2867-Pos Stabilizing and Destabilizing Interactions between Intrinsically Unstructured N and C Terminal Regions and Homeodomain of NK-2 Class Homeoproteins

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Board B170

NK-2 class homeoproteins are transcription factors that orchestrate organogenesis during embryonic development, and maintain organ cells in their differentiated state in adults, suppressing carcinogenesis. They consist of roughly 80% flexible random coil and a single, well-ordered DNA binding domain, the homeodomain. NMR and CD spectroscopy results for the NKX 2–1, NKX 2–5, and NKX 3–1 proteins are presented. Stabilizing interactions occur between the homeodomain and motifs in the N-terminal flexible random coil region, while destabilizing interactions occur with motifs in the C-terminal region. These motif interactions appear to be modulated by phosphorylation, affecting homeodomain stability and, consequently, protein turnover in the cell. Thus, these N and C-terminal motifs and their interacting kinases could be targets for therapies aimed at boosting tumor suppression by promoting stabilizing and inhibiting destabilizing homeodomain interactions, leading to increased protein levels in the cell.

Molecular Modeling

2868-Pos Polymers at High Solution Concentration, in Polymer Brushes, and in Mesoscopic Channels

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Board B171

In high-concentration solutions, stressed between lipid bilayers, squeezed into narrow channels, when does a polymer feel the walls? When is a polymer or oligomer so self-crowded that neither its length nor the walls matter? When will it act as it does in a des Cloizeaux regime where compressive properties depend only on the density of monomers (with its characteristic 9/4 power dependence on concentration)?

Measurements of forces between bilayers with attached PEG oligomers [1–3] together with measurements of polymer partitioning between a channel and the outside solution [4,5], show that within a small enough space, an oligomer or a polymer can even forget its length. At high enough monomer density it, paradoxically, forgets the walls that confine it. This becomes dramatically clear when large polymers are able to enter nanoscale pores.

A unity of thinking emerges between behaviors on the nanometer and centimeter scales.

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2870-Pos 3D Structure of the Unliganded gp120 Based on Structural Homology and Molecular Dynamics Symulations

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Board B173

Infection with HIV-1 begins with the docking of the viral envelope protein gp120 to CD4 receptors expressed on the the host cell surface. Understanding of the conformational states available to the HIV-1 envelope glycoprotein gp120 is critical to any rational attempt to design inhibitors and vaccines. The full-length HIV-1 gp120 has eluded structural analysis. However, deletion of the V1, V2 and V3 variable loops and the N- and C-termini of gp120 from HIV-1 and from simian immunodeficiency virus (SIV), resulting in gp120 core protein have been crystallized. The objective of gp120 molecular modeling is to derive the complete 3D structure of the free (unliganded) biomolecule using experimental elements belonging to different strains of the virus, characterized in different crystallization conditions and at different resolutions.

The reconstruction procedure is based on primary data experimentally derived from X-ray crystallography or NMR of certain major components from the gp120 molecular puzzle:

(i) the unliganded, completely glycosylated, V3 loop-missing SIV structure;
(ii) structures liganded to CD4 and antibody (HIV);
(iii) the liganded structure containing V3 loop (HIV);
(iv) V3 loop and C5 domain in solution.

The 3D structures were modelled by protein structure homology. The alignments between the primary structures of known states and the ∼60 strains modelled were manually adjusted and then each

alignment was used to generate the following 3D structures: the free gp120, the CD4-bound, the b12-bound, and the gp120 bound by both the CD4 receptor and the b12 antibody. All models included known 3D structures for C5 domain and V3 loop. The refinement of the ~240 structures and the determination of intermediary states were performed by intensive molecular dynamics simulation computations. We plan to use the output 3D structures for generating their fully glycosylated variants.

