This talk will describe recent in silico studies of amyloid beta-protein carried out by a group of computational physicists at Boston University in strong collaboration with a group at Harvard Medical School (now at UCLA). Recently published results [1–4] will be emphasized, and all papers can be downloaded from http://polymer.bu.edu/hes/ad/

References

[1]. S. Yun, B. Urbanc, L. Cruz, G. Bitan, D. B. Teplow, and H. E. Stanley, "Role of Electrostatic Interactions in Amyloid-Protein (A) Oligomer Formation: A Discrete Molecular Dynamics Study," Biophysical J. *92*, 4064–4077 (2007).

[2]. B. Urbanc, L. Cruz, D. B. Teplow, and H. E. Stanley, "Computer Simulations of Alzheimer's Amyloid-Protein Folding and Assembly," Current Alzheimer Research, invited review paper *3*, 493–504 (2006).

[3]. D. B. Teplow, N. D. Lazo, G. Bitan, S. Bernstein, T. Wyttenbach, M. T. Bowers, A. Baumketner, J. E. Shea, B. Urbanc, L. Cruz, J. M. Borreguero, and H. E. Stanley, "Elucidating Amyloid Beta-Protein Folding and Assembly: A multidisciplinary Approach," Accounts of Chemical Research *39*, 635–645 (2006).

[4]. B. Urbanc, J. M. Borreguero, L. Cruz, and H. E. Stanley, "Amyloid-Protein Aggregation: Ab initio Discrete Molecular Dynamics Approach to Protein Folding and Aggregation," invited review, Methods in Enzymology 412, Chapter *19*, 314–338 (2006).

1836-Symp Nucleation of Condensed Phases In Supersaturated Lysozyme Solutions

Martin M. Muschol

Univ. of South Florida, Tampa, FL, USA.

Proteins in aqueous suspensions can phase separate into highly concentrated liquid or solid phases. Protein phase separation shares many similarities with the thermodynamic and kinetics of phase separation in colloidal systems. Most prominently, metastable liquid phase separation predicted for colloids is readily observed in protein solutions. Similarly, the intricate kinetics of phase separation can prevent either system from reaching its equilibrium configuration.

Using static and dynamic light scattering, we have investigated phase nucleation in supersaturated solutions of hen egg white lysozyme. Solid-phase nucleation measurements in this system are easily distorted by the presence of frequently overlooked, contaminating lysozyme clusters. These clusters dramatically enhance nucleation rates, leading to large numbers of poor quality crystals. Nucleation rates with clean stock materials indicate that bulk crystal nucleation rates are negligible compared to nucleation at solution interfaces. In contrast, protein solutions readily undergo liquid-liquid phase separation. There are several potential explanations for the disparity in nucleation rates of ordered *vs.* disordered phases under identical solution conditions. One hypothesis is that only solid-phase nucleation requires shedding of parts of the protein's hydration layer. This kinetic barrier should be sensitive to the presence of either chaotropic or cosmotropic ions that disrupt or enhance water structure. To explore this possible connection, we investigated how miscellaneous chaotropic *vs.* cosmotropic salt-ion pairs affect vaious aspects of protein- and solution dynamics. Changes in bulk water structure were evaluated by viscosity measurements, while changes in protein hydration were monitored by measuring the protein's hydrodynamic radius. Using static and dynamic light scattering, salt-specific effect on both direct and hydrodynamic protein interactions were ascertained over a wide range of salt concentrations and solution temperatures.

Funded, in part, by the USF FMMD initiative.

