Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

An Atomistic View of Rigid Crystalline Supramolecular Polymers Derived from Short Amphiphilic, α, β Hybrid Peptide

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Methods:

Materials:

Lyophilized peptide Fmoc-Phe-Phe-OH was purchased from Bachem (Budendorf, Switzerland) Fmoc-F-βK hybrid peptide was synthesized by DGpeptides Co (China) (Figure S15-16). using conventional Fmoc solid-phase method, purified through HPLC and confirmed by mass spectrometry.

Preparation of peptide assemblies:

For self-assembly studies, 1 mg of the Fmoc-F-βK hybrid peptide was dissolved in ultrapure water by heating to 80^{o}C in a water bath until it completely dissolved and 30 seconds of vortexing in a micro vortex for 1 min. After approximately 2-10 minutes, crystalline supramolecular fibers appeared.

Capillary assay:

A sample of Fmoc-F-βK was prepared as described above, immediately inserted into a rectangular glass capillary, sealed, and then observed using Nikon Eclipse light microscope over time.

Transmission electron microscopy (TEM):

Fmoc-F-βK and composite hydrogel fibers in solution (10 μL) were drop casted onto 400-mesh copper grids. Samples were allowed to adsorb for 2 min before excess fluid was blotted off and further dried under ambient conditions. The micrographs were recorded using a JEM-1400Plus Transmission Electron Microscope (JEM) operating at 80 kV.

Thioflavin-T (ThT) binding assay:

For ThT binding analysis, 20 μl of 2 mM peptide solution was drop casted over a microscope glass slide. Then, 20 μl of 20 μM ThT was immediately added and stained samples were visualized using Nikon Intensilight C-HGFI fluorescent lamp and imaged using ZylascMOS camera. The ThT-bound peptide fibers were represented using a FITC fluorescence filter cube in green.

Circular dichroism (CD) spectroscopy:

CD spectra of the Fmoc-Phe-βLys-OH fibers (1mg/mL) were recorded using a Chirascan spectrometer (Applied Photophysics) fitted with a Peltier temperature controller set to 25 °C using quartz cuvettes with an optical path length of 0.1 mm (Hellma Analytics). Analysis was performed in steps of 1 nm in a wavelength range of 190-260 nm with a spectral bandwidth of 1.0 nm and an averaging time of 3 s. The spectrum of the sample was collected three times and averaged. The baseline was similarly recorded for water and subtracted from the sample spectra. Data processing was performed using Pro-Data Viewer software (Applied Photophysics).

Fourier-transform infrared (FTIR) spectroscopy:

A 30 μl aliquot of the Fmoc-F-βK-OH fibers (1mg/mL) was deposited on disposable KBr infrared sample cards (Sigma-Aldrich), then allowed to dry under vacuum. FTIR spectra were collected

using a nitrogen-purged Nicolet Nexus 470 FTIR spectrometer (Nicolet) equipped with a deuterated triglycine sulfate detector. Measurements were performed using a 4 cm^{-1} resolution and by averaging 64 scans. The absorbance maxima values were determined using an OMNIC analysis program (Nicolet). The background was subtracted using a control spectrum.

Wide-angle X-ray scattering (WAXS):

A 30 µl aliquot of the Fmoc-F-βK-OH fibers (1mg/mL) solutions was sealed in a quartz capillary 1.5 mm in diameter. WAXS measurements were conducted using an in-house X-ray scattering system, with a GeniX (Xenocs) low divergence Cu K_α radiation source (wavelength of 1.54 Å) and a scatterless slits setup.[1] Two-dimensional scattering data, with a momentum transfer wave vector (q) range of 0.07-2.4 $Å^{-1}$ at a sample-to-detector distance of ~170 mm, was collected on a Pilatus 300K detector (Dectris, Baden-Daettwil, Switzerland) and radially integrated using Matlab (MathWorks, Natick, MA, USA)-based procedures (SAXSi). Calibration was performed using silver behenate. The scattering data of water was collected as background and used to subtract the solvent and spurious scattering from the WAXS system itself, for example, Kapton vacuum windows and air gap.

Crystallization and structure determination:

Single crystals suitable for X-ray diffraction were grown by slow evaporation of the assembled fibers in MeOH/ water solvent combination at room temperature. Crystals of diffraction quality were obtained after 5 days of sample preparation. For data collection, crystals were coated in paratone oil (Hampton Research), mounted on a MiTeGen cryo-loop and flash-frozen in liquid nitrogen. Diffraction data were collected at 100 K on a Rigaku XtaLabPro with a Dectris 200 K detector using CuKα radiation at λ = 1.54184 Å. The structures were solved by direct methods using SHELXT-2013 and refined by full matrix least squares against F2 with SHELXL-2013. The crystallographic data are given in Table S3 and Data S3 S1. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre (CCDC), under deposition number CCDC 1569244.

Mechanical measurement of the fibers:

AFM nanomechanical measurements were performed in a MMAFM with Nanoscope 5 controller (Bruker, Santa Barbara CA). The measurements were performed using Peak Force Quantitative Nanomechanical Measurement (PF-QNM) which, after calibration, returns a value of the DMT modulus for each pixel, providing a direct correlation with the topographical mapping. Calibrated RTESPA-300-30 and RTESPA-525-30 probes (Bruker) were used, and sensitivity was determined on a sapphire substrate. Peak applied force was typically between 20 – 150 nN, resulting in sample deformations between 1 – 4 nm. Samples were prepared by diluting the stock fiber solution 1:40 in Millipore water and incubating on a freshly cleaved mica substrate for 2 minutes. The droplet was then largely aspirated away by contacting the droplet edge with filter paper, and the sample was blown dry under a gentle stream of clean nitrogen. Images were processed and analyzed using Gwyddion software.[2]

Proteolytic stability analysis:

Proteolytic stability analysis of the hybrid hydrogel was carried out as previously reported.[3] The hybrid peptide, Fmoc-F-βK-OH (1 mM), was dissolved in 1 mL of Tris HCl buffer (pH 8) containing 5% DMSO. Then, 200μL of 50 μM chymotrypsin enzyme was added to the peptide solution (200 μL) and incubated at 37 °C for ~48 hours. Afterward, 50 μL from the stock mentioned above solution was injected into RP-HPLC. The HPLC was carried out using ACN/H_2O water solvent.

Preparation of composite hydrogel:

The composite hydrogel was prepared at a concentration of 5 mg/mL. Fmoc-FF and Fmoc-F-βK-OH powders were separately dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL. Co-assembly solutions were prepared by combining the two peptide stock solutions at a 1:1 molar ratio (25 μL each). The co-assembled solutions were then diluted in 950 μL of double distilled water under vortex to a final concentration of 5 mg/mL.

Absorbance kinetics of formation of the hydrogel:

150 μL of hydrogel samples were transferred into a 96-well plate. Absorbance at 405 nm was measured every 2 min using a TECAN Infinite M200PRO plate reader for a total of 3 hours.

Rheology:

Rheology measurements were carried out using an ARG2 controlled-stress rheometer (TA Instruments, USA). The frequency sweep experiments were performed in 200 μL samples of pre-gelated hydrogel disks in the frequency range of 0.1-100 Hz at a constant strain of 0.1%. The strain sweep experiments were also performed in 200 μL samples of pre-gelated hydrogel disks by increasing the strain from 0.01-100% at a constant frequency of 1 Hz. For the time sweep experiment, 200 μL of the turbid solution which was formed after adding 25 μL FmocFF stock solution (100 mg mL−1 in DMSO) into 475 μL water, directly on the plate, and geometry was immediately set at a gap size of 0.6 mm, and measurement started. Time sweep oscillatory tests were performed for 1 h at a constant frequency of 1 Hz and strain of 0.1% to determine G′ and G″, the storage and loss moduli, respectively. All measurements were conducted at room temperature.

