Tuan A. Nguyen and Camkiintide faces and enzymatic activity of CaN to understand how congenital defects residue''. We are pursuing connections between these protein-protein interactions for CaMBDs clearly do not indicate energetic contributions at interface.

CaN acts by dephosphorylating NF-AT, promoting its nuclear localization to its target sequences in kinases, receptors and other channels. Support: NIH R01 GM57001, Carver Charitable Trust Grant 01-224.

Calcium-mediated regulation of early stages of heart development critically depends on calcineurin (CaN), a heterodimeric, calcium-activated phosphatase. CaN acts by dephosphorylating NF-AT, promoting its nuclear localization to launch transcription that controls cardiac muscle growth. Vulnerability of the embryonic heart to fluctuations in CaN activity propels our studies to understand calcium-dependent regulation of CaN. Calcium partially activates CaN by binding to CaNB, its intrinsic 4-EF-Hand subunit. Full activity occurs upon binding of apo CaN. Furthermore, the decrease in affinity for apo CaN is caused by loss of the Ile-Gln pair was 30-fold greater than that observed for loss of the Tyr-Tyr pair. Thus, the energy of interaction between the NaV1.2 IQ motif (2KXW.pdb). The contributions of these residues to binding energetics were determined by monitoring CaM-induced disruption of FRET in biosensors containing wild-type or mutant sequences of the IQ motif bracketed by auto-fluorescent proteins YFP and CFP. All mutations lowered affinity for calcium-saturated CaM, but they had uniformly more deleterious effects on the binding of apo CaM. Furthermore, the decrease in affinity for apo CaN caused by loss of the Ile-Gln pair was 30-fold greater than that observed for loss of the Tyr-Tyr pair. Thus, the energy of interaction between the NaV1.2 IQ motif and semi-open apo CaM is not accounted for primarily by the classical "aromatic anchors" that dominate interactions of apo-calcium-saturated CaM with its target sequences in kinases, receptors and other channels. Support: NIH R01 GM57001, Carver Charitable Trust Grant 01-224.

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Calcium-Mediated Regulation of Calcineurin by a Dynamic Duo of EF-Hand Proteins Madeleine A. Shea, Sean A. Klein, Susan E. O'Donnell, Brett C. Waite, Jesse B. Yoder. Biochemistry, Univ. of Iowa Carver College of Medicine, Iowa City, IA, USA.

Calcium-mediated regulation of early stages of heart development critically depends on calcineurin (CaN), a heterodimeric, calcium-activated phosphatase. CaN acts by dephosphorylating NF-AT, promoting its nuclear localization to launch transcription that controls cardiac muscle growth. Vulnerability of the embryonic heart to fluctuations in CaN activity propels our studies to understand calcium-dependent regulation of CaN. Calcium partially activates CaN by binding to CaNB, its intrinsic 4-EF-Hand subunit. Full activity occurs upon binding of apo CaN. Furthermore, the decrease in affinity for apo CaN is caused by loss of the Ile-Gln pair was 30-fold greater than that observed for loss of the Tyr-Tyr pair. Thus, the energy of interaction between the NaV1.2 IQ motif and semi-open apo CaM is not accounted for primarily by the classical "aromatic anchors" that dominate interactions of apo-calcium-saturated CaM with its target sequences in kinases, receptors and other channels. Support: NIH R01 GM57001, Carver Charitable Trust Grant 01-224.

CaMKII is a dimeric enzyme that regulates long-term potentiation in the hippocampus. The assembly and organization of 8-14 subunits in a holoenzyme is thought to be required for transduction of calcium spike frequencies, cooperative binding to calmodulin, persistent activation by T286 autophosphorylation, and translocation into synaptic spines. CaMKII assembles into a holoenzyme by virtue of its unique C-terminal association domain (AD). ADs form a central hub-like structure from which regulatory and catalytic domains project. Each AD interacts tightly with three other subunits, two laterally and one transversely, to form a stable core composed of two stacked rings. FRET and analytical centrifugation has indicated that individual catalytic domains of CaMKII lacking AD has lower affinity, no cooperativity, and reduced T286 autophosphorylation. In support of the hypothesis, our study shows that a CaMKII dimeric mutant, biophysical analysis suggests that the nature of the pairing might be altered. In this hypothesis, we mutated the lateral surface of the CaMKII AD. When expressed in HEK293 cells, the mutation generated a paired dimeric CaMKIIz. In support of the hypothesis, our study shows that the dimeric enzyme has the same affinity for calmodulin, a similar Hill coefficient for enzymatic activity, and T286 autophosphorylation comparable to that observed in the wild type holoenzyme. In contrast, the monomeric mutant CaMKII lacking AD has lower affinity, no cooperativity, and reduced T286 autophosphorylation. While catalytic domain pairing was observed in the dimeric mutant, biophysical analysis suggests that the nature of the pairing might be altered.
Assemblies and Aggregates II

