Beyond detergent micelles: the advantages and applications of non-micellar and lipid-based membrane mimetics for solution-state NMR

Kai Klöpfer[1,2] and Franz Hagn[1,2,*]

[1]Bavarian NMR Center at the Department of Chemistry, Technical University of Munich, Ernst-Otto-Fischer-Str. 2, 85747 Garching, Germany
[2]Institute of Structural Biology, Helmholtz Zentrum München, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany

[*]correspondence should be addressed to F.H. (Tel: +49-89-289-52624, Fax: +49-89-289-52669, e-mail: franz.hagn@tum.de)

# of pages: 32
# of figures: 6
# of tables: 3

Keywords: lipids, membrane proteins, nanodiscs, NMR, structure

Abstract (366/500 words)

Membrane proteins are important players in signal transduction and the exchange of metabolites within or between cells. Thus, this protein class is the target of around 60 % of currently marketed drugs, emphasizing their essential biological role. Besides functional assays, structural and dynamical investigations on this protein class are crucial to fully understanding their functionality. Even though X-ray crystallography and electron microscopy are the main methods to determine structures of membrane proteins and their complexes, NMR spectroscopy can contribute essential information on systems that (a) do not crystallize and (b) are too small for EM. Furthermore, NMR is a versatile tool for monitoring functional dynamics of biomolecules at various time scales. A crucial aspect of such studies is the use of a membrane mimetic that resembles a native environment and thus enables the extraction of functional insights. In recent decades, the membrane protein NMR community has moved from rather harsh detergents to membrane systems having more native-like properties. In particular, most recently phospholipid nanodiscs have been developed and optimized mainly for solution-state NMR but are now also being used for solid-state NMR spectroscopy. Nanodiscs consist of a patch of a planar lipid bilayer that is encircled by different (bio-)polymers to form particles of defined and tunable size. In this review, we provide an overview of available membrane mimetics, including nanodiscs, amphipols and bicelles, that are suitable for high-resolution NMR spectroscopy and describe how these advanced membrane mimetics can facilitate NMR studies on the structure and dynamics of membrane proteins. Since the stability of membrane proteins depends critically on the chosen membrane mimetic, we emphasize the importance of a suitable system that is not necessarily developed for solution-state NMR applications and hence requires optimization for each membrane protein. However, lipid-based membrane mimetics offer the possibility of performing NMR experiments at elevated temperatures and studying ligand and partner protein complexes as well as their functional dynamics in a realistic membrane environment. In order to be able to make an informed decision during the selection of a suitable membrane system, we provide a detailed overview of the available options for various membrane protein classes and thereby facilitate this often-difficult selection process for a broad range of desired NMR applications.

Table of Contents

1. Introduction

Membrane proteins (MPs) are essential players in cellular signaling [1]. Major efforts have been undertaken to obtain functional and in particular structural insights for this protein class [2]. MPs are positioned in strategic locations and control the exchange of information and metabolites across biological membranes. These processes are regulated by various stimuli, such as small molecule ligands, partner proteins and membrane potential. Since they have such a large variety of biological functions, MPs are of utmost importance for drug design, representing around 70 % of all current drug targets [3].

In order to obtain a better understanding of MP function and regulation, structural information is required. However, MPs only represent about 2% of all structures deposited in the protein data bank so far (https://blanco.biomol.uci.edu/mpstruc), a situation that is most likely caused by difficulties in the production and purification of MPs in a functional form and in a sufficient amount for structure determination. A major part of this problem is the time-consuming process of selecting an appropriate membrane-mimicking environment that can support both function and stability for a particular MP of interest. Most commonly, detergent micelles are used as a membrane mimetic for MP preparation and subsequent structural investigations [4]. The general advantages of using detergent micelles are easy handling and the availability of a large variety of different detergent scaffolds, offering the possibility to screen for an optimal choice for a given MP of interest. Detergent micelles have been widely used for solution-state NMR spectroscopy; however, only a small number has proven to be suitable for high-resolution work (**Tab. 1**). These detergents commonly form relatively small micelles providing the benefit of favorable NMR relaxation properties for inserted MPs. In many cases, this leads to a functionally folded MP preparation, in particular in case of high thermodynamic MP stability (**Fig. 1a**). However, the drawback is that these detergents generally tend to be quite harsh and thus are often not compatible with more labile MPs, i.e. detergents lower their thermodynamic stability and lead to incorrectly folded or unfolded protein (**Fig.1b**) [5]. In addition, detergent molecules often bind to hydrophobic cavities of MPs that are important for enzymatic activity (**Fig. 1c**). Another, and probably the most important, issue is that detergent micelles might interfere with partner protein binding, leading to unfolding or precipitation of soluble protein domains (**Fig. 1d**) [6, 7]. In addition, for studying complexes of two different MPs, detergents that have been individually optimized for each protein are not necessarily compatible with each other, leading to structural perturbations or unfolding of one or both proteins in the complex. In general, MP structures can be markedly modulated by a chosen membrane mimetic [8]. This dilemma has been recognized in past decades and was addressed by the introduction of more lipid-like membrane mimetics that have the potential to resolve most or all of those issues [9]. This process was further motivated by the fact that crystal structures of MPs solubilized in detergents often contain bound lipids, emphasizing the beneficial effect of a lipid environment for their structure and stability [10]. Here, we will summarize the most common non-micellar membrane mimetics that are relevant and suitable for NMR-based investigations on the

structure and dynamics of MPs. Even though the emphasis will be put on the more recent and most actively developed nanodisc technology, we will also provide a detailed comparison with other systems and highlight the advantages and drawbacks of each system for specific MP systems and NMR applications. Finally, we will give practical guidelines on relevant membrane mimetics for particular MP systems and their complexes. Furthermore, we will describe a straightforward strategy to obtain NMR resonance assignments for MPs in nanodiscs that, due to their large size, would not permit recording of most triple resonance experiments. In doing so, we aim to provide a thorough and critical overview of current developments in the field of membrane mimetics so as to enable an informed search for the most suitable system for NMR studies of a particular MP of interest.

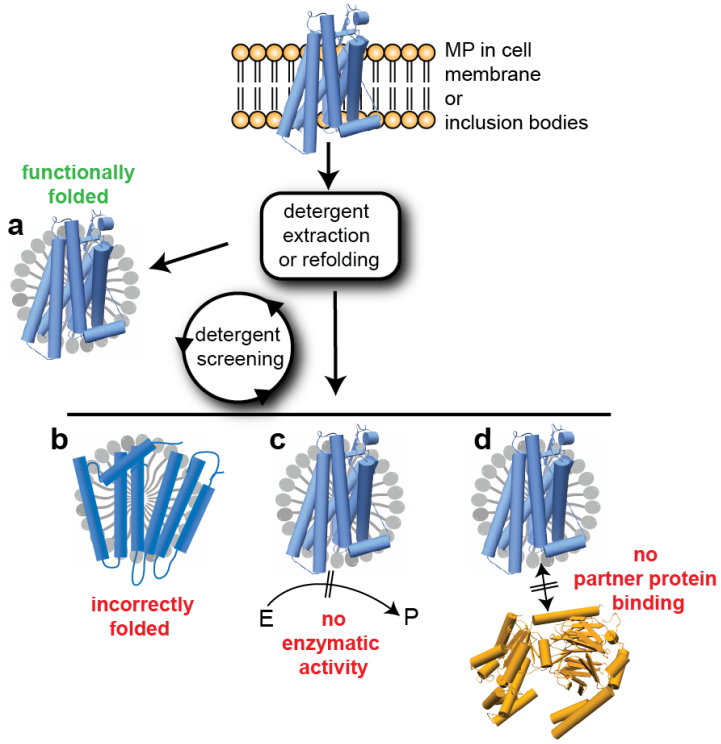

Fig. 1: Common problems with using detergents for membrane protein extraction and refolding. Extracting a membrane protein from cells or refolding of inclusion bodies using detergent micelles can lead to various scenarios that might require a tedious optimization procedure. (a) The best case is that the membrane protein tolerates the initial detergent used for extraction. Such preparations can then readily be investigated by NMR. Alternative, more likely options are that (b) the membrane protein is incorrectly folded in the chosen detergent, (c) a putative enzymatic activity is inhibited by detergent molecules or (d) the detergent prevents interaction with a soluble partner protein or even leads to its unfolding. Scenarios (b) – (d) would require an iterative screening effort to identify detergents that are compatible with the desired application and that are still suitable for NMR spectroscopy. Even though these problems can be solved individually, it is almost impossible to find a detergent that can be used for all applications and, in particular, for NMR experiments at elevated temperatures.

2. Non-micellar membrane mimetics

Since a large variety of membrane mimetics are available, it is important to obtain a rough overview of the particular systems and their characteristics. The most commonly used membrane mimetic are detergent micelles. As discussed in the introduction section, micelles can have adverse effects on MP stability and on that of their complexes. Even though suitable detergents can be found by screening, this membrane mimetic can be considered harsher than lipid-based systems (**Fig. 2**). A mixture of detergents and lipids, termed bicelles [11], shows a more lipid bilayer native-like behavior but, due to the presence of detergent molecules, can still lead to denaturation and destabilization of MPs and their partner proteins. A polymer-based amphipathic membrane mimetic, called amphipols [12], are more native-like and also seem to be suitable for use as a refolding system for certain MPs. The most native membrane mimetics are pure lipid systems. In particular, phospholipid nanodiscs [13], due to their tunable and defined size, are well-suited for solution-state NMR [14, 15]. The size of a nanodisc is defined by the length of a lipid binding protein or, more recently, a polymer [16] that wraps around the hydrophobic rim of the phospholipid bilayer. A pure liposome or immobilized lipid bilayer system would be the most native system for studying MPs structure and function. However, these assemblies are very large and thus not suitable for solution-state NMR or most other structural methods. For this reason, these systems will not be covered here, although we should point out that they can be used for solid-state NMR, as described in recent reviews [17-20].

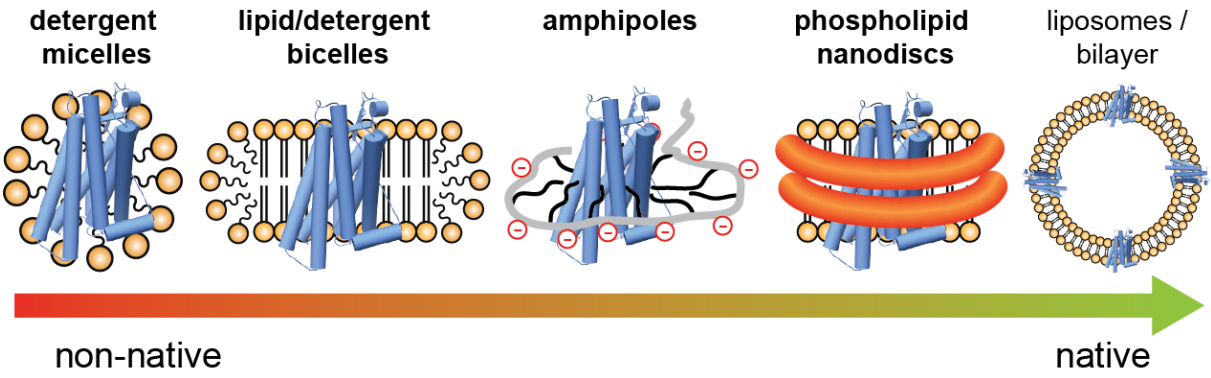

Fig. 2: Commonly used membrane mimetics for solution and solid-state NMR. Those written in bold letters have been demonstrated to be suitable for solution-state NMR. Liposomes or immobilized lipid bilayers are being used for solid-state NMR. As a rough guide, these membrane mimetics are sorted according to their ability to resemble a native membrane environment, with nanodiscs and liposomes being the most native. For details of the different mimetics, see main text.

In this review, we will focus on non-micellar membrane mimetics with an emphasis on phospholipid nanodiscs that can nowadays be assembled with a large variety of amphipathic molecules, such as proteins, peptides and polymers, and thus are a versatile tool for NMR as well as for other structural and biophysical and biochemical experiments.

