Trends in
Pharmacological Sciences

CellPress

Review

# Membrane protein production and formulation for drug discovery

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**Integral membrane proteins (MPs) are important drug targets across most fields of medicine, but historically have posed a major challenge for drug discovery due to difficulties in producing them in functional forms. We review the state of the art in drug discovery strategies using recombinant multipass MPs, and outline methods to successfully express, stabilize, and formulate them for small-molecule and monoclonal antibody therapeutics development. Advances in structure-based drug design and high-throughput screening are allowing access to previously intractable targets such as ion channels and transporters, propelling the field towards the development of highly specific therapies targeting desired conformations.**

## MPs are challenging drug targets

As gatekeepers to the cell, integral MPs fulfill many important functions, ranging from transport to signaling. MPs are implicated in a multitude of human ailments and are accessible to both small- and large-molecule drugs from their location at the cell surface (Box 1). Historically, there has been a bottleneck in the development of MP-targeting drugs due to difficulties in producing MPs recombinantly in sufficient quantities and stabilizing them in therapeutically relevant conformations. Many of the challenges associated with MP production are associated with their hydrophobicity due to multiple **transmembrane domains** (**TMDs**, see Glossary), which can lead to instability and aggregation during expression and purification.

Significant concerns associated with many existing drugs acting on MPs (e.g., opioids) include lack of specificity due owing to relatively similar ligand-binding pockets in related MPs, or undesired effects because of their participation in numerous different signaling pathways that can have a strong influence on overall cell equilibrium conditions, as with **ion channels** and transporters. These can manifest in adverse safety and side-effect profiles, demonstrating a need to develop therapeutics targeting specific MP conformations or signaling pathways, which will enable greater drug affinity, specificity, and potency, among other advantages. Targeting MPs by **monoclonal antibody (mAb)** therapeutics is one way to achieve target selectivity, especially when the mAb recognizes a conformational epitope, as when generated against a full-length MP. The potential impact of such strategies is illustrated by the novel mAb therapeutic leronlimab, that is under FDA review, which when approved would be the first licensed mAb developed by targeting a full-length MP. Leronlimab binds to multiple extracellular loops of its MP target CCR5 and blocks HIV entry while preserving normal signaling [1].

The next generation of MP-targeting drugs will rely on the ability to make adequate amounts of full-length MPs in stable, functionally distinct conformations, preferably associated with other proteins or lipid cofactors that constitute native-like complexes. We review here the status and recent advances in multipass MP production (expression, purification, and formulation), and the discovery methods that enable them to be targeted more effectively, including particularly difficult

## Highlights

Recent advances in multipass membrane protein (MP) production and formulation strategies have made them more accessible as drug targets.

Growing knowledge of MP function modulators (ligands, specific lipids/cholesterol, nanobodies stabilizing desired conformations, or protein interaction partners) can aid selection of more specific MP-targeting drugs in discovery assays.

Increased sophistication in structure determination [e.g., cryoEM (cryogenic electron microscopy)] and high-throughput assay technologies, such as the ability to monitor protein–protein interactions and conformational changes, complement advances in MP production.

Challenges for targeting MPs with monoclonal antibody therapeutics are being addressed by judicious choice of immunization host, *in vitro* discovery platforms, and implementation of activity assays to select function-modulating antibodies.

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## Box 1. Integral MP drug targets

MPs are widely expressed throughout the body and account for ~30% of the druggable proteome [88]. They contribute to numerous physiological processes and disorders including cancer, respiratory, metabolic, and autoimmune diseases [1,73,85]. Multipass MPs of the highest interest for pharmaceutical development include GPCRs, ion channels, and transporters. GPCRs and ion channels combined are targets for almost 50% of existing small-molecule drugs [88]. Transporters, primarily **ATP-binding cassette (ABC) transporters** and solute carrier (SLC) transporters, are significantly less explored; although there is much opportunity given their roles in cancers (including drug resistance) and in various metabolic and neurological disorders [89,90].

Structurally, MPs are composed of multiple α-helical TMDs and have variable extramembrane regions; representative examples of recently solved structures are depicted in Figure I. GPCRs are involved in signal transduction and are the most structurally homogeneous; all contain seven TMDs with an extracellular N-terminus, and they often participate in multiple different homo- or heterodimers with distinct signaling outcomes [91]. Ion channels are defined by their functional activation (e.g., voltage-or ligand-gated, mechanosensitive, acid-sensing) and are comprised of tri-, tetra- or pentameric repeat domains, each with an even number of TMDs (2–6) and cytoplasmic N- and C-termini. This pore-forming α-subunit can be a single chain (e.g., voltage-gated sodium and calcium channels) or formed by homo- or heteromeric subunit complexes (e.g., voltage-gated potassium channels, ligand-gated purinergic P2X channels), and may associate with single-TMD regulatory β/γ subunits. MP transporters, such as ABC/SLC transporters and aquaporins, allow the movement of molecules (e.g., amino acids, lipids, drugs, water) across cell membranes. Eukaryotic ABC transporters are composed of two subunits, each typically having six TMDs [89], and SLC transporters have 8–12 TMDs depending on the subfamily [92].

Aside from structural considerations, MPs are very dynamic and undergo continuous conformational changes as they carry out their function. For example, GPCRs experience various activation states upon association with downstream signaling factors such as G-proteins, while ion channels cycle through open, closed, and inactive conformations. The lipid bilayer in which MPs are embedded also plays a crucial role in both function and stability. Membrane components such as cholesterol can have both direct and indirect interactions with MPs [93]. The dynamic structure of MPs and their extensive interactions with lipids and other proteins (as oligomers or for regulation/signaling) provide more opportunities for drug development because specific epitopes exposed in a transitory conformational state or within a specific cell context can be targeted.

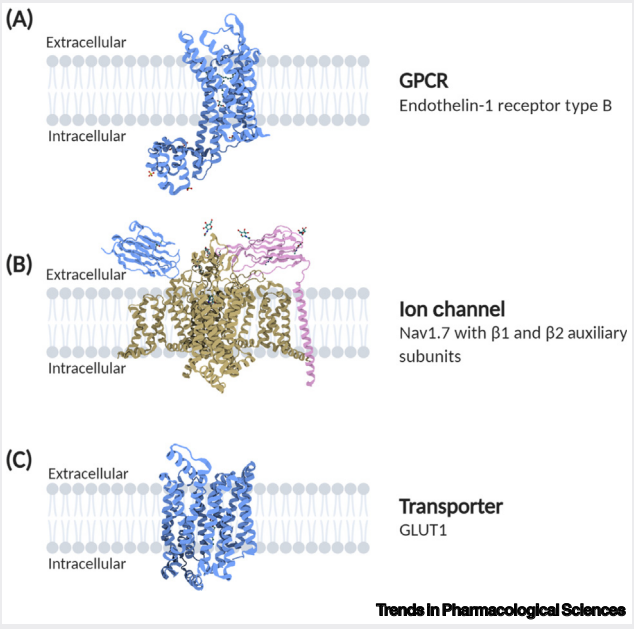



Figure I. Representative examples of therapeutically-relevant membrane protein structures. (A) Human G protein-coupled receptor (GPCR) endothelin receptor type B [Protein Data Bank (PDB) 5GLI [94]]. (B) Human $Na_v1.7$ ion channel [24 transmembrane domains (TMDs)] in complex with single-TMD auxiliary subunits β1 (pink) and β2 (blue) (PDB 6J8J [46]). The TMD of β2 was not resolved. (C) The human SLC glucose transporter GLUT1 (PDB 4PYP [95]), which has 12 TMDs. Figure generated in BioRender (https://biorender.com/).

