

Improved pendant-bearing glucose-neopentyl glycols (P-GNGs) for membrane protein stability

Taeyeol Youn,^{†a} Ganghee Kim,^{†a} Parameswaran Hariharan,^b Xianglan Li,^c Waqar Ahmed,^a Bernadette Byrne,^d Xiangyu Liu,^c Lan Guan,^b and Pil Seok Chae^{*a}

^a Department of Bionano Engineering, Hanyang University ERICA, Ansan 155-88, South Korea

^b Department of Cell Physiology and Molecular Biophysics, Center for Membrane Protein Research, School of Medicine, Texas Tech University Health Sciences Center, Lubbock, Texas 79430, United States

^c Tsinghua-Peking Center for Life Sciences, Beijing Frontier Research Center for Biological Structure, Beijing Advanced Innovation Center for Structural Biology, School of Medicine, School of Pharmaceutical Sciences, Tsinghua University, Beijing 100084, China

^d Department of Life Sciences, Imperial College London, London SW7 2AZ, U.K.

[†]These authors contributed equally.

Abstract

Membrane proteins are biologically and pharmaceutically significant, and determining their 3D structures requires a membrane-mimetic system to maintain protein stability. Detergent micelles are widely used as membrane mimetics; however, their dynamic structures often lead to the denaturation and aggregation of encapsulated membrane proteins. To address the limitations of classical detergents in stabilizing membrane proteins, we previously reported a class of glucose-neopentyl glycols (GNGs) and their pendant-bearing versions (P-GNGs), several of which proved more effective than DDM in stabilizing membrane proteins. In this study, we synthesized additional GNG derivatives by varying the lengths of the pendant (P-GNGs), and by introducing hemi-fluorinated pendants to the GNG scaffold (fluorinated pendant-bearing GNGs, or FP-GNGs). The synthetic flexibility of the GNG chemical architecture allowed us to create a diverse range of derivatives, essential for effective optimizing of detergent properties effectively. When tested with two model membrane proteins (a transporter and a G-protein coupled receptor (GPCR)), most of the new (F)P-GNGs demonstrated superior stabilization of these membrane proteins compared to DDM, original GNG (OGNG)), and a previously developed P-GNG (i.e., GNG-3,14). Notably, several P-GNGs synthesized in this study were as effective as or even better than lauryl maltose neopentyl glycol (LMNG) in stabilizing a human GPCR, beta2 adrenergic receptor (β_2 AR). Enhanced protein stability was particularly observed for the P-GNGs with a butyl (C4) or pentyl (C5) pendant, indicating that these pendant sizes are optimal for membrane protein stability. The volumes of these pendants appear to minimize the empty spaces in the micelle interiors, thereby enhancing *detergent-detergent* interactions in micelles complexed with the membrane proteins. Additionally, we identified one FP-GNG that was more efficient at extracting the transporter and more effective at stabilizing the GPCR than DDM. Thus, the current study demonstrates that both chain length and number of fluorine atoms in the pendants of the P-GNGs were crucial determinants for membrane protein stability. We not only developed a few (F)P-GNGs that are significantly more effective than maltoside detergents (LMNG/DDM) for protein extraction and stability, but we also provided an effective strategy for detergent design through the utilization of hemi-fluorinated pendants of varying length.

Introduction

Membrane proteins residing within biological membranes perform critical functions, including material transport, signal transduction and cell-cell communications.¹ In humans, malfunctions of these proteins are implicated in various diseases such as asthma, cancer, Alzheimer's, and Parkinson's, underscoring their additional importance in drug development.² Over 50% of current drugs target this class of bio-macromolecules, making detailed understanding of their high-resolution 3D structures essential for structure-based drug design.^{3,4} Despite their significant roles in biological research and drug development, progress in elucidating membrane protein structures remains slow, even with recent technical advances. As of October 30, 2024, only around 2,000 out of a total 210,000 known protein structures represent unique

membrane proteins.⁵ The challenges in determining membrane protein structures stem from their intrinsic amphiphilic nature. Unlike soluble proteins, membrane proteins have a hydrophobic region that is embedded in membranes, flanked by two hydrophilic regions that project into aqueous environment on either side of the membrane. When the hydrophobic regions are exposed to the aqueous environment, membrane proteins tend to denature and/or aggregate, complicating structural characterization.⁶⁻⁸ Therefore, *in-vitro* studies of membrane proteins require membrane mimetics capable of encapsulating the hydrophobic protein surface, thus maintaining membrane protein stability in aqueous conditions.^{9,10} The first crystal structure of membrane protein was obtained for *Rhodospseudomonas viridis* photosynthetic assembly using the detergent lauryldimethylamine-*N*-oxide (LDAO) in 1985.¹¹ Since then, micelles formed by conventional detergents, such as *n*-octyl- β -D-glucoside (OG) and *n*-dodecyl- β -D-maltoside (DDM)—small amphiphilic molecules—have been widely used for membrane protein structure determination.^{12,13} Similar to phospholipids in cell membranes, these conventional detergents possess both hydrophilic and hydrophobic moieties, enabling them to form self-assemblies in water. However, due to their conical shapes, they form globular micelles rather than planar bilayers.^{14,15} Detergent micelles can associate with the hydrophobic surfaces of membrane proteins in aqueous environments, resulting in protein-detergent complexes (PDCs) that serve as starting materials for *in-vitro* studies of membrane protein structures and functions.¹²

Despite the availability of more than 100 conventional detergents, they often fail to stabilize membrane proteins, particularly G protein-coupled receptors (GPCRs) and large membrane protein complexes.¹⁶ The highly dynamic nature of micelles formed by conventional detergents compared to lipid bilayers leads to the instability of encapsulated membrane proteins.¹⁷ Over the past two decades, various membrane mimetic systems have been developed to address the limitations of conventional detergents.^{18,19} Notable examples include bicelles,^{20,21} nanodiscs (NDs) derived from membrane scaffold protein (MSP),^{22,23} styrene-maleic acid (SMA) copolymer,^{24,25} or saposin A,²⁶ and peptide-based detergents such as lipopeptide detergents (LPDs)²⁷ and beta-peptides (BPs).²⁸ Those alternatives that closely mimic lipid bilayers have proven highly effective in maintaining the native structures of membrane proteins. However, they are typically inefficient at extracting proteins from the membranes and often show poor compatibility with protein crystallization.²⁹ In contrast, detergent micelles have the advantages of being applicable across all stages of *in-vitro* membrane proteins studies from protein extraction and solubilization to purification and structure determination.³⁰ Additionally, detergent micelles are compatible with various methods for protein structure determination, including X-ray crystallography, cryo-electron microscopy (cryo-EM), and nuclear magnetic resonance (NMR) spectroscopy. Therefore, developing new detergents with enhanced efficacy for protein stabilization is crucial to advancing membrane protein research.³¹ Significant efforts have been made over the past two decades to develop novel detergents, leading to the creation of various amphiphilic molecules, including facial amphiphiles (FAs),^{32,33} oligoglycerol detergents (OGDs),³⁴ neopentyl glycol-based amphiphiles [e.g., maltose neopentyl glycols (MNGs),^{35,36} and glucose neopentyl glycols (GNGs)^{37,38}], rigid hydrophobic group-bearing amphiphiles [e.g., chobimalt³⁹ and glyco-diosgenin (GDN)⁴⁰], tandem amphiphiles [tandem neopentyl glycol maltosides (TNMs),⁴¹ tandem malonate-derived glucoside (TMG),⁴² tandem facial amphiphiles (TFAs),⁴³ and tandem triazine-based maltosides (TZMs)⁴⁴], and triazine-based detergents [1,3,5-triazine-based di-maltosides (TEMs)⁴⁵ and melamine-based glucosides (MGs)⁴⁶]. These novel detergents have shown superiority to the gold standard DDM in terms of membrane protein stability. In particular, LMNG has been used in over 300 structural studies of membrane proteins, including more than 150 GPCRs, since its introduction in 2010, highlighting the significant impact of novel detergents in membrane protein structure determination.⁴⁷

