Supporting information

Graphene Oxide as a Dual Template for Induced Helicity of Peptides

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Experimental method:

Reagents and materials. Graphite was obtained from Asbury Carbons Inc.-USA and Carbon nanofiber. The exfoliation of graphite powder was carried out using following chemicals such as phosphorus pentoxide (P_2O_5, 98%) potassium persulphate ($K_2S_2O_8$, 99%) and potassium permanganate ($KMnO_4$, 99%), hydrochloric acid (HCl, 37%), sulphuric acid (H_2SO_4, 98%), hydrogen peroxide (H_2O_2, 30%), dibasic sodium phosphate heptahydrate (Na_2HPO_4 $7H_2O$, 95%) and mono basic sodium phosphate (NaH_2PO_4, 99%) dimethyl sulphoxide (DMSO, 99.5%), ethanol (EtOH, 99.9%) and N,N-dimethylformamide (DMF, 99.5 %). All the Fmoc and orthogonally protected amino acids, 1-hydroxybenzotriazole (HOBt), (1[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxid hexafluorophosphate) (HATU), 1-hydroxy-7-azabenzotriazole (HOAt) as well as Rink amide resins were purchased from GL Biochem, Shanghai, China. N, N'-diisopropylcarbodimide (DIC), N, N-diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA), triisopropylsilane (TIPS) and 5(6)-carboxyfluorescein were purchased

from Sigma-Aldrich. Piperidine was purchased from Spectrochem Private Ltd. All chemicals were used as received.

Synthesis and characterization of GO. GO was prepared using modified Hummer's method[1]. For preparation of small size GO, we have used Carbon nanofiber as discussed in our earlier report. In typical reaction, $K_2S_2O_8$ (1 g) and P_2O_5 (1 g) were added to 15 mL of conc. H_2SO_4 solution and stirred at 80 ^oC temperature until complete dissolution. Graphite powder (1 g) was added to the solution and kept at 80 ^oC for 3 h. Then the mixture was cooled and diluted with deionized water (100 mL), followed by filtered using filter paper grade 3. Further, the mixture was rinsed with 1.2 liter distilled water to remove residual salt or reactants and dried in air. The pre-treated graphite powder was collected and transferred into a 500 mL RB flask with 45 mL of conc. H_2SO_4 and chilled to 0 ^oC using an ice bath. Then $KMnO_4$ (6 g) was slowly added to the mixture while stirring, taking caution to keep the temperature below 10 ^oC. Then the RB flask was moved to 42 ^oC water bath and left for 2 h. Then, it was transferred back into the ice bath. 100 mL of distilled water was slowly added to the RB while stirring, taking caution that the temperature did not rise above 55 ^oC. After dilution, 4 mL of 30% H_2O_2 solution was added slowly (without overflowing) to the mixture to avoid violent bubble formation. The colour of the solution turned bright yellow. This was filtered and rinsed with 3.4% HCl solution to remove residual salts. The resulting wet solid mass was re-dispersed in distilled water and centrifuge at 1000 rpm for 15 min. The supernatant was taken and labelled as small size GO (average size distribution ~40-50 nm). To prepare very large size GO (average size distribution ~4-7 μm) we slightly modified our GO preparation. We have used 0.5 g graphite and 20 g of NaCl which was grinded for 30 mins then dissolved in distilled water followed by filtration and drying for 12 h. We have used that dried material for exfoliation using the above protocol.

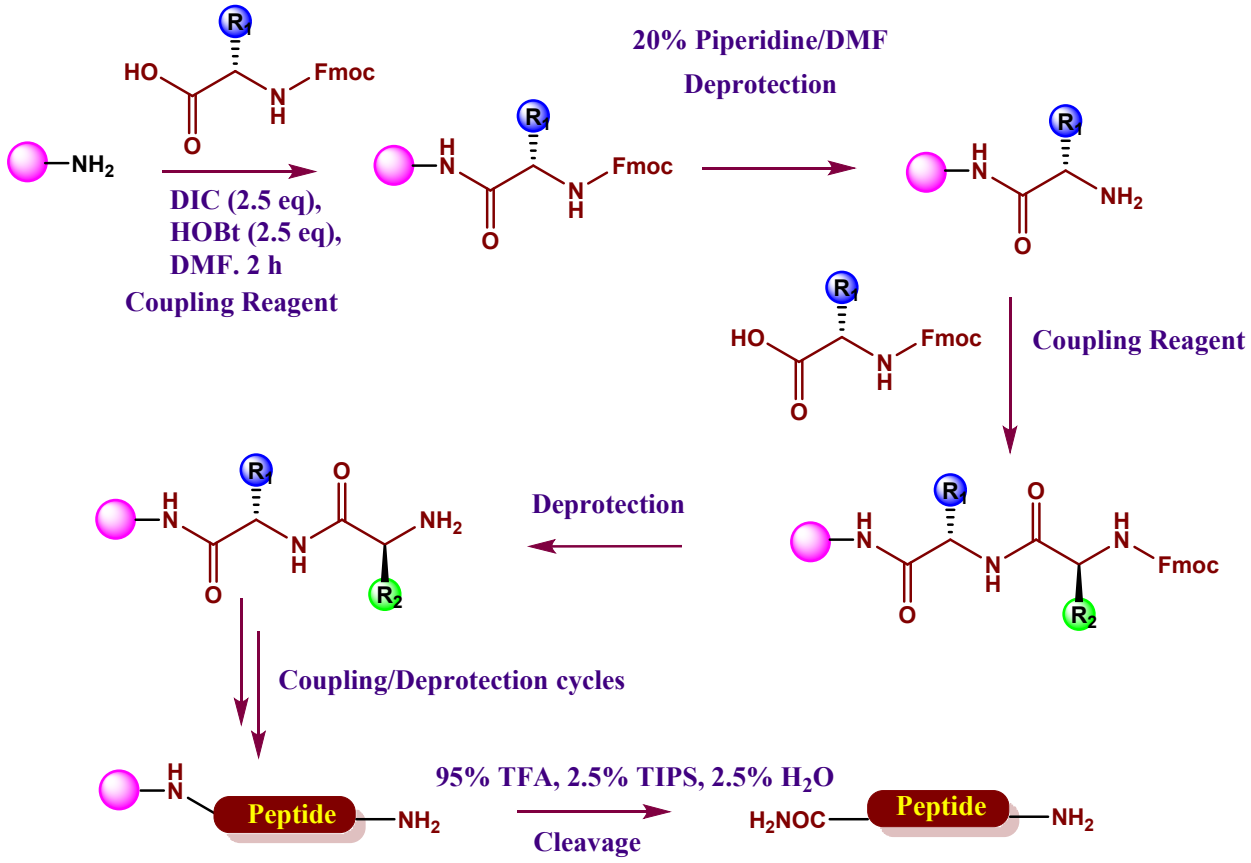
Fluorescence Spectroscopy study. Fluorescence measurement was performed on Varian Fluorescence Spectrophotometer. Fluorescence spectra of the Trp tagged peptide-2 with GO was measured in 5 mM sodium phosphate buffer, pH 7.4 using quartz cell (path length 1 cm) and emission wavelength range from 280 to 450 nm.

Circular Dichroism (CD) study. JASCO J-815 CD spectrophotometer was used to measure the CD spectra at room temperature. CD spectra of each of the native peptide and as well as GO-peptide complex were measured, to compare the conformational changes in secondary structure. The spectra were recorded over a range of 190 -250 nm using quartz cell of path length 0.1 cm. Each CD spectrum measured was an average of three scans.

Atomic Force Microscopy study. JPK Instrument was used for AFM sample analysis. For sample preparation we used freshly cleaned mica foil sheet, followed by very dilute solution (10 μg/mL) of GO and GO-peptides complexes were drop casted onto the mica foil and vacuum dried for overnight. We have used resonance energy 300 KHz and force constant 40 N/m in the measurement for imaging the samples.

