol}^{-1}$) and 217 nm (negative minimum, $-28.000 \text{ deg} \cdot \text{cm}^2 \cdot \text{dmol}^{-1}$)).

Microscopy:

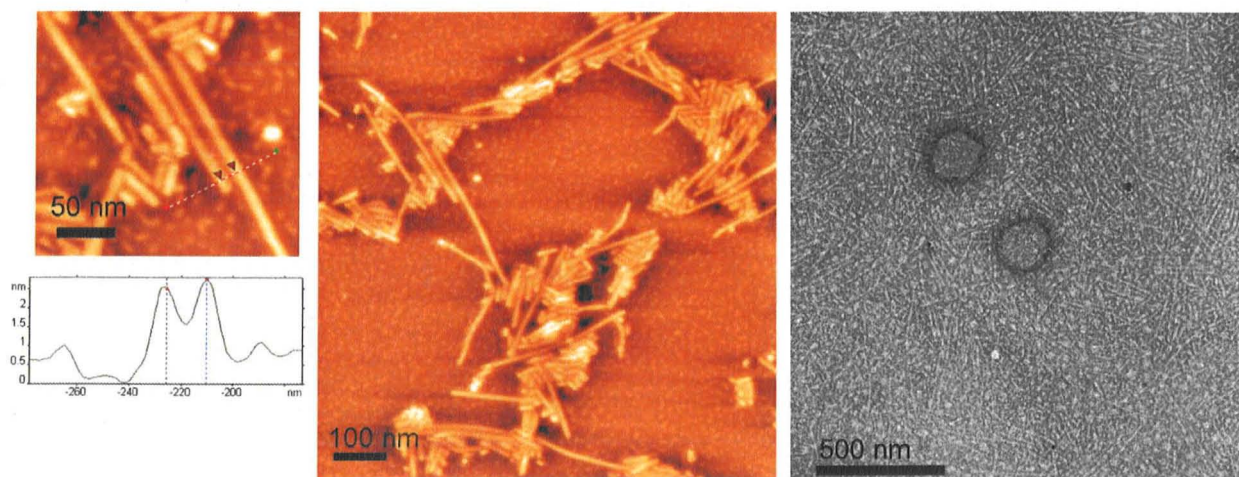


Figure S14. Micrographs of pentinoic acid modified fibres (IV b). AFM height image (middle), the corresponding height profile (left) (z range 3 nm, mica) and a TEM micrograph (right) on carbon coated copper grid (negative stained with uranyl acetate). (AFM: 15.3 nm width, 1.86 nm height; TEM: 16.7 nm width).

3. Introducing alkyne functionalized Pra-NTA onto the azide-functional nanotapes (II → V b)

2-[bis(carboxymethyl)amino]pent-4-inoic acid (V a) (Pra-NTA, propargylglycine-nitrilotriacetic acid)

A solution of propargylglycine (1.5 g, 0.014 mol) in 25 mL dry THF was slowly added to a THF solution of *tert*-butyl bromoacetate (10.34 g, 0.05 mol, 4 equiv.) and DIPEA (18 g, 10 equiv.). The mixture was heated to 55 °C for 17 h. After cooling to room temperature, the solvent was removed and the crude product was purified via column chromatography in cyclohexane: ethyl acetate (2:1 v/v) to yield 1.9 g (60%). Deprotection takes place by dissolving the compound in 30 vol.% TFA in DCM (0.1% TES). After 1 h the cocktail was distilled off and the remaining compound was lyophilized 3 times from water.

¹H-NMR (400 MHz, D₄-MeOH, RT, in ppm) δ = 2.11 (s, 1H, term. alkyne), 2.74 (bd, 2H, ^βCH Pra), 3.58 (overlaid resonances, 4H, CH₂-COOH), 4.11 (bs, 1H, ^αCH Pra).

