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Improved Glucose-Neopentyl Glycol (GNG) Amphiphiles for Membrane Protein Solubilization and Stabilization

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Abstract: Membrane proteins are inherently amphipathic and undergo dynamic conformational changes for proper function within native membranes. Maintaining the functional structures of these biomacromolecules in aqueous media is necessary for structural studies but difficult to achieve with currently available tools, thus necessitating the development of novel agents with favorable properties. This study introduces several new glucose-neopentyl glycol (GNG) amphiphiles and reveals some agents that display favorable behaviors for the solubilization and stabilization of a large, multisubunit membrane protein assembly. Furthermore, a detergent structure–property relationship that could serve as a useful guideline for the design of novel amphiphiles is discussed.

Keywords: amphiphiles · membrane proteins · molecular design · protein solubilization · protein stabilization

Introduction

Membrane proteins are amphipathic macromolecules that reside in lipid bilayers called membranes. It is estimated that a third of all open reading frames in the human genome encode membrane proteins that are distributed over several different types of membranes, including the plasma membrane, nuclear membrane, and inner and outer mitochondrial membranes.[1] Integral membrane proteins play central roles in a variety of cellular processes such as material transport, signal transduction, cell adhesion, and cell-to-cell communication. The importance of these biomacromolecules in normal and disease states is reflected by the fact that more than half of all pharmaceutical agents currently under development target membrane proteins.[2] Despite its biological and pharmaceutical importance, membrane protein research lags far behind that of its soluble counterpart, largely because of difficulty in handling these proteins.[3] The structures of only several hundred membrane proteins are known, in contrast to tens of thousands of soluble proteins with known structure. This slow progress is mainly due to a low natural abundance of these proteins, their instability in non-native environments, and their high flexibility caused by dynamic conformation changes within the lipid bilayer.[4] Maintaining native membrane protein conformations is a particularly formidable task because these macromolecules tend to aggregate and denature when extracted from native membranes for study.[5] Thus, a membrane-mimicking system must be used to prevent structural degradation of these biomacromolecules.

Detergents tend to self-assemble to form micelles. Detergent micelles are often illustrated having a globular or eclipsed shape with a hydrophobic interior and hydrophilic exterior. This architecture confers the micelles with an ability to interact with the hydrophobic portions of membrane proteins in an aqueous environment. Thus, detergent micelles serve as excellent media for membrane protein solubilization and stabilization, and are indispensable tools for membrane protein studies.[6] Membrane protein research starts with protein extraction from the membrane by detergent molecules. It is a prerequisite to keep the resulting protein–detergent complexes (PDCs) soluble in the subsequent multistep processes, including protein purification and crystallization.

A large number of conventional detergents with different combinations of hydrophilic and hydrophobic groups are available, but only a small number are widely used for membrane protein manipulation. For instance, the five conventional detergents, OG (*n*-octyl-β-D-glucopyranoside), NG (*n*-nonyl-β-D-glucopyranoside), DM (*n*-decyl-β-D-maltopyranoside), DDM (*n*-dodecyl-β-D-maltopyranoside), and LDAO (lauryldimethylamine-*N*-oxide), have facilitated crystal structure determinations of about 70% of α-helical membrane proteins.[7] It is interesting to note that these popular agents share their behaviors for membrane protein manipulation. They are not only efficient at extracting membrane

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proteins from membranes but also effective at stabilizing the solubilized proteins. Despite their wide utility, membrane proteins in detergent micelles often undergo structural degradation through denaturation and/or aggregation.[4] The absence of lateral pressure exerted by a membrane[8] is regarded as the main reason for limited membrane protein stability in detergent micelles. Protein extraction and purification involves loss of lipid molecules specifically bound to membrane protein surfaces.[9] Along with micelle inhomogeneity, this could contribute to the observed PDC instability. Furthermore, most membrane proteins with unknown structures are more difficult to solubilize and stabilize than those with known structures.[10] Thus, the development of novel amphiphiles with enhanced protein solubilization and stabilization efficacy is of great importance for the future success of membrane protein research.