Fluorescence Microscopy study. JPK Instrument was used for florescence imaging of sample. We have also used in previous set up the resonance energy 300 KHz and force constant 40 N/m in the measurement for imaging the samples. For sample preparation we have used glass slide, which cleaned with isopropanol three times followed by acetone washed then we kept drying for 30 min. A very dilute solution of GO-peptides complexes was drop casted onto the cleaned surface of glass slide and kept for vacuum dried. Then the experiment was performed using halogen lamp to excited the sample based on molecular fluorophore. To get FAM conjugated emission color, we have excited blue region in halogen lamp and then it emits green color wavelength of the sample which we have recorded.

Synthesis of Peptides. All Peptides were synthesized on Rink Amide AM resin (0.8 $mmolg^{-1}$) on 200 mg scale (0.16 mmol) using standard Fmoc-based strategy. The resin was swollen in DMF and deprotected with 20% piperidine in DMF (5 min x 1, 15 min x 1) followed by thorough washing with DMF (3 times). The C-teminal amino acid, Fmoc-Asp(tBu)-OH (2.5 eq.) was loaded onto the resin by using standard coupling reagents (2.5 equiv HOBt, 2.5 equiv DIC) in DMF for 2 hours at room temperature. The entire peptide was assembled with this same protocol as well.



N-terminal Acetylation of the peptides. After synthesis the desired sequences the final N-terminal Fmoc deprotection of the peptide 1**,** 2 and 3 was carried out. Following to this the free primary amine was acetylation taken place with acetic anhydride (2.5 equiv) and DIPEA (2.5

equiv) for 5 mins in DCM at room temperature. The resin was washed thoroughly with DMF (3 times) followed by DCM (2 times) to obtain the final acetylated product.

FAM Conjugation. A fraction of amine free product (peptide 1 and peptide 2) was coupled with 5(6)-carboxyfluorescein in the presence of HOAt (3 equiv), HATU (3 equiv) and DIPEA (6 equiv) as coupling reagents for 4 hours in the dark.

Global Deprotection and Cleavage from the Resin. Peptide**s** were cleaved off from the resin and globally deprotected by using the cleavage cocktail TFA: TIPS: H_2O (95:2.5:2.5) for 1.5 hours at room temperature. Peptide-containing supernatants were separated from the solid support by filtration and concentrated under a stream of nitrogen. Crude peptide was precipitated and washed twice with ice-cold diethyl ether.

Peptide purification and Characterization. The formation of peptide was confirmed by using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Germany) and ESI mass spectrometry. ESI mass spectra were recorded in positive ion mode on a HCTultra ETD II ion trap spectrometer (PTM Discovery System, Bruker Daltonics, Germany). Peptide purity was determined by a Shimadzu UFLC system equipped with Prominence Diode Array (PDA) UV Detector at 210 and 254 nm using an analytical column (Phenomenex C18, 250 mm x 4.6 mm I.D., 5μm) at a flow rate of 1 mL/ min. Purifications were performed using a semi-preparative column (Phenomenex C18, 250 mm x 10 mm I.D., 5 μm) at a flow rate of 4 mL /min.

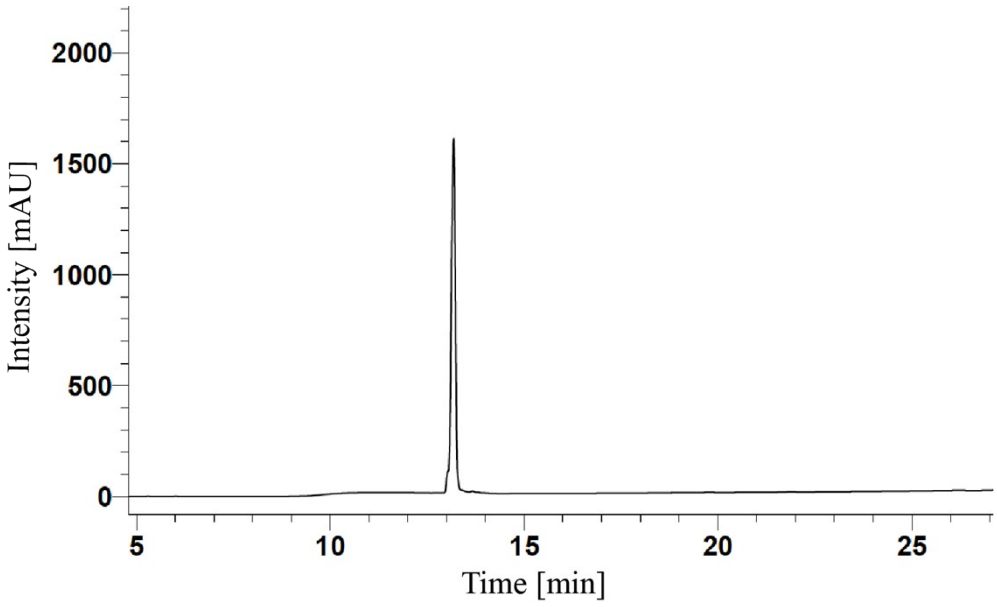
Molecular dynamics simulations. The all-atom molecular dynamics simulations of peptides and Graphene Oxide (GO) system in water were performed for 200 ns using amber14sb forcefield and GAFF with TIP3P water model[2, 3] . All simulations were carried out in the isothermal-isobaric

(NPT) ensemble and an integration time step of 2 fs was used for the unrestrained simulation. The pressure was controlled at 1 atm and temperature was retained at 300 K using Parrinelo-Rahman barostat and V-rescale thermostat[4-6] , respectively. The electrostatic interaction was calculated using Particle Mesh Ewald[7] with a non-bonded cut-off distance of 0.9 nm. All bonds involving hydrogens were constrained using the LINCS algorithm[8] and periodic boundary conditions are applied in all three dimensions. The simulation systems are neutralized at 150 mM salt (Na^+ and $H_2PO_4^-$) concentration. Before the production runs, a 5000-step minimization was carried out with the steepest descent algorithm followed by an NVT and NPT equilibration for 1 ns. The trajectories were saved every 100 ps and all the simulations are carried out using GROMACS 2020.4[9] and VMD[10] and UCSF Chimera[11] was used for the visualization.

Peptide 1

Ac-A-A-A-R-A-D-A-R-A-A-R-A-A-A-R-A-D-NH_2

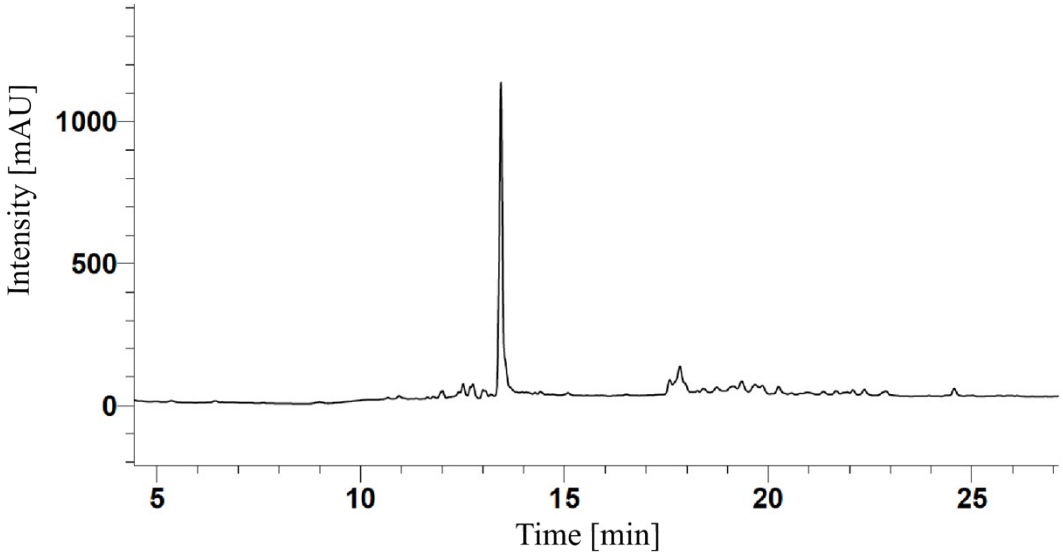
Purity check: 15-60% ACN, 20 min (detection at 220 nm)



