Supporting Information for

“Controlling the properties and self-assembly of helical nanofibrils by engineering zinc-binding β-hairpin peptides”

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Constructions of fibril models

The MAX1 β-hairpin peptide has been used as a ‘building block’ to design the nine new β-hairpin peptides that present self-assemble fibrils that bind Zn$^{2+}$ ions. The coordinates for the monomer peptide were taken from our previously study of the self-assembly MAX1 peptide. Two types of mutations were investigated along the sequence of MAX1 in order to bind the Zn$^{2+}$ ions (Scheme 1). In one type – type (I), two mutations of Val residues (Val1 and Val20) were performed, and in the second type – type (II), four mutations of Lys residues (Lys4, Lys6, Lys15 and Lys17) were produced. To design fibrils with various possible Zn$^{2+}$ ions coordination binding sites, in type (I), eight conformations of self-assembly fibrils were constructed: four conformations were organized in arrangement of M1 (Fig. 1) and four conformations in arrangement of M3 (Fig. 1): models E1-E4 for each arrangement. In this type, the Zn$^{2+}$ ions were bound to the ends of the peptides. Figures S1-S4 illustrate the initial and simulated conformations of these fibrils. In type (II), twenty conformations of self-assembly fibrils were constructed with various possible Zn$^{2+}$ ions coordination binding sites: each five conformations were organized in each one of the four arrangements M1, M2, M3 and M4: models C1-C5 for each arrangement. In this type, the Zn$^{2+}$ ions were bound to the central domain of the β-hairpin peptides. Fig. 2 demonstrates only two of the five conformations for each arrangement and Fig. S5-S24 exhibit the initial and simulated conformations of all five conformations.

Zinc ions bind to the incorporated His and/or Cys residues in these nine new designed peptides or in these 28 designed self-assembly fibrils. In type (I), each Zn$^{2+}$ ion within the fibril binds to two residues for each peptide in each layer, i.e. four coordination mode. In type (II), each Zn$^{2+}$ ion within the fibril binds to four residues, also forming four coordination model. Thus, in type (I) the ratio Zn$^{2+}$:peptide is 1:2 and in type (II) it is 1:1.

Molecular Dynamics (MD) Simulations Protocol

The MD simulations for each peptide model were performed in the NPT ensemble using NAMD with the CHARMM27 force-field. The peptides were energy minimized and explicitly solvated in a TIP3P water box with a minimum distance of 15 Å from each edge of the box. Each water molecule within 2.5 Å of the oligomers was removed and
the remaining water molecules were allowed to freely move. Counter ions were added at random locations to neutralize the peptides’ charge and were allowed to freely move in the solution. The Langevin piston method\textsuperscript{3, 7, 8} with a decay period of 100 fs and a damping time of 50 fs was used to maintain a constant pressure of 1 atm. A temperature of 310 K was controlled by a Langevin thermostat with a damping coefficient of 10 ps\textsuperscript{-1}. The short-range van der Waals interactions were calculated using the switching function, with a twin range cut-off of 10.0 and 12.0 Å. Long-range electrostatic interactions were calculated using the particle mesh Ewald method with a cutoff of 12.0 Å.\textsuperscript{9,10} The equations of motion were integrated using the leapfrog integrator with a step of 2 fs. The solvated systems were energy-minimized for 2,000 conjugated gradient steps, where the hydrogen bonding distance between β-sheets in each peptide oligomer was fixed in the range 2.2-2.5 Å. The hydrogen atoms were constrained to the equilibrium bond using the SHAKE algorithm.\textsuperscript{11} The force constant values for the metal-peptide (metal-binding atom) are in the range of 10–50 kcal/mol/Å\textsuperscript{2}. The minimized solvated systems were energy-minimized for 5,000 additional conjugate gradient steps and 20,000 heating steps at 250 K, with all atoms (including counter ions and water molecules) being allowed to move. Then, the system was heated from 250 K to 310 K for 300 ps and equilibrated at 310 K for a further 300 ps. All simulations were run at 310 K. Simulations time for every peptide model was 60 ns, with a total simulation time for all peptide models of 1.68 \(\mu\)s. These timescales of simulations were chosen after examining the convergence of the simulated models, using hydrogen bond analysis and root mean square deviation (RMSD) analysis. Therefore, in the current work, the timescale of the simulations is reasonable for the studied systems. The simulated structural models were saved every 10 ps for analysis.

