Ca^{2+} sparklets are local elevations in intracellular Ca^{2+} produced by the opening of a single or a small cluster of sarcolemmal L-type Cav1.2 Ca^{2+} channels. At present, however, the spatial organization and mechanisms of modulation of Ca^{2+} sparklets in cardiac myocytes is unknown. Here, we tested the hypothesis that Ca^{2+} sparklets activity varies within the sarcolemma of neonatal cardiac myocytes and that chemically-induced translocation of PKCα increases the Ca^{2+} sparklet activity in these cells. Consistent with this hypothesis we found that application of the PKC activator phorbol 12,13-dibutyrate (PDBu; 500 nM) increased Ca^{2+} sparklet activity in neonatal cardiac myocytes. Analysis of the spatial distribution Ca^{2+} sparklet activity was not random (i.e. did not have a Poisson distribution). Rather, as in reported in smooth muscle cells, Ca^{2+} sparklet activity was higher at specific regions of the cell. Translocation of PKCα to the sarcolemma of neonatal cardiac myocytes resulted in an increase in Ca^{2+} sparklet activity in specific regions of the cell. Data will be presented on the relationship between Ca^{2+} sparklet activity and Ca^{2+} release from the sarcoplasmic reticulum via ryanodine receptors (i.e. Ca^{2+} sparks) during excitation-contraction coupling. Our data suggest that Ca^{2+} sparklet (i.e. L-type Ca^{2+} channels) activity varies within the sarcolemma of neonatal cardiac myocytes and that they are modulated by PKCα, potentially regulating SR Ca^{2+} release during EC coupling in heart.

85-Plat

Reconstitution of PKA-Dependent Modulation of Cardiac $Ca_v1.2$ Channels

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L-type calcium currents through $Ca_v1.2$ channels initiate contraction in cardiac muscle. Their regulation by neurotransmitters and hormones through second messenger signaling cascades and protein kinase A (PKA) phosphorylation is a key controller of calcium signaling and contractile force. The α_1 subunit C-terminus contains binding sites for multiple regulatory proteins including the PKA/A kinase anchoring protein 15 (AKAP15) complex. The C-terminal domain is proteolytically cleaved but reassociates non-covalently with the truncated channel and acts as a potent autoinhibitor of channel activity. Relief of autoinhibition by cellular regulatory signals acting on the C-terminus provides an attractive mechanism for producing the large increases in calcium current that are observed physiologically. In fact, consistent reconstitution of PKA-dependent regulation of $Ca_v1.2$ channels in non-muscle has been difficult to achieve. To reconstitute such PKA regulation, we optimized the expression of truncated $Ca_v1.2$ channels, the distal C-terminal domain, the $\alpha_2\delta$ subunit, and the β_{2b} subunit to give a functional autoregulatory complex as assessed by whole-cell voltage clamp recordings of tsA-201 cells. Expression of the truncated $Ca_v1.2$ channel with the free distal domain resulted in large decreases in inward barium current and in coupling between voltage-dependent gating and pore opening. We hypothesized that AKAP15 expression would promote PKA association with the distal C-terminal of the channel and increase the likelihood of PKA-dependent phosphorylation. After optimizing AKAP15 expression, currents recorded in 5 μM forskolin were approximately 5-fold larger than those recorded in the presence of kinase inhibitor RO 31-8220 (1 uM). These findings show that the full-range of PKA-dependent modulation of $Ca_v1.2$ channels can be reproduced when an autoinhibitory complex is formed in this manner and provide a substrate for further studies of this physiologically important regulatory process.

