the NBD model MJ0796 (M. jannaschii NBD), we found complete dissociation of ATP-induced NBD dimers following ATP hydrolysis. However, it is still unknown if dissociation requires ATP hydrolysis by one or both sites in the NBD dimer. Here, we used LRET to study heterodimers formed by a normal (acceptor-labeled) and a catalytically defective (MJ0796-E171Q; donor-labeled) NBD. Rapid mixing experiments in a stop-flow chamber showed that NBD heterodimers with one functional and one inactive site dissociate at a rate undistinguishable from that of dimers with two hydrolysis-competent sites (+0.1 s⁻¹). These results indicate that one ATP hydrolysis event is sufficient to elicit dimer dissociation. Comparison with the rates of hydrolysis of normal dimers and heterodimers strongly suggests that in the dimers with two hydrolysis-competent sites, the two ATPs are hydrolyzed “simultaneously”, before NBD dissociation takes place. Additional LRET experiments forming heterodimers with mutants of ATP-interacting residues suggest an important role of the electrostatic balance for the dimer stability. Thus, charge changes after ATP hydrolysis might destabilize the dimer interface and promote NBD dissociation. This work is supported by CPRIT grant RP101073.

1140-Pos Board B32
FRET Quenching by a Hybrid Voltage Sensor (Hvs) Reveals that the Na/Glucose Cotransporter (SGLT1) Is a Disulfide-Bridged Homodimer with Re-Entrant 12-13 Loop
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Determining the structural characteristics of membrane transporters is essential to understanding their mechanisms. The sodium-glucose cotransporter 1 (SGLT1) has 14 transmembrane segments (TMs) and is a member of the LeuT structural family as demonstrated by the crystal structure of Vibrio SGLT. Despite numerous studies on SGLT1, its quaternary structure and the possibility of a re-entrant loop between TMs 12 and 13 (following LeuT numbering) are still uncertain. In this study, dipycrylamine (DPA), a lipophilic anion which distributes across the membrane according to the membrane potential, was used as a resonance-energy-transfer acceptor from donor molecules attached to SGLT1 via cysteine labelling. By randomly labelling SGLT1 C511 with Alexa 488-C5-maleimide and tetramethylrhodamine-C5-maleimide (TMR), we observed a clear DPA-sensitive FRET signal indicating molecular contact between SGLT1 monomers. Western blotting of myc-tagged SGLT1 in absence of the reducing agent β-mercaptoethanol revealed a 150 kDa band which disappeared in the presence of β-mercaptoethanol, leaving only a 75 kDa band as would be expected for monomeric SGLT1. Using a series of 18 mutants where individual cysteines were replaced by alanine, C355 was found to be responsible for the disulfide bridge that stabilizes SGLT1 dimers. We also investigated the loop between TMs 12 and 13 by measuring energy transfer between TMR-tagged SGLT1 E624C and DPA. We unambiguously found that this loop, which is poorly conserved in Vibrio SGLT and is assumed to be intracellular, lies significantly above the extracellular plane of the membrane. Taken together, these results establish structural characteristics of SGLT1 which could not be settled using the bacterial homolog structure.

1141-Pos Board B33
Gpa Dimerization in Plasma Membranes of CHO, HEK293T and A431 Cells
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Glycoporin A is the major sialoglycoprotein of the human erythrocyte membrane. Gpa dimerization has been extensively studied in detergents and in model lipid bilayers, but measurements in the plasma membrane of mammalian cells are rare. Using a FRET based technique, we have measured the free energy of Gpa dimerization in plasma membrane vesicles derived from Chinese Hamster Ovary (CHO), Human Embryonic Kidney (HEK293T) and A431 (epidermoid carcinoma) cells, using three different vesiculation methods. These measurements provide new insights into the effect of the environment on membrane protein interactions.

1142-Pos Board B34
Monitoring the CEACAM1 Monomer and Dimer Distribution upon Cell-Substrate Contact
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The carcinoembryonic antigen-related cellular adhesion molecule 1 (CEACAM1) is an intercellular adhesion molecule that plays a key role in processes related to cell growth, differentiation, and infection in normal and cancerous cells. Expressed on the cell surface, its extracellular domain participates in interactions with CEACAMs on neighbouring cells, as well as interacting with Opal proteins expressed on Neisseria gonorrhoeae and Neisseria meningitides in order to facilitate bacterial entry. While CEACAM1 is known to exist in both monomeric and dimeric states that are heterogeneously distributed at the cell surface, which form participares in the various inter-cellular and bacterial interactions remains a mystery. Resolving this uncertainty is fundamental to understanding the role of the receptor, as the monomer-dimer status of CEACAM1 has been shown to differentially affect its ability to bind certain downstream signalling molecules. We are using live cell fluorescence spectroscopy to investigate the distribution, dynamics, and monomer-dimer equilibrium of EYFP-labeled CEACAM1. Cells expressing the labeled receptor are monitored as they come into contact with CEACAM1-engaging surfaces, such as other cells or glass dishes that have been patterned with Opal + Neisseria gonorrhoeae. These approaches will allow us to directly examine the initial stages of CEACAM1 association with CEACAM1-interacting proteins and will answer critical questions regarding the nature of these interactions. Such insights are essential for understanding the nature of CEACAM1 signalling, function, and regulation, and ultimately targeting its functions for purposes such as cancer therapy or the treatment of infection.

Protein Dynamics II

1144-Pos Board B36
Protein Dynamical Transition at Cryogenic Temperatures
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Hydrated proteins are known to undergo a dynamical transition at around 200 K. Several mechanisms have been proposed but the underlying mechanism, physical origin and relation to water remains controversial. We studied protein dynamics inside protein crystals using a high pressure cryocooling technique [1] and X-ray diffraction at cryogenic temperatures (90 to 160 K). Water crystallization was bypassed by inducing an unusual form of water: high-density amorphous to low-density amorphous state [3]. The results provide new insights into the underlying mechanism of protein dynamical transition and its relationship with the unusual physical properties of supercooled water.