Electronic Supplementary Information

Discovery of Neuro-regenerative Peptoid from Amphibian Neuropeptide Inhibits Aβ Toxicity and Crossed Blood-Brain Barrier

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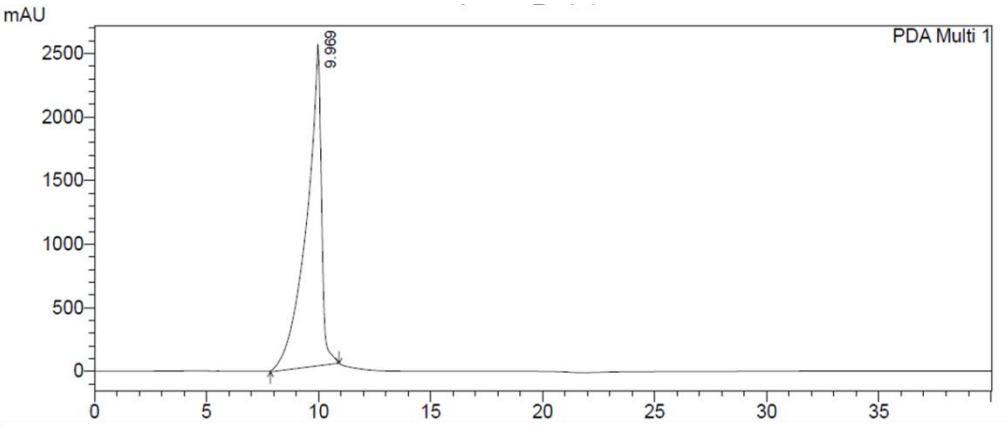
Supplementary Figures and Tables



PeakTable

PDA Ch1 210nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	9.969	116502330	2529666	100.000	100.000
Total		116502330	2529666	100.000	100.000

Figure S1: HPLC chromatogram of R-RFa peptide showing 100% purity.

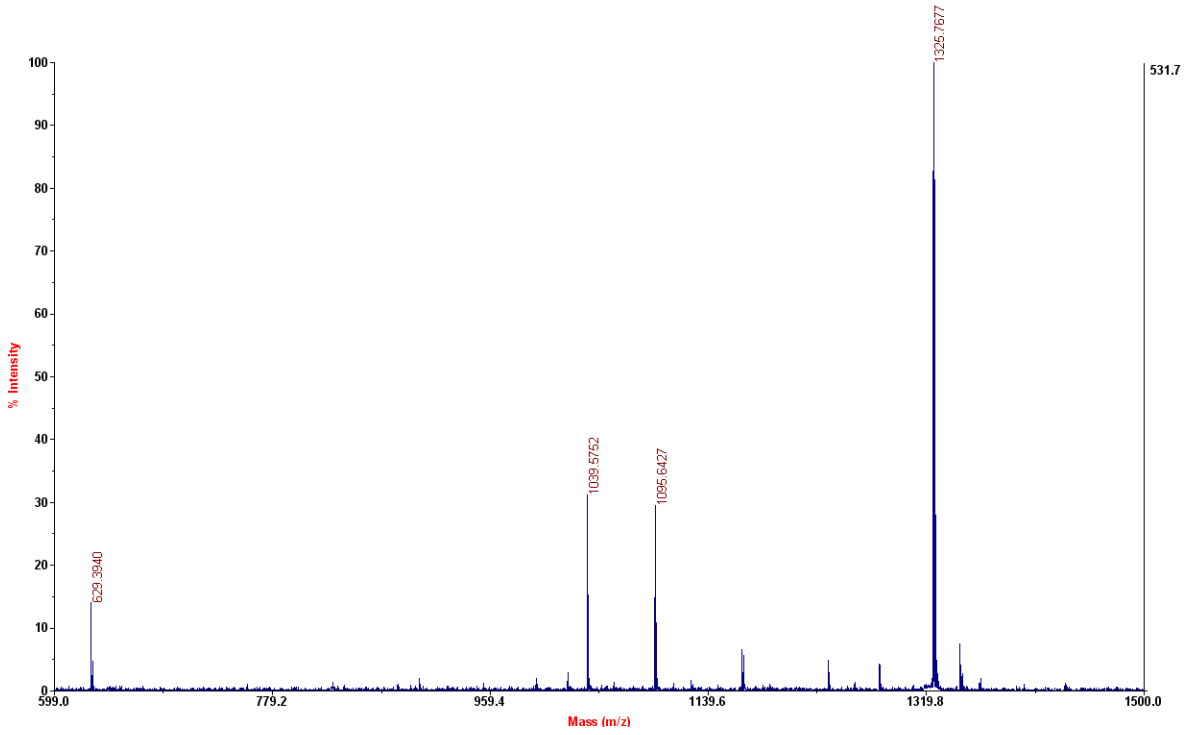
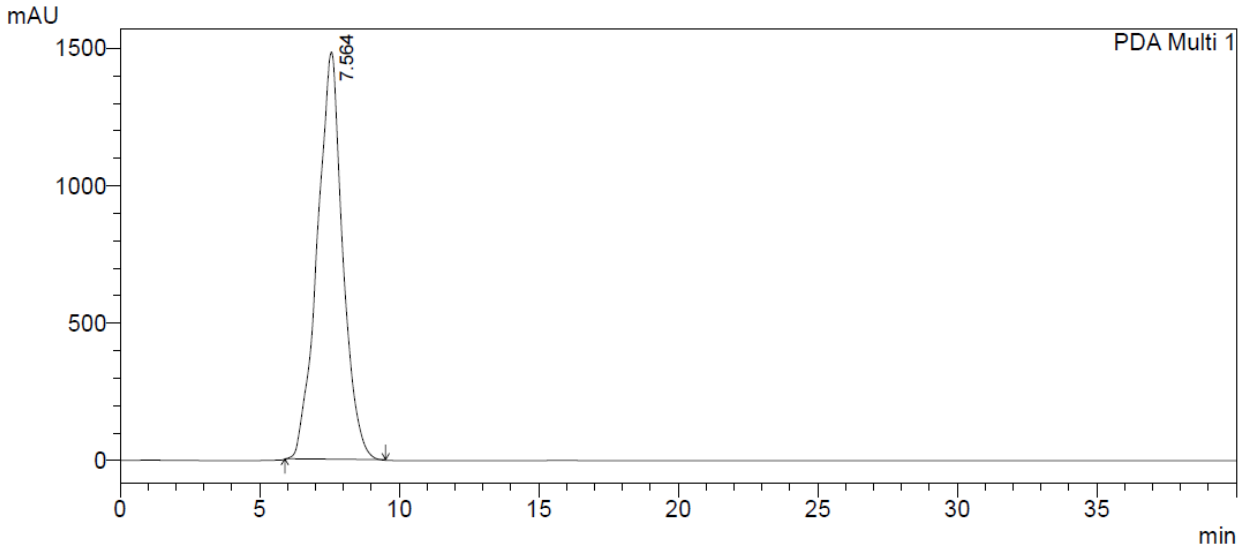


Figure S2: MALDI mass spectra of R-RFa peptide. Observed mass 1325 Da (M= 1325 Da).



Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.564	93962746	1482780	100.000	100.000
Total		93962746	1482780	100.000	100.000

Figure S3: HPLC chromatogram of SLKP peptide showing 100% purity.

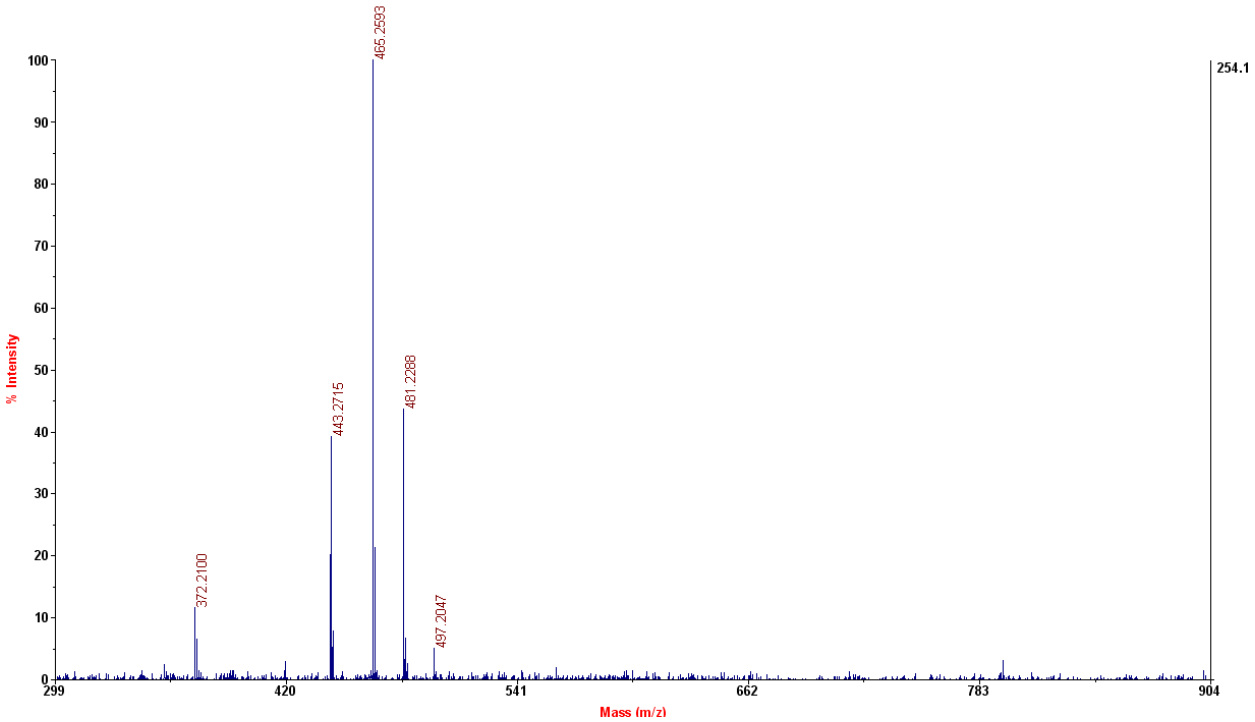
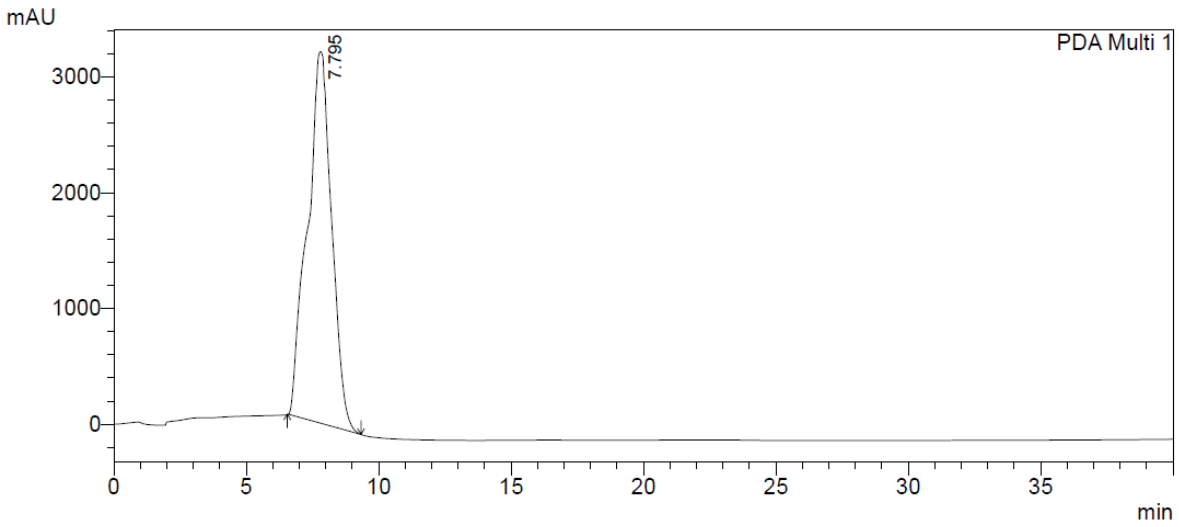


