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Review: Membrane protein nanodiscs for antibody discovery

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Membrane proteins play pivotal roles in cellular signaling, transport, and immune responses. Dysregulation of these proteins frequently underlies diverse disease states, making them appealing targets for drug development, including therapeutic antibodies. Traditionally, the extraction and stabilization of membrane proteins involve detergents, which may compromise the protein's native conformation, thus impeding antibody discovery. The shift toward detergent-free formulations using membrane protein nanodiscs formed by membrane scaffold proteins (MSPs), copolymers, saposins, or peptides has opened new avenues in membrane protein research and antibody discovery. They allow for the stabilization of membrane proteins in a more native-like environment, preserving structural integrity and function. This review discusses various membrane protein nanodiscs, and their applications in antibody discovery, alongside current advancements and challenges.

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Introduction

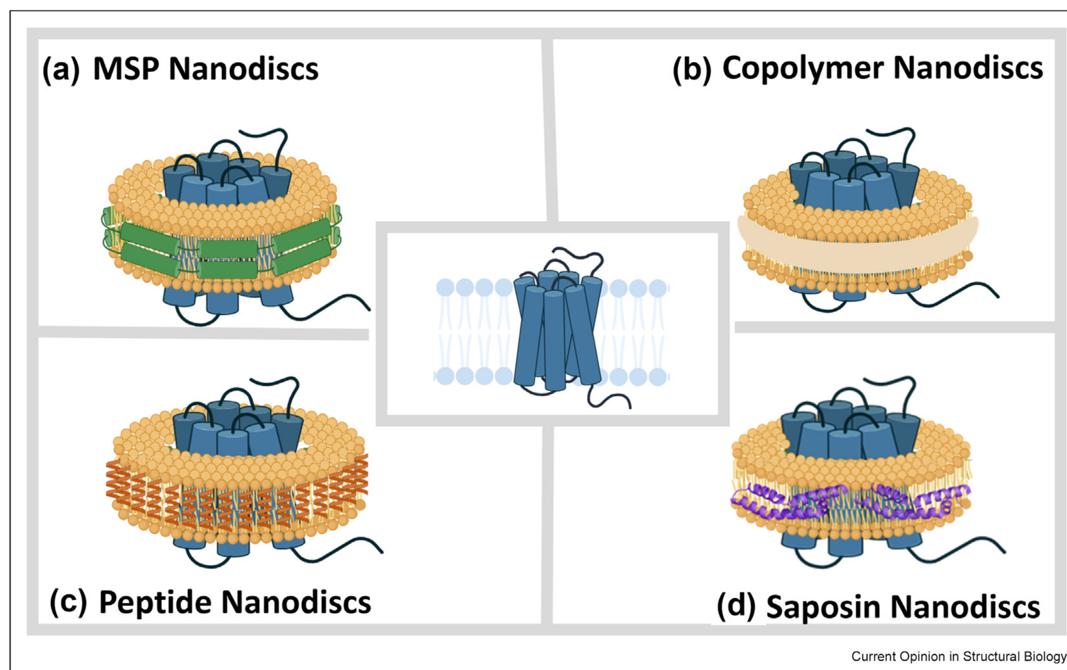
Membrane proteins, which make up approximately 30% of all proteins encoded by the human genome, represent more than 60% of drug targets [1,2]. However, antibody discovery against membrane proteins remains a significant challenge due to the difficulty in maintaining the structure and functionality of these proteins outside their native lipid environment. Additionally, their dynamic conformations, limited availability of accessible immunogenic regions, and the complexities of antibody screening further complicate the identification and targeting of specific epitopes for antibody binding [3].

While detergents are widely used to solubilize membrane proteins by mimicking the hydrophobic lipid bilayer environment, their use often destabilizes these proteins, potentially leading to aggregation or partial unfolding—as seen in cases like G-protein-coupled receptors (GPCRs) and transporters [4]. Additionally, detergents may strip away essential lipid molecules, distorting protein structure. These structural perturbations can sometimes compromise function and reduce antigenicity [5], limiting the effectiveness of detergents in antibody discovery applications. Detergent-based formulations have also been problematic for high-throughput antibody screening platforms such as phage display or hybridoma technologies, as conformational changes can alter epitope availability and affinity.

