The combination of increased urinary oxalate and attenuated urinary citrate concentration are a major risk factor for Ca\(^{2+}\)–oxalate kidney stone formation, even when urinary Ca\(^{2+}\) levels are not elevated. Nevertheless, the molecular mechanisms that regulate urinary oxalate and citrate levels are not well understood. We studied the role of the oxalate transporter, slc26a6, and the citrate transporter, NaDC-1, in regulating oxalate/citrate homeostasis. We found that slc26a6-null mice exhibited increased renal and intestinal sodium-dependent succinate uptake, which is attributed to NaDC-1 function. Furthermore, we monitored urinary hyperoxaluria and hypocitraturia, but no change in urinary pH, indicating enhanced transport activity of NaDC-1 in slc26a6-null mice. When co-expressed in xenopus oocytes, slc26a6 dramatically inhibited NaDC-1 transport activity in an activity dependent manner. Finally, biochemical and physiological analysis revealed that the STAS domain of Slc26a6 and the first intracellular loop of NaDC-1 mediated both the physical and functional interactions of these transporters. Our findings reveal a molecular pathway that tightly regulates oxalate and citrate concentration and may control biological Ca\(^{2+}\)–oxalate stone formation in the kidney and other organs like, for example, salivary glands.

### 2319-Pos Board B456
Assessing Protomer Independence of the Dimeric C4-Dicarboxylate Transporter, VcINDY
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The divalent anion:sodium symporter (DASS) family includes characterized representatives from bacteria and humans (SLC13 family). DASS family members have been implicated in key physiological roles, including fatty acid synthesis and the transport of Krebs cycle intermediates and sulfate across the cytoplasmic membrane. Disruption of genes encoding DASS family members in mice and flies reveal roles in energy homeostasis, affecting lifespan determination, insulin resistance and obesity in these organisms. VcINDY, from the bacterium Vibrio cholerae is the only DASS family member with which there is high resolution structural information available and functional characterization reveals VcINDY to share key functional characteristics with mammalian DASS family members. VcINDY is a functional dimer exhibiting a large buried surface area at the dimer interface; a feature likely shared by all DASS family members. Characterization of other bacterial members of the family suggests cooperativity between the protomers. This apparent interprotomer communication is likely transmitted via substrate-induced conformational changes at the dimer interface. If true, this has important mechanistic implications for the entire family.

To investigate whether transport by VcINDY is a cooperative process we have introduced cysteine residues at the dimer interface that, under oxidizing conditions or in the presence of crosslinking agents, will staple regions of the interfacial proteomicelles. This study suggests that these residues may help stabilize substrates within the transporter and imply a more complex interaction between substrates than simple competition for a single site.

2321-Pos Board B458
The Role of Annnular Lipids in the Mechanism of Membrane Protein Solubilization and Functionality
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The “solubilization” procedure in which membrane proteins are extracted from their native lipid membranes and transferred into detergent micelles (proteomicelles) is a critical step in the purification of the protein for subsequent structure/function studies. In some notable examples it has become clear, however, that the properties of the proteins in proteomicelles depend on the detergent type and its concentration relative to the residual membrane lipids originating from the preparation. To reveal the molecular mechanism of the solubilization process and the effect of various lipid-to-detergent (L:D) ratios on the properties of the system, we performed extensive atomistic molecular dynamics simulations. We previously explored the properties of micelles formed by dodecyl-β-maltoside (DDM) detergent around the leucine transporter (LeuT), a bacterial homolog of neurotransmitter:sodium symporter family, and showed how the DDM concentration determines the extent of detergent penetration into the functionally relevant secondary substrate (S2) binding site in LeuT. Here we present computational studies of LeuT embedded in proteomicelles at different L:D ratios exploring configurations of LeuT proteomicelles at different stages of the protein solubilization process. Our results reveal that at a sparse lipid annulus around the transporter, consisting of no more than ~10 lipids, DDM partitions into the LeuT, engaging with the residues in the S2 site. Higher lipid concentrations protect the S2 site from DDM penetration. In contrast to DDM, MNG-3 (lauryl maltosyl glycerol) detergent, an amphiphile known to better stabilize membrane proteins, does not penetrate the transporter even in the absence of lipids. Parallel experimental measurements of ligand binding to LeuT in the presence of increasing MNG-3 concentrations revealed that the functionality of the S2 site was fully preserved even at the highest (0.5%) MNG-3 concentration probed.

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The Environment Modulates the Conformation of Transmembrane Helix 1A in the Leucine Transporter (LEUT)
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Neurotransmitter transporter are found on the presynaptic neurons and on glial cells. The function of these transporters is termination of neurotransmission by the rapid removal of neurotransmitter molecules from the synaptic cleft. These transporters couple substrate transport to ion gradients of sodium and chloride. Structural studies often require the transporter to be removed from its physiological membrane, which can affect its structure or conformation. Crystal structures of the bacterial homolog LeuT (SLC6 family) were solved in three states of the transport cycle: occluded, outward and inward. The recent inward facing structure shows a conformation where the first helix (TM1A) did not seem to be compatible with a membrane environment. We carried out molecular dynamics simulations of LeuT in membrane and micelle environment to investigate the conformational behaviour of TM1A and combined the investigation with distance measurements using LRET. We used POPC as membrane lipids, and build the micelle systems with three different protein-detergent ratios (1:120, 1:140, or 1:160) using the detergent n-Octyl-β-D-Glucopyranoside (β-OG) molecules. We observed a rigid body motion of the TM1A helix, but the essential dynamic of the transmembrane helix was retained. In contrary, TM1A was stable in its position in the micelle simulations. We confirmed this observation by distance measurements of solubilized LeuT in micelles and reconstituted POPC liposomes. This study suggests that the polar part...