Peptide 2

Ac-A-A-A-E-A-D-A-E-A-A-E-A-A-A-E-A-D-NH_2

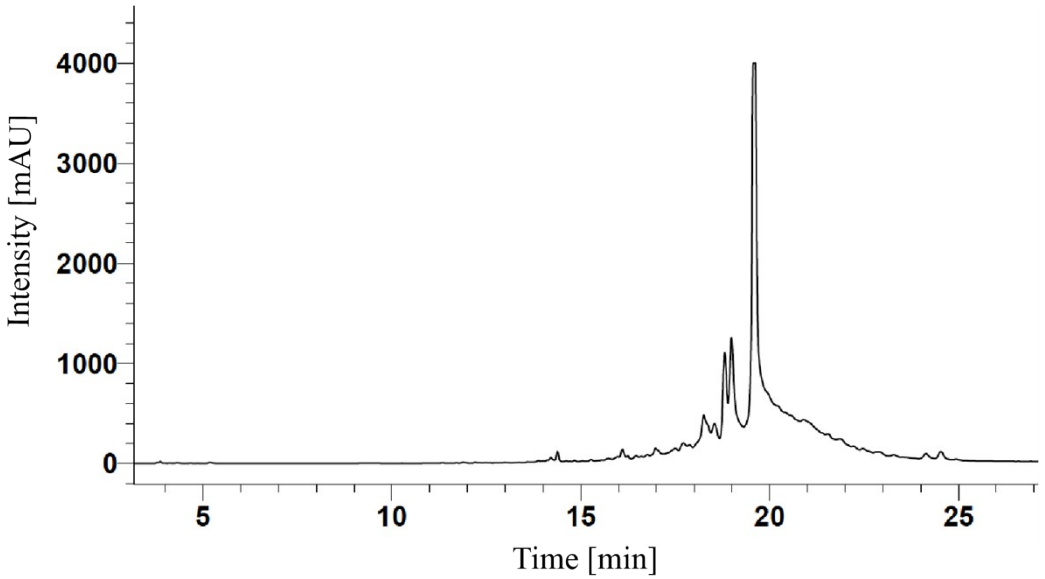
Purity check: 15-60% ACN, 20 min (detection at 220 nm)



Peptide 3

Ac-A-A-A-W-A-D-A-W-A-A-W-A-A-A-W-A-D-NH_2

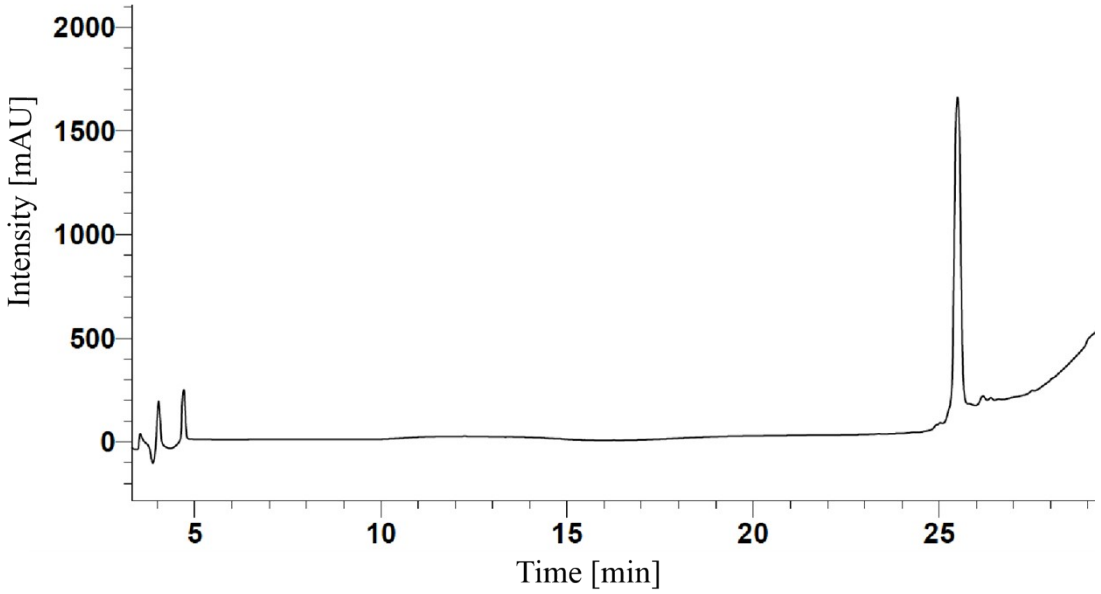
Purity check: 20-80% ACN, 20 min (detection at 220 nm)



FAM conjugated Peptide 1

FAM-A-A-A-R-A-D-A-R-A-A-R-A-A-A-R-A-D-NH_2

Purity check: 20-70% ACN, 20 min (detection at 220 nm)



Dynamic light scattering (DLS).

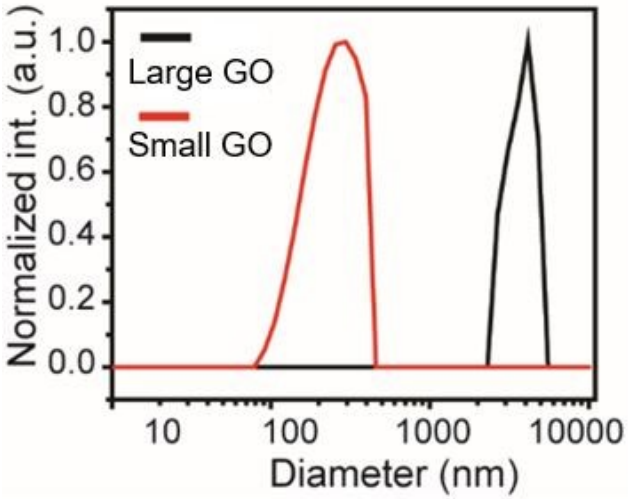


Figure S1. Dynamic light scattering (DLS) of different size GOs in 5 mM sodium phosphate buffer pH 7.4.

Zeta potential plot.

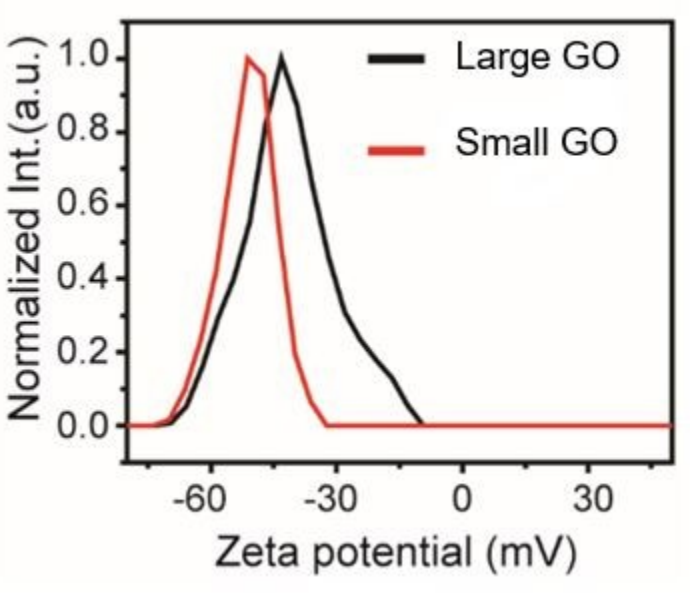


Figure S2. Zeta potential plot of different size GOs in 5 mM sodium phosphate buffer pH 7.4.

X-ray photoelectron spectroscopy (XPS).

