values, while at pH's lower than 4.0 the virus particles reversibly aggregate without any noticeable structural change, as analyzed under Transmission Electron Microscopy, Static and Dynamic Light Scattering and Fluorescence Spectroscopy. In this study, we also compute at different pH values TrV surface electrostatic potential and we postulate that the virus aggregation is due to electrostatic effects.

1128-Pos Rigidity of the Influenza Virus During its Infectious Cycle

Iwan A.T. Schaap, Frederic Eghiaian, Claudia Veigel

NIMR, London, United Kingdom.

Board B102.01

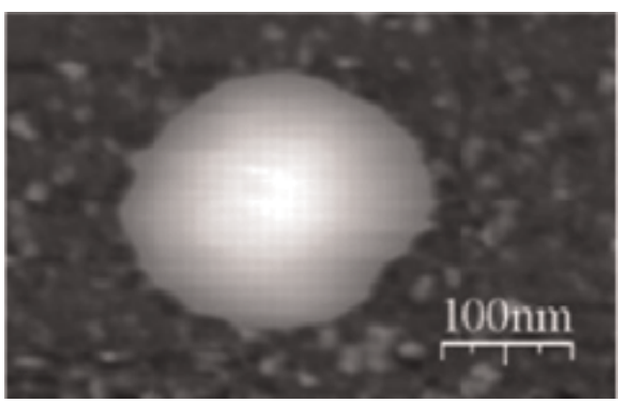
The influenza virus is an enveloped virus from the Orthomyxovirus family. The protein rich lipid membrane of the virus particle needs to persist in the often hostile extra-cellular environment when the virus transmits from host to host organism, but it also needs to permit membrane fusion with the target cell to allow infection.

We set out to investigate how this virus negotiates these apparently conflicting demands on its membrane rigidity during its life-cycle. We have developed methods to image this relatively large (diameter ~100 nm) virus and to measure its mechanical properties using an Atomic Force Microscope (AFM).

We have measured the stiffness of the viral membrane under conditions mimicking the different stages of the viral life-cycle including different pH levels. We have further compared the complex response of the viral membrane with the behavior of simplified model systems to understand the role of the various part of the viral structure for its mechanical properties.

(supported by MRC; I.A.T.S. is supported by the European Community through a Marie Curie Fellowship)

Figure, AFM scan of an influenza virus attached to a substrate



Membrane Fusion

1129-Pos Energetics And Dynamics Of SNAREpin Folding Across Lipid Bilayers

Feng Li[1], Frederic Pincet[1], Eric Perez[1], William Eng[2], Thomas J. Melia[2], James E. Rothman[2], David Tareste[2]

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Board B103

Membrane fusion occurs when SNAREpins fold up between lipid bilayers. How much energy is generated during SNAREpin folding and how this energy is coupled to the fusion of apposing membranes is still a mystery. We have utilized the Surface Forces Apparatus to determine the energetics and dynamics of SNAREpins formation and characterize the different intermediate structures sampled by cognate SNAREs in the course of their assembly. The force versus distance profiles of assembling SNAREpins reveal that SNARE motifs begin to interact when the membranes are 8 nm apart. Even after very close approach of the bilayers ($\sim$ 2–4 nm), the SNAREpins remain partly unstructured in their membrane-proximal region. The energy stabilizing a single SNAREpin in this configuration (35 kBT) corresponds closely with the energy needed to fuse outer but not inner leaflets (hemifusion) of pure lipid bilayers (40–50 kBT). The cooperative effect of a few SNAREpins at the site of exocytosis would thus be sufficient to overcome the high energetic barriers of membrane fusion.

1130-Pos SNAREs Drum Up In Silico: Molecular Dynamics Simulations Of The Synaptic Fusion Complex

