Supporting Information for

A Modular Approach for Organizing Dimeric Coiled Coils on Peptoid Oligomer Scaffolds

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1. Materials

Rink amide MBHA resin were purchased from Gyros Protein Technologies. Propargylamine was supplied by Oakwood Chemical. N,N'-diisopropylcarbodiimide (DIC) was purchased from Alfa Aesar. L-Ascorbic acid sodium salt was purchased from Acros Organics. Dichloromethane (DCM), acetonitrile (ACN) and HPLC grade water were supplied by Pharmco. 2-Chlorotrityl chloride resin, all amino acids used for Fmoc chemistry, 2-methoxyethylamine, N,N-diisopropylethylamine (DIEA), piperidine, trifluoroacetic acid (TFA), 2,2,2-trifluoroethanol (TFE), 2-azidoacetic acid, acetic acid, triisopropylsilane (TIPS), bromoacetic acid (BrAA), tris(benzyltriazolylmethyl)amine (THPTA), cupric sulfate, N,N-dimethylformamide (DMF), sodium chloride, sodium phosphate monobasic dihydrate, sodium phosphate dibasic dihydrate, aminoguanidine hydrochloride, HATU, HCTU and PyBOP were purchased from Milipore Sigma. Deionized water was produced in the lab by using Elga water purification system.

2. Methods

2.1 Synthesis

2.1.1 Synthesis of Linear Precursors of Macrocyclic Peptoids

The linear precursor was synthesized via the combination of Fmoc chemistry and sub monomer solid phase synthesis methods following a procedure we previously reported.[1-2] 2-chlorotrityl chloride resin was used as the solid support. The following procedure is optimized for a synthesis scale of 200 mg resin with a loading number of 1.4 mmol/g. Prior to synthesis, the resin was swelled in DCM for 45 minutes. The first residue was added through **1)** 45-minute shaking of the resin with 2 ml of 0.65 M BrAA/DCM solution and 0.216 ml of DIEA and **2)** 20-minute shaking with 2 ml of 1.3 M amine/DMF solution. The coupling of other N-substituted glycine residues was carried out by iterative steps of **1)** 20-minute shaking with 2 ml of 1.2 M BrAA/DMF solution and 0.4 ml of DIC and **2)** 20-minute shaking with 2 ml of 1.3 M amine/DMF solution. Proline residues were coupled using standard Fmoc chemistry. To the resin, 3 ml DMF solution containing 5 equivalents Fmoc-L-proline, 4.9 equivalents HATU and 10 equivalents DIEA was added. The reaction mixture was shaken for 45 minutes. To ensure the complete addition of proline to the solid support, this coupling procedure was repeated before deprotection of the Fmoc groups. Before coupling the next N-substituted glycine residue, Fmoc groups were deprotected by using 5 ml of 20 vol% piperidine/DMF solution. The deprotection step was also repeated twice. The resin was washed by DMF thoroughly after each step. Once the target sequence was reached, mild cleavage of linear precursor from the solid support was performed by using a cleavage cocktail containing 10 vol% acetic acid, 10 vol% TFE and 80 vol% DCM. After 1-hr shaking, the solution was dried by using nitrogen gas flow. The resulting oily product was dissolved in a water/acetonitrile (50:50 by volume) cosolvent. Lyophilization was used to freeze-dry the solution. Three cycles of dissolution-lyophilization were performed for complete removal of acetic acid.

2.1.2 Macrocyclization of Linear Peptoids

Cyclization of the linear oligomer precursors was conducted as follows: a 1 mM linear peptoid solution was prepared by dissolving the lyophilized powder in the proper amount of dry DCM, followed by the addition of 6 equivalents DIEA and 3 equivalents PyBOP. The reaction

mixture was stirred for 12 hrs under nitrogen gas. Afterwards, DCM was removed by rotary evaporation. The cyclized products were purified by reversed phase HPLC using water and ACN as mobile phases. The purified HPLC fractions were then lyophilized for three days. The molecular weight of each product was confirmed by using Agilent 6120 single quadrupole LC-MS spectrometer.

2.1.3 Synthesis of Linear Peptoid Scaffolds

Rink amide resin was used to synthesize the linear peptoid scaffolds. The resin was swelled in DMF for 40 min before the deprotection of Fmoc groups. Typically, for a synthesis scale of 0.28 mmole, Fmoc groups were deprotected by using 5 ml of 20 vol% piperidine/DMF solution. The deprotection was repeated twice to ensure the complete removal of Fmoc groups. The N-substituted glycine residues and proline residues were coupled in the same manner as for the synthesis of peptoid linear precursor as specified in section 2.1.1. The N-termini were acetylated via shaking the resin with 50 equivalents acetic anhydride and 8 equivalents DIEA for 45 min. A cleavage cocktail containing 2.5 vol% water, 2.5 vol% TIPS and 95 vol% TFA was used to cleave the linear peptoids from the resin. The cleaved crude products were then purified by reversed phase HPLC using water and acetonitrile as the mobile phase. Collected HPLC fractions were then lyophilized. The molecular weight of the products was determined by using Agilent 6120 single quadrupole LC-MS spectrometer.

2.1.4 Synthesis of Peptides

A Prelude model automated synthesizer (Gyros Protein Technologies) was used to synthesis the peptide through Fmoc-chemistry based solid phase peptide synthesis method, following prior literature protocols.[1, 3] Rink-amide MBHA resin was used as the solid support. For a typical synthesis at 0.05 mmol scale, the Fmoc groups were deprotected by using 5 ml of 20 vol% piperidine/DMF solution. The deprotection was repeated twice to ensure the complete removal of Fmoc groups. For amino acid coupling to the resin, 3 ml DMF solution containing 5 equivalents of Fmoc amino acid (or 2-azidoacetic acid for N-terminal capping), 4.9 equivalents of HCTU and 10 equivalents of DIEA were added. The reaction mixture was stirred for 45 minutes. The deprotection-coupling cycle was repeated until the target polypeptide sequence was obtained. The peptide was cleaved from resin by using a cleavage cocktail containing 2.5 vol%

water, 2.5 vol% TIPS and 95 vol% TFA. The side chain protection groups were removed simultaneously during the cleavage reaction (3-hr shaking). The cleaved product was washed by diethyl ether 3 times. The obtained white precipitate was then placed under vacuum for 12hrs. Reversed phase HPLC was then used to purify the crude product using water and acetonitrile as the mobile phase. Collected HPLC fractions were then lyophilized. The molecular weight of the product was confirmed by using Agilent 6120 single quadrupole LC-MS spectrometer.

2.1.5 Conjugation Reaction using CuAAC Click Chemistry

The conjugation reaction condition was a modification of published procedures.[4] Six solutions were prepared as following,

Solution A: The **N_3-GCNshSN** peptide lyophilized powder was dissolved in 100 mM potassium phosphate buffer (pH=7.5) to obtain 0.6 mM concentration.

Solution B: The peptoid oligomer lyophilized powder was dissolved in deionized water so that the concentration of propargyl oligomer side chain groups was 5 mM.

Solution C: A 20 mM copper (II) sulfate solution was prepared by using deionized water as solvent.

Solution D: A 100 mM tris-hydroxypropyltriazolylmethylamine (THPTA) solution was prepared by using deionized water as solvent.

Solution E: A 1 M aminoguanidine hydrochloride solution was prepared by using deionized water as solvent.

Solution F: A 1 M sodium ascorbate solution was prepared by using deionized water as solvent.

A 0.15 ml aliquot of **solution C** was pre-mixed with 0.15 ml of **solution D**, and the mixture was then placed aside. To 0.5 ml of **solution A**, 0.03 ml of **solution B** was added, followed by the addition of the pre-mixed **solution C** and **D**. Afterwards, 0.15 ml of **solution E** and 0.15 ml of **solution F** were added sequentially. The mixture was stirred for 24 hrs. Reversed phase HPLC was used to separate the desired product from other species using water and acetonitrile as the mobile phase. Collected HPLC fractions were then lyophilized. The molecular weight of the desired product was confirmed by using Agilent 6120 single quadrupole LC-MS spectrometer.

2.2 Experimental characterization

2.2.1 Circular Dichroism (CD) Spectroscopic Studies

All samples were prepared by using 10 mM sodium phosphate-150 mM sodium chloride buffer with pH of 7.5. The concentration of peptide was determined by UV-Vis spectroscopy using Beer's law and the molar extinction coefficient of tyrosine at 280 nm (1280 $cm^{-1}M^{-1}$).[5-6] A Jasco J-1500 circular dichroism spectrometer was used for all CD studies. The heating rate for thermal denaturation experiments was 1 ℃/minute. For conjugated molecules, the CD signals contributed by cyclic peptoid components were subtracted by using the corresponding CD data of pure cyclic peptoid as the background. The intensity in mDeg was converted to molar residue ellipticity through the following equation:

$$MRE = \frac{mDegx1000}{c \times n \times l}$$

where c is the concentration of peptide in mM, n is the number of residues in one peptide chain and l is path length of the cuvette in mm.

2.2.2 Dynamic Light Scattering Measurements

A Malvern Zetasizer (Nano ZS) was used for DLS measurements. Samples for DLS measurements were prepared in the same way as for CD studies. The concentration was adjusted to 0.03 mM. Prior to the DLS measurements, the solution was freshly prepared and subjected to centrifugation with a speed of 3,000 rpm for 5 minutes to sediment particulates that might be present in the solution. The upper-layer solution was then carefully transferred for measurements.

2.3 Molecular Modelling

*2.3.1 Construction of **Meta596-Conjugate***

The Meta596-Conjugate was created in three steps. **Step-1:** A conformational reservoir of Meta**596** peptoid macrocycle were generated by carrying out a 170 ns Molecular Dynamics simulation at 298 K (see details in the following paragraphs). **Step-2:** The crystal structure of **GCN4** peptides (pdb code: 2ZTA) was used to create the **GCNshSN** peptides with the N-terminus capped by an acetyl group and the C-terminus capped by amide group.[7] **Step-3:** An in-house Python script was used to search the conformational reservoir of **Meta596** peptoid to identify a particular conformation for which the N-termini of coiled coil structure could be

properly matched to the specific side chain of the cyclic peptoid without causing any steric clash (**Figure S8**). The two molecular models were then associated by installation of the proper covalent bonds in order to obtain the initial structure of **Meta596-Conjugate**.