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86-Plat

Biochemical and Functional Characterization of Crystallographic Ca2+/CaM-Cav1.2 A-C-IQ Complex Dimer

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The ubiquitous calcium sensor calmodulin (CaM) mediates two important voltage-gated calcium channel (CaV) calcium-dependent modulatory mechanisms through its interaction with the CaV C-terminal tail. Here, we report the structure of Ca2+/CaM bound to a portion of the CaV1.2 C-terminus with three consecutive CaM binding motifs (A-C-IQ domain). The structure reveals two channel chains dimerized via bridging CaMs and interactions between two long, antiparallel helices. Unlike the crystal structure, the CaM peptide complex is a monomer in solution that corresponds to a single channel chain and two CaMs. Disruption of the crystallographic inter-helix interactions had minimal effect on CDI and CDF of full-length CaV1.2. Moreover, subunit counting experiments using CaV1.2-EGFP fusion proteins clearly indicate that CaV1.2 is a monomer in cell membranes. Thus, contrary to previously proposed models, there appears to be no role for dimerization in channel function.

87-Plat

Enzyme-Inhibitor-Like Tuning of Calcium Channel Connectivity With Calmodulin

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Ca^{2+} channels and calmodulin (CaM) are prominent hubs of signaling networks, extensively coordinated by feedback control. For example, each channel associates with its own CaM (but see *PNAS***106**:5135), acting as a Ca^{2+} sensor that regulates Ca^{2+} entry through channels. Because channels bind CaM avidly, every channel should possess CaM and exhibit regulation, regardless of biological fluctuations of CaM concentration. This would represent a significant form of concentration independence between Ca^{2+} channels and CaM. Here, we reveal significant exceptions to this autonomy, by combining electrophysiology to characterize channel regulation, with concurrent optical FRET sensor determination of free apoCaM concentration in live cells. This approach translates quantitative CaM biochemistry from the traditional test-tube context, into the realm of functioning holochannels within intact cells. From this perspective, we find that long splice forms of $Ca_V1.3$ and $Ca_V1.4$ channels include a distal-carboxy-tail module that functions like an enzyme inhibitor to retune channel affinity for apoCaM. In this configuration, natural CaM variations alter Ca^{2+} feedback gain, and the strength of competitive retuning is customized across channel subtypes ($Ca_V1.3$ versus $Ca_V1.4$), and species (rat versus human $Ca_V1.3$). Given the ubiquity of these channels, the corresponding connections between ambient CaM levels and Ca^{2+} entry via channels are broadly significant for Ca^{2+} homeostasis—presumed alterations of apoCaM levels in neurodegenerative conditions like Parkinson's and Alzheimer's are predicted to increase Ca^{2+} entry, potentially explaining the Ca^{2+} dysfunction underlying these diseases. Mechanistically, our extensions of enzyme-inhibitor analysis argue well that the competitive retuning in holochannels indeed reflects competition between a single distal-carboxy-tail module and a single CaM molecule, both vying for IQ domain occupancy on channels. Finally, our overall approach may be generally useful for the *in situ* analysis of signaling molecules resistant to *in vitro* reconstitution, such as Ca^{2+} channels.

Platform H: Physical Chemistry of Proteins & Nucleic Acids

88-Plat

Single Molecule Observations of DNA Hybridization Kinetics

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Two 25 base-pair complementary DNA strands are encapsulated within an optically trapped nano-droplet, and we observe the kinetics of their hybridization in dynamic equilibrium via single molecule fluorescence resonance energy transfer (FRET) as a function of temperature and of the solution's NaCl concentration. We have directly observed binding and unbinding events between the two freely diffusing DNA strands, and our measurements resolve multiple conformational states at elevated temperatures and low concentrations of NaCl.