Figure S4: ESI mass spectra of SLKP peptide shows 443 Da ($442+H^+$), 465Da ($442+Na^+$), 481Da ($442+K^+$).



Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.795	203361917	3207611	100.000	100.000
Total		203361917	3207611	100.000	100.000

Figure S5: HPLC chromatogram of AANL peptide showing 100% purity.

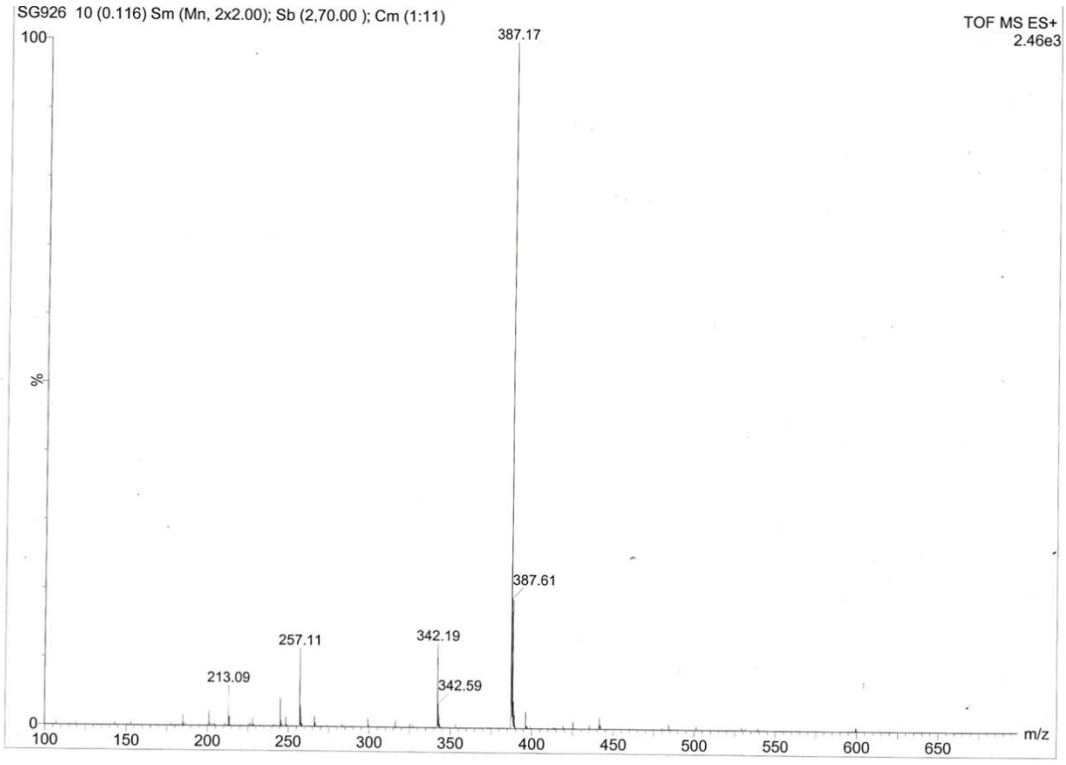
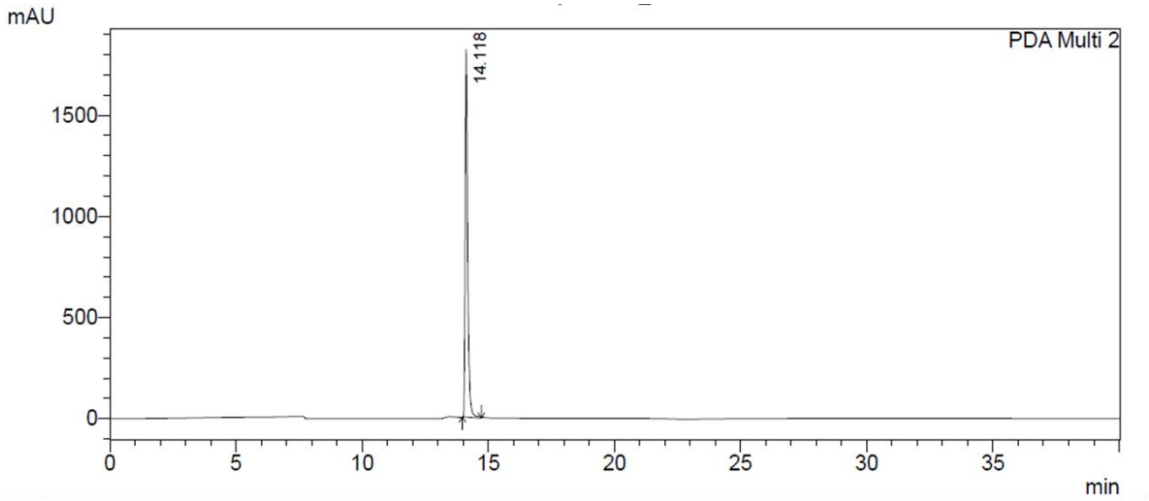


Figure S6: ESI mass spectra of AANL peptide shows 387 Da ($386+H^+$).



PeakTable

PDA Ch2 260nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	14.118	12454279	1817238	100.000	100.000
Total		12454279	1817238	100.000	100.000

Figure S7: HPLC chromatogram of PLRF peptide showing 100% purity.

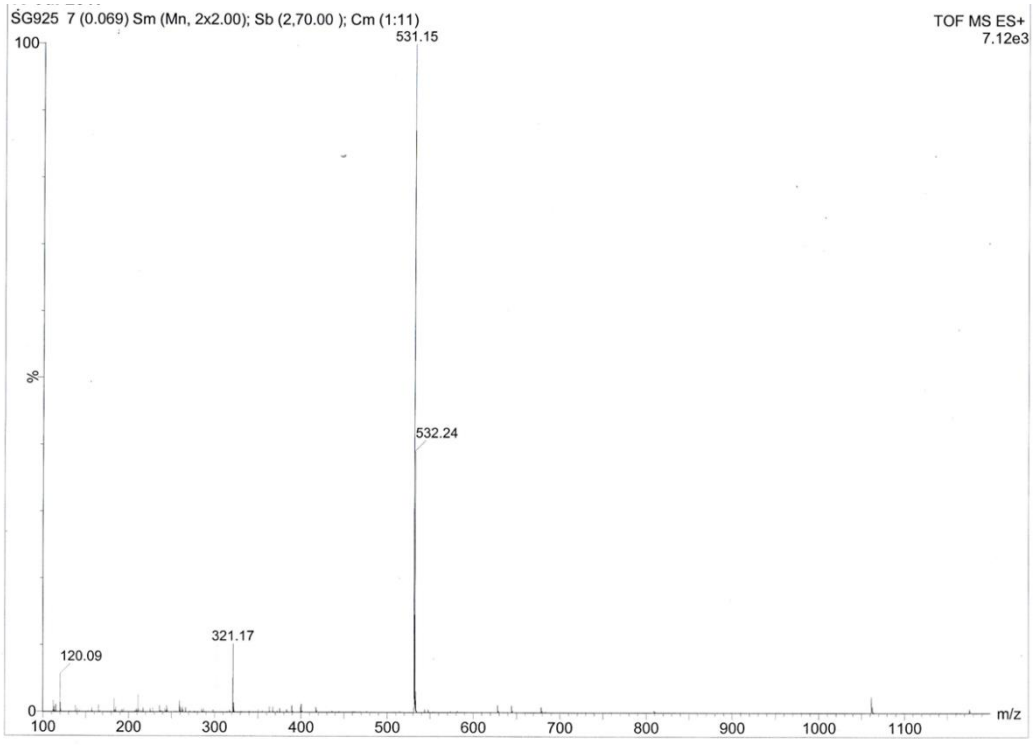


Figure S8: ESI mass spectra of PLRF peptide shows 531 Da (530+H^+).

