Electronic Supplementary Information

Influence of Dityrosine Nanotubes on the Expression of Dopamine and Differentiation in Neural Cells

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THERMAL CHARACTERIZATION. The dynamic thermal behavior of monomers was analyzed using differential scanning calorimeter, DSC Q1000 (TA Instruments, New Castle, DE, USA) equipped with autosampler, digital mass flow controller, and chromel/constantan Tzero thermocouple. The energy changes were recorded for the samples with a temperature range of 0 – 300 °C (Ramp of 1 °C/min).

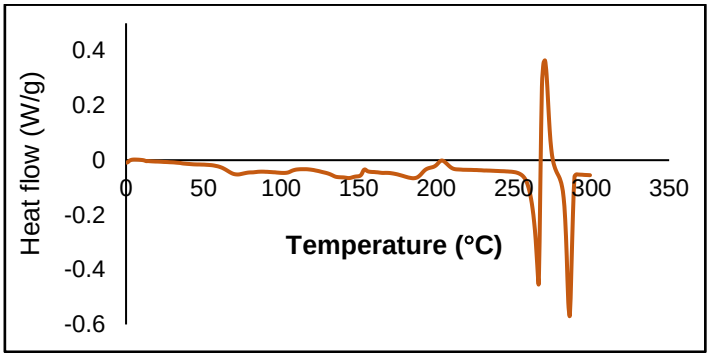


Figure S1. *DSC of YY linear monomer.*

LC-MS. Ultra high-performance liquid chromatography coupled with a hybrid quadrupole time-of-flight mass spectrometry was used to study the changes in the molecular weight of the cyclized and linear YY nanotubes (Waters inc. UHPLC-QTOF MS).

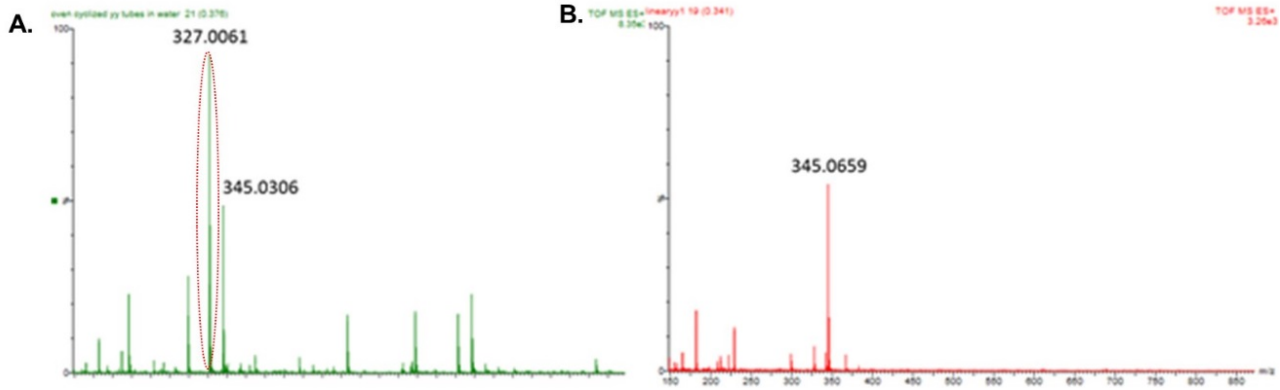


Figure S2. *LCMS spectra of a) Cyclized linear YY tubes and b) Linear YY tubes.*

HPLC. High-performance liquid chromatography was used to study the changes in the molecular weight of the cyclized YY nanotubes (Prominence I, LC 2030 C, Shimadzu) with a C18 column (50 * 4.6 mm) at

UV detection of 280 nm. A gradient elution was used with solvent A (99.9% water with 0.1% HFBA) and solvent (99.9% ACN). The results indicate that the oven cyclized YY nanotubes product consists of mainly cyclized YY peptide (92%) and the remaining was the starting material linear YY peptide (~ 8%). Both compounds were identified through comparison of their retention times. Retention times as obtained by HPLC were 4.058, and 2.498 for the linear YY peptide, and cyclic YY nanotubes respectively combined with a minimal retention at 4.058 for the linear YY peptide.