Platform AR: Calcium Signaling

1837-Plat Glycosylation Of The Inositol 1,4,5-trisphosphate Receptor Alters Whole Cell Calcium Signaling

Craig J. Gibson[1], Juliana Rengifo[1,2], Thibault Collin[3], Barbara E. Ehrlich[1]

[1] *Yale University, New Haven, CT, USA,*
[2] *University of Wisconsin- Madison, Madison, WI, USA,*
[3] *Universite Paris 5, Paris, France.*

Calcium is a ubiquitous intracellular signalling compound. The inositol 1,4,5-trisphosphate receptor (InsP3R) is an intracellular calcium channel found within the endoplasmic reticulum. Upon cell stimulation, extracellular signals are transduced through the cytosol leading to activation of the InsP3R and release of calcium from the endoplasmic reticulum. We previously found that the InsP3R is posttranslationally modified by the addition of O-linked β-N-acetylglucosamine (O-GlcNAc), and that this modification reduces the single channel open probability. It was unclear whether this alteration in single channel function would correlate to a change in the whole cell calcium response to an InsP3-generating agonist. To address this question, we examined the effect of O-GlcNAc on InsP3-dependent calcium signalling in SH-SY5Y cells, a cultured human neuroblastoma cell line. We first show that treatment for 72 h with 8 mM extracellular GlcNAc is sufficient to increase the O-GlcNAcylation state of the InsP3R. Using Fluo-4 AM, a calcium sensitive cell-permeant fluorophore, we show that after sugar treatment fewer cells generate a calcium signal in response to 50 nM ATP stimulation. However, all cells can be induced to generate a calcium response given sufficient stimulation. Of the cells that do respond to a higher stimulus (1 μM ATP), the peak amplitude of the calcium signal is reduced in GlcNAc-treated cells. To ensure that the response is due to an effect at the level of the InsP3R, we loaded cerebellar interneurons with Oregon green BAPTA, a green fluorescent calcium indicator, and caged InsP3. We show that cells loaded with UDP-GlcNAc have a dramatically reduced calcium response to photorelease of InsP3. These results show that O-GlcNAcylation is an important regulator of the $InsP_3R$ and suggest a mechanism for altered signalling under conditions where O-GlcNAc is high, such as diabetes.

1838-Plat Intracellular Calcium Dynamics Mediated By NMDA Receptors In Retinal Horizontal Cells

Xu-Long Wang, Pei-Ji Liang

Shanghai Jiao Tong University, Shanghai, China.

Intracellular calcium dynamics mediated by NMDA receptors was studied in carp retinal horizontal cells (HCs). Fura-2 fluorescent calcium imaging showed that H1 subtype horizontal cells responded to exogenously applied NMDA with a transient $[Ca^{2+}]_i$ increase which was reduced down to a sustained steady $[Ca^{2+}]_i$ level. Contributions of different Ca^{2+} flux pathways underlying this $[Ca^{2+}]_i$ dynamics were explored via experiment as well as using a computational model based on the biophysical properties of H1 cells. Relevant contributions of the NMDA receptors, voltage-gated calcium channels, Na^+/Ca^{2+} exchangers, Ca^{2+} pumps and intracellular calcium stores for the induction of $[Ca^{2+}]_i$ dynamics observed in our experiments were analysed.

1839-Plat Modulation Of Cytosolic Ca^{2+} Signalling By cADP-ribose Independent Of Ryanodine Receptors

Michiko Yamasaki, Angelo Demuro, Ian Parker

University of California, Irvine, Irvine, CA, USA.

The intracellular second messenger, cyclic ADP-ribose (cADPR) regulates Ca2+ release from internal Ca2+ stores in a wide range of cells, where it is thought to promote Ca2+ liberation through ryanodine receptors. However, recent studies suggest that cADPR may also modulate Sarco/Endoplasmic Reticulum Ca2+-ATPase (SERCA) pump activity. We used Xenopus oocytes, which lack ryanodine receptors, in order to examine the effects of cADPR on Ca2+ transients evoked by photoreleased IP3 and by influx through plasma membrane channels.

