

**1937-Pos****Transport of Cephalosporin Antibiotics Across the Outer Membrane**

Marcos Lovelle<sup>1</sup>, Mahendran Kozhinjampara R.<sup>2</sup>, Tivadar Mach<sup>1</sup>, Mathias Winterhalter<sup>2</sup>, Paula Gameiro<sup>1</sup>.

<sup>1</sup>REQUIMTE Faculdade de Ciências do Porto, Porto, Portugal, <sup>2</sup>Jacobs University, Bremen, Germany.

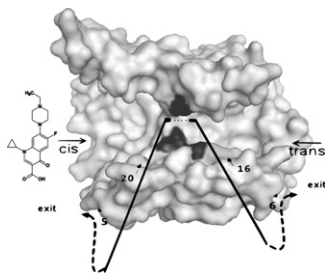
Antibiotic resistance is emerging in Gram-negative bacteria worldwide. The outer membrane of Gram-negative bacteria is a formidable selective barrier, and a major factor in broad-spectrum antibiotic resistance. Influx of antibiotics into the Periplasm of bacteria is facilitated by proteins that form channels in the outer membrane. We studied the influx of several cephalosporin antibiotics through the major *Escherichia coli* porins OmpF and OmpC.

Conductance measurements through purified single porins reconstituted in artificial lipid bilayers allowed us to count the passage of single antibiotic molecules. Statistical analysis of transport events yields the kinetic parameters at the single molecular level. Fluorescence steady-state measurements were used to quantify the interaction between the antibiotics and the porin channels and verify the calculation of translocation kinetics. For the first time, we have been able to characterize facilitated translocation of several  $\beta$ -lactams through OmpC (most expressed porin *in vivo*) and quantified the distinguishable permeation properties of ceftriaxone, ceftazidime and cefpirome through both outer membrane porins OmpF and OmpC - concluding a stronger interaction with OmpF than OmpC for all three cephalosporins -especially ceftazidime-. Our approach may be of great benefit to the understanding of porin-drug interaction at the molecular scale and may contribute to the rational design of more efficient antibiotics.

**1938-Pos****Molecular Basis of Enrofloxacin Affinity to a Membrane Channel of E. Coli - When Binding Does Not Imply Translocation**

Tivadar Mach<sup>1</sup>, Kozhinjampara R. Mahendran<sup>2</sup>, Eric Hajjar<sup>3</sup>, Isabel Sousa<sup>1</sup>, Marcos Lovelle<sup>1</sup>, Amit Kumar<sup>3</sup>, Paula Gameiro<sup>1</sup>, Mathias Winterhalter<sup>2</sup>, Matteo Ceccarelli<sup>3</sup>.

<sup>1</sup>REQUIMTE, Faculdade de Ciências, Porto, Portugal, <sup>2</sup>Jacobs University Bremen, Bremen, Germany, <sup>3</sup>University of Cagliari, Monserrato (CA), Italy. The molecular pathway of enrofloxacin, a fluoroquinolone antibiotic, across the main outer membrane channel OmpF of *E. coli* is investigated. Through high-resolution ion current fluctuation analysis we count single enrofloxacin channel penetrations, showing a binding to OmpF comparable to the affinity-enhanced translocation through substrate specific channels. A single point mutation D113N increases the dissociation rate 30 times, making the interaction comparable to other antibiotics, corresponding to their weaker binding in a non-specific channel. Molecular dynamics simulations elucidate translocation barriers: WT OmpF has two symmetric binding sites for enrofloxacin located at each channel entry separated by a large barrier in the centre, inhibiting antibiotic translocation. Removal of the negative charge on 113 removes the central barrier shifting both peripheral binding sites to one central site enabling translocation. Fluorescence steady-state measurements confirm simulations. Our results demonstrate that a single mutation of the porin results in a substantial modification of translocation. This example demonstrates how translocation through a channel depends not only on the strength of the substrate-channel interaction, but on a local affinity site counteracting the conformational entropy change at the smallest constriction.

**1939-Pos****Permeation of Antibiotics through Bacterial Porins: Screening for Influx on a Single Molecular Level**

Mahendran R. Kozhinjampara<sup>1</sup>, Mohamed Kreir<sup>2</sup>, Niels Fertig<sup>2</sup>, Jean-Marie Pages<sup>3</sup>, Eric Hajjar<sup>4</sup>, Matteo Ceccarelli<sup>4</sup>, Mathias Winterhalter<sup>1</sup>.

