Biochimica et Biophysica Acta 1818 (2012) 225–233

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Artificial membrane-like environments for *in vitro* studies of purified G-protein coupled receptors ☆

Eugene Serebryany, Gefei Alex Zhu, Elsa C.Y. Yan *

Department of Chemistry, Yale University, New Haven, CT 06520, USA

ARTICLE INFO

Article history:
Received 8 June 2011
Received in revised form 28 July 2011
Accepted 29 July 2011
Available online 9 August 2011

Keywords:
GPCR reconstitution
Bicelle
Nanodisc
Giant unilamellar vesicle
Planar lipid membrane
Lipidic cubic phase

ABSTRACT

Functional reconstitution of transmembrane proteins remains a significant barrier to their biochemical, biophysical, and structural characterization. Studies of seven-transmembrane G-protein coupled receptors (GPCRs) *in vitro* are particularly challenging because, ideally, they require access to the receptor on both sides of the membrane as well as within the plane of the membrane. However, understanding the structure and function of these receptors at the molecular level within a native-like environment will have a large impact both on basic knowledge of cell signaling and on pharmacological research. The goal of this article is to review the main classes of membrane mimics that have been, or could be, used for functional reconstitution of GPCRs. These include the use of micelles, bicelles, lipid vesicles, nanodiscs, lipidic cubic phases, and planar lipid membranes. Each of these approaches is evaluated with respect to its fundamental advantages and limitations and its applications in the field of GPCR research. This article is part of a Special Issue entitled: Membrane protein structure and function.

1. Introduction

The ability to sense environmental cues and respond to them appropriately is critical for the existence of any living cell [1]. A wide variety of receptors has evolved to fulfill this need, and the G-protein coupled receptors (GPCRs) have enjoyed particular evolutionary success. These receptors follow a common mechanism of action. First, the receptor is activated by the binding of a ligand from the extracellular side. Second, the seven transmembrane helices rearrange, transducing the conformational change associated with ligand binding from the extracellular side of the receptor protein to the cytoplasmic side. Finally, the binding of one or more types of heterotrimeric G-proteins on the cytoplasmic side allows downstream signaling cascades within the cell to take effect [2–4]. All GPCRs are metabotropic, *i.e.*, not directly linked to ion channels or pores. The versatility of GPCRs stems from the large variety of ligands that can be accommodated [5] – from photons of light, to ions, neurotransmitters, and even large polypeptide hormones – as well as from the vast possibilities for signal amplification and modulation through downstream signaling cascades [6,7]. At over 800 members, the GPCRs are the largest protein family in the human proteome. From a pharmacological standpoint, they are the target of some 50% of all drugs currently on the market [8]. Understanding molecular mechanisms of GPCR signaling is therefore of both fundamental scientific impact and high medical potential.

However, biophysical studies of these receptors have been particularly challenging because they are transmembrane proteins heavily dependent on the membrane environment for proper function. Purified protein samples are necessary for obtaining quantitative biophysical information on the molecular details of structure and function. Reconstitution into a native-like environment is important in the case of GPCRs to ensure that the sample represents a biologically relevant protein conformation, *i.e.*, as close as possible to the one present in living cells. Reconstitution is also needed to maintain stability. Furthermore, though crystal structures of several GPCRs have been solved [9–19], understanding the kinetics and dynamics of these molecules under physiological conditions remains challenging. Biophysical studies *in vitro*, such as NMR [20–23], infrared [24] and Raman vibrational spectroscopy [25,26], electron paramagnetic resonance (EPR) [27,28], circular dichroism [20–23], and fluorescence spectroscopy [29,30], provide detailed molecular insight into the function of these proteins. All of these functional methods, as well as structural ones like X-ray crystallography [9–19], solid-state NMR [20–23], or neutron scattering [31], require stable, purified GPCR sample reconstituted in a mimic of the native environment.

A fundamental challenge in this research field lies in the basic topology of GPCR signaling. A GPCR can be conceptualized as consisting

Abbreviations: GPCR, G-protein coupled receptor; SUV, small unilamellar vesicle; GUV, giant unilamellar vesicle; MSP, membrane scaffold protein; PLM, planar lipid membrane

☆ This article is part of a Special Issue entitled: Membrane protein structure and function.

* Corresponding author at: 225 Prospect St., Yale University, New Haven, CT 06520, USA. Tel.: +1 203 436 2509.
E-mail address: elsa.yan@yale.edu (E.C.Y. Yan).

0005-2736/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2011.07.047

of three parts: an extracellular ligand-binding surface, a transmembrane region, and an intracellular G-protein-binding surface (Fig. 1). Though any given experimental technique imposes its own constraints, a complete molecular-level study of receptor activation and signaling requires each of these surfaces to be accessible to experimental manipulation. Thus, the best reconstitution system for a given investigation permits easy and independent experimental access to the outside and inside surface of the membrane, as well as to the plane of the membrane. While reviews of GPCR structure [4], GPCR dimerization [32], the pharmacological potential of GPCRs [8,33], GPCR purification [34,35], and GPCR–lipid interactions are available [36], reconstitution of purified GPCRs into artificial membrane-like environments has not been reviewed to date. This article reviews various membrane mimics for reconstitution of functional GPCRs *in vitro* for detailed biophysical analysis of their function in relation to their structure and dynamics.

2. Overview of GPCR reconstitution

The general approach to prepare reconstituted samples is to first obtain a sufficient quantity of the GPCR under study, then purify it, and finally reconstitute it into a membrane-like environment. The GPCR must either be expressed recombinantly [34,37] and then purified or purified directly from natural sources [38,39]. For instance, bovine rhodopsin, the first GPCR to be studied *in vitro*, was initially purified from preparations of bovine retinas [38,39]. In general, however, purifying GPCRs from natural sources is difficult due to the low abundance of most GPCRs and the need to raise highly specific antibodies against each for immunopurification. A better strategy is to use an expression system to express a GPCR fused to a tag for purification [34,40]. The choice of expression system depends on the GPCR under study [37]. GPCRs are eukaryotic proteins. A small number of GPCRs can be expressed functionally in bacterial cells [41]; some can be expressed as nonfunctional polypeptides in inclusion bodies and subsequently refolded; others fail to express in a bacterial system at all. Yeast and insect cells [17,42,43] have been used to express some GPCRs, but others require the post-translational modification machinery only found in mammalian cells [44,45]. Robinson and colleagues provide a review of GPCR expression systems [46].

Regardless of the choice of expression system, three steps need be carried out to reconstitute GPCRs. The first step is to solubilize the plasma membranes of the expressing cells, typically with the help of detergents [35,40,47]. Afterward, affinity purification can separate the target GPCR from all other membrane components of the expressing cells. Finally, detergent is removed by dialysis [48,49], gel filtration [50], or adsorption onto a material such as Biobeads® [51]. At that time, the GPCR has been reconstituted and is available for quantitative biophysical investigations.

3. Membrane-like environments

Six basic types of membrane mimics will be considered in this review. These are (1) micelles, (2) unilamellar lipid vesicles, (3) bicelles, (4) nanodiscs, (5) planar lipid membranes, and (6) lipidic cubic phases. Conceptual considerations and examples of applications for each are discussed below.

3.1. Detergent and mixed micelles

The most basic strategy for *in vitro* studies of purified GPCRs is the use of detergent or mixed micelles [35,40]. Mixed micelles are prepared from a mixture of detergents or a mixture of detergent and lipid. Since the protein contains both hydrophobic and hydrophilic regions, amphipathic molecules such as detergents provide an environment that lends some stability to the GPCR molecules and prevents their precipitation (Fig. 2).

A variety of detergents is available for studies of transmembrane proteins, but the choice must fall on a detergent that is gentle enough to avoid immediate denaturation of the protein. For example, sodium dodecyl sulfate (SDS), a very common but highly denaturing detergent, is not suitable for this reason. Detergents such as cholate, dodecyl-β-D-maltoside (DDM) [52], and *n*-octyl-β-D-glucopyranoside (OG) [53], as well as mixed micelles have been used with greater success. For instance, the first crystals of rhodopsin that yielded a high-resolution structure were obtained from solutions of mixed micelles of nonyl-β-D-glucoside and heptanetriol [18,54].