2871-Pos Designing Effective Molecular Animations for Use in the Classroom

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Board B174

The use of animations in biochemistry, cell and molecular biology courses is widespread, but the question still exists as to their effectiveness. A project to answer this question is underway, and animations describing G protein signaling in the cell have been developed for this purpose. We have incorporated many of the design principles uncovered in our previous research studies, plus best practices gleaned from the fine arts. The procedure for making the animations included conceptual planning, story-boarding, researching aesthetic filming techniques, and finally modeling and animating the molecules in this pathway. Particular attention has been paid to key aspects of film/animation such as space, color, rhythm, and perspective. Likewise, the information about these signaling pathways has been layered and segmented to represent distinct aspects of pathway activation, deactivation, and adaptation. These design principles may help students better grasp underlying principles by reducing cognitive load. After formative evaluations of the animations by small groups of students, pre- and post-viewing assessments will be carried out in order to determine how well students learn from a given animation. The animations created and the results of the testing will give a greater understanding of the usefulness and potential of scientific/biological educational animations.

2872-Pos Ring Opening Dynamics Of The Sliding DNA Clamp PCNA

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Board B175

The eukaryotic proliferating cell nuclear antigen (PCNA) is a homotrimeric sliding clamp that encircles DNA and tethers the polymerase at the site of replication. Loading PCNA onto duplex DNA requires that the protein must interconvert between a closed planar ring and an open conformation that permits strand passage into its central channel. We have investigated ring opening using equilibrium and non-equilibrium molecular dynamics simulations. Removal of one subunit relaxes the closure constraint on the ring and allows for fluctuations at the dimer interface. Equilibrium simulations demonstrate that the dimer can relax into conformations consistent with both right- and left-handed spirals. We have also begun to elucidate the energetics of ring opening for the full timer using metadynamics non-equilibrium simulations.

2873-Pos Brownian Dynamics Simulations of Enzyme-Enzyme Interactions and Ligand Transfer

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Board B176

Functional protein-protein interactions are essential for many physiological processes. Some of these functional interactions have been hypothesized to play a role in substrate channeling or ligand transfer. Whether such interactions occur within the glycolytic pathway remains controversial. Glycolytic enzymes are known to compartment as a result of transient interactions with cytotskeletal structures. Glycolytic enzymes are also known to interact with each other. Brownian dynamics (BD) has successfully elucidated a possible mechanism of compartmentation in glycolysis; BD agrees with experiments that several glycolytic enzymes interact with F-actin to provide a platform for compartmentation. Herein, the interactions between the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with lactate dehydrogenase (LDH) and fructose-1,6-bisphosphate aldolase (ALD) with GAPDH are explored to examine potential channeling pathways. Both pairs of enzymes interact favorably in BD simulations where actives sites of the two enzymes are in reasonably close proximity. Enzyme-enzyme complexes between GAPDH and LDH involve three different binding modes that make use of mainly positively charged lysine residues and negatively charged glutamates and aspartates on both GAPDH and LDH. BD can also simulate the transfer of a ligand moving from the active site of one enzyme to the active site of the other enzyme; by comparing these simulations to computations of the ligand binding from solution, BD can determine if there is any gain in efficiency from complex formation. BD simulations show higher transfer efficiencies of the substrate glyceraldehyde-3-phosphate (GAP) from an aldolase active site to a GAPDH active site as compared to transfer efficiencies of GAP from solution. Similarly. BD simulations suggest a possibility of channeling the cofactor NAD(H) between the GAPDH and LDH.

2874-Pos Molecular Models of the Interface Between γ-Secretase Components Involving (small)xxx(small) xxx(small) Motifs

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Board B177

Gamma-secretase is an intra-membrane protease, improper functioning of which was found to be critical in the pathogenesis of Alzheimer's disease. The enzyme is a complex of four proteins, however, the detailed structure of the protease as well as its proteolytic mechanism remain poorly understood. Theoretical methods were used to build molecular models of helix - helix interface between two proteins forming γ-secretase: APH-1 and PS-1. The interaction is based on tight contact between highly conserved GxxxGxxxG motif in the fourth transmembrane helix of APH-1 and AxxxAxxxG motif in eighth transmembrane helix of PS1. Four binding modes based on two homologous structures involving GxxxG motifs in glycophorin (parallel orientation) and aquaporin (antiparallel) were proposed and validated. The most probable model employs antiparallel orientations of interacting helices and is in agreement with currently accepted membrane topology of both proteins in γ-secretase complex. The impact of PS-1 mutations associated with Familial forms of AD occurring within the proposed interface were also characterized. The model can be used in further structural characterization of γ-secretase and its components.

