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Review

Applications of Synthetic Polymer Discoidal Lipid Nanoparticles to Biomedical Research

Masafumi Tanaka

Laboratory of Functional Molecular Chemistry, Kobe Pharmaceutical University; 4–19–1 Motoyamakita-machi, Higashinada-ku, Kobe 658–8558, Japan.
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Liposomes are artificially prepared vesicular lipid nanoparticles with a bilayer structure, resembling cell membrane. Their ability to encapsulate various molecules along with excellent biocompatibility makes them ideal delivery vehicles for pharmaceuticals. They can also serve as platforms for membrane proteins to elucidate the structure and function in lipid membranes. Nascent high-density lipoproteins are discoidal lipid nanoparticles with a bilayer structure, which can be reconstituted with their constituents. Such reconstituted nanoparticles, nanodisks, were originally generated in terms of elucidation for mechanisms of lipoprotein metabolisms. At the same time, like liposomes, nanodisks have been developed as delivery vehicles and platforms for membrane proteins in structural biology. From a developmental background, apolipoproteins, their analogs, or fragment peptides were initially used as scaffolding molecules to wrap around the edge of the disk-shaped lipid bilayer. Since the discovery that styrene-maleic acid copolymers produce nanodisks instead of apolipoproteins, variously modified or novel polymers have been synthesized to broaden the applications of polymer nanodisks. This review provides an overview of the types of synthetic polymers used to produce nanodisks, and the biomedical applications of nanodisks to the developments of delivery vehicles and to the structural studies of membrane proteins.

Key words discoidal lipid nanoparticle; synthetic polymer; delivery vehicle; membrane protein

1. Introduction

Discoidal nanoparticles of the phospholipid bilayer architecture provide a novel lipidic platform distinct from well-defined conventional micelles and vesicles. Such discoidal particles are originally derived from the field of lipid biochemistry,[1] but have evolved in the field of structural biology.[2] The terms "nanodisc" and "nanodisk" are found in the literature when referring to discoidal phospholipid bilayer nanoparticles. The term "nanodisc" occasionally represents commercially available discoidal particles proposed by Dr. Stephen G. Sligar, which consists of membrane scaffold proteins (MSP), a tool for membrane protein studies. Thus, "nanodisk" is used in the present review.

Historically, studies of discoidal nanoparticles arose in lipoprotein research.[3] Native high-density lipoproteins (HDL) can be largely classified into nascent and matured HDL, whose morphologies are discoidal and spherical, respectively. In the case of the former, apolipoprotein (apo) A-I, which is the major protein constituent of HDL, wraps around the edge of the disk-shaped phospholipid bilayer with the long axis perpendicular to the acyl chains. To understand the structure and function of endogenous HDL in relation to the prevention of coronary artery diseases, reconstituted nanodisks mimicking nascent HDL have been extensively investigated.[4]

There are typically two methods to generate nanodisks: self-assembly method and detergent dialysis method (Fig. 1). Self-assembly method is simple and quick. Apolipoproteins—not only apoA-I but also apoE or apoCs in a lipid-free state incubated with phospholipid vesicles—under certain conditions spontaneously associate and form nanodisks. However, available lipids are limited at least in the preparation by apolipoproteins. In contrast, detergent dialysis method contains a relatively laborious process. Ternary mixtures of phospholipids, apolipoproteins, and detergents are temporarily prepared, after which detergents are removed to form nanodisks. This method has a broad spectrum of available lipids, but the time-consuming detergent removal step by exhaustive dialysis or adsorption onto hydrophobic beads is inevitable. In both methods, as it is likely that lipid-free or lipid-poor apolipoproteins and residual lipids unincorporated into nanodisks can contaminate, purification, such as by size exclusion chromatography, is desired according to the aim of use.