Newer, rationally designed detergents include amphipols [55,56], which can wrap around the hydrophobic region of a GPCR while exposing their hydrophilic side chains to the solvent. This feature is especially useful since detergent concentration does not have to exceed the critical micelle concentration (CMC), and therefore the interference with molecular conformation or spectroscopic measurements due to free detergent in solution is lower. Zhang and colleagues reported that addition of peptide surfactants to traditional detergents like DDM and OG enhances thermal stability of rhodopsin compared to what is attainable with detergent alone [57]. Recently, Welte and coworkers implemented a modified detergent system, using maltoside detergents with cyclohexyl or aromatic groups in place of the alkyl chains. This modification resulted in increased thermal stability for at least two human GPCRs compared to DDM [58]. A detailed review of the variety of detergents used to stabilize transmembrane proteins is available to the interested reader [49].

In spite of these successes, it is clear that a true lipid bilayer environment is best suited for biophysical studies of GPCRs in a functional and stable form. For instance, the photochemical properties of rhodopsin are sensitive to the composition of its lipid environment [59]. Indeed, a crystal structure of bovine rhodopsin retains tightly bound phospholipid molecules [19]. This tight binding suggests an important role for these molecules in maintaining the proper structure of the receptor.

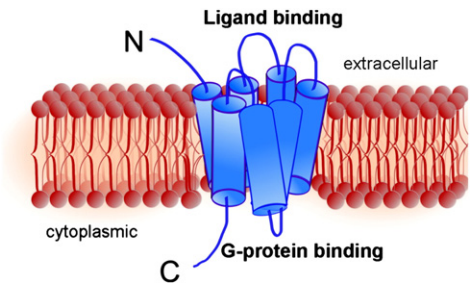

Fig. 1. Schematic overview of a GPCR (blue) embedded in the plasma membrane (red).

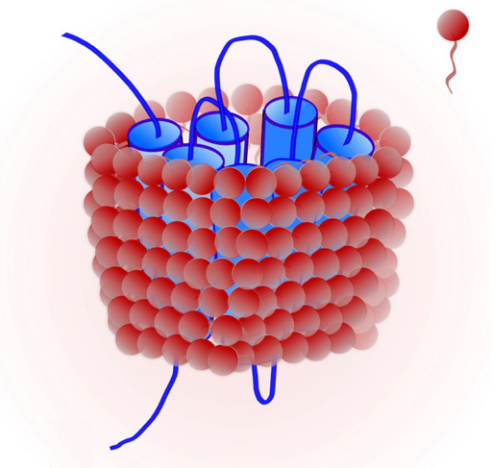

Fig. 2. A GPCR (blue) stabilized by a detergent micelle (red).

Moreover, reconstitution in micelles poses a fundamental challenge to functional studies of GPCRs due to the limited accessibility of the protein in the resulting system. Even if detergent and lipid composition were optimized such that a given receptor could be successfully kept in solution, the micelle is a highly disordered environment compared to the native membrane. Concentration of free detergent in solution is high because total detergent concentration must exceed the CMC at all times. The presence of high concentrations of detergent or lipid in solution may interfere with normal ligand and G-protein binding. Hydrophobic regions within the ligand-binding or G-protein-binding domain could also be destabilized or denatured by detergent. It is difficult in general to use a system of this kind for studying the interaction between a GPCR and its soluble protein interacting partners, since the fold and function of the soluble protein may not be preserved in detergent [60,61]. At the same time, hydrophobic binding partners, such as signaling proteins, lipids, and ligands, may partition into empty micelles, which reduces their effective concentration [62]. The micelle system is also unsuitable for investigating the oligomerization of GPCRs, which is thought to be critical to many of their normal functions [32,63,64]. This is because the bound detergent around the protein interferes with protein–protein contacts. Finally, the presence of lipids or detergents at high concentration freely diffusing in solution may lead to high background in optical measurements or NMR. All of these drawbacks make a more faithful mimic of the native membrane environment highly desirable.

3.2. Unilamellar lipid vesicles

GPCRs in detergent-solubilized form can be reconstituted in unilamellar lipid vesicles for biophysical studies (Fig. 3). This section discusses reconstitution in small unilamellar vesicles (SUVs) and giant unilamellar vesicles (GUVs).

Early on, Ross and colleagues reconstituted partially purified β_2-adrenergic receptor, along with G_s, into lipid vesicles. The vesicles were prepared by solubilizing the protein samples and phospholipids with cholate and subsequently removing the detergent by gel filtration and collecting the resulting vesicles. G_s binding to GTPγS was observed upon external application of catecholamines, which are agonists of the receptor [50]. Using a similar procedure, the entire primary pathway of cholinergic signaling, including the m1 muscarinic receptor, $G\alpha_{q/11}$ G-protein, and the enzyme phospholipase C, was later reconstituted [65]. The resulting vesicles were able to catalyze the formation of inositol triphosphate in response to the application of m1 receptor agonists. More recently, Grisshammer and coworkers carried out solid-state NMR on the neurotensin receptor reconstituted into lipid vesicles [66].

A variation of this approach uses giant unilamellar vesicles (GUVs), which have diameters comparable to those of eukaryotic cells [67]. Typically, GUVs are obtained in a procedure termed electroformation by applying alternating electric fields to dry lipid films as they are being rehydrated [68–70]. Though these conditions are denaturing to many membrane proteins, this limitation can be circumvented by fusing pre-made GUVs with small lipid vesicles containing reconstituted GPCRs. This was first demonstrated by Hoekstra and coworkers using SUVs with lipids covalently linked to a fusion peptide (WAE) to incorporate functional bacteriorhodopsin into GUVs [71]. Although bacteriorhodopsin is not a GPCR, it is a close structural homolog. So far, no GPCRs have been reconstituted using this method, but it remains promising.

The use of lipid vesicles has many advantages over micelles. They provide a well-defined, native-like environment for GPCRs, which increases receptor stability. Depending on the orientation of incorporation (*i.e.*, facing in or out of the vesicle), they allow easy access to either the ligand-binding or the G-protein-binding surface of the receptor, though not both. The diameter of GUVs is in the range of 10–100 μm, so they can be examined by visible microscopy. Finally, the reconstituted receptors are free to diffuse in two dimensions, allowing studies of their oligomerization, interactions with other transmembrane proteins, and, in principle, even their preference for specific membrane domains, such as lipid rafts [72]. Furthermore, GUV fusion with GPCR-containing SUVs allows a high degree of preferred orientation of the incorporated protein, as GPCRs are known to have preferred orientation in SUVs [73,74].

Despite these advantages, the enclosed topology of vesicles can present a challenge. Only membrane-permeable molecules can interact with both the extracellular and the intracellular surfaces of the receptor. GUVs offer a workaround: given these vesicles' size range, it is possible to use glass microneedles to inject molecules directly into the vesicle [75–77]. However, the microinjection approach limits the set of applicable biophysical techniques to those useful at the single-vesicle level. For biochemical and biophysical studies of ligand or effector binding and conformational dynamics of the receptor, it would be optimal to be able to probe all of the receptor's surfaces while retaining the ability to use bulk biophysical and structural techniques.

3.3. Bicelles

In the case of a GPCR sample solubilized in a mixed lipid/detergent micelle environment containing both long- and short-chain lipids, removal of detergent triggers formation of bicelles. Bicelles (Fig. 4) are fragments of lipid bilayer whose perimeter is stabilized by short-chain lipids or detergent molecules [78–80].

Khorana and coworkers were able to use a bicelle system to obtain the first detailed characterization of the kinetics of retinal binding to the opsin protein to form the GPCR rhodopsin [81]. Opella and coworkers reconstituted the CXCR1 chemokine receptor into bicelles by first incorporating it into lipid vesicles and subsequently disrupting those vesicles with the short-chain phospholipid dihexanoylphosphatidylcholine [82]. The resulting sample proved useful for NMR studies [80,82–84]. Recently, the Y-2 G-protein coupled receptor was reconstituted in a bicelle-like environment for solid-state NMR [85]. It has also

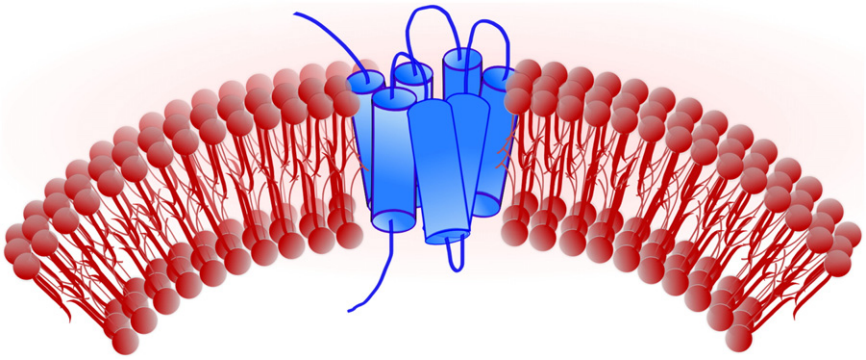

Fig. 3. A GPCR (blue) embedded in a unilamellar lipid vesicle (red).

