gating in human $Ca_V1.2$ channels, we have optically tracked the activation of the S4 helices of repeats I, III and IV by site-directed fluorescent labeling of introduced Cysteines with thiol-reactive probes. The channels were co-expressed with their modulatory subunits β_3 and $\alpha_2\delta$ in *Xenopus* oocytes. Ionic current and fluorescence emission were simultaneously recorded using the cut-open oocyte voltage clamp fluorometry technique. Prior voltage-clamp, oocytes were injected with 100 nl 80 mM BAPTA.4K, to prevent the activation of endogenous Ca^{2+}-gated Cl^- channels. The extracellular solution contained 2 mM Ba^{2+} or 10 mM Ca^{2+} as charge carrier. Gating currents were recorded by replacing Ca^{2+} or Ba^{2+} with Co^{2+}. 0.1 mM ouabain was added to abolish Na^+/K^+ ATPase non-linear charge movement. Voltage-dependent fluorescence changes (ΔF) were reported by fluorophores attached to substituted Cysteines at the extracelular end of the S4 segment in repeats I, III and IV, tracking local, voltage-dependent conformational rearrangements. The voltage dependence of observed fluorescence deflections preceded ionic activation, reporting repeat-specific VSD transitions taking place during shut states of the channel. Prolonged sojourns at depolarized potentials (+50 mV) shifted the voltage-dependence of reported ΔF to more hyperpolarized potentials by >50mV, recapitulating previously-characterized gating current properties. In summary, we report the first optical characterization of VSD conformational changes in a human voltage-gated Ca^{2+} channel, revealing repeat-specific voltage- and time-dependent properties.

1840-Plat

Engineering the Composition of L-Type (Cav1.2) Channels in Heart Cells using Split Intein-Mediated Protein Trans-Splicing

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Ca^{2+} influx through L-type ($Ca_V1.2$) channels in heart regulates excitation-contraction (EC) coupling, action potential duration, and gene expression. This versatility of L-type channels in heart is hypothesized to be mediated in part by differential sub-cellular compartmentalization and hormonal modulation of distinct $Ca_V1.2$ channel pools in cardiac myocytes. Such prevailing hypotheses are best tested directly in cardiac myocytes, since their unique cyto-architecture and signaling environment cannot be replicated in heterologous cells. There are two major hurdles: first, the large size of pore-forming α_{1C} subunits exceeds the packaging capacity of viral vectors, necessary to express exogenous $Ca_V1.2$ channels in adult cardiac myocytes; second, endogenous channels are a source of confounding contaminating signals. To overcome these limitations, we focused on functionally reconstituting two separately expressed moieties of the channel using a novel split-intein-mediated protein splicing approach. In HEK 293 cells, split-intein fragments were *trans* spliced to generate full-length α_{1C} as determined by Western blot. When co-expressed with auxiliary β subunits, *trans*-spliced α_{1C} trafficked normally to the cell surface and yielded robust whole-cell currents (I_{Ca}). The split-intein fragments were readily incorporated into adenoviral vectors which when used to infect adult myocytes yielded *trans*-spliced α_{1C} that was detected at the cell surface. To isolate exogenous I_{Ca}, we introduced mutations that reduced dihydropyridine sensitivity of *trans*-spliced α_{1C} (α_{1CDHP-}). In the presence of 10 μM nifedipine, myocytes expressing *trans*-spliced α_{1CDHP-} yielded significantly larger currents than controls expressing *trans*-spliced α_{1C}. The results demonstrate a novel approach to robustly engineer the pore-forming α_{1C} subunit composition of $Ca_V1.2$ channels in cardiac myocytes, removing a longstanding technical obstacle to L-type channel structure-function studies in heart.

