

## Symposium 1: Amyloids in Human Disease

### 14-Symp

#### Dysregulation of Intracellular Calcium in Alzheimer's Disease

**Brian J. Bacskai**, Kishore V. Kuchibhotla, Carli Lattarulo, Bradley T. Hyman.

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Alzheimer's disease is characterized by the deposition of senile plaques in the brain resulting in focal neurotoxicity that ultimately leads to neural network disruption. Intracellular calcium is a tightly regulated second messenger whose activation leads to numerous downstream events, including cell death. It has been suggested that dysregulation of calcium homeostasis plays a role in Alzheimer's disease, however this has not been demonstrated directly. We combined in vivo multiphoton cell-resolved calcium imaging to quantitatively image resting and dynamic calcium signaling in both neurons and astrocytes in the brains of mouse models of AD. We found that resting calcium was elevated in a subset of neurons and throughout the astrocytic network in mice with cortical plaques. This increase in calcium levels in neurons but not astrocytes, depended on the proximity to individual senile plaques. The neuronal calcium overload was not the result of presenilin mutations, and led to the loss of spino-dendritic compartmentalization, important for synaptic coordination. In astrocytes, we observed increased spontaneous calcium transients that were not dependent on neuronal activity. Astrocytes were functionally coupled across long distances in APP transgenic but not wildtype mice with evidence of in vivo intercellular calcium waves originating near plaques and spreading to astrocytes nearly 200 microns away. These data reveal disruptions in calcium homeostasis in both neurons and astrocytes in mouse models of AD with differing spatial ramifications. Together, the results demonstrate that the aberrant intracellular calcium levels in the brain provide insight into the pathophysiology of AD and that specific manipulation of calcium levels may lead to new drug targets.

### 15-Symp

#### Alzheimer's Presenilin Regulation of InsP3R Ca<sup>2+</sup> Release Channel Gating

**J. Kevin Foskett**.

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Familial Alzheimer's disease (FAD) is caused by mutations in amyloid precursor protein and presenilins (PS1, PS2). Many FAD-linked PS mutations affect intracellular calcium (Ca<sup>2+</sup>) homeostasis by proximal mechanisms independent of amyloid production, although the molecular details are controversial. Here, we demonstrate that several FAD-causing PS dramatically enhance gating of the inositol trisphosphate receptor (InsP3R) intracellular Ca<sup>2+</sup> release channel measured in native endoplasmic reticulum membranes by nuclear patch clamp electrophysiology. In contrast, wild type or mutant PS that cause frontotemporal dementia have no such effect. FAD PS alter InsP3R channel gating by modal switching to a high open probability burst mode. Single channel recordings of endogenous InsP3R in FAD patient B cells as well as cortical neurons of asymptomatic PS1-AD mice revealed they have higher occupancy in the burst mode than controls, resulting in enhanced intracellular Ca<sup>2+</sup> signals. These results indicate that exaggerated Ca<sup>2+</sup> signaling through InsP3R-PS interaction is a disease specific and robust proximal mechanism in AD.

### 16-Symp

#### Structural Diversity of Amyloid Oligomers

**Charles Glabe**.

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Recent evidence suggests that soluble amyloid oligomers may represent the primary pathological species of protein aggregates in neurodegenerative diseases. Conformation-dependent, aggregation specific antisera indicate that there are three widely distributed and distinct classes of oligomer structures: prefibrillar oligomers, fibrillar oligomers and pore like annular protofibrils. Fibrillar oligomers are soluble at 100,000 x g, rich in  $\beta$ -sheet structures but yet bind weakly to thioflavin T. EPR spectroscopy indicates that fibrillar oligomers display significantly more spin-spin interaction at multiple labeled sites than prefibrillar oligomers and are more structurally similar to fibrils. Fibrillar oligomers are approximately one half to one third the height of mature fibrils, suggesting that they may represent small pieces of a single fibril protofilament. Fibrillar oligomers seed the formation of fibrillar oligomers from A $\beta$  monomers, but do not seed the formation of fibrils. The fibrillar oligomers resulting from seeded reactions have the same dimensions and morphology as the initial seeds, suggesting that the seeds replicate by growing to a limiting size and then splitting. We have also isolated a number of monoclonal antibodies that recognize generic, sequence-independent epitopes associated with prefibrillar oligomers. Analysis of synthetic A $\beta$  oligomers by dot blots using prefibrillar oligomer

