Biochimie xxx (xxxx) xxx

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Travel light: Essential packing for membrane proteins with an active lifestyle

Nicolai Tidemand Johansen [a, *], Frederik Grønbæk Tidemand [a], Martin Cramer Pedersen [b], Lise Arleth [b]

[a] Section for Transport Biology, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, Frederiksberg C, 1871, Denmark

[b] Condensed Matter Physics, Niels Bohr Institute, University of Copenhagen, Universitetsparken 5, Copenhagen E, 2100, Denmark

ARTICLE INFO

Article history:
Received 6 May 2022
Received in revised form
29 June 2022
Accepted 23 July 2022
Available online xxx

Keywords:
Membrane protein
Carrier system
Nanodisc
Micelle
Amphiphilic polymers

ABSTRACT

We review the considerable progress during the recent decade in the endeavours of designing, optimising, and utilising carrier particle systems for structural and functional studies of membrane proteins in near-native environments. New and improved systems are constantly emerging, novel studies push the perceived limits of a given carrier system, and specific carrier systems consolidate and entrench themselves as the system of choice for particular classes of target membrane protein systems. This review covers the most frequently used carrier systems for such studies and emphasises similarities and differences between these systems as well as current trends and future directions for the field. Particular interest is devoted to the biophysical properties and membrane mimicking ability of each system and the manner in which this may impact an embedded membrane protein and an eventual structural or functional study.

Contents

* Corresponding author.
E-mail address: nicolai.johansen@plen.ku.dk (N.T. Johansen).

https://doi.org/10.1016/j.biochi.2022.07.014

Please cite this article as: N.T. Johansen, F.G. Tidemand, M.C. Pedersen *et al.*, Travel light: Essential packing for membrane proteins with an active lifestyle, Biochimie, https://doi.org/10.1016/j.biochi.2022.07.014

1. Introduction

Structural and biophysical characterisation of membrane proteins (MPs) remains a major challenge in biology. Under native conditions, MPs reside in complex biological membranes, but many experimental techniques require that MPs are isolated and purified under solution conditions in presence of much simpler agents. This implies that MPs must be handled in non-native environments provided by secondary entities, here termed carrier systems, which adds a layer of complexity to sample handling. In any experiment, the ultimate goal will be that an MP retains its native biochemical activity and structural stability. Given that MPs interact with their carrier systems, the activity and stability depend on the system as a whole, and consequently, MP experimentalists must often be experts in both MP chemistry and the carrier systems used for stabilising MPs in solution.

As for soluble proteins, different MPs will generally not behave identically under equal conditions. One MP may be stable and active in a given detergent micelle, whereas another MP will aggregate immediately when adding the same detergent to the native cell membrane. This protein dependent behaviour, and the so far unpredictable nature of the intricate relationship between an MP and a carrier system, is a major bottleneck in studies of MPs and makes the field highly empirical in practice. As a consequence, several different carrier systems have been developed to circumvent this problem and to facilitate a wide range of experiments.

Fig. 1 provides a general overview of common classes of carrier systems that are used for handling MPs in solution. In our presentation, we will use the term nanodisc for discoidal lipid particles wrapped in amphiphilic molecules. However, we have chosen to make an exception for binary detergent-lipid particles that are historically named bicelles and not detergent nanodiscs. We note that elsewhere, the term “native nanodisc” is used to refer to nanodisc- or bicelle-like particles formed directly with lipids from biological membranes [1,2]. In the context of this review, liposomes serve a reference for evaluating the membrane-environment of an MP (See section 5.2), and together with polymersomes [3], it remains the only system for studying membrane curvature and electrochemical potential [4]. However, because of their large size and relatively broad size-distribution, together with an often low abundance of MP compared to lipid, proteoliposomes are generally not the system of choice in structural studies, albeit with the exception that they are widely used in solid-state nuclear magnetic resonance (NMR) [5]. Thus, in this review, we will focus on smaller, soluble carrier systems formed with conventional, small molecule detergents, amphiphilic polymers (APols)/copolymers, and membrane scaffold protein (MSP) based nanodiscs (NDs) that are representatives for three rather distinct classes of carrier systems. We will focus on how various properties differ between these classes, but also on the “grey zones” in which their properties overlap; at the end of the day, all MP carrier systems share the most important property that they are amphiphilic molecules, i.e. surfactants. To avoid confusion with the literature, we use the term APol throughout for polymers that are based on the original template design proposed by the group of J.L. Popot [6] and the term copolymer for more recently developed polymers with two chemically diverse, repeating units in their backbone (Details in Section 3). For a comprehensive review of the field, we highly recommend J.L. Popot's book, which in detail describes historic developments and compares the advantages of different carrier systems, with focus on APols [7]. Our goal of this review is that the reader will be able to appreciate the properties of different carrier systems and thereby be successful in selecting the right carrier system for a given application and be able to handle it for reconstituting MPs. Finally, we provide our perspective for future developments that will facilitate novel MP research.

2. Detergent micelles

The word surfactant is a contraction of the words *surface active agent*. A surfactant is characterised by being a substance that lowers the surface tension of the medium in which it is dissolved, and/or the interfacial tension between the medium and other phases. In turn a detergent is defined as a surfactant with cleaning properties in solution [9]. While this implies that detergents are only a subset of the broader category of surfactants, it defines a clear distinction between detergents and other commonly encountered surfactants in MP research labs, namely lipids. The terms detergent and surfactant are often used interchangeably by biochemists and biophysicists to describe small amphiphilic micelle-forming molecules, whereas the term lipid is commonly used to describe molecules forming bilayer structures with a hydrophobic interior sandwiched between the hydrophilic headgroup region [10]. The self-assembly of detergents into micelles displays a high degree of cooperativity and occurs in a narrow concentration range [10–12] (Fig. 2A). For micelle forming agents, this is referred to as the critical micelle concentration (CMC), whereas for other surfactants, it is preferentially termed the critical aggregation concentration (CAC), indicating that the formed structures are not necessarily micelles.

Membrane solubilisation by surfactants is a ratio dependent process and commonly described by a three-stage model, involving first saturation and thereafter complete solubilisation [14] (Fig. 2B). The critical ratio for membrane saturation, R_{Sat}, signifies the actual onset of solubilisation, whereas the critical ratio for solubilisation, R_{Sol}, signifies where all species are found in another environment than the original lipid bilayer membrane. In general, surfactants may be classified according to their surfactant and lipid vesicle-to-micelle free energies, which are calculated from R_{Sat} and R_{Sol} [8]. This classification effectively describes the main thermodynamics involved in membrane solubilisation by structurally diverse surfactants, which fall into two major groups divided by some energetic threshold: i.e. head-tail type detergents and facial/sterol-based type detergents (Fig. 3). Due to their cone shaped molecular geometry, determined by the packing parameter [15], classic

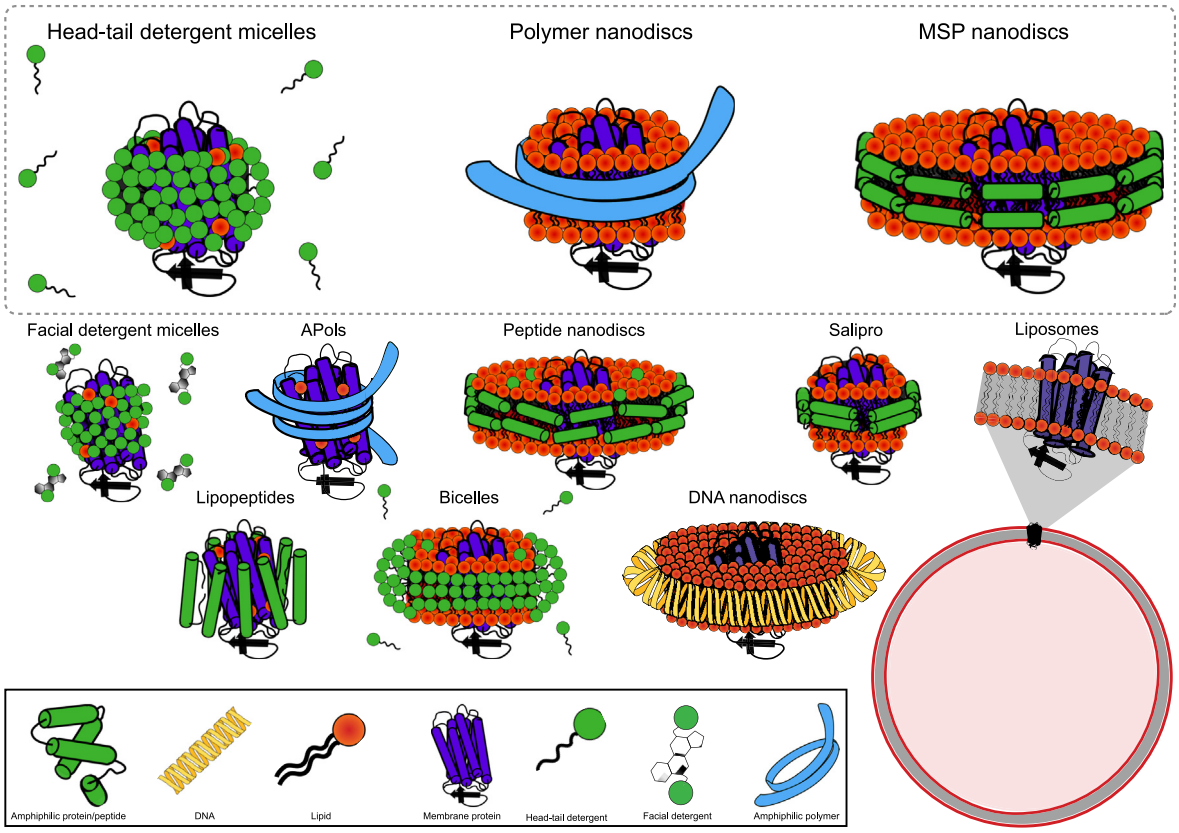

Fig. 1. Static, commonly perceived representations of carrier systems for extracting, solubilising, handling, and studying membrane proteins. This review focuses on the properties, advantages and drawbacks, and recent developments for the systems in the top row.

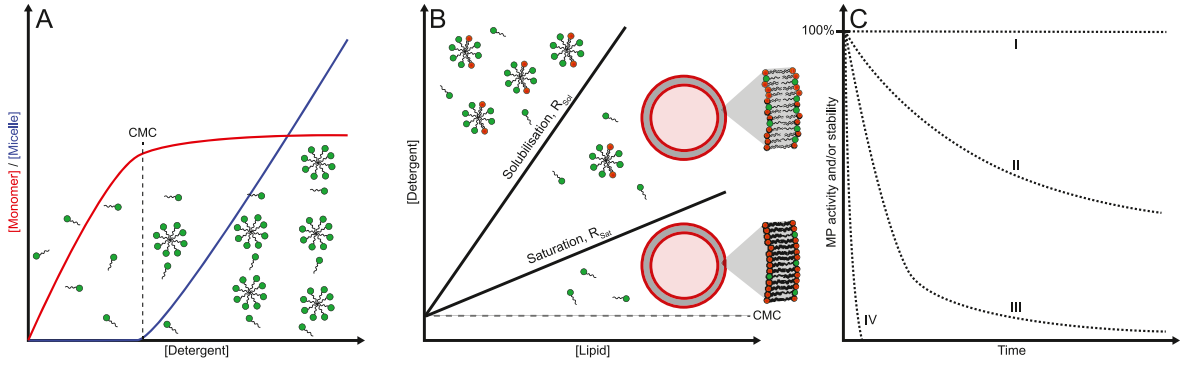

Fig. 2. Defining principles in membrane solubilisation and membrane protein stabilisation. A: Self-assembly of detergent micelles. B: Membrane solubilisation phase diagram. Below the saturation threshold, the lipid bilayer structure is retained. Saturation of the bilayer marks the beginning of solubilisation, entering a co-existence regime with lipid bilayers and mixed micelles present. Above the solubilisation threshold, all lipids are found in mixed micelles. Typically, biological membranes are solubilised around or above R_{Sol}. This principle holds for many other surfactants, and R_{Sol} and R_{Sat} depend on both surfactant properties and membrane composition [8]. C: Membrane protein activity and/or stability over time. Case I represents a membrane protein solubilised in a perfectly stabilising surfactant. Cases II and III are more common, where surfactants destabilise MP in a time-dependent manner. Several factors may influence loss of stability and/or activity, including surfactant concentration/ratio, surfactant type, temperature, disassociation of co-factors/lipids, etc. Finally, case IV represents MPs that do not tolerate removal from their native environment (with current technologies).

head-tail detergents have a modest preference for residing in micelles over bilayers, whereas lipids with roughly cylindrical geometry strongly prefer to reside in bilayers. Solubilisation therefore occurs at relatively high detergent-to-lipid ratios. On the other hand, facial/sterol-based detergents provide a more favorable environment for lipids, resulting in effective solubilsation at lower detergent-to-lipid ratios. Altogether, these observations reflect that facial/sterol-based detergents are less compatible with insertion in lipid bilayers and that they generally support disc-like structures that accommodate lipids better than globular micelles formed by most head-tail detergents [8]. The strength of the ability to solubilise membranes is termed detergency, which is an important property for any kind of surfactant beyond detergents [7,8]. Typically, solubilisation of MPs from biological membranes is done around or above R_{Sol} and with different surfactants to optimise yields. Here, it is obviously important to consider the kinetics of destabilisation/inactivation of the MP [16] (Fig. 2C), which depends on a large set of parameters, including detergency. In post-

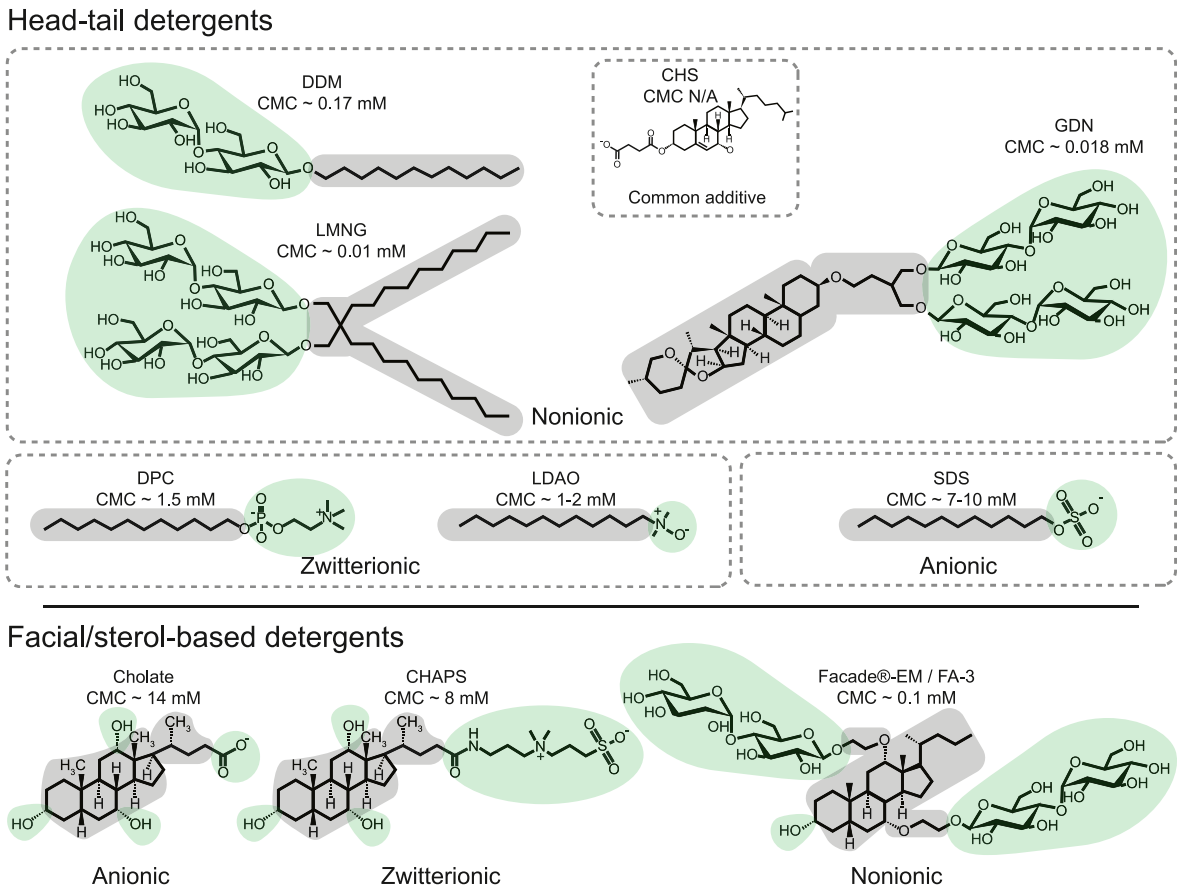

Fig. 3. Examples of and classification of small molecule detergents with hydrophobic regions having light grey background and hydrophilic regions having light green background. The CMC values are those reported from commercial sources and in pure water (www.sigmaaldrich.com and www.anatrace.com), except for GDN which is from Ref. [13]. We note that the CMC of ionic detergents depends on ionic strength. As an example, the CMC of SDS decreases to around 1 mM in presence of 100 mM NaCl.

solubilisation steps, the detergent concentration is typically reduced to around or slightly above CMC to ensure on one hand that micelles exist, and on the other hand to minimize the presence of excess micelles.

Head-tail detergents contain hydrophilic head-groups and hydrophobic tail-groups (Fig. 3). A further subdivision is typically done to distinguish detergents based on the charge of their head-group. Non-ionic detergents, such as n-dodecyl-β-D-maltoside (DDM), are considered mild and are commonly used for stabilising MPs and even MP complexes [17–19]. Ironically, for membrane solubilisation, DDM is classified as a strong detergent (high detergency) [20]. Ionic detergents are often referred to as harsh as they in most cases deactivate MPs [17,18]. The quintessential detergent (sodium) dodecyl sulfate (SDS) will even unfold soluble proteins [21], and tellingly, SDS-unfolded soluble proteins can refold in DDM or other nonionic detergents [22]. As such, the label as a mild detergent is merely given to DDM based on empiric observations that it better preserves MP structure and function compared to other detergents. Zwitterionic detergents, exemplified by e.g. dodecyl phosphocholine (DPC) and lauryldimethylaminoxid (LDAO) (Fig. 3), are typically better at solubilising biological membranes (high detergency) than nonionic detergents, and at the same time less deactivating to MPs than ionic detergents [17,18]. In practice, detergent screens are often applied to identify the optimal detergent in terms of high solubilisation efficiency and homogenous MP preparations (See e.g. Refs. [23–26]).

Maltose-neopentyl glucols (MNGs) is an interesting subclass of head-tail detergents [27]. Lauryl-MNG (LMNG) is basically two DDM molecules linked at a tertiary carbon in their tail-groups. Notably, the CMC of LMNG is an order of magnitude lower than for DDM, and MD simulations have shown that LMNG is more densely packed on MPs [28]. These properties explain the stabilisation of inherent MP dynamics [29 as well as] thermostabilisation of MPs in LMNG micelles [27,30], and that LMNG-stabilised MPs may be purified in detergent-free buffers [31]. Glyco-diosgenin (GDN) is likewise part of an interesting subclass with steroid-based tail-groups and maltoside-head groups [13]. GDN is a synthetic analogue of digitonin and both are used increasingly in structural studies of MPs, especially of eukaryotic origin [32].

Facial/sterol-based detergents form a distinct class, often referred to as facial amphiphiles (FA) [33,34]. As illustrated for three examples, a central, hydrophobic sterol-moiety is decorated on one side with polar groups (Fig. 3). Due to the facial character, these detergents are not expected to form standard micelles. Indeed, cholate, as an example, likely forms either dimers or small assemblies of dimers [35], and the detergent:MP ratio was only 37:1 for FA-1 compared to 219:1 for undecyl-β-*D*-maltoside (UDM) bound to the MP MsbA [33].

Certain types of detergents are compatible with the formation of discoidal lipid particles, called bicelles [36–39]. With their discoidal patch of lipid bilayer, bicelles bridge detergent micelles and liposomes. Bicelles spontaneously form when mixing certain long-chain lipids with detergents or short-chain phosphatidylcholines (PCs), such as 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC), under the right conditions [40]. The mixture of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and DHPC has

been the most popular choice for bicelle formation [38]. DMPC lipids form a central bilayer stabilised on the rim by DHPC that effectively functions as a detergent [41]. Facial detergents, in particular 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO), and more complex versions derived from cholate have also been proven well-suited for generation of stable bicelles [37,42]. The size of a bicelle is tuned by the ratio, q, of lipid:detergent. Importantly, only above a certain value of q, lipids and detergents are segregated spatially into an actual bicelle structure, whereas at low q, the system behaves rather as mixed micelles [43]. Bicelle formation is analogous to the formation of a range of other systems, including peptide-based nanodiscs and APol/copolymer-based nanodiscs (See Section 3).

The number of available detergents far exceeds what we are able to cover within the scope of this review. We note that detergents with extreme properties exist, especially fluorinated detergents that indeed form micelles in solution, but have extremely low detergency [7,8,44,45]. Furthermore, lipopeptides and peptergents have detergent-like characteristics (Fig. 1). Lipopeptides are alkyl-modified peptides that form micellar structures in solution and can be used to stabilise MPs with a cylindrical packing of the alkyl-tails towards the MP, making a structural interface resembling that of lipid tails in a bilayer [46,47]. α-helical lipopeptides are predominant [46–48] and only a single study has reported on β-strand lipopeptides for MP stabilisation [49]. Lipopeptides have not been used for direct solubilisation of MPs, seemingly because synthesis is expensive [47]. Detergent-like peptides, or peptergents, consist of repeating copies of hydrophobic amino acids and a terminal hydrophilic amino acid [50]. For peptergents, there are mixed reports regarding their ability to directly solubilise MPs, but they tend to improve the thermostability of MPs over detergents [50,51].

2.1. General advantages and drawbacks

Recent meta-data reviews highlight the importance of detergent micelles that remain the dominantly used carrier system in MP structural biology [32,52]. This reflects the versatility of detergent micelles, especially those that are able to effectively solubilise cell membranes to extract MPs, and not least that a wealth of different detergent types are commercially available in a high purity. Detergents are small molecules, and they will decorate the hydrophobic surface of an MP in a quite adaptable fashion; the detergent-to-MP ratio depends on the size of the MP [53]. In other words, many detergents are straight-forward to use.

In purification of MPs, protocols often utilise detergent concentrations well above the CMC to fully ensure that micelles are formed. One, if not the, main disadvantage of detergents is the presence of excess micelles that will compete for binding hydrophobic or amphiphilic molecules. This phenomenon has been termed the “hydrophobic sink” effect [44,54], and it will eventually lead to stripping of functionally relevant lipids or protein-partners from the MP of interest. The rate by which this happens naturally depends on the properties of the used detergent.

The micelle structure is another disadvantage, at least for head-tail detergents. The packing of hydrophobic tail-groups in a micelle does not resemble the packing of hydrophobic tails in the lipid bilayer of a cell membrane. Consequently, micelles do not exert a membrane-like lateral pressure on incorporated MPs, which might be important for e.g. mechano-sensitive channels [55]. The potentially destabilising effect of micelles may be alleviated by addition of exogenous lipids during MP purification. Recently, a group of researchers developed a high-throughput screen to identify such MP stabilising lipids [56]. A common stabiliser is the cholesterol analogue cholesterol hemisuccinate (CHS) (Fig. 3), which has been included in a substantial amount of MP preparations leading to high-resolution structures [32,52]. The beneficial effect of CHS has both been attributed to direct stabilising MP interactions and/or a more general remodelling of the overall micelle shape [57].