To further elucidate the structure–function relationship of apolipoproteins, fragments of apolipoprotein and mimetic peptides have been alternatively used instead of full-length apolipoproteins. For example, the ability to activate lecithin-cholesterol acyltransferase (LCAT) was tested using nanodisks formed with apoA-I deletion mutants.[5] Within the apoA-I molecule, carboxy terminus and additional regions were found to be important for lipid-binding and the direct activation of LCAT, respectively. Also, the ability of synthetic peptides to form discoidal nanoparticles was examined to reveal the structural requirements.[6] An amphipathic helical segment with high lipid affinity, or at least two helical segments punc-

e-mail: masatnk@kobepharma-u.ac.jp

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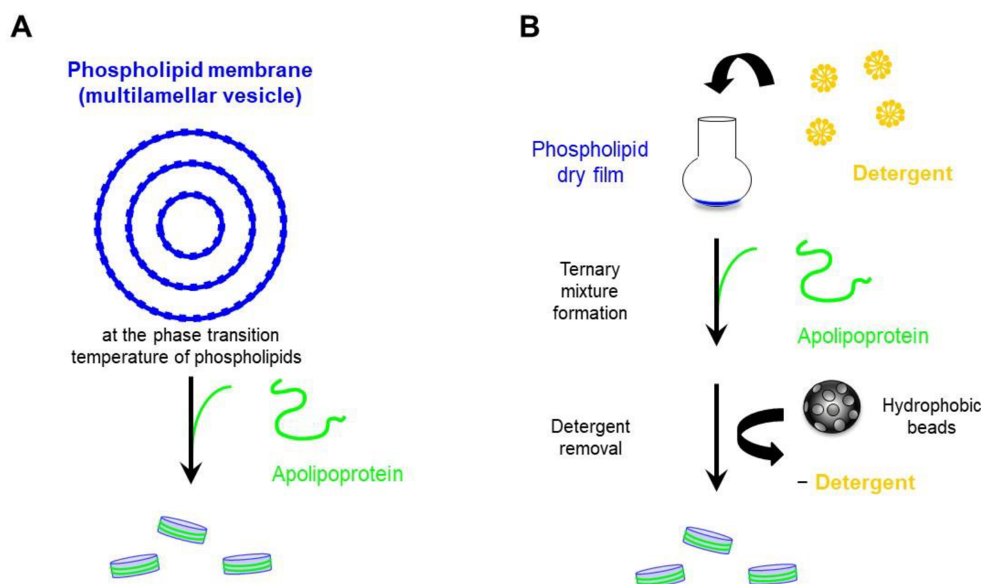

Fig. 1. Representative Reconstitution Methods for Lipid Nanodisks

(A) Self-assembly method. Incubation of apolipoproteins with phospholipid membranes (multilamellar vesicles in this case) at the gel to liquid-crystalline phase transition temperature spontaneously generates lipid nanodisks. (B) Detergent dialysis method. Temporary formation of ternary mixtures of phospholipids, apolipoproteins, and detergents followed by detergent removal generates lipid nanodisks. Hydrophobic beads are sometimes employed during the dialysis process to efficiently adsorb detergents. Note that the "detergent dialysis method" is occasionally represented as the "self-assembly method" in some literature because self-assembly of lipid nanodisks occurs during the detergent dialysis.

tuated by proline, is required for the stable reconstitution of nanodisks.[7] Conversely, based on these results, it became possible to enhance the functionality and improve the stability of reconstituted HDL by adding modifications to scaffolding molecules. Equally, since HDL is an inverse predictor of coronary artery diseases, intravenous infusion of reconstituted HDL has been attempted to bring about cardioprotective effects.[8] Additionally, the inherent properties of native HDL that deliver lipophilic molecules in blood circulation prompted researchers to develop lipid nanodisks as delivery vehicles, which would be biocompatible in nature.

