Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Carrier free Self-Build Aspirin Nanorods as Anti-Aggregation Agents towards Alpha Crystallin Derived Peptide Aggregates: Potential Implications in Non-Invasive Cataract Therapy

Anjali Bisht[a,1], Manju Sharma[a,1], Shikha Sharma[a], Md Ehesan Ali*[a], Jiban Jyoti Panda*[a]

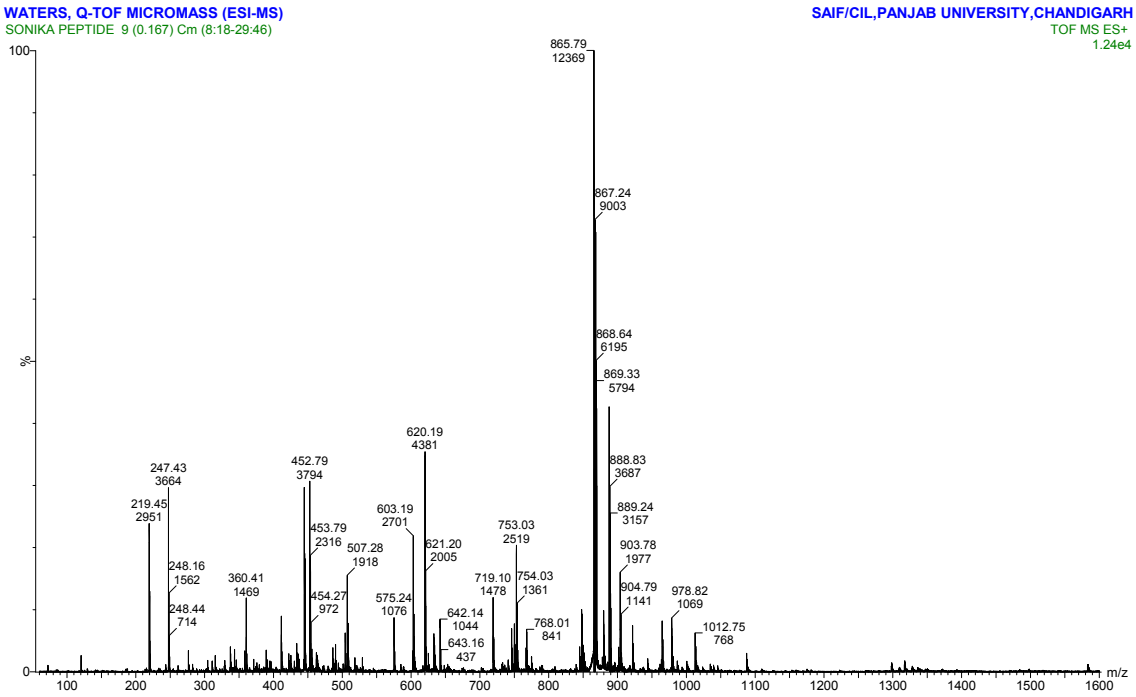

Figure S1. Mass spectrum of Pep 7.