All the computational work was carried out using NYU Prince high performance computing clusters. The conformational reservoir of **Meta596** peptoid macrocycle was generated as follows: The initial structure of **Meta596** peptoid macrocycle was constructed by properly aligning and then merging the reported crystal structure of an analogous peptoid macrocycle and ligand molecule containing a triazole unit (Ligand ID on RCSB: 1P0).[8] The atomic charge of the peptoid macrocycle was derived using am1bcc charge model through the Antechamber package implemented in Amber 18 suite.[9-10] The molecule was modeled by using the GAFF2[11] forcefield in explicit solvent using a TIP3P[12] water model. The PMEMD.MPI[9] module of Amber 18 was used for energy minimization, system equilibrium and production run. Force evaluation was set with a 12 Å cutoff for Lennard-Jones interactions and a 9 Å cutoff for electrostatic interactions (calculated by smooth PME[13-14] electrostatics) in a periodic boundary condition. The bond lengths involving hydrogen were constrained by SHAKE algorithm.[15] The simulation temperature was controlled with a Langevin thermostat with a collision frequency of 1 ps^{-1}.[16] During NPT runs, constant pressure was controlled by a Berendsen barostat with isotropic pressure scaling.[17]

Solvent molecules were relaxed by 100 steps of steep-descent minimization and 900 steps of conjugate gradient minimization with a restraint of 20 kcal/mol-$Å^2$ applied to the peptoid macrocycle. The entire simulation box was relaxed in the same manner without any restraint. A 50-ps run in constant volume was performed to gradually increase the temperature from 0.1 K to 298 K with 20 kcal/mol-$Å^2$ applied to the peptoid macrocycle at a 1 fs time step, followed by a 100-ps NPT run with the constant pressure of 1bar and the same restraint but using a 2 fs time step. The restraint was reduced to 0.5 kcal/mol-$Å^2$ for another 200-ps NPT run. The production run was propagated in NPT ensemble for 170 ns with no restraint. The trajectories with converged Cα-RMSD produced by the production run were used as the conformational reservoir of **Meta596** cyclic peptoid.

2.3.2 *Molecular Dynamics Simulation of Coiled Coil Formed by **GCNshSN** Peptides and **Meta596-Conjugate***

The simulation protocol used in this work was modified from the published literature.[18] Implicit solvent with GB-Neck2[19] model was used to increase the conformational sampling efficiency. Mbondi3 radii[19] and ff14SBonlysc[18, 20] forcefield were used to model the coiled coil tertiary structure and the GAFF2[11] forcefield was used to model the peptoid scaffold. The salt concentration was set to be 0.15 M for mimicking the experimental condition. The simulation temperature was controlled with a Langevin thermostat with collision frequency of 1 ps^{-1}.[16] The SHAKE algorithm was used to constrain bonds involving hydrogen.[15] The time step was set as 2 fs for all steps. Force evaluation was set with 999 Å cutoff.

The PMEMD.MPI module of Amber 18 was used for energy minimization and system equilibrium. The PMEMD.Cuda module of Amber 18 was used for the production run.[21] The initial structure was minimized and equilibrated as follows: **1)** The entire system was relaxed by 100 steps of steep-descent minimization and 900 steps of conjugate gradient minimization without any restraint. **2)** The system temperature was increased from 100 K to the target temperature with restraint of 10 kcal/mol-$Å^2$ on all heavy atoms over 500 ps. **3)** The restraint was reduced to 1 kcal/mol-$Å^2$ for another 500-ps run at constant temperature. **4)** The restraint was further reduced to 0.1 kcal/mol-$Å^2$ for an additional 500-ps run at constant temperature. The production run was carried out without any restraint. Five independent production runs were performed for each molecule at 303 K. All data analysis was done by using Cpptraj[22] package in Amber 18 suite.

3. Supplementary Data

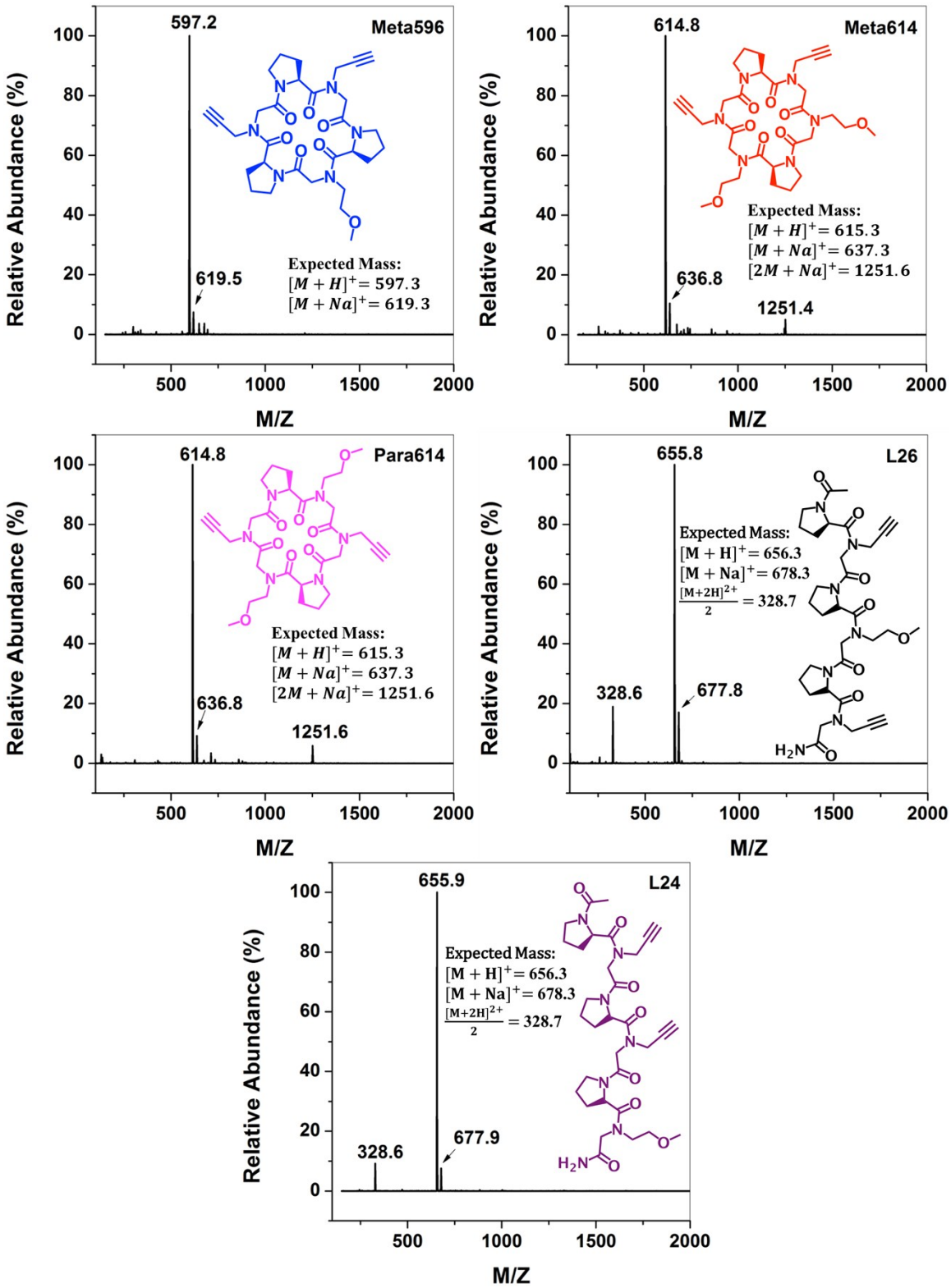

Figure S1. Mass spectrometry results of the five different peptoid oligomer scaffolds.

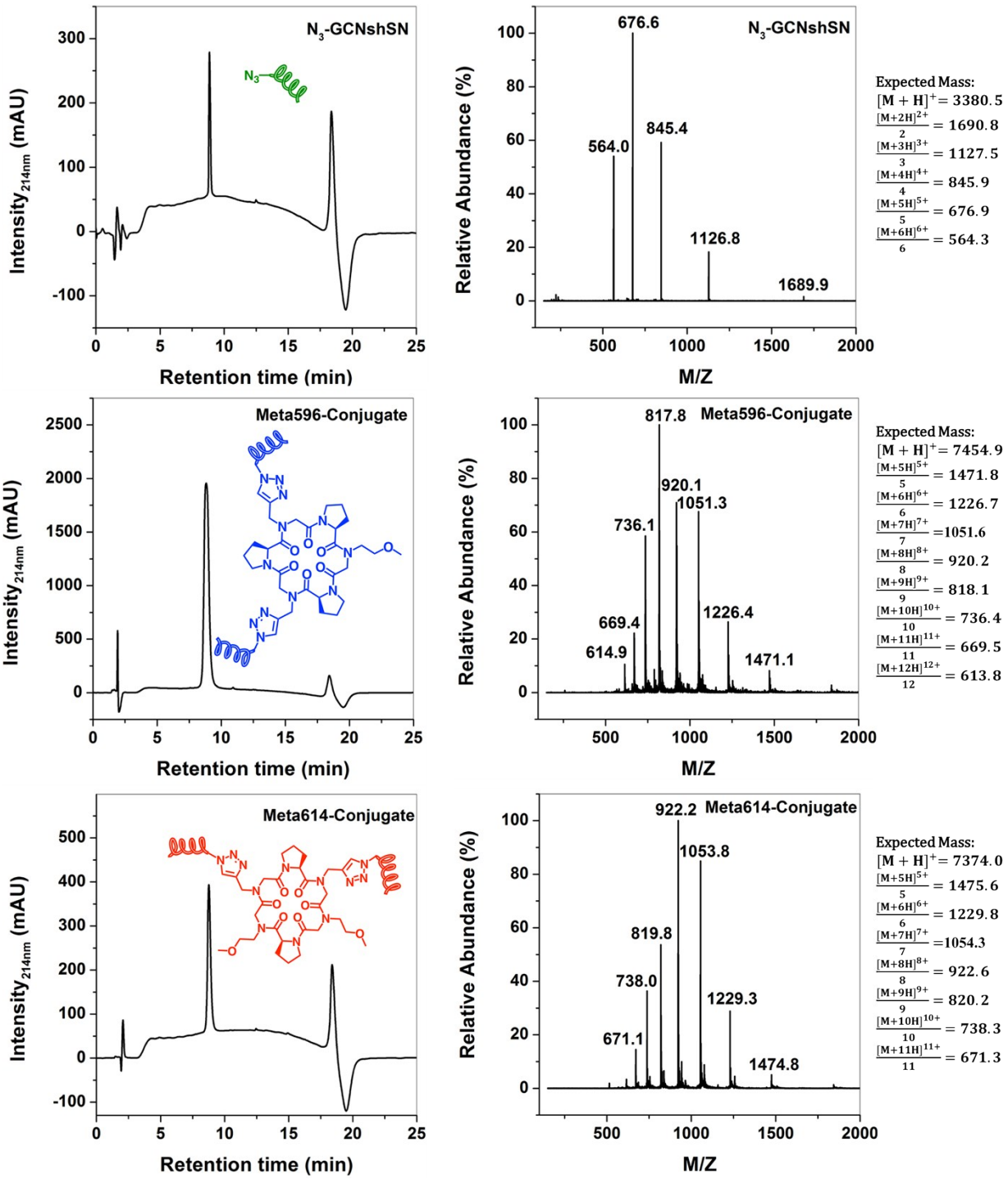

Figure S2. Mass spectrometry data and analytical HPLC traces of **GCNshSN** peptides, **Meta596-Conjugate** and **Meta614-Conjugate**.