Cell viability analysis:

NIH3T3 mouse fibroblast cells were purchased from ATCC and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 U Ml^{-1} penicillin, 100 U ml^{-1} streptomycin, and 2 mmol L^{-1} L-glutamine (all from Biological Industries, Israel). The cells were maintained in a Petri dish at 37 °C in a humidified atmosphere containing 5% CO_2. Composite hydrogels were formed in a 24-well plate and washed with a culture medium for 3 days to ensure complete removal of excess materials and solvent followed by UV sterilization. Subsequently, the pH of the final medium over the gels was measured to be 7.4-7.8, as also inferred from the medium color, which was observed to be the same as that of the naïve medium, indicating a suitable pH required for cell culture. After reaching a confluence of 90%, the cells were separated from the Petri dish using trypsin A, and 2×10^5 cells in 0.1 mL of fresh culture medium were seeded

per well on the prewashed gel samples and incubated for 24 hours. Fluorescent live–dead staining assay (Sigma-Aldrich) containing fluorescein diacetate (6.6 μg/mL) and propidium iodide (5 μg/mL) was then used to visualize the proportion of viable versus nonviable cells. The labeled cells were immediately viewed using a Nikon Eclipse Tifluorescent microscope, and images were captured by a ZylascMOS camera using Nikon Intensilight C-HGFI fluorescent lamp.

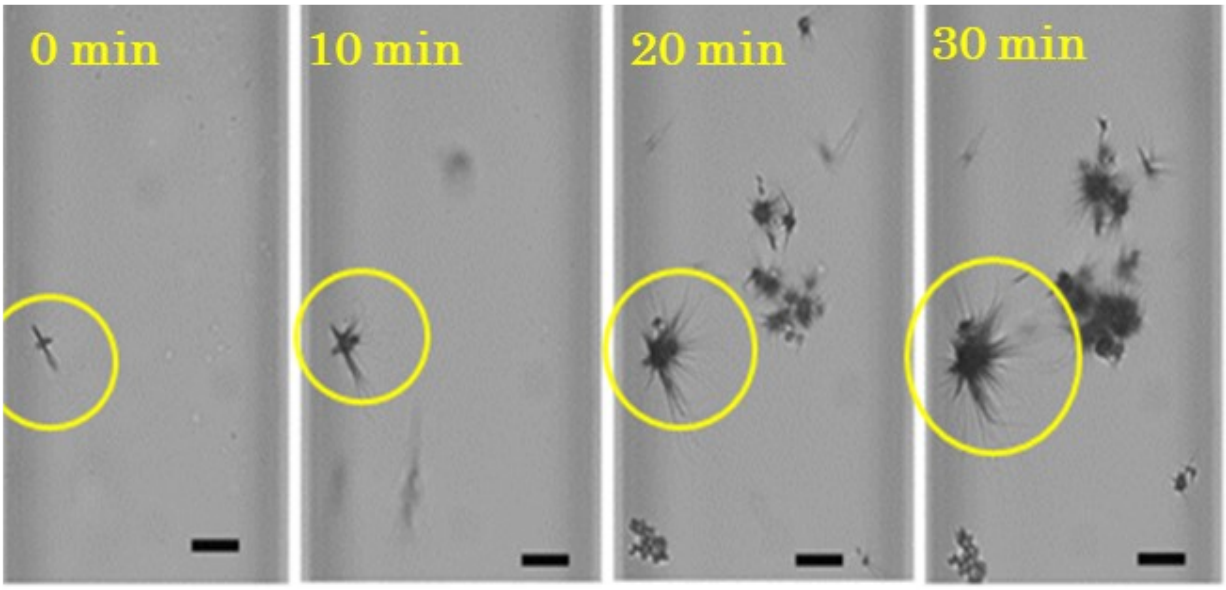


Figure S1: After dissolving with water, time-resolved light microscopy images of assemblies formed by the Fmoc-F-βK peptide amphiphile demonstrated the assembly kinetics. Original magnification, ×10. Bar=10 μm

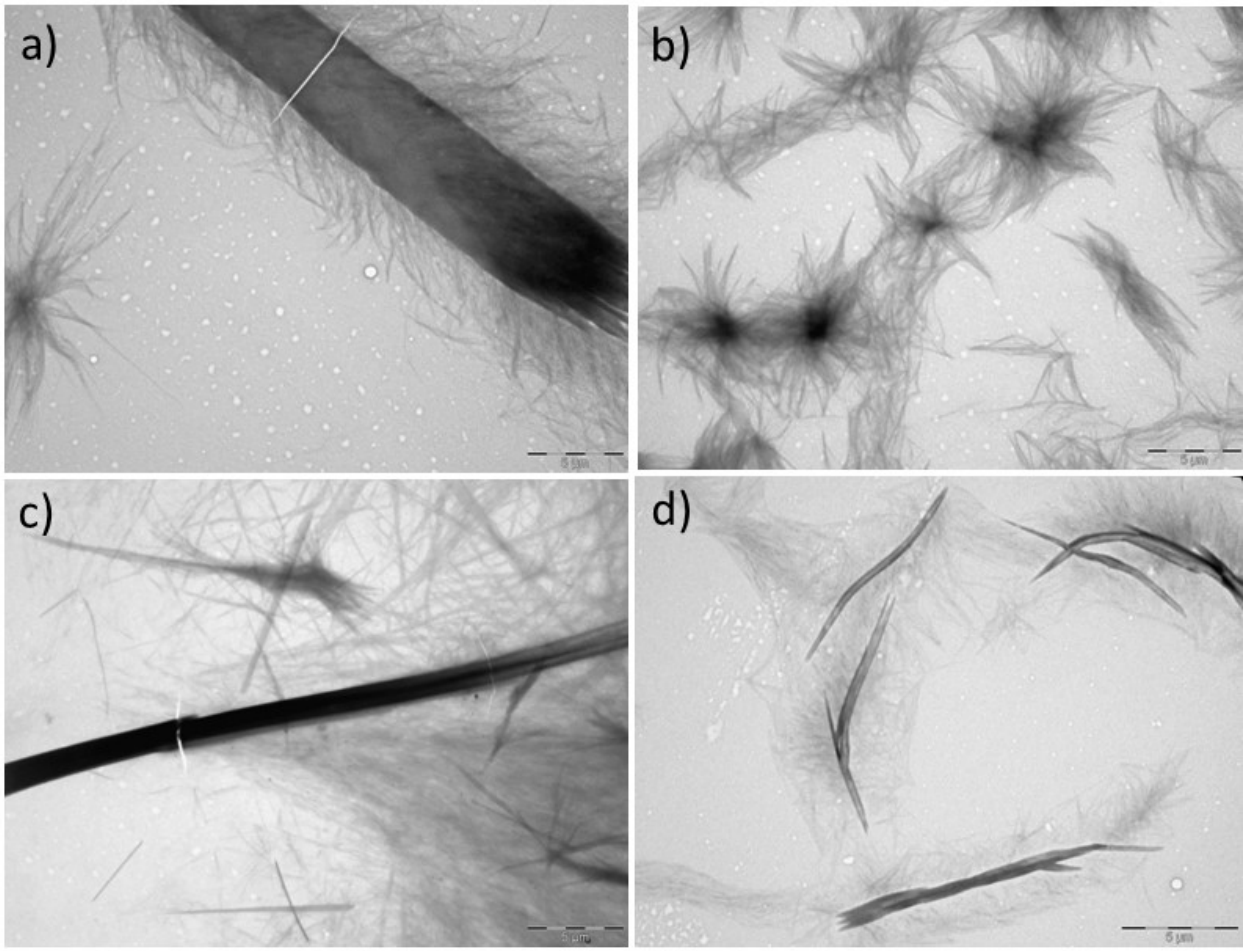


Figure S2: a, b, c, d**)** TEM images of Fmoc-F-βK-OH fibers and large crystalline assemblies in water (1 mg/ mL). Bar= 5 µm.

