Rationally engineered tandem facial amphiphiles for improved membrane protein stabilization efficacy

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Abstract: A new family of tandem facial glucoside/maltosides (TFG/TFMs) for membrane protein manipulation is reported. The best detergent varied depending on the hydrophobic thickness of the target protein, but TFM-C0E, TFM-C3E or TFM-C5E were notable for their ability to confer enhanced membrane protein stability compared to the previously developed TFA-1 (*JACS*, **2010**, *132*, 16750). These agents have potential for use in membrane protein research.

Membrane proteins are crucial for cellular physiology as they are directly involved in a large spectrum of cellular processes including transport of biological molecules and ions, intracellular signal transduction and are major human drug targets.[1] These bio-macromolecules are harder to study than soluble proteins because they are relatively unstable, often losing both structural and functional integrity, once extracted from the native membranes. Conventional detergents such as *n*-dodecyl-β-D-maltoside (DDM), *n*-octyl-β-D-glucoside (OG) and lauryldimethylamine-*N*-oxide (LDAO), which shield the large hydrophobic protein surfaces from the polar aqueous environment, are widely used in the extraction, purification and crystallization of membrane proteins.[2a] However, these mild detergents represent rather poor lipid bilayer mimetics.[2b] Hence, it is essential to develop new amphiphiles for structural and functional studies of membrane proteins. This is particularly important for the study of eukaryotic/human membrane proteins required to facilitate structure-based drug discovery. Recent representatives include neopentyl glycol (NG) class amphiphiles (MNGs/GNGs/NDT),[3a-c] glyco-diosgenin (GDN),[3d] fluorinated detergents (F_6OM),[3e] penta-saccharide amphiphiles (PSEs),[3f] mannitol-based amphiphiles (MNAs),[3g] dendronic trimaltosides (DTMs),)[3h] and glycosyl-substituted dicarboxylate detergents (DCODs)[3i]. A departure from the canonical 'polar head and nonpolar tail' design of conventional detergents are the cholate or deoxycholate derivatives which also displayed remarkable protein stabilization properties when used as scaffolds for facial detergents.[4] Zhang *et al*., for example, have reported cholate-based facial amphiphiles (FAs) intended to provide a match between the hydrophobic length of the detergent and half the hydrophobic width of a lipid bilayer. Hence, two FA molecules form a sheath across the hydrophobic surface of a membrane protein.[4b] By covalently linking two FA units using a spacer, we previously developed tandem facial amphiphiles (TFAs) where a single amphiphilic molecule can facially cover the ~30 Å hydrophobic width of membrane proteins.[5] This resulted in TFAs with improved properties for membrane protein stability.

The previously reported TFA-1 bears two deoxycholate-*bis*-maltoside building blocks connected by a propylene spacer. As amide linkages were used for the connection, this facial amphiphile produces two non-facial rotamers (i.e. *trans-cis* and *cis-cis* rotamers) in addition to a facial *trans-trans* isomer (Fig. S1a). The presence of two non-facial rotamers in TFA-1 was demonstrated by the ^{1}H NMR spectrum measured at room temperature (Fig. S1b). The spectrum indicates that the proportion of TFA-1 molecules with non-facial *trans-cis* configuration is comparable to that of the facial *trans-trans* configured molecules. This heterogeneity of TFA-1 seriously limits both the facial property of the agent in solution and likely its ability to stabilize membrane proteins. On the basis of this observation and our subsequent hypothesis, we modified the TFA-1 structure in three ways (Fig. 1). First, cholate-*tris*-maltoside was used as a building block for the new amphiphiles instead of deoxycholate-*bis*-maltoside to increase the micellar stability in water. TFA-1 tended to precipitate in water over time.[5] Second, non-polar ether-based linkers were used to connect two building blocks instead of the polar amide-functionalized linker used for TFA-1. This change of functional group in the linker region should strengthen detergent interactions with the hydrophobic protein surfaces. Because of the reduced likelihood of non-facial rotamer generation as described above, the ether-based facial amphiphiles would play an additionally favorable role in membrane protein stability. Third, a few different linkers were introduced to generate new facial amphiphiles with a range of hydrophobic lengths; direct linkage (C0), propylene (C3), pentylene (C5) and *para*-xylene spacers (PX) were used to generate TFG(M)-C0E, TFM-C3E, TFM-C5E and TFM-PXE, respectively (Fig. 1a,b). The hydrophobic lengths of the individual facial detergents were calculated from energy-minimized conformations obtained *via* density functional theory (DFT) (Fig. 1 & S2[†]). The length variation in the alkyl linker is important as most membrane proteins have the hydrophobic width ranging from 28 to 32 Å with various tilt angles (Fig 1d). Of the facial agents developed so far, the current study is the first report investigating an effect of systematic variation in the lipophilic group length on membrane protein stability. The previously described TFA-1 is a good control agent to compare the effect of amide *vs* ether linkages on protein stability, but this

