have demonstrated some of the effects of monovalent ions on phospholipid bilayers, including decreased area per lipid, higher ordering in head group vertical orientations, and decreased lateral lipid mobility [1-4]. MD simulations of porated membranes have also shown that the binding of monovalent cations to phospholipids can increase pore line tension, which leads to a decrease in pore lifetime [5]. In this work we employ MD simulations to systematically study the effects of varying the concentration of Na^+, K^+, and Cl^- in POPC lipid bilayer systems during different stages of electropore formation.

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886-Pos Board B655

Conduction and Selectivity of Ions through a Sodium Channel

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The NavAb (a bacterial voltage-gated Na^+ channel) atomic structure has been recently resolved. Because NavAb is a possible ancestor of the vertebrate Nav and Cav - voltage dependent Na^+ and Ca^{++} channels, respectively - such a structure provides a unique opportunity to deepen our understanding on these closely related channels. Strikingly, the NavAb structure displays a selectivity filter much wider than the one observed in the well characterized K^+ channels, and therefore raises relevant questions concerning the conduction and selectivity mechanism in this channel. We follow from an ongoing project aiming at studying the conduction mechanism and selectivity on sodium channels. For that purpose, the Free-Energy surface (FES) of the permeation events of two Na^+ ions was assessed by means of a 180 ns long metadynamics calculation. In such method, two reaction coordinates are defined for each ion: the axial distance (z) along the pore axis and the radial distance (x, y) plane. The resulting four-dimensional FES is employed to retrieve the minimum energy pathway covered by the ions. In order to investigate channel selectivity, the present work extends the above mentioned studies to the two-ions constructs: sodium-potassium and potassium-potassium. Our findings point to a dynamical process in which ions transit between favorable interaction sites. Also, subtle differences in the process free-energy landscape may lead to significantly altered permeation rates. Taken together, these results are likely to provide a rationalization for selectivity.

887-Pos Board B656

The Electric Fingerprint of Membrane Voltage Sensor Domains

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Voltage-sensor domains (VSDs) are electrically-charged constructs controlling the voltage-dependent activity of ion channels in excitable cells. Four packed transmembrane (TM) helices, S1 through S4, form the domain in which S4 contains 4 to 7 positively charged basic amino acids, mostly arginines. VSDs operate essentially by transferring the S4 charges across the transmembrane electric field (**E**), giving rise to the observable Q, the so-called ‘‘gating charge’’. Mostly supported by structure-function studies on voltage-gated potassium (Kv) channels, a focused **E** has been identified as one key electric property of the VSD machinery. The recent increasing availability of other VSD-containing ion channel structures, including the x-ray structures for the NavAb and NavRh voltage-gated Na^+ channels, provides us with the opportunity to extend the structure-based investigation of the domain electrostatic properties over a larger set of distinct conformations and isoforms. Using all-atom MD simulations in combination with electrostatic calculations, founded on an energetic formalism, we show that, over the entire set of available VSD structures, a specific hydration of the voltage sensor focuses **E** over a narrow TM region across the domain, at the vicinity of the so-called catalytic center. Furthermore, its focalization and shape is largely preserved over distinct conformations of the construct. Our results support that a focused and conformation-independent TM field is a robust electric feature of the VSD machinery, despite sequence variations or local structural modifications of the domain. This electric fingerprint seems to favor a highly conserved sensing mechanism for VSDs over the large family of voltage-gated cation channels.

888-Pos Board B657

Skeletal Calsequestrin - Calcium Interaction: Role of Acidic C-Terminus

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Skeletal isoform of calsequestrin (CASQ1) is expressed primarily in fast twitch skeletal muscles in all vertebrates and buffers Ca^{2+} inside the sarcoendoplasmic reticulum (SR), the intracellular Ca^{2+} store. CASQ1 has a very unique C-terminus composed sole of aspartic acid residues. Presence of more than 10 consecutive aspartic acid residues in a protein sequence [referred as consecutive aspartate stretch (CAS)] is a very rare feature that is found only in about 20 proteins in the human genome. However, the role of CAS in CASQ1 function has not been investigated. Here we applied computational approach to understand the role of CAS in Ca^{2+}-binding. The recent structure of CASQ1 has resolved the structure of its whole protein except for the CAS. We prepared the model by adding the CAS residues and performed molecular dynamics simulations for 50 nanoseconds in the presence of various Ca^{2+} concentrations. Our study shows that the CAS assumes a compact structure at higher Ca^{2+} concentrations and indicates that the CAS might work as a metal sensor. We found that the CAS undergoes maximal Ca^{2+}-binding before the rest of the surface is saturated. The study revealed various Ca^{2+}-binding sites with differing affinities and geometry. Interestingly, some sites are Ca^{2+}-concentrations dependent while some others are independent of Ca^{2+} concentration. The low affinity sites of CASQ1 bind Ca^{2+} transiently that is mediated by water molecules and can dissociate quickly to support Ca^{2+}-release during contraction. These studies collectively indicate the CAS works as a Ca^{2+}-sensor that may be a novel metal sensing motif. We propose the term ‘‘D_n-motif’’ for CAS.

