measurements, stopped-flow fluorescence and ITC. We are using pyrene labeled DNA, IκBα, or NF-κB to study the fluorescence changes occurring during the enhanced dissociation process. Our results show that IκBα increases the dissociation rate of the DNA from the NF-κB complex in a concentration-dependent manner and with high efficiency. We also repeated the experiments using different DNA chains and different mutants of IκB. We are also studying IκBβ, which appears to stabilize the NF-κB/DNA interaction. Both IκBα and IκBβ are able to form NF-κB/DNA/IκB ternary complexes. The rates of association and dissociation of DNA to form the ternary complexes was also measured and compared to interpret the kinetics of the enhanced dissociation process.

278-Pos Board B78
Revealing the Affinity of Individual and Combined Flig Domains for Filin in the Bacterial Flagellar Motor Switch Complex by CG-MALS
Sophia Kenrick, Armand Vartanian, Frederick Dahlquist.

Complex interactions between flagellar motor proteins modulate the rotational direction of the bacterial flagella. In particular, the middle and C-terminal domains of Flig (FligM and FligG, respectively) bind two different sites on the binding partner FlIM, as part of the flagellar motor switch. We extend previous NMR studies of the interactions between Flig domains with FlIM via composition gradient multi-angle static light scattering (CG-MALS) to confirm specific binding, quantify affinity and identify the stoichiometry of each complex. Though the equilibrium association constants between the individual FligG domains and FligM differ by >25x, both complexes appear to reach equilibrium on time scales faster than that of the CG-MALS measurement (~5 sec). By contrast, binding of the dual-domain protein FligM/FligG to FlIM exhibited unusually slow association kinetics with reaction time constants on the order of 30-60 minutes. CG-MALS revealed complex self-and hetero-association stoichiometries that could not be identified or quantified by means of NMR. Combined with structural studies, these data could provide insight to the mechanism of flagellar motor switching.

279-Pos Board B79
Multi-Functional Receptor Binding Activity of the Moraxella Catarrhalis Adhesin UspA1
Nathan R. Zaccai, Christopher Agnew, Elena Borodina, Rebecca Conners, Nicholas Burton, David K. Cole, Massimo Antognazzi, Mumtaz Vrijii, R. Leo Brady.

Moraxella catarrhalis, the causative agent of middle-ear infections, has a distinctive proliferation of fimbriop-like molecular structures protruding from its outer membrane and formed primarily by the bacterial adhesin protein UspA1. UspA1 forms trimeric, densely packed and extended structures that project up to 800 Å from the bacterial surface and is believed to play a major role in colonisation and infection by this pathogen. Using X-ray crystallography, here we show that the head region of UspA1 comprises a trimeric β-roll domain, mounted on an extended trimeric coiled-coil stalk. The head domain has micromolar affinity for the extracellular matrix (ECM) protein fibronectin, by forming an extended interaction surface, distinctly different from its binding to the CEACAM1 receptor which is a function of a specific site 400 Å distant on the stalk. Atomic force microscopy studies furthermore showed that binding of either ligand leads to changes in the mechanical elasticity of the bacterial outer surface. UspA1 therefore appears to provide a multi-functional and mechano-sensitive scaffold for Moraxella interactions with both ECM and cell surface receptors.

280-Pos Board B80
Characterization of Self-Assembled Peptide Porphyrin Complexes as Light Harvesting Antennas
Matthew Warner, Laura Hagens, Michael Reca, Darius Kuciauskas, Gregory A. Caputo.

Following a biomimetic approach, synthetic polypeptides designed to induce porphyrin self-assembly into nanostructures were created. Because of their stability and favorable spectroscopic properties, peptide-porphyrin aggregates could be applied as light harvesting antennas in dye-sensitized nanocrystalline solar cells. Using a designed porphyrin binding motif (PBM) consisting of 3 cationic lysine residues, we have developed and characterized a short, amphiphilic peptide that can reversibly bind meso-tetra-(4-sulfonatophenyl) porphine (TPPS) molecules and facilitate three-dimensional orientation into an excitonically coupled J-aggregate. The ability of the peptide to act as a scaffold for TPPS interactions was shown to be dependent on the sample pH and the resulting impacts on peptide secondary structure conformation using a combination of fluorescence, absorbance and circular dichroism spectroscopy. Under all conditions tested the peptide was shown to cooperatively bind three TPPS molecules per peptide with little change in Cd from pH 1.8 through pH 7.6. At low pH (3.6 and below) the peptide adopted a random coil conformation and allowed for the TPPS molecules to form the J-aggregate structure. Upon neutralization of the sample pH, the peptide adopts a predominantly alpha-helical structure which is concomitant with the loss of J-aggregate formation within the TPPS molecules. Using a series of peptides that contained Trp at different positions with respect to the PBMs we have initial measures of the sequence specificity of the cooperative binding event in terms of which PBMs sequence is more likely to be the FIRST in the peptide to bind a TPPS molecule.