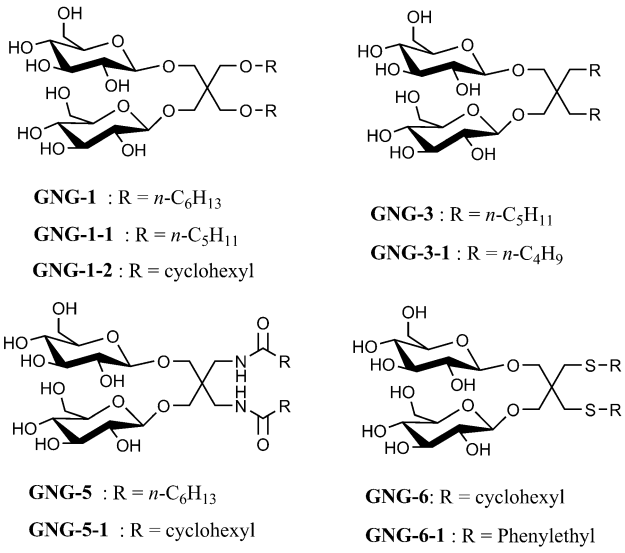
Many conventional detergents have the common structural features of a flexible tail group and polar head group such as a glucoside, maltoside, or *N*-oxide. A number of novel classes of amphiphiles with different architectures have been devised to overcome the limited performance of conventional detergents.[11] Representative examples include amphipathic polymers (amphipols),[11a–c] tripod amphiphiles (TPAs),[11d–f] hemifluorinated surfactants (HFSs),[11g,h] nanodiscs (NDs),[11c,i] rigid hydrophobic group-bearing amphiphiles,[11j–l] peptide-based amphipathic agents,[11m–o] facial amphiphiles (FAs),[11p–r] and neopentyl glycol-based amphiphiles (glucose-neopentyl glycol (GNG) and maltose-neopentyl glycol (MNG).[11s–u] Despite the structural diversity and innovative designs of these novel agents, only a few classes are successful in membrane protein crystallization, as exemplified by FAs,[11r] GNGs and MNGs.[12] It is particularly noteworthy that GNG and MNG agents have facilitated 10 new X-ray crystal structure determinations of membrane proteins over the last three years, including several G-protein coupled receptors (GPCRs) such as β_2 adrenergic receptors,[12a–c] acetylcholine receptors,[12d,e] and opioid receptors.[12f,g] This indicates that these class members are indispensable tools for membrane protein science. GNG and MNG class members share a quaternary carbon in the central region from which two hydrophobic and hydrophilic groups project. Our previous study showed that in addition to favorable protein stabilization behavior, GNG agents tend to form small PDCs relative to those formed by DDM, presumably due to their short tail- and head-groups.[11u] This attribute of GNG would provide a favorable environment for PDC-based membrane protein crystallization by facilitating nucleus and crystal lattice formation. The use of a GNG led to the generation of high-quality crystals of some membrane proteins, including sodium-pumping pyrophosphatase.[11u,12j]

Another favorable aspect of this class is its high structural flexibility and synthetic accessibility. Thus, detergent properties can be efficiently fine-tuned by preparing a number of family members. In the current study, several new GNG analogues were prepared based on previously reported GNGs.[11u] Some of the new GNG amphiphiles showed favorable solubilization and/or stabilization efficacy toward a large, multi-subunit membrane protein assembly.

Results and Discussion

Design and Characterization of New GNG Amphiphiles

GNG agents were first designated according to their functional groups in the central region (Scheme 1); GNG-1 and GNG-5 derivatives contain ether and amide linkages, respectively, while GNG-6 derivatives have thioether linkages.



Scheme 1. Chemical structures of previously described GNG amphiphiles (GNG-1 and GNG-3) and newly prepared GNG analogues (GNG-1-1, GNG-1-2, GNG-3-1, GNG-5, GNG-5-1, GNG-6, and GNG-6-1).

The hydrophobic groups of GNG-3 were directly attached to the central quaternary carbon. GNG agents were further designated according to their hydrophobic group variations. The hexyl chains (C6) of the previously reported GNGs, GNG-1 and GNG-3, were replaced with pentyl chains (C5) to give GNG-1-1 and GNG-3-1, respectively. These short alkyl chain GNGs were designed based on the idea that conventional detergents with a short alkyl chain (e.g., OG) are more favorable than their long alkyl chain counterparts (e.g., DDM) in PDC-based protein crystallization, provided that native protein structures can be effectively maintained.[13] Due to synthetic convenience, an amide coupling reaction was utilized to generate GNG-5 with C6 alkyl chains and GNG-5-1 with cyclohexane rings. Note that the three new GNGs, GNG-1-2, GNG-5-1, and GNG-6, bear cyclohexane rings as hydrophobic groups. These GNGs were designed based on the popular use of cyclohexane ring-containing conventional detergents (e.g., CYGLUs and CYMALs; Figure S1, Supporting Information). To investigate potential differences in detergent efficacy between cyclohexane- and benzene-bearing GNGs, 2-phenylethanethiol was used instead of cyclohexanethiol to produce GNG-6-1.

Except for GNG-1-2, all new GNG agents were prepared by straightforward chemical reactions (3 steps) in high overall yields (80–90%). The protocol allowed for the preparation of multi-gram quantities, thus supporting the availability of new agents. One of main reasons why many novel amphiphiles have not become popular within the membrane protein community is believed to be due to their impracticability. Most GNG agents are highly water-soluble (> 10 wt%), but the solubility of the cyclohexane-bearing GNGs in water was dependent on the functional group present in the central region. An amide-functionalized GNG, GNG-5-1, was water-insoluble and thus not studied further. Ether- and thioether-functionalized GNGs (GNG-1-2 and GNG-6, respectively) are water-soluble >10 wt%. Note that the cyclohexyl version of GNG-3 (GNG-3-2; Figure S2, Supporting Information) was not prepared because a previous study showed that the constitutional isomer of this agent, GNG-4, is barely water-soluble.[11u]