Platform: Protein Assemblies & Aggregates

1841-Plat

Toxic Intermediates in Islet Amyloid Formation: Analysis of IAPP Mutants Reveals a Correlation between Lag Time and Toxicity

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Islet amyloid polypeptide (IAPP, Amylin) is responsible for amyloid formation in type 2 diabetes and in islet cell transplants. The mechanism of IAPP toxicity is poorly understood. Here we identify and characterize the toxic species produced during amyloid formation by human IAPP. using a combination of time-dependent biophysical and biological assays, we show that the transiently populated pre-fibrillar intermediates formed in the early phase of amyloid formation are toxic and they are loosely packed with very modest amounts of secondary structure. The only known natural mutation found in mature human IAPP is a Ser20-to-Gly mis-sense mutation, which appears to be associated with an increased risk of early-onset type 2 diabetes. We demonstrate that the mutant accelerates amyloid formation under physiologically relevant conditions. We rationally design another variant at residue 20, S20K-IAPP, which is much slower to aggregate with an 18-fold longer lag phase and inhibits wild type amyloid formation. The pronounced effects of the Ser20 mutants highlight the sensitivity of amyloid formation to the identity of position 20. By comparing the kinetics and toxicity profiles of the two Ser20 mutants to wild type IAPP, we demonstrate that changes in the length of the lag phase directly correlate with changes in the onset and the duration of toxicity. Our findings provide direct evidence that the toxic species are transient intermediates and have implications for the treatment of type 2 diabetes and other amyloid related diseases.

1842-Plat

Solid-State NMR Study of Pathologically Relevant Amylioid Intermediate of 42-Residue Alzheimer'S Beta

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Accumulating evidences suggest that many neurodegenerative diseases including Alzheimer's disease (AD) are linked with cytotoxic diffusible aggregates of amyloid proteins, which are metastable intermediate species in protein misfolding. Despite increasing importance of the amyloid intermediates, very little has been known on their structures, relationship with amyloid fibril, and pathogenic functions. This work presents a site-specific structural study on an intermediate called amylo-spheroid (ASPD)(1) for 42-residue Alzheimer's β (Aβ(1-42)) by solid-state NMR (SSNMR), which offers means to characterize metastable amyloid intermediates.(2) As the ASPD level in a brain correlates with the severity of AD, ASPD is likely an intermediate pathogenically relevant to AD.(1) We demonstrate that detailed structural examination by ^{13}C SSNMR is possible on synthetic ASPD that well mimics native ASPD isolated from a brain extract from an AD patient. Electron micrograph and immunological analyses using an ASPD-specific ''conformational'' antibody confirm that morphologies and conformations of the synthetic ASPD used for the present NMR analysis are similar to native ASPD. ^{13}C SSNMR chemical-shift analysis over 20 residues demonstrated that ASPD is made of a homogeneous conformer that is largely composed of β-sheet structure. An inter-strand ^{13}CO-^{13}CO distance measurement suggests that the ASPD involves a parallel β-sheet arrangement despite the fact that ASPD does not bind to fibril-specific dyes such as thioflavin-T. The structural features of ASPD will be compared with those of amyloid fibril for Aβ(1-42), which were elucidated by SSNMR analysis. The approach presented here is likely to open an avenue to examine structural details of various amyloid intermediate species pathologically relevant to AD or other amyloid diseases, for which structures have been poorly characterized.

References
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1843-Plat

Single Molecule Fluorescence Studies of Amyloid Beta 1-42 Aggregation

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The proteolytic cleavage of the transmembrane amyloid precursor protein (APP) produces amyloid-β peptides (Aβ) that vary from 38 to 43 amino acids in length. Two of these peptides, $A\beta_{1-40}$ and $A\beta_{1-42}$, are the major components of the extracellular amyloid plaques characteristic of Alzheimer's disease (AD). Within these plaques, the Aβ is found aggregated into long polymeric assemblies rich in β-sheet structure that are known as amyloid fibrils. Although the correlation between plaque load and disease severity is poor there is strong evidence that small soluble oligomers of Aβ formed during the early stages of the aggregation process are the agents of AD-associated neurotoxicity (1). Single molecule fluorescence techniques have the potential to resolve the size and structural heterogeneity of these oligomers, which are often difficult to discern by ensemble methods. Most importantly, they allow the characterisation of small oligomeric species at the nucleation stage of the aggregation as the structures of amyloid seeds remain ambiguous (2). Equimolar mixtures of $A\beta_{1-42}$ singly labelled with either HiLyteFluor-488 or HiLyteFluor-647 were studied using