It is commonly observed that MPs possess low stability, especially thermostability, in detergent micelles formed by conventional detergents. In the specific case of alkyl PC detergents, β-barrel MPs are typically quite stable, whereas α-helical MPs are typically more dynamic in micelles than in their native cell membrane environment [58]. These properties of DPC stabilised MPs are of major concern in detergent screeing efforts, as clearly, MP activity is not correlated to maximum solubilisation and MP homogeneity. Increased structural dynamics can lead to unfolding events that promote aggregation, and this phenomenon reflects the dynamic properties of micelles, as indicated by temperature dependent CMCs and solubilsation efficiencies [59].

2.2. Recent developments

In a series of developments following the successes of LMNG [27], the lab of Pil Seok Chae and colleagues have synthesised and characterised branched detergents [60–65]. More specifically, these studies have aimed to investigate the effects of asymmetry in MNG detergents [60], head-group variation in monosaccharaide-cored glycoside detergents [61], variation of pendant chains of glucose-neopentyl glycol detergents [62], position of ether-linkages in tetra-glucoside detergents [63], pendant chain polarity in conformationally flexible maltoside-based detergents [64], and foldable maltoside detergents [65]. Notably, all of these studies have used equal or very similar experimental pipelines, i.e. the same MPs for characterisation, using the same techniques, which provides a solid basis for comparison of the different detergents. In all cases, the novel detergents were analysed for the solublisation and stabilisation of MPs compared to the gold standard DDM and either LMNG or n-octyl-β-D-glucoside (OG), and it was found that all design strategies could lead to improved MP stabilisation. As exemplified throughout for the melibiose transporter MelB, solubilisation efficiency was generally compromised compared to DDM at 0 °C. However, for many of the novel detergents, solubilisation efficiency was equal or better than DDM at 45 °C, whereas at 55 °C, where DDM solubilisation was almost none, a few novel detergents were still able to extract moderate amounts of MP. The most pronounced improvement in stability of the two GPCRs β_2AR and MOR was observed in the conformationally flexible detergent M-GTM-O12, where antagonist binding was effectively unaltered over the course of three to five days with incubation at room temperature [64]. M-GTM-O12, and other highly similar detergents in that study, have relatively low CMCs and small aggregate sizes, which support the explanation that improved stability of MPs is a result of a high hydrophobic density in the proteomicelle [28,62]. Altogether, these novel design strategies were aimed at pinpointing the key parameters to optimise in future synthesis strategies of MP stabilising detergents. However, as pointed out, different detergents performed better for different MPs, suggesting that there is no “one solution fits all”. For a comprehensive overview of the above and other novel detergents, we refer to a recent review [66].

In another recent development, a library of oligoglycerol detergents (OGD) with modular structures was developed for fine-tuning the properties towards different MP applications [67]. Regarding activity and stability, MP dependent effects were observed, but in native MS, the OGDs were in general found to have superior performance over DDM, due to being readily removed from proteomicelles by collisional activation, and in case of basic linkers (e.g. triazole) having the ability to reduce protein charge. Elsewhere, catalytically cleavable detergents (CatCD) that facilitate

complete detergent exchange were designed on a template resembling the structure of DDM [68]. A single CatCD (CatCD-1) was found to solubilise MsbA from *E. coli* membranes with comparable yields to LMNG and UDM. CatCD-1 solubilised MsbA was then diluted into a range of different detergents and treated with a catalyst that tracelessly removed CatCD-1. Finally, diglucoside (DG) detergents were shown to solubilise both model and biological membranes and form discoidal lipid particles [2]. Notably, the hemifluorinated F_6ODG showed appealing properties, with a comparatively stable lipid bilayer compared to copolymer nanodiscs and bicelles. A likely explanation is that the flourinated carbon chains better segregate from the hydrocarbon lipid tails in the discoidal structure compared to hydrogenated alternatives. Other fluorinated detergents are emerging as viable alternatives to DDM for extraction and improved stabilisation of MPs, including FLAC6, which is commercially available [69].

3. Amphiphilic polymers

In this section, we will focus mainly on the field of APols and copolymers, which is seeing tremendous development towards the isolation of MPs with native cell membrane lipids associated. Fig. 4 provides an overview of common polymers along with recent developments. While we will not go into details with all of these polymers, the figure serves to provide an idea of the extent of which the field is progressing in terms of chemical diversity. For more detailed descriptions of diverse polymers, we refer to reviews elsewhere [1,70–72]. Although all of the presented polymers here are amphiphilic, we will follow the consensus of the literature and use the term APol for only polymers based on the poly-acrylate template [6], while we will use the general term copolymers for amphiphilic polymers with two repeating units in their backbone, such as styrene-maleic acid (SMA) and diisobutylene maleic acid (DIBMA). Altogether, we will use the common term polymer for all amphiphilic polymers presented here, noting that this category does not include peptides and proteins that in principle are also polymers.

With successful developments in novel detergents towards densely packed hydrophobic cores and/or dampening of structural dynamics that leads to irreversible aggregation, the hydrophobic sink effect of excess micelles remains the major challenge to overcome [44]. APols were designed on the idea to replace detergents with a compound having such high affinity for MP hydrophobic surfaces that it could remain bound irrespective of free compounds in solution along with a rather dynamic nature allowing the adjustment to MP of different sizes [6,54]. Since the first APols, e.g. A8-35, a wealth of other polymers have followed; of special interest are the SMA and DIBMA copolymers that are produced easily on industrial scale (Fig. 4) [73,74]. In the common view of these systems, the original APols are generally visualised to bind directly to MP surfaces, whereas others are typically depicted to form nanodisc-like particles with a central, discoidal lipid bilayer. However, as for detergents, lipids can probably be stabilised in any of these polymer-based systems [54]. Rather, the main difference in performance is the ability of some polymers to solubilise lipid membranes directly into nanodisc-like particles, which is the case for e.g. SMA and DIBMA, but not for e.g. A8-35. In essence, these polymers behave as facial detergents that form bicelles [8], but with the advantage of being compatible with a wider range of lipids and forming discoidal particles directly from cell membranes [75]. Other carrier systems share these characteristics. Amphiphilic peptides, such as the 18 amino acid 18A can solubilise membranes and form rather heterogenous nanodisc-like particles, termed peptide nanodiscs [76,77]. The duplicated versions of 18A, termed beltides [78] or NSP_r [79] (reversed sequence), may also form nanodisc-like particles or bind directly to the surface of an MP (peptidisc).

SMA is a copolymer synthesised from styrene and maleic anhydride followed by hydrolysis [75]. The copolymer has wide applications in industry and science [80], but its applicability for stabilising MPs was first explored a decade ago [73]. SMA and other related copolymers, such as DIBMA, styrene-maleimide (SMI), and zwitter-ionic SMA (zSMA) are capable of directly solubilising lipid bilayers, both synthetic and native, producing discoidal lipid particles (SMALP, DIBMALP, SMILP, and zSMALP respectively) [74,80,81]. Thus, MPs can be extracted from biological membranes together with their native environment without the aid of other detergents [73,75], and the resulting lipoprotein particles have consequently been called native nanodiscs [82]. The use of SMALP and derivatives for MP handling has been reviewed elsewhere (see e.g. Refs. [72,75,83–85]).

The most common SMA copolymers are termed SMA(3:1) or SMA(2:1) given their ratio of styrene to maleic acid groups [75]. The average molecular weight of SMA copolymers used in studies of MPs has ranged from 7.5 kDa to 10 kDa [75], while the more recently explored DIBMA is larger and has an average molecular weight of 37 kDa [74]. SMALPs have been widely studied by electron microscopy (EM) and have been found to have an average diameter of 13 nm [86], whereas DIBMALPs are accordingly larger at approximately 18 nm [74]. For zSMA, the average diameter of zSMALPs was shown to be proportional to the molecular weight of the polymer, albeit also with drastically increasing size polydispersity [87]. A detailed structural description of SMALPs (SMA(2:1)) was pursued by small-angle neutron scattering (SANS), but the proposed discoidal core-shell model did not fit the data well [88]. In a more recent report, SMILPS formed with varying lipid ratios were analysed by small-angle X-ray scattering (SAXS) [81]: decent fits were obtained using a rather complex model including both a polydisperse bicelle model and a polydisperse ellipsoidal model. Finally, SMALPs (SMA(3:1) formed with different lipids DMPC and 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC)) were analysed in detail by SAXS, with the best fits obtained from a model describing nanodisc-like particles with additional polymers inserted in the central bilayer [89]. As such, the structure of copolymer-based nanodiscs resemble the characteristics of bicelles and peptide nanodiscs that are rather polydisperse [76,78]. Finally, the size of copolymer-based nanodiscs, as well as peptide nanodiscs, can be controlled by the ratio of lipid to surfactant (q), analogously to the formation of bicelles. Large, so-called macrodiscs with diameters around 50 nm can form at least with synthetic, well-defined lipid composition, typically based on DMPC [90–92]. As expected, the longer SMA(3:1) formed macrodiscs at a high q ($q = 49.1$), whereas the shorter SMA(2:1) formed macrodiscs at relatively lower q ($q = 27.7$), and it was further noted that macrodisc formation was a very slow process [92]. For comparison, the typical molar ratio is roughly $q = 2$ for SMA(2:1) to efficiently solubilise cell membranes over 2 h [93], while detailed structural studies revealed that also for SMA(3:1), $q = 2$ was required for complete solubilisation of DMPC liposomes into small nanodisc-like particles [89].

3.1. General advantages and drawbacks

SMA and later a wide range of other polymers are heralded for the “detergent-free” purification of MPs directly from cell membranes into stable discoidal lipid particles. This phrasing is somewhat delusive, given that these polymers behave like detergents from a biophysical point of view [8]. Rather, the advantage over traditional detergents comes from the elimination of the “hydrophobic sink”: after solubilisation of cell membranes, chromatographic purification

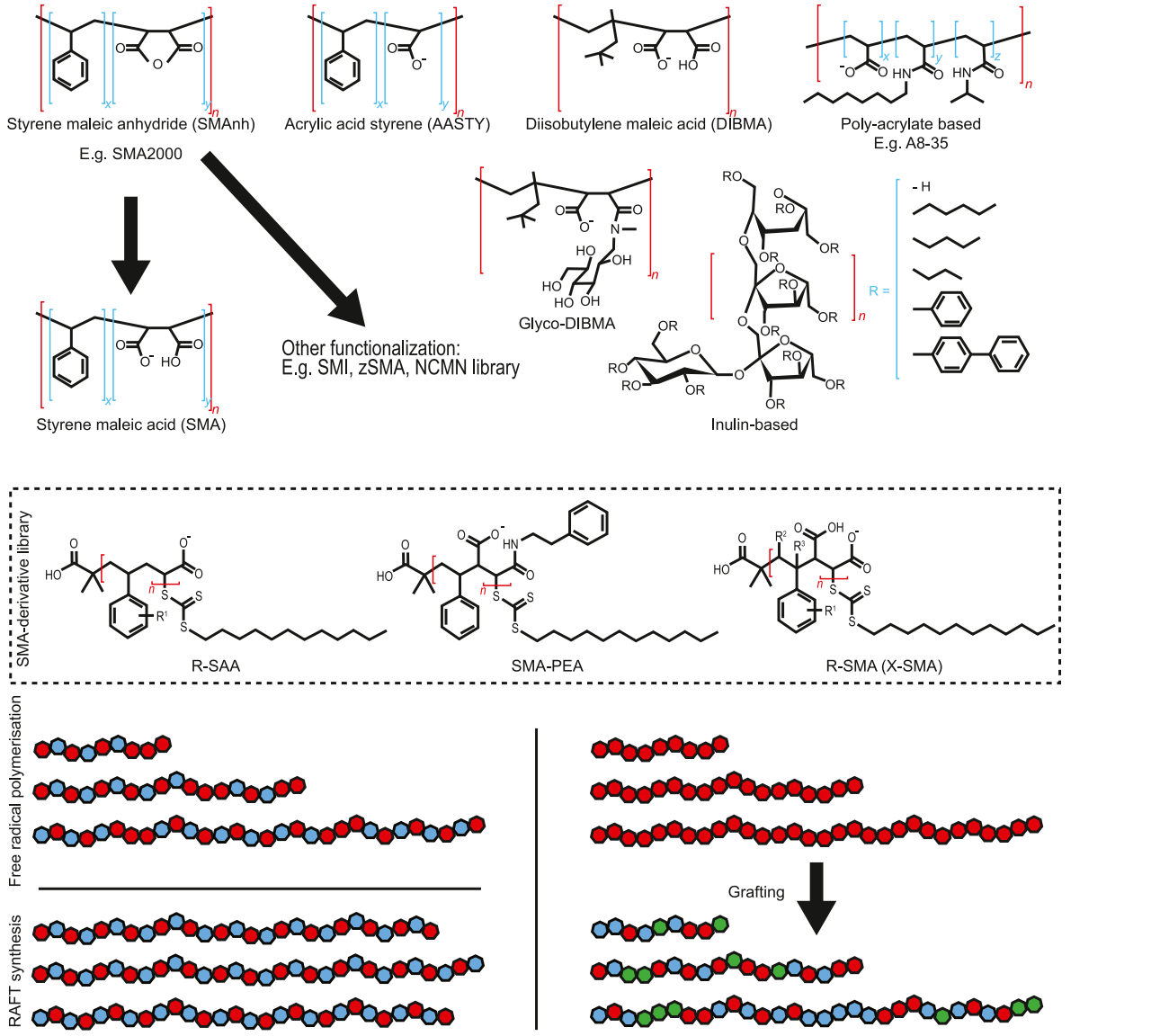

Fig. 4. Top: Overview of common and recently developed polymers and polymer libraries used for MP stabilisation. Inulin is obtained from biological sources, whereas the remaining polymers are synthesised chemically. Bottom: Chemical synthesis of polymer backbones is achieved by either free radical polymerisation, which yields polydisperse polymers, or RAFT, which yields relatively more homogenous polymers. Finally, side-groups are added by random grafting on to the backbone. In the case of e.g. the A8-35 polymer, functional groups will be randomly distributed along the polymer.

steps may be carried out in polymer-free buffer. Furthermore, polymer-based nanodiscs have comparatively high colloidal stability, tolerating in some cases up to around 60 °C [94], whereas bicelle morphology is sensitive to environmental effects, including temperature, hydration level, and ionic strength [36,95,96].

The synthesis of polymers presents advantages as well as drawbacks. On one hand, synthesis is rather simple and easily scaled up to large quantities [75,97]. On the other hand, free-radical polymerisation reactions are somewhat random by nature, resulting in polydisperse products. Reversible addition-fragmentation chain transfer (RAFT) synthesis is a more controlled variation of standard free radical polymerisation, which accordingly yields more homogenous products with low molar mass dispersity and more consistent "runs" of monomers [97,98] (Fig. 4). Finally, functionalisation of polymers is often achieved by grafting of chemical groups. In case of adding multiple reactive groups, pendant chains may be grafted randomly along the backbone, as is typically the case for e.g. poly-acrylate templates. Altogether, batch variation may arise, and note should be taken that polymers obtained from different sources may differ in their ability to solubilise membranes [99,100].

While RAFT synthesis yields relatively homogenous copolymers, it has to be noted that polymer length is a crucial parameter. For SMA, it has been shown that short/low MW species are much more efficient for solubilising membranes than long species [101]. Conversely, lipid particles formed with the short species are highly dynamic and do not even contain a discoidal lipid bilayer. Seemingly, there is a trade-off between the need for short SMA to solubilise membranes and long SMA to stabilise discoidal lipid particles. Perhaps, the actual mix of short and long species is the explanation for the successful generation of SMALPs that resemble nanodiscs: small species solubilise membranes and are gradually exchanged by longer species providing stability. Furthermore, short species are likely the first to disassociate from lipid particles and get separated during chromatographic purification [101]. Similar effects were observed for the DIBMA polymer, albeit that below some

critical polymer length, solubilisation was not achieved [102].

In general, polymer-based nanodiscs are quite dynamic particles that exchange both lipids and polymer components [103]. This enables further applications such as the formation of liposomes [75] and could potentially be extended to the formation of supported lipid bilayers (SLBs), as is the case for bicelles and 18A peptide nanodiscs [104,105]. Notably, these peptide nanodiscs appeared to have limited stability for MP purification, as visualised by fast growth in particle size and eventual aggregation upon exposure to room temperature during or after size-exclusion chromatrography (SEC) purification in absence of excess peptide [76]. However, this could be exploited in peptide nanodisc mediated formation of SLBs, as peptides were efficiently removed from the solid support, resulting in lipid bilayers with high surface coverage and oriented MPs [105]. Formation of SLBs opens up an entire new venue of experimental techniques, including atomic force microscopy and X-ray and neutron reflectrometry. The length and hydrophobicity, among other properties, of polymers influence key parameters such as solubilisation efficiency, particle stability and homogeneity, and lipid exchange dynamics [86,87,94,106,107]. In MP solubilisation, co-extracted lipids in polymer nanodiscs are native and may be functionally relevant, but intuitively, polymers that are efficient solubilisers will likely not form particles containing more than tightly bound annular lipids surrounding the MP.

So far, the SMA(2:1) is the most widely used copolymer for forming so-called “native nanodiscs”. This copolymer has some important limitations, including a low tolerance to divalent ions, insolubility at acidic pH, and strong UV absorption, and it performs best at room temperature or above [93]. Some of these limitations are overcome by other copolymers, such as DIBMA [74] or by different functionalisation of the SMAnh polymer, e.g. the case for the cationic SMI [81] and zwitter-ionic zSMA [87]. Interestingly, despite sharing the maleic acid units as a building block, divalent metal ions were found to impair membrane solubilisation by SMA [93], but to accelerate membrane solubilisation by DIBMA [106].

3.2. Recent developments

The development of new polymers takes place at high pace. A major reason for this is the commercial availability of e.g. the SMA precursor styrene maleic anhydride (SMAnh, for example SMA2000) that can be functionalised using different small chemicals to produce polymers with different properties. As such, variations in pendant chains constitute the major design strategy being pursued, which has led to the development of polymer libraries, such as the SMA-derivative library [107] and the native cell membrane nanoparticles (NCMN) library [84,108,109]. Rather confusing, it has been proposed that a NCMN has different properties from a SMALP [84], although SMALPs formed from cell membranes are indeed a subset of the NCMN library. The same goes for e.g. SMILPs that are also obtained from the common SMAnh precursor [81]. Altogether, the polymer field resembles that of conventional detergents, where it is well acknowledged that there is no “one solution fits all”. Rather, polymers encompassing different properties are continuously developed to serve specific purposes, which enables polymer screens, much as we know it from detergent screens. Concomitant with this development are the detailed studies of polymer biophysical properties, as reviewed recently [72]. These are of high importance for establishing generalised and specific protocols that will facilitate the general use of polymers in MP research. Noteworthy, two SMA preparations of the MP AcrB resulted in cryo-EM structures with markedly different characteristics [84]: first, a 8.8 Å resolution structure with no visible lipids [110], and more recently a 3.2 Å resolution structure with more than 30 lipids visible [108]. Despite the possibility of batch variation in the utilised SMA polymer, the only main difference in their protocols appeared to be the inclusion of a SEC purification step after affinity chromatography for the 8.8 Å structure [110], whereas the 3.2 Å structure was obtained from a grid prepared directly after the affinity chromatography step [108]. This could suggest that SMA, much like conventional detergents, form dynamic entities capable of e.g. stripping lipids during extended purification steps.

The group of Sandro Keller and colleagues has established an experimental pipeline for thoroughly characterising the biophysical properties of polymers and polymer lipid particles. As illustrated by their recent work on glyco-DIBMA, polymer-based nanodiscs were systematically characterised by a battery of methods, always in relation to the polymer:lipid ratio, which dictates the phase diagram of the system according to the critical ratios R_{Sat} and R_{Sol} [94]. Polymer-based nanodiscs were studied in terms of average diameter and polydispersity (dynamic light scattering and SEC), corroborated by elecron-miscroscopy (EM) to confirm nanodisc formation. Two additional biophysical measurements provided additional insight to the properties of these systems, that is differential scanning calorimetry (DSC) probing the thermotropic phase transition of DMPC-lipids and time-resolved Förster resonance energy transfer (FRET) probing lipid exchange dynamics. Later, we will compare such measurements between different carrier systems (Section 5.1 and Fig. 7), which are telling for the lipid bilayer integrity in common carrier systems. In the specific case of glyco-DIBMA, this polymer was developed to improve the performance of aliphatic copolymers, such as DIBMA, in terms of membrane solubilisation at physiological conditions, and to reduce charge density to improve tolerance to divalent cations and acidic pH. Compared to DIBMA, glyco-DIBMA indeed showed improved membrane solubilisation, and furthermore, it formed nanodisc-like lipid particles with a narrower size distribution.

In another recent development, novel cycloalkane modified APols (CyclApol) were shown to efficiently extract MPs into stable particles [111]. Like A8-35, CyclAPols are poly-acrylate based, and functionalisation with cycloalkanes instead of aliphatic chains was the key driver for the improved properties. The CyclAPol showed similar extraction efficiency for the MP bacteriorhodopsin (BR) in DMPC-fused purple membranes as compared to SMA(3:1), but BR showed much higher thermal and temporal stability in the CyclAPol extracted form. More recent work has suggested that CyclAPols can extract MPs at much lower concentrations than other types of polymers [112].

Until now, we have only considered polymers obtained from chemical synthesis. Recently, naturally extracted oligosaccharides, inulin, functionalised with hydrophobic pendant chains (Fig. 4) were reported as the first non-ionic polymers to solubilise membranes efficiently and form nanodiscs [113]. Being nonionic, the inulin-based polymer tolerated a wide range of pH and concentrations of divalent metal ions. Furthermore, the membrane solubilisation efficiency proved better than synthetic SMA-derivatives and was on par with the gold standard detergent, DDM. The degree of substituion, i.e. the extent of functionalisation with hydrophobic groups, was critical to balance in order to achieve efficient membrane solubilisation and at the same time keep the polymer soluble in aqueous solution. Thus, this study provided insights to guide future developments in nonionic polymers. However, we note that further experiments are required to establish the ability of inulin-based polymers to isolate and stabilise functional MPs.

Ultimately, the polymer-based toolbox is rapidly expanding, and based on recent developments highlighting beneficial side groups, synthesis-strategies, etc., even more and specialised polymers are expected to be devised in the near future. Thus, much like for chemically diverse conventional detergents, we can expect to see the availability of commercial polymer-screens that will improve the success rate and facilitate MP specific extraction.

4. MSP nanodiscs

So far, we have considered dynamic surfactants that self-assemble into well-ordered, albeit rather polydisperse, complexes. A key attribute of those systems is that they are adaptable, meaning that they can accommodate MPs of different sizes and that the diameter of nanodiscs/bicelles depend on the lipid:surfactant ratio. With long surfactants, at least the latter characteristic disappears. Instead, the diameter of discoidal lipid particles becomes dictated by the length of the surfactant. This is the case for MSP nanodiscs as well as DNA-encircled lipid bilayers (DEBs)/DNA-corralled nanodiscs (DCNDs) (Fig. 1). In this section, we will focus on MSP nanodiscs that are ultimately well-studied, whereas DEBs will be mainly discussed in terms of recent developments. Finally, we also introduce the saposin-lipoprotein nanoparticle system (Salipro), which shares some characteristics with MSP nanodiscs, including that saposin, like MSPs, is a protein.