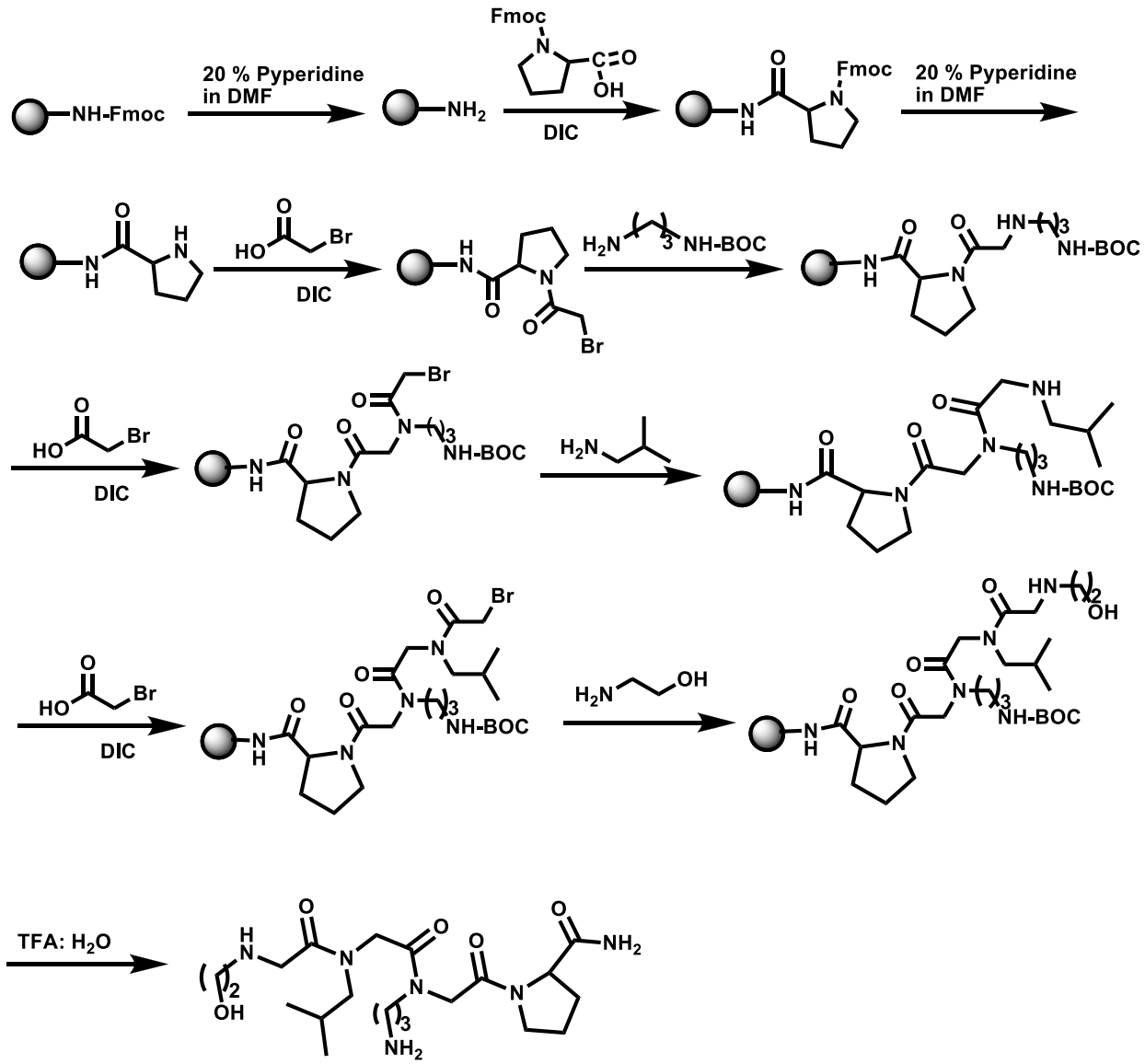
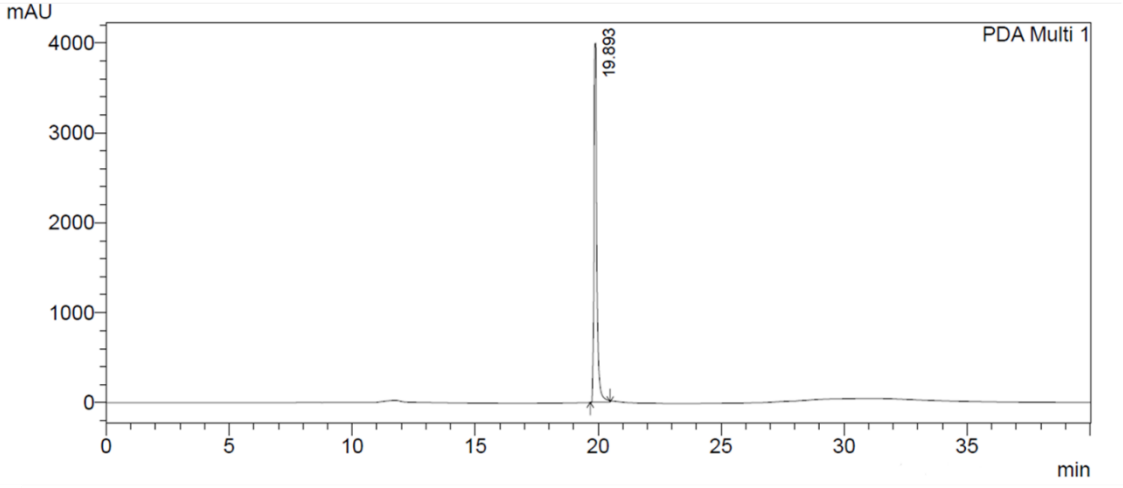


Figure S9: Schematic diagram of SLKP peptoid synthesis.



PeakTable

PDA Ch1 210nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	19.893	33030388	3991259	100.000	100.000
Total		33030388	3991259	100.000	100.000

Figure S10: HPLC chromatogram of SLKP peptoid showing 100% purity.

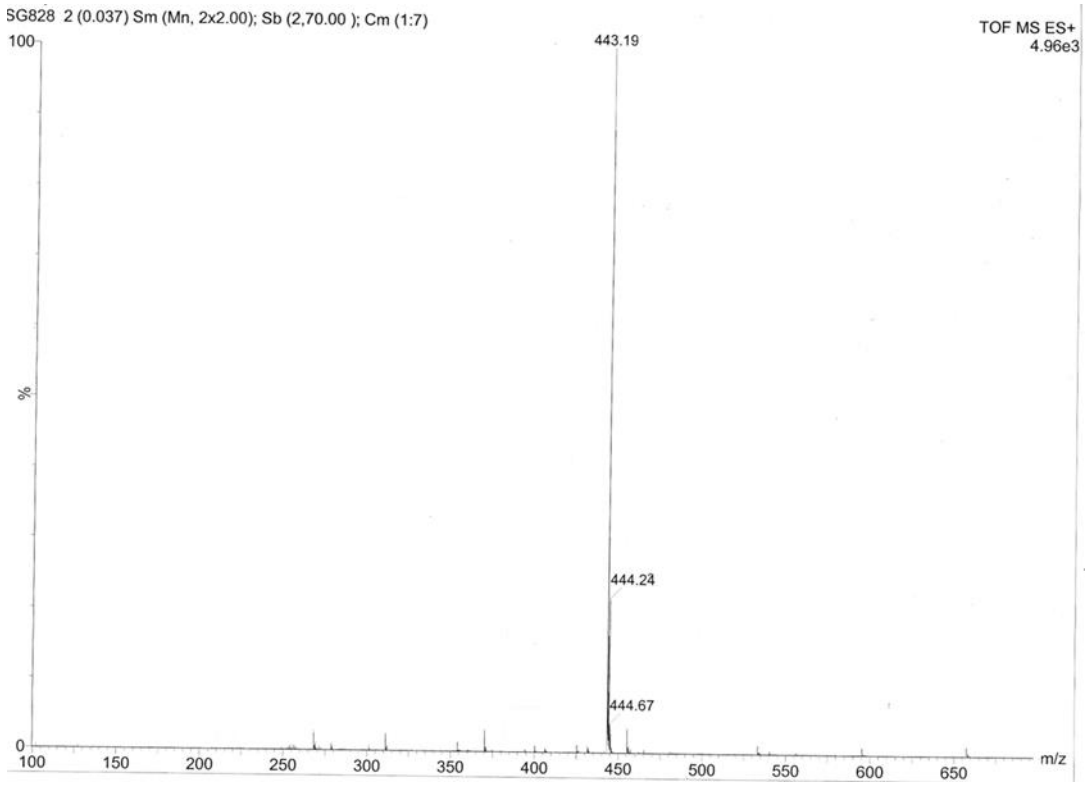
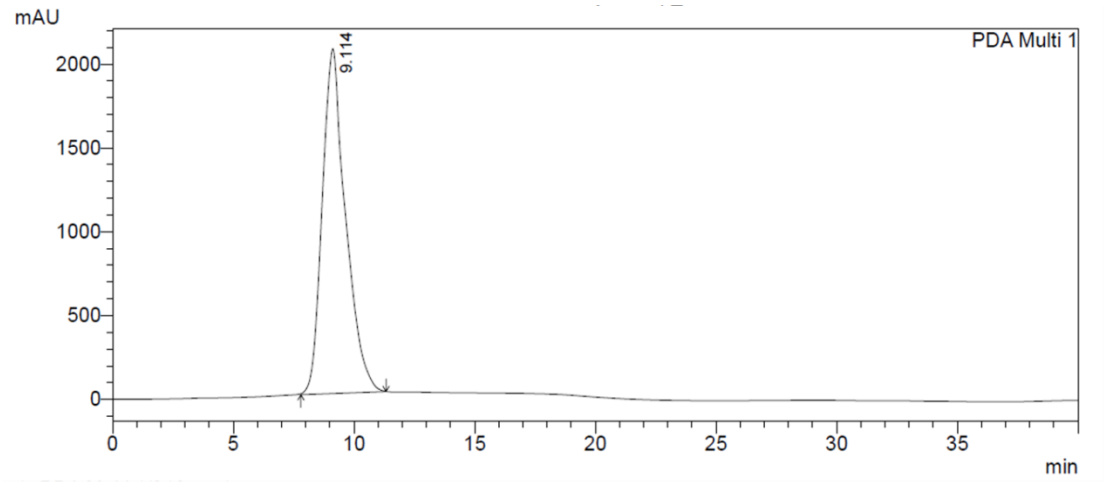


Figure S11: ESI mass spectra of SLKP peptoid shows 443Da ($442+H^+$).



Peak#	Ret. Time	Area	Height	Area %	Height %
1	9.114	138462505	2056872	100.000	100.000
Total		138462505	2056872	100.000	100.000

Figure S12. HPLC chromatogram of 5(6)-carboxyfluorescein attached SLKP peptoid showing 100% purity.