UV-Vis MEASUREMENTS. UV-Vis absorption of the peptides and nanotubes were measured using an AccuSkan GO spectrophotometer (Thermo-Fisher).

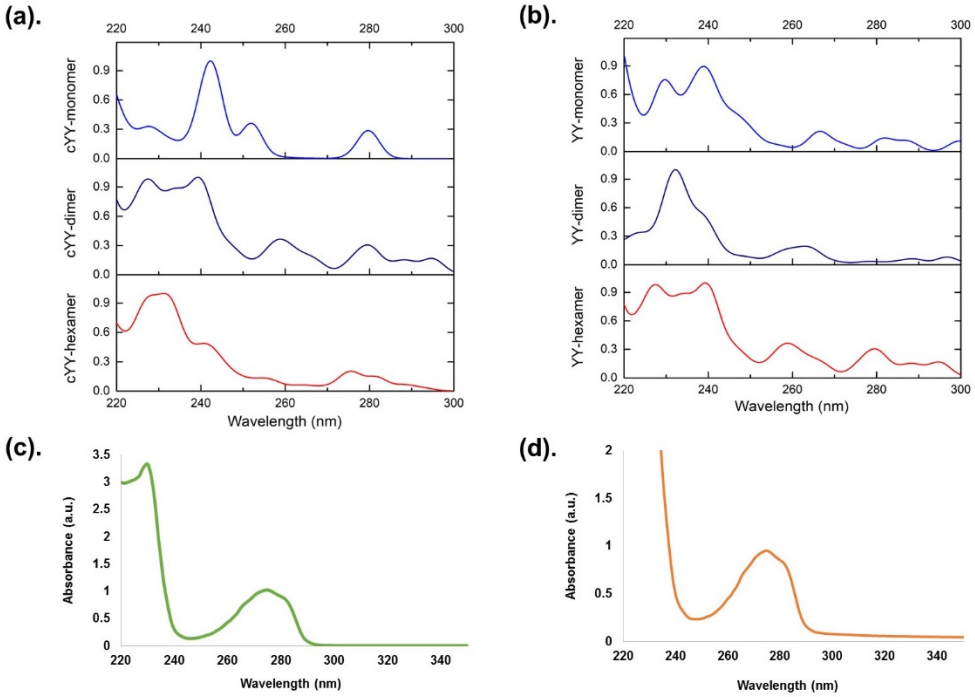


Figure S3. *Calculated UV-Vis peaks for (a). linear YY (monomer, dimer, hexamer); (b). cyclic YY (monomer, dimer, hexamer); Experimental UV-Vis absorption spectra of (c). linear YY nanotubes; (d). Cyclized YY nanotubes.*