Meanwhile, MSP, which is a modified form of apoA-I, was exploited as material for lipid nanodisks.[9] It was conceivable that if membrane proteins were included together with the ternary mixture during the detergent dialysis process, the membrane proteins would reside in the bilayer—their own location. Based on this assumption, from the very beginning, the purpose of MSP nanodisk production was dedicated to provide membrane proteins with a native-like membrane environment. Various uniformly sized MSP nanodisks, which can accommodate proteins possessing only one transmembrane helix, oligomeric proteins such as G-protein-coupled receptors, and larger molecular complexes, were prepared by selecting a combination of MSP construct, phospholipid species, and the compositional stoichiometry.[10] MSP nanodisks became one of the most promising platforms for membrane proteins, and the number of structural studies using MSP nanodisks is still increasing. However, the addition of detergent is prerequisite for the incorporation of membrane proteins into MSP nanodisks, which may perturb the native lipid environment, lead to a non-physiological conformation, and cause a loss in function of the proteins.

Apolipoproteins have an amphipathic nature, which enables them to wrap around the periphery of nanodisks, in which the hydrophobic faces orient themselves toward lipids, and the hydrophilic faces orient themselves toward the aqueous phase. Styrene-maleic acid copolymer (SMA), one of the amphiphilic synthetic polymers, has been shown to produce nanodisks in a similar manner to apolipoproteins.[11] In SMA nanodisks, the polymer encircles the edge of the disk-shaped phospholipid bilayer with the styrene moieties intercalating between the lipid acyl chains.[12] It is notable that nanodisk formation *via* the self-assembly method by apolipoproteins only occurs at the gel to liquid-crystalline phase transition temperature of phospholipids, which limits the available lipids. This is also the case with MSP, which compels detergent dialysis method for incorporation of membrane proteins into MSP nanodisks. Whereas, spontaneous nanodisk formation occurs merely by adding SMA into phospholipids, irrespective of the phase transition temperature, enabling detergent-free isolation of membrane proteins. Consequently, SMA is capable of extracting membrane proteins even from biological membranes.[13] Furthermore, for use in nanodisk formations, molecular structures of SMA were modified and other synthetic polymers have been successively discovered.

In the present review, recent advances in the development of polymer nanodisks are described initially. Subsequently, biomedical applications of lipid nanodisks as delivery vehicles for a wide variety of pharmaceuticals and membrane mimetics for the structural biology of membrane proteins are enumerated, comparing the conventional nanodisks comprised of apolipoprotein analogs with polymer nanodisks.

2. Types of Polymer Nanodisks

The idea that SMA can be used for the production of nanodisks was already introduced in review article as early as 2001,[14] but may have been spread by a report in 2009, which found that SMA/lipid particle (SMALP) preserved the integrity of transmembrane proteins.[15] The term "lipodisq" was also used to describe lipid-polymer complexes, but is now a

product name of SMA itself (provided from Sigma-Aldrich).[16] In the present review, the term "SMA nanodisk" is used to describe discoidal nanoparticles complexed with SMA.

Spontaneous formation of SMA nanodisks can be monitored by the turbidity clearance due to the conversion (solubilization) of lipid vesicles (in the order of hundreds of nm) into smaller discoidal particles (typically 10 nm). Changes in light scattering intensity upon the addition of SMA were spectroscopically investigated using artificial and biological lipid membranes. Upon solubilization, SMA showed no significant lipid preference from homogeneously mixed membranes, but had a strong preference to the fluid phase lipids from phase-separated membranes.[17] As SMA nanodisk formation is quite simple, only few parameters, such as buffer conditions (pH and ionic strength), SMA to lipid ratios, and incubation temperature can be variable. Once solubilized, SMA nanodisks can be easily purified using standard methods, such as size exclusion chromatography to remove excess lipids and SMA. However, there are some drawbacks of SMA nanodisks, especially when they are intended to incorporate membrane proteins. For example, SMA nanodisks are unstable under low pH conditions or in the presence of divalent metal ions, probably because carboxy groups in the maleic acid moiety are protonated by the acidification or chelated with divalent cations.