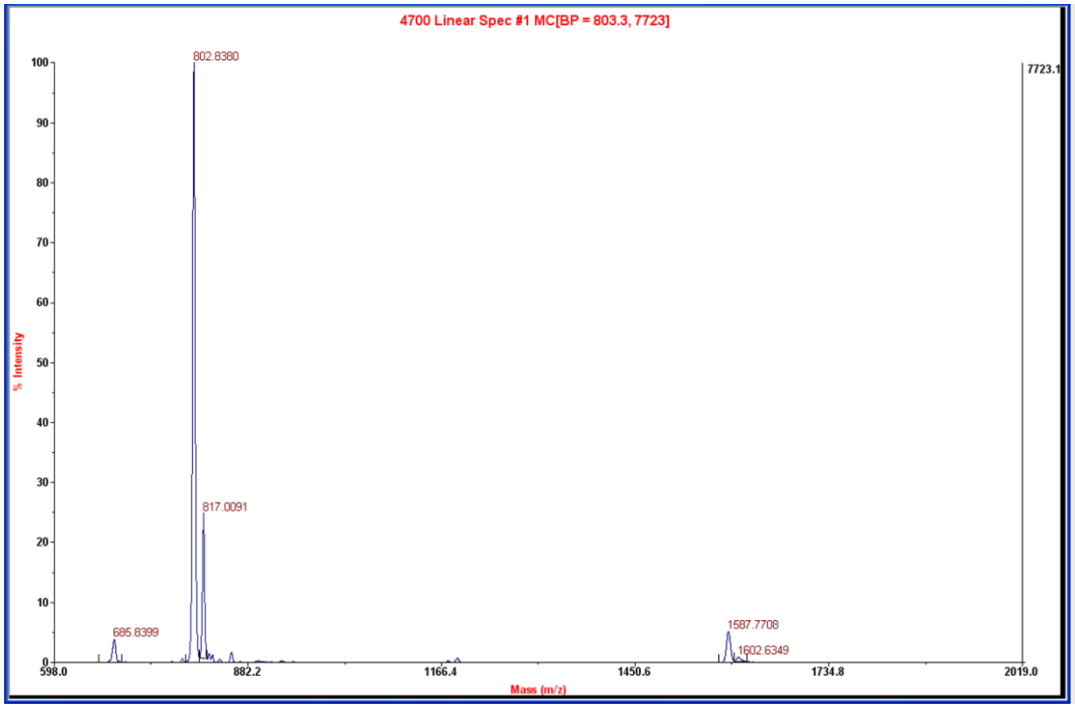


Figure S13: ESI mass spectra of 5(6)-carboxyfluorescein attached SLKP peptoid shows 802 Da ($801+H^+$).

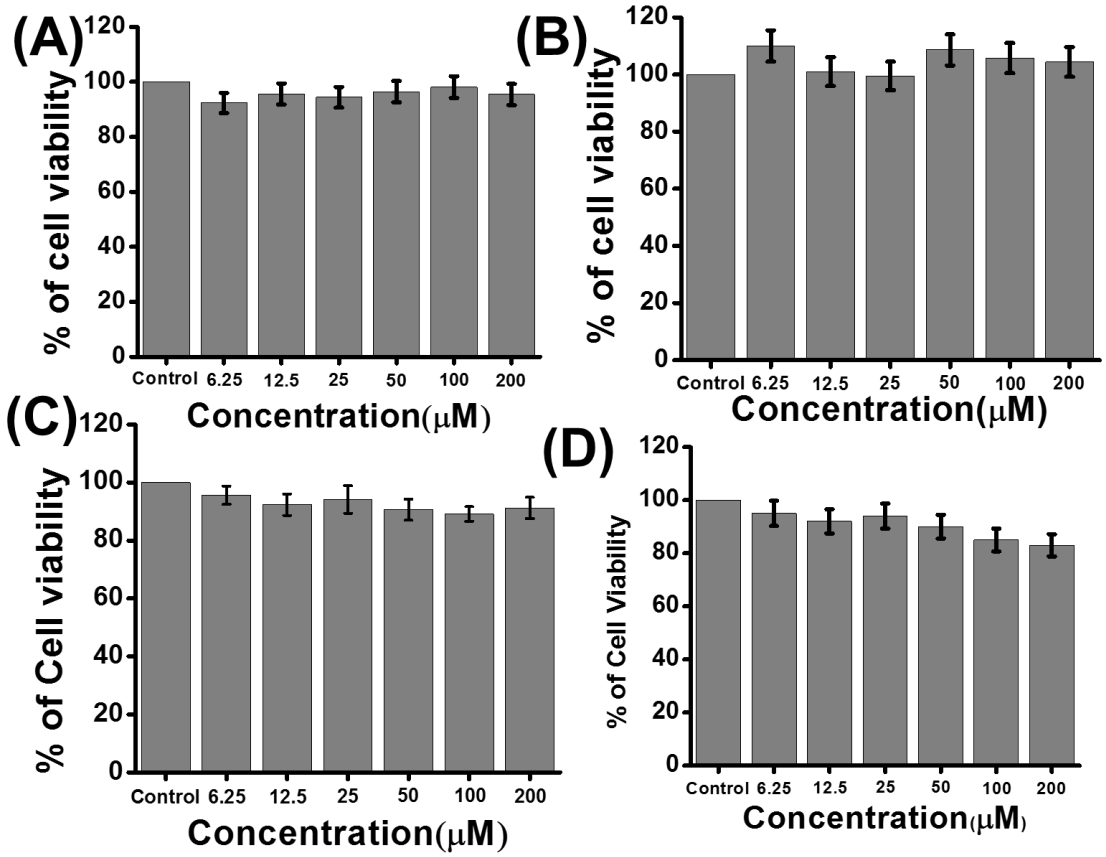


Figure S14: MTT assay of various peptide in PC12 derived neurons. (A) R-RFa (B) SLKP (C) AANL do not have any toxicity. (D) PRLF has slight toxicity in PC12 derived neurons.

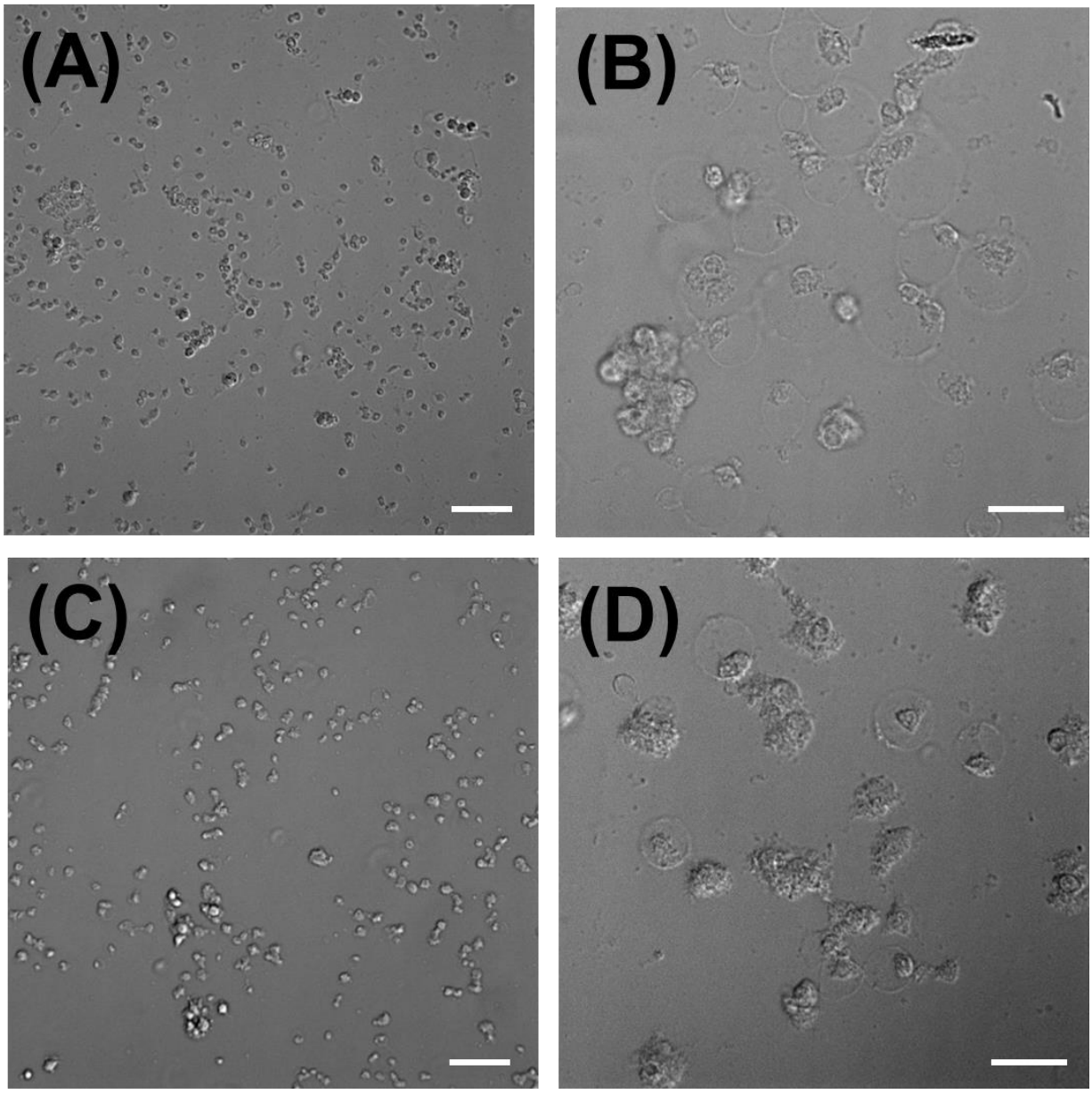


Figure S15: DIC images of neurite outgrowth in PC12 derived neurons. (A, B) Neurons treated with the AANL peptide. (C, D) Neurons treated with PRLF peptide. The images reveal that AANL and PRLF unable to provide the neurite out growth in PC12 derived neurons. (Scale bars of A and C corresponds to 100 μm; Scale bars of B and D correspond to 20 μm).

