Supporting information

Peptide-guided Organization of Peptide-polymer Conjugates: Expanding the Approach from Oligo- to Polymers.

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Materials: 2-Bromopropionic acid (Aldrich, 99+%), *n*-butyl acrylate (*n*BA, Aldrich, 99%) and *N,N*-dimethylformamide (DMF; Aldrich, 99+%) were distilled and stored at -15 ºC. THF was dried over Na/benzophenone and distilled prior to use. Dichloromethane (DCM; IRIS Biotech GmbH, peptide grade) was distilled from CaH$_2$. Diisopropylethylamine (Acros, peptide grade), piperidine (Acros, peptide grade), trifluoroacetic acid (TFA; Acros, peptide grade), *N,N*-diisopropylcarbodiimide (DIC; Fluka, 99%), 1-benzotriazoyloxy-tris(pyrrolidino)-phosphonium hexafluorophosphate (PyBOP, NovaBiochem), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro phosphate (HBTU, IRIS Biotech GmbH), *N*-methyl-2-pyrrolidone (NMP, 99.9% peptide synthesis grade), 1-hydroxybenzotriazole (HOBt, IRIS Biotech GmbH) and 1-methyl-imidazole (NMI; Fluka, 99+%) have been used as received. All other reagents were used as received from Aldrich.

Amino acid derivatives   Fmoc-Gly OH,   Fmoc-Val OH,   Fmoc-Val-Thr($\Psi^{Me,Me}$pro) OH,   Fmoc-Thr(*t*Bu) OH),   *t*Boc-Thr OH   and   polystyrene–(2-aminoethanol-2-chlorotrityl)resin   (loading: 0.74 mmol/g), were used as received from IRIS Biotech GmbH, Germany.

Instrumentation: The standard amino acid coupling leading to native amide bonds was performed on an ABI 433a peptide synthesizer (Applied Biosystems, Germany). Mass spectrometry was performed on a high performance liquid chromatograph electron spray ionization mass spectrometer (LC-ESI-MS) (Shimadzu, qp8000α, Duisburg, Germany) without chromatographic separation. Nuclear magnetic resonance spectra (NMR) were recorded on a Bruker DPX-400 Spectrometer at 400.1 MHz in CDCl$_3$, MeOH-d$_4$ or DMSO-d$_6$. GPC measurements were carried out in THF (flow rate 1 mL/min) using three 5 μ-MZ-SDV columns (10^3, 10^5 and 10^6 Å). The detection was performed with a RI- (Shodex RI-71) and a UV-Detector (TSP UV 1000; 260 nm) and calibration was based on linear pS-standards (PSS, Germany). Fourier transformation infrared spectroscopy (FT-IR) was performed on a BioRad FTS6000 spectrometer using a Golden Gate arrangement (Single Reflection Diamond ATR). Atomic force microscopy (AFM) was performed on a NanoScope IIIa microscope (Digital Instruments) with a 10x10 μm e-scanner and silicon tips (type NCR-W; tip radius <10 nm, spring constant of 42 N•m^{-1} at a resonance frequency of 285 kHz). All measurements were carried out in tapping mode. The samples

were spin-coated (3000 rpm) from solution (0.05-0.5 mg/mL) on freshly cleaved Mica substrates. TEM micrographs were obtained with a Zeiss EM 912 OMEGA instrument operating at an acceleration voltage of 120 kV. Samples were air-dried on 400-mesh carbon-coated copper grids (solution 0.5 mg/mL).

Synthesis of the switch-peptide precursor H-Thr(tBu)-(Val-Thr)^{pro}-Val-Thr(tBu)- (Val-Thr)^{switch}-(Val-Thr)^{pro}-Val-Gly-NHCH$_2$CH$_2$OH) (I)