Marc Baaden

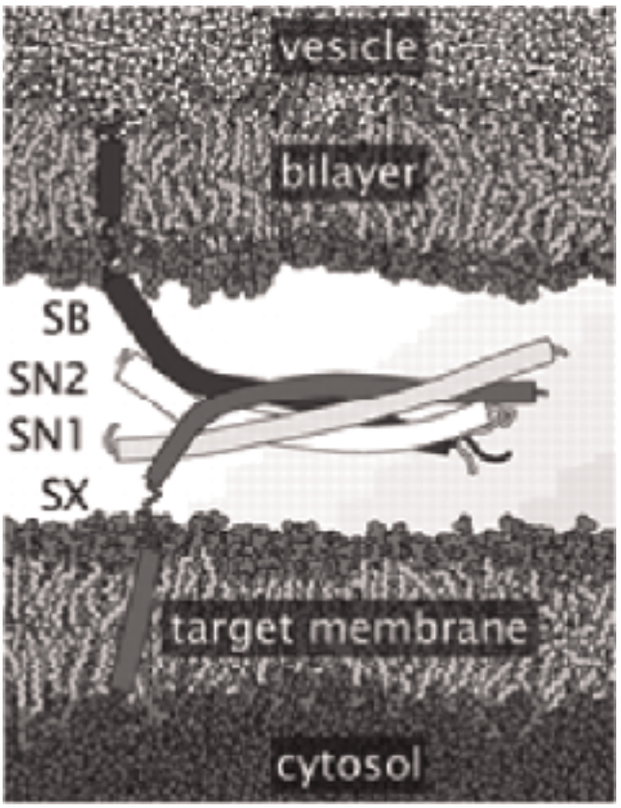
IBPC, CNRS UPR9080, Paris, France.

Board B104

The SNARE protein complex is central to membrane fusion, a ubiquitous process in biology. Modeling this system in order to better understand its guiding principles is a challenging task. This is mainly due to the complexity of the environment: two adjacent membranes and a central bundle of four helices made up by vesicular and plasma membrane proteins as shown in the Figure below.

We have modeled this system at several levels of detail, reaching from coarse grained representations of the Synaptobrevin transmembrane helix in a single lipid bilayer up to an atomistic model of the full membrane-embedded synaptic fusion complex shown above. Molecular dynamics simulations of these models were carried out to characterize the conformational dynamics and key interactions in these systems. No evidence for directionality in the

formation of these interactions was found. Our results support the metaphor of a "molecular Velcro strip" that has been used by others to describe the neuronal fusion complex. A crosssection of the most exciting results will be presented.



1131-Pos Free Energy Barriers In The Fusion Of Bilayer Membranes And Vesicles

Dina T. Mirijanian, Mark J. Stevens

Sandia National Laboratories, Albuquerque, NM, USA.

Board B105

Molecular dynamics simulations are used to study membrane fusion between vesicles and bilayers. We use a coarse-grained bead-spring model of lipids to treat the time scale involved in membrane fusion. The coarse-grained MARTINI forcefield is used. Free energy barriers to membrane fusion are calculated using the center of mass between the two fusing bodies as the internal reaction coordinate. The effect of different lipid types on the fusion tendency is examined and quantified in terms of the free energy calculations. The connection between the lipid conformations and their fusogenicity will also be discussed.

1132-Pos Interaction of the Pretransmembrane Domain of SARS-CoV. Implications for the Viral Fusion Mechanism

Jaime Guillén-Casas, Ana J. Pérez-Berná, Ana I. Gómez, Miguel R. Moreno, Alejandro Gónzalez-Álvarez, José Villalaín

IBMC-UMH, Elche - Alicante, Spain.

Board B106

A new infectious disease caused by a novel type of Coronavirus, SARS-CoV, emerged in Southern China in autumn of 2002, causing an atypical and often lethal pneumonia spread to more than twenty countries in Asia, North America, South America, and Europe in the spring of 2003. Domain S2 of the S glycoprotein is the responsible for the fusion between the viral and cellular membranes. It has been reported that a membrane-proximal region extremely rich in aromatic amino acids is essential for the membrane fusion mechanism of HIV-1 or Ebola virus and it is a common characteristic to other class 1 viral fusion proteins of several virus families. Furthermore, a recent work in our laboratory has shown the existence of this aromatic domain in the SARS-CoV S glycoprotein, domain which displayed a high partitioning into model biomembranes as well as it perturbed its integrity. We have also shown that this region represents a surface with high bilayer-to-water transfer free-energy values. It is known that viral-induced membrane fusion proceeds through a series of organized steps, i.e., close apposition of the involved bilayers, mixing between the outer leaflets of the opposing membranes (hemifusion) and finally mixing of both the outer and inner monolayers leading to mixing of the aqueous contents (fusion). In the present report we show that SARS-CoV PTM is able to facilitate these steps analyzing its biophysical properties through a series of complementary experiments, i.e. leakage, hemifusion, fusion and aggregation of vesicles. Moreover, we show that SARS-CoV PTM strongly partitions into phospholipid membranes and is localized at different depths depending on the lipidic composition of the vesicles.