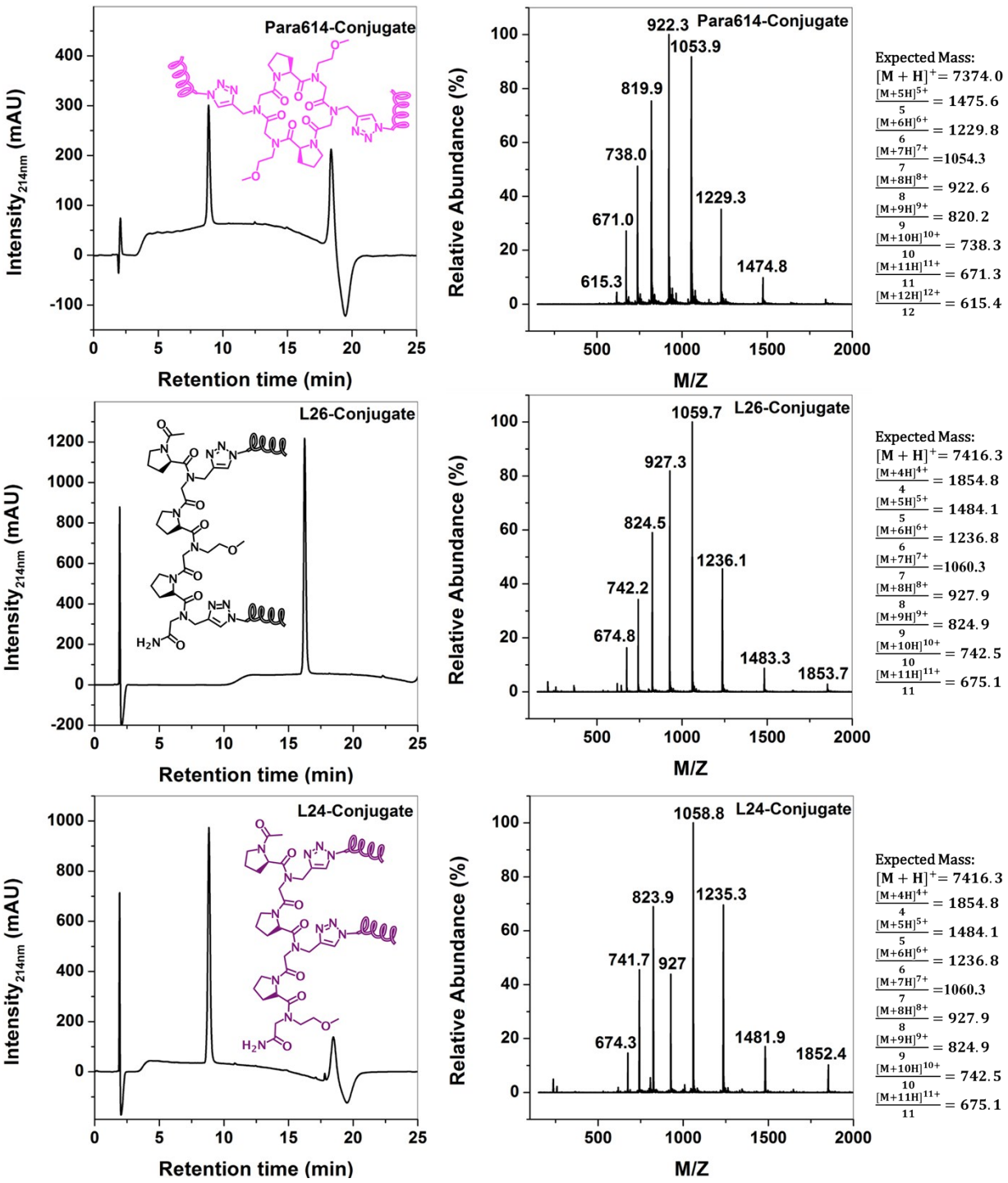

Figure S3. Mass spectrometry data and analytical HPLC traces of **Para614-Conjugate, L26-Conjugate** and **L24-Conjugate**.

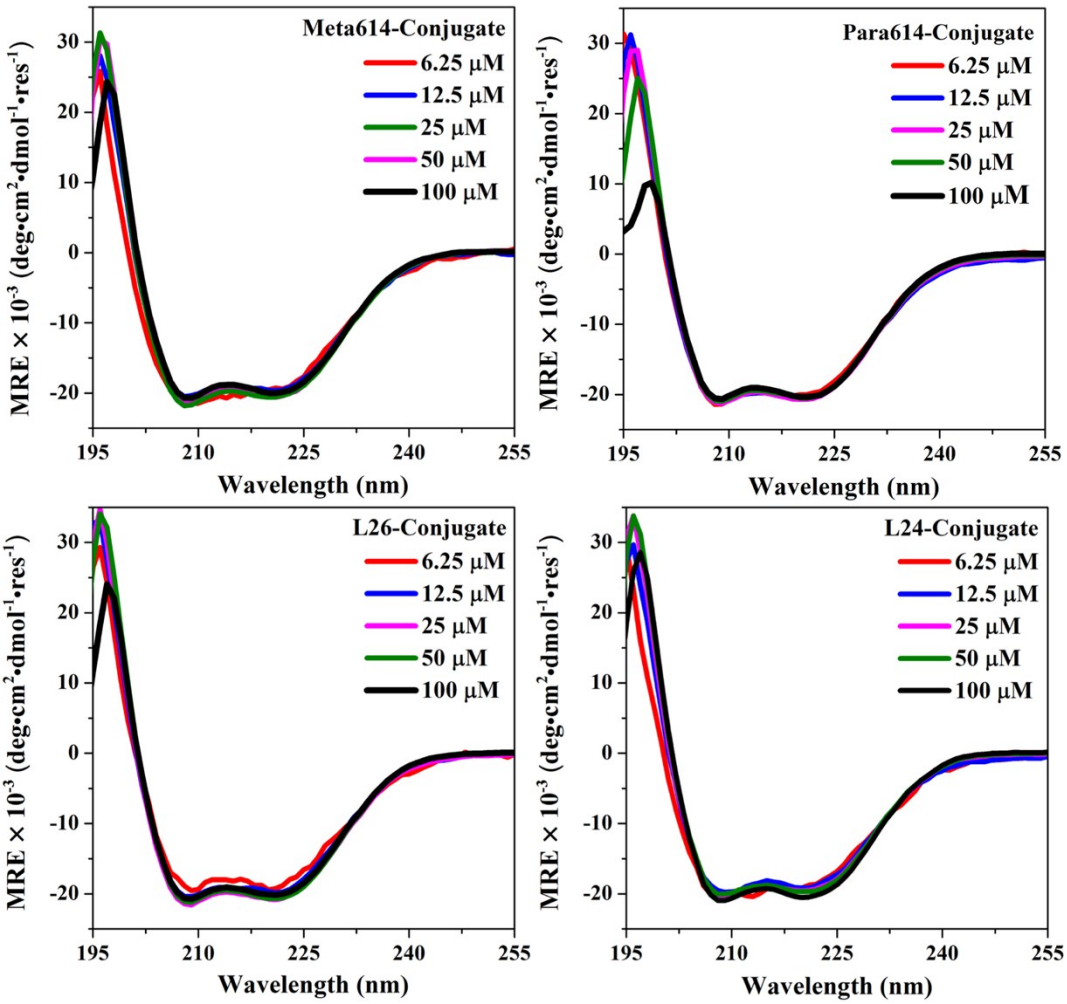

Figure S4. Circular dichroism spectra of **Meta614-Conjugate**, **Para614-Conjugate**, **L26-Conjugate** and **L24-Conjugate** at varying concentrations. Solvent: 10 mM sodium phosphate-150 mM sodium chloride buffer, pH 7.5. Path length of CD cuvette: 1mm. Temperature: 25 °C. MRE: molar residue ellipticity. The CD signal contributed by the peptoid macrocycle has been subtracted.

