Detecting Microparticles in Human Intestine with Synchrotron Based X-Ray Beamline

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In this work, we present for the first time, direct structural information for porcine Factor VIII (FVIII) membrane-bound organization differing from the human crystal structure and the side-chains were well resolved. 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 particle, and thus increase the resolution of the determined structure.

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.

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.

A 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 particle, and thus increase the resolution of the determined structure.
LRRK2 dimerization may be mediated primarily by the COR domain. Docking of a prokaryotic ROC-COR homologous structure suggests LRRK2 dimerization may be mediated primarily by the COR domain. Immuno precipitation experiments confirm the predicted COR-COR interaction. Furthermore, competition experiments showed the COR domain inhibits LRRK2 kinase activity in vitro. Our data reveal the COR domain to play a critical role at the dimerization interface and in the regulation of LRRK2 kinase activity.

Structural Studies of Dynamin-Related Protein 1 (DRP1) Provide Mechanistic Insight into Mitochondrial Fission.

Electron microscopy (cryo-EM) studies have been used to gain mechanistic insight into the mammalian mitochondrial fission complex. Several similarities and differences have been found between the yeast and mammalian systems. In solution, Drp1 forms stable tetramers, which represent the pre-assembled state of Drp1. The size of this complex (~330 kDa) provides a suitable target for 3D image reconstruction. Additional interactions with GTP analogs and/or synthetic liposomes promote Drp1 self-assembly into extended helical oligomers. The 3D structures of these helices will be determined to elucidate interactions that mediate Drp1 self-assembly. The effects of GTP hydrolysis on the Drp1 helical oligomers are also being studied to determine how Drp1 promotes outer mitochondrial membrane fission. Future studies will examine interactions between Drp1 and partner proteins in the mitochondrial fission complex.

Electron Microscopy Structure of Dimeric LRRK2 Reveals a Structural and Regulatory Role of the COR Domain

The resolution of the X-ray structure of LRRK2 suggested that altered GTPase and kinase activities may be implicated in pathogenesis. Biochemical experiments suggest LRRK2 kinase activity may be regulated by dimerization. Electron microscopy imaging and single-particle 3D reconstruction, at a resolution of 22 Å, reveal that LRRK2 purified from mouse brain forms elliptical homodimers with each monomer having a concave lune shape. Dimerization occurs via a single two-fold rotation axis, in which the two monomers interact via two main interfaces. Details in the electron microscopy map provide insight into the domain organization of LRRK2. Docking of a prokaryotic ROC-COR homologous structure suggests LRRK2 dimerization may be mediated primarily by the COR domain. Immuno precipitation experiments confirm the predicted COR-COR interaction. Furthermore, competition experiments showed the COR domain inhibits LRRK2 kinase activity in vitro. Our data reveal the COR domain to play a critical role at the dimerization interface and in the regulation of LRRK2 kinase activity.

**End databank: Unified Data Resource for 3DEM**

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3D cryo-electron microscopy (3DEM) is emerging as a powerful method for determining structures of large biological assemblies in solution and in the cell, enabling elucidation of complex biological interactions that are integral to understanding the inner workings of cellular machinery, and yielding novel insights into fundamental biological processes. Hundreds of 3DEM experiments are now reported in the literature each year and more than 1,500 structures are now available through EMDataBank. The project website, EMDataBank.org, serves as a “one-stop shop” resource for global deposition and retrieval of 3DEM data and model data in the 3DEM public archives that will be rolled out within the 3DEM module of the new wwPDB deposition & annotation tool. We will also provide an update on our current candidate methods for assessing reliability of 3DEM maps and map-derived models in collaboration with community scientists, with the goal of creating validation criteria that will permit independent assessment of 3DEM data by expert and non-expert scientists.

**Domain Organization of Membrane-Bound Factor VIII**

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Factor VIII (FVIII) is the blood coagulation protein which when defective or deficient causes for hemophilia A, a severe hereditary bleeding disorder. Activated FVIII (FVIIIa) is the co-factor to the serine protease Factor IXa (FIXa) within the membrane-bound Tenase complex, responsible for amplifying its proteolytic activity more than 100,000 times, necessary for normal blood clotting. FVIII is composed of two non-covalently linked peptide chains: a light chain holding the membrane interaction sites and a heavy chain holding the main FIXa interaction sites. The interplay between the light and heavy chains in the membrane-bound state is critical for FVIII biological efficiency.

Here, we present our cryo-electron microscopy and structure analysis studies of human FVIII light chain (LC), as helically assembled onto negatively charged single lipid bilayer nanotubes (LNT). The resolved FVIII-LC membrane-bound structure at 20 Å, supports aspects of our previously proposed FVIII structure from membrane-bound two-dimensional (2D) crystals, such as only the C2 domain interacts directly with the membrane [Stoilova-McPhie 2002]. The light chain is oriented differently in the FVIII membrane-bound helical and 2D crystal structures based on electron microscopy data and the 3D structure solved by X-ray [Ngo 2008]. The flexibility of the FVIII-LC domain organization in different crystal packing (3D, 2D and helical) is essential to understand the FVIII membrane-bound organization and its significance for hemostasis.

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