proteins, obtained from SMD simulations, hints towards the unknown mechanism of anion transport through these proteins.
[1] Liu et al., PNAS USA 102, 2192-2197 (2005)

1875-Pos Board B12
An Intra-Molecular Disulfide Cross-Link Stabilizes an Inward-Oriented Transport Intermediate Conformation of the Tonb-Dependent Transporters
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The Gram negative bacteria TonB-dependent transporter (TBDT) BtuB translocates vitamin B12 (cobalamin or Cbl) across the outer membrane. The amino-terminal lumenal domain fits within the 22-stranded ß-barrel and contains several determinants for Cbl binding. Current models suggest that the TBDT carries out active transport driven by energy from the proton motive force (pmf), which is transmitted via direct interactions between the inner membrane protein TonB and the BtuB amino-terminal Ton Box motif. The lumenal domain must undergo large conformational changes to accommodate passage of the substrate through the barrel, but the molecular features of the mechanism are unknown. We show that disulfide bonds can be induced to form in whole cells between three pairs of cysteines introduced at barrel-lumenal domain contact points, consistent with the x-ray crystallographic structures. However, we also find spontaneously formed, stoichiometric cross-links between cysteines in the Ton Box motif and in place of Ser120 that indicate a conformation different from the x-ray structures. The TBDT family members FecA and FhuA with cysteines in equivalent positions also form stoichiometric disulfide bonds. Cbl uptake through BtuB is blocked by the Ton Box Cys-S120C cross-link and activity is recovered upon reduction. Significantly, Cbl binds to the cross-linked BtuB in isolated outer membrane fragments but not to whole cells, indicating that the Cbl binding site of the cross-linked form is oriented towards the periplasmic side. In contrast, the transporter is oriented outwards in all other conditions, included in the absence of a pmf. These results suggest an alternating access transport mechanism.

1876-Pos Board B13
Live-Cell Measurements of the Conformational Rearrangements in Bax at the Initiation of Apoptosis
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The Bcl-2 family of proteins regulates the activation of apoptosis through the mitochondria pathway. Pro- and anti-apoptotic members of this family keep each other in check until the correct time to commit to apoptosis. The point of no return for this commitment is the permeabilization of the outer-mitochondrial membrane (OMM). Translocation of the pro apoptotic member, Bax, from the cytosol to the mitochondria is the molecular signature of this event. We employed a novel method to reliably detect Förster Resonance Energy Transfer (FRET) between pairs of fluorophores to identify intra-molecular conformational changes and inter-molecular contacts in Bax as this translocation occurs in live cells. In the cytosol, our FRET measurements indicated that the C-terminal helix is exposed instead of tucked away in the core of the protein. This coincided with measurements using fluorescence correlation spectroscopy (FCS) that showed that cytosolic Bax diffuses much slower than expected, suggesting possible complex formation or transient membrane interaction. We propose that this exposed helix allows for this contact to occur. Cross-linking the C-terminal helix (α9) to helix α4 reduced the instances of these interactions while at the same time yielded FRET measurements that are consistent with the α9 helix tucked into the core of the protein. After translocation, our FRET measurements showed that Bax molecules form homo-oligomers in the mitochondria through two distinct interfaces involving the BH3 domain (helix α2) and the C-terminal helix. These findings have implications for possible contacts with other Bcl-2 proteins to create pores to permeabilize the OMM, which would also be necessary for the regulation of apoptosis.

1877-Pos Board B14
FIS1 and DNM1L Cooperate in Mitochondrial Fission: Convergence of Evolution and Intelligent Design
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Mitochondrial fission helps to maintain proper mitochondrial homeostasis in a poorly understood manner despite its association with human disease. Several proteins have been identified in this process, but only two – FIS1 and DNM1L – are found in every species that contain mitochondria. We asked whether these proteins might cooperate together in this process. We found that FIS1 has the ability to directly recruit DNM1L, but is auto-inhibited by an N-terminal arm. A point mutant was rationally designed to relieve auto-inhibition and found to increase DNM1L binding and impair mitochondrial fission. This point mutant also increased the population of a latent dimeric state of FIS1 highlighting the complex nature of protein-protein interactions in fission. To address this, we devised an unbiased and general method to rapidly identify residues critical to protein interfaces and applied this technology to yeast Fis1 interactions. Of the >3000 Fis1 alleles screened, ~9% selectively disrupted interactions with one of the three protein partners including DNM1L. To test the functional consequences, each allele was parsed into its corresponding point mutation and tested for mitochondrial fission. Of 211 yeast Fis1 mutants tested to date, 97 resulted in nonfunctional fission indicating that our method identifies residues essential for mitochondrial fission. Orthologous mutations were introduced into human Fis1 and also found to impair interactions with DNM1L and mitochondrial morphology. Analysis of these data supports a new model for the assembly of the mitochondrial fission machinery.

1878-Pos Board B15
Structural Basis for Enhanced Hiv-1 Neutralization by a Dimeric Immunoglobulin G Form of the Glycan-Recognizing Antibody 2G12
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The human immunoglobulin G (IgG) 2G12 recognizes high-mannose carbohydrates on the HIV type 1 (HIV-1) envelope glycoprotein gp120. Its two antigen-binding fragments (Fabs) are intramolecularly domain exchanged, resulting in a rigid (Fab)2 unit including a third antigen-binding interface not found in antibodies with flexible Fab arms. We determined crystal structures of dimeric 2G12 IgG created by intermolecular domain exchange, which exhibits increased breadth and >50-fold increased neutralization potency compared with monomeric 2G12. The four Fab and two fragment crystalline (Fc) regions of dimeric 2G12 were localized at low resolution in two independent structures, revealing IgG dimers with two (Fab)2 arms analogous to the Fabs of conventional monomeric IgGs. Structures revealed three conformationally distinct dimers, demonstrating flexibility of the (Fab)2-Fc connections that was confirmed by electron microscopy, small-angle X-ray scattering, and SPR binding studies. We conclude that intermolecular domain exchange, flexibility, and bivalent binding to allow avidity effects are responsible for the increased potency and breadth of dimeric 2G12. In addition, we present this as the first known crystal structure of an IgG dimer.

1879-Pos Board B16
A Computational and Experimental Study of the Structure of FOXl1 Protein
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Mutated or unregulated FOX proteins have been linked with numerous human genetic diseases. Premature ovarian failure, mental retardation, and severe immune defects are just a few of the severe health problems linked with mutations in the FOXO3a, FOXP1, and FOXN1 proteins, respectively. Studying the structure of FOX proteins is crucial in uncovering the essential structural features that allow these proteins to function properly. However, the structure of the C-terminal domain is unknown for many FOX proteins including FOXL1. In this research, the structure of the C-terminal domain of FOXL1 protein is investigated using both computational and experimental methods. Computationally, first-principle molecular dynamic (MD) folding simulations were performed using replica exchange MD to provide a prediction of the native structure. Experimentally, the C-terminal domain of FOXL1 was expressed, purified, and then structurally characterized using circular dichroism.

1880-Pos Board B17
All-Alpha to All-Beta Structural Conversion in the Transcription Factor RfaH
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We used combination of replica exchange molecular dynamics simulations with implicit solvent and detailed all-atom simulations with explicit solvent to investigate the α-helix to β-structure transformation of RfaH-CTD. While interacting with the N-terminal domain (NTD), the C-terminal domain (CTD) of RfaH folds to an α-helix bundle but it undergoes an all-α to all-β