Ultrafast Folding and *Ab-initio* Molecular Dynamics of a Linear Hydrophobic β -hairpin

Upadhyayula Surya Raghavender*

National Centre for Biological Sciences, Tata Institute of Fundamental Research, GKVK Campus, Bellary Road, Bangalore, 560065, India.

Peptides used in the all-atom MD simulations are listed below:

Ace-Leu-Phe-Val-^DPro-^LPro-Leu-Phe-Val-NMA (1)

Ace-Leu-Phe-Val-^LPro-^LPro-Leu-Phe-Val-NMA (2)

Ace-Leu-Phe-Val-^LPro-^DPro-Leu-Phe-Val-NMA (3)

Ace-Leu-Phe-Val-^DPro-^DPro-Leu-Phe-Val-NMA (4)

Modeling and Simulation Methods

The peptide structures **1-4** were prepared in an all-*trans* (all residues) extended conformation in Maestro, protected by acetyl (Ace) and n-methylamide (NMA) groups at N- and C-termini, respectively. The peptides were incorporated in simulation box, using the System Builder module of Desmond. The periodic boundary conditions are set up by specifying the shape (cubic) and size (30 Å in vacuum, and 10 Å in water(SPC), methanol, DMSO) of the box. Volume minimization was carried out, resulting in a reorientation of the solute(peptides) in the box. The OPLS_2005 forcefield and NVT ensemble were employed for all the simulations.

The models prepared were subjected to production MD runs, which includes a series of equilibrating minimizations and short MD runs before subjecting to production runs. Briefly, the protocol of relaxation and MD is described below:

1. Minimize with the solute restrained.
2. Minimize without restraints.
3. Simulate in the NVT ensemble using a Berendsen thermostat with:
 - a simulation time of 12ps

- a temperature of 10K
 - a fast temperature relaxation constant
 - velocity resampling every 1ps
 - non-hydrogen solute atoms restrained
4. Simulate in the NVT ensemble using a Berendsen thermostat and a Berendsen barostat with:
 - a simulation time of 24ps
 - a temperature of 10K and a pressure of 1 atm
 - a fast temperature relaxation constant
 - a slow pressure relaxation constant
 - velocity resampling every 1ps
 - non-hydrogen solute atoms restrained
 5. Simulate in the NVT ensemble using a Berendsen thermostat and a Berendsen barostat with:
 - a simulation time of 24ps
 - a temperature of 300K and a pressure of 1 atm
 - a fast temperature relaxation constant
 - a slow pressure relaxation constant
 - velocity resampling every 1ps
 - non-hydrogen solute atoms restrained
 6. Simulate in the NVT ensemble using a Berendsen thermostat and a Berendsen barostat with:
 - a simulation time of 24ps
 - a temperature of 300K and a pressure of 1 atm
 - a fast temperature relaxation constant
 - a normal pressure relaxation constant

Step 5 is skipped during vacuum simulations.

The systems were subjected to production MD runs, only after being well equilibrated. All the simulations were run on a Desktop PC with Intel i7 processor (using a maximum 6 processors). Vacuum and bulk solvent simulations were performed for a period of 10.2 ns, with a

timestep of 1.2 ps, and the energy values were written at every 4.8 ps. Metadynamics simulations was also done with a similar protocol, but with explicit restraints in the form of “collective variables” specified at the start of the simulations. Two CVs were chosen, dihedral and h-bond length as described in the body of the Communication. The width and height of the Gaussians were set to 5.0 Å and 0.03 Å for torsional variables and 5.0 Å and 0.03 Å for distance variable.

The replica exchange MD runs were performed with the peptide(**1**) immersed in SPC water for a period of 1 ns. 4 replicas at different temperatures, $T = 300\text{K}$, 311.1K, 344.4K, 400K, were employed during the simulation in a NVT ensemble. The relaxation protocol is as described above.

At the end of each simulation, Simulation Quality Analysis was performed to check the parameters (Energy, Potential Energy, Temperature, Pressure and Volume). Few parameters calculated at the end of each simulation include:

Radius of gyration (Rg)

RMSD w.r.t to the initial structure (at $t = 0\text{ps}$)

Number of hydrogen bonds formed during the simulation (N-O)

Dihedral angles of select residues (diproline segment)

Native hydrogen bond distances (O3-N6, O6-N3, O8-N1, O1-N8)

Only those states in the simulations were considered for further analyses, where the conformations of the amino acid residues were those allowed for protein amino acids. States where the residues adopted (e.g. Leu(1) in **1**, Val(8) in **4**) unusual conformations were rejected.

