in larger amounts than does SDS across hairpin sequences. Pyrene excimer fluorescence data indicate that SPFO additionally retains more tertiary contacts vs. SDS for all hairpin sequences tested. Solubilization in SPFO therefore appears to favour protein-protein over lipid-protein interactions. Our overall results imply that the 'harshness' of a detergent is proportional to its protein sequence specificity upon binding, and consequent tendency not to disrupt intra-protein contacts during micelle formation.

## Membrane Protein Function I

**270-Pos**

**Anion Translocation in a Brush-Like Nanopore: Simulations of the Outer Membrnae Protein OprP**

**Prapasiri Pongprayoon**, Oliver Beckstein, Chze Ling Wee, Mark Sansom.
University of Oxford, Oxford, United Kingdom.

The outer membrane protein OprP from *Pseudomonas aeruginosa* forms an anion-selective pore, especially selective for phosphate ions. The protein is homotrimeric, with each pore lined by three positively charged loops (L3, L5, and T7) folded into its lumen. OprP plays a key role in high-affinity phosphate uptake under the condition of phosphate starvation. To better understand the mechanism of phosphate-selective permeation, we employed three simulation techniques: (i) equilibrium molecular dynamics simulations (MD); (ii) steered MD (SMD); (iii) umbrella sampling to calculate a potential of mean force (PMF) for phosphate and chloride ions. The PMFs reveal a deep energy well midway along the OprP channel. Two adjacent phosphate-binding sites (W1 and W2), each with a well depth of ~8$kT$, are identified close to the L3 loop in the most constricted region of the pore. The transfer of phosphate between sites W1 and W2 is correlated with changes in conformation of the sidechain of K121, which serves as a 'charged brush' to facilitate phosphate passage between the two subsites. The PMF for chloride has also been computed and can be compared with that of phosphate. Our simulations suggest that OprP does not conform to the conventional picture of a channel with a relatively flat energy landscape for permeant ions, but rather resembles a membrane-inserted binding protein with a high specificity that allows access to a centrally located binding site from both the extracellular and the periplasmic spaces.

**271-Pos**

**Regulation of Channel Function Due To Coupling With a Lipid Bilayer**

**Md. Ashrafuzzaman**[1], J. Tuszynski[1,2].
[1]Cross Cancer Institute, University of Alberta, Edmonton, AB, Canada,
[2]Department of Physics, University of Alberta, Edmonton, AB, Canada.

Regulation of membrane protein functions due to hydrophobic coupling with lipid bilayer is investigated. Binding energy between lipid bilayer and integral ion channel with different structures has been calculated considering 0$^{th}$ or 1$^{st}$, 2$^{nd}$, etc. order terms in the expansion of the screened Coulomb interaction $V_{sc}$(r)=integral of $d^3kExp\{i\mathbf{k}.\mathbf{r}\}V_{sc}(\mathbf{k})$ with $V_{sc}$(r) being the inverse Fourier transformation of the screened Coulomb interaction in Fourier space $V_{sc}(\mathbf{k})=V(\mathbf{k})(1+f(n,T)V(\mathbf{k}))^{-1}$ for bilayer thickness ($d_0$) channel length ($l$) mismatch ($d_0$-$l$) to be filled by none or single, double etc. lipids, respectively. $V(k)$ is the direct Coulomb interaction (in Fourier space) between channels and lipids on the bilayer, $f(n,T)=n/2k_BT$, $n$ is the lipid density, $T$ is absolute temperature and $k_B$ is Boltzmann's constant. We find that the hydrophobic bilayer thickness channel length mismatch $d_0$-$l$ induces channel destabilization exponentially while negative lipid curvature ($c_0$) linearly. Lipid charge appears with dominant effects in case of higher mismatch. Experimental parameters related to gramicidin A (gA) and alamethicin (Alm) channel dynamics in black lipid membranes inside NaCl aqueous phases are consistent with theoretical predictions. Our experimental results (with others) show that average gA channel lifetime decreases exponentially with increasing $d_0$-$l$ but linearly with increasing negative $c_0$. The Alm channel formation rate and relative free energy profiles between its different conductance levels follow identical trends as predicted by our theoretical results. This study provides a general framework for understanding the underlying mechanisms of membrane protein functions in biological systems.

**272-Pos**

**Properties of Liposomes With Complex Lipid Mixture**

Markus Schwiering, Antje Brack, Heinz Decker, **Nadja Hellmann**.
Institute for Molecular Biophysics, Mainz, Germany.

