Molecular Dynamics Simulations

To derive force field parameters for the substrate, electrostatic potential (ESP) calculations were carried out at the HF/6-31G(d) level, followed by a two-stage restrained electrostatic potential (RESP) charge fitting protocol (1). The bonded and non-bonded parameters, including bond lengths, angles, dihedral angles, and van der Waals radii, were generated using the Antechamber module within the AmberTools suite. Molecular docking calculations were performed using AutoDock Tools. In the constructed structural model, the central moiety of the PET trimer was positioned adjacent to the catalytic serine residue. Protonation states of titratable residues were assigned under the experimental pH conditions using PROPKA 3.0(2). Specifically, the histidine residue in the catalytic triad was protonated at the δ-nitrogen position. All MD simulations were conducted using the GPU-accelerated pmemd.CUDA engine in the Amber 22 package.

Before the production molecular dynamics (MD) simulations, a two-step energy minimization protocol was employed to eliminate atomic clashes and optimize the initial conformation. In the first step, water molecules were relaxed individually using the steepest descent algorithm while restraining the backbone atoms of the protein and ligand to prevent excessive structural distortion. This rapid energy minimization addressed solvent-solvent overlaps and abnormal bond lengths. The second step

involved full-system relaxation via the conjugate gradient method without restraints, continuing until energy convergence (defined as an energy change $\leq 1\times10^{-6}$ kcal/mol), ensuring the initial conformation reached a thermodynamically favorable low-energy state.Following energy minimization, the system underwent an NVT (canonical) ensemble equilibration for 50 ps. A Langevin thermostat (coupling time constant $\tau\_T = 1.0$ ps) was used to linearly increase the temperature from 0 K to 300 K (physiological temperature) while maintaining a constant volume. This step enabled thermal equilibration, minimizing structural perturbations caused by abrupt temperature changes.

Subsequently, the system transitioned to an NPT (isothermal-isobaric) ensemble for 100 ps of equilibration. A Parrinello-Rahman pressure controller (coupling time constant $\tau\_P = 2.0$ ps, target pressure 1 atm) adjusted the system volume to stabilize the density at ~1.0 g/cm³ (close to physiological conditions). This phase allowed the system to relax its volume under constant pressure, correcting any initial compaction or expansion artifacts and preparing it for production sampling.

To enhance sampling robustness, 10 independent replica simulations were conducted. Each replica followed the same energy minimization → heating → equilibration workflow, followed by 1 ns of production MD (time step 2 fs, trajectories saved every 1 ps). Based on

analyses of hydrogen bond network stability (standard deviation of key residue hydrogen bond occupancy $\sigma < 0.1$) and root-mean-square deviation (RMSD; protein backbone RMSD fluctuations ≤0.2 nm), 3 replicas with the best stability were selected.

To further sample conformational space, these 3 stable replicas were extended to 500 ns of production simulation. Among them, one trajectory—representing a key reaction conformation (e.g., optimal ligand-target active site binding, correct spatial arrangement of catalytic residues)—was extended to 500 ns to improve sampling of rare conformations (e.g., transition states or functionally relevant intermediates).

All simulations employed the particle mesh Ewald (PME) method for long-range electrostatic interactions and Lennard-Jones potentials for van der Waals interactions. This approach effectively avoided artificial truncation artifacts, ensuring accurate calculation of ionic bonds, hydrogen bonds, and dipole-dipole interactions (3).

References:

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