Supporting Information

Supramolecular Nanostructure Activates TrkB Receptor Signaling of Neuronal Cells by Mimicking Brain-Derived Neurotrophic Factor

Alexandra N. Edelbrock,[1,2,†] Zaida Àlvarez,[2,†] Dina Simkin,[3,4] Timmy Fyrner,[2] Stacey M. Chin,[5] Kohei Sato,[2,5,‡] Evangelos Kiskinis,[4] Samuel I. Stupp[1,2,5,6,7,#]

Affiliations:

[1]Department of Biomedical Engineering, Northwestern University, Evanston, Illinois 60208, USA;

[2]Simpson Querrey Institute, Northwestern University, Chicago, Illinois 60611, USA;

[3]Department of Pharmacology, Feinberg School of Medicine, Northwestern University, Chicago, IL, 60611, USA;

[4]The Ken & Ruth Davee Department of Neurology & Clinical Neurological Sciences, Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA;

[5]Department of Chemistry, Northwestern University, Evanston, Illinois 60208, USA;

[6]Department of Materials Science and Engineering, Northwestern University, Evanston, Illinois 60208, USA

[7]Department of Medicine, Northwestern University, Chicago, IL 60611, USA.

[†]*Authors contributed equally*

‡ Present address: Department of Life Science and Technology, Tokyo Institute of Technology, Yokohama 226-8501, Japan

[#] Corresponding author. Email: s-stupp@northwestern.edu

1. Synthesis

1.1 General.

Unless otherwise noted, all commercial reagents were used as received. The Fmoc-21-amino-4,7,10,13,16,19-hexaoxaheneicosanoic acid (Fmoc-NH-PEG_6-CH_2CH_2COOH) was purchased from ChemPep Inc. [CAS: 882847-34-9]. For column chromatography, SiliaFlash P60 (particle size 40-63 μm; silica; Silicycle) was used. Preparative reverse-phase high-performance liquid chromatography (RP-HPLC) was performed at 25 °C using a Phenomenex Kinetex column (C18 stationary phase, 5 μm, 100 Å pore size, 30.0 × 150 mm) on a Shimadzu model prominence modular HPLC system equipped with a DGU-20A5R degassing unit, two LC-20AP solvent delivery units, a SPD-M20A diode array detector and a FRC-10A fraction collector, using H_2O/CH_3CN gradient containing 0.1% NH_4OH (v/v) as an eluent at a flow rate of 75.0 mL min^{-1}. Electrospray ionization mass spectrometry (ESI-MS) was performed in positive or negative mode on an Agilent model 6520 Quadrupole Time-of-Flight (Q-ToF) LC/MS spectrometer using direct injection. Matrix-assisted laser deposition ionization time-of-flight (MALDI-ToF) mass spectrometry was performed in the reflector mode on a Bruker autoflex III smartbeam spectrometer using α-cyano-4-hydroxycinnamic acid as a matrix. High resolution mass spectroscopy (HRMS) was recorded on an Agilent 6210A LC-ToF High Resolution Time of Flight Mass Spectrometer connected to Agilent 1200 series HPLC, using electrospray ionization source (ESI). MassHunter Workstation Data Acquisition software was used for instrument operation and MassHunter Qualitative Analysis software for data analysis and processing.

Peptide Purification. All peptides and peptide amphiphiles (PAs) were purified using standard reversed-phase HPLC (Shimadzu), unless otherwise stated. The mass spectra for each fraction of PA after HPLC purification was verified using direct injected Q-ToF MS on an Agilent model 6520.

LCMS Purity Measurements. Analytical liquid chromatography-mass spectroscopy (LC-MS) was performed on an Agilent 1200 system with a Phenomenex Jupiter C-12 column (100 × 1.00 mm; 5 μm) for acidic conditions or Phenomenex Gemini C-18, (100 × 1.00 mm; 5 μm) for basic conditions. The mass detector (MS) was an Agilent 6520 quadrupole-time of flight Q-ToF MS. All gradient methods followed:

acetonitrile at 5% for 5 min at 50 µL/min, 5–95% over 25 min at 50 µL/min followed by 95% for 5 min at 50 µL/min. Ammonium hydroxide (0.1% v/v) for basic or formic acid (0.1% v/v) for acidic conditions was added to all solvents. Peaks were detected at λ = 220 nm.

NMR spectroscopy. Proton nuclear magnetic resonance (^{1}H) was recorded on a Varian model Inova 500 spectrometer BroadBand 5mm probe w/Z-Gradient, an Agilent 600 MHz DD2 w/HCN COLD Probe w/Z-Gradient, or a Bruker Avance III 600 MHz system w/BBFO Smart Probe w/ Z-Gradient. Carbon nuclear magnetic resonance (^{13}C) were recorded on a Bruker AVANCE III 500 MHz w/CryoProbe 5mm DCH w/Z-Gradient. NMR specta was recorded at 25 °C using CD_3OD, D_2O, or DMF-d_7 + 5% D_2O. Chemical shifts are reported in parts per million (ppm) with respect to tetramethylsilane (TMS), the solvent residual peak, or acetone (for D_2O) as internal reference. CD_3OD (^{1}H-NMR, δ = 3.31 ppm; ^{13}C NMR, δ = 49.0 ppm), D_2O (acetone, ^{1}H-NMR, δ = 2.22 ppm; ^{13}C NMR, δ = 30.9 ppm), DMF-d_7 (^{1}H-NMR, δ = 8.03 ppm; ^{13}C-NMR, δ = 163.2 ppm)]. Multiplicities are denoted as double of doublets (dd), triplets (t), apparent doublet (ad), apparent triplet (at), multiplet (m), and quartet (q). Structural assignment was performed using ^{1}H-^{1}H-gCOSY, ^{1}H-^{1}H-TOCSY, ^{1}H-^{13}C-gHSCQAD and ^{1}H-^{13}C-gHMBCAD. *Listed carbon peaks are those detected by ^{13}C-NMR or gHSQCAD, gHMBCAD, and have been assigned when possible.*

1.2 General PA and Peptide Synthesis Methods.

Loading Fmoc-Arg(Pbf)-OH to 2-chlorotrithyl chloride resin. DMF (15 mL) was used to swell the resin for 30 min. A DMF solution (15 mL) of Fmoc-Arg(Pbf)-OH (649 mg, 1.0 mmol) and *N*,*N*-diisopropylethylamine (DIPEA, 260 µL, 1.5 mmol) was added to the resin, and the reaction vessel was shaken for 2 hours at 25 °C. MeOH (400 µL) was then added and the reaction vessel was shaken for another 5 min to cap the free groups on beads. After the coupling solution was drained off, the resin was washed with DMF (3x30 mL).

Deprotection of Fmoc group. 4-methylpiperidine in DMF (30% v/v) was added to the peptidyl resin, and the reaction vessel was shaken for 10 min at 25 °C. After the reaction solution was drained off, this reaction was repeated one more time, and the resulting peptidyl resin was washed with DMF (3x30 mL) and CH_2Cl_2 (30 mL). Deprotection and subsequent coupling was confirmed with the use of a colorimetric Kaiser test.

Peptide coupling using HBTU as a coupling reagent. To a DMF solution (30 mL) of Fmoc-protected amino acid (4.0 mmol) and *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uranium hexafluorophosphate (HBTU, 1.5 g, 3.95 mmol) was added DIPEA (1.0 mL, 6 mmol), and the mixture was stirred for 1 min for activation. The mixture was then added to the peptidyl resin, and the reaction vessel was shaken for 2 hours at 25 °C. The coupling solution was then drained off and the peptidyl resin was washed with DMF (3x30 mL).

Peptide coupling using PyBOP as a coupling reagent. To a DMF solution (15 mL) of Fmoc-protected amino acids (1.1 mmol), Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP, 546 mg, 1.05 mmol) and DIPEA (290 µL, 1.65 mmol) was added and the mixture was stirred for 1 min for activation. The mixture was then added to the peptidyl resin and the reaction vessel was shaken for 12 hours at 25 °C. After the coupling was complete the solution was drained off and the resin was washed with DMF (3x30 mL).

Deprotection of Alloc group. A CH_2Cl_2 solution (15 mL) of tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$) (116 mg, 100 µmol) and phenylsilane (3.08 mL, 25 mmol) was added to the peptidyl resin, and the reaction vessel was shaken for 2 hours at 25 °C. After the reaction was complete, the solution was drained off and a DMF solution (10 mL) of sodium diethyldithiocarbamate trihydrate (1.0 g, 4.44 mmol) was added to the peptidyl resin and the reaction vessel was shaken for 20 min at 25 °C. This reaction was repeated one more time, and the resulting peptidyl resin no longer containing an Alloc was washed with DMF (3x30 mL) and CH_2Cl_2 (30 mL).

1.3 Synthesis of E_2 PA, E_4 PA and E_4PEG PA.

Molecules were synthesized using standard fluorenylmethyloxycarbonyl (Fmoc)-solid phase peptide chemistry. The peptide was synthesized on a 1 mmol scale on Rink amide MBHA resin (1.92g, 0.52 meq g^{-1}, 100-200 mesh) in the Peptide Synthesis Core at the Simpson Querrey Institute. The E_2 PA, E_4 PA, and E_4PEG PA were synthesized using a CEM Liberty microwave-assisted peptide synthesizer. The cleavage solution was prepared by mixing trifluoroacetic acid (TFA)/triisopropylsilane (TIPS)/dichloromethane (CH_2Cl_2) (2.5:2.5:95), which was added to the resin-bound peptide, the mixture was shaken for 3 hours

after which the solution was collected and concentrated followed by trituration in ether. The crude peptide was dissolved in 0.1% NH_4OH (aq.), filtered using a 0.2 μm syringe filter and subjected to HPLC purification. Freeze-drying gave the titled compounds as white solids.

Characterization data of E_4PEG PA. The **E_4PEG PA** was synthesized as described above. ^{1}H-NMR (600 MHz, DMF-d_7 + 5% D_2O): δ = 0.86 (t, 3H, J = 7.0 Hz, 3xPal-C$\underline{H}_3$), 0.93 (d, 3H, J = 6.8 Hz, 3xVal-C$_{(\gamma)}\underline{H}_3$), 0.96 (d, 3H, J = 6.6 Hz, 3xVal-C$_{(\gamma)}\underline{H}_3$), 0.97 (d, 3H, J = 6.8 Hz, 3xVal-C$_{(\gamma)}\underline{H}_3$), 1.00 (d, 3H, J = 6.8 Hz, 3xVal-C$_{(\gamma)}\underline{H}_3$), 1.26 – 1.29 (m, 24H, 24xPal-C$\underline{H}_2$), 1.40 (at, 6H, J = 7.0 Hz, 6xAla-C$_{(\beta)}\underline{H}_3$), 1.55 – 1.63 (m, 2H, 2xPal-C$\underline{H}_2$), 1.97 – 2.06 (m, 1H, Glu-C$_{(\beta)}\underline{H}_2$), 2.07 – 2.20 (m, 9H, 7xGlu-C$_{(\beta)}\underline{H}_2$, 2xVal-C$_{(\beta)}\underline{H}$), 2.29-2.61 (m, 12H, 8xGlu-C$_{(\gamma)}\underline{H}_2$, 2xPal-C$\underline{H}_2$, 2xOCH$_2C\underline{H}_2$CONH$_2$), 3.33 (t, 2H, J = 6.3 Hz, 2xNHC$\underline{H}_2$CH$_2$O), 3.52 (t, 2H, J = 6.3 Hz, 2xNHCH$_2$C$\underline{H}_2$O), 3.54 – 3.58 (m, 20H, 20xOC$\underline{H}_2$), 3.68 (t, 2H, J = 6.4 Hz, 2xOC$\underline{H}_2$CH$_2$CONH$_2$), 4.06 – 4.09 (at, 2H, J = 6.7, 7.6 Hz, 2xVal-C$_{(\alpha)}\underline{H}$), 4.17 – 4.21 (m, 2H, Ala-C$_{(\alpha)}\underline{H}$, Glu-C$_{(\alpha)}\underline{H}$), 4.22 – 4.26 (m, 2H, Ala-C$_{(\alpha)}\underline{H}$, Glu-C$_{(\alpha)}\underline{H}$), 4.28 (dd, 1H, J = 4.90, 9.7 Hz, Glu-C$_{(\alpha)}\underline{H}$), 4.33 (dd, 1H, J = 4.51, 9.8 Hz, Glu-C$_{(\alpha)}\underline{H}$); ^{13}C-NMR (125 MHz, DMF-d_7 + 5% D_2O): δ = 13.7 (Pal-$\underline{C}$H$_3$), 16.3 (2xAla-$\underline{C}_{(\beta)}$H$_3$), 18.5 (Val-$\underline{C}_{(\gamma)}$H$_3$), 18.8 (Val-$\underline{C}_{(\gamma)}$H$_3$), 18.9 (2xVal-$\underline{C}_{(\gamma)}$H$_3$), 22.5 (Pal-$\underline{C}$H$_2$), 25.6 (Pal-$\underline{C}$H$_2$), 26.4 (Glu-$\underline{C}_{(\beta)}$H$_2$), 26.5 (Glu-$\underline{C}_{(\beta)}$H$_2$), 26.7 (Glu-$\underline{C}_{(\beta)}$H$_2$), 27.2 (Glu-$\underline{C}_{(\beta)}$H$_2$), 29.0 – 30.1 (*Several carbons overlap with solvent*, multiple Pal-C$\underline{H}_2$, 2xVal-$\underline{C}_{(\beta)}$H$_2$), 30.4 (Glu-$\underline{C}_{(\gamma)}$H$_2$), 30.5 (Glu-$\underline{C}_{(\gamma)}$H$_2$), 30.6 (Glu-$\underline{C}_{(\gamma)}$H$_2$), 30.7 (Glu-$\underline{C}_{(\gamma)}$H$_2$), 31.7 (Pal-$\underline{C}$H$_2$), 35.5 (Pal-$\underline{C}$H$_2$), 36.0 (OCH$_2\underline{C}$H$_2$CONH$_2$), 38.9 (NH$\underline{C}$H$_2$CH$_2$O), 50.7 (Glu-$\underline{C}_{(\alpha)}$H/Ala-$\underline{C}_{(\alpha)}$H), 51.0 (Ala-$\underline{C}_{(\alpha)}$H), 53.0 (Glu-$\underline{C}_{(\alpha)}$H), 53.7 (Glu-$\underline{C}_{(\alpha)}$H), 54.4 (Glu-$\underline{C}_{(\alpha)}$H/Ala-$\underline{C}_{(\alpha)}$H), 54.7 (Glu-$\underline{C}_{(\alpha)}$H), 60.6 (Val-$\underline{C}_{(\alpha)}$H), 60.6 (Val-$\underline{C}_{(\alpha)}$H), 67.1 (O$\underline{C}$H$_2$CH$_2$CONH$_2$), 69.1 (NHCH$_2\underline{C}$H$_2$O), 69.9 ($\underline{C}$H$_2$O), 70.1 ($\underline{C}$H$_2$O), 70.1 ($\underline{C}$H$_2$O), 70.2 ($\underline{C}$H$_2$O), 70.2 – 70.3 (Several $\underline{C}$H$_2$O), 171.7 (CO), 171.9 (CO), 172.8 (CO), 173.1 (CO), 173.2 (CO), 173.3 (CO), 173.6 (CO), 174.2 (2xCO), 174.3 (CO), 174.3 (CO), 174.5 (CO), 174.6 (CO), 174.9 (CO); HRMS (ESI/Q-ToF): calcd for $C_{67}H_{118}N_{10}O_{24}$, 1446.8320; found 1446.8327.

1.4 Synthesis of BDNF PAs

PAs were synthesized in a 2.0 mmol scale on 2-chlorotrityl chloride resin (1.176 g, 1.70 meq g^{-1} 100–200 mesh) employing a standard Fmoc solid-phase peptide synthesis (SPPS) method (see **Scheme S1**).

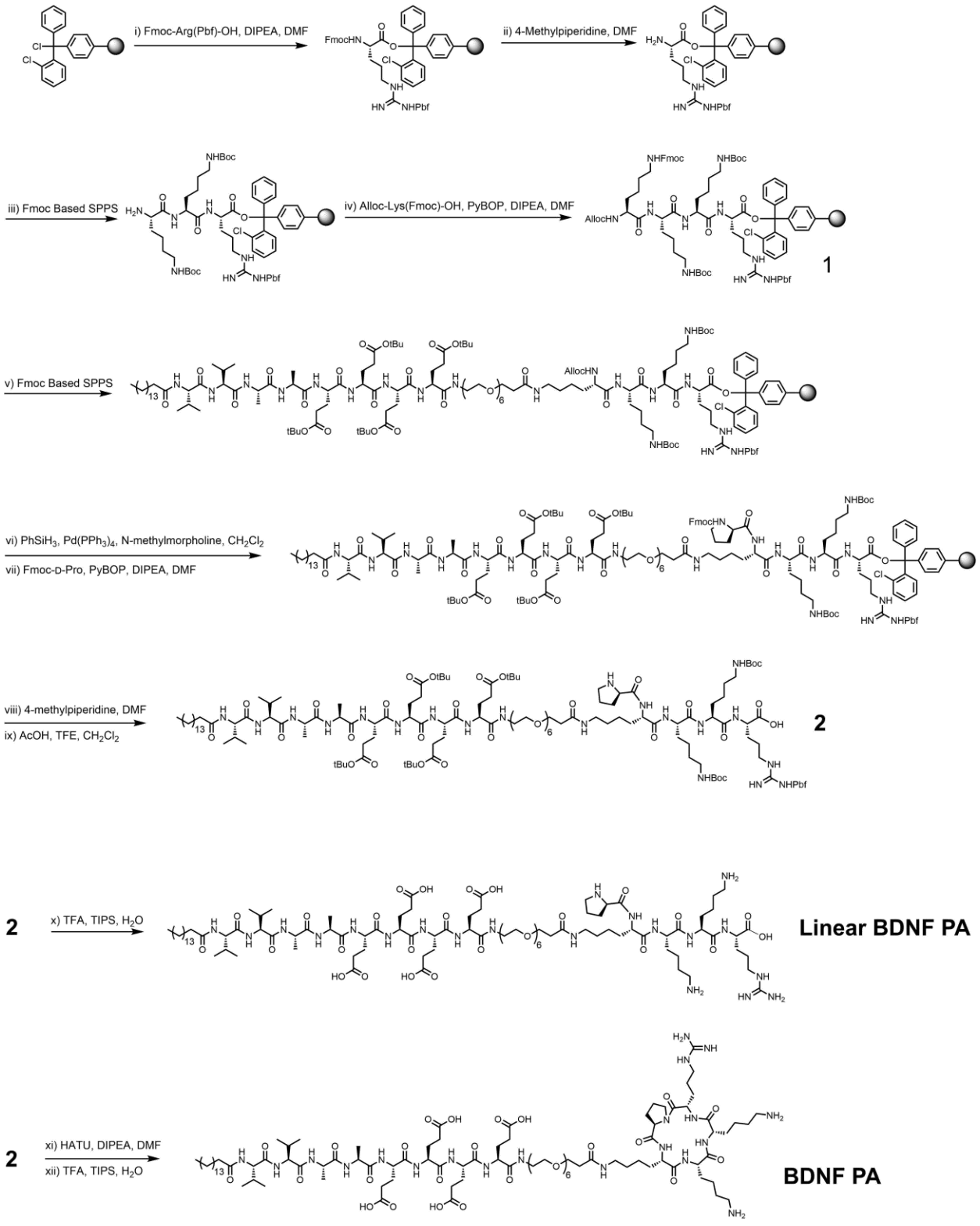
Synthesis of protected Linear BDNF PA. After addition of the Alloc-protected lysine to yield resin-bound peptide **1**, an Fmoc protected PEG_6 linker was added (633 mg, 1.1 mmol). Then, four glutamic acid residues, two alanine residues and two valine residues were sequentially added at 4 molar equivalence (Fmoc-glutamic acid; 1.70g, Fmoc-alanine; 1.25g, Fmoc-valine; 1.33g, 4.0 mmol) using the general procedures described above. Palmitic acid was added and shaken for 3 days at 25 °C after which the Alloc was deprotected and Fmoc-D-proline was attached (371 mg, 1.1 mmol) to form the fully protected resin-bound peptide. The Fmoc was deprotected following the general protocol above. A mixture of CH_2Cl_2/trifluoroethanol (TFE)/acetic acid (AcOH) (7:2:1, v/v/v) (20 mL) was added to selectively cleave the peptide from the 2-chlorotrithyl chloride resin without deprotecting the amino acids. After shaking for 2 hours, the cleavage mixture and two subsequent CH_2Cl_2 washings were filtered. The combined solution was evaporated to a viscous solution under reduced pressure. Then, hexanes were added, and solvent was removed with rotary evaporation and subsequently dried *in vacuo* for 3 days to remove any residual acetic acid giving the titled compound **2**. The batch was split, and half was set aside to synthesize the Linear BDNF PA.

Linear BDNF PA. Compound **2** was subjected to a cleavage solution of TFA/TIPS/H_2O in a ratio of 95:2.5:2.5 for 3 hours. The reaction mixture was concentrated by rotary evaporation and the remaining peptide solution was triturated with cold diethyl ether. The precipitate was collected with vacuum filtration and the crude precipitate was then stored at -20 °C until purification by HPLC. HPLC gave the **Linear BDNF PA** as a white powder after freeze-drying. ^{1}H-NMR (600 MHz, DMF-d_7 + 5% D_2O): δ = 0.86 (t, 3H, J = 7.0 Hz, 3xPal-C$\underline{H}_3$), 0.93 (d, 3H, J = 6.8 Hz, 3xVal-C$_{(\gamma)}\underline{H}_3$), 0.96 (ad, 6H, J = 6.7 Hz, 6xVal-C$_{(\gamma)}\underline{H}_3$), 1.01 (d, 3H, J = 6.8 Hz, 3xVal-C$_{(\gamma)}\underline{H}_3$), 1.20 – 1.31 (m, 24H, 24xPal-C$\underline{H}_2$), 1.33 – 1.84 (m, 26H, 6xAla-C$_{(\beta)}\underline{H}_3$, 4xLys-C$_{(\beta)}\underline{H}_2$, 4xLys-C$_{(\gamma)}\underline{H}_2$, 6xLys-C$_{(\delta)}\underline{H}_2$, 2xPal-C$\underline{H}_2$, 2xPro-C$_{(\beta)}\underline{H}_2$, 2xPro-C$_{(\gamma)}\underline{H}_2$), 1.85 – 1.93 (m, 2H, 2xLys-C$_{(\gamma)}\underline{H}_2$), 1.97 – 2.23 (m, 14H, 2xArg-C$_{(\gamma)}\underline{H}_2$, 2xLys-C$_{(\beta)}\underline{H}_2$, 8xGlu-C$_{(\beta)}\underline{H}_2$, 2xVal-C$_{(\beta)}\underline{H}$),

2.30 – 2.64 (m, 14H, 2xArg-$C_{(\beta)}\underline{H}_2$, 8xGlu-$C_{(\gamma)}\underline{H}_2$, 2xO$CH_2C\underline{H}_2$CONH, 2xPal-$C\underline{H}_2$), 2.98 – 3.05 (m, 4H, 4xLys-$C_{(\varepsilon)}\underline{H}_2$), 3.13 (t, 2H, *J* = 7.0 Hz, 2xLys-$C_{(\varepsilon)}\underline{H}_2$), 3.29 (at, 2H, *J* = 7.1, 7.7 Hz, 2xPro-$C_{(\delta)}\underline{H}_2$), 3.32 – 3.39 (m, 2H, 2xNHC$\underline{H}_2CH_2O$), 3.39 – 3.48 (m, 2H, 2xArg-$C_{(\delta)}\underline{H}_2$), 3.53 – 3.59 (m, 22H, 2xNH$CH_2C\underline{H}_2O$, 20xO$C\underline{H}_2$), 3.69 (t, 2H, *J* = 6.3 Hz, 2xO$C\underline{H}_2CH_2$CONH), 4.01 – 4.03 (m, 2H, 2xVal-$C_{(\alpha)}\underline{H}$), 4.08 – 4.12 (m, 1H, Pro-$C_{(\alpha)}\underline{H}$), 4.14 – 4.18 (m, 2H, Ala-$C_{(\alpha)}\underline{H}$, Glu-$C_{(\alpha)}\underline{H}$), 4.20 – 4.23 (m, 2H, Ala-$C_{(\alpha)}\underline{H}$, Glu-$C_{(\alpha)}\underline{H}$), 4.27 (dd, 1H, *J* = 5.1, 9.7 Hz, Glu-$C_{(\alpha)}\underline{H}$), 4.31 – 4.35 (m, 2H, Glu-$C_{(\alpha)}\underline{H}$, Lys-$C_{(\alpha)}\underline{H}$), 4.42 – 4.44 (m, 2H, 2xLys-$C_{(\alpha)}\underline{H}$), 4.44 – 4.49 (m, 1H, Arg-$C_{(\alpha)}\underline{H}$); ^{13}C-NMR (125 MHz, DMF-d_7 + 5% D_2O): δ = 13.7 (Pal-$\underline{C}H_3$), 16.2 (Ala-$\underline{C}_{(\beta)}H_3$), 16.2 (Ala-$\underline{C}_{(\beta)}H_3$), 18.7 (Val-$\underline{C}_{(\gamma)}H_3$), 18.9 (Val-$\underline{C}_{(\gamma)}H_3$), 18.9 (Val-$\underline{C}_{(\gamma)}H_3$), 19.1 (Val-$\underline{C}_{(\gamma)}H_3$), 22.3 – 31.8 (Arg-$\underline{C}_{(\beta)}H_2$, Arg-$\underline{C}_{(\gamma)}H_2$, 4xGlu-$\underline{C}_{(\beta)}H_2$, 4xGlu-$\underline{C}_{(\gamma)}H_2$, 3xLys-$\underline{C}_{(\beta)}H_2$, 3xLys-$\underline{C}_{(\gamma)}H_2$, 3xLys-$\underline{C}_{(\delta)}H_2$, multiple Pal-$\underline{C}H_2$, Pro-$\underline{C}_{(\beta)}H_2$, Pro-$\underline{C}_{(\gamma)}H_2$, 2xVal-$\underline{C}_{(\beta)}H$, *several carbon peaks with insufficient S/N and overlap with solvent*), 22.5 (Pal-$\underline{C}H_2$), 31.8 (Pal-$\underline{C}H_2$), 35.5 (Pal-$\underline{C}H_2$, *overlap with solvent*), 36.4 (O$CH_2\underline{C}H_2$CONH), 38.1 (Lys-$\underline{C}_{(\varepsilon)}H_2$), 39.0 (NH$\underline{C}H_2CH_2O$), 39.2 (Lys-$\underline{C}_{(\varepsilon)}H_2$), 39.3 (Lys-$\underline{C}_{(\varepsilon)}H_2$), 40.9 (Pro-$\underline{C}_{(\delta)}H_2$), 46.5 (Arg-$\underline{C}_{(\delta)}H_2$), 51.0 (Ala-$\underline{C}_{(\alpha)}H$), 51.3 (Ala-$\underline{C}_{(\alpha)}H$), 53.2 (Glu-$\underline{C}_{(\alpha)}H$), 53.2 (Lys-$\underline{C}_{(\alpha)}H$), 53.5 (Lys-$\underline{C}_{(\alpha)}H$), 53.6 (Lys-$\underline{C}_{(\alpha)}H$), 53.6 (Pro-$\underline{C}_{(\alpha)}H$), 53.9 (Glu-$\underline{C}_{(\alpha)}H$), 54.6 (Glu-$\underline{C}_{(\alpha)}H$), 55.1 (Glu-$\underline{C}_{(\alpha)}H$), 60.0 (Arg-$\underline{C}_{(\alpha)}H$), 61.1 (Val-$\underline{C}_{(\alpha)}H$), 61.1 (Val-$\underline{C}_{(\alpha)}H$), 67.1 (O$\underline{C}H_2CH_2$CONH), 69.1 (NH$CH_2\underline{C}H_2O$), 70.0 ($\underline{C}H_2O$), 70.1 ($\underline{C}H_2O$), 70.2 ($\underline{C}H_2O$), 70.2 – 70.2 ($\underline{C}H_2O$, *several carbon peaks*), 157.3 (Arg-$\underline{C}$=NH), 173.1 (CO), 173.6 (CO), 174.0 (CO), 174.0 (CO), 175.0 (CO), 175.2 (CO), *several carbonyl peaks are missing due to insufficient S/N*; HRMS (ESI/Q-ToF): calcd for $C_{96}H_{172}N_{20}O_{30}$, 2085.2548; found 2085.2516.