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2875-Pos The Mechanical Regulation of Filamins' Integrin Binding

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Board B178

Cellular actin-binding proteins are largely responsible for determining the dynamic and mechanical properties of the actin cytoskeleton. Among these actin-binding proteins, the filamins, are known to orthogonally orient the actin-cytoskeleton, but only recently are their mechanically regulated properties coming into question. Human filamin (FLN) is a modular protein, each monomer of the active dimer containing twenty-four tandem Immunoglobin (Ig) like repeats with two hinge domains. Human filamin can bind to integrins through tandem repeat 21 in its rod domain. Recent structural data by Lad et al. illustrates filamin is autoinhibited by repeat 20 associating and occluding the integrin binding site on repeat 21. The processes leading to disruption of binding of repeat 20 and repeat 21 and subsequent increased integrin binding are tested utilizing molecular dynamics simulations. These simulations illustrate how force and temperature can disrupt autoinhibition and promote integrin association of filamin.

2876-Pos Molecular dynamics simulations of the Alzheimer amyloid β-peptide (1–42)

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Board B179

The major components of plaques found in Alzheimer's disease (AD) are amyloid $^{\beta}$-peptides that range in length from 39 to 43 amino acids. The major form found in the plaques is Aβ (1–42). In this work, to study dynamics behavior of Aβ (1–42), we have performed 6 independent MD simulations (each 60ns) at 298k, 320k and 368k with explicit water using GROMACS package under GROMOS and OPLS force fields, respectively. MD simulations were run on a 44-CPU Lenovo Cluster. A "connubial phenomena" of β-sheet forming, most β-sheets were formed in parallel or antiparallel pairs, was observed, which may be origin of structure of the cross-β spine of amyloid-like fibrils (Nature, 2005, 435: 773). Most connubial β-sheets are composed of 5 or 6 residues, which show that Aβ (1–42) is easy to form pentamer or hexamer. The residues 27–42 are easy to form β-sheets. The helix in the region of 10–18 residues is the steadiest. The higher the temperature is, the more β-sheets were formed and the more helix disappeared. There are a few states with mixing helix and β-sheets. The MD results are consistent with experiments (PNAS, 2003, 100:330). In GROMOS and OPLS, there is some difference of MD results such as more β-sheets were formed and helix was steadier in GROMOS, which attribute to difference of parameter optimizing and accuracy of current force fields. The studies make us for understand of AD at the molecular level.

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2877-Pos Nonlinear Relaxation Phenomena in Elastic Network Models and Dynamical Features of Biomolecular Machines

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Board B180

We studied relaxation dynamics in elastic network models. By comparing models for protein machines and artificially generated networks, we discuss dynamical and structural features of biomolecular machines.

Elastic network models (networks of material particles and linear springs) are often adopted to study slow dynamics of proteins. Although chemical details are not considered, equilibrium fluctua-

tions and conformational changes between machine-states in various proteins can be reproduced fairly well by normal mode analysis (NMA) of these models. However, relevance of NMA to large scale nonlinear motion in these models is not trivial and should be sought.

In this study, instead of NMA, we compared relaxation dynamics in elastic networks corresponding to actual protein machines and those generated artificially (randomly or with certain criteria) [1], to highlight dynamical features of bio-molecular machines. First, we studied relaxation from randomly chosen initial deformations. Even starting from such unnatural initial conditions, elastic networks for machines usually show ordered motion along well-defined relaxation paths in the conformational space. In contrast, relaxation behavior of random elastic networks is generally complicated with many (meta)stable states around the original equilibrium conformation, and thus inconvenient for repeatable machine operations under fluctuations. Next, we simulated conformational motion between two machine-states by switching elastic networks, and discuss relevance of relaxation phenomena shown above to conformational changes in actual machine cycles. We also demonstrate simulated motion in Myosin V and KIF1A, and discuss the peculiarity of KIF1A in NMA and its relation to the mechanism of the motor [2].