89-Plat

Control of the Viscoelasticity of the Genome By Topoisomerase Type II and Anti-Cancer Drugs

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The mechanical, viscoelastic properties of the genome are important for our understanding of cell division and, indirectly cancer therapy. Topology controlling enzymes (topoisomerase type II) are thought to play an essential role, but so far quantitative measurements of the effect on the viscoelasticity of DNA are lacking. We report experiments showing how double strand passage facilitated by human topo II controls the disentanglement of DNA. For this purpose, we have measured the elastic storage and viscous loss modulus of a model system consisting of bacteriophage DNA in buffer solution using video tracking of the Brownian motion of colloidal probe particles. We found that the viscoelastic response is critically dependent on the formation of entanglements among the DNA molecules with relaxation times on the order of seconds. For the first time we observed that topo II effectively removes these entanglements and converts the system from an elastic gel to a viscous fluid depending on the dissipation of energy through the hydrolysis of ATP. A second aspect of this study is the effect of a generic topo II inhibitor on the viscoelasticity. Topo II inhibitors constitute an important class of anti-cancer drugs, because they impede

the division of cancerous cells by forming clamps between DNA segments. We continue interpreting our results in terms of a molecular model, which includes the reaction kinetics and functional mechanism. Our work contributes to the understanding of energy dependent, non-equilibrium dynamics of biomolecules, which is a key feature of life.

90-Plat

Photocontrol of Gene Expressions Systems Using Light-Induced Conformational Changes of Nucleic Acids

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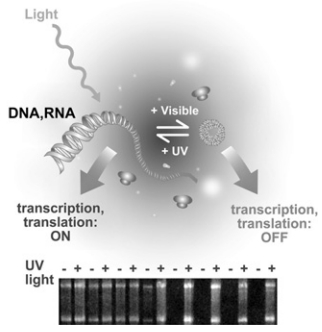
To understand non-trivial biological functions, it is crucial to develop minimal synthetic models that capture their basic features. Here, we demonstrate a sequence-independent, reversible control of transcription and gene expression using a photosensitive nucleic acid binder (pNAB). By introducing a pNAB whose affinity for nucleic acids is tuned by light, in vitro RNA production, EGFP translation, and GFP expression were successfully inhibited in the dark and recovered after a short illumination at 365 nm. Our results indicate that the accessibility of the protein machinery to one or several nucleic acid binding sites can be efficiently regulated by changing the conformational/condensation state of the nucleic acid (DNA conformation or mRNA aggregation), thus regulating gene activity in an efficient, reversible, and sequence-independent manner. The possibility offered by our approach to use light to trigger various gene expression systems in a system-independent way opens interesting perspectives to study gene expression dynamics as well as to develop photocontrolled biotechnological procedures.



Ref: A. Estévez-Torres, C. Crozatier, A. Diguet, T. Hara, H. Saito, K. Yoshikawa, D. Baigl, Proc. Natl. Acad. Sci. USA 2009, 106, 12219-12223.

91-Plat

Long-Range Electronic Couplings Observed in DNA By SR-EPR

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DNA can self-assemble into a wide variety of pre-programmed 2-dimensional and 3-dimensional structures. This property, combined with the ability to conduct charge, makes DNA an attractive material for the fabrication of nanoscale circuitry, sensors, and catalysts. Fermi's Golden Rule, an equation governing the rate of charge transfer, describes the ease with which DNA conducts electrons or ''holes'' (positive charges). However, the predictive power of Fermi's Golden Rule is limited by the difficulty of independently measuring the terms that appear in this equation. One of these terms, the square of the electronic coupling matrix element, is proportional to the scalar exchange coupling between the charge donor and acceptor. Using a nitroxide radical and the paramagnetic Dy(III) ion as surrogates for donor and acceptor, we have measured scalar exchange couplings and their distance dependence in a family of DNA duplexes via saturation-recovery electron paramagnetic resonance (SR-EPR). Scalar exchange couplings are observed at distances as great as 5.6 nm. The decay in the scalar exchange coupling parallels the decay in electron transfer rates recently measured in DNA. The SR-EPR methodology is general and provides a new tool for determining the electronic coupling matrix element in Fermi's Golden Rule. The knowledge gained from these measurements may prove useful in designing DNA-based electronic devices.

