Symposium 20: Single Molecules Meet Systems Biology

3919-Symp
Single Molecules in Single Cells: A System-Wide Quantification of Gene Expression
Sunney Xie.
Harvard University, Cambridge, MA, USA.
Our group studied gene expression in living bacterial cells with single-molecule sensitivity with millisecond time resolution and nm spatial precision. We reported the first movie of protein production, one molecule at a time, binding and unbinding kinetics of transcription factors on DNA, and their single-molecule events that change the cell's phenotype. We recently conducted system-wide studies of transcriptome and proteome with single-molecule sensitivity in a single cell. We are investigating the phenomenon of persisters, abnormal and rare bacterial cells that have the same genes as normal cells, but are phenotypically drug tolerant, hoping to provide clues for developing tuberculosis drugs.

3920-Symp
Probing Intracellular Kinetics at the Level of Single Molecules
Johan Elf.
Uppsala University, Uppsala, Sweden.
I will present our resent advancements in tracking individual freely diffusing fluorescent proteins molecules at high time resolution in the cytoplasm of bacterial cells. *In vivo* tracking of individual proteins molecules makes it possible to study kinetics high time resolution without synchronizing the population of molecules. For example by monitoring the kinetics of the response mediator RelA we have developed a single molecule assay to study stress response and starvation at the level of individual bacteria.
The RelA protein binds to a small fraction of ribosomes, where it synthesizes the global transcriptional regulator ppGpp in response to amino acids deprivation. This the ppGpp molecule binds to the RNAP and rapidly reprograms the cell for the new environment, in what is called the stringent response. While *E. coli* contains on average about 100 RelA molecules, using a photo-activatable fluorescent probe we can activate only a few fluorescent molecules per cell at any given time and track them at high time resolution. The procedure can be repeated many times to get accurate statistics for in individual cells.
When the cell grows exponentially, RelA trajectories closely resemble trajectories of fluorescently tagged ribosomal proteins (D~0.4um$\perp$2/sec as compared to D~0.3 um$\perp$2/sec for ribosomes). After nutritional downshift, RelA binding kinetics changes rapidly and the protein diffuses very fast (D~3.5um$\perp$2/sec) as if it only binds to ribosomes transiently. The assay has made it possible to study the rapid and transient stringent response in individual cell as well as the heterogeneity in the stress response over the population.

3921-Symp
Error Minization in Lateral Inhibition Circuits
Naama Barkai.
Weizmann Inst, Rehovot, Israel.
No Abstract.

3922-Symp
Signaling Dynamics at the Single Cell Level
Michael Elowitz.
California Inst Tech, Pasadena, CA, USA.
Our lab studies the dynamics of gene circuits at the single-cell level. This talk will focus on signal encoding schemes used in different prokaryotic and eukaryotic signal transduction systems. In particular, I will discuss new, dynamic encoding schemes and their possible functions.

Symposium 21: Mechanism of Electromechanical Coupling in Voltage-gated Ion Channels

3923-Symp
The Structural Basis of Voltage Sensing
Roderick MacKinnon.
Rockefeller Univ, HHMI, New York, NY, USA.
No Abstract.

3924-Symp
The Structural Basis of Gating Currents: Insights from Fluorescence Spectroscopy
Francisco Bezanilla.
Univ Chicago, Chicago, IL, USA.
The S4-based voltage sensor found in voltage-gated ion channels and in voltage dependent phosphatases plays a fundamental role in controlling cellular excitability. Although recently the combination of electrophysiology, mutagenesis, spectroscopy and X-ray crystallography have delineated the basic molecular correlates of the voltage sensor operation, we still are far from understanding the physical basis of the sensing (gating) currents. The study of sensing currents show that the sensor visits three main states: Resting, Active and Relaxed (inactivated). Furthermore, between the Resting and Activestatesthere is at least another major state that can be modulated by mutations. In addition, we know with a fair degree of precision, the kinetics and magnitude of the charge moving among the different states. However, the structural correlates of these states and transitions are for the most part unknown. Fluorescence spectroscopy , concomitant with electrophysiology, has been used to detect conformational changes. Thus, local changes within the sensor are detected by following modifications in the environment of a fluorophore probe situated in specified sites and changes in conformations have been detected and quantified as intramolecular distance changes using the LRET technique. These techniques are especially critical in characterizing the short-lived Active state that is populated by positive potentials and spontaneously decays to the Relaxed state within tens to hundreds of milliseconds. As the Relaxed state is stable, it is most likely the state that X-ray crystallography has caught. Fluorescence spectroscopy confirmed that indeed there are three main states and that they each correspond to different conformations of the voltage sensor. In addition, it has provided the kinetics of the conformational changes and estimates of the relative movements of the voltage sensor while evolving from Resting to Active to Relaxed. Support: NIHGM030376.

