stress conditions [1]. The transporter contributes to virulence of pathogens such as *Staphylococcus aureus* and *Helicobacter pylori*. We utilize PutP of *Escherichia coli* as a model to explore structure and molecular mechanism of function of SSSF proteins.

Here, we present a model of the helix bundle of PutP obtained by molecular modeling constrained by experimentally determined intramolecular distances and template restraints derived from the ten-helix core of the vSGLT crystal structure [2]. For this purpose, DEER distance measurements between spin labels attached to helix ends were conducted and mean interspin distances were determined. Fitting algorithm based on matrix geometry in combination with prediction of spin label conformations by a rotamer library approach [3] resulted in an ensemble of helix bundle structures. The central structure of the ensemble showed a core structure with a fold similar to that of the vSGLT template. Furthermore, analysis of spin label motility and environmental polarity by cwEPR yielded information on secondary structure elements and structural rearrangements of external loop (eL) 9 of PutP upon sodium and/or l-proline binding. The results support the idea that eL9 controls access to the sodium and/or l-proline binding site(s) similar as previously proposed for eL 4 of LeuT [4].

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doi:10.1016/j.bbabio.2012.06.103

4P6

Mitochondrial carrier structure and diseases

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To date eleven disorders are known to be caused by defects of mitochondrial carriers, a family of proteins that shuttle a variety of metabolites across the inner mitochondrial membrane. Mutations of mitochondrial carrier genes are responsible for carnitine/acylcarnitine carrier deficiency, ornithine carrier deficiency (HHH syndrome), aspartate/glutamate isoform 1 deficiency (global cerebral hypomyelination), aspartate/glutamate isoform 2 deficiency (CTLN2 and NICCD), Amish microcephaly, early epileptic encephalopathy, congenital sideroblastic anemia, PiC deficiency, ADP/ATP carrier isoform 1 deficiency, neuropathy with bilateral striatal necrosis and adPEO (autosomal dominant progressive external ophthalmoplegia). Structural, functional and bioinformatics studies have revealed the existence in mitochondrial carriers of a substrate-binding site in the internal carrier cavity, of two gates that close the cavity alternatively on the matrix or cytosolic side of the membrane, and of two sets of prolines and glycines in the six transmembrane a-helices located strategically between the substrate-binding site and the two gates. The key role played by these mitochondrial carrier areas is supported by the observation that they host most of the disease-causing missense mutations of the mitochondrial carriers.

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doi:10.1016/j.bbabio.2012.06.104

4P7

Insights into the mechanism of the Na^+/Ca^{2+} exchanger from atomistic molecular dynamics simulations

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Na^+/Ca^{2+} exchangers (NCX) and potassium-dependent Na^+/Ca^{2+} exchangers (NCKX) are two related families of transporters involved in Ca^{2+} signaling that function by extruding cytosolic Ca^{2+} (and K^+ for the potassium-dependent transporter) in exchange for extracellular Na^+ [1]. Previous studies have established that this exchange process is electrogenic and with a defined stoichiometry, and have identified specific acidic aminoacids believed to be crucial for ion binding and translocation [1–3]. Recently the crystal structure of the NCX from *Methanococcus jannaschii* was determined at 1.9 Å resolution [4], revealing an intriguing transmembrane topology consisting of inverted structural repeats, and the presence of four putative ion binding sites formed by highly conserved residues. Notwithstanding these groundbreaking insights, based on the structure alone several ion occupancy states can be hypothesized that would be compatible with the experimental exchange stoichiometry. Moreover, in the crystal the protein adopts a unique outward facing conformation, which does not immediately explain how ion binding to the protein facilitates the necessary outward-to-inward conformational transition. Here, we use extensive molecular simulations and molecular modeling to investigate the occupancy and specificity of the ion binding sites in NCX_Mj, and the microscopic mechanism by which Na^+ and Ca^{2+} are exchanged across the membrane.

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doi:10.1016/j.bbabio.2012.06.105

4P8

The evolutionary history of membrane-integral pyrophosphatases supports Na^+ as the ancestral coupling ion in membrane bioenergetics

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Membrane-integral pyrophosphatases (mPPases) are primary H^+- or Na^+-ion pumps directly energized by pyrophosphate, an abundant byproduct of anabolic reactions. mPPases are widespread in all domains of life and provide the host necessary energy reserves, particularly during stress and low-energy conditions. The enzyme holds promise in biotechnology insofar as agricultural plants that overexpress mPPase have salt- and drought-tolerant phenotypes.