<sup>1</sup>Jacobs university, Bremen, Bremen, Germany, <sup>2</sup>Nanion Technologies GmbH, Munich, Germany, <sup>3</sup>Université de la Méditerranée, Marseille, France, <sup>4</sup>University of Cagliari, Cagliari, Italy.

Chip based automated patch clamp technique provides an attractive biophysical tool to quantify solute permeation through membrane channels. Proteo-Giant Unilamellar Vesicles (GUVs) were used to form stable lipid bilayer across the micrometer sized hole and single channel recordings were achieved with

very low background noise. Influx of antibiotics into the periplasm of gram-negative bacteria is facilitated by porins that form channel in the outer membrane. Influx of two major class of antibiotics- cephalosporin and fluoroquinolones through major *E. coli* porins OmpF and OmpC was investigated. Ion current fluctuations through porins in the presence of penetrating antibiotics revealed thermodynamic and kinetic parameters of substrate binding from which we calculated flux. We have been able to show rapid and efficient screening of antibiotics through bacterial porins at a single-molecule scale. *In vitro* activity of antibiotics was determined by microbiological assays which correlates with the results obtained from lipid bilayer measurements. In addition, molecular modelling provided details on the interaction of the molecules with the channel surface, revealed the preferred orientation of the antibiotic along its pathway and the position of affinity sites. Our approach may contribute to the rational design of new antibiotics against clinical bacterial strains for the most efficient delivery to target sites.

**1940-Pos****Probing the Molecular Mechanism of Passive Transport**

Noah Malmstadt, Su Li, Peichi Hu.

University of Southern California, Los Angeles, CA, USA.

The passive transport of small molecules across the plasma membrane is the major pathway by which orally delivered drugs enter circulation and a major route of delivery into target cells. While molecular dynamics simulations suggest that molecules diffusing through lipid bilayers are subject to a complex, dynamic environment, the analytical framework for describing passive transport continues to treat the membrane as a uniform material. The study of passive transport is complicated by the shortcomings of current experimental techniques, in which transport tends to be dominated by diffusion through a stagnant layer adjacent to the membrane.

We have developed an approach to probing passive transport that eliminates these artifacts and allows for a study of relationships between molecular structure and transport properties. This approach consists of confocal microscopy of the diffusion of small molecules into giant unilamellar lipid vesicles (GUVs). Experiments and finite element models show that due to small size of GUVs relative to the characteristic diffusive length scale of transported molecules, no significant stagnant layer is established. In addition, confocal imaging allows for observation of the steady-state association of diffusing molecules with the membrane itself, while other technologies only allow for detection of transported molecules.

We have concurrently developed a fabrication technology that yields GUVs in which each leaflet of the bilayer has a different lipid composition. This allows a novel investigation of the relevance of the asymmetry of the plasma membrane to passive transport.

A series of fluorescent molecules of varying hydrophobicity was synthesized. Time-series images of these molecules crossing GUV membranes were captured, and these images were fit to both an analytical model of membrane permeation and a finite element model of permeation with diffusion. Lipid composition was varied to reproduce the range of compositions observed in human plasma membranes.

**1941-Pos****Supported Bilayers with Excess Membrane Reservoir (SUPER): Novel Templates for Vesicular and Non-Vesicular Transport Studies**

Thomas J. Pucadyil, Sandra L. Schmid.

The Scripps Research Institute, La Jolla, CA, USA.

Our understanding of membrane-localized processes has largely been gained from the use of liposome-based systems. However reactions involving budding and fission of membranes are difficult to analyze using liposome-based systems since their buoyancy imposes a fundamental limitation on separating end-products of such reactions. Supported bilayers formed by liposome fusion on glass represent an attractive solution. Conventional supported bilayers are however deficient in membrane reservoir necessary for membrane budding and fission reactions. We report a novel system of supported bilayers with excess membrane reservoir (SUPER) and have analyzed factors that contribute to their formation. The excess reservoir in this system originates from higher binding affinity of liposomes to glass and depends on the presence of anionic lipids in the membrane and high salt content in the buffer. This template formed on silica beads allows the seamless application of microscopy-based assays to analyze membrane-localized processes as well as sedimentation-based assays to isolate vesicular and non-vesicular products released from the membrane. We demonstrate the utility of SUPER templates by the direct visualization of amphiphile-induced membrane tubulation prior to solubilization and