3925-Symp
Dynamics of Voltage-Sensor Movement in Sodium Channels
Todd Scheuer.
Univ Washington, Seattle, WA, USA.
Voltage-gating of ion channels is driven by the S4 segment residing in a gating pore in proximity to S1, S2 and S3 segments of the voltage sensing module of the channels. Molecular modeling using Rosetta has proposed detailed movements of the S4 voltage sensor in the gating pore during gating. As it transits between deactivated and activated conformations, voltage sensor arginines are hypothesized to make sequential ion pairs with negatively-charged residues in surrounding transmembrane segments that catalyze the transmembrane movement of the voltage-sensor positive charges; the charge-charge pairs also prevent movement of solution ions through the gating pore. We have used two approaches to test this model. First, using mammalian sodium channels we have shown that substitutions of glutamine for the two most extracellular arginines allows current through the gating pore at hyperpolarized potentials where predicted charge-charge interactions are missing due to mutation, but not at depolarized potentials where the remaining more intracellular and unmutated arginines are predicted to form pairs and block the gating pore. Conversely, glutamine substitutions for arginines 2 and 3 allows gating pore current at depolarized but not hyperpolarized potentials. In a second approach, we have used bacterial NaChBac sodium channels and substituted pairs of cysteines for negatively-charged amino acids in S2 and positively-charged arginines in S4 that are predicted to interact during gating. We demonstrate rapid disulfide bond formation between the substituted cysteines that is voltage (and conformation) dependent. The time and voltage-dependence of these disulfide interactions provide experimental evidence for sequential formation of specific ion pairs as the voltage sensor moves within the gating pore during channel activation. The combination of these experimental approaches gives an increasingly detailed picture of the conformations and interactions of S4 in the gating pore during voltage-sensor activation.

3926-Symp
Molecular Movements within the Voltage-Sensor Domain of a Potassium Channel
Fredrik Elinder.
Linköping Univ, Linköping, Sweden.
Voltage-gated ion channels play critical roles in all biological systems, for instance in transmitting the nervous impulse and initiating the heart beat. Changes in the membrane potential are detected by a voltage sensor in the channel, leading to opening and closing of the ion-conducting pore. While the atomic structure is known of a voltage-gated K channel in an activated state, the chain of molecular events leading to channel gating is known in less detail. We have used disulfide-linking techniques in combination with electrophysiology to probe molecular motion within the channel protein. Two amino-acid residues are mutated to cysteins and if these residues are close enough to each other it is possible to make and break disulfide or cadmium bridges, detected as a change in channel function. The analysis also gives information about if

the residues are close to each other in closed, open or inactivated states. With this technique, we have detected and analyzed molecular movements within the voltage-sensor domain, between the voltage sensor and the pore domain, and within the pore domain.