The loading of a polystyrene–(2-aminoethanol-2-chlorotrityl) resin (1g, 0.74 mmol/g) was reduced to 0.25 mmol/g by coupling of an appropriate mixture of (Boc)-Gly OH and (Fmoc)-Gly OH (65:35). Exact loading was confirmed by analytical Fmoc tests, using UV spectroscopy. The coupling of standard amino acids, leading to native amide bonds was performed on a ABI 433a peptide synthesizer using NMP as solvent and standard ABI-Fastmoc protocols (double coupling & capping). Fmoc amino acid coupling was facilitated by HBTU/DIPEA in NMP. Quantitative coupling was verified after each coupling step with the colorimetric Kaiser test (Kaiser, E. *et al. Anal. Biochem.* **1970,** 34, 595). To couple the pseudoproline (Fmoc-Val-Thr($\Psi^{Me,Me}$pro) OH) (0.5 mmol, 2 *eq*.), the resin was transferred into a glass reactor. Two coupling cycles were performed in DMF using PyBOP/HOBt/DIPEA protocols (2/2/4 *eq*., 1 h). After the successful coupling (confirmed by Kaiser-test), the resin was washed carefully, transferred back to the peptide synthesizer and the synthesis of the native peptide segments was continued in an automated manner. The switch-ester defect was synthesized by coupling of Fmoc-Val OH (10 *eq*.) to the unprotected β-hydroxyl side-chain functionality of the previously attached *t*Boc-Thr OH. Enforced coupling procedures were applied, using bench top procedures as described previously (Carpino, L. A. *et al. Tetrahedron Lett.* **2004,** 45, 7519; Hentschel, J. *et al. J. Am. Chem. Soc.* **2006,** 128, 7722.). The coupling was facilitated by DIC/NMI (10/7.5 *eq*., 2 h) and carried out in a glass reactor, using DCM as solvent. Three coupling cycles were performed to drive the reaction to completion. Quantitative conversion was verified by analytical Fmoc tests, using UV spectroscopy. To ensure the absence of deletion sequences, capping steps were carried out, following

Ac$_2$O/NMI/NMP protocols, prior to the removal of the Fmoc amine protecting group. Subsequently the resin was washed, transferred back to peptide synthesizer and the remaining amino acids were coupled. After final removal of the Fmoc protecting group, a small amount of **I** was liberated from the support in order to characterize the precursor. The cleavage was accomplished by 60 min. treatment of the resin, using a cleavage mixture of TFA/DCM/triethylsilane (30/69/1 *vol.%*). The peptide was isolated by diethyl ether precipitation, centrifugation, and washing of the precipitate with diethyl ether, followed by lyophilization from 1,4-dioxane.

^{1}H NMR (MeOH-d$_4$ (3.30 and 4.84 ppm)): δ = 0.94-0.98 (m, 30 H, C(CH$_3$)$_2$ Val), 1.13-1.18 (m, 9 H, C(CH$_3$)OH Thr), 1.27-1.29 (d, 3 H, C(CH$_3$)OH Thr), 1.33-1.35 (d, 3 H, C(CH$_3$)O-CO Thr), 2.07-2.21 (m, 5 H, CH(CH$_3$)$_2$ Val), 3.61-4.74 (m, 20 H, 12 α-CH + 4 CH-OH Thr + HO-CH$_2$-CH$_2$), 5.30-5.33 (t, 1 H, CH-O-CO Thr), 8.10-8.46 (m, NH) ppm. ESI-MS m/z = 1119 ([M+H]$^+$), 560 ([M+2H]$^{2+}$), 551 ([(M−H$_2$O)+2H]$^{2+}$; Thr in source fragmentation)
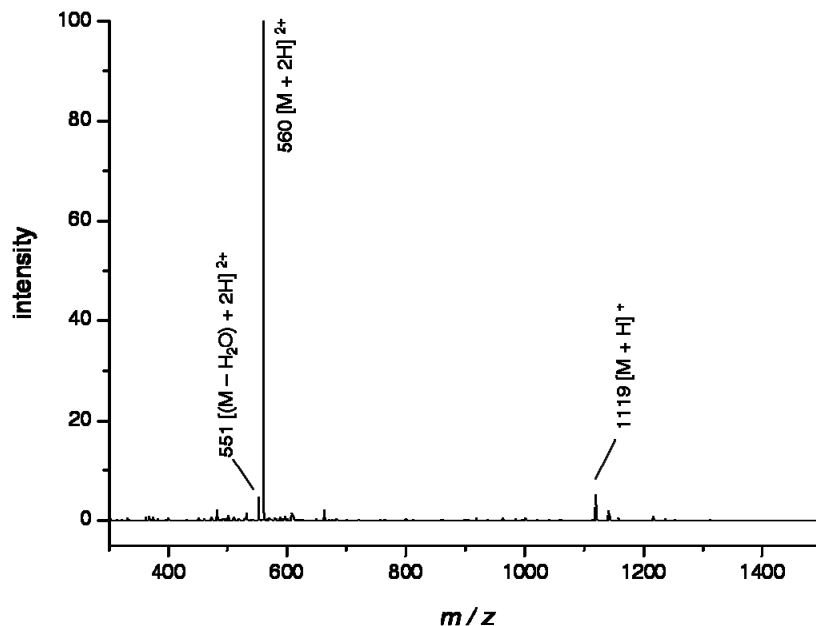

Figure S1. ESI-MS (relative detector intensity) of **I** in acetonitrile/water (1:1) (c = 0.1 mg/mL, M$_{theor}$ = 1118.3 g/mol).

