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Review

Zooming in on disordered systems: Neutron reflection studies of proteins associated with fluid membranes ☆

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ABSTRACT

Neutron reflectometry (NR) is an emerging experimental technique for the structural characterization of proteins interacting with fluid bilayer membranes under conditions that mimic closely the cellular environment. Thus, cellular processes can be emulated in artificial systems and their molecular basis studied by adding cellular components one at a time in a well-controlled environment while the resulting structures, or structural changes in response to external cues, are monitored with neutron reflection. In recent years, sample environments, data collection strategies and data analysis were continuously refined. The combination of these improvements increases the information which can be obtained from NR to an extent that enables structural characterization of protein–membrane complexes at a length scale that exceeds the resolution of the measurement by far. Ultimately, the combination of NR with molecular dynamics (MD) simulations can be used to cross-validate the results of the two techniques and provide atomic-scale structural models. This review discusses these developments in detail and demonstrates how they provide new windows into relevant biomedical problems. This article is part of a Special Issue entitled: Interfacially Active Peptides and Proteins. Guest Editors: William C. Wimley and Kalina Hristova.

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1. Introduction

Transmembrane and membrane-associated proteins play crucial roles in a broad range of cellular processes [1]. At least 30% of mammalian genes encode membrane proteins. Their roles in cells are indispensable, for example as mediators of cell signaling [2–4], information transduction and processing [5], as well as in cellular morphogenesis. Membrane proteins control selectivity of energy, material and information transfer into and out of the cell and between intracellular compartments, as well as vesicular transport within the cell [1]. Consequently, anomalies often result in disease states, ranging from cancer and premature senescence to neurological disorders [3]. In addition, since membranes provide the natural barrier between the cell and its environment, toxin and pathogen entry into cells inevitably involve protein–membrane interactions [6]. Yet, established techniques to determine molecular details of the association of proteins with lipid bilayers – the matrix they associate with – lag better-developed methods of structural biology such as protein crystallography and NMR spectroscopy dramatically. The leading reason is that proteins embedded or adsorbed to functionally intact, in-plane fluid lipid bilayers are notoriously difficult to study, as the classical crystal-based or solution-based characterization techniques are inadequate. As a result, our knowledge of high-resolution structures of membrane proteins in their natural membrane environment, and consequently also of mechanisms of their action and cellular control, is critically underdeveloped.

For more than 25 years, membrane protein structures have been determined by X-ray diffraction from crystals grown from detergent-solubilized protein solutions [7,8]. This technique provides atomic-scale 3D structures. However, it shows detergent molecules at those protein surfaces natively embedded in the membrane. While the art of crystal growth remains tedious, this technique still provides the bulk of the more than 400 unique high-resolution structures of transmembrane proteins know to date [9]. While membrane-peripheral proteins are usually not amenable to crystallization in detergent, many such membrane proteins are buffer soluble, because they shuttle between the cytosol and membrane surfaces within the cell, and can therefore be directly crystallized from detergent-free solutions. In both cases, protein–membrane association can only be estimated (transmembrane proteins) or is not known at all. In comparison to X-ray crystallography, electron diffraction from two-dimensional (2D) lipid/protein co-crystals [10,11], protein crystallization in cubic lipid phases [12] and solid-state NMR [13] or NMR on proteins solubilized in nanodiscs [14] have only played minor roles in the determination of high-resolution internal membrane protein structures so far. However, all these methods yield crucial information when it comes to determine the structure of protein/membranes complexes using scattering techniques.