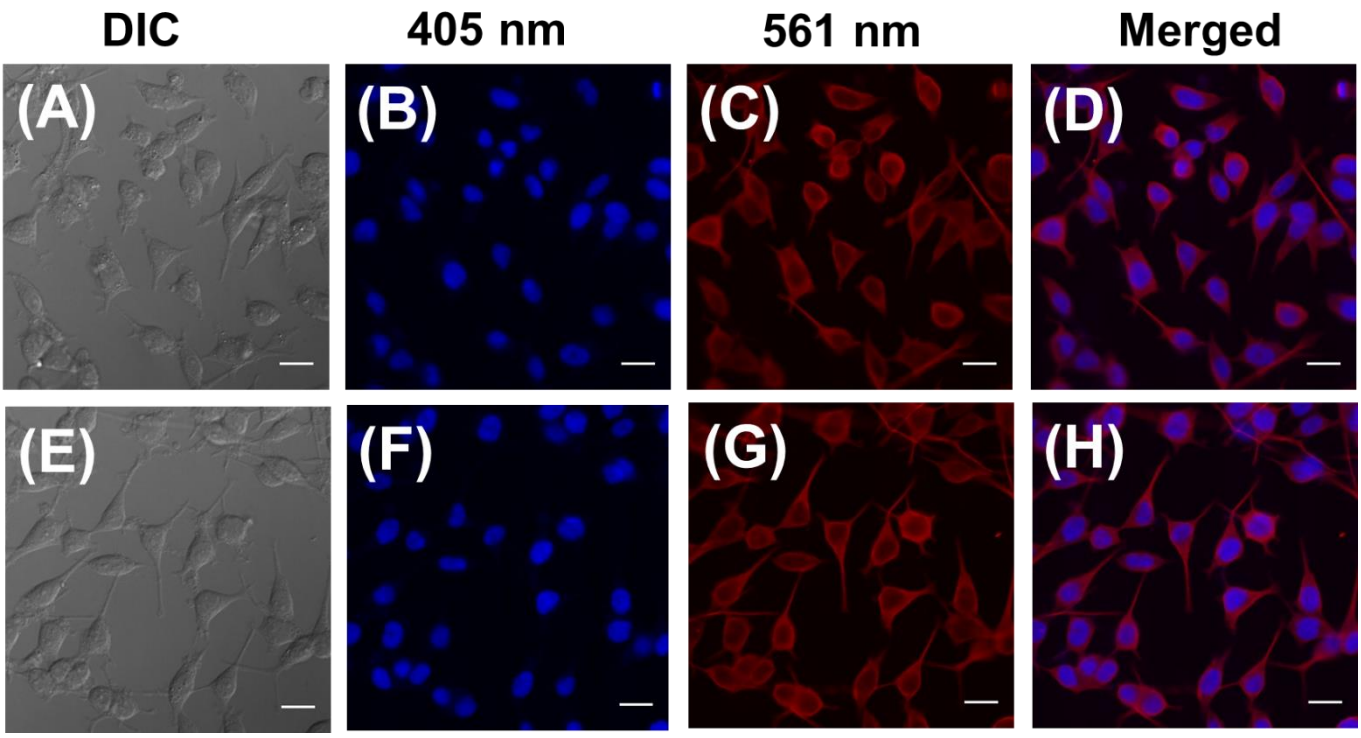


Figure S16: Microscopic images of neurite outgrowth in different channel. (A-D) neurons treated with R-RFa. (E-H) neurons treated with SLKP peptide. Microscopic images reveal that SLKP peptide gives better neurite outgrowth than R-RFa. (Scale bars correspond to 20 μm).

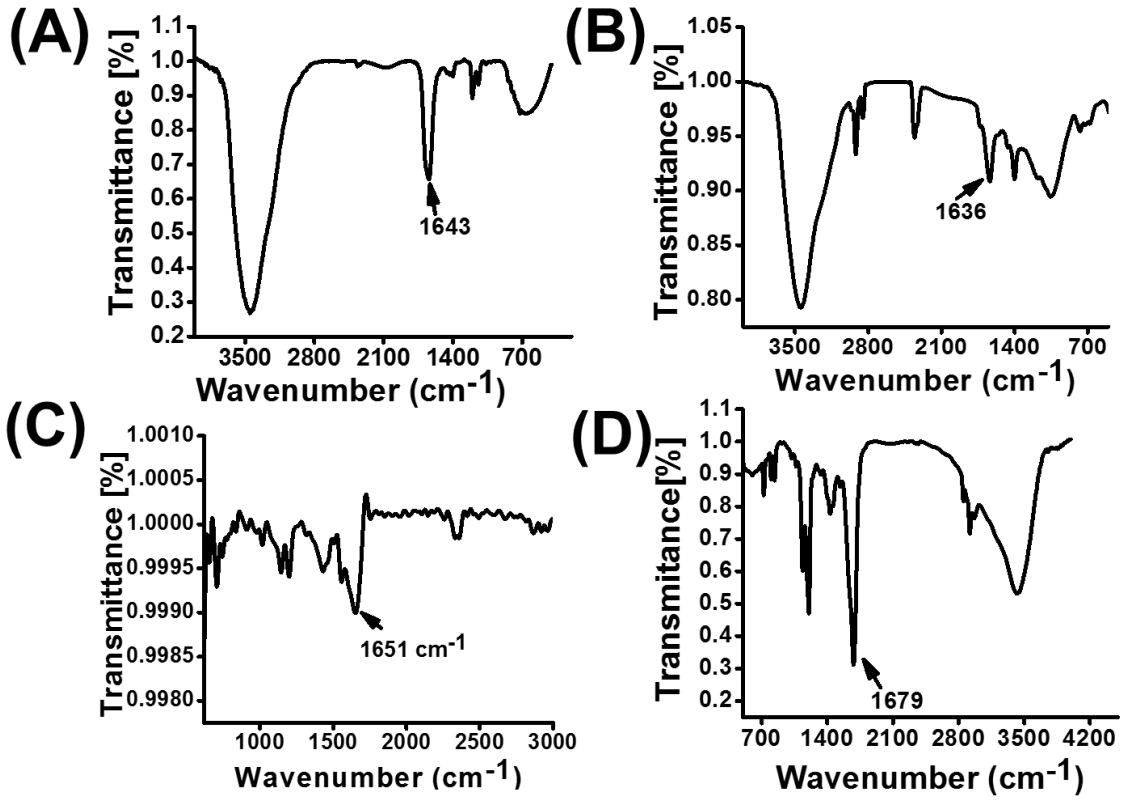


Figure S17: FT-IR study reveals inhibition β-sheet structure of Aβ peptide upon treatment of SLKP peptoid. (A) FT-IR spectra of only SLKP peptoid at 0 day incubation. (B) FT-IR spectra of only Aβ peptide after 7 days incubation. (C) FT-IR spectra of Aβ peptide with SLKP peptoid after 7 days incubation. (D) FT-IR spectra of only SLKP peptoid after 7 days incubation.

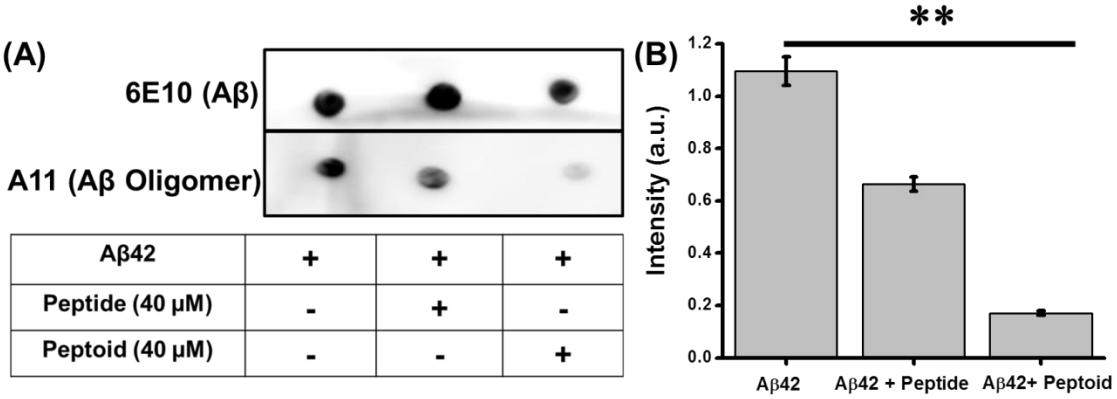


Figure S18: Dot blot experiment reveals that SLKP peptiod has better efficiency for inhibition of amyloid aggregation than it counter peptide. (A) Images of dot blot. (B) Bar diagram of dot blot experiment.

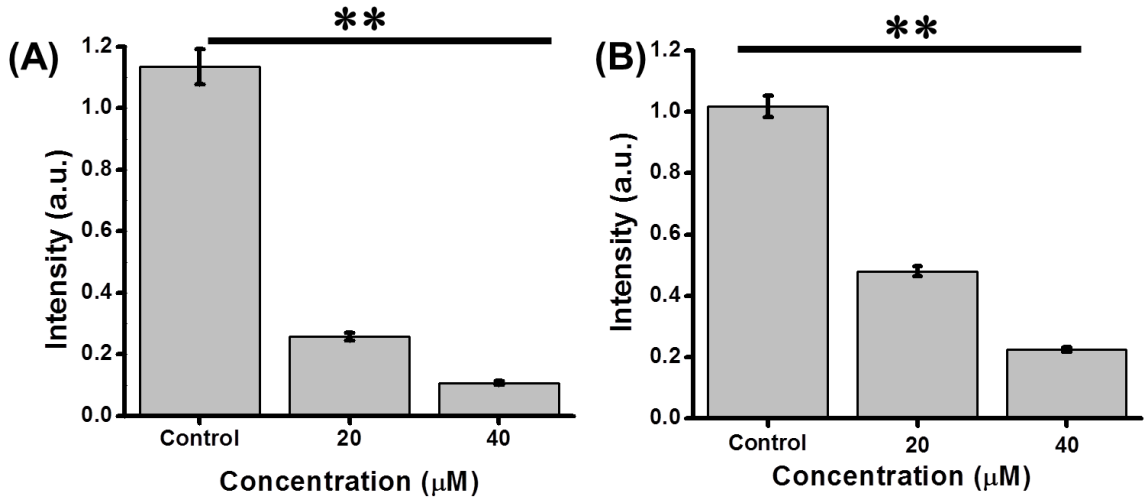


Figure S19: Dot blot experiment with SLKP peptiod in different concentrations. (A). Bar diagram of amyloid aggregation. (B) Bar diagram of amyloid fibrillation.