Synthesis of the switch-peptide chain transfer agent (Br-CH(CH3)-C(O)-Thr(tBu)-(Val-Thr)^{pro}-Val-Thr(tBu) -(Val-Thr)^{switch}-(Val-Thr)^{pro}-Val-Gly-NHCH₂CH₂OH) (II)

To access the oligopeptide-ATRP-macroinitiator a procedure was performed according to Ref (ten Cate, M. G. J. et al. *Macromolecules* **2005,** 38, 10643-10649). In brief summary, the coupling of 2-bromopropionic acid (10 *eq*.) to the *N*-terminal of the supported **I** was achieved using standard DCC (5 *eq*.) double-coupling protocols (2 × 4 h). The reaction was accomplished in a glass reactor using DMF as solvent. The resin was washed with DMF, NMP and a small amount was cleaved from the support with a cleavage mixture of TFA/DCM/triethylsilane (30/69/1 vol.%) in order to characterize **II**.

^{1}H NMR (MeOH-d_4 (3.30 and 4.84 ppm)): δ = 0.94-0.98 (m, 30 H, C(C$\underline{H}_3$)$_2$ Val), 1.17-1.35 (m, 15 H, 12 H C(C$\underline{H}_3$)OH Thr + 3 H C(C$\underline{H}_3$)O-CO Thr), 1.76-1.78 (d, 3 H, Br-C(C$\underline{H}_3$)-CO), 2.07-2.18 (m, 5 H, C$\underline{H}$(CH$_3$)$_2$ Val), 3.65-4.68 (m, 21 H, 12 H α-C$\underline{H}$ + 4 H C$\underline{H}$-OH Thr + 4 HO-C$\underline{H}_2$-C$\underline{H}_2$ + Br-C$\underline{H}$(CH$_3$)-CO), 5.27-5.30 (t, 1 H, C$\underline{H}$-O-CO Thr), 8.00-8.45 (m, N$\underline{H}$) ppm; ESI-MS *m/z* = 1255 ([M+H]$^+$), 1277 ([M+Na]$^+$), 647 ([M+H+K]$^{2+}$), 628 ([M+2H]$^{2+}$), 619 ([(M–H$_2$O)+2H]$^{2+}$; Thr in source fragmentation)

Ph-C(S)-S-CH(CH3)-C(O)-Thr(tBu)-(Val-Thr)^{pro}-Val-Thr(tBu) -(Val-Thr)^{switch}-(Val-Thr)^{pro}-Val-Gly-NHCH₂CH₂OH) (III)

To convert **II** into the macro chain transfer agent, dithiobenzoic acid was prepared from bromobenzene using the Grignard reaction and subsequently added to the resin-bound ATRP-macroinitiator **II**. Under argon atmosphere, bromobenzene (19 mmol, 1 *eq.*) was gently added to a dispersion of magnesium turnings (19 mmol, 1 *eq.*) in THF (10 mL) and stirred for 45 minutes. While cooling, the resulting phenylmagnesium bromide was filtered into a round bottom flask containing carbon disulfide (27 mmol, 1.5 *eq.*) and 5 mL of THF were added for rinsing purposes. The reaction mixture was stirred for 2 hours at room temperature to form the dithiobenzoate salt. Water (6 mL) was added carefully and the dithiobenzoate solution was concentrated *in vacuo*. Water (20 mL) and diethyl ether (40 mL) were added. The aqueous layer was acidified using HCl (1 M, 20 mL) and the product

was extracted twice with diethylether. The combined ether layers were dried over Na_2SO_4 and concentrated *in vacuo*. The oligopeptide precursor resin (0.1 mmol) was pre-swollen in THF (4 mL). The dithiobenzoic acid (315 mg, 2.05 mmol) was added, followed by the addition of pyridine (160 μL, 2.0 mmol). The reaction was stirred at 60 °C for 20 h. The reaction mixture was cooled to room temperature and washed thoroughly with THF. This procedure was repeated once more after which the washing steps were extended using THF, water, THF and DCM. The product was cleaved from the resin with 2% TFA in DCM (3 × 10 mL, 1 min.) and the resin was washed with DCM. All fractions were directly added to a large excess of dioxane and the combined organic fractions were concentrated *in vacuo*. The product was reprecipitated in diethyl ether and lyophilized from acetonitrile/benzene (1:1), resulting in 112 mg (0.07 mmol) of **III** as a pink powder.