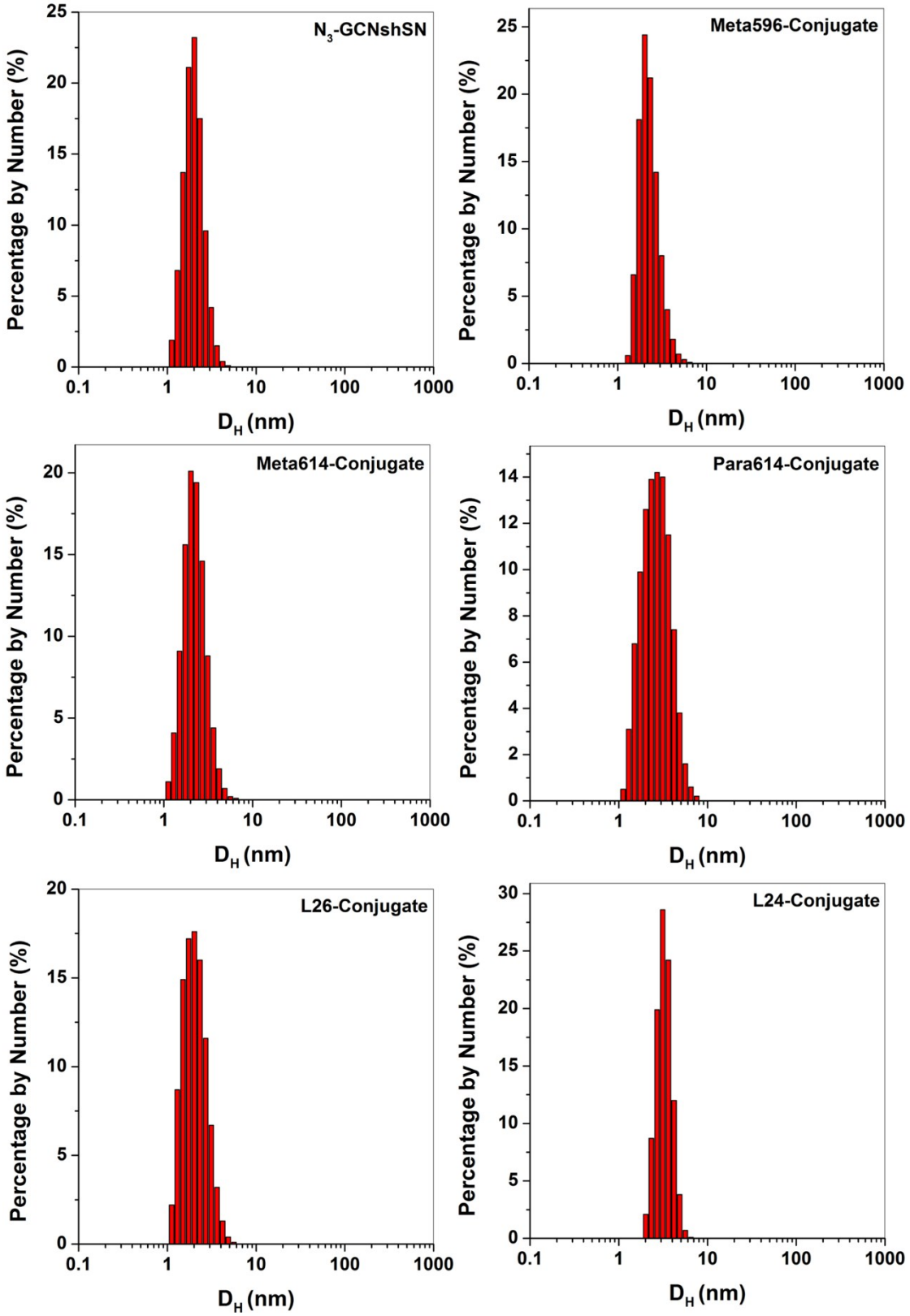

Figure S5. Hydrodynamic diameters of coiled coils formed by the unconjugated individual peptides and five different conjugated molecules measured by dynamic light scattering.

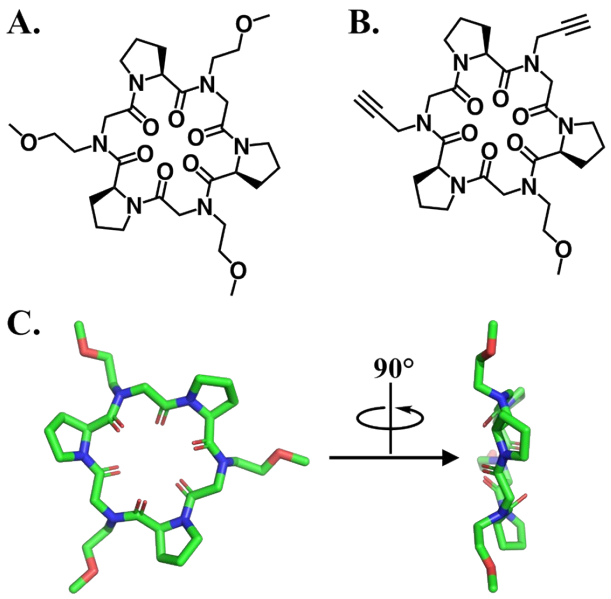

Figure S6. Structural comparison of **Meta596** peptoid macrocycle (B) used in this work and the analog (A) whose crystal structure[8] (C) has been reported by the De Riccardis group.

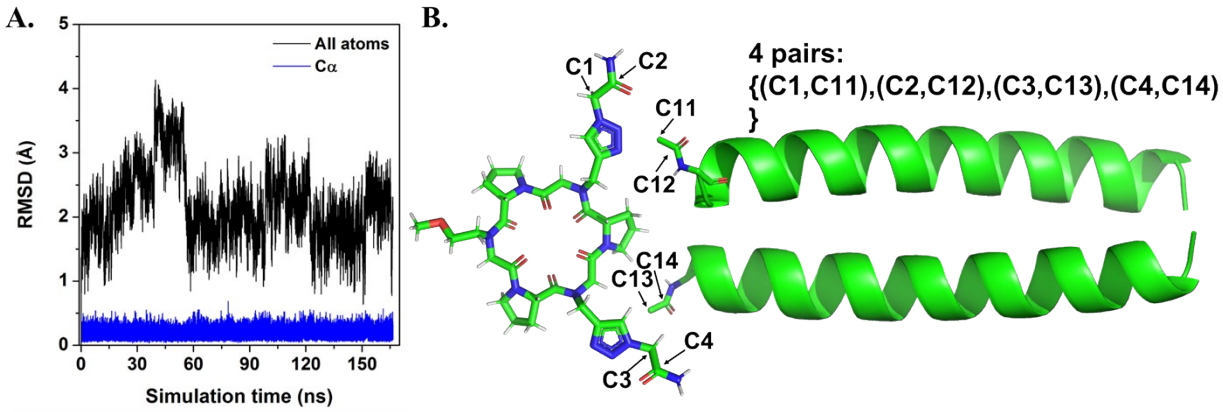

Figure S7. (A) RMSD plot of the macrocyclic peptoid scaffold (**Meta596** peptoid with triazole units installed) during the MD simulation for generating a conformational reservoir. (B) Illustration of conformational search process during which alignments were performed using the four groups of paired atoms. Carbon C1 was aligned to carbon C11, carbon C2 was aligned to carbon C12, carbon C3 was aligned to carbon C13 and carbon C4 was aligned to carbon C14 simultaneously. A search produced the alignment with RMS value lower than 0.1 Å was considered as a successful trial.

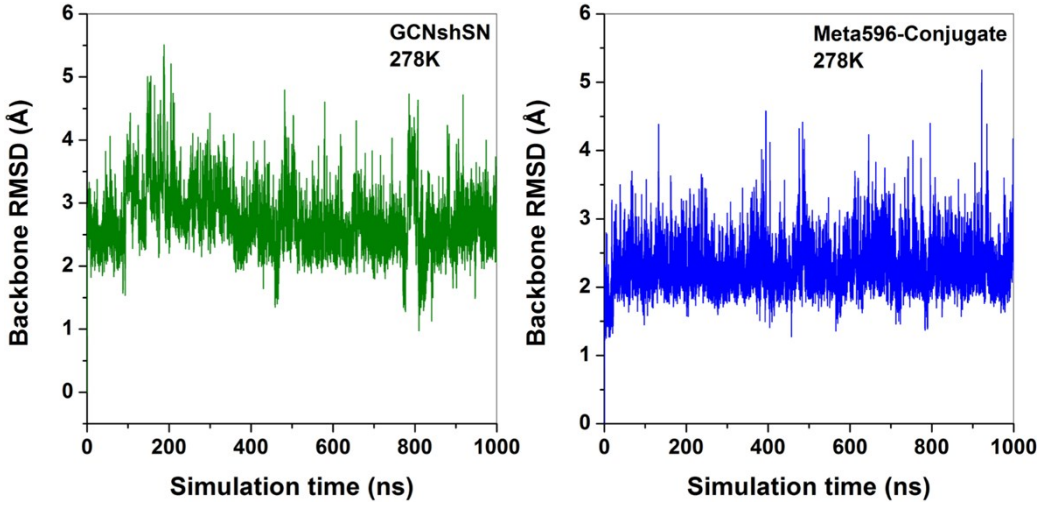

Figure S8. Mass-weighted backbone (N, Cα and C) root mean squared deviation (RMSD) of **GCNshSN** peptides and **Meta596-Conjugate** over the course of 1 μs simulation at 278 K.

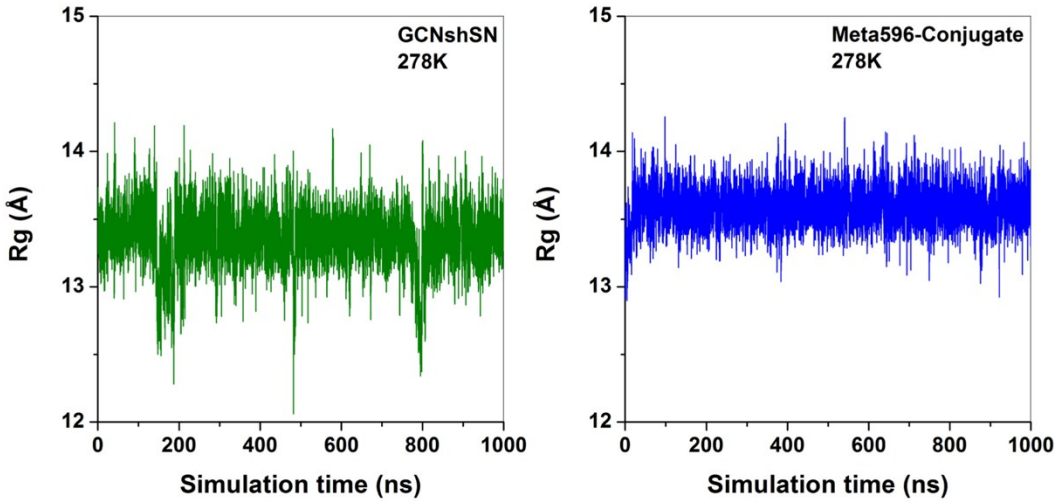

Figure S9. Radius of gyration (Rg) of coiled coils formed by individual **GCNshSN** peptides and **Meta596-Conjugate** over the course of 1 μs simulation at 278 K.

