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New Ganglio-tripod Amphiphiles (TPAs) for Membrane Protein Solubilization and Stabilization: Implications for Detergent Structure-Property Relationships

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Detergents are widely used for membrane protein research; however, membrane proteins encapsulated in micelles formed by conventional detergents tend to undergo structural degradation, necessitating the development of new agents with enhanced efficacy. Here we prepared several hydrophobic variants of ganglio-tripod amphiphiles (TPAs) derived from previously reported TPAs and evaluated for a multi-subunit, pigment protein superassembly. In this study, TPA-16 was found to be most efficient in protein solubilization while TPA-15 proved most favourable in long-term protein stability. The current study combined with previous TPA studies enabled us to elaborate on a few detergent structure-property relationships that could provide useful guidelines for novel amphiphile design.

Introduction

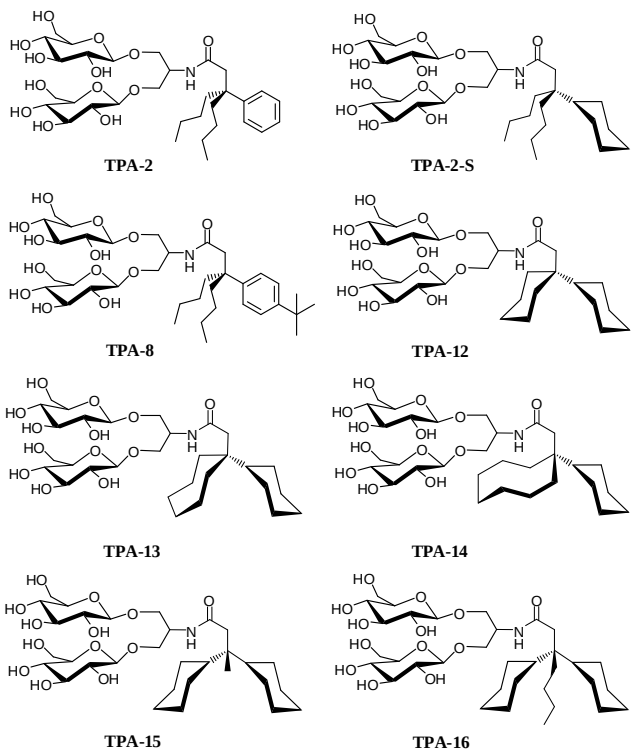
Membrane proteins are key players for a variety of cellular activities such as material transfer, signal transduction, and cell-to-cell communication. These bio-macromolecules account for a third of all human ORFs and are found in all membrane compartments including the plasma membrane, nuclear membrane, and mitochondrial membrane.[1] In addition, more than half of the pharmaceutical agents under current development target these membrane macromolecules, driving considerable attention to membrane protein research.[2] Despite such prevalence in biological systems and their importance in drug discovery, membrane protein structural study lags far behind that of soluble counterparts. The number of soluble proteins with known structure is approaching 100,000 while only hundreds of membrane protein structures are available from the Protein Data Bank (PDB).[3] Most of those structures were determined by X-ray crystallographic methods whereas some of them, particularly those with small molecular weights, were determined preferentially by nuclear magnetic resonance (NMR) spectroscopy.[4] Maintaining the native structure of membrane proteins in an aqueous medium is a prerequisite in these structural studies.[5] Nature evolved to utilize membrane architecture to ingeniously attain membrane protein stabilization; as such, membrane-inserted proteins are extremely stable. Native membranes exert not only lateral pressure on membrane proteins but also contain lipid molecules that specifically bind to protein surfaces, which play an essential role in preserving the native structures of membrane proteins.[6] Due to the large size of lipid-protein architectures, structure resolution of membrane proteins is not compatible with the analytical methods used for soluble protein structural studies (e.g., X-ray crystallography and NMR spectroscopy). Thus, membrane proteins must first be extracted from the native membrane for membrane protein structure research. Amphipathic molecules are widely used for this purpose as the micelles formed by these agents have the ability to interact with lipid molecules as well as membrane proteins.[7] Accordingly, the use of sufficient amounts of a detergent allows us to dismantle lipid bilayers and encapsulate a target membrane protein in micelles. The resulting protein-detergent complexes (PDCs) are the main entities used for membrane protein crystallization.

More than 100 conventional detergents are available, but only a few detergents are commonly used for membrane protein study, including lauryldimethylamine–*N*–oxide (LDAO), *n*–octyl–β–D–glucoside (OG), and *n*–dodecyl–β–D–maltoside (DDM).[8] Most of these conventional detergents have a single alkyl chain and a single hydrophilic group such as a glucoside, maltoside, or *N*-oxide. Along with the small number of popular detergents, the properties of these agents are too narrow to stabilize a large number of membrane proteins, which leads to serious limitations in detergent utility for membrane protein manipulation. As a result, many membrane proteins solubilized in these conventional detergents often suffer protein denaturation and aggregation.[7] Substantial efforts have been devoted to develop new amphipathic agents with enhanced properties.[9,10] A simple way to achieve this goal is to introduce structural modifications into conventional detergents based on detergent structure-property relationships. For instance, Chae's glyco-tritons (CGTs) bearing a carbohydrate headgroup, derived from Triton X-100, were developed based on the fact that