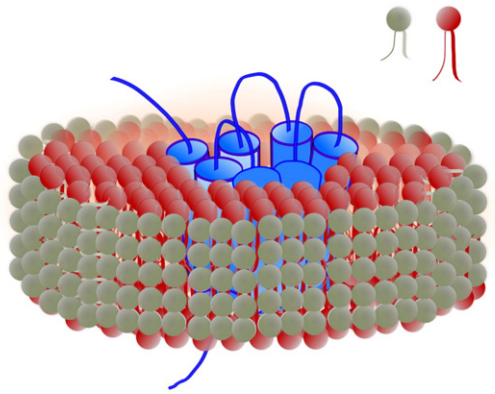

Fig. 4. A GPCR (blue) embedded in a bicelle composed of long-chain lipids (red) and short-chain lipids or detergent molecules (gray).

been possible to obtain a crystal structure of bacteriorhodopsin crystallized in lipid/detergent bicelles [86]. It should be noted that, due to the high sample requirements of NMR, both groups expressed the GPCRs in *Escherichia coli* and refolded them from exclusion bodies.

Depending on the ratio of long- to short-chain components (q value), bicelles have diameters of 10–30 nm, and up to 80 nm for some systems, enabling reconstitution of multiple GPCRs [79,87]. The diameter of a GPCR transmembrane core is ~3 nm. This means that, in theory, even the smallest (10 nm) bicelles or nanodiscs (discussed below) can accommodate multiple GPCR molecules. However, the number is limited for several reasons. First, the concentration of GPCRs is too low during the reconstitution procedure to make such a high density in a bicelle at all likely. Second, the perturbation to the structure of the lipid bilayer region is likely to be too great. Third, many GPCRs have intra- or extra-cellular domains whose diameters exceed that of the 7-transmembrane core.

The bicelle is a versatile system for GPCR reconstitution thanks to the wide range of lipid compositions it can accommodate [83,84,88]. Since it allows drastically reduced free detergent concentrations compared to mixed micelles, this strategy minimizes detergent-induced background and structural perturbation of soluble macromolecules [78]. Larger bicelles ($2.5<q<5$) are particularly useful for NMR studies of GPCRs because they align in a magnetic field [79]. This alignment greatly improves resolution in NMR spectra and enables measurement of protein orientation relative to the bilayer plane [84]. However, this system suffers from some of the drawbacks of micelles. Just as a concentration in excess of the CMC is needed to maintain micelles in solution, the long- and short-chain components of the bicelle structure need to be maintained above a critical concentration at all times. For GPCRs, which are often found in low abundance, this requirement can mean a large number of empty bicelles in the resulting solution, with the same consequences of high background and altered effective concentrations of protein or lipid interacting partners as noted above for micelles. Moreover, the bicelle components need to be maintained at a specific ratio at all times in order for bicelles to persist. Thus, addition of a sample of a detergent-solubilized protein interacting partner may alter the ratio and destabilize the bicelles. The use of nanodiscs in place of bicelles can overcome many of these drawbacks.

3.4. Nanodiscs

Nanodiscs – also referred to as nanoscale apolipoprotein bound bilayers (NABB) or high-density lipoprotein (HDL) particles – are the most novel paradigm for GPCR reconstitution. They resemble bicelles but provide a much more stable, well-defined membrane environment. Nanodiscs consist of a lipid bilayer center, which can incorporate a transmembrane protein, and two molecules of membrane scaffold protein (MSP), a helical repeat protein with a hydrophobic and a hydrophilic face. MSP wraps around the hydrophobic edge of the lipid disc and stabilizes it in an aqueous environment (Fig. 5).

Sligar and coworkers were able to make nanodiscs of controlled diameter by varying the number of α-helical repeats in the belt protein. As a result, particles with diameters of 9–13 nm could be prepared [89,90]. Sligar and coworkers have also been able to incorporate purified β_2-adrenergic receptor into nanodiscs [91], and Kobilka and coworkers demonstrated that this sample can activate G-proteins [92,93]. Purified rhodopsin has also been reconstituted using this system [94–96], demonstrating both light-induced activation of the receptor and activation of the G-protein transducin or arrestin as a result [95,97]. Recently, Sakmar and coworkers successfully reconstituted purified CCR5 chemokine receptor into nanodiscs [30].

Reconstitution of GPCRs into nanodiscs offers several advantages. The use of nanodiscs does not lead to high concentrations of freely diffusing lipids or detergent molecules, and the nanodisc structure is compact. These features permit relatively high concentrations of protein per volume, enabling bulk spectroscopic measurements. Furthermore, in the case of the nanodisc the presence of MSP permits additional labeling for spectroscopic analysis. MSP can be expressed in *Escherichia coli*, a system that allows genetic incorporation of a variety of unnatural amino acids, including fluorescent ones, by stop-codon suppression [98,99]. This provides a well-defined handle, for instance, for experiments using Forster resonance energy transfer (FRET) or fluorescence correlation spectroscopy (FCS) down to the single-particle level. In such experiments, conformational dynamics can be studied in the close-to-native environment of the planar lipid bilayer core. In addition, due to their stability and monodispersity, nanodiscs represent a promising avenue for structure determination of GPCRs, for instance by NMR. They may also become useful for crystallography, although crystallization may be challenging due to the rotational degree of freedom within the disc. Finally, unlike vesicles, nanodiscs do not have an enclosed topology, so both the extracellular and intracellular sides of the receptor are easily accessible. This property makes them very useful for reconstitution of GPCR-activated signaling cascades [91,94].

However, the topology of bicelles and nanodiscs can also be a disadvantage: though both binding surfaces of the protein are accessible to manipulation, they can be manipulated only simultaneously. Thus nanodiscs and bicelles cannot in principle reproduce the difference between the extracellular and intracellular environments — something that vesicles can do. For instance, concentrations of ions such as Ca^{2+} are tightly regulated at nerve cell synapses, where many GPCRs are expressed and where the concentration gradient of Ca^{2+} may be 1000–10,000-fold between the external and internal surfaces of the membrane. This may make whatever buffer is used during studies of bicelle- or nanodisc-bound GPCRs physiologically realistic for only one side of the receptor. Any measured effect resulting from a change in the buffer conditions must be attributed to a combination of

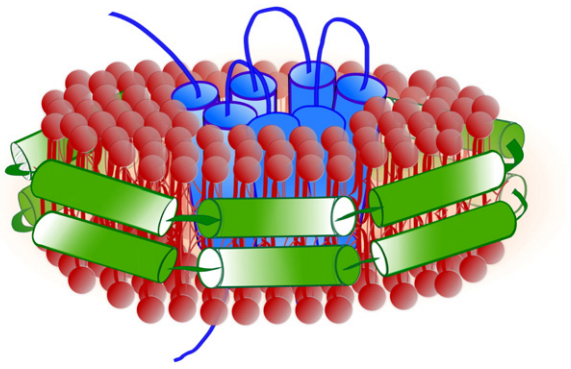

Fig. 5. A GPCR (blue) embedded in a nanodisc composed of a lipid bilayer (red) and membrane scaffold protein (green).

effects on the extra- and intracellular surfaces unless the effects on each can be resolved by further experiments.

A further potential drawback of nanodiscs is the strict limit on the diameter of the particle. The nanodisc severely constrains the protein's diffusion, making investigations of protein–protein interactions difficult. Thus, if only one GPCR is incorporated per nanodisc, any information resulting from interactions among diffusing GPCR molecules in the cell membrane will be lost. If, on the other hand, multiple copies of the receptor (or the receptor and a potential interacting partner) are incorporated, there is no guarantee against artifactual interactions due to the high local protein concentration in the lipid bilayer core. Moreover, in the latter scenario two GPCR molecules in close proximity may be antiparallel to each other, giving rise to biologically spurious protein–protein interactions [96]. While other systems, depending on how they are prepared, may also suffer from non-uniform incorporation topology, this problem is most pronounced for nanodiscs due to their constricted bilayer regions.