Oocytes were injected either with Ca2+ indicator (Fluo-4) and caged-IP3; or with these compounds plus caged-cADPR. Ca2+ transients evoked by photoreleased IP3 showed no change in amplitude with concomitant photorelease of cADPR, but their decay rate was accelerated. The result was confirmed using non-metabolic cADPR analog, 3-deaza-cADPR together with Fluo-4 and caged-IP3. This change in kinetics appears to result from modulation of Ca2+ sequestration rather than a direct action on IP3 receptors because the decay of signals evoked by transient Ca2+ influx through nicotinic receptor/channels (nAChR) expressed in the oocyte membrane was similarly accelerated by both photoreleased cADPR and 3-deaza-cADPR. To further validate this hypothesis we examined the effects of blocking SERCA activity with thapsigargin. In the presence of thapsigargin (20 ÂµM for 30 min) the decays of Ca2+ signals evoked by both photoreleased IP3 and influx through nAChR were no longer accelerated by 3-deaza-cADPR. Our results suggest that cADPR acts through multiple pathways to regulate cellular Ca2+ signaling, via actions on both RyR calcium release channels and sequestration mechanisms.

1840-Plat Gap Junction-Mediated Calcium Waves in Differentiating Embryonic Stem Cells

Seth Malmersjo, Isabel Liste, Ernest Arenas, Per Uhlén

Karolinska Institutet, Stockholm, Sweden.

We have examined the implication of calcium (Ca2+) signaling for differentiation of embryonic stem (ES) cells into dopamine neurons. ES cells are defined by their capacity of self-renewal and their potential to differentiate into any cell type. Calcium is an important intracellular second messenger that regulates many different cell functions. We observed that both human and mouse ES cells exhibit spontaneous intracellular Ca2+ waves during their differentiation process. To determine which type of cells responded with spontaneous Ca2+ waves we first monitored intracellular Ca2+ changes followed by immunocytochemistry using grided coverslips. We found that neural precursor cells showed spontaneous Ca2+ waves whereas mature neurons and undifferentiated ES cells did not. By applying various pharmacological inhibitors we could conclude that the Ca2+ waves that propagated in the cell clusters were mediated via gap junctions. Blockers of gap junctions abolished the spontaneous Ca2+ signaling in virtually all cells. Expression of gap junctions in undifferentiated ES cells is well documented, but there are few reports about their function for Ca2+ signaling and their role in the differentiation process. Fluorescent dyes were used to show functional gap junction coupling between differentiating cells. In summary, we reveal that ES cells exhibit gap junction-mediated spontaneous intracellular Ca2+ waves during their differentiation process, which might have vital implications for differentiation of ES cells into dopamine neurons.

1841-Plat Analysis of PIP2 Signaling in Cerebellar Purkinje Spines

Sherry-Ann Brown, Frank Morgan, James Watras, Leslie Loew

University of Connecticut Health Center, Farmington, CT, USA.

Cerebellar ataxia is a debilitating disease that affects approximately 150,000 Americans. The disease results from dysfunctional Purkinje cells or the obliteration of the cells altogether. Ataxia therefore may manifest in the absence of cerebellar synaptic plasticity, particularly long term depression, mediated primarily by the Purkinje cell. The Purkinje cell lacks NMDA receptors, which in other cell types is critical for long term depression. Instead, the Purkinje cell depends on coincidence detection of climbing fiber stimulus invoking instantaneous extracellular calcium flux into the cell and parallel fiber stimulus invoking calcium flux from the endoplasmic reticulum into the cytosol. The parallel fibers induce calcium flux via production of IP3 upon hydrolysis of PIP2. With dysfunction of any point along the PIP2 pathway, the parallel fiber stimulus is disrupted. We understand the dynamics of mGluR1 stimulation of PIP2 hydrolysis, and a little of the supralinear calcium response to IP3 concentration, but we do not know how the cell instantaneously provides sufficient PIP2 significantly greater than basal levels to produce the IP3 necessary for the calcium spike observed. We have developed a 3D model that allows us to explore both dynamics and kinetics of PIP2 signaling in the Purkinje cell. We have explored stimulated synthesis and local sequestration, among other possibilities, as candidates for sufficiently increased instantaneous levels of PIP2. Stimulated synthesis leads to adequate IP3 amplitude and a long signal duration for a robust mass of both IP3 and calcium. Elevated spine PIP2 via a local sequestering protein produces an adequate amplitude of IP3, but a short duration. Our simulation results indicate that stimulated synthesis produces sufficient IP3

under all circumstances, while local sequestration produces sufficient IP3 if climbing fiber induced calcium influx is optimally timed.

