An important early molecular recognition event that triggers T cell mediated immune responses is the interaction of a T cell receptor (TCR) on the surface of the T cell with a heterodimeric complex displayed on the surface of pathogen-infected cells. This heterodimeric complex consists of a peptide, 8-10 amino acids in length, bound to the highly polymorphic major histocompatibility complex (MHC). A TCR binds to this heterodimeric complex (peptide-MHC) with sufficient affinity only if interactions between the TCR and certain key regions located on both the MHC and the bound peptide are favorable. On the MHC, these key regions or ''hotspots'' are restricted to just a few amino acids. The molecular mechanisms by which mutations of these MHC ''hotspot'' residues influence TCR/peptide-MHC binding are not well understood.

Molecular Dynamics simulations coupled with free energy calculations based on the inverse form of the Potential Distribution Theorem were carried out to evaluate the effect of single-amino acid mutations of the MHC ''hotspot'' residues on the binding affinity of the A6 TCR to the HLA-A2 MHC complexed with the Tax peptide of the Type I T lymphotropic virus. In agreement with experimental observations, this analysis reveals a strong influence of the MHC ''hotspot'' residue mutations on TCR/peptide-MHC binding affinity. Also, the changes in TCR binding affinities resulting from the MHC ''hotspot'' mutations are compared to those resulting from mutations of key amino acids in the bound peptide of the peptide-MHC complex in order to provide a quantitative comparison of the relative contributions of the peptide and the MHC to the TCR binding affinity. These comparisons permit a detailed thermodynamic analysis of the effect of mutations on TCR molecular recognition of peptide-MHCs.

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Free Energy Calculation And Decomposition Of Hiv-1 Protease-Darunavir Binding By MM-PB/GBSA And Thermodynamic Integration Method

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Darunavir (DRV) is a novel HIV-1 protease inhibitor which has very high binding affinity with the enzyme (Kd=4.5 X 10-12 M, ΔG=-15.2Kcal/M). Two drug-resistant protease variants MD4 (L10I, G48V, I54V, V82A) and MD2 (V82T, I84V) have been found to decrease the binding affinity with DRV by 1.0kcal/M and 1.5kcal/M respectively. In this study the absolute binding energy of DRV with wild-type protease, MD4 and MD2 is calculated by MM-PB/GBSA method. Relative binding energy of wild-type protease and MD2 with DRV is also calculated by thermodynamic integration method. Free energy decomposition is performed to investigate the mutations' influence on the protease-DRV binding and how the DRV responses to these mutations. The results suggest that the mutations have distorted the binding pocket of the protease so that the protease residues contributing to the loss of binding energy is not limited to the sites of mutations. The bis-tetrahydrofuranylurethane moiety of DRV is found to maintain its very favorable interaction with the protease atoms even for the MD4 and MD2 variants. On the contrast the amino-benzyl group of DRV has sampled larger conformational space in MD4 and MD2 than in the Wild-type protease that could be the source of the loss of binding energy. Free energy calculations can therefore be an effective way of evaluating relative binding affinities of similar complexes.

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Analyzing drug-resistance in terms of substrate recognition by Hepatitis C Virus NS3 protease

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Hepatitis C virus NS3 protease is essential to the viral lifecycle by cleaving at least four sites along the viral polyprotein, and for this reason, has been viewed as an attractive therapeutic target. Although several protease inhibitors have shown promise in clinical trials, drug resistance has occurred both in replicon studies and in treated patient populations. The goal of this study is to use molecular modeling approaches to investigate the balance between substrate recognition and the occurrence of drug resistance. Peptides corresponding the NS3 substrates 4A4B, 4B5A and 5A5B were modeled in the active site of full-length single-chain NS3 structure (1CU1). The crystal structure (2OC8) of the NS3 protease domain in complex with the protease inhibitor boceprevir (SCH503034) was then superposed separately onto the 1CU1 structure to determine regions where the inhibitor bound relative to NS3 substrates. We found that most primary active site mutations do not extensively contact substrates, but are critical to inhibitor binding. This implies that future NS3 protease inhibitors that fit better within the substrate binding region should be less susceptible to drug resistant mutations. We believe that drug design strategies can be utilized in the development of NS3 protease inhibitors, which are less susceptible to resistance and therefore more robust for HCV treatment.

