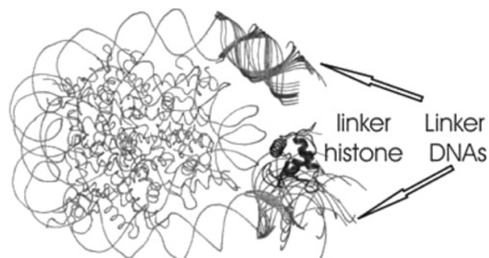
The linker histone is a protein that binds to the nucleosome and determines how the nucleosomes are linked to each other. To simulate the nucleosome-linker histone interactions, we applied a Brownian Dynamics (BD) technique together with normal mode analysis (NMA). NMA of the nucleosome revealed the most prominent modes of motion of its two linker DNAs. The results were used to generate conformations of the linker DNAs which were used in BD simulations of docking of a linker histone and its mutants to the nucleosome. From the simulations, two distinct binding sites on the linker histone were identified. The residues found to be most important for binding in the simulations with the linker histone mutants are consistent with experimental data. Moreover, a unique binding mode of the linker histone to the nucleosome was found for a wide range of conformations of the linker DNAs. As well as providing insights into the determinants of linker histone-nucleosome binding, the results are valuable for higher-order modelling of the chromatin.



431-Pos Board B310

Exploring The Spatiotemporaloral Dynamics of DNA Binding and Cleavage by Restriction Endonucleaes

Wei-Ting Wang, Jing-Shin Tsai, Chien-Ting Hsu, Tzu-Sen Yang*.
Taipei Medical University, Taipei, Taiwan.

Using restriction endonucleases to catalyze the double-stranded DNA (dsDNA) breakage at certain recognition sequences is an important molecular biology technique. The restriction endonucleases constitute an important defense mechanism of bacteria against viral attacks; this mechanism is to destroy invading foreign DNA molecules via cleaving a specific site (phosphodiester bond) of a dsDNA. By cleaving recognition sites on dsDNA with extraordinary specificity can lead to the DNA double strand breaks (dsb). We presented a novel single-molecule approach to investigate the interaction between DNA and restriction endonucleases, including DNA recognition and cleavage. To elucidate how fast restriction endonucleases recognize and cleavage DNA sequence, we constructed a high resolution dual-beam laser tweezers system to manipulate single DNA molecule, together with the site-specific restriction enzymes, namely, EcoRI (one-site endonuclease) and Cfr9I (two-site endonuclease), conjugated to nanometer-sized fluorescence particle. Because most endonucleases work in the presence of magnesium ions, we will apply optically based reaction mechanism to control and synchronize the restriction endonuclease activity in this study. Furthermore, both laser tweezers and fluorescence particle imaging will be used to probe whether the DNA double strand breaks occurred due to the molecular cutting. Hence, this single-molecule approach allows us to directly observe and visualize the spatiotemporal dynamics of DNA binding and cleavage by restriction endonucleases, and can be further applied to determine the DNA cleavage rate due to the presence of EcoRI and Cfr9I. Finally, we extend this approach, together with the light-induced molecular cutting, to investigate the DNA binding and cleavage by restriction endonucleases under tension at different temperatures.

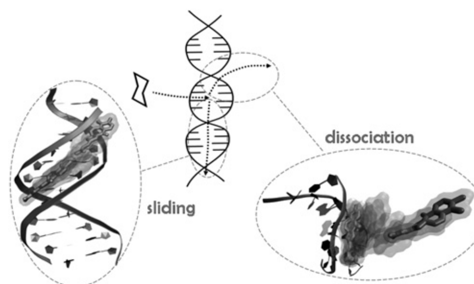
432-Pos Board B311

Molecular Recognition Routes Of DNA By Anticancer Ligands: Mechanisms and Free Energies Explored Via Molecular Dynamics Simulations

Attilio Vittorio Vargiu[1,2], Alessandra Magistrato[3], Paolo Carloni[3], Paolo Ruggerone[1].
[1]Universitá di Cagliari, Cagliari, Italy, [2]CNR-INFM-SLACS, Cagliari, Italy, [3]SISSA/ISAS, Trieste, Italy.

Molecular recognition of the DNA minor groove is a multi-route process which can involve many steps before the formation of the most stable adduct. In particular, many studies have pointed out the importance of events like sliding along the groove and dissociation (which is a relevant step in the translocation among different sequences) for the affinity and the specificity of minor groove binders.

