Reporting for MD simulations according to JCIM

Coordinates of the starting conformation

The Crystal Structures for the HADs PA0810, RSC1362, ST2570, BPRO0530 and RHA0230 are downloaded from RCSB. For PDB files, which contain selenomethionine, selenomethionine is exchanged with methionine.

For the second set of simulations, the amino acid sequences of the HADs were downloaded and then the PDB files were generated using alphafold2 with amber to relax the top 1 structure in each iteration.

Force Field

For all simulations the force field amber99sb-ildn was used. For a subset of simulations, the crystal structures of PA0810, RSC1362, ST2570, BPRO0530 and RHA0230, the force field OPLS/AA was used for an additional set of simulations. Amber99sb-ildn was used because of its superior performance on protein folding experiments compared to OPLS/AA.

Simulation conditions

a. Conjugate Gradient energy minimization (cg) with 50 step steepest descent and a maximum of 1000 steps to integrate or the Energy of the system reaches below 1000 kJ/mol/nm. The initial EM step size is 0.01 nm and the cut-off scheme is Verlet. For the MD integrator the leap-frog algorithm was used (md) with a time step of 2 fs and a total simulation length of 100 ns and 10 replicas and 1 ms and 2 replicates, for each simulation.

b. The LINear Constrains Solver (LINCS) was used to constrain all bonds. Tip3p was used for water geometry.

c. Modified Berendsen thermostat was used (Berendsen) with a target temperature of 300K and a time constant of 0.1 ps and Protein & non-Protein coupling groups.

d. Berendsen pressure coupling algorithm was used with a target pressure of 1 bar, isotropic pressure coupling in x-y-z directions, a time constant of 1 ps, compressibility of 4.5\*10^(-5) bar^(-1).

e. Long-Range neighbourlist cut-off of 1 nm and Particle Mesh Ewald (PME) with a cubic interpolation (pme order = 4) and fourierspacing of 0.16 nm.

f. GROMACS 2021.4 was used to perform the simulations.

g. The boxsize is 1.2 nm bigger than the protein in each direction, the box is cubic and the protein is solvated in explicit water. The Protein’s net charge is neutralized with Na+ or Cl- Ions.

h. The variance of the 100 ns simulations with 10 replicates ranged between 0.015 and 0.08 nm for all simulated proteins. !!! add 1 ms simulation data and RMSD change during the run and comparison between Crystal Structure and AF2.!!!

(input/output files and data availability)

(experimental validation)

Other publication differences:

Covalent bonds of water and other molecules were constrained with SETTLE (20) and P-LINCS (21), respectively

Coulombic interactions were calculated using the smooth particle-mesh Ewald (PME) method (16,17) with a real-space cut-off of 1.2 Å and a Fourier grid spacing of 1.4 Å.

During 500-step steepest-descent energy minimizations performed initially, all heavy atoms of the enzymes were kept restrained to their crystallographic structure using a force constant of 1000 kJ×mol-1×nm-2.

Simulation in the isothermalisobaric (NpT) ensemble was achieved by isotropic coupling to a Berendsen barostat (18) at 1 bar with coupling constants of 4 ps and temperature coupling using velocity Langevin dynamics (19) at 300 K with a collision frequency of 1 ps-1

The integration time step was 2 fs and the non-bonded pair-list was updated every 20 fs. The time trajectories were saved every 25 ps. The first 50 ns of each trajectory were removed from analysis as equilibration based on the analysis of backbone RMSD vs. time (Figure S4) and