carbohydrate headgroups (e.g., maltose) were shown to be superior to polyoxyethylene in membrane protein stabilization.[9a] Cholate and deoxycholate-based *N*-oxide agents (CAOs and DCAOs) were also prepared by replacing the sulfobetaine headgroup of 3-[(3-cholamindopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) with an *N*-oxide group, noting that *N*-oxide-containing agents (e.g., LDAO) are more commonly used than anzergents with a sulfobetaine headgroup in most membrane protein studies.[9b] These modified analogs were shown to be superior to their parent detergents in terms of membrane protein solubilization and stabilization. Continued efforts have been devoted to invent novel amphiphiles with significantly distinct architectures from those of conventional agents.[10] Examples include tripod amphiphiles (TPAs),[10a-d] facial amphiphiles (FAs),[10e,f] rigid hydrophobic group bearing amphiphiles (Chobimalt and glycosylated diosgenin-based amphiphile (GDN)),[10g-i] NG class amphiphiles (glucose neopentyl glycols (GNGs),[10j,k] maltose neopentyl glycols (MNGs)[10l,m]), and calixarene-based surfactants.[10n] Recently, a number of polymeric nanoparticles, including amphipols[10o,p] and nanoassemblies (e.g., nanodiscs (NDs)[10q] and nanolipodisq[10r]) have been successfully applied to various membrane protein studies. Also, note that a number of peptide-based agents such as α-helices (e.g., peptitergents[10s] and lipopeptides[10t]), β-peptides[10u], and short peptides[10v] with no specific secondary structure have been shown to be promising in membrane protein studies. However, neither these nanoassemblies nor the peptide-based detergents have proved successful for membrane protein structural studies as of yet.

We have studied tripod amphiphiles with three hydrophobic groups in the lipophilic region. The use of modular synthetic approaches toward these agents has allowed us to introduce large scope of structural variations into the lipophilic and hydrophilic region. The original of this class, designated TPA-0 (commercially TRIPAO), has an *N*-oxide headgroup and were shown to be particularly promising in solubilization and stabilization of rather robust membrane proteins such as bacteriorhodopsin (bR) and bovine rhodopsin (rho) proteins.[10a] Accordingly, this agent proved successful in obtaining high quality crystals of these proteins.[11] The rather harsh nature of this agent prompted us to prepare glyco-tripod amphiphiles with various carbohydrate headgroups, noting that carbohydrate-containing detergents (e.g., DDM and OG) are superior to *N*-oxide-bearing agents (e.g., LDAO) in terms of membrane protein stabilization efficacy.[10b] When we evaluated a set of TPAs with various headgroups for the fragile superassembly, two branched diglucoside-bearing TPAs, TPA-2 and TPA-2-S (commercially Trip-Pheglu and Trip-Cyglu, respectively; **Scheme 1**), were superior to the original TPA (TPA-0) in terms of protein stabilization efficacy. This result encouraged us to prepare hydrophobic variants of TPA-2 by introducing an alkyl appendage on a benzene ring at the *para* position.[10d] Here we made continuous effort in a different direction to further improve protein solubilization efficiency and stabilization efficacy by preparing several TPA-2-S variants (**Scheme 1**). We found a set of TPAs that are more efficient at membrane protein solubilization than a conventional detergent (DDM) and the other TPAs. Among these efficient TPAs, TPA-15 displayed the most promising behavior for the long-term protein stability. In addition, detergent structure-property relationships proposed in a previous report were refined based on current TPA results.

Results and Discussion



Scheme 1. Chemical structures of previously reported TPAs (TPA-2, TPA-2-S, and TPA-8) and newly prepared TPAs (TPA-12, TPA-13, TPA-14, TPA-15 and TPA-16). TPA-12, TPA-13, and TPA-14 have two rings in the lipophilic portion without an alkyl chain. This set of TPAs has one cyclohexyl ring, but vary in the size of the other ring (cyclohexane for TPA-12, cycloheptane for TPA-13, and cyclooctane for TPA-14). On the other hand, TPA-15 and TPA-16 include two cyclohexane rings and one alkyl chain varying in chain length (methyl for TPA-15 and butyl for TPA-16). All of these TPAs share the same, branched diglucoside headgroup with TPA-2.

The new agents designed here were based on the TPA-2-S compound with one cyclohexyl ring and two butyl chains (**Scheme 1**). All the new variants share a hydrophilic group (i.e., a branched diglucoside) and a hydrophobic cyclohexane moiety with TPA-2-S, but vary in the other parts of the hydrophobic group. The structural modification was first made by connecting two TPA-2-S alkyl chains to produce a set of TPAs with a different ring size (cyclohexane for TPA-12, cycloheptane for TPA-13, and cyclooctane for TPA-14). The second set of TPAs was prepared by substituting one butyl chain of TPA-2-S for another cyclohexyl ring, thus commonly bearing two cyclohexyl rings but with a different alkyl chain (methyl for TPA-15 and butyl for TPA-16). These two sets of TPAs were synthesized in seven reaction steps with moderate overall yield (~20%; see supporting information for details).