Apo-A1 is the major constituent in high-density lipoproteins in the human reverse cholesterol transport system [114,115] and has been shown to form discoidal particles with lipids in solution [116,117]. MSP nanodiscs are discoidal particles made with amphiphilic MSPs that are derivatives of Apo-A1 to comprise only its lipid-binding helical-repeat domain [118]. The MSP nanodisc field has produced an overwhelming number of structural and functional studies, as reviewed several times by Stephen Sligar's group (see e.g. Refs. [119–121] and others [122]). Furthermore, recent analyses of an MP database revealed a steady increase in cryo-EM structures of MPs stabilised in nanodiscs [32,52].

The most utilised MSP for generation of nanodiscs has been the so-called MSP1D1 (overview of common MSPs is provided in Fig. 5). MSP1D1 was directly derived from human Apo-A1 by truncation of the 110 amino acid N-terminal domain as well as 11 extra residues from the first helix shown not to be involved in lipid binding [123]. MSP1D1-based nanodiscs have a slightly elliptical cross-section of roughly 10 nm in diameter [124]. Another popular MSP was generated from insertion of three extra helices inside MSP1D1, yielding the construct MSP1E3D1, which lead to nanodiscs of roughly 13 nm in diameter [123]. Truncated MSP1D1 constructs form smaller nanodiscs, for instance MSP1D1ΔH5, which yields nanodiscs of roughly 9 nm in diameter that are just small enough to be compatible with solution state NMR (sNMR) [125]. Because of this, MSP1D1ΔH5 nanodiscs facilitated the first high-resolution structure of a nanodisc as determined in a recent NMR-based study [126]. This structure showed that each nanodisc contained two MSPs oriented as anti-parallel belts, laying orthogonal to the bilayer normal, and surrounding a central lipid bilayes. Furthermore, the two copies of the MSPs were stabilised by specific ionic interactions between side chains [126]. In terms of size, the creation of very short and very long MSP constructs have revealed limitations and provided indirect insight to stability and/or flexibility of the nanodisc system as a lipid carrier system. MSP truncations, MSP1D1ΔH4H5H6 and MSP1D1ΔH4H5 yield nanodiscs with only 10 to 20 DMPC lipids inside that are unstable and tend to form larger particles than expected as well as increasing in size over time [125]. MSP repeats, such as MSP2N2, form large, but relatively polydisperse nanodiscs [127], suggesting a high flexibility towards the number of lipids loaded. Notably, MSP2N2 is the most widely used MSP for cryo-EM studies [52], and it turns out that MP incorporation aids to forming rather homogenous particles as opposed to such large nanodiscs containing only lipids inside.

MSPs are typically expressed in high yield in *E. coli* with reported levels of about 250 mg per liter bacterial culture [118] and relatively straight forward purification procedures are well established [128]. The crystal structure of a truncated Apo-A1 shows that MSP alone likely folds on itself or in dimers in solution to shield hydrophobic regions [129]. In our experience, the yield of MSP varies between different constructs. The short MSP1D1ΔH5 yields roughly 50 mg per liter culture (shake flask and TB medium), whereas MSP1D1 and MSP1E3D1 yield in the range of 100 mg–150 mg per liter culture.

Compared to other soluble, small carrier systems, the MSP nanodisc is unique in not being an equilibrium particle. DSC experiments on MSP-DMPC nanodiscs yielded a broad transition due to uneven packing of lipids inside, but after being heated to high temperature, the transition became sharp and reminiscent to that of pure DMPC bilayers, suggesting that the nanodisc structure disintegrated and formed larger, lipid-rich particles [130]. As such, MSP nanodisc assembly, occurring when the detergent component is removed from a mix of lipids, MSP and detergent, is a kinetically trapped out-of-equilibrium process that does not reach a global

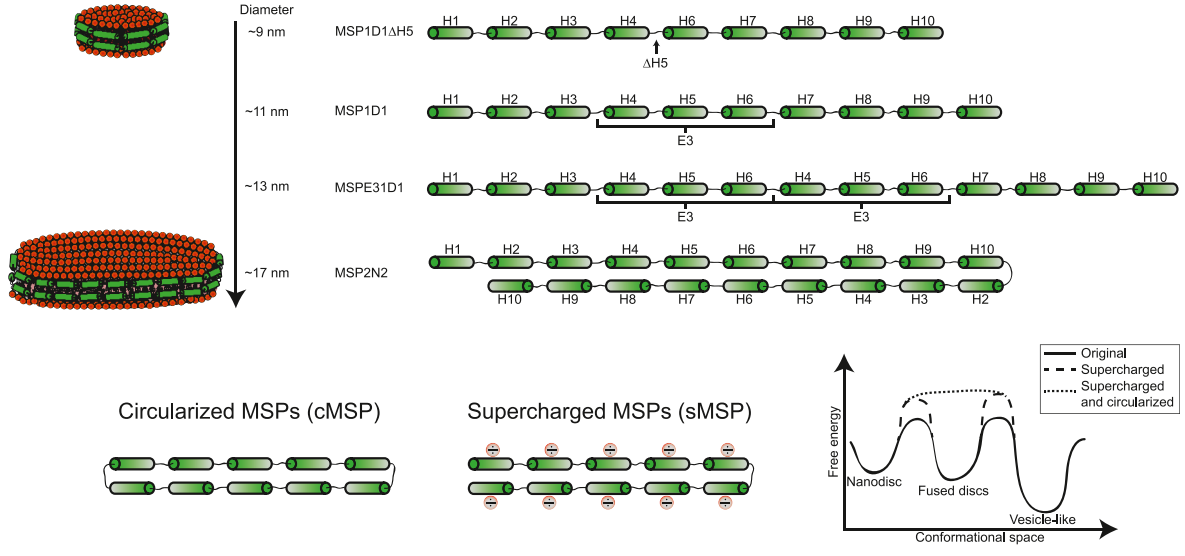

Fig. 5. Overview of popular variants of MSPs arranged by increasing length. As sketched below, recent developments include the covalently linked termini producing what is known as circularised MSPs [133] as well as the negatively charged mutations in the sequence to produce more soluble (supercharged) MSPs [143]. The nanodisc energy landscape and the effect of these alterations is sketched in the bottom right (details in Ref. [143]).

energy minimum [131]. Accordingly, MSP nanodiscs are stable particles with no exchange of the MSP with neither the solution nor other nanodiscs. While kinetically trapped, MSP nanodisc formation is thermodynamically reversible and controllable via the removal and reintroduction of detergent [132].

The nanodisc circumference is proportional to the length of the MSP [123,127]. Thus, by tuning the length of the MSP, the space available in the nanodisc can be controlled for incorporation of MPs in a controlled oligomeric state [133]. Another consequence is that nanodiscs have a maximum loading capacity for lipids [123]. This effect was studied systematically for MSP1D1 nanodiscs, finding an optimal POPC:MSP stoichiometry of approximately 70:1 [131]. Starting from a stoichiometry well below this resulted in lipid-poor and more elliptical nanodiscs, whereas starting well above it resulted in the formation of nanodiscs alongside larger undefined particles. Thus, the lipid:MSP stoichiometry is often optimised to ensure that nanodisc are made at this maximum capacity [118,123,127]. The type of detergent used for nanodisc reconstitution is critical. Facial detergents, especially cholate, facilitate the formation of homogenous nanodiscs with maximal lipid loading, whereas other common detergents, such as DDM, yields lipid-poor nanodiscs [131]. To insert an MP into nanodiscs, a detergent solubilised MP is added to the initial mix of MSP and detergent solubilised lipids. As such, detergent compatibility is two-fold in that it has to stabilise the MP and be compatible with proper nanodisc formation. We have introduced the notions of storage detergent for the former, and reconstitution detergent for the latter [131]. Notably, a mixture of DDM and cholate as reconstitution detergents results in nanodisc formation with high efficiency, which mimics the reconstitution of a DDM stabilised MP into nanodiscs formed from cholate solubilised lipids [131,134].

In a more rare approach, MPs can be inserted into nanodiscs directly from detergent solubilised cell membranes [135–139] or whole cells [140]. Furthermore, a single study has showcased the ability to form nanodiscs from MSP and triblock-polymers, which had comparatively higher long-term stability to phospholipid nanodiscs without compromising MP activity [85,141]. Finally, other amphiphilic proteins can be used for nanodisc formation, e.g. α-synuclein which has been shown to form nanodiscs with improved properties for reconstituting negatively charged lipids compared to MSP nanodiscs [142].

4.1. General advantages and drawbacks

Unlike the dynamic systems presented so far, MSP nanodiscs are restricted in size by the encircling protein belt. Thus, they are controllable in size and highly homogenous compared to other carrier systems. The kinetically trapped structure of the nanodiscs is highly stable and tolerates a wide range of experimental conditions, making them applicable to most biochemical, biophysical, and structural techniques [119–121]. The well-defined stoichiometry and size of nanodiscs allow control of e.g. MP oligomeric state [128,133] and the well-defined bilayer patch can serve as interaction surface for intra/extracellular lipid-binding MP domains, peripheral MPs, or MP interaction partners.

In cryo-EM, *in silico* purification refers to high quality particle reconstruction from a heterogeneous sample, either in terms of composition [139] or conformation [144], suggesting that particle homogeneity is not a prerequisite for this method *per se*. Yet, for lipid containing systems, the well-defined, single-particle nature of the MSP nanodisc has been heralded as a major advantage [145]. Other structural methods are more sensitive to homogeneity, as indicated for solution state NMR by the improved resolution of MP spectra obtained in circularised nanodiscs compared to standard nanodiscs [133,146]. Furthermore, SAXS and SANS techniques are notoriously sensitive to size and shape polydispersity, and here, carrier systems with well-defined stoichiometry and shape are critical for increasing structural resolution and improving model robustness, as exemplified for the detailed analysis of bacteriorhodopsin (BR) and proteorhodopsin inside nanodiscs [147,148]. Altogether, MSP nanodiscs are compatible with most biophysical and structural techniques.

The main problem for MSP nanodiscs is the rather limited understanding of the biophysics of the assembly process. Several components, i.e. the MSP, the MP, lipids, and detergents must be carefully optimised in terms of properties and ratios to form these small particles. Although systematic studies on the assembly of MP-free nanodiscs exist [131], functional MP reconstitution can in most cases not be directly extrapolated due to the extra hydrophobic interface introduced by the addition of an MP. Furthermore, the MP storage detergent should be taken into account in the reconstitution. Hitherto, insertion of MPs into MSP nanodiscs remains a trial-and-error process that requires careful optimisation of conditions and with a rather high sample consumption. Efforts have been made to alleviate this, for instance by utilising microfluidics to form nanodiscs at different conditions and in small volumes to facilitate screening of conditions [149]. Still, nanodiscs are formed by detergent-mediated assembly, which ultimately requires expertise in handling an MP in detergent prior to attempting nanodisc reconstitution. Thus, obtaining homogenous samples of MPs in MSP nanodiscs represent a relatively difficult task compared to dynamic systems that self-assemble into defined aggregates.

4.2. Recent developments

The stability and homogeneity of MSP nanodiscs was improved by the introduction of circularised MSPs (cMSP) [133]. Circularisation refers to covalent linkage of the MSP's N- and C-termini (Fig. 5), which can be achieved by a number of methods, including sortase-mediated ligation [133,143,150], split-intein mediated ligation [146], and SpyCatcher mediated ligation [151]. In addition, we developed circularised supercharged MSPs (csMSPs) with solubility-increasing mutations, forming csMSP nanodiscs (csND) [143] (Fig. 5). These mutations were devised to prevent nanodisc aggregation in D_2O-based buffers used for SANS experiments. Similar improvements were recently described for nanodiscs with high content of negatively charged lipids [152]. The improvement in homogeneity for circularised nanodiscs ranging up to 11 nm in diameter has been observed by electron microscopy [133,146]. However, for MSP1E3D1-based nanodiscs, we observed no significant improvement in homogeneity from circularisation using SAXS [143], which reflects that nanodisc flexibility increases with size and that non-circularised supercharged nanodiscs are already homogenous. A notable benefit of circularisation is that long, circularised MSPs form fairly homogenous nanodiscs even without MPs loaded inside, which range from 50 nm to 100 nm in diameter and are compatible with studying e.g. viral entry on a membrane surface [133,153] and fusion pore formation with liposomes [151].

Both circularisation and supercharging of the MSPs have stabilising effects on the nanodisc structure [143] (Fig. 5). However, we observed that cholate-assisted assembly of nanodiscs with sMSP1D1ΔH5 and sMSP1D1 and POPC lipids resulted in multiple peaks in the SEC purification [134]. This was to some degree circumvented by circularisation, but ultimately, the best conditions were achieved by reconstituting with a mixture of cholate and DDM, which presumably alleviated charge repulsions between the sMSPs and the anionic cholate. Of note, however, assembly with DMPC lipids instead of POPC posed no problems for the sMSP1D1 using the standard-cholate assisted formation [154], suggesting lipid-dependent effects. In line with this, we note that MSP

nanodiscs formed with DOPC tend to be unstable and form larger lipid-MSP species over time [155]. Altogether, these effects provide another view on why MSP nanodisc formation is considered technically challenging: there are several parameters to optimise for all components, including detergent type, MSP length, lipid-type, and detergent:lipid:MP:MSP ratios. We note that despite possessing additional negative charges, our csNDs are compatible with incorporation of negatively charged phospholipids, as demonstrated in studies of Tissue Factor and its interaction with blood coagulation factors [156].

Finally, it was recently shown that MSPs can stabilise MPs in solution without incorporating lipids [157]. In this study, detergent was exchanged for MSP, which yielded homogenous preparations of two different MPs, OprM and MexB, suitable for negative stain EM and cryo-EM, respectively. In our own studies, we have observed that even csMSP can stabilise the Mg^{2+} channel CorA directly without exogenous lipids added [158]. While the presence of lipids is desired in many applications, this direct stabilisation resembles that obtained with e.g. APols and amphipathic peptides, which in turn are widely popular systems for handling MPs.

4.2.1. DNA-based nanodiscs

Alongside the development of large, circularised MSP nanodiscs, DNA scaffolds are emerging as an alternative tool [159–161]. In one approach, a 147 nucleotide (nt) oligonucleotide was circularised and hybridised to seven 21 nt alkylated oligonucleotides to produce a so-called double-stranded DNA minicircle (dsMC) that formed DEBs at an optimised lipid ratio of 450 DMPC per dsMC using detergent assisted assembly and corresponding to a nanodisc diameter of roughly 16 nm [159]. Further biophysical investigations and studies on MP incorporation and activity inside DEBs are warranted to fully establish the potential of this system. In another approach, the goal was to form nanodiscs larger than the apparent size limitation around 50 nm for MSP based circularised nanodiscs [160]. Using DNA origami design, DNA barrels with 60 nm or 90 nm in outer diameter were created to form DCNDs. Rather than binding directly to lipids, the DNA barrels were decorated with 11 nm MSP nanodiscs through binding of single-stranded DNA handles present on both the MSP nanodiscs and the DNA barrel. Upon addition of excess detergent solubilised lipids and subsequent dialysis, the DCNDs constituted a single, large lipid bilayer with a diameter of ~40 nm or ~70 nm. Such large diameter nanodiscs allowed the formation and visualisation of large MP complexes and clusters inside a lipid bilayer as well as visualisation of poliovirus interactions with a partner protein mediated by the lipid bilayer. Further elaborate DNA origami designs were devised to build various DNA-tethered nanodiscs (DNDs) as well as building spatial arrangements of DNDs [162]. This proof of concept highlights the precise programmable nature of DNA nanostructures, which could facilitate future studies on MPs residing in different lipids bilayers spatially arranged with e.g. a well-defined distance apart.

4.2.2. Salipro

Finally, we note that MP reconstitution in Salipro is typically achieved by similar protocols to MSP nanodiscs [163]. Salipro particles are formed with saposin-like proteins [164], commonly saposin A (SapA), which contains three disulfide bridges that stabilise its overall helix-turn-helix structure [165]. Free in solution and at neutral pH, SapA is a soluble and compact protein with a hydrophobic core, but at pH 4.8, the protein is only soluble in the presence of lipids, likely because it partially unfolds and expose hydrophobic residues [165,166]. SapA can form soluble salipro particles down to sizes of about 3.2 nm in diameter when mixed with liposomes [165]. For the typical detergent-assisted reconstitution of salipro particles, increasing the lipid:SapA ratio leads to larger, but also somewhat polydisperse particles [166]. The stability, size, and stoichiometry of Salipro are pH-dependent, producing small but stable assemblies at pH 4.8, but larger and more unstable assemblies at neutral pH [167]. Salipro can adapt in size to the incorporated MP, using the MP and a layer or two of lipids around as scaffold to make a compact assembly [163,168]. As such, the particle characteristics of salipro are reminiscent of both APols/polymers (dynamic and adaptable) and MSP nanodiscs (require detergents in formulation). Interestingly, a method for extraction of MPs from crude cell membranes into salipro, DirectMX, was recently published and shown feasible for two human MPs: a solute carrier family (SLC) 1 transporter and an undisclosed GPCR [169]. The key to successful reconstitution was membrane solubilisation with the mild detergent digitonin (See section 2), albeit at a relatively high molar ratio. After adding SapA to the solubilised membranes, affinity chromatography and SEC were carried out in detergent-free buffer, rendering stable salipro particles that tolerated freeze-thawing. As such, at least for MPs that tolerate digitonin solubilisation from cell membranes, DirectMX provides superior properties as compared to using detergent-buffers throughout purification.

5. Functional reconstitution of membrane proteins

Here, we will change our focus from the individual properties of the carrier systems themselves to a comparison of the practical aspects of functional MP reconstitution along with a discussion of the resulting MP environments.

5.1. Sample preparation cases

In Fig. 6, we reconcile the different paths to obtaining functional reconstitution of MPs in the three main groups of carrier systems presented. Initially MPs reside in complex, biological membranes, and for the purpose of simplicity, we here consider the extraction of an affinity-tagged MP (Fig. 6, purple tag).

A MP solubilisation

Initially, biological membranes are solubilised in excess detergent or surfactant with detergent properties, such as polymers. At this stage, to ensure complete solubilisation, the detergent concentration must be above CMC and, more importantly, exceed the critical solubilisation ratio, R_{Sol} (Fig. 2B), which depends on membrane lipid composition and detergent type [14,107,170]. Accordingly, it is critical to first estimate the actual concentration of lipids to solubilise. In practice, the detergent:membrane ratio is varied and solubilisation efficiency is empirically determined. Typical detergent screens vary only the detergent/membrane weight-ratio, but the problem is multi-dimensional and equally critical factors in this step are temperature and time: the presence of excess surfactant can act as a “hydrophobic sink”, which has the potential to gradually strip functionally relevant co-factors such as specific lipids from MPs or dissolve functionally relevant MP complexes [44]. As such, solubilisation should ideally be evaluated both on yield in terms of amount and specific activity where possible [16]. Stabilising additives may be added during MP solubilisation. In the case of detergent mediated solubilisation, these could be exogenous lipids, especially cholesterol-derivatives, or certain low CMC detergents [16,57]. Polymer mediated solubilisation is typically done without any external agents and rely on endogenous lipids for MP stabilisation.

B Detergent micelles

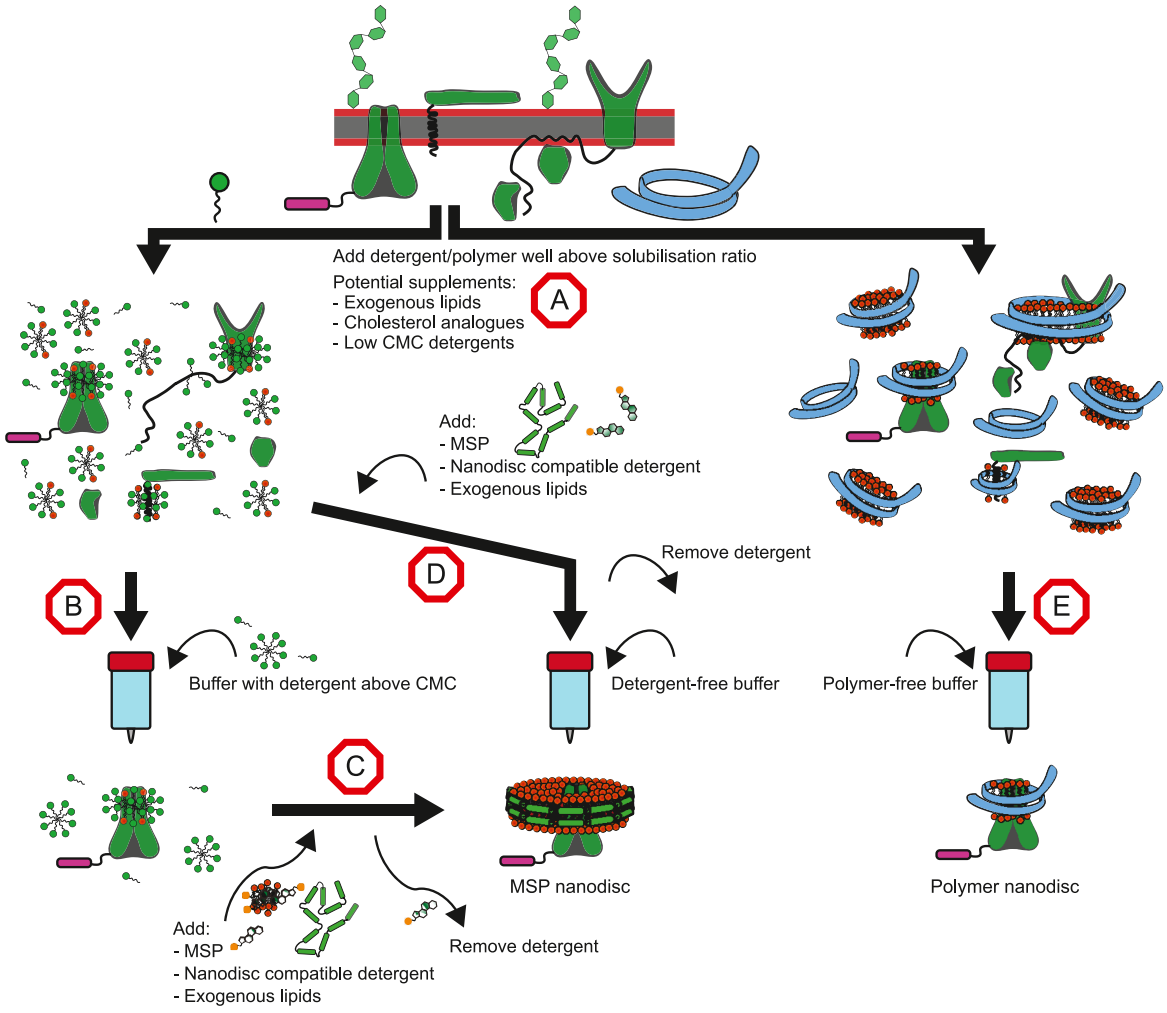

Fig. 6. Overview of membrane protein extraction into small, soluble carrier systems. Due to redundant sample preparation between different systems, examples are shown for specific cases only. A) A cell membrane is solubilised by addition of detergent or polymer above the critical solubilisation ratio. B) Classic detergent-solubilisation and purification of the target MP in presence of excess micelles. This step also encompass the general strategy of exchanging a detergent with high detergency for an alternative system with low detergency, including facial amphiphiles/detergents, the peptidisc, APols, and peptergents. C) Incorporation of detergent-purified MP into nanodiscs with synthetic lipids. This strategy is utilised for a number of systems, including bicelles, peptide nanodiscs, and DNA-based nanodiscs. D) Direct formulation of MSP nanodiscs from detergent-solubilised biological membranes. This approach can be used for the Salipro system as well. E) Direct extraction with polymer, e.g. SMA. The main difference from detergents (B) is that subsequent purification is done in polymer-free buffer.

