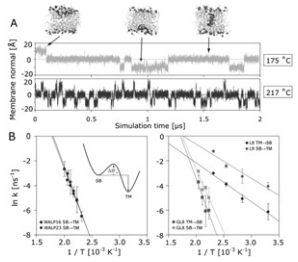
2704-Pos Board B690
Thermodynamic Analysis of Protegrin-1 insertion and Permeation through a Lipid Bilayer
Victor Vivcharuk, Yiannis Kaznessis.
The molecular dynamics (MD) simulations are used to study the pathway for the insertion of the cationic antimicrobial peptide protegrin-1, (PG1) into anionic POPG and neutral POPE lipid bilayers at 310 K. We calculate the potential of mean force (PMF) during transfer of protein from the bulk of aqueous phase to the transmembrane (TM) configuration using the the adaptive-biasing-force method which is implemented as a part of the collective Variables Module of NAMD. We find that the PMF has two energy minima and one potential barrier. One minimum corresponds to full insertion TM configuration. The second PMF minimum corresponds for adsorption to the membrane surface. The PMF exposes the existence of the potential barrier for the insertion process.Using Langevin equation for Kramers theory and generalized Langevin equation for Grote-Hynes theory we calculated the transmission coefficient for PG1 diffusion through the potential barrier. We focus on use of the PMF and time correlation function of the fluctuation instantaneous force to calculate the rate constants for insertion of PG1 into the POPG and POPE membranes. The influence of the activation free energy barrier to the dynamic of the insertion and permeation for peptides through the membranes are discussed.

2705-Pos Board B691
Adsorption and Insertion Kinetics of Membrane Active Peptides from Fully Atomistic Simulations
Jakob P. Ulmschneider.
We show here that the insertion and translocation kinetics of peptides into and across lipid bilayers can be fully described by all-atomistic simulations. Peptides quickly fold, adsorb, and subsequently insert into the membrane. The partitioning equilibrium consists of flipping back and forth between folded interfacial and transmembrane inserted orientations, with the secondary structure remaining fully helical. Transition rates increase rapidly with temperature: Arrhenius plots of the insertion and expulsion rates show sequence-dependent first-order kinetics with activation barriers for insertion of ~6 kcal/mol for pure hydrophobic sequences, ~23 kcal/mol for peptides containing aromatics (Trp translocation) and ~20 kcal/mol for peptides containing helix-breaking Gly and Pro residues. The simulations demonstrate for the first time in full atomic detail how membrane active peptides assemble and integrate into lipid bilayers. Transition rates at ambient temperature can be accurately predicted from the first-order kinetics, resulting in the first computational quantification of this process: At 30 °C, average insertion times of τ = 100-200 ms are seen for a variety of synthetic (e.g. WALP, GL*n*) as well as viral (Vpu, FluA-M2) sequences.



2706-Pos Board B692
Correlation between Properties of pHLIP Peptide-Lipid Interaction and Tumor Targeting In Vivo
Dhammika A. Weerakkody, Alexander G. Karabadzhak, Mak S. Thakur, Bethany Rossi, Donald M. Engelman, Oleg A. Andreev, Yana K. Reshetnyak.
We have discovered a way to target acidity *in vivo,* which is a hallmark of many pathological states. The tumor targeting is based on the pH-dependent transmembrane insertion and folding of the water-soluble membrane peptide, pHLIP - pH (Low) Insertion Peptide. Here we present the result of the sequence variation study of pHLIP, which was carried out with the main goal to improve blood clearance and tumor targeting at low pH. We have investigated more than 10 pHLIP variants with various mutations in transmembrane and C-terminal parts, including peptides with significantly truncated transmembrane part. Currently our library of pHLIP variants, contains peptides inserting into bilayer with pKa ranging from 4.5 to 6.5, which have different affinity to lipid bilayer at neutral and low pHs. Kinetics measurements indicate that all investigated variants containing truncated or no C-terminal flanking sequence demonstrate fast insertion into lipid bilayer. The results of biophysical studies are in excellent agreement with the tumor targeting and blood clearance data obtained in vivo. Our data contribute in understanding of main principles of peptide-lipid interactions.