281-Pos Board B81
The Effects of LFA-1 Antagonism on Protein Sorting and T Cell Triggering

The large-scale spatial patterning of receptor-ligand pairs at intermembrane spaces are increasingly studied due to their contribution to cell signaling and downstream events. One example, the immunological synapse (IS), is a key event in the immune response. Crucial to the overall pattern at the T cell/antigen presenting cell (APC) interface are submicron clusters that become spatially organized to initiate T cell triggering. The T cell receptor (TCR) and its ligand, activating peptide-bound major histocompatibility complex (pMHC), form a large central cluster surrounded by a peripheral ring of lymphocyte function-associated antigen-1 (LFA-1) bound to its ligand intercellular adhesion molecule-1 (ICAM-1). These receptor-ligand interactions catalyze submicron clustering and subsequent centripetal transport by the actin cytoskeleton. In this work, we replace the APC with a biomimetic, fluid supported membrane with easily controlled protein composition. Using fluorescence microscopy visualization techniques, we have developed a reliable and robust assay to test the effects of LFA-1 inhibition. We hypothesize that LFA-1 antagonism through the use of a small-molecule inhibitor or an anti-LFA-1 antibody will alter protein sorting and T cell triggering. Unexpectedly, the small-molecule antagonist affects TCR spatial patterning in addition to the predicted disruption of LFA-1 ring formation. We further investigate this by studying the effects on actin morphology, calcium signaling, and IL-2 secretion. Our results provide a fundamental understanding of LFA-1 antagonism on the IS, TCR signaling, and actin behavior. In addition, our research can impact the development and analysis of drugs for immune-mediated disorders, which frequently target LFA-1 and inhibit ligand binding.

282-Pos Board B82
Structural Studies of Calmodulin Activation of Estrogen Receptor Alpha
Jeffrey Urbauer, Carrie Jolly, Brian Jones, Erik Henderson, Ramona Bieber Urbauer.

The goal is to determine the mechanism of calcium-dependent activation of estrogen receptor alpha (ERα) by calmodulin (CaM) and to ascertain how oxidative stress and oxidative modifications mediate the interactions between CaM, ERα and antiestrogens. Systemic endocrine/antiestrogen therapy is among the most common treatments for estrogen-dependent breast cancers. ERα is the primary target for antiestrogen therapies, and antiestrogen drugs such as tamoxifen and its metabolites (4-hydroxytamoxifen, endoxifen) bind tightly to ERα and inhibit its ability to activate transcription. Recently, it was demonstrated that CaM is an obligate ERα activator. Interestingly, antiestrogens that bind tightly to ERα also bind tightly to CaM. It has been suggested that therapeutic benefit of antiestrogens for estrogen-dependent breast cancers may derive partially from CaM antagonism. Towards our goal of understanding how CaM activates ERα, we have localized the CaM binding region of ERα and initiated structural studies to determine the structure of the complex of CaM with the ERα CaM binding region. Using NMR and SAXS we find that CaM bound to ERα is somehow assembled structurally compared to high affinity CaM complexes. Circular dichroism and fluorescence studies indicate high affinity between CaM and the CaM binding domain of ERα and that upon binding to CaM the CaM binding region of ERα adopts only partial helical structure which is concomitant with the loss of J-aggregate formation and allowed for the TPPS molecules to form the J-aggregate structure. Upon neutralization of the sample pH, the peptide adopts a predominantly alpha-helical structure which is concomitant with the loss of J-aggregate formation within the TPPS molecules. Using a series of peptides that contained Trp at different positions with respect to the PBMs we have initial measures of the sequence specificity of the cooperative binding event in terms of which PBMs sequence is more likely to be the FIRST in the peptide to bind a TPPS molecule.