3.5. Planar lipid membranes

Planar lipid membranes (PLMs) can be subdivided into two broad classes. The first, a lipid bilayer covering a small aperture between two aqueous phases, is commonly referred to as a black membrane; the second class consists of solid-supported PLMs (Fig. 6).

PLMs have been known for decades. First reported in the 1960s by Wescott and colleagues, they immediately became important tools for electrophysiology of membrane-spanning proteins [100]. The original black membranes were prepared using organic solvents. For this reason, many researchers considered them unsuitable for investigations of complex transmembrane proteins, since organic solvents might denature them. However, PLM technology has advanced, and PLMs are now prepared without organic solvents. For instance, Tamm and McConnell first reported PLMs made by sequential deposition of lipid monolayers onto a solid support [101–103]; Boxer and coworkers prepared PLMs by direct fusion of SUVs on a glass surface [104,105]; and Wagner and Tamm prepared PLMs supported on polymer spacers by depositing lipid vesicles onto the polymer support [106].

Recently, Saavedra and colleagues incorporated purified rhodopsin into a solid-supported PLM; they also showed that the protein stayed functional upon photoinduced cross-linking of the phosopholipids [107]. The latter result holds the promise of highly stable planar lipid membranes for protein-based biosensors and other technological applications. In another recent study, Leutenegger et al. designed an experimental system consisting of *in vitro* translation machinery in proximity of a supported PLM to incorporate a GPCR (the olfactory receptor OR5) with fully uniform orientation [108]. In that study, a polymer spacer was placed between the PLM and the solid surface in order to accommodate the extracellular loops of the receptor. Receptor diffusion and aggregation within the PLM could then be tracked.

Although solid-supported PLMs are certainly an important technology, they represent a trade-off between stability and versatility. Interaction of intra- or extracellular loops or domains of GPCRs with the solid support or with the spacer polymer may interfere with their mobility and even activity [67]. Furthermore, placing the membrane on a solid support makes access to the bottom side of the lipid bilayer difficult, which negates the advantage of PLMs over the enclosed topology of vesicles. Finally, irregularities in the solid support may create irregularities in the bilayer structure.

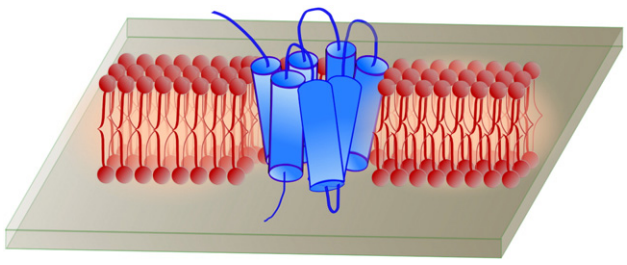

Fig. 6. A GPCR (blue) embedded in a planar lipid membrane (red) on a solid support (gray).

In recent years, some groups have sought alternatives to strictly solid-supported PLMs. Spacer polymers extend the water layer between the membrane and the solid support. Another approach is to deposit the PLM onto a porous substrate. For instance, Nishiya and coworkers created PLMs on a silicon surface with pores averaging 20 nm in diameter [109]. Brozick and coworkers successfully reconstituted the $5HT_3$ serotonin receptor into a PLM supported on a surface with >10 nm pores [110]. As these diameters exceed the diameter of the GPCR transmembrane core, a GPCR is in principle free to diffuse and even oligomerize within the area of the pore. Pores also make the GPCR accessible from both sides of the membrane. In other words, this type of membrane is functionally equivalent to the black membrane, but does not require organic solvents.

Another promising solution to the accessibility problem posed by solid-supported PLMs is the use of S-layer membranes [111]. This system is based on the observation that certain archaea as well as Gram-positive bacteria manage to survive extreme environmental conditions without a cell wall thanks to a crystalline lattice of S-proteins overlaid on their plasma membranes. The S-layer is highly porous (30–70%) with pore diameters reaching 8 nm [112]. Putting an S-layer on one or both sides of the lipid bilayer in lieu of a solid support creates essentially a mesh of lipid discs of uniform size, and capable of incorporating most transmembrane proteins. In contrast to bicelles and nanodiscs, the two aqueous phases in contact with the membrane can be manipulated independently. This allows experimenters to reproduce physiological gradients of ions and other substances across the artificial membrane, as well as physiological membrane potentials. However, the crystalline lattice of the S-layer can constrain mobility of any incorporated GPCRs and prevent their oligomerization. Continued development of porous-supported or S-layer PLMs with greater pore sizes will alleviate this disadvantage.

3.6. Lipidic cubic phases

In recent years, lipidic cubic phases, or meso-phases, have also emerged as a tool for GPCR reconstitution. These sponge-like, multilamellar, continuous lipidic phases perforated by aqueous channels supply an ordered hydrophobic matrix that permits free diffusion and encourages GPCR crystallization in three dimensions (Fig. 7). They were first prepared to crystallize membrane proteins by Landau and Rosenbusch by centrifugation of a defined mixture of monoolein and water [113]. These phases can also be doped with cholesterol, phosphatidylserine, or other lipids to make the reconstitution system as native-like as possible [114]. Ordinary cubic phases are less ordered than a solid but more ordered than a liquid; related sponge phases, characterized by greater inner aqueous volume, are liquids [115].

A lipidic cubic phase containing cholesterol has been used to reconstitute and crystallize the β_2-adrenergic receptor [9,17,42,43,116]. Other GPCRs crystallized to date using this system are the adenosine A2A receptor [10,117], the dopamine D_3 receptor [13], and the CXCR4 chemokine receptor [14]. This method has the advantage of allowing a high concentration of protein in a given volume. Like vesicles, cubic phases permit free diffusion of reconstituted proteins and native-like interactions among them. Access to the ligand-binding and G-protein-binding surfaces of a GPCR is more difficult in a cubic phase than in nanodiscs or PLMs. However, at least one group has reported the coupling of rhodopsin to transducin within a lipidic cubic phase [118].

The use of lipidic cubic phases in the field of GPCR research has been mostly limited to preparing crystals. Nonetheless, this system

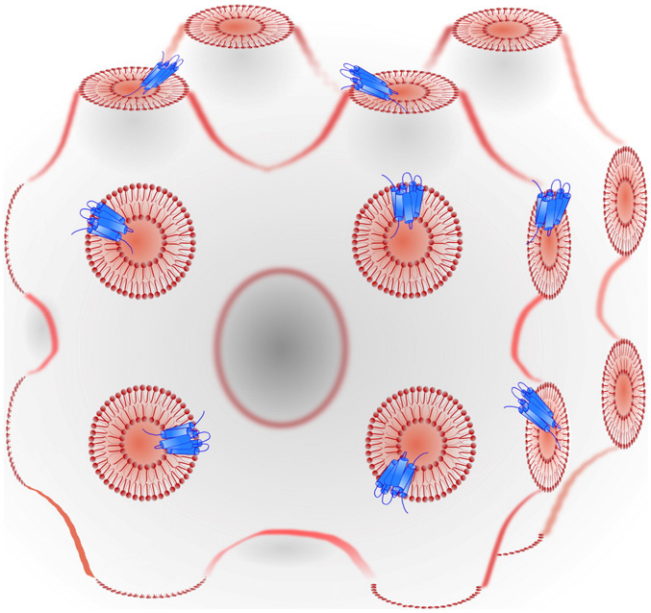

Fig. 7. GPCRs (blue) embedded in a lipidic cubic phase (red).

represents an intriguing possibility both as a possible vehicle for reconstituted GPCRs and as a replacement for detergents in the difficult process of GPCR purification.

4. Discussion

It should be noted that all the approaches described above require purification in detergent prior to reconstitution. Purification is a major challenge in the field of GPCR research because many GPCRs are denatured by detergents. Rational design of improved detergents may address this issue [119]. Alternatively, it may be possible to circumvent detergent solubilization altogether. One option is the use of electroformation to obtain GUVs directly from dried cell membranes, together with all the embedded membrane proteins [67]. However, the resulting "ghost" cells are bound to suffer from the same overabundance of complexity in the native membrane that makes reconstitution necessary in the first place. An ambitious solution, as proposed by Leutenegger et al., is to synthesize GPCRs by *in vitro* translation in close proximity to a PLM [108]. This method allows functional GPCR incorporation directly into an artificial membrane. One disadvantage of this approach, however, is the absence of quality-control mechanisms. GPCRs expressed in live cells are normally retained in the cytosol if they are misfolded; an *in vitro* system lacks this checkpoint and may yield samples of reduced functionality.