BDNF PA. A DMF solution (15 mL) of *N*,*N*,*N'*,*N'*-tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate (HATU) (210 mg, 0.55 mmol) and DIPEA (270 µL, 1.5 mmol) was added to compound **2**. After 30 min at 25 °C, the mixture was concentrated and co-evaporated with toluene followed by drying under vacuo for 3 days giving a crude fully protected cyclic BDNF PA. The remaining protecting groups were removed using with a mixture of TFA/TIPS/H_2O in a ratio of 95:2.5:2.5 for 3 hours. The reaction mixture was concentrated by rotary evaporation and the remaining peptide solution was triturated with cold diethyl ether. The precipitate was collected with vacuum filtration and the crude precipitate was then stored at -20 °C until purification by HPLC. HPLC gave the cyclic **BDNF PA** as a white powder after

freeze-drying. ^{1}H-NMR (600 MHz, DMF-d_7 + 5% D_2O): δ = 0.85 (t, 3H, *J* = 7.0 Hz, 3xPal-C$\underline{H}_3$), 0.92 (d, 3H, 3xVal-C$_{(\gamma)}\underline{H}_3$), 0.96 (at, 6H, 6xVal-C$_{(\gamma)}\underline{H}_3$), 1.00 (d, 3H, *J* = 6.8 Hz, 3xVal-C$_{(\gamma)}\underline{H}_3$), 1.25 – 1.30 (m, 24H, 24xPal-C$\underline{H}_2$), 1.35 – 2.25 (m, 32H, 6xAla-C$_{(\beta)}\underline{H}_3$, 2xArg-C$_{(\beta)}\underline{H}_2$, 2xArg-C$_{(\gamma)}\underline{H}_2$, 8xGlu-C$_{(\beta)}\underline{H}_2$, 6xLys-C$_{(\beta)}\underline{H}_2$, 6xLys-C$_{(\gamma)}\underline{H}_2$, 6xLys-C$_{(\delta)}\underline{H}_2$, 2xPal-C$\underline{H}_2$, 2xPro-C$_{(\beta)}\underline{H}_2$, 2xPro-C$_{(\gamma)}\underline{H}_2$, 2xVal-C$_{(\beta)}\underline{H}$), 2.36 – 2.63 (m, 12H, 8xGlu-C$_{(\gamma)}\underline{H}_2$, 2xOCH$_2C\underline{H}_2$CONH, 2xPal-C$\underline{H}_2$), 3.01 – 3.07 (m, 4H, 4xLys-C$_{(\varepsilon)}\underline{H}_2$), 3.15 (t, 2H, *J* = 6.9 Hz, 2xLys-C$_{(\varepsilon)}\underline{H}_2$), 3.26 – 3.39 (m, 4H, 2xNHCH$_2$C$\underline{H}_2$O, 2xPro-C$_{(\delta)}\underline{H}_2$), 3.54 (t, 2H, *J* = 6.2 Hz, 2xNHC$\underline{H}_2$CH$_2$O), 3.55 – 3.59 (m, 22H, 20xOC$\underline{H}_2$, 2xArg-C$_{(\delta)}\underline{H}_2$), 3.70 (t, 1H, *J* = 6.3 Hz, 2xOC$\underline{H}_2$CH$_2$CONH), 3.95 (dd, 1H, *J* = 6.1, 9.7 Hz, Lys-C$_{(\alpha)}\underline{H}$), 3.99 – 4.02 (m, 2H, 2xVal-C$_{(\alpha)}\underline{H}$, *overlap with HDO-peak*), 4.12 – 4.23 (m, 5H, 2xAla-C$_{(\alpha)}\underline{H}$, 2xGlu-C$_{(\alpha)}\underline{H}$, Lys-C$_{(\alpha)}\underline{H}$), 4.26 – 4.33 (m, 3H, 2xGlu-C$_{(\alpha)}\underline{H}$, Lys-C$_{(\alpha)}\underline{H}$), 4.55 (t, 1H, *J* = 5.9 Hz, Arg-C$_{(\alpha)}\underline{H}$), 4.61 (dd, 1H, *J* = 4.3, 9.6 Hz, Pro-C$_{(\alpha)}\underline{H}$); *Several carbons are missing due to insufficient S/N;* ^{13}C-NMR (125 MHz, DMF-d_7 + 5% D_2O): δ = 13.6 (Pal-C$\underline{H}_3$), 16.0 (Ala-C$_{(\beta)}\underline{H}_3$), 16.1 (Ala-C$_{(\beta)}\underline{H}_3$), 18.6 (Val-C$_{(\gamma)}\underline{H}_3$), 18.8 (Val-C$_{(\gamma)}\underline{H}_3$), 18.8 (Val-C$_{(\gamma)}\underline{H}_3$), 19.0 (Val-C$_{(\gamma)}\underline{H}_3$), 22.4 (Pal-C$\underline{H}_2$), 22.6 – 30.8 (*several carbon peaks, partial overlap with solvent*, Arg-C$_{(\beta)}\underline{H}_2$, Arg-C$_{(\gamma)}\underline{H}_2$, 4xGlu-C$_{(\beta)}\underline{H}_2$, 4xGlu-C$_{(\gamma)}\underline{H}_2$, 3xLys-C$_{(\beta)}\underline{H}_2$, 3xLys-C$_{(\gamma)}\underline{H}_2$, 3xLys-C$_{(\delta)}\underline{H}_2$, multiple Pal-C$\underline{H}_2$, Pro-C$_{(\beta)}\underline{H}_2$, Pro-C$_{(\gamma)}\underline{H}_2$, 2xVal-C$_{(\beta)}\underline{H}$), 31.6 (Pal-C$\underline{H}_2$), 35.6 (Pal-C$\underline{H}_2$), 36.3 (OCH$_2C\underline{H}_2$CONH), 38.5 (Lys-C$_{(\varepsilon)}\underline{H}_2$), 38.8 (NHC$\underline{H}_2CH_2$O), 39.2 (Lys-C$_{(\varepsilon)}\underline{H}_2$), 39.4 (Lys-C$_{(\varepsilon)}\underline{H}_2$), 40.5 (Pro-C$_{(\delta)}\underline{H}_2$), 46.6 (Arg-C$_{(\delta)}\underline{H}_2$), 50.3 (Pro-C$_{(\alpha)}\underline{H}$), 51.0 (Ala-C$_{(\alpha)}\underline{H}$), 51.2 (Ala-C$_{(\alpha)}\underline{H}$), 53.1 (Glu-C$_{(\alpha)}\underline{H}$), 53.1 (Lys-C$_{(\alpha)}\underline{H}$), 53.8 (Glu-C$_{(\alpha)}\underline{H}$), 54.1 (Lys-C$_{(\alpha)}\underline{H}$), 54.5 (Glu-C$_{(\alpha)}\underline{H}$), 54.9 (Glu-C$_{(\alpha)}\underline{H}$), 57.0 (Lys-C$_{(\alpha)}\underline{H}$), 60.1 (Arg-C$_{(\alpha)}\underline{H}$), 60.9 – 61.6 (2xVal-C$_{(\alpha)}\underline{H}$), 67.1 (OC$\underline{H}_2CH_2$CONH), 69.0 (NHCH$_2C\underline{H}_2$O), 69.6 – 70.8 (*Several carbon peaks*, C$\underline{H}_2$O), 171.5 (CO), 173.5 (CO), 173.6 (CO), 174.2 (CO), 174.3 (CO), 175.0 (CO), 175.1 (CO), *several carbonyl and Arg-C=NH peaks are missing due to insufficient S/N*; HRMS (ESI/Q-ToF): calcd for $C_{96}H_{170}N_{20}O_{29}$, 2067.2443; found 2067.2427.



Scheme S1. Synthesis of BDNF PA

1.5 Synthesis of BDNF Peptide and PEG_6-BDNF peptide.

The free **BDNF peptide** and **PEG_6-BDNF peptide** were synthesized in a 1.0 mmol scale on 2-chlorotrityl chloride resin (0.588 g, 1.7 meq g^{-1} 100-200 mesh) and a 0.125 mmol scale (0.0825 g, 1.52 meq g^{-1} 100-200 mesh) respectively, employing a standard Fmoc solid-phase peptide synthesis (SPPS) method (see **Scheme S2 and S3**).

Synthesis of BDNF peptide (Scheme S2). Standard Fmoc solid-phase peptide procedures yielded resin-bound peptide **3**.

Synthesis of PEG_6-BDNF Peptide (Scheme S3). After addition of the Alloc-protected lysine to yield resin-bound peptide **1**, a standard Fmoc deprotection was performed. Fmoc protected PEG_6 linker was added (79 mg, 0.14 mmol) to a solution of DMF (15 mL) and PyBOP (34 mg, 0.066 mmol) and shaken for 2 hours at 25 °C. The Fmoc-protected PEG_6 group was deprotected using standard methods. A solution of acetic anhydride (Ac_2O) in pyridine (2:3 v/v) (10 ml) was added to the resin to N-acetylate the PEG_6. After shaking for 0.5 hours at 25 °C, the solution was drained off, and the resin was washed with DMF (5x15 mL). Afterwards the Alloc was deprotected and Fmoc-D-proline was attached (23 mg, 0.07 mmol) to form fully protected resin-bound peptide **5**. The Fmoc was deprotected following the general protocol above.

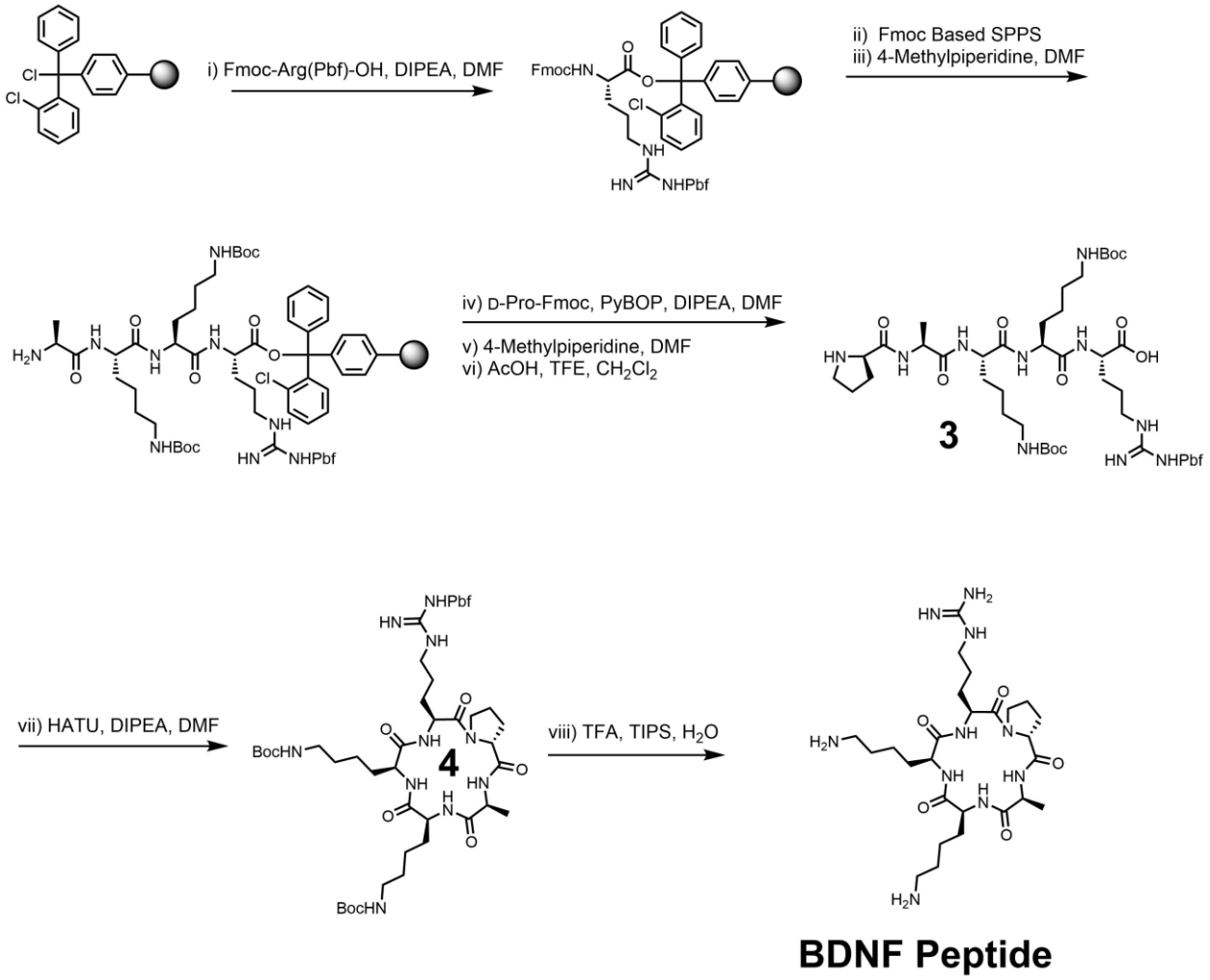
Cleavage reaction of peptide from the peptidyl resin (Scheme S2 & S3). In a typical reaction, a mixture of CH_2Cl_2/TFE/AcOH (7/2/1, v/v/v) (20 mL) was added to the peptidyl resin (1.0 mmol). After shaking for 1.5 hours, the cleavage mixture and two subsequent CH_2Cl_2 (30 mL) were filtered. The combined solution was evaporated to a viscous solution under reduced pressure. Then, cold diethyl ether was added to the solution and the resulting precipitate was washed with cold Et_2O (3x30 mL) to remove any residual acetic acid. The precipitate was dried under reduced pressure to afford peptide **3, 6**, which were used in the next step without further purification.

Cyclization of peptides (Scheme S2 & S3). In a typical reaction, a DMF solution (15 mL) of HATU (210 mg, 0.55 mmol) and DIPEA (270 µL, 1.5 mmol) was added to peptide **3, 6** for 30 min at 25 °C to form the protected peptide **4, 7**. The reaction mixture was evaporated to dryness using rotary evaporation. Remaining protecting groups were removed to form the crude BDNF peptide and crude PEG_6-BDNF peptide with a

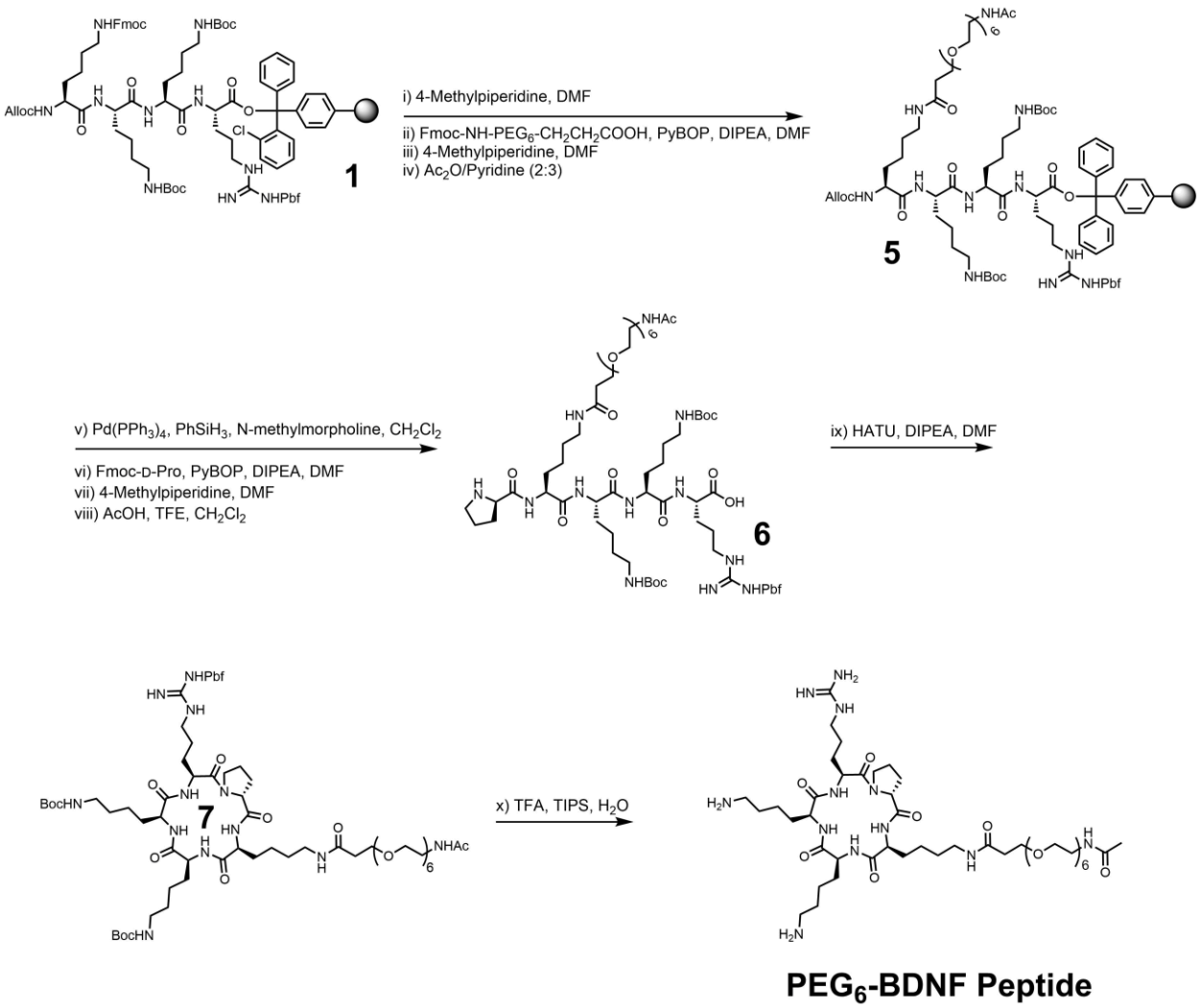
mixture of TFA/TIPS/H_2O in a ratio of 95:2.5:2.5 for 3 hours. Excess TFA was removed by rotary evaporation. The remaining peptide solution was triturated with cold diethyl ether and the precipitate was collected with vacuum filtration. The crude precipitate was then stored at -20 °C until purified. HPLC gave the **BDNF peptide** and **PEG$_6$-BDNF peptide** both as white powders after freeze-drying.

BDNF peptide: ^{1}H-NMR (600 MHz, D_2O): δ = 1.15 – 1.22 (m, 1H, Lys-$C_{(\gamma)}\underline{H}$), 1.23 – 1.37 (m, 3H, 3xLys-$C_{(\gamma)}\underline{H}$), 1.28 (d, 3H, J = 7.2 Hz, Ala-$C_{(\beta)}\underline{H}$), 1.37 – 1.44 (m, 1H, Lys-$C_{(\beta)}\underline{H}$), 1.46 – 1.60 (m, 6H, 2xArg-$C_{(\gamma)}\underline{H}$, 4xLys-$C_{(\delta)}\underline{H}$), 1.63 – 1.70 (m, 2H, 2xLys-$C_{(\beta)}\underline{H}$), 1.71 – 1.78 (m, 2H, 2xArg-$C_{(\beta)}\underline{H}$), 1.80 – 1.85 (m, 3H, Lys-$C_{(\beta)}\underline{H}_2$, Pro-$C_{(\beta)}\underline{H}_2$, Pro-$C_{(\gamma)}\underline{H}_2$), 1.99 – 2.04 (m, 2H, Pro-$C_{(\beta)}\underline{H}_2$, Pro-$C_{(\gamma)}\underline{H}_2$), 2.78 – 2.86 (m, 4H, 4xLys-$C_{(\varepsilon)}\underline{H}_2$), 3.07 (t, 2H, J = 7.0 Hz, 2xArg-$C_{(\delta)}\underline{H}_2$), 3.41 – 3.45 (m, 1H, Pro-$C_{(\delta)}\underline{H}_2$), 3.54 – 3.58 (m, 1H, Pro-$C_{(\delta)}\underline{H}_2$), 3.86 (t, 1H, J = 8.0 Hz, Arg-$C_{(\alpha)}\underline{H}$), 4.11 (q, 1H, J = 7.2 Hz, Ala-$C_{(\alpha)}\underline{H}$), 4.20 (dd, 1H, J = 5.3, 7.1 Hz, Pro-$C_{(\alpha)}\underline{H}$), 4.39 (dd, 1H, J = 4.0, 11.2 Hz, Lys-$C_{(\alpha)}\underline{H}$), 4.44 (dd, 1H, J = 3.2, 10.3 Hz, Lys-$C_{(\alpha)}\underline{H}$); ^{13}C-NMR (125 MHz, D_2O): δ = 15.9 (Ala-$\underline{C}_{(\beta)}H_3$), 21.8 (Lys-$\underline{C}_{(\gamma)}H_2$), 22.1 (Lys-$\underline{C}_{(\gamma)}H_2$), 23.8 (Arg-$\underline{C}_{(\gamma)}H_2$), 24.6 (Pro-$\underline{C}_{(\gamma)}H_2$), 25.7 (Lys-$\underline{C}_{(\delta)}H_2$), 25.8 (Lys-$\underline{C}_{(\delta)}H_2$), 27.1 (Lys-$\underline{C}_{(\beta)}H_2$), 27.6 (Pro-$\underline{C}_{(\beta)}H_2$), 28.5 (Arg-$\underline{C}_{(\beta)}H_2$), 28.7 (Lys-$\underline{C}_{(\beta)}H_2$), 38.8 (Lys-$\underline{C}_{(\varepsilon)}H_2$), 38.9 (Lys-$\underline{C}_{(\varepsilon)}H_2$), 39.8 (Arg-$\underline{C}_{(\delta)}H_2$), 47.0 (Pro-$\underline{C}_{(\delta)}H_2$), 50.1 (Arg-$\underline{C}_{(\alpha)}H$), 50.1 (Lys-$\underline{C}_{(\alpha)}H$), 51.5 (Lys-$\underline{C}_{(\alpha)}H$), 56.6 (Ala-$\underline{C}_{(\alpha)}H$), 60.6 (Pro-$C_{(\alpha)}H$), 156.4 (Arg-$\underline{C}$=NH), 171.2 (Lys-$\underline{C}$O), 172.4 (Lys-$\underline{C}$O), 174.5 (Ala-$\underline{C}$O), 174.7 (Pro-$\underline{C}$O), 175.0 (Arg-$\underline{C}$O); HRMS (ESI/Q-ToF): calcd for $C_{26}H_{48}N_{10}O_5$, 580.3809; found 580.3811.

PEG$_6$-BDNF peptide: ^{1}H NMR (600 MHz, MeOH-d_4): δ = 4.61 (dd, 1H, J = 4.3, 9.1 Hz), 4.49 (dd, 1H, J = 5.3, 7.1 Hz), 4.33 (dd, 1H, J = 5.2, 9.9 Hz), 4.22 (dd, 1H, J = 4.5, 9.3 Hz), 3.93 (dd, 1H, J = 6.6, 9.2 Hz), 3.72 (t, 2H, J = 6.1 Hz), 3.66 – 3.56 (m, 22H), 3.54 (t, 2H, J = 5.6 Hz), 3.35 (t, 2H, J = 5.6 Hz), 3.31 – 3.16 (m, 4H), 2.96 – 2.91 (m, 4H), 2.44 (t, 2H, J = 6.1 Hz), 2.26 – 2.19 (m, 1H), 2.10 – 1.36 (m, 28H); HRMS (ESI/Q-ToF): calcd for $C_{46}H_{87}N_{12}O_{13}$ [M + H], 1015.6517; found 1015.6508.



Scheme S2. Synthesis of BDNF Peptide.