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Modeling Orientation-Constrained Reactions: A Study Of Crowding Effects With Brownian Dynamics Simulation

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Specific protein-binding is essential for biochemical reactions in cell signaling. To understand the effects of the intracellular crowding environment on the kinetics of such specific binding, we studied anisotropic interactions in a simple crowded model system composed of hard spheres with effective charges interacting through a Yukawa-type potential, to model the orientation-constrained specific protein binding process. Utilizing Brownian dynamics simulations, our studies on a monodisperse system indicate that although the diffusion of each molecule is slowed down in the crowded environment, the rate constant for the diffusion-limited orientation-constrained reaction is increased. For a charged-molecule system this speed-up could reach up to 4-fold at about 38% solute volume fraction, compared to the same reaction in the dilute solution. Crowding not only reduces the time to the ''first binding'' event, but also greatly reduces the average re-binding time, thus increasing the chemistry-limited reaction rate. For the same charged-molecule system, the re-binding time is also reduced significantly, up to 100-fold. Assuming 0.001 ~ 0.01 reaction probability for each specific binding, these simulation results imply a full order of magnitude enhancement for the rate constant of the chemistry-limited reactions. These significant effects of the crowding environment on the reaction rate depend both on the direct interactions between the tracer molecule and the crowding molecules, and on the interactions among the crowding molecules. In general, we find that the repulsive interaction between the tracer and crowding molecules has stronger ''caging'' effects on the acceleration of the reaction. The quantitative information obtained even from such a simple set of model systems indicates the directions and expected range of changes in the magnitude of important parameters used in the quantitative study of corresponding processes involving complex proteins, and advances the realistic modeling of cellular processes.

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Parameter Effects Of Crowding On Binding Chemistry Using Stochastic Off-lattice Simulations

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The intracellular environment imposes a variety of constraints on biochemical reaction systems that can substantially change reaction rates and equilibria relative to an ideal solution-based environment. One of the most significant constraints in the intracellular environment is the dense macromolecular crowding in the cell, which tends to strongly enhance binding and assembly reactions among many other effects. In order to develop more realistic models of assembly reactions in the cell, we have implemented a stochastic off-lattice model of binding reactions based on the Green's function reaction dynamics (GFRD) method. In the present work, we describe a simulation study intended to determine how various parameter values of an assembly system influence the magnitude and direction of crowding effects on assembly kinetics. We used this model to test the influence of relative volumes of assembly subunits in bound and unbound forms, relative volumes of inert crowding agents, solution temperature and viscosity, and degrees of crowding. Consistent with prior theory, the model showed enhanced binding under conditions of high temperature, low solution viscosity, and large volume reductions upon binding. The model also showed unexpected effectives of the sizes of inert crowding agents on binding kinetics. These results and other ongoing work in this direction will be useful in developing more accurate quantitative models of large-scale assembly processes in the cell for which we currently lack suitable experimental or simulation methods.

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Interface Volume As A Possible Diagnostic Of The Quality Of Protein-protein Docking

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The correlation between the volumes of the interface between decoy structures with the RMSD from the experimental structure will be presented for a set of 84 protein dimers with known crystal structure. The interface volume is calculated with a so-called crude Monte Carlo technique: uniformly distributed random points are generated in a rectangle around the interface and the fraction of these points fond to lie in the interface will give the volume. The filters for being in the interface include simultaneous proximity of atoms belonging to both proteins and a filter based on the circular variance that was shown to be a effective diagnostic of being inside/outside of set of points of irregular shape.