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Steered Molecular Dynamics Simulations of NAD Unbinding from GAPDH and LDH

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Protein-ligand interactions play an important role in understanding biophysical processes including the glycolytic pathway. Calculation of the energy profile of ligand unbinding is essential for understanding possible substrate channeling of nicotinamide adenine dinucleotide (NAD) between lactate dehydrogenase (LDH) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Herein, steered molecular dynamics (SMD) simulations elucidate the process of NAD unbinding from LDH and GAPDH. Brownian dynamics (BD) simulate, using the energy reaction criterion, NAD diffusion towards the binding site of GAPDH or LDH to identify potential residues where strong protein-ligand coulombic interactions exist. These residues are used to design several dissociation pathways for the SMD simulations. Simulations either apply a harmonic guiding potential or a constant force SMD to perform center of mass (COM) pulling of the NAD. The two ligands in the tetrameric GAPDH protein are successfully released from the binding pocket using a force constant $k \geq 5000$ kJ/mol/nm^2 or a constant force $F \geq 600$ pN, within the first 4.2 ns of simulation time. A constant force of 600 pN is enough to pull out three of the four ligands from their corresponding LDH binding sites within the first 0.5 to 1.2 ns of simulation time. Upon releasing the ligand from the binding site, NAD conformational changes are traced, starting with a stretched, open conformation in the binding site and ending with a bent structure in solution. The bent structure is consistent with previous experimental and simulation

data of NAD free in solution. The unbinding free energies associated with the NAD release along the proposed pathways are calculated using the Jarzynski equality, in the stiff-spring approximation of pulling.