specific monoclonal antibodies indicates that structural polymorphisms exist in A $\beta$  prefibrillar oligomers that vary in their reactivity with monoclonal antibodies. These results suggest that distinct structural variants of soluble A $\beta$  oligomers exist, analogous to different strains of prions. These structural polymorphisms may contribute to disease heterogeneity. This work was supported by NIH NS 38298, AG00538, the Cure Alzheimer Fund and a grant from the Larry L. Hillblom Foundation.

### 17-Symp

#### Mechanisms Underlying Neuronal "hyperactivity" in a Mouse Model of Alzheimer's Disease

**Bianca Brawek**, Gerhard Eichhoff, **Olga Garaschuk**.

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Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disorder characterized by deposition of amyloid plaques and accumulation of intraneuronal neurofibrillary tangles. Mouse models of the disease, expressing human amyloid precursor protein and/or presenilins with mutations typically causing early onset AD in humans, recapitulate many hallmarks of the human disease. They develop senile plaques and neurofibrillary tangles; exhibit dysregulation of the intracellular Ca<sup>2+</sup> homeostasis, brain inflammatory response and memory impairment. Because the dysregulation of the intracellular Ca<sup>2+</sup> homeostasis was postulated to act as an important progenitor of AD, we studied intraneuronal Ca<sup>2+</sup> dynamics in APP23xPS45 mutant mice. In vivo two-photon Ca<sup>2+</sup> imaging in these mice revealed 3 different classes of layer 2/3 cortical neurons: "silent cells" showing no spontaneous Ca<sup>2+</sup> transients during a prolonged recording period, neurons with normal frequency of Ca<sup>2+</sup> transients (< 4/min) and "hyperactive" cells with unusually high frequency of Ca<sup>2+</sup> transients (Busche et al., 2008). Here we studied the mechanisms underlying Ca<sup>2+</sup> transients in normal and hyperactive cells. The Ca<sup>2+</sup> transients in both cell types had similar amplitudes and kinetics. They were tetrodotoxin-sensitive and thus caused by action potential firing. Moreover, these transients were of synaptic origin because they were completely and reversibly blocked by a mixture of glutamate receptor blockers CNQX and APV. Surprisingly, APV alone was sufficient to block the transients in both cell types, suggesting a key contribution of NMDA receptor-channels. According to our data, the increased frequency of Ca<sup>2+</sup> transients in hyperactive cells was not due to an increase in intrinsic excitability but rather to a relative loss of synaptic inhibition. Thus, neuronal hyperactivity in APP23xPS45 mice is caused by a local synaptic rewiring with a relative increase in the excitation vs. inhibition.

## Symposium 2: The Cytoskeleton: Variations on a Theme

### 18-Symp

#### Function and Regulation of the Bacterial Cytoskeleton

**Christine Jacobs-Wagner**.

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During the fast paced multiplication of bacteria, cell growth, DNA replication, DNA segregation and cell division must occur and these essential processes must be coordinated temporally and spatially to achieve homeostasis. How this is achieved is not well understood. In the Gram-negative bacterium *Caulobacter crescentus*, the assembly and cellular positioning of the cytokinetic ring made of the tubulin homolog FtsZ is coordinated with chromosome origin segregation through the *parS*/ParB/MipZ kinetochore complex. We found that the polarity factor TipN plays a major role in the segregation of the *parS*/ParB/MipZ complex by affecting the dynamics of the ParA cytoskeletal element. This in turn governs the timing and placement of FtsZ ring formation, which ultimately affects cell growth, division and the size of the progeny.