All new agents except TPA-12 were water-soluble well up to 10%. TPA-12 was initially soluble well but tended to form insoluble aggregates with time and thus not studied further. The critical micelle concentrations (CMCs) of the new agents were determined by absorption of the fluorescent dye, diphenylhexatriene (DPH),[12] and their micelle sizes were estimated by dynamic light scattering (DLS) experiments. The summarized data of these results along with the solubilization yields (SYs) for the *Rhodobacter* (*R.*) *capsulatus*

superassembly are presented in **Table 1**. The molecular weights of TPA-14 and TPA-15 were similar to that of TPA-2 and their CMC values were also comparable. A similar trend was seen for TPA-8 and TPA-16; these two TPAs are comparable in molecular weight (716 and 692) and CMC value (0.42 mM and 0.64 mM). The relationship between the molecular weight and CMC value of the detergents observed here is consistent with the general notion that detergent CMC value decreases with the molecular weight of detergent lipophilic group. This is due to the fact that an enlarged lipophilic group increases detergent hydrophobicity, thereby facilitating micelle formation at a lower concentration. Consistent with this notion, TPA-13 with the lowest molecular weight among the current TPA series gave the largest CMC value of 14 mM. Note that, in addition to the hydrophobicity of detergent lipophilic group, other factors (e.g., lipophilic group volume) could strongly influence the CMC values of detergents, as can be found in the literature.[13] These TPAs except TPA-8 and TPA-16 have relatively large CMC values ranging from ~1.8 mM to ~14 mM, indicating of the high capability of these TPAs for dialysis; detergent removal via dialysis is reported to be much more efficient for detergents with large CMC values (e.g., OG; ~ 18 mM) than detergents with small CMC values (e.g., DDM).[14] Micelle sizes formed by TPAs tend to increase with alkyl chain length of the lipophilic groups. For example, micelles formed by TPA-8 with a *t*-butylphenyl group gave a three-fold increase in hydrodynamic radius (R_h) than those formed by TPA-2 with a phenyl group, which corresponds to a 27-fold increase in terms of micellar volume. A similar degree of difference in the hydrodynamic radii was observed for TPA-15 (methyl) vs. TPA-16 (*n*-butyl). Micelles formed by TPA-14 with a cyclooctyl (C8) ring were also larger than those formed by TPA-13 with a cycloheptyl (C7) ring, suggesting an effect of ring size present in the hydrophobic portion on detergent micelle size. Increase in alkyl chain length or ring size is likely to make a TPA molecule more cylindrical, thus leading to the formation of larger self-assemblies.[16] Most of the TPAs designed here showed one set of micelle distributions in the DLS experiments, as did DDM. On the other hand, two kinds of aggregates were observed for TPA-8, TPA-13, and TPA-14. The R_h values of these aggregates were ~6.0 nm and ~334 nm for TPA-8, ~2.2 nm and ~184 nm for TPA-13, and ~2.6 nm and ~174 nm for TPA-14 (**Figure S1**). The ratios for the two sets of aggregates were calculated to be > 10^{11} in the *number* distribution-based calculations, indicating the exclusive dominance of small micelles for these TPAs (TPA-8, TPA-13, and TPA-14).

Table 1. Critical micelle concentrations (CMC) and hydrodynamic radii (R_h) of the micelles (mean ± SD, n = 4), solubilization yields (SYs) for previously reported TPAs (TPA-2, TPA-2-S, and TPA-8), newly synthesized TPAs (TPA-13, TPA-14, TPA-15, and TPA-16), and conventional detergents (DDM and LDAO).

	M_W [a]	CMC (mM; wt%)	R_h (nm)[b]	SY (%)
TPA-2	659.8	~3.6; ~0.24	2.0 ± 0.1	~50
TPA-2-S	665.8	~1.8; ~0.12	3.3 ± 0.1	~70
TPA-8	715.9	~0.42; ~0.030	6.0 ± 0.2[c]	~80
TPA-13	635.7	~14.0; ~0.88	2.6 ± 0.2[c]	~80
TPA-14	649.8	~4.5; ~0.29	3.8 ± 0.3[c]	~80
TPA-15	649.8	~4.7; ~0.31	2.2 ± 0.0	~80
TPA-16	691.8	~0.64; ~0.044	6.8 ± 0.1	~90
DDM	510.6	~0.17; ~0.0087	3.5 ± 0.0	~70
LDAO	229.4	~1.0; ~0.023[d]	2.0[d]	~100

[a]Molecular weight of detergents. [b]Hydrodynamic radius of micelles, except TPA-15 was determined at 1.0 wt % by dynamic light scattering. TPA-15 was used at 2.0 wt % to obtain a strong signal. [c]Two forms of aggregates were observed with hydrodynamic radii of ~6.0 nm and ~334 nm for TPA-8, ~2.2 nm and ~184 nm for TPA-13, and ~2.6 nm and ~174 nm for TPA-14 (see **Figure S1**).[d] These values were obtained from the literature.[15]