2.1 Phospholipid bicelles

Bicelles are discoidal lipid aggregates composed of long-chain phospholipid and detergent [11]. The morphology of bicelles depends on the total phospholipid concentration, the ratio of the constituents, and the temperature [21, 22]. In contrast to detergent micelles, bicelles contain a planar lipid bilayer formed by the long-chain phospholipid. This planar region is surrounded by a rim of detergent that shields the long-chain lipid tails from water [22-24]. As is the case for any phospholipid bilayer system, the lipid composition in the planar region of bicelles can be adjusted to the particular requirements of the MP under investigation. This is particularly useful for membrane-attached proteins that require certain lipid head-group charges [25] of chemical scaffolds for their specific interaction. Suitable detergents for bicelle formation are bile salts, such as CHAPS, or short-chain lipids, such as dihexanoylphosphatidylcholine (DHPC). The advantage of this system is its size scalability by simple variations of the parameter q which represents the molar ratio between the long-chain and short-chain lipid (or detergent) component. A simple equation can be used to roughly estimate the radius of the bilayer region of the bicelle R by using the parameter q and half the bilayer thickness r: $R = ½\, rq\, [\pi + (\pi^2 + 8/q)^{½}]$ [24] (**Fig. 3**). The overall diameter D of a bicelle can thus be expressed as: $D = 2R + 2r$. A typical value for the phospholipid bilayer membrane thickness is 4 nm, i.e. $r = 2$ nm. For a q value of 0.5, i.e. a two-fold excess of short-chain lipid, bicelles with a diameter $2R$ of the planar region of 8 nm and an overall diameter $2R + 2r$ of 12 nm are obtained. Bicelles that are suitable for solution-state NMR experiments typically have q-values between 0.15 and 0.5, resulting in a diameter of between 6.4 and 12 nm.

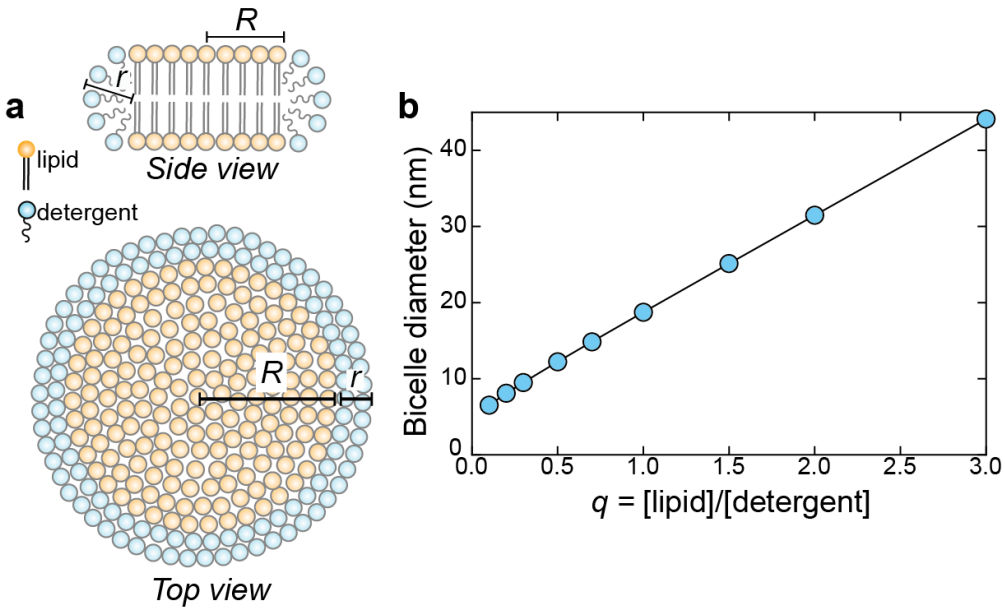

Fig. 3: Structure and size dependency of phospholipid bicelles. (a) The planar region consists of phospholipids (orange) and the hydrophobic rim of the bilayer is comprised of short-chain lipids or detergents (blue). The size of a bicelle can be controlled by the ratio q of the two components (lipids *versus* detergent). R: radius of the planar region, r: radius of the hydrophobic rim (half the thickness of the lipid bilayer). The resulting bicelle diameter is: $2R + 2r$. (b) Plot of the bicelle diameter against the value of q, using an empirical formula for R as mentioned in the main text. Bicelles with q > 2.5 (diameter > 40 nm) spontaneously align in the magnetic

field. For solution-state NMR, bicelles with *q*-values between 0.15 and 0.5 (6.4 - 12 nm diameter) are most commonly used.

Due to their large size and the anisotropic magnetic properties, bicelles with $q > 2.5$ (diameter of > 40 nm) spontaneously align in the magnetic field and can be used as an alignment medium for the measurement of residual dipolar couplings (RDCs) of soluble proteins [26, 27]. However, due to the presence of a short-chain lipid or detergent, similar problems arise as with detergent micelles, leading to the destabilization of MPs and their binding partners. Despite this limitation, due to their planar structure, bicelles provide a more native-like environment than detergent micelles. Since their size can be fine-tuned in an easy manner, this system is suitable for a wide range of MPs and has been extensively used for solution NMR studies [28-31]. More recently, an optimal *q*-value of 0.5 was determined that leads to the most realistic bilayer properties for solution-state NMR applications [32].

2.2 Amphipols

Amphipols are polymers with amphipathic, i.e. hydrophobic and polar, properties (**Fig. 4a**) that can solubilize MPs and form stable and homogenous particles [33] suitable for solution-state NMR [34]. Amphipols stabilize inserted MPs and can be used for MP refolding and thus facilitate biochemical and biophysical studies. In particular, NMR [34, 35] and cryo-electron microscopy [36-39] of MPs has been conducted in amphipols. Here, we will emphasize the use of amphipols in MP folding and stabilization and their benefits for solution-state NMR.

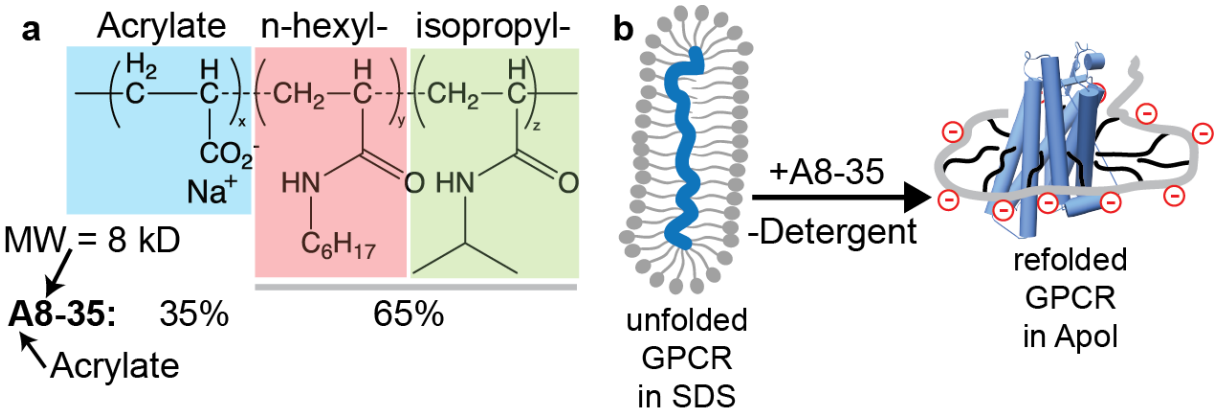

Fig. 4: Chemical structure, nomenclature and applications of amphipols. (a) Amphipols are acrylate polymers that have been grafted at the carboxylate position with alkanes such as n-hexyl- or isopropyl- moieties to yield an amphipathic polymer (amphipol). The nomenclature of amphipols consists of a capital letter (A: acrylate-derived), followed by a number indicating the average molecular weight of the polymer in kDa and a negative number that indicates the content (in %) of negative charges (i.e. acrylate side chains). (b) A major application of amphipols is the refolding of MPs from an SDS-solubilized form. Addition of amphipols followed by detergent removal with Biobeads leads to gentle refolding in relatively high yields.

2.2.1 Chemical structure and properties of amphipols

The most commonly used amphipol is A8-35, where a relatively short polyacrylate chain (~ 35 %) is randomly grafted with around 9 octylamine and 14 isopropylamine units [12, 33] (**Fig. 4a**). The nomenclature of amphipols begins with a capital letter, where A stands for acrylate, followed by a number that indicates the molecular weight of the monomeric modified polymer. The latter number represents the content of the charged monomer, where -35 indicates the percentage of the negatively charged acrylate moieties. Thus, A8-35 stands for an acrylate polymer with a molecular weight of 8 kDa that contains, after grafting the acrylate side chains with octylamine and isopropylamine, around 35 % negative charge. A large variety of modified amphipols have been introduced, ranging from zwitterionic amhipols [40], fluorophore adducts [41] to phosphocholine-based [42], sulfonated [43] and glycosylated non-ionic variants [44]. Unfortunately, A8-35 is the only amphipol that is commercially available, restricting the use of this system. However, for NMR applications, A8-35 can be purchased in a per-deuterated form, a prerequisite for high-resolution NMR. Despite the restricted access to amphipols through commercial sources, these polymers can be quite easily produced in house, as described recently [45].

2.2.2 Applications of amphipols

The initial idea behind the development of amphipols was to stabilize MPs that do not tolerate a detergent environment or where a detergent micelle is too flexible for structural studies. More specific applications came up over the years, such as immobilization to surfaces [46] or controlled delivery of solubilized MPs to preformed membranes [47]. Transfer of MPs from detergent into amphipols is simply achieved by the addition of amphipol followed by dialysis or treatment with hydrophobic polystyrene beads (Biobeads or Amberlite) to remove residual amounts of detergent [48]. Amphipols can be easily produced in a hydrocarbon deuterated form (i.e. with C-H universally replaced by C-D) for subsequent high-resolution NMR experiments of inserted MPs [34, 49]. The quality of solution-NMR spectra of the model protein bacteriorhodopsin (bR) has been recently compared in various membrane mimicking media. It was found that amphipols promoted the long-term stability of bR and permitted the acquisition of high-quality 2D-[^{1}H,^{15}N]-TROSY experiments and enabled partial resonance assignment [35].

In particular, amphipols gained attention through the idea that they are suitable for refolding studies of MPs and generally lead to higher yields than detergent micellar systems. A prominent example is the application to G-protein coupled receptors (GPCRs) [50]. These essential MPs mediate signal transduction across the cell membrane by allosteric conformational changes that are induced by binding of an agonist ligand to the extracellular side. An activated GPCR is then able to stimulate GTP

exchange within the associated G-protein, thus mediating the activation of a multitude of signaling pathways [51, 52]. However, production of this protein class is very challenging and often only possible in eukaryotic cell systems [53]. For NMR applications, with the requirement for high-level isotope labeling, bacterial expression hosts, such as *E. coli*, are desirable. Since production of GPCRs in a functionally folded form is very difficult in *E. coli*, efforts have been made to optimize production in the form of insoluble inclusion bodies and to refold the protein into a suitable membrane environment [54]. Unfortunately, the refolding step into detergent micelles is very inefficient for GPCRs resulting in very low refolding yields [50]. Amphipols seem to be better suited for refolding of this protein class GPCRs (**Fig. 4b**) with reported overall yields of up to 50 % for human BLT1 receptor [50]. Thus, since cutting-edge isotope labeling is possible in *E. coli*, refolding from inclusion bodies appears to be a suitable route for the preparation of functional GPCRs for NMR.

2.3 Phospholipid nanodiscs

Despite the advantages and the progress in the development of non-micellar membrane mimetics, the described systems do not provide a planar and detergent-free phospholipid bilayer surface. Such native lipid bilayer properties and the associated lateral pressure characteristics are in many cases essential for proper MP functionality. Furthermore, binding of peripheral MPs to their partner proteins or a plain membrane surface is only possible in a detergent-free lipid environment (see **Fig. 1**).

Since pure lipid systems such as liposomes are too big for solution-state NMR experiments, novel strategies are required for the stabilization of homogenous lipid particles of defined size. This issue has been resolved by the use of lipid binding proteins, peptides or polymers that encircle a patch of lipid bilayer and lead to the formation of lipid discs in the nanometer diameter range, so called nanodiscs [16, 55, 56].

Here, we will focus on the four major nanodisc systems: membrane scaffold protein (MSP), styrene-maleic acid (SMA) copolymer, Saposin and amphiphilic peptide nanodiscs. We will describe these components as well as the assembly procedure of these nanodisc systems and provide additional information on the advantages in each case as well as their specific applications with a focus on solution NMR studies.