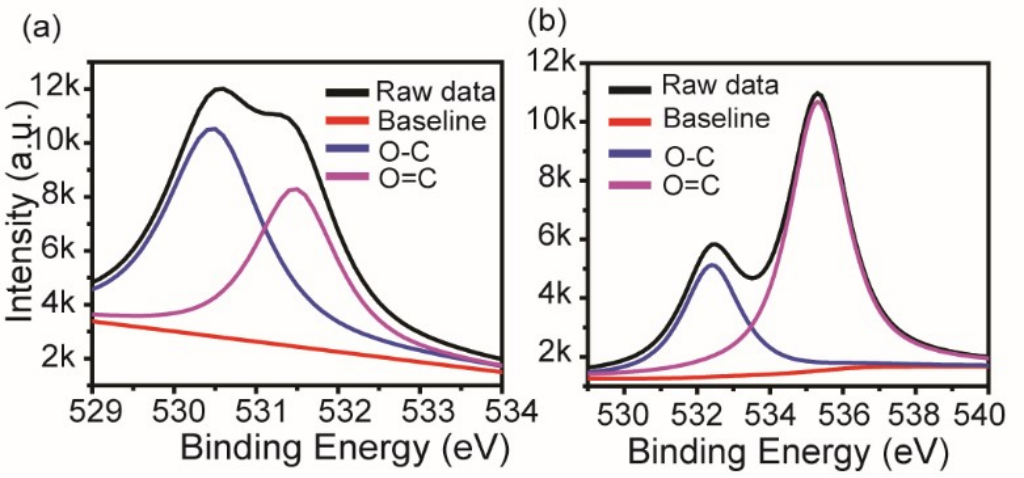


Figure S3. High resolution X-ray photoelectron spectra of different size GOs. O1s spectra of large GO (a) and small GO (b).

Circular Dichroism Study.

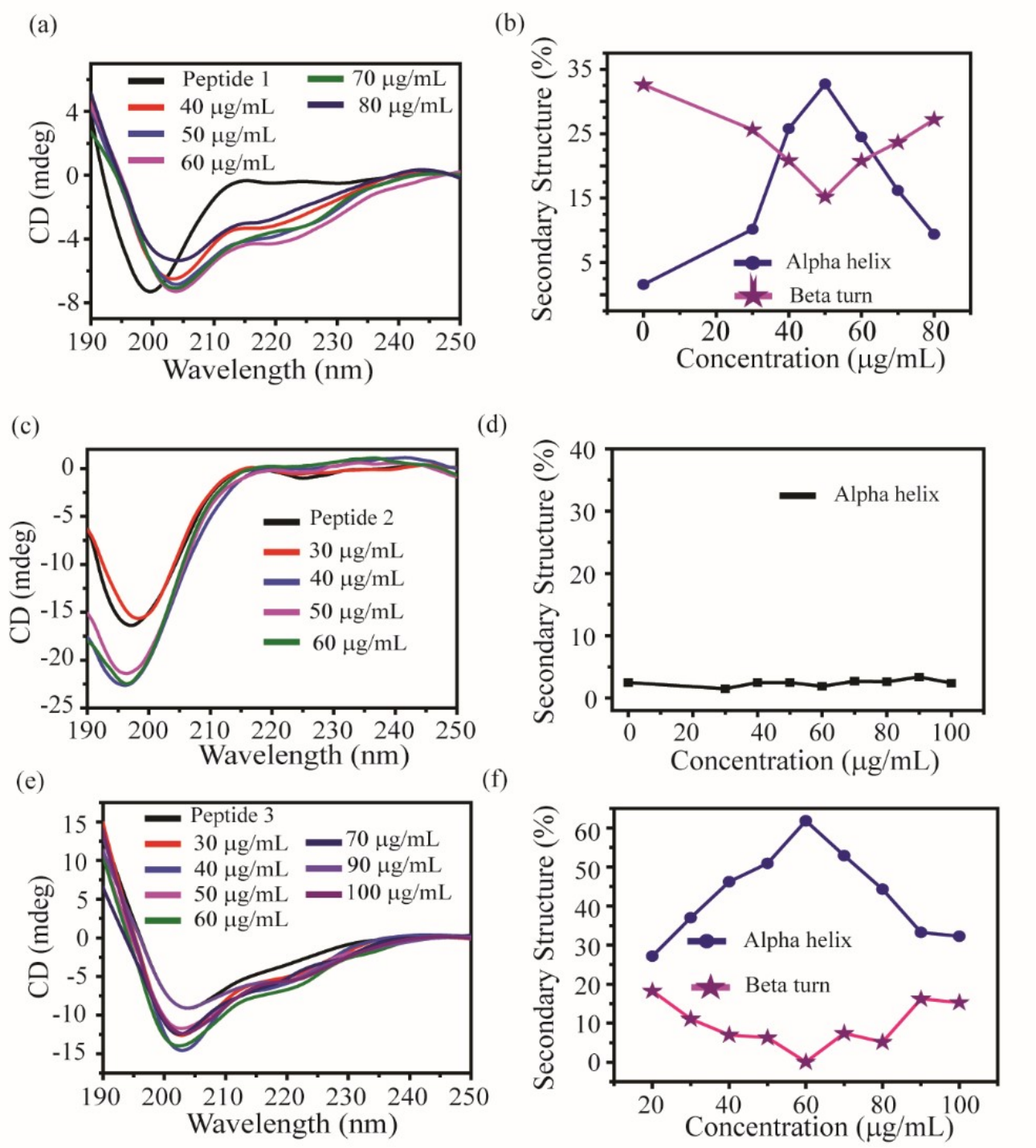
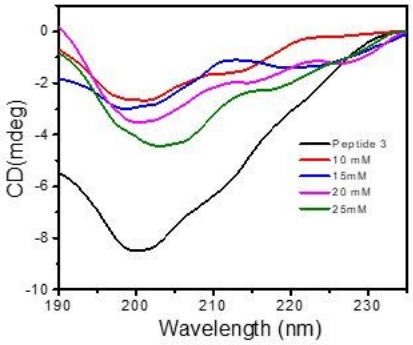


Figure S4. CD study of peptide-1, Peptide-2 and Peptide-3 in the presence of large size GOs: (a), (c) and (e); percentage of secondary structure (b), (d) and (f) of respective peptides in 5 mM sodium phosphate buffer pH 7.4.



Salt study of peptide 3.

Figure S5: Salt study of large sizes GO incubated with peptide 3 at different concentration of salt.

Secondary structure of FAM conjugated peptide 1.

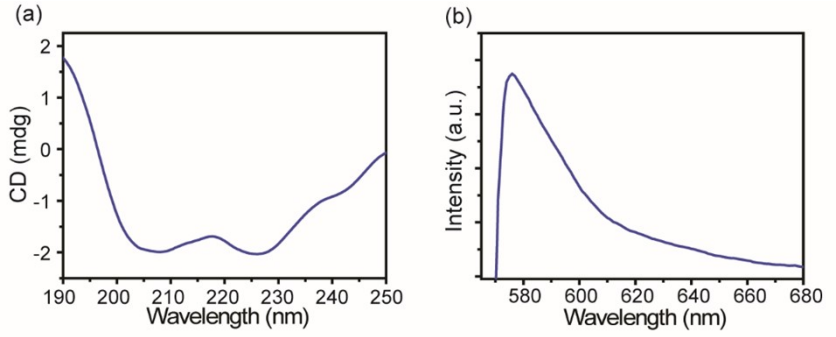


Figure S6. Circular dichroism study (a) and Fluorescence spectra (b) of peptide 1 with large GO in 5 mM sodium phosphate buffer at pH 7.4.

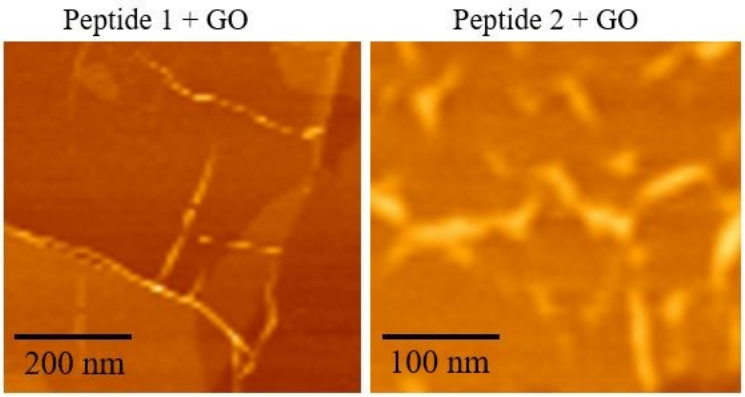


Figure S7. The magnified images of the peptide 1 and Peptide 2 in the presence of GO, the wrinkles observed at the edges for peptide 1 and surface for peptide 2.