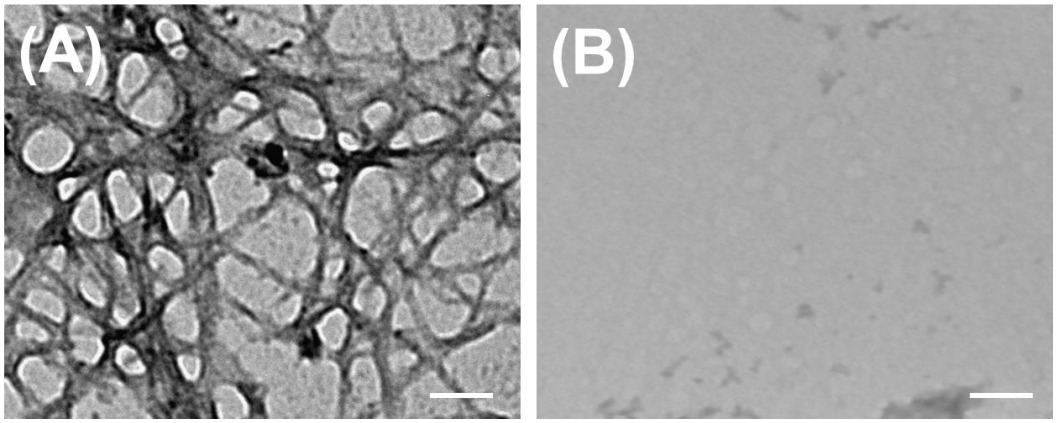


Figure S20: TEM images of Aβ fibril inhibition. (A) Only Aβ42, (B) Aβ42 in presence of SLKP peptoid. TEM study reveals that SLKP peptoid inhibits the fibrillation of Aβ. (Scale bars correspond to 200 nm)

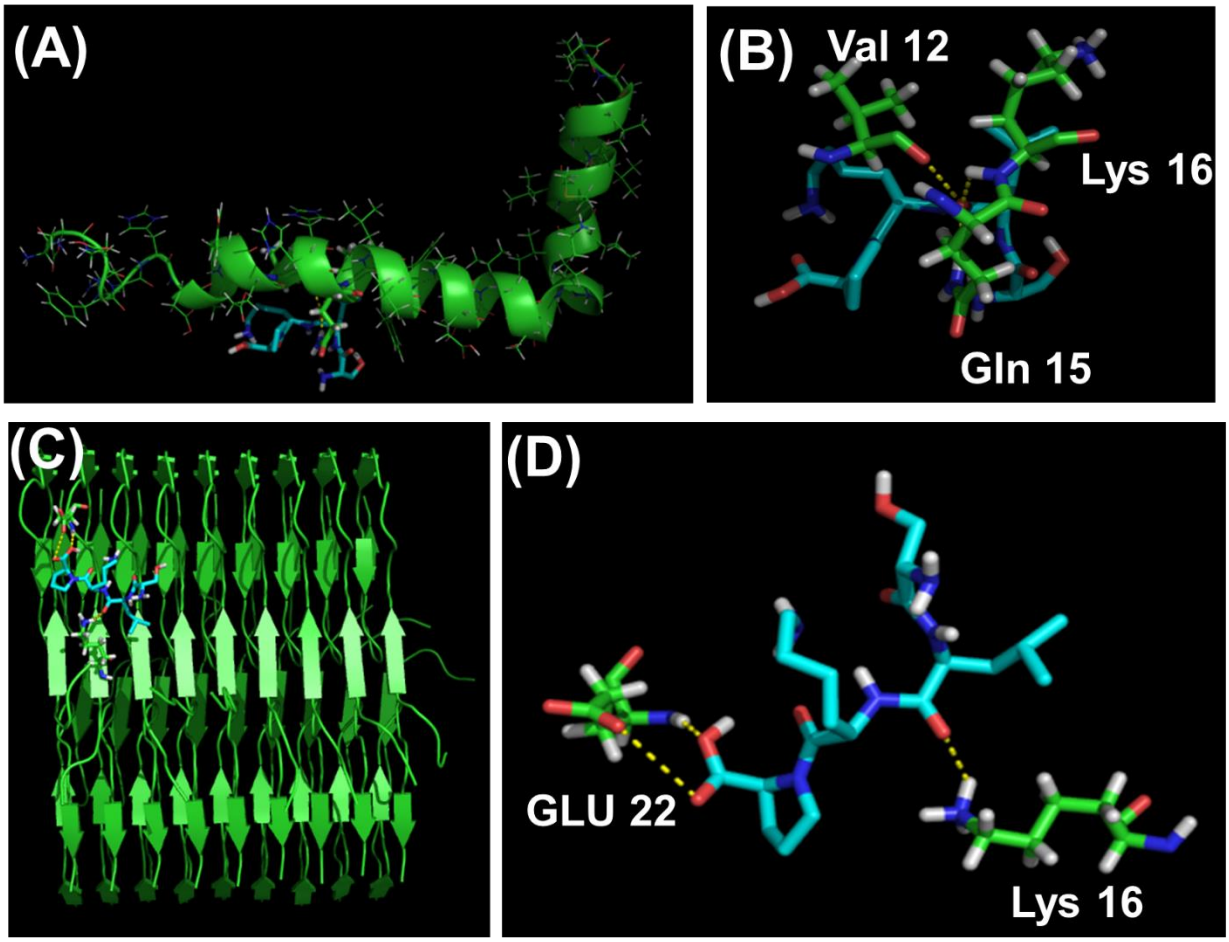


Figure S21: (A) Molecular docking study of SLKP peptide with Aβ peptide reveals that SLKP peptide binds with $A\beta_{12\text{-}16}$ region. (B) Polar binding partner of SLKP peptide with Aβ peptide. (C) Molecular docking study of SLKP peptide with Aβ fibril structure reveals that SLKP peptide binds with $A\beta_{14\text{-}22}$ region. (D) Polar binding partner of SLKP peptide with Aβ fibril structure.

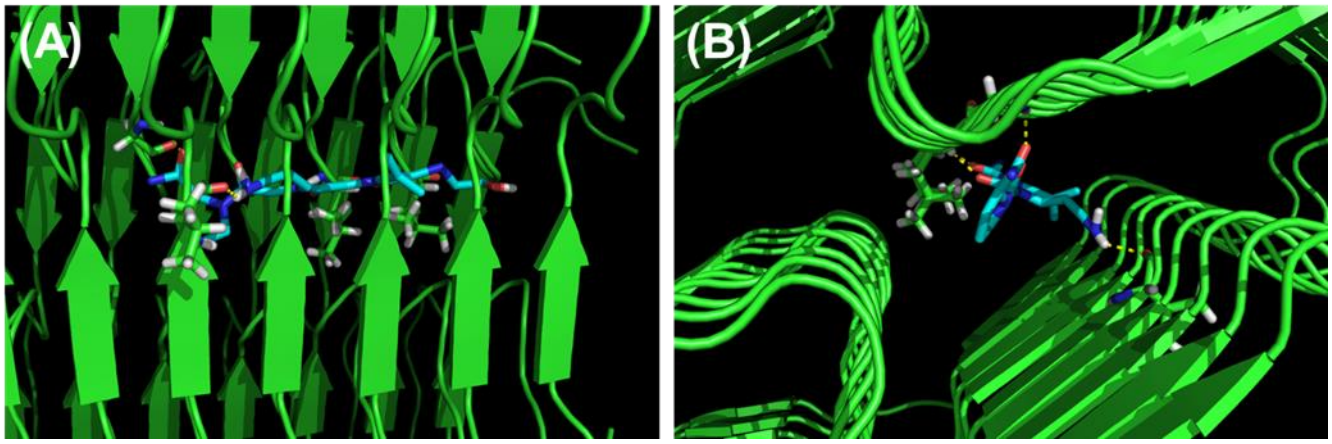
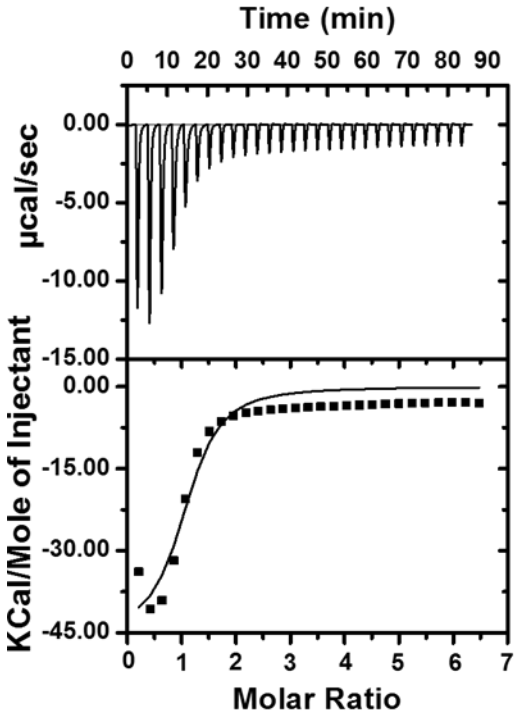


Figure S22: Molecular docking of SLKP peptoid with Aβ fibril. (A) and (B) are images of interaction in different angle.



K_a	$(2.35 \pm 1.03)\times10^5$ M^{-1}
ΔH	-45.31 ± 4.2 Kcal/mol
ΔS	-128 cal/mol/deg

Figure S23: ITC experiment of SLKP peptoid showing strong interaction with Aβ42 peptide.