2.3.1 Membrane scaffold protein (MSP) nanodiscs

The recent introduction of phospholipid bilayer nanodiscs as membrane mimetics [55, 57, 58] appears promising for studying membrane proteins in a native-like environment by solution NMR. The formation of nanodiscs is based on the observation that apolipoproteinA-I (ApoA-I) can wrap around small lipid bilayer patches creating small membrane-like particles of defined size [59, 60] (**Fig. 5a**). In living cells, ApoA-I is a main constituent of high-density lipoprotein particles that is essential for the transport of lipids and cholesterol to the liver. This task is facilitated by the amphipathic nature

of ApoA-I that is used to stabilize these hydrophobic assemblies [61]. Different versions of ApoA-I have been engineered for biophysical studies, and these are called membrane-scaffolding proteins (MSPs) [55]. Ideally, two copies of MSP form a disc-like particle or nanodisc [55, 62] of a well-defined diameter. The size of these nanodiscs is governed by the length of the MSP. Nanodisc assembly can be easily achieved by mixing detergent-solubilized lipids with the MSP and the to-be-incorporated MP solubilized in detergent micelles. After an incubation time of 1-2 hours, stable assembly is initiated by detergent removal, using dialysis or treatment with hydrophobic beads (Biobeads or Amberlite). The temperature during assembly should be chosen to be above the gel-to-liquid-crystal phase transition temperature of the lipid being used (**Tab. 2)** to ensure pre-formation of the MSP-lipid-MP complex. For assembly, any kind of bilayer-forming lipid can be used, depending on the requirements of the particular MP of interest or of particular experiments. Nanodiscs with a diameter of around 10 nm and higher have been widely used for 2D heteronuclear NMR spectroscopy [35, 63-65] as well as for ligand binding experiments [66]. However, due to their relatively large size for solution state NMR, an in-depth NMR characterization, such as for resonance assignment and structure determination, of MPs in these 10 nm nanodiscs remains very challenging. To address this issue, an interesting approach has been proposed to use nanodiscs as a reference system [67] and screen for detergents that give rise to an (almost) identical 2D NMR spectrum of an inserted membrane protein. This provides the opportunity to perform multidimensional NMR experiments and structure determination in smaller detergent systems and extrapolate the findings to a membrane bilayer environment. Since chemical shifts are very sensitive probes of structural changes, it seems very likely that the structure in lipids is rather similar to that determined in detergent micelles. In order to solve the size problem associated with the nanodisc system, a set of truncated MSP variants has been introduced that form nanodiscs of diameters ranging from 6-8 nm [14, 15] (**Fig. 5b,c**). The use of smaller nanodiscs led to a marked improvement of NMR spectral quality (**Fig. 5d**) and enabled the first structure determination of a MP in nanodiscs [14, 68] (**Fig. 5e**). Since then other MPs have been characterized in nanodiscs by NMR, e.g. refs [69, 70].

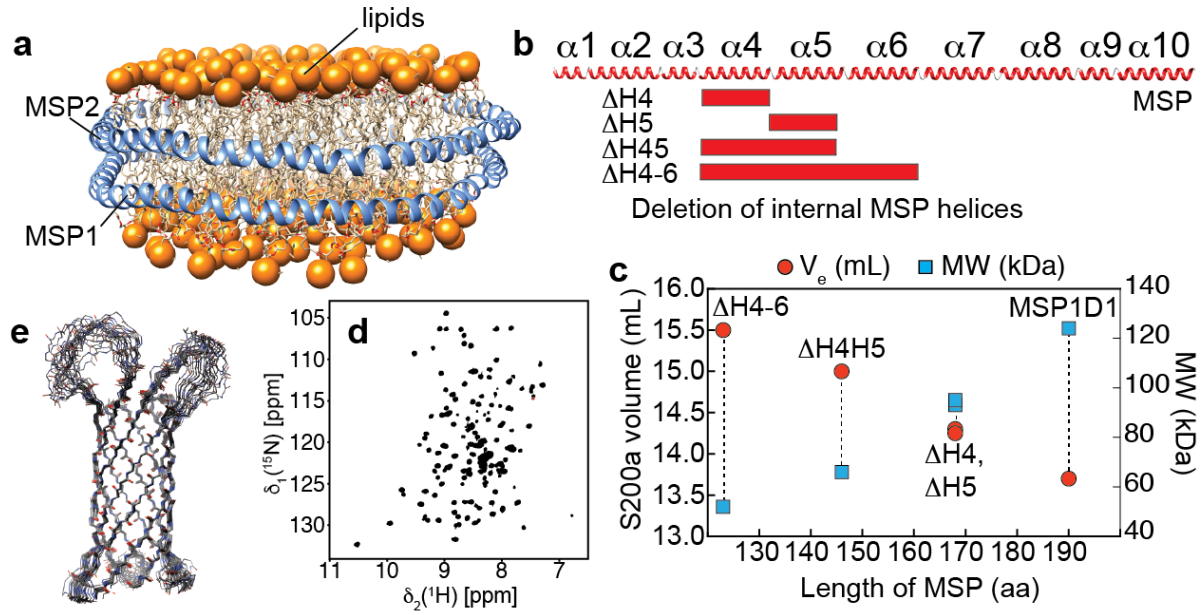

Fig. 5: Phospholipid nanodiscs assembled with ApoA-1 or membrane scaffold protein (MSP). (a) Structure of a MSP nanodisc, where two copies of MSP (MSP1 and MSP2) wrap around a patch of lipid bilayer in an antiparallel manner [55, 71]. (b,c) Truncation of internal segments of the MSP leads to nanodiscs of successively smaller size [15]. (d) The quality of a 2D-[^{15}N,^{1}H]-TROSY spectrum of ^{2}H,^{15}N-labeled OmpX in MSP1D1ΔH5 nanodiscs is suitable for multidimensional NMR spectroscopy and subsequent structure determination [14]. (e) NMR structural ensemble of OmpX determined in smaller MSP1D1ΔH5 nanodiscs showing an r.m.s.d. of 0.32 Å for ordered secondary structure elements [14, 68].

Furthermore, one of the truncated MSP variants, MSP1D1ΔH5 (lacking helix 5 of the MSP), has been recently used for the structure determination of an empty nanodisc by solution-state NMR spectroscopy [71]. This structure revealed important insights into the features of the MSP protein double belt surrounding a patch of lipid bilayer in an antiparallel manner and the interaction between the two MSP copies.

More recently, the MSP nanodisc field has been further advanced by the introduction of circularized MSPs for nanodisc assembly [72]. In order to achieve covalent circularization, a C-terminal SortaseA recognition site (LPETG) was fused to the MSP to be ligated to an N-terminal Glycine residue of the MSP using SortaseA enzyme [73, 74]. Intramolecular circularization can be facilitated by using diluted MSP solutions of around 10 µM concentration and by adding mild detergents, such as Triton-X100 [75]. Furthermore in another study, the properties of the MSP were engineered to optimize solubility, an approach that further enhanced the yields of circular MSP after ligation with SortaseA [76]. In order to speed up the production workflow of circularized MSPs, a split-intein approach was established whereby intein fragments were fused to MSPs of various lengths to allow for *in vivo* production of circular MSP in *E. coli* at high yields [77] without the need for an enzymatic reaction step.

Nanodisc assembly with circularized MSPs can be achieved in a similar manner as with linear MSPs with a slightly increased incubation time before detergent removal to allow for efficient MP incorporation into the somewhat more restricted circular MSP belt. In contrast to linear MSP nanodiscs, the circularized versions show improved size homogeneity and thermal stability [72, 77]. In particular, since small nanodiscs tend to disassemble over time due to the higher strain in the MSP belt [14, 77], circularization is an elegant way to stabilize these nanodiscs for long-term structural studies. Thus, these small circular nanodiscs are promising for high-resolution NMR investigations of small to medium sized MPs due to their shorter rotational correlation times, in particular with the very small variants, approaching those found in small detergent micelles [77]. In addition, small circular MSPs can be used for long-term NMR experiments at elevated temperatures due to their enhanced stability, further facilitating high-resolution NMR work.

In addition to their widespread use in solution [15, 78, 79] and solid-state NMR [80-83], MSP nanodiscs are particularly useful in (cryo-) electron microscopy [84-86], small-angle X-ray scattering (SAXS) [87, 88] and small-angle neutron scattering (SANS) [89] studies of membrane proteins.

2.3.2 Polymer nanodiscs

Polymer nanodiscs were discovered through the observation that amphipathic polymers are able to solubilize lipid bilayers and form discoidal particles [90], which led to the development of nanodiscs composed of an amphipathic styrene and maleic-acid (SMA) co-polymer (**Fig. 6a**) [16]. The mechanism of nanodisc formation is based on the interaction of the styrene phenyl rings of the polymer with the hydrophobic edge of a planar lipid bilayer. This interaction leads to a stabilization of a disc-shaped particle, similar to what can be obtained with a lipid binding protein. The maleic acid moieties are oriented towards the solvent and thus mediate the solubility of the obtained particles. The most commonly used SMA co-polymer has a styrene-to-maleic-acid-anhydride ratio of 2:1. The maleic acid anhydride can subsequently be hydrolyzed to expose the di-maleic-acid moieties. This composition seems to provide the most native-like membrane properties [91]. The molecular weight of the polymer is around 10 kDa, which translates to 62 styrene and 31 maleic acid building blocks. As with any chemical polymer, the polydispersity of the chain has to be considered. For SMAs, a typical polydispersity of ~ 2.5 [92] can be observed, which means that the polymer chains have a broad size distribution over more than one order of magnitude. Thus, the structure of an individual SMA lipid particle (SMALP) cannot exactly be described, since it is not known which parts of the polymer are actually in contact with the lipid bilayer. Interestingly, the size of a SMALP seems to depend on the net charge of the polymer. A 2:1 molar ratio of maleic acid to styrene in the SMA polymer yields SMALPs of around 30 nm diameter, whereas a 3-to-1-ratio results in smaller discs of around 10 nm in diameter [93]. However, the dependence of the SMALP diameter on polymer composition has not yet been investigated systematically. The relative ratio of polymer to lipids seems to have a more pronounced effect on SMALP size, as demonstrated recently [94, 95], where diameters between 10

and 30 nm could be obtained with SMA-to-lipid ratios of 1.5 or 0.25, respectively. 30 nm SMALP have been shown to align in the magnetic field, as shown by ^{31}P and ^{14}N NMR spectra [94].

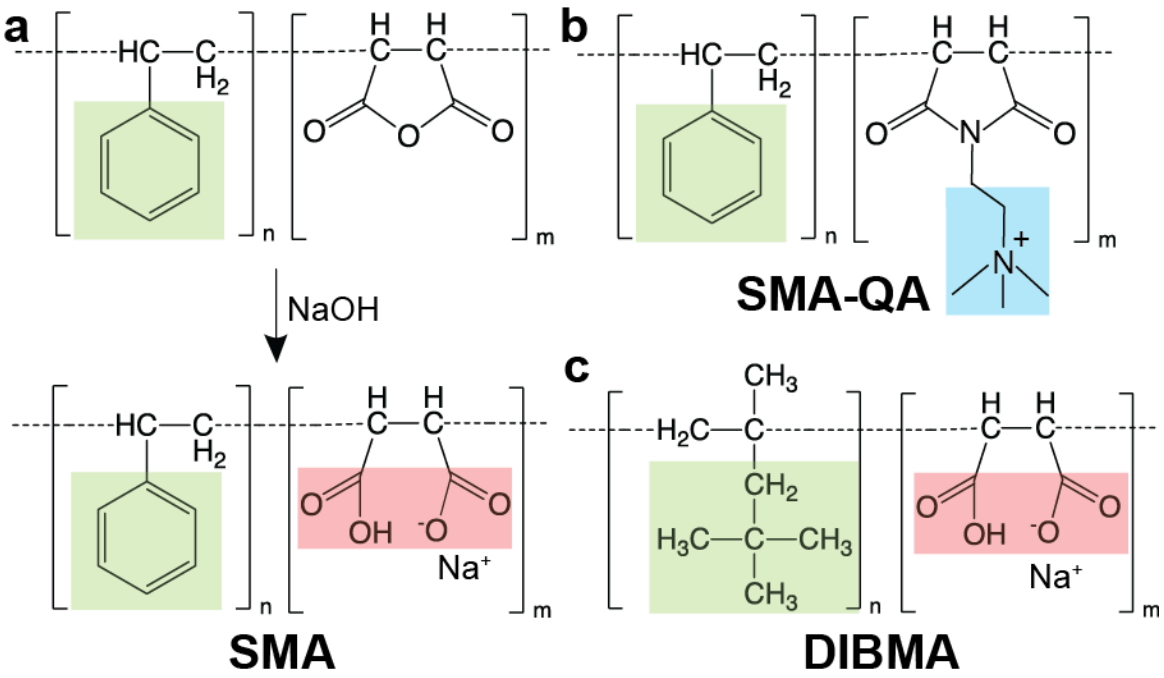

Fig. 6: Commonly-used polymers that form nanodiscs. (a) Styrene-maleic acid (SMA) copolymer can be obtained by hydrolysis of poly-styrene-maleic acid anhydride yielding a negatively charged polymer. A positively-charged derivative of SMA, called SMA-QA (quaternary ammonium), can be obtained after reaction with (2-aminoethyl) trimethylammonium chloride. This polymer shows high stability in the presence of divalent cations over a wide pH range of 2.5 to 10. (c) Another polymer, DIBMA, or di-isobutylene maleic acid, shows high stability against divalent cations and lacks UV absorption at 280nm, which would interfere with membrane protein quantification.