Thereafter, to improve the colloidal properties depending on the aim of the study, various polymers to form nanodisks have been newly synthesized. Even SMA itself has some varieties, such as molar ratio of styrene to maleic acid, the order of their alignment, length, *etc*. For example, hydrophobicity of SMA can be adjusted by changing the ratio of styrene to maleic acid, which affects the membrane solubilization efficiencies. Although the styrene to maleic acid ratio between 2 : 1 and 3 : 1 is typically selected, the ratio of 2 : 1 is considered the most efficient in solubilization.[18] Nanodisks with approximately the same size were prepared by SMA possessing different lengths. On the other hand, shorter SMA solubilized lipid vesicles with the highest efficiency, and the resulting nanodisks formed by longer SMA were more stable than those formed by shorter SMA.[19] Charge of SMA also plays significant roles in nanodisk formation. SMA modified with ethylenediamine to yield zwitterionic form solubilized lipid vesicles to produce stable nanodisks, except in the pH range of 5–7.[20] By varying the ratio of lipid to polymer, SMA modified with ethanolamine produced not only typical 10 nm nanodisks but also 50 nm nanodisks that can spontaneously align in an external magnetic field.[21]

To avoid spectroscopic absorbance interference in the UV region by styrene aromatic group, diisobutylene-maleic acid copolymer (DIBMA) was presented to show equal performance to SMA in solubilizing lipid vesicles to form nanodisks.[22] It was supposed that DIBMA becomes applicable to the solubilization of biological membranes by increasing the ionic strength or lowering the pH of the solvent.[23] Similarly to SMA, the influence of DIBMA length on nanodisk formation was investigated further and the optimum length was suggested.[24] Another copolymer with maleic acid subunit, stilbene-maleic acid copolymer, which contains a single repeating unit of diphenylethylene and maleic acid, solubilized biological membranes to form homogeneous nanodisks by increasing the rigidity of the polymer.[25]

Other polymers than randomly alternating copolymers containing maleic acid residues, as mentioned above, have also been used for nanodisk formation. Although poly(2-ethylacrylic acid) (PEAA) was suggested to form nanodisks a few decades ago,[26] there has been limited progress in the development of PEAA nanodisks for a while since then. In order to suppress strong UV absorption in a similar way to DIBMA, polymethacrylate copolymer (PMA) was employed to form polymer nanodisks with no aromatic groups.[27] The design was further expanded to polyacrylic acid (PAA) with varying hydrophobic groups, and it was found that the choice of hydrophobic group greatly affects solubilization properties.[28] Moreover, PAA carrying cyclic rather than linear aliphatic side groups enhanced the ability to solubilize membranes.[29] The development of a variety of polymers to form nanodisks will broaden the possibility of selecting the suitable membrane mimetics for individual experiments.

All amphiphilic polymers do not necessarily form nanodisks. For example, amphiphilic polymers, such as polyvinylpyrrolidone (PVP) and Pluronic F-127, which are widely employed in the pharmaceutical field, did not form nanodisks, at least under our experimental conditions.[30] We also failed to use cationic polymers, such as poly-L-lysine and poly-L-arginine, for nanodisk formation. However, it is plausible that the buffer composition—such as salt concentration and pH or incubation temperature—were inappropriate for membrane solubilization. Indeed, it has been reported previously that PMA did not solubilize dimyristoylphosphatidylcholine (DMPC) vesicles in a 10 mM sodium phosphate buffer (pH 7.4), which is inconsistent with the success mentioned above.[27] Also, membrane components and their charges affect solubilization. In fact, the degree of solubilization induced by SMA was unchanged by the mixing ratio of negatively charged lipids, whereas it was inversely correlated to the mixing ratio of positively charged lipids.[31] Molecular mechanisms of nanodisk formation pathways are currently being elucidated computationally using molecular dynamics simulations[32] or experimentally, such as by small-angle X-ray scattering.[33] Even though it still requires trial and error to find the optimal conditions for solubilization, rational strategy to reconstitute polymer nanodisks using different types of polymers for different purposes would be possible in the future.