Here, we consider the purification of an MP initially solubilised in detergent micelles. For most common conventional detergents, chromatographic purification is done in buffer containing detergent above CMC, suggesting that excess micelles are present. As such, the hydrophobic sink effect persists during purification, albeit to a lesser extent than in the initial conditions used for solubilisation. Depending on the detergency of the utilised detergent, tightly bound lipids or co-factors may remain associated to the MP in the purified sample. It is also possible to exchange detergent before or during chromatographic purification, which is desired e.g. when using expensive detergents or peptides/APols that can not solubilise membranes. When exchanging detergents on-column, one should allow sufficient time for complete exchange, which will depend mainly on the CMC of the detergent themselves. From our studies with deuterated detergents for SANS, we had to apply slow flow-rates in SEC (<0.3 ml/min) at 4 °C to observe complete exchange of DDM for deuterated DDM [171], corresponding to roughly 35 min for at MP eluting at 11 ml on a Superdex 200 column. Accordingly, slow flow-rates or extensive washes of several column volumes should be applied in affinity chromatography to ensure complete exchange. After purification, the MP is amenable to a wide range of experiments, but typically with the caveat of relatively low MP stability in detergent micelles.

C Reconstitution from micelles

Exemplified with MSP nanodiscs, MPs purified in detergent micelles may be reconstituted into lipid carrier systems with well-defined composition. This allows the detailed study of specific lipid compositions on MP structure and function. In the case of kinetically-trapped systems such as MSP nanodiscs and DNA-based nanodiscs, it sets the highest experimental requirements in terms of optimisation, but the reward is well-defined and stable samples for further experiments.

D Reconstitution from mixed micelles

In an alternative approach to Step C, MPs can be inserted more directly into lipid carrier systems, bypassing purification in detergent micelles (Step B) and maintaining a larger proportion of

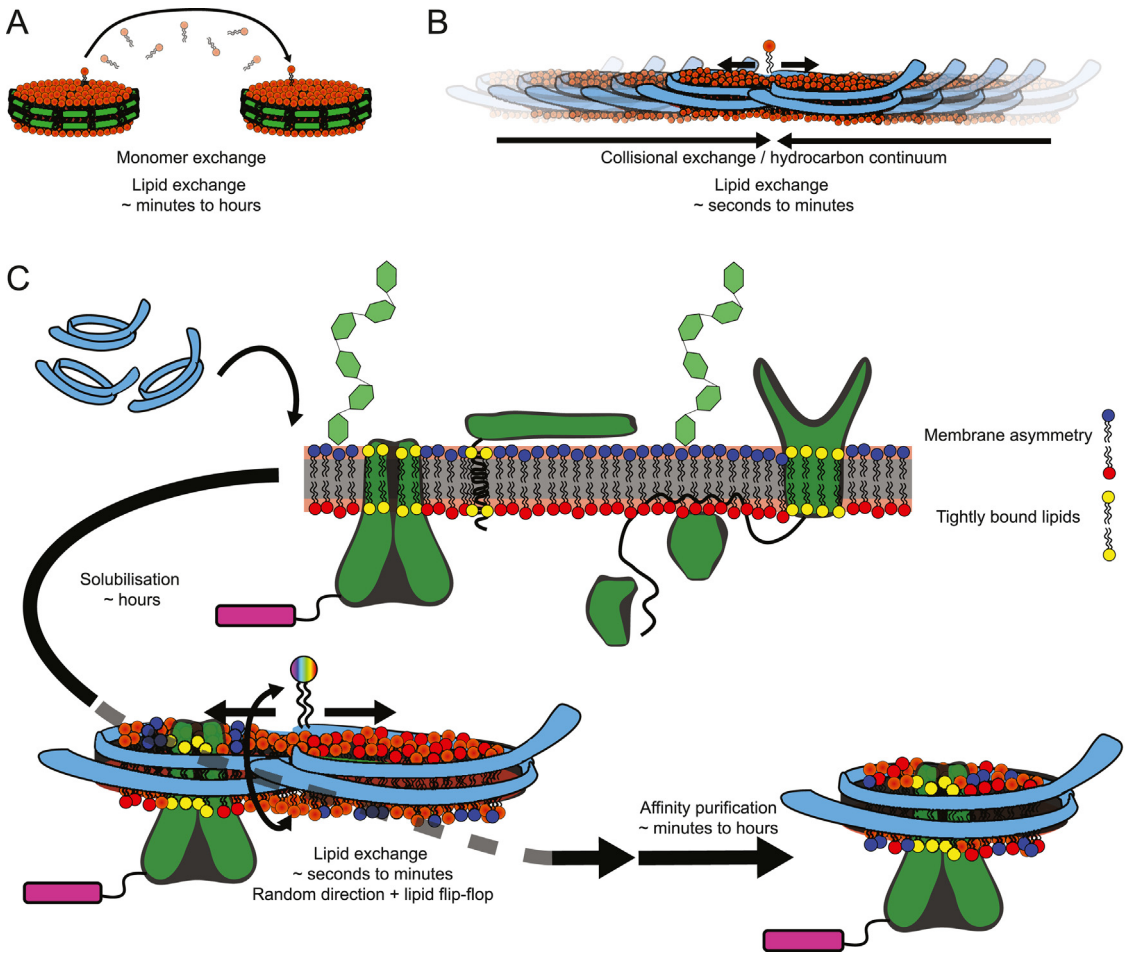

Fig. 7. Lipid exchange dynamics. A: Monomer exchange mechanism as illustrated for MSP nanodiscs [116,154]. B: Collisional exchange with a hydrocarbon continuum mechanism as illustrated for SMALPs [173]. C: Dynamic considerations during solubilisation of biological membranes and subsequent purification as illustrated for polymers. The time scales of different steps and events are indicated.

natively associated lipids and co-factors. The literature contains only a few examples of this approach, which probably reflects several experimental hurdles. First, a large excess of MSP would be required to obtain equal amounts of isolated MP compared to Step C. Second, sample preparation is more demanding due to the complex composition of the biological membrane. Finally, the initial detergent solubilisation step (Step A) is still required, and to our knowledge, the vast majority of MPs that have been stabilised in MSP nanodiscs have also been successfully stabilised in detergent micelles. In other words, if the MP of interest does not tolerate detergents at any stage, it is also unlikely to insert in MSP nanodiscs with this approach.

E Direct extraction to APol lipid particles

Here, we depict polymer solubilisation of biological membranes as an alternative path to detergent solubilisation. However, the commonly presented idea of extracting membrane patches, including MPs, in a “cookie/pastry-cutter” fashion into stable well-defined nanodiscs [55,83,172] must be revised: like detergents, APol solubilisation results in a highly heterogeneous mixture of dynamically exchanging particles. In this regard, biophysical measurements of lipid exchange rates are for the actual integrity of the embedded lipid bilayer and the nature of the bilayer-wrapping molecules. Only the MSP nanodisc system displays predominantly monomer exchange, i.e. where lipid exchange occurs from a single lipid jumping into the solution and thereafter into another neighbouring disc [116,154] (Fig. 7A). Polymer-based nanodiscs and peptide nanodiscs display concentration dependent lipid exchange rates on time scales of seconds to minutes [94,106,173–175], suggesting collisional events where particles temporarily fuse and form a hydrophobic continuum allowing redistribution of lipids, similarly to mixed micelles of detergents and lipids (See Ref. [173] and references therein) (Fig. 7B). Furthermore, rapid polymer exchange between nanodiscs has been directly measured as well [176]. Thus, during solubilisation, which is typically carried out at low lipid:polymer ratios and on the timescale of hours, the system is likely to reach equilibrium in terms of random lipid distribution (Fig. 7C). Recently, it has been proposed an advantage of polymers that they can preserve membrane leaflet asymmetry [55,97]. However, even in the thought experiment that the lipids within a single polymer nanodisc remain asymmetrically distributed, collisional events are still of random orientation, resulting in loss of asymmetry at equilibrium (Fig. 7C). Thus, the same principles apply as for detergents that solubilisation should be carefully executed, considering molar ratios, temperature, and time. The main difference is that membrane solubilising polymers appear to generally have negligible CACs when bound to lipid particles, referred to distinctively as nanodisc CAC, although it depends on polymer length and hydrophobic/hydrophilic balance in composition [177].

As such, the "hydrophobic sink" effect is limited in post-solubilisation chromatographic purification.

5.2. What is a membrane-like environment?

Common to the different paths for reconstitution of MP in carrier systems is the extraction from the native environment of a complex biological membrane. While small carrier systems enable experiments, their local environment may be stress-inducing and compromise the well-being (i.e. functionality) of an isolated MP. In this regard, the native-likeness of carrier systems is usually discussed in the context of experiments [55,94,178–180]. Later, we discuss typical experimental measures for probing the well-being of isolated MPs with specific examples, but first we consider the frequent yet blurry discussion of what a membrane-like environment is.

5.2.1. Pure lipid membranes

Due to their pure composition of lipid species, liposomes and/or SLBs are often described as the most native-like membrane systems [179], and consequently lipid bilayer properties in small, soluble lipid carrier systems are typically benchmarked against these systems [36]. Indeed, pure lipid bilayers possess some key properties of biological membranes, that is a relevant lateral pressure profile, and for liposomes also curvature stress and membrane potential gradients. However, they lack the large lipid variation of biological cell membranes. Furthermore, cell membranes are asymmetric in terms of different lipid composition in the inner and outer leaflets [181]; a feature which is generally not present in pure lipid bilayer systems and that by all means is not expected to be preserved during solubilisation into small carrier systems. Some protocols and insight are available for generation of asymmetric liposomes [182,183], but it has to our knowledge not been investigated for small, soluble carrier systems. Finally, the cytoskeleton of eukaryotic cells interacts with and regulates membranes and MPs [181], which would be difficult to mimick even in artificial cells [184]. As such, a first realisation is that MP carrier systems will never be fully native-like on all parameters: we can only aim to support MPs in their presumably native state as evaluated by preservation of their native activity.

5.2.2. Small discoidal membrane patches

Arguments have been raised that MSP nanodiscs, in which the small piece of membrane experience packing stress from the surrounding MSP [116,154,185–187], may be more native-like than pure lipid systems, as they better mimic the environment in a densely packed biological membrane [130,188]. Indeed, it can be estimated that MPs in biological membranes are on average separated by a layer of only four lipids [7]. Asymmetric lipid composition has been mentioned as a future possibility using large DNA corralled nanodiscs, potentially with incorporated lipid flippases to maintain the asymmetry [153].

5.2.3. Detergency

In a thermodynamic classification, surfactants that favor lipid insertion in their micelles are the most efficient solubilisers and generally support bicelle/nanodisc-like structures [8]: the favorable lipid insertion could be interpreted as a membrane-like environment. In general, the concept of detergency presents a good way of thinking about MP carrier systems, yet many studies use the phrasing "detergent-free" [84,189,190] to distinguish alternative systems from conventional detergents, although the solubilisation of membranes by e.g. polymers resemble the action of (facial) detergents from a biophysical point of view [8]. Furthermore, common polymers form supramolecular assemblies in solution, resembling micelles [191]. In this regards, some polymers could be equally well described as polymeric detergents. For any solubilising agent, there will be a trade-off between solubilisation efficiency (high surfactant/membrane ratio) and retention of biological lipids (low surfactant/membrane ratio). Thus, while the co-extracted lipids in polymer nanodiscs are native and may be functionally relevant, it is unlikely that such efficient solubilisers will result in particles containing more than tightly bound lipids surrounding the MP [174,176]. Indeed, we demand two inversely proportional properties of our carrier systems: the ability to extract MPs from their native environment (possibly alongside accompanying lipids from this environment), and the ability to stabilise these MPs in solution over experimentally relevant timescales for subsequent studies afterwards. The former requires high detergency, which implies competition with the native MP interactions with e.g. the membrane lipids or other proteins and destabilise the MP in question [7,71]. In the common view of different carrier systems (Fig. 1), some systems are typically depicted with a central lipid bilayer patch. However, polymers, amphiphilic peptides, and even MSPs, can directly stabilise MPs in solution, i.e. in rather lipid-poor particles [54,79,157]. The inability of some of these systems to solubilise membranes testifies to their low detergency and thereby mild environments for MPs compared to conventional detergents. Detergency is a universal property for surfactants and should be considered for any carrier system, including those that are typically viewed as a non-interacting, stable belt around a lipid bilayer patch. In this context, MSPs are generally viewed as being almost inert towards membranes, but under specific circumstances, partial solubilisation has been observed [192].

5.3. What is the membrane protein's environment

Reviews on MP structure databases show that detergents, especially DDM and DDM in combination with CHS, remain among the most popular systems for handling MPs [32,52]. This is despite the well-acknowledged fact that DDM micelles do not resemble the lipid bilayer environment of the cell membrane. Perhaps, in many cases, the micelle environment is membrane-like *enough*, as also indicated by the sometimes successful approach of refolding MPs into non-bilayer systems [193]. A recent example highlights that detergents are not the pure evil they are often depicted to be. The yeast proton pump Pma1 was solubilised in DDM + CHS and exchanged into LMNG + CHS for cryo-EM, which revealed a central lipid bilayer patch of 57 well-ordered lipid inside the hexameric structure [194]. No lipids were added in the sample preparation, suggesting that these lipids were natively associated to Pma1 in the cell membrane. Unfortunately, the study did not detail the ratio of DDM to membrane in the solubilisation step, but clearly, the authors reached a condition in which DDM did not break neither native protein/protein nor protein/lipid contacts. Exchanging to LMNG (low CMC, Fig. 3) in purification steps likely further reduced the "hydrophobic sink" effects, rendering the central bilayer intact in the final sample. Thus, the statement "be cautious with crystal structures of membrane proteins or complexes prepared in detergents" [195] should perhaps be generalised to "be cautious with sample preparation of MPs". Analogously to the example above for detergent solubilised Pma1, preservation of tightly bound lipids is likely, in many cases, enough for preserving MP stability, activity, functional regulation etc. once isolated from cell membranes, as is the case for also the peptidisc [79,196] and Salipro [163,169].

In classic protein science, stability relates to the free energy of folding. However, for MPs, the stability is unsurprisingly influenced by the nature of the carrier system as well, and the term "stability" is commonly used in a broad and qualitative sense for evaluating how well an MP tolerate different experimental conditions, for

example exposure to elevated temperature. Improved MP stability (compared to a detergent micelle reference) is a common trait for carrier systems developed as alternatives to classic detergents: improved MP stability has been the key driver for this development. In line of this, the literature is full of examples of improved MP stability in detergent-alternatives compared to MP stability in detergent micelles. Here, we will not go into these specific examples, but rather comment on how MP stability can be conceived.

5.3.1. Activity

Arguably, the best measure of stability is that the activity and regulation of an MP is intact. Intact activity indicates that an MP remains in its native fold, is not degraded, damaged, or inhibited by the utilised surfactant. However, it remains difficult to measure actual native activity, as it requires a reference of the activity in native sources, i.e. biological membranes. For many MPs, including many channels, it is entirely impossible, because small soluble carrier systems are non-compartmentalised. A further complication is that activity and functional regulation can be inversely related, as seen for the plant H^+-pump AHA2 that is misregulated and hyperactivated in presence of detergent [197]. Purification history must also be considered. The A2A adenosine receptor retained full activity in DDM/CHAPS/CHS micelles when CHS was included in all purification steps [198]: purified in DDM alone, the receptor had low activity, and reconstitution into the mix of DDM/CHAPS/CHS did not regain full activity, despite the fact that the receptor appeared to retain its native fold in all tested micelles.

The bacterial ABC transporter MsbA is a well-studied MP that serves an interesting basis for comparing the impact of different carrier system on its structure and activity: it is an active transporter that utilises ATP hydrolysis and undergoes large structural rearrangements in the transport cycle [199,200]. Several structures of MsbA, in absence or presence of regulatory ligands, have been solved to near-atomic resolution in different carrier systems, ranging from maltoside detergents, facial amphiphiles (detergents), MSP nanodiscs, to peptidiscs (see ref. [200]) as well as more recently in Salipro [201]. From these, it was concluded that lipid environments were favorable compared to detergent micelles, as potentially artificial widely-open states were observed in the latter [200,202]. The abundance of activity data on MsbA further exceeds that of structural studies, and several studies have reported activity in different carrier systems. Comparing the activity of detergent purified (*E. coli*, Ec or *S. typhimurium*, St) MsbA reported in such comparative studies, there is a big variation in activity, at 37 °C ranging from 0.003 μmol/min/mg to 14.6 μmol/min/mg with a median of 0.8 μmol/min/mg (Fig. 8A, see figure legend for references). It was suggested that long-term solubilisation with DDM (12 h), but not short-term (1 h), effectively removed the natural substrate, lipid A, from a binding pocket of MsbA, which had a large impact on the transporter's activity [203]. As such, the large variation in activity might partially reflect variations in protocols, including solubilisation time, detergent:membrane ratio in solubilisation, and detergent concentration in purification. Fig. 8B compares the absolute activity of MsbA in different carrier systems as well as this activity normalised to a detergent purified reference. While we note that the activity, as well as the relative change, is in some cases temperature dependent [178], this analysis still yields some key observations. First, the absolute activity does not correlate with the relative change, which in part reflects the large variation in reference activities discussed above. Second, in general, the lipid environment of MSP nanodiscs, Salipro, and liposomes induces high relative activity. Indeed, although endogenous lipids were carefully removed in purification, reconstitution in salipro and MSP nanodiscs resulted in 10–27 fold activity compared to the lipid free reference in APol A8-35 [155]. Third, MsbA directly extracted from bacterial membranes or from *E. coli* lipid liposomes with certain zSMA polymers showed modest relative activity compared to the other lipid carrier systems, and notably, SMA extracted samples showed no activity due to Mg^{2+}-induced precipitation (low Mg^{2+} concentrations required for ATP hydrolysis). For MSP nanodiscs, MsbA activity depends on the size of the nanodisc [204,205], which might reflect the different bilayer properties in nanodiscs of different size, more specifically that lipids close to the MSP are less tightly packed than those in the center of the nanodisc [154,185–187]. Altogether, the presence of lipids is important for MsbA activity, but secondary effects, including MP-surfactant interactions and lipid packing, might have an influence as well.

5.3.2. Thermostability

Most often, MP stability is evaluated based on resistance towards heat-induced unfolding/aggregation, also termed thermostability. In its simplest form, samples are heated at set time and temperature, centrifuged, and analysed by gel electrophoresis to visualise the relative amount of soluble MP, providing a rather qualitative measure of stability. Differential scanning fluorimetry (DSF) is of particular interest due to quantitative evaluation of the melting temperature (T_M) and low sample consumption, which e.g. allows high-throughput detergent screening [213]. Increased thermostability is desired in the sense that it can be extrapolated to low temperatures and will result in long-term stability. In lipid carrier systems, EcMsbA had T_Ms in the range 52.2–60.8 °C compared to 40.9 °C in lipid-free APol A8-35 [155]. Elsewhere, the T_M was reported for EcMsbA in micelles of DDM (46.3 °C) and LMNG (50.4 °C) [68], and for StMsbA in micelles of UDM (53 °C), LMNG (58.6 °C), and FA-3 (63.3 °C) [210]. Clearly, increased thermostability is generally not equal to increased activity for MsbA. Rather, increased thermostability can arise from stabilisation of particular protein states, which is a main strategy in many structural studies to achieve high resolution. As an example, the melting temperature of MsbA increased by almost 20 °C inside Salipro particles upon binding of non-hydrolysable ATP-γS that stabilised a compact state of the protein [201]. Thus, depending on the experimental question, high thermostability is not necessarily desired, but in cases of aggregation prone MPs, it may be required.

5.3.3. MP carrier system interactions and structural dynamics

Both activity and thermostability are related to underlying structural dynamics of MPs. NMR is the main method for direct observations of structural dynamics with atomic resolution, and the method has been widely used to compare MPs in different carriers [214,215]. In one example, OmpX was measured in DPC micelles, DHPC/DMPC bicelles and in DMPC nanodiscs, and it was found that the inherent flexibility of OmpX was compromised in the detergent micelles compared to the other systems [216]. A generalised view of detergent mediated pertubation of MP structural dynamics is presented in Ref. [58]. Other NMR studies have pointed out differences in membrane protein structural dynamics between detergent micelles and MSP nanodiscs [217,218], and also APols [217]. In all cases, major differences were observed between the different systems. For BR, the core structure was intact in all tested carrier systems, but the surfactant-exposed surface of the protein showed carrier-dependent behaviour, including specific interactions of loops with the polar regions of surfactants; notably, the APol A8-35 preserved the presumably native conformational dynamics of an important loop in BR, but the stability of BR was much higher in DMPC-NDs [217]. Again, such studies show that the carrier system should be carefully optimised, and that different system might favor distinct conformations. This argument is repeated from time to time and emerging near-resolution

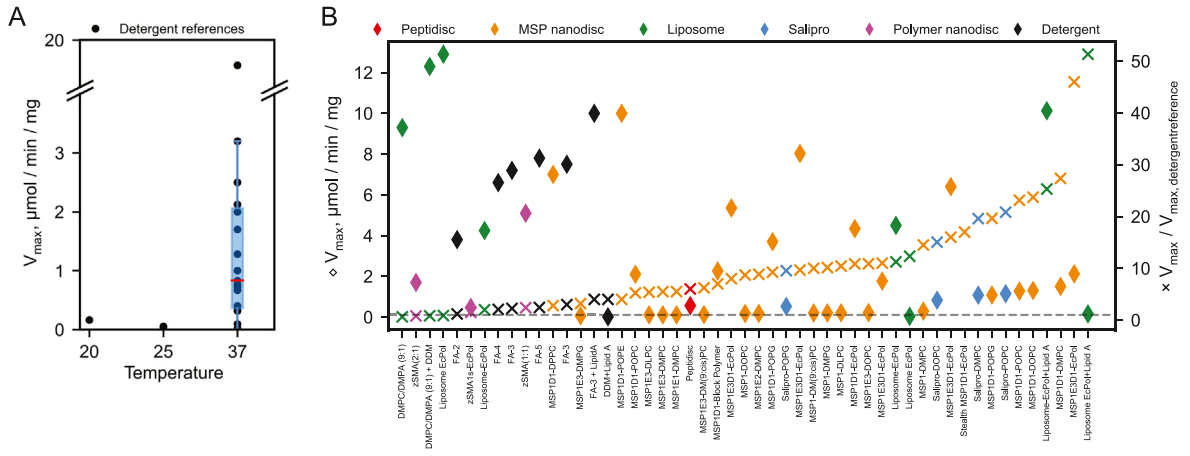

Fig. 8. MsbA ATPase activity in different membrane-like environments. A: Maximum ATP hydrolysis rate in detergent micelles. B: Maximum ATP hydrolysis rate in alternative carrier systems together with the fold change compared to internal detergent micelle references for each respective study. The dashed line indicates a fold change of 1. Samples that were added the natural substrate Lipid A are indicated. EcPol = *E. coli* polar lipids, SoyPol = Soy bean polar lipids. Data were taken from Refs. [34,87,141,155,178,196,199,201,203–212]. Data are for both EcMsbA and StMsbA, which show similar activity in detergent [206]. For data in Ref. [155], MsbA in APol A8-35 was used as reference as there was no measurement in detergent micelles reported.

structures of MPs in different carrier systems highlight this as well [55,84,195,200].