¹³C-NMR (100 MHz, D₄-MeOH, RT, in ppm) δ = 21.3 (^βC, Pra), 61.1 (CH₂-COOH), 63.5 (^αC, Pra), 63.5 (^δC, Pra), 82.2 (^γC, Pra), 172.3, 173.5, 173.9 (COOH).

ESI-MS: 252.2 Da [M+Na]⁺ (M_{th.} = 229.19 Da)

Modification of the azido-functional nanotapes with Pra-NTA (V b)

The introduction of V a onto the nanotapes of II was performed by following the standard protocol of click ligation as described above. To counterbalance the ligand properties of Pra-NTA that might partially complex copper ions and reduce the effective concentration of the click catalyst, the equivalent of copper catalyst has been increased from 2 equiv. per azide to 4 equiv. per azide.

Characterization of V b

FTIR-ATR (ν in cm^{-1}): 3269(s), 3086(w), 2961(w), 2872(s), 1706(m), 1664(w), 1627 (s), 1546(s), 1466(s), 1465(s), 1456(w), 1392(w), 1365(m), 1342(s), 1303(w), 1278(m), 1240 (s), 1230 (s), 1141 (m), 1097(s), 1060(m), 960(s), 945(m), 841(s), 7967(w), 748 (w), 708(m).

Note: A strong decrease of the azide vibration band at 2100 cm^{-1} indicates that the click ligation proceeds very well.

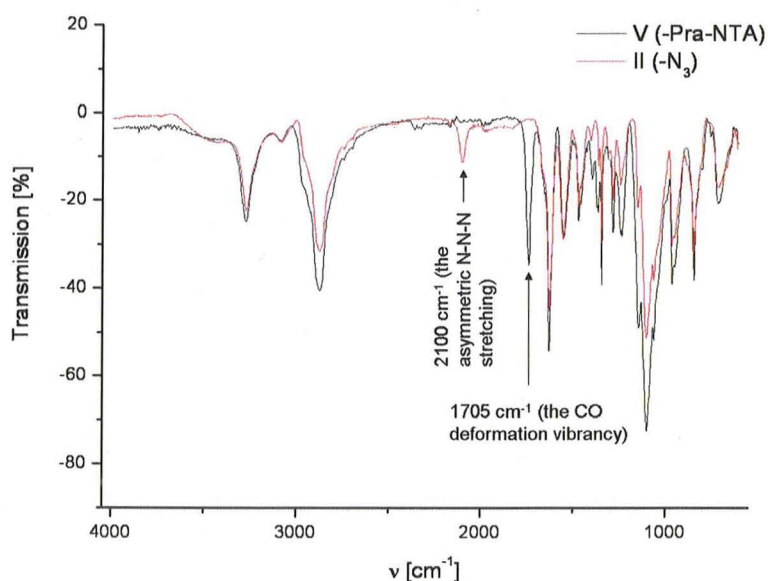


Figure S15. ATR-FT IR of the Pra-NTA functional conjugate (V b).

10

¹H-NMR (400 MHz, D₆-DMSO, δ in ppm): δ = 0.76-0.86 (m, 36H, CH₃-Val), 0.97-1.04 (m, 12H, CH₃-Thr), 1.37 (not assigned, EtOH), 1.40-2.13 (multiple resonances, m, ~20H, γ CH₂-Lys, δ CH₂-Lys, β CH₂-Lys, CH₂-template, CH₂-template), 2.23 (br. s, 12H, CH₃-dmG), 2.64-2.78 (m, 6H, β CH-Val), 2.98 (d, 4+4H, CH₂-template, β CH₂ Pra-NTA), 3.41 (br. s, 300 H, O-CH₂-CH₂-O), 3.72, 3.82, 3.96, 4.02, 4.11,

4.20, 4.28** (multiple resonances m, ~22H, α CH-Val, α CH-Lys, α CH-Thr, β CH-Thr, PEO-O-CH₂-CH₂-NH-Gly-template), 4.42 (s, 4H, ϵ CH-Lys), 4.54 (br. s, 2H, N-CH₂-carbazole) (Integral 4.42+4.54 equals 6H), 4.93 (s, 2H, α CH-Pra), 7.22 (s, 2H, CH-Ar template), 7.36 (s, 2H, CH-Ar template), 7.66 (m, 2+2H, CH-Ar template, NH-amid), 7.99 (2H, 1,2,3-triazole).

