A method for atom probe analysis of lipid bilayers and membrane proteins

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The cell membrane envelops all living cells and acts as a barrier between the cell and its surroundings. It consists of a phospholipid bilayer, which hosts a class of proteins referred to as membrane proteins. Membrane proteins control the transport of ions, nutrients, signaling molecules in and out of the cell, and are the target of roughly 60% of all pharmaceutical drugs today[1]. It is estimated that about one third of the human genome encodes for membrane proteins.

The consensus in structural biology is that the three dimensional structure of a protein is intimately linked to its function. Thus, if the structure of a membrane protein is known, a candidate drug that will interact with the protein may be developed[2]. In spite of this, to date only 1% of this class of proteins has been structurally resolved, mainly due to inherent limitations in the preparation and data acquisition of samples for the two most common techniques for protein structure analysis; X-ray crystallography and NMR. While cryo electron microscopy has had remarkable success in recent years[3], the structure of most membrane proteins remains elusive, and a fast and reliable method for structure determination is still lacking.

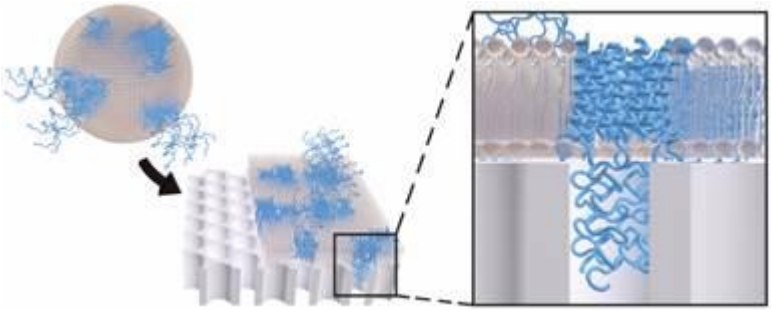

Figure 1: Schematic illustration depicting protein-containing supported lipid bilayer formation from protein-containing vesicles on a mesoporous silica substrate. The pores host parts of the proteins (enlargement).

We have developed a new type a methodology for structural analysis of membrane proteins in their native lipid bilayer environment, using atom probe tomography (APT). Atom probe tomography has some unique virtues for nanoscale analysis, such as equal sensitivity for all elements across the periodic table and near-atomic resolution in three dimensions.

The most critical step for atom probe analysis is the sample preparation, whereby ultra-sharp needles that can withstand vary large mechanical stresses (on the order of GPa[4]) without mechanical fracture must be prepared. At the same time care must be taken to ensure that the proteins do not denature due to the change in environment. This has been accomplished by immobilizing protein-containing lipid bilayers in silica. Protein-containing lipid vesicles were introduced to a mesoporous silica substrate, upon which they readily formed supported lipid bilayers through spontaneous vesicle adsorption and rupture.[5] The porous substrate is of great importance to the setup by simultaneously providing the proteins with accessible space underneath the bilayer (as in the native cell membrane) and additional robustness. The lipid bilayers were then silicified (captured in glass), yielding a silica monolith wherein the membrane protein is stabilized and encapsulated through the exchange of aqueous surroundings for silica. This structure was sufficiently strong to permit atom probe analysis, while still maintaining the native protein structure.

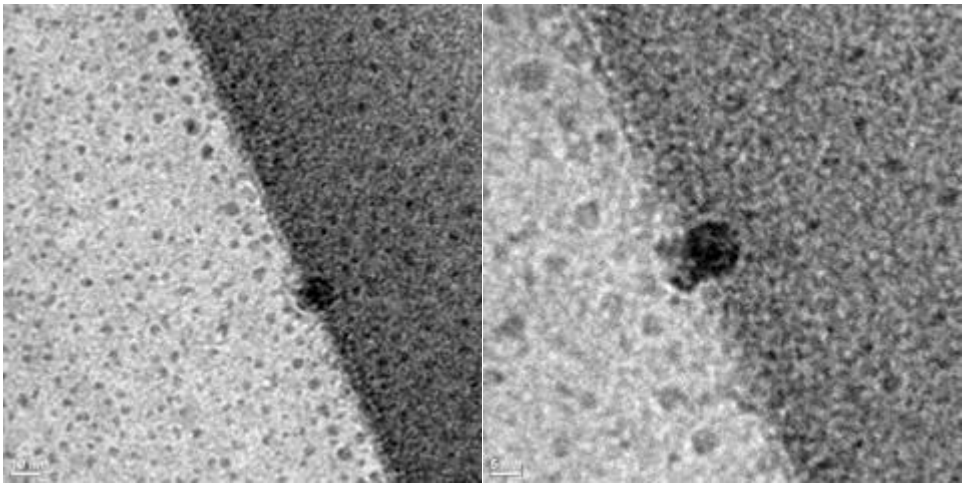

Figure 2: TEM micrographs of a silica-embedded lipid bilayer containing an aquaporin complex.

Herein, we present results from atom probe studies of a silicified lipid bilayer and discuss the possibilities and limitations of this approach.

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