Scheme S3. Synthesis of PEG_6-BDNF Peptide.

1.6 Synthesis of BDNF Peptide and PEG_6-BDNF Peptide for Immobilization

BDNF Peptide and PEG_6-BDNF Peptide for immobilization. The BDNF Peptide **11a** for immobilization was synthesized in a 2.0 mmol scale on 2-chlorotrityl chloride resin (1.32 g, 1.52 meq g^{-1} 100–200 mesh). The PEG_6-BDNF Peptide **11b** for immobilization was synthesized in a 0.25 mmol scale on 2-chlorotrityl chloride resin (0.165 g, 1.52 meq g^{-1} 100-200 mesh). Both **11a** and **11b** were synthesized by using standard Fmoc solid-phase peptide synthesis (SPPS) method (see **Scheme S4**).

Coupling compound 1 with Fmoc-NH-PEG_6-CH_2CH_2COOH. To a DMF solution (15 mL) of Fmoc-NH-PEG_6-CH_2CH_2COOH (158 mg, 0.28 mmol), PyBOP (137 mg, 0.26 mmol), and DIPEA (72 µL, 0.41 mmol) was added, and the mixture was stirred 1 min for activation. The reaction mixture was then added to the peptidyl resin and the reaction vessel was shaken for 2h at 25 °C. The coupling solution was then drained off and the peptidyl resin was washed with DMF (3x30 mL) and CH_2Cl_2 (2x30 mL).

Coupling with succinic acid monobenzyl ester to form 8a and 8b. The Fmoc was deprotected following general procedures mentioned above. In a typical reaction, a DMF solution (15 mL) of succinic acid monobenzyl ester (416 mg, 2.0 mmol), PyBOP (989 mg, 1.9 mmol), and DIPEA (520 µL, 3.0 mmol) was added, and the mixture was stirred 1 min for activation. The mixture was then added to the peptidyl resin, and the reaction vessel was shaken for 12 hours at 25 °C. After the coupling solution was drained off, the peptidyl resin was washed with DMF (3x30 mL) to afford resin bound **8a** and **8b**. The peptidyl resins were then subjected to Alloc deprotection, coupling with Fmoc D-proline, and Fmoc deprotection following the general procedures mentioned above.

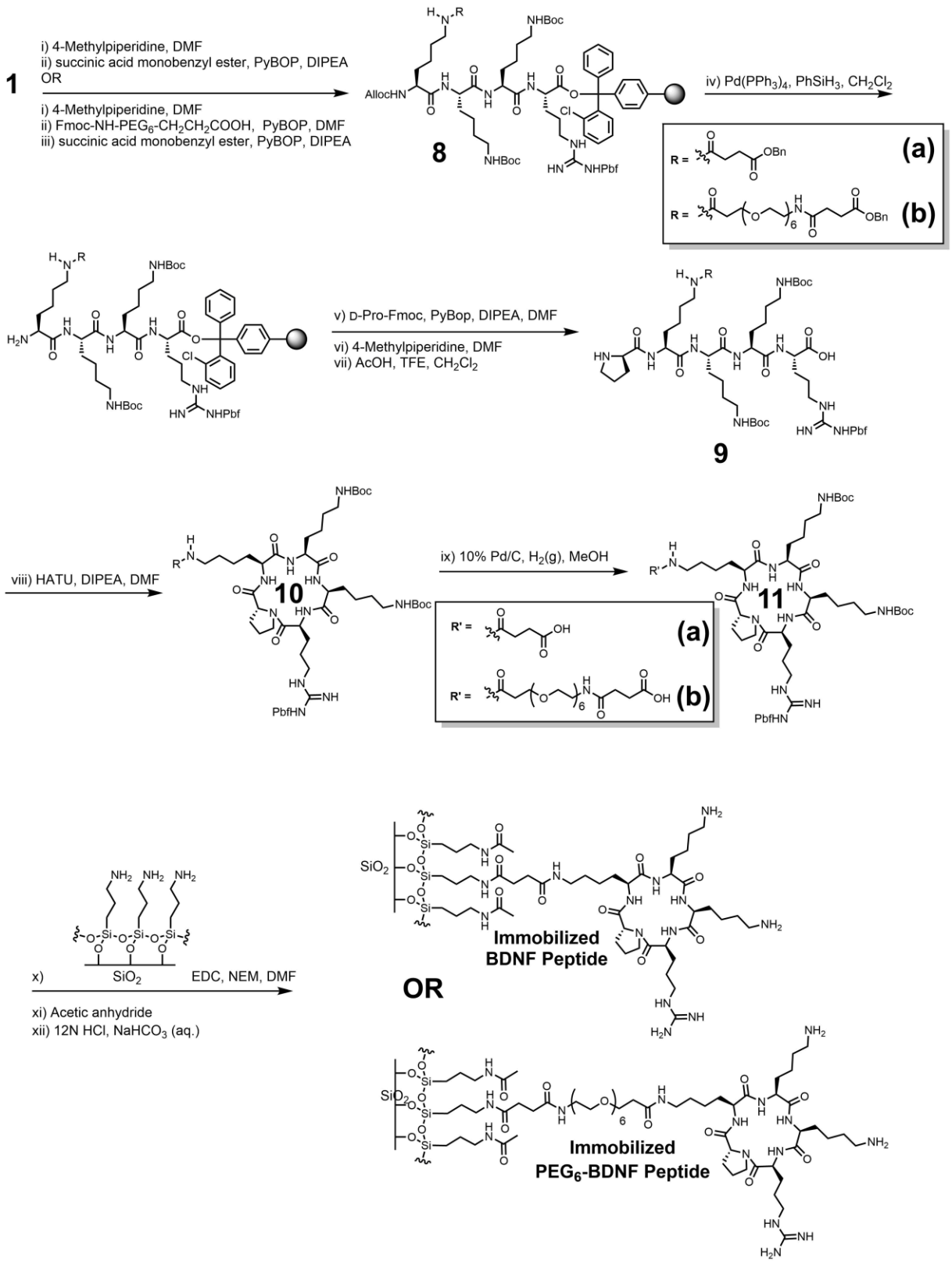
Cleavage reaction of peptide from the peptidyl resin. In a typical reaction, a mixture of CH_2Cl_2/TFE/AcOH (7/2/1, v/v/v) (20 mL) was added to the peptidyl resin (1.0 mmol). After shaking for 1.5 hours, the cleavage mixture and was washed with CH_2Cl_2 (2x30 mL) and filtered. The combined solution was evaporated to a viscous solution under reduced pressure. Then, cold diethyl ether was added to the solution and the resulting precipitate was washed with cold Et_2O (3x30 mL) to remove any residual acetic acid. The precipitate was dried under reduced pressure to afford peptide **9a** and **9b**, which was used in the next step without further purification.

Synthesis of BDNF peptide for immobilization 11a. To a DMF solution (30 mL) of peptide **9a** (890 mg, 685 μmol) was added HATU (1.67 g, 8.0 mmol) and DIPEA (4.2 mL, 24 mmol) under N_2 (g) at 25 °C. After stirring for 17 hours, EtOAc (100 mL) was added and the mixture was washed successively with H_2O (3x100 mL) and brine (100 mL). The combined organic extract was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was subjected to column chromatography on silica gel using CH_2Cl_2/MeOH (19/1 => 7/1 v/v), leaving cyclic peptide **10a** as a pale-yellow foam (570 mg) which was used for the next step without further purification. MALDI-ToF-MS *m/z* calcd for $C_{63}H_{97}N_{11}O_{15}SNa$ ([M + Na]$^+$) 1302.68, found 1302.30. To a MeOH solution (20 mL) of cyclic peptide **10a** (570 mg) was added 10% Pd/C (120 mg) at 0 °C, and the mixture was stirred under atmospheric pressure of H_2 for 5 hours at 25 °C. The reaction mixture was then filtered through a pad of Celite and eluted with MeOH. The filtrate was evaporated to dryness under reduced pressure to yield peptide **11a**. The residue was purified by RP-HPLC to allow isolation of the **BDNF peptide for immobilization 11a** as a white solid (101 mg, 0.848 mmol, overall yield 8%). ^{1}H NMR (500 MHz, MeOH-d_4): δ 6.57 (s, 1H), 4.58 (m, 1H), 4.50 (t, J = 5.5 Hz, 1H), 4.30 (t, J = 6.8 Hz, 1H), 4.20 (dd, J = 9.9, 4.1 Hz, 1H), 3.89 (m, 1H), 3.60 (m, 1H), 3.53 (m, 1H), 3.25–3.15 (m, 5H), 3.10–2.95 (m, 8H), 2.60–2.40 (m, 14H), 2.17 (m, 1H), 2.10–2.00 (m, 6H), 2.00–1.85 (m, 6H), 1.85–1.65 (m, 4H), 1.65–1.30 (m, 51H); MALDI-ToF mass *m/z* calcd. for $C_{56}H_{91}N_{11}O_{15}S$ [M]$^-$: *m*/*z* = 1189.64, found: 1190.30.

Synthesis of PEG$_6$-BDNF peptide for immobilization 11b. To a DMF (15 mL) of peptide **9b** was added HATU (0.21 g, 1.0 mmol) and DIPEA (0.53 mL, 3 mmol) under N_2 (g) at 25 °C. After stirring for 1h the reaction mixture was evaporated to dryness under reduced pressure. The crude residue was subjected to column chromatography on silica gel using CH_2Cl_2/MeOH (19/1 => 6/1 v/v), leaving the cyclic peptide **10b** as an off-white solid (274 mg). To a MeOH solution (10 mL) of the cyclic peptide **10b** (274 mg) was added 10% Pd/C (50 mg), and the mixture was stirred under pressure of H_2 overnight at 25 °C. The reaction mixture was purged with N_2 and then filtered through a pad of Celite and washed with MeOH. The filtrate was evaporated to dryness under reduced pressure to yield peptide **11b**. RP-HPLC afforded the **PEG$_6$-BDNF peptide for immobilization 11b.** as a white powder after freeze-drying. ^{1}H NMR (600 MHz,

MeOH-d_4): δ 4.59 – 4.57 (m, 1H), 4.47 (dd, 1H, J = 5.3, 7.0 Hz), 4.27 (dd, 1H, J = 6.7, 8.2 Hz), 4.20 (dd, 1H, J = 4.2, 9.9 Hz), 3.86 (dd, 1H, J = 6.8, 8.6 Hz), 3.72 (t, 2H, J = 6.2 Hz), 3.64 – 3.61 (m, 21H), 3.54 – 3.51 (m, 3H), 3.35 (t, 2H, J = 5.5 Hz), 3.23 – 3.20 (m, 4H), 3.07 – 3.01 (m, 6H), 2.58 (s, 3H), 2.55 (t, 2H, J = 7.0 Hz), 2.52 (s, 3H), 2.48 (t, 2H, J = 7.0 Hz), 2.43 (t, 2H, J = 6.1 Hz), 2.23 – 2.16 (m, 1H), 2.09 (s, 3H), 2.07 – 1.87 (m, 7H), 1.83 – 1.66 (m, 4H), 1.64 – 1.29 (m, 38H); HRMS (ESI/Q-ToF): calcd for $C_{71}H_{121}N_{12}O_{22}S$ [M+H], 1525.8439; found 1525.8417.

Fabrication of BDNF-mimetic monolayer. The purified **BDNF peptide for immobilization 11a** and **PEG$_6$-BDNF Peptide for immobilization 11b** were immobilized onto the surface of glass coverslips or 6 μM silica beads (Spherotech). Surfaces were washed with 2% (v/v) micro-90 detergent (Sigma Aldrich) for 30 min at 60 °C, then rinsed six times with distilled water. Surfaces were then rinsed with ethanol, and dried. Surfaces were plasma-etched (Harrick Plasma PDC-001-HP) with O_2 for 30 s, then immediately incubated in a 2% (v/v) solution of (3-aminopropyl)triethoxysilane (Sigma Aldrich) in ethanol for 15 min. They were then rinsed twice with ethanol and twice with water. Coverslips or beads were dried in the oven. BDNF peptide was attached to surfaces following an established protocol reported previously.[1] COOH-functionalized BDNF peptide or Peg$_6$-BDNF Peptide was then prepared at 50 nmol/mL in a 1.25 mg/mL solution of 1-ethyl-3-(dimethyl-aminopropyl)carbodiimide (Arcos Organics) with 2% ethyl morpholine (NEM) in DMF. Surfaces were incubated with this solution for 3.5 hours at 40 °C with perturbation. After incubation, samples were washed with 100% acetic anhydride (Fisher Chemical), 12 N hydrochloric acid (Fisher Chemical), and 0.2 M sodium bicarbonate in succession. After rinsing with copious amounts of water, samples were sonicated in 4 M urea for 10 min followed by 1 M NaCl for 10 min. Samples were rinsed with copious amounts of water and dried at 100 °C for 1 h.



Scheme S4. Synthesis of Immobilized BDNF Peptide and PEG_6-BDNF Peptide.

2. Materials and Methods

2.1. Material Preparation.

2.1.1. PA Preparation. PAs were co-assembled at different percentages with C_{16}-$V_2A_2E_2$ PA (E_2) by dissolving the lyophilized powder in hexafluoroisopropanol (HFIP) and mixing for 15 min. Samples were frozen in liquid N_2, and HFIP was removed *in vacuo* for 2 h. Samples were then re-dissolved using several microliters of 1N NaOH in distilled deionized water. These solutions were frozen in liquid N_2 and lyophilized to remove any residual HFIP. The co-assembled peptide amphiphile powder was reconstituted in 125 mM NaCl and 3 mM KCl solution. It was then adjusted to a pH of 7.4 using 1 µL additions of 1 N NaOH. Samples were annealed at 80 °C for 30 min, then slowly cooled at 1° per minute to reach a final temperature of 27 °C.

2.1.2. PA Gel Preparation. Gels were made using annealed PAs prepared with methods described in section 2.1.1. BDNF native protein or BDNF peptide were incorporated by mixing at 10 nM or 0.5 uM respectively, with E_2 PA gel at 1 wt% while it was in its liquid state. Silicon isolators with adhesive (Invitrogen) were placed on PDL coated glass coverslips. 70 µL of PA liquid was pipetted into the circular silicon well and a Transwell insert was placed flush with the top of the well of the silicon insert. This step ensured a flat, even thickness for all gels. Gelling solution comprised of 125 mM NaCl, 3 mM KCl, and 25 mM $CaCl_2$ was pipetted into the Transwell insert and allowed to slowly soak down into the material from the top. The insert prevented the gel from swelling more than the thickness of the silicon isolator. All gels were incubated at 37 °C for 5 min before removing the Transwell insert.

2.2. Material Characterization

2.2.1. Cryogenic-Transmission Electron Microscopy. Samples were plunge-frozen using a Vitrobot Mark IV (FEI) vitrification robot. Samples at 1 w/v% were tenfold diluted to 0.1 w/v% immediately before 7.5 µL of sample solutions were transferred to plasma-cleaned 300-mesh copper grids with lacey carbon support (Electron Microscopy Science). Samples were blotted at room temperature with 95-100% humidity and plunge frozen into liquid ethane. Samples were transferred into a liquid nitrogen bath and placed into a Gatan 626 cryo-holder through a cryo-transfer stage. Cryo-TEM was performed using a liquid nitrogen-

cooled JEOL 1230 TEM working at 100 kV accelerating voltage. Images were acquired using a Gatan 831 CCD camera.

2.2.2. Dynamic Light Scattering (DLS). Measurements were performed on a Malvern Zetasizer Nano ZSP light scattering spectrometer. Samples were prepared at 1 wt% as previously described. During the sample measurement, the temperature was kept at 25 °C. The sample was equilibrated for 30 seconds before each measurement was taken. The duration of each measurement was 10 seconds and the measurement angle was 173° backscatter. The attenuator was determined by the instrument automatically, as was the number of accumulations for each run. Each measurement run was repeated 3 times.

2.2.3. Small-Angle X-ray Scattering (SAXS). Experiments were performed at beamline 5-ID-D of the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) Synchrotron Research Center at the Advanced Photon Source, Argonne National Laboratory. PA samples were prepared at 1 w/v% in 1.5 mm quartz capillaries (Charles Supper) and irradiated for 2 seconds. Data was collected with an X-ray energy at 17 keV (l = 0.83 Å), and the SAXS CCD detector (MAR) was positioned 245 cm behind the samples to record the scattering intensity in the interval $0.001 < q < 0.20$ Å^{-1}. The wave vector q is defined as $= (4\pi/\lambda) \sin(\theta/2)$, where θ is the scattering angle. Azimuthal integration (Fit2D) was used to average 2D scattering images to produce 1D profiles of intensity versus *q*. A capillary containing only solvent was tested as well and this graph was subtracted from the corresponding data using IgorPro software. Using the NCNR analysis macro in IgorPro, E_2 PA, 100% BDNF PA, 10% BDNF PA and 10% Linear BDNF PA were fitted to a lamellar head-to-tail form factor model, spherical model, and cylindrical core shell model respectively.

2.2.4 Fourier Transform Infrared (FT-IR). Samples were prepared by co-assembling with HFIP as previously described in section 2.1.1. The sample powder was reconstituted at 1 wt% using D_2O and placed between two CaF_2 windows with a 50 μm separation. Infrared spectra were recorded on a Bruker Tensor 37 FTIR Spectrometer. Spectra were averaged over 25 scans with a resolution of 1 cm^{-1}.

2.2.5 Circular Dichroism (CD). Samples were prepared as previously described in section 2.1.1. Immediately before the measurement was taken, each sample was diluted to 0.03-0.04 wt% in H_2O. CD spectra were recorded on a JASCO model J-815 spectropolarimeter using a quartz cell of 1.0 mm optical

path length. Continuous scanning mode was used with a scanning speed of 100 nm per minute with the sensitivity set to standard mode. High Tension (HT) voltage was recorded for each sample to ensure that the measurement was not saturated. An accumulation of 3 measurements was used and a buffer sample was background-subtracted to obtain final spectra. The final spectra were normalized to final concentration of each sample.

2.2.6. Scanning Electron Microscopy (SEM). PA gels or sample coverslips were fixed in 4% paraformaldehyde (PFA) for 20 minutes. They were rinsed with PBS and dehydrated by incubation in a series of ethanol solutions of increasing concentration. Ethanol was subsequently removed by critical point drying (Tousimis Samdri-795). Dehydrated samples were mounted on stubs using carbon glue and coated with 16 nm of osmium (Filgen, OPC-60A) to create a conductive sample surface. All SEM images were taken using a Hitachi SU8030 or LEO 1525 instrument operating at an accelerating voltage of 2 kV.

2.2.7. Rheological Measurements. PA materials were prepared using methods described above. An Anton Paar MCR302 Rheometer with a 25 mm cone plate was used for all rheological studies. 150 µL of PA liquid was placed on the sample stage and 30 µL of 150 mM $CaCl_2$ solution (final concentration 25 mM) was placed on the sample plunger positioned above the material. The instrument was set to 37 °C. The plunger was lowered to the measuring position and a humidity collar was added to prevent sample evaporation. The sample was equilibrated for 30 minutes with a constant angular frequency of 10 rad/s and 0.1% strain. The angular frequency was then decreased incrementally from 100 rad/s to 1 rad/s over 21 points and the storage and loss modulus were reported. Lastly, the % strain was increased incrementally from 0.1 to 100% over 31 points and the storage and loss modulus were recorded.

2.3. *In vitro* Studies

2.3.1. Animal Protocol All animal housing and procedures were performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. All procedures were approved by the Northwestern University Institutional Animal Care and Use Committee. Timed pregnant (E16) CD1 mice were supplied by Charles River Laboratories (Wilmington, MA).

2.3.2. Dissection of Embryonic Primary Cortical Neurons. Neurons were obtained from embryonic brains as described elsewhere.[2] Briefly, time-pregnant mouse was sacrificed by cervical dislocation and the embryos were extracted at embryonic day 16 (E16). Cerebral cortices were dissected from the mouse embryos and meninges were removed in a solution of Hank's Balanced Salt Solution (HBSS) with 1% pen-strep (Invitrogen) and then digested with trypsin (Invitrogen) and DNAse (Sigma-Aldrich) for 10 min at 37 °C. The tissue was mechanically dissociated, centrifuged at 1000 g for 5 min, and resuspended in CO_2-equilibrated Neurobasal (NB, Invitrogen) neuronal culture medium supplemented with 10% normal horse serum (NHS, Invitrogen), 1% pen-strep (Invitrogen), 0.5 mM L-glutamine (Invitrogen), and 5.8 µL $NaHCO_3$/mL (Sigma-Aldrich). The cell suspension was pre-plated at 37 °C for 30 min. Afterwards, the supernatant was collected, passed through a cell strainer with 100 µm pore size and centrifuged at 1000 g for 5 min. The pellet was resuspended in NB neuronal culture medium (1% NHS, 1% pen-strep, 0.5 mM L-glutamine, 22 µM glutamic acid (Sigma-Aldrich), 2% B27 (Gibco), and 5.8 µL $NaHCO_3$/mL (Sigma-Aldrich)), and plated at different densities (depending on the type of experiment, see below) directly on tissue culture plates coated with poly-D-lysine (Sigma-Aldrich). After 24 h, the medium was replaced with serum-free neuronal culture medium (1% pen-strep, 0.5 mM L-glutamine, 2% B27, 5.8 µL $NaHCO_3$/mL). Under these conditions, we obtained a neuron-enriched culture with a composition of approximately 10% glial cells.

2.3.3. BDNF PA Treatments and Cell Culture Procedures. Treatments were prepared by dissolving PA (including E_2, E_2 + E_4PEG, E_2 co-assembled with Linear BDNF, E_2 co-assembled with BDNF PAs) and BDNF peptide at different concentrations (0.5, 1.0 or 5.0 µM), in media without serum or B27 supplement or starvation media. Unless otherwise noted, BDNF PA, Linear BDNF PA, or E_4PEG PA was co-assembled at 10 mol % with E_2 PA (total concentration of PA was 5.0, 10.0, or 50.0 µM respectively). For the co-assembly ratio study, BDNF PA was co-assembled at 10, 20, 50, 70, 90, and 100 mol %. Human/Murine/Rat BDNF protein (Peprotech) was resuspended at different concentrations (0.25 and 1 nM) in starvation media.

For morphometric analysis and cell viability assays, cells were cultured in 24 or 48 well plates at a density of 25,000 or 40,000 cells/well respectively, for three days before being treated. Cells were then treated for 24 hours, 3 days or 7 days *in vitro*. Samples were fixed in 4% PFA for 15 min (20 min for gels) at room temperature (RT) for immunofluorescence studies.

For Western blot, cells were cultured in 6 well plates at a density of approximately 900,000 cells/well for 14 days *in vitro* (DIV) before being treated. Treatments were added for 2, 4, 6, 8, 12 or 24h *in vitro* before protein was harvested. For TrkB inhibitor studies, cells were cultured for 14 DIV and then treated with a TrkB specific inhibitor, K-252a (Sigma Aldrich), for 1 hour prior to being treated with the different conditions mentioned above. Protein was extracted after 6 h of treatment.

2.4. Biological Assays

2.4.1. Western Blot. Protein was extracted from primary neuronal cultures with Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Scientific) and a BCA assay (Thermo Scientific) was performed to determine protein content of each sample. Cell protein was loaded into and separated using a 4-20% SDS-PAGE gel (Bio-Rad). It was then electrotransferred from the gel to a nitrocellulose membrane (Bio-Rad). The membranes were blocked with 5% milk solution (Bio-Rad) for 30 minutes followed by an overnight incubation with primary antibodies at 4 °C. The following primary antibodies were used: rabbit anti-pTrkB (1:1000, Cell Signaling), rabbit anti-TrkB (1:1000, Cell Signaling), rabbit anti-Actin (1:2000, Sigma Aldrich), mouse anti-Actin (1:2000, Sigma Aldrich), rabbit anti-pPLCγ (1:1000, Cell Signaling), rabbit anti-PLCγ (1:1000, Cell Signaling), rabbit anti-pAKT (1:2000, Cell Signaling), mouse anti-AKT (1:2000, Cell Signaling), rabbit anti-pERK1 + ERK2 (1:1000, Abcam), rabbit anti-ERK ½ (1:1000, Cell Signaling), rabbit anti-PSD95 (1:1000, Abcam), rabbit anti-MAP-2 (1:2000, BioLegend), and mouse anti-TUJ-1 (1:1000, BioLegend). Membranes were then incubated with their corresponding secondary HRP-conjugated antibodies (1:1000; ThermoFisher). Protein signals were detected using Radiance Bioluminescent ECL substrate (Azure Biosystems). Densitometry analysis, standardized to total receptor content or Actin as a control for protein loading, was performed using ImageJ software.[3] For quantification, triplicate samples were analyzed and at least two different experiments were conducted.

2.4.2. Immunofluorescence. For immunofluorescence, fixed samples (4% PFA for 15 min at RT) were incubated with primary antibodies overnight and Alexa 488 or Alexa 555 secondary antibodies (1:2000, Invitrogen) were used for 2 h at room temperature. The following primary antibodies were used: mouse anti-TUJ-1 (neuronal marker 1:4000, BioLegend), rabbit anti-MAP-2 (marker for mature neurons 1:2000, BioLegend), mouse anti-SMI312 (axonal filament marker 1:1000, BioLegend), and DAPI (nuclear stain, 1:2000, Invitrogen). If not already in a glass bottom plate (MatTek), the preparations were mounted with Immu-Mount (Thermo Scientific) for imaging.

2.4.3. Imaging and Morphometric Analysis. Throughout the experiments, a digital camera (Nikon) mounted on a tissue culture microscope was used to take bright field images of cells. Nikon A1R confocal laser-scanning microscope with GaAsP detectors was used to visualize and image fluorescent preparations. Images were processed using an ImageJ (National Institutes of Health) plugin. Confocal images were reconstructed by NIS Elements Advanced Research Microscope Imaging software (version 4.20) or Imaris program (version 8.1, Bitplane Scientific software) for 3D interactive data viewing with normal or shadow projections of cells screened under Nikon A1R confocal laser-scanning microscope with GaAsP detectors. For morphometric analysis, NeuronJ was used to trace and measure neuronal extensions.[4] For quantification of number of primary neurites and neurite length, a minimum of 60 randomly selected cells per condition were analyzed with a minimum of 2 independent experiments. The images were arranged in Adobe Photoshop (v.7.0), with adjustments for contrast, brightness and color balance to obtain optimal visual reproduction of data.