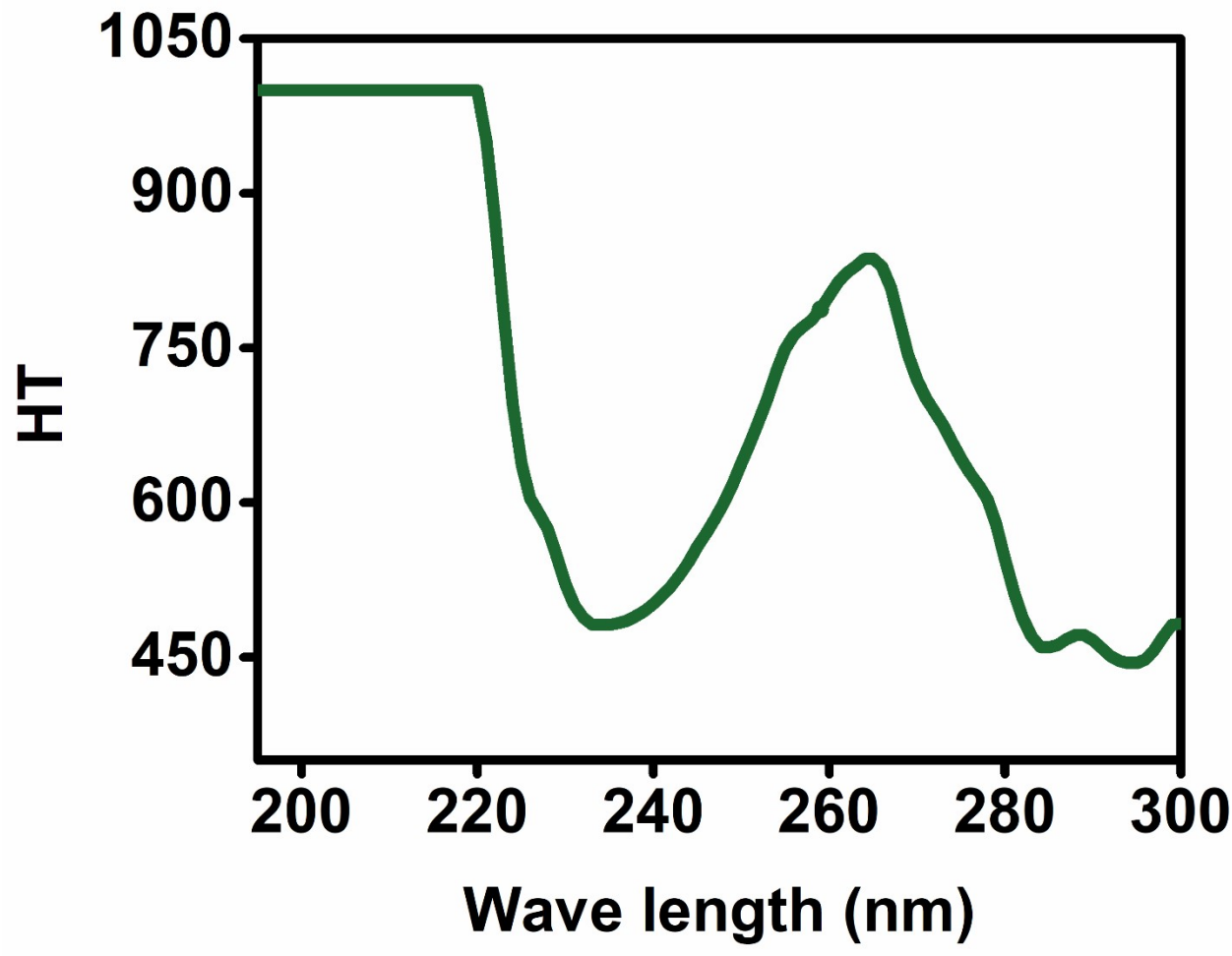


Figure S3: a, b) High Tension (HT) Voltage plot of CD spectra for Fmoc-F-βK-OH in water (1 mg/ mL).

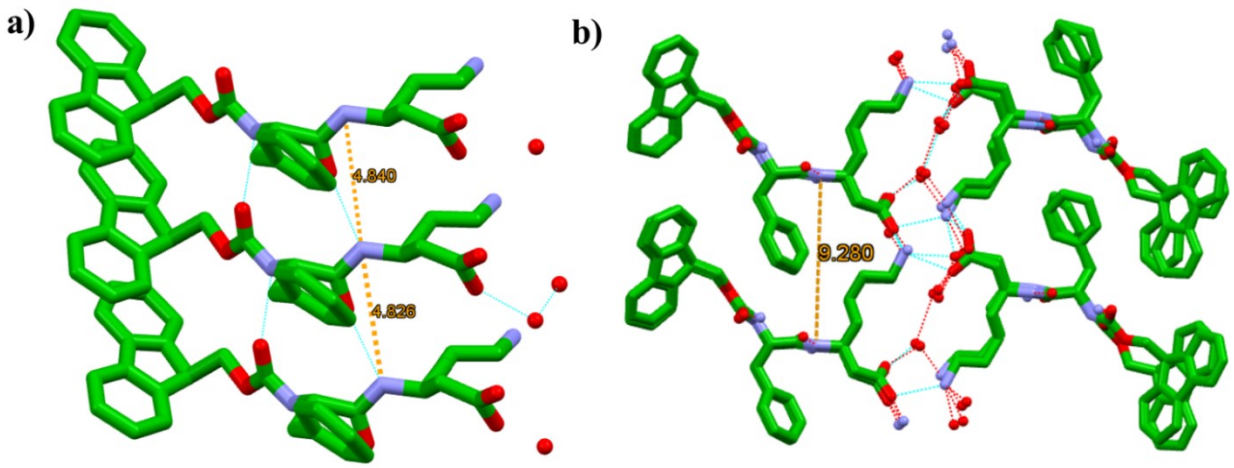


Figure S4: a) The distance between two β-strands (4.8 Å obtained from single crystal). b) The distance between two β-sheets obtained from single crystal (9.2 Å obtained from single crystal).