Minisymposium 4: New Chemical Modulators by Rational Design

3927-MiniSymp
Optimizing Ligand-Protein Interactions Via Silcs: Site Identification by Ligand Competitive Saturation
Alexander MacKerell.
Univ Maryland, Baltimore, MD, USA.
Fragment-based methods for drug optimization have great potential; however, time, expense and sensitivity considerations associated with NMR and x-ray crystallographic based methods limit their applicability. As an alternative we have developed a computational approach, SILCS: Site Identification by Ligand Competitive Saturation, that uses explicit solvent all-atom molecular dynamics to identify binding sites on protein surfaces for functional groups. Information from the SILCS approach may then be combined with structural information on an inhibitor-protein complex to facilitate modification of the ligand to improve its binding affinity. An overview of SILCS and its application to inhibitor-ligand optimization will be presented.

3928-MiniSymp
Structure of P-Glycoprotein Reveals a Molecular Basis for Poly-Specific Drug Binding
Stephen G. Aller.
Univ. Alabama at Birmingham, Birmingham, AL, USA.
P-glycoprotein (Pgp) detoxifies cells by exporting hundreds of chemically unrelated toxins but causes multidrug resistance in the treatment of cancers. Substrate promiscuity is a hallmark of Pgp activity, thus a structural description of polyspecific drug-binding is vital for the rational design of anticancer drugs and MDR inhibitors. The x-ray structure of apo-Pgp at 3.8 Å reveals an internal cavity of ~6,000 Å3 with a 30 Å separation of the two nucleotide binding domains (NBD). Two additional Pgp structures with novel cyclic peptide inhibitors demonstrate distinct drug binding sites in the internal cavity capable of stereo-selectivity that is based on hydrophobic and aromatic interactions. Apo- and drug-bound Pgp structures have portals open to the cytoplasm and the inner leaflet of the lipid bilayer for drug entry. The inward-facing conformation represents an initial stage of the transport cycle that is competent for drug binding.

3929-MiniSymp
Chemical Synthesis of a Highly Selective Probe of the Renal Outer Medullary Potassium Channel (ROMK)
Jerod S. Denton, Brian A. Chauder, Rishin Kadakia, Eric S. Dawson, Craig W. Lindsley, C. David Weaver, Gautam Bhave.
Vanderbilt University School of Medicine, Nashville, TN, USA.
The renal outer medullary potassium channel, ROMK, critically regulates salt and water balance and may be a drug target for a novel class of diuretic. However, the molecular pharmacology of the inward rectifier potassium channel family is virtually undeveloped, precluding assessment of ROMK's therapeutic potential. We therefore performed a high-throughput screen of approximately 225,000 small molecules for modulators of ROMK function, from which several novel antagonists were identified. One compound, termed VU590, inhibits ROMK with a half-inhibition concentration (IC_{50}) of 300 nM, has no effect on Kir2.1 or Kir4.1, but inhibits Kir7.1 at low micromolar concentrations. Two structurally related compounds were identified in the screen, but were found to be comparatively weak ROMK inhibitors. Using a molecular mechanics-based knowledge of VU590, medicinal chemistry was employed to improve the potency of one compound 33-fold (IC_{50} from 8 μM to 240 nM). This novel probe, termed VU591, is highly selective for ROMK over Kir2.1, Kir2.3, Kir4.1, Kir6.2/SUR1B, Kir7.1 and a panel of more than 65 other potential off-targets, including voltage-gated sodium and calcium channels and hERG. Functional studies suggest the VU591 binding site is located in the cytoplasmic pore of ROMK. VU591 will be instrumental in mapping the location and topographical features of this selective binding site and could pave the way for animal studies assessing the therapeutic potential of ROMK.

