Protein-ligand dissociation rate constant from all-atom simulation

Supplementary information

MOLECULAR DYNAMICS SIMULATION DETAILS

Molecular dynamics simulations were performed using GROMACS 2020.1 package with implemented τ-RAMD method [1]. CHARMM36 force field [2] was chosen for the protein and SwissParam server [3] was used to generate a consistent topology file for the ligand. The system was placed in dodecahedral box at a distance of 1 nm from the edges, solvated with TIP3P water molecules. Na^+ and Cl^- ions were added at physiological concentration of 150 mM. After that, the energy of the system was minimized using steepest descent method and the complex was equilibrated consecutively in NVT and NPT ensembles for 100 ps at 300 K and 1 bar with velocity rescaling thermostat and Berendsen barostat. The system was then subjected to 230 ns of MD simulation with velocity rescaling thermostat and Parrinello-Rahman barostat in order to achieve a stable starting structure (RMSD reached the value of 0.25 nm) for further τ-RAMD calculations. The time step was equal to 2 fs.

[1] D. B. Kokh, B. Doser, S. Richter, F. Ormersbach, X. Cheng, and R. C. Wade, A workflow for exploring ligand dissociation from a macromolecule: Efficient random acceleration molecular dynamics simulation and interaction fingerprint analysis of ligand trajectories, The Journal of Chemical Physics **153**, 125102 (2020).

[2] A. D. MacKerell Jr, D. Bashford, M. Bellott, R. L. Dunbrack Jr, J. D. Evanseck, M. J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, *et al.*, All-atom empirical potential for molecular modeling and dynamics studies of proteins, The journal of physical chemistry B **102**, 3586 (1998).

[3] V. Zoete, M. A. Cuendet, A. Grosdidier, and O. Michielin, Swissparam: a fast force field generation tool for small organic molecules, Journal of computational chemistry **32**, 2359 (2011).

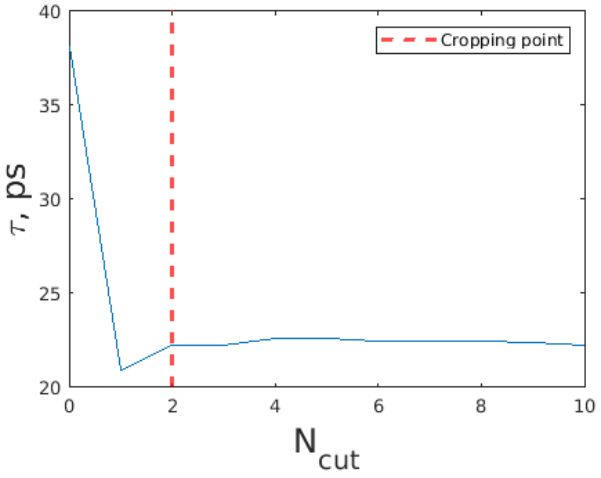
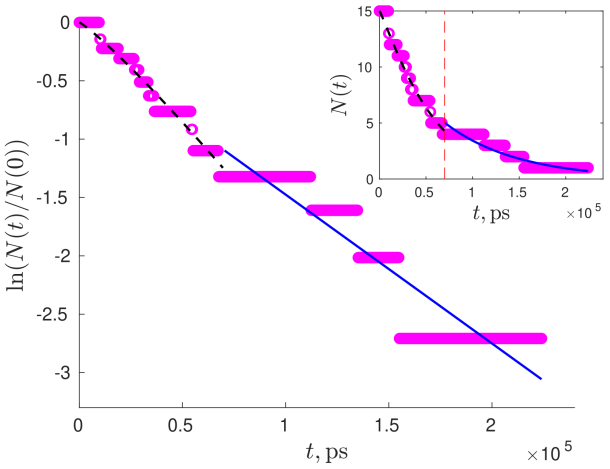
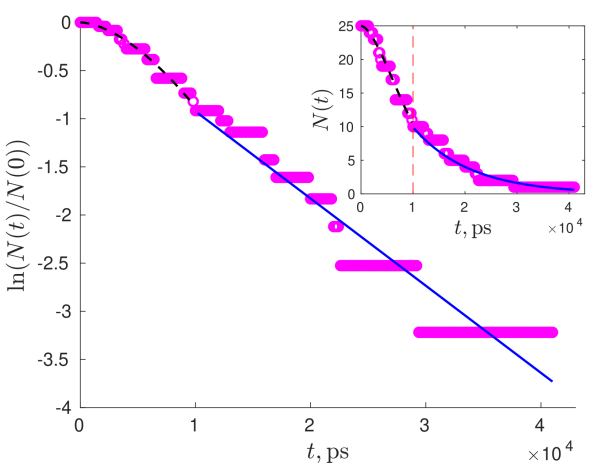