1133-Pos The Membrane-Active Regions of the Hepatitis C Virus E1 and E2 Envelope. Glycoproteins. Structural and Biophysical Characterization of the Fusogenic Region

Ana J. Perez-Berna[1], Jaime Guillen Casas[1], Miguel Moreno[1], Ana I. Gomez Sanchez[1], Alejandro Gonzalez-Alvarez[1], Georg Pabst[2], Peter Laggner[2], Jose Villalain[1]

[1] *IBMC-UMH, Elche-Alicante, Spain*

[2] *Institut für Biophysik und Nanosystemforschung, Graz, Austria.*

Board B107

We have identified the membrane-active regions of the full sequences of the HCV E1 and E2 envelope glycoproteins by perform-

ing an exhaustive study of membrane leakage, hemifusion, fusion, calorimetry, solid-state NMR and X-ray diffraction induced by 18-mer peptide libraries on membrane model systems. The data and their comparison have led us to identify different E1 and E2 membrane-active segments which might be implicated in local perturbation in the bilayer such as viral membrane fusion or membrane interaction, as well as protein-protein binding. Moreover, it has permitted us to suggest that the fusion peptide might be located in the E1 glycoprotein and, more specifically, the segment comprised by amino acid residues 274–296; meanwhile, other membrane-active regions along proteins E1 and E2 could contribute to the membrane desestabilization during the fusion process. We have studied by calorimetry, infrared and fluorescence spectroscopies, solid-state NMR and X-ray diffraction the structural and conformational changes of the membranes during the peptide-membrane association. The identification of these membrane-active segments from the E1 and E2 envelope glycoproteins, as well as their capability of modifying the biophysical properties of model membranes, support their direct role in HCV-mediated membrane fusion, sustain the notion that different segments provide the driving force for the merging of the viral and target cell membranes and define those segments as attractive targets for further development of new anti-viral compounds.

1134-Pos Strong Stimulation of SNARE-mediated Membrane Fusion by Complexin and Calcium

Xiaobing Lu[1], Tae-Young Yoon[2], Jiajie Diao[2], Soo-Min Lee[1], Yeon-Kyun Shin[1], Taekjip Ha[2]

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Board B108

Ca^{2+}-triggered, synchronized fusion of synaptic vesicles to the presynaptic plasma membrane underlies inter-neuronal communication. Complexin is a major binding partner of the SNARE complex-the core fusion machinery of neuronal presynaptic parts-and has been considered an important fusion regulator. The physiological data on complexin, however, have been at odds with each other, making delineation of its molecular function difficult. Here we report on direct observation of two-faceted functions of complexin using the single-vesicle fluorescence fusion assay. We show that full length complexin I has two opposing effects on *trans*-SNARE assemblies: inhibition of SNARE complex formation and stabilization of assembled SNARE complexes. SNARE-mediated fusion is markedly stimulated by complexin, and further accelerated by two orders of magnitude in response to an externally applied Ca^{2+} wave. We suggest that SNARE assemblies, complexin, and lipid membranes collectively form a 'primed' complex, providing a substrate for Ca^{2+} and Ca^{2+}-sensing fusion effectors in the neurotransmitter release. Our approach provides a unique avenue to unravel the function of the presynaptic fusion regulators.

1135-Pos SNARE-driven Single-vesicle Fusion Assay With Simultaneous Detection of Content Release and Lipid Mixing at 5-ms Time Resolution

Tingting Wang, Izzy A. Smith, James C. Weisshaar

UW-Madison, Madison, WI, USA.