3. Application of Nanodisks to the Development of Delivery Vehicles

A variety of substances including drugs, nucleic acids, signal emitting molecules, or dyes can be loaded into lipid nanodisks (Table 1). Particularly, bioactive compounds with poor water solubility, such as amphotericin B, all-*trans*-retinoic acid, and curcumin, were successfully incorporated into nanodisks stabilized by apoA-I or apoE.[34–36] Although loading efficiencies of the compounds depends on the compatibility with lipid compositions of nanodisks, they appear to be generally high (>70%). In addition, drugs incorporated into nanodisks normally exert biological activity. For example, nanodisks containing amphotericin B were less toxic toward erythrocytes and cultured hepatoma cells, but were effective in mice infected with *Candida albicans*. Likewise, nanodisks containing curcumin inhibited hepatoma cell growth more effectively than free curcumin. Furthermore, nanodisks containing all-*trans*-retinoic acid were relatively stable to long-term storage at 4 °C in the dark. These advantages offer the poten-

Table 1. Application Examples of Lipid Nanodisks for Delivery Vehicles

Payload	Scaffolding molecule	Lipid	Reference
Amphotericin B	ApoA-I	DMPC/DMPG (7/3)	34)
All-*trans*-retinoic acid	ApoE	DMPC/DMPG (7/3)	35)
Curcumin	ApoA-I	DMPC	36)
siRNA	ApoA-I	DMPC/DMTAP (7/3)	38)
Self-replicating mRNA	ApoA-I or apoE	Zwitterionic lipids with cationic lipids	39)
Gd^{3+}	Apolipoproteins purified from human HDL	POPC	40)
^{64}Cu	MSP	POPC	41)
^{89}Zr	ApoA-I	DMPC	42)
Gadolinium chelates fluorescent dyes	ApoA-I	DPPC	43)
10-Hydroxycamptothecin	ApoA-I mimetic peptide	Egg sphingomyelin	44)
Neoantigen	ApoA-I mimetic peptide	DMPC	46)
Doxorubicin	SMA	DMPC	49)

DMPG; dimyristoylphosphatidylglycerol, DMTAP; dimyristoyltrimethylaminopropane, POPC; 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, DPPC; dipalmitoylphosphatidylcholine.

tial for future therapeutic applications of nanodisks as novel drug delivery vehicles. However, it must be remembered that the loading capacity of drugs in lipid nanodisks is intrinsically limited because it is impossible to keep the discoidal shape with a bilayer structure when hydrophobic drugs partition into the bilayer interior.

Systemic delivery of nucleic acids, such as small interfering RNA (siRNA), has attracted significant attention for therapeutic applications. However, the use of RNA-based drugs has been hampered mainly by the instability of naked RNA. Thus, the development of delivery systems of RNA is necessary to overcome this problem. Because native HDLs have been found to carry endogenous microRNA,[37] it is hypothesized that lipid nanodisks can also be delivery vehicles for RNA. In general, cationic vehicles have been shown to increase siRNA stability. In fact, incorporation of synthetic cationic lipid into nanodisks enhanced the binding capabilities to siRNA and the resulting complexes showed target gene knockdown activity.[38] Recently, cationic nanodisks complexed with large self-replicating mRNAs achieved a higher *in vivo* transfection efficiency.[39] Continuous advances will lead to the clinical use of lipid nanodisks as a promising platform for gene therapy and vaccine development in the future.

In addition, lipid nanodisks can be a platform for medical imaging modalities for diagnostic purposes. Magnetic resonance imaging (MRI) was performed using apoA-I nanodisks labeled with Gd^{3+}, a paramagnetic metal ion frequently involved in clinically approved contrast agents, to visualize the atherosclerotic lesions of apoE knockout mice.[40] Enhancement of MR signals was observed successfully depending on the plaque macrophage content. Positron emission tomography (PET) imaging was performed using MSP nanodisks labeled with ^{64}Cu to analyze the biodistribution in tumor-bearing mice.[41] The accumulation of intravenously administered nanodisks into tumor tissue was achieved by a long blood circulation allowing extravasation through the leaky tumor vasculature. ApoA-I nanodisks labeled with ^{89}Zr were developed to selectively target tumor-associated macrophages (TAMs), which are a major component of the tumor microenvironment, contributing to tumor progression and metastasis.[42] Specificity for TAM was confirmed by *ex vivo* histologic analysis after PET imaging in an orthotopic mouse model of breast cancer. Optical imaging is highly sensitive although its use is usually restricted to dissected tissues or tissues near the surface of small animals due to the limited penetration depth of light. To satisfy both high sensitivity and excellent spatial and temporal resolution for multimodal imaging, apoA-I nanodisks with a high payload of gadolinium chelates and fluorescent dyes were reconstituted using the advantages of facile integration into versatile nanoplatform.[43] *In vivo* multimodal imaging and targeting of tumor blood vessels were achieved by functionalization of nanodisks with Arg-Gly-Asp (RGD) peptides, which was corroborated by the *ex vivo* confocal microscopy.