2.4.4. Flow Cytometry and Cell Viability. Cells were carefully washed three times with 1X PBS. Each well was incubated with 200 µL trypsin until cells detached (did not exceed 10 min of incubation). Cells were then resuspended in 600 µL of media to neutralize the trypsin and were centrifuged for 15 min at 1.2 rpm. Supernatant was removed, and the pellet was re-suspended in approximately 40 µL of media before Flow Cytometry (BD LSRFortessa) was performed. DAPI (Sigma, 5 µg/mL) was added 1-3 min before measurements were taken to determine the absolute number of dead cells (excitation wavelength of 405 nm). Cell populations were gated based on cell size (FSC), granularity of the cytoplasm (SSC), multiple

cells (FSC-W), UV fluorescence (DAPI filter). Percentage of positive cells was calculated from alive cells (DAPI negative). All the samples were measured in triplicates. Results were analyzed using FlowJo software.

2.4.5. Cell Culture on Immobilized Peptide and Viability Assay. For immobilized peptide studies, cells were cultured on blank, BDNF peptide, PEG_6-BDNF peptide, or APTES coated glass coverslips for 5 DIV. Media was removed, and cells were rinsed once with HBSS. A calcein-AM/ethidium homodimer-1 live/dead assay (Invitrogen) was used to assess cell viability. Calcien-AM/ethidium homodimer-1 solution in HBSS was added to each well for 20 min at RT. The solution was removed, and samples were rinsed 1 time with HBSS before coverslips were mounted for imaging.

2.4.6. Characterization of Peptide Immobilization on Silica Microspheres and Cell Culture. To visualize the attachment of immobilized peptide to the surface of silica microspheres, an activated fluorescein isothiocyanate (FITC) dye (Thermo Scientific) was used to detect the presence of free amines. Blank, APTES coated (with capped amines), BDNF peptide and PEG_6-BDNF peptide functionalized microspheres. 20 uL of microspheres at 1 wt% were added to 180 uL of 50 mM borate buffer (pH 8.4). 4 uL of FITC dye (10 mg/mL in DMF) was added to this solution and incubated at room temperature, in the dark, on a shaker for 1 h. After incubation, the beads were washed with borate buffer 10 times using centrifugation to remove unattached FITC from the supernatant. Afterwards, beads were resuspended in borate buffer and mounted on glass coverslips. Samples were analyzed using confocal microscopy (refer to SI 2.4.3). Laser intensity was kept constant for all images. For immobilized peptide studies on silica microspheres, cells were treated with blank, APTES, BDNF peptide, and PEG_6-BDNF peptide at 0.01 and 0.05 wt% for 6 h *in vitro*. BDNF protein, PEG6-BDNF peptide in solution, and starvation media were used as controls. After 6 h, protein was harvested, and Western blot analysis was performed following section 2.4.1.

2.4.7. Electrophysiology - Multi Electrode Array (MEA) Plates. For 2D electrophysiology studies, 12 well MEA plates with 64 electrodes per well were coated with PEI and laminin according to Axion Biosystems protocols.[5] Embryonic primary neurons were seeded at a density of 60,000 cells/well and

cultured during 14 DIV. On day 14, cells were treated with BDNF protein, BDNF PA, BDNF Peptide, or starvation media. Every 5 days, half of the media was removed from each well and replaced with fresh media containing additional treatment. Spontaneous network and synchronized activity was recorded using Axion Biosystems Maestro 768 channel amplifier and Axion Integrated Studios (AxIS) v2.4 software. The amplifier recorded from all channels simultaneously using a gain of 1200× and a sampling rate of 12.5 kHz/channel. After passing the signal through a Butterworth band-pass filter (300–5000 Hz) on-line spike detection (threshold = 6× the root-mean-square of noise on each channel) was done with the AxIS adaptive spike detector. All recordings were conducted at 37 °C with appropriate 5% CO_2/95% O_2. Spontaneous network activity was recorded for 5 min each day starting at 10 DIV. Active electrodes were defined as having >5 spikes/min and only wells with over 10 active electrodes during the baseline-recording period were used in the analysis. Synchronized activity was defined as spike and burst activity that occurred on 25% of the electrodes or more in a well within 100 ms of each other. The mean firing rate (Hz), network burst duration (sec), and number of spikes per network burst were used as a measure of neuronal activity as this demonstrates maturity of neuronal functional properties. All data reflects well-wide averages, where the reported value of *n* represents the number of wells per condition.

2.4.8. Infiltration Study. Gels were prepared as described in 2.1.2. Gels were then rinsed with media one time before cells were seeded on top at a density of approximately 42,000 cells/well. Cells were cultured for one week *in vitro*. Half of the gels were analyzed by patch clamp analysis (see section 3.4.9. Other gels were fixed with 4% PFA to perform infiltration studies. Cell were stained for MAP-2, and TUJ-1 using immunocytochemistry methods described previously. Confocal microscopy was used to make z-stack reconstructions of each gel. The MultiMeasure function in the ROI manager of ImageJ was used to measure the depth of pixels for each channel which was then plotted as intensity vs depth in the gel. The average intensity of MAP-2 was measured by averaging the mean intensities obtained from maximum intensity projections of z-stacks images of gels.

2.4.9. Patch Clamp. Whole-cell current-clamp recordings were made from visually identified primary cultured neurons using inverted Olympus IX51 microscope equipped with a 40X objective. Recording

pipettes were made of glass capillaries using a horizontal Sutter P-1000 puller yielding a 3-5 MΩ resistance pipette when filled with standard intracellular solution containing (in mM): 120 K-$MeSO_4$, 10 KCl, 10 HEPES, 10 Na_2-phosphocreatine, 4 Mg-ATP, 0.4 Na_3-GTP, pH 7.3; 285-290 mOsm. Gels with neurons were continuously perfused with standard oxygenated aCSF bath solution (in mM): 125 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgSO_4$, 25 glucose, 2 $CaCl_2$, pH 7.4 at 32-34 °C. Whole-cell current-clamp data was acquired using an Multiclamp 200B amplifier (Molecular Devices, USA) and digitized at 10 kHz (filtered at 3kHz) with the neurons held at -65 mV. Resting membrane potential was measured immediately after breaking into the cell. Input resistance was calculated as the slope of the voltage-current curve using 500 ms current steps from -50 pA to 30 pA at 10 pA steps. There were no differences in resting membrane potentials or input resistance between the three groups. AP amplitudes, thresholds, half-widths and fAHP measures were taken from ramp current injection steps. AP threshold was calculated where the first derivative of the up phase of the trace equals 5mV/ms. Neurons meeting our quality criteria were used: series resistance < 30 MΩ, membrane resistance > 100 MΩ, resting potential < -40 mV, and AP amplitude > 65mV from holding. Data was analyzed using MATLAB protocols designed specifically for these experiments.

2.5. Statistical Analysis. All error bars indicate the standard error of the mean. Statistical analysis was performed using Graphpad Prism v.6 software. Analysis of variance (ANOVA) with a Bonferroni post hoc test was used for all multiple group experiments. P-values <0.05 were deemed significant.

FIGURES

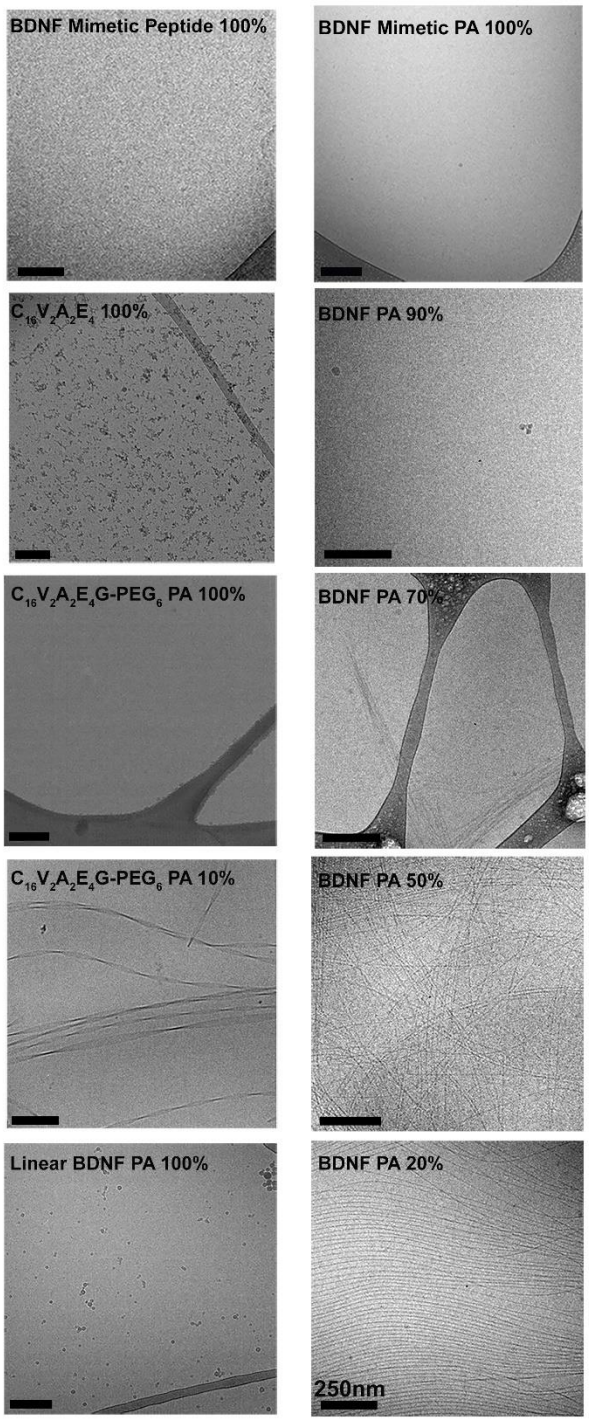


Figure S1. Cryo-TEM of synthesized materials and different co-assembly ratios. BDNF Peptide, E_4, E_4PEG, E_4PEG PA co-assembled with E_2 at 10 mol%, and Linear BDNF PA at 100%. Co-assembled ratios of BDNF PA with E_2 PA 100% to 20%.

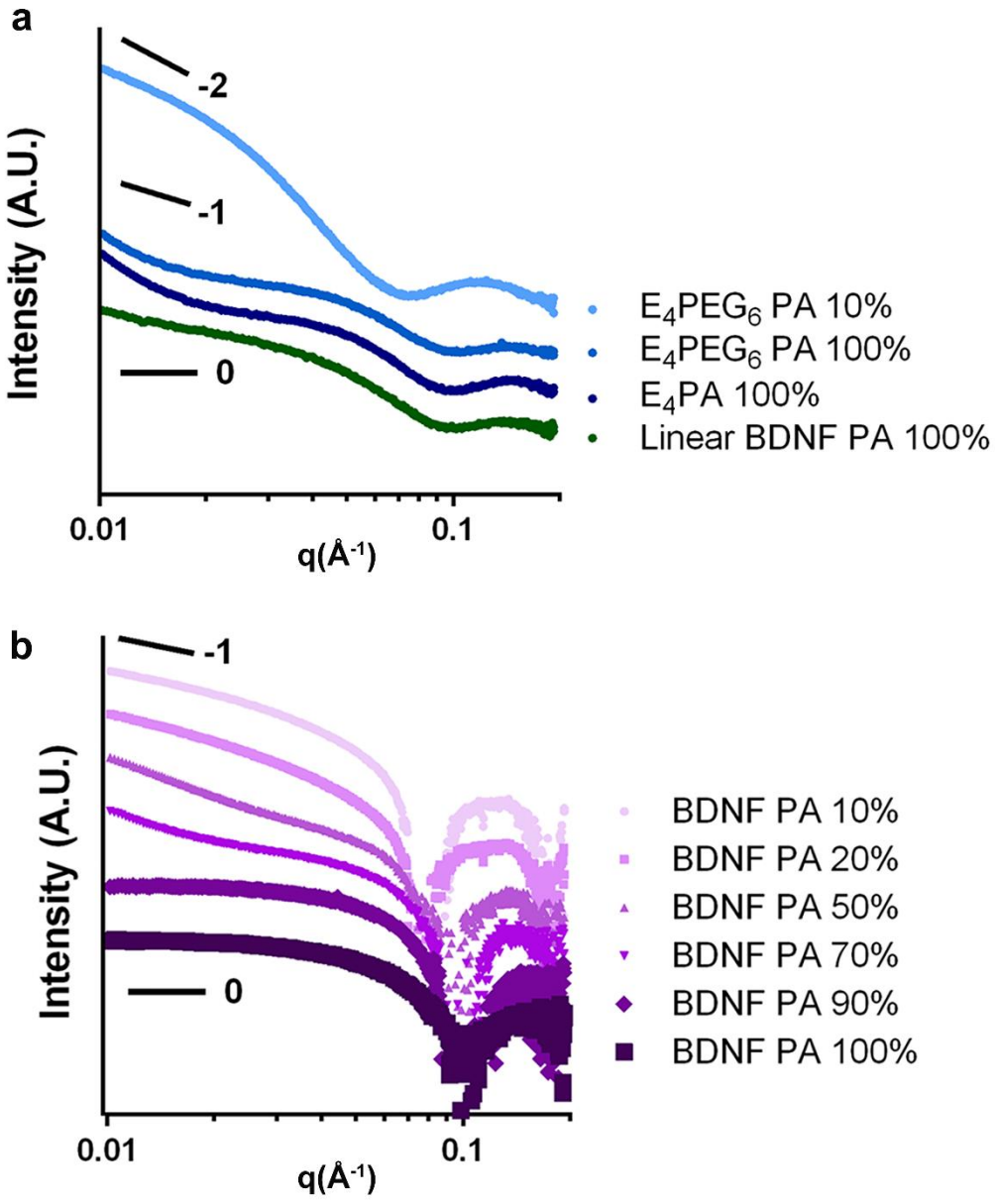


Figure S2. Small Angle X-Ray Scattering data of various PA conditions. (a) BDNF Peptide, PA backbones; E_4, E_4PEG, E_4PEG PA co-assembled with E_2 at 10 mol%, and Linear BDNF PA at 100%. (b) Co-assembly ratios of BDNF PA at 10-100 mol% with E_2 PA.

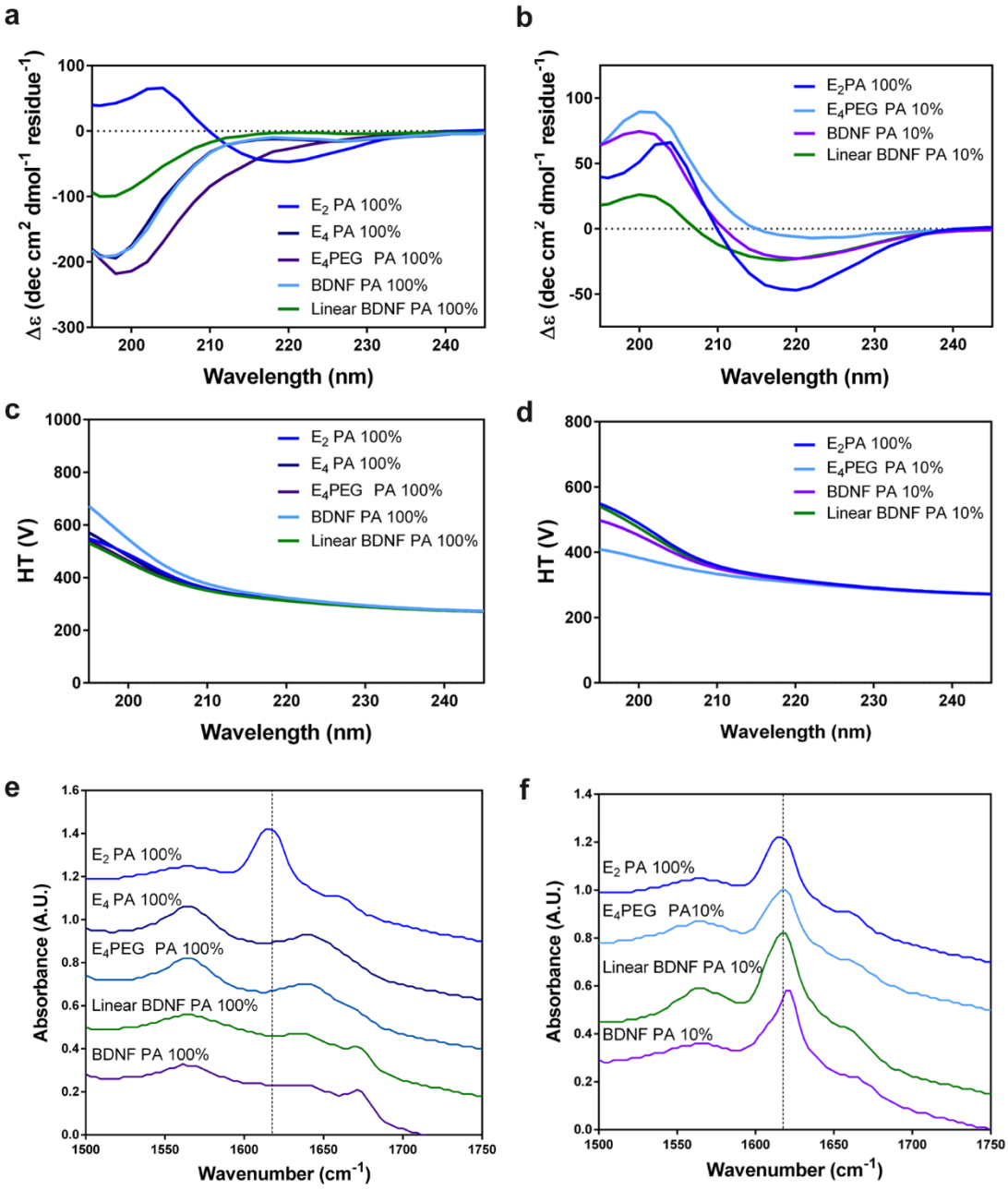


Figure S3. Circular Dichroism (CD), High Tension (HT), and Fourier-Transform Infrared (FTIR) spectra of all PA conditions. (a, c) CD and HT curves of E_2 PA, E_4 PA, E_4PEG PA, Linear BDNF PA, and BDNF PA at 100 mol% and (b, d) CD and HT curves of E_4PEG, Linear BDNF and BDNF PAs co-assembled with E_2 PA at 10 mol%. (e, f) FTIR spectra for conditions referred in (a, b). Dashed line denotes position of β-sheet peak.

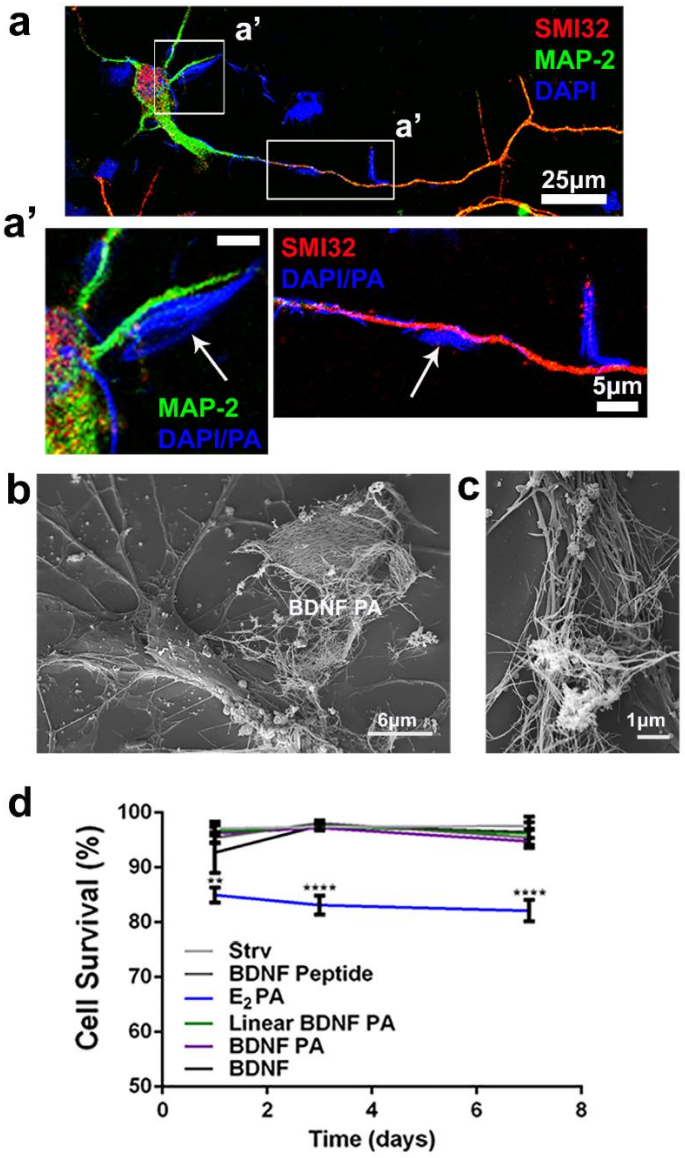


Figure S4. PA fiber-cell interaction and cell viability assay of primary cortical neurons. (a) Confocal micrograph showing a cortical neuron treated with PA nanofibers (in blue). Staining for SMI312 (axonal marker, red), MAP-2 (dendritic marker, green), and DAPI (nuclei, blue). (a') Inset of (a) showing PA fibers interacting with the dendrites (left) and axon (right) of a neuron after being treated for 72 h *in vitro*. (b) SEM micrograph of BDNF PA interacting with primary cortical neurons at 72 h *in vitro*. (c) A zoomed in image of (b) showing BDNF PA fiber mesh. (d) Percentage of neuronal cell survival treated with various conditions for 24 h, 3 and 7 days *in vitro*. *P <0.05, **P<0.01, ***P<0.001, and ****P<0.0001, LSD test (d) (n=3).

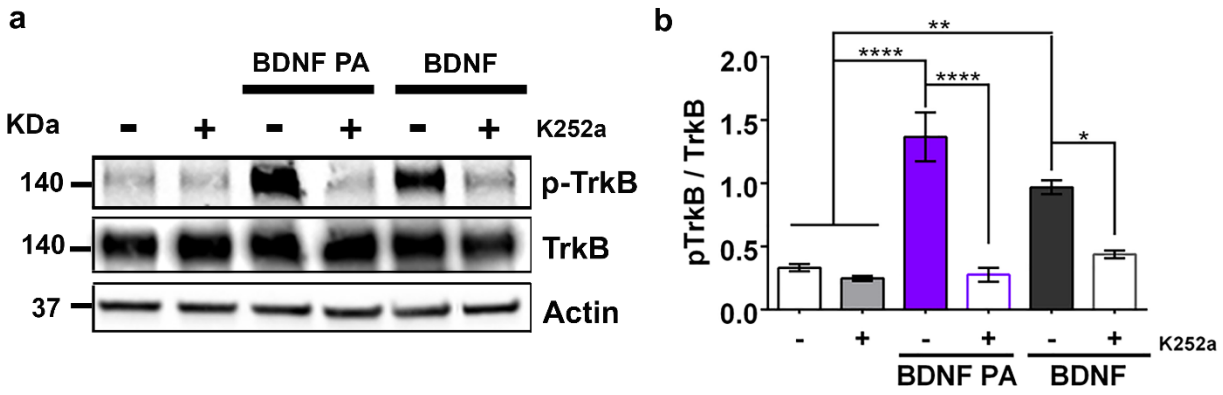


Figure S5. K252a inhibitor study. (a) Western blot of phosphorylated TrkB (p-TrkB) and total TrkB receptor in neural cells treated with (-) starvation media and (+) K252a, in the presence of BDNF PA or BDNF native protein at 6 h. Cells were treated with K252a 2 h prior to treatment. (b) Densitometry analysis of p-TrkB of the conditions shown in (a). (Intensity values normalized to TrkB). *P <0.05, **P<0.01, ***P<0.001, and ****P<0.0001, LSD test (n=3).

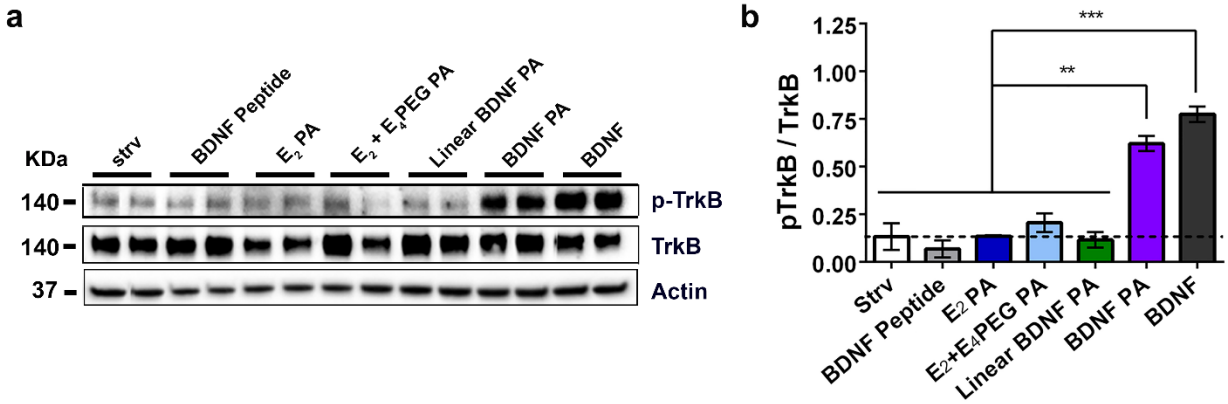


Figure S6. TrkB receptor activation study at 4 hours. (a) Western blot of p-TrkB and total TrkB receptor in neural cells treated with BDNF peptide, E_4PEG PA co-assembled at 10 mol% with E_2 PA, Linear BDNF PA, BDNF PA, and BDNF native protein during 4 h *in vitro*. (b) Densitometry analysis of p-TrkB of the conditions shown in (a). (Intensity values normalized to TrkB). *P <0.05, **P<0.01, and ***P<0.001, LSD test (n=3).