Note: Comparing the integral intensities of the resonance $\delta = 0.76$ - 0.86 (CH₃ group of the Val residues) with the Pra resonance at $\delta = 4.93$ (α CH-Pra) and the template resonances at $\delta = 7.22$ ppm (Ar template) indicates a ratio of 36.00:1.75:2.05 protons, suggesting an high conversation of the click reaction.

* Resonances are not baseline separated and can only be integrated cumulatively.

** Complex resonances could not be assigned individually.

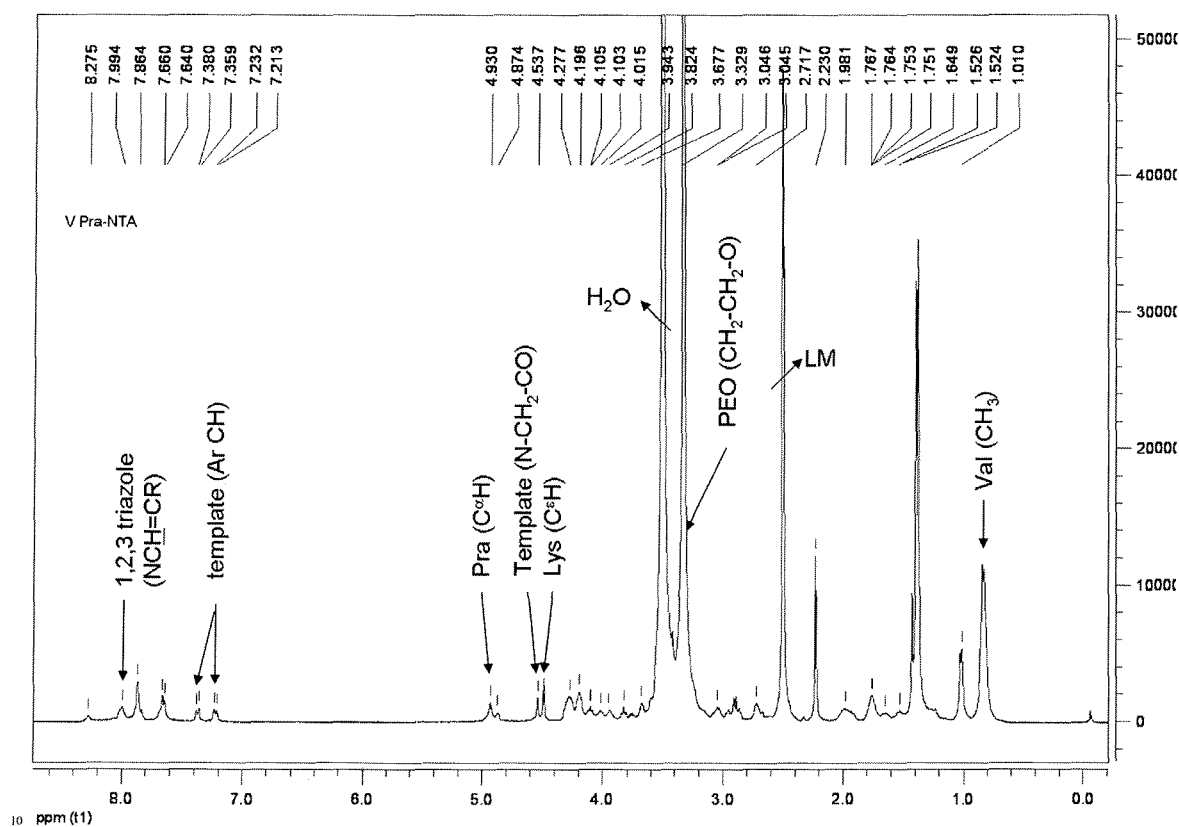


Figure S16. ¹H-NMR of the Pra-NTA in DMSO functional conjugate (V b).