## Glossary

**ATP-binding cassette (ABC) transporters:** a category of ~48 human active transporters that utilize ATP hydrolysis to move substrates across a membrane, most often for efflux.
**Chinese hamster ovary (CHO) cells:** an immortalized epithelial cell line commonly used for the expression of mammalian recombinant proteins
**Fluorescence/bioluminescence resonance energy transfer (FRET/BRET):** when an excited fluorescent or bioluminescent donor molecule comes into proximity with, and transfers energy to, an acceptor molecule, the acceptor then emits light at a particular wavelength, as when two donor/acceptor labeled sites on a protein come together during conformational changes.
**Fluorescence-detection size-exclusion chromatography (fSEC):** a size-based separation method where the protein of interest is detected using a fluorescent marker introduced by fusion with a fluorescent protein or by chemical fluorescent labeling. fSEC reports on the aggregation and homogeneity of a protein sample.
**G protein-coupled receptors (GPCRs):** membrane proteins (MPs) with seven transmembrane domains that undergo a conformational change when bound by an extracellular ligand, triggering activation of intracellular G-proteins (guanine nucleotide-binding proteins) and downstream signaling pathways.
**Human embryonic kidney 293 (HEK293) cells:** a robust human cell line that is commonly used for production of recombinant proteins
**Ion channels:** pore-forming MPs that transport ions through a membrane down their electrochemical gradient; the open/closed states of ion channels are often gated by membrane potential, ligand binding, or mechanical activation.
**Monoclonal antibody (mAb):** a type of protein produced by the immune system that recognizes and binds to a particular epitope, or a highly specific protein sequence or conformation, and that can be used as a therapeutic. The canonical bivalent immunoglobulin fold is composed of two heavy chains and two light chains, each comprising a constant domain and a variable domain that is responsible for binding.
**Nanobody:** a monomeric single-domain antibody fragment

MP subclasses such as ion channels and transporters. Although monotopic (one TMD) MPs and membrane-associated proteins are also of high pharmacological interest (e.g., viral envelope proteins), they are outside the scope of this review.

## Recombinant expression systems for MPs

Some recent reviews describe in detail the various expression platforms used for the successful production of MPs [2–5], the advantages and disadvantages of which are summarized in Table 1. In short, strategies to generate MPs for drug discovery must balance, at a minimum, the requirements of physiological relevance and yield, where the importance of the former typically outweighs the latter. Generally speaking, expression platforms that most resemble the native host cell generate the highest-quality recombinant protein owing to similar folding environments and cofactors as well as access to related trafficking and **post-translational modification (PTM)** machinery. However, MP overexpression in such mammalian cells [e.g., **Chinese hamster ovary (CHO) cells** or **human embryonic kidney (HEK293) cells**] is often accompanied by cytotoxicity owing to the high energy load for folding transmembrane segments and their potential for aggregation, or by disrupted equilibrium upon overexpression of some types of MPs such as ion channels and transporters. Potential solutions to this expression bottleneck include the use of insect cells such as *Spodoptera frugiperda* (Sf9, Sf21) and *Trichoplusia ni* (Hi5), which have enabled the determination of ~85% of solved **G protein-coupled receptor (GPCR)** structures [6], or microbial expression platforms (e.g., bacteria, yeast, protists). Alternative expression platforms include **virus-like particles (VLPs)**, where high densities of recombinant MPs are incorporated into lipoparticles that bud from host cell membranes[i], and cell-free expression technologies, which are gradually becoming viable methods to generate functional MPs [7].

Given the variability in PTM capabilities (e.g., only mammalian cells are able to introduce complex glycans such as sialic acid) and differences in lipid membrane composition (e.g., lack of cholesterol), as outlined in Table 1, the known properties of the particular target should inform the choice of expression system. For example, bacteria have limited utility and are only able to express some GPCRs, and cannot be used for larger mammalian MPs (>7 TMs), ion channels with large extracellular domains that require extensive glycosylation for function (e.g., P2X receptors), or MPs that require sterol interactions for function. On the other hand, yeast have more sophisticated eukaryotic PTM and trafficking mechanisms, and have successfully produced MPs of all subclasses for structure/function studies, even having been engineered to recapitulate GPCR signaling pathways [8,9], despite the potential handicap of introducing non-mammalian hyper-mannosylated glycans (Table 1, and see S1 in the Supplemental information online). Microbial platforms do share some key advantages for drug discovery applications beyond increased expression levels, including the ability to screen large libraries of mutations (i.e., directed evolution) for enhanced MP expression levels/thermostability [10–12], which enables subsequent expression of the optimized target in conventional mammalian/insect cell systems and *in vitro* testing. Furthermore, microbial expression is often necessary for NMR structural studies which require cell growth in minimal media for isotopic labeling. When alternative expression platforms are used for MP-targeted drug discovery, more native-like cell types must be integrated in downstream assays to validate lead molecules for modulation of the native MP conformation.

## Purification, stabilization, and formulation of full-length MPs for drug discovery

Once a MP target is successfully expressed, the recombinant host cell itself, membrane preparations, purified protein, or a combination of these can be utilized for drug discovery and development (Figure 1A, Key figure). Although whole cells overexpressing MPs have been used extensively in the discovery process (e.g., for screening chemical libraries, reviewed for GPCRs in [13]; or as antigens for mAb generation, reviewed in [2]), in some cases low concentrations

corresponding to the variable domain of the camelid heavy-chain-only immunoglobulin. A nanobody can be used to recognize and stabilize a specific protein conformation for structural studies or therapeutic applications.

**Post-translational modifications (PTMs):** chemical modifications to a protein that can regulate its function and/or stability, most commonly the addition of carbohydrates (glycosylation) or phosphate groups (phosphorylation), but also encompassing the addition of peptides (e.g., ubiquitination) or hydrophobic groups such as fatty acids (e.g., palmitoylation).

**Solute carrier (SLC) transporters:** a group of >400 transporters that use facilitative transport or exchange of solutes to move substrates across a membrane.

**Structure-based drug design (SBDD):** a strategy where the structure of the protein target is used to predict and develop chemical binders with a desired functional effect.

**Transmembrane domain (TMD):** a hydrophobic, membrane-embedded α-helix; a defining structural element of an integral MP.

**Virus-like particles (VLPs):** self-assembling structures obtained by cotransfecting cells expressing a MP of interest together with a viral capsid protein. VLPs mimic the overall structure of a virus and display MPs at their surface, but do not contain the viral genome.