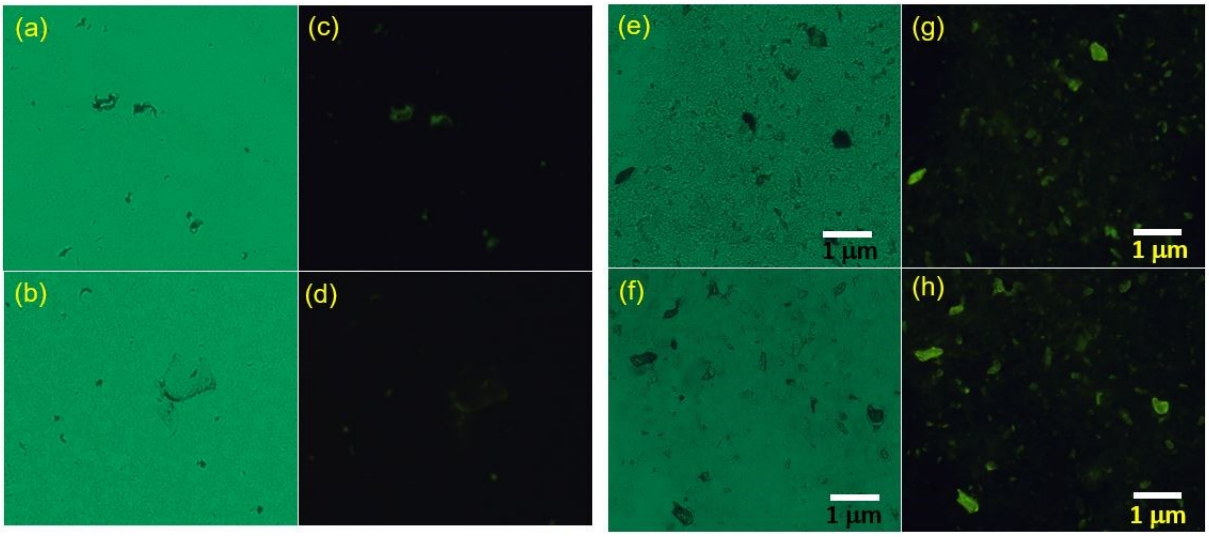


Figure S8. Fluorescence microscopy image of FAM conjugated peptide 1 incubated with GO (a); (b) bright field images both are same sample but different region and (c); (d) corresponding fluorescence images, and FAM conjugated peptide 2 incubated with GO (e); (f) bright field images and (g); (h) corresponding fluorescence images.

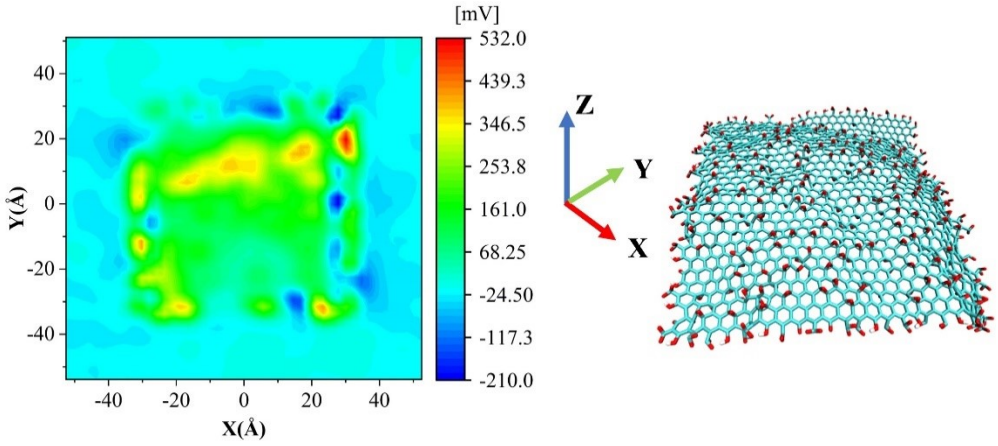
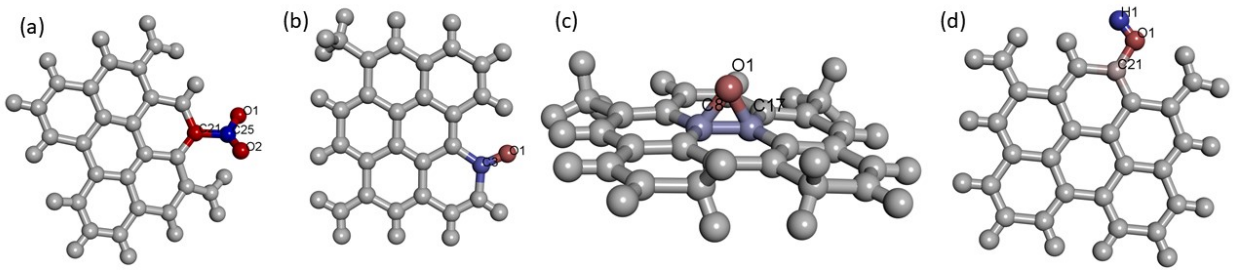


Figure S9. Electrostatic potential of GO in water solution along the normal of the plane. The green region is mainly comprised of GO. Near the GO edges (blue region), the potential varies between -20 meV to -60 meV.

Table S1: The partial charges of individual units of the GO sheet. The calculations are performed at HF/6-31G*. The diagram shows the representation of small units and corresponding atoms.

Atom name	Partial charge
O1	-1.4201
C25	3.2293
O2	-1.4201
C21	-1.3891
O1	-0.3959
C3	0.3959
C8	0.1682
C17	0.1682