MALDI-TOF-MS: MALDI of **V b** was difficult to be obtained with acceptable quality. Attempts to optimize the matrix or change the mode of acceleration did not provide conditions in which **V b** could be desorbed properly. Due to the presents of the Pra-NTA-substituents four negative net-charges are generated per molecule. This hampers desorption due to strong matrix interactions. However, a rather noisy spectrogram could be obtained provided some evidence for a PEO distribution with characteristic repeats of ~44 Da.

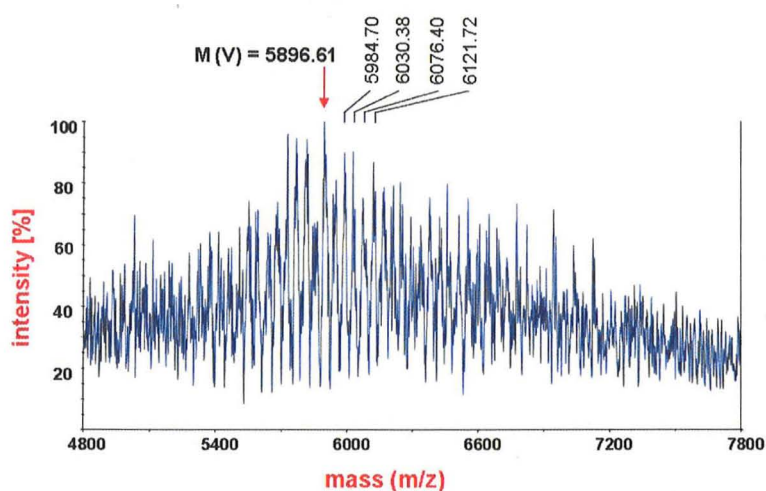


Figure S17. MALDI-TOF-MS of **V b** (DHB as matrix).

Despite the signal to noise ratio a preliminary assignment of signals was possible. However the authors would like to state that the data should be seen only as additional indication for the chemical structure not as proof. The m/z signals could be assigned to **V b** within ± 2 Da accuracy. For instance the experimentally found signal at m/z 5896.61 can be assigned to $[(M-2H+2K)+Na]^+ = 5896.53$ Da by assuming a $M_{[EO]}$ of 44.04 Da, $n = 80$ units, mass of the template of $M_{[template]} = 335.19$ Da, mass of each peptide chain of $M_{[peptide]} = 969.07$ Da, mass of polymer end group (1 H = 1 Da), two protons of the NTA-ligand exchanged by potassium ions and the mass of a sodium counter ion ($M_{[counter\ ion]} = 23$ Da). The symmetric exchange of protons with metal ions (e.g. $H \leftrightarrow K$) is commonly in MALDI-mass spectrometry of peptides with good ligand properties.

CD-spectroscopy: Cotton effects characteristic for the β -sheet motif could be observed (195 nm (positive maximum) and 218 nm (negative minimum)).

Microscopy:

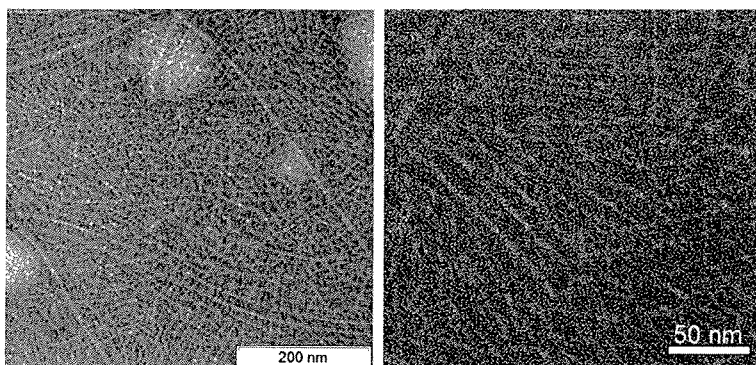


Figure S18. TEM micrographs of Pra-NTA modified fibres (V b; Evaluation: 17.0 nm width, 3.88 nm unstained core).