single molecule fluorescence confocal microscopy and FRET, allowing the characterisation of oligomers present during aggregation of monomers and disaggregation of fibrils. Additionally, we have extended our single-molecule studies to examine the species formed during the co-aggregation of $A\beta_{1-40}$ and $A\beta_{1-42}$ to understand the interaction at physiological concentrations and ratios. The thorough detection and characterisation of these potentially toxic oligomeric species provides a basis with which to screen therapeutic agents and other modulators of aggregation *in vitro* which could inform *in vivo* studies in the future.

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1844-Plat
The pH-Dependence of Amylin Fibrillization
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In type 2 diabetics, the hormone amylin misfolds into amyloid plaques implicated in the destruction of the pancreatic β-cells that make insulin and amylin. The pH-dependence of fibril formation affects fibrillization kinetics, fibril morphology, and possibly toxicity. Amylin is stored in β-cell secretory granules at pH 5.5 and is released into the extracellular matrix at pH 7.4. There are two ionizable residues in amylin: the α-amino group and His 18. Our approach to measuring the pK_a values for these sites has been to look at the pH dependence of fibrillization in amylin variants that have only one of the groups: NAc-amylin (for His18) and H18R (for the N-terminus). The fibril-specific dye thioflavin-T (ThT) was used to monitor kinetics. The pK_a of His18 is lowered to 5.1 in the fibrils, compared to the random coil value of 6.5. The lowered pK_a is due to the unfavorable burial of a charge, and electrostatic repulsion between positively charged His18 residues on neighboring amylin molecules in the fibril. For H18R, which can only be protonated at the N-terminus, we see a pK_a ~8 due to the N-terminus but a second pK_a of ~4.5 due to protonation of ThT. We confirmed this with the amylin-derived peptide NAc-SNNFGAILSS-NH_2 which has no titratable groups but still shows a pK_a of 4.5 due to ThT. A pK_a of 1.9 for free ThT was determined by NMR but with fluorescence we are monitoring the excited-state of the amyloid-bound dye which has a perturbed pK_a. By using alternative methods to follow fibrillization such as the dye Nile Red or turbidimetry we were able to distinguish between the effects. Large differences in reaction kinetics were observed between the different methods, due to charges on the dyes, which affect fibril formation much like charges on the protein.

1845-Plat
The Deltak280 Mutation in the Tau Protein may Induce the Formation of Stable Abeta-Tau Aggregates in FTDP-17
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One of the main pathological symptoms for Frontotemportal Dementia and Parkinsonism linked to chromosome 17 (FTDP-17) is the accumulation of neurofibrillary tangles in the brain via amyloid aggregation of the Tau protein. It is well known that this process is accelerated in the presence of Aβ. However, the molecular mechanisms underlying the interactions between Aβ and Tau protein are currently not well understood. One of the most well-known mutations in the tau protein that is related to FTDP-17 is the ΔK280 mutant. In our work, we have studied the effect of this mutation on the tau protein's propensity for aggregation in the absence and with the presence of Aβ. We constructed a set of molecular oligomer models based on previous solid-state NMR measurements and molecular dynamics simulations. Our results suggest that with the presence of Aβ, the mutated tau - Aβ complex occupies a rugged conformational landscape. The mutated tau oligomers that are stabilized by Aβ oligomers, preferred to interact as double layer structures instead of single layer structures. However, in the absence of Aβ, the conformational landscape of mutated tau is characterized only by one preferred structure. These new insights cast light on the interactions between amyloids in FTDP-17(as well as in other neurodegenerative diseases) and may offer new strategies for future pharmacological studies that aim to ameliorate the interactions between amyloids in FTDP-17. The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2011) under grant agreement 303741.