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agent contains deoxycholate-*bis*-maltoside as a building block rather than the cholate-*tris*-maltoside used in the new agents. Thus, we prepared an additional amide-based tandem cholate-*tris*-maltoside control molecule, designated TFM-C3Am (Fig. 1c). When the new ether-based facial amphiphiles were evaluated with a few membrane proteins including the human β_2 adrenergic receptor (β_2AR), we found some of these agents conferred enhanced stability to all the target proteins compared to TFA-1 and TFM-C3Am.

The novel agents were synthesized from cholic acid using a straightforward synthetic pathway (see ESI† for details). Because of the high synthetic efficiency of each step, the final amphipathic compounds were prepared with good overall yields. High anomeric purities of all the new detergents, confirmed by their individual ^{1}H NMR spectra (Fig. S3†, S4†, S5† and S6†), were achieved using stereo-selective β-glycosylation as a key step. For example, the axial anomeric protons of TFM-C3Am, designated H_a, gave rise to three separate ^{1}H NMR peaks as doublets around 4.4 ppm (Fig. 2a,b and S3†). In addition, the vicinal coupling constants ($^3J_{aa}$) for these anomeric protons (H_a) were 8.0 Hz, typical of a β-anomer, demonstrating the exclusive formation of a β-glycosidic bond in the glycosylation step. Note that an α-anomeric proton produces a peak shifted downfield to 5.19 ppm with a smaller coupling constant ($^3J_{ae}$ = 4.0 Hz), as detected for anomeric protons (H_e) of the terminal saccharide units in TFM-C3Am (Fig. 2b).