^{1}H NMR (MeOH-d$_4$ (3.30 and 4.84 ppm)): δ = 0.96-1.67 (m, 87 H, 30 H C(C$\underline{H}_3$)$_2$ Val + 12 H C(C$\underline{H}_3$)OH Thr + 3 H C(C$\underline{H}_3$)O-CO Thr + 3 H S-C(C$\underline{H}_3$)-CO + 18 H C(C$\underline{H}_3$)$_3$ ^{t}Bu + 12 H C(C$\underline{H}_3$)$_2$ ^{Me,Me}pro + 9 H C(C$\underline{H}_3$)$_3$ Boc), 2.07-2.18 (m, 5 H, C$\underline{H}$(CH$_3$)$_2$ Val), 3.56-4.60 (m, 21 H, 12 H α-C$\underline{H}$ + 4 H C$\underline{H}$-OH Thr + 4 H HO-C$\underline{H}_2$-C$\underline{H}_2$ + 1 H S-C$\underline{H}$(CH$_3$)-CO), 5.26 (t, 1 H, C$\underline{H}$-O-CO Thr), 7.42 (t, 2 H, ArH$_{meta}$), 7.58 (m, 1 H, ArH$_{para}$), 7.96 (d, 2 H, ArH$_{ortho}$), 7.90-8.62 (m, N$\underline{H}$) ppm. FT-IR: ν = 3284 (w, amide A), 2973 − 2880 (m, C-H), 1783 (w, C=O ester), 1636 (s, C=O amide I), 1513 (s, amide II), 1392-1368 (m, amide III), 1162 (s, O-H) cm^{-1}; ESI-MS m/z = 1643 ([M+Na]$^+$), 1603 ([(M-H$_2$O)+H]$^+$; Thr in source fragmentation), 812 ([M+2H]^2.
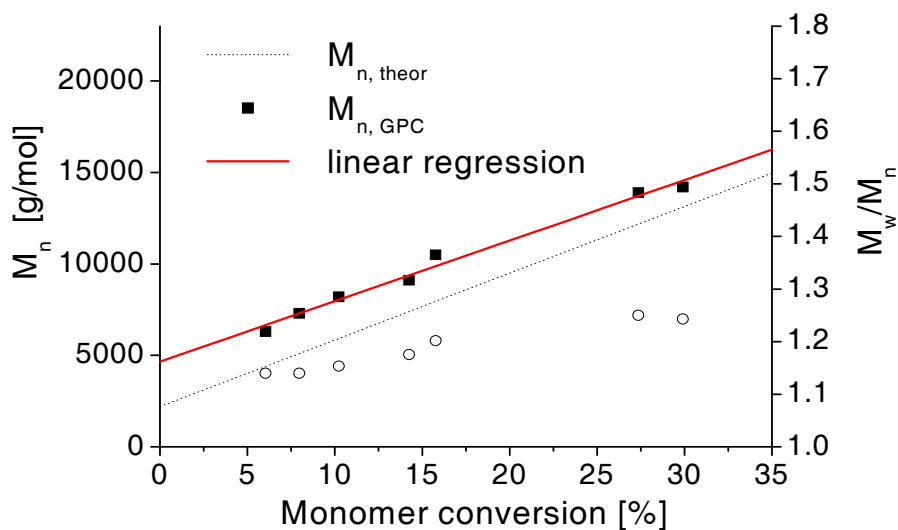
Figure S2. RAFT radical polymerization of *n*BA mediated by **III** at 60 °C: plot of average molecular weight (M_n, determined from GPC) *vs.* monomer conversion (Conditions: $[nBA]_0/[III]_0/[AIBN]_0$ = 285/1/0.1, DMF = 90 *vol.%*) (circles: M_w/M_n; black squares: experimental M_n). The number-average molecular weight exhibits a linear increase with monomer conversion. The course of the experimental molecular-weight plot is matched by the calculated values ($M_{n,th} = ([nBA]_0/[III]_0) \times MW_{nBA} \times$ conversion + MW_{III}). However, an unusual parallel shift is evident between the calculated and the experimental molecular-weight plots. Similar behavior has been observed previously with related peptide-polymer conjugates (H. Rettig *et al. Macromol. Rapid Commun.* **2004**, *25*, 1251–1256) and is probably be due to an overestimation of the molecular weight of the oligopeptide by GPC.
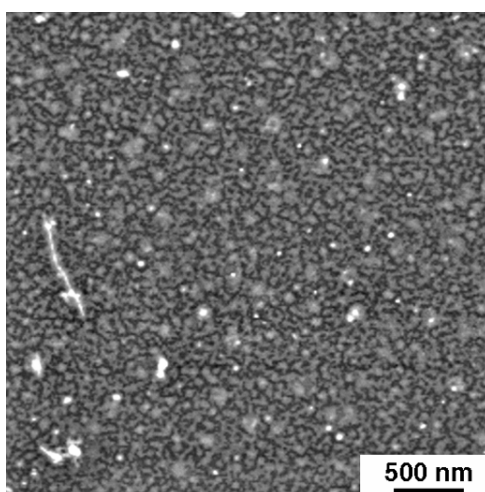
Figure S3. IV-8k stabilized by TFA in Et$_2$O/MeOH (7:3) spin coated after 15 days from solution (1 mg/mL), AFM height image (z = 8 nm).
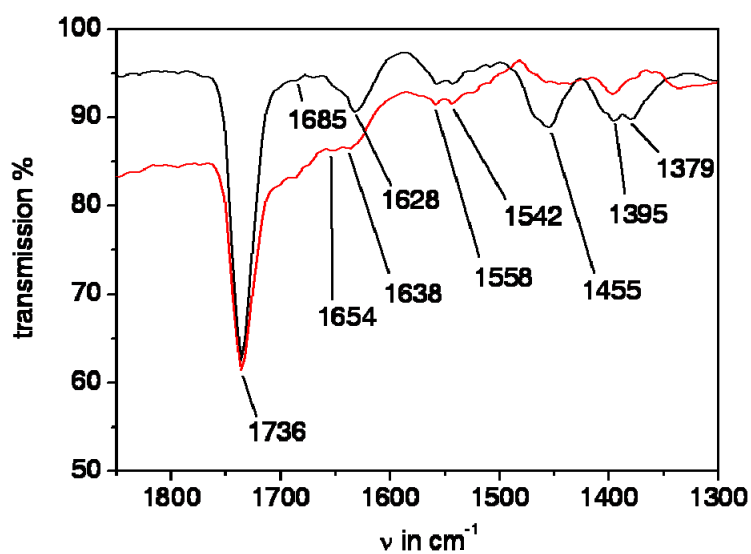