Board B109

We have previously reported a cell free assay that exhibits SNARE-dependent fusion of 50-nm proteoliposomes with a supported planar lipid bilayer. Fusion is fast and efficient, with rate constants of $k_{dock} = 2.2 \times 10^7\ M^{-1}\text{-}s^{-1}$ and $k_{fuse} = 40\ s^{-1}$. Recently, we have reconstituted VAMPII-containing proteoliposomes with a liquid-phase marker, calcein dye, in addition to a lipid phase marker, R18. We utilized through-the-objective TIRF and a CCD camera to make two-color movies of single vesicles docking and fusing onto the supported bilayer with 1–5 ms frame rates. By incorporating self-quenching concentrations of both calcein and R18, fusion events were marked by a rapid increase in fluorescence intensity. Intensity traces reveal that the both dyes dequench within 1 ms, the temporal resolution of the camera. Lipids with negative intrinsic curvature, DOPE, were included in the VAMPII-containing proteoliposomes, to encourage hemifusion. For hemifusion events, content release coincided with the dequenching of the bilayer's inner leaflet, suggesting the extended hemifusion diaphragm does not contain a fusion pore. Careful comparison of experimental fusion traces with theoretical models allowed us to conclude that the majority of the calcein dye is released above the bilayer, implying significant content leakage during fusion.

1136-Pos The C-terminal SNARE Motif of Yeast SNAP-25 Analog Sec9 Controls the Transition from Docking to Fusion

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Board B110

Association between v- (vesicle associated) and t- (target membrane) SNARE proteins results in the formation of a four helical bundle, which brings two membranes together to facilitate membrane fusion. Although the general topology of the SNARE complex is known, the process of complex formation has been elusive. We used site-directed spin labeling EPR to investigate the structure of a yeast SNARE system (Sso1/Sec9/Snc2p). Unexpectedly, we found that the C-terminal SNARE motif (SN2) of Sec9 was mostly unstructured in the complex bound to the membrane. However, the single fusion assay showed that the SN2 of Sec9 was necessary for the membrane fusion, although not essential for liposome docking. On the other hand, we also found that the C-terminal half of Sso1 was partially unstructured. Thus, we propose a structural model for the folding intermediate of the SNARE complex that might explain how SN2 controls the transition from the docking to the fusion.

1137-Pos Syncytia Formation Mediated By Viral Fusogens: From Early Fusion Pores To Cell-size Diameter Cytoplasmic Bridges

Evgenia Leikina[1], Andrew Chen[1], Jean-Philippe Richard[1], Kamran Melikov[1], Michael M. Kozlov[2], Leonid V. Chernomordik[1]

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Board B111

Cell fusion is a critical step in fertilization and development of diverse tissues such as muscles and bones. Fusion is also a process used by many viruses to infect cells. Formation of an initial fusion pore mediated by best characterized viral protein fusogens has been explored in many studies. However, the subsequent pore expansion to fully merge the volumes of the fusing cells and form a new cellular entity, is poorly understood. In this work we have studied late stages of cell-cell fusion mediated by baculovirus protein gp64 and influenza virus hemagglutinin. Syncytia formation progresses through opening, expansion and merger of multiple pores; or by an expansion of a single pore. Fusion pore expansion does not leave behind small vesicles and is accompanied by an increase in the cell contact area that compensates for the open lumen area. Pore growth is driven neither by membrane tension, nor by microtubule cytoskeleton as neither hypertonic shock nor microtubule stabilization by taxol affect syncytia formation. Effects of actin-modifying agents indicate that fusion pore(s) expansion is not driven by actin cytoskeleton but rather requires local F-actin cortex dissociation. We suggest that the driving force for the pore growth comes from the factors generating the negative line tension of the pore rim such as membrane bending proteins existing within the cell and involved in generation of highly curved membrane compartments such as endocytic vesicles, and intracellular transport intermediates.

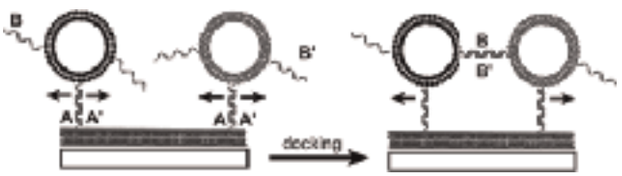
1138-Pos Kinetics Of A DNA-mediated Docking Reaction Between Tethered Vesicles

Yee-Hung M. Chan[1], Peter Lenz[2], Steven G. Boxer[1]

[1] *Department of Chemistry, Stanford University, Stanford, CA, USA*
[2] *Philipps University-Marburg, Marburg, Germany.*