Nanodiscs, which provide a native-like lipid bilayer environment for embedding membrane proteins, have emerged as a powerful tool in membrane protein research, including antibody discovery. They stabilize membrane proteins by wrapping around their hydrophobic regions, thus keeping membrane proteins soluble in aqueous solutions while retaining their functional and structural properties. These self-assembling structures are typically formed using membrane scaffold proteins (MSPs) derived from apolipoprotein A-I along with synthetic or natural lipids, allowing fine-tuning of lipid−protein interactions in the target protein [6]. The term "nanodiscs" is now broadly used to refer to lipid nanoparticles stabilized by other amphipathic structures, including saposin−lipoprotein nanoparticles (Salipro), a lipid-binding protein-based belt system, peptides, or synthetic polymers [7−10] (Figure 1). Protein engineering of the amphipathic α-helical repeats in MSP or adjusting the saposin A to lipid ratio allows for tunable nanodisc size with high versatility toward various membrane protein families [11,12]. Synthetic polymers like styrene-maleic acid (SMA) copolymers can directly extract membrane proteins, forming nanodiscs with native lipids known as SMA lipid particles (SMALPs) [13].

Nanodiscs have been used in various antibody discovery platforms. By preserving the structural and functional integrity of membrane proteins, nanodiscs enable the display of native epitopes, which is crucial for antibody generation and binding. Moreover, nanodiscs offer a homogenous presentation of membrane proteins, which is essential for screening and antibody selection.

Figure 1



Visual representation of four classes of nanodiscs that stabilize membrane proteins to mimic cell membranes. (**a**) Membrane scaffold protein (MSP) nanodiscs assembled with engineered variants of apolipoprotein A-1 that form discoidal bilayers. (**b**) Copolymer nanodiscs generated by using amphipathic copolymers to directly solubilize membrane proteins into polymer-lipid-membrane protein complexes. (**c**) Peptide nanodiscs stabilized with amphipathic peptides that wrap around lipid bilayers. (**d**) Salipro derived from saposin proteins that encapsulate membrane proteins in a lipid environment. (Icons used in figure were generated using BioRender.)

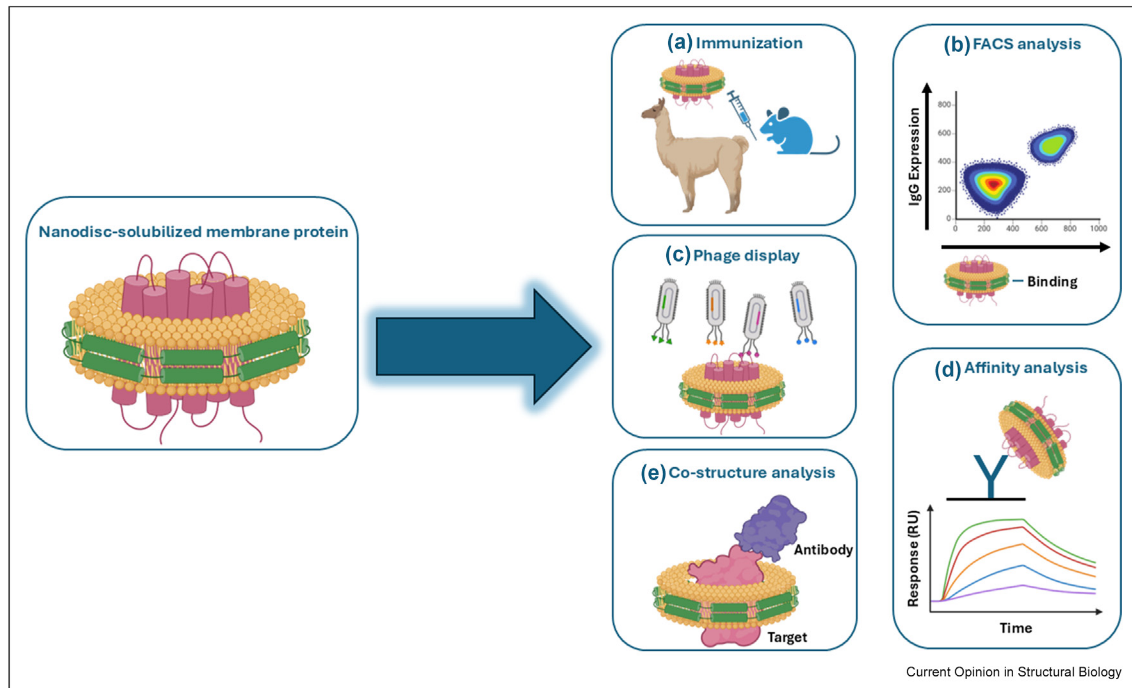
Nanodiscs also provide the stability required for large multisubunit complexes, such as ion channels and multisubunit receptors, allowing antibody discovery against targets previously thought too complex [14]. Additionally, the ability to work with native-like proteins can streamline the drug discovery process, as antibodies identified using these formulations are more likely to retain efficacy *in vivo*.