Micelles formed by all amphiphiles were characterized by solubilization experiments using a hydrophobic fluorescent dye, diphenylhexatriene (DPH),[14] and by dynamic light scattering (DLS). Table 1 summarizes the critical micelle concentrations (CMCs) and hydrodynamic radii (R_h) of micelles formed by these new detergents. The table includes data for two conventional detergents (DDM and OG) and two previously described GNGs (GNG-1 and GNG-3) for comparison. The CMC values of GNG-1-1 and GNG-3-1 were around seven times larger than those of their parent molecules, GNG-1 and GNG-3, respectively. Interestingly, GNG-1-2 had a 10-fold larger CMC value than its constitutional isomer, GNG-1. In spite of the similar hydrophobicity, the large difference in the CMC value between GNG-1 and GNG-1-2 is likely due to the bulkiness of the cyclohexyl group. The presence of a bulky group in the lipophilic region hinders micelle formation.[15] The amide-containing GNG, GNG-5, had a weak tendency to self-associate, thereby giving a large CMC value (~18 mM; ~1.2 wt%). The thioether-bearing GNGs, GNG-6 and GNG-6-1, showed high propensity to aggregate, giving small CMC values (~2.1 mM and ~0.82 mM, respectively). The relative polar and nonpolar characteristics of amide and thioether groups, respectively, are responsible for their different self-association behaviors. Micelles formed by all new GNGs and previously reported GNGs are smaller than those formed by DDM, which is likely related to their property of small PDC formation.[11u] Notably, short alkyl chain GNGs (GNG-1-1 and GNG-3-1) formed even smaller micelles than their original compounds, GNG-1 and GNG-3, respectively. This is because a decrease in the length of the aliphatic tail group causes molecules to be more cone-shaped, thereby leading to the formation of smaller micelles. This result is consistent with the idea that the relative size of hydrophilic and hydrophobic groups (i.e., molecular geometry) is responsible for the self-association behavior of detergents.[16] Micelles formed by the amide-functionalized GNG (GNG-5; R_h = ~2.40 nm) and two thioether-functionalized GNGs (GNG-6 and GNG-6-1; R_h = ~2.66 nm and ~2.98 nm, respectively) were comparable to those formed by ether-functionalized GNG-1 (R_h = ~2.64 nm). Thus, functional group differences in the central region have only a minimal effect on the micelle size. The micelle size range displayed by the new GNGs (1.9–3.1 nm) overlaps with the reported range of OG micelles (1.5–2.3 nm),[17] thus suggesting that these GNGs resemble OG with respect to small PDC formation. GNGs and DDM micelles were further characterized by their size distribution. All agents except GNG-1-1 showed one micelle distribution, as did DDM (Figure S3, Supporting Information). The DLS diagram for GNG-1-1 indicates the presence of two sets of micelles with hydrodynamic radii of about 2.3 nm and 59 nm, respectively. Scattering intensity analysis indicates that the set of small micelles is an almost exclusive entity in the solution (see the caption of Figure S3 for details, Supporting Information).

Table 1. Molecular weight (MW), critical micelle concentration (CMC), and hydrodynamic radius (R_h) of micelles (mean ± SD, n = 4), and solubilization yields (SYs) for GNGs (GNG-1, GNG-1-1, GNG-1-2, GNG-3, GNG-3-1, GNG-5, GNG-6, and GNG-6-1) and conventional detergents (DDM and OG).

Detergents	MW[a]	CMC [mM; wt%]	R_h [nm][b]	SY [%]
GNG-1	628.8	~1.6; ~0.10	2.64 ± 0.04	~90
GNG-1-1	600.7	~11; ~0.65	2.28 ± 0.01[c,d]	~40
GNG-1-2	624.7	~17; ~1.1	2.30 ± 0.02[c]	~70
GNG-3	568.7	~1.0; ~0.058	3.07 ± 0.01	~80
GNG-3-1	540.6	~6.9; ~0.37	1.96 ± 0.04[c]	~60
GNG-5	682.8	~18; ~1.2	2.40 ± 0.03[c]	~80
GNG-6	656.8	~2.1; ~0.13	2.66 ± 0.09	~90
GNG-6-1	700.9	~0.82; ~0.057	2.98 ± 0.11	~95
DDM	510.1	~0.17; ~0.0087	3.47 ± 0.04	~80
OG	292.4	~25; ~0.73[e]	1.5–2.3[e]	>95

[a] Molecular weight of detergents. [b] Hydrodynamic radius of micelles determined by dynamic light scattering at 0.5 wt%. [c] These agents were tested at higher concentration (2.0 wt% for GNG-1-1 and GNG-3-1, or 4.0 wt% for GNG-1-2 and GNG-5) to obtain a strong signal. [d] Two forms of aggregates were found with hydrodynamic radii of about 2.3 nm and 59 nm (Figure S3, Supporting Information). [e] This value was obtained from ref. [17].