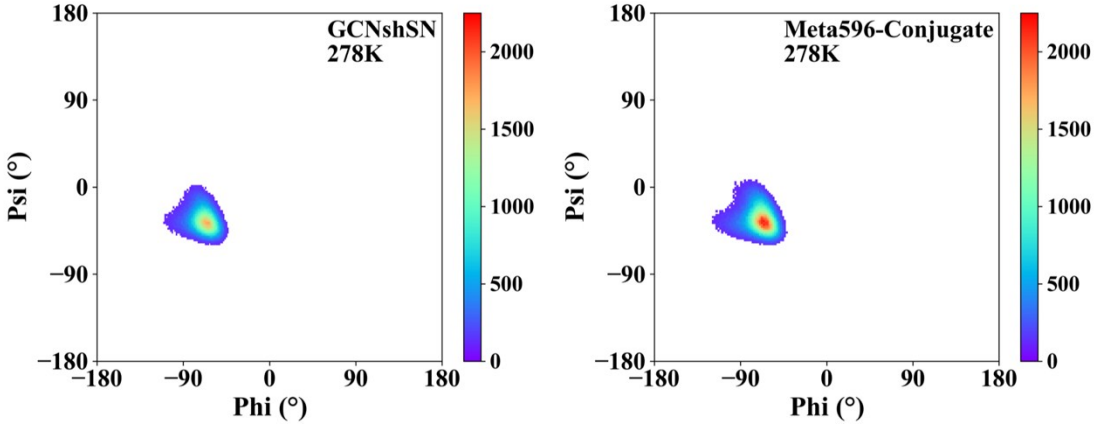

Figure S10. Ramachandran maps of the conformational space sampled by individual **GCNshSN** peptides and **Meta596-Conjugate** over the course of 1μs simulation at 278 K.

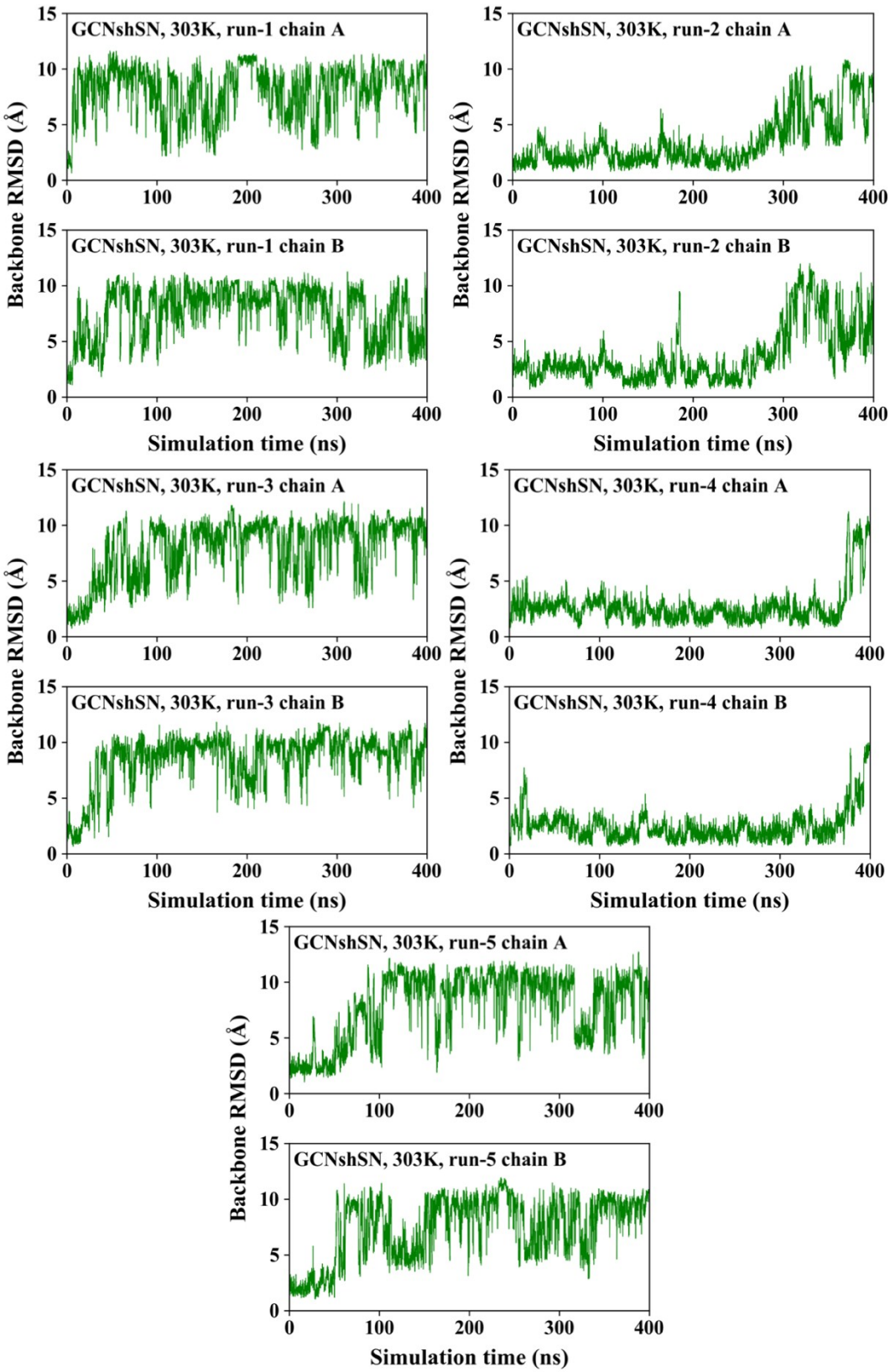

Figure S11. Mass-weighted backbone RMSD of each chain from the coiled coils formed by the **GCNshSN** peptides over the course of five independent 400 ns simulation runs at 303 K.

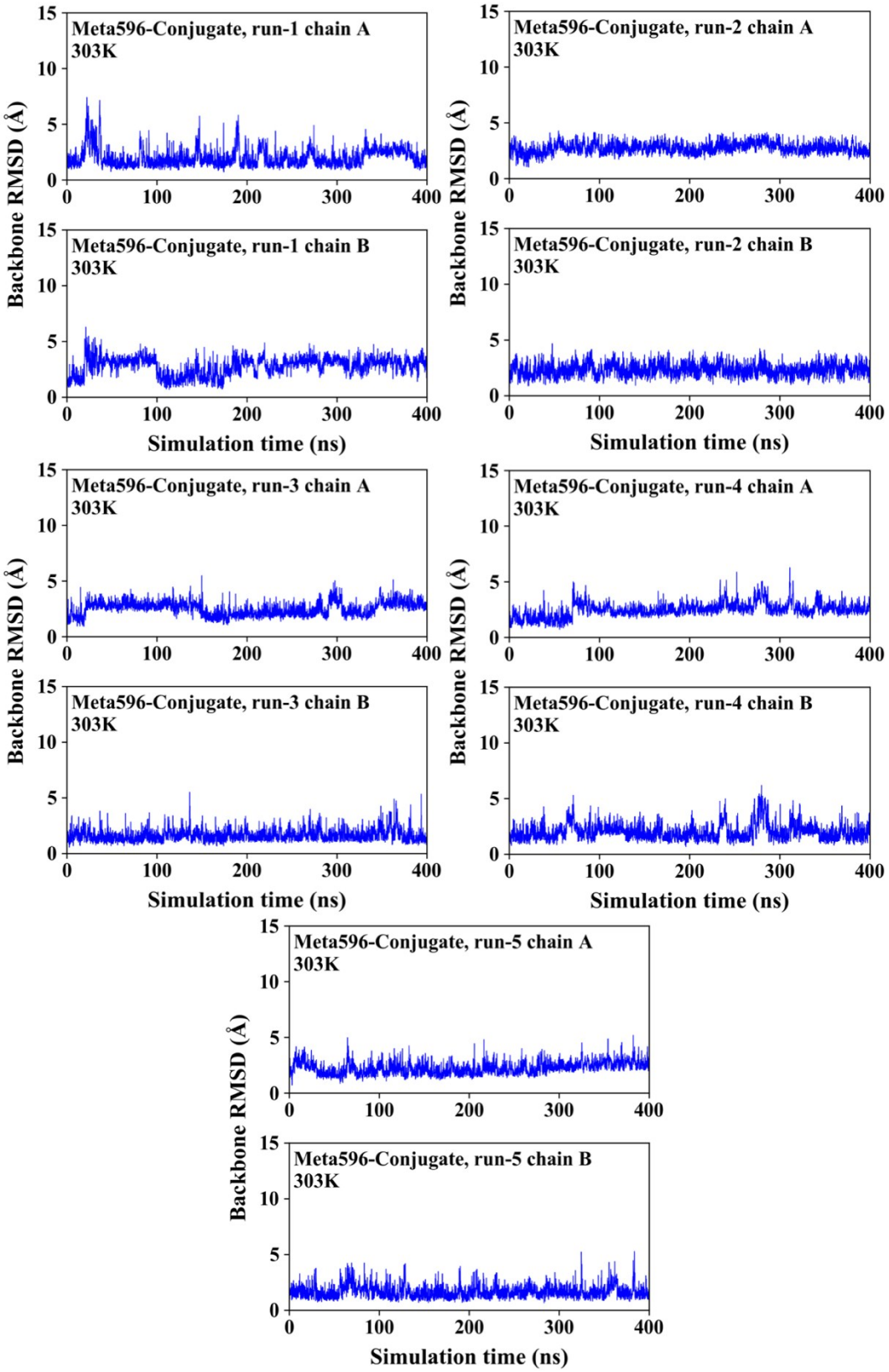

Figure S12. Mass-weighted backbone RMSD of each chain from the coiled coils formed by the **Meta596-Conjugate** over the course of five independent 400 ns simulation runs at 303 K.