Figure S1. Illustration of how the slope of the exponential regime (τ^{-1}) in semi-logarithmic scale can change depending on the number of the values cut off from the end (N_{cut}) for force $F = 550$ kJ/(nm · mol). The last point in the data, when $N(t) = 1$, forms a long horizontal plateau which significantly increases τ. After $N_{cut} = 2$, τ stabilizes and cropping of the 2 points ensures reliability of the fitting.

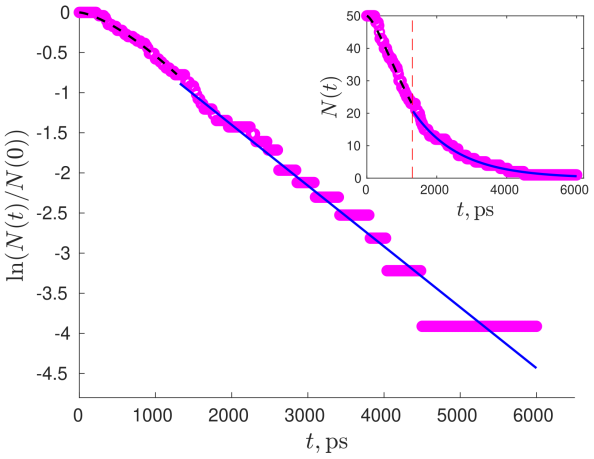
(a) $F = 250$ kJ/(nm · mol)



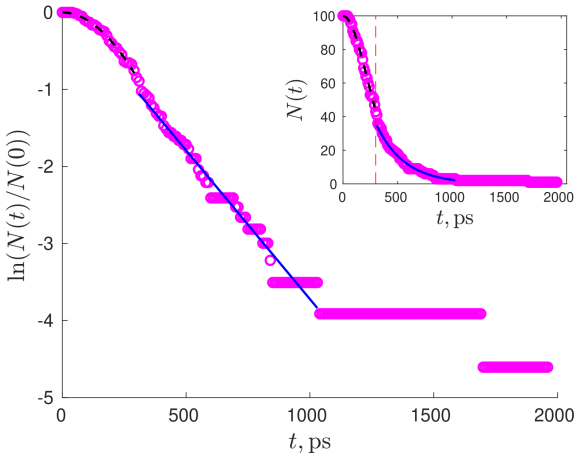
(b) $F = 300$ kJ/(nm · mol)



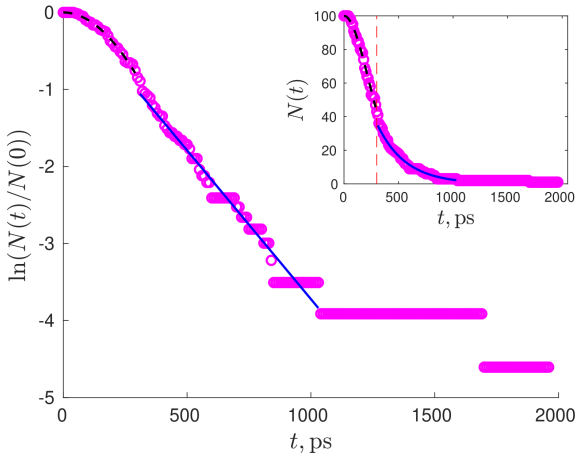
(c) $F = 350$ kJ/(nm · mol)