Evaluation of GNGs for Membrane Protein Solubilization and Stabilization

To evaluate the solubilization efficiency and stabilization efficacy of new GNG agents, the photosynthetic superassembly of *Rhodobacter (R.) capsulatus* was employed. This system comprises a resilient reaction center complex (RC) and a labile light-harvesting complex I (LHI). The robust light-harvesting complex II (LHII), which is present in the natural source of the superassembly, was genetically deleted for the purpose of detergent evaluation.[18] The LHI-RC superassembly is a pigment–protein complex that contains 30–40 subunits and various types of cofactors, including bacteriochlorophylls and carotenoids. The high susceptibility of LHI to denaturation, which is due to its large size and multiple quaternary structures, enabled us to discriminate between the efficacies of a set of mild detergents. LHI tends

to gradually denature even in mild conventional detergents such as DDM and OG. Detergent efficacies with a medium range of strength could be differentiated by virtue of the resilient character of RC. Only harsh detergents such as sodium dodecyl sulfate (SDS) completely destroyed the RC complex during solubilization. Accordingly, we unambiguously assessed detergent efficacies in a graded way based on the denaturation extent of the LHI-RC superassembly. This complex is also a convenient test bed for detergent evaluation because the multiple cofactors embedded in the complex interior produce a characteristic UV/Vis spectrum. This enabled us to utilize spectrophotometry to determine protein quantity and integrity. The native conformation of the LHI-RC complex has an intense peak at 875 nm, while intact RC with denatured LHI, and denatured LHI and RC have characteristic peaks at around 800 nm and 760 nm, respectively.

This study included two conventional detergents (DDM and OG) and two previously described GNGs (GNG-1 and GNG-3) for comparison. For the initial solubilization of LHI-RC complexes, intracytoplasmic *R. capsulatus* membranes were treated with CMC + 1.0 wt % individual GNGs or conventional detergents. The insolubilized parts of membranes and cellular debris were then removed by ultracentrifugation. The resulting supernatant containing detergent-solubilized superassembly was collected while the pellet containing insolubilized parts was resuspended in an aqueous medium. UV/Vis spectra of these two portions were obtained to assess detergent efficacy regarding protein solubilization and stabilization. Protein solubilization yield (SY; Table 1) for each detergent was calculated from the difference between the initial amount of superassembly and the protein amount of homogenized pellets following solubilization (Table 1 and Figure S4, Supporting Information). A previous study showed that DDM is one of the best conventional detergents for solubilization and stabilization of the complexes, which is in agreement with the popular use of this agent in membrane protein studies.[19] Consistent with this observation, DDM efficiently extracted the complexes from the membrane (~80 %) without structural degradation (Figure 1 a and Figure S4, Supporting Information). Strong absorbance at 875 nm indicates the intact nature of DDM-solubilized complexes. The other non-ionic conventional detergent, OG, extracted protein almost quantitatively (> 95 %), but the resulting LHI structure was partially degraded in the course of protein solubilization. A characteristic peak at 760 nm indicates this partial protein denaturation (Figure 1 a and Figure S5 a, Supporting Information). Remarkably, all GNG agents solubilized the superassembly without denaturation, thus revealing their generally favorable property with respect to membrane protein stabilization. However, a large variation in detergent solubilization efficiency was observed. The new GNG derivatives with short alkyl chains (C5) (GNG-1-1 and GNG-3-1) were substantially inferior to their parent GNGs with C6 alkyl chains (GNG-1 and GNG-3) (Figures S4 and S5 a, Supporting Information). A similar result was found for cyclohexane-containing GNG-1-2. The high CMC values of these agents (GNG-1-1, GNG-1-2, and GNG-3-1; 7–17 mM) relative to those of other GNGs may be a good indication for such poor protein solubilization efficiency (Table 1). Detergents with high CMC values have a low tendency of self-association mainly because of reduced lipophilicity. Thus, the tail groups of high CMC detergents are likely to weakly interact with the hydrophobic portion of membrane proteins. GNG-5 with amide linkages in the central region was less efficient than DDM in the complex solubilization (Figure S4, Supporting Information). By contrast, thioether-functionalized GNGs (GNG-6 and GNG-6-1) were superior to DDM and other GNGs in this regard (Table 1 and Figure S4; ~90 % and ~95 % vs. ~80 %). Overall, the new GNG amphiphiles showed intriguing behaviors in this initial evaluation with LHI-RC complexes.

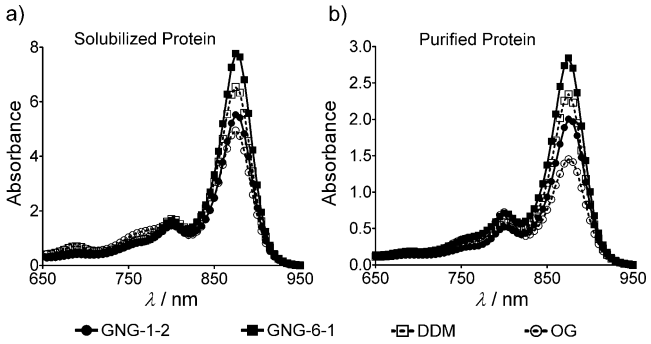


Figure 1. a,b) Absorbance spectra of *R. capsulatus* superassembly solubilized (a) and purified (b) in representative GNGs (GNG-1-2 and GNG-6-1; solid lines) and two conventional detergents (DDM and OG; dotted lines). Detergents were used at CMC + 1.0 wt % for superassembly solubilization. For protein purification, Ni-NTA affinity chromatography was performed and protein complexes were collected from the column by using 1xCMC detergent concentration. Absorbance values were calculated from dilution factors (normally 1:5 or 1:10).

