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Review

Encapsulated membrane proteins: A simplified system for molecular simulation☆

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ABSTRACT

Over the past 50 years there has been considerable progress in our understanding of biomolecular interactions at an atomic level. This in turn has allowed molecular simulation methods employing full atomistic modelling at ever larger scales to develop. However, some challenging areas still remain where there is either a lack of atomic resolution structures or where the simulation system is inherently complex.

An area where both challenges are present is that of membranes containing membrane proteins. In this review we analyse a new practical approach to membrane protein study that offers a potential new route to high resolution structures and the possibility to simplify simulations.

These new approaches collectively recognise that preservation of the interaction between the membrane protein and the lipid bilayer is often essential to maintain structure and function. The new methods preserve these interactions by producing nano-scale disc shaped particles that include bilayer and the chosen protein. Currently two approaches lead in this area: the MSP system that relies on peptides to stabilise the discs, and SMALPs where an amphipathic styrene maleic acid copolymer is used. Both methods greatly enable protein production and hence have the potential to accelerate atomic resolution structure determination as well as providing a simplified format for simulations of membrane protein dynamics. This article is part of a Special Issue entitled: Biosimulations edited by Ilpo Vattulainen and Tomasz Róg.

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1. Introduction — the membrane protein dichotomy

Atomic resolution structures of proteins have revolutionised our understanding of many of the processes that underpin biology. The same structures have also enabled the growth of molecular simulation studies that have done much to uncover the complex structural dynamics that underpin protein function. However, progress across all protein classes has not been equal, with atomic resolution studies of membrane proteins, in particular, lagging significantly behind soluble proteins. For example extraction and purification of the first membrane protein (glycophorin A) did not occur until more than 30 years after a similar feat was achieved for soluble proteins. The first high resolution structure of a membrane protein, that of the photosynthetic reaction centre, was not solved until 1984 [1], nearly 20 years after this landmark was reached with soluble proteins [2]. This slow progress in the study of membrane proteins continues to the present day with only 1700 high resolution structures of membrane proteins compared to more than 110,000 of soluble proteins being found in the PDB in 2015 [3]. This is despite membrane proteins having a clear and significant importance in biology The obvious question posed by this comparison is *Why has success in studying membrane proteins been so limited cf. that of soluble proteins*?

1.1. The challenge of membrane protein extraction and purification

The real challenge in the production and study of membrane proteins occurs at the first step of the purification process: the need to separate the target protein from all other proteins present in the membrane. Over-expression of the chosen protein helps by increasing its proportion in the membrane, but to continue the purification process the membrane must be solubilised. Solubilisation of biological membranes is trivial using a detergent (or strictly speaking surface active agent, "surfactant") will lead to a rapid fragmentation of the membrane. The resulting solution contains a heterogeneous mix of 'detergent/lipid', 'detergent/protein' and 'detergent only' micelles. However, the general issue that has challenged membrane protein scientists for more than 40 years is that the target protein contained in the 'membrane protein/detergent' micelles often possesses low activity and/or stability. This makes the study of these proteins after purification very challenging.

1.2. Complexity of the bilayer: why are detergents an imperfect solution?

At first sight the 'detergent/protein' micelle seems like the perfect solution. The protein is contained in a small particle made up of a reagent that has both hydrophobic and hydrophilic character. The hydrophobic moieties replace the acyl chains of the lipid in membrane stabilising the surfaces of the protein that naturally interact with the hydrophobic part of the membrane. The hydrophilic moieties ensure that the resulting mixed micelle is soluble in water. The real situation however, is somewhat different.

The first issue is that the membrane itself is a very complex structure made up of a number of physico-chemically distinct layers. It is in this complex layered environment that membrane proteins have evolved to function. This means that the outer surface of the membrane protein that is in contact with the membrane is not necessarily simply hydrophobic, but instead may contain polar amino acids which engage in complex interactions with the membrane in order to stabilise the conformation of the protein; perhaps most well-known of these being the positively charged arginine "snorkel" [4]. It is therefore clear that to maintain the activity of the protein any detergent system has to replicate this complex membrane structure as closely as possible. Such replication is seldom possible using the available detergents. This effect is further exacerbated when it is considered that most natural membranes are not made up of a single lipid type, but are mixtures of many different types of lipids. Such mixtures include phospholipids with different head-groups and acyl chains (varying in length and degree of unsaturation) as well as non-phospholipid constituents such as cholesterol. To replicate this is an almost impossible task for any single detergent or indeed combination of various detergents.

A second major issue with detergent solubilisation is that detergents themselves have the potential to disrupt the intramolecular interactions within a membrane protein that are essential for structural integrity. The choice of a detergent therefore always involves maintaining a delicate balance between successful solubilisation and retention of the target membrane protein's native structure. In practise this is very rarely achieved using detergents.

1.3. A new dawn in membrane protein solubilisation

In this review we discuss a range of new approaches to the production of membrane proteins that recognise the importance of the lipid bilayer in determining the correct structure and function of said species. These methods aim to preserve the lipid bilayer in the locality of the protein with the aim of preserving 'native-like' activity and stability. We describe how these techniques have developed from early proofs-of-concept that still require some use of detergent through to the most recent methods that dispense with detergent entirely. In the latter part of the review we describe how a wide range of structural and biochemical analytical techniques can be applied to the study of SMALP-encapsulated membrane proteins, and finally argue that the time is now ripe for these new particles to be the basis for molecular simulation studies.

2. The role of nanoparticles in membrane protein purification

In recent years, the basic format of an amphipathic molecule capable of solubilising a lipid bilayer and/or maintaining membrane proteins (MPs) in solution has been radically reinterpreted. The first development was amphipols (APs), amphipathic polymers invented by Popot [5] and coworkers that have been shown to bind tightly to MPs and preserve their solubility in water. Like detergents, APs largely replace the lipid bilayer leaving a protein/AP particle that is analogous to the protein/detergent micelle. More recently methods have been developed that attempt to preserve the membrane context around the protein. By achieving this scientists aim to more effectively preserve protein stability, structure and function. At the forefront of this new movement are groups that have developed systems that extract disc shaped particles from membranes. These new particles share a common format: the MP sits in a disc-shaped piece of membrane (generally close to 10 nm in diameter) that is stabilised by a reagent that interacts with the edge of the disc. Two systems currently lead in this area and utilise different methods for stabilising the particles. First to show utility were groups that used membrane scaffold proteins (MSPs) as stabilising agents while later on the scene have been groups that use an organic polymer based on styrene maleic acid (SMA). In the next section both systems will be analysed.

2.1. Membrane scaffold protein (MSP) nanodiscs

The development of nanodiscs as a method for MP encapsulation was first reported in 2002 by Stephen Sligar and co-workers. Their nanodisc system contains three components: the MP, a lipid bilayer