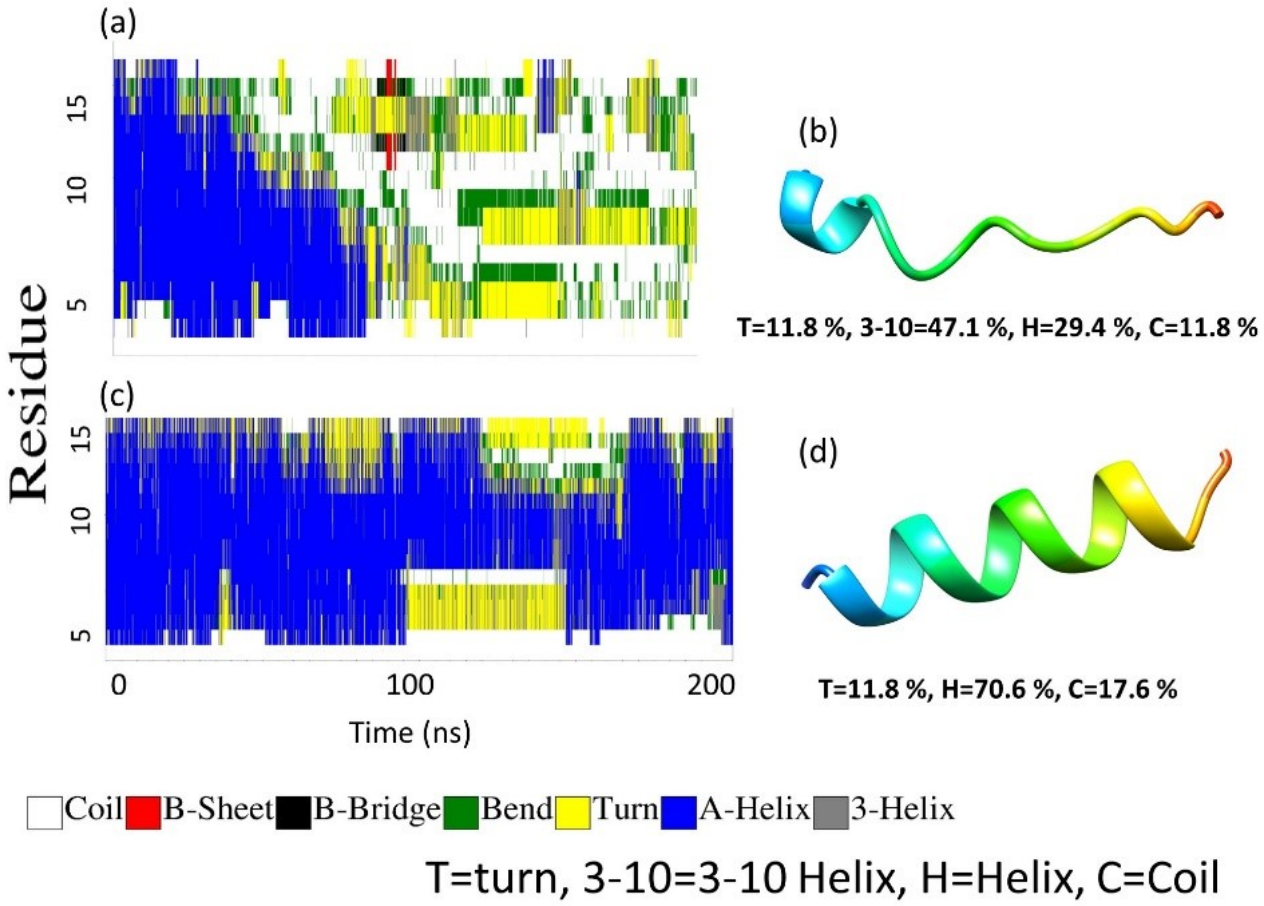


Figure S10. The secondary structure transition of peptide-1 during the 200 ns simulation. The top panel represents the peptide-1 in water and the bottom panel represent the peptide-1 with graphene oxide (a & c). The average peptide structure during the 200 ns simulation time is provided on the right side (b & d).

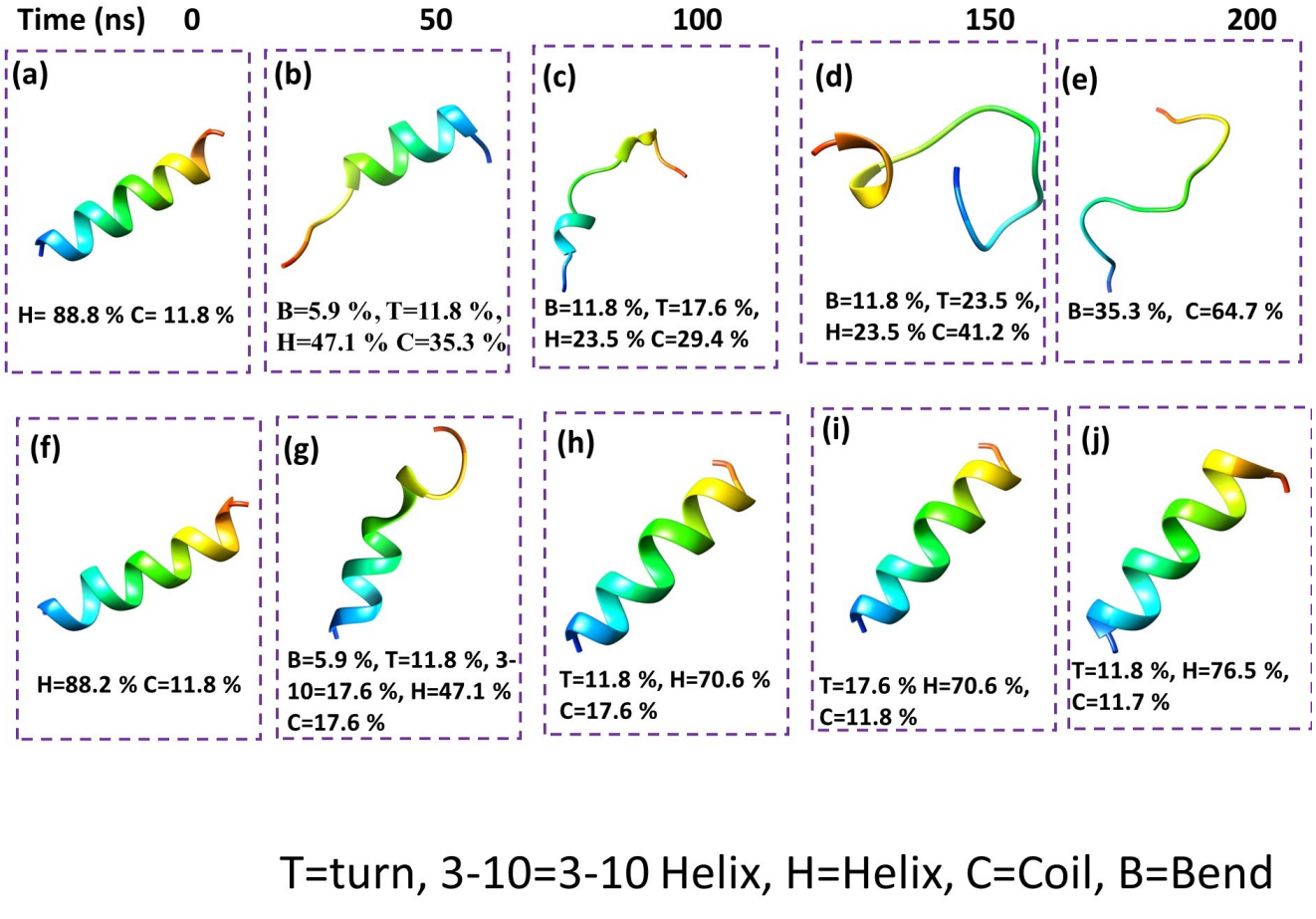


Figure S11. The time-dependant secondary structural transition of peptide-1 in 50 ns time interval (0 ns to 200 ns). The top panel (a-e) represents the transition in water and the bottom panel (f-j) represent the transition in presence of graphene oxide.

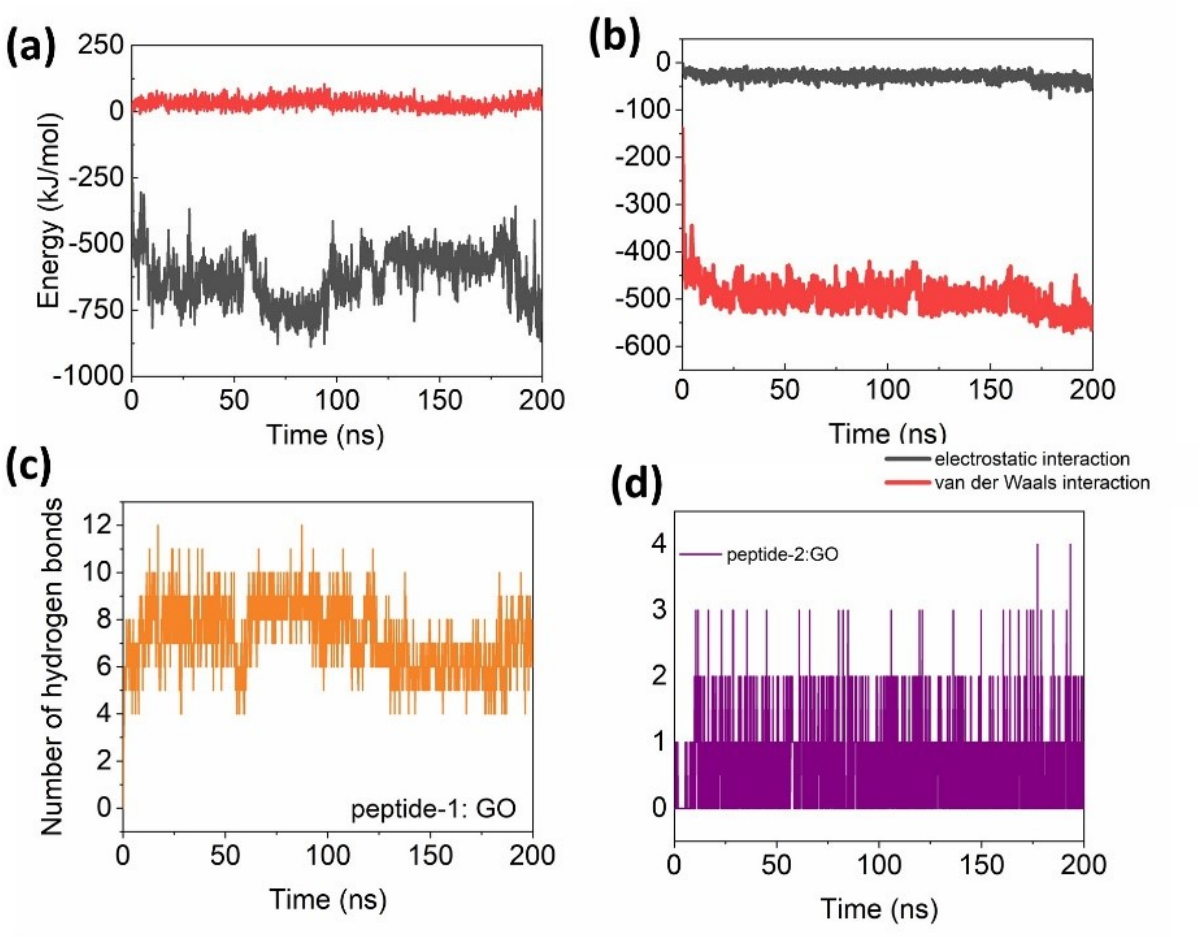


Figure S12. The interaction energy and the number of hydrogen bonds between the peptide-1 and peptide-2 with GO. (a) and (b) represent the electrostatic and van der Waals interaction energy between peptide-1 and peptide-2 with graphene oxide, respectively. (c) and (d) represent the number of hydrogen bonds between peptide-1 and peptide-2 with graphene oxide, respectively.