The first class of GNG amphiphiles we reported previously contains a branched diglucoside head group and two identical alkyl chains.³⁷ Although these GNGs, glucoside versions of LMNG, were generally not as effective as DDM in stabilizing membrane proteins, one representative (OGNG) has facilitated structure determinations of 10 membrane proteins since its introduction in 2012.⁴⁸⁻⁵¹ We recently reported a second generation of GNGs featuring two different alkyl chains — one long main chain and a small pendant (methyl, ethyl, or propyl).⁵² These pendant-bearing GNGs (P-GNGs) demonstrated superior performance compared to DDM in stabilizing various membrane proteins, with the best

Commented [BB1]: This phrase is not clear. I think your work and that of others has changed the field and so if we talk about conventional detergents I think they need to be defined (single hydrophilic head group and single hydrophobic tail)

results observed for propyl pendant-bearing GNGs (e.g., GNG-3,14). In the current study, we prepared additional P-GNG derivatives by introducing longer pendants (butyl, pentyl, or hexyl) into the detergent scaffold. This design was based on our hypothesis that increasing the pendant length of P-GNGs would enhance the hydrophobic density in the micelle interiors, thereby promoting protein stabilization. We also synthesized a new set of GNGs by incorporating a hemi-fluorinated alkyl chain as a pendant into the P-GNG scaffold, designated fluorinated pendant-bearing GNGs (FP-GNGs). When tested with two model membrane proteins [a transporter and a GPCR], several (F)P-GNGs outperformed DDM, the original OGNG, and previously developed GNG-3,14 in stabilizing these proteins. Notably, some butyl or pentyl pendant-bearing GNGs prepared in this study were even superior or comparable to LMNG in stabilizing the GPCR. The enhanced *detergent-detergent* interactions achieved through pendant elongation likely contribute to the significantly improved efficacy of these P-GNGs for protein stabilization.

Results

Rationale for new detergent design

Original GNG (OGNG) tends to form smaller PDCs compared to DDM.³⁷ This property endows OGNG with high compatibility for membrane protein crystallization, as evidenced by the fact that all 10 membrane protein structures determined using this detergent were obtained via the *in surfo* crystallization method.⁴⁷⁻⁴⁹ However, due to its limited efficacy in stabilizing membrane proteins, OGNG has primarily been useful in the structural studies of relatively robust membrane proteins, such as channels and transporters, rather than aggregation-prone GPCRs. The limited protein stabilization efficacy of OGNG is believed to originate, at least in part, from its shorter hydrophobic length compared to DDM and LMNG (C8 vs. C12), the two most widely used detergents for membrane protein structural studies. For this reason, we strategically lengthened the hydrophobic region of GNGs in our previous study by introducing two different alkyl chains: one long main chain (C11/C12/C13/C14) and one short pendant (methyl [C1]/ethyl [C2]/propyl [C3]).⁵² When tested with membrane proteins, these P-GNGs, which have sufficient hydrophobic lengths, generally outperformed DDM in terms of protein stability. The efficacy of the P-GNGs for protein stabilization tended to improve with increasing pendant length, with the best performance detected for the C3 pendant-bearing GNGs (GNG-3,13 and GNG-3,14). We hypothesized that the propyl pendant effectively fills empty spaces in the micelle interiors, thereby increasing the alkyl chain density of micelle interiors and enhanced protein stability. However, it is not possible to determine *a priori* which chain — propyl (C3) or a longer alkyl chain — will provide the best fit in these micellar spaces. Thus, in the current study, we prepared new P-GNGs having longer alkyl pendants (butyl [C4], pentyl [C5] and hexyl [C6]) than the propyl chain (C3). The main chains of the new P-GNGs varied from C12 to C15 based on our previous findings, which indicates that membrane protein stability increased with longer main chain lengths from C11 to C14. By varying both the main (C12 to C15) and pendant chain lengths (C4 to C6), we synthesized nearly 20 new P-GNGs. The structures of these new detergents, along with those of previously developed P-GNGs, are depicted in [Figure S1](#), according to their main and pendant chain lengths. The newly prepared P-GNGs were designated based on lengths of their main and pendant chains, as exemplified by GNG-4,15, which has a C4 (butyl) pendant and a C15 main chain. Additionally, we prepared a second set of P-GNGs with hemi-fluorinated pendants (FP-GNGs) to investigate the impact of this fluorine-bearing pendant on membrane protein stability within the P-GNG architecture. This set of detergents belongs to hemifluorinated surfactants (HFSs),⁵³ but the FP-GNGs represent the first examples that incorporate a hemi-fluorinated chain as a detergent pendant. HFSs are known to maintain the subtle integrity of membrane protein complexes due to the reduced tendency of hemi-fluorinated chains to dissolve lipophilic cofactors embedded in protein interiors or lipids specifically bound onto membrane protein surfaces, compared to their hydrocarbon counterparts.^{54,55} The unique properties of HFSs arise from the hydrophobic yet lipophobic nature of fluorinated chains. The three FP-GNGs prepared in this study contain the same number of total carbons (C21) in the hydrophobic region, allowing us to derive instructive detergent design principles from their comparative evaluation with membrane proteins. Of note, these detergents were designated to indicate the lengths of both the fluorinated and hydrogenated components within the pendant chain, as well as the main chain length. For instance, GNG-F1,2,15 contains a trifluoromethyl ethyl (CF₃-CH₂-

CH₂-) pendant and a C15 main chain.

The hydrophilic-hydrophobic balance (HLB) is a primary property influencing membrane protein stability. It has been reported that detergents with a narrow range of HLB (11.5 to 13.5) are favorable for membrane protein stability, as exemplified by those of OG (12.3), DDM (13.4) and LMNG (13.6).^{56, 57} When calculated according to Griffin's method,⁵⁸ the new P-GNGs yielded HLB values ranging from 10.5 to 11.5, lower than those of conventional detergents (OG and DDM), except for the monopod GNGs (GNG-0,12, GNG-0,13, GNG-0,14, and GNG-0,15) (Figure 1). It is important to note that HLB values were not calculated for the FP-GNGs, as Griffin's method is inapplicable to a fluorinated chain-bearing detergent. The low HLB values indicate that the new P-GNGs are substantially more hydrophobic than DDM and LMNG. As we will discuss shortly, several P-GNGs prepared in this study were comparable to or more effective than DDM and LMNG in stabilizing membrane proteins (*vide infra*). Therefore, based on the current results, the established range of optimal HLB values (11.5 to 13.5) known to promote membrane protein stability should be revised to include the HLB values of these P-GNGs [i.e., 10.5 to 13.5]. The previously recognized optimal HLB range (11.5 to 13.5) for protein stability were based on the results of conventional detergents with simple structures and thus is unlikely apply to novel detergents with more complex architecture. The high hydrophobicity of the new P-GNGs compared to DDM and LMNG is likely favorably associated with membrane protein stability through the promotion of effective hydrophobic interactions between detergent molecules in PDCs.