The new TPAs were evaluated with the photosynthetic superassembly from *R. capsulatus*, comprised of light harvesting complex I (LHI) and reaction center complex (LC).[17] The LHI complex contains multiple tertiary and quaternary structures, thus being highly sensitive to protein denaturation while the LC complex is rather resilient.[10b] Only mild detergents such as DM and DDM were shown to maintain the native conformation of the LHI-RC complexes in the course of protein solubilization and purification. The use of OG and Triton X-100 with intermediate strength led to degradation of the LHI with an intact RC. When we introduced harsh detergents such as LDAO and sodium dodecyl sulfate (SDS), both components, LHI and RC, underwent fast structural degradation. Hence, the LHI-RC complex allowed us to evaluate a set of detergents in a gradable way according to their efficacy for membrane protein stabilization, thus being a good litmus test for detergent evaluation in terms of protein *stabilization* efficacy. The LHI-RC complex is also a convenient system for detergent evaluation on membrane protein *solubilization* efficiency thanks to the presence of multiple cofactors such as chlorophylls and carotenoids in the interior of the complex. These cofactors, when embedded in the native conformation, give rise to a featured UV-Visible spectrum showing intense absorption at 875 nm. The partial or complete degradation of LHI and/or RC complexes can be readily detected by the appearance of peaks at ~760 nm and ~800 nm and decrease of a peak at 875 nm. Therefore, we can make an unambiguous assessment on protein quantity and integrity via simple optical spectrophotometry.

Detergent evaluation started with the addition of individual detergents to the intracytoplasmic *R. capsulatus* membranes enriched in LHI-RC complexes. Due to the large variation in CMC values, different detergent concentrations were used for protein solubilization (2xCMC for TPA-13, 4xCMC for TPA-14 and TPA-15, and 30xCMC for TPA-16). For comparison, we included three previously reported TPAs (TPA-2, TPA-2-S, and TPA-8 with 5xCMC, 10xCMC, and 30xCMC, respectively) and two conventional detergents (DDM and LDAO with 60xCMC and 20xCMC, respectively). The insolubilized portion including cellular debris and membrane-inserted LHI-RC complexes following detergent treatment was separated from the detergent-solubilized portion via ultracentrifugation. The supernatant portion including detergent micelles and detergent-solubilized complexes was directly used for spectroscopic measurements while detergent-insolubilized portions obtained as pellets were suspended in an aqueous buffer before spectroscopic measurements. Consistent with previous results, DDM solubilized the LHI-RC complexes in ~70% yield and previously reported TPAs (TPA-2, TPA-2-S, and TPA-8) solubilized

in ~50%, ~70%, and ~80% yields, respectively (**Figure 1a** & **S2a**).[10b,d] The first set of the new TPAs, TPA-13 and TPA-14, with cycloheptyl and cyclooctyl rings, respectively, was not much successful in increasing solubilization efficiency. Rather, these agents were compared to TPA-8 in this regard (~80%; **Figures S2**). When we evaluated the second set of TPAs including those with two cyclohexyl rings and one alkyl appendage, however, detergent solubilization efficiency was increased to ~90% for TPA-16 whereas TPA-15 was just comparable to other well-behaving TPAs (TPA-8, TPA-13, and TPA-14) (**Figure 1a**). The intense peak at 875 nm in the spectrum of TPA-16-solubilized complex indicates the strong power of this agent in disrupting the lipid bilayer and extracting complexes from that layer, yet with little detrimental effect on protein structure deformation (**Figure S2b**,**c**). In contrast, LDAO-solubilized complex lost most of its structural integrity during protein solubilization although most of the protein complexes could be solubilized by the use of this agent (**Figure 1a**,**b**). Achieving detergent efficacy corresponding such high solubilization and favorable stabilization is challenging because detergents with high solubilization efficiency tend to have strong propensity to denature fragile membrane protein complexes, as exemplified by Triton X-100, OG, and LDAO.[9a,10c]

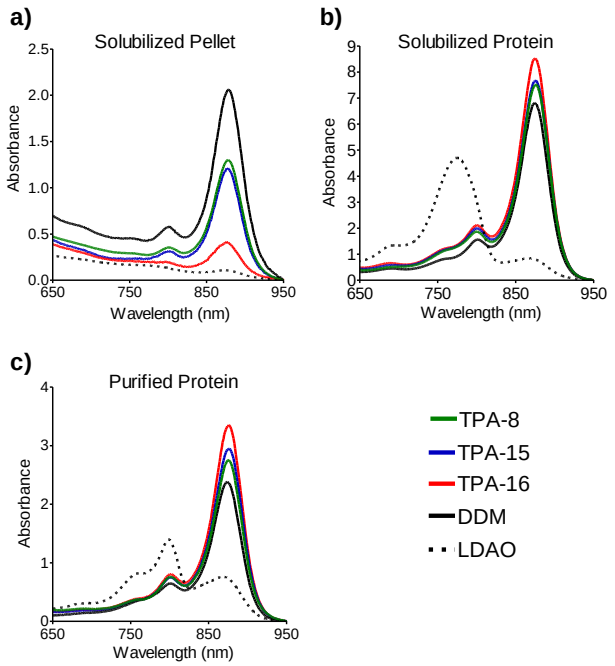


Figure 1. Absorbance spectra of *R. capsulatus* superassembly (a) insolubilized, (b) solubilized, and (c) purified in three TPA agents (TPA-8, TPA-15, and TPA-16) and two conventional detergents (DDM and LDAO). For protein solubilization, individual detergents were used at different concentrations due to the large variation in their CMC values (60xCMC for DDM, 30xCMC for TPA-8 and TPA-16, 20xCMC for LDAO, and 4xCMC for TPA-15). Detergent solubilized and insolubilized portions were separated via ultracentrifugation and used for spectroscopic measurements. Protein purification was performed via Ni-NTA affinity column chromatography with an elution buffer including 1xCMC detergent and 1.0 M imidazole. All spectra were measured ranging from 650 nm to 950 nm.