Another important feature of SMALPs is their pH stability. Due to protonation of the maleic acid moiety at low pH, SMALPs tend to precipitate at pH values below 6.5, limiting their use in applications for membrane protein structure and function at lower pH regimes. Furthermore, divalent cations like Ca^{2+} and Mg^{2+} that are often used for nucleotide binding or signaling assays are chelated by the polymer, rendering the particle insoluble. These issues have been addressed by the design of SMA polymers in which the maleimide carboxylates are chemically modified with enthanolamine or positively charged quaternary ammonium compounds (SMA-QA, **Fig. 6b**) [94, 95]. Due to the absence of a carboxyl moiety, these SMALPs are stable over a wider pH range from 2.5 to 10. In addition, a non-styrene-containing SMA co-polymer named DIBMA (diisobuthylene/maleic acid) (**Fig. 6c**) shows better stability in the presence of divalent cations and, due to the absence of an aromatic moiety, DIBMA is less harsh than SMA and does not interfere with far-UV optical spectroscopy [96].

A very interesting feature of SMALPs is their ability to be used for the direct extraction of MPs from cellular membranes [97]. The standard procedure for MP extraction involves usage of detergent micelles, leading to commonly observed problems of MP unfolding or misfolding (**Fig. 1**). In contrast, SMALPs provide a very mild environment for MPs but are nevertheless capable of efficient solubilization of lipids. This feature makes SMALPs suitable to be used as a carrier system for MPs for subsequent transfer to lipidic cubic phase systems for crystallography [98] or for the direct usage for structure determination with cryo-electron microscopy [99]. Macrodiscs assembled with SMA polymers have turned out to be useful for solid-state NMR [100, 101]. Solution-state NMR experiments involving SMALPs have been limited to membrane attached proteins [102] with no integral MPs reported so far. The reason for this might be the larger size inhomogeneity of SMALPs compared to protein based nanodisc systems [103]. Such variations would not affect the spectral quality of membrane attached proteins, since the soluble domains do not interact directly with the nanodisc.

2.3.3 Saposin nanodiscs

Saposins are lipid-binding proteins that are derived from a precursor protein, called pro-Saposin. Saposins are modulators of the lipid membrane that are located in lysosomes and are active at acidic pH [104]. Individual members of the saposin family have lipid binding and lipid transport activity and structures of individual members have been solved recently [105, 106]. In a systematic study, the capability of a large set of Saposin variants to form homogenous nanodiscs has been investigated, showing that Saposin A (SapA) is the most widely applicable member of the family, tolerating a broad range of lipids and lipid-to-Saposin ratios [107]. SapA is a sphingolipid activator protein in lysosomes at a pH of 4.8. Crystal structures of SapA revealed that in the apo form a closed conformation is present which can be transformed into an open form upon the addition of detergents, such as lauryldimethylamine-N-oxide (LDAO) [108], even at neutral pH. In this open state, two SapAs surrounding the bilayer are in a head-to-tail arrangement. The assembly of nanodiscs with SapA, termed Salipro nanodiscs, has been recently confirmed and characterized by cryo-EM [56]. Furthermore, the structure of Salipro nanodiscs containing target membrane proteins, such as the mechanosensitive channel T2 and the bacterial peptide transporter $PepT_{So2}$, could characterized at high resolution [56].

In comparison to MSP nanodiscs, where the membrane scaffold protein completely encloses a patch of lipid bilayer membrane in an extended helical conformation, SapA nanodiscs need to form oligomers to stabilize a nanodisc assembly. This feature offers the possibility of adjusting the size of the nanodisc simply by variations in the SapA-to-lipid ratio. As a minimum, two SapA molecules are sufficient to form a nanodisc particle. However, by increasing the amount of lipid in the system, a larger number of SapA molecules are required to bind to the hydrophobic rim of the bilayer resulting in larger nanodiscs. Such an open system is particularly helpful for MP oligomers that would not insert

into an MSP nanodisc due to their strictly defined diameter. Furthermore, SapA nanodiscs loaded with a MP tend to contain only a limited number of lipid molecules, leading to a tightly packed particle that facilitated analysis by single particle cryo-EM [56]. In addition, solution-state NMR has also been reported with SapA nanodiscs. In this study, isotope-labeled OmpX was inserted yielding 2D-NMR spectra of decent quality [109]. Furthermore, the functionality of β1-adrenergic receptor in this nanodisc system could be proven with NMR chemical shift perturbation experiments with various ligands as well as a nanobody G-protein mimetic [109].

Despite the above-mentioned advantages, the production of SapA, containing a disulfide bridge, is less straightforward than for MSP proteins that can easily be obtained in quantities exceeding 50 mg per liter growth medium. Thus, this system might become more popular for cryo-EM than for NMR in the future, where sample quantity requirements are less demanding and a low amount of lipids might be beneficial for high-resolution structure determination.

2.3.4 Peptide nanodiscs

Peptide nanodiscs are based on short amphipathic peptides that can bind to the hydrophobic rim of a phospholipid bilayer membrane. The most common peptide, called 18A, is derived from ApolipoproteinA-1 [110-112]. 18A (Ac-DWLKAFYDKVAEKLKEAF-NH_2) is an 18 amino acid peptide with amphipathic properties of the so-called class A type (i.e. derived from ApolipoproteinA-1) that forms a single α-helix of approximately the same length as a single helix within the ApoA-1 protein. A large set of structural data [110, 113] suggests that 18A peptides are aligned in an antiparallel manner at the hydrophobic rim of the bilayer membrane of the nanodisc.

An advantage of peptide nanodiscs is the possibility of adjusting the nanodisc size simply by variations in the peptide-to-lipid ratio, a feature that can also be achieved by using the saposin nanodiscs system or with bicelles. For DMPC nanodiscs, it was shown that the diameter can be increased from 10 to 30 nm if the lipid-to-peptide molar ratio is increased from 1.67 to 13.3 [114]. A drawback of the peptide nanodisc approach is the relative high cost of custom peptide synthesis as compared to recombinant production of ApoA-1 proteins. In addition, due to their non-covalent assembly, peptide nanodiscs are less stable than other nanodisc systems. However, this issue has been addressed by chemical methods, where covalent ligation of individual peptides in an assembled nanodiscs was performed e.g. by native chemical ligation [115].

3. Practical hints for finding a suitable membrane mimetic for a particular membrane protein

The availability of a large number of different membrane mimetics can be overwhelming, making it difficult to identify a suitable system, in particular for structural studies, where homogeneity

and long-term stability is a key factor. In addition, for solution-state NMR spectroscopy, the overall size of the complex of a MP and a membrane mimetic is a critical parameter. In the following sections, we will discuss the use of membrane mimetics for different MP types, such as integral MPs, single-spanning transmembrane helices, tail-anchored MPS and, finally, their suitability for studying complexes between MPs and soluble or lipidated partner proteins. The properties of the membrane mimetics that we discuss and their (dis-)advantages are outlined in **Table 3**.

3.1 Integral membrane proteins

For folded integral MPs, a membrane mimetic needs to fulfill two main requirements for high-resolution NMR: (a) to promote the functional form of the MP and (b) have a minimum size. The classical approach is to screen for a suitable detergent micelle system [4]. Since a large variety of detergents is available, the above-mentioned main objectives for a membrane mimetic can in principle be achieved. As a rule of thumb, detergents harboring a net charge are harsher than zwitterionic species or non-charged detergents such as alkyl maltosides. The diameter of a detergent micelle is dependent on the chain length of the hydrophobic part and the bulkiness of the head group. These few parameters can quite efficiently guide the way to finding a good detergent system. In general, harsher detergents can be used for β-barrel MPs and single transmembrane helices, whereas milder detergents are required for α-helical bundles, such as GPCRs. A selection of NMR-suitable detergents is commercially available in per-deuterated or tail-deuterated forms. However, due to their spherical shape, detergent micelles do not realistically resemble a bilayer membrane. Thus, the use of non-micellar bilayer systems is generally preferred. The easiest way to deviate from a spherical shape is by using bicelles. This enables embedding of the MP in a real bilayer environment, but still with large amounts of detergents present in the sample. The benefit of bicelles for solution-state NMR is their tunable size [24]. In a recent study, it was concluded that bicelles with a *q*-value of 0.5 or above can be considered to be a realistic membrane mimic [32].

Amhipols represent a non-lipid-containing alternative to micelles. These relatively large molecules (≈ 8 kDa) are less denaturing as compared to detergent micelles and still have an acceptable size for solution-state NMR [35]. Despite their use for refolding of MPs from inclusion bodies [50] and subsequent cryo-EM studies [36-39], the application for solution state NMR has only been demonstrated with model integral MPs (OmpA [34], OmpX [116], bR [35]). This is complicated by the fact that the most common amphipol A8-35 can only be used at pH > 7.

In cases where MP stability is a critical issue, the use of pure lipid systems is advantageous. Thus, nanodisc systems are often the best choice to study such MP systems in a more native-like environment by solution-state NMR. Since SMALPs have not been used for solution NMR studies of integral MPs, it remains unclear whether the homogeneity of these nanodiscs is sufficient for high-resolution structural work. However, membrane associated proteins have been studied by NMR in SMALPs [117], corroborating their native-like properties.

The largest pool of NMR data is available for protein-based nanodiscs, confirming their versatility and suitability for integral MPs. Among these, MSP nanodiscs are most often used, especially since the diameter of these nanodiscs can be adapted to account for the size of the inserted MP [14, 15]. Reasonable MSP constructs for medium to small size proteins are MSP1D1 [55] (10 nm diameter) and the truncated variants MSP1D1ΔH5 (8 nm) or ΔH4H5 (7 nm) [14]. Larger nanodiscs (e.g. with MSP1E3D1, 13 nm [55]) might be useful to obtain fingerprint 2D NMR spectra for larger MPs, but, due to their large size, the spectral properties are not sufficient for more sophisticated multidimensional NMR experiments. Smaller nanodiscs (down to 6 nm with MSP1D1ΔH4-6) have been reported [14] but their long-term stability is rather limited. Thus, circularization of MSP nanodiscs [72, 75-77] seems to be a good way to stabilize these very small particles. Saposin A nanodiscs, which have recently been reported to be a suitable tool for EM studies, have also been shown to be applicable for solution-state NMR experiments of integral MPs [109]. Since variations in the saposin-to-lipid ratio lead to nanodiscs containing three ($Salipro_3$) or four ($Salipro_4$) SapA molecules, the size of these nanodiscs can easily be adapted to account for the loaded MP [109]. Using ^{15}N-labeled OmpX in $Salipro_3$ most backbone resonance signals could be resolved. However, the overall spectral quality was lower as compared to OmpX in MSP1D1ΔH5 nanodiscs. This is presumably caused by the lack of OmpX deuteration in this study or the lower number of lipid molecules (~ 5-10 DMPC per SapA) in OmpX-loaded $Salipro_3$ nanodiscs. As discussed recently [15], two layers of lipids around the inserted membrane protein are commonly required for a native lipid environment and a stable NMR sample, as demonstrated with OmpX in MSP nanodiscs of subsequently decreasing diameters [14]. Finally, despite promising results from solid-state NMR [114], the only uses of peptide nanodiscs in high-resolution solution-state NMR studies reported so far have been in studies of membrane attached proteins [118], presumably because the limited homogeneity of these more loosely assembled nanodiscs renders studies of integral MPs difficult.