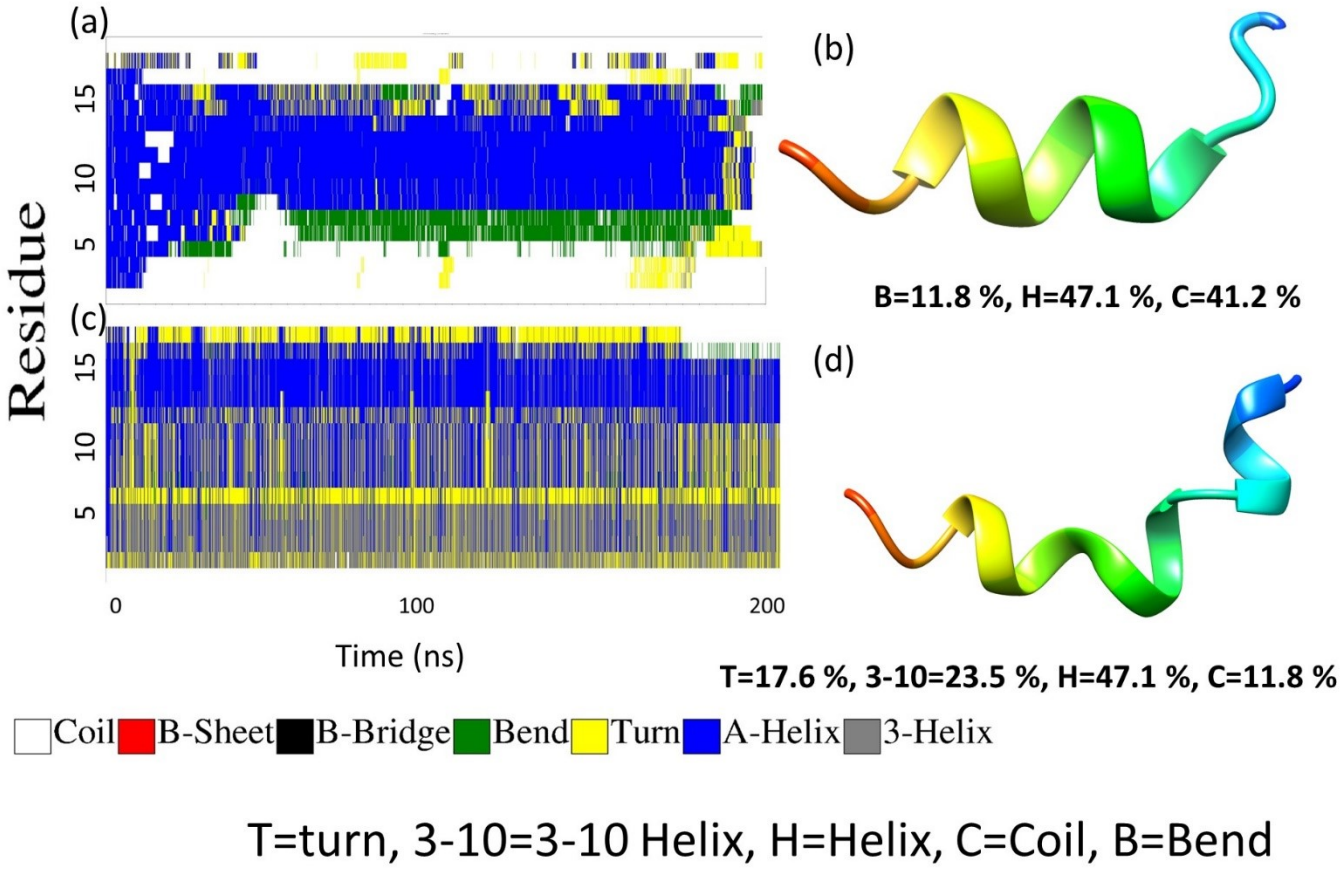


Figure S13. The secondary structure transition of peptide-2 during the 200 ns simulation. The top panel represents the peptide-2 in water and the bottom panel represent the peptide-2 with graphene oxide. The average peptide structure during the 200 ns simulation time is provided on the right side.

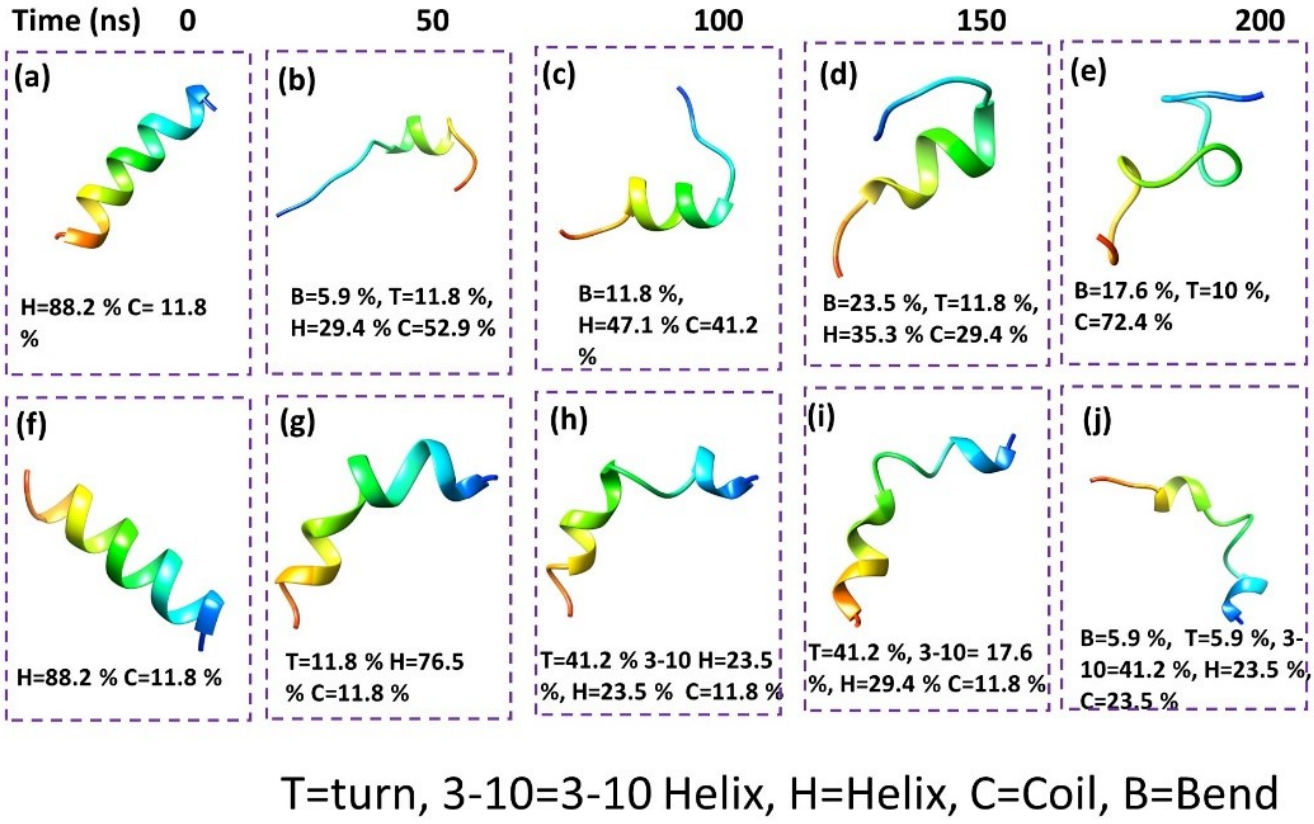


Figure S14. The time-dependent secondary structural transition of peptide-2 in 50 ns time interval (0 ns to 200 ns). The top panel (a-e) represents the transition in water and the bottom panel (f-j) represent the transition in presence of graphene oxide.