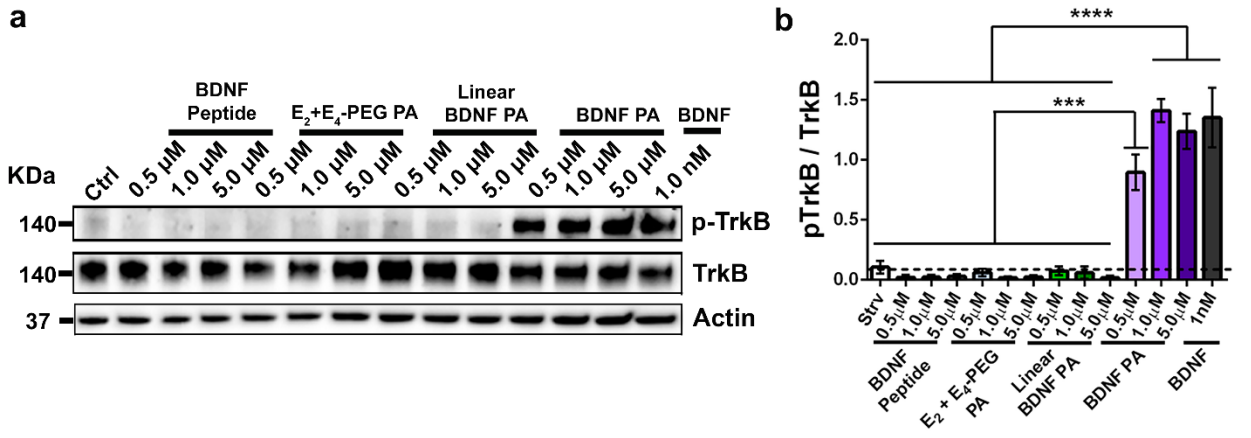


Figure S7. TrkB receptor activation concentration dependence study. (a) Western blot of p-TrkB and total TrkB receptor in neural cells treated with 0.5, 1.0 and 5.0 μM of BDNF peptide, E_4PEG PA co-assembled at 10 mol% with E_2 PA, Linear BDNF PA, BDNF PA, and BDNF native protein during 6 h *in vitro*. (b) Densitometry analysis of p-TrkB of the conditions shown in (a). (Intensity values normalized to TrkB). *P <0.05, **P<0.01, ***P<0.001, and ****P<0.0001, LSD test (n=3).

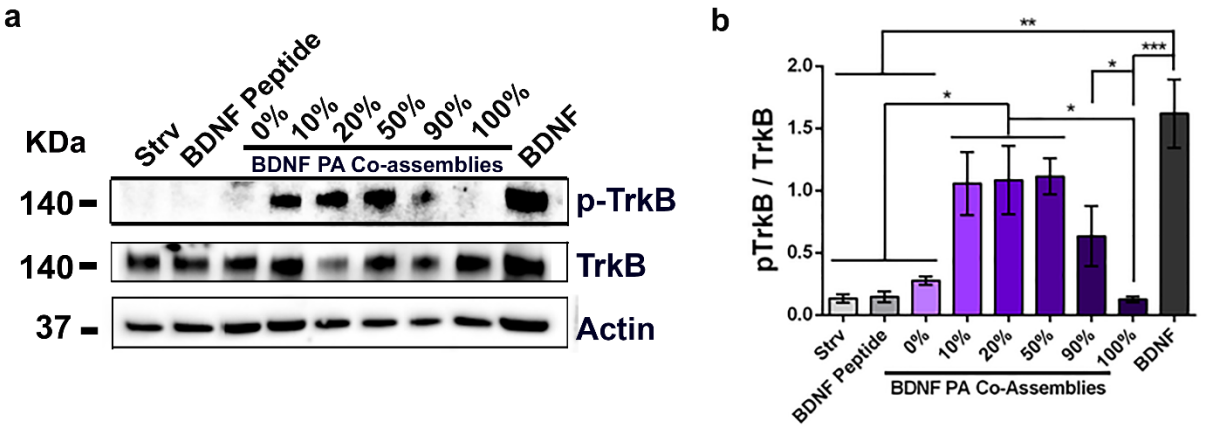


Figure S8. TrkB receptor activation study with different co-assembly ratios of BDNF PA to E_2 PA. (a) Western blot of phosphorylated TrkB (p-TrkB) and total TrkB receptor in neural cells treated with starvation media, BDNF Peptide, and BDNF PA co-assembled with E_2 PA at 0, 10, 20, 50, 90, 100 mol%. (b) Densitometry analysis of p-TrkB of the conditions shown in (a). (Intensity values normalized to TrkB). *P <0.05, **P<0.01 and ***P<0.001, LSD test (n=3).

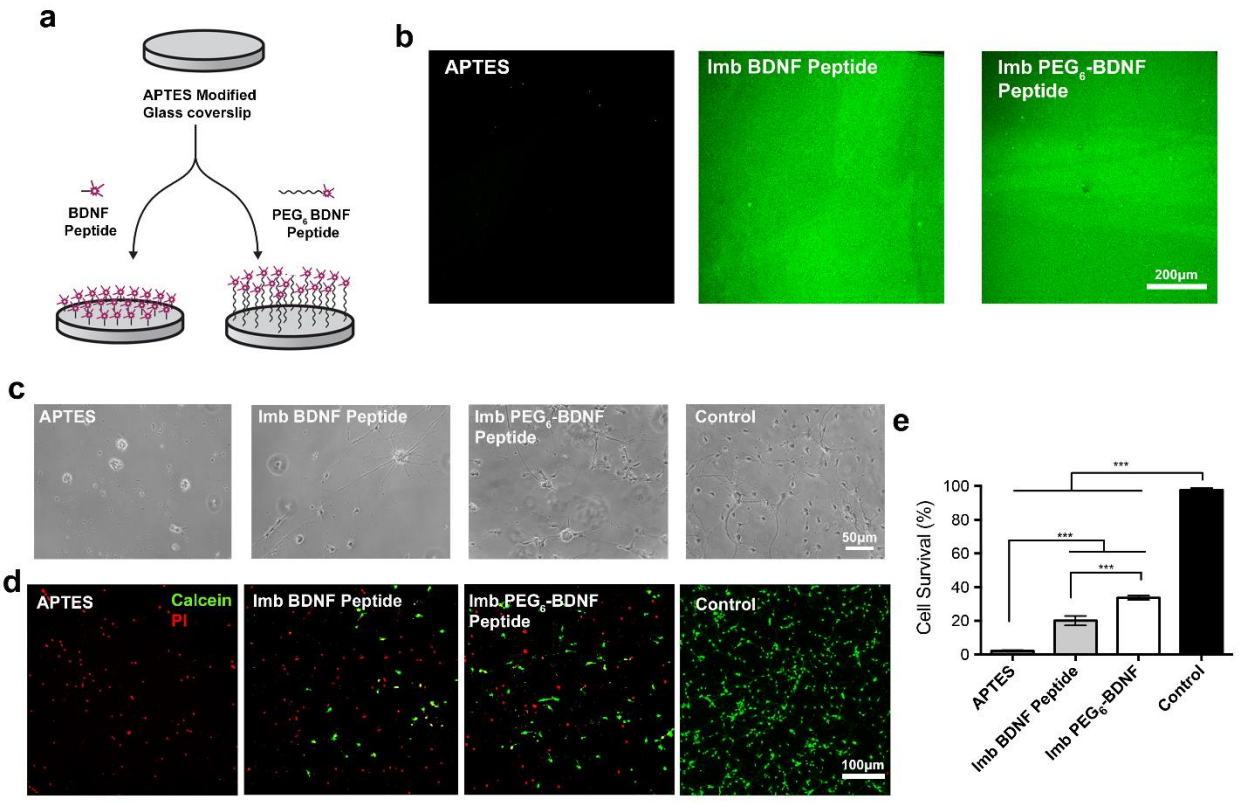


Figure S9. Viability assay of cells cultured on surfaces coated with the immobilized BDNF peptide. (a) Schematic showing BDNF Peptide and PEG_6-BDNF Peptide functionalized glass coverslips. (b) Confocal micrographs of glass coverslips coated with APTES, immobilized BDNF and PEG_6-BDNF peptides. FITC conjugated BDNF Peptide and PEG_6-BDNF Peptide are shown in green. (c) Bright field images of cells cultured on surfaces coated with APTES, the immobilized BDNF peptide, the immobilized PEG_6-BDNF peptide, and control poly-D-lysine (PDL) for 5 days *in vitro*. (d) Confocal micrographs of neuronal cells stained with calcein (live marker, green) and propidium iodide (dead marker, red) cultured on the coatings referred in (c). (e) Quantification of cell survival under conditions shown in (d). (values normalized to total number of cells).

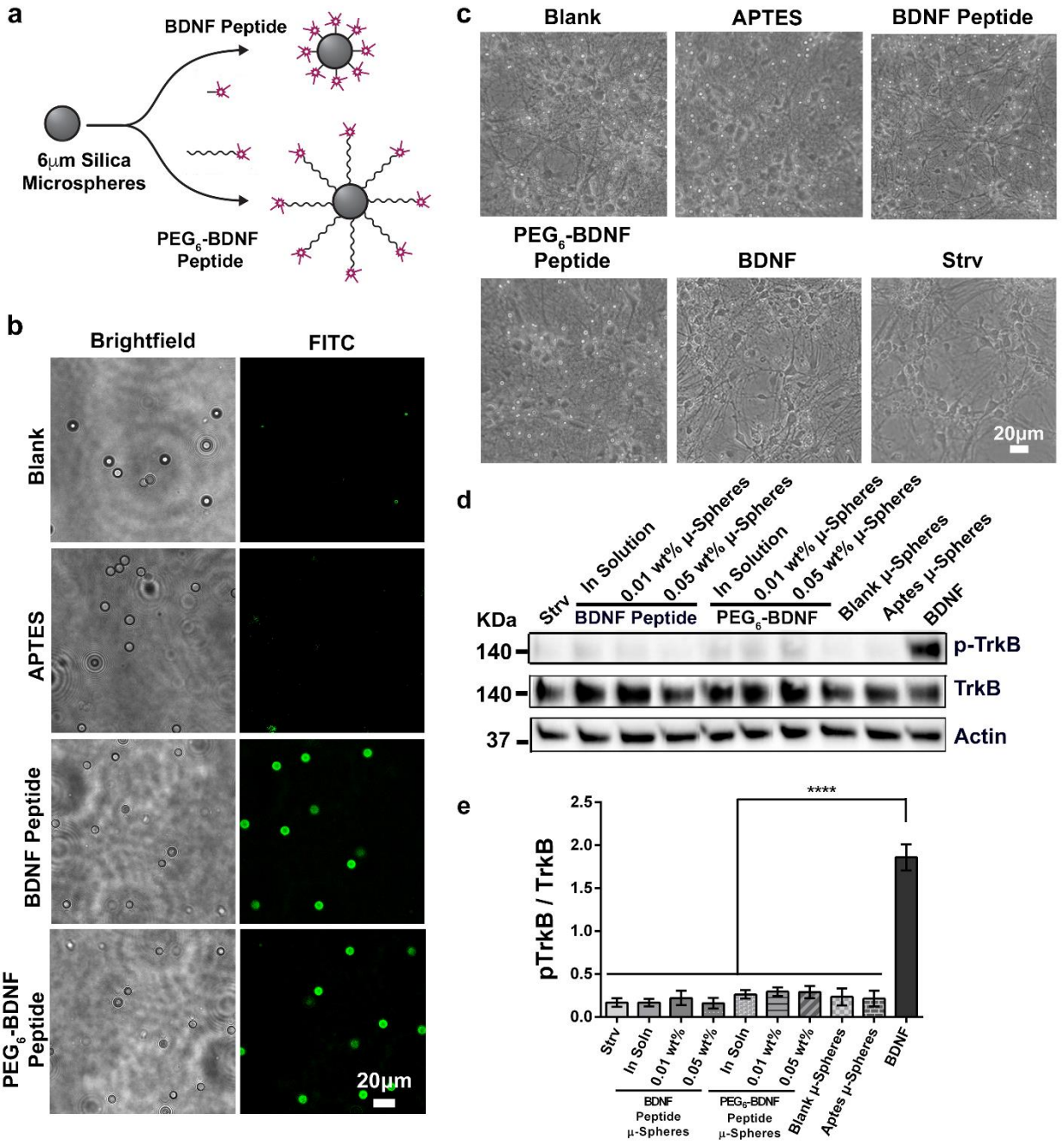


Figure S10. BDNF peptide immobilized on silica microsphere surfaces to probe TrkB receptor activation. (a) Schematic of BDNF mimetic and PEG_6-BDNF peptides attached to silica microspheres. (b) Confocal micrographs of silica microspheres coated with APTES, immobilized BDNF and PEG_6-BDNF peptides. Blank microspheres were used as control. (Left: Brightfield images of microspheres, Right: Fluorescent images of FITC conjugated peptide). (c) Bright field images of primary cortical neurons treated

with silica microparticles functionalized with BDNF peptide, PEG_6-BDNF peptide, and APTES coated microparticles for 6 h. Blank particles, BDNF native protein, PEG_6-BDNF peptide in solution and starvation media were used as controls. (d) Western blot of p-TrkB and total TrkB receptor in neuronal cells treated using the conditions shown in (c). (e) Densitometry analysis of p-TrkB of the conditions shown in (d). (Intensity values normalized to TrkB). *P <0.05, **P<0.01, ***P<0.001, and ****P<0.0001 LSD test (n=3).

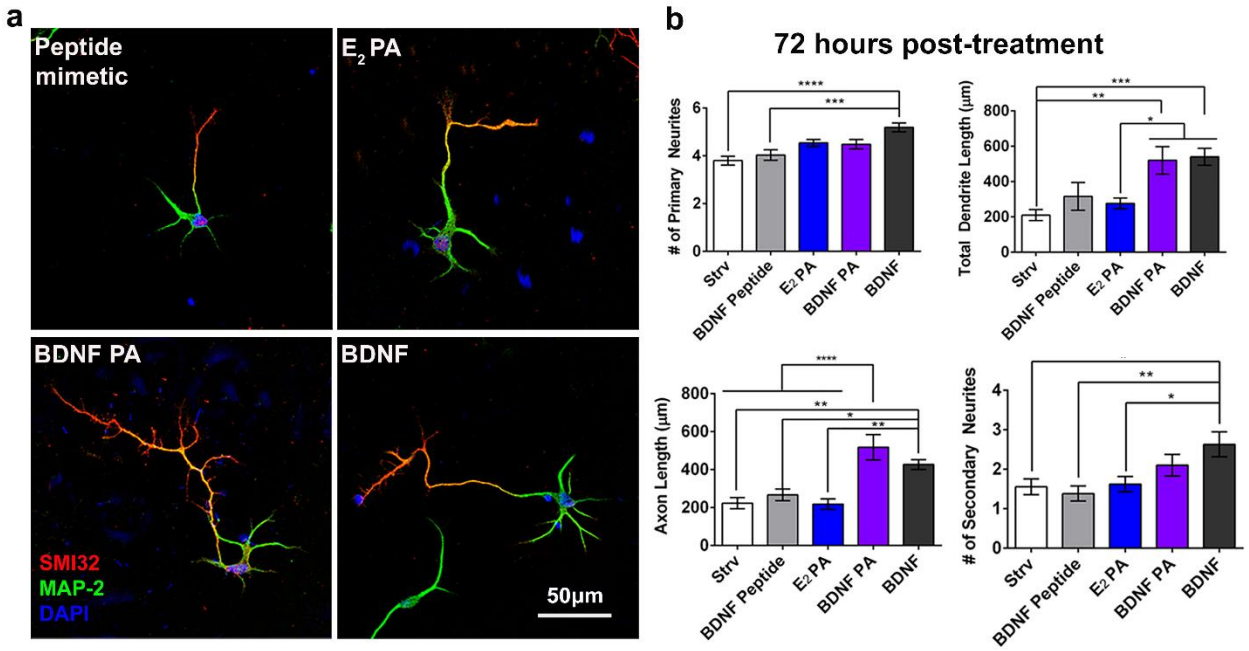


Figure S11. Morphometric analysis of primary cortical neurons after 72h treatment with BDNF PA. (a) Confocal images of neuronal cells treated BDNF Peptide, E_2 PA, BDNF PA and BDNF for 72 h *in vitro*. Cells were stained with SMI312 (axonal marker, red), MAP-2 (dendritic marker, green) and DAPI (nuclei). (b) Morphometric analysis of number of primary neurites, total dendrite length, average axon length, and number of secondary neurites for neuronal cells treated with starvation media (strv), BDNF peptide, E_2 PA, BDNF PA and BDNF for 72 h. *P <0.05, **P<0.01, ***P<0.001, and ****P<0.0001, LSD test (b) (n=60).

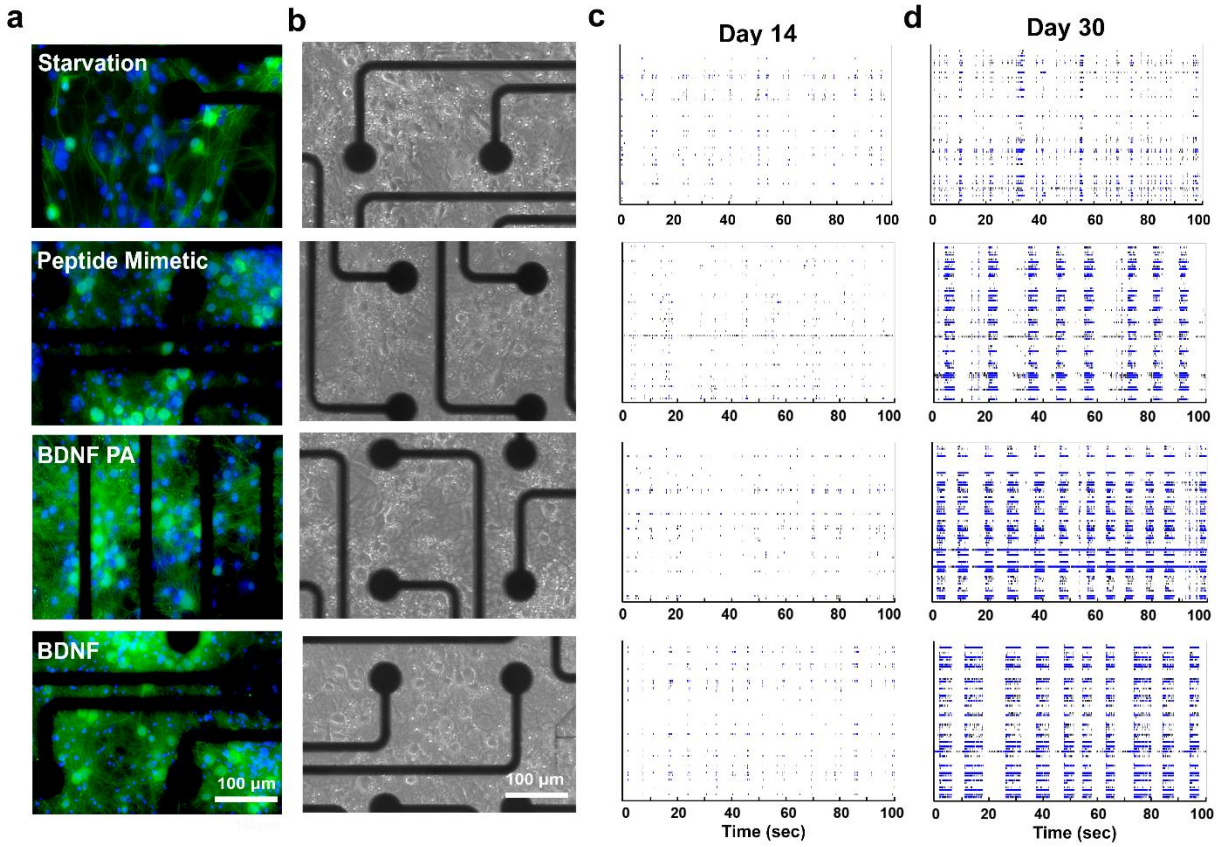


Figure S12. Electrophysiology studies of neuronal cultures treated with different conditions. (a) Fluorescent micrographs of neurons cultured on an MEA plate treated with starvation media, BDNF Peptide, BDNF PA, or BDNF protein for 30 days *in vitro*. Cells stained with MAP-2 (maturation marker, green) and DAPI (nuclei, blue). (b) Bright field images of conditions referred to in (a). (c, d) Raster plots showing electrical activity of culture at 14 and 30 days *in vitro* for wells treated with conditions in (a).

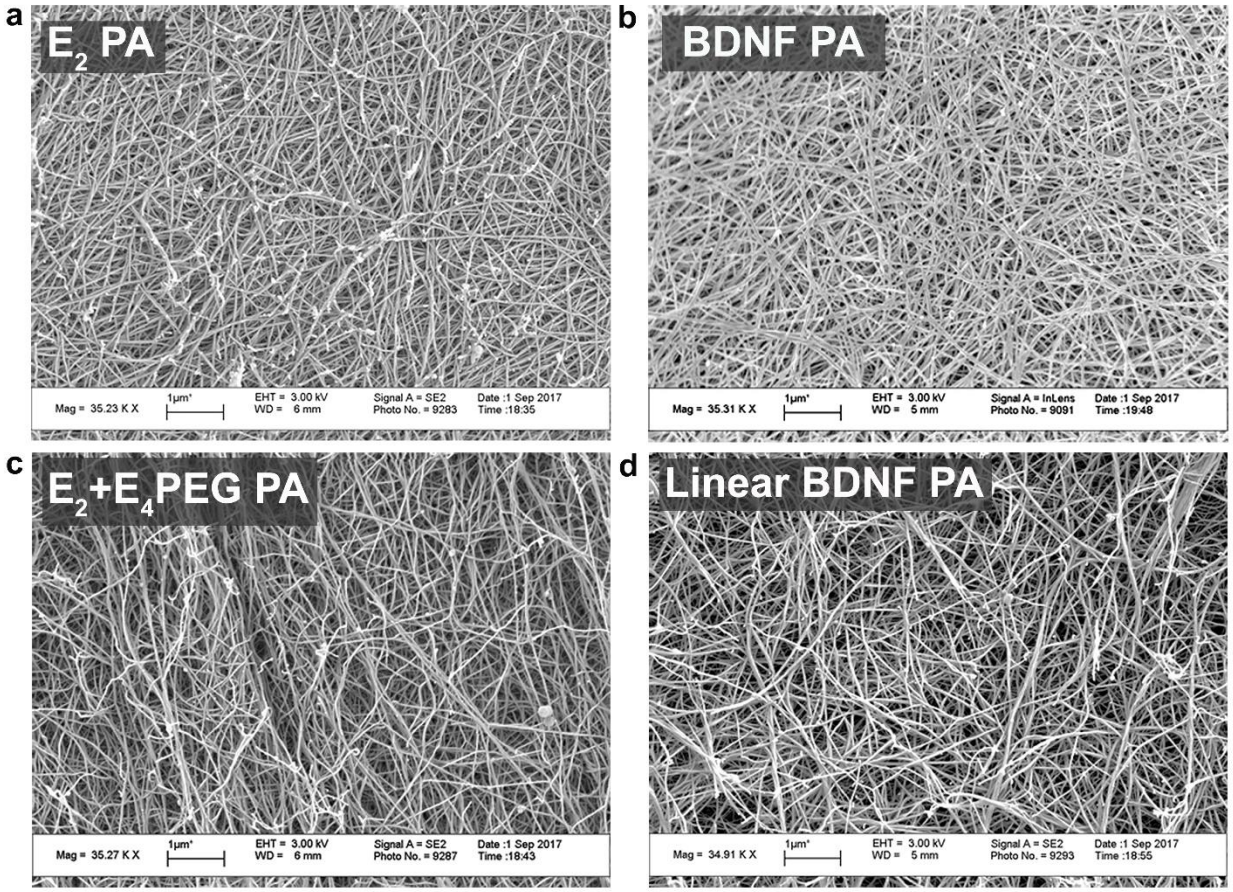


Figure S13. SEM micrographs of PA gels. SEM images of E_2 PA 100 mol%, E_4PEG PA, Linear BDNF PA, and the BDNF PA co-assembled with the E_2 PA at 10 mol% (Gelation conditions described in supplementary section 2.1.2).

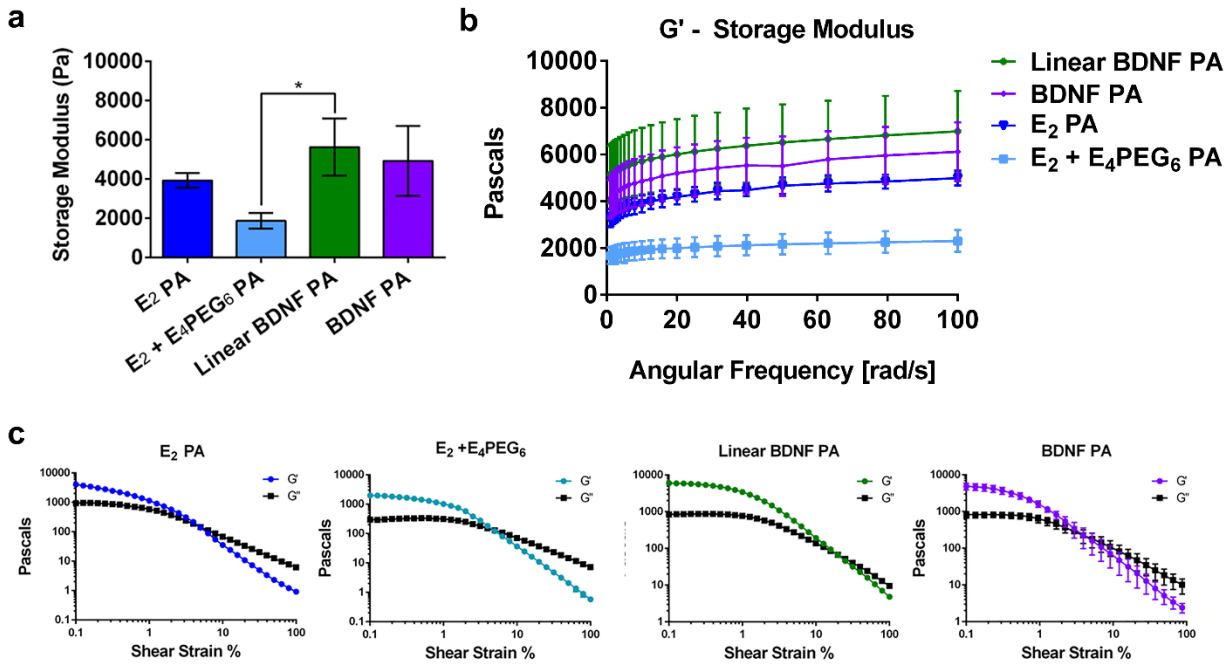


Figure S14. Rheological studies of PA gels. (a) Storage modulus of E_2 PA 100 mol%, E_4PEG PA, Linear BDNF PA, and the BDNF PA co-assembled with the E_2 PA at 10 mol%. (b) Frequency sweep showing the storage modulus, G', for angular frequencies ranging from 0-100 rad/s, of the conditions referred in (a). (c) Strain sweep showing the storage modulus, G', and loss modulus, G'', at shear strain ranging from 0-100 of conditions referred in (a). *P <0.05, LSD test (n=3).