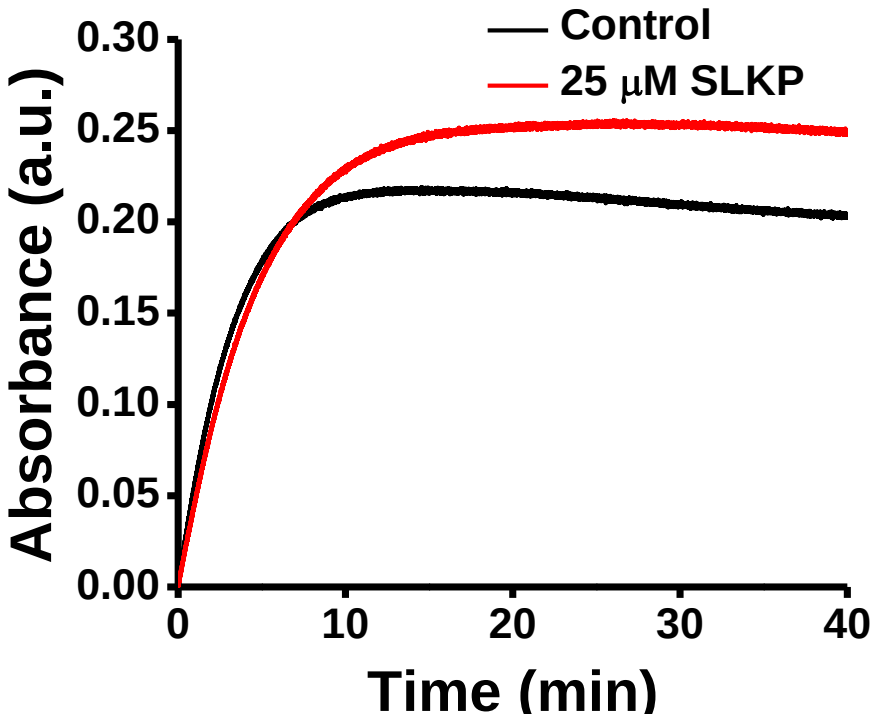


Figure S24: Turbidity assay of SLKP peptoid with the tubulin showing polymerization of microtubule.

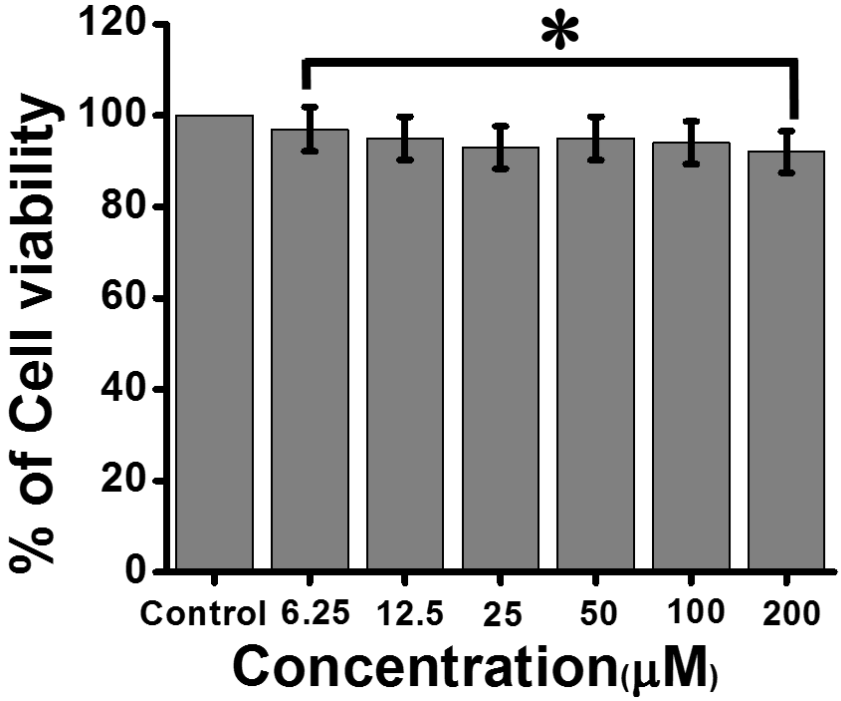


Figure S25: MTT assay of SLKP peptoid showing no cytotoxicity in PC12 derived neurons.

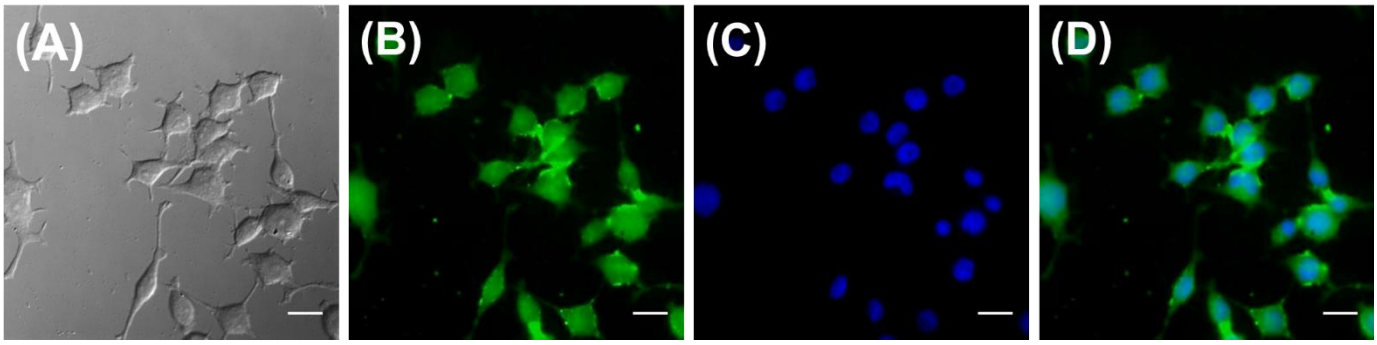


Figure S26: Microscopic images of 5(6)-carboxyfluorescein attached SLKP peptoid in PC12 derived neurons reveal that a significant cellular uptake in the neurons.

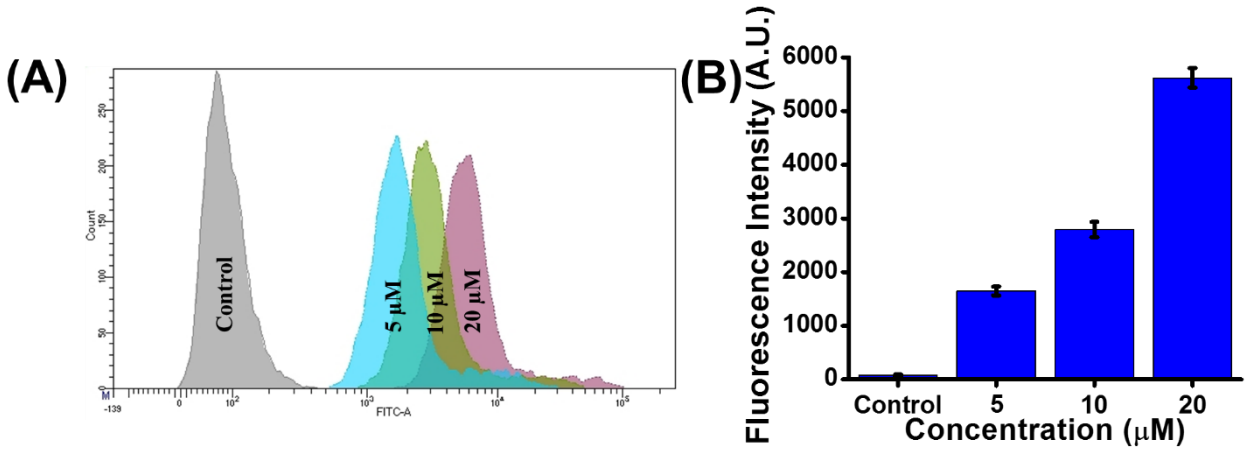


Figure S27: (A) Cellular uptake study of (6)-carboxyfluorescein attached SLKP peptoid through FACS. (B) Quantitative analysis of FACS study.

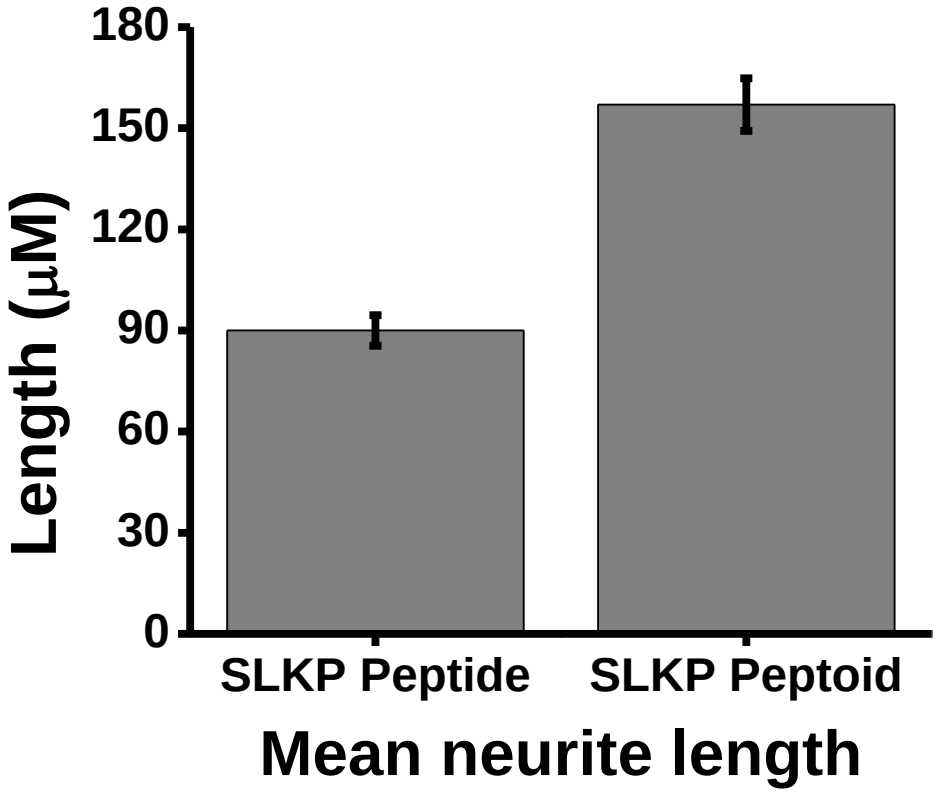


Figure S28: Comparative study of neurite outgrowth for SLKP peptide and peptoid.