To investigate the utility of these new agents for membrane protein purification, individual detergent-solubilized LHI-RC complexes were subjected to immobilized nickel affinity chromatography. The complex contains a nickel binding moiety (i.e., hepta-histidine tag) on the C-terminus of the M-subunit of RC. A highly pure LHI-RC in solution was obtained by eluting the resin-bound complexes from the affinity column using an elution buffer containing 1 M imidazole and 1xCMC individual amphiphiles. As shown in Figure 1 b and Figure S5 b in the Supporting Information, the spectra of GNGs-, DDM-, or OG-purified complexes resemble those of the complexes solubilized with the individual agents. This data indicates that all GNGs and DDM were successful in retaining native complex conformations in the course of protein purification. Conversely, the rather harsh nature of OG further destroyed the complexes during purification, as indicated by the small shoulders at 760 nm and 800 nm (Figure S5 b, Supporting Information).

Long-Term Stabilization Efficacy of GNG Agents

The initial short-term solubilization and purification protocol was not satisfactory in discerning the membrane protein stabilization efficacy of individual amphiphiles except for OG. For this reason, these mild agents (DDM and GNGs) were further evaluated for long-term stabilization efficacy. LHI-RC complexes were solubilized with 1.0 wt% DDM and purified in 1xCMC DDM through metal affinity chromatography according to the protocol described above. The DDM-purified protein solution was then diluted into individual amphiphile-containing solutions to reach a final DDM concentration far below its CMC and individual amphiphile concentrations of CMC+0.04 wt% or CMC+1.0 wt%. Protein integrity was monitored over a 20 day period by measuring the absorbance at 875 nm during the storage at room temperature (Figure 2 and Figure S6, Supporting Information). Consistent with a previous study,[11u] at relatively low detergent concentration (i.e., CMC+0.04 wt%), GNG-1- and GNG-3-solubilized protein complexes preserved the native conformation as effectively as DDM while OG-purified complexes gradually lost their integrity with time (Figure 2a and Figure S6a, Supporting Information). The stabilization efficacies of new GNGs (short alkyl chain GNGs, and amide- and thioether-functionalized GNGs) were comparable to that of DDM. Notably, the cyclohexane-bearing GNG-1-2 was clearly superior to the conventional detergents (OG and DDM) and other GNGs (Figure 2a). When the detergent concentration was increased to CMC+1.0 wt%, the difference in detergent efficacy was more prominent (Figure 2b and Figure S6b, Supporting Information). Under these harsh conditions, OG-solubilized proteins completely lost their integrity after a few days, while DDM-solubilized complexes gradually underwent structural degradation. Only 10% of the LHI-RC complexes retained their native conformation after 20 days of storage. Every GNG agent displayed a more favorable behavior than DDM, with the best performance of GNG-1-2. In this cyclohexane-containing GNG, approximately 50% of LHI-RC complexes retained their native conformation after 20 days (vs. ~10% in DDM, Figure 2b). The difference in the detergent stabilization efficacies of GNG-1-2 and DDM is also well illustrated by time-course changes in the absorption spectra of the complexes solubilized in each detergent (Figure 2c,d).

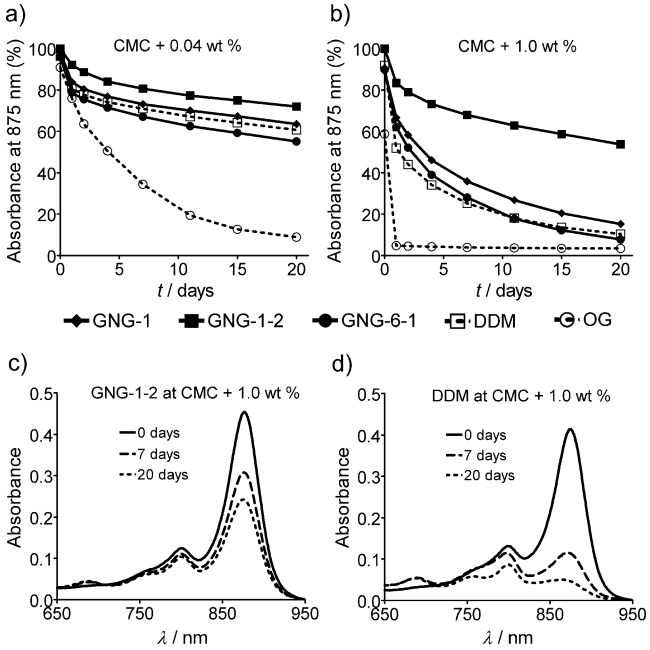


Figure 2. a,b) Time course stability of *R. capsulatus* superassembly at CMC+0.04 wt% (a) and CMC+1.0 wt% (b). Absorption spectra were taken over a 20 day incubation period at room temperature for complexes solubilized in GNGs (GNG-1, GNG-1-1, GNG-1-2, GNG-3, GNG-3-1, GNG-5, GNG-6, and GNG-6-1) and conventional detergents (DDM and OG). Results are expressed as % absorbance at 875 nm (A_{875}) relative to measurements at day 0. c,d) Time course change in the absorption spectra of GNG-1-2-solubilized (c) and DDM-solubilized (d) protein samples. Detergents were used at CMC+1.0 wt%. A binding buffer (10 mM Tris, pH 7.8, containing 100 mM NaCl) was used for long-term protein storage.