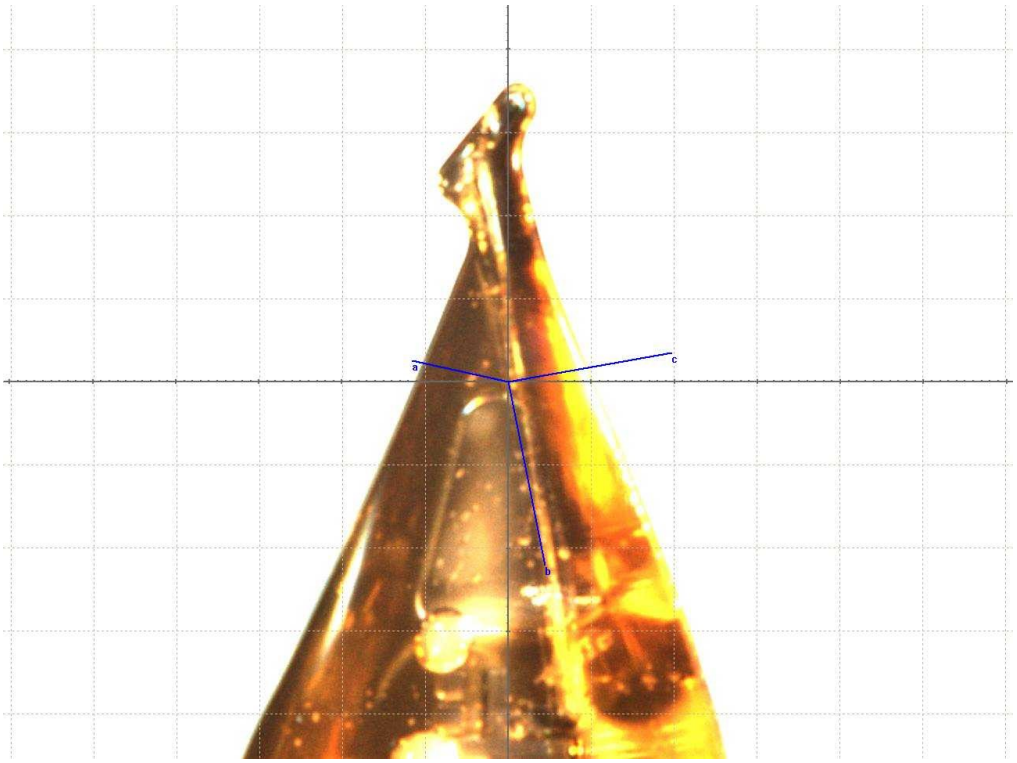


Figure S5: Unit cell measurement of the crystal with respect to crystal morphology. A single crystal is shown mounted on a MiTeGen loop. The cell axes are shown in blue. The morphological long axis of the crystal is aligned along the crystallographic b axis of the unit cell.

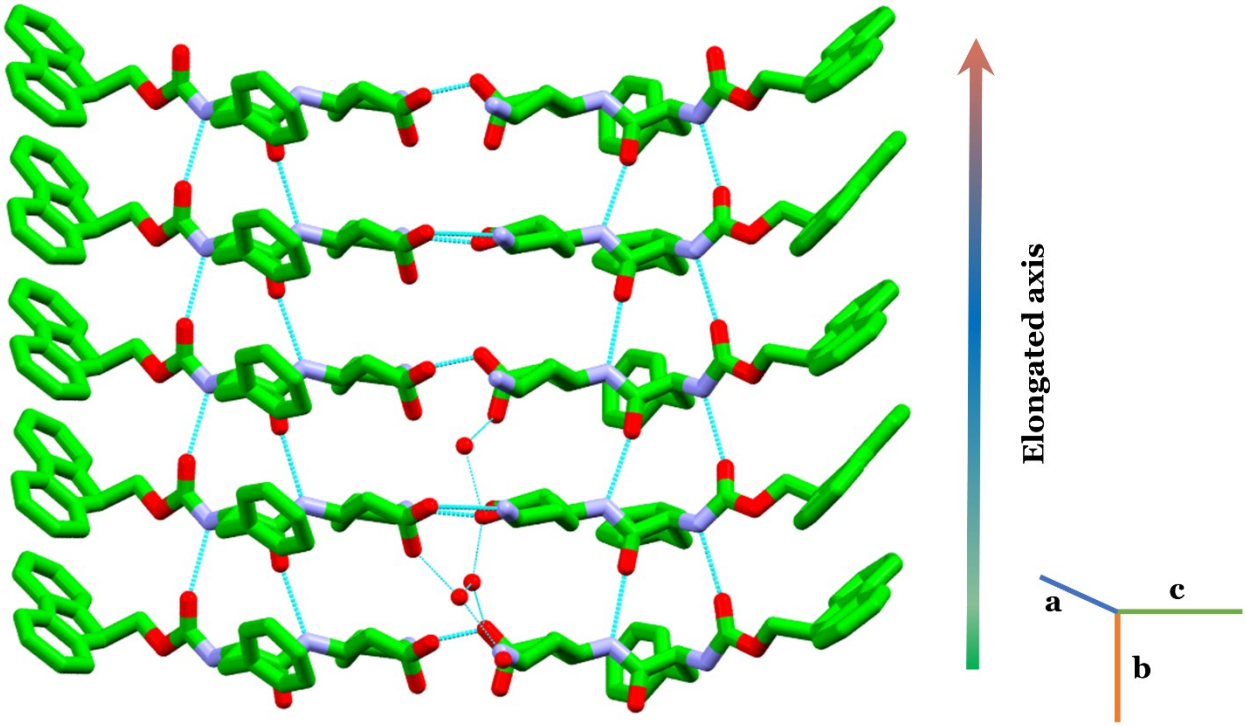


Figure S6: Hydrogen bonding and π-π stacking between the β-strand along the long morphological b axis of the needle-shaped crystal.