Recent work has uncovered significant functional divergence among members of the mPPase protein family. Notably, mPPases differ in pumping specificity and sensitivity to K^+ ions [1,2]. All K^+-independent mPPases operate as H^+-pumps, whereas most K^+-dependent mPPases are primary Na^+-pumps. However, several types of K^+-dependent, H^+-pumping mPPases are known. One particular mechanism that allows a change in transport specificity from Na^+ to H^+ is spatial repositioning of a glutamate residue that forms part of the cytoplasmic gate in the ion transport channel [3].

The reconstructed evolutionary history of mPPases suggests that the ancestral enzyme operated as a Na^+-pump and the transition to H^+-pumping occurred in several independent enzyme lineages [3]. These data lend support to the hypothesis of primordial Na^+-based membrane bioenergetics [4]. Na^+- and H^+-pumping mPPases are structurally very similar [5], supporting the concept, first proposed for a rotating ATP-synthase/ATPase, that switching between Na^+ and H^+ transport specificities requires only subtle changes in structure [4].

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doi:10.1016/j.bbabio.2012.06.106

4P9

Characterization and purification of the multi subunit type Na^+/H^+ antiporter from alkaliphilic *Bacillus pseudofirmus* OF4

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Mrp antiporters are monovalent cation/proton antiporters which exchange cytoplasmic Na^+, Li^+ and/or K^+ ions for extracellular H^+. They are widespread among bacteria and archaea. Mrp antiporters have seven or six hydrophobic proteins that are encoded in the *mrp* operons, in contrast to most of bacterial Na^+/H^+ antiporters which are single gene products. Interestingly, the entire Na^+/H^+ antiport activity requires all of these proteins, suggesting that Mrp antiporters function as a hetero-oligomeric protein complex in the cytoplasmic membrane. Purification and functional reconstitution of the Mrp antiporter have not been reported. Therefore, we purified and reconstituted the Mrp antiporter from alkaliphilic *Bacillus pseudofirmus* OF4, because purification of target proteins and their complex with the native conformation is required for further functional and structural research. The Mrp antiporter expressed in major Cation/H^+ antiporter-defective *Escherichia coli* strain KNabc cells was purified by immobilized metal ion adsorption chromatography (IMAC). The purified Mrp samples were reconstituted into artificial membrane vesicles (liposomes) with F_oF_1-ATPase from *Bacillus* sp. PS3 as the "power supply" to generate a proton motive force required for activation of the Mrp antiporter. The Na^+/H^+ antiport activity of the purified Mrp antiporter was measured in the constructed proteoliposomes (protein-inserted liposomes). Using TALON resin, all of the Mrp subunits could be purified from the *E. coli* membrane fraction expressing the Mrp antiporter and seemed to be present as predominantly a MrpABCDEFG complex dimer. Apparent Na^+/H^+ antiport activity was observed in the proteoliposomes into which purified Mrp and F_oF_1-ATPase were reconstituted. After elution of Mrp proteins, they remained as Mrp complexes and most of which were present as the MrpABCDEFG complex dimer. This suggested that MrpABCDEFG complexes detectable by BN-PAGE are the active forms. It was also speculated that the Mrp complex dimer is more stable than the complex monomer. This is the first report of the purification and functional reconstitution of a Mrp antiporter.

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doi:10.1016/j.bbabio.2012.06.107

4P10

Crystal structure of the heterotrimeric EGC_{head} complex from yeast vacuolar ATPase

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The eukaryotic vacuolar ATPase (V-ATPase) is a rotary molecular motor and dedicated proton pump found on the endomembrane system of all eukaryotic cells and the plasma membrane of specialized cells in higher organisms [1]. The enzyme is composed of a soluble catalytic subcomplex (V1) and a membrane integral complex (Vo) involved in proton translocation. Linking the soluble and membrane sectors are the stator subunits (E, G, C, H and aNT) which absorb the torque generated during rotary catalysis. The unique mode of V-ATPase regulation, known as reversible dissociation, involves the release of V1-ATPase from the membrane integral Vo, and the activity of both domains is silenced [2]. Regulated release of V1-ATPase requires breaking of protein interactions mediated by three peripheral stalks, each composed of a heterodimer of subunits E and G. Two of the peripheral stalks (EG1 and EG2) connect the top of the V1 to the membrane bound a subunit while the third (EG3) is bound to subunit C, which is released from both V1 and Vo during enzyme dissociation. We have previously characterized and quantified the affinities of some of these interactions and have found that the globular "head" domain of subunit C (C_{head}) binds to one EG heterodimer with high affinity [3,4].

Here, we present X-ray crystal structures of two conformations of the EGC_{head} complex from *Saccharomyces cerevisiae* at 2.91 and 2.82 Å