Supporting Information

Biotin-avidin interaction triggers conversion of triskelion peptide nanotori into nanochains

Vikas Kumar#, Ramesh Singh# and Khashti Ballabh Joshi*, [a]

Department of Chemistry, School of Chemical Science and Technology, Dr. Harisingh Gour Central University, Sagar, MP, 470003, India
E-mail: kbjoshi77@gmail.com; kbjoshi@dhsgsu.ac.in

Experimental Section

General- Dichloromethane, N, N-dimethylformamide, methanol and triethylamine was distilled following standard procedures prior to use. N, N'-dicyclohexylcarbodiimide, N-hydroxybenzotriazole, D-Biotin, L-tryptophan, and N-hydroxysuccinimide were purchased from Spectrochem, Mumbai, India, and used without further purification. Fluorescein and Hydrochloric acid were purchased from sd fine-chem limited. Tris-(2-aminoethyl) amine was purchased from Aldrich. \(^{1}\)H and \(^{13}\)C NMR spectra were recorded on JEOL-JNM LAMBDA 500 model operating at 500 and 125 MHz, respectively. HRMS spectra were recorded at IIT Kanpur, India, on Waters, Q-Tof Premier micromass HAB 213 mass spectrometer using capillary voltage 2.6-3.2 kV.

Peptide synthesis - All N-biotinyted peptide were synthesized by simple solution phase fragment condensation methodologies using \(^{5}\)Boc chemistry and in the presence of HOBT. Purity of final product (Fig S1) was checked by analytical RP-HPLC (conditions for all purifications: 0.1% trifluoroacetic acid in water (eluent A) to 0.1% trifluoroacetic acid in acetonitrile (eluent B) with the gradient (0-60% of B in 45 min), flow rate: 1 mL/min, column specification: Agilent’s Eclipse XDB-C18, 4.6x250 mm at RT). Concentration of all peptide conjugates for a typical analytical run was ~ 1 mg/mL. (Figure S1).
Atomic Force Microscopy (AFM) – Neat and co-incubated ethanolic solution of biotinylated triskelion peptide (1) and (1) with avidin was imaged with an atomic force microscope. The samples were placed on freshly cleaved muscovite mica surfaces followed by imaging with an atomic force microscope (INNOVA, ICON analytical equipment, Bruker, operating under the acoustic AC mode (AAC or tapping mode), with the aid of a cantilever (NSC 12(c) from MikroMasch, Silicon Nitride Tip) by NanoDrive™ version 8 software. The force constant was 2.0 N/m, while the resonant frequency was ~285 kHz. The images were taken in air at room temperature, with the scan speed of 1.2-1.5 lines/sec. The data analysis was done using of nanoscope analysis software. The sample-loaded substrates were dried at dust free space under 60W lamp for 30 minutes followed by high vacuum drying and subsequently examined under AFM.

Scanning Electron Microscopy (SEM) - A solution of 5 μL aliquots of the fresh samples (1) and (1) with avidin, were dried at room temperature on a freshly cleaved muscovite mica surfaces. Scanning electron microscopy images were made in low vacuum mode using high resolution scanning electron microscope dual beam system (NOVA 450 NANOLAB, FEI) operating at WD 10.6 mm and 20 kV.

Transmission Electron Microscopy (TEM) – The samples were placed on a 400 mesh carbon coated copper grid for 1 minute, excess fluid was removed and the grid was negatively stained with 2% uranyl acetate solution. Excess stain was removed from the grid and the samples were viewed using a FEI Technai 20 U Twin Transmission Electron Microscope, operating at 80 kV. The microscope is a STEM and is also equipped with a EDS detector, HAADF detector and Gatan digital imaging system.

Fluorescence Microscopy – Peptide self-assembled structures were examined under a fluorescent optical microscope (Leica DM2500M), in a dark field. 1 mM solution of the triskelion peptide was incubated with 1 mM solution of fluorescein dye for 1 hour. 10 μL of this solution was spread over glass slide and dried under in dust-free space at room
temperature followed by imaging under optical microscope. The dried samples were thoroughly washed with distilled water 3 X 5s each followed by ethanol for removal of excess and unbounded dye. The samples were analyzed under fluorescent optical microscope.

**NMR titrations** – The purified samples of (1) was prepared by dissolving it into DMSO-$d_6$ followed by the addition of increasing amount of EtOH solution. The $^1$H NMR experiments were carried out at 25 °C on 500 MHz JEOL ECX spectrometer.

![Scheme 1: Synthesis scheme of triskelion biotinylated ditryptophan peptide (1)](image)

**Synthesis of N-Biotinyl-L-tryptophanyl-L-tryptophan-N-hydroxysuccinimide ester (1a).** Compound N-Biotinyl-L-tryptophanyl-L-tryptophan (150 mg, 0.24 mmol, 1 eq) and N-hydroxysuccinimide (30 mg, 0.26 mmol, 1.1 eq) were dissolved in dimethylformamide (5
mL) in two neck round bottom flask and reaction mixture was cooled to 0ºC under nitrogen atmosphere. Solution of N, N’-dicyclohexylcarbodiimide (60 mg, 0.29 mmol, 1.2 eq) in dichloromethane (5 mL) was added into the reaction mixture drop-wise and reaction mixture was stirred for 2 hours at 0 ºC followed by an overnight incubation at 25ºC. Next, the reaction mixture was filtered and filtrate was concentrated under reduced pressure. The white solid was washed with diethyl ether several time and dried under high vacuum pump. The crude product (183 mg, 0.29 mmol) was directly used for synthesis of compound 1.