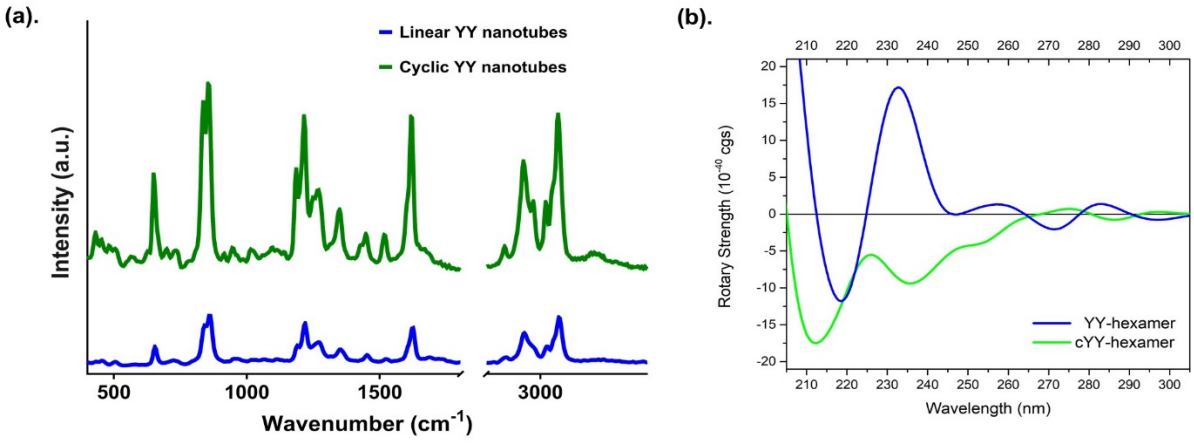


Figure S4. *(a). Raman analysis of the linear and cyclic YY nanotubes; (b). Molar ellipticity calculation using TD-DFT.*

NMR analysis. The proton NMR of oven cyclic YY nanotubes showed a singlet peak at δ 10.26 corresponding to phenolic hydroxy (-OH) proton. The significant amide proton peaks (-CONH) were observed at 7.69 ppm as a doublet whereas the aromatic proton peaks of phenyl ring showed up at 6.77 and 6.60 ppm with defined doublets. The sharp signals at 3.78 ppm indicated the presence of protons of chiral carbon attached to carbamides. The benzylic protons with a value of 3.25 ppm appeared as a sharp singlet.

The ^{13}C NMR of oven cyclic YY nanotubes revealed the presence of amide carbon signal at 166.73 ppm and the phenolic hydroxy attached carbon peaks at 156.54. The aromatic phenyl ring carbon peaks were at 131.22, 127.02, and 115.50 ppm. In addition, benzylic carbons and chiral carbon appeared at 39.27 and 56.21ppm, respectively. The chemical structure of oven cyclic YY nanotubes resulted in our experiment being confirmed based on the ^{1}H and ^{13}C NMR spectra.