5.3.4. Carrier system dynamics and homogeneity

Regarding carrier system dynamics, DDM and LMGN serve as a great case for comparison, given that LMNG corresponds to two DDM molecules linked at a tertiary carbon. LMNG is consistently reported to improve MP thermostability [27] and to dampen structural dynamics compared to DDM [29]. The CMC of LMNG (~ 10 µM) is one order of magnitude lower than DDM (~ 0.17 mM), which, as discussed in Ref. [30], translates roughly into off rates of similar relative magnitude. Like-wise, glycosylated sterols, such as FA-3 (Facade-EM) and GDN [13], with low CMCs provide improved MP stability. Analogies can be drawn towards APols and peptides. Once bound to MPs, several molecular contacts aid in stabilising the complexes and likely render slow off rates. Furthermore, recent molecular dynamics simulations suggest that not only the CMC, but also the denser packing of LMNG micelles explain its stabilising behaviour on MPs over DDM micelles [28], which agrees with conclusions from studies of conformationally flexible detergents [64] and asymmetric detergents [62].

Also for lipid carriers, the packing and dynamics of the surfactant plays an important role for homogeneity. Bicelles typically form at specific conditions, established by first scanning temperatures, composition, and hydration to establish pseudo-phase diagrams [36]. In this regards, nonionic facial detergents with low CMC showed improved temperature and concentration ranges for bicelle formation compared to the ionic CHAPS with rather high CMC [37]. It has been shown that increasing the length of the polymer/peptide leads to more stable polymer/peptide-based nanodiscs

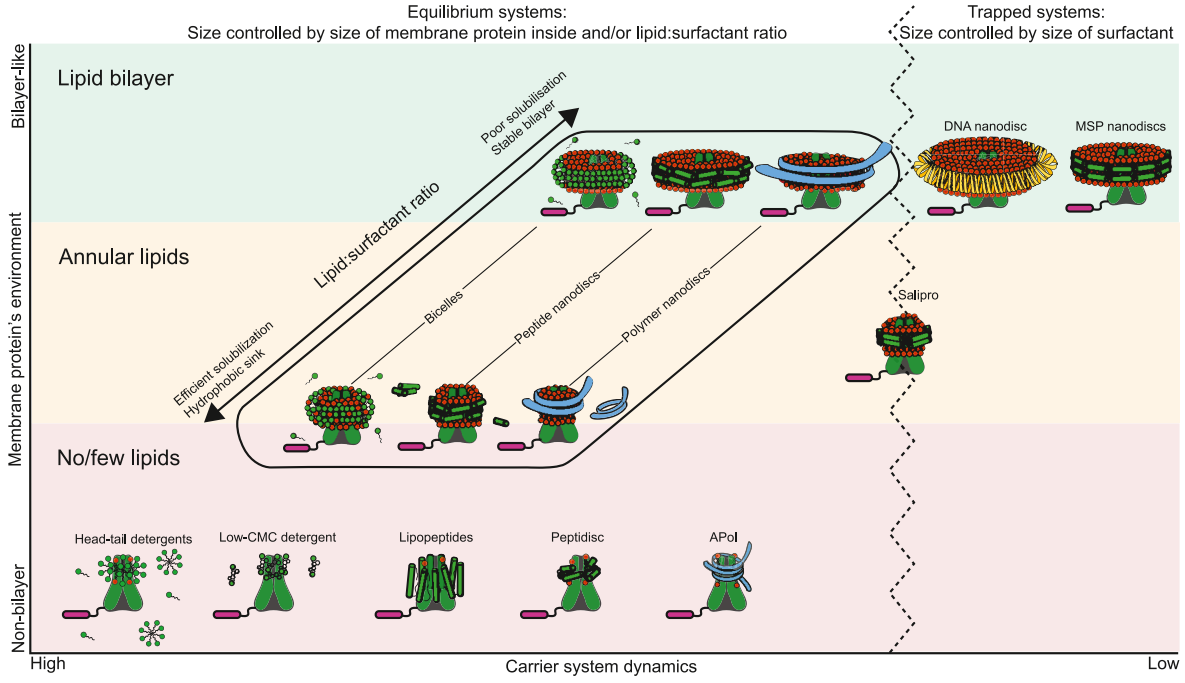

Fig. 9. An overview of membrane protein environment and particle dynamics between different carrier systems. See the main text for details.

[78,101]. We can further consider MSP1D1 as a decamer of the 18A peptide: MSP1D1 nanodiscs have roughly the same diameter as 18A-based nanodiscs at optimised ratios, but MSP nanodiscs possess high colloidal stability, whereas 18A-based nanodiscs are dynamic and relatively polydisperse [76,78].

5.4. Classification of carrier systems

In Fig. 9, we attempt to rank the different MP carrier systems according to two biophysical properties: particle dynamics and the MP's local environment. As discussed section 5.2, whether or not a system has membrane-like properties is a rather philosophical discussion, and thus we only consider i) whether or not a given carrier system has the characteristics of an actual lipid bilayer, ii) is more likely to only contain lipids closely associated to the embedded MP (annular lipids), or iii) if no (or few very tightly bound) lipids are present. On the other axis of Fig. 9, particle dynamics is a rather broad descriptor. Surfactant geometry, hydrophobic/hydrophilic balance, number of hydrophobic groups per surfactant, CMC, surfactant:lipid ratio, and many other biophysical properties influence particle stability and exchange dynamics. In the case of direct MP stabilisation (i.e. with no/few lipids), our proposed ranking is based on the length of the surfactant, and for detergents also on CMC. In the case of lipid containing carrier systems, we have attempted to depict the ratio-dependent characteristics of bicelles, peptide nanodiscs, and polymer nanodiscs. These span the entire range of membrane environments, from mixed micelle-like structures at low lipid:surfactant ratios to large (~50 nm in diameter) nanodisc-like structures at high lipid:surfactant ratios. Once again, the length of the surfactant is important for the dynamics of the particles, thus ranking polymer nanodiscs generally less dynamic than peptide nanodiscs, which in turn are less dynamic and environmentally sensitive than bicelles. Furthermore, the balance of free surfactant to lipid-complexed surfactant changes along with the lipid:surfactant ratio, which alters the dynamic exchange of e.g. lipids between particles. Finally, above some threshold of surfactant length, kinetically trapped systems form. A key difference in characteristics between equilibrium systems and kinetically trapped systems is the manner in which the size of the particles is determined. Equilibrium systems are dynamic in nature and can adapt to MPs of different sizes, whereas for kinetically trapped systems, the long surfactant forms a well-defined outer rim that controls and constrains the maximum size of the particles. With our current biophysical understanding, Salipro particles traverse this threshold. On one hand, SapA is somewhat competent at extracting lipids from membranes and can adapt to MPs of different sizes, but on the other hand, Salipro particles are typically formed by detergent-assisted assembly, and the resulting particles possess annular lipids around MPs and have high long-term stability [155].

6. Conclusion

Previously, high-resolution structures were mainly obtained from MPs tolerating significant delipidation and specific detergent micelles favoring crystal formation. The resolution revolution in cryo-EM [219] now allows MP structure determination in dynamic carrier systems with complex composition, resulting in significantly increased numbers of solved MP structures and with focus on their interactions with the surrounding carrier system [55,84,195,220,221]. This increase in MP structures reveals key observations on sample preparation [32,52]: DDM, LMNG, MSP nanodiscs, digitonin, and GDN were by far the most successful systems in recent years, followed by polymers. Notably, MSP nanodiscs were the most widely used system in recent years for cryo-EM [32,52], highlighting the increased attention on MP-lipid interactions [55].

Despite having well-acknowledged destabilising and inactivating effects on some MPs, DDM is the gold standard detergent for membrane solubilisation. Thermodynamically, DDM can be classified as a “strong” detergent [222], but membrane solubilisation has comparatively slow kinetics to other types of detergent [14]. In practice, DDM solubilises membranes with relatively high efficiency at manageable time scales, it is relatively cheap to obtain from commercial sources, and it often preserves MP-lipid interactions better than other common detergents [223]. The CMC of DDM (0.17 mM) is relatively low, but not too low to efficiently exchange for other, more stabilising surfactants, including MSP nanodiscs. Furthermore, other surfactants, e.g. low-CMC detergents, can readily be added during solubilisation with DDM to improve MP integrity [224] and stability [13]. Here, it is noteworthy to mention CHS, which is often included with either DDM or LMNG [32,52]. Ultimately, more focus should be directed towards the most critical step in MP sample preparation, that is the solubilisation step in which an MP is extracted from its native environment. Many parameters are crucial in this step, including surfactant concentration, surfactant:lipid ratio, temperature, ionic strength, pH, and not at least time. Many biologists will approach solubilisation of MPs according to standardised protocols, i.e. 1 % DDM at 4 °C for 1 h, followed by purification at slight excess CMC of DDM. Clearly, this ignores many aspects of the underlying biophysics.

MSP nanodiscs were first reported in 2002 [118], whereas SMA was first reported in 2009 [73]. Perhaps this gap in development reflects the lower number of cryo-EM structures solved in polymer-based nanodiscs. The direct extraction into discoidal lipid particles for cryo-EM offered by certain polymers is a highly desired feature. While initial development was limited to abundant bacterial MP targets [83], there are ongoing developments towards e.g. GPCR stabilisation in SMALPs [172,225]. It will be interesting to follow the field in coming years, and especially to see whether polymers emerge as capable of stabilising MPs that have hirtherto avoided stabilisation in other systems. Comparative studies on MP activity as well as direct structural dynamics measured by NMR between polymer-based nanodiscs and other lipid carrier systems will prove valuable for evaluating further pros and cons of this system. Finally, the high level of control in constructing DNA-based membrane scaffolds of different sizes and shapes holds a great potential for future studies of MP interactions. For these, further detailed biophysical investigations are required as well as further comparative studies on the stability and activity of reconstituted MPs to other lipid carrier systems. Indeed, the fundamental biophysical understanding of carrier systems is important in modern integrative structural biology approaches that harness the power of combining a wide array of experimental techniques that each require application-specific carrier systems [226,227].

6.1. Unexplored opportunities

From developments in polymers, cycloalkyl and aromatic side chains have been identified to improve membrane solubilisation compared to linear alkyl-chains [111]. It would be interesting to see whether this trend is general and could be translated to other carrier systems, including MSP and DNA scaffolds.

Surprisingly, only few studies have reported direct extraction from cell membranes into MSP nanodiscs since it was first suggested almost a decade ago [135]. Assembly into nanodiscs from GDN (or digitonin) solubilised membranes seems like an obvious strategy to pursue given that a similar protocol has been applied for Salipro [169]. The bottleneck might be the relatively high consumption of MSP combined with expensive MSP from commercial sources. Indeed, in general, many carrier systems, ranging from structurally complex detergents, homogeneous polymers, to MSP

and Salipro proteins suffer from high costs and limited commercial availability to fulfill their potential.

Regulatory lipid-binding domains, often unstructured N- and/or C-termini, are frequent in MPs [26,228], and many receptors, including GPCRs, contain large, flexible regulatory termini or loops and that serve as binding hubs [229]. Besides lipid-MP interactions, two recent cryo-EM structures have also highlighted the importance of a membrane surface extending far from the MP to support functional interactions of auxiliary proteins and lipids as well [230,231]. With our current knowledge, large MSP nanodiscs seems the most obvious choice of carrier system for these such MPs, but it remains interesting to see whether e.g. polymers can extract and sustain a bulk lipid bilayer outside tightly bound annular lipids for supporting such more diffuse interactions.

Finally, more complex solutions could be proposed towards overcoming the seemingly inverse relationship between detergency and membrane-environment [7], with the goal of achieving highly efficient solubilisation and ending up in a stable (kinetically trapped) membrane-like environment. Ultimately, this would require the development of molecular switches that upon some stimulation change their properties. As an example, 18A peptides were functionalised with chemical groups enabling native chemical ligation to form peptide concatamers once lipid solubilisation had been achieved [77]. This process resembled a conversion from 18A peptide discs into MSP nanodiscs, albeit that concatamers of varying length were assembled. Likewise, detergents or polymers could be designed with UV/heat/redox reactive groups, facilitating either crosslinking and/or a reduction in detergency once bound to lipids.

6.2. Final remarks

The toolbox for extracting, handling, and studying MPs is growing at a remarkable pace. Future MP research will certainly benefit from these developments, but to fully realise the potential of novel carrier systems, a thorough understanding of their biophysical properties and impact on MP functionality is of utmost importance.

Acknowledgements

We thank the Villum Foundation (project numbers 35955 and 40960) and the Lundbeck Foundation (project number R155-2015-2666) for financial support.

References

[1] M. Overduin, M. Esmaili, Native nanodiscs and the convergence of lipidomics, metabolomics, interactomics and proteomics, Appl. Sci. 9 (6). doi: 10.3390/app9061230.

[2] F. Mahler, A. Meister, C. Vargas, G. Durand, S. Keller, Self-assembly of protein-containing lipid-bilayer nanodiscs from small-molecule amphiphiles, Small 17 (49) (2021), 2103603, https://doi.org/10.1002/smll.202103603. URL, https://onlinelibrary.wiley.com/doi/10.1002/smll.202103603.

[3] P.A. Beales, S. Khan, S.P. Muench, L.J. Jeuken, Durable Vesicles for Reconstitution of Membrane Proteins in Biotechnology, 2, 2017, https://doi.org/10.1042/BST20160019.

[4] J.-L. Rigaud, Membrane proteins: functional and structural studies using reconstituted proteoliposomes and 2-D crystals, Braz. J. Med. Biol. Res. 35 (7) (2002) 753–766, https://doi.org/10.1590/S0100-879X2002000700001. URL, http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-879X2002000700001&lng=en&tlng=en.

[5] V. Ladizhansky, Applications of solid-state NMR to membrane proteins, Biochim. Biophys. Acta, Proteins Proteomics 1865 (11) (2017) 1577–1586, https://doi.org/10.1016/j.bbapap.2017.07.004. URL.

[6] C. Tribet, R. Audebert, J.-L. Popot, Amphipols: polymers that keep membrane proteins soluble in aqueous solutions, Proc. Natl. Acad. Sci. USA 93 (26) (1996) 15047–15050, https://doi.org/10.1073/pnas.93.26.15047. URL, http://www.pnas.org/cgi/doi/10.1073/pnas.93.26.15047.

[7] J.-L. Popot, Membrane Proteins in Aqueous Solutions, Biological and Medical Physics, Biomedical Engineering, Springer International Publishing, Cham, 2018, https://doi.org/10.1007/978-3-319-73148-3. URL, https://link.springer.com/content/pdf/10.1007http://link.springer.com/10.1007/978-3-319-73148-3.

[8] C. Vargas, R.C. Arenas, E. Frotscher, S. Keller, Nanoparticle self-assembly in mixtures of phospholipids with styrene/maleic acid copolymers or fluorinated surfactants, Nanoscale 7 (48) (2015) 20685–20696, https://doi.org/10.1039/C5NR06353A. URL, http://xlink.rsc.org/?DOI=C5NR06353A.

[9] A.D. McNaught, A. Wilkinson, IUPAC. Compendium of Chemical Terminology; "The Gold Book", Blackwell Scientific Publications, Oxford, 1997 https://doi.org/10.1351/goldbook.ST06784.

[10] R.M. Garavito, S. Ferguson-Miller, Detergents as tools in membrane biochemistry, J. Biol. Chem. 276 (35) (2001) 32403–32406, https://doi.org/10.1074/jbc.R100031200. URL, http://www.jbc.org/lookup/doi/10.1074/jbc.R100031200.

[11] H. Wennerström, Micelles. Physical chemistry of surfactant association, Phys. Rep. 52 (1) (1979) 1–86, https://doi.org/10.1016/0370-1573(79)90087-5. URL, http://linkinghub.elsevier.com/retrieve/pii/0370157379900875.

[12] C. Tanford, The Hydrophobic Effect: Formation of Micelles and Biological Membranes, second ed., John Wiley and Sons, Somerset, NJ, 1980. URL, http://pubs.acs.org/doi/abs/10.1021/ed058pA246.1.

[13] P.S. Chae, S.G. Rasmussen, R.R. Rana, K. Gotfryd, A.C. Kruse, A. Manglik, K.H. Cho, S. Nurva, U. Gether, L. Guan, C.J. Loland, B. Byrne, B.K. Kobilka, S.H. Gellman, A new class of amphiphiles bearing rigid hydrophobic groups for solubilization and stabilization of membrane proteins, Chem. Eur J. 18 (31) (2012) 9485–9490, https://doi.org/10.1002/chem.201200069.

[14] D. Lichtenberg, H. Ahyayauch, F.M. Goñi, The mechanism of detergent solubilization of lipid bilayers, Biophys. J. 105 (2) (2013) 289–299, https://doi.org/10.1016/j.bpj.2013.06.007. URL, https://linkinghub.elsevier.com/retrieve/pii/S0006349513006814.

[15] J. Israelachvili, Intermolecular & Surface Forces, Academic Press, London, 1991, https://doi.org/10.1017/CBO9781107415324.004.

[16] P. Champeil, S. Orlowski, S. Babin, S. Lund, M. le Maire, J. Møller, G. Lenoir, C. Montigny, A robust method to screen detergents for membrane protein stabilization, revisited, Anal. Biochem. 511 (2016) 31–35, https://doi.org/10.1016/j.ab.2016.07.017. URL, https://linkinghub.elsevier.com/retrieve/pii/S0003269716301932.

[17] M. le Maire, P. Champeil, J.V. Møller, Interaction of membrane proteins and lipids with solubilizing detergents, Biochim. Biophys. Acta Biomembr. 1508 (1–2) (2000) 86–111, https://doi.org/10.1016/S0304-4157(00)00010-1.

[18] A.M. Seddon, P. Curnow, P.J. Booth, Membrane proteins, lipids and detergents: not just a soap opera, Biochim. Biophys. Acta Biomembr. 1666 (1–2) (2004) 105–117, https://doi.org/10.1016/j.bbamem.2004.04.011. URL, http://linkinghub.elsevier.com/retrieve/pii/S0005273604001610.

[19] A. Stetsenko, A. Guskov, An overview of the top ten detergents used for membrane protein crystallization, Crystals 7 (7) (2017) 197, https://doi.org/10.3390/cryst7070197. URL, http://www.mdpi.com/2073-4352/7/7/197.

[20] H. Heerklotz, J. Seelig, Correlation of membrane/water partition coefficients of detergents with the critical micelle concentration, Biophys. J. 78 (5) (2000) 2435–2440, https://doi.org/10.1016/S0006-3495(00)76787-7. URL.

[21] F.G. Tidemand, A. Zunino, N.T. Johansen, A.F. Hansen, P. Westh, K. Mosegaard, L. Arleth, Semi-empirical analysis of complex ITC data from protein-surfactant interactions, Anal. Chem. 93 (37) (2021) 12698–12706, https://doi.org/10.1021/acs.analchem.1c02558.

[22] J.D. Kaspersen, A. Søndergaard, D.J. Madsen, D.E. Otzen, J.S. Pedersen, Refolding of SDS-unfolded proteins by nonionic surfactants, Biophys. J. 112 (8) (2017) 1609–1620, https://doi.org/10.1016/j.bpj.2017.03.013. URL.

[23] M. Hattori, R.E. Hibbs, E. Gouaux, A fluorescence-detection size-exclusion chromatography-based thermostability assay for membrane protein precrystallization screening, Structure 20 (8) (2012) 1293–1299, https://doi.org/10.1016/j.str.2012.06.009.

[24] D. Sjöstrand, R. Diamanti, C.A. Lundgren, B. Wiseman, M. Högbom, A rapid expression and purification condition screening protocol for membrane protein structural biology, Protein Sci. 26 (8) (2017) 1653–1666, https://doi.org/10.1002/pro.3196.

[25] F.B. Bjørkskov, S.L. Krabbe, C.N. Nurup, J.W. Missel, M. Spulber, J. Bomholt, K. Molbaek, C. Helix-Nielsen, K. Gotfryd, P. Gourdon, P.A. Pedersen, Purification and functional comparison of nine human Aquaporins produced in Saccharomyces cerevisiae for the purpose of biophysical characterization, Sci. Rep. 7 (16899) (2017) 1–21, https://doi.org/10.1038/s41598-017-17095-6. URL, http://www.nature.com/articles/s41598-017-17095-6.

[26] N. Kassem, M.M. Kassem, S.F. Pedersen, P.A. Pedersen, B.B. Kragelund, Yeast recombinant production of intact human membrane proteins with long intrinsically disordered intracellular regions for structural studies, Biochim. Biophys. Acta Biomembr. 1862 (6) (2020), 183272, https://doi.org/10.1016/j.bbamem.2020.183272. URL.

[27] P.S. Chae, S.G. Rasmussen, R.R. Rana, K. Gotfryd, R. Chandra, M.A. Goren, A.C. Kruse, S. Nurva, C.J. Loland, Y. Pierre, D. Drew, J.L. Popot, D. Picot, B.G. Fox, L. Guan, U. Gether, B. Byrne, B. Kobilka, S.H. Gellman, Maltose-neopentyl glycol (MNG) amphiphiles for solubilization, stabilization and crystallization of membrane proteins, Nat. Methods 7 (12) (2010) 1003–1008, https://doi.org/10.1038/nmeth.1526.

[28] S. Lee, S. Ghosh, S. Jana, N. Robertson, C.G. Tate, N. Vaidehi, How do branched detergents stabilize GPCRs in micelles? Biochemistry 59 (23) (2020) 2125–2134, https://doi.org/10.1021/acs.biochem.0c00183.

[29] I.R. Möller, P.S. Merkle, D. Calugareanu, G. Comamala, S.G. Schmidt, C.J. Loland, K.D. Rand, Probing the conformational impact of detergents on the integral membrane protein LeuT by global HDX-MS, J. Proteonomics 225 (December 2019) (2020), 103845, https://doi.org/10.1016/j.jprot.2020.103845. URL.

[30] C. Breyton, W. Javed, A. Vermot, C.A. Arnaud, C. Hajjar, J. Dupuy, I. Petit-Hartlein, A. Le Roy, A. Martel, M. Thépaut, C. Orelle, J.M. Jault, F. Fieschi, L. Porcar, C. Ebel, Assemblies of lauryl maltose neopentyl glycol (LMNG) and LMNG-solubilized membrane proteins, Biochim. Biophys. Acta Biomembr. 1861 (5) (2019) 939–957, https://doi.org/10.1016/j.bbamem.2019.02.003. URL.

[31] F. Hauer, C. Gerle, N. Fischer, A. Oshima, K. Shinzawa-Itoh, S. Shimada, K. Yokoyama, Y. Fujiyoshi, H. Stark, GraDeR: membrane protein complex preparation for single-particle cryo-EM, Structure 23 (9) (2015) 1769–1775, https://doi.org/10.1016/j.str.2015.06.029. URL.

[32] B.C. Choy, R.J. Cater, F. Mancia, E.E. Pryor, A 10-year meta-analysis of membrane protein structural biology: detergents, membrane mimetics, and structure determination techniques, Biochim. Biophys. Acta Biomembr. 1863 (3) (2021), 183533, https://doi.org/10.1016/j.bbamem.2020.183533. URL.

[33] Q. Zhang, X. Ma, A. Ward, W.X. Hong, V.P. Jaakola, R.C. Stevens, M.G. Finn, G. Chang, Designing facial amphiphiles for the stabilization of integral membrane proteins, Angew. Chem. Int. Ed. 46 (37) (2007) 7023–7025, https://doi.org/10.1002/anie.200701556.