Two trajectories (three in the case of vacuum simulations) were generated for each structure. The results of different trajectories are in general agreement with each other. The results of the best run are reported here.

References

Desmond Molecular Dynamics System, version 3.0, D. E. Shaw Research, New York, NY, 2011.
Maestro-Desmond Interoperability Tools, version 3.0, Schrödinger, New York, NY, 2011.

Ace-Leu-Phe-Val-^DPro-^LPro-Leu-Phe-Val-NMA (DL)(1)

(a) 10 ns Vacuum

(b) 10 ns water

(c) 10 ns Methanol

(d) 10 ns DMSO

Ace-Leu-Phe-Val-^LPro-^LPro-Leu-Phe-Val-NMA (LL)(2)

Ramachandran Plot

(a) 10 ns Vacuum

Ramachandran Plot

(b) 10 ns water

Ace-Leu-Phe-Val-^LPro-^DPro-Leu-Phe-Val-NMA (LD)(3)

Ace-Leu-Phe-Val-^DPro-^DPro-Leu-Phe-Val-NMA (DD)(4)

10 ns Vacuum

Ace-Leu-Phe-Val-^DPro-^LPro-Leu-Phe-Val-NMA (DL)(1)

1(a)

1(b)

10 ns Metadynamics

Ace-Leu-Phe-Val-^DPro-^LPro-Leu-Phe-Val-NMA (DL)(1)

1(a)

1(b)

Metadynamics

Ace-Leu-Phe-Val-^DPro-^LPro-Leu-Phe-Val-NMA (DL)(1)

Vacuum : 10ns

Ace-Leu-Phe-Val-^LPro-^LPro-Leu-Phe-Val-NMA (LL)(2)

Vacuum : 10ns

Ace-Leu-Phe-Val-^DPro-^LPro-Leu-Phe-Val-NMA (DL)(1)

Replica Exchange MD in water : 1ns

Replica 3 [777.6 ps]

Replica 3 [787.6 ps]

N3...O6

Replica3 @ T = 344.4K

Replica 3 [811.2 ps]

Replica 3 [820.8 ps]

Replica 3 [825.6 ps]

Replica 3 [830.4 ps]

Ace-Leu-Phe-Val-^DPro-^LPro-Leu-Phe-Val-NMA (DL)(1)

Methanol : 10ns

DMSO : 10ns

Ace-Leu-Phe-Val-^DPro-^LPro-Leu-Phe-Val-NMA (DL)(1)

Metadynamics : 10 ns

#Chain A:
0.0%Helix 9.4%Sheet 54.9%Turn

Res	H(%)	S(%)	T(%)				Xxx
2	0.00	0.00	51.64	0.00	0.00	0.00	LEU
3	0.00	10.80	65.73	0.00	0.00	0.00	PHE
4	0.00	26.76	69.01	0.00	0.00	0.00	VAL
5	0.00	0.00	96.24	0.00	0.00	0.00	DPRO
6	0.00	0.00	95.31	0.00	0.00	0.00	PRO
7	0.00	27.23	44.60	0.00	0.00	0.00	LEU
8	0.00	10.33	15.02	0.00	0.00	0.00	PHE
9	0.00	0.00	1.41	0.00	0.00	0.00	VAL

DPro-LPro

Chi1 vs Chi2

Chi2 vs Chi3

Chi3 vs Chi4

Chi4 vs Chi5

Chi1 vs Chi5

LPro-LPro

Chi1 vs Chi2

Chi2 vs Chi3

Chi3 vs Chi4

Chi4 vs Chi5

Chi1 vs Chi5

LPro-DPro

Chi1 vs Chi2

Chi2 vs Chi3

Chi3 vs Chi4

Chi4 vs Chi5

Chi1 vs Chi5

DPro-DPro

Chi1 vs Chi2

Chi2 vs Chi3

Chi3 vs Chi4

Chi4 vs Chi5

Chi1 vs Chi5