S15. PA Gel formation process. (a) PA gel solution is placed on glass coverslip within silicon isolator. (b) Transwell membrane is placed on top of PA solution and gelling solution is added through membrane. (c) PA gel molded to silicon isolator.

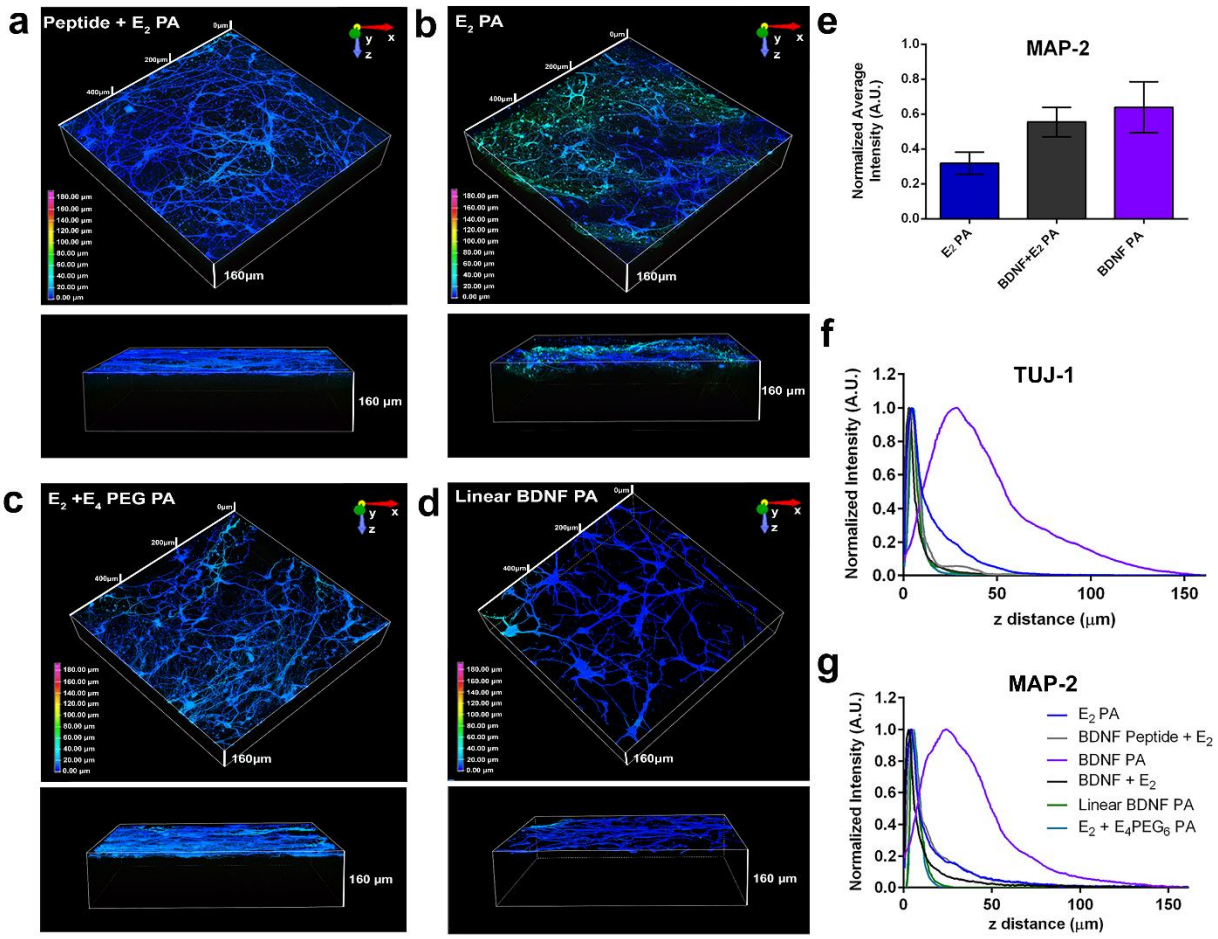
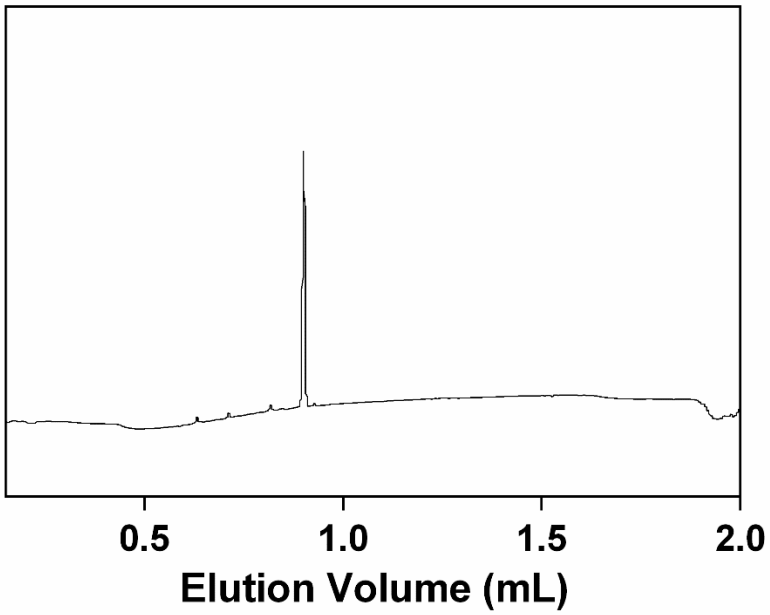
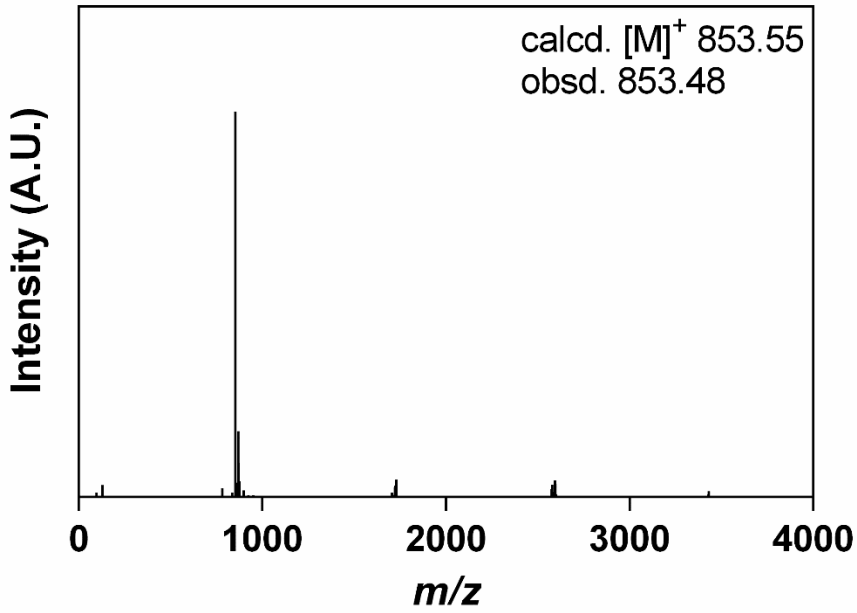


Figure S16. Cell infiltration of primary cortical neurons on PA gels. (a-d) Depth-coded z-stack reconstructions showing cell infiltration on E_2 PA, E_2 PA gel + BDNF peptide, E_4PEG co-assembled at 10 mol% with E_2 PA, and Linear BDNF PA after 1 week *in vitro*. (e-f) Pixel depth analysis of TUJ-1 (e) and MAP-2 (f) in the conditions referred in (a). (g) Normalized average intensity of MAP-2 in E_2 PAs, BDNF + E_2 PA and BDNF PA.

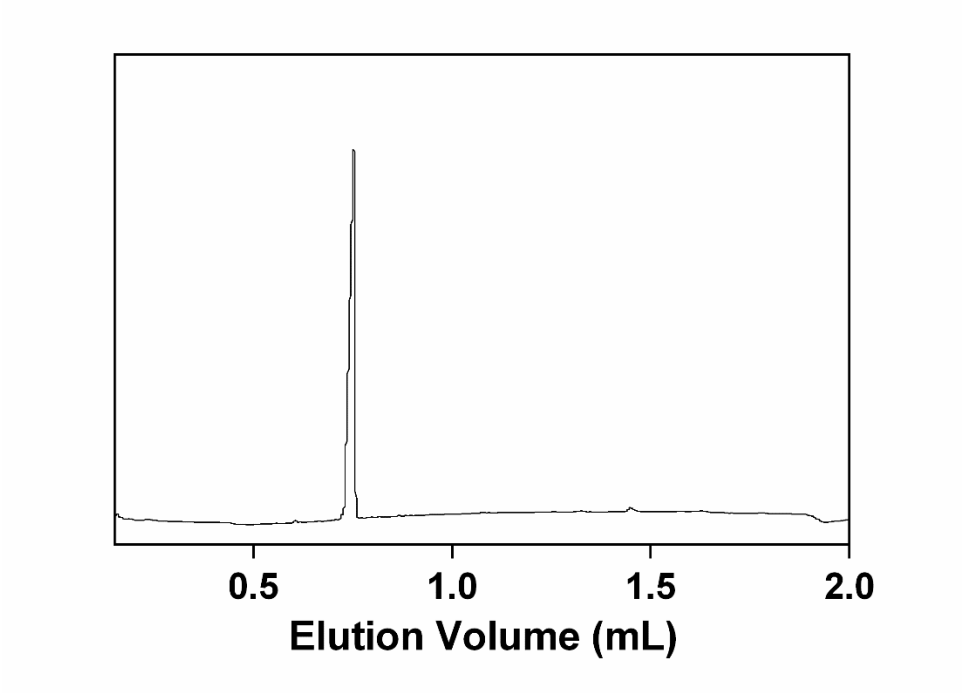
Spectroscopic Data



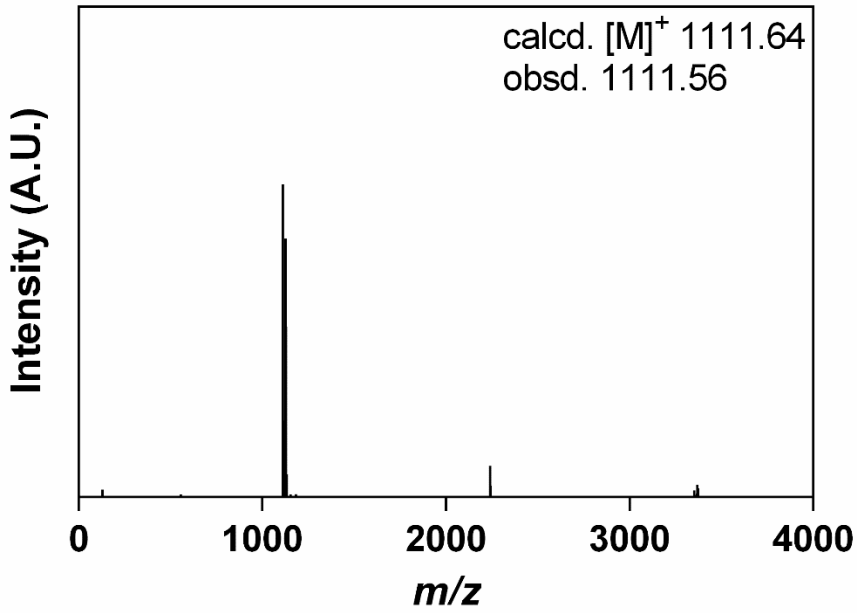
LCMS trace of **E_2 PA**. [**E_2 PA**] = 1 mg/mL, loading solvent; H_2O with 0.1% NH_4OH (v/v), eluent; H_2O–CH_3CN gradient containing 0.1% HCOOH (v/v), column; Phenomenex Gemini 5 µm C18 110 Å LC column 150 x 1 mm.



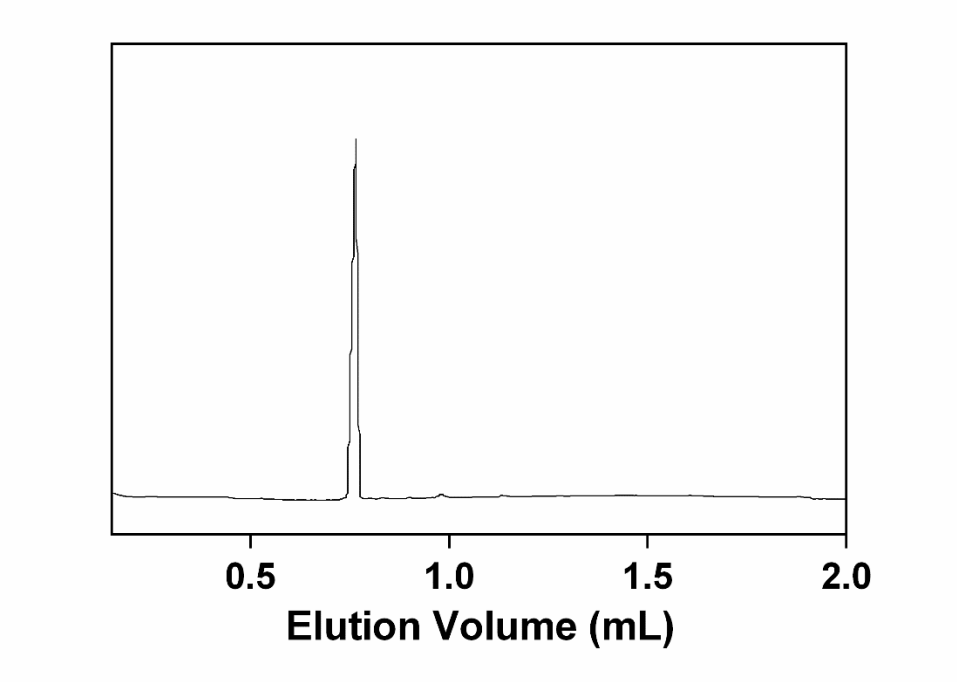
ESI-mass spectra of **E_2 PA**. Elution volume 0.895-0.905 mL.



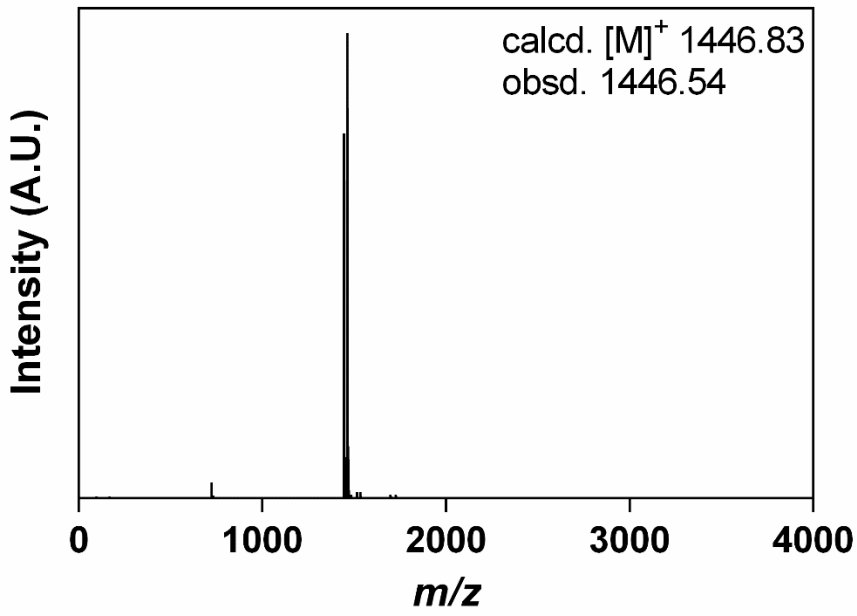
LCMS trace of **E_4 PA**. [E_4 PA] = 1 mg/mL, loading solvent; H_2O with 0.1% NH_4OH (v/v), eluent; H_2O–CH_3CN gradient containing 0.1% HCOOH (v/v), column; Phenomenex Gemini 5µm C18 110 Å LC column 150 x 1 mm.



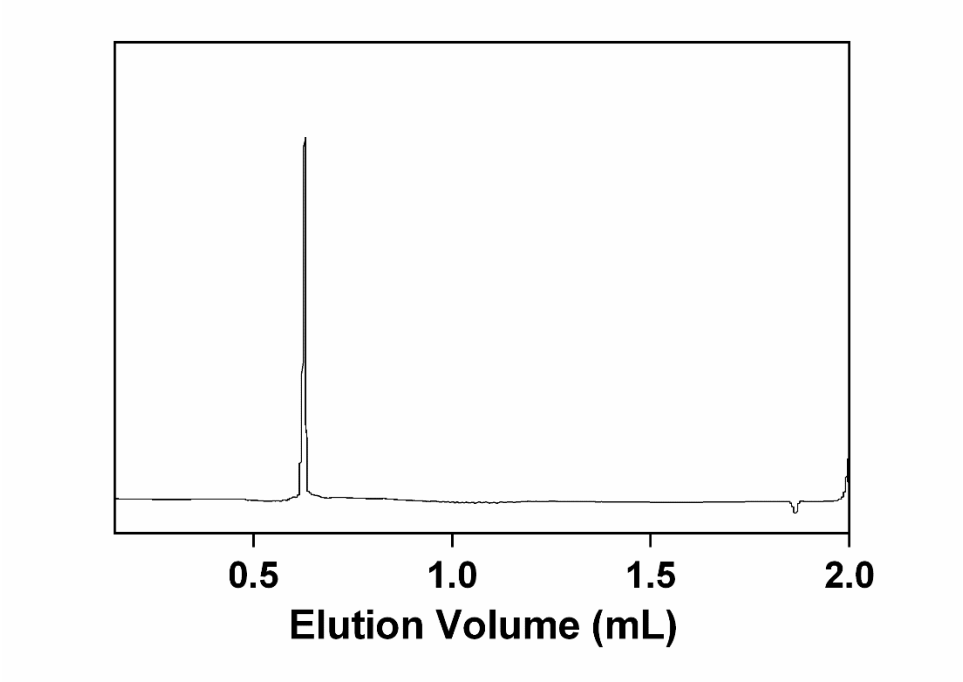
ESI-mass spectra of **E_4 PA**. Elution volume 0.725-0.760 mL.



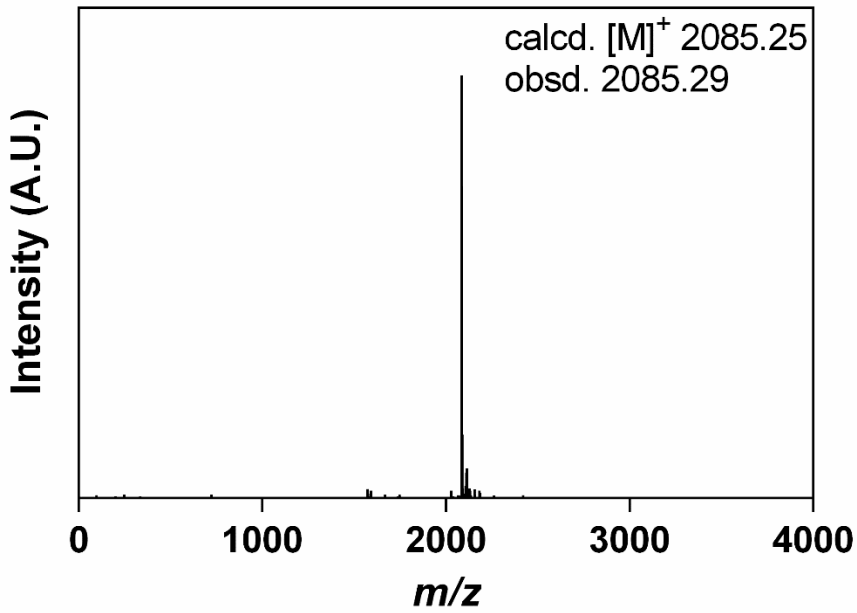
LCMS trace of **E_4PEG PA**. [**E_4PEG PA**] = 1 mg/mL, loading solvent; H_2O with 0.1% NH_4OH (v/v), eluent; H_2O–CH_3CN gradient containing 0.1% HCOOH (v/v), column; Phenomenex Gemini 5 µm C18 110 Å LC column 150 x 1 mm.



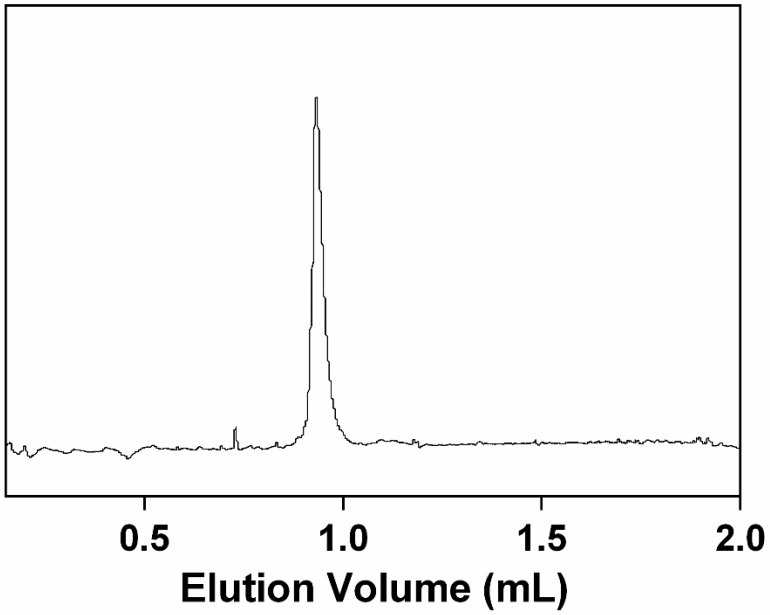
ESI-mass spectra of **E_4PEG PA**. Elution volume 0.745-0.775 mL.



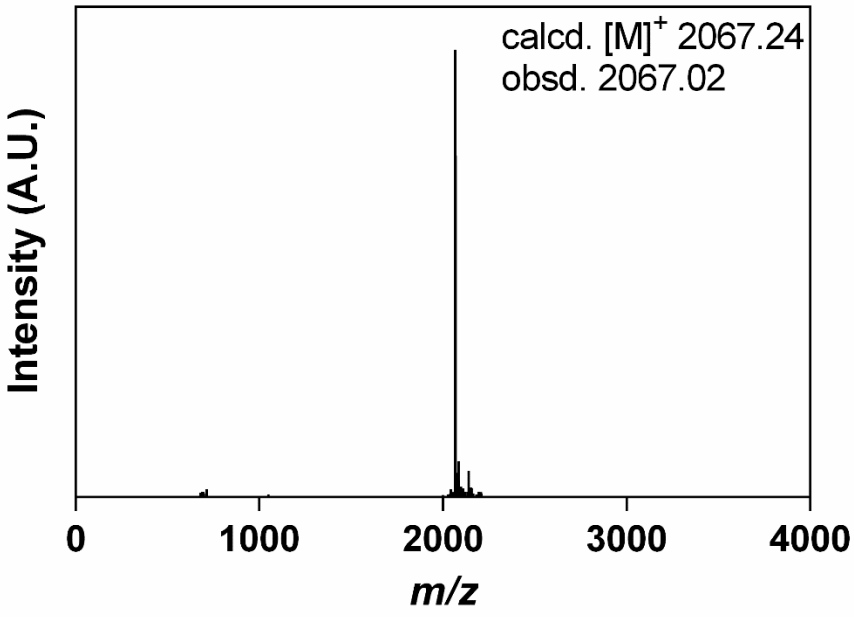
LCMS trace of **Linear BDNF PA**. [**Linear BDNF PA**] = 1 mg/mL, loading solvent; H_2O, eluent; H_2O–CH_3CN gradient containing 0.1% HCOOH (v/v), column; Jupiter 4 µm Proteo 90Å LC column 150 x 1 mm.



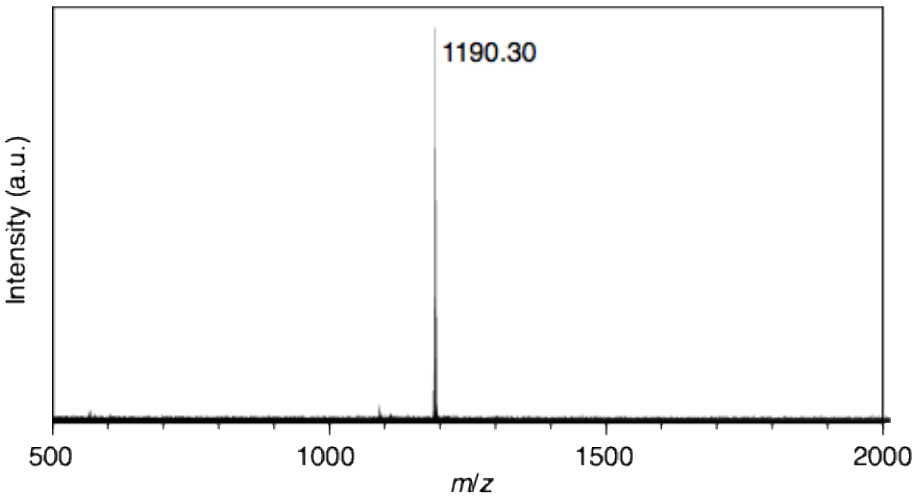
ESI-mass spectra of the **Linear BDNF PA**. Elution volume 0.585-0.675 mL.