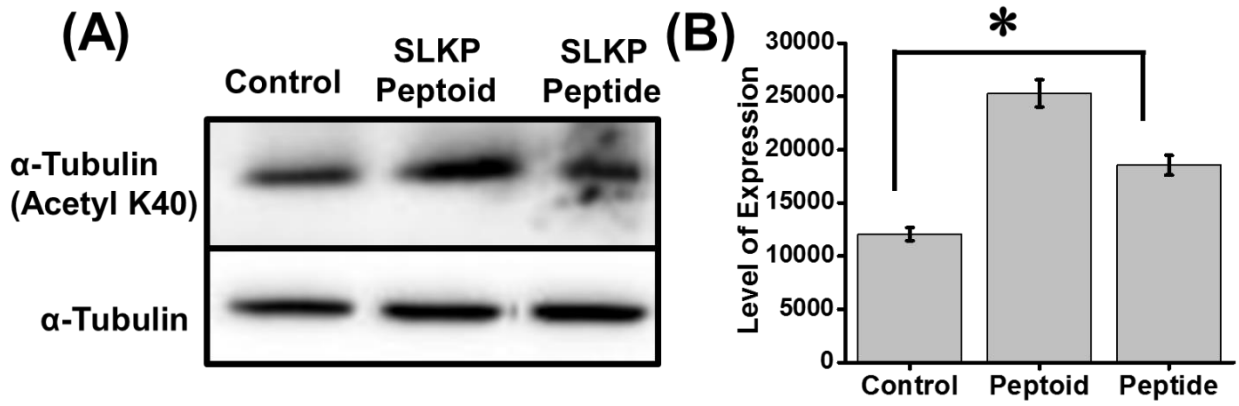


Figure S29: Western blot experiment of acetylated tubulin with the treatment of SLKP peptoid and peptide. (A) Images of the western blot experiment. (F) Quantitative analysis of western blot experiment of acetylated tubulin.

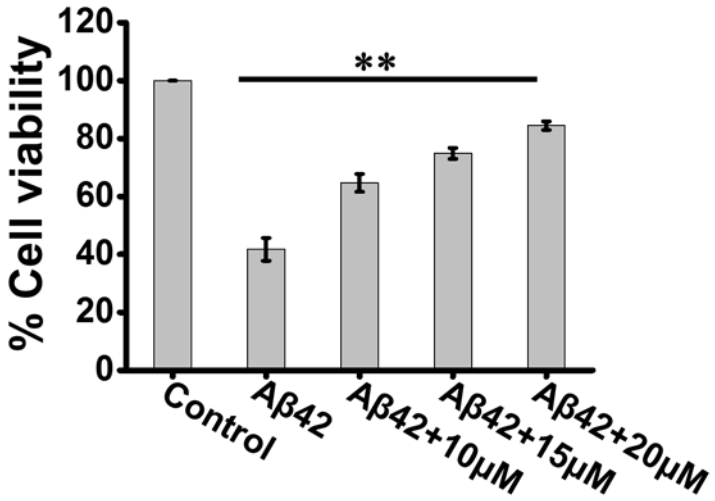


Figure S30: Cell toxicity assay of SLKP peptoid in presence of Aβ42.

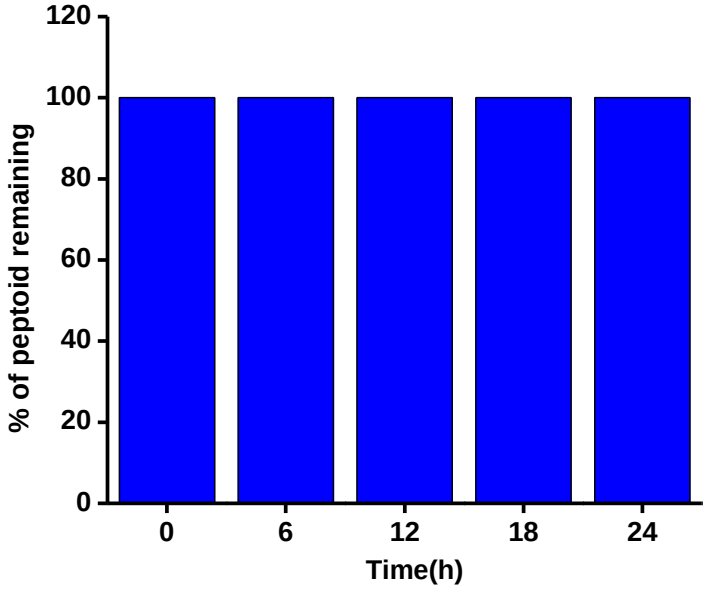


Figure S31: Serum stability of SLKP peptoid showing the SLKP peptoid is stable in human serum.

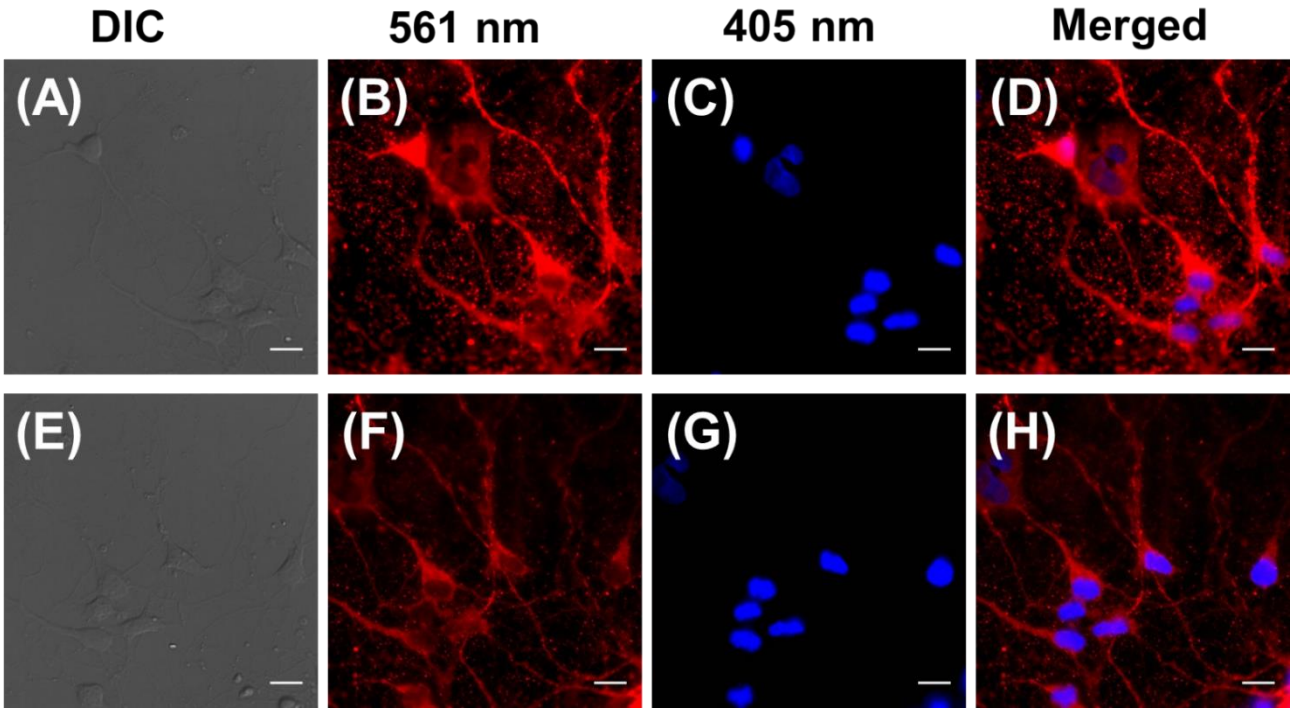


Figure S32: Microscopic images of primary cortical neurons in different channels (A-D) control cells. (E-H) Cells treated with SLKP peptoid. Images reveal that SLKP peptoid maintains healthy morphology of primary cortical neurons. (Scale bars correspond to 20 μm).

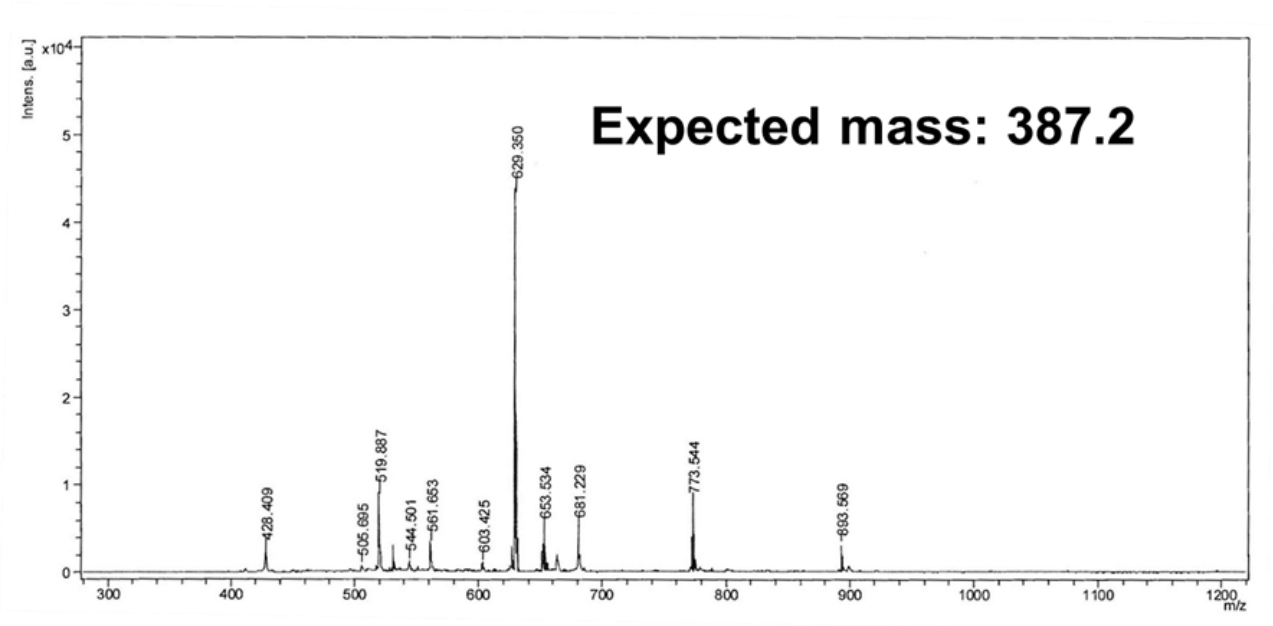


Figure S33. MALDI-TOF mass spectra of mice brain extract of sucrose. Expected mass is 387.2.