In order to investigate the utility of these new TPAs for the next step of protein purification, each TPA-solubilized complex was incubated with Ni-NTA resins for resin binding as the complexes contain a hepta-histidine tag at the C-terminus of the RC M subunit. Detergent-purified protein samples were obtained in high purity by eluting the protein complexes from IMAC column. A buffer containing 1.0 M imidazole and 1xCMC of the individual detergents was used for this elution. The UV-Visible spectra of these protein samples were then obtained in the range of 650 nm to 950 nm. Overall, these spectra were similar to those of detergent-solubilized counterparts, with one exception with the LDAO sample, indicating the continual effectiveness of these TPAs and DDM at preserving the native structure of the complexes in the course of complex purification (**Figure 1c**). On the other hand, LDAO-solubilized complexes were further degraded during the purification.

Among dozens of TPAs developed so far, several TPAs (TPA-8, TPA-13, TPA-14, TPA-15 and TPA-16) showed high membrane protein solubilization efficiency (≥80%). However, the current protocol failed to discriminate between their efficacies for membrane protein stabilization because all these agents are mild enough to stabilize the superassembly in the course of protein solubilization and purification. In order to differentiate their stabilization efficacies, we evaluated these agents for *long-term* stability of the superassembly. In this evaluation, the superassembly was first solubilized with 1.0 wt% DDM and purified via IMAC column as described above. The purified protein solution was 50-fold diluted with individual TPA-containing solutions, so that the amount of residual DDM is far less than its CMC. We selected three amphiphile concentrations to investigate detergent concentration effect on proteins stability: CMC+0.04 wt%, CMC+0.2 wt%, and CMC+1.0 wt%. Protein stability was monitored over 20 days of incubation period at room temperature by tracking absorbance value at 875 nm. At the low amphiphile concentration, TPA-14 and TPA-15 showed a better ability to stabilize the superasembly as compared to other TPAs (TPA-8, TPA-13, TPA-16) and conventional detergents (DDM and LDAO) (**Figure 2a**). When we increased the amphiphile concentration to CMC+1.0 wt%, the efficacy differences between TPAs became more prominent. At this high concentration, TPA-15 was superior to other TPAs and conventional detergent (DDM) (**Figure 2b**), followed by TPA-14 and TPA-13. A similar trend was obtained at CMC+0.2 wt% (**Figure S3**). Note that we used the protein complexes at a low concentration of ~ 0.2 μM for this stability study mainly because many detergents could be evaluated in a number of conditions with small amounts of protein samples. A preliminary study showed that the detergent efficacy order for protein stabilization was little changed when the concentration of this complex was increased to ~10 μM. This result implies that the stability of LHI-RC complex is minimally influenced by protein concentration under the condition. The combined results of protein solubilization, purification and long-term stabilization reveal that TPA-16 is most powerful at solubilizing the complex from the membrane while TPA-15 is most outstanding in terms of long-term stability of the protein.

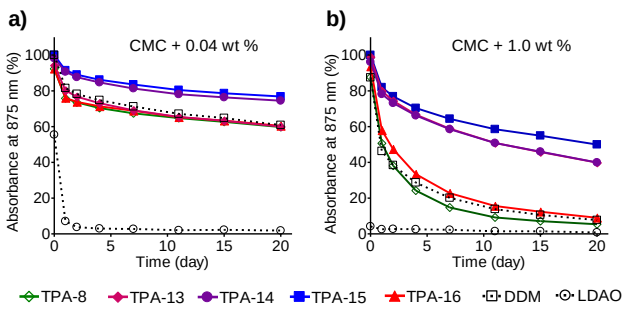


Figure 2. Long-term stability of *R. capsulatus* superassembly at two different amphiphile concentrations: (a) CMC + 0.04 wt% and (b) CMC + 1.0 wt%. Five tripod amphiphiles showing high solubilization power were selected for this stability assay. DDM-purified protein sample was mixed with solutions containing these individual TPAs and then protein stability was monitored by taking absorbance value at 875 nm at regular interval during 20 days of incubation at room temperature.