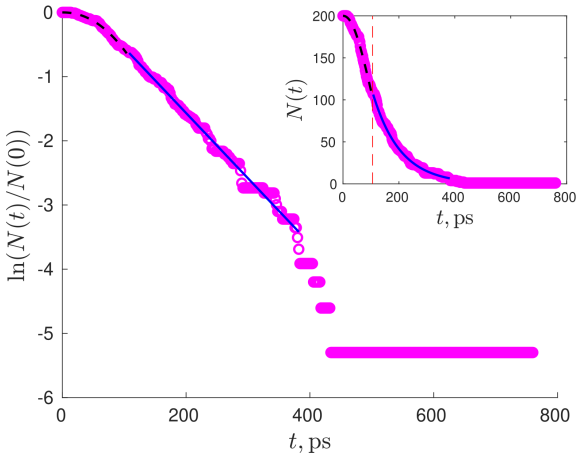
(d) $F = 400$ kJ/(nm · mol)



(e) $F = 450$ kJ/(nm · mol)



(f) $F = 500$ kJ/(nm · mol)



(g) $F = 550$ kJ/(nm · mol)

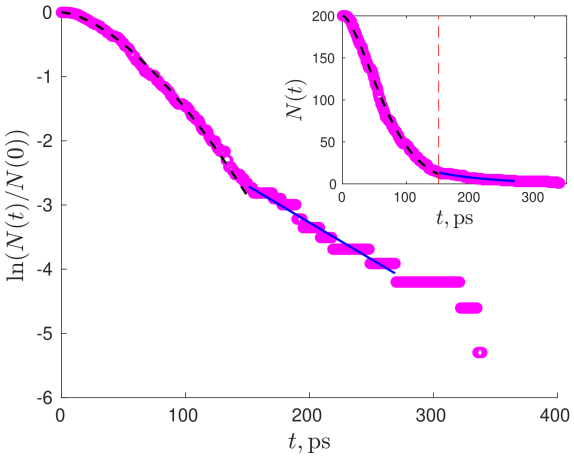


Figure S2. Fitting the survival probabilities of the protein-ligand complex with 2 regimes at different external forces: black dashed line – non-exponential regime, blue line – exponential regime, black dash-dot line – the moment of switching between the regimes. The "shelves" at large times were cut off according to the procedure shown in Fig. S1.

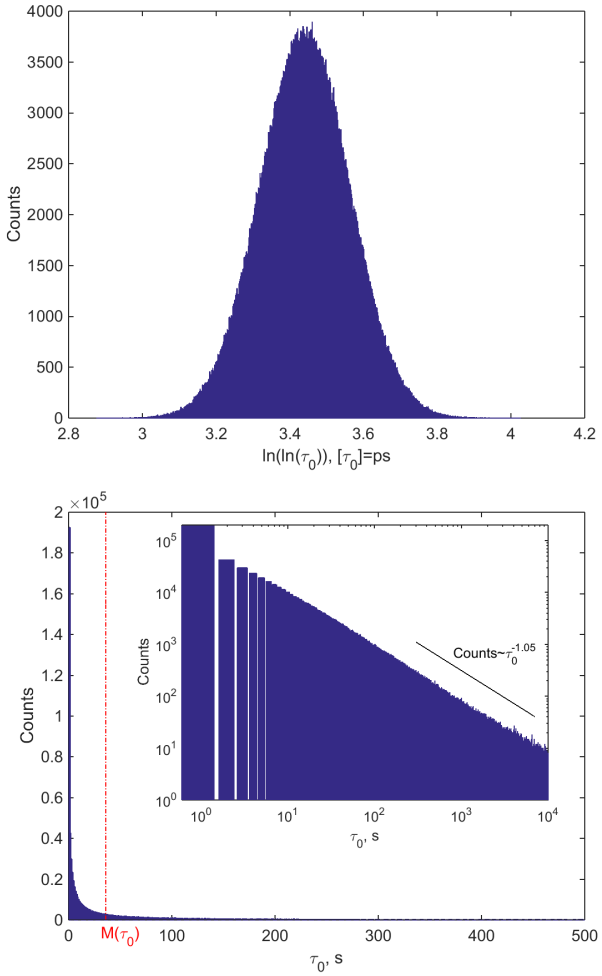


Figure S3. Monte-Carlo simulated distributions of the fitted parameter $\ln(\ln(\tau_0))$ (top) and transformed τ_0 (the long tail at large values is cut off to show the statistically significant part).