Table 1. Expression platforms for human MP drug targets, with recent examples[a]

| Expression platform | Features | Limitations | PTMs | Lipids | Drug target type | Recent examples | Refs[b] |
|---|---|---|---|---|---|---|---|
| Mammalian cells (e.g., HEK293, CHO) | Provides most native-like PTMs, lipid environment, and folding factors (e.g., chaperones)<br><br>Inducible expression by transient transfection<br><br>Stable cell lines can be generated and optimized for expression via FACS | Potentially low expression levels and yields; some targets may be toxic to the cell when overexpressed (e.g., ion channels)<br><br>Scale-up can be difficult<br><br>Long timescales for expression and optimization | Native-like | Native-like: phospholipids (PLs) include predominantly PC with smaller amounts of PE, PS, and PI<br><br>Additional important membrane components include sphingolipids and cholesterol | GPCR<br>Ion channel<br>SLC transporter | CXCR2 CCR1 AT1R<br>TRPV1 hERG<br>ENT1 | [79,80,83, S1–S3] |
| Insect cells (e.g., Hi5, Sf9) | Often higher yields than mammalian expression (~mg/l) | Robust expression from the strong polyhedrin promoter can cause misfolding, aggregation, or cell lysis<br><br>Long timescales for expression and optimization | Simplified *N*-glycosylation (lack sialic acids) | Mammalian-like PLs are present but in different proportions (more PE and PI, less PC)<br><br>Low sterol synthesis<br><br>Cholesterol and other lipids can be incorporated by addition to the culture media | GPCR<br>Ion channel<br>SLC transporter | APJ GLP1R<br>TASK1 P2X4<br>GlyT1 | [79,86, S4–S7] |
| Bacteria (e.g., *Escherichia coli*) | Well-studied and simple genetic engineering<br><br>Inexpensive<br><br>High-density cultures<br><br>Fast growth/ optimization<br><br>High yields (~mg/l)<br><br>Amenable to directed evolution for expression/ stability optimization<br><br>Can grow in minimal media to introduce isotopes for NMR structure determination | MP instability and aggregation is common<br><br>For denatured MPs expressed as inclusion bodies, refolding procedures are difficult/unreliable<br><br>Endotoxin removal is required for immunization | Not native-like<br><br>No glycosylation<br><br>Correct disulfide bond formation can be challenging | Not native-like (no PC, mostly PE)<br><br>No cholesterol | GPCR<br>SLC transporter | CB2 NTS1<br>ASCT2 BoAT1 | [10,S8,S9] |
| Yeast (e.g., *Pichia pastoris* and *Saccharomyces cerevisiae*) | Easily amenable to genetic manipulation<br><br>Inexpensive<br><br>High cell densities<br><br>Fast growth/ optimization<br><br>High yields (~mg/l) | Cell wall may hinder purification | Non-native N-glycosylation (hyper-mannosylated), although strains have been glycoengineered to incorporate more human-like structures | Most mammalian-like PLs are present (PC, PE, PS, PI) but in different proportions<br><br>Lack cholesterol, although cholesterol synthesis has been engineered into yeast strains<br><br>Non-mammalian lipids are present, for example ergosterol (cholesterol analog) | GPCR<br>Ion channel<br>SLC transporter | 5HTR-4 A2a κOR<br>Pannexin1 TREK-1<br>GLUT1 | [8,12, S10–S15] |

Table 1. (continued)

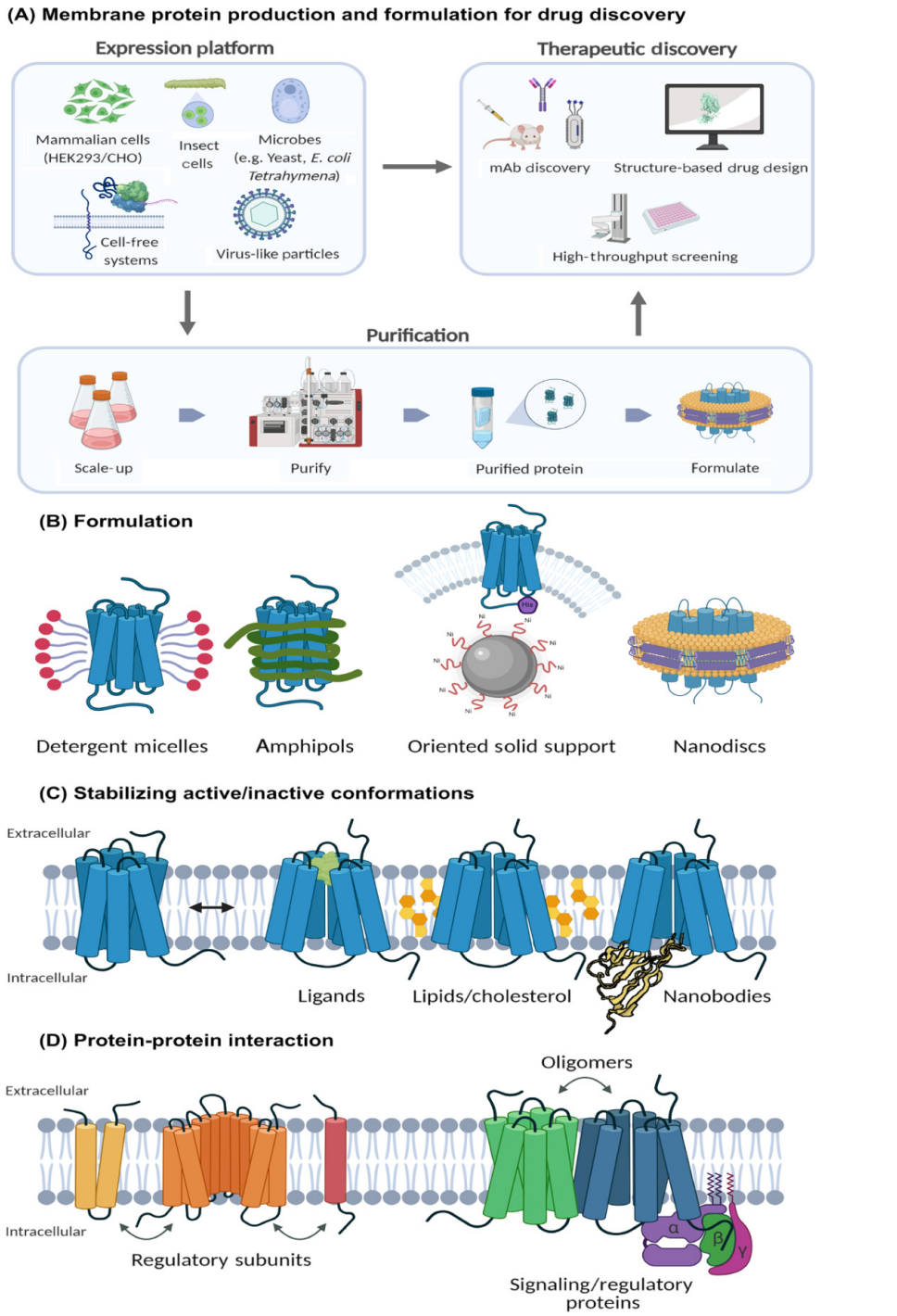
| Expression platform | Features | Limitations | PTMs | Lipids | Drug target type | Recent examples | Refs[b] |
|---|---|---|---|---|---|---|---|
| | Amenable to directed evolution for expression/ stability optimization<br><br>Can grow in minimal media to introduce isotopes for NMR structure determination | | | | | | |
| *Tetrahymena thermophila* | Cell metabolism is geared towards MP expression<br><br>Inexpensive<br><br>Fast growth/ optimization<br><br>High yields (~mg/l) | Specialized methods and vectors | Non-native *N*-glycosylation (noncomplex, terminal mannose) | Some mammalian-like PLs are present (PC, PE), but in different proportions<br><br>Lacks cholesterol<br><br>Non-mammalian lipids are present, e.g., tetrahymenol<br><br>Lipid composition can be modified by addition of cholesterol and other lipids into the growth media | Ion channel | Kv1.3 | [33,S16] |
| Virus-like particles (VLPs) | Native-like conformation of proteins expected when derived from mammalian cells<br><br>Present a high density of MPs<br><br>Able to produce strong B cell response for mAb generation | Require sufficient expression in host cells | PTMs depend on the host cell | Lipids/cholesterol depend on the host cell and the viral protein used to create the VLPs | GPCRs<br>Ion channels<br>SLC transporter | CB1[i]<br>P2X3<br>GLUT4 | [75,76] |
| Cell-free expression | Small reaction volumes<br><br>Fast reaction rates – the most rapid expression method<br><br>Toxic targets are not an issue<br><br>Highly controlled environment: can control membrane properties and add chaperones | Expensive low yields – limited by the availability of reaction substrates<br><br>Often lack PTM machinery and other *in vivo* factors that may help with expression/folding | PTMs/glycosylation require additional components (e.g., endoplasmic reticulum microsomes) | Synthetic/natural lipids or cholesterol can be added as desired, although additives must be tested for inhibition of transcription/translation reactions | GPCRs<br>Ion channels | ET-B µOR<br>Kv1.1 hERG | [S17–S20] |