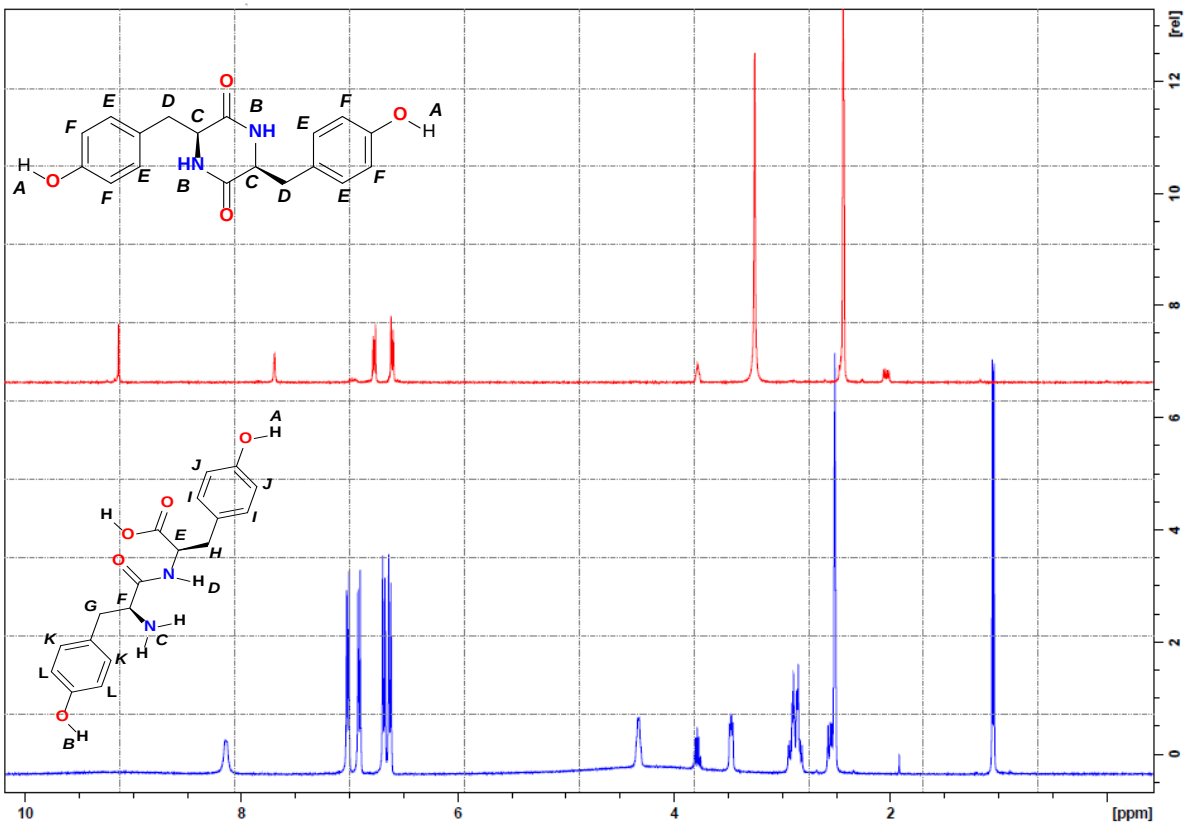


Figure S5. *NMR spectra and chemical structure of YY monomer (blue, commercial from Bachem) and cyclized YY nanotubes following heat-treatment in the oven (red).*

NMR Analysis of heat treated cyclic YY nanotubes:

^{1}H NMR (400 MHz, DMSO) δ 9.13 (s, 2H), 7.69 (d, J = 2.7 Hz, 2H), 6.77 (d, J = 8.4 Hz, 4H), 6.60 (d, J = 8.5 Hz, 4H), 3.78 (s, 2H), 3.25 (s, 4H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 166.73, 156.54, 131.22, 127.02, 115.50, 56.21, 39.27.

Primers (SHSY-5Y)	*Forward*	*Reverse*
AADC	5'-GAAGCCCTGGAGAGAGACAA-3'	5'-CCTTGTTGCAGATAGGACCG-3'
MAOA	5′-GCCCTGTGGTTCTTGTGGTATGT-3′	5′-TGCTCCTCACACCAGTTCTTCTC-3′
VMAT-2	5'-TGAAGAGAGAGGCAACGTCA-3'	5'- CGTCTTCCCCACAAACTCAT-3'
DβH	5'-GCCTTCATCCTCACTGGCTA-3'	5'- TTCTCCCAGTCAGGTGTGTG-3'
GAPDH (control)	5'-ATGAAGGGGTCATTGATGG-3'	5'-AAGGTGAAGGTCGGAGTCAA-3'
RPII (control)	5'-TTGGTGACGACTTGAACTGC-3'	5'-CCATCTTGTCCACCACCTCT-3'

Table S1. *Primers used in SH-SY5Y cells*

Primers (PC12)	*Forward*	*Reverse*
COMT	5' – CCTGACTTCCTGGCGTATG - 3'	5'-TTCTCCAAGCCGTCTACAAC-3'
β III-Tubulin	5'-TCTACGACATCTGCTTCCGC-3'	5'-GTCGAACATCTGCTGGGTGA-3'
GAPDH (control)	AACCTGCCAAGTATGATG	GGAGTTGCTGTTGAAGTC