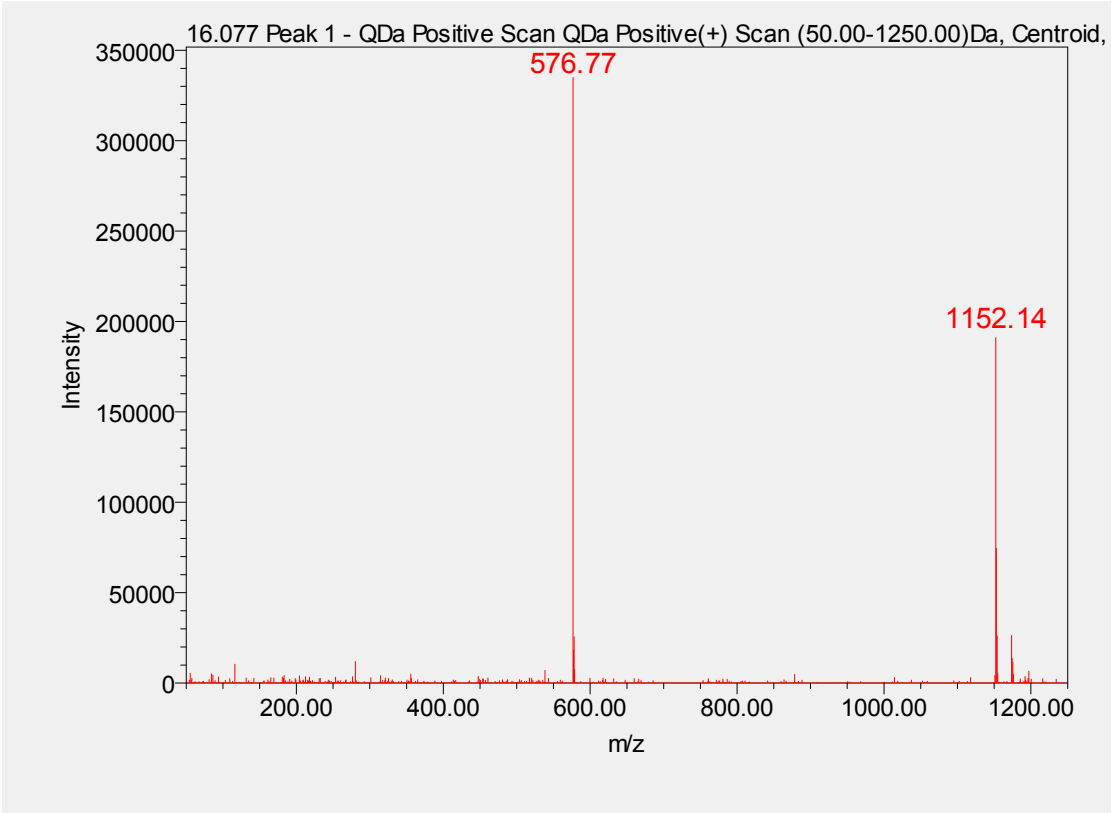

Figure S2. Mass Spectrum of Pep 9.

Information S1: Homology Modelling

Primary sequence alignments of the model peptides were initially generated using the program BLAST. The template determination of sequence Pep-7 and Pep-9 revealed ~50 different crystallin sequences from the PDB database producing significant alignments. The Blast result showed 80-100% sequence identity with these peptides. The homology models of peptides were derived from the crystallographic structure of the protein (PDB ID: 3L1E, 3L1F, 5LUQ and 5W1R) which showed 100 % of structural similarity with target sequence (supply info Figure S3). Further sequence alignment between the target peptides and template protein were performed by using Modeller program. Out of the 4 models generated by Modeller, the one with the highest Discrete Optimized Protein Energy (DOPE) score was selected. The best model was selected on the basis of DOPE assessment score and GA341 score which was 37481.64 and 1.00 respectively, that validated for a good model.