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2878-Pos A new Force Field for Lignin

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Board B181

We present a new force field for lignin to enable accurate Molecular Dynamics (MD) simulations of this compound. Lignin is a complex and highly heterogeneous plant cell wall biopolymer which plays a major role in biomass recalcitrance, the natural resistance of plant cells to enzymatic and chemical degradation . A detailed computational study of lignin might shade further light to the molecular origins of biomass recalcitrance, which is known to be the bottle neck in production of bioethanol fuel from biomass degradation. The first crucial step in modeling biophysical systems is deriving the empirical force field to be used in MD. As in previous CHARMM force filed parameterizations our effort concentrated on reproducing as closely as possible properties of lignin derived from QM calculations, while maintaining compatibility with the existing CHARMM force field. Equilibrium geometries were derived from accurate QM optimized structures. Then partial atomic charges were obtaining by reproducing minimum interaction energies and distances between water and models of lignin. Dihedral constants were then obtained by considering potential energy surfaces of the relevant dihedral angles. Finally, bond and angle constants were derived by reproducing vibrational frequencies and normal modes of lignin models. The above procedure was repeated three times until convergence with the target data was archived. As a final step the new force field was validated by checking how well it reproduces experimentally known condensed properties of lignin. For this reason MD simulations were done on a lignin dimer and the force field was shown to reproduce the experimental structure well.

2879-Pos Peptide-mediated TCR/MHC Interactions as the Molecular Basis for Characterizing Immunodominant Peptides in Adaptive Immune Response: A Molecular Dynamics Study

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Board B182

T cells are lymphocytes that play a key role in the adaptive immune response to pathogenic infection. 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 bimolecular complex displayed on the surface of pathogen-infected cells. This complex consists of an antigen (a small oligopeptide extracted from the infecting pathogen) bound to a major histocompatibility complex (MHC) molecule. The interaction of this complex (peptide-MHC, or pMHC) with the TCR is mediated by the antigen, whose amino acid sequence determines the interactionâ™s specificity. Very often, an immune response to a pathogen appears to be specific to a small fraction of all potential peptide antigens from that particular pathogen, which is referred to as immunodominance.

Here, we examine TCR/pMHC molecular recognition as the basis for immunodominance of certain peptides by comparing the peptide-mediated stability of this complex for the influenza-specific, immunodominant M1 peptide to that of the Epstein-Barr Virus-specific immunodominant BMLF1 peptide. The structural basis of the TCR-M1-MHC interaction has been established using X-ray crystallography. We use this information and molecular dynamics simulations to study conformational properties of the TCR/pMHC interface for M1 and BMLF1, and to compute binding free energies. The measurement of changes in binding energy and stability that result from the mutation of residues at the interface sheds light on the roles these residues play in the binding interaction. The simulations also reveal that interfacial water plays an important role in facilitating binding by forming an extensive and dynamic hydrogen-bond network, which serves to enhance the geometric complementarity of the interface and, thereby, binding affinity of the TCR to the pMHC.

2880-Pos Density Functional Study of Intein Reaction Mechanisms

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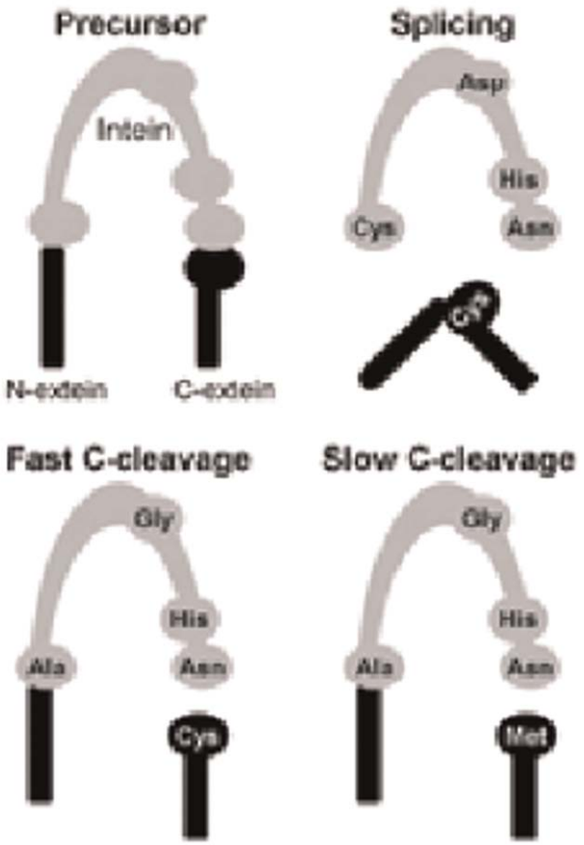
Board B183