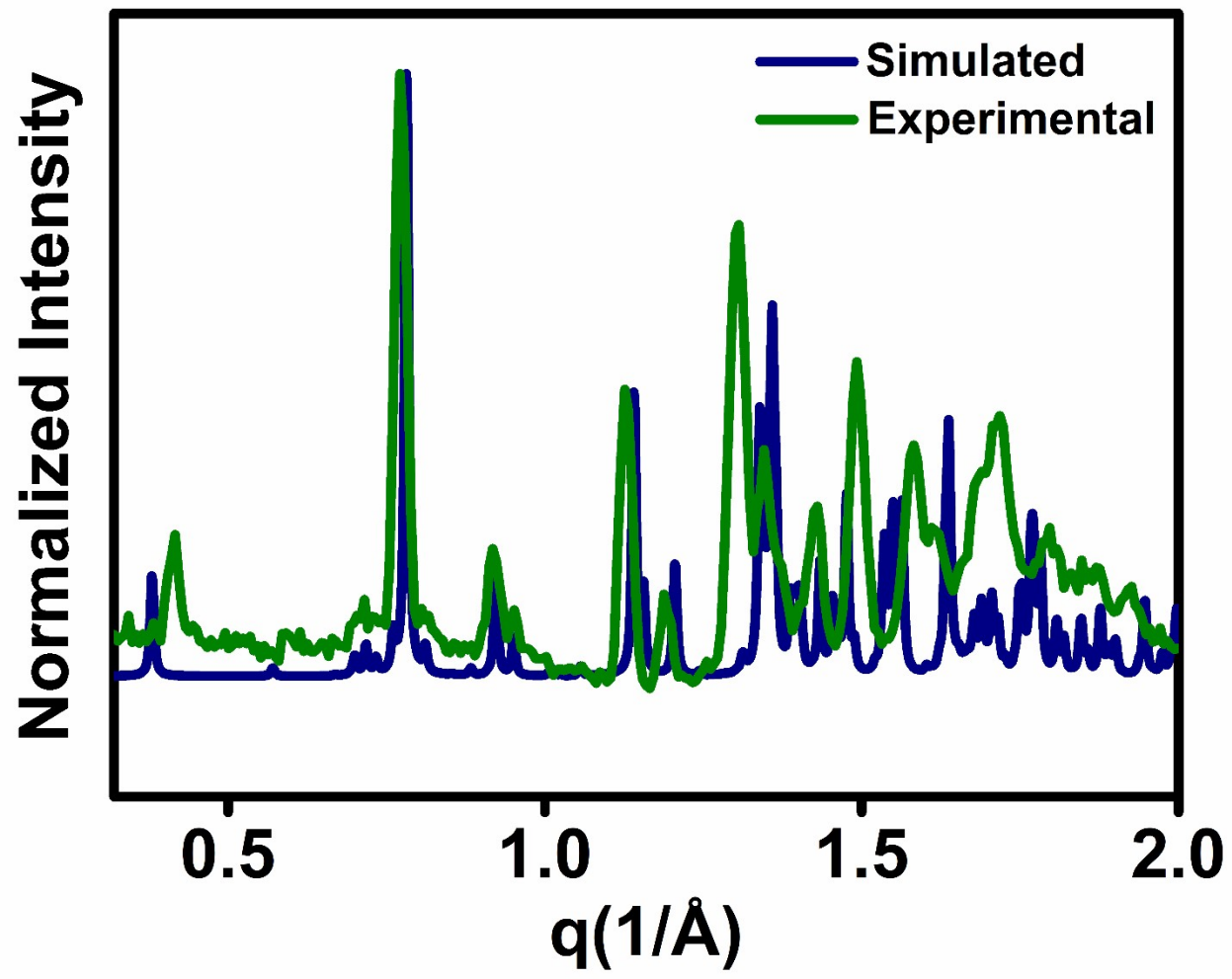


Figure S7: Comparison of wide-angle X-ray diffraction (WAXS) of the assembly's solution (green) and radially integrated powder pattern obtained from single crystal analysis (blue).

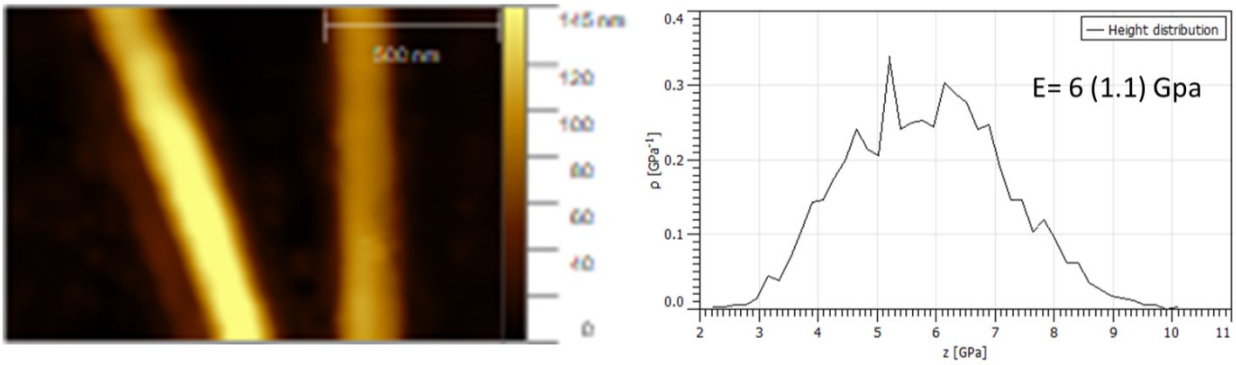


Figure S8: a) Representative AFM image of the crystalline fibers. Bar= 500 nm b) Histogram of the DMT modulus obtained for the fibers using the PF-QNM method.

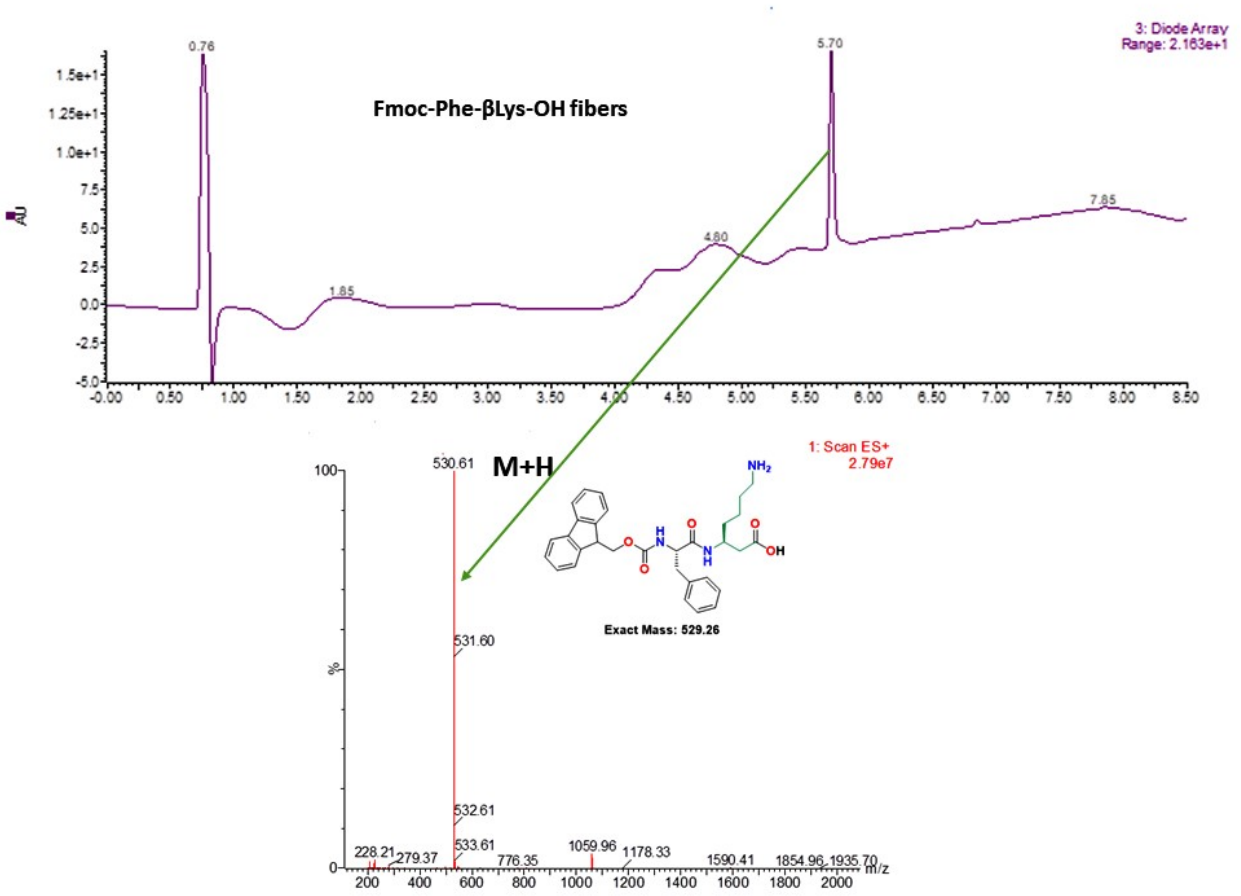


Figure S9: HPLC and mass spectrometry analysis of Fmoc-F-βK before proteolytic degradation studies.