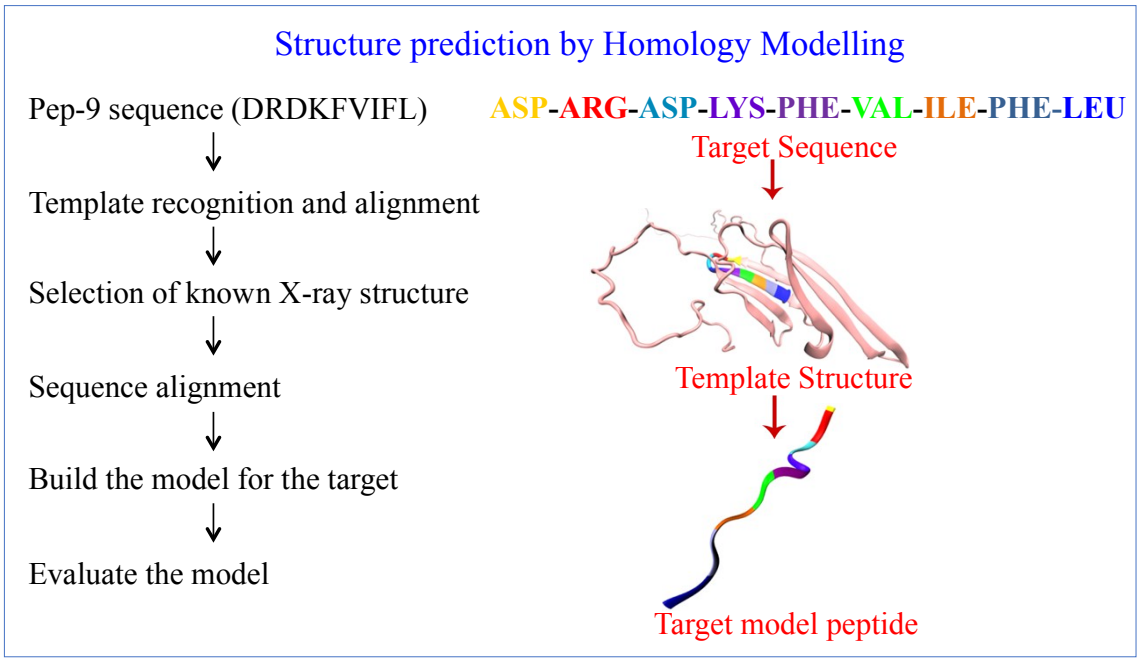

Figure S3. Steps of structure prediction of Pep-9 (DRDKFVIFL) by homology modelling. Alignment results show 100% sequence similarity with Bovine α-crystallin Zinc Bound (PDB: 3L1E). Modeller program performs further sequence alignment and model refinement.

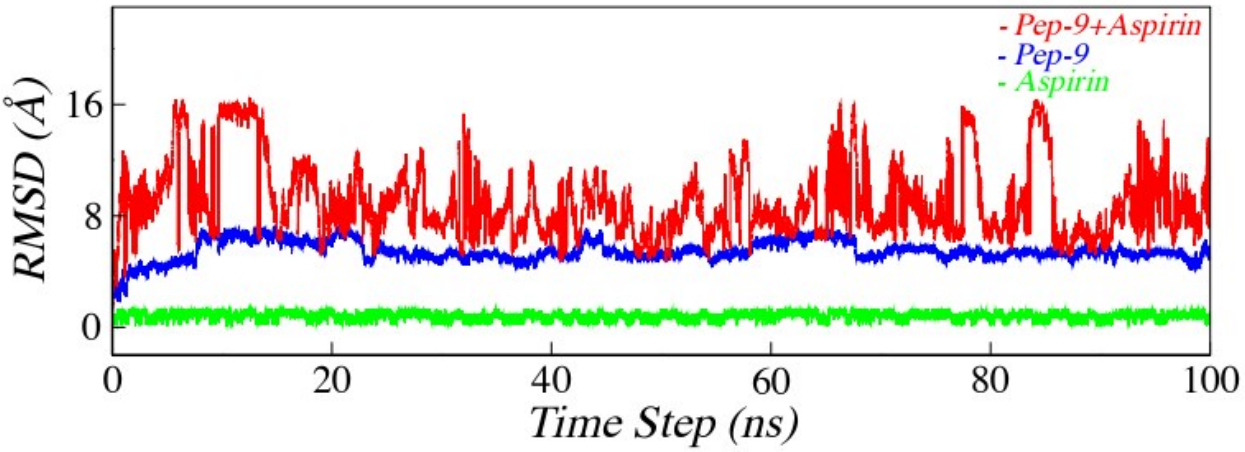

Figure S4**.** The RMSD of the structural fluctuations for aspirin (Green), Pep-9 (Blue), Pep-9+Aspirin (Red) with respect to the initial structures during 100 ns MD simulations. Structural fluctuations of aspirin molecule were quite small because of its rigid molecular structure. However, the structural fluctuations for Pep-9 were comparatively larger because of its large size and owing to the flexibility of peptide molecules. The RMSD fluctuations were the largest in case of peptide along with aspirin as it moved faster over various amino acids of the peptides.