Once the challenge of purifying the GPCR under study is overcome, a variety of reconstitution systems is available for biophysical studies. The choice depends on the requirements of the particular experiment, as none of the strategies described here is optimal for all experimental questions. Nevertheless, it is possible to offer two general considerations.

First, we may evaluate artificial membrane environments based on the set of relevant biological processes they support. GPCRs are sensitive to lipid composition and may require specific interacting partners, so it is preferable to reconstitute them into environments most closely resembling the native one. Micelles, though easy to prepare, are not a faithful mimic of the native environment; in addition, they allow the least degree of GPCR stabilization. GUVs are the most faithful membrane mimics, followed by PLMs and cubic phases; all of these support native-like lipid compositions and open the extracellular or cytoplasmic surfaces for ligand binding or interaction with downstream signaling proteins while allowing lateral diffusion within the membrane. Nanodiscs and bicelles also open the extra- and intracellular surfaces, but only simultaneously, and they impede proper interactions within the membrane.

Second, we may evaluate artificial membrane environments based on the relevant biological processes they make available for biochemical and biophysical investigations. For instance, both lipid vesicles and PLMs permit reconstitution of a very wide swath of GPCR biology, including ligand-binding, G-protein activation, GPCR oligomerization, and the influence of lipid rafts or other membrane components on receptor function. Nonetheless, porous-supported or S-layer PLMs may be seen as more advantageous thanks to the greater ease of access to the receptor surfaces that they allow. Bicelles and nanodiscs support a more limited set of biological processes; however, they are better suited for bulk spectroscopic measurements of conformational changes in GPCRs. Feasibility of some classes of biophysical investigations for various membrane mimics is summarized in Table 1.

Finally, stability of these reconstitution systems is of practical concern to researchers. In general, planar lipid bilayer fragments tend to be unstable. Bicelles are more fragile than micelles. Nanodiscs are more stable than bicelles thanks to the MSP belt around the relatively fragile planar bilayer core. Liposomes and cubic phases are much more stable than PLMs, which usually require solid support or other stabilizing modifications. There are various approaches to stabilize membrane mimics, including ether-linked lipids [120], various types of support for planar bilayer regions [107–112], and the use of photo-crosslinking to prevent bilayer dissociation [107]. Such modifications are often a trade-off between greater stability and more native-like function [67,121].

Due to the difficulty of purifying GPCRs, only a small number of them have been successfully reconstituted to date. Nonetheless, membrane mimics reviewed here provide a range of choices for GPCR reconstitution and biophysical characterization. Many of them are only beginning to be applied to GPCR research, and detailed biophysical data remain scarce for receptors other than rhodopsin. However, if purification and reconstitution can be combined in a single step, the field of GPCR biophysics will advance more rapidly. At the same time, continuing development and refinement of reconstitution environments should make GPCRs amenable to the powerful biophysical techniques available for soluble proteins.

Table 1
Biophysical characterization of GPCRs in membrane mimics.

	Micelle	Bicelle	Vesicle	Nanodisc	PLM	Cubic phase
Structure determination	+++	+++	−	+++	+	+++
Ligand-binding	+++	+++	+++	+++	+++	+
Activation mechanism/conformational dynamics	+++	+++	+	+++	+	+++
Coupling to downstream signaling proteins	+	+	+++	+++	+++	+
Diffusion/oligomerization	−	−	+++	−	+++	+++
Lipid interactions	−	+	+++	+	+++	+++
Trafficking/endocytosis	−	−	+++	−	+++	−

+++ : possible.
+ : possible in principle.
− : not possible.

Acknowledgements

We would like to thank Prof. Patrick Sung (Yale University) for discussion and comments on the manuscript, and Li Fu (Yale University) for preparing the figures. E.Y. is the recipient of the NSF Career Award (MCB-0955407) and the ACS Petroleum Research Fund Grant Award (ACS PRF#49557-DNI5). E.S. and A.Z. are the recipients of the Yale–HHMI Future Scientist fellowship. E.S. is the recipient of the Sackler Undergraduate fellowship.