Detergent synthesis and physical characterization

The new P-GNGs were prepared with slight modifications to a previously reported protocol.⁵² The FP-GNGs were synthesized using hemi-fluorinated alkyl chains instead of hydrocarbon chains. All P-GNGs exhibited a single doublet peak at 4.31/4.32 ppm in their individual NMR spectra, with a vicinal coupling constant of $J = 8.0$ Hz (Figures 2a & S2a), confirming β -stereochemistry of the two glycosidic bonds present in these GNGs. The ¹H NMR spectra of the FP-GNGs were similar to those of the P-GNGs, with the most notable difference being the presence of resonance signals corresponding to the CF₂-neighboring protons (CH₂-CF₂/CF₃ or CH₂-CH₂-CF₂/CF₃). These signals appeared as multiplets in the ranges of 2.00 to 2.20 ppm and 1.40 to 1.68 ppm (Figure S3a), respectively. We then measured the water-solubility of the new detergents at room temperature, as summarized in Table 1. The (F)P-GNGs with relatively short pendants (C1 to C4) exhibited more than 5.0 wt% water solubility. However, as the pendant length increased to C5/C6, the water-solubility of the detergents tended to decrease to less than 5.0 wt%. For instance, the pentyl pendant-bearing GNG-5,13, GNG-5,14 and GNG-5,15 gave approximately 5.0 wt% water-solubility, while the hexyl pendant-bearing GNG-6,13, GNG-6,14 and GNG-6,15 had water-solubility of around 1.0 % or less. Interestingly, the monopod GNGs (GNG-0s) exhibited varying water-solubility depending on the main chain length. GNG-0,12 and GNG-0,13 were 10 wt% water-soluble, whereas GNG-0,14 and GNG-0,15 were around 1.0 wt% soluble in water. The three FP-GNGs demonstrated good water-solubility (5.0/10 wt%). We did not evaluate the poorly water-soluble P-GNGs (less than 5.0 wt%) for membrane protein stability as these GNGs are unlikely to be useful for membrane protein studies.

Assemblies formed by the (F)P-GNGs were investigated using NMR spectroscopy. When we changed the NMR solvent from MeOD to D₂O, the proton signals of GNG-4,15 were largely broadened, accompanied by small downfield-shifts, indicating the formation of self-assemblies in water (Figure 2a). Interestingly, the methyl peaks between the main and pendant chains were well differentiated upon self-assembly formation, with the methyl signal of the pendant shifting further down-fielded than that of the main chain. Similar changes were observed in the ¹H NMR spectra of GNG-5,14 and GNG-F1,2,15 in MeOD and D₂O (Figures S2 & S3). The resolution of NMR peaks for GNG-5,14 was rather poor compared to GNG-4,15 and GNG-F1,2,15, suggesting that this GNG forms rather large self-assemblies in water. The (F)P-GNG assemblies were then further characterized by their critical micelle concentrations (CMC) and self-assembly sizes in water at room temperature, determined using the hydrophobic dye [diphenylhexatriene (DPH)] and dynamic light scattering (DLS), respectively.⁵⁹ As summarized in Table 1, the new P-GNGs exhibited a narrow range of CMCs (0.01 to 0.025 mM) despite substantial variations in the lengths of their main and pendant chains. These CMCs are comparable to those of LMNG (0.01 mM) and the previously reported GNG-3,14 (0.025 mM), but significantly lower

than those of DDM (0.17 mM) and OGNG (1.0 mM). The CMCs of the monopod GNGs were higher than those of pendant-bearing counterparts and tended to decrease with increasing main chain length. Specifically, GNG-0,12, GNG-0,13, GNG-0,14 and GNG-0,15 were estimated to have CMCs of 0.20, 0.08, 0.03, and 0.02 mM, respectively. The CMCs of the FP-GNGs were higher than or comparable to those of their hydrocarbon counterparts depending on the number of fluorine atoms in the pendant region. The pentafluoroethyl (CF_3CF_2)-ended pendant-bearing GNG (GNG-F2,2,14) exhibited a CMC of 0.035 mM, higher than that of GNG-4,14 (0.025 mM). The FP-GNGs with the trifluoromethyl (CF_3)-terminated pendant (GNG-F1,3,14 and GNG-F1,2,15) showed CMCs of 0.025 and 0.020 mM, respectively, comparable to their hydrocarbon counterparts (GNG-4,14 at 0.025 mM and GNG-3,15 at 0.015 mM). Previous studies have indicated that the presence of a fluorinated chain/segment in the hydrophobic region tends to increase detergent CMC due to its lipophobic nature;⁶⁰ however, this effect was not observed for the trifluoromethyl (CF_3)-ended pendant-bearing GNGs in this study. The incorporation of a relatively small fluorinated group (CF_3) at the hydrophilic-lipophilic interface is likely a reason for their distinctive behaviors.

Next, we measured micelle sizes formed by the new detergents at 1.0 wt%, estimated by the hydrodynamic radii (R_h) of the micelles. Micelles formed by the new P-GNGs tended to increase in size with the main chain length; R_h values were determined to be 3.2 nm (GNG-4,12), 3.2 nm (GNG-4,13), 3.4 nm (GNG-4,14), and 3.7 nm (GNG-4,15). In contrast, the pendant chain length appeared to have little effect on micelle size unless it increased beyond a C4 pendant (Figure 2b). For example, the micelle sizes formed by the C15 main chain P-GNGs remained relatively unchanged (3.7 to 3.8 nm) as the pendant chain length extended from C0 (no pendant) to C1 (methyl) to C2 (ethyl) to C3 (propyl) to C4 (butyl). A similar trend was observed for previously described P-GNGs with the C1/C2/C3 pendant.⁵² However, when the pendant length was increased to C5 (pentyl) or C6 (hexyl), we noted a dramatic increase in micelle sizes (Figure 2b). For example, the C5 pendant-bearing GNGs (GNG-5,13, GNG-5,14 and GNG-5,15) exhibited R_h values of 24, 27 and 73 nm, respectively, significantly larger than their C4 pendant-bearing counterparts (GNG-4,13 at 3.2, GNG-4,14 at 3.4 and GNG-4,15 at 3.7 nm). This interesting trend can be explained by the relative size/volume of the pendants compared to the empty spaces in the micelle interiors formed by the corresponding monopod GNGs (Figure 1c; $m = -3$). In other words, the micelle sizes remained relatively constant for the GNGs with the C1/C2/C3/C4 pendants, as the empty spaces in the micelle interiors formed by the monopod GNGs were large enough to accommodate these relatively small pendant chains. However, these empty spaces appear limited to accommodate larger pendants (C5/C6). The size/volume mismatch between the empty spaces in the micelle interiors and the larger pendants (C5/C6) would induce micelle restructuring to relieve steric hindrance exerted by the large pendants, resulting in micelle expansions. Together, the butyl (C4) pendant appears optimal for filling the empty spaces within the micelle interiors, thereby maximizing alkyl chain density in this region, while maintaining a small micelle size. For the FP-GNGs, GNG-F1,3,14 and GNG-F1,2,15 formed small micelles with R_h values of 3.3 and 3.8 nm, respectively, comparable to their hydrocarbon counterparts (GNG-4,14 at 3.4 nm and GNG-3,15 at 3.7 nm). This result indicates that the empty spaces in the micelle interiors formed by GNG-0,14 or GNG-0,15 are sufficient to accommodate the CF_3 -ended butyl or propyl pendant. In contrast, GNG-F2,2,14 formed substantially larger micelles (9.6 nm) than its hydrocarbon counterpart (GNG-4,14 at 3.4 nm), suggesting that the CF_3CF_2 -ended butyl pendant is too large to fit into the empty spaces of the micelle interior formed by the corresponding monopod GNG-0,14. When we investigate the size distribution of micelle populations, micelles formed by all the (F)P-GNGs showed unimodal distributions (Figures S4 and S5). The sizes of micelles formed by the (F)P-GNGs were further examined by varying the detergent concentration and solution temperature, using the selected detergents (GNG-4,15, GNG-5,14, and GNG-F1,2,15), along with DDM and LMNG as references. As expected, the micelle size formed by DDM showed little variation with changes in detergent concentration from 0.5 to 2.0 wt% or solution temperature from 15 to 65 °C (Figure 2c,d). Similar behavior was observed for the small micelle-forming GNGs (GNG-4,15 and GNG-F1,2,15). Interestingly, micelle sizes formed by GNG-4,15 tended to slightly increase with increasing solution temperature. In contrast, the micelle size formed by LMNG tended to increase with increasing detergent concentration and decrease with increasing solution temperature, consistent with previous results.^{46,47} Similar trends were observed for the large micelle-forming detergent, GNG-5,14. These results suggest that a strong dependency

of micelle size on detergent concentration and/or solution temperature is a typical characteristic of detergents that forms large micelles.

