of detergent micelle resonances, a problem overcome by the selective excitation approach.

3507-Pos Board B554

An Important Double Bond: Effects of 22:5n-6 vs. 22:6n-3 on Visual Signal Transduction

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In a normal, healthy retinal rod outer segment 40% to 50% of the phospholipid acyl chains consist of docosahexaenoic acid (DHA, 22:6n-3). Diets that are deficient in n-3, or ω-3, fatty acids lead to the replacement of 22:6n-3 with 22:5n6. Dietary n-3 deficiency leads to a spectrum of developmental disorders associated with learning, memory, intelligence, and visual function. We examined rhodopsin, transducin (G_t) and phosphodiesterase (PDE) function and acyl chain packing in large unilamellar proteoliposomes consisting of phosphatidylcholines with *sn-1* = 18:0, and *sn-2* = 22:6n-3, 22:5n-6 or 22:5n-3. Rhodopsin activation and binding to G_t was assayed with steady-state and time-resolved UV/vis spectroscopy, acyl chain packing was assessed via time-resolved fluorescence of diphenylhexatriene (DPH) and PDE activity was determined from the change in pH due to hydrolysis of cyclic GMP. The motion of DPH in the membrane was slower in 22:5n-6 than in 22:6n-3 and overall acyl chain packing was more constrained. The most significant structural difference between the 22:5n-6 containing bilayer and bilayers containing both n-3 polyunsaturates was in the bilayer mid plane where 22:5n-6 produced much higher DPH orientational order. At physiological temperature the formation of both the active metarhodopsin II conformation (MII) and the MII-G_t complex was much slower in 18:0,22:5n-6 PC than in 18:0,22:6n-3 PC and the equilibrium amount of MII formed was 50% higher in 18:0,22:6n-3 PC. In 18:0,22:5n-6 PC PDE activity at a physiologically relevant level of rhodopsin activation is only about 60% of that observed in either 18:0,22:6n-3 PC or 18:0,22:5n-3 PC. Taken together, these results demonstrate that the subtle change in bond configuration from 22:6n-3 to 22:5n-6 produces more structured acyl chain packing in the bilayer midplane, leading to delayed and reduced MII-G_t interaction and PDE function.

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Dynamics of the Internal Water Molecules in Squid Rhodopsin

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G-protein coupled receptors (GPCRs) are major parmaceutical targets because of their key role in a diverse array of physiological functions. The visual rhodopsin is the prototype for the family A of GPCRs. Upon photoisomerization of the covalently-bound retinal chromophore, visual rhodopsins undergo a large-scale conformational change that prepares the receptor for a productive interaction with the G protein. The mechanism by which the local perturbation of the retinal cis-trans isomerization is transmitted throughout the protein is not well understood. The recently reported crystal structure of squid rhodopsin (M. Murakami and T. Kouyama, Nature 453, 363, 2008) displays new features that may provide additional insight into the mechanism of the signal transduction in GPCRs. It has been suggested, based on the location of water molecules in the interhelical region extending from the retinal towards the cytoplasmic side, that a water-mediated hydrogen-bond network may play a role in the activation process. As a first step towards understanding the role of water in rhodopsin function, we have performed a molecular dynamics simulation of squid rhodopsin embedded in a hydrated bilayer of polyunsaturated lipid molecules. Here we report results from the simulation that show that the water molecules present in the crystal structure participate in favorable interactions with side chains in the interhelical region, and form a persistent hydrogen-bond network in connecting Tyr315 to Trp274 via Asp80. We also present preliminary results from a simulation study of the changes in the structure and dynamics of the hydrogen-bond network that accompany the photoisomerization of the retinal chromophore.

3509-Pos Board B556

Single-Molecule Photoactivation and Localization of Signaling Complexes in T cells

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In T cells, membrane receptor and ligand engagement initiates a signaling cascade. Ligand binding is relayed through specific membrane receptors, protein tyrosine kinases, and critical adaptors to regulate downstream activation of transcription factors, cytokine production and cell proliferation. The membrane segregation and relocalization of these signaling components display highly dynamic protein networks in response to distinct stimulations. However, the membrane-protein and protein-protein interactions involved in the formation of signaling complexes are still poorly understood. We are currently using a high-resolution imaging technique to visualize the early stage of signal transduction events at the single-molecule level with photoactivatable or photoconvertable fluorescent markers. The photoactivation and localization of T-cell signaling components allow measurements of molecular spatial and temporal correlation in response to different stimulations. With dual-color application, molecular correlation functions will enhance the understanding of the protein-protein interactions in signaling complexes.

3510-Pos Board B557

Molecular un-stability of peptide-MHC complex and activation of T cell

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CD4+ T cell responses require the recognition of specific peptide-MHC complexes displayed by APC. It is important to determine how Ag presentations affect the ensuing T cell response. Imunization of B10.BR mice with immunodominant peptide 48-61 of Hen egg lysozyme elicit two different types of T cell responses. First type of T cell (type A termed by Unanue et. al) respond to APC pulsed with either peptide or whole HEL protein. Second type of T cell (termed type B) respond to APC incubated with peptide but showed no response to APC with whole protein. Some of the type B T cell clone exhibit unusual response to the variant of 48-61 peptide and responded better to poor MHC binding peptide that to strong MHC binding peptide. In contrast, reactivity of the type A of T cell clones correlate well with the affinity of the peptide to the MHC molecules. Since weak MHC binding peptides form unstable complex, we hypothesize that T cells, like type B T cell, respond well to unstable MHC peptide complex by interacting with one of multiple transitional conformations. To test this hypothesis, we observed the movement of peptide/MHC complex at the single molecular level by using diffracted X-ray tracking (DXT) method. It was found that movement of the low affinity peptide MHC complex was greater than that of high affinity peptide MHC complex. However, the difference between two complexes was mainly due to the overall movement of the molecule rather than the different movement of the peptides in the MHC groove. Thus, peptide bound to the MHC greatly influence the movement of the whole MHC peptide complex and this movement strongly affect the recognition by T cell.

3511-Pos Board B558

The Influence Of Plasma Membrane Order On TCR Signalling

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The signalling events that follow T-cell receptor triggering are mediated by multi-molecular complexes consisting of both membrane-associated and cytosolic proteins. Formation of these complexes is driven by a network of protein-protein and protein-lipid interactions. We study how the selective partitioning of signalling components into separate ordered/raft or disordered phases of the plasma membrane controls the assembly and activity of these signalling complexes.

Previous work from our laboratory shows that T cell plasma membrane domains engaged in TCR signalling specifically undergo condensation, and that treatment of Jurkat T-cells with polyunsaturated fatty acids specifically disrupts condensed membrane rafts at TCR activation sites. We have also shown that disruption of membrane order by 7-ketocholesterol leads to reduced recruitment of key TCR signalling components as measured biochemically and by TIRF microscopy of fixed cells. We aim to understand the influence of this biophysical phenomenon on the formation and composition of TCR signalling protein/lipid complexes.

To this end, we are currently studying the effects of raft disruption by 7-ketocholesterol and polyunsaturated fatty acids on the kinetic behaviour of TCR signalling components. We employ single molecule real time TIRF microscopy of fluorescently labelled TCR signalling proteins as well as biochemical and proteomic analysis of TCR activation domains upon raft disruption.

3512-Pos Board B559

Dissecting T cell receptor nanocluster signaling

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The early steps of T cell activation involve the spatial rearrangement of T cell receptors (TCR) and associated molecules into small nanoclusters at the