Since pure apolipoproteins and MSP are usually manufactured either through plasma purification or recombinantly, protein-based nanodisks are undesirable for clinical applications because of safety and productivity. Instead, nanodisks comprised of synthetic peptide were developed as potential alternatives. Nanodisks stabilized by apoA-I mimetic peptide exhibited loading efficiency of 10-hydroxycamptothecin (HCPT) up to 70% by the selection of lipid composition.[44] Consequently, IC_{50} of HCPT-loaded nanodisks were approximately three-fold lower than that of free HCPT. To enhance the affinity to the target receptor by increasing the number of ligands per particle, peptide-based nanodisks were prepared using a shorter form of apoE including low-density lipoprotein receptor-binding region (LDLRBR) instead of the full-length apoE.[45] As anticipated, the uptake of LDLRBR nanodisks into LDL receptor-expressing cells was higher than that of apoE nanodisks, suggesting enhanced targeting efficiency. Peptide-based nanodisks carrying tumor-specific neoantigens have been developed for a novel immunotherapeutic approach against gliomas.[46] Used in combination with immune checkpoint inhibitor, neoantigen-loaded nanodisks elicited potent anti-tumor effects in an orthotopic murine glioma model. Synthetic peptide could enable the manufacture of homogeneous, stable, safe, and functional nanodisks of pharmaceutical grade.

To date, few studies have investigated the development of SMA or other polymer nanodisks as delivery vehicles. One of the advantages of SMA nanodisks for potential therapeutic utility is that they are vulnerable to pH reduction, which may serve to disintegrate and release payload at acidic microenvironments. Control of the pH at the disintegration occurs may be possible by altering the lipid composition; as succeeded in SMA-containing lipid emulsions.[47] Furthermore, SMA nanodisks injected into mice behaved in a manner similar to

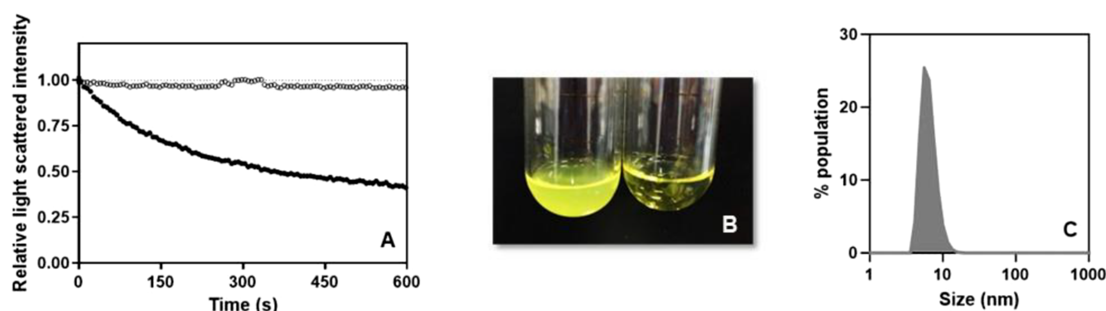

Fig. 2. Formation of SMA Nanodisks Containing Curcumin Induced by Solubilization of 1-Palmitoyl-2-oleoyl-phosphatidylcholine (POPC) Vesicles