(Supported by NIH Grant RR013186)

1842-Plat Dynamic Physical Coupling Of Stim1 To Orai1 Is Mediated By The C-terminus Of Orai1 Rather Than By Its N-terminus

Isabella Derler[1], Martin Muik[1], Irene Frischauf[1], Marc Fahrner[1], Petra Eder[2], Rainer Schindl[1], Clemens Hesch[1], Judith Bergsmann[1], Reinhard Fritsch[1], Heike Kahr[1], Josef Madl[1], Klaus Groschner[2], Christoph Romanin[1]

[1] *Institute for Biophysics, Linz, Austria,*
[2] *Institute for Pharmacology and Toxicology, Graz, Austria.*

Recently STIM1 and ORAI1 were identified as essential components of the classical Ca^{2+} release activated calcium (CRAC) current, based on RNAi interference studies. STIM1 is an ER located single transmembrane protein acting as a luminal Ca^{2+} sensor, whereas ORAI1 is supposed to form the CRAC pore, based on mutations in its transmembrane regions. However the detailed mechanism of STIM1 activating ORAI1 remained elusive. Using Förster Resonance Energy Transfer (FRET) microscopy we were able to demonstrate a dynamic, physical coupling of STIM1 to ORAI1 that results in activation of inward recifying Ca^{2+} currents. Various coiled-coil prediction programs identified a putative coiled coil motif, a common interaction domain, in the C-terminus of ORAI1 that is conserved with even a higher probability in ORAI2 and ORAI3. Accordingly we elucidated the putative coiled-coil structure in the C-terminus of ORAI1 as the relevant domain for this physical coupling. An ORAI1 C-terminal deletion mutant as well as a mutant (L273S) impeded in coiled-coil domain formation lacked both physical interaction and functional communication with STIM1 and failed to generate Ca^{2+} inward currents. In contrast an N-terminal deletion mutant as well as the ORAI1 R91W, linked to severe combined immune deficiency syndrome (SCID), was still able to physically interact with STIM1, however, current activation was similarly impaired. Consequently the N-terminus of ORAI1 seems to be rather important for gating of ORAI1 whereas the putative coiled-coil region represents a key domain for dynamic, physical coupling to STIM1.

(Supported by Austrian Academy of Science to I. D., PhD-Program W1201 “Molecular Analytics” from the FWF to M. M. and J. M., FWF P18169 to C. R.)

1843-Plat ML-9 Inhibits Store-operated Calcium Entry by Reversing Store Depletion-induced Stim1 Rearrangement

Jeremy Smyth, Wayne DeHaven, Gary Bird, James Putney

National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA.