**Generalized Born Method with Molecular Volume (GBMV)**

The GBMV method\textsuperscript{12, 13} was used to obtain the solvation energies for each simulated fibril model. For each fibril model, the solvation energy was computed from the last 500 frames of each simulation (accounting for the last 5 ns of each run), while excluding the water molecules from each of one of these frames. For GBMV calculations, the dielectric constant was set to 80 (in accordance with the expected value for water) and the hydrophobic solvent-accessible surface area (SASA) term factor was set to 0.00592
kcal/(mol*Å²). Each conformation was minimized using 1,000 iterations, and the solvation energy was evaluated by grid-based GBMV. Then, the mean energies and standard deviations of the solvation energies for each model were calculated.

**Population analysis using Monte Carlo simulations**

In each of the studied systems the GBMV energies were computed from the last 500 frames (i.e. last 5ns) of each model’s simulation. By taking into account all the computed values from all the simulated models for each system, the energy landscape of each system was constructed and evaluated for its respective conformer probabilities by using Monte Carlo (MC) simulations. In the first step, one conformation of conformer i and one conformation of conformer j were randomly selected. Then, the Boltzmann factor was computed as $e^{-(E_j - E_i)/kT}$, where $E_i$ and $E_j$ are the conformational energies evaluated from the GBMV calculations for conformations i and j, respectively, $k$ is the Boltzmann constant and $T$ is the absolute temperature (298 K used here). If the value of the Boltzmann factor was larger than the random number, then the move from conformation i to conformation j was allowed. After 1 million steps, the conformations ‘visited’ for each conformer were counted. Finally, the relative probability of model n was evaluated as $P_n = N_n/N_{total}$, where $P_n$ is the population of model n, $N_n$ is the total number of conformations visited for model n, and $N_{total}$ is the total steps. The advantages of using MC simulations to estimate conformer probability lie in their good numerical stability and the control that they allow of transition probabilities among several conformers. It should be noted that the relative conformational energies can be compared only for models that have the same sequence and number of peptides.

**Structural analysis details**

We determine the structural stabilities of the models by following the changes in the average percentage of hydrogen bonds between β-strands, with the hydrogen bond cutoff being set to 2.5 Å. This examination was performed by following the root-mean square deviations (RMSDs). The RMSD is computed along the trajectory of the simulations. Each RMSD value is an averaged value over atoms as a function of time. The RMSD value is the difference between two structures for a specific set of atoms:
snapshot along MD simulations from a simulated structure to a reference structure (e.g.,
the starting point of a simulation). This analysis provides us information about the
dissimilarity between the structures.

To estimate the inter-sheet distance for each fibrillar model, the distances between the
Ca atoms of Val5 residue peptide in one layer and the Val5 residue of the peptide in
second layer were computed. These calculations were performed for each one of the
four central peptides within the fibril and for each frame of the last 5 ns.

Finally, the secondary structure of peptides was determined by the standard dictionary
of protein secondary structure pattern (DSSP) algorithm,\textsuperscript{14} which is integrated in the
CHARMM program. Given the atomic-resolution coordinates of a peptide, the
algorithm identifies hydrogen bonds and determines the appropriate secondary
structure. The DSSP algorithm provides information on specific domains that illustrate
α-helix and β-sheet structures. In the secondary structure analyses, we computed the
potential β-sheet regions (K2-K8, K13-K19) of all peptides in the fibril for the last 5ns
of the simulation for each fibril.

