

BINDING TO A LIPID LAYER: A LOW MATERIAL CONSUMING APPROACH FOR STRUCTURAL ANALYSIS OF MEMBRANE PROTEINS.

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Difficulties of over-expression of membrane proteins represent a bottleneck for their structural analysis. We have developed strategies based on the specific binding of membrane proteins to a lipid layer spread at the air/water interface, which allowed decreasing to 1-2 microgram the amount of proteins per structural experiment. A first approach is the production of 2D crystals that can be analysed by electron crystallography. It involves a binding step of the micellar proteins to the lipid layer, followed by reconstitution of the proteins into a lipid bilayer¹ (Fig. 1). 2D crystals of different his-tagged proteins have been obtained after specific binding to a Ni-chelating lipid layer, and of charged proteins after electrostatic binding to a charged lipid layer. Since the first 2D crystals obtained in 1999, up to 13 different membrane proteins have been crystallized by the lipid layer approach by us or other groups revealing the general use of this approach. A second strategy consists to bind and to orient single proteins onto the lipid layer. The unique orientation of bound proteins provided rapidly an averaged projection useful *per se* or a missing projection crucial for a 3D reconstruction by single particles analysis. This has been successfully used in the case of YvcC, a multidrug transporter from *Bacillus subtilis*, member of the ATP-binding cassette superfamily, highly homologous to each half of human multidrug-resistance P-glycoprotein and to several other bacterial half-ABC transporters. Controlled and partial detergent removal from YvcC-lipid micelles allowed the production of particularly interesting lipid-detergent-YvcC ring-shaped particles, about 40 nm in diameter, well suited for single particle analysis. Analysis reveals a preferred orientation of the particle onto the grid leading to a reconstruction with a large missing cone. Binding of these histidine-tagged ring-shaped particles to lipid layers functionalized with a Ni²⁺-chelating head group generated a preferential perpendicular orientation, eliminating the missing cone in the final three-dimensional reconstruction. From such analysis, a computed volume has been determined to 2.5 nm resolution giving a detailed insight into the structural organization of this half-ABC transporter within a membrane. (fig. 2)

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Fig. 1: 2D crystallization of membrane protein by the lipid layer methods. a) binding of micella lipid/protein/detergent micelles to a functionalised lipid layer at the air/water interface. b) Reconstitution of proteins into a lipid bilayer upon detergent removal. c) 2D crystallization.

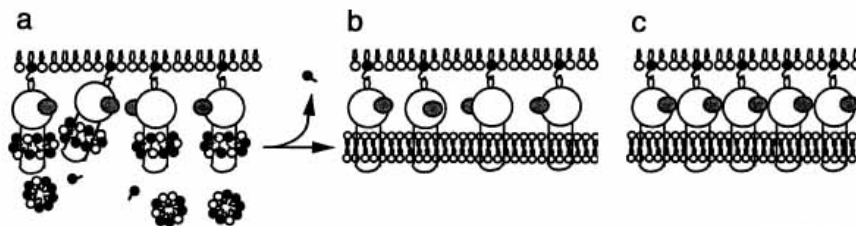


Figure 2 3D reconstruction of the bacterial ABC transporter YvcC at 2.5nm resolution by cryo-EM. A) ring-like structures of 24 homodimers obtained by partial detergent removal. The average of particles spread onto a holey grid reveals mainly top views. B) side views obtained by binding onto an Ni-NTA lipid layer. C) 3D reconstruction. D) NBD domains. E) transmembran domains