Figure S4. FT-IR spectra (transmission) of **IV-8k** dried on the ATR-crystal, from TFA-stabilized solution after 25 days (1 mg/mL in Et$_2$O/MeOH/TFA (68:30:2)) (red) and from a solution 25 days after titration (1 mg/mL in Et$_2$O/MeOH (70%) at pH$_{app.}$ = 6.0 (black).
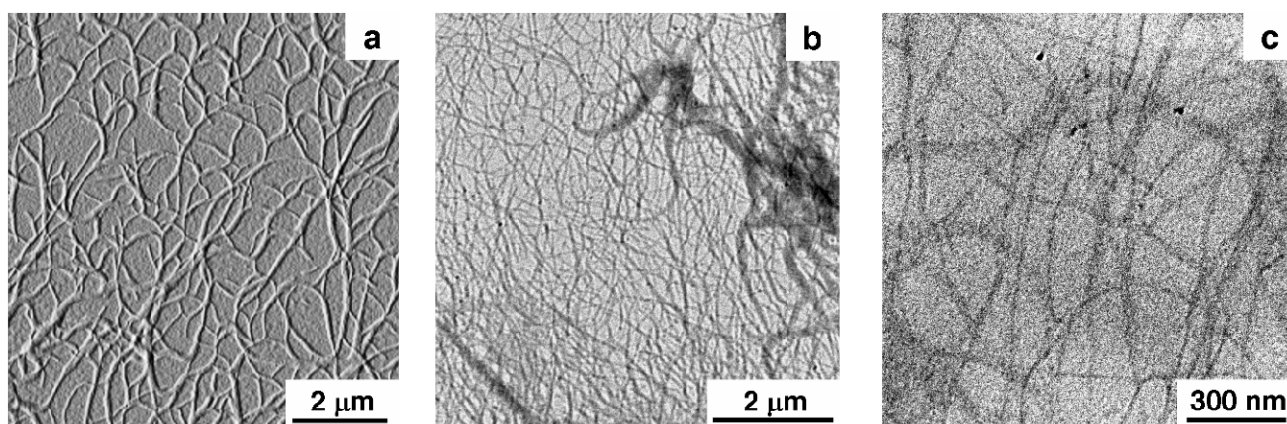
Figure S5. Fibril-network (a, b) and individual fibrils (c), formed by the peptide-guided organization of **IV-8k** in solution, 12 days after titration (1 mg/mL in Et$_2$O/MeOH (70%) at pH$_{app.}$ = 6.0, AFM tapping mode, amplitude z = 0.4 V (a); TEM (b, c)).
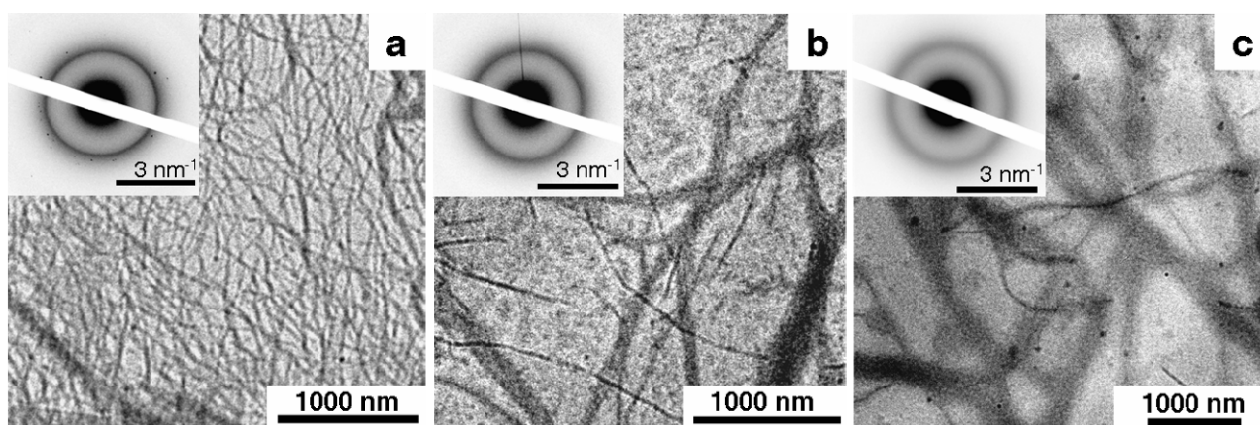