Protein splicing consists of the excision of an internal insertion sequence (intein) from two flanking proteins (exteins) and their ligation. Also, it is auto-catalyzed mostly by the intein's internal

sequences. This process is being use to develop molecular switches and sensors and for controlled drug delivery. Combining quantum mechanics and classical molecular mechanics (QM/MM), as well as gas phase and implicit solvent calculations, we have investigated the initial reaction steps of intein splicing, namely the N-S shift of the N-terminal Cys and constructed energy barriers for an all-atom reaction profile within the context of the full protein. The mutation of Asp to Gly, located between and hypothesized to link the N- and C-terminal active sites, was experimentally shown to inhibit splicing activity, and we have studied the effect of this mutation by comparing computational energy barriers. These results indicate that certain mutations either inhibit or enhance specific reaction steps of the overall splicing reaction. Thus, intermediate states may be isolated and studied in the context of altering the molecular triggers and inhibitors that impact protein splicing with inteins.



2881-Pos Molecular Modeling Of Proteins Confined In AOT Reverse Micelles: Horse Cytochrome C

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Cytochrome *c* (CYTC) is a small (110 residues) soluble hemoprotein associated with the inner membrane of the mitochondrion. It plays a role in the electron transfer (ET) within the respiratory chain and in cellular mechanisms such as apoptosis. CYTC interactions with detergents have been investigated previously to explore physiological aspects of ET in various membrane mimetic environments such as reverse micelles (RM). It has been shown by spectroscopic studies (fluorescence, circular dichroism, etc.) that in RM, the ternary structure of the protein and the heme environment can be affected when the micellar water pool radius is close the protein size (2.5 nm x 2.5 nm x 3.7 nm).

To explore the conformation change of the CYTC, we use Molecular Dynamics Simulations (MD) with a RM model of sodium bis-(2-ethylhexyl) sulfosuccinate (AOT) in isooctane (Abel, Waks, Urbach and Marchi, J. Am. Chem. Soc. (2006) ***128***, 382)). Since the size and the quantity of water in the RM can be controlled by the parameter W_o=[H_2O]/[AOT], RM provide a simple but accurate model for the study of peptide or protein membrane environment.

For this work, we simulate two sizes of RM at W_o=5.6 and 9.1 with large amounts of solvent (> 80 % w/w) to simulate a L_2 phase. We analyze the MD trajectories in terms of the micelle and protein structures, hydration and water dynamics and compare the confined protein conformation with that of the CYTC in water. The results obtained show that the protein size, hydration and conformational state are significantly affected by the micellar environment. For the heme moiety, we observe that the bond between Fe and methionine-80 is disrupted, in contrast to the protein in the bulk water, in good agreement with experiments.

2882-Pos On the use of Multiple Templates to Improve the Quality of Homology Models

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Board B185

The most crucial part in modeling a protein structure based on homologous proteins is the selection of a good template and the quality of the corresponding alignment between the target and template sequences. Many methods exist for identifying templates and for making alignments. In many cases, several possible template molecules are found, which in theory would help to produce better models. Existing methods for utilizing multiple templates in the modeling process include Modeller and Nest. In this study, we have used the targets from the most recent CASP experiment as well as a larger dataset, to carry out a systematic investigation of the impact of multiple templates in improving the quality of homology-based models. In addition to Modeller and Nest, a novel method for multiple template based modeling is used, based on the segment matching method originally used in the SegMod program. It was found that for models for which there exist a number of high-quality alignments, model quality can indeed be improved, whereas it is more difficult to improve upon more difficult targets.

2883-Pos Automatic Modelling Of Olfactory Receptor 3D Structures And Classification Of Odor - Receptor Affinities

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Olfaction is a complex process involving the recognition of odor molecules by olfactory receptors (ORs), the largest G protein coupled receptor (GPCR) category. They account for more than 1% of total mammalian genome. A receptor can recognize different odor molecules and one odor molecule can bind different receptors: these observed cross recognitions define the combinatorial coding.