(A) Effect of SMA addition on the light scattering intensity of POPC vesicles containing curcumin at 25 °C (open circles for no SMA, closed circles for with SMA). Time-dependent changes in the right-angle light scattering intensity was monitored at a wavelength of 600 nm. (B) POPC vesicles containing curcumin before (left) and after (right) the addition of SMA. (C) Size distribution of SMA nanodisks containing curcumin at 25 °C.

apoA-I nanodisks, suggesting that SMA nanodisks are biocompatible.[48] However, until now, the incorporation of drugs into SMA nanodisks has not been well researched in comparison with nanodisks comprised of apolipoproteins or their mimetic peptides. Recently, one study investigated the formation of SMA nanodisks containing doxorubicin, and their cytotoxicity and biodistribution were examined.[49] Preliminary data also suggests that curcumin and camptothecin can be successfully incorporated into SMA nanodisks with an approximate size of 10 nm by the self-assembly method (Fig. 2).

4. Application of Nanodisks to Structural Studies of Membrane Proteins

Membrane proteins perform essential biological functions *in vivo*, thus continue to be primary targets of small-molecule drugs. Structural determination of membrane proteins in membranous environments are required to understand their functions and for the structure-based drug design. Reconstitution into liposomes or bicelles is one of the most commonly-used methods for reconstructing membrane proteins. However, during their reconstitution process, detergents or detergent-like short chain lipids, which tend to denature many membrane proteins, are required. They also destabilize the intrinsic interactions of membrane proteins with membrane lipids. In addition, instability, heterogeneity, and low reproducibility of liposomes are often problematic when structurally determining membrane proteins. Consequently, lipid nanodisks, which can provide a native bilayer environment, are useful for structural studies of membrane proteins.

NMR is a powerful technique for the structural determination of membrane proteins. The first solid-state NMR study of an integral membrane protein embedded in MSP nanodisks was reported in 2007.[50] The secondary structure of uniformly ^{13}C-,^{15}N- labeled CYP3A4 in nanodisks, which remained enzymatically active, was consistent with that reported by crystallography. Subsequently, a membrane-spanning fragment of human CD4 incorporated into MSP nanodisks was investigated by solution NMR, which relies on rapid isotropic overall tumbling of the sample.[51] Controlling the size of nanodisks is essential to obtain resonances with the narrowest line widths. Using peptide-based nanodisks, the effects of size on NMR spectroscopy were compared by changing the lipid to peptide molar ratio.[52] For solution NMR, depending on the target protein, a protocol on the nanodisk assembly was further optimized by constructing a set of truncated MSP variants that produce smaller nanodisks.[53]

Recent advances in single-particle cryo-electron microscopy (cryo-EM) enable the structural determination of membrane proteins at near-atomic resolution without crystallization. To obtain high resolution cryo-EM images, samples should be as homogeneous as possible. For this purpose, lipid nanodisks are the favorable platform to accommodate a single target protein. Cryo-EM structure of the bacterial ribosome-SecYEG complex incorporated into apoA-I nanodisks is one of the first examples elucidated at sub-nanometer resolution.[54] General approaches for reconstituting membrane proteins into MSP nanodisks for single-particle cryo-EM are established and well summarized in the literature.[55] For an optimal reconstitution, not only the selection of MSP construct, lipids, and detergents but also their molar ratios to the membrane protein need to be taken care of. Coupled with the development of hardware and software, structures of a wide variety of membrane proteins have been revealed by cryo-EM studies one after another. The structure of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) ORF3a in MSP nanodisks recently determined by cryo-EM in conjunction with its non-selective cation channel activity observed *in vitro* could provide a new target for treating coronavirus disease 2019 (Covid-19).[56]

The use of polymer nanodisks for structural studies of membrane proteins was initiated since the first introduction of SMALP in 2009. One of the advantages of SMA as scaffolding molecules is that spontaneous nanodisk formation occurs without detergent, irrespective of lipid compositions, suggesting that it can be applied to the extraction of native-like membrane proteins from biological membranes. Several factors influence the solubilization of membrane proteins by SMA, but the optimized conditions for solubilization from *Escherichia coli* membranes are tentatively recommended.[57] SMA nanodisks are used not only for the structural determination of membrane proteins but also for identification and quantification of the lipid composition surrounding membrane proteins.[58] While NMR is still useful for structural determination of membrane proteins in polymer nanodisks, its frequency of use is relatively low mainly due to the limitations derived from the still quite large size and inhomogeneity of the nanodisks.[59] Polymer nanodisks play a key role in a growing number of the membrane protein structures deposited in the Protein Data Bank solved by single-particle cryo-EM, as summarized in the literature.[60]