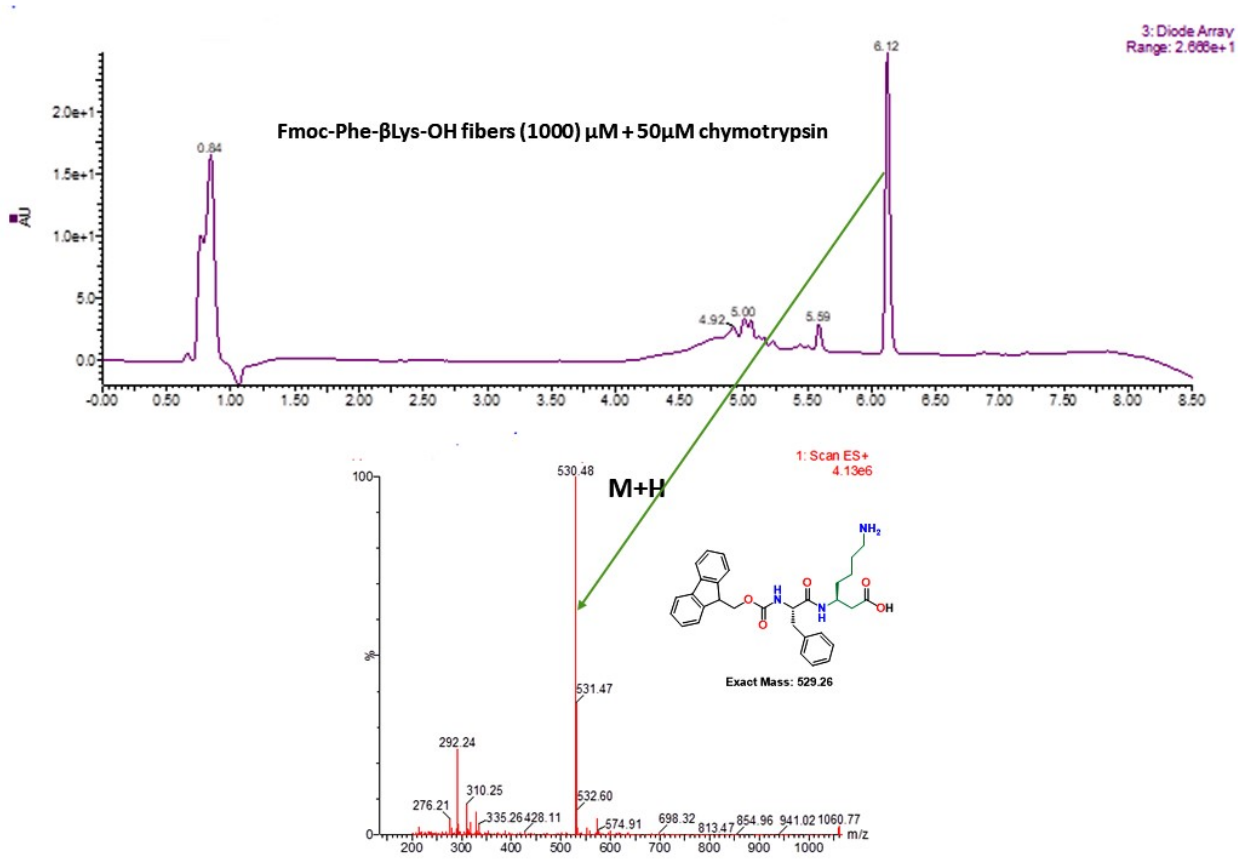


Figure S10: HPLC and mass spectroscopy analysis depicting the stability of Fmoc-F-βK after 2 days of chymotrypsin treatment.

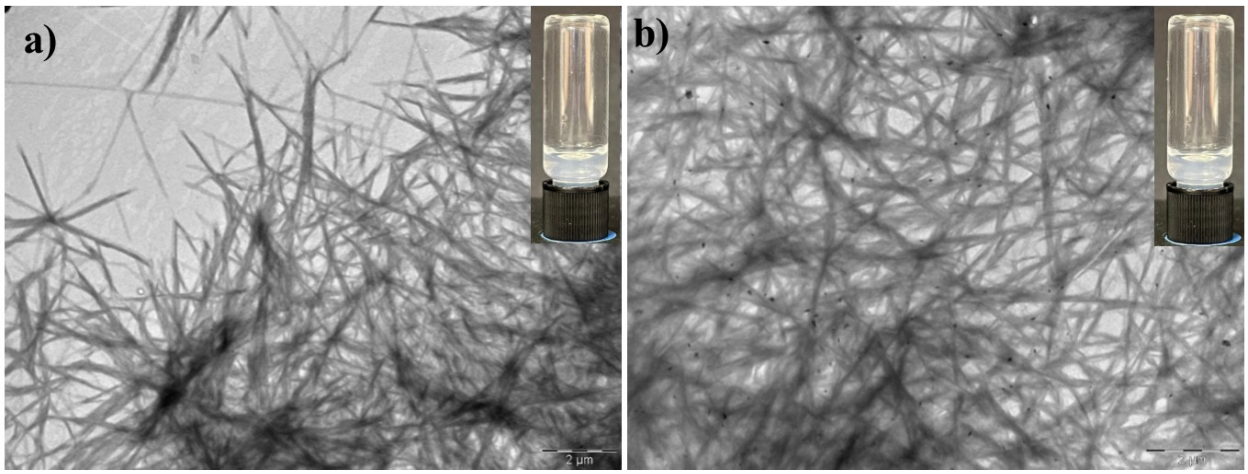


Figure S11: a, b) TEM images of Fmoc-F-βK-OH fibers in 5% DMSO/water at 5mg/mL. Bar= 2 µm