In this contribution we present our recent work on the subject. Umbrella sampling and metadynamics were used to characterize mechanisms and free energy profiles of molecular recognition routes by the antitumoral agents anthramycin, duocarmycin and distamycin. Our results are in very good agreement with the available experimental data, and provide insights on the influence of factors like size, charge and flexibility on the molecular recognition process.



433-Pos Board B312

Computational Studies of Substrate Binding and Conformational Change in the Glycine Betaine Symporter BetP

Kamil Khafizov, Christine Ziegler, Lucy Forrest.
Max-Planck Institute of Biophysics, Frankfurt am Main, Germany.

The glycine betaine symporter BetP is an important protein for regulation of osmotic pressure in the microbe *Corynebacterium glutamicum*, a bacterium used extensively in biotechnology. BetP responds to changes in external osmolality by regulation of its transport activity. The recently solved X-ray crystallographic structure of this protein reveals that it is a homotrimer and that each monomer possesses its own substrate binding pocket. Available structural data for several secondary transporters suggest that these proteins may adopt one of several structurally-distinct states, namely outward- and inward-facing state conformations, as well as a so-called occluded state. Since the monomers in the BetP X-ray structure do not show any apparent substrate pathways, they are likely to represent an occluded state. To identify structural features of alternate states, which may have relevance for a range of secondary transporters, we constructed 3D models of outward- and inward-facing states of BetP using secondary transporters of known structure as templates and validated the modelling results through the rigid-body fitting of these models to low-resolution cryo-EM maps. In addition, to address several other remaining questions, including: the location of glycine betaine and Na^+ binding sites; the effect of the headgroup size and net charge of lipid molecules; and the importance of the trimeric state of the protein, we have performed all-atom molecular dynamics simulations of BetP. Finally, we combined the results of structural and simulation studies with those from sequence analysis of BCCT transporters in order to identify structural and functional roles for several important residues. The results of our computational studies may lead to a better understanding of key events in the transport cycle and they are being validated experimentally.

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Substrate Binding Directs the Functional Hinge Bending Motion of Human 3-Phosphoglycerate Kinase

Erika Balog[1], Zoltan Palmai[1], Laurent Chaloin[2], Corinne Lionne[2], Judit Fidy[1], David Perahia[3].
[1]Semmelweis University, Budapest, Hungary, [2]CNRS–Université Montpellier 1—Université Montpellier 2, Montpellier, France, [3]Université Paris-Sud, Orsay, France.

3-Phosphogycerate kinase (PGK) is a two domain enzyme, with a binding site of the 1,3-bisphosphoglycerate on the N-domain and of the ADP on the C-domain. In order to transfer a phosphate group the enzyme has to undergo a hinge bending motion from open to closed conformation to bring the substrates to close proximity. Molecular dynamics simulation was used to elucidate the effect of ligand binding onto the domain motions of this enzyme. The simulation results indicate the presence of a relatively small amplitude hinge bending motion of ns timescale in the apo form while the time period of the hinge bending motion of the complex form is clearly over the 20 ns simulation time. Upon binding the ligands, the hinge bending shows more directed characteristics with one dominant hinge point in the vicinity of the substrates while the apo form exhibits several hinge points that contribute to the hinge bending motion. The correlation of interdomain atomic movements also increased upon substrates binding.

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Molecular Dynamics Simulation Study of T Cell Receptor Molecular Recognition of Peptide-Major Histocompatibility Complexes

Ghalib A. Bello, Michael E. Paulaitis.
The Ohio State University, Columbus, OH, USA.

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.

436-Pos Board B315

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

Yufeng Cai.
University of Massachusetts Medical School, Worcester, MA, USA.

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

Keith Romano.
University of Massachusetts Medical School, Worcester, MA, USA.

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.

438-Pos Board B317

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

Jian Sun[1], Harel Weinstein[1,2].
[1]Weill Medical College of Cornell University, New York, NY, USA, [2]The HRH Prince Alwaleed Bin Talal Bin Abdulaziz Alsaud Institute for Computational Biomedicine, New York, NY, USA.

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.

439-Pos Board B318

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

Byoungkoo Lee, Philip R. LeDuc, Russell Schwartz.
Carnegie Mellon University, Pittsburgh, PA, USA.

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

Mihaly Mezei.
Mount Sinai School of Medicine, New York, NY, USA.

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.