3.2 Single-pass transmembrane helices

Single-pass transmembrane helices (TMHs) are often used to anchor soluble domains to the external leaflet of the cell membrane or organelles. In many cases, protein-protein interactions are regulated by a fine-tuned interaction between transmembrane helices. Since in TMHs no tertiary structure is present, the use of (often harsh) detergent micelles, such as DPC or DHPC, does not lead to unfolding or inhibition of relevant interactions, as has been shown e.g. for the assembly of subunits of the influenza M2 proton channel [119] or the dimerization of glycophorin [120]. Thus, detergent micelles are the most common membrane mimetic used for TMHs. Since the step from detergent micelles to the preparation of bicelles is rather small, NMR structural information is also abundantly available in this system, e.g. the complex of TMHs of the α and β chains of integrins [29]. In addition to detergent-based systems, TMHs have been inserted and structurally characterized in MSP nanodiscs. In recent years, the structure of the TMH of BclxL was investigated in DPC micelles [79]

and phospholipid nanodiscs assembled with MSP1D1ΔH5 [121]. These studies showed that the structure of BclxL-TMH is very similar in both membrane mimetics. It remains to be seen whether the type of membrane mimetic has a significant impact on functional dynamics of TMHs. Recent data on a TMH of an intra-membrane protease substrate in detergent micelles indicate that functional insights can sometimes be obtained even without a bilayer membrane [122].

3.3 Membrane-anchored proteins

A more challenging MP class are tail-anchored MPs. The dilemma with these proteins is that harsh detergents that would be suitable for solubilizing the TMH for NMR studies often unfold or destabilize the folded soluble domain. Thus, screening for suitable detergents that preserve both parts of the structure is very difficult. For structural studies, TMHs are usually removed, focusing on the soluble domain only. A prominent protein family are Bcl2 proteins that regulate membrane pore formation and subsequently the induction of apoptosis at the outer mitochondrial membrane [123]. It has been shown that lyophilized full-length BclxL can be solubilized in DPC micelles and transferred into phospholipid nanodiscs [79]. In DPC, the soluble domain of BclxL lacks tertiary structure, as shown previously by NMR [7]. However, upon detergent removal during nanodisc assembly, in this case the soluble domain folds back to its native structure, as indicated by a well-dispersed 2D-[^{15}N,^{1}H]-TROSY spectrum [79]. Another example is tail-anchored cytochrome b_5 ($Cytb_5$), where the TMH binds to the soluble domain in solution. Membrane insertion can be induced by the addition of detergent to dissociate the TMH [124]. In this detergent-bound state, it has been shown that $Cytb_5$ can be inserted into MSP nanodiscs [125]. For other membrane attached proteins that show a high degree of detergent sensitivity, the approach to solubilize the protein in detergent prior to nanodisc incorporation might be more difficult, calling for a detergent-free workflow of nanodisc assembly. For this scenario, a SortaseA-based method has been introduced for the production of full-length membrane-anchored proteins [121]. In this strategy, BclxL-TMH is produced separately, purified in detergent micelles and subsequently inserted into nanodiscs. The TMH in nanodiscs is then ligated in a detergent-free environment to the soluble domain of BclxL containing a C-terminal SortaseA recognition motif (LPXTG, X: any amino acid). In this approach, the soluble domain is never in contact with detergents and thus adopts a well-folded state in the full-length context. This functional feature was demonstrated by binding assays with canonical peptide ligand, which found binding affinities very similar to those obtained for the soluble domain alone [121]. The advantage of this approach is the possibility of segmental isotope labeling, which can be beneficial for visualizing the TMH in the full-length protein, since its resonances usually appear in the random-coil region and overlap with other signals originating from the soluble domain whose lines are much sharper due to faster tumbling outside the membrane. Another benefit is the possibility of producing large amounts of ligated protein using optimized SortaseA variants [74], a prerequisite for NMR structural studies. Furthermore, refolding of disulfide bond-containing ectodomains, such as CD3 subunits of the T-cell

receptor [126], for NMR is very difficult by itself. The presence of a TMH during refolding would be detrimental for the overall refolding yields. Thus, separating refolding from membrane insertion is a promising approach for difficult members of this protein class.

3.4 Complexes of integral and membrane associated membrane proteins

In order to understand functional aspects of MP signaling, structural investigations of complexes between receptors and soluble partner proteins are essential. Detergents are often too harsh to stabilize such interactions as well as the properly folded state of the soluble part of the complex. Thus, lipid-based membrane mimetics are in many cases better suited to performing structural studies of such complexes in a more native-like environment. For membrane binding domains of soluble proteins, the lipid composition can be adjusted appropriately to enable a stable and native interaction mode. Due to their tunable size and the absence of detergent molecules, MSP nanodiscs are often used to monitor lipid binding [127-129]. Furthermore, lipids with modified headgroups that contain a cysteine-selective maleimide moiety can be used to produce membrane-anchored proteins, as demonstrated for Ras proteins [130], and probe their membrane location. Incorporation of paramagnetic Gd^{3+}-labeled lipids is an excellent tool for deriving distance information between a membrane-attached protein, or selected parts of a MP, and the membrane surface [68, 121, 130]. MSP nanodiscs are the most suitable membrane mimetic for studying complexes of soluble proteins with an integral MP by NMR. In particular, MSP nanodiscs have been used to study complexes between G-proteins and GPCRs in a native bilayer membrane environment [72, 131, 132].

4. Conclusions

In this review, we have aimed to provide a general overview of commonly used membrane mimetics, with a focus on nanodisc systems and their use in the investigation of membrane proteins and their complexes by solution-state NMR. Despite the fact that detergent micelles are the most commonly used membrane mimetic, non-conventional membrane mimetics are often a better choice for probing structural and functional features of inserted membrane proteins. The information presented herein should enable an informed decision on what membrane mimetics might be suitable for a particular system of interest. The overall aim is to select a membrane system that provides a functional and native-like environment necessary for the extraction of structural and dynamical features and to be able to study complexes with partner proteins. In addition, the homogeneity and overall size of the final particles need to be optimized for high-resolution solution-state NMR and balanced with functional aspects, which need to be characterized using accompanying biochemical or cell-based assays.

Acknowledgements

The authors want to thank the Institute for Advanced Study funded by the German Excellence Initiative and the European Union Seventh Framework Program under grant agreement n° 291763, and the Department of Chemistry, Technical University of Munich, the Deutsche Forschungsgemeinschaft (SFB1035, project B13) and the Helmholtz Zentrum München and Helmholtz Society (VH-NG-1039) for support.

Tables

Table 1: Critical micelle concentration (cmc) and micellar molecular weights of detergents used for NMR spectroscopy[133] and lipid solubilization for subsequent nanodisc assembly.

Detergent	CMC* (mM)	Micellar Molecular Weight
Sodium cholate[55]	9-15	900-1,300
Sodium deoxycholate[134]	2-6	1,200-5,000
CTAB	1	62,000
LysoPC	0.007	92,000
CHAPS[135]	6	6,150
Octyl-*β*-D-glucopyranoside[136]	23-25	25,000
n-Dodecyl-*β*-D-Maltoside (DDM)[137]	0.15	50,000
Digitonin[138]	< 0.5	70,000
Triton X-100[139]	0.2-0.9	80,000
Decyl-*β*-D-Maltoside (DM)[131]	1.6	33,000
LDAO[63]	1-2	17,000
Cymal-5	2.4-5	32,600
Cymal-6	0.56	32,000
Dodecyl-phosphocholine (DPC)[14]	1.5	19,000
N-lauroylsarcosine[140]	14.6	600
SDS[141]	7-10	18,000

* CMC in water at 20-25 °C

Table 2. **Phase transition temperatures for lipids used in membrane protein studies** (derived from the membrane protein data bank: http://www.lipidat.tcd.ie and Ref. [142]). Commonly used lipid mixtures are DMPC/DMPG (T_m are 24 and 23 °C, respectively) and POPC/POPG (T_m is -2 °C for each). For these systems, handling is done at room temperature and over ice, respectively. Mixtures of lipids with significantly different T_m values consequently show two transition steps. Handling and nanodisc assembly is then done at a temperature close to the T_m of the major lipid component.

Lipid	T_m (°C)
POPC	-2°C
POPS	14°C
POPG	-2°C
DPPC	41°C
DMPC	24°C
DMPG	23°C
DOPG	-18°C
DPPC	41°C
DLPC	-2°C
DOPC	-17°C
DLPG	-3°C
DMPS	35°C
DOPS	-11°C
DOPA	-4°C
DOPE	-16°C
POPE	25°C

Table 3: Pros and cons of various membrane mimetics in general and for their use in solution-state NMR

Membrane mimetic	PROs	CONs
Detergent micelles	• Small size • Large number of detergents available	• Denaturation of membrane protein or partner protein • CMC needs to be considered
Bicelles	• Easy to prepare • Tunable size	• Denaturing effect of detergent component
Amphipols	• Solubilization of membrane proteins • Suitable for refolding of GPCRs	• Only one amphipol type is commercially available • Sometimes too harsh for maintaining complexes
MSP nanodiscs	• Tunable size • Lipid-only system • Any lipid can be incorporated • Stable after assembly • Homogenous size	• Optimization of assembly conditions can be time consuming • Not suitable for large MP oligomers • Dynamics of lipids affected by protein 'belt'
Polymer nanodiscs	• Easy handling • Commercially available • Extraction of membrane proteins from membrane is possible without use of detergents • Stable	• Size not tunable in an easy manner • Stability issues at low pH and in presence of divalent cations • Less homogenous as compared to MSP nanodiscs
Saposin nanodiscs	• Size tunable by saposin:lipid ratio • Tight packing of Sap with MP, small size • Open system that can adapt to larger MPs and their oligomers	• Less stable than other nanodisc systems • Production of saposins requires some experience • Low amount of lipids leads to reduced NMR spectral quality
Peptide nanodiscs	• Size tunable by peptide:lipid ratio • Suitable for MP oligomers	• Lower stability • Size homogeneity lower

References

[1] Z. Cournia, T.W. Allen, I. Andricioaei, B. Antonny, D. Baum, G. Brannigan, N.V. Buchete, J.T. Deckman, L. Delemotte, C. Del Val, R. Friedman, P. Gkeka, H.C. Hege, J. Henin, M.A. Kasimova, A. Kolocouris, M.L. Klein, S. Khalid, M.J. Lemieux, N. Lindow, M. Roy, J. Selent, M. Tarek, F. Tofoleanu, S. Vanni, S. Urban, D.J. Wales, J.C. Smith, A.N. Bondar, Membrane Protein Structure, Function, and Dynamics: a Perspective from Experiments and Theory, J Membr Biol 248(4) (2015) 611-40.

[2] R.M. Bill, P.J. Henderson, S. Iwata, E.R. Kunji, H. Michel, R. Neutze, S. Newstead, B. Poolman, C.G. Tate, H. Vogel, Overcoming barriers to membrane protein structure determination, Nat Biotechnol 29(4) (2011) 335-40.

[3] M. Rask-Andersen, M.S. Almen, H.B. Schioth, Trends in the exploitation of novel drug targets, Nat Rev Drug Discov 10(8) (2011) 579-90.

[4] D. Linke, Detergents: an overview, Methods Enzymol 463 (2009) 603-17.

[5] A.M. Seddon, P. Curnow, P.J. Booth, Membrane proteins, lipids and detergents: not just a soap opera, Biochim Biophys Acta 1666(1-2) (2004) 105-17.

[6] T.J. Malia, G. Wagner, NMR structural investigation of the mitochondrial outer membrane protein VDAC and its interaction with antiapoptotic Bcl-xL, Biochemistry 46(2) (2007) 514-25.

[7] J.A. Losonczi, E.T. Olejniczak, S.F. Betz, J.E. Harlan, J. Mack, S.W. Fesik, NMR studies of the anti-apoptotic protein Bcl-xL in micelles, Biochemistry 39(36) (2000) 11024-33.

[8] H.X. Zhou, T.A. Cross, Influences of membrane mimetic environments on membrane protein structures, Annu Rev Biophys 42 (2013) 361-92.

[9] G. Pabst, N. Kucerka, M.P. Nieh, J. Katsaras, Liposomes, Lipid Bilayers and Model Membranes From Basic Research to Application Preface, Liposomes, Lipid Bilayers and Model Membranes: From Basic Research to Application BOOK_DOI 10.1201/b16617 (2014) Ix-X.

[10] A.G. Lee, Lipid-protein interactions in biological membranes: a structural perspective, Biochim Biophys Acta 1612(1) (2003) 1-40.