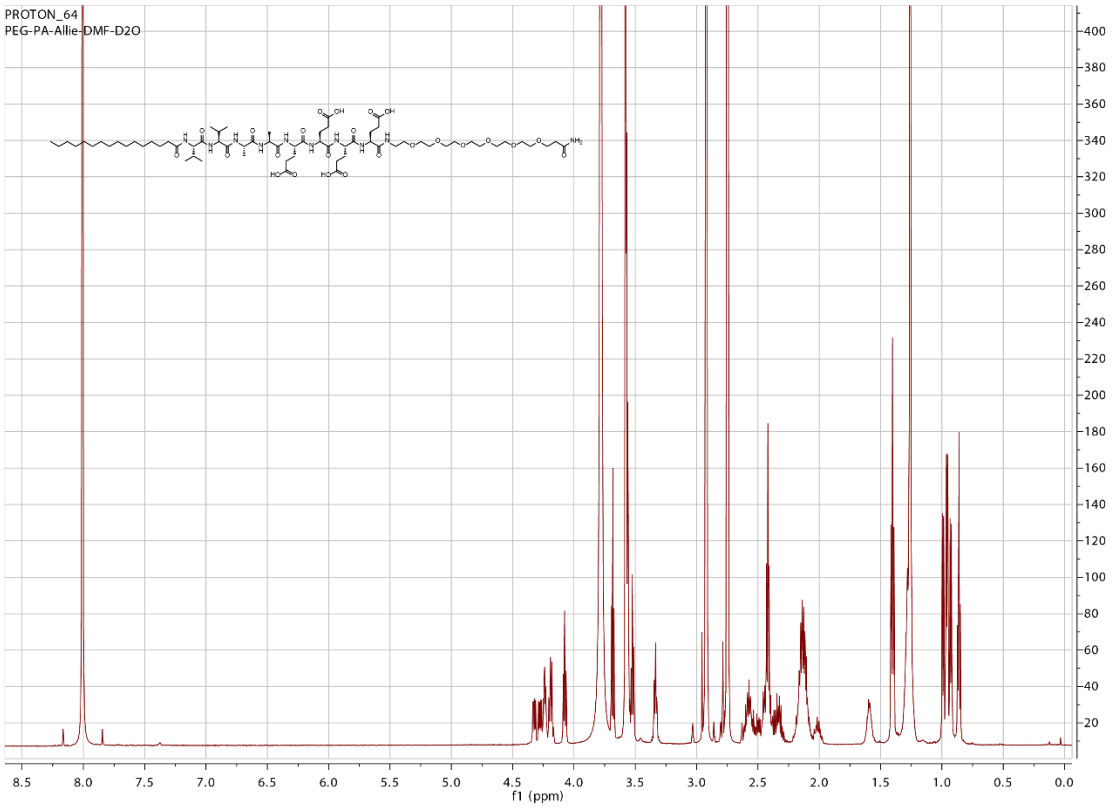
LCMS trace of **BDNF PA**. [**BDNF PA**] = 1 mg/mL, loading solvent; H_2O, eluent; H_2O–CH_3CN gradient containing 0.1% HCOOH (v/v), column; Jupiter 4 µm Proteo 90 Å LC column 150 x 1 mm.



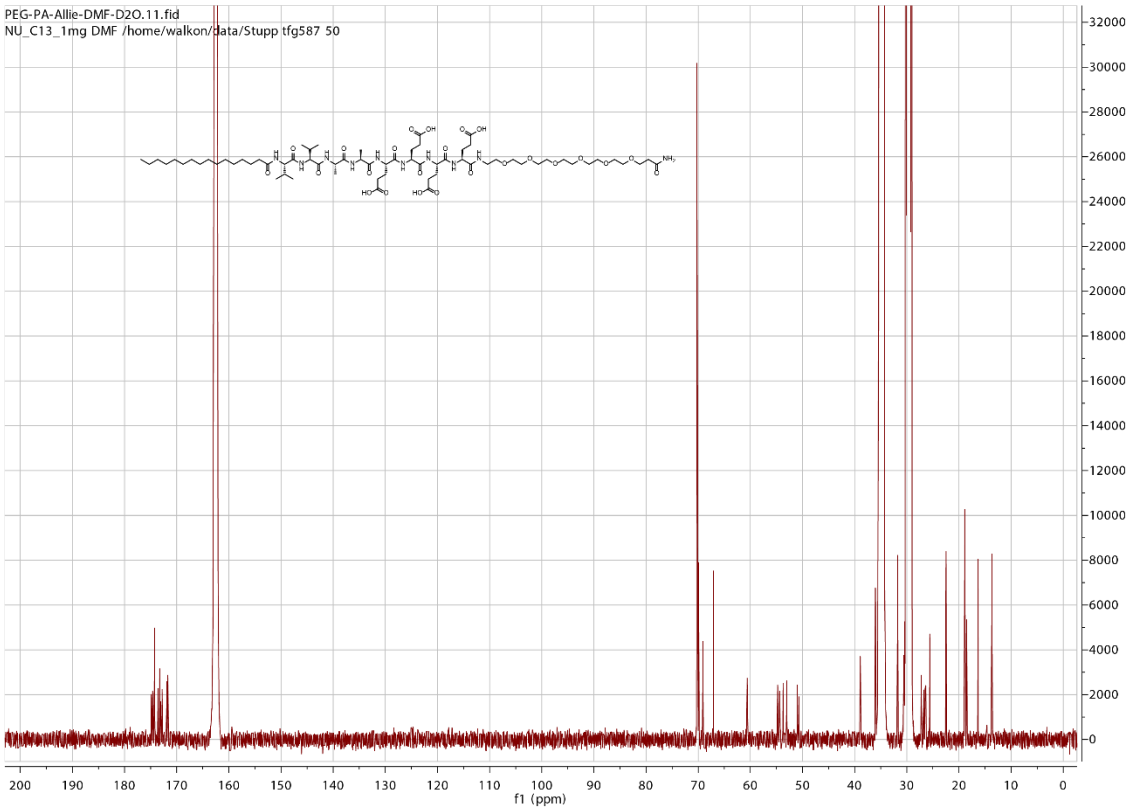
ESI-mass spectra of the **BDNF PA**. Elution volume 0.910-0.970 mL.



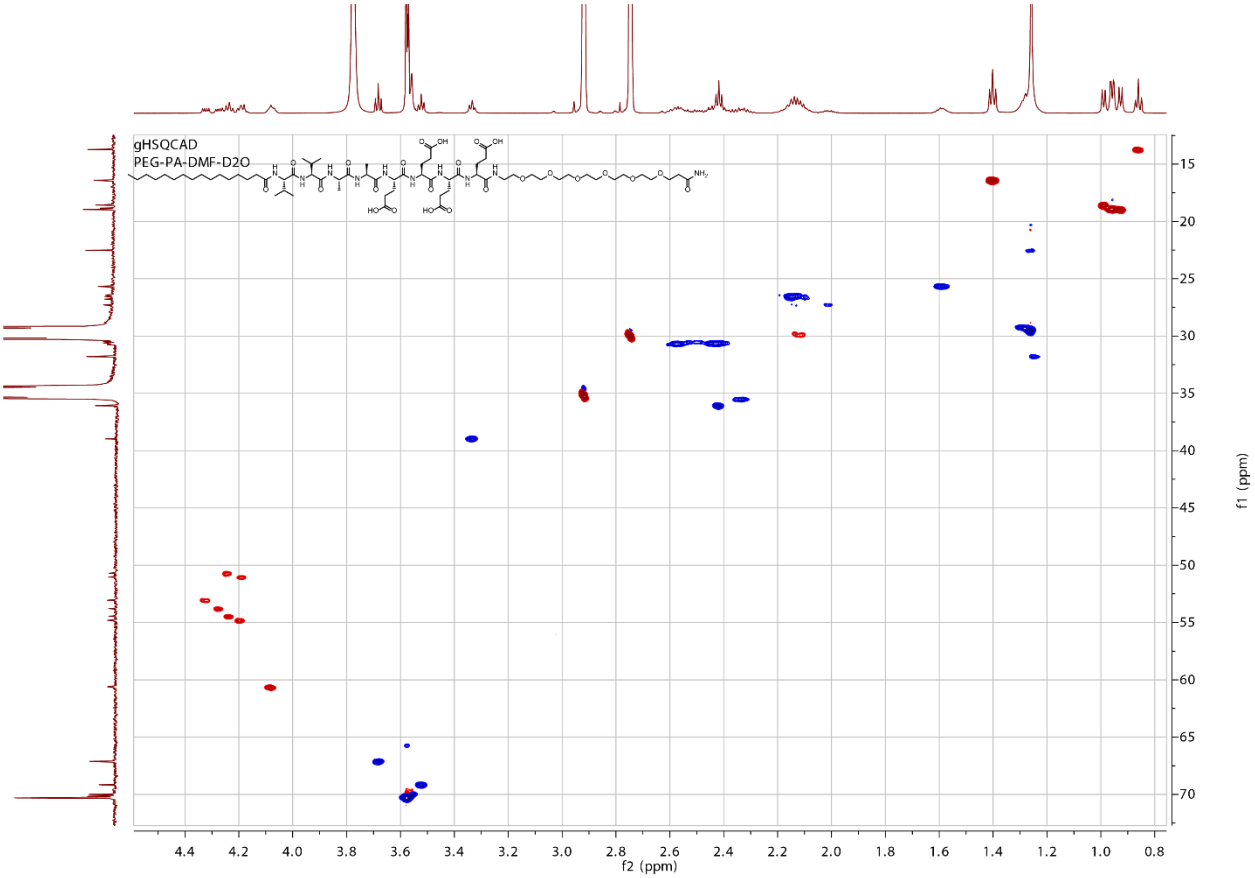
MALDI-ToF mass spectrum of the **BDNF Peptide for Immobilization 11a.**



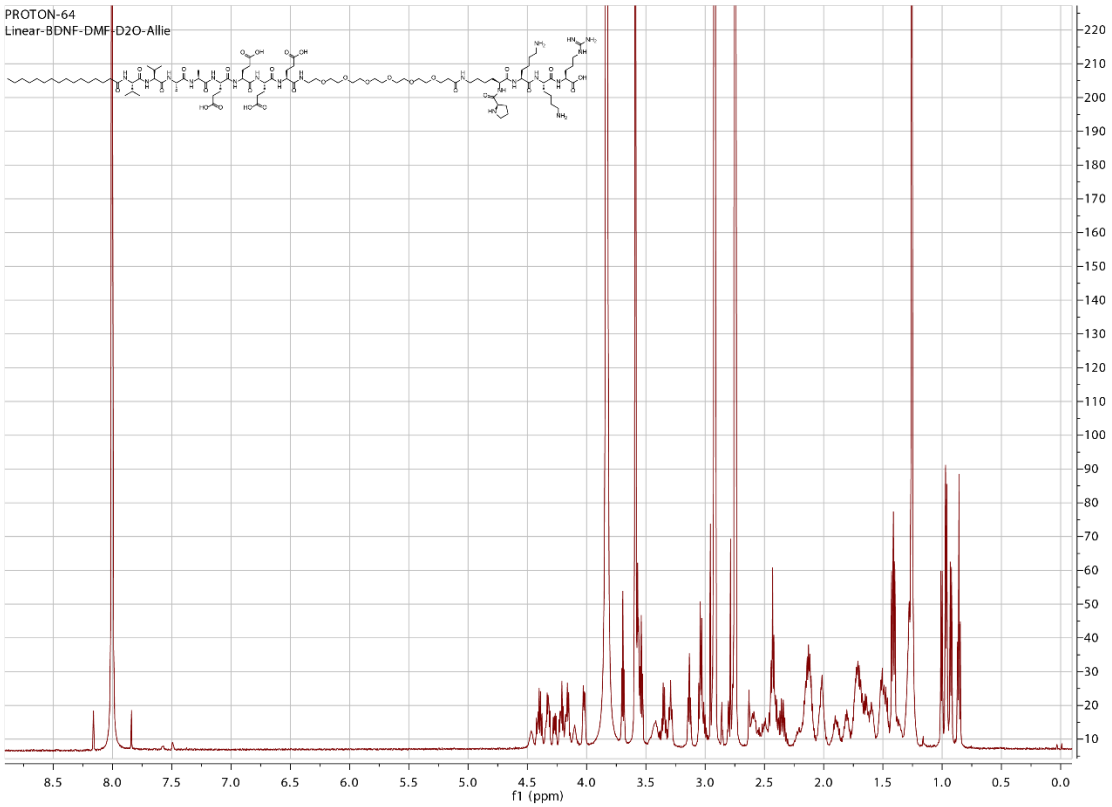
^{1}H-NMR spectrum of **E_4PEG PA** in DMF-d_7 + 5 % D_2O at 25 °C.



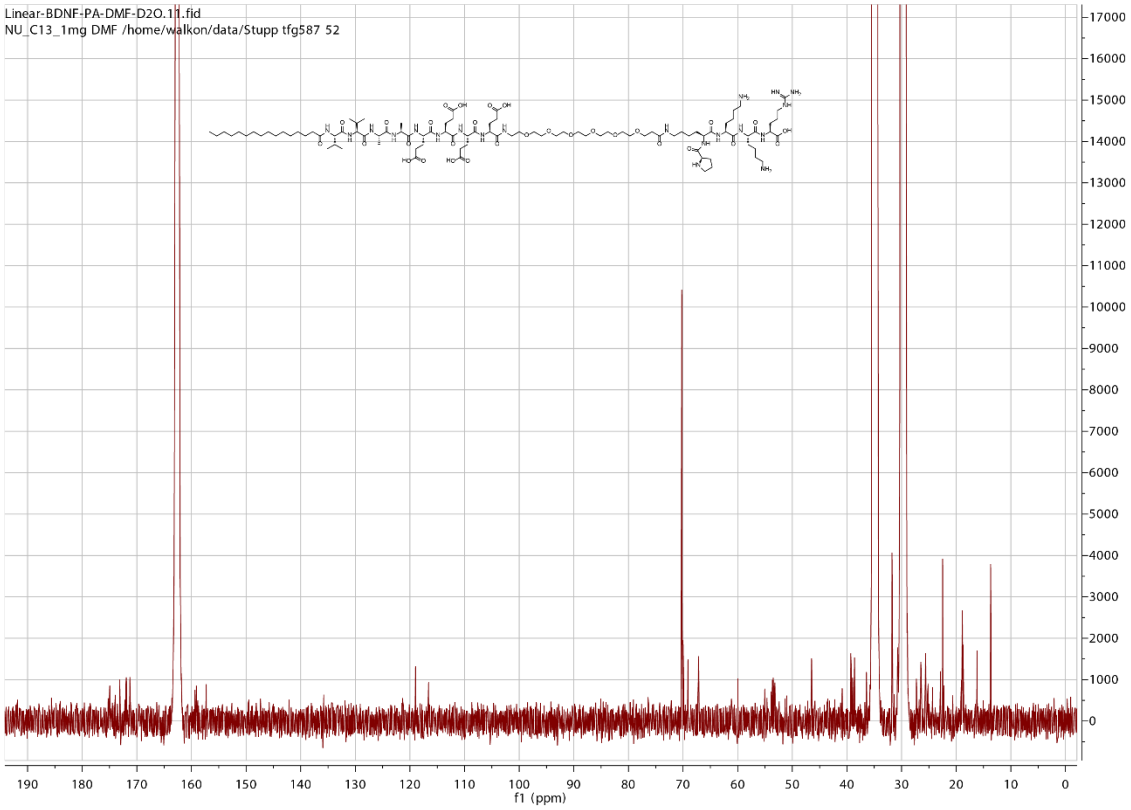
^{13}C-NMR spectrum of **E_4PEG PA** in DMF-d_7 + 5 % D_2O at 25 °C.



gHSQCAD spectrum of **E_4PEG PA** in DMF-d_7 + 5 % D_2O at 25 °C.



^{1}H-NMR spectrum of **Linear BDNF PA** in DMF-d_7 + 5 % D_2O at 25 °C.



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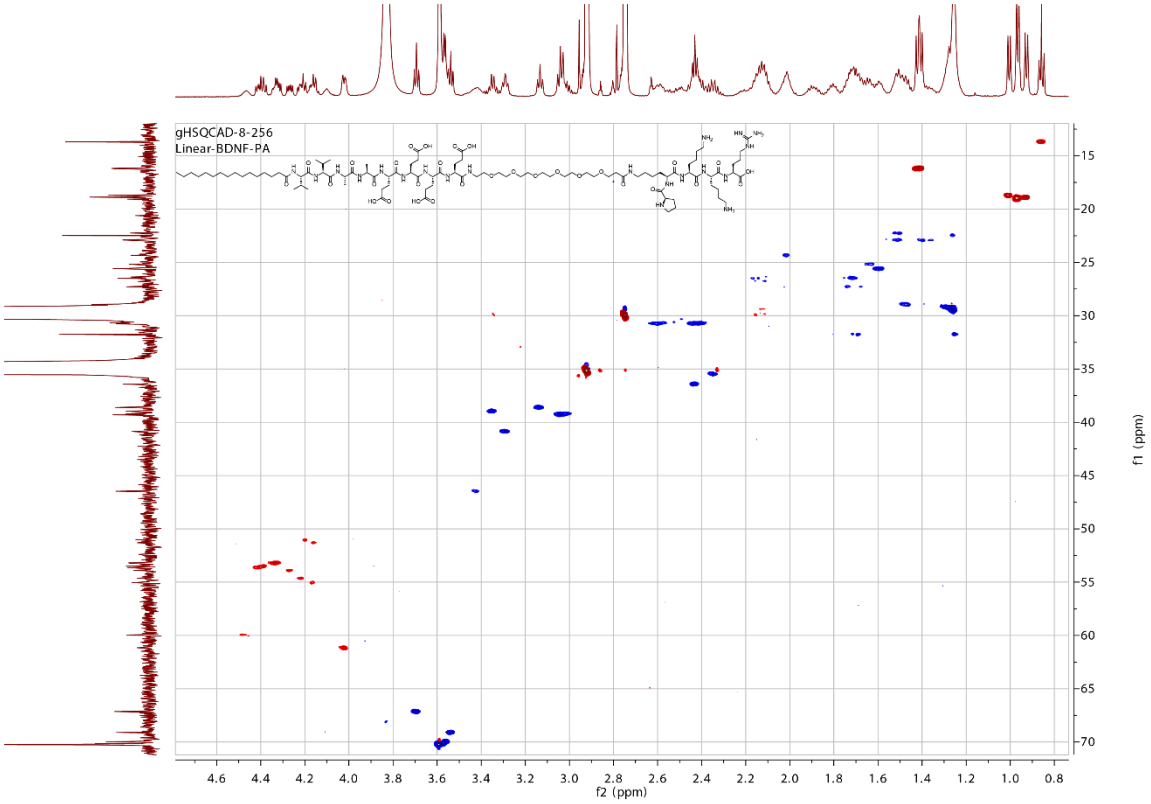
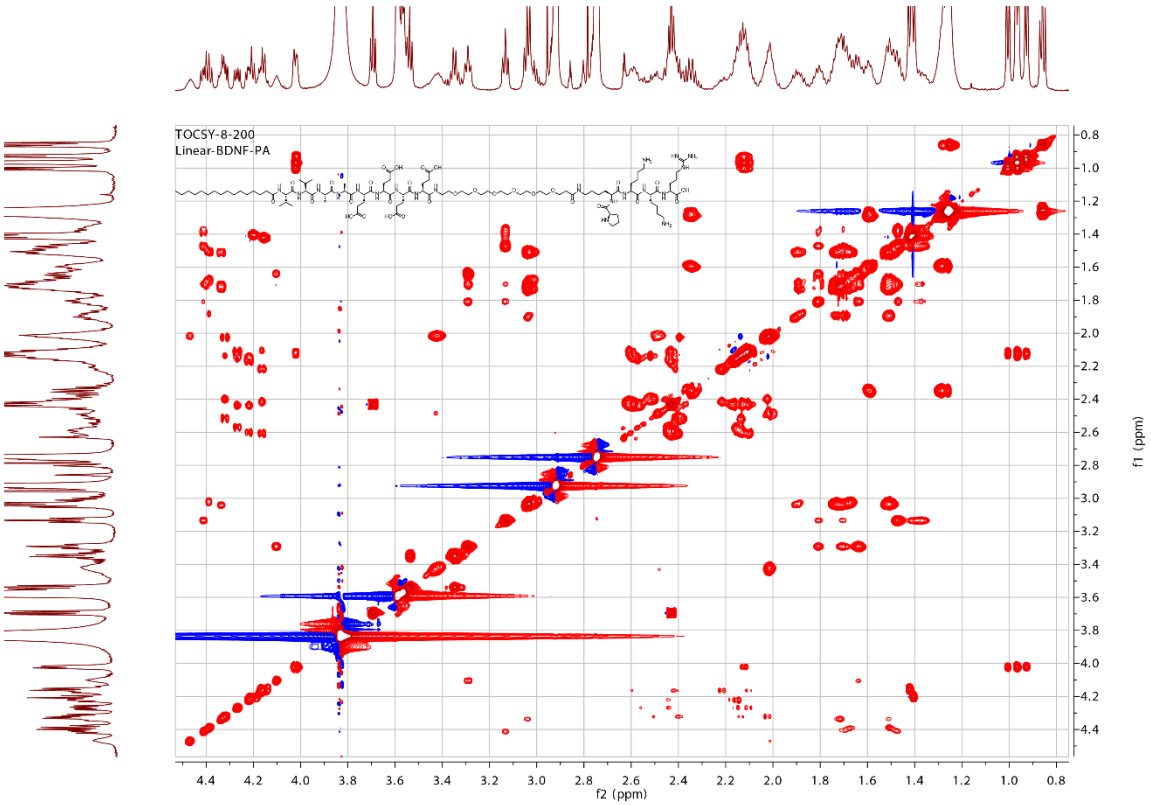
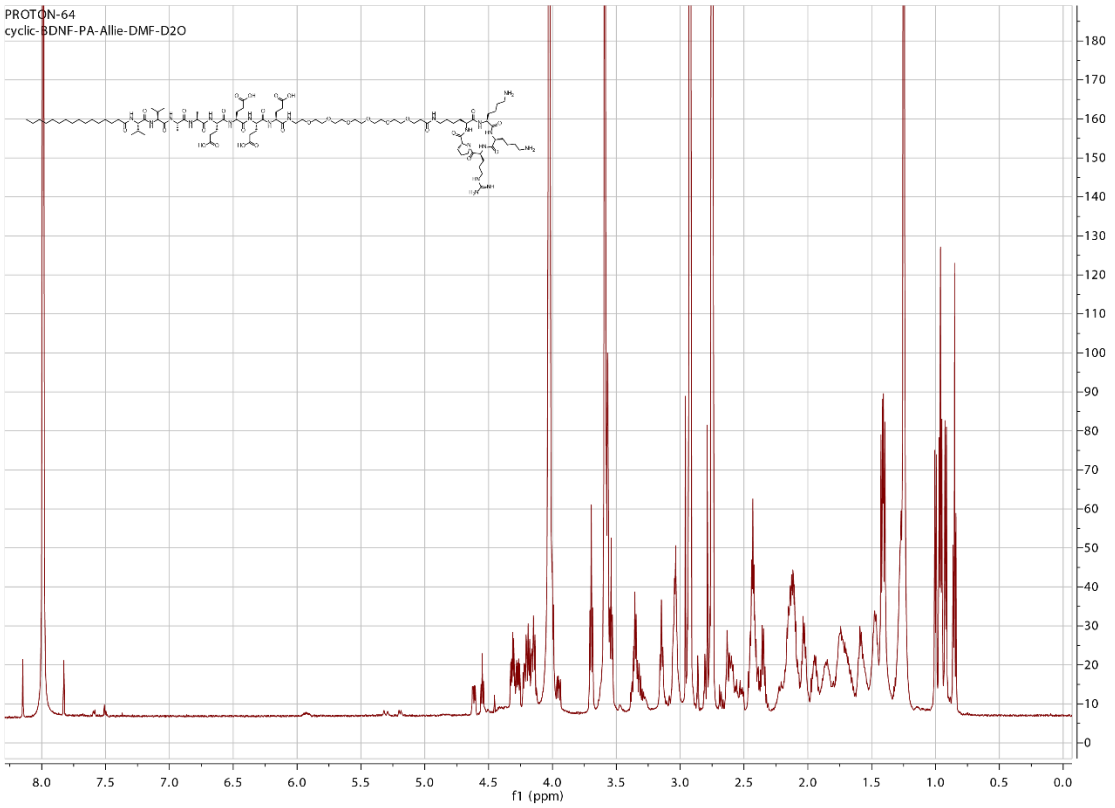


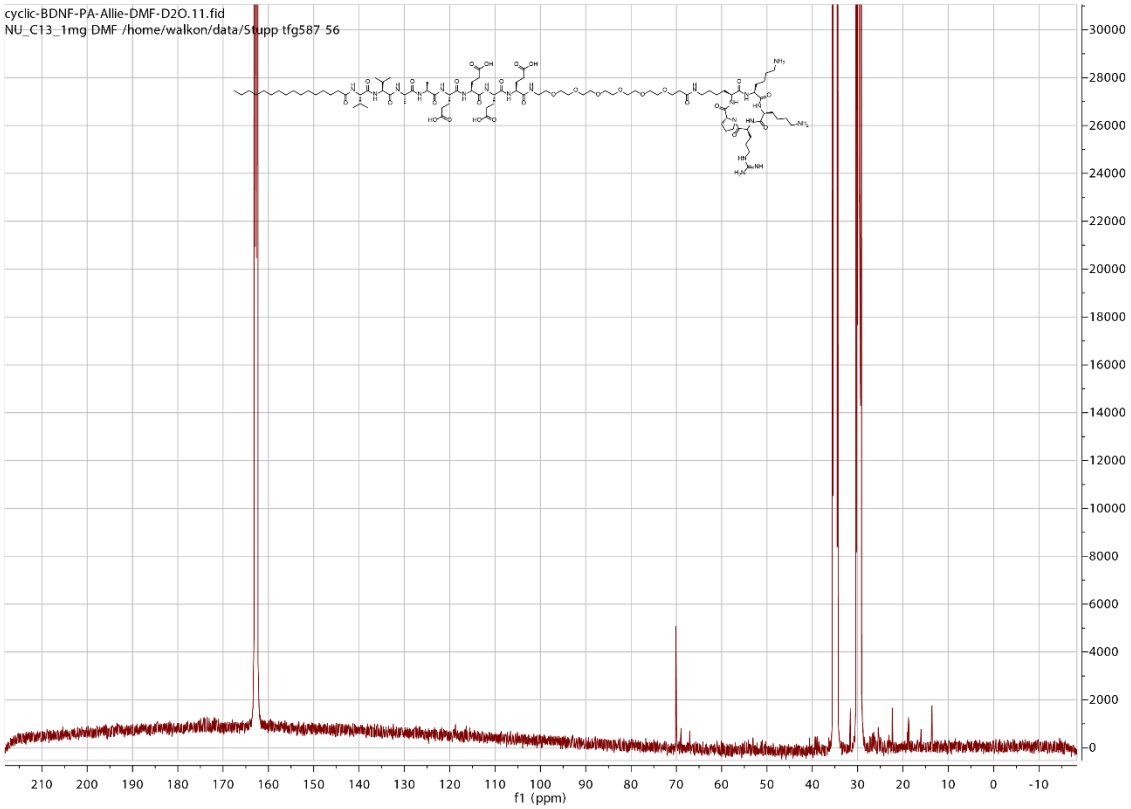
gHSQCAD spectrum of **Linear BDNF PA** in DMF-d_7 + 5 % D_2O at 25 °C.