[a]Abbreviations: FACS, fluorescent-activated cell sorting; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine.
[b]Additional references related to this table can be found in the Supplemental material online.

of the MP target in cell membranes and/or potentially immunodominant nontarget proteins can create challenges. Purified MPs in functional conformations mitigate these issues, while providing customizability of *in vitro* assays to potentially target chosen conformations, or even MP complexes with soluble or membrane-associated regulatory/signaling proteins, for drug discovery.

## Key figure

Membrane protein (MP) expression, purification, and formulation for therapeutic drug discovery: stabilizing specific conformations




*(See figure legend at the bottom of the next page.)*

Table 2 compiles recent studies where lead therapeutic molecules were discovered using purified human MPs, highlighting the choice of expression host, formulation, and discovery method. VLPs are included as a unique formulation; although the MP target itself is not purified from other membrane components, it is at higher concentrations than in cell membranes.

## Successful MP stabilization and purification strategies

One strategy to stabilize MPs is to modify the protein sequence by mutagenesis to optimize thermostability or desired functional activity, a method that has been applied by Heptares for GPCR-targeted drug discovery (StaR technology[ii]). MPs can also be made as fusion proteins with stabilizing soluble proteins or additional tags, although beneficial fusions are better-known for GPCRs than for other MP types (e.g., T4 lysozyme inserted into intracellular loop 3 [14]).

For purified MPs to maintain a physiologically relevant conformation necessary for productive drug discovery, their transmembrane regions require a hydrophobic environment that sufficiently mimics the native membrane (see Figure 1B and Box 2 for example formulations). Detergents are commonly used to extract MPs from recombinant cell membranes and maintain them in solution during purification, but can compromise protein quality over time and are not stable upon dilution. Therefore, although MPs within detergent micelles have been used in *in vitro* drug discovery programs (Table 2), there are distinct advantages of reconstituting MPs into lipid-incorporating stabilizing formulations such as liposomes or nanodiscs, or dispensing with the use of detergents altogether (e.g., styrene maleic acid lipoparticles, SMALPs).

During MP purification and reconstitution, care must be taken to avoid denaturation and maintain functionality, as monitored by analytical methods that evaluate ligand-binding ability and/or activity. In some cases, functional MPs can be directly isolated by ligand affinity chromatography, which has been applied to recover active GPCRs [15], although elution can be difficult and not all MP targets have known ligands. For targets such as ion channels, where it is difficult to measure activity in the absence of a membrane, or understudied proteins for which ligands are uncharacterized, general measures of protein quality, such as aggregation and mono-dispersity, can be measured by **fluorescence-detection size-exclusion chromatography (fSEC)** [16,17] or thermostability (e.g., differential scanning fluorimetry [18]). Given the large number of empirical factors that can influence the stability of an MP during purification (e.g., the presence of ligands, detergent type, pH, buffer components), many recently developed methods focus on optimizing conditions in high-throughput assays [18]. Among the detergents typically used for MP purification, the current consensus identifies nonionic carbohydrate-based detergents, such as dodecyl maltoside (DDM) and lauryl maltose neopentyl glycol (LMNG) [19], as the most able to maintain functional conformations, and lipids or a detergent-soluble cholesterol analog, cholesteryl hemisuccinate (CHS), are often added for further stability.

Figure 1. (A) MPs produced in mammalian, insect, or microbial cells and cell-free systems (top left box) can be purified (bottom box) and subsequently used for monoclonal antibody (mAb) drug discovery, high-throughput drug screening, or structure determination for structure-based drug design (top right boxes), or used directly in cells or membranes for mAb discovery and high-throughput drug screening. In addition, virus-like particles containing recombinant MPs can be used directly in mAb discovery and high-throughput drug screening. (B) Example formulations for purified MPs. (C) Tunable factors that can enrich desired MP conformations for drug discovery (ligands, lipids/cholesterol, and the addition of nanobodies). (D) Various protein–protein interactions occurring within the membrane: MPs associate with and can be modulated by regulatory subunits, other multipass MPs (as homo- or hetero-oligomers), or signaling interaction partners (e.g., G-proteins). Figure generated in BioRender (https://biorender.com/). Abbreviations: CHO, Chinese hamster ovary; *E.coli, Escherichia coli*; HEK293, human embryonic kidney.

Table 2. Examples of recent drug discovery programs using full-length formulated human MPs[a]