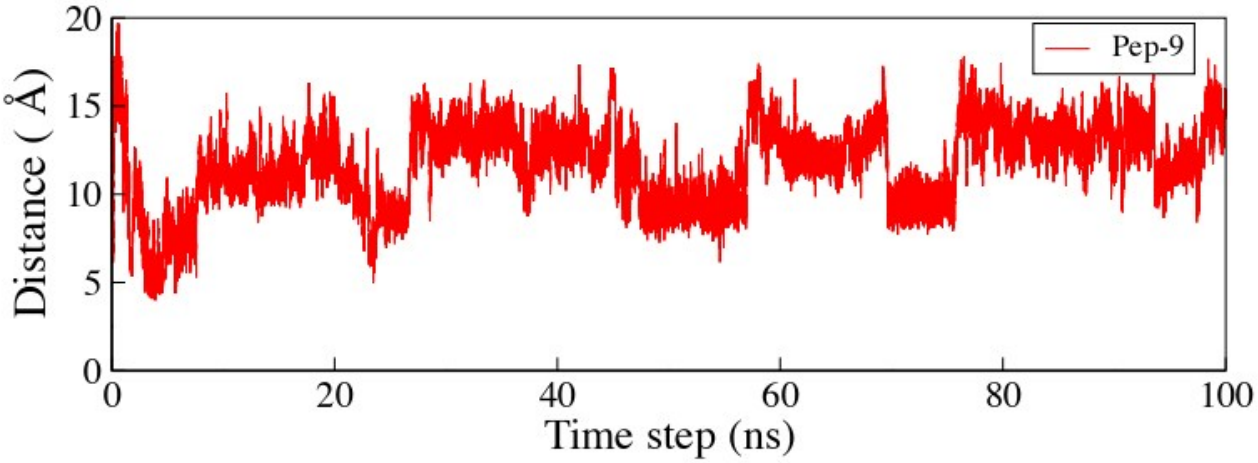

Figure S5. Variations of distance between the N to C terminus of Pep-9. This distance of peptide was about 20 Å for the initial unfolded beta-strand. We observed turns in the starting trajectory of peptide which formed due to intra chain hydrogen bond interaction between the residues. However, it was clearly visible that this distance was much lower and was approximately 4 Å that indicated that the peptides became more folded in presence of aspirin.

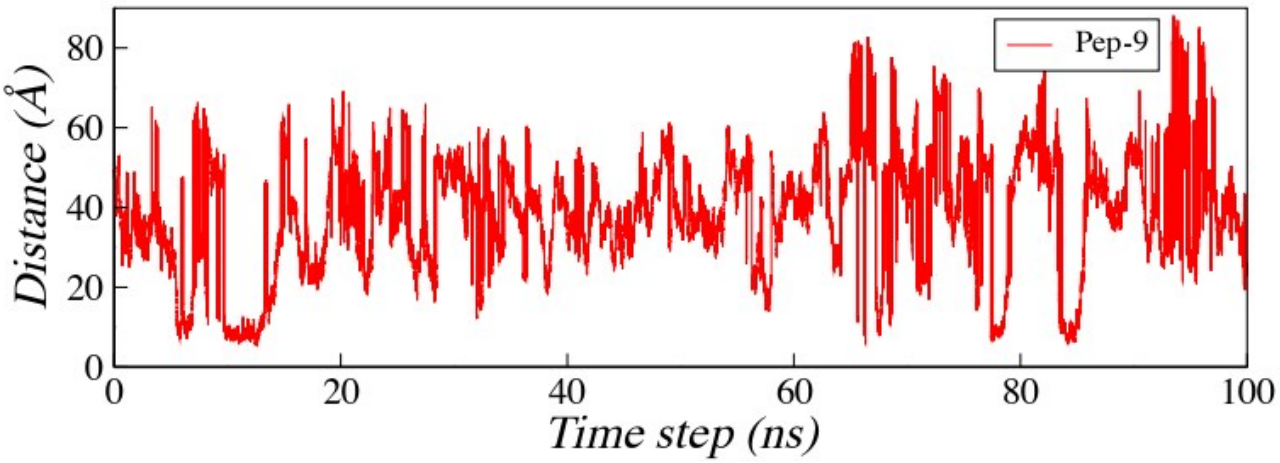

Figure S6**.** The distance between centre of mass of peptide and aspirin in Pep-9. Centre of mass of peptide resided inside the folded region of peptide and the minimum binding distance between the peptide and aspirin was up to 5 Å. Moreover, the minimum distance between individual residue of peptide and aspirin was up to 1.5 Å which was generally observed in case of formation of hydrogen bond between individual residues. The graph depicts large fluctuations in the distance of peptide and aspirin.

Information S2: MD simulation of Pep-7

A strong interaction between the peptide and aspirin was observed during the simulation studies. The secondary structure populations of the peptide showed β-sheets, random coils

and turns as observed in Pep-9. Aspirin as an inhibitor changed the structure into alpha-helical form. A strong hydrogen bond interaction was observed with PHE1, VAL2 and VAL7 in the 100 ns trajectory. Approx. 0.35 ns effective time was observed between VAL7 and aspirin (Table S1).

Table S1. The cumulative time spent by the aspirin molecule with the different peptides in the 100ns long trajectory.