Endoplasmic reticulum Ca^{2+} store depletion causes Stim1 rearrangement from a fibrillar configuration into near-plasma membrane (PM) puncta, where it activates members of the Orai family of store-operated Ca^{2+} entry (SOCE) or Ca^{2+} release-activated Ca^{2+} current (I_{crac}) channels. The mechanism of Stim1 rearrangement remains unclear. It is known that ML-9, a myosin light chain kinase (MLCK) inhibitor, antagonizes SOCE and I_{crac}. We confirmed that ML-9, added prior to or following store depletion, reversibly inhibits SOCE and I_{crac} in wildtype HEK293 cells with an IC_{50} of 10 μM. Store depletion induces an increase in enhanced yellow fluorescent protein (EYFP-Stim1) fluorescence measured by total internal reflection fluorescence microscopy (TIRFM) due to rearrangement of EYFP-Stim1 into near-PM puncta. ML-9 reversibly inhibited this TIRFM response, again added prior to or following store depletion, with an IC_{50} of 51 μM. Although this was higher than the IC_{50} for SOCE inhibition in wildtype cells, EYFP-Stim1 overexpression increased the IC_{50} for inhibition of SOCE to 66 μM. This is consistent with the idea that inhibition of SOCE by ML-9 is due to inhibition of Stim1 rearrangement. By confocal microscopy, addition of ML-9 to store-depleted cells caused reversal of punctate EYFP-Stim1 localization into filamentous structures indistinguishable from those observed prior to store depletion. Cells expressing EYFP-Stim1 in which two aspartic acids within the Ca^{2+}-sensing EF-hand are converted to asparagines (DDNN-Stim1) exhibit constitutive SOCE, and DDNN-Stim1 is arranged in near-PM puncta even when Ca^{2+} stores are full. ML-9 also caused rearrangement of DDNN-Stim1 into filamentous structures and reversed constitutive SOCE. ML-9 inhibits Stim1 activity independently of MLCK, since neither wortmannin nor siRNA knockdown of MLCK recapitulated ML-9 effects. Thus, ML-9 prevents formation of near-PM punctae by Stim1 and therefore inhibits SOCE and I_{crac}; however, the mechanism is unclear.

1844-Plat Oligomerization Of STIM1 Couples ER Calcium Depletion To CRAC Channel Activation

Riina M. Luik[1], Bin Wang[2], Murali Prakriya[3], Minnie M. Wu[1], Elizabeth D. Covington[1], Richard S. Lewis[1]

[1] *Stanford University, Stanford, CA, USA,*
[2] *University of Texas Health Science Center at San Antonio, San Antonio, TX, USA,*
[3] *Feinberg School of Medicine, Northwestern University, Chicago, IL, USA.*

The mechanism that couples Ca^{2+} depletion from the ER lumen to the activation of store-operated channels (SOCs) in the plasma membrane (PM) has been a longstanding mystery. Recent studies have revealed that STIM1 senses the loss of ER Ca^{2+}, whereupon it multimerizes and redistributes within the ER to sites adjacent to the PM where Orai1, a component of the CRAC channel, a prototypic SOC, accumulates and brings about Ca^{2+} entry. Here we show that STIM1 multimerization is the critical event that triggers all the subsequent rearrangements leading to store-operated Ca^{2+} entry. Using an ER-targeted Ca^{2+} reporter protein, we find that STIM1 redistribution is a steep function of ER [Ca^{2+}], with a $K_{1/2}$ of ~200 μM and a Hill coefficient of ~4. Because STIM1 binds only a single Ca^{2+} ion, the high cooperativity indicates that STIM1 multimerization is necessary to enable target binding and accumulation at ER-PM junctions. CRAC channel activation displays a similar sensi-

tivity and cooperative dependence on ER [Ca^{2+}], suggesting that the accumulation and activation of CRAC channels at the junctions are driven entirely by STIM1 accumulation. To multimerize STIM1 independently of store depletion, we replaced the lumenal domain of STIM1 with FRB or FKBP. In cells coexpressing these two chimeric proteins, addition of a rapamycin analog caused STIM1 multimerization and redistribution to ER-PM junctions, as well as the activation of CRAC channels even though the ER was replete with Ca^{2+}. These results demonstrate that the multimerization of STIM1 is both necessary and sufficient to activate CRAC channels and therefore constitutes the critical event that couples store depletion to the activation of Ca^{2+} entry.

Platform AS: Single Molecule Biophysics II

1845-Plat Observing Individual Protein Conformation In Live Cells With Single Molecule FRET

keith weninger, John Sakon

North Carolina State University, Raleigh, NC, USA.