92-Plat

Overcharging Below the Nanoscale: Multivalent Cations Reverse the Ion Selectivity of a Biological Channel

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We report charge inversion within a nanoscopic biological protein ion channel in salts of multivalent ions. The presence of positive divalent and trivalent counterions reverses the cationic selectivity of the bacterial porin OmpF into anionic. We discuss the conditions under which charge inversion can be inferred from the change of sign of the measured quantity, the channel zero current potential. By comparing experimental results in protein channels whose charge has been modified after site-directed mutagenesis, the predictions of current theories of charge inversion are critically examined. It is emphasized that charge inversion does not necessarily increase with the bare surface charge density of the interface and that even this concept of surface charge density may become meaningless in some biological ion channels. Thus, any theory based on electrostatic correlations or chemical binding should explicitly take into account the particular structure of the charged interface.

93-Plat

Optical Proteomics Combining Nonlinear Electrokinetics and Coherent Two-Dimensional Infrared Spectroscopy

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We have previously demonstrated that multidimensional optical spectroscopy (EVV 2DIR) can conveniently and easily quantify tryptophan (W), tyrosine (Y), phenylalanine (F) and the total methyl (CH_3) content of a protein , but more independent variables need to be quantified in order to uniquely identify a higher proportion of the proteome. Bioinformatics analysis shows that only a small number of amino acids need be quantified to uniquely identify a substantial proportion of the human proteome. For example, if the approximate mass ($\pm$ 10%) of a protein is also known as well as the W, F, Y and CH_3 content, then the number of proteins uniquely identified increases from 2% to 15% of the entries in the human protein database (Ensemble release 44) containing 33100 proteins.

In this paper, we demonstrate a comprehensive protein identification and characterisation strategy based on the combination and principles of nonlinear electrokinetics together with EVV-2DIR spectroscopy.

A complex mixture is spatially resolved via Capillary Zone Electrophoresis (CZE) and the electrophoretic mobilities of the resulting fractions are analysed in a manner that facilitates the assignment of values to a substantial range of physicochemical properties such as the molecular weight and surface charge densities to each fraction with an accuracy larger than 80%[4]. The two analysis methods are joined up with a specially-devised CZE-EVV interface platform which enables the preservation of the spatial resolution of the fractions and facilitates upstream optical interrogation of each individual fraction[5].

94-Plat

Denatured-State Conformation As Regulator of Amyloid Assembly Pathways?

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Deposits of insoluble protein fibrils with cross beta-sheet structure are the hallmark feature of numerous human disorders, including Alzheimer's disease and type II diabetes. Using correlated dynamic light scattering (DLS) and atomic force microscopy (AFM) we investigated amyloid formation with hen egg white lysozyme at acidic pH values. We found that there was a pronounced transition in the aggregation behavior at low *vs* near physiological salt concentrations. At low salt concentrations ($<$ 100 mM), DLS indicated the near simultaneous nucleation of three distinct aggregate populations. For elevated salt concentrations, only a single aggregate peak nucleated. AFM imaging shortly after nucleation further indicated distinct aggregate morphologies and sizes. Low salt concentrations yielded polymeric aggregates of varying dimensions but consistent with the three aggregate peaks observed in DLS. Aggregation near physiological salt concentrations, in contrast, yielded oligomeric intermediates that nucleated into protofibril strands. It is commonly accepted that amyloid fibril growth by native proteins requires a partially denatured conformation. We wondered, however, whether these changes in aggregation behavior could be related to *differences* in the conformation of denatured lysozyme monomers. Using DLS to measure lysozyme's diffusivity, we found that lysozyme assumes a noticeably more extended conformation at low vs. high salt concentrations. These latter measurements were carefully corrected for the effects of protein interaction and variable solution viscosity on protein diffusivity. ANS fluorescence measurements revealed a similar trend towards increased solution exposure of lysozyme monomers at low salt concentrations compared to high salt concentrations. These observations suggest that amyloid fibril assembly pathways might depend on the conformation of the denatured state from which they grow. This has potentially significant implications for our understanding of amyloid fibril formation in general and how to control the emergence of toxic intermediates, in particular.