References

[1] T. Hunter, Signaling—2000 and beyond, Cell 100 (2000) 113–127.
[2] K.L. Pierce, R.T. Premont, R.J. Lefkowitz, Seven-transmembrane receptors, Nat. Rev. Mol. Cell Biol. 3 (2002) 639–650.
[3] P.S.H. Park, D.T. Lodowski, K. Palczewski, Activation of G protein-coupled receptors: beyond two-state models and tertiary conformational changes, Annu. Rev. Pharmacol. 48 (2008) 107–141.
[4] D.M. Rosenbaum, S.G.F. Rasmussen, B.K. Kobilka, The structure and function of G-protein-coupled receptors, Nature 459 (2009) 356–363.
[5] R. Fredriksson, M.C. Lagerström, L.-G. Lundin, H.B. Schiöth, The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints, Mol. Pharmacol. 63 (2003) 1256–1272.
[6] E.J. Neer, D.E. Clapham, Roles of G-protein subunits in transmembrane signaling, Nature 333 (1988) 129–134.
[7] H.E. Hamm, The many faces of G protein signaling, J. Biol. Chem. 273 (1998) 669–672.
[8] M.C. Lagerstrom, H.B. Schioth, Structural diversity of G protein-coupled receptors and significance for drug discovery, Nat. Rev. Drug Discov. 7 (2008) 339–357.
[9] M.A. Hanson, V. Cherezov, M.T. Griffith, C.B. Roth, V.-P. Jaakola, E.Y.T. Chien, J. Velasquez, P. Kuhn, R.C. Stevens, A specific cholesterol binding site is established by the 2.8 Å structure of the human β2-adrenergic receptor, Structure 16 (2008) 897–905.
[10] V.-P. Jaakola, M.T. Griffith, M.A. Hanson, V. Cherezov, E.Y.T. Chien, J.R. Lane, A.P. IJzerman, R.C. Stevens, The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist, Science 322 (2008) 1211–1217.
[11] J.H. Park, P. Scheerer, K.P. Hofmann, H.-W. Choe, O.P. Ernst, Crystal structure of the ligand-free G-protein-coupled receptor opsin, Nature 454 (2008) 183–187.
[12] T. Warne, M.J. Serrano-Vega, J.G. Baker, R. Moukhametzianov, P.C. Edwards, R. Henderson, A.G.W. Leslie, C.G. Tate, G.F.X. Schertler, Structure of a β1-adrenergic G-protein-coupled receptor, Nature 454 (2008) 486–491.
[13] E.Y.T. Chien, W. Liu, Q. Zhao, V. Katritch, G. Won Han, M.A. Hanson, L. Shi, A.H. Newman, J.A. Javitch, V. Cherezov, R.C. Stevens, Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist, Science 330 (2010) 1091–1095.
[14] B. Wu, E.Y.T. Chien, C.D. Mol, G. Fenalti, W. Liu, V. Katritch, R. Abagyan, A. Brooun, P. Wells, F.C. Bi, D.J. Hamel, P. Kuhn, T.M. Handel, V. Cherezov, R.C. Stevens, Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptide antagonists, Science 330 (2010) 1066–1071.
[15] H.-W. Choe, Y.J. Kim, J.H. Park, T. Morizumi, E.F. Pai, N. Krausz, K.P. Hofmann, P. Scheerer, O.P. Ernst, Crystal structure of metarhodopsin II, Nature 471 (2011) 651–655.
[16] S.G.F. Rasmussen, H.-J. Choi, D.M. Rosenbaum, T.S. Kobilka, F.S. Thian, P.C. Edwards, M. Burghammer, V.R.P. Ratnala, R. Sanishvili, R.F. Fischetti, G.F.X. Schertler, W.I. Weis, B.K. Kobilka, Crystal structure of the human β2 adrenergic G-protein-coupled receptor, Nature 450 (2007) 383–387.
[17] V. Cherezov, D.M. Rosenbaum, M.A. Hanson, S.r.G.F. Rasmussen, F.S. Thian, T.S. Kobilka, H.-J. Choi, P. Kuhn, W.I. Weis, B.K. Kobilka, R.C. Stevens, High-resolution crystal structure of an engineered human β2-adrenergic G protein coupled receptor, Science 318 (2007) 1258–1265.
[18] K. Palczewski, T. Kumasaka, T. Hori, C.A. Behnke, H. Motoshima, B.A. Fox, I.L. Trong, D.C. Teller, T. Okada, R.E. Stenkamp, M. Yamamoto, M. Miyano, Crystal structure of rhodopsin: a G protein-coupled receptor, Science 289 (2000) 739–745.
[19] J. Li, P.C. Edwards, M. Burghammer, C. Villa, G.F. Schertler, Structure of bovine rhodopsin in a trigonal crystal form, J. Mol. Biol. 343 (2004) 1409–1438.
[20] Q. Tan, J.H. Lou, B. Borhan, E. Karnaukhova, N. Berova, K. Nakanishi, Absolute sense of twist of the C12 – C13 bond of the retinal chromophore in bovine rhodopsin based on exciton-coupled CD spectra of 11,12-dihydroretinal analogues, Angew. Chem. Int. Ed. 36 (1997) 2089–2093.
[21] P.L. Yeagle, A.D. Albert, G-protein coupled receptor structure, BBA—Biomembranes 1768 (2007) 808–824.
[22] S. Ahuja, S.O. Smith, Multiple switches in G protein-coupled receptor activation, Trends Pharmacol. Sci. 30 (2009) 494–502.
[23] G.F.J. Salgado, A.V. Struts, K. Tanaka, N. Fujioka, K. Nakanishi, M.F. Brown, Deuterium NMR structure of retinal in the ground state of rhodopsin, Biochemistry—US 43 (2004) 12819–12828.
[24] R. Vogel, F. Siebert, Conformations of the active and inactive states of opsin, J. Biol. Chem. 276 (2001) 38487–38493.
[25] P. Kukura, D.W. McCamant, S. Yoon, D.B. Wandschneider, R.A. Mathies, Structural observation of the primary isomerization in vision with femtosecond-stimulated Raman, Science 310 (2005) 1006–1009.
[26] E.C.Y. Yan, Z. Ganim, M.A. Kazmi, B.S.W. Chang, T.P. Sakmar, R.A. Mathies, Resonance Raman analysis of the mechanism of energy storage and chromophore distortion in the primary visual photoproduct, Biochemistry—US 43 (2004) 10867–10876.
[27] D.L. Farrens, C. Altenbach, K. Yang, W.L. Hubbell, H.G. Khorana, Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin, Science 274 (1996) 768–770.
[28] C. Altenbach, A.K. Kusnetzow, O.P. Ernst, K.P. Hofmann, W.L. Hubbell, High-resolution distance mapping in rhodopsin reveals the pattern of helix movement due to activation, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 7439–7444.
[29] P. Ghanouni, J.J. Steenhuis, D.L. Farrens, B.K. Kobilka, Agonist-induced conformational changes in the G-protein-coupling domain of the beta(2) adrenergic receptor, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 5997–6002.
[30] A.M. Knepp, A. Grunbeck, S. Banerjee, T.P. Sakmar, T. Huber, Direct measurement of thermal stability of expressed CCR5 and stabilization by small molecule ligands, Biochemistry—US 50 (2011) 502–511.
[31] M. Yeager, B. Schoenborn, D. Engelman, P. Moore, L. Stryer, Neutron-diffraction analysis of the structure of rod photoreceptor-membranes in intact retinas, J. Mol. Biol. 137 (1980) 315–348.
[32] G. Milligan, G protein-coupled receptor dimerisation: molecular basis and relevance to function, BBA—Biomembranes 1768 (2007) 825–835.
[33] P.J. Conn, A. Christopoulos, C.W. Lindsley, Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders, Nat. Rev. Drug Discov. 8 (2009) 41–54.
[34] V. Sarramegna, I. Muller, A. Milon, F. Talmont, Recombinant G protein-coupled receptors from expression to renaturation: a challenge towards structure, Cell. Mol. Life Sci. 63 (2006) 1149–1164.
[35] R. Grisshammer, Purification of recombinant G-protein-coupled receptors, Second Edition, Guide to Protein Purification, vol. 463, Elsevier Academic Press Inc., San Diego, 2009, pp. 631–645.
[36] P.V. Escriba, P.B. Wedegaertner, F.M. Goni, O. Vogler, Lipid–protein interactions in GPCR-associated signaling, BBA—Biomembranes 1768 (2007) 836–852.
[37] V. Sarramegna, F. Talmont, P. Demange, A. Milon, Heterologous expression of G-protein-coupled receptors: comparison of expression systems from the standpoint of large-scale production and purification, Cell. Mol. Life Sci. 60 (2003) 1529.
[38] T. Okada, K. Takeda, T. Kouyama, Highly selective separation of rhodopsin from bovine rod outer segment membranes using combination of divalent cation and alkyl(thio)glucoside, Photochem. Photobiol. 67 (1998) 495–499.
[39] F.D. Collins, R.M. Love, R.A. Morton, Studies in rhodopsin. 4. Preparation of rhodopsin, Biochem. J. 51 (1952) 292–298.
[40] M.L. Chiu, C. Tsang, N. Grihalde, M.P. MacWilliams, Over-expression, solubilization, and purification of G protein-coupled receptors for structural biology, Comb. Chem. High Throughput Screen. 11 (2008) 439–462.
[41] S. Marullo, C. Delavier-Klutchko, Y. Eshdat, A.D. Strosberg, L. Emorine, Human beta 2-adrenergic receptors expressed in *Escherichia coli* membranes retain their pharmacological properties, Proc. Natl. Acad. Sci. 85 (1988) 7551–7555.
[42] S.G.F. Rasmussen, H.-J. Choi, J.J. Fung, E. Pardon, P. Casarosa, P.S. Chae, B.T. DeVree, D.M. Rosenbaum, F.S. Thian, T.S. Kobilka, A. Schnapp, I. Konetzki, R.K. Sunahara, S.H. Gellman, A. Pautsch, J. Steyaert, W.I. Weis, B.K. Kobilka, Structure of a nanobody-stabilized active state of the β2 adrenoceptor, Nature 469 (2011) 175–180.
[43] D.M. Rosenbaum, C. Zhang, J.A. Lyons, R. Holl, D. Aragao, D.H. Arlow, S.G.F. Rasmussen, H.-J. Choi, B.T. DeVree, R.K. Sunahara, P.S. Chae, S.H. Gellman, R.O. Dror, D.E. Shaw, W.I. Weis, M. Caffrey, P. Gmeiner, B.K. Kobilka, Structure and function of an irreversible agonist-β2 adrenoceptor complex, Nature 469 (2011) 236–240.
[44] P.J. Reeves, J.-M. Kim, H.G. Khorana, Structure and function in rhodopsin: a tetracycline-inducible system in stable mammalian cell lines for high-level expression of opsin mutants, Proc. Natl. Acad. Sci. 99 (2002) 13413–13418.
[45] P.J. Reeves, N. Callewaert, R. Contreras, H.G. Khorana, Structure and function in rhodopsin: high-level expression of rhodopsin with restricted and homogeneous N-glycosylation by a tetracycline-inducible N-acetylglucosaminyltransferase I-negative HEK293S stable mammalian cell line, Proc. Natl. Acad. Sci. 99 (2002) 13419–13424.
[46] E.C. McCusker, S.E. Bane, M.A. O'Malley, A.S. Robinson, Heterologous GPCR expression: a bottleneck to obtaining crystal structures, Biotechnol. Prog. 23 (2007) 540–547.
[47] M. le Maire, P. Champeil, J.V. Moller, Interaction of membrane proteins and lipids with solubilizing detergents, BBA—Biomembranes 1508 (2000) 86–111.
[48] J.R. Silvius, Solubilization and functional reconstitution of biomembrane components, Annu. Rev. Biophys. Biomol. Struct. 21 (1992) 323–348.
[49] A.M. Seddon, P. Curnow, P.J. Booth, Membrane proteins, lipids and detergents: not just a soap opera, Biochim. Biophys. Acta (BBA)—Biomembranes 1666 (2004) 105–117.
[50] D.R. Brandt, T. Asano, S.E. Pedersen, E.M. Ross, Reconstitution of catecholamine-stimulated guanosinetriphosphatase activity, Biochemistry—US 22 (1983) 4357–4362.
[51] J.L. Rigaud, G. Mosser, J.J. Lacapere, A. Olofsson, D. Levy, J.L. Ranck, Bio-Beads: an efficient strategy for two-dimensional crystallization of membrane proteins, J. Struct. Biol. 118 (1997) 226–235.
[52] R.R. Franke, T.P. Sakmar, R.M. Graham, H.G. Khorana, Structure and function in rhodopsin. Studies of the interaction between the rhodopsin cytoplasmic domain and transducin, J. Biol. Chem. 267 (1992) 14767–14774.