The unique capabilities of the single molecule approach are likely to provide insights into a wide variety of cellular signaling pathways. Single particle tracking experiments in live cells are beginning to realize this potential. Here we present progress in our efforts to combine single particle tracking with single molecule spectroscopy to allow fluorescence resonance energy transfer (FRET) to report the real time conformational dynamics of individual protein molecules inside live cells. Recombinantly expressed proteins are dye-labeled and microinjected into cells for in vivo tracking using total internal reflection microscopy. Simultaneous spectroscopy allows the degree of FRET to be determined. We present initial results detecting single molecule FRET in live cells.

(supported by NIH GM076039)

1846-Plat Single Molecule Imaging with Self-Assembled Colloidal Lenses

Stavros Stavrakis, Jerrod Schwartz, Stephen Quake

Stanford University, Stanford, CA, USA.

Single molecule spectroscopy has grown into a powerful technique that allows for the study of the behavior of individual molecules in complex local environments, effectively removing the ensemble averaging of bulk measurements. Wide-field single molecule imaging techniques such as epifluorescence and total internal reflection microscopy often employ high numerical aperture (NA) objective lenses to maximize their photon collection ability. These objectives are challenging to use because they are expensive and have small fields of view, short working distances, and require fluidic contact via an index-matching liquid. The principle of colloidal lensing overcomes these limitations by incorporating a focusing element in immediate proximity to the emitting molecule or nanoparticle. The colloid acts as a lens and dramatically improves the photon collection efficiency of the optical system. By self-assembling a high index of refraction colloid in close proximity to a fluorophore, we have shown that it is possible to image single DNA molecules using an inexpensive objective with a large field of view, long working distance, and low light collection ability (20x 0.5 NA air).

1847-Plat Differential Traveling Wave Technique with Ångstrom Resolution for Tracking Fast Bio-molecular Processes

Irene Dujovne, Jacob W. J. Kerssemakers, Cees Dekker

Delft University of Technology, Delft, The Netherlands.

We have developed a new experimental optical technique that provides extremely high temporal (microseconds) and spatial resolution (with less than 0.1 nm noise at 10kHz) that are key to studying bio-molecular processes and Brownian dynamics at unprecedented resolution.

In this work I will present the new optical experimental technique that is based on a differential measurement of a traveling wave which is highly sensitive and minimally invasive. This technique allows the study of real time dynamics of molecular motors in the single-molecule limit. I will present our results of the application of the technique to the study of kinesin-microtubule systems and Brownian dynamics in confined dimensions

1848-Plat Multicolor Single Quantum Dot-DNA Dimers As A Nanometer Ruler

Joshua Antelman, Shimon Weiss, Xavier Michalet

UCLA, Los Angeles, CA, USA.

Single Quantum Dot-dsDNA dimers (QD-dimers) have been constructed for use as an optical ruler in ultrahigh-resolution colocalization experiments. By varying the length of the dsDNA, the length of the ruler can be precisely controlled and multiple distance measurements can be accomplished. This technique makes use of an advantageous property of quantum dots (QD's) which allows QD's of different emission wavelengths to be excited by a single laser. A closed loop piezo scanning confocal microscope was used which allows for the simultaneous imaging of multiple colored QD's, free of chromatic aberrations. This is achieved by scanning the sample pixel by pixel using the piezo scanner and collecting the emission wavelengths and intensities of each pixel with two avalanche photodiodes. The QD images are then fit to the excitation point-spread function of the laser to precisely locate their respective (x, y) 2-dimensional positions. Using this method, we demonstrate distance measurements with sub-nanometer resolution.

1849-Plat High-resolution Magnetic Tweezers For Single-molecule Measurements

Kipom Kim[1], Omar A. Saleh[1,2]

[1] *Materials Department, University of California, Santa Barbara, CA, USA,*
[2] *Biomolecular Science and Engineering Program, University of California, Santa Barbara, CA, USA.*