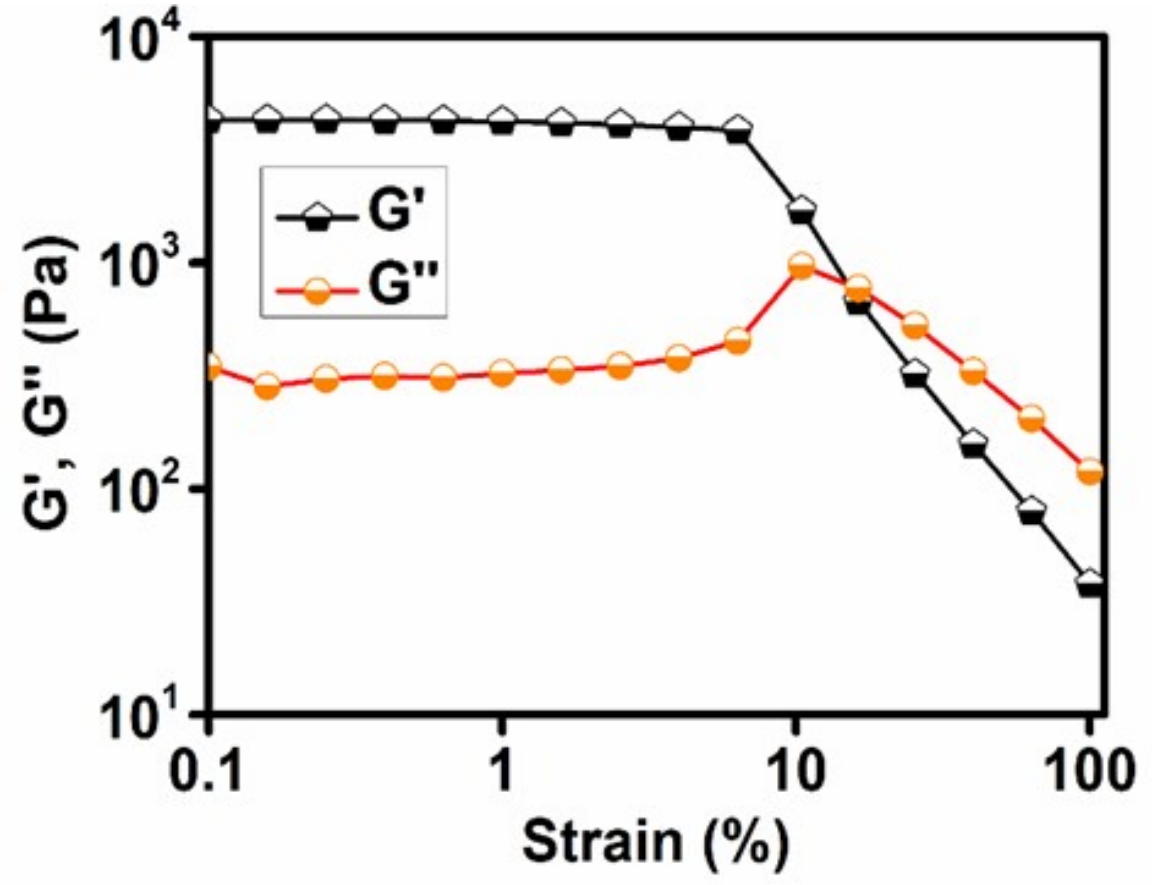


Figure S12: Strain sweep analysis of a 0.5% w/v Fmoc-F-βK and Fmoc-FF composite hydrogel at a constant frequency of 1 Hz.

Figure S13: Stability of the Fmoc-F-βK and Fmoc-FF composite hydrogel incubated with the cell media (DMEM)

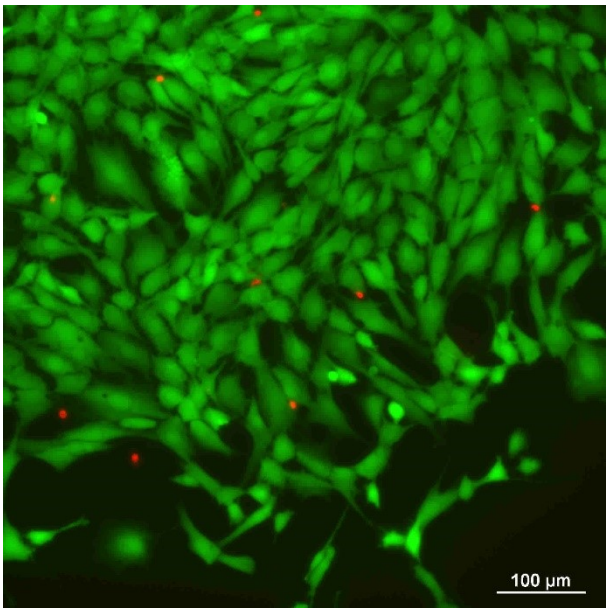


Figure S14: Live/Dead imaging showing 2D cell cultures of 3T3 fibroblasts on the 0.5% composite hydrogel, 20x image.

Table S1: Comparison of Young's moduli of Fmoc-F-βK-OH fibers and several amyloid fibril types as measured via the PF-QNM technique.

Amyloid fibrils	Young's modulus[c]
Amphiphilic α,β hybrid peptide[b]	6.0 ±1.1
Insulin[a]	3.2 ± 0.6
CH_3CONH-βAβAKLVFF-$CONH_2$[a]	2.3 ± 0.6
β-Lactoglobulin[a]	3.7 ± 0.8
Lysozyme[a]	2.8 ± 0.9
Tau protein[a]	3.4 ± 0.7
Aβ(1–42)[a]	3.2 ± 0.8
α-Synuclein[a]	2.2 ± 0.6
Bovine serum albumin[a]	3.0 ± 0.6
Ovalbumim[a]	2.7 ± 0.8

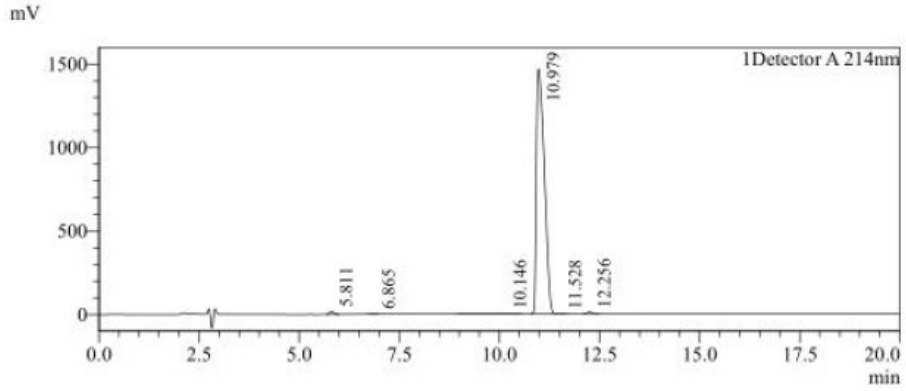
[a]Obtained from reference no 41, [b]present study, [c]values are in GPa.

Table S2: Crystal data and structure refinement of the Fmoc-F-βK-OH hybrid peptide.

CCDC number	2122681
Crystal description	Colourless needle
Diffractometer	Rigaku Synergy R
Empirical formula	C_{31} H_{37} N_3 O_6
Formula weight (g/mol)	547. 63
Temperature (K)	100 (2)
Wavelength (Å)	1.54184
Crystal system	Triclinic
Space group	*P1*
a, Å	9.2497(6)
b, Å	9.6644(2)
c, Å	32.9380(7)
α°	90.062(2)
β°	92.440(3)
γ°	104.738(4)
Volume (Å3)	2844.7(2)
Z	4
dcalc (mg/cm3)	1.279
μ (mm-1)	0.725
F(000)	1168
Theta range for data collection (°)	4.030 to 58.933
Reflections collected (unique)	10714 (7787)
Rint	0.0362
Completeness	99.7
Data/restraints/parameters	10714/1457/232
Final R [I > 2 σ (I)]	R1=0.1174 wR2=0.3321
R (all data)	R1=0.1422 wR2=0.3486
Goodness of Fit	1.398
Largest diff. peak and hole (e· $Å^{-3}$)	0.766 -0.554

Sample:	T-3954		Analyzed date:	2021-08-27
Sequence:	Fmoc-Phe-beta-HomoLys-OH			
Lot. No.:	DG-97902			
Column:	4.6×250mm,Sinochrom ODS-BP 5μm			
Solvent A	A: 0.1% Trifluoroacetic Acid in 100% Acetonitrile			
Solvent B	B: 0.1% Trifluoroacetic Acid in 100% Water			
Gradient:		A	B	
	0.0min	22%	78%	
	25.0min	47%	53%	
	25.1min	100%	0%	
	30.0min	Stop		
Volume:	5μl			
Wavelength:	220nm			
Flow rate:	1.0ml/min			

Chromatogram



Peak Table

Detector A 214nm

Peak#	Ret. Time	Area	Height	Area%
1	5.811	117667	17303	0.572
2	6.865	19778	2794	0.096
3	10.146	117346	4180	0.571
4	10.979	20200008	1466488	98.272
5	11.528	11445	1270	0.056
6	12.256	89060	11105	0.433
Total		20555305	1503141	100.000