In this review we will summarize recent advances in antibody discovery using nanodiscs to display the target membrane protein antigen in a soluble format. We will use the terms antibody and nanobody interchangeably, with nanobodies representing heavy -chain -only antibodies from camelids or display libraries. The focus is on key antibody discovery processes that enable lead antibody selection, including immunization, binder identification, affinity, and costructure analysis (Figure 2). In contrast with cell- and detergent-based methods, we aim to showcase the potential of nanodiscs to expedite therapeutic antibody discovery.

Immunization

Discovery of target specific antibodies often relies on the generation of an immune response in animals. Multipass membrane proteins pose unique challenges as immunogens due to their often low expression, limited solvent exposure of loop epitopes, and the potential for structural perturbation caused by detergent extraction [15,16]. Whole-cell immunization allows the protein to be presented in a native environment but is dependent on expression level, the size of the extracellular region displayed on the cell surface, and the downstream screening strategy to determine specificity over other proteins displayed by the cell [17,18]. DNA-based immunization offers a cleaner approach but is reliant on robust protein expression [19]. Virus-like particles (VLPs), display antigens in a multivalent format and are used in vaccines to elicit a robust response, such as with the human papillomavirus vaccine [20,21]. Like cell or DNA immunizations, VLPs can present membrane proteins in their native conformation and are reliant on the expression levels of the target membrane protein [22]. GLUT4, a membrane protein with small extracellular loops, has historically presented challenges in discovery of loop-specific antibodies. Immunization of transgenic animals with GLUT4 VLPs resulted in the successful generation of conformation-specific antibodies to be used as tools to monitor function and trafficking [23]. As an alternative to full-length protein immunogens, peptide immunogens representing extracellular domains are a common immunization method, but peptides may not be conformationally relevant and are epitope-dependent [24,25]. Protein engineering can

Figure 2



Nanodiscs enable diverse screening platforms used in antibody discovery. When conformationally relevant membrane proteins are incorporated into nanodiscs (left panel), they can be used in many antibody discovery processes historically reliant on soluble proteins. Shown here are representative methods utilizing membrane mimetics in antibody discovery. **(a) Immunization** of animals with nanodisc-incorporated membrane proteins stimulates an immune response and generation of antigen-specific antibodies. To identify antibodies that bind to the target membrane proteins, fluorescence-activated cell sorting (**FACS) analysis (b)** of cells displaying antibodies on their surface is performed. Cell types can include primary B cells, hybridoma, and yeast display libraries. **(c) Phage display** is an alternative method used to identify antibodies that bind specifically to the target membrane protein. Nanodiscs can be utilized for **affinity analysis (d)** to understand the strength of the binding interaction between the antibody and target membrane protein (e.g. surface plasmon resonance - SPR). **(e) Costructural analysis** allows for further understanding of antibody-target protein interactions through the identification of amino acid residues of the antibody that interact with the target (i.e. the paratope) and residues on the target protein that are bound by the antibody (i.e. epitope). (Icons used in figure were generated using BioRender.)

display known epitopes as fusion proteins, but prior structural knowledge is needed [26].

Immunization of membrane proteins in soluble mimetics can overcome expression, specificity and conformation concerns. For example, the human apelin receptor (APJ) is a class A GPCR with a short N-terminal domain and normally intractable to functional antibody discovery. Using conformationally stabilized APJ protein reconstituted into nanodiscs to immunize camels, scientists identified functional nanobodies that engaged ECL2 of APJ [27]. Similarly, the influenza matrix-2 (M2) protein and HIV gp41 protein membrane-proximal external region (MPER) are infectious disease targets that have seen limited success with peptide-focused approaches due to poor antibody titers. By leveraging the lipid-bilayer of nanodiscs to present these antigens, neutralizing antibodies were discovered where soluble peptide approaches failed [28,29]. It is important to note that the ability of nanodisc material to induce an immune response with antibodies targeting relevant epitopes is reliant on selection of a membrane mimetic that displays structurally relevant protein approximating a native environment. Immunization of animals with membrane proteins in a non-native form risks generation of an immune response to irrelevant epitopes.