**N, N’, N”-tris-[N-Biotinyl-L-tryptophanyl-L-tryptophan]-[2-aminoethyl] amine (1).** Compound 1a (150 mg, 0.21 mmol, 1 eq) was dissolved in dry N, N-dimethylformamide (2.5 ml) at room temperature under nitrogen atmosphere. The tris-(2-aminoethyl) amine (TREN) (9 mg, 9.4µl, 0.06 mmol) was dissolved in dry N, N-dimethylformamide (0.5 ml) followed by drop-wise into the reaction mixture under nitrogen atmosphere at room temperature. The reaction mixture was set aside for 24 hours at room temperature under nitrogen atmosphere. Reaction was monitored by analytical TLC, which was performed on Merck silica gel 60 F254 covered aluminum sheets. When the reaction was complete, the solvent was evaporated under reduced pressure and the residue was dissolved in dichloromethane. Organic layer was washed with 1N HCl (3 × 10 mL), 10% NaHCO₃ (3 × 10 mL) and finally brine solution (10 mL). The organic layer was dried over anhydrous sodium sulphate and concentrated to give crude compound 1. The crude compound was purified through a silica gel column chromatography by using dichloromethane methanol (90: 10) solvent system to give pure compound 1 (81 mg, yield 20%). M.P = 181-185°C, ¹H NMR (400 MHz, DMSO-d₆, TMS, δ ppm): 1.06-1.13(m, 6H); 1.28-1.36 (m, 12H); 1.49-1.58 (m, 3H); 1.90-2.01 (m, 6H); 2.46-2.53 (m, 6H); 2.71-2.75(m, 3H); 2.80-2.86 (m, 3H); 2.90-2.92(m, 3H); 3.05-3.12, (m, 6H); 3.13-3.18 (m, 6H); 3.32 (m, 6H, merged signal); 3.98( m, 3H); 4.21, (m, 3H); 4.42 (m, 3H); 4.54 (m, 3H); 6.33 (m, 6H); 6.92-7.11 (overlapped aromatic signals, 18H); 7.27, (m, 6H); 7.47-7.49 (m, 3H), 7.52 (m, 3H); 7.85-7.87, (d, 3H, J = 8 Hz); 8.01-7.03 (d, 3H, J = 6.9 Hz); 10.73 (s, 3H-indolic
NH); 10.82 (s, 3H-indolic NH); $^{13}$C NMR (100 MHz; DMSO-$d_6$, δ ppm): 25.57, 27.46, 28.26, 28.32, 35.50, 52.40, 53.53, 53.66, 55.80, 59.80, 61.49, 109.64, 110.50, 111.80, 112.01, 118.45, 118.79, 119.07, 121.44, 121.60, 124.09, 124.26, 127.81, 128.01, 136.55, 163.50, 172.48, 172.63, 172.95; HRMS: Calculated [M+H]$^+$ = 1941.8696, found 1941.8696(Fig. S4).

**Figure S1:** HPLC of compound 1. Conditions for purifications: 0.1% trifluoroacetic acid in water (eluent A) to 0.1% trifluoroacetic acid in acetonitrile (eluent B) with the gradient (0-60% of B in 45 min), flow rate: 1 mL/min,

**Figure S2:** 500 MHz- $^1$H NMR spectrum of compound 1 in DMSO-$D_6$. 
Figure S3: 125 MHz-\textsuperscript{13}C-NMR spectrum of compound 1 in DMSO-$D_6$. 
Figure S4: HRMS of HPLC purified compound 1.

Figure S5: Fluorescence titration spectra of avidin with 1, \( \lambda_{\text{ex}} = 270 \) nm and \( \lambda_{\text{em}} = 350 \) nm
Figure S6: Fluorescence titration spectra of 1 with avidin, $\lambda_{\text{ex}} = 270$ nm and $\lambda_{\text{em}} = 350$ nm.

Figure S7: AFM images of A) self-assembled structures of 1 depicts formation of intact multiple nanotorus which is further confirmed by corresponding D) high resolution AFM image. Image B) in the presence of 0.1 equiv. of avidin and C) 0.2 equiv. of avidin showing dimer and chain formation respectively. Images E) and F) representation of number 080, and F) random connectivity of nanotorus at increased amount of avidin.
Figure S8: SEM images of A) self assembled structures of 1, Image B) in the presence of 0.1 equiv. of avidin and C) 0.2 equiv. of avidin showing dimer and chain formation respectively D) and E) random connectivity of nanotorus at increased amount of avidin.

Figure S9: TEM images of nanotorus formation.
Figure S10: Fluorescence optical micrograph of fluorescein dye stained sample of nanotori and magnified images of nanotorus.

Scheme-2: Depicts the formation of nanotorus initiated via the formation of nanotube/fibril by pi-pi stacking interactions where the biotin groups are extruded from the tube which further interact to each other in the presence of ethanol and guide the bending followed by torus formations. The shape transformation into dimer and chain in the presence of avidin can easily be achieved also.
Figure S11- AFM images depicts the effect of other solvents in the self assembly process of 1. (A) in the presence of methanol, (B) in the presence of 50% aqueous methanol/ethanol and (C) in the presence of DCM. (D, E and F) are the corresponding 3D images of (A, B and C).