Tripod amphiphiles were shown to be favorable in membrane protein solubilization. It is likely that the multiple alkyl chains present in the lipophilic region are responsible for the high solubilization efficiency. Conceivably, each of these alkyl chains noncovalently interacts with membrane proteins, giving rise to a strong binding between detergent molecules and membrane proteins. Conventional detergents typically having a single alkyl chain cannot achieve such multiple-point interactions. This advantageous effect was well illustrated by the comparative study of TPAs and monopod amphiphiles (MPAs).[10b] When TPA-2 variants were used instead of conventional MPA-2 variants for the solubilization of the superassembly (**Figure S4**), a significant increase in membrane protein solubilization yield was observed (from ~30% to ~80%).[10b] In the current study, the two rings-bearing TPAs with no alkyl chain (the first TPA set) or the two cyclohexyl rings-containing TPAs with one alkyl chain (the second TPA set) were evaluated for membrane protein solubilization and purification. Consistent with the previous observations, these TPAs displayed favourable behaviour in terms of membrane protein solubilization efficiency, with the best performance of TPA-16. The favourable behaviour of TPA-16 is likely attributed to the presence of three alkyl groups with similar chain length (two cyclohexyl rings and one butyl chain) because such architecture would facilitate three-point interactions with the hydrophobic segment of membrane proteins. A similar result was observed in a previous study,[10d] suggesting that this design strategy can be generally useful for the development of novel agents with high solubilisation efficiency. Despite the high solubilization power (~90%), however, TPA-16 turned out to be sub-optimal for the long-term protein stabilization. Rather TPA-15 with a dipodal structure was superior in the stabilization of LHI-RC complexes, indicating that the tripod architecture may not be ideal for membrane protein stabilization. Note that detergent structural features for membrane protein solubilization don't generally match with those for membrane protein stabilization. This is a reason why a novel detergent showing both high solubilization efficiency *and* an excellent stabilization efficacy (e.g., TPA-15) is rare.

DDM, OG, and LDAO are three conventional detergents most widely-used for membrane protein crystallization.[8,18] It is notable that these popular agents are commonly promising at membrane protein solubilization and stabilization. This notion indicates that, in addition to membrane protein *stabilization* efficacy, protein *solubilization* efficiency is a crucial factor in determining the outcome of membrane protein crystallization attempts. We have little knowledge about the precise reason for the correlation between these detergent properties: membrane protein solubilization efficiency and membrane protein crystallization efficacy. Conceivably, detergents with strong binding affinity toward the hydrophobic segment of membrane proteins would favour protein-detergent micelle interaction over protein-protein interaction, thereby effectively preventing protein aggregation. Thus, detergents showing high solubilization efficiency and reasonably good stabilization efficacy would facilitate membrane protein crystallization by minimizing protein aggregation. Protein aggregation can be particularly problematic in membrane protein structure studies via X-ray crystallography and NMR spectroscopy because these analytical methods commonly utilize high protein concentrations for crystal formation or strong spectral signal. The scrutiny for a number of novel amphiphile studies also reaches a similar conclusion. Strong solubilizing agents such as TPAs, FAs, and NG-based agents (GNGs and MNGs) have facilitated the determination of membrane protein structures.[10e,11,19] In contrast, conventional detergents (e.g., CHAPS) and novel agents (e.g., amphipols and HFSs[20]) with low solubilization efficiency have not yet been successful in these studies. Owing to the promising protein stabilization efficacy and high protein solubilization efficiency, TPA-15 holds promise in membrane protein structural studies. It is notable that other TPAs such as TPA-14 and TPA-16 can be useful as well because these agents are excellent in either membrane protein stabilization or membrane protein solubilization.

Detergent hydrophilic groups play a pivotal role in determining membrane protein stabilization efficacy. In general, non-ionic detergents are known to be least destabilizing membrane proteins, followed by zwitterionic and ionic detergents. For instance, carbohydrate containing agents (e.g., OG and DDM) are known to be excellent in maintaining the native structures of membrane proteins while *N*-oxide group agents (e.g. LDAO) and an anionic SDS have a medium and strong protein-destabilizing effects, respectively.[18] Amongst non-ionic detergents, maltoside-bearing agents (e.g. DDM) are better than glucoside-bearing agents (e.g. OG) in this regard, indicating the superiority of maltose to glucose as a headgroup. Through a couple of systematic studies of TPAs, the branched diglucoside headgroup has emerged as a new promising hydrophilic group in membrane protein stabilization because all TPAs with this hydrophilic group developed thus far favourably maintained the native structure of the complexes while a few maltoside-bearing TPAs (e.g., TPA-4) substantially destroyed the complexes during the protein solubilization; only one maltoside-bearing TPA, TPA-4, has been reported thus far,[10b] but we have a few hydrophobic variants of TPA-4 that display detergent behaviours similar to that of TPA-4 (**Figure S5**). These results along with previous TPA studies have led us to find a strategy to maximize detergent efficacy for membrane protein solubilization and/or stabilization. First, multiple hydrophobic groups in detergent lipophilic region tend to increase membrane proteins solubilization efficiency. Second, hydrophobicity of detergent molecules is necessary to be high enough for strong interaction with the hydrophobic segment of membrane proteins, but should be modulated because exceedingly hydrophobic detergents could give a

harmful effect on protein stability, as can be seen in TPA-8 and TPA-16. Third, use of the branched diglucoside headgroup will confer detergents on favourable membrane protein stabilization efficacy. Note that detergent properties such as membrane protein solubilisation efficiency and stabilization efficacy are not determined by a single part of a molecule but by the cooperation of both hydrophobic and hydrophilic groups. It is also important to note that an appropriate balance between these groups is essential for the best performance of novel amphiphiles, indicating that there is no magic bullet in detergent hydrophobic or hydrophilic group. In this context, the lipophilic groups of TPAs appeared well compatible with the branched diglucoside headgroup for favourable detergent properties, making TPA class members suited for membrane protein science.