[53] M.L. Jackson, B.J. Litman, Rhodopsin phospholipid reconstitution by dialysis removal of octyl glucoside, Biochemistry—US 21 (1982) 5601–5608.
[54] T. Okada, I. Le Trong, B.A. Fox, C.A. Behnke, R.E. Stenkamp, K. Palczewski, X-ray diffraction analysis of three-dimensional crystals of bovine rhodopsin obtained from mixed micelles, J. Struct. Biol. 130 (2000) 73–80.
[55] C.L. Pocanschi, T. Dahmane, Y. Gohon, F. Rappaport, H.J. Apell, J.H. Kleinschmidt, J.L. Popot, Amphipathic polymers: tools to fold integral membrane proteins to their active form, Biochemistry—US 45 (2006) 13954–13961.
[56] T. Dahmane, M. Damian, S. Mary, J.L. Popot, J.L. Baneres, Amphipol-assisted in vitro folding of G protein-coupled receptors, Biochemistry—US 48 (2009) 6516–6521.
[57] X.J. Zhao, Y. Nagai, P.J. Reeves, P. Kiley, H.G. Khorana, S.G. Zhang, Designer short peptide surfactants stabilize G protein-coupled receptor bovine rhodopsin, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 17707–17712.
[58] J. Hovers, M. Potschies, A. Polidori, B. Pucci, S. Raynal, F. Bonnete, M.J. Serrano-Vega, C.G. Tate, D. Picot, Y. Pierre, J.L. Popot, R. Nehme, M. Bidet, I. Mus-Veteau, H. Busskamp, K.H. Jung, A. Marx, P.A. Timmins, W. Welte, A class of mild surfactants that keep integral membrane proteins water-soluble for functional studies and crystallization, Mol. Membr. Biol. 28 (2011) 170–180.
[59] M.F. Brown, Modulation of rhodopsin function by properties of the membrane bilayer, Chem. Phys. Lipids 73 (1994) 159–180.
[60] B. Jastrzebska, T. Maeda, L. Zhu, D. Fotiadis, S. Filipek, A. Engel, R.E. Stenkamp, K. Palczewski, Functional characterization of rhodopsin monomers and dimers in detergents, J. Biol. Chem. 279 (2004) 54663–54675.
[61] M.A. O'Malley, M.E. Helgeson, N.J. Wagner, A.S. Robinson, The morphology and composition of cholesterol-rich micellar nanostructures determine transmembrane protein (GPCR) activity, Biophys. J. 100 (2011) L11–L13.
[62] O.P. Ernst, V. Gramse, M. Kolbe, K.P. Hofmann, M. Heck, Monomeric G protein-coupled receptor rhodopsin in solution activates its G protein transducin at the diffusion limit, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 10859–10864.
[63] S. Bulenger, S. Marullo, M. Bouvier, Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation, Trends Pharmacol. Sci. 26 (2005) 131–137.
[64] S.C. Prinster, C. Hague, R.A. Hall, Heterodimerization of G protein-coupled receptors: specificity and functional significance, Pharmacol. Rev. 57 (2005) 289–298.
[65] G. Berstein, J.L. Blank, A.V. Smrcka, T. Higashijima, P.C. Sternweis, J.H. Exton, E.M. Ross, Reconstitution of agonist-stimulated phosphatidylinositol 4,5-bisphosphate hydrolysis using purified m1 muscarinic receptor, Gq/11, and phospholipase C-beta 1, J. Biol. Chem. 267 (1992) 8081–8088.
[66] S. Luca, J.F. White, A.K. Sohal, D.V. Filippov, J.H. van Boom, R. Grisshammer, M. Baldus, The conformation of neurotensin bound to its G protein-coupled receptor, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 10706–10711.
[67] N. Kahya, Protein–protein and protein–lipid interactions in domain-assembly: lessons from giant unilamellar vesicles, Biochim. Biophys. Acta (BBA)—Biomembranes 1798 (2010) 1392–1398.
[68] R.B. Gennis, Biomembranes: Molecular Structure and Function, Springer-Verlag, New York, 1989.
[69] A. Fischer, T. Oberholzer, P.L. Luisi, Giant vesicles as models to study the interactions between membranes and proteins, BBA—Biomembranes 1467 (2000) 177–188.
[70] L.R. Montes, A. Alonso, F.M. Goni, L.A. Bagatolli, Giant unilamellar vesicles electroformed from native membranes and organic lipid mixtures under physiological conditions, Biophys. J. 93 (2007) 3548–3554.
[71] N. Kahya, E.I. Pecheur, W.P. de Boeij, D.A. Wiersma, D. Hoekstra, Reconstitution of membrane proteins into giant unilamellar vesicles via peptide-induced fusion, Biophys. J. 81 (2001) 1464–1474.
[72] O. Wesolowska, K. Michalak, J. Maniewska, A.B. Hendrich, Giant unilamellar vesicles—a perfect tool to visualize phase separation and lipid rafts in model systems, Acta Biochim. Pol. 56 (2009) 33–39.
[73] J.R. Keefer, J. Nunnari, I.H. Pang, L.E. Limbird, Introduction of purified alpha(2a)-adrenergic receptors into uniformly oriented, unilamellar, phospholipid-vesicles — productive coupling to G-proteins but lack of receptor-dependent ion-transport, Mol. Pharmacol. 45 (1994) 1071–1081.
[74] L. Niu, J.M. Kim, H.G. Khorana, Structure and function in rhodopsin: asymmetric reconstitution of rhodopsin in liposomes, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 13409–13412.
[75] R. Wick, M.I. Angelova, P. Walde, P.L. Luisi, Microinjection into giant vesicles and light microscopy investigation of enzyme-mediated vesicle transformations, Chem. Biol. 3 (1996) 105–111.
[76] P. Bucher, A. Fischer, P.L. Luisi, T. Oberholzer, P. Walde, Giant vesicles as biochemical compartments: the use of microinjection techniques, Langmuir 14 (1998) 2712–2721.
[77] M.I. Angelova, N. Hristova, I. Tsoneva, DNA-induced endocytosis upon local microinjection to giant unilamellar cationic vesicles, Eur. Biophys. J. Biophys. Lett. 28 (1999) 142–150.
[78] C.R. Sanders, R.S. Prosser, Bicelles: a model membrane system for all seasons? Structure 6 (1998) 1227–1234.
[79] K.J. Glover, J.A. Whiles, G.H. Wu, N.J. Yu, R. Deems, J.O. Struppe, R.E. Stark, E.A. Komives, R.R. Vold, Structural evaluation of phospholipid bicelles for solution-state studies of membrane-associated biomolecules, Biophys. J. 81 (2001) 2163–2171.
[80] R.R. Vold, R.S. Prosser, Magnetically oriented phospholipid bilayered micelles for structural studies of polypeptides. Does the ideal bicelle exist? J. Magn. Reson. B 113 (1996) 267–271.
[81] P.J. Reeves, J. Hwa, H.G. Khorana, Structure and function in rhodopsin: kinetic studies of retinal binding to purified opsin mutants in defined phospholipid-detergent mixtures serve as probes of the retinal binding pocket, Proc. Natl. Acad. Sci. 96 (1999) 1927–1931.
[82] S.H. Park, S. Prytulla, A.A. De Angelis, J.M. Brown, H. Kiefer, S.J. Opella, High-resolution NMR spectroscopy of a GPCR in aligned bicelles, J. Am. Chem. Soc. 128 (2006) 7402–7403.
[83] I. Marcotte, M. Auger, Bicelles as model membranes for solid- and solution-state NMR studies of membrane peptides and proteins, Concepts Magn. Reson. A 24A (2005) 17–37.
[84] R.S. Prosser, F. Evanics, J.L. Kitevski, M.S. Al-Abdul-Wahid, Current applications of bicelles in NMR studies of membrane-associated amphiphiles and proteins, Biochemistry—US 45 (2006) 8453–8465.
[85] P. Schmidt, C. Berger, H.A. Scheidt, S. Berndt, A. Bunge, A.G. Beck-Sickinger, D. Huster, A reconstitution protocol for the in vitro folded human G protein-coupled Y-2 receptor into lipid environment, Biophys. Chem. 150 (2010) 29–36.
[86] S. Faham, J.U. Bowie, Bicelle crystallization: a new method for crystallizing membrane proteins yields a monomeric bacteriorhodopsin structure, J. Mol. Biol. 316 (2002) 1–6.
[87] C. McKibbin, N.A. Farmer, C. Jeans, P.J. Reeves, H.G. Khorana, B.A. Wallace, P.C. Edwards, C. Villa, P.J. Booth, Opsin stability and folding: modulation by phospholipid bicelles, J. Mol. Biol. 374 (2007) 1319–1332.
[88] Y. Gohon, J.L. Popot, Membrane protein–surfactant complexes, Curr. Opin. Colloid Interface Sci. 8 (2003) 15–22.
[89] T.K. Ritchie, Y.V. Grinkova, T.H. Bayburt, I.G. Denisov, J.K. Zolnerciks, W.M. Atkins, S.G. Sligar, Reconstitution of membrane proteins in phospholipid bilayer nanodiscs, Methods Enzymol. Liposomes Pt F 464 (2009) 211–231.
[90] T.H. Bayburt, S.G. Sligar, Membrane protein assembly into nanodiscs, FEBS Lett. 584 (2010) 1721–1727.
[91] A.J. Leitz, T.H. Bayburt, A.N. Barnakov, B.A. Springer, S.G. Sligar, Functional reconstitution of beta2-adrenergic receptors utilizing self-assembling nanodisc technology, Biotechniques 40 (2006) 601–602, 604, 606, passim.
[92] M.R. Whorton, M.P. Bokoch, S.G.F. Rasmussen, B. Huang, R.N. Zare, B. Kobilka, R.K. Sunahara, A monomeric G protein-coupled receptor isolated in a high-density lipoprotein particle efficiently activates its G protein, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 7682–7687.
[93] X.J. Yao, G.V. Ruiz, M.R. Whorton, S.G.F. Rasmussen, B.T. DeVree, X. Deupi, R.K. Sunahara, B. Kobilka, The effect of ligand efficacy on the formation and stability of a GPCR-G protein complex, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 9501–9506.
[94] T.H. Bayburt, S.A. Vishnivetskiy, M.A. McLean, T. Morizumi, C.C. Huang, J.J.G. Tesmer, O.P. Ernst, S.G. Sligar, V.V. Gurevich, Monomeric rhodopsin is sufficient for normal rhodopsin kinase (GRK1) phosphorylation and arrestin-1 binding, J. Biol. Chem. 286 (2011) 1420–1428.
[95] H. Tsukamoto, A. Sinha, M. DeWitt, D.L. Farrens, Monomeric rhodopsin is the minimal functional unit required for arrestin binding, J. Mol. Biol. 399 (2010) 501–511.
[96] S. Banerjee, T. Huber, T.P. Sakmar, Rapid incorporation of functional rhodopsin into nanoscale apolipoprotein bound bilayer (NABB) particles, J. Mol. Biol. 377 (2008) 1067–1081.
[97] T.H. Bayburt, A.J. Leitz, G. Xie, D.D. Oprian, S.G. Sligar, Transducin activation by nanoscale lipid bilayers containing one and two rhodopsins, J. Biol. Chem. 282 (2007) 14875–14881.
[98] D. Mendel, V.W. Cornish, P.G. Schultz, Site-directed mutagenesis with an expanded genetic-code, Annu. Rev. Biophys. Biomol. Struct. 24 (1995) 435–462.
[99] L. Wang, J. Xie, P.G. Schultz, Expanding the genetic code, Annu. Rev. Biophys. Biomol. Struct. 35 (2006) 225–249.
[100] P. Mueller, D.O. Rudin, H.T. Tien, W.C. Wescott, Reconstitution of cell membrane structure in vitro and its transformation into an excitable system, Nature 194 (1962) 979–980.
[101] L.K. Tamm, H.M. Mcconnell, Supported phospholipid-bilayers, Biophys. J. 47 (1985) 105–113.
[102] A.A. Brian, H.M. McConnell, Allogeneic stimulation of cyto-toxic t-cells by supported planar membranes, Proc. Natl. Acad. Sci. U.S.A.—Biol. Sci. 81 (1984) 6159–6163.
[103] H.M. McConnell, T.H. Watts, R.M. Weis, A.A. Brian, Supported planar membranes in studies of cell–cell recognition in the immune-system, Biochim. Biophys. Acta 864 (1986) 95–106.
[104] J. Salafsky, J.T. Groves, S.G. Boxer, Architecture and function of membrane proteins in planar supported bilayers: a study with photosynthetic reaction centers, Biochemistry-Us 35 (1996) 14773–14781.
[105] P.S. Cremer, S.G. Boxer, Formation and spreading of lipid bilayers on planar glass supports, J. Phys. Chem. B 103 (1999) 2554–2559.
[106] M.L. Wagner, L.K. Tamm, Tethered polymer-supported planar lipid bilayers for reconstitution of integral membrane proteins: silane-polyethyleneglycol-lipid as a cushion and covalent linker, Biophys. J. 79 (2000) 1400–1414.
[107] V. Subramaniam, I.D. Alves, G.F.J. Salgado, P.W. Lau, R.J. Wysocki, Z. Salamon, G. Tollin, V.J. Hruby, M.F. Brown, S.S. Saavedra, Rhodopsin reconstituted into a planar-supported lipid bilayer retains photoactivity after cross-linking polymerization of lipid monomers, J. Am. Chem. Soc. 127 (2005) 5320–5321.
[108] M. Leutenegger, T. Lasser, E.-K. Sinner, R. Robelek, Imaging of G protein-coupled receptors in solid-supported planar lipid membranes, Biointerphases 3 (2008) FA136–FA145.
[109] O. Worsfold, N.H. Voelcker, T. Nishiya, Biosensing using lipid bilayers suspended on porous silicon, Langmuir 22 (2006) 7078–7083.
[110] R.W. Davis, A. Flores, T.A. Barrick, J.M. Cox, S.M. Brozik, G.P. Lopez, J.A. Brozik, Nanoporous microbead supported bilayers: stability, physical characterization, and incorporation of functional transmembrane proteins, Langmuir 23 (2007) 3864–3872.