Board B112

Recognition and binding between biological membranes are generally tightly regulated processes, and characterizing these reactions is important for many problems including cell adhesion and vesicle fusion. A model system consisting of tethered vesicles was used to measure the kinetics of docking of vesicle populations displaying complementary DNA oligonucleotides which are linked to the lipid anchor at the 5′ end.* Vesicles were first tethered to a supported bilayer by using a complementary sequence pair A/A′ by using a microfluidic device which creates physically separated populations of tethered vesicles. After the vesicles diffuse and mix, epifluorescence microscopy can capture collisions between pairs of vesicles, and docking mediated by B/B′ results in the vesicles' colocalization and tandem diffusion as shown schematically. The probability, P_{dock}, that a collision leads to docking was extracted from the data using a lattice-diffusion model of the system, and shows a dependence on the sequence and the number density of DNA per vesicle. These trends are explained by a reaction model which gives a scaling relationship between P_{dock} and relevant parameters.



1139-Pos Lipid Vesicle Fusion Event Studied By Molecular Dynamics At The Atomistic Scale

David J. Michel, Helmut Grubmueller

MPI for biophysical chemistry, Goettingen, Germany.

Board B113

Fusion of lipid vesicles and membranes is a fundamental intra- and inter-cellular transport mechanism, which lies at the heart of secretion, synaptic signal transmission, and viral infections. However, the cell membrane is by principle, a formidable barrier to fusion events and the ingenious biological systems designed by nature to enable cell fusion in a regulated and site-specific fashion is a challenge to understand.

Experimental studies suggest that is mediated, controlled, and triggered by a complex machinery of fusion proteins. However, the energetics of the actual fusion event must be essentially dictated by the phyico-chemistry of bilayer contact and merger. Hourglass-shaped stalk intermediates, which are believed to occur prior to the formation of a fusion pore, have been characterised by x-rays and electron tomography. However, the molecular basis and driving forces of these primary fusion steps are still largely unresolved.

Using different triggers such as, applying electric field, varying ion concentration and lipid composition, we aim at understanding the molecular mechanism of protein-free lipid vesicle fusion at the atomistic scale by means of molecular dynamics simulation.

1140-Pos Ca-dependent Non-secretory Vesicle Fusion In Secretory Cell

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*Yoshina-Ishii *et al.,JACS,**125,**3696 (2003); **127**, 1356 (2005); *Langmuir*, **22**, 2384 & 5682 (2006).

Board B114

We describe Ca-dependent exocytosis in the rat secretory cell line, RBL, using both excised patch and whole cell recording. Stable excised patches of 2–4 pF are easily obtained after disrupting the actin cytoskeleton with latrunculin A. Membrane fusion is triggered by solution containing 100–200uM free Ca. Capacitance and amperometric recording reveal that the large secretory granules (SGs, filled with serotonin) tend to be lost in membrane patches. The major vesicle pool on the excised patch is with much smaller size (non-SGs). The non-SG fusion is ATP-dependent and blocked by non-hydrolysable ATP analogue, AMP-PNP. This dependency is not neutralized by pre-incubating the patches with N-ethylmaleimide, implying that the ATP mechanism is not coupled to SNARE cycling. Application of high affinity PIP2 binding ligands, neomycin and PIP2 antibodies, cannot block non-SG fusion, indicating that ATP is not being used to maintain PIP2 levels. High concentrations of wortmannin and adenosine, which block PI3 and PI4 kinases, can however block the ATP effect. But the PI3 kinase inhibitor, LY294002, antibodies against PI3P and PI4P, and a PI-transfer protein that removes PI from membranes, do not block non-SG fusion. These results together raise significant doubts about the targets of the inhibitors in these experiments and tend to negate any role for phosphatydialinositides in non-SG fusion. We also examined some of the candidate Ca sensors for the non-SG fusion, including Dysferlin (DYSF), and Synaptotagmin 7 (Syt7). DYSF antibodies failed to block fusion, and fusion remains robust in syt7−/− mouse embryonic fibroblasts, indicating that these popular Ca sensors are not required for non-SG fusion. In summary, we have established new approaches for studying membrane fusion in excised patches. For the most part, the results negate prevalent hypotheses about non-SG fusion that may be related to the cell wound response.