| Target | Target type | Disease Indication(s) | Expression host | Formulation | Therapeutic type | Discovery platform | Notes | Refs[b] |
|---|---|---|---|---|---|---|---|---|
| Bacteria | | | | | | | | |
| NTS1 | GPCR: neurotensin receptor | Inflammation, cancer, addiction | *Escherichia coli* | Detergent (MNG-3) | Small molecule | HTS fluorescence polarization assay | Rat protein was used | [53] |
| Mammalian cells | | | | | | | | |
| CCR5 | GPCR: chemokine receptor | HIV | Unspecified | Liposomes | mAb | DNA immunization (chicken) boosted w/purified protein | | [75] |
| CB1 | GPCR: cannabinoid receptor | Various | HEK293 or CHO | Liposomes | mAb | Immunization (mouse hybridoma) plus phage display | | [S21] |
| CCR1 | GPCR: chemokine receptor | Autoimmune diseases | HEK293 | Detergent (DDM/CHS) | mAb | Naïve library (yeast-based) screened with cells; purified protein was used for affinity maturation | | [83] |
| AT1R | GPCR: angiotensin II receptor | Hypertension, kidney disease | HEK293 | Detergent (LMNG/CHS) | mAb | Synthetic library (yeast-based) screened with purified protein | Screening in the presence of ligands | [S22] |
| ASIC1a | Acid-sensing ion channel | Stroke | HEK293 BacMam | Nanodiscs | mAb | scFv phage library | | [96] |
| P2X4 | Ligand-gated ion channel | Neuropathic pain | HEK293 | Detergent (DDM/CHS/CHAPS) | mAb | Naïve scFv phage library | | [S7] |
| P2X3 | Ligand-gated ion channel | Neuropathic pain | HEK293 | Detergent (DDM/CHS) | mAb | Immunization (mouse hybridoma) | | [S23] |
| TRPM8 | Ion channel | Neuropathic pain | HEK293S GnTI- for structures | Detergent (digitonin) Amphipols (PMAL-C8) | Small molecule | SBDD/molecular modeling using cryo-EM structures | TRPM8 structures were from birds | [42, S24–S25] |
| ENT1 | SLC transporter | Ischemia, pancreatic cancer | HEK293 | Detergent (LMNG) | mAb | Synthetic library (ribosome display) screened with purified protein | | [S26] |
| ASCT2 | SLC transporter | Tumors | Melanoma cell line + HEK293 for functional assays<br><br>HEK293 for EAAT1 structure | Detergent (decanoyl sucrose/CHS) precrystallization (EAAT1) | Small molecule | SBDD/virtual screening using a homology model based on EAAT1 structure | | [43,S27] |
| Insect cells | | | | | | | | |
| APJ | GPCR: apelin receptor | Chronic heart failure | Sf9 | Nanodiscs and liposomes | mAb | immunization (camel) with SBDD | | [86] |
| Orexin receptors (OX1R and OX2R) | GPCR | Insomnia | CHO for functional assay<br><br>Sf9 (insect cells) for structure | DDM/CHS and LMNG precrystallization | Small molecule | SBDD | | [37,38,S28] |
| MC4R | GPCR | Obesity | HEK293 for functional assay<br><br>Sf9 (insect cells) for structure | DDM/CHS precrystallization | Small molecule | SBDD | | [39,S29] |

Table 2. (continued)

| Target | Target type | Disease Indication(s) | Expression host | Formulation | Therapeutic type | Discovery platform | Notes | Refs[b] |
|---|---|---|---|---|---|---|---|---|
| CGRP receptor | GPCR | Migraine | Sf21 | Unspecified | Small molecule | SBDD | | [S30–S31] |
| TREK-1 | Ion channel | Depression | CHO for functional assays<br><br>Sf9 (insect cells) for TREK-2 structure | OGNG/CHS precrystallization (TREK-2) | Small molecule | SBDD using molecular dynamics to model an intermediate conformational state based on TREK-2 structures | | [44,S32] |
| GLUT5 | SLC transporter | Cancers | Sf21 | Liposomes | Small molecule | SBDD (virtual screening) plus liposome assay | | [S33] |
| GlyT1 | SLC transporter | Nervous system disorders | Sf9 | Detergent (LMNG/CHS) | mAb | Synthetic library (ribosome display) screened w/purified protein | | [S26] |
| Yeast | | | | | | | | |
| various $K^+$ channels (mouse GIRK2, TRAAK) | Ion channel | Various | *Pichia pastoris* | Liposomes | Small molecule | HTS of compound library | | [55] |
| TrpV5 | Ion channel | kidney diseases | *Saccharomyces cerevisiae* | Detergent (DMNG) | Small molecule | Structure-based virtual screening | Rabbit protein was used | [49,S34] |
| *Tetrahymena thermophila* | | | | | | | | |
| Kv1.3 | Voltage-gated ion channel | Autoimmune diseases | *Tetrahymena thermophila* | Liposomes or oriented solid support | mAb | Immunization (chicken) with proteoliposomes; screening of B cells with magnetic beads | | [33] |
| Virus-like particles | | | | | | | | |
| GCGR | GPCR: glucagon receptor | Metabolic disorders | HEK293 | VLPs | mAb | DNA immunization (llamas), phage selections with VLPs | | [S35] |
| CXCR4 | GPCR: chemokine receptor | Cancer, fibrosis | Unspecified | VLPs | mAb | Phage library of single-domain antibodies | | [S36] |
| P2X3 | ligand-gated ion channel | Neuropathic pain | Unspecified | VLP | mAb | Immunization (chicken) | | [75] |
| GLUT4 | SLC transporter | Type 2 diabetes | HEK293T | VLPs | mAb | Immunization (chicken) | | [76] |
| Cell-free expression | | | | | | | | |
| DRD1 | GPCR: dopamine receptor | Cerebral meningioma | Wheat-germ extract | Liposomes | mAb | Immunization (mouse) | | [S37] |
| Claudin-5 | Structural (tight junctions) | Neurodegenerative disorders | Wheat-germ extract | Liposomes | mAb | Immunization (mouse) | | [S38] |

[a]Abbreviations: CHAPS, 3-((3-cholamidopropyl) dimethylammonio)-1-propanesulfonate); CHS, cholesteryl hemisuccinate; DDM, dodecyl maltoside; DMNG, decyl maltose neopentyl glycol; MNG-3, maltose neopentyl glycol-3; LMNG, lauryl maltose neopentylglycol; OGNG, octyl glucose neopentyl glycol; PMAL-C8, poly(maleic anhydride-*alt*-1-decene), 3-(dimethylamino)-1-propylamine.
[b]Additional references related to this table can be found in the Supplemental information online.

### Box 2. Lipid membrane and membrane-mimetic formulations for purified MPs

To impart further stability and a more native-like environment, detergent-solubilized MPs can be reconstituted into lipid membrane or membrane-mimetic formulations (see examples in Figure 1B in the main text). Although all have been used in structure–function studies, they each confer different properties and limitations for drug discovery assays, as summarized in Table I. An important factor to consider is whether functional lipids should be included to access desired conformations; for example, PIP2 regulates the function of TRP channels [97] and some GPCRs [98], cholesterol modulates many MPs [93,99,100], and two-pore potassium channels (e.g., TRAAK) are activated by phosphatidic acid (PA) and phosphatidylethanolamine (PE) lipids [101]. In some cases lipids copurify with the MP target, but may be diluted upon reconstitution into liposomes, a typical spherical lipid bilayer formulation. More recently developed formulations utilize engineered lipoproteins (nanodisc, peptidisc [102]) or saposins (Salipro [103]) to encircle lipid-membrane discs containing the embedded MP target, resulting in small particles (~10 nm) that are amenable to structural studies with reduced nonspecific membrane interactions [104]. In addition, novel surfactants such as calixarene-based detergents have emerged as effective formulations for a range of MP drug targets[x], as have modular oligoglycerol detergents that maintain lipid interactions [105].

Among the considerations listed in Table I, the discovery of MP-targeting therapeutic mAbs has its own challenges owing to the need to target the extracellular surface (see Box 3 in the main text) as well as the potential for a formulation to introduce irrelevant, nontarget epitopes. For example, target MP-containing nanodiscs made with MSP (membrane scaffold protein) require counter-selection with empty nanodiscs without the MP target to eliminate mAbs that bind to MSP and not the target. Nevertheless, nanodiscs have demonstrated ability to isolate higher-affinity mAbs from a phage library compared with detergent-solubilized antigens [106] and recently enabled the discovery of ASICa1 blockers [96]. Salipro technology preserves interactions with native lipids and is currently being applied to stabilize MP targets for mAb discovery[xi].