[111] P.C. Gufler, D. Pum, U.B. Sleytr, B. Schuster, Highly robust lipid membranes on crystalline S-layer supports investigated by electrochemical impedance spectroscopy, BBA-Biomembranes 1661 (2004) 154–165.
[112] B. Schuster, U.B. Sleytr, Composite S-layer lipid structures, J. Struct. Biol. 168 (2009) 207–216.
[113] E.M. Landau, J.P. Rosenbusch, Lipidic cubic phases: a novel concept for the crystallization of membrane proteins, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 14532–14535.
[114] V. Cherezov, J. Clogston, Y. Misquitta, W. Abdel-Gawad, M. Caffrey, Membrane protein crystallization in meso: lipid type-tailoring of the cubic phase, Biophys. J. 83 (2002) 3393–3407.
[115] L.C. Johansson, A.B. Wohri, G. Katona, S. Engstrom, R. Neutze, Membrane protein crystallization from lipidic phases, Curr. Opin. Struct. Biol. 19 (2009) 372–378.
[116] D.M. Rosenbaum, V. Cherezov, M.A. Hanson, S.r.G.F. Rasmussen, F.S. Thian, T.S. Kobilka, H.-J. Choi, X.-J. Yao, W.I. Weis, R.C. Stevens, B.K. Kobilka, GPCR engineering yields high-resolution structural insights into β2-adrenergic receptor function, Science 318 (2007) 1266–1273.
[117] F. Xu, H. Wu, V. Katritch, G.W. Han, K.A. Jacobson, Z.-G. Gao, V. Cherezov, R.C. Stevens, Structure of an agonist-bound human A2A adenosine receptor, Science 332 (2011) 322–327.
[118] J. Navarro, E.M. Landau, K. Fahmy, Receptor-dependent G-protein activation in lipidic cubic phase, Biopolymers 67 (2002) 167–177.
[119] P.S. Chae, S.G.F. 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 (2010) 1003–1008.
[120] M.G.L. Elferink, J.G. Dewit, R. Demel, A.J.M. Driessen, W.N. Konings, Functional reconstitution of membrane-proteins in monolayer liposomes from bipolar lipids of *Sulfolobus acidocaldarius*, J. Biol. Chem. 267 (1992) 1375–1381.
[121] K. Bertelsen, B. Vad, E.H. Nielsen, S.K. Hansen, T. Skrydstrup, D.E. Otzen, T. Vosegaard, N.C. Nielsen, Long-term-stable ether-lipid vs conventional ester-lipid bicelles in oriented solid-state NMR: altered structural information in studies of antimicrobial peptides, J. Phys. Chem. B 115 (2011) 1767–1774.