Detergent evaluation with model membrane proteins

We began detergent evaluation with the prokaryotic transporter melibiose permease from *Salmonella typhimurium* (MelB_{St}).⁶¹⁻⁶³ MelB_{St}, overexpressed in *E. coli* membranes, was extracted using 1.5 wt% of DDM, GNG-3,14, and the individual GNGs for 90 minutes at 0 °C. The MelB extracts were then incubated at elevated temperatures (45, 55, or 65 °C) for an additional 90 minutes. The amounts of solubilized MelB_{St} were measured using Western blot and density quantification following ultracentrifugation and presented as percentages of the total MelB_{St} present in the untreated membranes. The amounts of soluble MelB_{St} extracted at low temperature (0 °C) directly indicate how efficiently a detergent extracts proteins from the membranes. On the other hand, the amounts of soluble MelB_{St} incubated at increased temperatures (45, 55, or 65 °C) correlates with the effect of detergents on protein stability. Thus, this temperature-ramp protocol effectively estimates a detergent's ability to both extract membrane protein and maintain it in a stable state, two crucial properties for membrane protein studies. At 0 °C, DDM yielded a quantitative amount of soluble MelB_{St}, consistent with its extensive use for membrane protein extraction. The previously reported GNG-3,14 resulted in approximately 75% soluble MelB_{St}. All new P-GNGs, except for GNG-0,12, were more efficient than GNG-3,14 at extracting MelB_{St}, indicating that the P-GNG architecture is highly compatible with membrane protein extraction. When the incubation temperature increased to 45 °C, the monopod GNG (GNG-0,12 and GNG-0,13) showed poor solubilization yields of MelB_{St} (~10 and ~20%, respectively), suggesting that the transporter encapsulated by these monopod GNGs may have denatured or aggregated. The other P-GNGs, including the previous GNG-3,14, yielded amounts of soluble MelB_{St} comparable to DDM (~100%), except for GNG-4,14 (~70%). When the incubation temperature further increased to 55 °C, the amounts of soluble MelB_{St} varied significantly, primarily depending on pendant chain length. At this high temperature, DDM and the monopod GNGs failed to stabilize the transporter, resulting in less than 10% soluble MelB_{St}, whereas previous GNG-3,14 yielded around 50% of soluble MelB_{St}. Notably, most P-GNGs with C13/C14/C15 main chains and C4/C5 pendant chains were generally more effective than DDM and GNG-3,14 at stabilizing the transporter. For instance, GNG-4,13, GNG-5,13, GNG-5,14, and GNG-4,15 retained MelB solubility in a range of 60 to 90%. The use of the C6 pendant-bearing GNG (i.e., GNG-6,12) or C12 main chain P-GNGs (e.g., GNG-4,12) resulted in less than 10 or 30% soluble MelB_{St}, respectively. These results suggest that the optimal lengths for MelB_{St} stability lie in the range of C13 to C15 for the main and C4 to C5 for the pendant chain. Importantly, the optimal length of the pendant chain for MelB_{St} stability was nearly the same irrespective of the main chain length: the C4 pendant length was optimal for the P-GNGs with the C12, C13, or C15 main chains (e.g., GNG-4,13 and GNG-4,15), while the C5 pendant length was most suitable for the P-GNGs with the C14 main chain. The significant dependency of detergent efficacy on pendant length for protein stabilization highlights the importance of alkyl chain density at the hydrophilic-lipophilic interfaces of detergent micelles in maintaining membrane protein stability.

When we examined the fluorinated pendant-bearing GNGs under the same conditions, these FP-GNGs exhibited a wide range of behaviors depending on the specific structures of the pendants. GNG-F2,2,14 yielded approximately 60% soluble MelB_{St} following protein extraction at 0 °C, but increasing the incubation temperature above 45 °C completely diminished MelB_{St} solubility and stability. This suggests that this CF₃CF₂-ended pendant-bearing GNG is unlikely to be suitable for membrane protein manipulation. In contrast, when GNG-F1,3,14 and GNG-F1,2,15 were used at 0 °C, these CF₃-ended pendant-bearing GNGs proved to be more efficient at extracting the transporter than DDM and the other P-GNGs from the membranes. This is notable, as many hemifluorinated surfactants (HFSs) have been shown to be less effective than their hydrocarbon counterparts for membrane protein extraction.⁶⁴ When the incubation temperature was increased to 55 °C, the transporter solubilized in GNG-F1,3,14 underwent denaturation/aggregation, resulting in approximately 10% solubility, similar to that observed with DDM. In contrast, the transporter solubilized by GNG-F1,2,15 fully retained its solubility even at this high temperature, demonstrating superior efficacy for MelB stabilization compared to DDM and the other P-GNGs. Overall, this CF₃-based GNG-F1,2,15 outperformed both the other (F)P-

GNGs in terms of extraction and stabilization of MelB_{St}. The presence of a small number of fluorine atoms (-CF₃) in the pendant chain likely contributes to the markedly improved performance of GNG-F1,2,15, suggesting that a well-designed HFS can serve as an effective tool for both protein extraction and stabilization.

The promising properties of the new detergents with MelB_{St} led us to evaluate their abilities to stabilize a more challenging membrane protein, β_2 AR.⁶⁵ This human G protein-coupled receptor (GPCR) was extracted using DDM and purified in 0.01% LMNG. The LMNG-purified β_2 AR was then diluted into buffer solutions containing the individual detergents (DDM, GNG-3,14 and (F)P-GNGs) at a final concentration of 0.1 wt%. OGNG and LMNG served as additional controls. Receptor stability in each detergent was assessed by monitoring the binding of the radio-labeled antagonist ([³H]-dihydroalprenolol (DHA)) at regular intervals over an 8-day incubation period at room temperature.⁶⁶ The receptor in DDM showed poor binding ability to DHA after detergent exchange from LMNG and lost this ability rapidly over time. Consistent with previous results, OGNG was also not effective at retaining GPCR ligand-binding capability.^{67,68} As expected, LMNG allowed the receptor to bind DHA effectively at day 0, but this activity was gradually lost over time even for this gold standard. The previously described GNG-3,14 demonstrated greater receptor activity than DDM and OGNG over time, though it was slightly less effective than LMNG. The new P-GNGs exhibited variable receptor stability based on the lengths of their pendant and main chains. For the P-GNG series with identical pendant groups, receptor stabilization peaked with the main chain length of C14/C15. Among the C4 pendant-bearing GNGs, for instance, GNG-4,14 and GNG-4,15 were the most effective, followed by GNG-4,13 and GNG-4,12. A similar trend was observed for the P-GNGs bearing the C3/C5 pendant; GNG-3,14 and GNG-5,14 were the most effective of the C3 and C5 pendant-bearing GNGs, respectively. More importantly, detergent efficacy for receptor stabilization improved with pendant chain length increasing from C1 to C5 within the same main chain series. For instance, among the C14 main chain GNGs, GNG-5,14 was the most effective, followed by GNG-4,14 and GNG-3,14. A similar pattern was found with the C15 main chain GNG, though GNG-5,15 was slightly less effective than GNG-4,15. Across all main chain length (C12/C13/C14/C15), the optimal pendant length for the P-GNGs for receptor stability were C4 or C5, indicating that the C14/C15 main chains combined with the C4/C5 pendants were the best for receptor stability. The exact same trend was observed for the evaluation of the P-GNGs for MelB stability. The best P-GNG for long-term β_2 AR stability was GNG-5,14, followed by GNG-4,15, GNG-5,13, and GNG-4,14. These detergents showed comparable or superior receptor stability compared to LMNG, a significant achievement given the prevalent use of LMNG in GPCR structural studies.⁴⁷ When examining the FP-GNGs under the same conditions, we observed considerable variation in detergent efficacy based on their specific structure. Among the FP-GNGs, GNG-F2,2,14 exhibited the least effectiveness for long-term receptor stabilization, comparable to DDM. However, the CF₃-ended pendant-bearing GNGs (GNG-F1,3,14 and GNG-F1,2,15) demonstrated significantly improved efficacy. GNG-F1,3,14 was notably better than DDM, but less effective than its hydrocarbon counterpart, GNG-4,14. Intriguingly, GNG-F1,2,15 excelled at stabilizing the receptor, performing significantly better than both DDM and its hydrocarbon counterpart (GNG-3,15), and was nearly comparable to LMNG. This result is particularly noteworthy given the scarcity of HFSs that support GPCR stability.⁶⁹