Sl. No	Amino-acids	Time step (ns)
1	PHE	0.20
2	VAL	0.25
3	ILE	0.12
4	PHE	0.05
5	LEU	0.15
6	ASP	0.08
7	VAL	0.35

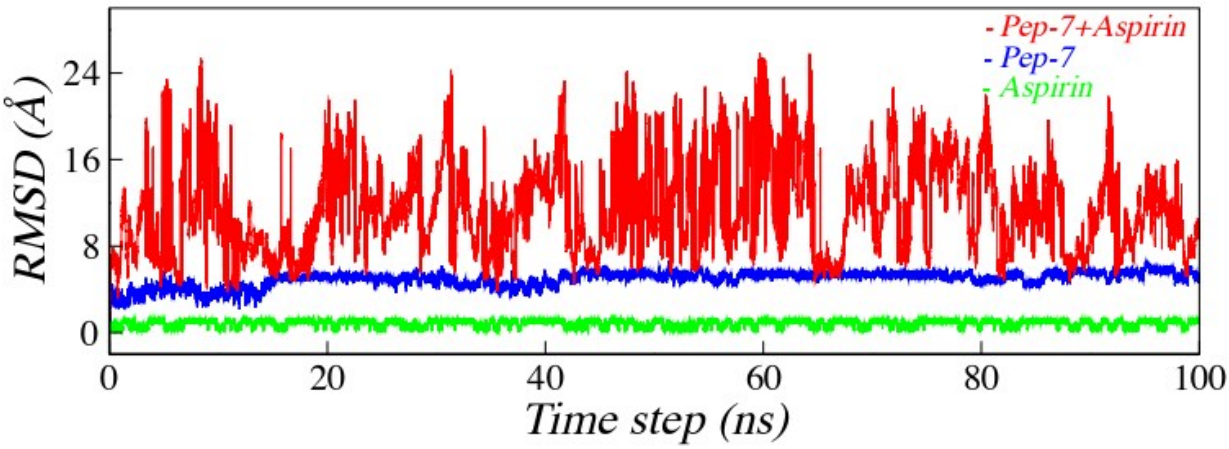

Figure S7. The RMSD of structural fluctuations for aspirin (Green), Pep-7 (Blue), Pep-7+Aspirin (Red) with respect to the initial structures for the 100 ns MD simulations. The structural fluctuations of aspirin molecule was quite small because of its rigid molecular structure. However, the structural fluctuations for Pep-7 were comparatively larger because

of the large and flexible peptide molecules. The RMSD fluctuations were the largest in case of peptide along with the aspirin as it moved faster over the various amino acids of the peptides. It reached maximum at 24 Å which implied that large conformational change occurred at the structure.

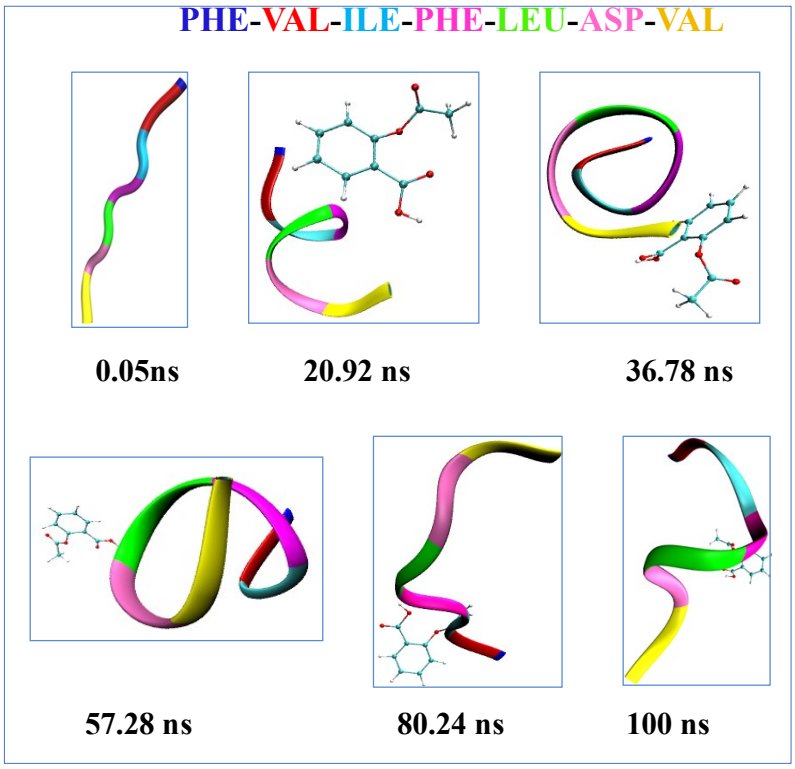

Figure S8. Different conformations of Pep-7 as captured in various snapshots along the 100 ns MD simulation trajectory. The first frame was obtained at 0.05 ns. Interactions between aspirin and peptide were quite small from 0.0 - 0.05 ns and during this period the peptide mostly preserved its beta-sheet structure with regular dynamical fluctuations. However, as the simulation progressed, a clear change of beta-sheets of the peptide to various coils and helical forms were observed.

