1793-Pos Board B685
Detecting Microparticles in Human Intestine with Synchrotron Based X-Ray Beamline
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Purpose: Microparticles (MP) are non-biological inorganic bacterial-sized particles (0.1-0.7μm) from endogenous formation (calcium phosphate) or dietary intake (AlSi and Ti02), which may contribute to the pathogenesis of inflammatory bowel disease (IBD). This study was to establish the method for MP detection in human intestine.
Methods: Biopsy tissue samples were collected from 5 IBD and 5 cancer screening patients undergoing colonoscopy.Tissue sections were cut at variable thickness (5, 10 and 20 um) prior to analysis on the Very Sensitive Elemental and Structural Probe Employing Radiation from a Synchrotron (VESPERS) microprobe beamline at the Canadian Light Source. They were mapped with micro-X-ray fluorescence spectroscopy to determine the spatial location of the MPs at an X-ray excitation energy of 13 keV. The beam had a spot size of 4 μm with an 8 μm step-size and 15 second dwell time. Tested sample conditions include formalin fixation, section thickness, mounting substrate (regular glass slide and Lexan film), and embedding medium (OCT).
Results: Randomly selected areas in the mucosa or submucosa were observed. There was significant contamination in formalin-fixed tissue samples causing non-specific background. VESPERS exhibited greatest sensitivity with tissue sections of 20 um in thickness and demonstrated the best signal versus noise ratio. Specific signals of MPs from samples on Lexan film were much better with relatively lower background than those on glass slide. The OCT was free of contamination.
Conclusion: Synchrotron-based X-ray microprobe techniques can be used for detecting and mapping MPs in normal and IBD affected human intestine. This will allow us further elucidation of the immunopathological role of MPs in IBD patients. Frozen fresh tissue sections of 20 um thickness mounted on Lexan film is the superior method of sample processing.

Cryo Electron Microscopy & Reconstruction

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Structure of Membrane-Bound Porcine Factor VIII
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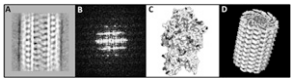
Human Factor VIII (FVIII) is the co-factor of the serine protease Factor IXa (FIXa) in the membrane-bound tenase complex. Defects or deficiency of FVIII cause Hemophila A. Recombinant FVIII concentrate is the most effective cure for Hemophilia A. Porcine FVIII is highly homologous to human FVIII (84% sequence identity).
In this work, we present for the first time, direct structural information for porcine FVIII, helically organized on lipid nanotubes (LNT) at closest to physiological conditions. This was achieved by combining direct structural data from Cryo-EM and molecular modeling. The 3D-structure at 15Å resolution supports our hypothesis that the functional FVIII membrane-bound organization differs from that of the 3D-crystal structure in solution. Fitting the FVIII domains form the human crystal structure and the porcine homology model, within the 3D-Cryo-EM map, will allow us to define the inter- and intra-protein interfaces important for the FVIII function.
Resolving the membrane-bound porcine FVIII structure by Cryo-EM will provide a scaffold for mapping functionally important FVIII interaction sites.



A: 2D-Class average of porcine-FVIII, B: FFT of A. C: surface-representation of human-FVIII crystal-structure. The side-chains different with porcine-FVIII are shown in black. D: 3D-reconstruction of porcine-FVIII molecules onto LNT.

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A Platform for Studying Structures of Membrane Proteins Inserted into Nanodiscs
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Membrane proteins are critical to many biological processes and account for 70% of all know pharmacological targets. However, there are only a few hundred unique membrane protein structures available in protein data bank due to the difficulty in forming crystals for x-ray crystallography or electron crystallography. Several methods have been developed to study structures of membrane proteins in lipid membrane environments (e.g. helical, planar, or spherical membranes). Among those, insertion of membrane proteins in lipid nanodiscs offers some benefits in terms of size, homogeneity, and curvature. In the studied structures, the nanodisc is considered part of the whole complex. Here, we proposed to develop a platform to computationally remove the nanodisc contributions. This is essential for the proteins not inserted at a fixed position in nanodisc (i.e. proteins are floating in the nanodisc). In addition, the geometry of the nanodisc will be used to aid the determination of two of the three Euler angles. This will decrease the computational requirement, increase accuracy of the determined orientation of each individual protein particles, and thus increase the resolution of the determined structure.

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Cryo-EM Studies of an Engineered Small Interfering RNA Nano-Ring used as a Gene Silencing Therapeutic
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Specific small interfering RNAs (siRNAs) designed to silence oncogenic pathways can be used for cancer therapy. Three-dimensional nanoscale RNA scaffolds functionalized with therapeutic siRNAs have the potential for broad use in nanotechnological and biomedical applications. The design strategies of RNA scaffolds employ assembly principles borrowed from natural RNA structures. We functionalized an RNA nanoscaffold with six therapeutic siRNAs, visualized the structure with electron cryo microscopy, and tested the therapeutic constructs in vitro and in cell lines. Our Cryo-EM reconstruction was in agreement with the nanoscaffold design and confirmed the correct formation of the siRNA functionalized nanoring. Cell culture experiments showed significant gene silencing with the siRNA nanoscaffold. Our results demonstrate that RNA-based therapeutic nanoparticle have high potential for siRNA silencing.

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9 Å Structure of Trimeric HIV-1 Envelope Glycoprotein in an Activated State
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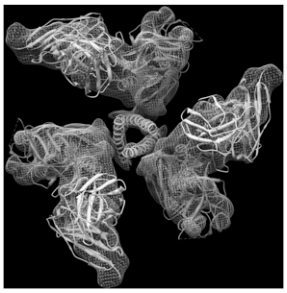
The HIV envelope glycoprotein (Env) binds to CD4 and a chemokine co-receptor to mediate fusion of viral and target-cell membranes required for infection. We have previously shown that the unbound Env is a mushroom-shaped trimer with a stem composed of three membrane-anchored gp41 subunits capped by three gp120 subunits. We also showed that binding to CD4 and the co-receptor site binding Fab 17b leads to a significant outward movement of gp120 and to a rearrangement of density in gp41, resulting in an "open" conformation. Soluble constructs bearing the complete ectodomain of Env (gp140) display conformations that closely resemble the structure of native unliganded trimeric Env, and importantly, show identical ligand-induced transitions. Here we show that a key structural signature of the open Env conformation is a three-helix motif composed of α-helical segments derived from highly conserved, non-glycosylated N-terminal regions of the gp41 trimer. The three N-terminal helices in this novel, pre-fusion intermediate are much less compactly packed than in the post-fusion, six-helix bundle state. These findings suggest a new structural template for designing immunogens that can elicit antibodies targeting HIV at a vulnerable, pre-entry stage.



1798-Pos Board B690
Computational Separation of Conformational Heterogeneity using Cryo-Electron Tomography and 3D Sub-Volume Averaging
Gabriel A. Frank[1], Oleg Kuybeda[2], Alberto Bartesaghi[1], Mario J. Borgnia[1], Guillermo Sapiro[3], Sriram Subramaniam[1].