1141-Pos Stability Of The HIV-1 Env Six-helix Bundle Affects Formation And Enlargement Of Fusion Pores

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Board B115

Fusion proteins of many viruses, including the HIV-1 envelope protein (Env), fold into a thermally stable trimer of hairpins, also known as a six-helix bundle. The bundle consists of three N-terminal helices that form a central coiled coil and three C-terminal helices that pack in an antiparallel orientation into the grooves of the coiled coil. The stability of the bundle is largely determined by the interaction of residues at positions “e” and “g” of the N-terminal helices with residues at positions “a” and “d” of the C-terminal helices. Conserved residues at these positions as well as mutations not participating in bundle stability were mutated to alanine to elucidate the relationship between fusion activity and bundle stability. The effect of reduced bundle stability was evaluated by measuring the extent of fusion, the kinetics of fusion, and pore enlargement for each mutant in a cell-cell fusion system. Mutations at positions expected to affect bundle stability were of much greater functional consequence to fusion than mutations on an external face of the bundle that should not participate in stability. Furthermore, substitution of residues near the turn region of the hairpins suppressed pore formation and enlargement more than residues removed from this region. We conclude that bundle formation is initiated by binding of C-terminii to the grooves in the turn region, followed by a zippering of the C-terminii along the length of the grooves until the bundle is complete.

Supported by NIH GM 27367.

1142-Pos Refinement of the Stalk Structure, the Intermediate State of Membrane Fusion, by X-Ray Anomalous Diffraction

Wangchen Wang[1], Lin Yang[2], Huey W. Huang[1]

[1] *Rice University, Houston, TX, USA*

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Board B116

The lipidic intermediate state called a stalk has been proven to be a critical step involved in membrane fusion. This state was long speculated, but was experimentally verified in DPhPC multilayers only recently. Its structure and energy are crucial for the energy pathway of membrane fusion and are of particular interest to fusion theorists. Here we attempt to refine the stalk structure by using a bromine-labeled phospholipid di18:0(6,7Br)PC and the multiwavelength anomalous diffraction (MAD) method (BJ 91, 736, 2006). We used the MAD method to extract the diffraction intensities from Br atoms alone and used the swelling method to determine their phases. The electron density distributions of Br atoms and of the whole lipids were constructed and the results are consistent with the stalk structure discovered previously. However the liquid-crystalline nature of the lipid assembly limits the diffraction resolution, in particular the spatial resolution of the bending region of the lipid monolayers. We used the experimental data to determine the Debye-Waller factors by model fittings and monte-carlo simulations. The analysis shows that the Debye-Waller factors for the in-plane positions are considerably larger than that for the vertical positions. We believe that the in-plane disorders include the variability of the center of the stalk position within the unit cell. By artificially removing the Debye-Waller factors, we obtained a refined stalk structure. This work demonstrated a method for refining complex lipidic structures by using low-resolution diffraction data.

1143-Pos Direct Measurements of the Rate of HIV-Cell Fusion by an Enzyme Delivery Assay

Kosuke Miyauchi, Gregory Melikian

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Board B117

Human Immunodeficiency Virus (HIV) envelope glycoprotein (Env) mediates virus entry by inducing fusion between the virus

and cell membrane. Binding of the Env surface subunit, gp120, to CD4 receptor exposes cryptic gp120 sites that, in turn, bind co-receptors, CXCR4 or CCR5. Formation of gp120/CD4/co-receptor complexes triggers conformational changes in the transmembrane gp41 subunit leading to fusion. The kinetics of HIV-1 fusion is one of the determinants of the virus resistance to entry inhibitors and neutralizing antibodies. To directly measure the rate of HIV fusion, pseudoviruses expressing HIV Env and bearing the viral core-incorporated enzyme, beta-lactamase (BlaM), were generated. The viruses were pre-bound to cells in the cold, and fusion was triggered by warming cells to 37°C. HIV-cell fusion was measured by the delivery of BlaM into the cytosol of susceptible target cells, using a fluorogenic substrate. The kinetics of fusion was measured by time-of-addition of a high concentration of the gp41-derived C34 peptide. Both CCR5- and CXCR4-using Env exhibited almost identical rates of fusion that proceeded after a short lag time of a few minutes. This lag time was completely eliminated after pre-incubation of viruses and cells at 22°C, consistent with creation of a temperature-arrested stage observed in a cell-cell fusion model. We found that fusion of CXCR4-using viruses was more sensitive to C-peptide inhibition than fusion of viruses that utilized CCR5 as co-receptor. These results suggest that, despite the identical fusion rates, the folding of CCR5-using Env into a 6-helix bundle is faster than that of CXCR4-using Env and that, thus, helical bundle formation is not the rate-limiting step of HIV-cell fusion. Our data demonstrate the applicability of a BlaM assay for mechanistic studies of HIV entry.