Discussion

We initiated our research with an aim of enhancing detergent efficacy for protein stabilization by either extending the pendant length of previous P-GNGs or incorporating fluorinated alkyl groups into the pendant region. The newly prepared GNGs feature pendants have varying chain lengths (P-GNGs) or differing numbers of fluorine atoms (FP-GNGs). The synthetic simplicity (only four synthetic steps) and flexibility for structural modification allowed us to efficiently prepare a large number of P-GNGs with diverse structural variations, enabling the optimization of detergent properties for membrane protein studies. In the evaluation with MelB_{St} and β_2 AR, we observed the generally favorable effect of the new P-GNGs on protein stability as the main chain length increased from C12/C13 to C14 or C15. This is likely due to the improved compatibility of these longer main chain lengths (C14/C15) with the hydrophobic dimensions of the membrane proteins. Notably, detergent efficacy for protein stabilization tended to improve significantly as the

pendant chain length gradually increased from C0 to C4, with a clear, consistent trend observed for both MelB_{SI} and β_2 AR. In contrast, despite variations in pendant chain length, micelle sizes formed by the P-GNGs were similar, ranging from 3.1 to 3.7 nm. These results suggest that the pendant chain length, rather than micelle size, plays a dominant role in determining membrane protein stability for this scaffold. When the pendant chain length increased further from C4 to C5 or C6, detergent efficacy showed little improvement (C5) or decreased (C6). Together, the results from both the current study and our previous reports demonstrate that the optimal pendant length for effective membrane protein stabilization by the P-GNGs lies between C4 and C5.

A question arises when comparing the effects of pendant chain length on micelle size versus protein stability. Micelle size formed by the P-GNGs increased abruptly when the pendant chain length increased from C4 to C5, suggesting that the size of the C4 pendant is comparable to the available space in the micelle interiors formed by the monopod GNGs. Thus, it is reasonable to expect that the C4 pendant-bearing P-GNGs are most suitable for stabilizing membrane proteins compared to other pendant variants. This is supported by the best performance observed for protein stability in the P-GNGs with the C13/C15 main chain, specifically with the C4 pendant-bearing GNGs, as demonstrated by GNG-4,13 (MelB_{SI}), GNG-4,15 (MelB_{SI}), and GNG-4,15 (β_2 AR). However, in some cases, the P-GNGs with the C5 pendant exhibited overall superior stability for membrane proteins, as seen with GNG-5,13 (β_2 AR) and GNG-5,14 (MelB_{SI} and β_2 AR). We propose a couple of possible explanation for this unexpected result. First, since the membrane protein stability assays were conducted in buffered solutions rather than pure water, the micelle sizes of the P-GNGs in the buffered solutions rather than in pure water are more relevant to the discussion. We measured the micelle sizes of the P-GNGs in HEPES buffer (20 mM, pH 7.4) and observed a similar trend in micelle size variation with pendant length, as seen in pure water. Therefore, the variation in optimal pendant length between dense micelle formation (C4) and enhanced protein stability (C4/C5) is unlikely to stem from difference in the experimental medium (pure water vs. buffered solution). Second, the micellar architecture in PDCs may differ substantially from that of pure detergent micelles. Upon association with membrane protein surfaces, detergent molecules in micelles rearrange to find optimal positions and conformations. This micelle remodeling could generate empty spaces in the micelle interiors that differ in size from those in pure micelles. In other words, the C5 pendant might fit better into the interiors of micelles associated with membrane proteins, even though the C4 pendant was optimal for pure GNG micelles. Thus, information from pure detergent micelles may only partially reflect the detergent properties relevant to membrane proteins. As another example, the sizes of PDCs are often unrelated to the sizes of detergent micelles, as exemplified by DDM and LMNG. These two detergents exhibit micelles of substantially different sizes (6.8 vs. 19.6 nm), yet their membrane protein complexes were of similar size.⁷⁰ This further supports the notion that micelle size alone may not fully explain the detergent's behavior in the context of membrane proteins. Further studies will be necessary to pinpoint exact origins of the variation in optimal pendant length observed for dense micelle size (C4) versus membrane protein stability (C4/C5).

Hemifluorinated alkyl chains have been used as hydrophobic groups in the previously described HFSs, exemplified by fluorinated carbon chains with either an ethyl or propyl group at the tip.^{60, 71} This class of detergents was designed to study membrane proteins while overcoming the high tendency of fully fluorinated surfactants (FSs) to induce protein aggregation. The ethyl/propyl group at the end of the hydrophobic chain strengthens detergent interactions with membrane protein surfaces (*protein-detergent* interactions), thereby playing a favorable role in reducing protein aggregation. Conversely, the presence of a fluorinated chain in the detergent hydrophobic region can enhance protein stability by improving hydrophobic interactions between detergent tail groups (*detergent-detergent* interactions) in aqueous solutions. Building on the unique behaviors of HFSs, which differ from fully fluorinated or hydrocarbon-based detergents, we prepared three FP-GNGs with different hemifluorinated pendant groups. Generally, the CF₃CF₂-ended pendant-bearing GNG (GNG-F2,2,14) was inferior to its hydrocarbon counterpart (GNG-4,14) in stabilizing MelB_{SI} and β_2 AR. This suggests that the pendant chain of GNG-F2,2,14 may be too bulky to fit into the empty spaces within the micelle interiors or too lipophobic to promote strong *detergent-detergent* interactions. This is supported by its larger micelle size (9.6 vs 3.4 nm) and higher CMC (0.035 vs 0.025 mM) compared to the hydrocarbon equivalent (GNG-

Commented [BB2]: This is a bit confusing-I think it could be better explained so it is really clear which detergents are best overall and which are also significantly stabilising relative to the controls.