Figure S15: HPLC characterization of Fmoc-F-βK

Mass spec

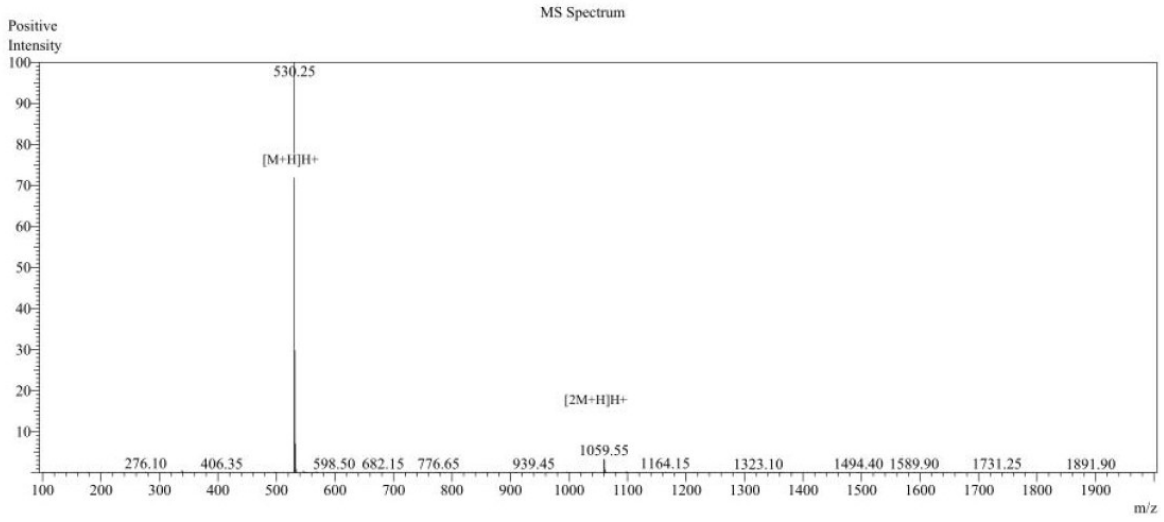
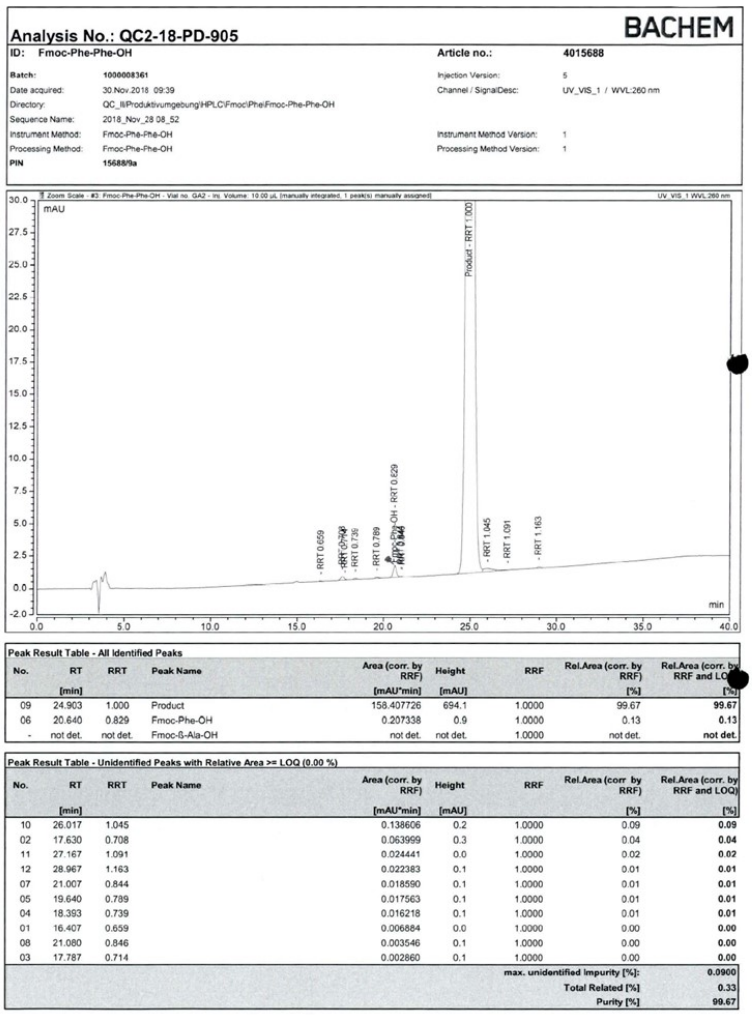


Figure S16: Mass spectrometry analysis of Fmoc-F-βK



Analysis No.: QC2-18-PD-905 — BACHEM

ID: Fmoc-Phe-Phe-OH — Article no.: 4015688

Batch:	1000008361	Injection Version:	5
Date acquired:	30.Nov.2018 09:39	Channel / SignalDesc:	UV_VIS_1 / WVL:260 nm
Directory:	QC_IlilProduktivumgebung\HPLC\Fmoc\Phe\Fmoc-Phe-Phe-OH		
Sequence Name:	2018_Nov_28 08_52		
Instrument Method:	Fmoc-Phe-Phe-OH	Instrument Method Version:	1
Processing Method:	Fmoc-Phe-Phe-OH	Processing Method Version:	1
PIN	15688/9a		

Peak Result Table - All Identified Peaks

No.	RT [min]	RRT	Peak Name	Area (corr. by RRF) [mAU*min]	Height [mAU]	RRF	Rel.Area (corr. by RRF) [%]	Rel.Area (corr. by RRF and LOQ) [%]
09	24.903	1.000	Product	158.407726	694.1	1.0000	99.67	**99.67**
06	20.640	0.829	Fmoc-Phe-OH	0.207338	0.9	1.0000	0.13	**0.13**
-	not det.	not det.	Fmoc-ß-Ala-OH	not det.	not det.	1.0000	not det.	**not det.**

Peak Result Table - Unidentified Peaks with Relative Area >= LOQ (0.00 %)

No.	RT [min]	RRT	Peak Name	Area (corr. by RRF) [mAU*min]	Height [mAU]	RRF	Rel.Area (corr by RRF) [%]	Rel.Area (corr. by RRF and LOQ) [%]
10	26.017	1.045		0.138606	0.2	1.0000	0.09	**0.09**
02	17.630	0.708		0.063999	0.3	1.0000	0.04	**0.04**
11	27.167	1.091		0.024441	0.0	1.0000	0.02	**0.02**
12	28.967	1.163		0.022383	0.1	1.0000	0.01	**0.01**
07	21.007	0.844		0.018590	0.1	1.0000	0.01	**0.01**
05	19.640	0.789		0.017563	0.1	1.0000	0.01	**0.01**
04	18.393	0.739		0.016218	0.1	1.0000	0.01	**0.01**
01	16.407	0.659		0.006884	0.0	1.0000	0.00	**0.00**
08	21.080	0.846		0.003546	0.1	1.0000	0.00	**0.00**
03	17.787	0.714		0.002860	0.1	1.0000	0.00	**0.00**
							max. unidentified impurity [%]:	**0.0900**
							Total Related [%]	**0.33**
							Purity [%]	**99.67**

Figure S17: HPLC characterization of Fmoc-F-F-OH

References:

1. Y. Li, R. Beck, T. Huang, M. C. Choi and M. Divinagracia, *J. Appl. Cryst.* 2008, **41**, 1134-1139
2. http://gwyddion.net/
3. R. M. Reja, R. Patel, V. Kumar, A. Jha, and H. N. Gopi, *Biomacromolecules,* 2019, **20**, 3, 1254–1262