It is evident that conformation of a membrane protein is af-

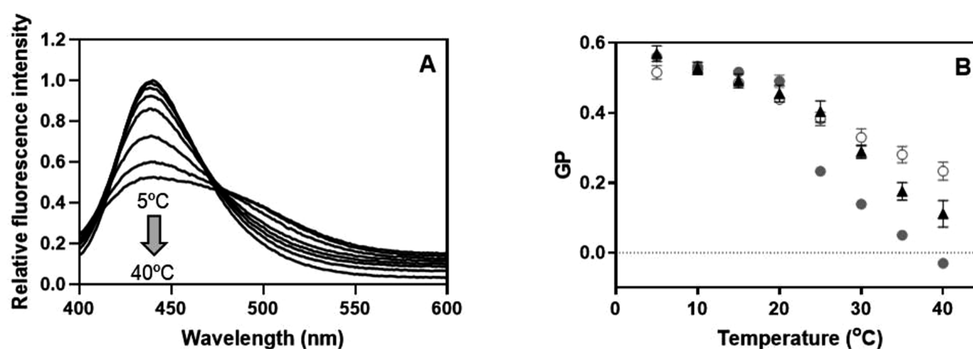

Fig. 3. Phase Transition Behavior of DMPC

(A) Laurdan emission spectra, which is sensitive to the polarity and dynamics of the environment, in PEAA nanodisks. (B) Generalized polarization (GP) values of Laurdan measured in PEAA nanodisks (closed triangles) as a function of temperature. These values were obtained by calculating the dual wavelength ratio of the emission intensities at 440 nm and 490 nm. Data of SMA nanodisks (open circles) and DMPC vesicles (closed circles) are presented for comparison.[30)]

fected by membrane fluidity, which can be controlled by lipid composition of the lipid nanodisks.[61)] Electron paramagnetic resonance experiments with spin-labeled lipids clearly indicate that lipids within MSP nanodisks are more tightly packed than those in liposomes.[62)] Thus, it is conceivable that membrane fluidity can be altered by the changes in the lipid composition and also the selection of scaffolding molecules. In fact, PEAA nanodisks exhibited a phase transition behavior intermediate between liposomes and SMA nanodisks (Fig. 3). It should also be noted that polymer nanodisks rapidly exchange lipids much faster than other conventional membrane mimetics.[63)] Even the polymer themselves are readily exchanged among nanodisks.[64)] Such dynamic properties of polymer nanodisks need to be fully understood for in-depth structural studies of membrane proteins.

5. Perspectives

Nanodisk technology is becoming increasingly important for the provision of excellent platforms for the development of delivery vehicles and for structural studies of membrane proteins. To overcome new technical obstacles and meet endless demands, different materials suitable to reconstitute nanodisks have been produced and are still being researched and developed. For example, MSP was covalently linked its amino and carboxy termini to produce circularized nanodisks, which exhibited high homogeneity in size and shape and significantly improved stability.[65)] A similar idea can be applied to synthetic polymers as well. Even small-molecule amphiphiles have been found to directly assemble lipids and membrane proteins to form native nanodisks.[66)] These results could prove inspirational for novel polymer design. Although such recent pioneering works on scaffolding molecules are generally for structural studies of membrane proteins, these nanodisks would change the drug loading capability or the *in vivo* metabolic pathway as delivery vehicles. Transdisciplinary research ranging from polymer chemistry, lipid biophysics, structural biology, to pharmaceutics create a synergetic effect, which allows the options to be increased for the selection of nanodisks appropriate for individual biomedical research.

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Conflict of Interest The author declares no conflict of interest.

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