4,14). In contrast, the CF₃-ended pendant-bearing GNG-F1,3,14 and GNG-F1,2,15, generally favored membrane protein stabilization, with GNG-F1,2,15 showing the best performance. Notably, GNG-F1,2,15 outperformed DDM at extracting MelB_{St} from the membranes and was significantly more effective at stabilizing β_2 AR (a GPCR). The favorable behaviors of the FP-GNG for protein extraction and GPCR stabilization have not been observed with other HFSs. Consequently, this FP-GNG can be effectively used in all stages of membrane protein manipulation - extraction, purification, and structural study. The unique properties of GNG-F1,2,15 compared to other HFSs appear to stem from its optimal structural features, including the number (3) and position (at the hydrophilic-lipophilic interface) of fluorine atoms, in addition to the main chain (C15) and pendant chain lengths (C3) in the GNG architecture.

Conclusion

Three sets of GNG detergents—monopod GNGs, P-GNGs and FP-GNGs—were prepared through structural variations in the pendant chain. Our comparison of their abilities to stabilize membrane protein demonstrated that a sufficiently large pendant size in this scaffold is critical for membrane protein stability, with the best performance observed for the GNGs bearing the C4 or C5 pendant. Additionally, the FP-GNG study indicates that the sizes of fluorinated carbons (i.e., the number of fluorine atoms) in the pendant region significantly affect the detergent's ability to stabilize membrane proteins. We identified several P-GNGs (GNG-5,13, GNG-5,14, and GNG-4,15) and one F-GNG (GNG-F1,2,15) as new detergent tools that are remarkably effective for membrane protein stability. Notably, GNG-5,14 outperformed LMNG in stabilizing β_2 AR, while GNG-F1,2,15 was superior to DDM for the extraction and stabilization of MelB_{St}. The markedly enhanced efficacy of these two glucoside detergents is attributed to the incorporation of multiple favorable factors, such as hydrophilic-lipophilic balance (HLB), pendant size and main chain length, into a compact detergent structure. Therefore, the current study not only suggests detergent tools for membrane protein research but also provides guidelines for detergent design aimed at enhancing membrane protein stability. This contribution will advance membrane protein research and facilitate the development of novel detergents for structural study.

Author Contributions

P.S.C., T.Y. and G.K. designed the new amphiphiles. T.Y. and G.K. synthesized the amphiphiles. T.Y., G.K., P.H., X.L., and W.A. designed and performed the research and interpreted the data. P.S.C., L.G., X.L., and B.B. contributed to experimental design and data interpretation. P.S.C. and T.Y. wrote the manuscript, with oversight from all the other authors. All authors have given approval to the final version of the manuscript.

Conflicts of interest

The authors declare the following competing financial interest(s): P.S.C., T.Y. and G.K. are inventors on a patent application that covers the (F)P-GNGs.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) (2021R1A2C2006067 and 2018R1A6A1A03024231 to P.S.C.), and the National Institutes of Health (NIH) (R35GM153222 to L.G.).

References

- (1) Cho, W.; Stahelin, R. V. Membrane-protein interactions in cell signaling and membrane trafficking. *Annu. Rev. Biophys. Biomol. Struct.* **2005**, *34* (1), 119-151.
- (2) Overington, J. P.; Al-Lazikani, B.; Hopkins, A. L. How many drug targets are there? *Nature reviews Drug discovery*

2006, 5 (12), 993-996.

(3) Sanders, C. R.; Myers, J. K. Disease-related misassembly of membrane proteins. *Annu. Rev. Biophys. Biomol. Struct.* **2004**, 33 (1), 25-51.

(4) Gong, J.; Chen, Y.; Pu, F.; Sun, P.; He, F.; Zhang, L.; Li, Y.; Ma, Z.; Wang, H. Understanding membrane protein drug targets in computational perspective. *Current Drug Targets* **2019**, 20 (5), 551-564.

(5) <https://www.rcsb.org/> (accessed).

(6) Newstead, S.; Ferrandon, S.; Iwata, S. Rationalizing α -helical membrane protein crystallization. *Protein Science* **2008**, 17 (3), 466-472.

(7) Serrano-Vega, M. J.; Magnani, F.; Shibata, Y.; Tate, C. G. Conformational thermostabilization of the β 1-adrenergic receptor in a detergent-resistant form. *Proceedings of the National Academy of Sciences* **2008**, 105 (3), 877-882.

(8) He, Y.; Wang, K.; Yan, N. The recombinant expression systems for structure determination of eukaryotic membrane proteins. *Protein & cell* **2014**, 5 (9), 658-672.

(9) Privé, G. G. Detergents for the stabilization and crystallization of membrane proteins. *Methods* **2007**, 41 (4), 388-397.

(10) Newstead, S.; Hobbs, J.; Jordan, D.; Carpenter, E. P.; Iwata, S. Insights into outer membrane protein crystallization. *Molecular membrane biology* **2008**, 25 (8), 631-638.

(11) Deisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. Structure of the protein subunits in the photosynthetic reaction centre of *Rhodospseudomonas viridis* at 3 Å resolution. *Nature* **1985**, 318 (6047), 618-624.

(12) Garavito, R. M.; Ferguson-Miller, S. Detergents as tools in membrane biochemistry. *Journal of Biological Chemistry* **2001**, 276 (35), 32403-32406.

(13) Choy, B. C.; Cater, R. J.; Mancina, F.; Pryor Jr, E. E. A 10-year meta-analysis of membrane protein structural biology: detergents, membrane mimetics, and structure determination techniques. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **2021**, 1863 (3), 183533.

(14) Lacapere, J.-J.; Pebay-Peyroula, E.; Neumann, J.-M.; Etchebest, C. Determining membrane protein structures: still a challenge! *Trends in biochemical sciences* **2007**, 32 (6), 259-270.

(15) Phillips, R.; Ursell, T.; Wiggins, P.; Sens, P. Emerging roles for lipids in shaping membrane-protein function. *Nature* **2009**, 459 (7245), 379-385.

(16) Zhang, Q.; Tao, H.; Hong, W.-X. New amphiphiles for membrane protein structural biology. *Methods* **2011**, 55 (4), 318-323.

(17) Guo, Y. Be cautious with crystal structures of membrane proteins or complexes prepared in detergents. *Crystals* **2020**, 10 (2), 86.

(18) Guan, L. The rapid developments of membrane protein structure biology over the last two decades. *BMC biology* **2023**, 21 (1), 300.

(19) Vénien-Bryan, C.; Fernandes, C. A. Overview of membrane protein sample preparation for single-particle cryo-electron microscopy analysis. *International Journal of Molecular Sciences* **2023**, 24 (19), 14785.

(20) Lind, J.; Nordin, J.; Mäler, L. Lipid dynamics in fast-tumbling bicelles with varying bilayer thickness: effect of model transmembrane peptides. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **2008**, 1778 (11), 2526-2534.

(21) Smrt, S. T.; Draney, A. W.; Singaram, I.; Lorieau, J. L. Structure and dynamics of membrane proteins and membrane associated proteins with native bicelles from eukaryotic tissues. *Biochemistry* **2017**, 56 (40), 5318-5327.

(22) Moon, S.; Kong, B.; Jung, Y.-H.; Kim, Y.; Yu, S.; Park, J.-b.; Shin, J.; Kweon, D.-H. Endotoxin-free purification of recombinant membrane scaffold protein expressed in *Escherichia coli*. *Process Biochemistry* **2018**, 66, 230-236.

(23) Bayburt, T. H.; Grinkova, Y. V.; Sligar, S. G. Self-assembly of discoidal phospholipid bilayer nanoparticles with membrane scaffold proteins. *Nano letters* **2002**, 2 (8), 853-856.

(24) Guo, Y. Detergent-free systems for structural studies of membrane proteins. *Biochemical Society Transactions*

2021, *49* (3), 1361-1374.

(25) Knowles, T. J.; Finka, R.; Smith, C.; Lin, Y.-P.; Dafforn, T.; Overduin, M. Membrane proteins solubilized intact in lipid containing nanoparticles bounded by styrene maleic acid copolymer. *Journal of the American Chemical Society* **2009**, *131* (22), 7484-7485.