1846-Plat
Binding of FDDNP Biomarker to Alzheimer's Disease Aβ Peptide
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Molecular biomarkers such as FDDNP can provide early diagnosis of Alzheimer's disease (AD). These biomarker ligands are designed to interact with aggregation-prone Aβ peptides implicated in AD onset. While numerous experimental observations have demonstrated FDDNP binding to Aβ *in vitro* and *in vivo*, molecular details of binding mechanism are currently unavailable. In our study, we use explicit solvent replica exchange molecular dynamics to examine FDDNP binding to $A\beta_{10-40}$ monomer in all-atom detail. We found that at both high and low concentrations, FDDNP binds with high affinity to Aβ sequence locations near the central hydrophobic cluster and the C-terminal. Analysis of ligand-Aβ interactions at both concentrations identifies hydrophobic effect as a main binding factor. However, with the increase in ligand concentration the interactions between FDDNP molecules also become important due to strong FDDNP self-aggregation propensity and few specific binding locations. As a result, FDDNP ligands partially penetrate the core of Aβ monomer forming large self-aggregated clusters. Ligand self-aggregation does not affect hydrophobic interactions as a main binding factor or the location of binding sites in Aβ. Lastly, using Aβ conformational ensemble in ligand-free water as a reference we show that FDDNP induces minor changes in Aβ secondary structure. However, FDDNP significantly alters the tertiary fold in a concentration dependent manner by redistributing long-range side chain interactions. Because FDDNP does not change Aβ secondary structure, we argue that it is likely to provide unbiased imaging of Aβ aggregates.

1847-Plat
The Local Mechanical Properties of Lipid Bilayers are Altered by Amyloid-Forming Proteins
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A vast number of protein misfolding diseases are characterized by the formation of nanoscale protein aggregates generally termed amyloids. Such diseases include Alzheimer's disease (AD), Huntington's disease (HD), and type 2 diabetes. While the precise mechanism by which amyloidogenic aggregates are toxic remains unclear, various amyloid-forming proteins interact strongly with lipid membranes. We investigated how mechanical properties of model total brain lipid extract bilayers are altered when exposed to different amyloid-forming proteins (Aß, huntingtin, synthetic polyQ peptide, and amylin) utilizing in situ tapping mode atomic force microscopy (AFM) and scanning probe acceleration microscopy (SPAM). The advantage of the SPAM technique is that provides nanoscale spatially resolved maps of tip/sample tapping forces, which are directly correlated to mechanical properties of the surface. As a result, mechanical changes of lipid membranes can be mapped and correlated with changes in surface topography associated with protein aggregation. using this technique, we demonstrate that lipid bilayer structure is disrupted by amyloid-forming proteins. Disrupted regions of the bilayer were associated with decreased compression modulus and reduced adhesion to the AFM probe. Both of these observed mechanical changes are consistent with a decrease in the packing efficiency of the lipids within the bilayer. The interpretation of the mechanical changes in the lipid bilayers as measured by the SPAM technique were validated via numerical simulations of the tip/surface force interaction under a variety of conditions. These changes in bilayer mechanical properties associated with exposure to amyloid forming proteins may represent a common mechanism leading to membrane dysfunction in protein misfolding diseases.

1848-Plat
Real-Time Visualization of a Pore-Forming Toxin Assembling on a Model Membrane
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The assembly of the sphingomyelin (SM)-binding pore-forming toxin (PFT), lysenin, on SM/cholesterol bilayer was examined by high-speed atomic force microscopy (HS-AFM). Previous studies suggest that lysenin oligomerizes after binding to SM and forms a honeycomb structure. The HS-AFM images of SM/ cholesterol bilayer preincubated with lysenin exhibited the honeycomb assembly of the lysenin oligomers. The time-lapse AFM images revealed that the honeycomb formation took place quickly. During honeycomb formation most of the oligomers underwent reorganization either by dissociating into monomers or by rapidly diffusing along the membrane in less than a second. In the period of reorganization, the mobile oligomers arranged into a static, well-ordered lattice. Once this static layer was formed, the lysenin molecules were firmly bound to the SM/cholesterol bilayer and the oligomers neither dissociated nor diffused. Our results revealed the dynamic nature of the oligomers of a lipid binding toxin during honeycomb formation.