Biomaterial Interfaces Division
Room: 105 - Session BI-TuA

Protein-Membrane Interactions

Moderator: L.J. Gamble, University of Washington

4:00pm **BI-TuA7 Membrane Binding, Structure and Regulation of the PTEN Phosphatase**, ***M. Lösche***, Carnegie Mellon University and National Institute of Standards and Technology **INVITED**

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is an important regulatory protein and tumor suppressor that performs its phosphatase activity as an interfacial enzyme at the plasma membrane-cytoplasm boundary. Acting as an antagonist to phosphoinositide-3-kinase (PI3K) in cell signaling, it is deleted in many human cancers. Despite its importance in regulating the levels of the phosphatidylinositoltriphosphate $PI(3,4,5)P_3$, there is little understanding of how PTEN binds to membranes, is activated and then acts as a phosphatase. The interaction of the protein with membranes is highly dynamic and is at least partially controlled by the in-plane fluidity of the bilayer. PTEN function requires multiple, lipid-specific interactions with the target membrane. These interactions regulate enzyme activity as well as lateral and subcellular distribution of the enzyme. From studies of the membrane association of PTEN under well-defined conditions in model systems, we report recent insights in the structural and functional basis for PTEN membrane binding and regulation.

4:40pm **BI-TuA9 Biomimetic Lipid Membrane Systems Applied to HIV-1 Neutralization**, ***G. Hardy***, *M. Alam*, *S. Zauscher*, Duke University

Evidence suggests that lipid membrane interactions with rare, broadly neutralizing antibodies (NAbs), 2F5 and 4E10, play a critical role in HIV-1 neutralization. The **objective** of this research is to understand how lipid membrane properties, such as chemical head groups, lipid domain organization, and lipid diffusivity contribute to 2F5/4E10 membrane interactions and antigen localization at the membrane interface, with the ultimate vision of guiding immunogen designs. Recent immunization studies have shown that induction of antibodies that avidly bind the gp41-MPER antigen is not sufficient for neutralization. Rather, it is required that antigen designs induce polyreactive antibodies that recognize MPER antigens as well as the viral lipid membrane. However, the mechanistic details of how membrane properties influence NAb-lipid and NAb-antigen interactions remain unknown. **Methods:** To understand how membrane properties contribute to 2F5/4E10 membrane interactions, we have engineered biomimetic supported lipid bilayers (SLBs) and have developed a surface plasmon resonance (SPR) spectroscopy based assay that monitors antibody binding to thiol monolayers, which mimic salient surface chemical properties of lipid membranes.

Our **results** showed that 2F5 and 4E10 bound preferentially on charged and hydrophobic thiol surfaces. This supports the theory that NAbs interact with lipid head groups before embedding into hydrophobic tail regions. We have also engineered supported lipid bilayers (SLBs) whose compositions mimic both the host cell membrane and the HIV-1 envelope. These SLBs have planar surfaces that facilitate quantitative surface-characterization techniques such as high-resolution scanning-probe imaging, detection of fluorescence recovery after photobleaching, and neutron reflection measurements. Using these characterization techniques we have begun to i) visualize domains of lateral membrane organization; ii) determine SLB domain diffusivity; iii) determine differences in adhesion force (surface energy) of domains; and iv) correlate these membrane properties with NAb-membrane binding and NAb/antigen localization.

Our research is **significant** in that it provides a biologically relevant system to screen interactions of lipid-reactive antibodies with a broad range of diagnostic tools. Because current 2F5/4E10 immunogens have not yet elicited antibodies with the required membrane reactivity it is important to reveal the role of lipids underlying antibody-antigen binding. This information will elucidate how membrane properties can enhance antigen recognition and thus enable the design of next generation HIV-1 immunogens.

5:00pm **BI-TuA10 Interactions between the Norovirus and Glycosphingolipids Studied with Cell Membrane Mimics**, ***M. Bally***, Chalmers University of Technology, Sweden, *G. Larson*, University of Gothenburg, Sweden, *F. Höök*, Chalmers University of Technology, Sweden

The determining initial step of viral infection is mediated by highly specific recognition events between the viral shell and ligands on the host cell surface. Detailed understanding of virus-membrane interactions is therefore of central importance to the development of new antiviral therapies, new vaccines and high-performance diagnostics platforms. In this context, assays based on cell-membrane mimics have a considerable potential, as they offer the possibility to study interactions between controlled ligand mixtures with surface-sensitive techniques, while presenting the ligand in a more native configuration. Potentially relevant characteristics such as membrane fluidity, ligand mobility and their ability to organize into microdomains can be preserved [1].