(26) Frauenfeld, J.; Löving, R.; Armache, J.-P.; Sonnen, A. F.; Guettou, F.; Moberg, P.; Zhu, L.; Jegerschöld, C.; Flayhan, A.; Briggs, J. A. A saposin-lipoprotein nanoparticle system for membrane proteins. *Nature methods* **2016**, *13* (4), 345-351.

(27) McGregor, C.-L.; Chen, L.; Pomroy, N. C.; Hwang, P.; Go, S.; Chakrabartty, A.; Privé, G. G. Lipopeptide detergents designed for the structural study of membrane proteins. *Nature biotechnology* **2003**, *21* (2), 171-176.

(28) Tao, H.; Lee, S. C.; Moeller, A.; Roy, R. S.; Siu, F. Y.; Zimmermann, J.; Stevens, R. C.; Potter, C. S.; Carragher, B.; Zhang, Q. Engineered nanostructured β -sheet peptides protect membrane proteins. *Nature methods* **2013**, *10* (8), 759-761.

(29) Nath, A.; Atkins, W. M.; Sligar, S. G. Applications of phospholipid bilayer nanodiscs in the study of membranes and membrane proteins. *Biochemistry* **2007**, *46* (8), 2059-2069.

(30) Allen, J. P. Recent innovations in membrane-protein structural biology. *F1000Research* **2019**, *8*.

(31) Wycisk, V.; Wagner, M. C.; Urner, L. H. Trends in the Diversification of the Detergentome. *ChemPlusChem* **2024**, *89* (1), e202300386.

(32) Lee, S. C.; Bennett, B. C.; Hong, W.-X.; Fu, Y.; Baker, K. A.; Marcoux, J.; Robinson, C. V.; Ward, A. B.; Halpert, J. R.; Stevens, R. C. Steroid-based facial amphiphiles for stabilization and crystallization of membrane proteins. *Proceedings of the National Academy of Sciences* **2013**, *110* (13), E1203-E1211.

(33) Hussain, H.; Du, Y.; Tikhonova, E.; Mortensen, J. S.; Ribeiro, O.; Santillan, C.; Das, M.; Ehsan, M.; Loland, C. J.; Guan, L. Resorcinarene-Based Facial Glycosides: Implication of Detergent Flexibility on Membrane-Protein Stability. *Chemistry—A European Journal* **2017**, *23* (28), 6724-6729.

(34) Urner, L. H.; Liko, I.; Yen, H.-Y.; Hoi, K.-K.; Bolla, J. R.; Gault, J.; Almeida, F. G.; Schweder, M.-P.; Shutin, D.; Ehrmann, S. Modular detergents tailor the purification and structural analysis of membrane proteins including G-protein coupled receptors. *Nature communications* **2020**, *11* (1), 564.

(35) Chae, P. S.; Rasmussen, S. G.; Rana, R. R.; Gotfryd, K.; Chandra, R.; Goren, M. A.; Kruse, A. C.; Nurva, S.; Loland, C. J.; Pierre, Y. Maltose-neopentyl glycol (MNG) amphiphiles for solubilization, stabilization and crystallization of membrane proteins. *Nature methods* **2010**, *7* (12), 1003-1008.

(36) Bae, H. E.; Du, Y.; Hariharan, P.; Mortensen, J. S.; Kumar, K. K.; Ha, B.; Das, M.; Lee, H. S.; Loland, C. J.; Guan, L. Asymmetric maltose neopentyl glycol amphiphiles for a membrane protein study: effect of detergent asymmetry on protein stability. *Chemical Science* **2019**, *10* (4), 1107-1116.

(37) Chae, P. S.; Rana, R. R.; Gotfryd, K.; Rasmussen, S. G.; Kruse, A. C.; Cho, K. H.; Capaldi, S.; Carlsson, E.; Kobilka, B.; Loland, C. J. Glucose-neopentyl glycol (GNG) amphiphiles for membrane protein study. *Chemical communications* **2013**, *49* (23), 2287-2289.

(38) Cho, K. H.; Bae, H. E.; Das, M.; Gellman, S. H.; Chae, P. S. Improved glucose-neopentyl glycol (GNG) amphiphiles for membrane protein solubilization and stabilization. *Chemistry—An Asian Journal* **2014**, *9* (2), 632-638.

(39) Howell, S. C.; Mittal, R.; Huang, L.; Travis, B.; Breyer, R. M.; Sanders, C. R. CHOBIMALT: a cholesterol-based detergent. *Biochemistry* **2010**, *49* (44), 9572-9583.

(40) Chae, P. S.; Rasmussen, S. G.; Rana, R. R.; Gotfryd, K.; Kruse, A. C.; Manglik, A.; Cho, K. H.; Nurva, S.; Gether, U.; Guan, L. A new class of amphiphiles bearing rigid hydrophobic groups for solubilization and stabilization of membrane proteins. *Chemistry—A European Journal* **2012**, *18* (31), 9485-9490.

(41) Bae, H. E.; Mortensen, J. S.; Ribeiro, O.; Du, Y.; Ehsan, M.; Kobilka, B. K.; Loland, C. J.; Byrne, B.; Chae, P. S. Tandem neopentyl glycol maltosides (TNMs) for membrane protein stabilisation. *Chemical Communications* **2016**, *52* (81),

12104-12107.

(42) Hussain, H.; Mortensen, J. S.; Du, Y.; Santillan, C.; Ribeiro, O.; Go, J.; Hariharan, P.; Loland, C. J.; Guan, L.; Kobilka, B. K. Tandem malonate-based glucosides (TMGs) for membrane protein structural studies. *Scientific Reports* **2017**, *7* (1), 3963.

(43) Chae, P. S.; Gotfryd, K.; Pacyna, J.; Miercke, L. J.; Rasmussen, S. G.; Robbins, R. A.; Rana, R. R.; Loland, C. J.; Kobilka, B.; Stroud, R. Tandem facial amphiphiles for membrane protein stabilization. *Journal of the American Chemical Society* **2010**, *132* (47), 16750-16752.

(44) Ghani, L.; Kim, S.; Wang, H.; Lee, H. S.; Mortensen, J. S.; Katsube, S.; Du, Y.; Sadaf, A.; Ahmed, W.; Byrne, B.; et al. Foldable Detergents for Membrane Protein Study: Importance of Detergent Core Flexibility in Protein Stabilization. *Chemistry – A European Journal* **2022**, *28* (21), e202200116. DOI: <https://doi.org/10.1002/chem.202200116>.

(45) Ghani, L.; Munk, C. F.; Zhang, X.; Katsube, S.; Du, Y.; Cecchetti, C.; Huang, W.; Bae, H. E.; Saouros, S.; Ehsan, M. 1, 3, 5-Triazine-cored maltoside amphiphiles for membrane protein extraction and stabilization. *Journal of the American Chemical Society* **2019**, *141* (50), 19677-19687.

(46) Ghani, L.; Kim, S.; Ehsan, M.; Lan, B.; Poulsen, I. H.; Dev, C.; Katsube, S.; Byrne, B.; Guan, L.; Loland, C. J. Melamine-cored glucosides for membrane protein solubilization and stabilization: importance of water-mediated intermolecular hydrogen bonding in detergent performance. *Chemical Science* **2023**, *14* (45), 13014-13024.

(47) Lee, H. J.; Lee, H. S.; Youn, T.; Byrne, B.; Chae, P. S. Impact of novel detergents on membrane protein studies. *Chem* **2022**, *8* (4), 980-1013.