Conclusions

Hydrophobic variations of previously reported GNG amphiphiles were prepared by introducing various lipophilic groups and/or functional groups to the central region. Their ability to solubilize and stabilize a challenging membrane protein complex, *R. capsulatus* superassembly, was assessed by spectrophotometry. The new GNG agents displayed medium to excellent efficiency at solubilizing the superassembly from the membrane, with the best performance of a thioether-functionalized agent, GNG-6-1. We have developed a few novel classes of amphiphiles with satisfactory protein stabilization efficacy, exemplified by TPAs, MNGs, and TFAs. Among these agents, GNG-6-1 is one of the best agents in terms of superassembly solubilization efficiency. Amphiphiles with such high solubilization efficiency tend to partially destroy the LHI-RC complexes, as observed for conventional detergents (Triton X-100, OG, and LDAO)[11u,20] and a tripod amphiphile (TPA-4).[11e] The favorable solubilization behavior likely results from the high affinity of these agents to the hydrophobic surface of the superassembly. This strong interaction could serve as a favorable role in membrane protein crystallization by preventing protein aggregation, which is one of the main mechanisms of protein structural degradation. Based on the fact that the three most widely used conventional detergents, DDM, OG, and LDAO, are efficient in membrane protein solubilization, we believe that the new GNG agents have potential utility in membrane protein research. Membrane protein stability is probably the most important determinant for the success of membrane protein structural studies. In this context, GNG-1-2 showed promising behavior as this agent was outstanding at maintaining the native structure of the photosyn-

thetic superassembly at both low and high detergent concentrations.

Despite their wide use in membrane protein studies, the currently available conventional detergents have limitations. DDM tends to form large PDCs while OG and LDAO are often too harsh to stabilize delicate membrane proteins.[13] New tools with favorable solubilization and stabilization efficacy and the ability to form small PDCs are needed. The GNG amphiphiles introduced here are excellent candidates for meeting these criteria because this class tends to form small PDCs, as shown in a previous study,[11u] while displaying favorable solubilization efficiency and stabilization efficacy, as described here. The short-chain detergents, GNG-1-1 and GNG-3-1, may also find use in membrane protein structural studies; these agents have alkyl chains as short as that of OG, but they showed significantly enhanced stabilization efficacy. Although it is unclear whether the results of the current study will translate to other membrane protein systems as well, this initial evaluation reveals that some GNG members are promising alternatives to conventional detergents for membrane protein study.

The current study could provide insightful information on which detergent moiety plays a crucial role in determining protein solubilization efficiency and stabilization efficacy. Here, we prepared several hydrophobic variations of GNG agents with the same hydrophilic group of a branched diglucoside. Evaluation for the the superassembly showed that the hydrophobic variations resulted in small changes in protein stabilization efficacy, but a wide range in protein solubilization efficiency (Table 1; 40–95%). This result indicates that detergent hydrophobic group dictates protein solubilization efficiency rather than protein stabilization efficacy. In previous studies,[11s,u] MNGs with a branched dimaltoside outperformed GNGs at maintaining membrane protein stability. The MNG architecture bears a resemblance to that of GNGs except for the hydrophilic group. Thus, these findings suggest that the detergent hydrophilic group mainly determines protein stabilization efficacy. A similar trend was also observed for the TPA architecture.[21] Therefore, these observations strongly support an important detergent structure–property relationship: protein stabilization efficacy is most effectively altered by modifying the detergent hydrophilic group, while protein solubilization efficiency is mainly affected by the detergent hydrophobic group. Because both detergent solubilization and stabilization properties play essential roles in membrane protein crystallization, neither the hydrophobic nor the hydrophilic group can be ignored when designing novel amphiphiles.

Experimental Section

Details regarding the synthesis and characterization of new GNG amphiphiles and detergent screening protocols for membrane protein solubilization and stabilization are given in the Supporting Information.