[34] S.C. Lee, B.C. Bennett, W.X. Hong, Y. Fu, K.A. Baker, J. Marcoux, C.V. Robinson, A.B. Ward, J.R. Halpert, R.C. Stevens, C.D. Stout, M.J. Yeager, Q. Zhang, Steroid-based facial amphiphiles for stabilization and crystallization of membrane proteins, Proc. Natl. Acad. Sci. U. S. A 110 (13) (2013) 1203–1211, https://doi.org/10.1073/pnas.1221442110.

[35] V.A. Maslova, M.A. Kiselev, Structure of sodium cholate micelles, Crystallogr. Rep. 63 (3) (2018) 472–475, https://doi.org/10.1134/S1063774518030173. URL, http://link.springer.com/10.1134/S1063774518030173.

[36] E.J. Dufourc, Bicelles and nanodiscs for biophysical chemistry, Biochim. Biophys. Acta Biomembr. 1863 (1) (2021), 183478, https://doi.org/10.1016/j.bbamem.2020.183478. URL.

[37] K.S. Mineev, K.D. Nadezhdin, S.A. Goncharuk, A.S. Arseniev, Façade detergents as bicelle rim-forming agents for solution NMR spectroscopy, Nanotechnol. Rev. 6 (1) (2017) 93–103, https://doi.org/10.1515/ntrev-2016-0069.

[38] U.H.N. Dürr, M. Gildenberg, A. Ramamoorthy, The magic of bicelles lights up membrane protein structure, Chem. Rev. 112 (11) (2012) 6054–6074, https://doi.org/10.1021/cr300061w. URL, http://pubs.acs.org/doi/10.1021/cr300061w.

[39] J.M. Hutchison, K.-C. Shih, H.A. Scheidt, S.M. Fantin, K.F. Parson, G.A. Pantelopulos, H.R. Harrington, K.F. Mittendorf, S. Qian, R.A. Stein, S.E. Collier, M.G. Chambers, J. Katsaras, M.W. Voehler, B.T. Ruotolo, D. Huster, R.L. McFeeters, J.E. Straub, M.-P. Nieh, C.R. Sanders, Bicelles rich in both sphingolipids and cholesterol and their use in studies of membrane proteins, J. Am. Chem. Soc. 142 (29) (2020) 12715–12729, https://doi.org/10.1021/jacs.0c04669. URL, https://pubs.acs.org/doi/10.1021/jacs.0c04669.

[40] C.R. Sanders, R.S. Prosser, Bicelles: a model membrane system for all seasons? Structure 6 (10) (1998) 1227–1234, https://doi.org/10.1016/S0969-2126(98)00123-3. URL, http://linkinghub.elsevier.com/retrieve/pii/S0969212698001233.

[41] P.A. Luchette, T.N. Vetman, R. Prosser, R.E. Hancock, M.-P. Nieh, C.J. Glinka, S. Krueger, J. Katsaras, Morphology of fast-tumbling bicelles: a small angle neutron scattering and NMR study, Biochim. Biophys. Acta Biomembr. 1513 (2) (2001) 83–94, https://doi.org/10.1016/S0005-2736(01)00358-3. URL, http://linkinghub.elsevier.com/retrieve/pii/S0005273601003583.

[42] R. Matsui, N. Uchida, M. Ohtani, K. Yamada, A. Shigeta, I. Kawamura, T. Aida, Y. Ishida, Magnetically alignable bicelles with unprecedented stability using tunable surfactants derived from cholic acid, ChemPhysChem 17 (23) (2016) 3916–3922, https://doi.org/10.1002/cphc.201600897. URL.

[43] T.A. Caldwell, S. Baoukina, A.T. Brock, R.C. Oliver, K.T. Root, J.K. Krueger, K.J. Glover, D.P. Tieleman, L. Columbus, Low- q bicelles are mixed micelles, J. Phys. Chem. Lett. 9 (15) (2018) 4469–4473, https://doi.org/10.1021/acs.jpclett.8b02079.

[44] J.-L. Popot, Amphipols, nanodiscs, and fluorinated surfactants: three nonconventional approaches to studying membrane proteins in aqueous solutions, Annu. Rev. Biochem. 79 (1) (2010) 737–775, https://doi.org/10.1146/annurev.biochem.052208.114057. URL, http://www.annualreviews.org/doi/10.1146/annurev.biochem.052208.114057 http://www.ncbi.nlm.nih.gov/pubmed/20307193.

[45] G. Durand, M. Abla, C. Ebel, C. Breyton, New Amphiphiles to Handle Membrane Proteins: “Ménage à Trois” Between Chemistry, Physical Chemistry, and Biochemistry, in: I. Mus-Veteau (Ed.), Membrane Proteins Production for Structural Analysis, Springer New York, New York, NY, 2014, pp. 205–251, https://doi.org/10.1007/978-1-4939-0662-8_8. URL, http://link.springer.com/10.1007/978-1-4939-0662-8_8.

[46] C.-L. McGregor, L. Chen, N.C. Pomroy, P. Hwang, S. Go, A. Chakrabartty, G.G. Privé, Lipopeptide detergents designed for the structural study of membrane proteins, Nat. Biotechnol. 21 (2) (2003) 171–176, https://doi.org/10.1038/nbt776. URL, http://www.nature.com/articles/nbt776.

[47] G.G. Privé, Lipopeptide detergents for membrane protein studies, Curr. Opin. Struct. Biol. 19 (4) (2009) 379–385, https://doi.org/10.1016/j.sbi.2009.07.008. URL, http://linkinghub.elsevier.com/retrieve/pii/S0959440X09001055.

[48] I. Sauer, H. Nikolenko, S. Keller, K. Abu Ajaj, M. Bienert, M. Dathe, Dipalmitoylation of a cellular uptake-mediating apolipoprotein E-derived peptide as a promising modification for stable anchorage in liposomal drug carriers, Biochim. Biophys. Acta Biomembr. 1758 (4) (2006) 552–561, https://doi.org/10.1016/j.bbamem.2006.03.017. URL, http://linkinghub.elsevier.com/retrieve/pii/S0005273606000964.

[49] H. Tao, S.C. Lee, A. Moeller, R.S. Roy, F.Y. Siu, J. Zimmermann, R.C. Stevens, C.S. Potter, B. Carragher, Q. Zhang, Engineered nanostructured β-sheet peptides protect membrane proteins, Nat. Methods 10 (8) (2013) 759–761, https://doi.org/10.1038/nmeth.2533. URL, http://www.nature.com/articles/nmeth.2533.

[50] J.I. Yeh, S. Du, A. Tortajada, J. Paulo, S. Zhang, Peptergents: peptide detergents that improve stability and functionality of a membrane protein, glycerol-3-phosphate dehydrogenase, Biochemistry 44 (51) (2005) 16912–16919, https://doi.org/10.1021/bi051357o.

[51] K. Veith, M. Martinez Molledo, Y. Almeida Hernandez, I. Josts, J. Nitsche, C. Löw, H. Tidow, Lipid-like peptides can stabilize integral membrane proteins for biophysical and structural studies, Chembiochem 18 (17) (2017) 1735–1742, https://doi.org/10.1002/cbic.201700235.

[52] C. L. Bon, B. Michon, J. L. Popot, M. Zoonens, Amphipathic environments for determining the structure of membrane proteins by single-particle electron cryo-microscopy, Q. Rev. Biophys.:10.1017/S0033583521000044.

[53] V. Chaptal, F. Delolme, A. Kilburg, S. Magnard, C. Montigny, M. Picard, C. Prier, L. Monticelli, O. Bornert, M. Agez, S. Ravaud, C. Orelle, R. Wagner, A. Jawhari, I. Broutin, E. Pebay-Peyroula, J.-M. Jault, H.R. Kaback, M. le Maire, P. Falson, Quantification of detergents complexed with membrane proteins, Sci. Rep. 7 (1) (2017), 41751, https://doi.org/10.1038/srep41751. URL, http://www.nature.com/articles/srep41751.

[54] J.-L. Popot, T. Althoff, D. Bagnard, J.-L. Banères, P. Bazzacco, E. Billon-Denis, L. Catoire, P. Champeil, D. Charvolin, M. Cocco, G. Crémel, T. Dahmane, L. de la Maza, C. Ebel, F. Gabel, F. Giusti, Y. Gohon, E. Goormaghtigh, E. Guittet, J. Kleinschmidt, W. Kühlbrandt, C. Le Bon, K. Martinez, M. Picard, B. Pucci, J. Sachs, C. Tribet, C. van Heijenoort, F. Wien, F. Zito, M. Zoonens, Amphipols from A to Z, Annu. Rev. Biophys. 40 (1) (2011) 379–408, https://doi.org/10.1146/annurev-biophys-042910-155219. URL, http://www.annualreviews.org/doi/10.1146/annurev-biophys-042910-155219.

[55] R.Q. Notti, T. Walz, Native-like environments afford novel mechanistic insights into membrane proteins, Trends Biochem. Sci. xx (xx) (2022) 1–9, https://doi.org/10.1016/j.tibs.2022.02.008. URL, https://linkinghub.elsevier.com/retrieve/pii/S0968000422000494.

[56] C. Cecchetti, J. Strauss, C. Stohrer, C. Naylor, E. Pryor, J. Hobbs, S. Tanley, A. Goldman, B. Byrne, A novel high-throughput screen for identifying lipids that stabilise membrane proteins in detergent based solution, PLoS One 16 (7 July) (2021) 1–20, https://doi.org/10.1371/journal.pone.0254118. URL.

[57] A.A. Thompson, J.J. Liu, E. Chun, D. Wacker, H. Wu, V. Cherezov, R.C. Stevens, GPCR stabilization using the bicelle-like architecture of mixed sterol-detergent micelles, Methods 55 (4) (2011) 310–317, https://doi.org/10.1016/j.ymeth.2011.10.011. URL.

[58] C. Chipot, F. Dehez, J.R. Schnell, N. Zitzmann, E. Pebay-Peyroula, L.J. Catoire, B. Miroux, E.R. Kunji, G. Veglia, T.A. Cross, P. Schanda, Perturbations of native membrane protein structure in alkyl phosphocholine detergents: a critical assessment of NMR and biophysical studies, Chem. Rev. 118 (7) (2018) 3559–3607, https://doi.org/10.1021/acs.chemrev.7b00570.

[59] M. da Graça Miguel, O. Eidelman, M. Ollivon, A. Walter, Temperature dependence of the vesicle-micelle transition of egg phosphatidylcholine and octyl glucoside, Biochemistry 28 (22) (1989) 8921–8928, https://doi.org/10.1021/bi00448a035.

[60] H.E. Bae, Y. Du, P. Hariharan, J.S. Mortensen, K.K. Kumar, B. Ha, M. Das, H.S. Lee, C.J. Loland, L. Guan, B.K. Kobilka, P.S. Chae, Asymmetric maltose neopentyl glycol amphiphiles for a membrane protein study: effect of detergent asymmetricity on protein stability, Chem. Sci. 10 (4) (2019) 1107–1116, https://doi.org/10.1039/c8sc02560f.

[61] A. Sadaf, M. Ramos, J.S. Mortensen, Y. Du, H.E. Bae, C.F. Munk, P. Hariharan, B. Byrne, B.K. Kobilka, C.J. Loland, L. Guan, P.S. Chae, Conformationally restricted monosaccharide-cored glycoside amphiphiles: the effect of detergent headgroup variation on membrane protein stability, ACS Chem. Biol. 14 (8) (2019) 1717–1726, https://doi.org/10.1021/acschembio.9b00166.

[62] H.E. Bae, C. Cecchetti, Y. Du, S. Katsube, J.S. Mortensen, W. Huang, S. Rehan, H.J. Lee, C.J. Loland, L. Guan, B.K. Kobilka, B. Byrne, P.S. Chae, Pendant-bearing glucose-neopentyl glycol (P-GNG) amphiphiles for membrane protein manipulation: importance of detergent pendant chain for protein stabilization, Acta Biomater. 112 (2020) 250–261, https://doi.org/10.1016/j.actbio.2020.06.001. URL.

[63] M. Ehsan, S. Katsube, C. Cecchetti, Y. Du, J.S. Mortensen, H. Wang, A. Nygaard, L. Ghani, C.J. Loland, B.K. Kobilka, B. Byrne, L. Guan, P.S. Chae, New malonate-derived tetraglucoside detergents for membrane protein stability, ACS Chem. Biol. 15 (6) (2020) 1697–1707, https://doi.org/10.1021/acschembio.0c00316.

[64] A. Sadaf, S. Kim, H.E. Bae, H. Wang, A. Nygaard, Y. Uegaki, Y. Du, C.F. Munk, S. Katsube, H. Sung Lee, J. Bae, C.W. Choi, H.J. Choi, B. Byrne, S.H. Gellman, L. Guan, C.J. Loland, B.K. Kobilka, W. Im, P.S. Chae, Conformationally flexible core-bearing detergents with a hydrophobic or hydrophilic pendant: effect of pendant polarity on detergent conformation and membrane protein stability, Acta Biomater. 128 (2021) 393–407, https://doi.org/10.1016/

j.actbio.2021.04.043. URL.
[65] L. Ghani, S. Kim, H. Wang, H. S. Lee, J. S. Mortensen, S. Katsube, Y. Du, A. Sadaf, W. Ahmed, B. Byrne, L. Guan, C. J. Loland, B. K. Kobilka, W. Im, P. S. Chae, Foldable detergents for membrane protein study: importance of detergent core flexibility in protein stabilization, Chem. Eur J. 28 (21). doi:10.1002/chem.202200116.
[66] H.J. Lee, H.S. Lee, T. Youn, B. Byrne, P.S. Chae, Impact of novel detergents on membrane protein studies, Chem 8 (4) (2022) 980–1013, https://doi.org/10.1016/j.chempr.2022.02.007. URL, https://linkinghub.elsevier.com/retrieve/pii/S2451929422000924.
[67] L. H. Urner, I. Liko, H. Y. Yen, K. K. Hoi, J. R. Bolla, J. Gault, F. G. Almeida, M. P. Schweder, D. Shutin, S. Ehrmann, R. Haag, C. V. Robinson, K. Pagel, Modular detergents tailor the purification and structural analysis of membrane proteins including G-protein coupled receptors, Nat. Commun. 11 (1). doi:10.1038/s41467-020-14424-8. URL https://doi.org/10.1038/s41467-020-14424-8.
[68] L. Liu, Z. Zhu, F. Zhou, D. Xue, T. Hu, W. Luo, Y. Qiu, D. Wu, F. Zhao, Z. Le, H. Tao, Catalytically cleavable detergent for membrane protein studies, ACS Omega 6 (32) (2021) 21087–21093, https://doi.org/10.1021/acsomega.1c02894.
[69] C. Faugier, S. Igonet, D. Cornut, R. Besson, G. Durand, A. Jawhari, Lactobionamide-based fluorinated detergent for functional and structural stabilization of membrane proteins, Methods 180 (December 2019) (2020) 19–26, https://doi.org/10.1016/j.ymeth.2020.02.005. URL.
[70] M.D. Farrelly, L.L. Martin, S.H. Thang, Polymer nanodiscs and their bioanalytical potential, Chem. Eur J. 27 (51) (2021) 12922–12939, https://doi.org/10.1002/chem.202101572.
[71] G. Ratkeviciute, B.F. Cooper, T.J. Knowles, Methods for the solubilisation of membrane proteins: the micelle-aneous world of membrane protein solubilisation, Biochem. Soc. Trans. 49 (4) (2021) 1763–1777, https://doi.org/10.1042/BST20210181. URL, https://portlandpress.com/biochemsoctrans/article/49/4/1763/229623/Methods-for-the-solubilisation-of-membrane.
[72] P. S. Orekhov, M. E. Bozdaganyan, N. Voskoboynikova, A. Y. Mulkidjanian, M. G. Karlova, A. Yudenko, A. Remeeva, Y. L. Ryzhykau, I. Gushchin, V. I. Gordeliy, O. S. Sokolova, H. J. Steinhoff, M. P. Kirpichnikov, K. V. Shaitan, Mechanisms of formation, structure, and dynamics of lipoprotein discs stabilized by amphiphilic copolymers: a comprehensive review, Nanomaterials 12 (3). doi:10.3390/nano12030361.
[73] 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) 7484–7485, https://doi.org/10.1021/ja810046q. URL, http://pubs.acs.org/doi/abs/10.1021/ja810046q.
[74] 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. Ed. 56 (7) (2017) 1919–1924, https://doi.org/10.1002/anie.201610778. URL.
[75] J.M. Dörr, S. Scheidelaar, M.C. Koorengevel, J.J. Dominguez, M. Schäfer, 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, https://doi.org/10.1007/s00249-015-1093-y. URL, http://link.springer.com/10.1007/s00249-015-1093-y.
[76] S.R. Midtgaard, M.C. Pedersen, J.J.K. Kirkensgaard, K.K. Sørensen, K. Mortensen, K.J. Jensen, L. Arleth, Self-assembling peptides form nanodiscs that stabilize membrane proteins, Soft Matter 10 (5) (2014) 738–752, https://doi.org/10.1039/C3SM51727F. URL, http://xlink.rsc.org/?DOI=C3SM51727F.
[77] 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) 423–430, https://doi.org/10.1016/j.colsurfb.2016.06.040. URL, https://linkinghub.elsevier.com/retrieve/pii/S0927776516304702.
[78] A.N. Larsen, K.K. Sørensen, N.T. Johansen, A. Martel, J.J.K. Kirkensgaard, K.J. Jensen, L. Arleth, S.R. Midtgaard, Dimeric peptides with three different linkers self-assemble with phospholipids to form peptide nanodiscs that stabilize membrane proteins, Soft Matter 12 (27) (2016) 5937–5949, https://doi.org/10.1039/C6SM00495D. URL, http://xlink.rsc.org/?DOI=C6SM00495D.
[79] M.L. Carlson, J.W. Young, Z. Zhao, L. Fabre, D. Jun, J. Li, J. Li, H.S. Dhupar, I. Wason, A.T. Mills, J.T. Beatty, J.S. Klassen, I. Rouiller, F. Duong, The Peptidisc, a simple method for stabilizing membrane proteins in detergent-free solution, Elife 7 (2018) 1–23, https://doi.org/10.7554/eLife.34085. URL, https://elifesciences.org/articles/34085.
[80] S. Tonge, B. Tighe, Responsive hydrophobically associating polymers: a review of structure and properties, Adv. Drug Deliv. Rev. 53 (1) (2001) 109–122, https://doi.org/10.1016/S0169-409X(01)00223-X. URL, http://linkinghub.elsevier.com/retrieve/pii/S0169409X0100223X.
[81] S.C.L. Hall, C. Tognoloni, J. Charlton, E.C. Bragginton, A.J. Rothnie, P. Sridhar, M. Wheatley, T.J. Knowles, T. Arnold, K.J. Edler, T.R. Dafforn, An acid-compatible co-polymer for the solubilization of membranes and proteins into lipid bilayer-containing nanoparticles, Nanoscale 10 (22) (2018) 10609–10619, https://doi.org/10.1039/C8NR01322E. URL, http://xlink.rsc.org/?DOI=C8NR01322E.
[82] J.M. Dörr, M.C. Koorengevel, M. Schäfer, A.V. Prokofyev, S. Scheidelaar, E.A.W. van der Cruijsen, T.R. Dafforn, M. Baldus, J.A. Killian, Detergent-free isolation, characterization, and functional reconstitution of a tetrameric K + channel: the power of native nanodiscs, Proc. Natl. Acad. Sci. USA 111 (52) (2014) 18607–18612, https://doi.org/10.1073/pnas.1416205112. URL, http://www.pnas.org/lookup/doi/10.1073/pnas.1416205112.
[83] K.S. Simon, N.L. Pollock, S.C. Lee, Membrane protein nanoparticles: the shape of things to come, Biochem. Soc. Trans. 46 (6) (2018) 1495–1504, https://doi.org/10.1042/BST20180139.
[84] Y. Guo, Detergent-free systems for structural studies of membrane proteins, Biochem. Soc. Trans. 49 (3) (2021) 1316–1374, https://doi.org/10.1042/BST20201080.
[85] A. Chen, E.J. Majdinasab, M.C. Fiori, H. Liang, G.A. Altenberg, Polymer-Encased nanodiscs and polymer nanodiscs: new platforms for membrane protein research and applications, Front. Bioeng. Biotechnol. 8 (November) (2020) 1–8, https://doi.org/10.3389/fbioe.2020.598450.
[86] R. Cuevas Arenas, J. Klingler, C. Vargas, S. Keller, Influence of lipid bilayer properties on nanodisc formation mediated by styrene/maleic acid copolymers, Nanoscale 8 (32) (2016) 15016–15026, https://doi.org/10.1039/C6NR02089E. URL, http://xlink.rsc.org/?DOI=C6NR02089E.
[87] M.C. Fiori, Y. Jiang, G.A. Altenberg, H. Liang, Polymer-encased nanodiscs with improved buffer compatibility, Sci. Rep. 7 (1) (2017) 1–10, https://doi.org/10.1038/s41598-017-07110-1. URL.
[88] M. Jamshad, V. Grimard, I. Idini, T.J. Knowles, M.R. Dowle, N. Schofield, P. Sridhar, Y. Lin, R. Finka, M. Wheatley, O.R.T. Thomas, R.E. Palmer, M. Overduin, C. Govaerts, J.-M. Ruysschaert, K.J. Edler, T.R. Dafforn, Structural analysis of a nanoparticle containing a lipid bilayer used for detergent-free extraction of membrane proteins, Nano Res. 8 (3) (2015) 774–789, https://doi.org/10.1007/s12274-014-0560-6. URL, http://link.springer.com/10.1007/s12274-014-0560-6.
[89] V.A. Bjørnestad, M. Orwick-Rydmark, R. Lund, Understanding the structural pathways for lipid nanodisc formation: how styrene maleic acid copolymers induce membrane fracture and disc formation, Langmuir 37 (20) (2021) 6178–6188, https://doi.org/10.1021/acs.langmuir.1c00304.
[90] 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) 8983–8985, https://doi.org/10.1021/bi201289c. URL, http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3236031&tool=pmcentrez&rendertype=abstract http://pubs.acs.org/doi/abs/10.1021/bi201289c.
[91] T. Ravula, N.Z. Hardin, S.K. Ramadugu, S.J. Cox, A. Ramamoorthy, formation of pH-resistant monodispersed polymer-lipid nanodiscs, Angew. Chem. Int. Ed. 57 (5) (2018) 1342–1345, https://doi.org/10.1002/anie.201712017. URL.
[92] 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, https://doi.org/10.1016/j.bpj.2018.05.024. URL, https://linkinghub.elsevier.com/retrieve/pii/S0006349518306271.
[93] A.H. Kopf, J.M. Dörr, M.C. Koorengevel, F. Antoniciello, H. Jahn, J.A. Killian, Factors influencing the solubilization of membrane proteins from Escherichia coli membranes by styrene–maleic acid copolymers, Biochim. Biophys. Acta Biomembr. 1862 (2) (2020), 183125, https://doi.org/10.1016/j.bbamem.2019.183125. URL.
[94] B. Danielczak, M. Rasche, J. Lenz, E. Pérez Patallo, S. Weyrauch, F. Mahler, M.T. Agbadaola, A. Meister, J.O. Babalola, C. Vargas, C. Kolar, S. Keller, A bioinspired glycopolymer for capturing membrane proteins in native-like lipid-bilayer nanodiscs, Nanoscale 14 (5) (2022) 1855–1867, https://doi.org/10.1039/d1nr03811g.
[95] M.-P. Nieh, C.J. Glinka, S. Krueger, R.S. Prosser, J. Katsaras, SANS study on the effect of lanthanide ions and charged lipids on the morphology of phospholipid mixtures, Biophys. J. 82 (5) (2002) 2487–2498, https://doi.org/10.1016/S0006-3495(02)75591-4. URL, http://linkinghub.elsevier.com/retrieve/pii/S0006349502755914.
[96] K. Yamamoto, R. Soong, A. Ramamoorthy, Comprehensive analysis of lipid dynamics variation with lipid composition and hydration of bicelles using nuclear magnetic resonance (NMR) spectroscopy, Langmuir 25 (12) (2009) 7010–7018, https://doi.org/10.1021/la900200s.
[97] A.A. Smith, H.E. Autzen, B. Faust, J.L. Mann, B.W. Muir, S. Howard, A. Postma, A.J. Spakowitz, Y. Cheng, E.A. Appel, Lipid nanodiscs via ordered copolymers, Chem 6 (10) (2020) 2782–2795, https://doi.org/10.1016/j.chempr.2020.08.004. URL, https://linkinghub.elsevier.com/retrieve/pii/S2451929420304149.
[98] S. Perrier, 50th anniversary perspective: RAFT polymerization - a user guide, Macromolecules 50 (19) (2017) 7433–7447, https://doi.org/10.1021/acs.macromol.7b00767.
[99] O. Korotych, J. Mondal, K.M. Gattás-Asfura, J. Hendricks, B.D. Bruce, Evaluation of commercially available styrene-co-maleic acid polymers for the extraction of membrane proteins from spinach chloroplast thylakoids, June 2018, Eur. Polym. J. 114 (2019) 485–500, https://doi.org/10.1016/j.eurpolymj.2018.10.035. URL.
[100] K.A. Morrison, A. Akram, A. Mathews, Z.A. Khan, J.H. Patel, C. Zhou, D.J. Hardy, C. Moore-Kelly, R. Patel, V. Odiba, T.J. Knowles, M.U.H. Javed, N.P. Chmel, T.R. Dafforn, A.J. Rothnie, Membrane protein extraction and purification using styrene-maleic acid (SMA) copolymer: effect of variations in polymer structure, Biochem. J. 473 (23) (2016) 4349–4360, https://doi.org/10.1042/BCJ20160723.
[101] J.J. Domínguez Pardo, M.C. Koorengevel, N. Uwugiaren, J. Weijers, A.H. Kopf, H. Jahn, C.A. van Walree, M.J. van Steenbergen, J.A. Killian, Membrane solubilization by styrene-maleic acid copolymers: delineating the role of