Amphipathic polymer surfactants, namely amphipols and SMA polymers, pose a few advantages over protein-based surfactants, including low immunogenicity and simple synthesis, functionalization, and reconstitution protocols. Amphipols have stabilized many therapeutically relevant MPs [107,108,110] including bacterial MPs used for immunization-based mAb generation [109,111]. MPs can be solubilized directly from crude membranes using SMA to form SMALPs [112] which retain lipids from the host membrane, including mammalian cells expressing human ion channels [113]. Second-generation polymeric surfactants, nonionic amphipols [114], and DIBMA (diisobutylene/maleic acid) [115] provide greater tolerance to divalent cations.

Table I. Characteristics of MP formulations for various drug discovery assays/applications

| | Detergent/mixed micelles | Liposome | Solid support | Nanodisc/peptidisc | Amphipol | SMALP | VLP |
|---|---|---|---|---|---|---|---|
| General considerations | | | | | | | |
| Stable upon dilution | No | Yes | Yes | Yes | Yes | Yes | Yes |
| Good shelf life; (i.e., can be frozen and maintain protein quality) | No | Yes | No | Yes | Yes | Yes | Yes |
| Can include functional lipids/cholesterol | Yes[a] | Yes | Yes | Yes | Yes[a] | Yes[b] | Yes[b] |
| Can be analyzed/purified to homogeneity by size exclusion chromatography (SEC; e.g., for structural studies) | Yes | No | No | Yes | Yes | Yes | Yes[c] |
| Closed compartment enables functional assays for ion channels/transporters | No | Yes | Yes | No | No | No | Yes |
| Functionalization available for binding assays or detection (e.g., biotinylation, fluorescence) | No[d] | Yes | Yes | Yes | Yes | Yes | Yes |
| Considerations for mAb discovery | | | | | | | |
| Can control orientation of MP | No | Yes[e] | Yes | No | No | No | Yes |
| Amenable to immunization for mAb discovery | Yes[f] | Yes | Yes | Yes | Yes | Yes | Yes |
| Can be used for *in vitro* mAb discovery (e.g., phage selection) | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

[a]The cholesterol analog CHS can be added during purification; lipids may co-purify with MPs but cannot be controlled.
[b]Lipid composition depends on the expression host.
[c]VLPs can be purified by SEC, but are not often used in structural studies.
[d]Functionalized detergents are not commonly used, but they can be used in binding assays if the formulated MP is functionalized.
[e]Lipid composition can be tuned to favor an orientation; often empirical and MP-dependent.
[f]Detergent micelles may dissociate when diluted during immunization.

## Isolation and formulation of desired conformations

In recent years the judicious design of cell-based assays has enabled the discovery of MP-targeting drugs with specific properties, for example, drugs that preferentially target a particular signaling pathway (i.e., biased agonism/antagonism) or that bind to a MP target at an allosteric site [20]. However, these assays require that the MP target is expressed at a sufficiently high

concentration at the cell surface, especially when a relatively rare conformation is being targeted, and results can vary from one cell type to another because of differences in the expression levels of interacting proteins [21]. Although purified MPs or MP complexes can provide higher concentrations of the target of interest, they can also present a mixed population of conformations based on the expression host and design of the purification procedure. Following advances in structural biology that make it possible to isolate specific active conformations [22,23] and complexes of MPs as homo- or hetero-oligomers [24] or with soluble/membrane-associated interaction partners (e.g., GPCRs with β-arrestin [25,26] or G-proteins [24,26,27]), we foresee the development of novel conformation- and state-specific therapeutics. In addition to greater mechanistic/structural insight guiding drug design, the biochemical methods developed to capture MP conformations and complexes can be applied to provide material for drug discovery assays (e.g., antigens for mAb generation), where demands for sample homogeneity are not as rigorous as for typical structure determination methods.

Some modulators of MP conformation (Figure 1C,D) can be controlled during the production of purified MPs and in *in vitro* drug discovery assays, for example, by the addition of known ligands (e.g., agonists, antagonists), **nanobodies** that stabilize a particular conformation [28,29], or purified interaction partners (e.g., mini G-proteins [30] that favor a GPCR active state, and regulatory proteins such as calmodulin that modulate some ion channels [31] and affect the subcellular localization of aquaporins [32]). Although nanobodies have been extensively applied in determining protein structures, they are now starting to be applied to drug discovery for GPCR targets (e.g., Confo Therapeutics[iii]). In addition, given the growing body of knowledge surrounding protein–lipid interactions, formulation of MPs with function-modulating lipids (Box 2) can also enable selection of a desired conformation. A formulation method particularly useful for therapeutic mAb discovery, where extracellular binders are desired (discussed in

Box 3. mAb discovery strategies: targeting the MP extracellular surface

With few exceptions, recognition of accessible epitopes on the exposed extracellular (EC) surface of MPs is a prerequisite for therapeutic mAb discovery because of their inability to traverse the cell membrane. For many MP targets this poses a challenging endeavor owing to the relative paucity of epitope targets at the cell surface – voltage-gated ion channels being a prime example (see Figure I in Box 1). A simple strategy to focus discovery efforts on EC epitopes is to utilize predicted surface loop peptides or chemically constrained peptides that mimic native loop conformations for immunization and/or screening. Although modulating antibodies targeting, for example, the EC3 loop of ion channels have been successfully generated [116], the lack of developable monoclonal antibodies using this approach suggests that a more comprehensive strategy is required.

A notable drawback of using predicted EC loops for mAb discovery is that they lack the contextual environment of the native MP surface. This is especially true for MPs consisting of homo- or hetero-complexes composed of one or more subunits that offer the possibility of targeting discontinuous or conformationally distinct epitopes created by subunit interactions (see Figure 1D in main text). Utilizing conformationally relevant full-length protein enables targeting these complex surface epitopes.

In many cases, exposure of epitope-rich intracellular surfaces to immune repertoires, either *in vivo* or *in vitro*, requires careful consideration of discovery strategies that will preferentially recover mAbs that recognize surface epitopes. An immunization strategy using nonoriented formulations such as membrane fragments, purified MPs in mixed micelles, liposomes, nanodiscs, or amphipols (see Figure 1B in the main text and Table I in Box 2) would be expected to induce an immune response that generates antibodies to all accessible epitopes. In fact, immune responses may be skewed toward intracellular immunodominant epitopes for challenging targets with a small extracellular target space, leaving sought-after surface binders relatively rare. Subsequent screening strategies should then incorporate cell lines expressing the target of interest on the cell surface, and have a significant advantage in that they identify mAbs that recognize the surface of the MP in a native or near-native context. On the downside, challenging targets are often associated with poor expression in recombinant systems, making analytics such as flow cytometry on live cells routinely difficult. In this case, an oriented MP tethered to a solid support and encapsulated in a lipid bilayer presents external epitopes and can be very useful in initial screens (e.g., panning an antibody display library) to isolate EC-binding antibodies before subsequent cell-based screens of functionality.

Box 3), is to control the orientation of a MP by anchoring its intracellular surface to a solid support (e.g., a magnetic bead; Figure 1B) via an affinity tag before adding lipids to stabilize the MP target in the desired orientation. This formulation has proved to be successful for panning phage libraries for mAb discovery [33], and, compared with a conventional immunization/hybridoma approach using overexpressing cells, it isolated a larger number of GPCR-targeting mAbs with different modes of action [34].