Binder identification

Following successful generation of an immune response, antibodies secreted from isolated B cells are screened for antigen binding, and the responsible VH (variable region of the heavy chain) and VL (variable region of the light chain) genes sequenced for recombinant antibody production. Given the often-poor immune response to membrane proteins, screening of hundreds of thousands of B cells to find rare antigen-specific clones is generally required. Today there exists B cell technologies that expedite the identification of antigen-specific B cells and allow for the screening of thousands to millions of cells. These include microfluidic platforms, microencapsulation, nanowell capture, hybridoma technology, and fluorescence-activated cell sorting (FACS) [30–35].

Display technologies, such as phage and yeast display, offer an alternative to direct interrogation of B cells. Combinatorial libraries used in display, contain $>10^9$ antibody fragments generated from naïve, immunized, or synthetic VH and VL genes and enable identification of rare binders not amenable to B cell screening [36,37].

B cell screening and display methods generally rely on a soluble probe to identify antigen-specific cells through binding of labeled target protein. Although detergent-solubilized membrane proteins or cells expressing complex membrane targets can be used, the success depends on the expression level of the protein, its stability, counter-screening for nonspecific binding to cellular components, and the amenability of the assay for use of whole cells. The ability of nanodiscs to display complex membrane proteins in a conformationally relevant soluble format enables screening platforms and overcomes many of the issues encountered by use of cells or protein fragments [28,38,39]. This was demonstrated by Dominik et al. [38], who compared the use of nanodisc-based phage sorting to detergent-based phage sorting of their target membrane protein. The nanodisc phage sorting was more robust and rapid compared to detergent-based phage sorting, which required extensive optimization on a per-target basis. Importantly, nanodisc phage sorting identified more high-affinity binders and binders whose epitope was not impacted by the presence of detergent. Qiang et al. [40] similarly incorporated a voltage-insensitive cation channel into nanodiscs and used phage to identify a functional antibody of high specificity. Beyond phage display, specific binding of nanodiscs to antibodies displayed on yeast and hybridoma cells has been demonstrated, providing a framework for antibody screening by FACS [14,41]. For example, nanodiscs encompassing a model ion channel (VSD4-NavAb) were used to perform flow cytometric sorting of VSD4-NavAb-specific hybridoma cells [14]. Following the single-cell sorting of the hybridoma, the resultant antibodies were screened for binding to VSD4-NavAb proteoliposomes to demonstrate specific binding to the VSD4-NavAb protein and not to the nanodisc material itself. Velappan et al. [42], used nanodiscs containing the M2 protein from influenza A to assess domain-specific binding by yeast display. They showed by FACS that the M2-containing nanodiscs bound to yeast displaying M2 binding antibodies and not to an irrelevant control antibody. The inclusion of negative controls for counter-screening are key to the correct identification of target-specific antibodies. This is especially important if the nanodisc material was the immunogen as the resultant antibody response could be against the target membrane protein or against the nanodisc material (e.g. MSP, lipids, polymers). Ideally, target-specific binding of lead antibodies is confirmed by FACS of cells expressing target versus nonexpressing control cells.

Affinity characterization

Following binder discovery, characterization of the antibody—antigen interaction aids selection of lead molecules. Affinity measurements are used to determine the strength of interaction, but common techniques such as surface plasmon resonance (SPR) or biolayer interferometry (BLI) rely on the availability of monodispersed soluble proteins to generate high quality data. Affinity values using whole cells can be accurately determined using the equilibrium-based kinetic exclusion fluorescence immunoassay (KinExA) [45]. However, this approach can be limited by throughput and tends to determine the avidity of interaction based on two-arm binding of the antibody.

The ability of nanodiscs to present multipass membrane proteins as soluble proteins provides the opportunity to investigate affinity using methods such as SPR or BLI. Indeed, Sarkar et al., [46], demonstrated that their SMALP-solubilized GPCR protein could be stably immobilized on an SPR chip and used to characterize the binding affinity of their extracellular domain (ECD)-specific antibody. Similarly, Ma et al. [27] captured their membrane protein-embedded nanodiscs onto an SPR chip to identify key residues involved in binding and to determine the relative affinity of their nanobody mutants. The opposite orientation was used by Yu et al. [28], whereby their lead nanobody was immobilized onto an SPR chip and the nanodisc-incorporated target injected as analyte to determine the dissociation constant (K_D) of the monovalent interaction. To facilitate affinity studies of small molecule binding to a target membrane protein, Nakagawa et al. [47] developed an antibody that binds to the MSP protein of nanodiscs. Using the MSP-specific antibody, they demonstrated capture of their protein-embedded nanodiscs on an SPR chip and subsequent kinetic analysis of ligand binding to their target membrane protein.

