Millisecond Time-scale Ligand (Un)binding Event Studied using Accelerated Molecular Dynamics Simulations

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Insulin, a protein hormone responsible for glucose homeostasis, self-associates to form torus-shaped hexameric complexes at physiological concentrations. Therapeutic formulations containing these hexamers are stabilized against degradation and denaturation by antimicrobialpreservatives such as phenols. Dissociation of hexameric species (on minutes to days time-scale) into biologically active monomers isfacilitated by rapid unbinding of phenols (on milliseconds time-scale). However, the dissolution kinetics of hexamers and determinants of the rates of phenol unbinding remain poorly understood due to unresolved ambiguities in NMR results. We identify and characterize a variety of potential ligand dissociation mechanisms for phenol using randomacceleration molecular dynamics (RAMD). The protocol under RAMD speeds up the dissociation kinetics, thereby making it feasible to study at nanosecond timescales, and also allows unbiased pathway search through various escape routes. We observe three distinct exit routes for the ligand and resolve potentials of mean force (PMFs) along them by performing free energy calculations. Free energy profiles for each mechanism are computed with the help of second order cumulant expansion of Jarzynski's equality and non-equilibrium work statistics gathered from multiple independent steered molecular dynamics (SMD) simulations. We suggest a plausible preferred mechanism for the ligand exchange based on energetic barriers and structural properties. The most likely pathway with the lowest free energy barrier involves a leap over the "gate" formed byHisF5 and IleA10, with simultaneous passage of the ligand through a narrow channel existing between LeuA13, LeuH17, and the "gate". Several weakly-bound metastable states are also observed for phenolic ligands during entry and exit from R6 insulin hexamer.