2707-Pos Board B693
A Fresh Look at Cochleate Cylinder by Freeze-Fracture Electron Microscopy: From Fusion Intermediate to Carrier of Antibiotics
Brigitte Papahadjopoulos-Sternberg, Raquel F. Epand, Liran Livne, Richard M. Epand, Ahram Mor.
Cochleate cylinders are tightly wrapped, micrometer-size tubes of concentric bilayers folded in a spiral configuration. They were discovered during Ca^{2+}-induced fusion of small unilamellar vesicles made of phosphatidylserine [1]. Now they are used as platform technology for antigen delivery for vaccination and for oral drug delivery.
Here we report the formation of cochleate cylinder by treating PS-free lipid films with cationic antimicrobial peptides without the requirement for divalent cations. The lipid films employed are mimicking the lipid pattern of cytoplasmic membranes of Gram negative bacteria. The antimicrobial peptides chosen contain the oligo-acyl-lysyl (OAK) sequence C_{12}K-7α_8. Due to their cationic nature they replace the divalent cations in the cochleate cylinder forming process. Furthermore, this new type of cochleate cylinder has been proven to encapsulate and transport traditional, essentially ineffective antibiotics such as erythromycin to resistant bacteria cells [2]. The double-function of OAK-based antimicrobial peptides in forming cochleate cylinder and promoting encapsulation and effective delivery of traditional antibiotics in/by these assemblies provides an interesting approach to overcome antibiotic resistance in bacteria.
References
[1] D. Papahadjopoulos et al, BBA 394 (1975) 483
[2] L. Livne et al, FASEB J., in press

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2708-Pos Board B694
A strong Correlation Between Ordered Plasma Membrane Domains and Actin Filaments in Live Cells
Jelena Dinic, Ingela Parmryd.
There is a relationship between ordered plasma membrane nanodomains, lipid rafts, and actin filaments. The nature of this relationship was the target of this study. Using laurdan we assessed the relative proportions of ordered and disordered domains in live Jurkat and primary human T cells at 37°C upon treatments affecting actin dynamics, causing aggregation of membrane components or modifying cell lipid content. Disrupting actin polymerization, using latrunculin B, decreased the fraction of ordered membrane domains while stabilizing actin filaments with jasplakinolide had the opposite effect in both cell types. Membrane blebs, which are detached from the underlying actin filaments, contained a lower fraction of ordered domains than the rest of the plasma membrane. Treatment with phenylarsine oxide, which decreases the PI(4,5)P2 content in the plasma membrane and therefore disrupts the link between the plasma membrane and the underlying actin filaments, resulted in a lower fraction of ordered plasma membrane domains. Patching of the lipid raft components GM1 and CD59, the non lipid raft component CD45 and the general cross-linker Concanavalin A all produced an increase the fraction of ordered domains. This increase was strongly correlated with an increase in polymerized actin at the plasma membrane. Patching of the T cell receptor did not alter the overall membrane order, although the actual patches contained a larger fraction of ordered domains than the surrounding plasma membrane, strongly suggesting that small ordered domains coalesce upon patch formation. Non-lethal limited cholesterol depletion using methyl-beta-cyclodextrin triggered peripheral actin polymerization and resulted in an increase in the fraction of ordered plasma membrane domains. Our study suggests that actin polymerisation at the plasma membrane, rather than lipid-lipid interactions, cause the formation of ordered plasma membrane domains/lipid rafts and calls for a conceptual change.

2709-Pos Board B695
Chasing Raft Localisation Signals: FLIM-FRET Reveals CRAC Mediated Microdomain Association of the Human Immunodeficiency Virus Glycoprotein gp41
Roland Schwarzer.
The glycoprotein gp41 is the only transmembrane protein of the human immunodeficiency virus (HIV). In concert with gp120 it mediates host cell infection by binding cellular receptors and later on triggering membrane fusion. Hitherto, little is known about the role of gp41 during assembly and budding of the newly synthesized virus particles. It has been reported that membranes of HIV-infected cells as well as virions are enriched in cholesterol, hence it was suggested that HIV-1 may take advantage of cholesterol and sphingolipids during