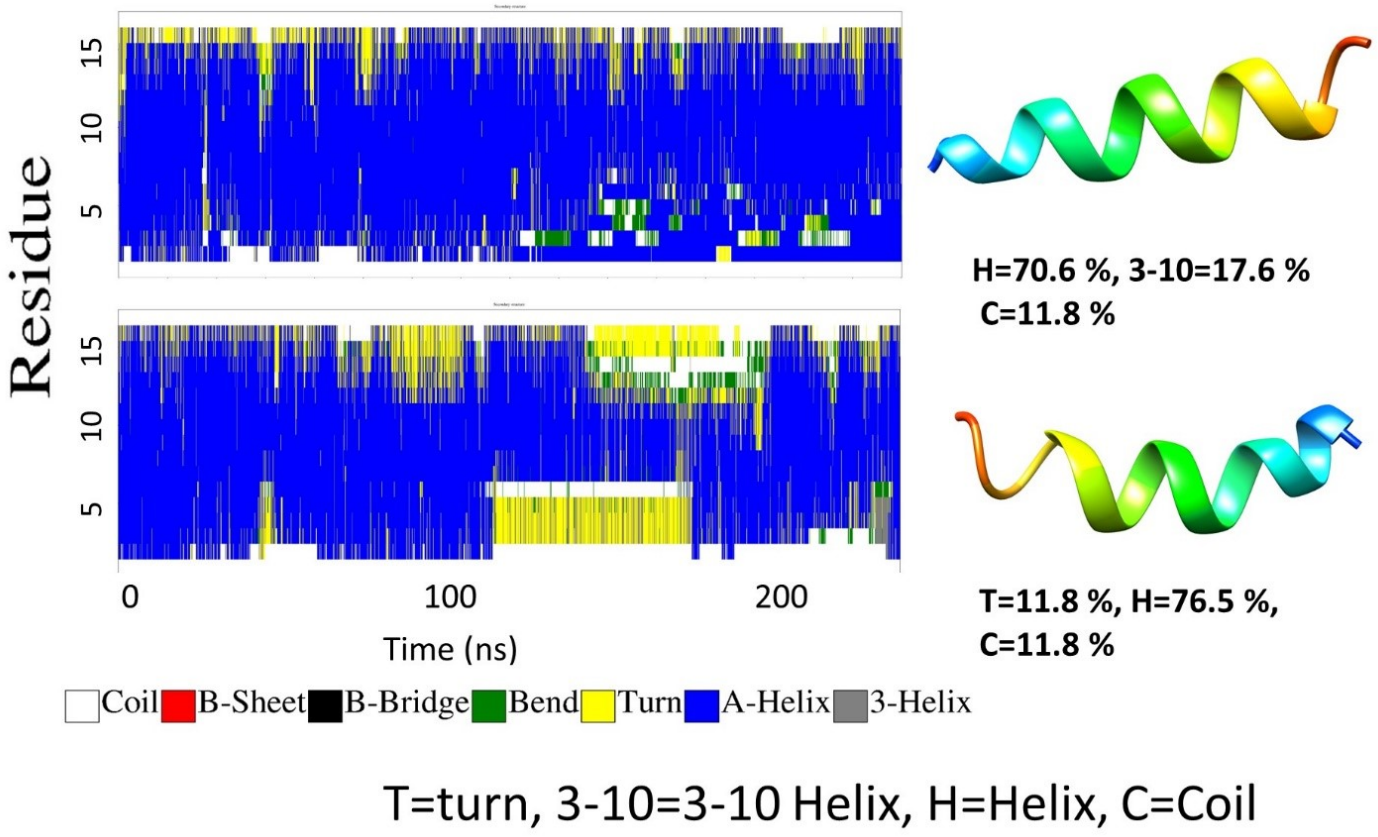


Figure S15. The secondary structure transition of peptide-3 during the 200 ns simulation. The top panel represents the peptide-1 in water and the bottom panel represent the peptide-1 with graphene oxide. The average peptide structure during the 200 ns simulation time is provided on the right side.

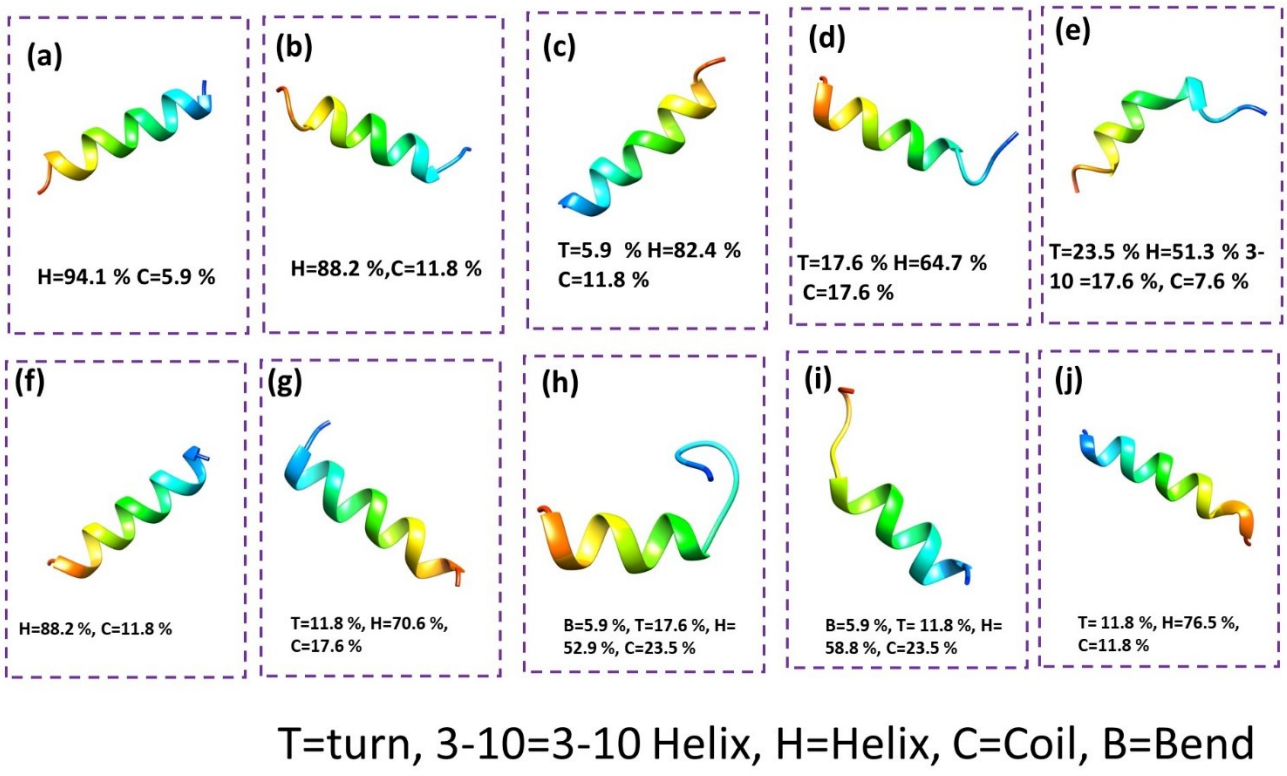


Figure S16. The time-dependent secondary structural transition of peptide-3 in 50 ns time interval (0 ns to 200 ns). The top panel (a-e) represents the transition in water and the bottom panel (f-j) represent the transition in presence of graphene oxide.

References.

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