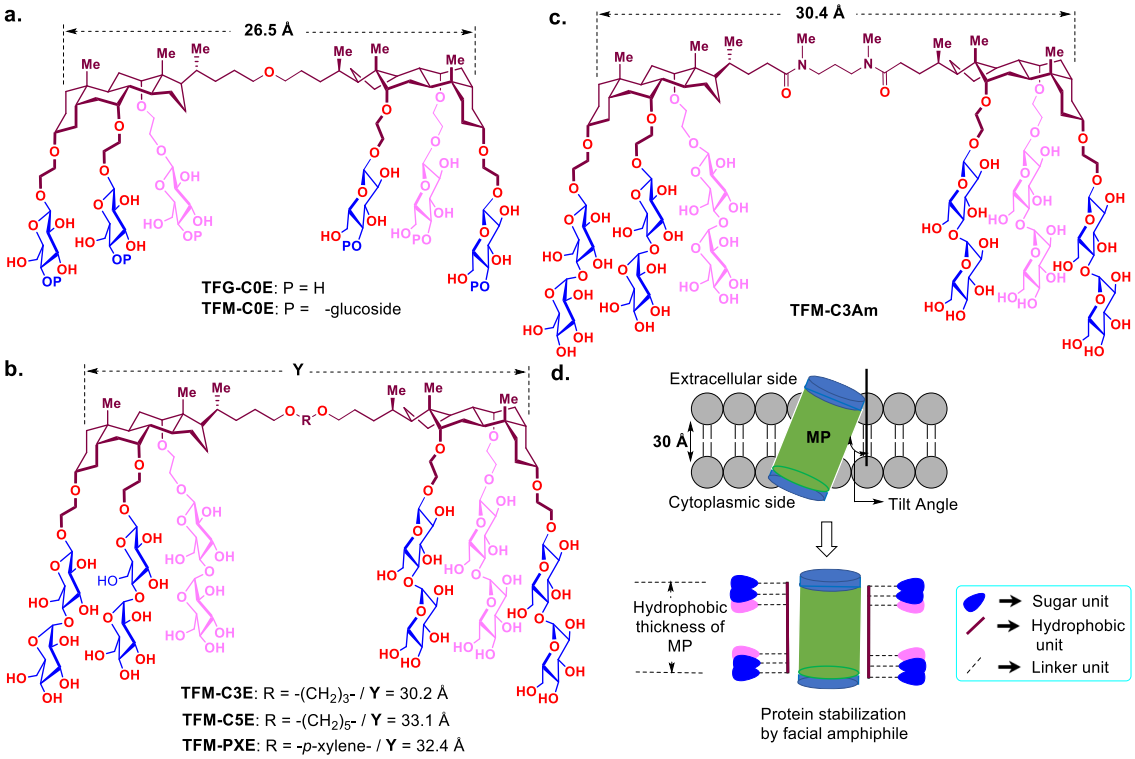

Fig. 1. Chemical structures of (*a,b,c*) newly prepared facial amphiphiles and (*d*) schematic representation of a membrane protein interacting with a facial detergent after extraction from the membrane (~30-Å hydrophobic thickness). The tilt angle provided information on actual hydrophobic thickness of a membrane protein and its orientation in the lipid bilayer. Two cholate-*tris*-glucoside/maltoside blocks were connected either directly (TFG/M-C0E) or using a spacer (propylene (TFM-C3E), pentylene (TFM-C5E), or *para*-xylene spacer (TFM-PXE)) *via* ether linkages. The lengths of the hydrophobic groups of the new agents (TFG/TFMs) are shown at the top of the respective chemical structures, estimated from the optimized conformations obtained *via* DFT calculations at energy level B3LYP/6-31G*. Size-tunable TFMs facially bind to a membrane protein surface, allowing complete coverage of the variable hydrophobic width of different membrane proteins. MP stands for membrane protein.

The correct TFM-C3Am structure was further supported by the through-space interactions seen in the 2D NOESY spectrum (Fig. 2c). Because of their close proximity in space, distinctive NOE correlation signals were observed between the H_8 proton with the methyl protons at the C_{19} position or between methyl protons at the C_{18} and C_{21} positions) in this detergent. Detailed H^1 NMR analyses for the other new amphiphiles (TFG/M-C0E, TFM-C3E/C5E and TFM-PXE) are given in Fig. S4, S5, and S6, respectively. Most significantly, due to restriction in bond rotation of an amide group at room temperature, the six methyl protons (2x$C\underline{H}_3$) on the amide nitrogen atoms in TFM-C3Am gave four peaks at 2.77, 2.79, 2.94 and 2.96 ppm in DMSO-*d*6 solvent (Fig. S7, *top*). These peaks could be assigned based on the relative stability and molecular symmetry of individual rotamers. The highest peak at 2.94 ppm corresponds to the methyl protons in the symmetric *trans-trans* configured rotamer while the two peaks at 2.96 and 2.77 ppm with the same intermediate heights correspond to two different sets of the methyl protons (*cis* and *trans* $C\underline{H}_3$) in the asymmetric *trans-cis* configuration. TFM-C3Am with the symmetric *cis-cis* configuration has two sets of methyl protons in an identical environment, yielding a single peak with the lowest intensity at 2.79 ppm. The population of the *cis-cis* configured TFA-C3Am is likely the smallest due to this form having the lowest stability. When the H^1 NMR spectrum was measured at 65 °C, these four peaks were converted into two broad single peaks centered at 2.80 or 2.96 ppm (Fig. S7, *bottom*), due to an accelerated interconversion of the three rotamers within an NMR time scale. A similar shape change was

observed for the corresponding peaks when MeOH-*d*4 instead of DMSO-*d*6 was used as an NMR solvent (Fig. 2b, *bottom*). However, peak broadening was less obvious in this case mainly due to the lower temperature (55 °C). Taken together, these results strongly indicate the presence of significant amounts of the non-facial rotamers in the case of amide-linked facial amphiphiles (TFA-1 and TFA-C3Am) which directly decreases overall detergent faciality and hence has the potential to negatively affect membrane protein stability.[5] Due to the lack of an amide functional group, the ether-based TFG/TFMs are unlikely to show such potential faciality-related negative features.