### 19-Symp

#### Actin and Bacterial Actin-Like Proteins: Insights Into Evolution

**Edward Egelman**.

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Protein polymers are ubiquitous in biology, from cytoskeletal filaments to bacterial pili, and in many cases contain most of the protein in the cell. While it has been assumed that each polymer has a defined structure, we can show that many polymers exist in a multiplicity of states. Conserved subunits, such as bacterial flagellin or Type IV pilin, can be assembled in different ways, giving rise to abrupt changes in quaternary structure. As new quaternary structures emerge, these can have very new functions. For example, the bacterial ParM protein, a homolog of eukaryotic actin, forms filaments that are very different in structure than F-actin, and have a very different function, being involved in DNA segregation. New insights have emerged about the structural plasticity

within F-actin, which may help explain the exquisite conservation of actin's sequence. We can show at better than 10 Å resolution that within the actin filament subdomain 2 can undergo significant structural alterations from an ordered position to complete disorder. We show that the DNase I-binding loop of actin can exist in multiple conformations, as can the N-terminal region of actin. Overall, these insights into structural polymorphism within protein polymers suggest an under-appreciated mechanism for evolutionary divergence.

## 20-Symp

### Actin Filament Nucleation: Structure-Function Relationships

Malgorzata Boczkowska, Suk Namgoong, Grzegorz Rebowski, Roberto Dominguez.

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The actin cytoskeleton is intimately involved in most cellular functions, including cell motility, endo/exocytosis and intracellular trafficking. These processes are characterized by rapid oscillations of actin polymerization/depolymerization under tight temporal and spatial regulation. Hundreds of G- and F-actin-binding proteins, along with signaling and scaffolding proteins regulate the assembly of actin networks. Among these proteins, *filament nucleators* play a critical role by determining the time and location for actin polymerization, as well as the specific structures of the actin networks that they generate. Eukaryotic cells and certain pathogens use filament nucleators to stabilize actin nuclei (small oligomers of 2-4 actin subunits), whose formation is rate-limiting. Known filament nucleators include the Arp2/3 complex and its large family of Nucleation Promoting Factors (NPFs), Formins, Spire, Cobl, Lmod, VopL/VopF and TARP. Structural and functional studies are beginning to shed light on the diverse mechanisms used by these molecules to stabilize actin nuclei. Thus, with the exception of Formins known filament nucleators use the WASP-Homology 2 domain (WH2 or W), a small and versatile actin-binding motif, for interaction with actin. A common architecture, found in Spire, Cobl and VopL/VopF, consists of tandem W domains that bind three to four actin subunits to form a nucleus. Structural considerations suggest that NPFs-Arp2/3 complex can also be viewed as a specialized form of tandem W-based nucleator. The nucleation activities of these proteins vary significantly, and the most effective nucleators are not necessarily those with the largest number of W domains. We show that the inter-W linkers play a critical role in determining the nucleation activities of filament nucleators and the structures of the actin nuclei that they generate. Furthermore, we present evidence that a previously neglected factor, oligomerization, is a major determinant of filament nucleation activity and nuclei structure.

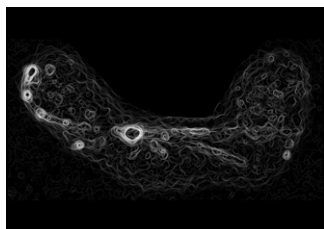
## 21-Symp

### Collective Action of Motor Proteins on Microtubules Regulates Large-Scale Forces in the Cell

Iva Tolic-Norrelykke.

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How do living cells deal with basic concepts of physics such as length and force? Cell interior is neatly yet dynamically organized through constant movements of organelles, which is to a large extent based on microtubules and motor proteins. Two concepts are emerging as key to the regulation of organelle movement: preferred disassembly of longer microtubules and preferred detachment of motors under high load. We have studied both experimentally and theoretically the role of these mechanisms in nuclear centering and nuclear oscillations in fission yeast. These universal concepts may be crucial for a variety of cell processes, including nuclear and mitotic spindle positioning, control of spindle length, and chromosome congression on the metaphase plate.