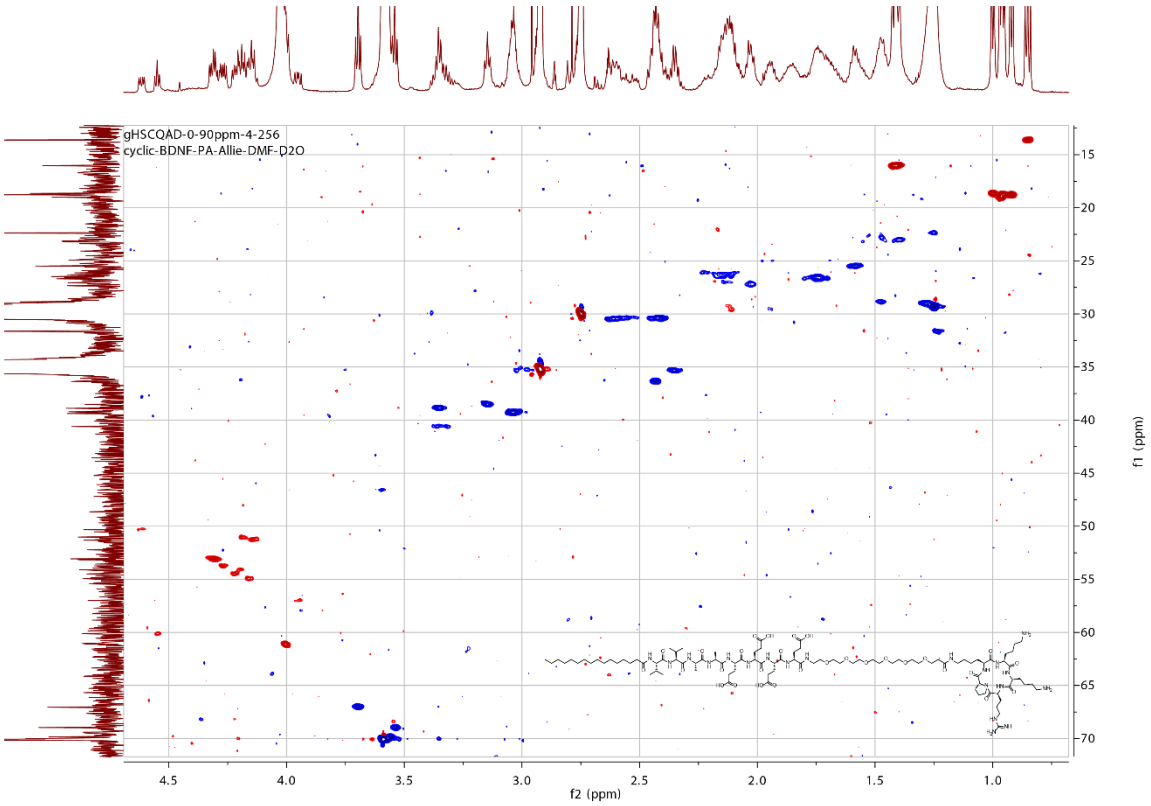
TOCSY spectrum of **Linear BDNF PA** in DMF-d_7 + 5 % D_2O at 25 °C.



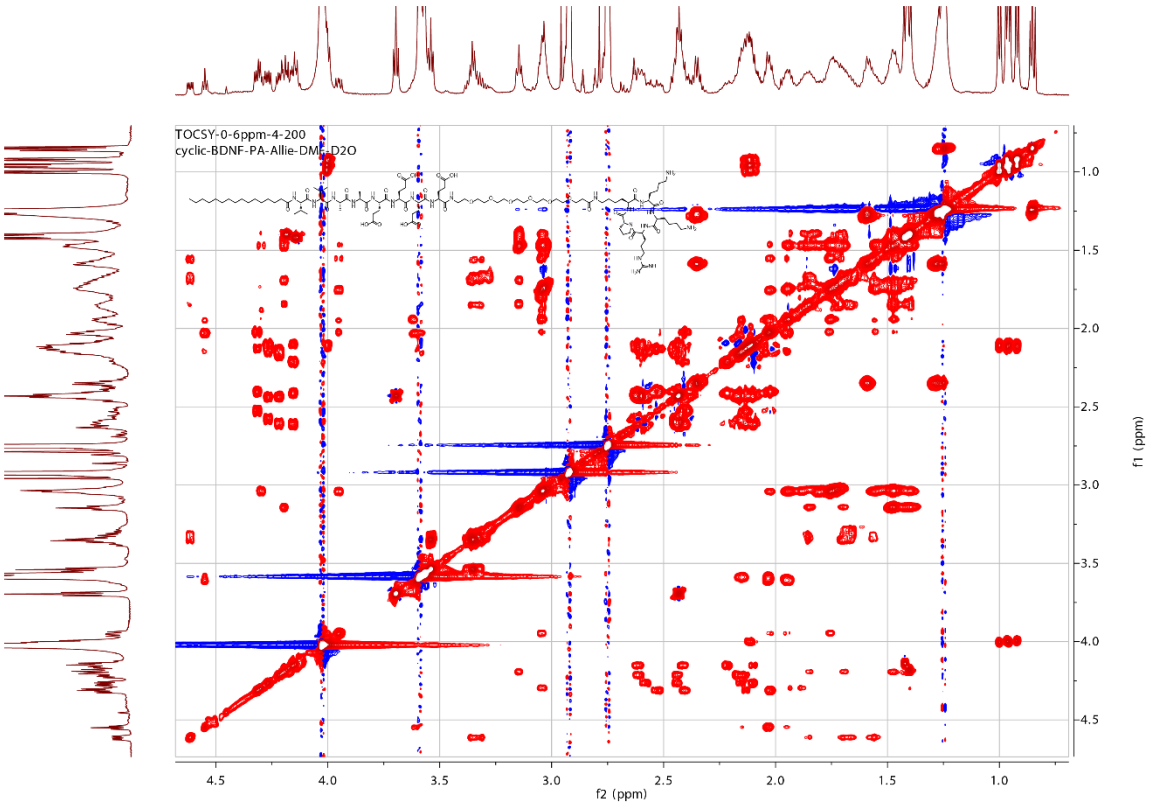
^{1}H-NMR spectrum of **BDNF PA** in DMF-d_7 + 5 % D_2O at 25 °C.



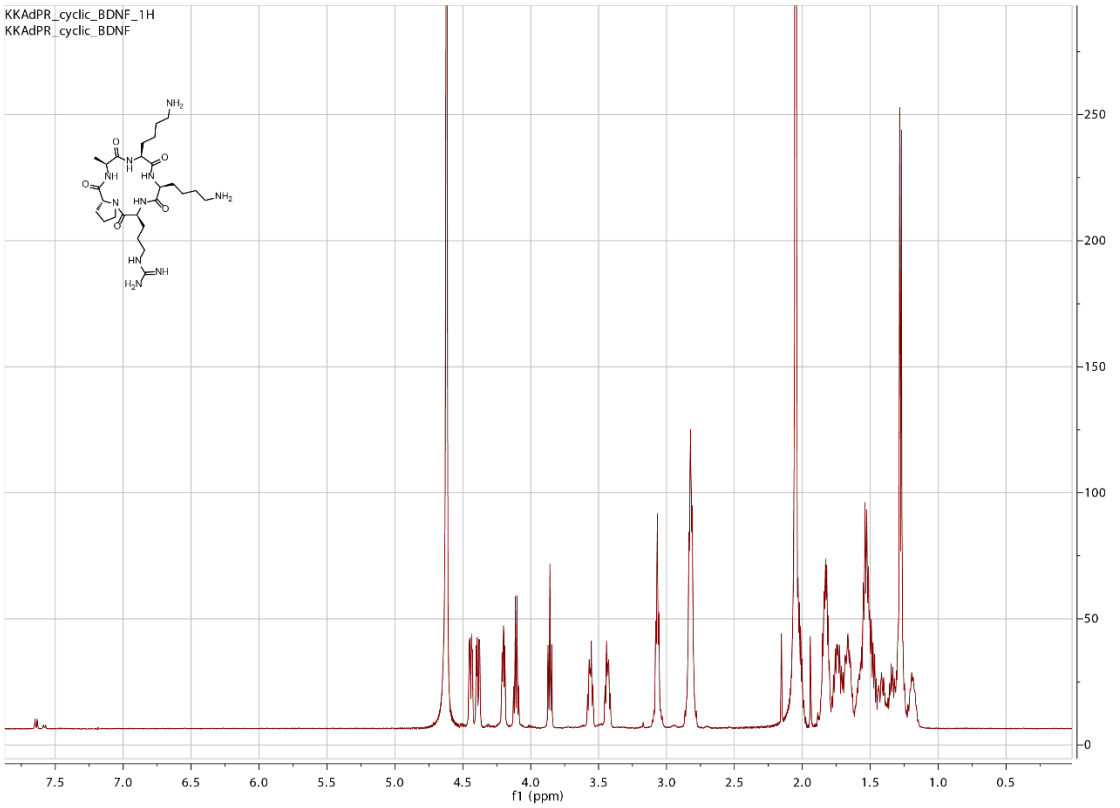
^{13}C-NMR spectrum of **BDNF PA** in DMF-d_7 + 5 % D_2O at 25 °C.



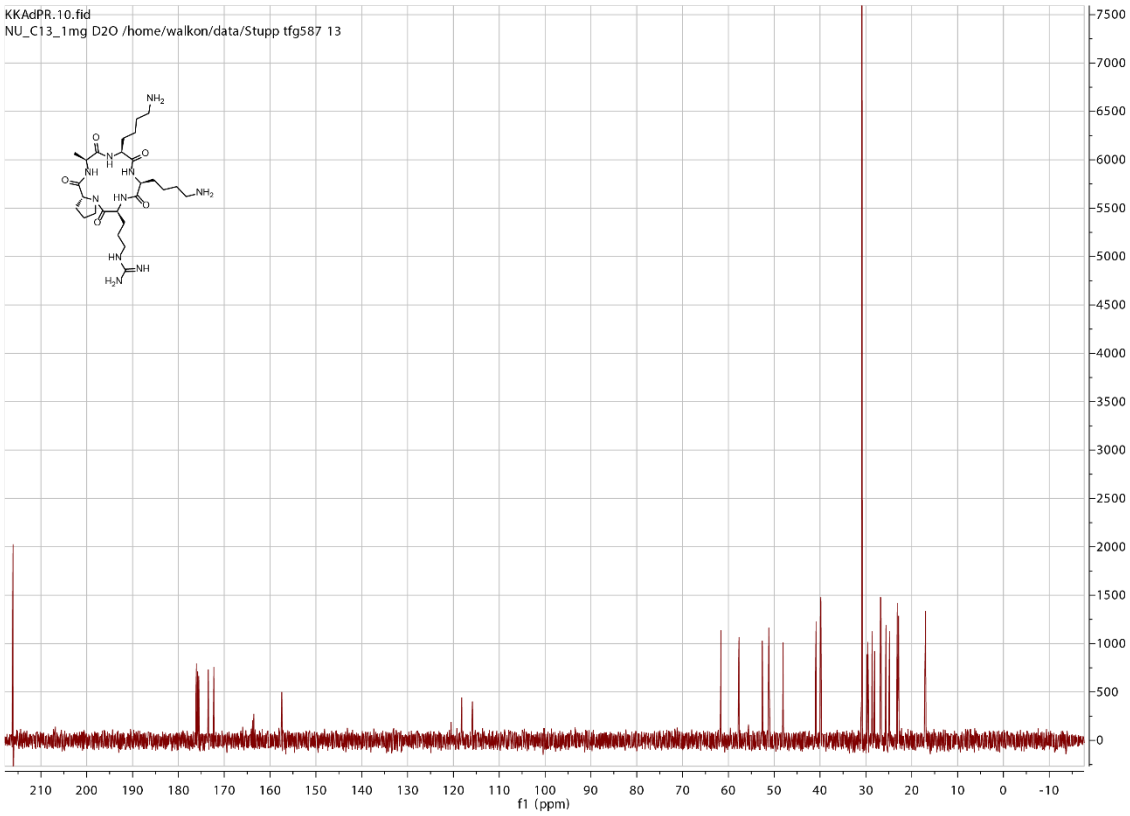
gHSQCAD spectrum of **BDNF PA** in DMF-d_7 + 5 % D_2O at 25 °C.



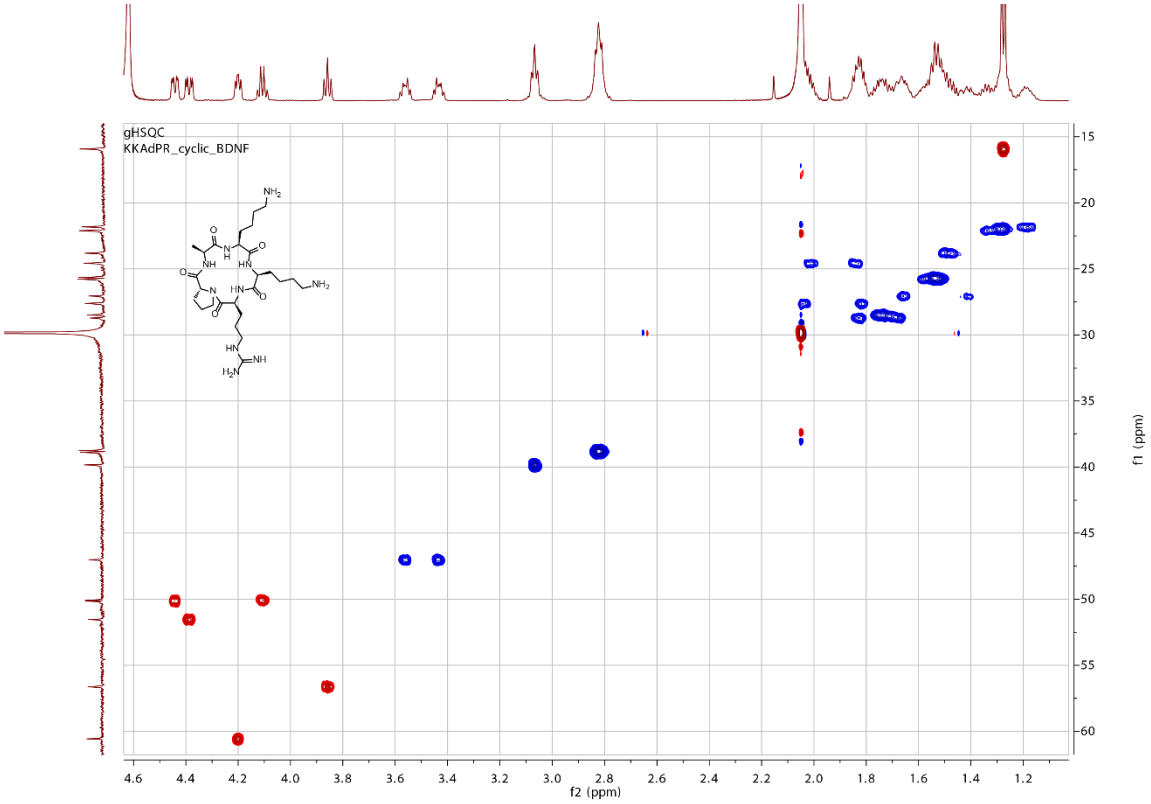
TOCSY spectrum of **BDNF PA** in DMF-d_7 + 5 % D_2O at 25 °C.



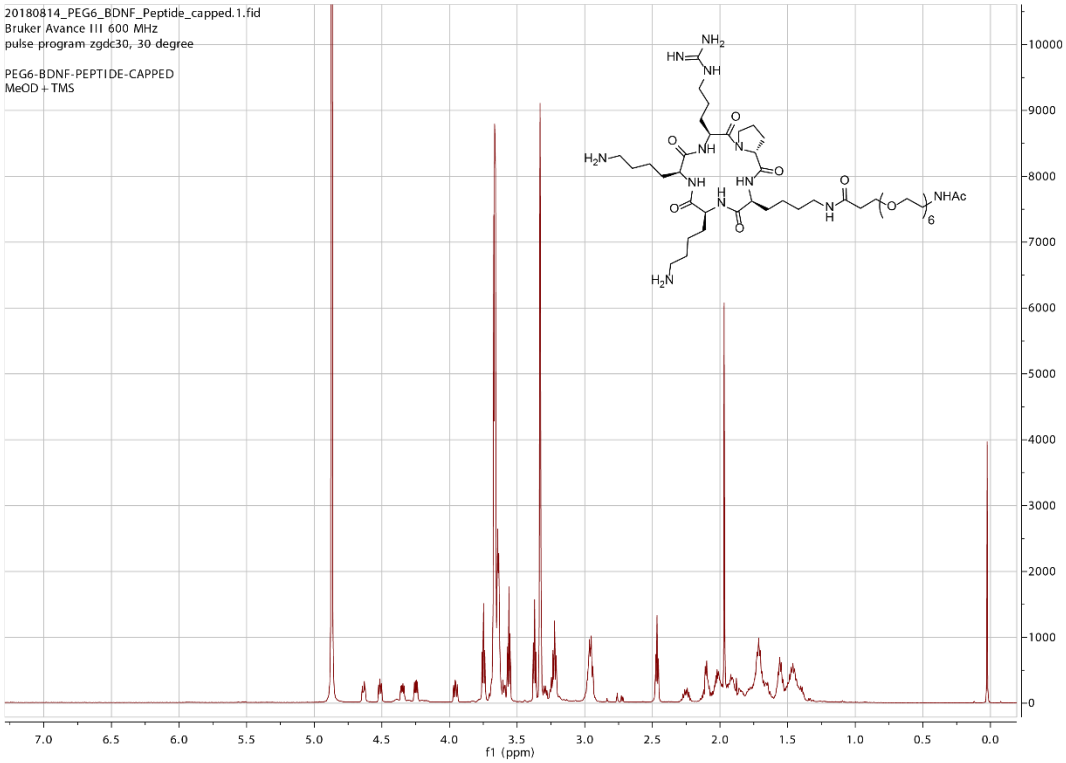
^{1}H-NMR spectrum of **BDNF Peptide** in D_2O + Acetone at 25 °C.



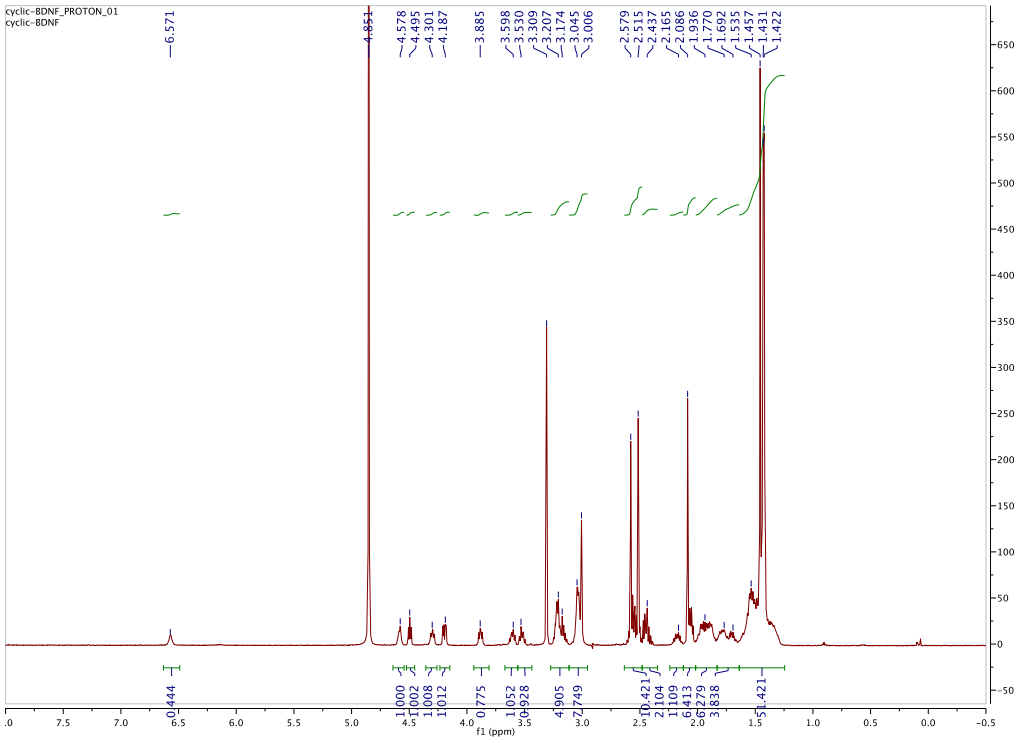
^{13}C-NMR spectrum of **BDNF Peptide** in D_2O + Acetone at 25 °C.



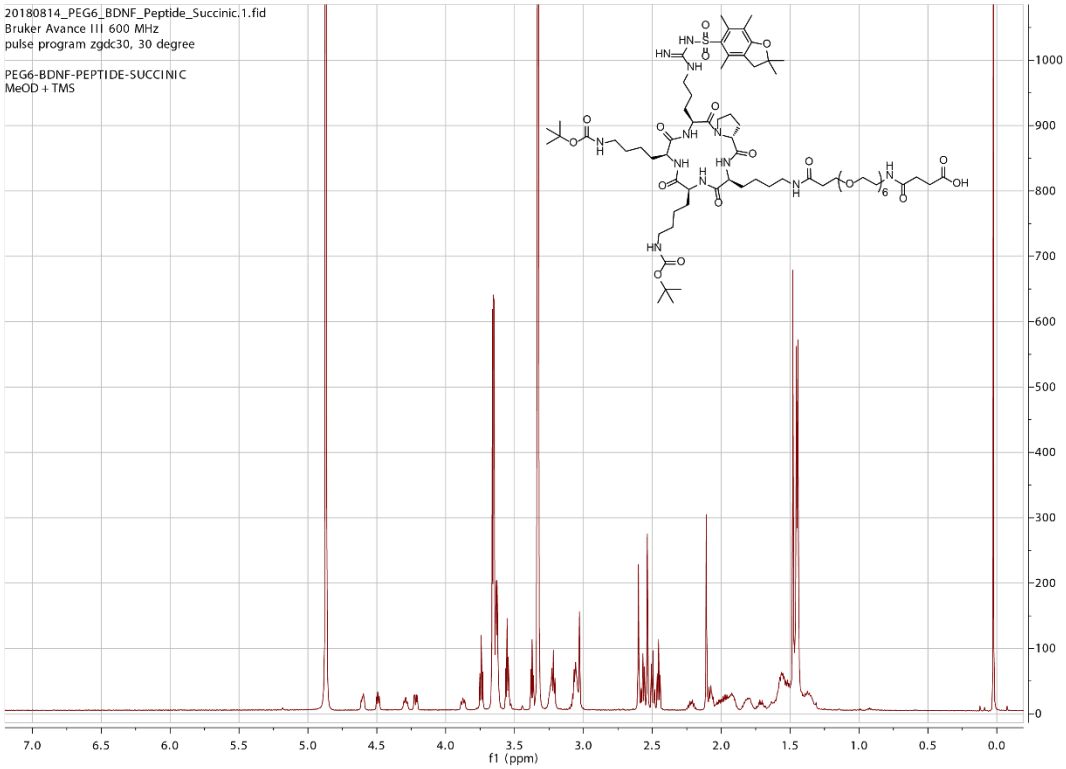
gHSQCAD spectrum of **BDNF Peptide** in D_2O + Acetone at 25 °C.



^{1}H-NMR spectrum of **PEG_6-BDNF Peptide** in MeOH-d_4 at 25 °C.



^{1}H NMR spectrum of the **BDNF Peptide for Immobilization 11a** in MeOH-d_4 at 25 °C.



^{1}H NMR spectrum of the **PEG$_6$-BDNF Peptide for Immobilization 11b** in MeOH-d_4 at 25 °C.

4. References

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