## State-of-the-art tools for drug discovery and development for MP targets

The ability to produce high-quality MP targets is accompanied by many compelling innovations in drug discovery methodologies. Although purified MPs are required for **structure-based drug design (SBDD)**, a diversity of MP formats can be used for high-throughput screening (HTS) assays and for therapeutic mAb discovery (Figure 1A). However, the recent examples in Table 2 illustrate that most successful drug discovery programs hinge on producing MPs in multiple formats and formulations, and ultimately a lead molecule must demonstrate function modulation in native-like cells.

### Structure-based drug design

The rapid rise in the number of unique MPs with structures in the Protein Data Bank, that has nearly doubled over the past 5 years (mpstruc database[iv]), has set the foundation for new MP-targeted drug development via SBDD, which relies on computational methods such as virtual screening and molecular modeling (software reviewed in [35]) to develop small-molecule drugs with high affinity and selectivity. Given the prevalence of GPCR crystal structures over other MP classes, SBDD for GPCRs is the most advanced, with three small-molecule candidates from Sosei Heptares currently in clinical trials [36]. SBDD studies from the past 2 years (Table 2) demonstrate that solved crystal structures of GPCRs with ligands are enabling the design of more selective drugs with novel chemical structures [37–39]. When multiple active-state structures are available, as in the case of the β2-adrenergic receptor (β2AR), virtual screening can enable the design of sought-after agonists [40,41]. SBDD for ion channels and transporter targets, for which fewer high-resolution structures are available, still relies on homology/molecular modeling with analogous proteins for which structures have been solved. For example, TRPM8 inhibitors were discovered using homologous structures from birds [42], whereas inhibitors of the **solute carrier (SLC) transporter** ASCT2 were found using the structure of a structurally similar MP, EAAT1 [43]. Computational modeling also allows the design of drugs against intermediate or dynamic conformational states that are difficult to capture physically, but can be simulated from active/inactive structures, as was done recently for the TREK-1 potassium channel [44].

Aside from advances in expression and biochemical methods that allow the purification and stabilization of MPs for traditional X-ray crystallography or NMR structure determination, the emergence of cryogenic electron microscopy (cryo-EM) is enabling unprecedented insight into the structure and function of particularly difficult MP targets such as ion channels and transporters. Cryo-EM resolves large MP/surfactant complexes with comparatively small amounts of protein, some notable examples being voltage-gated sodium channels ($Na_v$s) [45,46] and tetrameric TRPA1 [47] ion channels (both classes with 24 TMDs), and surpasses crystallography in its ability to capture multiple, native-like conformations. Although most MP cryo-EM structures to date lack the resolution required for SBDD (<3Å), the number of higher-resolution structures is steadily increasing [48]. For instance, inhibitors have been designed from a cryo-EM structure of the TRPV5 ion channel expressed in yeast, which had high local resolution in a potential drug-binding pocket [49]. Even lower-resolution cryo-EM structures are being used as a basis to inform next-generation drug design via homology modeling, as in the discovery of TRPM8 inhibitors [42], or in docking studies to gain mechanistic information, as for the Ach receptor ion channel [50].

Despite the revolution in MP structural biology, a comprehensive analysis of all GPCR structures and GPCR-targeting drugs published in April 2020 [36] found no SBDD-developed therapeutic yet on the market because of long timelines for drug approval; although ~30 molecules in clinical trials had target structures available in a timeframe where they may have informed discovery, the use of SBDD was difficult to verify other than three from Sosei Heptares[ii]. Building on this information, we surveyed the list of novel FDA-approved therapeutic drugs for 2020–2021[v] and found 10 that target MPs, only one of which applied an element of SBDD in its discovery. Relugolix, the first orally available drug for advanced prostate cancer[vi], was designed in 2011 from docking studies utilizing a homology model of the human gonadotropin-releasing hormone (GnRH) receptor target based on the structure of rhodopsin (the first GPCR structure to be solved), resulting in a more specific antagonist molecule than previous generations [51]. Given the exponential growth and sophistication of available MP structures in the intervening 10 years, we anticipate that many more SBDD-derived drugs will reach the clinic in the near future.

### HTS approaches

Automated HTS assays are designed to interrogate libraries of small molecules, fragments, peptides, or mAbs to identify binders or modulators of MP targets, usually expressed at a cell surface [13]. For MP targets, HTS has thus far been most productive for characterized GPCRs that trigger well-understood signaling pathways resulting in a cAMP/calcium response that can be detected by fluorescent/luminescent reporters [13,52]. Although background binding to recombinant host cells or off-target effects can be alleviated by using purified MPs, which have been integrated in screens to identify binding entities (e.g., fluorescent ligands [53] and mass spectrometry [54]) or ion channel/transporter-modulating compounds (e.g., liposome flux assays [55]), cell-based assays are still more commonly employed because they more readily enable functional readout. Along these lines, yeast expression, with its simple genetics, provides an interesting solution for cell-based assays when target expression levels are insufficient in mammalian/insect cells, or if there is high background from native signaling pathways. Yeast engineered to express human GPCRs and report on function via coupling to a yeast/human chimeric G-protein have enabled the discovery of GPCR modulators [8,9]. Functional assays for the GLUT5 transporter [56] and the Kir3.2 potassium channel [57] were also developed in yeast, where inhibition of cell growth indicates drug activity.

HTS technologies established specifically for difficult ion-channel targets include the use of radioactive ions (e.g., $Rb^{2+}$ as a substitute for $K^{+}$) or fluorescent voltage- or ion-sensitive dyes [58]. The advent and continuing improvement of automated patch-clamp/high-throughput electrophysiology (e.g., SyncroPatch, Qube platforms) is facilitating unprecedented drug discovery for many ion channel targets. The SyncroPatch 384PE, for example, recently identified >10 small-molecule modulators of the hetero-oligomeric NMDA receptor from a total of 1920 compounds, whereas a parallel fluorescence-based assay only identified one molecule that was not a false positive [59].

Towards the aim of developing the next generation of conformation- or pathway-specific therapeutics for MP targets, some novel assay designs show promise in HTS discovery. A particular conformation of a MP can be stabilized by a **nanobody** during HTS, as demonstrated in a study where β2AR locked in its active state by fusion to a G protein-mimicking nanobody demonstrated a different binding affinity for various ligands compared with the basal state [60]. In addition, dual protein–protein interactions can be monitored by split luciferase assays where luminescence reports on the proximity of two interaction partners. This type of assay was originally applied to discover biased ligands that favor G-protein signaling for opioid receptors [61], ultimately resulting in the first drug designed as a biased agonist, oliceridine (FDA-approved in August 2020), which has reduced adverse effects compared with morphine. Similar assays

have facilitated the discovery of biased ligands for the nociception opioid peptide (NOP) [62] and serotonin HT1A [63] receptors. Although ion channel/transporter interactions with function-modulating proteins are not as well characterized compared with GPCRs, there is great potential to apply similar assays to those systems, as in a recent example where a peptide disrupting the $Na_V1.6$/fibroblast growth factor interaction was discovered [64]. In addition, intramolecular **fluorescence/bioluminescence resonance energy transfer (FRET/BRET)** sensors have been developed to monitor MP conformational changes in response to drug binding/activation [65,66]. Recent optimization specifically for HTS has delineated a universal BRET sensor design for GPCRs, and this was applied to the discovery of novel histamine receptor ligands [67,68]. Although ion channels are not as structurally homogenous, BRET assays have been developed for Kir3 [69] and TRPV [70] channels, pointing to the possibility of applying this method more widely.