## Platform A: Member-Organized Session: Biopolymer Dynamics in Cell-like Environment

### 22-Plat

#### Protein Structure, Stability and Folding in the Cell - in Silico Biophysical Approaches

Margaret S. Cheung.

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How the crowded environment inside a cell affects the structural conformation of a protein with aspherical shape is a vital question because the geometry of

proteins and protein-protein complexes are far from globules in vivo. Here we address this question by combining computational and experimental studies of a spherical protein (i.e. apoflavodoxin), a football-shaped protein (i.e., Borrelia burgdorferi VlsE) and a dumbbell-shaped protein (i.e. calmodulin) under crowded, cell-like conditions. The results show that macromolecular crowding affects protein folding dynamics as well as an overall protein shape associated with changes in secondary structures. Our work demonstrates the malleability of "native" proteins and implies that crowding-induced shape changes may be important for protein function and malfunction in vivo.

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## 23-Plat

### Molecular Modeling of the Bacterial Cytoplasm

Adrian Eloco.

University of Iowa, Iowa City, IA, USA.

Advances in the fields of structural biology and quantitative proteomics mean that it is now possible to consider developing working molecular models of intracellular environments. This talk focuses on the construction of such a model for the bacterial cytoplasm, describes the use of Brownian dynamics simulations to model diffusion and association of macromolecules, and shows that calculations of protein stability in the model cytoplasm are in excellent agreement with those measured experimentally in vivo.

## 24-Plat

### Enthalpic Vs. Entropic Effects of Crowded Cellular Environments

Michael Feig.

Michigan State University, East Lansing, MI, USA.

The role of crowded cellular environments on biomolecular energetics and dynamics is often only considered from an entropic point of view in the form of excluded volume effects. Here, the enthalpic contribution of dense cellular environments is considered with two different models. 1) Dense cellular environments are modeled as reduced dielectric continua. 2) Biomolecular sampling in the presence of explicit protein crowders is explored with a new coarse-grained model.

## 25-Plat

### Protein Diffusion and Macromolecular Crowding

Gary Pielak, Yaqiang Wang, Conggang Li.

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Having recently quantified how crowding can affect equilibrium protein stability (1), we have turned our attention to diffusion. Our test molecule is the small globular protein, chymotrypsin inhibitor 2. Our crowding molecules are both the synthetic polymer polyvinylpyrrolidone (PVP) and several globular proteins. We assessed both translational and rotational diffusion by using nuclear magnetic resonance spectroscopy (2). Whereas crowding by PVP results in negative deviations from the Stokes laws, crowding by globular proteins leads to positive deviations. I will discuss our results in terms of what can be learned from in vitro, versus in cell (3) studies.

1. Charlton LM, et al. (2008) Macromolecular crowding effects on protein stability at the residue level. *Journal of the American Chemical Society* 130: 6826-6830.
2. Li C, Wang Y, Pielak GJ (2009) Translational and rotational diffusion of a small globular protein under crowded conditions. *Journal of Physical Chemistry* 113: in press.
3. Slade KM, Steele BL, Pielak GJ, Thompson NL (2009) Quantifying GFP diffusion in Escherichia coli by using continuous photobleaching with evanescent illumination. *Journal of Physical Chemistry* 113: 4837-4845.

## 26-Plat

### Understanding How the Crowded Interior of Cells Stabilises DNA/DNA and DNA/RNA Hybrids - in Silico Predictions and in vitro Proof

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Amplification of DNA in vivo occurs in intracellular environments characterized by macromolecular crowding (MMC). In vitro Polymerase-chain-reaction (PCR), however, is non-crowded and requires thermal cycling to effect melting of DNA strands, primer-template hybridization and enzymatic primer extension. The temperature optima for primer annealing and extension are strikingly disparate which predicts primers to dissociate from the template during extension thereby compromising PCR efficiency. We hypothesised that MMC is not only important for the extension phase in vivo but also during PCR by stabilising nucleotide hybrids. Novel atomistic Molecular Dynamics simulations revealed that MMC stabilises hydrogen