Supported by NIH grant GM054787.

1144-Pos The Membrane Potential Across The Target Membrane Regulates Fusion For Class II Viral Fusion Proteins

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Board B118

We have recently shown that cell-cell fusion induced by Semliki Forest Virus (SFV) E1 protein, a class II fusion protein, is blocked by trans-positive potential across the target cell membrane. In contrast, fusion induced by class I viral fusion proteins was independent of the transmembrane voltage. We hypothesized that, virus itself as well as viruses containing other class II viral fusion proteins utilize, in addition to a low pH trigger, transmembrane voltage of target membranes for fusion. To test this hypothesis, fluorescently labeled pseudoviruses bearing SFV E1 were generated and their fusion with a cell plasma membrane was monitored by confocal microscopy. Consistent with our previous cell-cell fusion data, a trans-positive (+40 mV) potential strongly blocked viral fusion with a plasma membrane. On the other hand, virus-cell hemifusion (lipid mixing) was independent of transmembrane voltage and occurred immediately after lowering the pH. That is pore formation is dependent on voltage, but hemifusion is not. These observations were extended by testing the voltage-dependence of fusion induced by another class II viral protein - from venezuelan equine encephalitis virus (VEEV) - and a protein similar to, but distinctly different from, class II proteins - the G protein of vesicular stomatitis virus (VSV). Similar to SFV E1, fusion induced by these viral glycoproteins exhibited a clear dependence on transmembrane potential. These results suggest that the inhibition of fusion by trans-positive potentials across the target membrane is common for class II and related viral fusion proteins.

Supported by NIH GM27367 and GM057454.

1145-Pos DNA-mediated Fusion of Lipid Vesicles

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Board B119

Hybridization of DNA-oligonucleotides coupled to lipids at the 5′ end can be used tether liposomes to supported lipid bilayers* and to mediate docking**, but fusion has not been observed because the duplex DNA acts as a spacer between the two membranes. A modified synthesis allows coupling of the lipid to the 3′ end of the DNA strand. Now, hybridization between 5′ and 3′ DNA-lipids on different vesicles may bring the membrane surfaces closer together. This approximates the geometry believed to be relevant in SNARE protein-mediated neuronal fusion. Reaction of vesicles displaying complementary DNA linked at the 5′ and 3′ ends leads to both lipid and content mixing, indicating DNA-mediated vesicle fusion is occurring as illustrated schematically. The rate of mixing shows a dependence on the copy number and sequence of the complimentary DNA. Progress towards visualizing individual fusion events using mobile vesicles tethered to supported bilayers by fluorescence microscopy is described.

1145.01-Pos Role of Cholesterol in HIV-1 gp41 Fusion Peptide Interaction with Plasma Cell Membranes

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[1] *Illinois Institute of Technology, Chicago, IL, USA*
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Board B120

Interaction between HIV-1 and the host cell membrane is an essential step in retrovirus proliferation. The N-terminal gp41

*Yoshina-Ishii *et al., JACS*, ***125***, 3696 (2003); **127**, 1356 (2005); *Langmuir*, **22**, 2384 & 5682 (2006).