Although the alpha-toxin from *S. aureus* was the first pore-forming toxin identified, its mode of interaction with membranes is still not fully understood. The toxin forms heptameric pores on cellular and artificial membranes. The observation that artificial membranes are permeabilized by this toxin indicates that no protein receptor is mandatory. Efficient permeabilisation is only possible in presence of cholesterol and sphingomyelin, which could be interpreted as a preference of the toxin for raft-like structures. However, the extent of oligomer formation as monitored by pyrene-fluorescence depends in a complex way on the lipid-composition of the liposomes which in our studies contain different amounts of eggPE, brainPS, eggSM and Cholesterol. Thus, we employed thin-layer chromatography in order to check whether the lipid composition as found finally in the liposomes correspond to the original mixture in chloroform. The results show that in case of extruded vesicles the deviation from the original mixture is not significant, but that in case of GUVs completely different relative amounts of the different lipid components can be obtained. Thus any comparison of liposome properties or toxin/liposome interactions between different liposome types has to be done very cautiously if these types of mixture are employed.
We thank the DFG (SFB 490) for financial support, S.Bhakdi and A.Valeva for production of the toxin and helpful discussions and G. Gimpl for help with fluorescence microscopy.

**273-Pos**

**Functional Reconstitution Into Liposomes of Purified Human RhCG Ammonia Channel**

**Isabelle Mouro-Chanteloup**[1], Sylvie Cochet[1], Mohamed Chami[2], Sandrine Genetet[1], Nedjma Zidi-Yahiaoui[1], Andreas Engel[2], Yves Colin[1], Olivier Bertrand[1], Pierre Ripoche[1].
[1]INSERM UMR_S 665/INTS/Université Paris Diderot-Paris 7, Paris, France,
[2]C-CINA, Center for Imaging and Nanoanalytics, .E. Müller Institute for Structural Biology, Biozentrum, University of Basel, Basel, Switzerland.

Rh glycoproteins (RhAG, RhBG, RhCG) are members of the Amt/Mep/Rh family which facilitate movement of ammonium across plasma membranes. Changes in ammonium transport activity following expression of Rh glycoproteins have been described in different heterologous systems: yeasts, oocytes and eukaryotic cell lines. However, in these complex systems, a contribution of endogenous proteins to this function cannot be excluded. To demonstrate that Rh glycoproteins by themselves transport $NH_3$, human RhCG was purified to homogeneity and reconstituted into liposomes, giving new insights into its channel functional properties.

An HA-tag introduced in the second extracellular loop of RhCG was used to purify to homogeneity the HA-tagged RhCG glycoprotein from detergent-solubilized recombinant HEK293E cells. Electron microscopy analysis of negatively stained purified RhCG-HA revealed, after image processing, homogeneous particles of 10 nm diameter with a trimeric protein structure. Reconstitution was performed with sphingomyelin, phosphatidylcholine and phosphatidic acid lipids in the presence of the $C_{12}E_8$ detergent which was subsequently removed by Biobeads. Control of protein incorporation was carried out by freeze-fracture electron microscopy. Particle density was a function of the Lipid/Protein ratio. When compared to empty liposomes, ammonium permeability was increased two and three fold in RhCG-proteoliposomes, depending on the Lipid/Protein ratio (1/300 and 1/150, respectively). This strong $NH_3$ transport was reversibly inhibited by mercuric and copper salts and exhibited a low Arrhenius activation energy.

This study allowed the determination of ammonia permeability, showing that the apparent $Punit_{NH3}$ per RhCG monomer (around $1x10^{-3}$ $\mu m^3.s^{-1}$) is close to the permeability measured in HEK293E cells expressing a recombinant human RhCG ($1.60x10^{-3}$ $\mu m^3.s^{-1}$), and in red blood cells endogenously expressing RhAG ($2.18x10^{-3}$ $\mu m^3.s^{-1}$). The major finding of this study is that RhCG protein is active as an $NH_3$ channel and that this function does not require any protein partner.

**274-Pos**

**Tracking Single Protein Translocation Complexes in the Membranes of Living Bacteria**

**Yves Bollen**, Siet van, den Wildenberg, Erwin Peterman.
Vrije Universiteit, Amsterdam, Netherlands.

The Twin Arginine Translocation (Tat) system transports fully folded en sometimes even oligomeric proteins across the inner membrane of bacteria. Its mechanism is largely unknown. Remarkably, a stable translocation complex has not been observed. Instead, the three components of the system, i.e., TatA, TatB and TatC, are isolated from the membrane of Escherichia coli in various complexes of different sizes, which suggests that a complete and active Tat complex is formed only transiently. We have used single particle tracking in living bacteria to gain more insight into the dynamics of the Tat proteins. TatA has been genetically fused tot enhanced Green Fluorescent Protein (eGFP). Living bacteria expressing low levels of TatA-eGFP have been immobilized on glass slides and imaged with a sensitive wide-field fluorescence microscope. Mobile fluorescent spots are observed, and their intensity and location have been tracked by fitting a 2D Gaussian function to successive frames. Analysis of the data shows that diffusion of TatA-eGFP is heterogeneous, and that the average diffusion coefficient of fluorescent TatA particles decreases when excess substrate is expressed. When the electrochemical potential, which is known to drive protein transport via the Tat system, is removed the diffusion coefficient of TatA-eGFP increases again. The latter suggests