As expected from the use of cholate-*tris*-maltoside as a building block, all new agents were water-soluble at more than 5.0 wt%, with no precipitation observed over a month at room temperature. Good water-solubility and micellar stability were even exhibited by TFG-C0E, the most hydrophobic of the new agents. The CMC values of all new agents (from 0.002 to 0.008 mM) turned out be much smaller than that of DDM (0.17 mM), indicating stronger tendencies to form self-assemblies than DDM *via* effective face-to-face packing of the detergent molecules (Table S1†). The CMC values of the new TFMs tended to decrease as the chain length of the spacer between the two cholate-*tris*-maltoside units increased. TFM-C0E, TFM-C3E and TFM-C5E with a direct linkage and propylene and pentylene spacers, respectively, gave steadily reduced CMCs (from 0.006 to 0.005 to 0.004 mM), probably due to the increased hydrophobicity caused by the hydrocarbon extension in the spacers. Interestingly, the CMCs of all the ether-based TFMs (from 0.002 to 0.006 mM) are lower than those of the amide-based agents (TFM-C3Am (0.008 mM) and TFA-1 (0.013 mM)), supporting their enhanced faciality and hydrophobicity. Detergent micelle size represented by hydrodynamic radii (R_h) increased from 2.4 to 2.6 to 2.7 nm with increasing chain length of the spacers from C0 to C3 to C5, as can be seen in the data for TFM-C0E/C3E/C5E (Table S1†). Interestingly, of the new agents, glucoside TFG-C0E gave the lowest CMC and the largest micelles. When we investigated micelle populations in terms of number-weighted size distribution, all TFMs/TFG and DDM showed a single set of populations, indicative of high micellar homogeneity (Fig. S8).