In this work we investigate the interactions between norovirus capsids and phospholipid bilayers containing glycosphingolipids (GSL). The norovirus is well known as the major causative agent of acute viral gastroenteritis, but its human target cells and the precise mechanism for viral entry are still poorly understood.

In a first example, we identify galctosylceramide - a major glycosphingolipid in the small intestine- as a ligand for Norovirus-like particles (VLP) from the Dijon strain. Quartz Crystal Microbalance and Atomic Force Microscopy studies on GalCer-containing supported lipid bilayers reveal that a clustered arrangement of the glycosphingolipids plays a crucial role in promoting a firm attachment of the pathogen to the lipidic membrane, most likely via the establishment of multiple contacts between the particle and the membrane [2].

We further investigated the interaction between individual fluorescent GSL liposomes and surface immobilized VLPs with single virus particle sensitivity. Besides representing the ultimate sensitivity for diagnostics purposes, our method makes it possible to study weak interactions. Kinetics analysis of vesicle residence times over large time scales reveals a highly heterogeneous behavior and yields information on multivalency, on the presence of domains and the role of cell-membrane curvature.

As exemplified here, simplified membrane models have a unique potential in providing fundamental understanding on the contribution of individual components to complex biological processes [3].

[1] Bally et al. "Liposome and Lipid Bilayer Arrays Towards Biosensing Applications", SMALL, 2010.

[2] Bally et al. "Interaction of Single Virus-like Particles with Vesicles Containing Glycosphingolipids", submitted.

[3] Bally et al. "Norovirus GII.4 virus-like particles recognize galactosylceramides in microdomains of planar supported lipid bilayers", submitted.

5:20pm **BI-TuA11 Binding of C-reactive Protein to Lipoprotein Nanoparticle Mimics: A Gel Electrophoresis Study**, ***M.S. Wang***, *S.M. Reed*, University of Colorado Denver

C-reactive protein (CRP) is an acute phase serum protein involved in inflammation that recognizes pathogenic agents, and activates complement. Because CRP levels can increase by over 1000-fold from basal levels within 72 hr in response to inflammation, it has been used as a biomarker to predict the risk for cardiovascular disease (CVD). In addition, CRP has been shown to bind oxidized low density lipoprotein (oxLDL) through the phosphatidylcholine (PC) headgroup that is exposed on the surface of oxLDL and has been found to co-localize with atherosclerotic lesions. It is suggested that CRP was deposited in these lesions when engulfed by macrophage while it is still bound to oxLDL. Because LDL and the other lipoprotein particles (LPPs) have diameters (d) in the nanometer range, they can be considered as biological nanoparticles. In fact, LPPs are classified into four major categories according to their size and density: high density lipoprotein (d=8-13nm), LDL (d=20-30nm), intermediate density lipoprotein (d=30-40nm), and very low density lipoprotein (d>40nm). Moreover, the physiological functions of LPPs are greatly influenced by the size. For example, the presence of sdLDLs (d<25.5nm) are associated with increased risk of coronary artery disease (CAD) and diabetes; while increased levels of HDL are considered to be atheroprotective. Therefore, LPP profiling has emerged as a tool to more accurately assess the development of metabolic risk factors such as CAD. While the PC moieties on oxLDL are likely ligands for CRP recognition, it is still unclear why CRP binds only oxLDL although PC is expressed on native LDL and on all cell membrane. In this work, we correlated the effects of particle size (i.e. membrane curvature) to CRP binding using LPP mimics. To this end, we engineered LPP mimics using lipid-coated gold nanoparticles (PC-AuNPs) to explore the influence of LPP size on CRP binding. Binding analysis of CRP to the mimics was performed using gel electrophoresis (GE). The migration of CRP and PC-AuNP was directly visualized after electrophoresis, and the presence of CRP was confirmed using Western blots. The overlapping bands from the gel and Western blot confirmed that CRP bound to the PC-AuNPs and co-migrated during GE. Together, we demonstrated that 1) PC-AuNPs are size-separable, 2) the lipid layer around

the PC-AuNP remained intact during GE, and 3) CRP binds to a relevant LDL-sized PC-AuNP mimic. This is, to our knowledge the first use of GE to separate lipid-coated nanoparticle and to evaluate non-covalent binding of protein-nanoparticle interactions.

Authors Index

Bold page numbers indicate the presenter