polymer length, Biophys. J. 115 (1) (2018) 129–138, https://doi.org/10.1016/j.bpj.2018.05.032. URL, https://linkinghub.elsevier.com/retrieve/pii/S0006349518306714.
[102] L.E. Ball, L.J. Riley, W. Hadasha, R. Pfukwa, C.J. Smith, T.R. Dafforn, B. Klumperman, Influence of DIBMA polymer length on lipid nanodisc formation and membrane protein extraction, Biomacromolecules 22 (2) (2021) 763–772, https://doi.org/10.1021/acs.biomac.0c01538.
[103] S. C. Hall, C. Tognoloni, R. A. Campbell, J. Richens, P. O'Shea, A. E. Terry, G. J. Price, T. R. Dafforn, K. J. Edler, T. Arnold, The interaction of styrene maleic acid copolymers with phospholipids in Langmuir monolayers, vesicles and nanodiscs; a structural study, J. Colloid Interface Sci.doi:10.1016/j.jcis.2022.03.102. URL https://doi.org/10.1016/j.jcis.2022.03.102.
[104] T.N. Sut, B.K. Yoon, S. Park, J.A. Jackman, N.J. Cho, Versatile formation of supported lipid bilayers from bicellar mixtures of phospholipids and capric acid, Sci. Rep. 10 (1) (2020) 1–10, https://doi.org/10.1038/s41598-020-70872-8. URL.
[105] A. Luchini, F.G. Tidemand, N.T. Johansen, M. Campana, J. Sotres, M. Ploug, M. Cárdenas, L. Arleth, Peptide disc mediated control of membrane protein orientation in supported lipid bilayers for surface-sensitive investigations, Anal. Chem. 92 (1) (2020) 1081–1088, https://doi.org/10.1021/acs.analchem.9b04125.
[106] B. Danielczak, A. Meister, S. Keller, Influence of Mg 2+ and Ca 2+ on nanodisc formation by diisobutylene/maleic acid (DIBMA) copolymer, November 2018, Chem. Phys. Lipids 221 (2019) 30–38, https://doi.org/10.1016/j.chemphyslip.2019.03.004. URL.
[107] A. H. Kopf, O. Lijding, B. O. Elenbaas, M. C. Koorengevel, J. M. Dobruchowska, C. A. Van Walree, J. A. Killian, Synthesis and evaluation of a library of alternating amphipathic copolymers to solubilize and study membrane proteins, Biomacromoleculesdoi:10.1021/acs.biomac.1c01166.
[108] W. Qiu, Z. Fu, G.G. Xu, R.A. Grassucci, Y. Zhang, J. Frank, W.A. Hendrickson, Y. Guo, Structure and activity of lipid bilayer within a membrane-protein transporter, Proc. Natl. Acad. Sci. U. S. A 115 (51) (2018) 12985–12990, https://doi.org/10.1073/pnas.1812526115.
[109] L. Yang, C. Catalano, Y. Xu, W. Qiu, D. Zhang, A. McDermott, Y. Guo, P. Blount, A native cell membrane nanoparticles system allows for high-quality functional proteoliposome reconstitution, BBA Advances 1 (April) (2021), 100011, https://doi.org/10.1016/j.bbadva.2021.100011. URL.
[110] M. Parmar, S. Rawson, C.A. Scarff, A. Goldman, T.R. Dafforn, S.P. Muench, V.L. Postis, Using a SMALP platform to determine a sub-nm single particle cryo-EM membrane protein structure, Biochim. Biophys. Acta Biomembr. 1860 (2) (2018) 378–383, https://doi.org/10.1016/j.bbamem.2017.10.005. URL, https://linkinghub.elsevier.com/retrieve/pii/S0005273617303152.
[111] A. Marconnet, B. Michon, C. Le Bon, F. Giusti, C. Tribet, M. Zoonens, Solubilization and stabilization of membrane proteins by cycloalkane-modified amphiphilic polymers, Biomacromolecules 21 (8) (2020) 3459–3467, https://doi.org/10.1021/acs.biomac.0c00929.
[112] A.J. Higgins, A.J. Flynn, A. Marconnet, L.J. Musgrove, V.L. Postis, J.D. Lippiat, C.w. Chung, T. Ceska, M. Zoonens, F. Sobott, S.P. Muench, Cycloalkane-modified amphiphilic polymers provide direct extraction of membrane proteins for CryoEM analysis, Communications Biology 4 (1) (2021) 1–9, https://doi.org/10.1038/s42003-021-02834-3.
[113] T. Ravula, A. Ramamoorthy, Synthesis, characterization, and nanodisc formation of non-ionic polymers, Angew. Chem. Int. Ed. 60 (31) (2021) 16885–16888, https://doi.org/10.1002/anie.202101950.
[114] W.S. Davidson, T.B. Thompson, The structure of apolipoprotein A-I in high density lipoproteins, J. Biol. Chem. 282 (31) (2007) 22249–22253, https://doi.org/10.1074/jbc.R700014200. URL, http://www.jbc.org/cgi/content/abstract/282/31/22249 http://www.jbc.org/lookup/doi/10.1074/jbc.R700014200.
[115] G.H. Rothblat, M.C. Phillips, High-density lipoprotein heterogeneity and function in reverse cholesterol transport, Curr. Opin. Lipidol. 21 (3) (2010) 229–238, https://doi.org/10.1097/MOL.0b013e328338472d. URL, https://insights.ovid.com/crossref?an=00041433-201006000-00011.
[116] M. Nakano, M. Fukuda, T. Kudo, M. Miyazaki, Y. Wada, N. Matsuzaki, H. Endo, T. Handa, Static and dynamic properties of phospholipid bilayer nanodiscs, J. Am. Chem. Soc. 131 (23) (2009) 8308–8312, https://doi.org/10.1021/ja9017013. URL, http://pubs.acs.org/doi/abs/10.1021/ja9017013.
[117] S.R. Midtgaard, M.C. Pedersen, L. Arleth, Small-angle X-ray scattering of the cholesterol incorporation into human ApoA1-POPC discoidal particles, Biophys. J. 109 (2) (2015) 308–318, https://doi.org/10.1016/j.bpj.2015.06.032. URL, https://linkinghub.elsevier.com/retrieve/pii/S0006349515006153.
[118] 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, https://doi.org/10.1021/nl025623k. URL, http://pubs.acs.org/doi/abs/10.1021/nl025623k.
[119] I.G. Denisov, S.G. Sligar, Nanodiscs for structural and functional studies of membrane proteins, Nat. Struct. Mol. Biol. 23 (6) (2016) 481–486, https://doi.org/10.1038/nsmb.3195. URL, http://www.nature.com/doifinder/10.1038/nsmb.3195.
[120] I.G. Denisov, S.G. Sligar, Nanodiscs in membrane biochemistry and biophysics, Chem. Rev. 117 (6) (2017) 4669–4713, https://doi.org/10.1021/acs.chemrev.6b00690. URL, http://pubs.acs.org/doi/abs/10.1021/acs.chemrev.6b00690 http://pubs.acs.org/doi/10.1021/acs.chemrev.6b00690.
[121] S.G. Sligar, I.G. Denisov, Nanodiscs: a toolkit for membrane protein science, Protein Sci. 30 (2) (2021) 297–315, https://doi.org/10.1002/pro.3994. URL, https://onlinelibrary.wiley.com/doi/10.1002/pro.3994.
[122] J.E. Rouck, J.E. Krapf, J. Roy, H.C. Huff, A. Das, Recent advances in nanodisc technology for membrane protein studies (2012-2017), FEBS (Fed. Eur. Biochem. Soc.) Lett. 591 (14) (2017) 2057–2088, https://doi.org/10.1002/1873-3468.12706. URL.
[123] 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) 3477–3487, https://doi.org/10.1021/ja0393574. URL, http://pubs.acs.org/doi/abs/10.1021/ja0393574.
[124] N. Skar-Gislinge, J.B. Simonsen, K. Mortensen, R. Feidenhans'l, S.G. Sligar, B. Lindberg Møller, T. Bjørnholm, 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, https://doi.org/10.1021/ja1030613. URL, http://pubs.acs.org/doi/abs/10.1021/ja1030613.
[125] 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) 1919–1925, https://doi.org/10.1021/ja310901f. URL, http://ieeexplore.ieee.org/document/818105/, http://pubs.acs.org/doi/10.1021/ja310901f.
[126] S. Bibow, Y. Polyhach, C. Eichmann, C.N. Chi, J. Kowal, S. Albiez, R.A. McLeod, H. Stahlberg, G. Jeschke, P. Güntert, 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, https://doi.org/10.1038/nsmb.3345. URL.
[127] Y.V. Grinkova, I.G. Denisov, S.G. Sligar, Engineering extended membrane scaffold proteins for self-assembly of soluble nanoscale lipid bilayers, Protein Eng. Des. Sel. 23 (11) (2010) 843–848, https://doi.org/10.1093/protein/gzq060. URL, https://academic.oup.com/peds/article-lookup/doi/10.1093/protein/gzq060.
[128] T. Ritchie, Y. Grinkova, T. Bayburt, I. Denisov, J. Zolnerciks, W. Atkins, S. Sligar, Reconstitution of membrane proteins in phospholipid bilayer nanodiscs, Ch. Methods in, in: N. Düzgünes (Ed.), Methods in Enzymology, vol. 464Academmic Press, 2009, pp. 211–231, https://doi.org/10.1016/S0076-6879(09)64011-8. URL, http://www.ncbi.nlm.nih.gov/pubmed/19903557 http://linkinghub.elsevier.com/retrieve/pii/S0076687909640118.
[129] X. Mei, D. Atkinson, Crystal structure of C-terminal truncated apolipoprotein A-I reveals the assembly of high density lipoprotein (HDL) by dimerization, J. Biol. Chem. 286 (44) (2011) 38570–38582, https://doi.org/10.1074/jbc.M111.260422. URL, http://www.jbc.org/cgi/doi/10.1074/jbc.M111.260422 https://linkinghub.elsevier.com/retrieve/pii/S002192582050700X.
[130] A.W. Shaw, M.A. McLean, S.G. Sligar, Phospholipid phase transitions in homogeneous nanometer scale bilayer discs, FEBS (Fed. Eur. Biochem. Soc.) Lett. 556 (1–3) (2004) 260–264, https://doi.org/10.1016/S0014-5793(03)01400-5.
[131] N. Skar-Gislinge, N.T. Johansen, R. Høiberg-Nielsen, L. Arleth, Comprehensive study of the self-assembly of phospholipid nanodiscs: what determines their shape and stoichiometry? Langmuir 34 (42) (2018) 12569–12582, https://doi.org/10.1021/acs.langmuir.8b01503.
[132] T. Camp, S.G. Sligar, S.G. Sligar, S.G. Sligar, Nanodisc self-assembly is thermodynamically reversible and controllable, Soft Matter 16 (24) (2020) 5615–5623, https://doi.org/10.1039/d0sm00336k.
[133] M.L. Nasr, D. Baptista, M. Strauss, Z.-Y.J. Sun, S. Grigoriu, S. Huser, A. Plückthun, 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, https://doi.org/10.1038/nmeth.4079. URL, http://www.nature.com/articles/nmeth.4079.
[134] F.G. Tidemand, S. Blemmer, N.T. Johansen, L. Arleth, M.C. Pedersen, Non-ionic detergent assists formation of supercharged nanodiscs and insertion of membrane proteins, Biochim. Biophys. Acta Biomembr. 1864 (6) (2022), 183884, https://doi.org/10.1016/j.bbamem.2022.183884. URL.
[135] M.T. Marty, K.C. Wilcox, W.L. Klein, S.G. Sligar, Nanodisc-solubilized membrane protein library reflects the membrane proteome, Anal. Bioanal. Chem. 405 (12) (2013) 4009–4016, https://doi.org/10.1007/s00216-013-6790-8. URL, http://www.ncbi.nlm.nih.gov/pubmed/23400332 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3628400.
[136] J. Roy, H. Pondenis, T.M. Fan, A. Das, Direct capture of functional proteins from mammalian plasma membranes into nanodiscs, Biochemistry 54 (41) (2015) 6299–6302, https://doi.org/10.1021/acs.biochem.5b00954.
[137] N. Shirzad-Wasei, J. Van Oostrum, P.H. Bovee-Geurts, L.J. Kusters, G.J. Bosman, W.J. DeGrip, Rapid transfer of overexpressed integral membrane protein from the host membrane into soluble lipid nanodiscs without previous purification, Biol. Chem. 396 (8) (2015) 903–915, https://doi.org/10.1515/hsz-2015-0100.
[138] Y. Cai, Y. Liu, K.J. Culhane, B.T. DeVree, Y. Yang, R.K. Sunahara, E.C. Yan, Purification of family B G protein-coupled receptors using nanodiscs: application to human glucagon-like peptide-1 receptor, PLoS One 12 (6) (2017) 1–18, https://doi.org/10.1371/journal.pone.0179568.
[139] C.C. Su, M. Lyu, C.E. Morgan, J.R. Bolla, C.V. Robinson, E.W. Yu, A 'Build and Retrieve' methodology to simultaneously solve cryo-EM structures of membrane proteins, Nat. Methods 18 (1) (2021) 69–75, https://doi.org/10.1038/s41592-020-01021-2. URL.
[140] S. Mak, R. Sun, M. Schmalenberg, C. Peters, P.B. Luppa, Express incorporation of membrane proteins from various human cell types into phospholipid bilayer nanodiscs, Biochem. J. 474 (8) (2017) 1361–1371, https://doi.org/

10.1042/BCJ20161110.
[141] M.C. Fiori, Y. Jiang, W. Zheng, M. Anzaldua, M.J. Borgnia, G.A. Altenberg, H. Liang, Polymer nanodiscs: discoidal amphiphilic block copolymer membranes as a new platform for membrane proteins, Sci. Rep. 7 (1) (2017) 1–9, https://doi.org/10.1038/s41598-017-15151-9. URL.
[142] C. Eichmann, S. Bibow, R. Riek, α-Synuclein lipoprotein nanoparticles, Nanotechnol. Rev. 6 (1) (2017) 105–110, https://doi.org/10.1515/ntrev-2016-0062. URL, https://www.degruyter.com/document/doi/10.1515/ntrev-2016-0062/html.
[143] 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 J. 286 (9) (2019) 1734–1751, https://doi.org/10.1111/febs.14766. URL, https://febs.onlinelibrary.wiley.com/doi/abs/10.1111/febs.14766.
[144] M. Serna, Hands on methods for high resolution cryo-electron microscopy structures of heterogeneous macromolecular complexes, Front. Mol. Biosci. 6 (MAY) (2019) 1–8, https://doi.org/10.3389/fmolb.2019.00033.
[145] K. Mio, C. Sato, Lipid environment of membrane proteins in cryo-EM based structural analysis, Biophysical Reviews 10 (2) (2018) 307–316, https://doi.org/10.1007/s12551-017-0371-6. URL, http://link.springer.com/10.1007/s12551-017-0371-6.
[146] J. Miehling, D. Goricanec, F. Hagn, A split-intein-based method for the efficient production of circularized nanodiscs for structural studies of membrane proteins, Chembiochem 19 (18) (2018) 1927–1933, https://doi.org/10.1002/cbic.201800345. URL.
[147] S.A.R. Kynde, N. Skar-Gislinge, M.C. Pedersen, S.R. Midtgaard, J.B. Simonsen, R. Schweins, K. Mortensen, L. Arleth, Small-angle scattering gives direct structural information about a membrane protein inside a lipid environment, Acta Crystallogr. Sect. D Biol. Crystallogr. 70 (2) (2014) 371–383, https://doi.org/10.1107/S1399004713028344. URL, http://scripts.iucr.org/cgi-bin/paper?S1399004713028344.
[148] N.T. Johansen, M.C. Pedersen, L. Porcar, A. Martel, L. Arleth, Introducing SEC–SANS for studies of complex self-organized biological systems, Acta Crystallogr. D: Struct. Biol. 74 (12) (2018) 1178–1191, https://doi.org/10.1107/S2059798318007180.
[149] J.H. Wade, J.D. Jones, I.L. Lenov, C.M. Riordan, S.G. Sligar, R.C. Bailey, Microfluidic platform for efficient Nanodisc assembly, membrane protein incorporation, and purification, Lab Chip 17 (17) (2017) 2951–2959, https://doi.org/10.1039/C7LC00601B. URL, http://xlink.rsc.org/?DOI=C7LC00601B.
[150] 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, https://doi.org/10.1021/acs.langmuir.8b00025. URL.
[151] S. Zhang, Q. Ren, S.J. Novick, T.S. Strutzenberg, P.R. Griffin, H. Bao, One-step construction of circularized nanodiscs using SpyCatcher-SpyTag, Nat. Commun. 12 (1) (2021) 5451, https://doi.org/10.1038/s41467-021-25737-7. URL, https://www.nature.com/articles/s41467-021-25737-7.
[152] D.T. Sweeney, S. Krueger, K. Sen, J.C. Hackett, Structures and dynamics of anionic lipoprotein nanodiscs, acs.jpcb.2c00758, J. Phys. Chem. B (2022), https://doi.org/10.1021/acs.jpcb.2c00758. URL, https://pubs.acs.org/doi/10.1021/acs.jpcb.2c00758.
[153] K.M. Padmanabha Das, W.M. Shih, G. Wagner, M.L. Nasr, Large nanodiscs: a potential game changer in structural biology of membrane protein complexes and virus entry, Front. Bioeng. Biotechnol. 8 (June) (2020) 1–7, https://doi.org/10.3389/fbioe.2020.00539.
[154] N.T. Johansen, A. Luchini, F.G. Tidemand, S. Orioli, A. Martel, L. Porcar, L. Arleth, M.C. Pedersen, Structural and biophysical properties of supercharged and circularized nanodiscs, Langmuir 37 (22) (2021) 6681–6690, https://doi.org/10.1021/acs.langmuir.1c00560.
[155] D.M. Kehlenbeck, I. Josts, J. Nitsche, S. Busch, T. Forsyth, H. Tidow, Comparison of lipidic carrier systems for integral membrane proteins - MsbA as case study, Biol. Chem. 400 (11) (2019) 1509–1518, https://doi.org/10.1515/hsz-2019-0171.
[156] F.G. Tidemand, H. Østergaard, M. Ploug, B.B. Kragelund, L. Arleth, Efficient refolding and reconstitution of tissue factor into nanodiscs facilitates structural investigation of a multicomponent system on a lipid bilayer, Biochim. Biophys. Acta Biomembr. 1862 (6) (2020), 183214, https://doi.org/10.1016/j.bbamem.2020.183214. URL.
[157] D. Salvador, M. Glavier, G. Schoehn, G. Phan, J.C. Taveau, M. Decossas, S. Lecomte, S. Mongrand, C. Garnier, I. Broutin, L. Daury, O. Lambert, Minimal nanodisc without exogenous lipids for stabilizing membrane proteins in detergent-free buffer, Biochim. Biophys. Acta Biomembr. 1861 (4) (2019) 852–860, https://doi.org/10.1016/j.bbamem.2019.01.013. URL.
[158] N.T. Johansen, Towards an Optimal Basis for Structural Analysis of Membrane Proteins in Solution by Small-Angle Scattering: Optimized Membrane Protein Carrier Systems and Online Size-Exclusion Chromatography Coupled to Small-Angle Neutron Scattering, Ph.D. thesis, University of Copenhagen, 2018.
[159] K. Iric, M. Subramanian, J. Oertel, N.P. Agarwal, M. Matthies, X. Periole, T.P. Sakmar, T. Huber, K. Fahmy, T.L. Schmidt, DNA-encircled lipid bilayers, Nanoscale 10 (39) (2018) 18463–18467, https://doi.org/10.1039/c8nr06505e.
[160] Z. Zhao, M. Zhang, J.M. Hogle, W.M. Shih, G. Wagner, M.L. Nasr, DNA-corralled nanodiscs for the structural and functional characterization of membrane proteins and viral entry, J. Am. Chem. Soc. 140 (34) (2018) 10639–10643, https://doi.org/10.1021/jacs.8b04638.
[161] S. Zhang, F. Tao, R. Qing, H. Tang, M. Skuhersky, K. Corin, L. Tegler, A. Wassie, B. Wassie, Y. Kwon, B. Suter, C. Entzian, T. Schubert, G. Yang, J. Labahn, J. Kubicek, B. Maertens, QTY code enables design of detergent-free chemokine receptors that retain ligand-binding activities, Proc. Natl. Acad. Sci. U. S. A 115 (37) (2018) E8652–E8659, https://doi.org/10.1073/pnas.1811031115.
[162] Z. Zhang, E.R. Chapman, Programmable nanodisc patterning by DNA origami, Nano Lett. 20 (8) (2020) 6032–6037, https://doi.org/10.1021/acs.nanolett.0c02048.
[163] J. Frauenfeld, R. Löving, J.-P. Armache, A.F. Sonnen, F. Guettou, P. Moberg, L. Zhu, C. Jegerschöld, A. Flayhan, J.A.G. Briggs, H. Garoff, C. Löw, Y. Cheng, P. Nordlund, A saposin-lipoprotein nanoparticle system for membrane proteins, Nat. Methods 13 (4) (2016) 345–351, https://doi.org/10.1038/nmeth.3801. URL, http://www.nature.com/articles/nmeth.3801.
[164] H. Bruhn, A short guided tour through functional and structural features of saposin-like proteins, Biochem. J. 389 (2) (2005) 249–257, https://doi.org/10.1042/BJ20050051. URL, http://biochemj.org/lookup/doi/10.1042/BJ20050051.
[165] K. Popovic, J. Holyoake, R. Pomes, G.G. Prive, Structure of saposin A lipoprotein discs, Proc. Natl. Acad. Sci. USA 109 (8) (2012) 2908–2912, https://doi.org/10.1073/pnas.1115743109. URL, http://www.pnas.org/cgi/doi/10.1073/pnas.1115743109.
[166] C.-T.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, https://doi.org/10.1021/jacs.7b06730. URL, http://pubs.acs.org/doi/10.1021/jacs.7b06730.
[167] J. Li, M.R. Richards, D. Bagal, I.D.G. Campuzano, E.N. Kitova, Z.J. Xiong, G.G. Privé, J.S. Klassen, Characterizing the size and composition of saposin A lipoprotein picodiscs, Anal. Chem. 88 (19) (2016) 9524–9531, https://doi.org/10.1021/acs.analchem.6b02097. URL, http://pubs.acs.org/doi/10.1021/acs.analchem.6b02097.
[168] A. Flayhan, H.D. Mertens, Y. Ural-Blimke, M. Martinez Molledo, D.I. Svergun, C. Löw, Saposin lipid nanoparticles: a highly versatile and modular tool for membrane protein research, Structure 26 (2) (2018) 345–355, https://doi.org/10.1016/j.str.2018.01.007. URL, https://linkinghub.elsevier.com/retrieve/pii/S0969212618300078.
[169] P. Lloris-Garcerá, S. Klinter, L. Chen, M.J. Skynner, R. Löving, J. Frauenfeld, DirectMX — one-step reconstitution of membrane proteins from crude cell membranes into salipro nanoparticles, Front. Bioeng. Biotechnol. 8 (March) (2020) 1–9, https://doi.org/10.3389/fbioe.2020.00215.
[170] A. Grethen, A.O. Oluwole, B. Danielczak, C. Vargas, S. Keller, Thermodynamics of nanodisc formation mediated by styrene/maleic acid (2:1) copolymer, Sci. Rep. 7 (1) (2017) 1–14, https://doi.org/10.1038/s41598-017-11616-z.
[171] S.R. Midtgaard, T.A. Darwish, M.C. Pedersen, P. Huda, A.H. Larsen, G.V. Jensen, S.A.R. Kynde, N. Skar-Gislinge, A.J.Z. Nielsen, C. Olesen, M. Blaise, J.J. Dorosz, T.S. Thorsen, R. Venskutonytė, C. Krintel, J.V. Møller, H. Frielinghaus, E.P. Gilbert, A. Martel, J.S. Kastrup, P.E. Jensen, P. Nissen, L. Arleth, Invisible detergents for structure determination of membrane proteins by small-angle neutron scattering, arXiv:0608246v3, FEBS J. 285 (2) (2018) 357–371, https://doi.org/10.1111/febs.14345. URL.
[172] M. Wheatley, J. Charlton, M. Jamshad, S.J. Routledge, S. Bailey, P.J. La-Borde, M.T. Azam, R.T. Logan, R.M. Bill, T.R. Dafforn, D.R. Poyner, GPCR-styrene maleic acid lipid particles (GPCR-SMALPs): their nature and potential, Biochem. Soc. Trans. 44 (2) (2016) 619–623, https://doi.org/10.1042/BST20150284.
[173] R. Cuevas Arenas, B. Danielczak, A. Martel, L. Porcar, C. Breyton, C. Ebel, S. Keller, Fast collisional lipid transfer among polymer-bounded nanodiscs, Sci. Rep. 7 (1) (2017), 45875, https://doi.org/10.1038/srep45875. URL, http://www.nature.com/articles/srep45875.
[174] B. Danielczak, S. Keller, Collisional lipid exchange among DIBMA-encapsulated nanodiscs (DIBMALPs), Eur. Polym. J. 109 (August) (2018) 206–213, https://doi.org/10.1016/j.eurpolymj.2018.09.043. URL.
[175] 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. 54 (69) (2018) 9615–9618, https://doi.org/10.1039/C8CC04184A. URL, http://pubs.rsc.org/en/Content/ArticleLanding/2018/CC/C8CC04184A http://xlink.rsc.org/?DOI=C8CC04184A.
[176] V. Schmidt, J.N. Sturgis, Modifying styrene-maleic acid co-polymer for studying lipid nanodiscs, Biochim. Biophys. Acta Biomembr. 1860 (3) (2018) 777–783, https://doi.org/10.1016/j.bbamem.2017.12.012. URL.
[177] G.M. Di Mauro, C. La Rosa, M. Condorelli, A. Ramamoorthy, Benchmarks of SMA-copolymer derivatives and nanodisc integrity, Langmuir 37 (10) (2021) 3113–3121, https://doi.org/10.1021/acs.langmuir.0c03554.
[178] M.E. Zoghbi, R.S. Cooper, G.A. Altenberg, The lipid bilayer modulates the structure and function of an ATP-binding cassette exporter, J. Biol. Chem. 291 (9) (2016) 4453–4461, https://doi.org/10.1074/jbc.M115.698498. URL.
[179] K. Klöpfer, F. Hagn, Beyond detergent micelles: the advantages and applications of non-micellar and lipid-based membrane mimetics for solution-state NMR, Prog. Nucl. Magn. Reson. Spectrosc. 114–115 (2019) 271–283, https://doi.org/10.1016/j.pnmrs.2019.08.001.
[180] S. Ganapathy, L. Opdam, Y. Hontani, S. Frehan, Q. Chen, K.J. Hellingwerf, H.J. de Groot, J.T. Kennis, W.J. de, Grip, Membrane matters: the impact of a