[11] C.R. Sanders, 2nd, G.C. Landis, Reconstitution of membrane proteins into lipid-rich bilayered mixed micelles for NMR studies, Biochemistry 34(12) (1995) 4030-40.

[12] C. Tribet, R. Audebert, J.L. Popot, Amphipols: Polymers that keep membrane proteins soluble in aqueous solutions, Proc Natl Acad Sci U S A 93(26) (1996) 15047-15050.

[13] I.G. Denisov, S.G. Sligar, Nanodiscs in Membrane Biochemistry and Biophysics, Chem Rev 117(6) (2017) 4669-4713.

[14] F. Hagn, M. Etzkorn, T. Raschle, G. Wagner, Optimized phospholipid bilayer nanodiscs facilitate high-resolution structure determination of membrane proteins, J Am Chem Soc 135(5) (2013) 1919-25.

[15] F. Hagn, M.L. Nasr, G. Wagner, Assembly of phospholipid nanodiscs of controlled size for structural studies of membrane proteins by NMR, Nat Protoc 13(1) (2018) 79-98.

[16] T.J. Knowles, R. Finka, C. Smith, Y.P. Lin, T. Dafforn, M. Overduin, Membrane proteins solubilized intact in lipid containing nanoparticles bounded by styrene maleic acid copolymer, J Am Chem Soc 131(22) (2009) 7484-5.

[17] O.C. Andronesi, S. Becker, K. Seidel, H. Heise, H.S. Young, M. Baldus, Determination of membrane protein structure and dynamics by magic-angle-spinning solid-state NMR spectroscopy, J Am Chem Soc 127(37) (2005) 12965-74.

[18] M. Hong, Y. Zhang, F. Hu, Membrane protein structure and dynamics from NMR spectroscopy, Annu Rev Phys Chem 63 (2012) 1-24.

[19] V. Ladizhansky, Applications of solid-state NMR to membrane proteins, Biochim Biophys Acta Proteins Proteom 1865(11 Pt B) (2017) 1577-1586.

[20] D.T. Murray, J. Griffin, T.A. Cross, Detergent optimized membrane protein reconstitution in liposomes for solid state NMR, Biochemistry 53(15) (2014) 2454-63.

[21] N.E. Gabriel, M.F. Roberts, Spontaneous Formation of Stable Unilamellar Vesicles, Biochemistry 23(18) (1984) 4011-4015.

[22] C.R. Sanders, B.J. Hare, K.P. Howard, J.H. Prestegard, Magnetically-Oriented Phospholipid Micelles as a Tool for the Study of Membrane-Associated Molecules, Prog Nucl Mag Res Sp 26 (1994) 421-444.

[23] P. Ram, J.H. Prestegard, Magnetic-Field Induced Ordering of Bile-Salt Phospholipid Micelles - New Media for Nmr Structural Investigations, Biochim Biophys Acta 940(2) (1988) 289-294.

[24] R.R. Vold, R.S. Prosser, Magnetically oriented phospholipid bilayered micelles for structural studies of polypeptides. Does the ideal bicelle exist?, J Magn Reson Ser B 113(3) (1996) 267-271.

[25] J. Struppe, J.A. Whiles, R.R. Vold, Acidic phospholipid bicelles: A versatile model membrane system, Biophys J 78(1) (2000) 281-289.

[26] M. Ottiger, A. Bax, Characterization of magnetically oriented phospholipid micelles for measurement of dipolar couplings in macromolecules, J Biomol NMR 12(3) (1998) 361-372.

[27] N. Tjandra, A. Bax, Direct measurement of distances and angles in biomolecules by NMR in a dilute liquid crystalline medium (vol 278, pg 1111, 1997), Science 278(5344) (1997) 1697-1697.

[28] E.V. Bocharov, Y.E. Pustovalova, K.V. Pavlov, P.E. Volynsky, M.V. Goncharuk, Y.S. Ermolyuk, D.V. Karpunin, A.A. Schulga, M.P. Kirpichnikov, R.G. Efremov, I.V. Maslennikov, A.S. Arseniev, Unique dimeric structure of BNip3 transmembrane domain suggests membrane permeabilization as a cell death trigger, J Biol Chem 282(22) (2007) 16256-66.

[29] T.L. Lau, C. Kim, M.H. Ginsberg, T.S. Ulmer, The structure of the integrin alphaIIbbeta3 transmembrane complex explains integrin transmembrane signalling, EMBO J 28(9) (2009) 1351-61.

[30] J. Lind, A. Graslund, L. Maler, Membrane interactions of dynorphins, Biochemistry 45(51) (2006) 15931-40.

[31] A. Gautier, H.R. Mott, M.J. Bostock, J.P. Kirkpatrick, D. Nietlispach, Structure determination of the seven-helix transmembrane receptor sensory rhodopsin II by solution NMR spectroscopy, Nat Struct Mol Biol 17(6) (2010) 768-U147.

[32] A. Piai, Q.S. Fu, J. Dev, J.J. Chou, Optimal Bicelle Size q for Solution NMR Studies of the Protein Transmembrane Partition, Chem-Eur J 23(6) (2017) 1361-1367.

[33] J.L. Popot, T. Althoff, D. Bagnard, J.L. Baneres, P. Bazzacco, E. Billon-Denis, L.J. Catoire, P. Champeil, D. Charvolin, M.J. Cocco, G. Cremel, T. Dahmane, L.M. de la Maza, C. Ebel, F. Gabel, F. Giusti, Y. Gohon, E. Goormaghtigh, E. Guittet, J.H. Kleinschmidt, W. Kuhlbrandt, C. Le Bon, K.L. Martinez, M. Picard, B. Pucci, J.N. Sachs, C. Tribet, C. van Heijenoort, F. Wien, F. Zito, M. Zoonens, Amphipols from A to Z, Annu Rev Biophys 40 (2011) 379-408.

[34] M. Zoonens, L.J. Catoire, F. Giusti, J.L. Popot, NMR study of a membrane protein in detergent-free aqueous solution, Proc Natl Acad Sci U S A 102(25) (2005) 8893-8.

[35] M. Etzkorn, T. Raschle, F. Hagn, V. Gelev, A.J. Rice, T. Walz, G. Wagner, Cell-free expressed bacteriorhodopsin in different soluble membrane mimetics: biophysical properties and NMR accessibility, Structure 21(3) (2013) 394-401.

[36] T. Althoff, D.J. Mills, J.L. Popot, W. Kuhlbrandt, Arrangement of electron transport chain components in bovine mitochondrial supercomplex I1III2IV1, EMBO J 30(22) (2011) 4652-64.

[37] T.L. Cvetkov, K.W. Huynh, M.R. Cohen, V.Y. Moiseenkova-Bell, Molecular architecture and subunit organization of TRPA1 ion channel revealed by electron microscopy, J Biol Chem 286(44) (2011) 38168-76.

[38] M. Liao, E. Cao, D. Julius, Y. Cheng, Structure of the TRPV1 ion channel determined by electron cryo-microscopy, Nature 504(7478) (2013) 107-12.

[39] P. Lu, X.C. Bai, D. Ma, T. Xie, C. Yan, L. Sun, G. Yang, Y. Zhao, R. Zhou, S.H.W. Scheres, Y. Shi, Three-dimensional structure of human gamma-secretase, Nature 512(7513) (2014) 166-170.

[40] B.M. Gorzelle, A.K. Hoffman, M.H. Keyes, D.N. Gray, D.G. Ray, C.R. Sanders, Amphipols can support the activity of a membrane enzyme, J Am Chem Soc 124(39) (2002) 11594-5.

[41] F. Giusti, J.L. Popot, C. Tribet, Well-defined critical association concentration and rapid adsorption at the air/water interface of a short amphiphilic polymer, amphipol A8-35: a study by Forster resonance energy transfer and dynamic surface tension measurements, Langmuir 28(28) (2012) 10372-80.

[42] C. Diab, C. Tribet, Y. Gohon, J.L. Popot, F.M. Winnik, Complexation of integral membrane proteins by phosphorylcholine-based amphipols, Biochim Biophys Acta 1768(11) (2007) 2737-47.

[43] T. Dahmane, F. Giusti, L.J. Catoire, J.L. Popot, Sulfonated amphipols: synthesis, properties, and applications, Biopolymers 95(12) (2011) 811-23.

[44] P. Bazzacco, E. Billon-Denis, K.S. Sharma, L.J. Catoire, S. Mary, C. Le Bon, E. Point, J.L. Baneres, G. Durand, F. Zito, B. Pucci, J.L. Popot, Nonionic homopolymeric amphipols: application to membrane protein folding, cell-free synthesis, and solution nuclear magnetic resonance, Biochemistry 51(7) (2012) 1416-30.

[45] J.L. Popot, E.A. Berry, D. Charvolin, C. Creuzenet, C. Ebel, D.M. Engelman, M. Flotenmeyer, F. Giusti, Y. Gohon, Q. Hong, J.H. Lakey, K. Leonard, H.A. Shuman, P. Timmins, D.E. Warschawski, F. Zito, M. Zoonens, B. Pucci, C. Tribet, Amphipols: polymeric surfactants for membrane biology research, Cell Mol Life Sci 60(8) (2003) 1559-74.

[46] D. Charvolin, J.B. Perez, F. Rouviere, F. Giusti, P. Bazzacco, A. Abdine, F. Rappaport, K.L. Martinez, J.L. Popot, The use of amphipols as universal molecular adapters to immobilize membrane proteins onto solid supports, Proc Natl Acad Sci U S A 106(2) (2009) 405-10.

[47] J.K. Nagy, A. Kuhn Hoffmann, M.H. Keyes, D.N. Gray, K. Oxenoid, C.R. Sanders, Use of amphipathic polymers to deliver a membrane protein to lipid bilayers, FEBS Lett 501(2-3) (2001) 115-20.

[48] C.L. Pocanschi, T. Dahmane, Y. Gohon, F. Rappaport, H.J. Apell, J.H. Kleinschmidt, J.L. Popot, Amphipathic polymers: tools to fold integral membrane proteins to their active form, Biochemistry 45(47) (2006) 13954-61.

[49] F. Giusti, J. Rieger, L.J. Catoire, S. Qian, A.N. Calabrese, T.G. Watkinson, M. Casiraghi, S.E. Radford, A.E. Ashcroft, J.L. Popot, Synthesis, characterization and applications of a perdeuterated amphipol, J Membr Biol 247(9-10) (2014) 909-24.

[50] T. Dahmane, M. Damian, S. Mary, J.L. Popot, J.L. Baneres, Amphipol-assisted in vitro folding of G protein-coupled receptors, Biochemistry 48(27) (2009) 6516-21.

[51] B. Kobilka, The structural basis of G-protein-coupled receptor signaling (Nobel Lecture), Angewandte Chemie 52(25) (2013) 6380-8.

[52] D. Hilger, M. Masureel, B.K. Kobilka, Structure and dynamics of GPCR signaling complexes, Nat Struct Mol Biol 25(1) (2018) 4-12.

[53] C.R. Midgett, D.R. Madden, Breaking the bottleneck: eukaryotic membrane protein expression for high-resolution structural studies, J Struct Biol 160(3) (2007) 265-74.

[54] J.L. Baneres, J.L. Popot, B. Mouillac, New advances in production and functional folding of G-protein-coupled receptors, Trends Biotechnol 29(7) (2011) 314-22.

[55] I.G. Denisov, Y.V. Grinkova, A.A. Lazarides, S.G. Sligar, Directed self-assembly of monodisperse phospholipid bilayer Nanodiscs with controlled size, J Am Chem Soc 126(11) (2004) 3477-87.

[56] J. Frauenfeld, R. Loving, J.P. Armache, A.F. Sonnen, F. Guettou, P. Moberg, L. Zhu, C. Jegerschold, A. Flayhan, J.A. Briggs, H. Garoff, C. Low, Y. Cheng, P. Nordlund, A saposin-lipoprotein nanoparticle system for membrane proteins, Nat Methods 13(4) (2016) 345-51.

[57] A. Nath, W.M. Atkins, S.G. Sligar, Applications of phospholipid bilayer nanodiscs in the study of membranes and membrane proteins, Biochemistry 46(8) (2007) 2059-69.

[58] T.K. Ritchie, Y.V. Grinkova, T.H. Bayburt, I.G. Denisov, J.K. Zolnerciks, W.M. Atkins, S.G. Sligar, Chapter 11 - Reconstitution of membrane proteins in phospholipid bilayer nanodiscs, Methods Enzymol 464 (2009) 211-31.