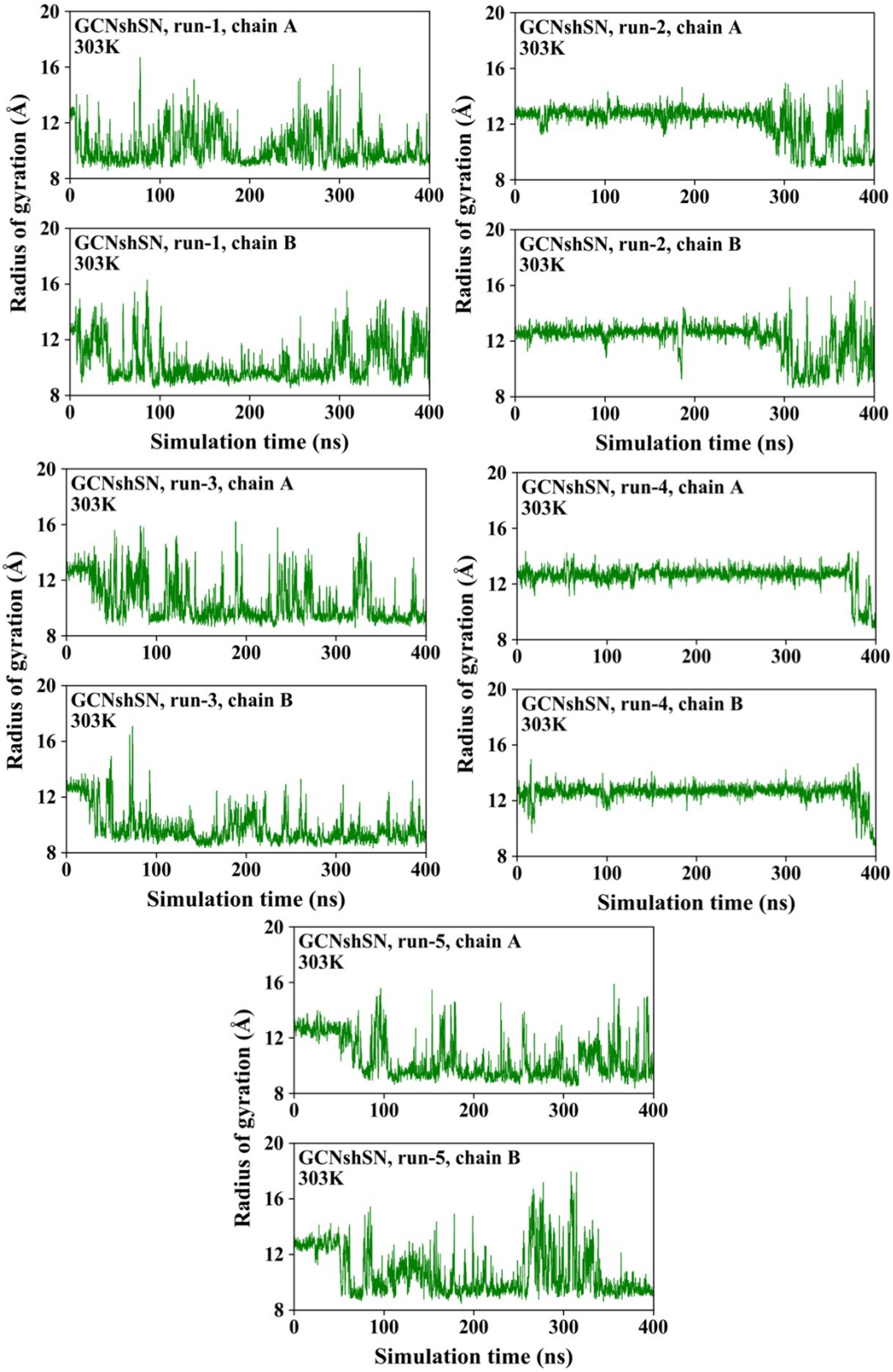

Figure S13. Radius of gyration of each chain from the coiled coils formed by **GCNshSN** peptides over the course of five independent 400 ns simulation runs at 303 K.

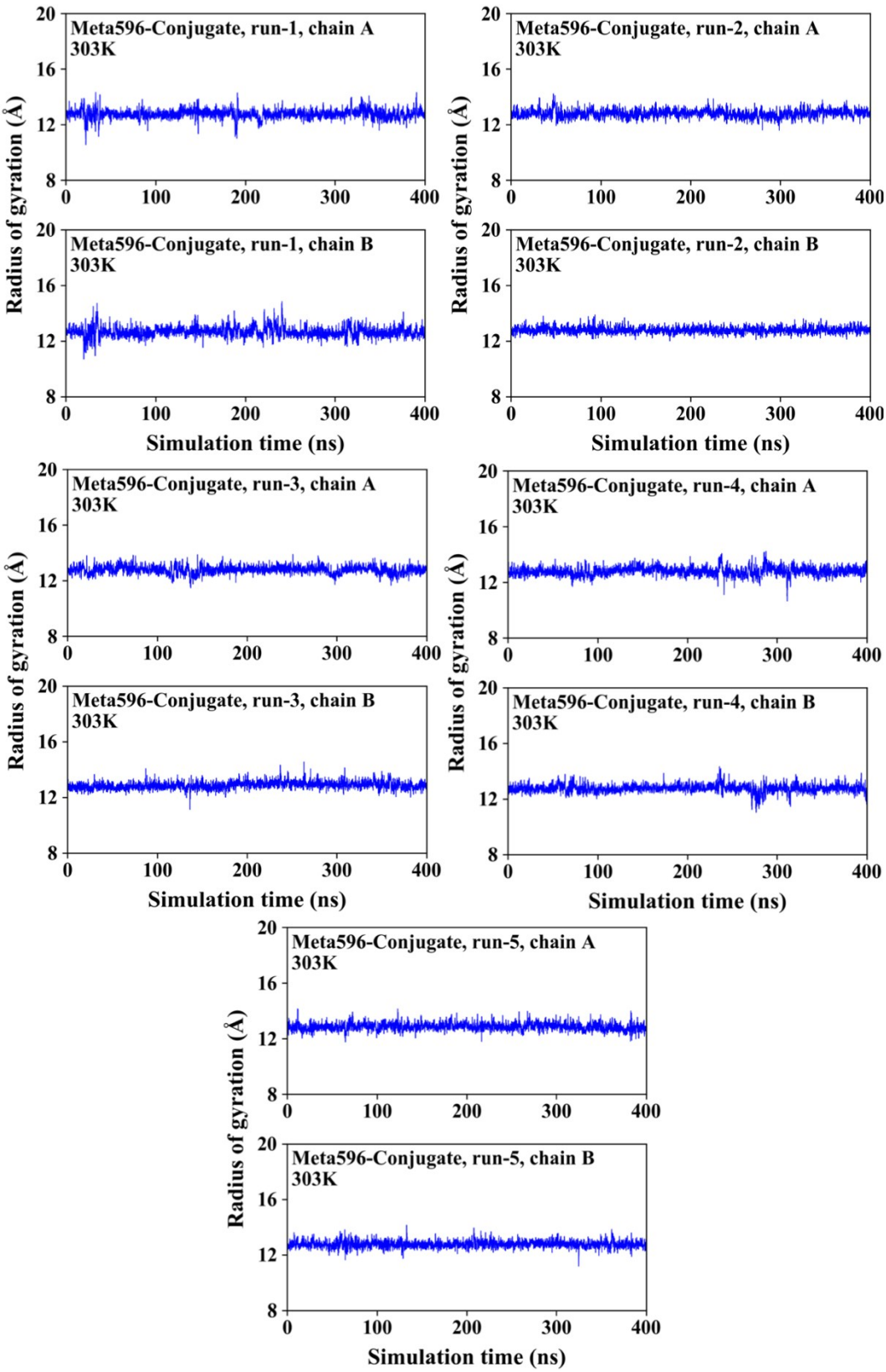

Figure S14. Radius of gyration of each chain from the coiled coils formed by **Meta596-Conjugate** over the course of five independent 400 ns simulation runs at 303 K.

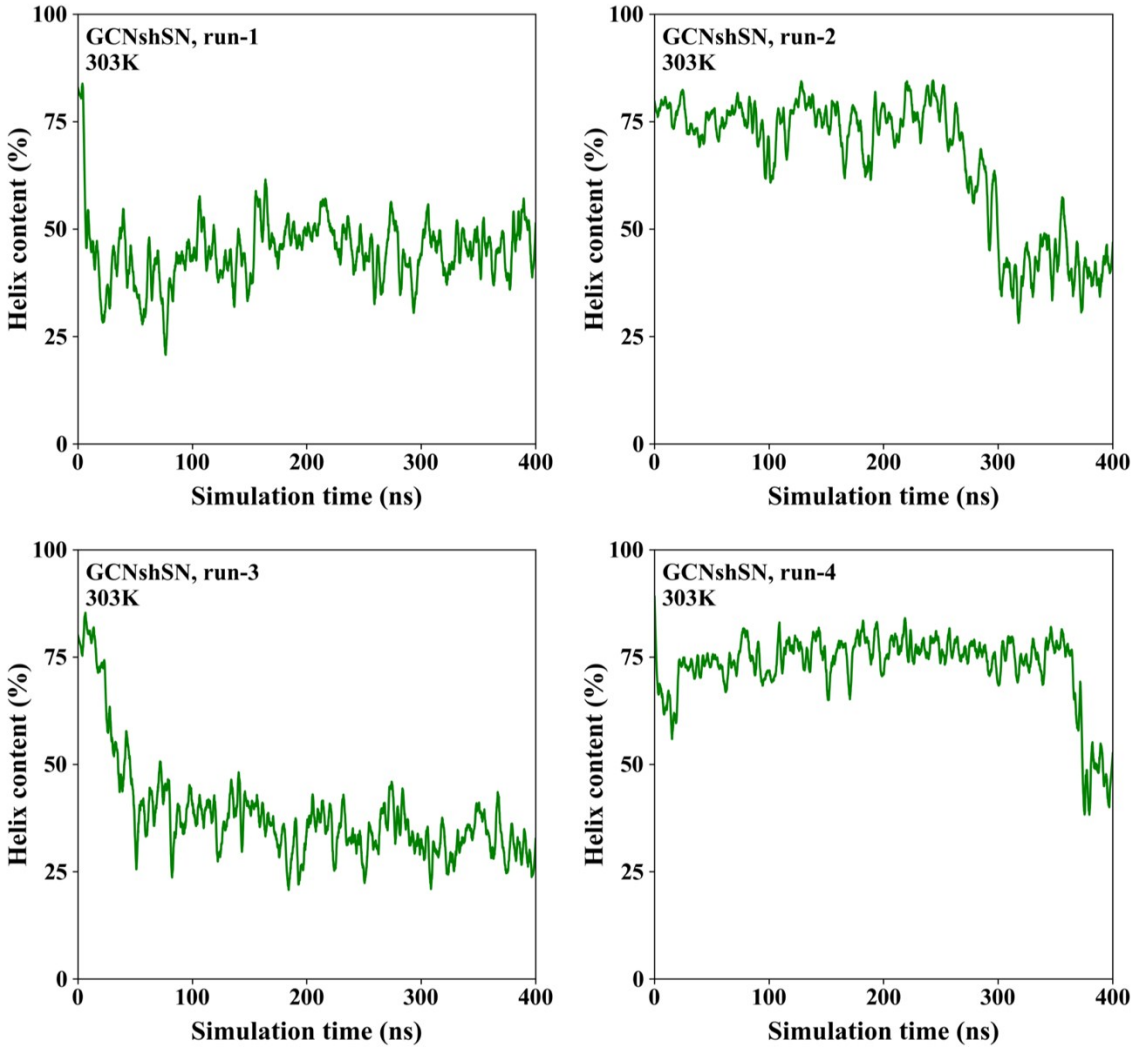

Figure S15. Helix content in the coiled coils formed by **GCNshSN** peptides over the course of additional four independent 400 ns simulation runs at 303 K.