Structural analysis

Structural information of antibody—membrane protein complexes can be leveraged for antibody optimization and discovery. For instance, structure-guided rational design strategy has successfully led to the conversion of an orthosteric single-domain antibody (sdAb) antagonist to an sdAb with potent agonist activity [27]. Cryo-electron microscopy (cryo-EM) has recently emerged as a powerful tool in antibody discovery, from epitope mapping to elucidation of mechanisms of action [48,49]. While membrane proteins in detergent are routinely used in cryo-EM studies, nanodiscs hold significant promise especially in studying interactions between the membrane protein and lipid bilayer [50—52]. High-resolution structures of membrane proteins in nanodiscs have also been pivotal for understanding their functional mechanisms, which were previously not

evident in structures solved in detergent, including but not limited to, ion channels, transporters, and GPCRs [53–55]. Recent structural determination of tetrameric forms of the serotonin-gated ion channel 5-HT3AR stabilized in Salipro further demonstrated the potential of nanodisc in stabilizing physiological relevant membrane protein conformation or assembly, presenting great opportunities for innovative antibody discovery [56]. Building on recent advances in deep learning and computational structure-based antibody design [57], cryo-EM structures of membrane proteins in nanodiscs are poised to provide unprecedented advantages in antibody discovery, given that the understanding of membrane protein conformations in native lipid bilayers is critical to rational drug design.

Future directions and challenges

The development of nanodiscs has allowed scientists to develop novel strategies to interrogate antibody binding to complex membrane proteins. One such assay, SMA-PAGE, is a method that combines SMALP polymer nanodiscs with native gel electrophoresis to detect membrane protein complexes extracted from their native state in cells [58]. In using SMA-PAGE and target-specific antibodies, Clark et al. [59] probed the oligomerization state of their target membrane protein when extracted from human platelet cells. To further enable antibody screening, direct labeling of the nanodisc protein or chemical moiety prior to membrane target incorporation spares labeling of the target protein antigen and potential masking of key epitopes [38,43,44]. The ability to directly label nanodiscs, the publishing of detailed protocols [38], and the commercial availability of common target proteins imbedded in nanodiscs will see a rapid increase in the use of nanodiscs for antibody binder discovery.

While membrane protein nanodiscs have proven successful in antibody discovery [60], challenges remain. Preparing membrane protein nanodiscs can be complex, labor-intensive, expensive, and hard to scale up. Reconstitution of membrane proteins into MSP nanodiscs requires solubilization and purification of the membrane protein first in detergents, while screening of optimal reconstitution conditions must be performed. Despite the promising advancements in synthetic copolymers for directly extracting and purifying membrane proteins, low solubilization efficiency and the lack of a universal solution remain significant challenges. Although membrane protein nanodiscs aim to replicate the natural environment, they are still artificial systems. One must be cautious about the potential deviations of a nanodisc system from the native cellular membrane. Limited nanodisc size and altered lipid packing may affect membrane curvature and structural integrity of membrane proteins, potentially leading to the discovery of antibodies that behave differently *in vivo* [61–64]. Inappropriate lipid composition in nanodiscs may affect accessibility of epitopes and bias conformations of membrane proteins, resulting in the loss of key epitopes during antibody discovery or distortion of the epitope–paratope interface [65,66].

Future directions include tailoring target specific lipid composition, developing next-generation synthetic polymers, and integrating artificial intelligence into screening processes. Although new nanodisc technologies such as circularized nanodiscs and DNA-corralled nanodiscs [67–69] have enabled the accommodation of larger and more complex membrane protein assemblies, their application in antibody discovery remains underexplored. As nanodisc preparation methods improve and more high-throughput, automated platforms become available, nanodiscs will likely become an essential tool in antibody discovery for a wider array of membrane proteins.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Copilot to improve the language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

The authors declare that they have no financial interests or personal relationships that could cause a conflict of interest regarding this article except as disclosed. XY and CT are employees of Amgen and own Amgen stock.

Data availability

No data was used for the research described in the article.

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