**Chan *et al.* submitted

fusion peptide of HIV-1 is a crucial player in viral entry into a target cell. Emerging evidence of the presence of cholesterol-enriched microdomains in a plasma membrane makes it important to understand the role of cholesterol in fusion peptide - membrane interactions. In this work we investigate the effect of cholesterol concentration on the capability of HIV-1 gp41 peptide to insert into lipid monolayers using insertion assays, epifluorescence microscopy, and synchrotron X-ray reflectivity (XR) and grazing incident-angle X-ray diffraction (GIXD) techniques. Lipid monolayer formed at the air-liquid interface models an approximate environment of the outer leaflet of a target cell membrane where viral fusion occurs. A target membrane was mimicked with 1,2-Dipalmitoyl-sn-glycero-3-phophocholine (DPPC), Cholesterol, and binary mixtures of Cholesterol:DPPC (with molar ratio between 0.15 and 0.85). In the binary mixture cholesterol has been found to be shielded from aqueous environment with DPPC headgroups at every cholesterol concentration used, in agreement with the *umbrella* model. The fusion peptide was found to insert readily into all lipid monolayers without destroying the morphology of a membrane. Binary mixture monolayers with low content of cholesterol were found to be the most vulnerable to the peptide insertion among all examined membrane mimics. XR results indicate that decrease in thickness of a membrane induced by gp41 fusion peptide insertion is more pronounced for monolayers with higher cholesterol content suggesting an important role of cholesterol in fusion pore formation. FP was found to penetrate the pure cholesterol and DPPC monolayers fully, while in mixed cholesterol:DPPC monolayers the fusion peptide inserts only partially, interacting strongly with cholesterol benzene rings.

1146-Pos New Tools For Biophysical Studies Of Hemagglutinin-mediated Membrane Fusion

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Medical Research Council, NIMR, Mill Hill, United Kingdom.

Board B121

The influenza Hemagglutinin (or flu HA) is responsible for immobilising influenza virus particles on the surface of the host cell and for fusion between the host cell and the viral membranes. Following endocytosis, HA-mediated membrane fusion occurs during intracellular transport, when viral surface proteins are being exposed to low pH in the endosomes. Low pH triggers a conformational change upon which the functional segment of HA named "fusion peptide" becomes solvent exposed and contacts the host's membrane. The mechanisms by which the flu HA facilitates subsequent merging of lipid membranes and opening of a fusion pore in the merged membrane are unclear: in particular it is not known how many HA molecules are needed for membrane fusion or whether structural intermediates of HA contribute to different stages of fusion. In order to obtain a more detailed understanding of the basic mechanisms underlying HA-mediated membrane fusion we use a combination of molecular biology and single molecule biophysical techniques. We are expressing mutant HA for single molecule imaging of the fusion process. We also use a photoactivatable caged proton to allow for a fast pH jump, in order to study the fusogenic conformational change of single HA molecules at high time resolution. The caged proton did not affect HA-induced membrane fusion. Also our biochemical studies indicate that the known low-pH refolding of HA2 correlates with a structural rearrangement of HA2 C-terminal region.

(Supported by MRC and The Royal Society)

1147-Pos Possible Mechanism Of Endosomal Escape Of Tat: Membrane Fusion Of Intraluminal Vesicles With Late Endosomes

Sung-Tae Yang, Leonid V. Chernomordik, Kamran Melikov

NICHD, Bethesda, MD, USA.

Board B122

Cellular uptake of the HIV Tat-derived peptide enables to deliver a variety of the bioactive molecules including proteins and nucleic acids. It has recently been recognized that endocytosis/pinocytosis plays a major role in the mechanism of Tat-mediated internalization. Following the endocytosis, however, the mechanism of escape of Tat from endosomes remains elusive. Here we suggest a model for its endosomal escape. We demonstrate that Tat induces both fusion and leakage of the liposomes containing lysobisphosphatidic acid (LBPA), which is a characteristic lipid of intraluminal vesicles of multivesicular body. In contrast, liposomes in which LBPA was replaced by its stereoisomer phosphatidylglycerol were unaffected by Tat. Both Tat-induced fusion and leakage of LBPA containing liposomes were promoted by acidic pH and elevated Ca^{2+} concentration. In addition, Tat efficiently induced membrane fusion of LBPA with the liposomes mimicking the late endosomal lipid composition. Based on these results, we propose that the electrostatic trans-interaction of anionic LBPA with cationic Tat initiates the membrane fusion of intraluminal vesicles with late endosome and enables the escape of Tat from the endosome.

Membrane Structure - I

1148-Pos Electroelastic Coupling between Membrane-Embedded Charges and Membrane Fluctuations: Continuum Multi-Dielectric Treatment

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There is growing interest in understanding effects due to electrostatically promoted membrane-water fluctuations, which may influence voltage gating in proteins. Membrane geometry is significantly influenced by electrostatic interactions with membrane-