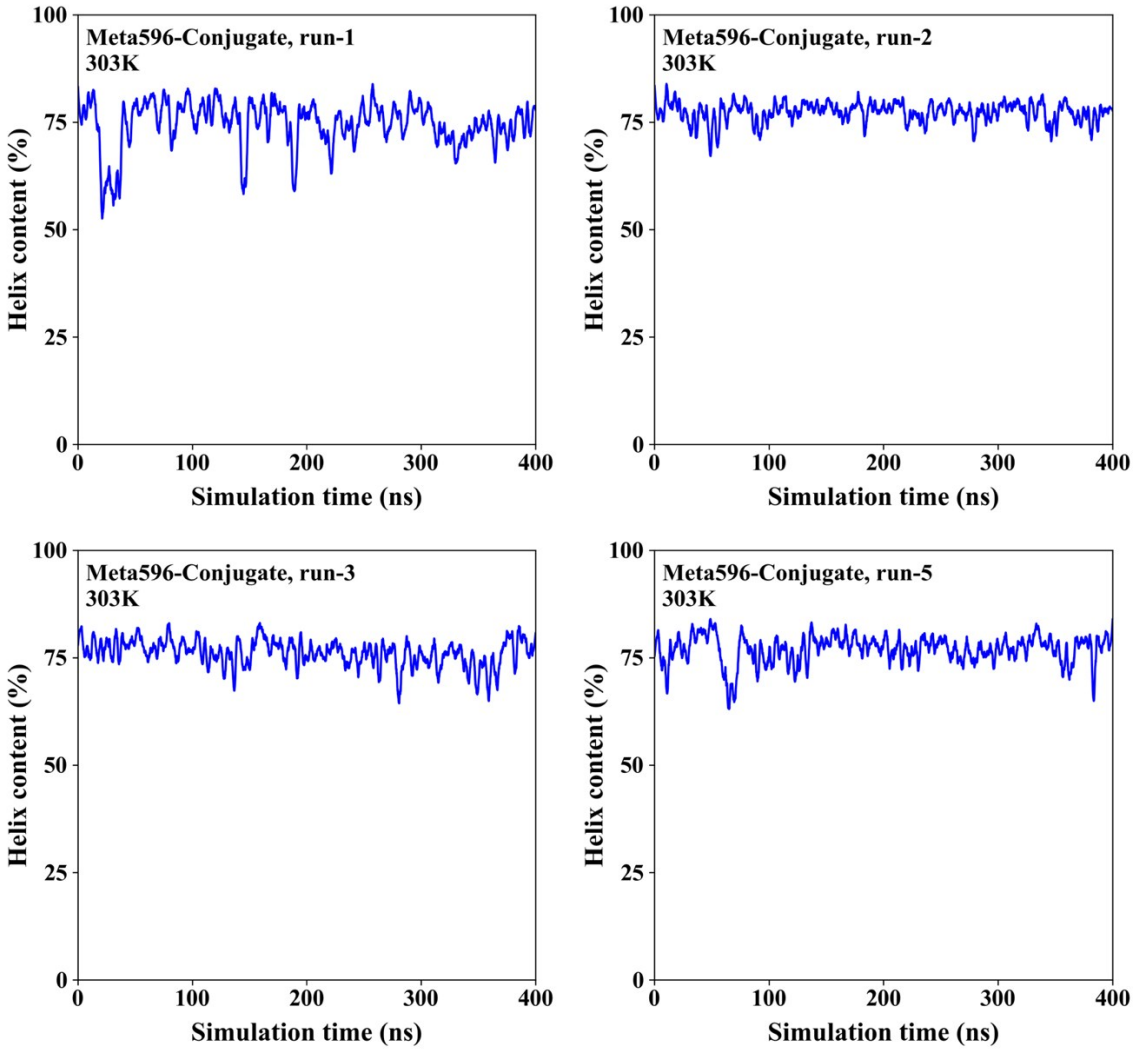

Figure S16. Helix content in the coiled coils formed by **Meta596-Conjugate** over the course of additional four independent 400 ns simulation runs at 303 K.

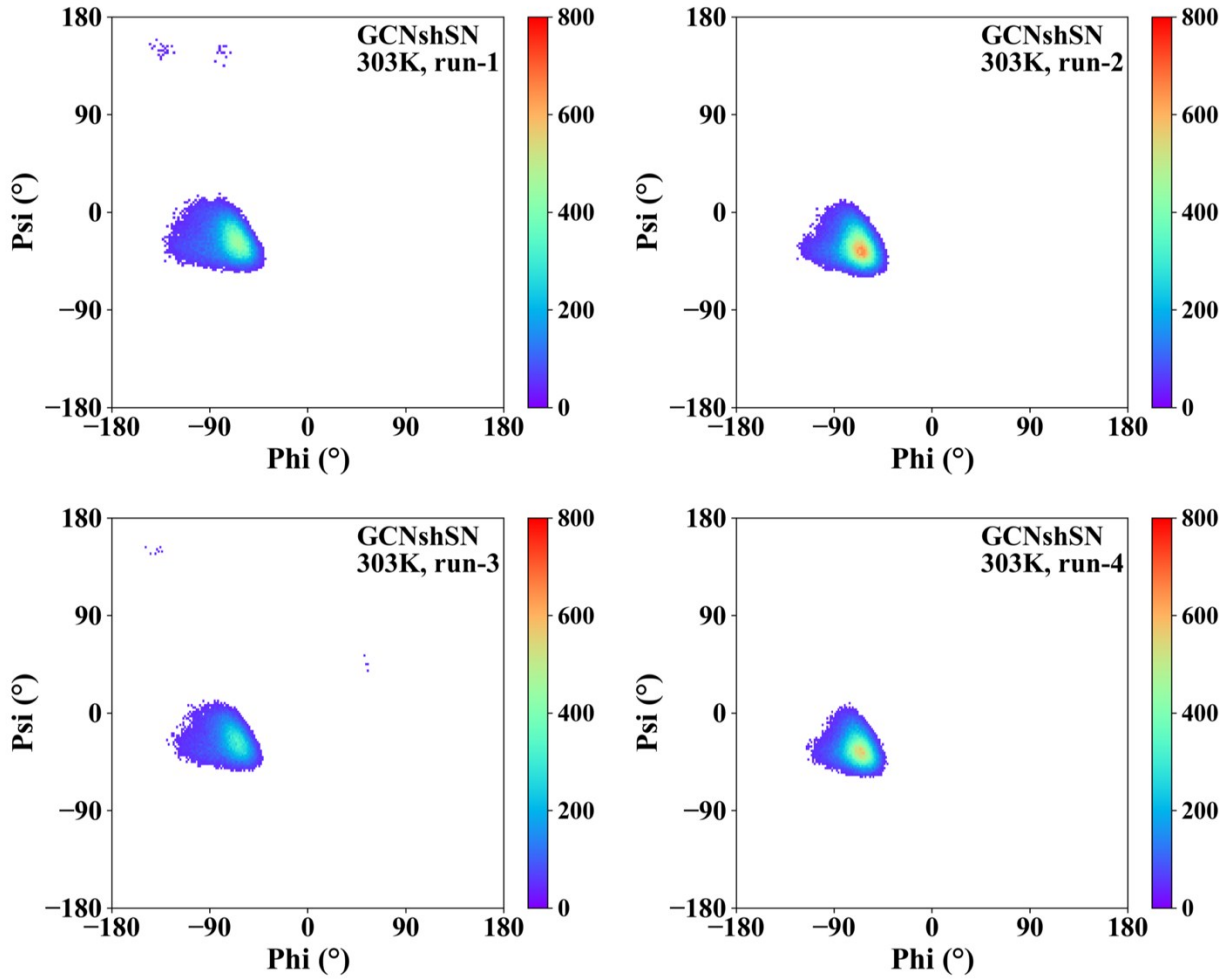

Figure S17. Ramachandran maps of conformational space sampled by **GCNshSN** peptides over the course of additional four 400 ns simulation runs at 303 K.