4. Introducing alkyne functionalized oligopeptide onto the azide-functional nanotapes (II → VI b).

Synthesis of alkyne functionalized aspartic acid hexapeptide (D₆, VI a).

A hexapeptide, composed of six repeats of aspartic acid was synthesized by automated SPPS on a support with a S-Ram linker. The N-terminal amino group of the last aspartic acid residue was capped by coupling with pentynoic acid via bench-top protocols with DCC according to the previous protocols.[Hentschel, J. *et al.* *Macromolecules*, **2007**, *40*, 9224] Quantitative coupling was confirmed by a negative Kaiser test. The alkynated hexapeptide VI a was cleaved from resin with 30% TFA in DCM, precipitated in diethyl ether and isolated by lyophilization from water.

Characterization of VI a

10 **ESI-MS:** Alkyne functionalized aspartic acid hexapeptide: $m(\text{IV a})_{\text{th.}} = 787.65 \text{ Da}$

Experimentally found: $m = 788.5 [\text{M}+\text{H}]^+$; $m = 810.5 [\text{M}+\text{Na}]^+$ and $m = 826.8 [\text{M}+\text{K}]^+$.

¹H-NMR (400 MHz, D₂O, RT, in ppm): $\delta = 2.10$ (s, 1H, alkyne H), 2.51 (br. m, 4H, pentynoic acid), 2.8-3.1 (m, 12 H, ^βCH₂-Asp), 4.23-4.74 (m, 6H ^αCH-Asp).

FTIR-ATR (ν in cm⁻¹): 3278 (s), 3076 (w), 2933 (w), 1710 (s), 1643 (s), 1533 (s), 1406 (s), 1340 (w),
15 1286 (m), 1224 (w), 1180 (s), 914 (m), 833(w).

Modification of the azido-functional nanotapes with alkyne functionalized D₆ (VI b).

The D₆-decorated nanotapes (VI b) could be synthesised by following the standard click protocol with the alkyne equals pentynoic acid-D₆

FTIR-ATR (ν in cm^{-1}): 3278 (s), 3076 (w), 2933 (w), 1710 (s), 2100(w)⁺⁺, 1705(s), 1625 (s), 1549(s), 1466(s), 1454(s), 1398(m), 1357(m), 1342(s), 1303(w), 1278(m), 1240 (s), 1143 (w), 1097(s), 1058(m), 960(s), 840(s), 711(m)

⁺⁺ Note: A residual band at 2100 cm^{-1} indicated that the conversion of the click reaction was not quantitative. A comparison of the area of the remaining azide vibration in the product with that in the IR spectrum of the starting material showed a residual azide band of about 7% signal intensity. This suggests a roughly estimated conversion of <90%.