3445-Pos Board B172

Two-Dimensional Infrared Spectroscopy and Electron Microscopy of Seeded and Non-Seeded Amyloid β Peptide Fibrils
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Seeded and non-seeded fibrils from amyloid β 40-residue (Aβ40) peptides have been studied with two-dimensional infrared (2D-IR) spectroscopy and electron microscopy (EM). For the former, 13C=15O isotope labels were placed in various residues to probe residue-specific amide-I' vibrations of Aβ40 peptides. 2D-IR spectroscopy (2D-IR photon echo) of both kinds of fibrils shows distinct linear chain excitons of amide units due to the interamide vibrational coupling. Structural differences between the seeded and the non-seeded fibrils vary across the sequence. However, seeded fibrils tend to have a better aligned tertiary structure than non-seeded fibrils. Negatively stained EM images indicate that non-seeded fibrils had no discernible narrowing or nodes. In comparison, seeded fibrils tend to appear twisted like a ribbon, with periodic narrowing or nodes. The distances between nodes (the “inter-nodal” distances) were relatively homogeneous distributions (~75 nm) and the same in all isotopically labeled peptides. The apparent width of the fibrils in these images indicate that seeded fibrils are two kinds and that the width of the narrower fibrils is the same as the width of non-seeded fibrils. The mass-per-length evaluated from dark-field EM images indicates that the most prevalent number of filaments in seeded fibrils is 6, which is twice as many as the number in non-seeded fibrils (3 filaments per fibril). Overall, these results demonstrate that seeded and non-seeded fibrils have distinctly different tertiary structures.

3446-Pos Board B174

Structural and Hydration Properties of Huntington Aggregates Determined by Small-Angle Neutron Scattering
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Huntington’s disease involves an abnormally expanded polyglutamine sequence in huntingtin protein (Htt-exon1) making it highly susceptible to aggregate formation. A current challenge is to map out the aggregation pathway by identifying the various precursor structures and establishing their roles in the disease. Here, we are using time-resolved small-angle neutron scattering (SANS) to follow the aggregation kinetics of both wild type and pathological Htt-exon1, where we obtain snapshots of the structures formed as the kinetic reaction ensues. An advantage of neutrons for these long time-scale measurements is that they are non-damaging to the biological sample. The scattering data can be fit using a multiple Guinier-Porod empirical model to account for the presence of multiple species, and this provides quantitative information on the size and shape of the precursors and internal structure of the resulting fibrils. Importantly, both the early-formed oligomers and mature fibrils of pathological Htt-exon1 exhibit distinct structural differences compared to wild type. Neutron contrast variation affords us with the ability to also probe corresponding hydration differences. This research is providing new insights into the pathway of Htt-exon1 aggregation and should later assist in determining the role that precursors play in neuronal toxicity.

3447-Pos Board B175

Enhancement, Equal Fluorescence Efficiency, and Quenching in the Interpretation of Fluorescence Anisotropy Data
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Decreases in fluorescence anisotropy from homo-FRET processes can report on the number of dyes on a protein or the size of a cluster. Fluorescent dyes exhibit a range of behaviours from fluorescence enhancement to quenching when assembled into clusters. The degree of enhancement and/or quenching depends on the number of dyes in close proximity. When interpreting anisotropy, an assumption of equal fluorescence intensity is widely applied. This assumption predicts, for example, that 3 fluorophores in a cluster have the same fluorescence intensity as the same three fluorophores outside of a cluster. This assumption will give incorrect predictions in cases where either quenching or enhancement of fluorescence occurs. Additionally, existing theory typically assumes that all positions within a cluster are equivalent. Application of these assumptions affects interpretation in two ways. It will tend to under-predict the anisotropy of a stochastic mix of individual species and, depending on whether the dye system is quenched or enhanced, will either under-predict or over-predict cluster sizes. Based on computations exploring the impact of enhancement, quenching, and inhomogeneous clustering, a number of conclusions may be reached. As fractional labeling approaches one all models converge to the same value. Inhomogeneous labeling tends to increase anisotropy at low fractional labeling. Applying equal fluorescence intensity assumptions to a fluorescence enhanced system of dyes will over-predict cluster size. Applying it to a quenched system will under-predict cluster size. These cases will be illustrated with simulations and experimental data.

3448-Pos Board B176

Charge Crowding Promotes Self-Assembly of Collagen Heterotrimers
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The design of heterospecific collagen like peptides presents a large challenge in the field of protein design. Many rational and computational approaches have been used to achieve this goal. Our model suggests that crowding of charged