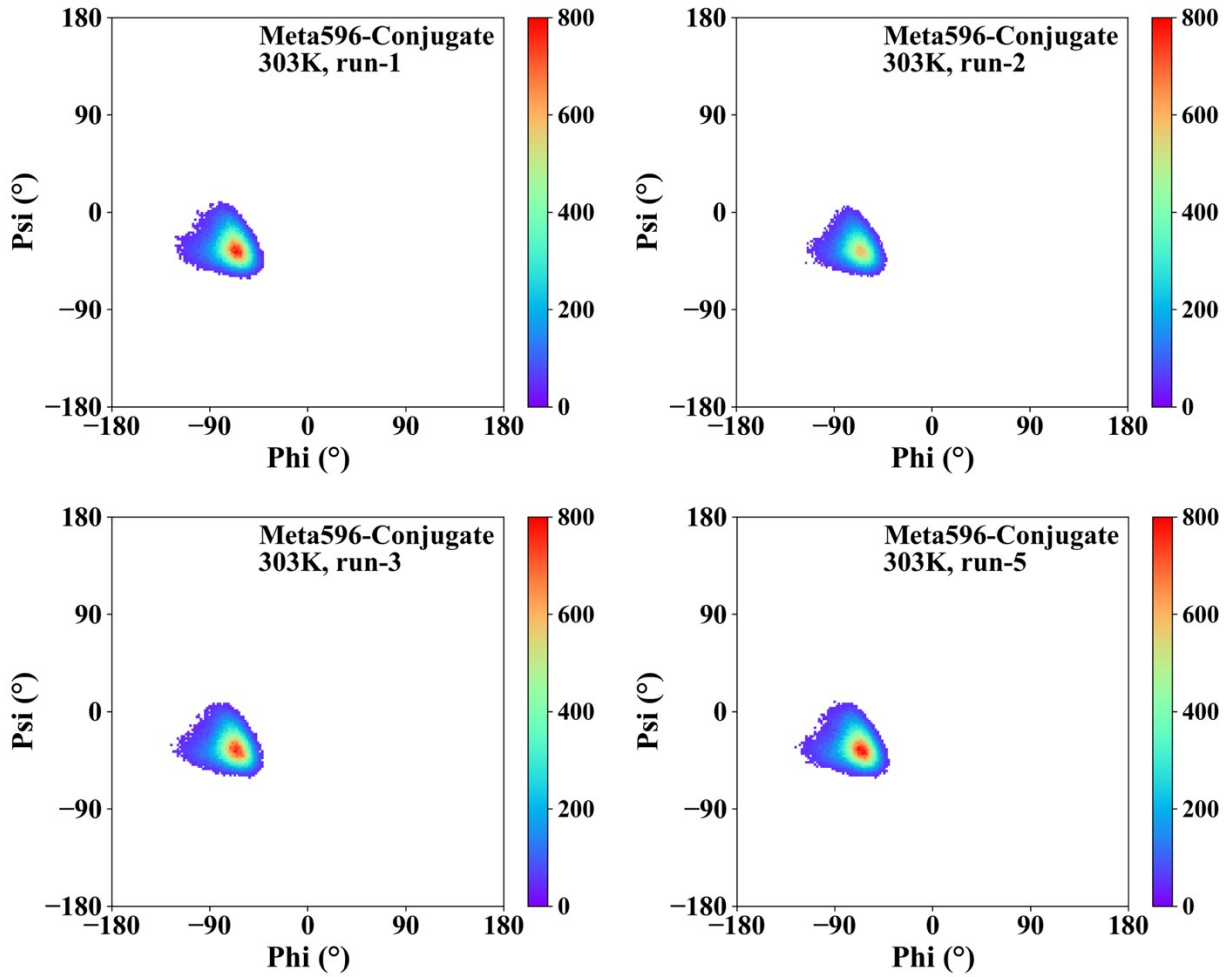

Figure S18. Ramachandran maps of conformational space sampled by **Meta596-Conjugate** over the course of additional four 400 ns simulation runs at 303 K.

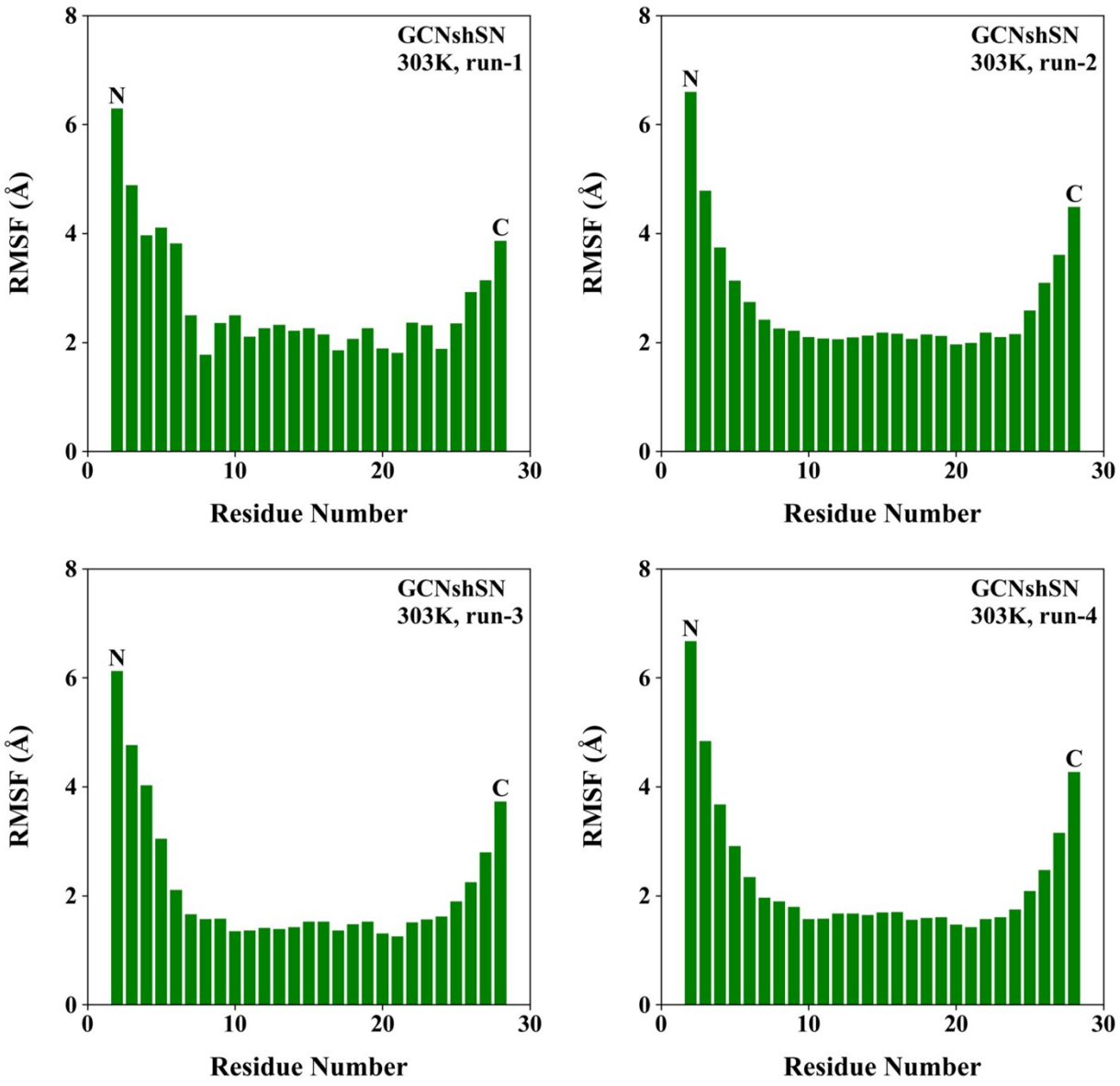

Figure S19. Residue-based root mean squared fluctuation of coiled coils formed by individual **GCNshSN** peptides within the folded regions of five 400 ns MD simulations at 303 K. N- and C-termini are labelled within the bar plots. RMSF values are averaged from two chains using only atomic fluctuations of the backbone atoms.

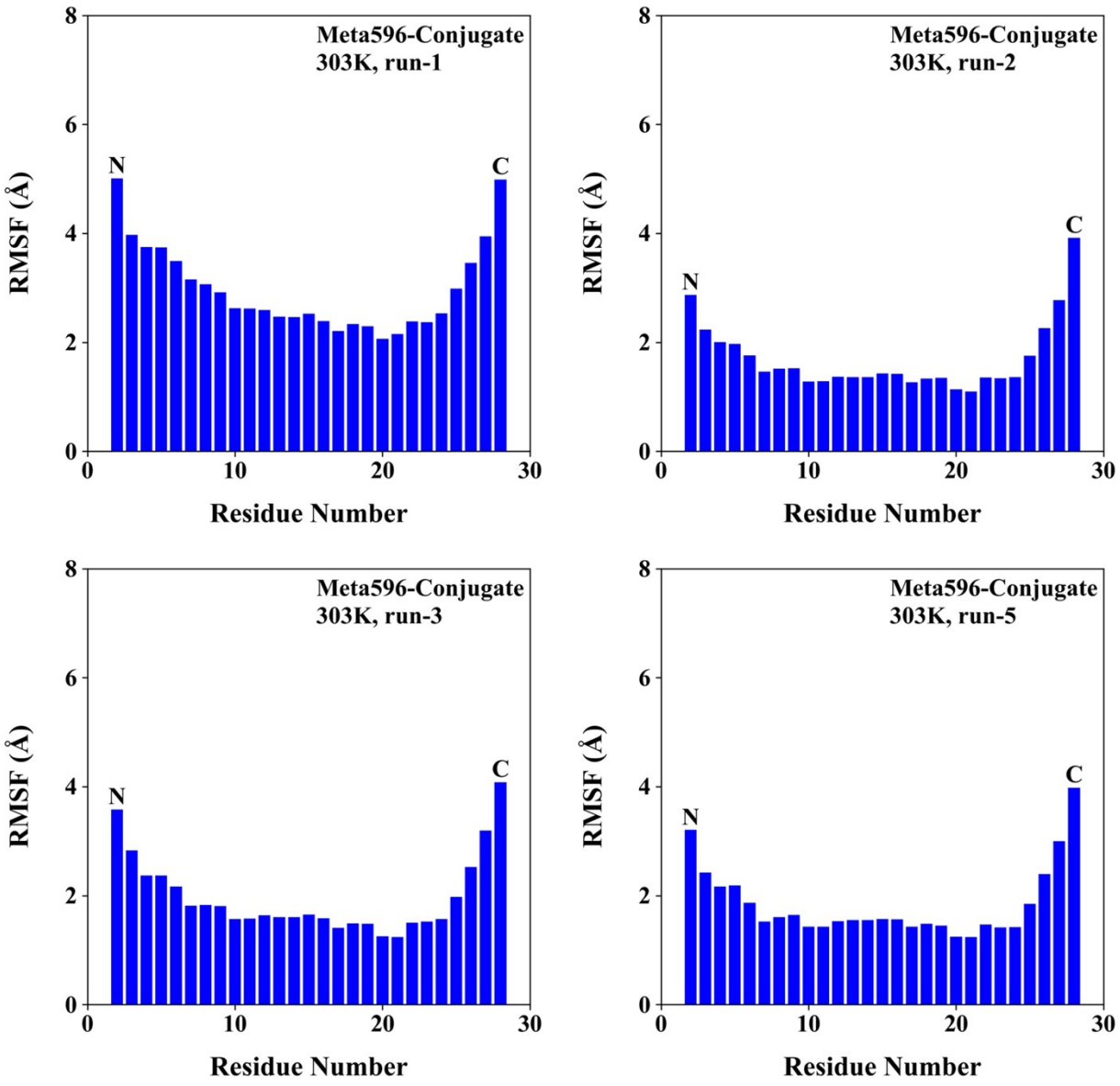

Figure S20. Residue-based root mean squared fluctuation of coiled coils formed by **Meta596-Conjugate** over the course of five 400 ns MD simulations at 303 K. N- and C-termini are labelled for the bar plots. RMSF values are averaged from two chains using only atomic fluctuations of the backbone atoms.

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