The crystal structure of B. pertussis BrkA is an important B. pertussis virulence factor. Whooping cough (pertussis) is a highly contagious, acute respiratory illness. Yujia Zhai of Autotransporter Hydrophobic Patch is Indispensable for Passenger Domain Translocation transport between these sites. Metal ions. These structures suggest a stepwise shuttle mechanism for from the cytosol, utilizing these methionine pairs/clusters to bind and export assays suggest that CusA is capable of actively picking up metal ions. Binding of Cu(I) and Ag(I) triggers significant conformational CusA-Ag(I) structures, which directly suggests a plausible pathway for ion cated within the cleft region of the periplasmic domain. Intriguingly, this that the metal binding sites, formed by a three-methionine cluster, are lo- cated within the cleft region of the periplasmic domain. Intriguingly, this cleft is closed in the apo-CusA form but open in the CusA-Cu(I) and CusA-Ag(I) structures, which directly suggests a plausible pathway for ion export. Binding of Cu(I) and Ag(I) triggers significant conformational changes in both the periplasmic and transmembrane domains. The crystal structure indicates that CusA has, in addition to the three-methionine metal binding site, four methionine pairs - three located in the transmembrane region and one in the periplasmic domain. Genetic analysis and transport assays suggest that CusA is capable of actively picking up metal ions from the cytosol, utilizing these methionine pairs/clusters to bind and export metal ions. These structures suggest a stepwise shuttle mechanism for transport between these sites.

Novel Amphiphilic Molecules Mediate Membrane Protein Crystal Contacts Qingzhai Zhang There have been quite a few approaches developed over the last two decades towards the creation of structurally novel amphiphiles. Some of these reagents have found broad applications in membrane protein biochemistry, being used for the solubilization and stabilization of membrane proteins for functional studies. However, success has been very limited in using these novel amphiphiles to crystallize IMPs. The lack of success exemplifies the significant degree of challenge and makes it clear that other types of amphiphiles are needed. We have contributed hundreds of new detergents through our previous efforts in this area. These detergents have been tested for various purposes (e.g. solubilization, stabilization, NMR, crystallization), and several were found to perform as well or better than the most popular commercial detergents. In particular, the use of our newly designed cholesterol-like facial amphiphiles has facilitated the 3D crystallization and structural determination of several membrane proteins. Interestingly, we have found evidence that facial amphiphiles mediated protein crystal contact. These results open up the possibility for future investigations of using intelligently designed stabilization reagents to mediate membrane protein surface interactions so as to increase crystallization propensity and improve crystal diffraction.

On the Interaction of Large Amounts of C12E8 on Na,K-ATPase Alpha Subunits: A Small Angle X-Ray Scattering Study Leandro R.S. Barbosa, Carolina Rigos, Juliana Y. Sakamoto, Rosangela Itri, Pietro Ciancaglini. In the current work, we studied the effect of the non-ionic detergent dodecyl- taethyleneglycol, C12E8, on the structure and oligomeric form of Na,K-ATPase membrane enzyme (sodium-potassium pump) in aqueous suspension, by means of small angle X-ray scattering (SAXS). Solubilized samples composed of 2 mg/ ml of Na,K-ATPase, extracted from rabbit kidney medulla, in the presence of small C12E8 amount (0.005 mg/ml) and in larger concentrations ranging from 2.7 mg/ml to 27 mg/ml did not present catalytic activity. Under this condition, an oligomerization of the alpha sub-units is expected. SAXS data were analyzed by means of a global fitting procedure supposing that the scattering is due to two independent contributions: one coming from the enzyme and the other one from C12E8 micelles. In the small detergent content (0.005 mg/ml), the SAXS results evidenced that Na,K-ATPase is associated into aggregates larger than alpha2 form. When 2.7 mg/ml of C12E8 is added, the data analysis revealed the presence of alpha4 aggregates in the solution and some free micelles. Increasing detergent amount up to 27 mg/ml does not disturb the alpha4 aggregate, just more micelles of same size and shape are proportionally formed in solution. We believe that our results shed light to better understanding on how non-ionic detergents induce sub-units dissociation and reassembling to minimize the exposure of hydrophobic residues to the aqueous solvent. Acknowledgments: the authors thank FAPESP CAPES and CNPq for financial support.