Figure S6. TEM images of **IV-8k** (12 days after titration) (a)**, IV-14k** (15 days after titration) (b) and **IV-38k** (80 days after titration; unstained, 10x diluted) (c), including SAED from a representative area as inset.

$$h_{tape} = b_2 \left(\frac{2\pi}{\gamma_\theta}\right) \left(1 + \left(\frac{\gamma_v}{\gamma_\theta}\right)^2\right)^{-1}$$ (Eq.1)

$$r_{tape} = b_2 \left(\frac{\gamma_v}{\gamma_\theta^2}\right) \left(1 + \left(\frac{\gamma_v}{\gamma_\theta}\right)^2\right)^{-1}$$ (Eq.2)

Equations used to describe a distorted stiff tape (β-sheet tape) consisting of a 2D-assembly of chiral rods (β-strands). The pitch height of the tape h_{tape} and the radius r_{tape} can be described by equations Eq.1 and Eq.2, where b_2 is the distance between adjacent rods in the tape (typical β-strand distances in β-sheets are 4.63-4.75Å) and γ_v as well as γ_θ are respectively the tape bend and twist angles (in radians) per unimers rod along the tape growth director. (Aggeli, A.; Nyrkova, I. A.; Bell, M.; Harding, R.; Carrick, L.; McLeish, T. C. B.; Semenov, A. N.; Boden, N. Proc. Natl. Acad. Sci. U. S. A. **2001**, *98*, 11857-11862)
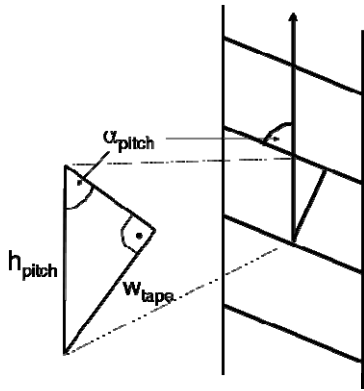
$$\sin \alpha_{pitch} = \frac{w_{tape}}{h_{pitch}}$$ (Eq.3)

Calculation of the maximum tape width in the helical superstructure, where α_{pitch} is the pitch angle, h_{pitch} corresponds to the pitch height and w_{tape} is the tape width.