Single Molecule Techniques I

889-Pos Board B658

Single-Molecule DNA Curtains Reveals the Details of KOPS Targeting, Translocation, and Collision with Protein Roadblocks of DNA Translocase FtsK

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In E coli cell division, two daughter chromosomes often form a dimer, which impedes a proper segregation of chromosomes. The chromosome dimer can be resolved by XerCD-mediated site specific recombination at dif site. The alignment of two dif sites and activation of XerCD require FtsK translocation, which is directed by a short DNA sequence called KOPS (FtsK Orienting Polar Sequences). KOPS targeting and translocation activities of FtsK were examined using single-molecule DNA curtains, which enables to visualize the protein-DNA interaction in real time. We show that FtsK preferentially locates KOPS through 3D collision within our resolution and non-hydrolysable nucleotides enhance the FtsK loading on KOPS. We also reveal that KOPS determines the orientation of FtsK translocation, but only upon initial binding to KOPS. During the translocation, FtsK abruptly pauses and/or changes its direction independently of KOPS, suggesting that FtsK cannot identify KOPS once it begins to translocate. Next we investigated the collision of FtsK with various protein roadblocks including XerCD through two-color labeling in DNA curtains. Interestingly, the FtsK, which has a hexameric ring structure, changes its direction and bypasses the roadblocks, and can also push them along the DNA. Our single-molecule results help reveal how FtsK might function in the crowded environments expected to be found in physiological settings.

890-Pos Board B659

Single-Molecule Dissection of KRas and EGFR Signaling Dynamics in Individual Cancers

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At the molecule level, individual cancers are driven by their own sets of dysregulated protein-protein interactions. Due to the lack of PCR technique for

proteins, these protein-protein interactions cannot be amplified. Single-molecule detection could thus be unique, ideal route for characterizing these 'inherently heterogeneous and tiny' protein-protein interactions. We here report on an antibody-based, label-free method that characterizes the signaling dynamics of native proteins. We have used our technique to characterize two core oncoproteins, KRas and EGFR in different samples including cancer cell lines, xenograft tumors, cancer-patient biopsy samples. We have characterized the single-molecule signaling kinetics and the proportion of single proteins that are actively binding with the downstream proteins tagged with fluorescent proteins. We also compare our analysis results, which are at the protein-protein interaction level, with the genotyping results of samples, which is the current standard for personalized medicine. Our results demonstrate the possibility that the single-molecule techniques could be a real analysis tool for clinically important samples, beyond cutting-edge tool for the curiosity-based science.

891-Pos Board B660

Solubilisation of Lipid Membranes by Detergents: Probing the Three-State Model at the Single Vesicle Level

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The solubilisation of lipid membranes by detergents is a common technique in the purification and isolation of proteins and in the study of membrane proteins. Biophysical studies of these solubilisation processes using ensemble-averaging techniques have provided an integrated picture of the solubilisation mechanism that involves three states: the detergent is taken up in stage I without solubilisation (vesicle regime); stage II refers to the coexistence of detergent-saturated membranes and mixed detergent-lipid micelles and finally stage III corresponds to complete membrane solubilisation leading to the formation of mixed micelles (micellar regime). Although the three-state hypothesis is a didactic and simple thermodynamic model, it is known that detergent lipid-interactions induce a much more complex and diverse dynamic transition between both regimes. These dynamic changes include transmembrane lipid motion (flip-flop), swelling and breakdown of the membrane permeability barrier and fusion between vesicles. To date, whether these processes are independent of each other and take place sequentially, or if they are somehow interconnected is an open question. Here, we have used single-molecule FRET to monitor the solubilisation dynamics of single POPC/POPS vesicles induced by the non-ionic detergent Triton-X 100. Using this approach we have been able to unambiguously separate within a single FRET trajectory the swelling, permeabilization and lysis steps of the solubilisation process and their kinetic details above and below the critical micellar concentration. The present strategy should help in the design of more efficient applications of vesicle solubilisation.