that TatA forms larger complexes upon substrate binding in the presence of a membrane potential.

**275-Pos**
**Solvation and Binding of the Membrane Enzyme Pagp By Detergents and Lipids**
**Chris Neale**[1,2], Régis Pomès[1,2].
[1]University of Toronto, Toronto, ON, Canada, [2]SickKids Hospital, Toronto, ON, Canada.
The solvation of membrane proteins by detergents is a necessary step for structure determination by NMR and X-ray crystallography, and yet this process remains poorly understood. The severe under-representation of membrane proteins amongst proteins of known structure is a direct consequence of the difficulties associated with the solubilization of the large hydrophobic faces presented by this important class of proteins. The prominence of these membrane proteins as important drug targets provides a strong impetus for the rational design of new detergents or cofactors to assist the solubilization of hydrophobic faces while maintaining structural integrity. To this end, we begin by investigating the process of detergent self-aggregation, utilizing molecular dynamics simulations to characterize the atomic interactions that underlie the dynamic aggregation of detergents in aqueous solution. We determine the critical micelle concentration and the equilibrium aggregation number based on generalized ensemble methods and, separately, thermodynamic cycles involving non-physical order parameters that are more computationally efficient. Next, we apply similar methods to study the aggregation of detergents around the bacterial outer membrane enzyme and virulence factor PagP. These studies shed light on the process of protein self aggregation in solution and the atomistic mechanisms by which detergents may prevent protein precipitation at high concentrations. Finally, we draw functional conclusions for PagP, an acyltransferase that binds and catalyses its own lipid solvent.

**276-Pos**
**Protein Secondary Structure Prediction Using Knowledge-Based Potentials and An Ensemble of Classifiers**
**Saraswathi Sundararajan**[1], Pawel Gniewek[2], Robert L. Jernigan[1], Andrzej Kolinski[3], Andrzej Kloczkowski[1].
[1]Department of Biochemistry, Biophysics and Molecular Biology, L.H. Baker Center for Bioinformatics and Biological Statistics, Ames, IA, USA, [2]Faculty of Chemistry, Warsaw University, Laboratory of Theory of Biopolymers, Warszawa, Poland, [3]Faculty of Chemistry, Warsaw University, Laboratory of Theory of Biopolymers, Warszawa, Poland.
A novel method is proposed for predicting protein secondary structure using knowledge-based potentials and Neural Networks. Potential energies for amino acid sequences in proteins are calculated using protein structures in the CATH database. The data consists of energy information calculated for a reduced set of three secondary structures: alpha-helix, beta-strand and coil. An Extreme Learning Machine (ELM) classifier, based on Neural Network, is used to model and predict protein secondary structure from this data.
Other classification techniques such as Support Vector Machines (SVM) and Naïve Bayes (NB) are also used and show comparable performance. Preliminary results show that an ensemble of various techniques can collectively improve prediction results.

**277-Pos**
**Determination of the Mechanism of Selectivity and Ammonia Conduction By AmtB Using MD Simulations**
**Shahram Khademi**, Ugur Akgun.
University of Iowa, Iowa city, IA, USA.
The transport of ammonia, fundamental to the nitrogen metabolism in all domains of life, is carried out by the Rh/Amt/MEP membrane protein superfamily. The first structure of this family (AmtB from E. coli) shows a pathway for ammonia that includes two vestibules connected by a long and narrow hydrophobic lumen. The accepted mechanism for AmtB is to recruit NH4+ and conduct neutral NH3 by deprotonation of NH4+ at the end of the periplasmic vestibule. We conducted several MD simulations (total of more than 0.3μs) using a model of trimeric AmtB embedded into POPE lipid bilayer to determine the mechanism of ligands conduction in the ammonia channels.
To determine the AmtB's selectivity, we added 100 ligand molecules to our solvated protein-lipid system and conducted unconstrained MD simulations for each ligand. The probability distribution for each ligand along the normal of the lipid plane shows that the periplasmic vestibule prefers NH4+ over NH3 and CO2. Our long MD simulations reveal that two stacking phenyl rings of F107 and F215 (located at the bottom of the periplasmic vestibule) simultaneously flip open and close with a frequency of ~108 flip-open events per second. The frequency of flip open/close events is independent of the presence of NH4+ at the vestibule. This indicates that the rate of this channel is controlled not only by the concentration gradient of ammonia but also by the frequency of phenyl rings open/close events.
Our simulations show that D160 along with the aromatic rings are essential for recruitment of NH4+ at the phenyl rings gate. In each ammonia conduction cycle, NH4+ stays behind the gate long enough for the gate to flip open and let ammonium enters the lumen.