\textbf{Coordination number measurements}

We measured the distances between the atoms involved in metal-binding (Ne2 and S
atoms for His and Cys residues, respectively) and the appropriate zinc ions. A
coordination interaction was counted if the distance was below 3.5 Å. The count of all
metal-residue interactions per frame was divided by the number of ions (8 or 16,
depending on the type of the model – type (I) and type (II)). The calculations were
performed for each frame of the last 5 ns.
Figure S1: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model E1 with arrangements of M1 (a) and M3 (b). One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S2: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillar model E2 with arrangements of M1 (a) and M3 (b). One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S3: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillar model E3 with arrangements of M1 (a) and M3 (b). One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S4: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model E4 with arrangements of M1 (a) and M3 (b). One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S5: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C1 with arrangements of M1. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S6: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C1 with arrangements of M2. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
**Figure S7:** Initial and simulated self-assembly Zn\(^{2+}\)-peptide fibrillary model C1 with arrangements of M3. One of the Zn\(^{2+}\)-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn\(^{2+}\) ions are seen along the fibril axis, but the other residues that do not bind Zn\(^{2+}\) ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
**Figure S8:** Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillar model C1 with arrangements of M4. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S9: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C2 with arrangements of M1. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S10: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C2 with arrangements of M2. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S11: Initial and simulated self-assembly Zn\(^{2+}\)-peptide fibrillary model C2 with arrangements of M3. One of the Zn\(^{2+}\)-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn\(^{2+}\) ions are seen along the fibril axis, but the other residues that do not bind Zn\(^{2+}\) ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
**Figure S12:** Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C2 with arrangements of M4. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S13: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C3 with arrangements of M1. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S14: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C3 with arrangements of M2. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S15: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C3 with arrangements of M3. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S16: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillar model C3 with arrangements of M4. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S17: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C4 with arrangements of M1. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
**Figure S18:** Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C4 with arrangements of M2. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
**Figure S19:** Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C4 with arrangements of M3. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S20: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C4 with arrangements of M4. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
**Figure S21**: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C5 with arrangements of M1. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
**Figure S22:** Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C5 with arrangements of M2. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S23: Initial and simulated self-assembly Zn\(^{2+}\)-peptide fibrillary model C5 with arrangements of M3. One of the Zn\(^{2+}\)-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn\(^{2+}\) ions are seen along the fibril axis, but the other residues that do not bind Zn\(^{2+}\) ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S24: Initial and simulated self-assembly Zn$^{2+}$-peptide fibrillary model C5 with arrangements of M4. One of the Zn$^{2+}$-binding site within the simulated fibril models are seen in top right image. The residues that bind all Zn$^{2+}$ ions are seen along the fibril axis, but the other residues that do not bind Zn$^{2+}$ ions of each one of the eight fibrils are seen only for the front peptide. Color of residues: Lys (blue), Val (red), Pro (pink), Thr (purple), Cys (orange), His (green).
Figure S25: The root-mean-square deviations (RMSDs) for the fibrillary models of peptides E1-E4 that are arranged in conformation M1 (red) and conformation M3 (brown).
Figure S26: The percentage of hydrogen bond analyses for the fibrillary models of peptides E1-E4 that are arranged in conformation M1 (red) and conformation M3 (brown).
Figure S27: The secondary structure of the simulated fibrillary models E1-E4 according to the DSSP analyses for conformation M1 (left, red) and conformation M3 (right, brown).
Figure S28: Populations for fibrillary models E1-E4 for conformation M1 (red) and conformation M3 (brown).
Figure S29: The root-mean-square deviations (RMSDs) for the fibrillar models of peptides C1-C5 that are arranged in conformation M1 (red), conformation M2 (blue), conformation M3 (brown) and conformation M4 (green).
Figure S30: The secondary structure of the simulated fibrillary models C1-C5 according to the DSSP analyses that are arranged in conformation M1 (red), conformation M2 (blue), conformation M3 (brown) and conformation M4 (green).
Figure S31: The percentage of hydrogen bond analyses for the fibrillary models of peptides C1-C5 that are arranged in conformation M1 (red), conformation M2 (blue), conformation M3 (brown) and conformation M4 (green).
Figure S32: Populations for fibrillary models C1-C5 for conformation M1 (red), conformation M2 (blue), conformation M3 (brown) and conformation M4 (green).
References