nanodisc-bilayer or a detergent microenvironment on the properties of two eubacterial rhodopsins, Biochim. Biophys. Acta Biomembr. 1862 (2) (2020), 183113, https://doi.org/10.1016/j.bbamem.2019.183113. URL.

[181] G.L. Nicolson, The Fluid—mosaic Model of Membrane Structure: still relevant to understanding the structure, function and dynamics of biological membranes after more than 40years, Biochim. Biophys. Acta Biomembr. 1838 (6) (2014) 1451–1466, https://doi.org/10.1016/j.bbamem.2013.10.019. URL, http://www.sciencedirect.com/science/article/pii/S0005273613003933 https://linkinghub.elsevier.com/retrieve/pii/S0005273613003933.

[182] D. Marquardt, B. Geier, G. Pabst, Asymmetric lipid membranes: towards more realistic model systems, Membranes 5 (2) (2015) 180–196, https://doi.org/10.3390/membranes5020180. URL, http://www.mdpi.com/2077-0375/5/2/180.

[183] M. Doktorova, F.A. Heberle, B. Eicher, R.F. Standaert, J. Katsaras, E. London, G. Pabst, D. Marquardt, Preparation of asymmetric phospholipid vesicles for use as cell membrane models, Nat. Protoc. 13 (9) (2018) 2086–2101, https://doi.org/10.1038/s41596-018-0033-6.

[184] J.A. Vance, N.K. Devaraj, Membrane mimetic chemistry in artificial cells, J. Am. Chem. Soc. 143 (22) (2021) 8223–8231, https://doi.org/10.1021/jacs.1c03436.

[185] I.G. Denisov, M.A. McLean, A.W. Shaw, Y.V. Grinkova, S.G. Sligar, Thermotropic phase transition in soluble nanoscale lipid bilayers, J. Phys. Chem. B 109 (32) (2005) 15580–15588, https://doi.org/10.1021/jp051385g. URL, http://pubs.acs.org/doi/abs/10.1021/jp051385g.

[186] T. Bengtsen, V.L. Holm, L.R. Kjølbye, S.R. Midtgaard, N.T. Johansen, G. Tesei, S. Bottaro, B. Schiøtt, L. Arleth, K. Lindorff-Larsen, Structure and dynamics of a nanodisc by integrating nmr, saxs and sans experiments with molecular dynamics simulations, Elife 9 (2020) 1–24, https://doi.org/10.7554/eLife.56518.

[187] C.A. López, M.F. Swift, X.P. Xu, D. Hanein, N. Volkmann, S. Gnanakaran, Biophysical characterization of a nanodisc with and without BAX: an integrative study using molecular dynamics simulations and cryo-EM, Structure 27 (6) (2019) 988–999, https://doi.org/10.1016/j.str.2019.03.013.

[188] K. Mörs, C. Roos, F. Scholz, J. Wachtveitl, V. Dötsch, F. Bernhard, C. Glaubitz, Modified lipid and protein dynamics in nanodiscs, Biochim. Biophys. Acta Biomembr. 1828 (4) (2013) 1222–1229, https://doi.org/10.1016/j.bbamem.2012.12.011. URL, https://linkinghub.elsevier.com/retrieve/pii/S0005273612004439.

[189] N.L. Pollock, S.C. Lee, J.H. Patel, A.A. Gulamhussein, A.J. Rothnie, Structure and function of membrane proteins encapsulated in a polymer-bound lipid bilayer, Biochim. Biophys. Acta Biomembr. 1860 (4) (2018) 809–817, https://doi.org/10.1016/j.bbamem.2017.08.012. URL.

[190] J.D. Hothersall, A.Y. Jones, T.R. Dafforn, T. Perrior, K.L. Chapman, Releasing the technical 'shackles' on GPCR drug discovery: opportunities enabled by detergent-free polymer lipid particle (PoLiPa) purification, Drug Discov. Today 25 (11) (2020) 1944–1956, https://doi.org/10.1016/j.drudis.2020.08.006. URL.

[191] N.G. Brady, S. Qian, B.D. Bruce, Analysis of styrene maleic acid alternating copolymer supramolecular assemblies in solution by small angle X-ray scattering, June 2018, Eur. Polym. J. 111 (2019) 178–184, https://doi.org/10.1016/j.eurpolymj.2018.11.034. URL.

[192] S. Scheidelaar, M.C. Koorengevel, J.D. Pardo, J.D. Meeldijk, E. Breukink, J.A. Killian, Molecular model for the solubilization of membranes into nanodisks by styrene maleic acid copolymers, Biophys. J. 108 (2) (2015) 279–290, https://doi.org/10.1016/j.bpj.2014.11.3464. URL, https://linkinghub.elsevier.com/retrieve/pii/S0006349514046773.

[193] J.L. Popot, D.M. Engelman, Membranes do not tell proteins how to fold, Biochemistry 55 (1) (2016) 5–18, https://doi.org/10.1021/acs.biochem.5b01134.

[194] P. Zhao, C. Zhao, D. Chen, C. Yun, H. Li, L. Bai, Structure and activation mechanism of the hexameric plasma membrane H+-ATPase, Nat. Commun. 12 (1) (2021) 1–11, https://doi.org/10.1038/s41467-021-26782-y.

[195] Y. Guo, Be cautious with crystal structures of membrane, Crystals 10 (2) (2020) 86.

[196] G. Angiulli, H.S. Dhupar, H. Suzuki, I.S. Wason, F.D. Van Hoa, T. Walz, New approach for membrane protein reconstitution into peptidiscs and basis for their adaptability to different proteins, Elife 9 (2020) 1–20, https://doi.org/10.7554/eLife.53530.

[197] A.G. Wielandt, J.T. Pedersen, J. Falhof, G.C. Kemmer, A. Lund, K. Ekberg, A.T. Fuglsang, T.G. Pomorski, M.J. Buch-Pedersen, M. Palmgren, Specific activation of the plant P-type plasma membrane H+-ATPase by lysophospholipids depends on the autoinhibitory N- and C-terminal domains, J. Biol. Chem. 290 (26) (2015) 16281–16291, https://doi.org/10.1074/jbc.M114.617746.

[198] A.N. Naranjo, P.M. McNeely, J. Katsaras, A.S. Robinson, Impact of purification conditions and history on A2A adenosine receptor activity: the role of CHAPS and lipids, Protein Expr. Purif. 124 (2016) 62–67, https://doi.org/10.1016/j.pep.2016.05.015. URL.

[199] W.T. Doerrler, C.R. Raetz, ATPase activity of the MsbA lipid flippase of Escherichia coli, J. Biol. Chem. 277 (39) (2002) 36697–36705, https://doi.org/10.1074/jbc.M205857200. URL.

[200] C. Bonifer, C. Glaubitz, MsbA: an ABC transporter paradigm, Biochem. Soc. Trans. 49 (6) (2021) 2917–2927, https://doi.org/10.1042/BST20211030.

[201] D.M. Kehlenbeck, D.A. Traore, I. Josts, S. Sander, M. Moulin, M. Haertlein, S. Prevost, V.T. Forsyth, H. Tidow, Cryo-EM structure of MsbA in saposin-lipid nanoparticles (Salipro) provides insights into nucleotide coordination, FEBS J. (2021) 1–12, https://doi.org/10.1111/febs.16327.

[202] J. Wright, S.P. Muench, A. Goldman, A. Baker, Substrate polyspecificity and conformational relevance in ABC transporters: new insights from structural studies, Biochem. Soc. Trans. 46 (6) (2018) 1475–1484, https://doi.org/10.1042/BST20180146.

[203] H. Kaur, A. Lakatos, R. Spadaccini, R. Vogel, C. Hoffmann, J. Becker-Baldus, O. Ouari, P. Tordo, H. McHaourab, C. Glaubitz, The ABC exporter MsbA probed by solid state NMR - challenges and opportunities, Biol. Chem. 396 (9–10) (2015) 1135–1149, https://doi.org/10.1515/hsz-2015-0119.

[204] T. Kawai, J.M. Caaveiro, R. Abe, T. Katagiri, K. Tsumoto, Catalytic activity of MsbA reconstituted in nanodisc particles is modulated by remote interactions with the bilayer, FEBS (Fed. Eur. Biochem. Soc.) Lett. 585 (22) (2011) 3533–3537, https://doi.org/10.1016/j.febslet.2011.10.015. URL.

[205] W. Mi, Y. Li, S.H. Yoon, R.K. Ernst, T. Walz, M. Liao, Structural basis of MsbA-mediated lipopolysaccharide transport, Nature 549 (7671) (2017) 233–237, https://doi.org/10.1038/nature23649. URL.

[206] A. Ward, C.L. Reyes, J. Yu, C.B. Roth, G. Chang, Flexibility in the ABC transporter MsbA: alternating access with a twist, Proc. Natl. Acad. Sci. U. S. A 104 (48) (2007) 19005–19010, https://doi.org/10.1073/pnas.0709388104.

[207] M.R. Arana, M.C. Fiori, G.A. Altenberg, Functional and structural comparison of the ABC exporter MsbA studied in detergent and reconstituted in nanodiscs, Biochem. Biophys. Res. Commun. 512 (3) (2019) 448–452, https://doi.org/10.1016/j.bbrc.2019.03.069. URL.

[208] M.C. Fiori, W. Zheng, E. Kamilar, G. Simiyu, G.A. Altenberg, H. Liang, Extraction and reconstitution of membrane proteins into lipid nanodiscs encased by zwitterionic styrene-maleic amide copolymers, Sci. Rep. 10 (1) (2020) 1–13, https://doi.org/10.1038/s41598-020-66852-7. URL.

[209] 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) 1072–1079, https://doi.org/10.1016/j.str.2018.05.007. URL, https://linkinghub.elsevier.com/retrieve/pii/S0969212618301710.

[210] P.S. Padayatti, S.C. Lee, R.L. Stanfield, P.-C. Wen, E. Tajkhorshid, I.A. Wilson, Q. Zhang, Structural insights into the lipid A transport pathway in MsbA, Structure 27 (7) (2019) 1114–1123, https://doi.org/10.1016/j.str.2019.04.007. URL, https://linkinghub.elsevier.com/retrieve/pii/S0969212619301297.

[211] P. Zou, H.S. Mchaourab, Alternating access of the putative substrate-binding chamber in the ABC transporter MsbA, J. Mol. Biol. 393 (3) (2009) 574–585, https://doi.org/10.1016/j.jmb.2009.08.051. URL.

[212] P. Zou, H.S. Mchaourab, Increased sensitivity and extended range of distance measurements in Spin-labeled membrane proteins: Q-band double electron-electron resonance and nanoscale bilayers, Biophys. J. 98 (6) (2010) L18–L20, https://doi.org/10.1016/j.bpj.2009.12.4193. URL.

[213] V. Kotov, K. Bartels, K. Veith, I. Josts, U.K. Subhramanyam, C. Günther, J. Labahn, T.C. Marlovits, I. Moraes, H. Tidow, C. Löw, M.M. Garcia-Alai, High-throughput stability screening for detergent-solubilized membrane proteins, Sci. Rep. 9 (1) (2019) 1–19, https://doi.org/10.1038/s41598-019-46686-8.

[214] K.S. Mineev, K.D. Nadezhdin, Membrane mimetics for solution NMR studies of membrane proteins, Nanotechnol. Rev. 6 (1) (2017) 15–32, https://doi.org/10.1515/ntrev-2016-0074.

[215] V. Yeh, A. Goode, B.B. Bonev, Membrane protein structure determination and characterisation by solution and solid-state nmr, Biology 9 (11) (2020) 1–23, https://doi.org/10.3390/biology9110396.

[216] L. Frey, N.-A. Lakomek, R. Riek, S. Bibow, Micelles, bicelles, and nanodiscs: comparing the impact of membrane mimetics on membrane protein backbone dynamics, Angew. Chem. Int. Ed. 56 (1) (2017) 380–383, https://doi.org/10.1002/anie.201608246. URL.

[217] 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, https://doi.org/10.1016/j.str.2013.01.005. URL, https://linkinghub.elsevier.com/retrieve/pii/S0969212613000099.

[218] T. Viennet, S. Bungert-Plümke, S. Elter, A. Viegas, C. Fahlke, M. Etzkorn, Reconstitution and NMR characterization of the ion-channel accessory subunit barttin in detergents and lipid-bilayer nanodiscs, Front. Mol. Biosci. 6 (MAR) (2019) 1–12, https://doi.org/10.3389/fmolb.2019.00013.

[219] W. Kuhlbrandt, The resolution revolution, Science 343 (6178) (2014) 1443–1444, https://doi.org/10.1126/science.1251652. URL, http://link.springer.com/10.1007/s12551-019-00558-7 https://www.sciencemag.org/lookup/doi/10.1126/science.1251652.

[220] H.E. Autzen, D. Julius, Y. Cheng, Membrane mimetic systems in CryoEM: keeping membrane proteins in their native environment, Curr. Opin. Struct. Biol. 58 (2019) 259–268, https://doi.org/10.1016/j.sbi.2019.05.022. URL.

[221] A.J. Jones, F. Gabriel, A. Tandale, D. Nietlispach, Structure and dynamics of GPCRs in lipid membranes: physical principles and experimental approaches, Molecules 25 (20) (2020) 1–39, https://doi.org/10.3390/molecules25204729.

[222] H. Heerklotz, J. Seelig, Detergent-like action of the antibiotic peptide surfactin on lipid membranes, Biophys. J. 81 (3) (2001) 1547–1554, https://doi.org/10.1016/S0006-3495(01)75808-0. URL, https://linkinghub.elsevier.com/retrieve/pii/S2210271X12003854 http://linkinghub.elsevier.com/retrieve/pii/S0006349501758080.

[223] H. Ilgü, J.M. Jeckelmann, M.S. Gachet, R. Boggavarapu, Z. Ucurum, J. Gertsch, D. Fotiadis, Variation of the detergent-binding capacity and phospholipid content of membrane proteins when purified in different detergents, Biophys. J. 106 (8) (2014) 1660–1670, https://doi.org/10.1016/j.bpj.2014.02.024.

[224] J. Dauvergne, E.M. Desuzinges, C. Faugier, S. Igonet, M. Soulié, E. Grousson, D. Cornut, F. Bonneté, G. Durand, E. Dejean, A. Jawhari, Glycosylated amphiphilic calixarene-based detergent for functional stabilization of native membrane proteins, ChemistrySelect 4 (19) (2019) 5535–5539, https://doi.org/10.1002/slct.201901220.

[225] S. J. Routledge, M. Jamshad, H. A. Little, Y. P. Lin, J. Simms, A. Thakker, C. M. Spickett, R. M. Bill, T. R. Dafforn, D. R. Poyner, M. Wheatley, Ligand-induced conformational changes in a SMALP-encapsulated GPCR., Biochim. Biophys. Acta Biomembr. 1862 (6). doi:10.1016/j.bbamem.2020.183235.

[226] N. Kassem, R. Araya-Secchi, K. Bugge, A. Barclay, H. Steinocher, A. Khondker, Y. Wang, A. J. Lenard, J. Bürck, C. Sahin, A. S. Ulrich, M. Landreh, M. C. Pedersen, M. C. Rheinstädter, P. A. Pedersen, K. Lindorff-Larsen, L. Arleth, B. B. Kragelund, Order and disorder—an integrative structure of the full-length human growth hormone receptor, Sci. Adv. 7 (27). doi:10.1126/sciadv.abh3805.

[227] N.T. Johansen, M. Bonaccorsi, T. Bengtsen, A.H. Larsen, F.G. Tidemand, M.C. Pedersen, P. Huda, J. Berndtsson, T. Darwish, N.R. Yepuri, A. Martel, T.G. Pomorski, A. Bertarello, M. Sansom, M. Rapp, R. Crehuet, T. Schubeis, K. Lindorff-Larsen, G. Pintacuda, L. Arleth, Mg2+dependent conformational equilibria in CorA and an integrated view on transport regulation, Elife 11 (2022) 1–35, https://doi.org/10.7554/ELIFE.71887.

[228] R. Hendus-Altenburger, J. Vogensen, E.S. Pedersen, A. Luchini, R. Araya-Secchi, A.H. Bendsoe, N.S. Prasad, A. Prestel, M. Cardenas, E. Pedraz-Cuesta, L. Arleth, S.F. Pedersen, B.B. Kragelund, The intracellular lipid-binding domain of human Na+/H+ exchanger 1 forms a lipid-protein co-structure essential for activity, Communications Biology 3 (1) (2020) 1–18, https://doi.org/10.1038/s42003-020-01455-6.

[229] V.V. Gurevich, E.V. Gurevich, GPCR signaling regulation: the role of GRKs and arrestins, Front. Pharmacol. 10 (FEB) (2019) 1–11, https://doi.org/10.3389/fphar.2019.00125.

[230] D.P. Staus, H. Hu, M.J. Robertson, A.L. Kleinhenz, L.M. Wingler, W.D. Capel, N.R. Latorraca, R.J. Lefkowitz, G. Skiniotis, Structure of the M2 muscarinic receptor–β-arrestin complex in a lipid nanodisc, Nature 579 (7798) (2020) 297–302, https://doi.org/10.1038/s41586-020-1954-0. URL.

[231] M. Zhang, M. Gui, Z.F. Wang, C. Gorgulla, J.J. Yu, H. Wu, Z.y.J. Sun, C. Klenk, L. Merklinger, L. Morstein, F. Hagn, A. Plückthun, A. Brown, M.L. Nasr, G. Wagner, Cryo-EM structure of an activated GPCR–G protein complex in lipid nanodiscs, Nat. Struct. Mol. Biol. 28 (3) (2021) 258–267, https://doi.org/10.1038/s41594-020-00554-6. URL.