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[1] a) E. Wallin, G. von Heijne, *Protein Sci.* **1998**, *7*, 1029–1038; b) A. Krogh, B. Larsson, G. von Heijne, E. L. Sonnhammer, *J. Mol. Biol.* **2001**, *305*, 567–580.
[2] J. P. Overington, B. Al-Lazikani, A. L. Hopkins, *Nat. Rev. Drug Discovery* **2006**, *5*, 993–996.
[3] http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html.
[4] J. J. Lacapère, E. Pebay-Peyroula, J. M. Neumann, C. Etchebest, *Trends Biochem. Sci.* **2007**, *32*, 259–270.
[5] a) S. H. White, W. C. Wimley, *Annu. Rev. Biophys. Biomol. Struct.* **1999**, *28*, 319–365; b) J. V. Møller, M. le Maire, *J. Biol. Chem.* **1993**, *268*, 18659–18672.
[6] a) R. M. Garavito, S. Ferguson-Miller, *J. Biol. Chem.* **2001**, *276*, 32403–32406; b) J. U. Bowie, *Curr. Opin. Struct. Biol.* **2001**, *11*, 397–402.
[7] a) M. J. Serrano-Vega, F. Magnani, Y. Shibata, C. G. Tate, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 877–882; b) S. Newstead, S. Ferrandon, S. Iwata, *Protein Sci.* **2008**, *17*, 466–472.
[8] T. C. Anglin, J. C. Conboy, *Biophys. J.* **2008**, *95*, 186–193.
[9] D. Marsh, *Biochem. Biophys. Acta* **2008**, *1778*, 1545–1575.
[10] A. Alexandrov, M. Mileni, E. Y. Chien, M. A. Hanson, R. C. Stevens, *Structure* **2008**, *16*, 351–359.
[11] a) C. Tribet, R. Audebert, J.-L. Popot, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 15047–15050; b) J.-L. Popot, *Annu. Rev. Biochem.* **2010**, *79*, 737–775; c) J.-L. Popot, T. Althoff, D. Bagnard, J.-L. Banères, P. Bazzacco, E. Billon-Denis, L. J. Catoire, P. Champeil, D. Charvolin, M. J. Cocco, G. Crémel, T. Dahmane, L. M. de la Maza, C. Ebel, F. Gabel, F. Giusti, Y. Gohon, E. Goormaghtigh, E. Guittet, J. H. Kleinschmidt, W. Kühlbrandt, C. Le Bon, K. L. Martinez, M. Picard, B. Pucci, J. N. Sachs, C. Tribet, C. van Heijenoort, F. Wien, F. Zito, M. Zoonens, *Annu. Rev. Biophys.* **2011**, *40*, 379–408; d) D. T. McQuade, M. A. Quinn, S. M. Yu, A. S. Polans, M. P. Krebs, S. H. Gellman, *Angew. Chem.* **2000**, *112*, 774–777; *Angew. Chem. Int. Ed.* **2000**, *39*, 758–761; e) P. S. Chae, M. J. Wander, A. P. Bowling, P. D. Laible, S. H. Gellman, *ChemBioChem* **2008**, *9*, 1706–1709; f) P. S. Chae, P. D. Laible, S. H. Gellman, *Mol. BioSyst.* **2010**, *6*, 89–94; g) C. Breyton, E. Chabaud, Y. Chaudier, B. Pucci, J.-L. Popot, *FEBS Lett.* **2004**, *564*, 312–318; h) M. Abla, G. Durand, B. Pucci, *J. Org. Chem.* **2011**, *76*, 2084–2093; i) A. Nath, W. M. Atkins, S. G. Sligar, *Biochemistry* **2007**, *46*, 2059–2069; j) S. C. Howell, R. Mittal, L. Huang, B. Travis, R. M. Breyer, C. R. Sanders, *Biochemistry* **2010**, *49*, 9572–9583; k) J. Hovers, M. Potschies, A. Polidori, B. Pucci, S. Raynal, F. Bonete, M. J. Serrano-Vega, C. G. Tate, D. Picot, Y. Pierre, J. L. Popot, R. Nehme, M. Bidet, I. Mus-Veteau, H. Busskamp, K. H. Jung, A. Marx, P. A. Timmins, W. Welte, *Mol. Membr. Biol.* **2011**, *28*, 171–181; l) P. S. Chae, S. G. F. Rasmussen, R. R. Rana, K. Gotfryd, A. C. Kruse, S. Nurva, U. Gether, L. Guan, C. J. Loland, B. Byrne, B. K. Kobilka, S. H. Gellman, *Chem. Eur. J.* **2012**, *18*, 9485–9490; m) C. E. Schafmeister, L. J. W. Miercke, R. M. Stroud, *Science* **1993**, *262*, 734–738; n) C.-L. McGregor, L. Chen, N. C. Pomroy, P. Hwang, S. Go, A. Chakrabartty, G. G. Privé, *Nat. Biotechnol.* **2003**, *21*, 171–176; o) X. Zhao, Y. Nagai, P. J. Reeves, P. Kiley, H. G. Khorana, S. Zhang, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 17707–17712; p) Q. Zhang, X. Ma, A. Ward, W.-X. Hong, V.-P. Jaakola, R. C. Stevens, M. G. Finn, G. Chang, *Angew. Chem.* **2007**, *119*, 7153–7155; *Angew. Chem. Int. Ed.* **2007**, *46*, 7023–7025; q) P. S. Chae, K. Gotfryd, J. Pacyna, L. J. W. Miercke, S. G. F. Rasmussen, R. A. Robbins, R. R. Rana, C. J. Loland, B. Kobilka, R. Stroud, B. Byrne, U. Gether, S. H. Gellman, *J. Am. Chem. Soc.* **2010**, *132*, 16750–16752; r) S. C. Lee, B. C. Bennett, W.-X. Hong, Y. Fu, K. A. Baker, J. Marcoux, C. V. Robinson, A. B. Ward, J. R. Halpert, R. C.

Stevens, C. D. Stout, M. J. Yeager, Q. Zhang, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, E1203–1211; s) P. S. Chae, S. G. F. Rasmussen, R. R. Rana, K. Gotfryd, R. Chandra, M. A. Goren, A. C. Kruse, S. Nurva, C. J. Loland, Y. Pierre, D. Drew, J.-L. Popot, D. Picot, B. G. Fox, L. Guan, U. Gether, B. Byrne, B. Kobilka, S. H. Gellman, *Nat. Methods* **2010**, *7*, 1003–1008; t) K. H. Cho, B. Byrne, P. S. Chae, *ChemBioChem* **2013**, *14*, 452–455; u) P. S. Chae, R. R. Rana, K. Gotfryd, S. G. F. Rasmussen, A. C. Kruse, K. H. Cho, S. Capaldi, E. Carlsson, B. K. Kobilka, C. J. Loland, U. Gether, S. Banerjee, B. Byrne, J. K. Lee, S. H. Gellman, *Chem. Commun.* **2013**, *49*, 2287–2289.