(48) Quigley, A.; Dong, Y. Y.; Pike, A. C.; Dong, L.; Shrestha, L.; Berridge, G.; Stansfeld, P. J.; Sansom, M. S.; Edwards, A. M.; Bountra, C. The structural basis of ZMPSTE24-dependent laminopathies. *Science* **2013**, *339* (6127), 1604-1607.

(49) Frick, A.; Eriksson, U. K.; de Mattia, F.; Öberg, F.; Hedfalk, K.; Neutze, R.; de Grip, W. J.; Deen, P. M.; Törnroth-Horsefield, S. X-ray structure of human aquaporin 2 and its implications for nephrogenic diabetes insipidus and trafficking. *Proceedings of the National Academy of Sciences* **2014**, *111* (17), 6305-6310.

(50) Karakas, E.; Furukawa, H. Crystal structure of a heterotetrameric NMDA receptor ion channel. *Science* **2014**, *344* (6187), 992-997.

(51) Dong, Y. Y.; Pike, A. C.; Mackenzie, A.; McClenaghan, C.; Aryal, P.; Dong, L.; Quigley, A.; Grieben, M.; Goubin, S.; Mukhopadhyay, S. K2P channel gating mechanisms revealed by structures of TREK-2 and a complex with Prozac. *Science* **2015**, *347* (6227), 1256-1259.

(52) Bae, H. E.; Cecchetti, C.; Du, Y.; Katsube, S.; Mortensen, J. S.; Huang, W.; Rehan, S.; Lee, H. J.; Loland, C. J.; Guan, L. Pendant-bearing glucose-neopentyl glycol (P-GNG) amphiphiles for membrane protein manipulation: Importance of detergent pendant chain for protein stabilization. *Acta biomaterialia* **2020**, *112*, 250-261.

(53) Park, K.-H.; Berrier, C.; Lebaupain, F.; Pucci, B.; Popot, J.-L.; Ghazi, A.; Zito, F. Fluorinated and hemifluorinated surfactants as alternatives to detergents for membrane protein cell-free synthesis. *Biochemical Journal* **2007**, *403* (1), 183-187.

(54) Popot, J.-L. Amphipols, nanodiscs, and fluorinated surfactants: three nonconventional approaches to studying membrane proteins in aqueous solutions. *Annual review of biochemistry* **2010**, *79* (1), 737-775.

(55) Soulié, M.; Deletraz, A.; Wehbie, M.; Mahler, F.; Bouchemal, I.; Le Roy, A.; Petit-Härtlein, I.; Keller, S.; Meister, A.; Pebay-Peyroula, E. Zwitterionic fluorinated detergents: From design to membrane protein applications. *Biochimie* **2023**, *205*, 40-52.

(56) Chae, P. S.; Kruse, A. C.; Gotfryd, K.; Rana, R. R.; Cho, K. H.; Rasmussen, S. G.; Bae, H. E.; Chandra, R.; Gether, U.; Guan, L. Novel tripod amphiphiles for membrane protein analysis. *Chemistry–A European Journal* **2013**, *19* (46), 15645-15651.

(57) Urner, L. H.; Junge, F.; Fiorentino, F.; El-Baba, T. J.; Shutin, D.; Nölte, G.; Haag, R.; Robinson, C. V. Rationalizing the optimization of detergents for membrane protein purification. *Chemistry–A European Journal* **2023**, *29* (30),

e202300159.

- (58) Griffin, W. C. Calculation of HLB values of non-ionic surfactants. *J. Soc. Cosmet. Chem.* **1954**, *5*, 249-256.
- (59) Oliver, R. C.; Lipfert, J.; Fox, D. A.; Lo, R. H.; Doniach, S.; Columbus, L. Dependence of micelle size and shape on detergent alkyl chain length and head group. *PLoS one* **2013**, *8* (5), e62488.
- (60) Abila, M.; Durand, G.; Pucci, B. Propyl-ended hemifluorinated surfactants: synthesis and self-assembling properties. *The Journal of Organic Chemistry* **2011**, *76* (7), 2084-2093.
- (61) Guan, L.; Hariharan, P. X-ray crystallography reveals molecular recognition mechanism for sugar binding in a melibiose transporter MelB. *Communications Biology* **2021**, *4* (1), 931.
- (62) Guan, L.; Parameswaran, H. Crystal Structures of a Melibiose Symporter MelB with Bound Sugar Substrate. *Biophysical Journal* **2021**, *120* (3), 212a.
- (63) Hariharan, P.; Shi, Y.; Katsube, S.; Willibal, K.; Burrows, N. D.; Mitchell, P.; Bakhtiari, A.; Stanfield, S.; Pardon, E.; Kaback, H. R. Mobile barrier mechanisms for Na⁺-coupled symport in an MFS sugar transporter. *Elife* **2024**, *12*, RP92462.
- (64) Wehbie, M.; Onyia, K. K.; Mahler, F.; Le Roy, A.; Deletraz, A.; Bouchemal, I.; Vargas, C.; Babalola, J. O.; Breyton, C.; Ebel, C. Maltose-based fluorinated surfactants for membrane-protein extraction and stabilization. *Langmuir* **2021**, *37* (6), 2111-2122.
- (65) Rosenbaum, D. M.; Cherezov, V.; Hanson, M. A.; Rasmussen, S. G.; Thian, F. S.; Kobilka, T. S.; Choi, H.-J.; Yao, X.-J.; Weis, W. I.; Stevens, R. C. GPCR engineering yields high-resolution structural insights into β 2-adrenergic receptor function. *science* **2007**, *318* (5854), 1266-1273.
- (66) Yao, X.; Parnot, C.; Deupi, X.; Ratnala, V. R. P.; Swaminath, G.; Farrens, D.; Kobilka, B. Coupling ligand structure to specific conformational switches in the β 2-adrenoceptor. *Nature Chemical Biology* **2006**, *2* (8), 417-422. DOI: 10.1038/nchembio801.
- (67) Ehsan, M.; Katsube, S.; Cecchetti, C.; Du, Y.; Mortensen, J. S.; Wang, H.; Nygaard, A.; Ghani, L.; Loland, C. J.; Kobilka, B. K. New Malonate-derived tetraglucoside detergents for membrane protein stability. *ACS chemical biology* **2020**, *15* (6), 1697-1707.
- (68) Lee, H. J.; Ehsan, M.; Zhang, X.; Katsube, S.; Munk, C. F.; Wang, H.; Ahmed, W.; Kumar, A.; Byrne, B.; Loland, C. J. Development of 1, 3-acetonedicarboxylate-derived glucoside amphiphiles (ACAs) for membrane protein study. *Chemical Science* **2022**, *13* (19), 5750-5759.
- (69) Nehmé, R.; Joubert, O.; Bidet, M.; Lacombe, B.; Polidori, A.; Pucci, B.; Mus-Veteau, I. Stability study of the human G-protein coupled receptor, Smoothed. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **2010**, *1798* (6), 1100-1110.
- (70) Breyton, C.; Javed, W.; Vermot, A.; Arnaud, C.-A.; Hajjar, C.; Dupuy, J.; Petit-Hartlein, I.; Le Roy, A.; Martel, A.; Thépaut, M. Assemblies of lauryl maltose neopentyl glycol (LMNG) and LMNG-solubilized membrane proteins. *Biochimica et Biophysica Acta (BBA)-Biomembranes* **2019**, *1861* (5), 939-957.
- (71) Abila, M.; Durand, G.; Pucci, B. Glucose-Based Surfactants with Hydrogenated, Fluorinated, or Hemifluorinated Tails: Synthesis and Comparative Physical-Chemical Characterization. *The Journal of Organic Chemistry* **2008**, *73* (21), 8142-8153. DOI: 10.1021/jo801379e.