X-ray and neutron scattering techniques, in distinction from crystal diffraction, provide capabilities to characterize disordered systems but lack the intrinsic resolution to study protein–membrane complexes on length scales shorter than nanometers. Nevertheless, in connection with complementing information from other sources they provide a novel window into high-resolution structures. In particular, neutron reflectometry as a surface-sensitive scattering technique has the potential to characterize protein–membrane complexes with unprecedented resolution, following the development of carefully engineered sample formats and dedicated data evaluation and modeling techniques. Indeed, recent progress in this area has been encouraging. Measuring the neutron reflection (NR) from engineered planar membrane mimics which retain their in-plane lipid fluidity [15], we showed that the out-of-plane localization of transmembrane proteins with known internal structures can be achieved with Ångstrom precision [16]. Extending work by Schlossman and collaborators using X-ray reflectometry [17,18], it was recently demonstrated that both the penetration depth into the lipid membrane and orientation on the bilayer can be determined for membrane-associated peripheral proteins with high precision using NR [19–21]. Using molecular dynamics (MD) simulations to interpret NR results we showed that the PTEN tumor suppressor, a lipid phosphatase whose structure was partially determined with X-ray crystallography [22], has slightly different atomic-scale structures in the crystal, in solution and in its membrane-bound state [23]. Finally, since NR characterizes protein structures on single bilayers, this technique is capable to determine structural changes that follow external cues *in situ*. This was demonstrated by the recent discovery that membrane-bound full-length gag from HIV-1 undergoes a dramatic structural reorganization upon nucleic acid binding [24]. Similarly, extensive studies of the conformation of HIV-1 Nef determined the impact of the composition and structure of the lipid membrane on protein organization [25,26]. In this review we describe recent accomplishments and discuss the technological developments that lay the basis for these advances.

2. Methods

2.1. Artificial lipid bilayer membranes

Current structural studies of lipid bilayer membranes and associated proteins with NR require the preparation of the biological interface on solid supports that are flat and atomically smooth over a large area (tens of square centimeters) [27]. This excludes investigations of natural membranes *in vivo* but can be readily achieved with artificial membrane systems. Types of artificial, supported membrane systems relevant for NR are solid-supported membranes [28], hybrid membranes [29–33], tethered membranes [34,35], polymer-cushioned membranes [36–39], and floating membranes [40,41]. Comprehensive reviews on supported lipid bilayer membranes can be found in the literature [42–45]. Langmuir monolayers of lipids at the air–water interface constitute a separate class of model systems [46].

A versatile lipid model system, optimized to meet several crucial requirements for high-resolution NR studies, is the sparsely tethered lipid bilayer membrane (stBLM, see Fig. 1). Grafted onto a planar solid support (typically a Si wafer or glass slide) that is terminated with a 10 nm to 200 nm thick gold film, stBLMs are excellent mimics of natural membranes with respect to lipid fluidity and structure [15]. They can be prepared using a large variety of lipids, including zwitterionic (phosphatidylcholine or phosphatidylethanolamin) or anionic lipids (phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, or phosphatidic acid) with saturated or unsaturated chains, sterols, and functional components such as phosphatidylinositol phosphate lipids. Spacing of the synthetic tether lipids, grafted to the terminal gold film of the substrate through thiol chemistry and (typically) an oligo(ethyleneoxide) linker [47–49] (Fig. 1), is achieved by co-adsorption with β-mercaptoethanol (βME). stBLMs can be prepared virtually defect-free [50], which prohibits unspecific protein adsorption to exposed support areas that would interfere with structural characterization of the membrane-associated protein. Of similar importance, stBLMs are stable for the time scale of NR experiments which can be on the order of days with current technology [51]. The lipid membrane in an stBLM is separated from the solid support which otherwise might interact with incorporated proteins [47]. stBLMs exhibit low interfacial roughness, because of their proximity to the substrate (≈20 Å). From a scattering point of view, this is important for achieving high resolution of the underlying structures [27]. On the other hand, the proximity of the substrate to the membrane and interference with the tethering chemistry – typically a molar fraction of 50% of the lipids located in the inner lipid leaflet are tether lipids – may limit the reconstitution of membrane proteins with large extramembraneous domains.

A variety of surface-sensitive techniques can be applied to the stBLM platform, aiding the characterization of biological systems of interest. For example, the gold-coated solid support allows for surface plasmon resonance (SPR) spectroscopy [21] and electrical impedance spectroscopy (EIS) [47–50]. Yet, the gold films are so thin that the system remains amenable to characterization with fluorescence techniques, for