Conclusion

The evaluation for a number of hydrophobic variants of TPA-2 and TPA-2-S allowed us to find a few TPA agents with high efficiency for membrane protein solubilisation, as exemplified by TPA-8, TPA-13, TPA-14, TPA-15 and TPA-16. Among these powerful TPAs, TPA-15 was most promising in the long-term stabilization of the complexes, followed by TPA-14. Detergent evaluation for both membrane solubilization efficiency and membrane protein stabilization efficacy is important because both factors are critical for the successful outcome of membrane protein crystallization. In addition, detergent structure-property-efficacy relationships discussed here will provide useful guidelines in novel amphiphile design, thereby facilitating advance in membrane protein research. Note that high flexibility in the structural variation of TPAs allowed us to disclose these relationships. Therefore, TPA class is an ideal system for the invention of promising amphipathic agents as well as the investigation of detergent structure-property-efficacy relationships.

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Notes and references

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Electronic Supplementary Information (ESI) available: Experimental details of the amphiphiles' synthesis and characterization, and their evaluation for membrane protein solubilisation and purification: See DOI: 10.1039/b000000x/

1 (a) E. Wallin and G. von Heijne, *Protein Sci.* 1998, **7**, 1029-1038; (b) *A.* Krogh, B. Larsson, G. von Heijne and E. L. Sonnhammer, *J. Mol. Biol.* 2001, **305**, 567-580.
2 J. P. Overington, B. Al-Lazikani and A. L. Hopkins, *Nat. Rev. Drug Discovery* 2006, **5**, 993-996.
3 http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html
4 (a) A. Arora and L. K. Tamm, *Curr. Opin. Struct. Biol*. 2001, **11**, 540-547; (b) S. -J. Kang, D. -H. Kim and B. -J. Lee, *Molecules* 2013, **18**, 13410-13424; (c) A. H. Kwan, M. Mobli, P. R. Gooley, G. F. King and J. P Mackay, *FEBS J.* 2011, **278**, 687-703.
5 J. J. Lacapere, E. Pebay-Peyroula, J. M. Neumann and C. Etchebest, *Trends Biochem. Sci.* 2007, **32**, 259-270.
6 (a) S. H. White and W. C. Wimley, *Annu. Rev. Biophys. Biomol. Struct.* 1999, **28**, 319-365; b) J. U. Bowie, *Curr. Opin. Struct. Biol*. 2001, **11**, 397-402.
7 (a) R. M. Garavito and S. Ferguson-Miller, *J. Biol. Chem*. 2001, **276**, 32403-32406; (b) M. J. Serrano-Vega, F. Magnani, Y. Shibata and C. G. Tate, *Proc. Natl. Acad. Sci. U. S. A.* 2008, **105**, 877-882; (c) J. V. Moller and J. le Maire, *J. Biol. Chem*. 1993, **268**, 18659-18672.
8 (a) S. Newstead, S. Ferrandon and S. Iwata, *Protein Sci.* 2008, **17**, 466–472; (b) S. Newstead, J. Hobbs, D. Jordan, E. P. Carpenter and S. Iwata, *Mol. Membr. Biol.* 2008, **25**, 631–638.
9 (a) P. S. Chae, M. J. Wander, K. H. Cho, P. D. Laible and S. H. Gellman, *Mol. BioSyst.* 2013, **9**, 626-629; (b) P. S. Chae, A. Sadaf and S. H. Gellman, *Chem. Asian J.* 2014, **9**, 110-116.
10 (a) S. M. Yu, D. T. McQuade, M. A. Quinn, C. P. R. Hackenberger, M. P. Krebs, A. S. Polans and S. H. Gellman, *Protein Sci*. 2000, **9**, 2518–2527; (b) P. S. Chae, M. J. Wander, A. P. Bowling, P. D. Laible and S. H. Gellman, *ChemBioChem* 2008, **9**, 1706-1709; (c) P. S. Chae, A. C. Kruse, K. Gotfryd, R. R. Rana, K. H. Cho, S. G. F. Rasmussen, H. E. Bae, R. Chandra, U. Gether, L. Guan, B. K. Kobilka, C. J. Loland, B. Byrne and S. H. Gellman, *Chem. Eur. J*. 2013, **19**, 15645-15651; (d) P. S. Chae, K. H. Cho, M. J. Wander, H. E. Bae, S. H. Gellman and P. D. Labile, *Biochim. Biophys. Acta* 2014, **1838**, 278-286; (e) 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 and Q. Zhang, *Proc. Natl. Acad. Sci. U. S. A.* 2013, **110**, E1203-1211; (f) 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 and S. H. Gellman, *J. Am. Chem. Soc.* 2010, **132**, 16750-16752; (g) S. C. Howell, R. Mittal, L. Huang, B. Travis, R. M. Breyer and C. R. Sanders, *Biochemistry* 2010, **49**, 9572-9583; (h) 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 and W. Welte, *Mol. Membr. Biol.* 2011, **28**, 171-181; (i) 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 and S. H. Gellman, *Chem. Eur. J.* 2012, **18**, 9485-9490; (j) 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 and S. H. Gellman, *Chem. Commun*. 2013**, 49**, 2287-2289; (k) K. H. Cho, H. E. Bae, M. Das, S. H. Gellman and P. S. Chae, *Chem. Asian J.* 2014, **9**, 632-638; (l) 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 and S. H. Gellman, *Nat. Methods* 2010, **7**, 1003-1008; (m) K. H. Cho, B. Byrne and P. S. Chae, *ChemBioChem* 2013, **14**, 452-455; (n) R. Matar-Merheb, M. Rhimi, A. Leydier, F. Huche, C. Galian, E. Desuzinges-Mandon, D. Ficheux, D. Flot, N. Aghajari, R. Kahn, A. Di Pietro, J. M. Jault, A. W. Coleman and P. Falson, *PLoS One* 2011, **6**, e18036-e18036; (o) C. Tribet, R. Audebert and J.-L. Popot, *Proc. Natl. Acad. Sci. U. S. A.* 1996, **93**, 15047-15050; (p) J.-L. Popot, *et al*., *Annu. Rev. Biophys.*2011, **40**, 379–408; (q) A. Nath, W. M. Atkins and S. G. Sligar, *Biochemistry* 2007, **46**, 2059-2069; (r) M. Orwick-Rydmark, J. E. Lovett, A. Graziadei, L. Lindholm, M. R. Hicks and A. Watts, *Nano Lett.* 2012, **12**, 4687-4692; (s) C. E. Schafmeister, L. J. W. Miercke and R. M. Stroud, *Science* 1993, **262**, 734-738; (t) C.-L. McGregor, L. Chen, N. C. Pomroy, P. Hwang, S. Go, A. Chakrabartty and G. G. Privé, *Nat. Biotechnol*. 2003, **21**, 171-176; (u) H. Tao, S. C. Lee, A. Moeller, R. S. Roy, F. Y. Siu, J. Zimmermann, R. C. Stevens, C. S. Potter, B. Carragher and Q. Zhang, *Nat. Methods* 2013, **10**, 759-761; (v) X. Zhao, Y. Nagai, P. J. Reeves, P. Kiley, H. G. Khorana and S. Zhang, *Proc. Natl. Acad. Sci. U. S. A.* 2006, **103**, 17707-17712.
11 P. S. Chae, P. D. Laible and S. H. Gellman, *Mol. BioSyst*. 2010, **6**, 89-94.
12 H. Schott, *J. Phys. Chem.* 1966, **70**, 2966–2973.
13 Y. Li, J. Reeve, Y. Wang, R. K. Thomas, J. Wang and H. Yan, *J. Phys. Chem. B* 2005, **109**, 16070-16074.
14 G. A. Signorell, T. C. Kaufmann, W. Kukulski, A. Engel and H.-W. Rémigy, *J.*