[59] Y. Fang, O. Gursky, D. Atkinson, Lipid-binding studies of human apolipoprotein A-I and its terminally truncated mutants, Biochemistry 42(45) (2003) 13260-8.

[60] M. Krieger, M.S. Brown, J.R. Faust, J.L. Goldstein, Replacement of endogenous cholesteryl esters of low density lipoprotein with exogenous cholesteryl linoleate. Reconstitution of a biologically active lipoprotein particle, J Biol Chem 253(12) (1978) 4093-101.

[61] S.E. Panagotopulos, E.M. Horace, J.N. Maiorano, W.S. Davidson, Apolipoprotein A-I adopts a belt-like orientation in reconstituted high density lipoproteins, J Biol Chem 276(46) (2001) 42965-70.

[62] T.H. Bayburt, Y.V. Grinkova, S.G. Sligar, Self-assembly of discoidal phospholipid bilayer nanoparticles with membrane scaffold proteins, Nano Lett 2(8) (2002) 853-856.

[63] T. Raschle, S. Hiller, T.Y. Yu, A.J. Rice, T. Walz, G. Wagner, Structural and functional characterization of the integral membrane protein VDAC-1 in lipid bilayer nanodiscs, J Am Chem Soc 131(49) (2009) 17777-9.

[64] T.Y. Yu, T. Raschle, S. Hiller, G. Wagner, Solution NMR spectroscopic characterization of human VDAC-2 in detergent micelles and lipid bilayer nanodiscs, Biochim Biophys Acta 1818(6) (2012) 1562-9.

[65] J.M. Gluck, M. Wittlich, S. Feuerstein, S. Hoffmann, D. Willbold, B.W. Koenig, Integral membrane proteins in nanodiscs can be studied by solution NMR spectroscopy, J Am Chem Soc 131(34) (2009) 12060-1.

[66] K. Fredriksson, P. Lottmann, S. Hinz, I. Onila, A. Shymanets, C. Harteneck, C.E. Muller, C. Griesinger, T.E. Exner, Nanodiscs for INPHARMA NMR Characterization of GPCRs: Ligand Binding to the Human A2A Adenosine Receptor, Angewandte Chemie 56(21) (2017) 5750-5754.

[67] Z.O. Shenkarev, E.N. Lyukmanova, A.S. Paramonov, L.N. Shingarova, V.V. Chupin, M.P. Kirpichnikov, M.J. Blommers, A.S. Arseniev, Lipid-protein nanodiscs as reference medium in detergent screening for high-resolution NMR studies of integral membrane proteins, J Am Chem Soc 132(16) (2010) 5628-9.

[68] F. Hagn, G. Wagner, Structure refinement and membrane positioning of selectively labeled OmpX in phospholipid nanodiscs, J Biomol NMR 61(3-4) (2015) 249-60.

[69] L. Susac, R. Horst, K. Wuthrich, Solution-NMR characterization of outer-membrane protein A from E. coli in lipid bilayer nanodiscs and detergent micelles, Chembiochem 15(7) (2014) 995-1000.

[70] R. Puthenveetil, O. Vinogradova, Optimization of the design and preparation of nanoscale phospholipid bilayers for its application to solution NMR, Proteins 81(7) (2013) 1222-31.

[71] S. Bibow, Y. Polyhach, C. Eichmann, C.N. Chi, J. Kowal, S. Albiez, R.A. McLeod, H. Stahlberg, G. Jeschke, P. Guntert, R. Riek, Solution structure of discoidal high-density lipoprotein particles with a shortened apolipoprotein A-I, Nat Struct Mol Biol 24(2) (2017) 187-193.

[72] M.L. Nasr, D. Baptista, M. Strauss, Z.J. Sun, S. Grigoriu, S. Huser, A. Pluckthun, F. Hagn, T. Walz, J.M. Hogle, G. Wagner, Covalently circularized nanodiscs for studying membrane proteins and viral entry, Nat Methods 14(1) (2017) 49-52.

[73] J.M. Antos, J. Ingram, T. Fang, N. Pishesha, M.C. Truttmann, H.L. Ploegh, Site-Specific Protein Labeling via Sortase-Mediated Transpeptidation, Curr Protoc Protein Sci 89 (2017) 15 3 1-15 3 19.

[74] I. Chen, B.M. Dorr, D.R. Liu, A general strategy for the evolution of bond-forming enzymes using yeast display, Proc Natl Acad Sci U S A 108(28) (2011) 11399-11404.

[75] Y. Yusuf, J. Massiot, Y.T. Chang, P.H. Wu, V. Yeh, P.C. Kuo, J. Shiue, T.Y. Yu, Optimization of the Production of Covalently Circularized Nanodiscs and Their Characterization in Physiological Conditions, Langmuir 34(11) (2018) 3525-3532.

[76] N.T. Johansen, F.G. Tidemand, T.T.T.N. Nguyen, K.D. Rand, M.C. Pedersen, L. Arleth, Circularized and solubility-enhanced MSPs facilitate simple and high-yield production of stable nanodiscs for studies of membrane proteins in solution, Febs Journal 286(9) (2019) 1734-1751.

[77] J. Miehling, D. Goricanec, F. Hagn, A Split-Intein-Based Method for the Efficient Production o f Circularized Nanodiscs for Structural Studies of Membrane Proteins, Chembiochem 19(18) (2018) 1927-1933.

[78] I. Kucharska, T.C. Edrington, B. Liang, L.K. Tamm, Optimizing nanodiscs and bicelles for solution NMR studies of two beta-barrel membrane proteins, J Biomol NMR 61(3-4) (2015) 261-74.

[79] Y. Yao, L.M. Fujimoto, N. Hirshman, A.A. Bobkov, A. Antignani, R.J. Youle, F.M. Marassi, Conformation of BCL-XL upon Membrane Integration, J Mol Biol 427(13) (2015) 2262-70.

[80] K. Mors, C. Roos, F. Scholz, J. Wachtveitl, V. Dotsch, F. Bernhard, C. Glaubitz, Modified lipid and protein dynamics in nanodiscs, Biochim Biophys Acta 1828(4) (2013) 1222-9.

[81] A.Z. Kijac, Y. Li, S.G. Sligar, C.M. Rienstra, Magic-angle spinning solid-state NMR spectroscopy of nanodisc-embedded human CYP3A4, Biochemistry 46(48) (2007) 13696-703.

[82] Y. Ding, L.M. Fujimoto, Y. Yao, F.M. Marassi, Solid-state NMR of the Yersinia pestis outer membrane protein Ail in lipid bilayer nanodiscs sedimented by ultracentrifugation, J Biomol NMR 61(3-4) (2015) 275-86.

[83] N.A. Lakomek, L. Frey, S. Bibow, A. Bockmann, R. Riek, B.H. Meier, Proton-Detected NMR Spectroscopy of Nanodisc-Embedded Membrane Proteins: MAS Solid-State vs Solution-State Methods, J Phys Chem B 121(32) (2017) 7671-7680.

[84] R. De Zorzi, W. Mi, M. Liao, T. Walz, Single-particle electron microscopy in the study of membrane protein structure, Microscopy (Oxf) 65(1) (2016) 81-96.

[85] Y. Gao, E. Cao, D. Julius, Y. Cheng, TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action, Nature 534(7607) (2016) 347-351.

[86] R.G. Efremov, C. Gatsogiannis, S. Raunser, Lipid Nanodiscs as a Tool for High- Resolution Structure Determination of Membrane Proteins by Single-Particle Cryo-EM, Method Enzymol 594 (2017) 1-30.

[87] N. Skar-Gislinge, S.A.R. Kynde, I.G. Denisov, X. Ye, I. Lenov, S.G. Sligar, L. Arleth, Small-angle scattering determination of the shape and localization of human cytochrome P450 embedded in a phospholipid nanodisc environment, Acta Crystallogr D 71 (2015) 2412-2421.

[88] N. Skar-Gislinge, J.B. Simonsen, K. Mortensen, R. Feidenhans'l, S.G. Sligar, B.L. Moller, T. Bjornholm, L. Arleth, Elliptical Structure of Phospholipid Bilayer Nanodiscs Encapsulated by Scaffold Proteins: Casting the Roles of the Lipids and the Protein, J Am Chem Soc 132(39) (2010) 13713-13722.

[89] I. Josts, J. Nitsche, S. Maric, H.D. Mertens, M. Moulin, M. Haertlein, S. Prevost, D.I. Svergun, S. Busch, V.T. Forsyth, H. Tidow, Conformational States of ABC Transporter MsbA in a Lipid Environment Investigated by Small-Angle Scattering Using Stealth Carrier Nanodiscs, Structure 26(8) (2018) 1072-+.

[90] S.R. Tonge, B.J. Tighe, Responsive hydrophobically associating polymers: a review of structure and properties, Adv Drug Deliv Rev 53(1) (2001) 109-22.

[91] S.C. Lee, T.J. Knowles, V.L.G. Postis, M. Jamshad, R.A. Parslow, Y.P. Lin, A. Goldman, P. Sridhar, M. Overduin, S.P. Muench, T.R. Dafforn, A method for detergent-free isolation of membrane proteins in their local lipid environment, Nat Protoc 11(7) (2016) 1149-1162.

[92] J.M. Dorr, S. Scheidelaar, M.C. Koorengevel, J.J. Dominguez, M. Schafer, C.A. van Walree, J.A. Killian, The styrene-maleic acid copolymer: a versatile tool in membrane research, Eur Biophys J 45(1) (2016) 3-21.

[93] M.C. Orwick, P.J. Judge, J. Procek, L. Lindholm, A. Graziadei, A. Engel, G. Grobner, A. Watts, Detergent-free formation and physicochemical characterization of nanosized lipid-polymer complexes: Lipodisq, Angewandte Chemie 51(19) (2012) 4653-7.

[94] T. Ravula, N.Z. Hardin, S.K. Ramadugu, S.J. Cox, A. Ramamoorthy, Formation of pH-Resistant Monodispersed Polymer-Lipid Nanodiscs, Angewandte Chemie 57(5) (2018) 1342-1345.

[95] T. Ravula, S.K. Ramadugu, G. Di Mauro, A. Ramamoorthy, Bioinspired, Size-Tunable Self-Assembly of Polymer-Lipid Bilayer Nanodiscs, Angewandte Chemie 56(38) (2017) 11466-11470.

[96] A.O. Oluwole, B. Danielczak, A. Meister, J.O. Babalola, C. Vargas, S. Keller, Solubilization of Membrane Proteins into Functional Lipid-Bilayer Nanodiscs Using a Diisobutylene/Maleic Acid Copolymer, Angew Chem Int Edit 56(7) (2017) 1919-1924.

[97] A.R. Long, C.C. O'Brien, K. Malhotra, C.T. Schwall, A.D. Albert, A. Watts, N.N. Alder, A detergent-free strategy for the reconstitution of active enzyme complexes from native biological membranes into nanoscale discs, BMC Biotechnol 13 (2013) 41.

[98] J. Broecker, B.T. Eger, O.P. Ernst, Crystallogenesis of Membrane Proteins Mediated by Polymer-Bounded Lipid Nanodiscs, Structure 25(2) (2017) 384-392.

[99] C. Sun, S. Benlekbir, P. Venkatakrishnan, Y. Wang, S. Hong, J. Hosler, E. Tajkhorshid, J.L. Rubinstein, R.B. Gennis, Structure of the alternative complex III in a supercomplex with cytochrome oxidase, Nature 557(7703) (2018) 123-126.

[100] J. Radoicic, S.H. Park, S.J. Opella, Macrodiscs Comprising SMALPs for Oriented Sample Solid-State NMR Spectroscopy of Membrane Proteins, Biophys J 115(1) (2018) 22-25.

[101] B. Bersch, J.M. Dorr, A. Hessel, J.A. Killian, P. Schanda, Proton-Detected Solid-State NMR Spectroscopy of a Zinc Diffusion Facilitator Protein in Native Nanodiscs, Angewandte Chemie 56(9) (2017) 2508-2512.

[102] T. Ravula, N.Z. Hardin, J. Bai, S.C. Im, L. Waskell, A. Ramamoorthy, Effect of polymer charge on functional reconstitution of membrane proteins in polymer nanodiscs, Chem Commun (Camb) 54(69) (2018) 9615-9618.

[103] M. Esmaili, M. Overduin, Membrane biology visualized in nanometer-sized discs formed by styrene maleic acid polymers, Biochim Biophys Acta Biomembr 1860(2) (2018) 257-263.