[12] a) S. G. F. Rasmussen, H.-J. Choi, J. J. Fung, E. Pardon, P. Casarosa, P. S. Chae, B. T. DeVree, D. M. Rosenbaum, F. S. Thian, T. S. Kobilka, A. Schnapp, I. Konetzki, R. K. Sunahara, S. H. Gellman, A. Pautsch, J. Steyaert, W. I. Weis, B. K. Kobilka, *Nature* **2011**, *469*, 175–180; b) D. M. Rosenbaum, C. Zhang, J. Lyons, R. Holl, D. Aragao, D. H. Arlow, S. G. F. Rasmussen, H.-J. Choi, B. T. DeVree, R. K. Sunahara, P. S. Chae, S. H. Gellman, R. O. Dror, D. E. Shaw, W. I. Weis, M. Caffrey, P. Gmeiner, B. K. Kobilka, *Nature* **2011**, *469*, 236–240; c) S. G. F. Rasmussen, B. T. DeVree, Y. Zou, A. C. Kruse, K. Y. Chung, T. S. Kobilka, F. S. Thian, P. S. Chae, E. Pardon, D. Calinski, J. M. Mathiesen, S. T. A. Shah, J. A. Lyons, M. Caffrey, S. H. Gellman, J. Steyaert, G. Skiniotis, W. I. Weis, R. K. Sunahara, B. K. Kobilka, *Nature* **2011**, *477*, 549–555; d) A. C. Kruse, J. Hu, A. C. Pan, D. H. Arlow, D. M. Rosenbaum, E. Rosemond, H. F. Green, T. Liu, P. S. Chae, R. O. Dror, D. E. Shaw, W. I. Weis, J. Wess, B. K. Kobilka, *Nature* **2012**, *482*, 552–556; e) K. Haga, A. C. Kruse, H. Asada, T. Y. Kobayashi, M. Shiroishi, C. Zhang, W. I. Weis, T. Okada, B. K. Kobilka, T. Haga, T. Kobayashi, *Nature* **2012**, *482*, 547–551; f) A. Manglik, A. C. Kruse, T. S. Kobilka, F. S. Thian, J. M. Mathiesen, R. K. Sunahara, L. Pardo, W. I. Weis, B. K. Kobilka, S. Granier, *Nature* **2012**, *485*, 321–326; g) S. Granier, A. Manglik, A. C. Kruse, T. S. Kobilka, F. S. Thian, W. I. Weis, B. K. Kobilka, *Nature* **2012**, *485*, 400–404; h) J. F. White, N. Noinaj, Y. Shibata, J. Love, B. Kloss, F. Xu, J. Gvozdenovic-Jeremic, P. Shah, J. Shiloach, C. G. Tate, R. Grisshammer, *Nature* **2012**, *490*, 508–513; i) S. E. Rollauer, M. J. Tarry, J. E. Graham, M. Jaaskelainen, F. Jager, S. Johnson, M. Krehenbrink, S. M. Liu, M. J. Lukey, J. Marcoux, M. A. McDowell, F. Rodriguez, P. Roversi, P. J. Stansfeld, C. V. Robinson, M. S. Sansom, T. Palmer, M. Hcgbom, B. C. Berks, S. M. Lea, *Nature* **2012**, *492*, 210–214; j) J. Kellosalo, T. Kajander, K. Kogan, K. Pokharel, A. Goldman, *Science* **2012**, *337*, 473–476.

[13] a) G. G. Privé, *Methods* **2007**, *41*, 388–397; b) Q. Zhang, H. Tao, W.-X. Hong, *Methods* **2011**, *55*, 318–323.

[14] A. Chattopadhyay, E. London, *Anal. Biochem.* **1984**, *139*, 408–412.

[15] Y. Li, J. Reeve, Y. Wang, R. K. Thomas, J. Wang, H. Yan, *J. Phys. Chem. B* **2005**, *109*, 16070–16074.

[16] J. N. Israelachvili, D. J. Mitchell, B. W. Ninham, *J. Chem. Soc. Faraday Trans. 2* **1976**, *72*, 1525–1568.

[17] B. Lorber, J. B. Bishop, L. Delucas, *Biochim. Biophys. Acta Biomembr.* **1990**, *1023*, 254–265.

[18] P. D. Laible, C. Kirmaier, C. S. Udawatte, S. J. Hofman, D. Holten, D. K. Hanson, *Biochemistry* **2003**, *42*, 1718–1730.

[19] M. Caffrey, D. Li, A. Dukkipati, *Biochemistry* **2012**, *51*, 6266–6288.

[20] P. S. Chae, M. J. Wander, K. H. Cho, P. D. Laible, S. H. Gellman, *Mol. BioSyst.* **2013**, *9*, 626–629.

[21] P. S. Chae, K. H. Cho, M. J. Wander, H. E. Bae, S. H. Gellman, P. D. Labile, *Biochim. Biophys. Acta Biomembr.* **2014**, *1838*, 278–286.

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