### Therapeutic mAb discovery for MP targets

Compared with small molecules and peptides, targeting MPs with mAbs presents several advantages including superior selectivity/safety and long circulating half-life, thus reducing the dose required. The modular nature of antibodies also allows a variety of engineering options (reviewed in [71]), including multivalent formats, single-chain fragments (scFvs), nanobodies, and peptide fusions (e.g., knotbodies – ion-channel toxins [72]), as well as modified Fc domains to enhance or mitigate effector functions. Recent compilations of MP-targeting mAbs can be found in [1,2,73]. Progress for GPCR-targeting mAbs is most advanced compared to other MP types, with two currently approved (mogamulizumab for T cell lymphoma and erenumab for migraine) and >40 in clinical trials [1].

Identifying mAbs that target and modulate MP function is typically challenging in part because of the relatively small accessible extracellular surfaces of some MPs (see Figure I in Box 1 and Box 3, and the potentially high homology (and therefore low immunogenicity) of these regions in the orthologs of host immune animals. Some of these challenges can be remedied by the choice of animal for immunization. For example, chickens often have lower homology to human sequences compared with rodents, and have yielded functional antibodies against GPCRs [74], ion channels (Kv1.3 [33], P2X3 [75]), and the GLUT4 transporter [76]. As an alternative, hyperimmune mice have been developed (Diversimab[vii]), as well as mice with broken tolerance (IMG-AbS[viii]), or animals where the target ortholog is knocked out. Although nucleic acid (DNA/mRNA) immunization strategies are interesting options that do not require a protein antigen at all [77], they are often boosted with purified MP [75] or overexpressing cells to mitigate poor immune responses.

The most recent examples of mAb discovery using purified MPs (Table 2) reveal a trend towards use of *in vitro* mAb discovery methods, which allow speed and customizability, for example, by controlling the conformation of an antigen by the addition of a known inhibitor. If desired, antibody libraries and associated display platforms (e.g., phage [78], ribosomes [79], yeast [80]) can be screened directly without immunization, while representing entire immune repertoires in either naïve, synthetic, or semi-synthetic formats [78]. Purified MPs are often used during panning because nonspecific binding may be observed with overexpressing adherent cells, although novel and more robust flow cytometry-based methods have been developed to alleviate this [81]. Moreover, advances in antibody library design have mitigated the developability of early iterations, such as manufacturing liabilities due to instability or aggregation caused by nonnatively paired light and heavy chains [82]. Many studies highlight the importance of having multiple antigen formats available for screening. In one illustrative example, the GPCR target CCR1 in overexpressing cells isolated more specific hits than CCR1 in VLPs [83], although the low affinity of

the antibodies required affinity maturation with purified protein to avoid the avidity effect from having multiple antigens close together in the cell membrane [83].

Although binding can be detected by various methods [e.g., fluorescence-activated cell sorting (FACS) and ELISA], selecting mAbs with inhibition or activation activity necessitates more discriminating functional assays. Cutting-edge single-cell sorting methods (i.e., Beacon optofluidics platform[ix]) promise to allow binding and functional screening to occur in parallel, greatly accelerating the mAb discovery timeline [84]. Another innovative functional screen was recently developed whereby single-domain antibodies were anchored to the cell membrane together with the apelin receptor GPCR target and downstream signaling (β-arrestin recruitment) was monitored via FACS assay. Using this approach, functional antibodies were identified, including a rarely found agonist, which were not identified by phage display of the same immune library [85].

## Concluding remarks and future perspectives

From our review of state-of-the-art MP production for drug discovery, we foresee that expanding ability to screen MP function and achieve conformational heterogeneity, for example, by directed evolution or nanobody stabilization, is likely to hasten the development of state-specific therapeutics. This includes selection of the mode of action (e.g., allosteric inhibitors), facilitated by the development of fully *in vitro* discovery platforms, where known modulators can be introduced during selection. Recent work illustrates that strategies combining multiple methodologies for a MP target can result in novel developments, for example, SBDD enabled the design of the first nanobody agonist for a class A GPCR from a structure of the receptor bound to a nanobody antagonist discovered via *in vitro* mAb discovery methods [86].

We expect that therapeutics targeting historically difficult ion channels and transporters will soon advance to the level of GPCRs because expression/purification obstacles are being overcome and sophisticated functional assays are emerging. The example of cryo-EM, which has demonstrated remarkable success in resolving large ion-channel structures using less material than traditional structural techniques, together with increased miniaturization for HTS (e.g., the Beacon platform for mAb discovery), leads us to predict that reduced protein quantity requirements will further expand the range of viable MP targets.

A significant challenge remaining is the generation of recombinant native-like complexes of target MPs with interacting proteins (Figure 1D) because hetero-/homo-oligomeric interactions are a primary driver of MP conformation, function, and stability for most classes of therapeutically relevant MPs. Oligomer-specific therapeutics would potentially allow discrimination between cell types or specific conformations, and are advantageous when the druggable interface spans multiple proteins – as exemplified for the approved mAb therapeutic, erenumab, which targets a GPCR hetero-oligomer with the single-TMD receptor activity-modifying protein (RAMP1) [87].

Overall, the outlook for MP-targeting therapeutics is very positive, and future efforts are expected to accelerate the drug discovery process and enable the development of more specific and potent therapeutic options (see Outstanding questions).

### Outstanding questions

Will cryo-EM, potentially with computational methods, routinely achieve the resolution required for SBDD for large MP complexes?

Will the number of approved therapeutics for ion channels and transporters increase as expression/stabilization obstacles are overcome and companion discovery platforms are further developed for HTS of small-molecule and ion transporters?

Can expression systems generate consistent, conformationally distinct, MP formulations for the discovery of state-specific therapeutics, particularly for non-GPCR targets such as ion channels where conformational states may be more subtle or difficult to control?

Can native MP oligomeric complexes be generated in recombinant form and formulated effectively to enable discovery of function-modulating therapeutics with higher potency and specificity compared with targeting a single MP?

### Acknowledgments

The authors would like to thank Joanna Cardarelli (TetraGenetics Inc.) and Levy Te for helpful input on the manuscript.

### Declaration of interests

All authors are employees of TetraGenetics Inc.

## Supplemental information

Supplemental information associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tips.2021.05.006

## Resources

[i]www.integralmolecular.com

[ii]https://soseiheptares.com/

[iii]www.confotherapeutics.com/

[iv]https://blanco.biomol.uci.edu/mpstruc/query

[v]www.fda.gov/drugs/new-drugs-fda-cders-new-molecular-entities-and-new-therapeutic-biological-products/novel-drug-approvals-2020

[vi]www.fda.gov/news-events/press-announcements/fda-approves-first-oral-hormone-therapy-treating-advanced-prostate-cancer

[vii]www.abveris.com/abveris-antibody-discovery-overview

[viii]http://immunogenes.com/

[ix]www.berkeleylights.com/

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