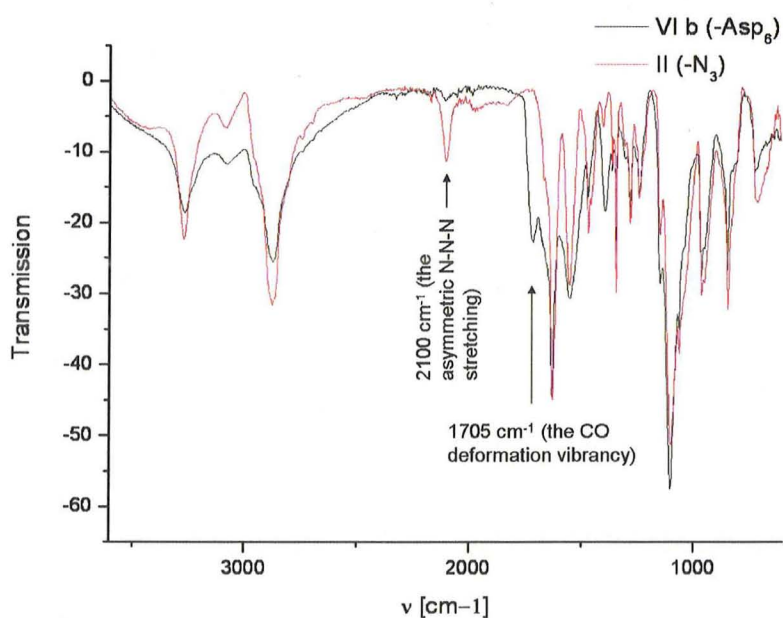


Figure S19. ATR-FT IR of the Asp₆ functional conjugate (VI b).

¹H-NMR (400 MHz, D₁-TFA, RT, δ in ppm): δ = 0.48-0.53 (m, 36H, CH₃-Val), 0.82-0.86 (m, 12H, CH₃-Thr), 1.02, 1.18, 1.39, 1.52-1.78* (multiple resonances m, 24H, ^γCH₂-Lys, ^δCH₂-Lys, ^βCH₂-Lys, CH₂-template, CH₂-template, CH₂- pentinoic acid amide), 2.00-2.16 (m, 4H, CH₂- pentinoic acid amide), 1.95-2.26 (m, 6H, ^βCH-Val), 2.55-2.68 (m, 12+24H, CH₃-dmGly, ^βCH₂-Asp), 3.39 (br. s, ~300H, O-CH₂-CH₂-O), 3.79, 3.84, 3.97, 4.04, 4.18, 4.31, 4.36** (multiple resonances, ^βCH Thr, ^εCH Lys, ^αCH Lys, ^αCH Val, ^αCH Thr, N-CH₂-carbazole), 4.53-4.68 (m, 13H, ^αCH Asp), 6.74-6.83 (m, 4H, 2×CH-Ar template), 7.38 (s, 2H, CH-Ar template), 7.66 (s, 2H, CH-1,2,3 triazole).

* Resonances are not baseline separated and can only be integrated cumulatively.

** Complex resonances could not be assigned individually.

10 Note: Comparing the integral intensities of the resonance δ = 0.48-0.53 (CH₃ group of Val) with the Asp resonance at δ = 4.53-4.68 and the template resonances at δ = 6.74-6.83 (Ar template) indicates a ratio of 36.0:12.8:4.4 protons, suggesting a quantitative conversion of the click reaction.