Struct. Biol. 2007, **157**, 321-358.

15 M. Osawa, K. I. Tong, C. Lilliehook, W. Wasco, J. D. Buxbaum, H. Y. M. Cheng, J. M. Penninger, M. Ikura and J. B. Ames, *J. Mol. Chem*. 2001, **276**, 41005-41013.

16 J. N. Israelachvili, D. J. Mitchell and B. W. Ninham, *J. Chem. Soc. Faraday Trans. II* 1976, **72**, 1525-1568.

17 P. D. Laible, C. Kirmaier, C. S. Udawatte, S. J. Hofman, D. Holten and D. K. Hanson, *Biochemistry* 2003, **42**, 1718-1730.

18 (a) G. G. Privé, *Methods* 2007, **41**, 388-397; (b) Q. Zhang, H. Tao and W.-X. Hong, *Methods* 2011, **55**, 318-323.

19 (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 and 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 and 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 and 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 and 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 and 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 and S. Granier, *Nature* 2012, **485**, 321-326; (g) S. Granier, A. Manglik, A. C. Kruse, T. S. Kobilka, F. S. Thian, W. I. Weis and 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 and 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 and S. M. Lea, *Nature* 2012, **492**, 210–214; (j) J. Kellosalo, T. Kajander, K. Kogan, K. Pokharel and A. Goldman, *Science* 2012, **337**, 473-476; (k) A. Frick, U. K. Eriksson, F. de Mattia, F. Oberg, K. Hedfalk, R. Neutze, W. J. de Grip, P. M. T. Deen and S. Tornroth-Horsefield, *Proc. Natl. Acad. Sci. U. S. A*. 2014, **111**, 6305-6310; (l) E. Karakas and H. Furukawa, *Science* 2014, **344**, 992-997; (m) A. C. Kruse, et al., *Nature* 2013, **504,** 101-106; (n) A. M. Ring, A. Manglik, A. C. Kruse, M. D. Enos, W. I. Weis, K. C. Garcia and B. K. Kobilka, *Nature* 2013, **502**, 575-579; (o) H. Suzuki, T. Nishizawa, K. Tani, Y. Yamazaki, A. Tamura, R. Ishitani, N. Dohmae, S. Tsukita, O. Nureki and Y. Fujiyoshi, Science 2014, **344**, 304-307; (p) A. K. Shukla, et al., *Nature* 2014, **512**, 218-222.

20 (a) C. Breyton, E. Chabaud, Y. Chaudier, B. Pucci and J.-L. Popot, *FEBS Lett*. 2004, **564**, 312-318; (b) M. Abla, G. Durand and B. Pucci, *J. Org. Chem*. 2011, **76**, 2084-2093.