[104] T. Kolter, K. Sandhoff, Lysosomal degradation of membrane lipids, FEBS Lett 584(9) (2010) 1700-12.

[105] V.E. Ahn, P. Leyko, J.R. Alattia, L. Chen, G.G. Prive, Crystal structures of saposins A and C, Protein science : a publication of the Protein Society 15(8) (2006) 1849-57.

[106] M. Rossmann, R. Schultz-Heienbrok, J. Behlke, N. Remmel, C. Alings, K. Sandhoff, W. Saenger, T. Maier, Crystal structures of human saposins C andD: implications for lipid recognition and membrane interactions, Structure 16(5) (2008) 809-17.

[107] A. Flayhan, H.D.T. Mertens, Y. Ural-Blimke, M. Martinez Molledo, D.I. Svergun, C. Low, Saposin Lipid Nanoparticles: A Highly Versatile and Modular Tool for Membrane Protein Research, Structure 26(2) (2018) 345-355 e5.

[108] K. Popovic, J. Holyoake, R. Pomes, G.G. Prive, Structure of saposin A lipoprotein discs, Proc Natl Acad Sci U S A 109(8) (2012) 2908-12.

[109] C.H. Chien, L.R. Helfinger, M.J. Bostock, A. Solt, Y.L. Tan, D. Nietlispach, An Adaptable Phospholipid Membrane Mimetic System for Solution NMR Studies of Membrane Proteins, J Am Chem Soc 139(42) (2017) 14829-14832.

[110] G.M. Anantharamaiah, J.L. Jones, C.G. Brouillette, C.F. Schmidt, B.H. Chung, T.A. Hughes, A.S. Bhown, J.P. Segrest, Studies of synthetic peptide analogs of the amphipathic helix. Structure of complexes with dimyristoyl phosphatidylcholine, J Biol Chem 260(18) (1985) 10248-55.

[111] R.M. Epand, A. Gawish, M. Iqbal, K.B. Gupta, C.H. Chen, J.P. Segrest, G.M. Anantharamaiah, Studies of Synthetic Peptide Analogs of the Amphipathic Helix - Effect of Charge-Distribution, Hydrophobicity, and Secondary Structure on Lipid Association and Lecithin, Cholesterol Acyltransferase Activation, Journal of Biological Chemistry 262(19) (1987) 9389-9396.

[112] S.R. Midtgaard, M.C. Pedersen, J.J.K. Kirkensgaard, K.K. Sorensen, K. Mortensen, K.J. Jensen, L. Arleth, Self-assembling peptides form nanodiscs that stabilize membrane proteins, Soft Matter 10(5) (2014) 738-752.

[113] V.K. Mishra, G.M. Anantharamaiah, J.P. Segrest, M.N. Palgunachari, M. Chaddha, S.W. Sham, N.R. Krishna, Association of a model class A (apolipoprotein) amphipathic alpha helical peptide with lipid: high resolution NMR studies of peptide.lipid discoidal complexes, J Biol Chem 281(10) (2006) 6511-9.

[114] S.H. Park, S. Berkamp, G.A. Cook, M.K. Chan, H. Viadiu, S.J. Opella, Nanodiscs versus macrodiscs for NMR of membrane proteins, Biochemistry 50(42) (2011) 8983-5.

[115] H. Kondo, K. Ikeda, M. Nakano, Formation of size-controlled, denaturation-resistant lipid nanodiscs by an amphiphilic self-polymerizing peptide, Colloids Surf B Biointerfaces 146 (2016) 423-30.

[116] L.J. Catoire, M. Zoonens, C. van Heijenoort, F. Giusti, E. Guittet, J.L. Popot, Solution NMR mapping of water-accessible residues in the transmembrane beta-barrel of OmpX, Eur Biophys J 39(4) (2010) 623-30.

[117] B. Liang, L.K. Tamm, Solution NMR of SNAREs, complexin and alpha-synuclein in association with membrane-mimetics, Prog Nucl Magn Reson Spectrosc 105 (2018) 41-53.

[118] E. Prade, M. Mahajan, S.C. Im, M. Zhang, K.A. Gentry, G.M. Anantharamaiah, L. Waskell, A. Ramamoorthy, A Minimal Functional Complex of Cytochrome P450 and FBD of Cytochrome P450 Reductase in Nanodiscs, Angewandte Chemie 57(28) (2018) 8458-8462.

[119] J.R. Schnell, J.J. Chou, Structure and mechanism of the M2 proton channel of influenza A virus, Nature 451(7178) (2008) 591-5.

[120] K.R. MacKenzie, J.H. Prestegard, D.M. Engelman, A transmembrane helix dimer: structure and implications, Science 276(5309) (1997) 131-3.

[121] K. Raltchev, J. Pipercevic, F. Hagn, Production and Structural Analysis of Membrane-Anchored Proteins in Phospholipid Nanodiscs, Chemistry 24(21) (2018) 5493-5499.

[122] R. Linser, N. Salvi, R. Briones, P. Rovo, B.L. de Groot, G. Wagner, The membrane anchor of the transcriptional activator SREBP is characterized by intrinsic conformational flexibility, Proc Natl Acad Sci U S A 112(40) (2015) 12390-5.

[123] J. Kale, E.J. Osterlund, D.W. Andrews, BCL-2 family proteins: changing partners in the dance towards death, Cell Death Differ 25(1) (2018) 65-80.

[124] S. Ahuja, N. Jahr, S.C. Im, S. Vivekanandan, N. Popovych, S.V. Le Clair, R. Huang, R. Soong, J. Xu, K. Yamamoto, R.P. Nanga, A. Bridges, L. Waskell, A. Ramamoorthy, A model of the membrane-bound cytochrome b5-cytochrome P450 complex from NMR and mutagenesis data, J Biol Chem 288(30) (2013) 22080-95.

[125] K.A. Gentry, E. Prade, C. Barnaba, M. Zhang, M. Mahajan, S.C. Im, G.M. Anantharamaiah, S. Nagao, L. Waskell, A. Ramamoorthy, Kinetic and Structural Characterization of the Effects of Membrane on the Complex of Cytochrome b 5 and Cytochrome c, Sci Rep 7(1) (2017) 7793.

[126] R.J. Mallis, K.N. Brazin, J.S. Duke-Cohan, W. Hwang, J.H. Wang, G. Wagner, H. Arthanari, M.J. Lang, E.L. Reinherz, NMR: an essential structural tool for integrative studies of T cell development, pMHC ligand recognition and TCR mechanobiology, J Biomol NMR 10.1007/s10858-019-00234-8 (2019).

[127] M.M. Reif, M. Fischer, K. Fredriksson, F. Hagn, M. Zacharias, The N-Terminal Segment of the Voltage-Dependent Anion Channel: A Possible Membrane-Bound Intermediate in Pore Unbinding, J Mol Biol 431(2) (2019) 223-243.

[128] D.C. Rodriguez Camargo, K.J. Korshavn, A. Jussupow, K. Raltchev, D. Goricanec, M. Fleisch, R. Sarkar, K. Xue, M. Aichler, G. Mettenleiter, A.K. Walch, C. Camilloni, F. Hagn, B. Reif, A. Ramamoorthy, Stabilization and structural analysis of a membrane-associated hIAPP aggregation intermediate, Elife 6 (2017).

[129] X. Jian, W.K. Tang, P. Zhai, N.S. Roy, R. Luo, J.M. Gruschus, M.E. Yohe, P.W. Chen, Y. Li, R.A. Byrd, D. Xia, P.A. Randazzo, Molecular Basis for Cooperative Binding of Anionic Phospholipids to the PH Domain of the Arf GAP ASAP1, Structure 23(11) (2015) 1977-88.

[130] M.T. Mazhab-Jafari, C.B. Marshall, P.B. Stathopulos, Y. Kobashigawa, V. Stambolic, L.E. Kay, F. Inagaki, M. Ikura, Membrane-dependent modulation of the mTOR activator Rheb: NMR observations of a GTPase tethered to a lipid-bilayer nanodisc, J Am Chem Soc 135(9) (2013) 3367-70.

[131] D. Goricanec, R. Stehle, P. Egloff, S. Grigoriu, A. Pluckthun, G. Wagner, F. Hagn, Conformational dynamics of a G-protein alpha subunit is tightly regulated by nucleotide binding, Proc Natl Acad Sci U S A 113(26) (2016) E3629-38.

[132] Y. Kofuku, T. Ueda, J. Okude, Y. Shiraishi, K. Kondo, T. Mizumura, S. Suzuki, I. Shimada, Functional dynamics of deuterated beta2 -adrenergic receptor in lipid bilayers revealed by NMR spectroscopy, Angewandte Chemie 53(49) (2014) 13376-9.

[133] T.H. Bayburt, S.G. Sligar, Membrane protein assembly into Nanodiscs, FEBS Lett 584(9) (2010) 1721-7.

[134] C.R. Morgan, C.M. Hebling, K.D. Rand, D.W. Stafford, J.W. Jorgenson, J.R. Engen, Conformational transitions in the membrane scaffold protein of phospholipid bilayer nanodiscs, Mol Cell Proteomics 10(9) (2011) M111 010876.

[135] D.R. McDougle, A. Palaria, E. Magnetta, D.D. Meling, A. Das, Functional studies of N-terminally modified CYP2J2 epoxygenase in model lipid bilayers, Protein science : a publication of the Protein Society 22(7) (2013) 964-79.

[136] T.H. Bayburt, A.J. Leitz, G. Xie, D.D. Oprian, S.G. Sligar, Transducin activation by nanoscale lipid bilayers containing one and two rhodopsins, J Biol Chem 282(20) (2007) 14875-81.

[137] K. Dalal, F. Duong, Reconstitution of the SecY translocon in nanodiscs, Methods Mol Biol 619 (2010) 145-56.

[138] S. Eggensperger, O. Fisette, D. Parcej, L.V. Schafer, R. Tampe, An annular lipid belt is essential for allosteric coupling and viral inhibition of the antigen translocation complex TAP (transporter associated with antigen processing), J Biol Chem 289(48) (2014) 33098-108.

[139] K. Gawrisch, O. Soubias, M. Mihailescu, Insights from biophysical studies on the role of polyunsaturated fatty acids for function of G-protein coupled membrane receptors, Prostaglandins Leukot Essent Fatty Acids 79(3-5) (2008) 131-4.

[140] G. Ma, M. Wei, L. He, C. Liu, B. Wu, S.L. Zhang, J. Jing, X. Liang, A. Senes, P. Tan, S. Li, A. Sun, Y. Bi, L. Zhong, H. Si, Y. Shen, M. Li, M.S. Lee, W. Zhou, J. Wang, Y. Wang, Y. Zhou, Inside-out Ca(2+) signalling prompted by STIM1 conformational switch, Nat Commun 6 (2015) 7826.

[141] Z.O. Shenkarev, E.N. Lyukmanova, I.O. Butenko, L.E. Petrovskaya, A.S. Paramonov, M.A. Shulepko, O.V. Nekrasova, M.P. Kirpichnikov, A.S. Arseniev, Lipid-protein nanodiscs promote in vitro folding of transmembrane domains of multi-helical and multimeric membrane proteins, Biochim Biophys Acta 1828(2) (2013) 776-84.

[142] J.R. Silvius, Thermotropic Phase Transitions of Pure Lipids in Model Membranes and Their Modifications by Membrane Proteins, John Wiley & Sons, Inc.1982.

Glossary

Amphipol: amphipathic polymer

ApoA-1: apolipoproteinA-1

Bicelles: bilayer micelles

bR: bacteriorhodopsin

CHAPS: 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate

$Cytb_5$: cytochrome b_5

DHPC: 1,2-dihexanoyl-sn-glycero-3-phosphocholine

DIBMA: diisobuthylene/maleic acid

DMPC: 1,2-dimyristoyl-sn-glycero-3-phosphocholine

DPC: n-dodecylphosphocholine (also termed Fos-Choline-12)

GPCR: G-protein coupled receptor

LDAO: lauryldimethylamine-N-oxide

MP: membrane protein

MSP: membrane scaffold protein

OmpA: outer membrane protein A

OmpX: outer membrane protein X

RDCs: residual dipolar couplings

r.m.s.d.: root mean square deviation

Salipro: saposin nanodisc

SapA: saposinA

SMA: styrene-maleimide copolymer

SMALP: styrene-maleimide lipid particle

SMA-QA: styrene-maleimide quaternary ammonium copolymer

TMH: transmembrane helix

TROSY: transverse relaxation-optimized spectroscopy