3930-MiniSymp
Gold Nanoparticles as a Platform for Designing Protein-Protein Interaction Inhibitors: Application to Ubiquitin-Like Modifications
Yang Su, Angela Perkins, Yi-Jia Li, Yuelong Ma, David Horne, Yuan Chen.
Beckman Research Institute of the City of Hope, Duarte, CA, USA.
Inhibitors of non-covalent protein-protein interactions hold much promise as useful probes to our understanding of human biology and disease mechanisms, as well as leads for developing new therapies. Developing such inhibitors, however, continues be a significant challenge. Protein-protein interactions mediated by ubiquitin-like (Ubl) modifications are among the most important signalling and regulatory mechanisms that control nearly every aspect of cellular functions. A unique feature of these post-translational modifications is the formation of poly-Ubl chains; however, strategies to target these poly-Ubl chain modified proteins are lacking. In this study, we show that gold nanparticles (AuNPs) conjugated with small molecule inhibitors can selectively target such poly-Ubl chains. Virtual ligand screening was carried out to identify small molecule mimmetics of the Small Ubiquitin-like MOdifier (SUMO) interaction motif in order to inhibit SUMO-mediated down-stream effects. Virtual ligand screening was based on the NMR structure of SUMO in complex with a peptide containing the SUMO-interaction motif. Interactions of the hit compounds with SUMO were investigated by NMR methods. One of the hits was modified for conjugation to an AuNP by adding a thiol tail. While the individual compounds do not have high affinity for SUMO (having K_d of 2 mM), conjugation of approximately 100 compounds to one AuNP allows for multi-valent interactions between AuNP and multiple SUMO proteins in a poly-SUMO chain; thus efficiently inhibits poly-SUMO-chain-mediated protein-protein interactions. This study demonstrates a viable approach to creating highly effective inhibitors by using AuNPs as a platform for multivalent interactions. This is the first application of AuNP for inhibiting Ubl modifications and provides a novel approach to specifically and effectively address such types of Ubl modifications for future research and therapeutic applications.

3931-MiniSymp
New Approaches to Anti-Infective and Anti-Cancer Therapeutics Targeting Metalloproteins
Eric Oldfield.
university of illinois at urbana, urbana, IL, USA.
I will give an account of recent progress in the development of novel anti-infectives targeting isoprenoid biosynthesis. Topics to be covered include: carotenoid biosynthesis as a target in staph and malaria; novel inhibitors of bacterial farnesyl and undecaprenyl diphosphate synthase; and the mechanism of action of the Fe4S4 cluster-containing proteins, GcpE (IspG) and LytB (IspH). We have investigated the mechanism of action and inhibition of Aquifex aeolicus LytB using a combination of site-directed mutagenesis (KM, Vmax), EPR and 1H, 2H, 13C, 31P and 57Fe-ENDOR. The EPR and ENDOR results support formation of an initial pi/sigma ''metallacycle'' complex similar to that observed previously with allyl alcohol bound to a nitrogenase FeMo cofactor. The complex is poised to interact with the E126 CO2H group, resulting in loss of H2O and formation of eta1 and/or eta3-allyl complexes. The IPP and DMAPP products are then formed in a second H+/e- reduction step. We also report that alkyne diphosphates are inhibitors of IspH and likewise form pi or pi/sigma metallacycle complexes, as evidenced by 1H, 2H, and 13C ENDOR. I will also give an update on the mechanism of action of GcpE, and the discovery of potent, mechanism-based, inhibitors of this enzyme.

Platform BF: Cardiac Muscle II

3932-Plat
Proteomics of the Human Cardiac Intercalated Disc: A More Complex Multi-Functional Structure than was Previously Thought
Cristobal G. dos Remedios[1], Colleen Estigoy[2], Darryl Cameron[3], Joshua W.K. Ho[3], Benjamin Herbert[4], Matthew Padula[4], Russel Pickford[5], Michael Guilhaus[5], Jacob Odeberg[6], Fredrik Ponten[7].
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The intercalated disc (ICD) of cardiac muscle joins one cardiomyocyte to several others. It transmits contractile force between these heart muscle cells, but it also must allow action potentials, ions and even small molecules to cross the junctions, it contains multiple receptors, and must permit the cardiomyocytes to grow. The literature contains 142 proteins in mammalian hearts that were identified using a wide range of techniques. Here we employ two technologies that nearly double this number. We use Fourier transform mass spectrometry to identify 84 proteins based on an analysis of their tryptic peptides using purified (but not pure) ICDs from human left ventricles from four non-failing hearts, only about half of which (43) were previously known. We then explore the Human Proteome Atlas (HPA) database to identify 162 ICD proteins using