892-Pos Board B661

Long-Term Single-Molecule TIRF Observation of Biomolecules without Immobilization

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Observation of individual molecules for long stretches of time aids in extracting dynamics and reveal hidden heterogeneity in biological systems. Commonly this is achieved by utilizing the high optical sectioning power of total internal reflection (TIR) microscopy, which involves complex immobilization procedures that cannot be generally applied and often perturb the native state of the biological sample. Here we present an easily adaptable and low-cost nanofluidic technology that enables us to perform long-term observation of freely diffusing single molecules. The microfluidic device is fabricated with soft lithography and molding techniques out of an elastomeric material, which allows highly reproducible production of several devices per day. Biomolecules flow through channels that are less than 100 nm deep, which keeps them within the TIR field. The nanochannels are actively generated by collapsing 1 µm deep flow channels using pressurized nitrogen gas in a control channel, which additionally increases photo stability of fluorophores by removing oxygen. We demonstrate that several second long time trajectories of thousands of molecules can be recorded with millisecond time resolution, illustrating the potential for long-term automated and high throughput experiments. We also show that the compatibility of the device with large biological complexes by showing multi-channel FRET imaging of freely diffusing single nucleosomes.

893-Pos Board B662

Accurate Intramolecular Distances by Single Molecule Confocal Spectroscopy: A Monte Carlo Markov Chain Analysis of Fluorescence Data from Freely Diffusing Biomolecules

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Förster Resonance Energy Transfer (FRET) is a powerful technique for studying the conformational dynamics of biological systems at the level of single molecules. FRET experiments on single molecules (smFRET) in solution have the potential to recover accurate intramolecular distances. However, established methods for event selection and de-noising of smFRET data, are *ad hoc* and cause systematic biases in the results obtained.

We introduce a novel method for the analysis of smFRET data, suitable for the study of protein folding or molecular association. The technique is based on a simple stochastic model of the physical FRET process. Based on this model, we devise a Bayesian inference technique to infer directly all key features of a smFRET dataset, using Markov Chain Monte Carlo (MCMC) sampling methods. The proposed technique is computationally fast, requires only a few minutes of data collection, yields reliable confidence intervals, and requires no subjective choice of parameters for de-noising.

We evaluate the technique's performance using a combination of synthetic and experimental data, demonstrating that the method:

i. effectively identifies fluorescence events;

ii. distinguishes noise and fluorescence contributions to fluorescence events;

iii. infers simultaneously the rate parameters for the noise distribution, the FRET efficiencies, and relative sizes of one or more fluorescent populations.

We validate the technique on smFRET data from mixtures of well characterised dual-labelled DNA duplexes. Our analysis method is able to infer both the mean FRET efficiencies of multiple FRET populations and the relative sizes of these populations. Results will be presented demonstrating that our Bayesian inference technique obtains accurate intramolecular distance information from smFRET experiments, whereas established techniques fail.

894-Pos Board B663

Optimal Estimation of Diffusion Coefficients from Noisy Single-Particle Trajectories

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Super-resolution microscopy allows us to track single molecules in cells. There, diffusion is ubiquitous, as many cellular processes rely on diffusion for transport. A precise understanding of such processes requires a precise determination of diffusion constants. Less than that may miss process-specific details by lumping them into a single, simple diffusive process as we demonstrate here. We present a simple, optimal, unbiased estimator of diffusion coefficients of freely diffusing particles in one, two, or three dimensional homogeneous media. It takes time-lapse recorded single-particle trajectories as input, is vastly superior to estimates based on the mean squared displacement as function of time, and is superior to Maximum Likelihood estimation for short trajectories. We extend the method to diffusion on one-dimensional fluctuating substrates. As a pertinent practical illustration of the power of these tools, we use them to reveal multi-state kinetics in the diffusion of a protein on flow-stretched DNA, a fluctuating substrate.

895-Pos Board B664

In Silico, In Vitro, and In Vivo Estimation of J-Factors for LAC Repressor-Mediated DNA Loop Formation

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Transcription is often regulated by regulatory proteins with specific binding sites that can be many kilo-bases away from promoters. This is made possible by long range, DNA looping interactions. According to the ''loop domain model'', the separation of promoters and binding sites into separate topological domains can block promoter-regulator communication and this has been shown in bulk, *in vitro* experiments. However, the efficiency and regulation of long-range DNA looping are not well understood. To investigate looping, simulations, tethered particle motion (TPM) experiments, and measurements of reporter gene repression were used to estimate *J*-factors (J_{loop}) for lac