**278-Pos**
**Electrophysiology of Viral Envelope Protein Ion Channels in Lipid Membranes Across Apertures in Polystyrene and Silicon**
Nipun Chaplot, Latrice Faulkner, Sasha Daskalova, Brenda Hogue, **Michael Goryll**.
Arizona State University, Tempe, AZ, USA.
Viral ion channels, such as the coronavirus envelope proteins (E protein), belong to a family of channels that have attracted a considerable amount of interest during recent years. However, not many studies on the electrophysiology have been performed; mainly due to the fact that these channels are membrane proteins that do not easily express in the outer membrane of bacteria. Moreover, the channel currents are small (on the order of 10-300 pS) when compared with bacterial outer membrane channels. In our studies, we reconstituted the full-length channel-forming E protein from murine hepatitis coronavirus (MHV-A59) into 3:1:1 POPE:POPS:POPC lipid bilayers that were suspended either across 150 μm diameter apertures in polystyrene cups or across a 50 μm diameter aperture in silicon. Lipid bilayers were formed using the painting method on all substrates, resulting in reproducible Gigaseal formation. The aperture in silicon was prepared using photolithography and dry reactive ion etching, resulting in excellent reproducibility of the pore geometry. The surface was coated hydrophobically to allow lipid bilayer attachment.
Bilayers created in the presence of E-protein in solution showed reproducible ion channel activity, independent of the substrate used. We were able to identify the signature conductance steps of E ion channels. Similar to what has been shown previously using the OmpF ion channel of E. coli, the ion channel activity on the silicon substrate was identical to that measured using the polystyrene cup, indicating the feasibility of the silicon substrate for the investigation of ion channels with conductances in the range of tens of picosiemens. Using silicon apertures for ion channel reconstitution experiments in array geometry provides an opportunity to increase measurement throughput.

**279-Pos**
**WITHDRAWN**

**280-Pos**
**Single Amino Acid Substitutions Change the Sodium/Iodide Symporter (NIS) Selectivity and Stoichiometry**
**Nancy Carrasco**.
Albert Einstein College of Medicine, Bronx, NY, USA.
The $Na^+/I^-$ symporter (NIS) is a key plasma membrane protein that mediates active $I^-$ transport in the thyroid and such other tissues as salivary glands, stomach, and lactating breast. NIS-mediated $I^-$ uptake is the first step in thyroid hormone biosynthesis. NIS mediates the inward simultaneous movement of $Na^+$ and $I^-$ with a 2:1 stoichiometry, thus resulting in a net transfer of positive charge into the cell (i.e., electrogenic transport). We recently reported that NIS translocates different anion substrates with different stoichiometries, as $Na^+$/perchlorate (or perrhenate) transport is electroneutral. Valuable mechanistic information on NIS has been obtained by the characterization of NIS mutants that cause congenital $I^-$ transport defect in patients. Here we provide a detailed study of the G93R NIS mutant. As we substituted neutral amino acids at this position, we observed that the longer the side chain of the substituted residue, the lower the protein's activity. G93T and G93N NIS exhibited significantly higher $K_m$ values for $I^-$ than WT NIS, the first time that such a change has been observed in any NIS mutants. Strikingly, we show by kinetic analysis that G93T-mediated $Na^+$/perrhenate symport is electrogenic with a 2:1 stoichiometry, a discovery confirmed by the detection of currents elicited by perrhenate (or perchlorate) in G93T NIS-expressing *X. laevis* oocytes in electrophysiological experiments. These observations demonstrate that a single amino acid substitution at position 93 converts NIS-mediated $Na^+$/perchlorate (or perrhenate) transport stoichiometry from electroneutral to electrogenic. Based on the 3-D structure of the bacterial $Na^+$/galactose transporter, we built a 3-D homology model of NIS and we propose a mechanism in which changes from an outwardly open to an inwardly open conformation during the transport cycle use G93 as a pivot.