To decipher its complexity, we focus on producing a generalized GPCR structure modelling tool and to link it to a scoring function measuring odor - receptor affinities. To date, no experimental three-dimensionnal (3D) structure of an olfactory receptor is available. In order to study precisely how the recognition is performed between the receptor and its ligand, it is thus necessary to build a model structure. The only known structure of a GPCR is the one of bovine rhodopsine that thus serves as a template for the modelling. Molecular modelling is based on the homology relationship existing between the query protein whose 3D model is needed and a protein template. A sequence alignement is carried out between the two sequences to find residues that occupy similar positions in the 3D structures and the template 3D is used to build the query protein model. The alignment accuracy is very important to obtain a reliable model. Unfortunately, for remote homologs, such as ORs and rhodopsine, the sequences are highly divergent and the alignment is error prone.

We will present the strategy we have developed in order to have a robust modelling approach, using a fold recognition method developed in our laboratory. This approach has been extensively tested using available experimental data. Detailed results of the model construction and its comparison to the available experimental data will be presented.

Chromatin & Nucleic Acids

2884-Pos Dynamics Of Individual Nucleosomes Analysed With Single Molecule Spectroscopy And Multiparameter Detection

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The structure and dynamics of nucleosomes affect the subsequent compaction into the higher order chromatin structure, which is an important regulatory mechanism of gene activity. We utilize a custom build confocal SMD system to investigate the dynamics within individual nucleosomes diffusing free in solution. Special focus is laid on the effect of histone acetylation and nucleosome remodelling. DNA fragments of the ribosomal 5S and the artificial 601 sequence from J. Widom are labelled with Alexa488 and Alexa594 and reconstituted onto histone octamers to form nucleosomes with 20 – 80 % average FRET, depending on the dye position. To ensure sample stability under SMD concentrations, an excess of inert protein and unlabeled nucleosomes was added to the solution.

In spFRET experiments heterogeneities were observed between the different nucleosome positioning sequences, which agree with PAGE gel analyses. Nucleosomes formed with the natural 5S sequence lead to broader distributions and larger dissociation compared to the highly affine 601 sequence. For both sequences acetylation of all histones caused a significant change in the linker DNA distribution of the nucleosome, but had only minor effect on DNA sites close to the dyad axis. Besides acetylation caused a destabilisation of the nucleosome which we monitored in a SMD based dissociation assay.

To further probe the intrinsic dynamics of the DNA we applied the method of Probability Distribution Analysis (PDA) recently developed in the Seidel lab. For 601 nucleosomes we found a salt-induced redistribution between at least two FRET conformations, pobbibly reflecting a symmetric and asymmetric nucleosome positioning. This approach is currently extended to investigate the activity of remodelling factors on the mononucleosome substrate.

2885-Pos AFM Imaging Of Chromatin Remodeling Complex Action : Mapping The Nucleosome Sliding

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Board B188

The organization of DNA into nucleosome represents a physical barrier for the transcription factors binding to their target DNA sequences and interferes with several basic cellular processes. Histone modifications, ATP-remodeling machines and the incorporation of histone variants within chromatin are used by the cell to overcome the nucleosomal barrier and modulate DNA accessibility by the control of nucleosome dynamics. Here we use a single molecule technique (Atomic Force Microscopy, AFM) to visualize isolated nucleosomes, to quantify the influence of histone octamer composition (H2A-Bbd variant) on the equilibrium nucleosome conformation and to map nucleosome mobility induced by a remodeling complex (SWI/SNF, RSC).

AFM allows to determine simultaneously the DNA complexed length and nucleosome position distributions in various contexts [Montel et al., Biophys. J *93*, 566 (2007)]. Using a linear energetic model for the distribution of DNA complexed length, we extract the net wrapping energy of DNA onto the histone octamer, and compare it to previous studies. Similar mapping of the nucleosome position and DNA complexed length is used to quantify the impact of ATP-activated remodeling and sliding on nucleosomes. The results