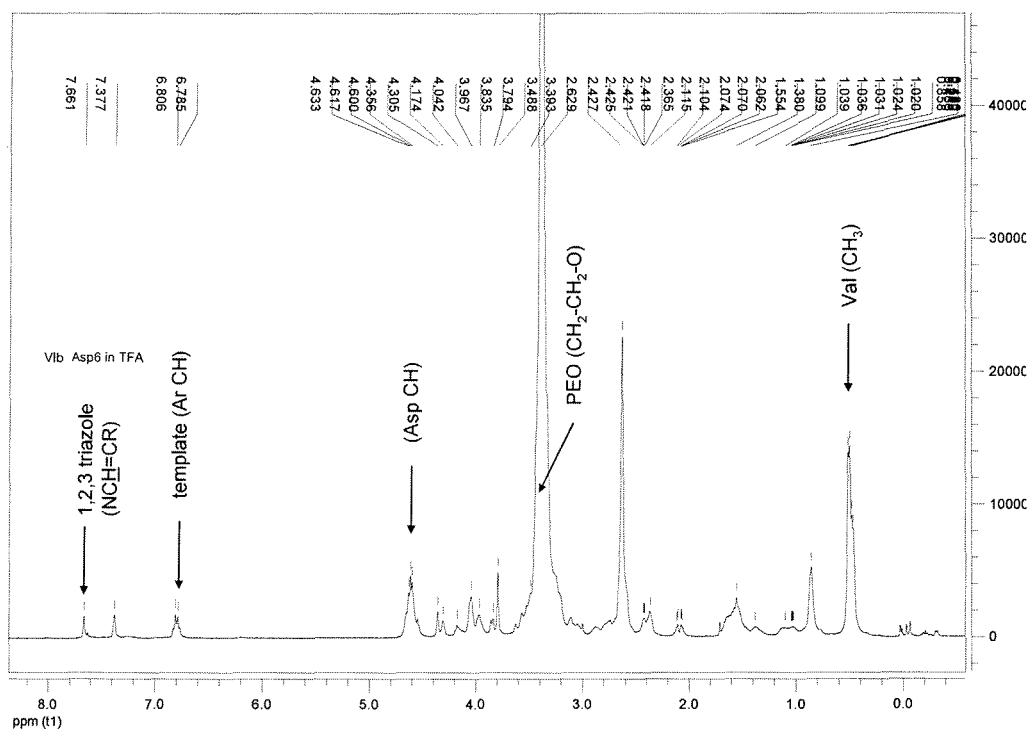


Figure S20. ¹H-NMR of the Asp₆ functional conjugate (VI b).

MALDI-TOF-MS: MALDI of **IV b** could not be obtained. Attempts to optimize the matrix or change the mode of acceleration did not provide conditions in which **IV b** could be desorbed properly. This might be explained by the presents of the Asp₆-substituents, providing ten negative net-charges per molecule, which could hamper desorption due to strong matrix interactions.

CD-spectroscopy: Cotton effects characteristic for the β -sheet motif could be found at 195 nm (positive max, 31.000 deg · cm² · dmol⁻¹) and 218 nm (negative minimum, -26.000 deg · cm² · dmol⁻¹).

Microscopy:

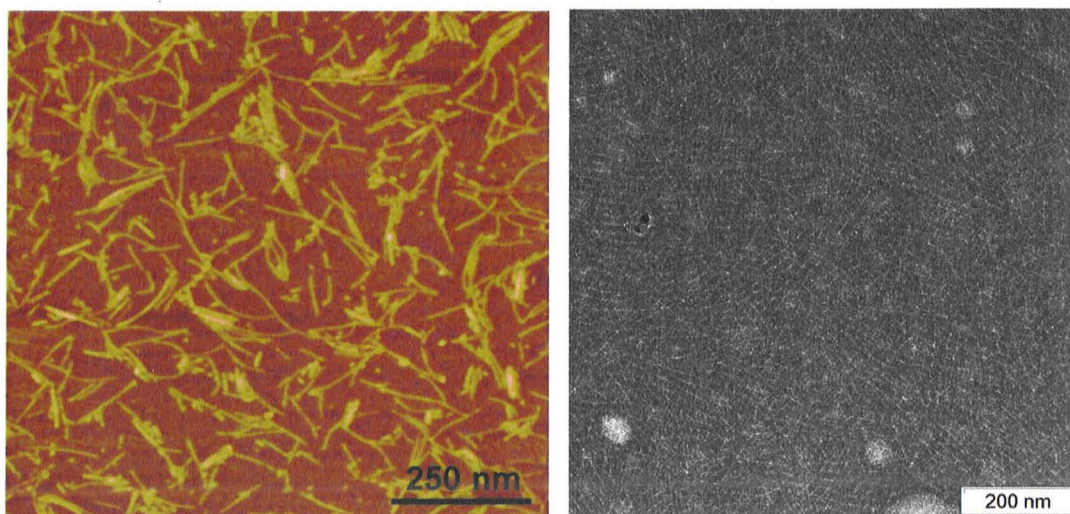


Figure S21. Micrographs of asparagine acid hexapeptide modified fibres (**VI b**). AFM height image (left
10 z range 3 nm, mica) and TEM micrograph (right) on a carbon coated copper grid (negative stained with
Uranyl acetate). (Evaluation: AFM: 1.8 nm height; TEM 15 nm width).