II. METHODOLOGIES

A. MD Simulations

The crystal structure of SIRT3/Ac-CS2/Carba-NAD+ (pdbID: 4FVT) was used as the starting structure to construct all the structures in SIRT3 MD simulations. Ternary complex was constructed by simply modifying Carba-NAD+ to NAD+. SIRT3/NAD+ binary structure was obtained by removing the peptide substrate from the ternary complex. For complex also including nicotinamde (NAM) as inhibitor, NAD+ and NAM coordinates were taken directly from crystal structure of Sir2Af2/NAD+ complex (pdbID: 1YC2 chain A) after structural alignment between 4FVT and 1YC2:A. Complex including iso-nicotinamide (isoNAM) was built by modifying NAM to isoNAM in the above structure. All other molecules including waters were removed. Extra sodium and chloride ions were added to neutralize the system, followed by solvation using boxes of TIP3P water molecules with a margin of 12.0 Å from solute atoms in all three dimensions.

Models for SIRT3, Ac-CS2 and NAD+ alone were also constructed by taking the coordinates of from 4FVT crystal structure, followed by the same neutralization and solvation process.

The crystal structure of Sir2TM/Ac-p53/NAD+ (pdbID: 2H4F) was used as that starting structure to construct all the structures in SIRT3 MD simulations. The missing loop of residue 37-42 was modeled using an ab initio loop prediction method in Prime version 3.2 (Schrödinger, LLC) during the protein preparation stage. Sir2TM/NAD+ binary structure was obtained by removing the peptide substrate from the ternary complex. Complex of Sir2TM/Ac-p53/NAD+/NAM was obtained by first removing the NAD+, followed by superimposing the NAD+ and NAM from 1YC2:A after structural alignment. All other molecules including waters were removed, and extra sodium and chloride ions were added to maintain system neutrality. The complexes were then solvated using boxes of TIP3P water molecules with a margin of 12.0 Å from solute atoms.

The Amber99SB force field[1,2] was used for all the molecular mechanics calculations. Extra parameters were adapted from the following sources: parameters for Zn developed by Luo’s group;[3] parameters for NAD+ developed by Walker et al [4] and Pavelites et al;[5] parameters for acetylated lysine developed by Papamokos et al.[6] Charges for nicotinamide and iso-nicotinamide were derived from RESP fits to electrostatic potentials obtained at HF/6-31(d) level of theory that is consistent with Amber99SB force field.

All MD simulations were performed with the periodic boundary condition to produce isothermal-isobaric ensembles (NPT) at 300 K using the NAMD program.[7] The Particle Mesh Ewald (PME) method [8] was used to calculate the electrostatic energy. The covalent bonds involving hydrogen atoms were frozen with the SHAKE algorithm.[9] Temperature was regulated using the Langevin dynamics with the collision frequency of 1 ps-1. Pressure regulation was achieved with isotropic position scaling and the pressure relaxation time was set to 1.0 picosecond. The integration of the equations of motion was conducted at a time step of 2 femtoseconds.

There are three phases in MD simulations. First in the relaxation phase, the system underwent a 2000-step minimization before a short 200 ps NPT MD simulation, with the main chain atoms of protein restrained to the positions of crystal structures with force constants of 5 kcal mol-1 Å-2. Next, the systems ran for various lengths of time up to 22ns in the equilibration phase. Last, the sampling phase includes a 10ns of MD simulation.

B. MM-PB/GBSA Calculations

Binding free energies were calculated using the Molecular Mechanics Poisson−Boltzmann Surface Area (MM-PBSA) and the Molecular Mechanics Generalized Born Surface Area (MMGBSA) methods as implemented in the AMBER package.[10] In MM-PBSA and MM-GBSA, binding free energy is evaluated as:

ΔGbind = ΔEMM + ΔGsolv − TΔS

where ΔEMM, ΔGsolv and TΔS are the changes of gas-phase interaction energy, solvation free energy, and conformational entropy upon binding. ΔEMM includes internal energy in bonded terms, electrostatic and van der Waals energies. ΔGsolv is the sum of polar contributions calculated using PB or GB model, and nonpolar contributions estimated from solvent-accessible surface area (SASA).

All the calculations were carried out using the MMPBSA.py module with AmberTools13. [10] The polar contribution of the solvation free energy was calculated by GB model developed by Onufriev et al.[11] and by PB method implemented in pbsa program. The solvent-accessible surface area was evaluated using the LCPO method.[12] Because relative free energy trends were of interest, solute entropy was neglected. Energies were evaluated using 10000 snapshots extracted from the last 10 ns at time interval of 1 ps of each trajectory after ensuring that each one of these trajectories was completely stable. One exception is the highly dynamic SIRT3/NAD+ binary system, where 20000 snapshots from last 20 ns were used in energy evaluations.

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