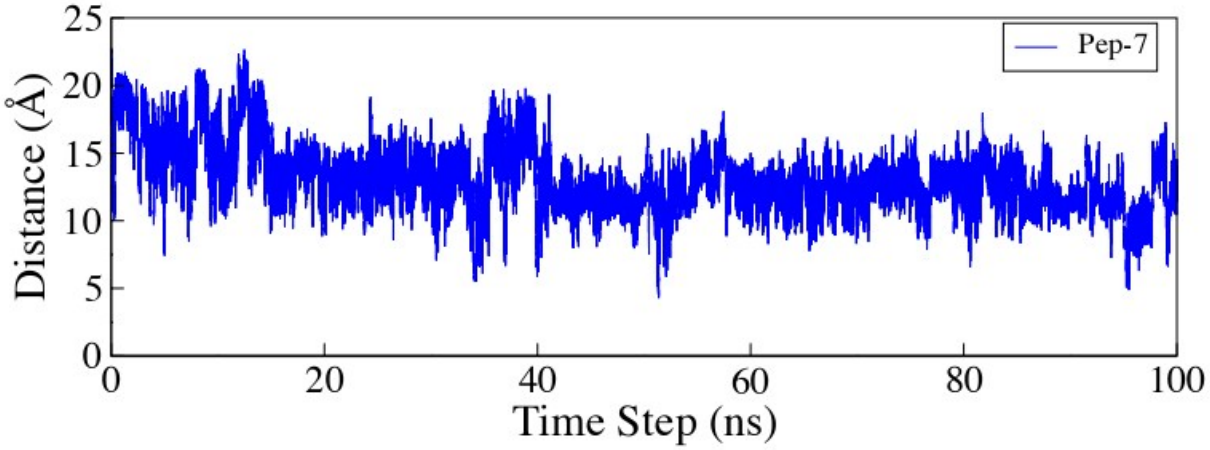

Figure S9. Variations of distance between the N to C terminus of peptide in Pep-7. The normal N to C terminus distance of peptide is about 20 Å. However, simulation studies demonstrated a lower distance indicating that the peptide to be more folded in presence of aspirin.

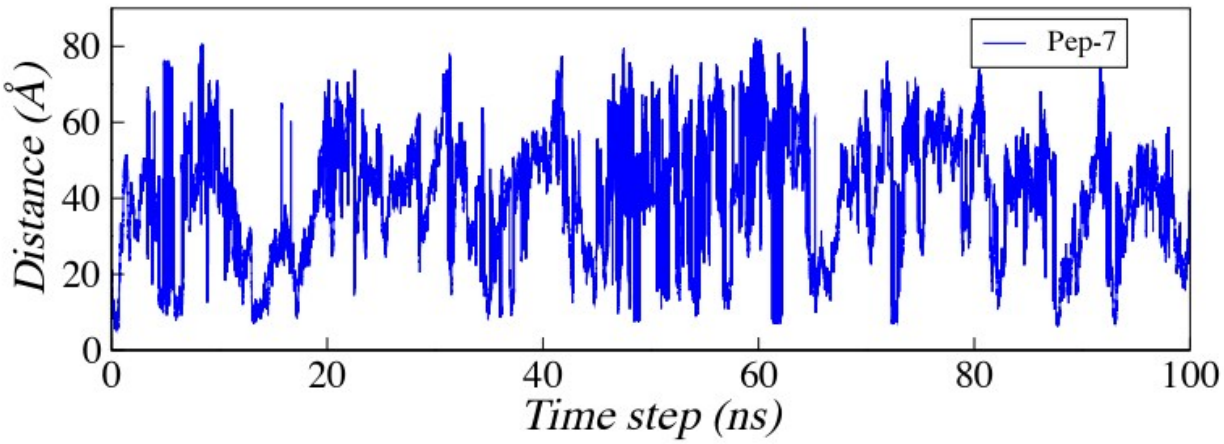

Figure S10**:** The distance between centre of mass of peptide and aspirin in Pep-7. As centre of mass of peptide was inside the folded region of peptide so the average binding distance between the peptide and aspirin was up to 4 Å. Moreover, the distance between individual

residue of peptide and aspirin was up to 2 Å which depicts hydrogen bonded interaction of between individual residue.