Table S2. *Primers used in PC12 cells*

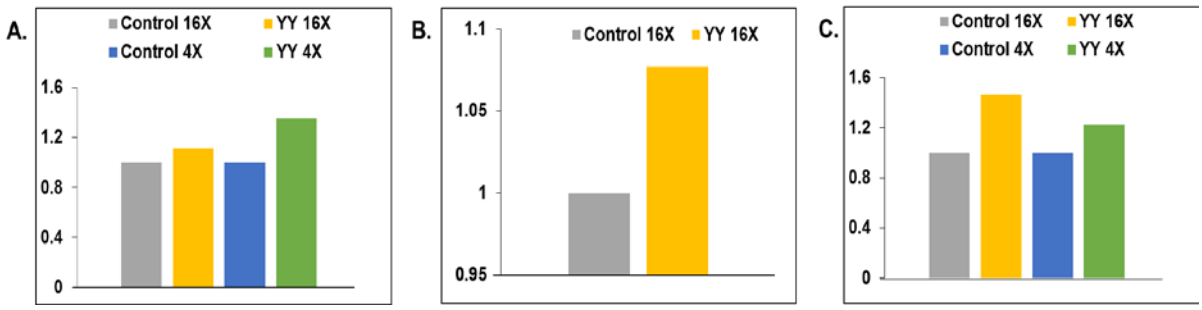


Figure S6. *Fold differences in expression of SHSY5Y cells in (a). MAOA, (b). AADC, (c). DβH (dilutions x/4, x/16)*

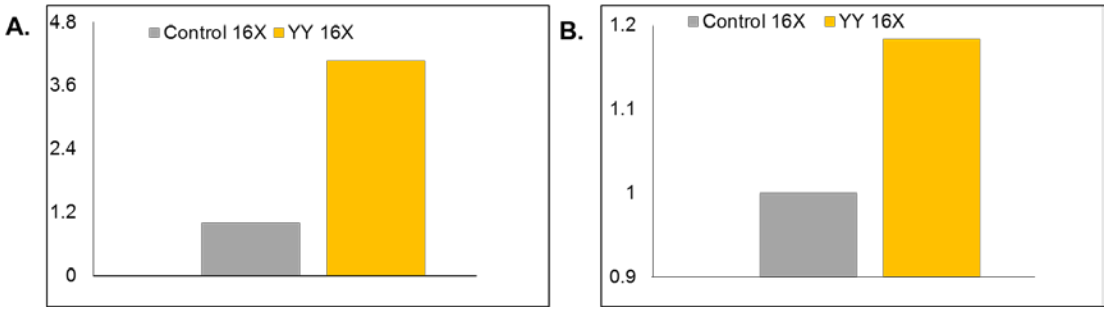
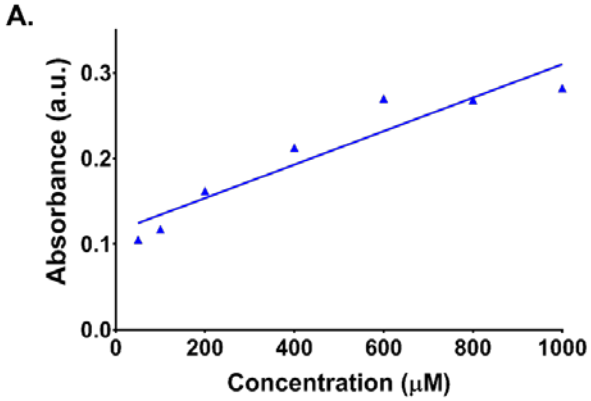


Figure S7. *Fold differences in gene expression of PC12 cells in (a). β III-Tubulin, (b). COMT, (dilution x/16)*

TYROSINE ASSAY. The release of tyrosine into the cell culture media from the cyclized YY nanotubes was tested using a colorimetric assay that measures free tyrosine presence in biological samples from Cell Bio Labs (MET-5073). Glass coverslips coated with 500 μl of 3.3 mg/mL of cyclized YY nanotubes were tested after 3 days of immersion in media.

A.



B.

Samples	Measured Data	Concentration (μM)
YY nanotubes (3 mg/ml)	0.1256	55.2583
YY monomer (3.33 mg/ml)	0.2105	489.9349
L-tyrosine (3.33 mg/ml)	0.216	518.0942

Figure S8. *Standard concentration curve (0 – 1000 μM) and interpolated concentration of tyrosine released in cyclized YY nanotubes.*