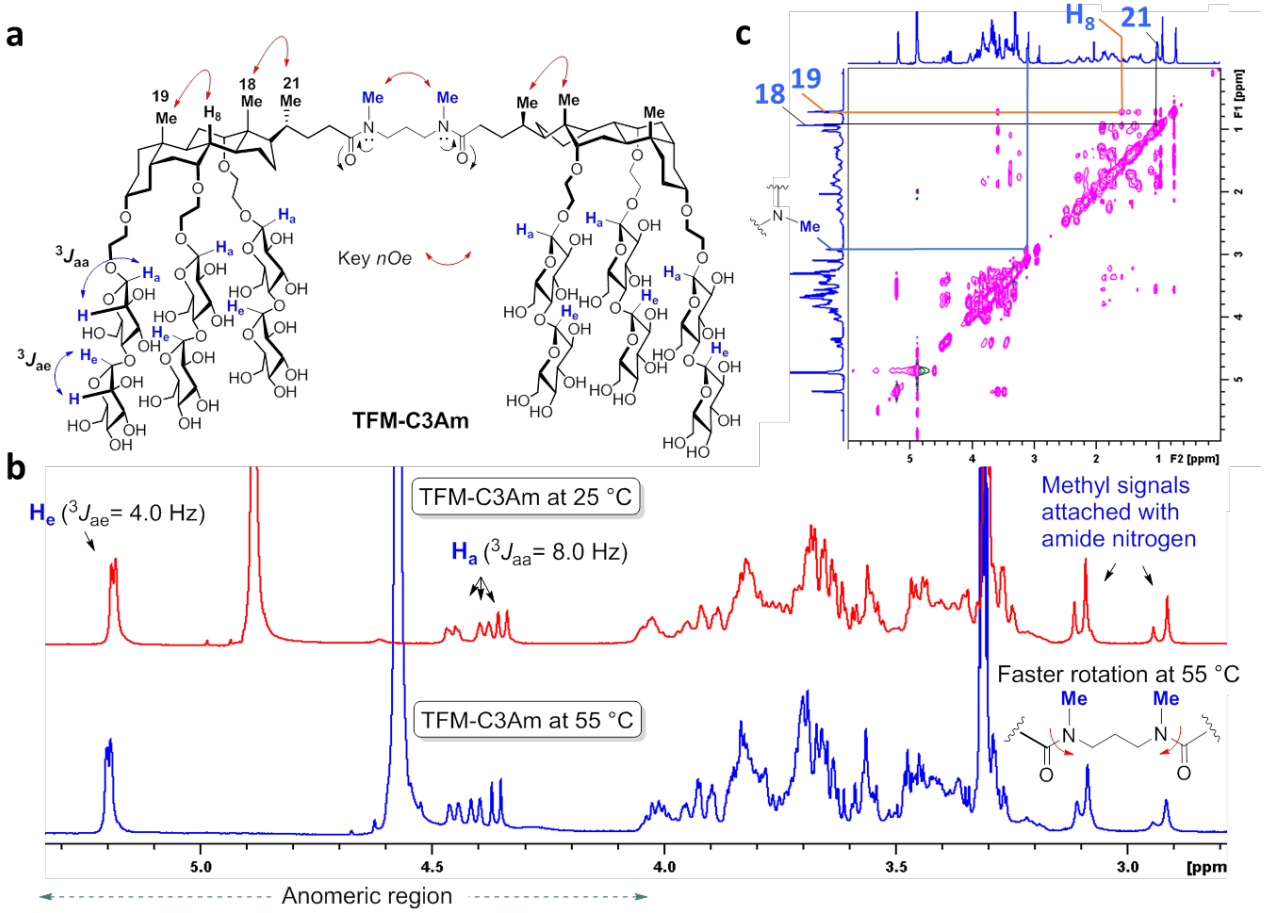

Fig. 2. (a) Chemical structure of TFM-C3Am to illustrate axial (H_a) and equatorial (H_e) anomeric protons, their couplings with neighboring protons (H in blue) and a set of protons of interest responsible for key NOE correlation signals. (b, *top spectrum*) Partial ^{1}H NMR spectrum for TFM-C3Am in MeOH-*d*4 at room temperature, showing high anomeric purity (see Fig. S3 for the full ^{1}H NMR spectrum). This ^{1}H NMR spectrum gave three doublets at 4.46, 4.39 and 4.35 ppm, along with the coupling constant ($^3J_{aa}$) of 8.0 Hz, typical peak characteristics for β-anomeric protons (H_a). TFM-C3Am also contains six α-anomeric protons (H_e) in the terminal saccharide, yielding peaks which appeared together at ~5.19 ppm with a reduced coupling constant ($^3J_{ae}$ = ~4.0 Hz) in the NMR spectrum. (b, bottom *spectrum*) Partial ^{1}H NMR spectrum for TFM-C3Am in MeOH-*d*4 at 55 °C, showing shape changes of peaks corresponding to the methyl protons on the amide nitrogen atoms. The partial peak broadening is due to an increased rate of interconversion between the multiple rotamers. (c) 2D NOESY NMR spectrum of TFM-C3Am showing key NOE patterns.

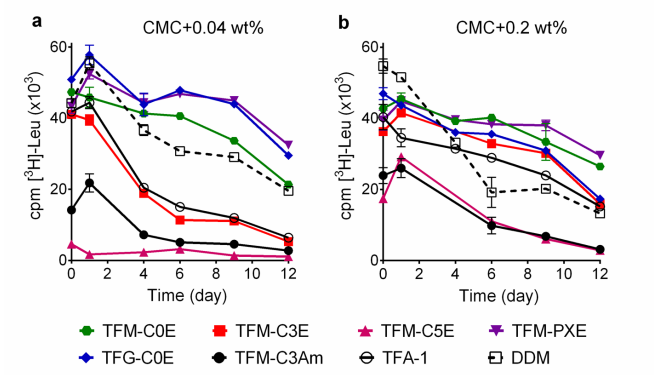

Fig. 3. Time course stability of LeuT solubilized in individual TFMs/TFG (TFM-C0E, TFM-C3E, TFM-C5E, TFM-PXE, TFG-C0E, TFM-C3Am). DDM and TFA-1 were used as controls. Each detergent was used at CMC+0.04 wt% (a) and CMC+0.2 wt% (b). Protein stability was assessed by monitoring the substrate binding ability of the transporter using [^{3}H]-leucine (Leu) at regular intervals during a 12-day incubation at room temperature. The substrate binding ability of the transporter was measured *via* scintillation proximity assay (SPA). Error bars, SEM, n = 3.

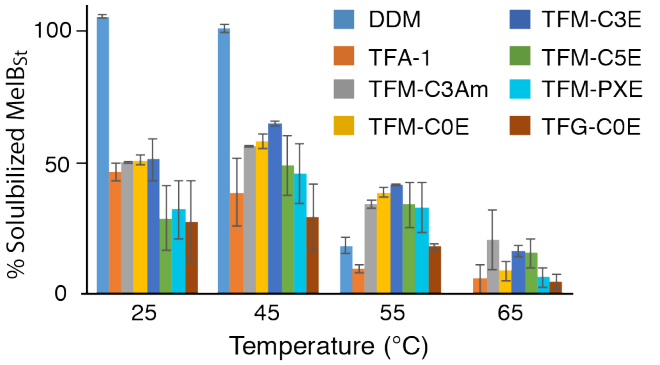

Fig. 4. Thermostability of MelB$_{St}$ solubilized in individual facial derivatives (TFM-C0E, TFM-C3E, TFM-C5E, TFM-PXE, TFG-C0E and TFM-C3Am). DDM and TFA-1 were used as controls. For protein extraction, membranes containing MelB$_{St}$ were incubated with 1.5 wt% individual detergents at four different temperatures (25, 45, 55, and 65 °C) for 90 min. The amounts of soluble MelB$_{St}$ were analyzed by SDS-PAGE and Western blotting (Fig. S9) and represented in a histogram as percentages of total MelB$_{St}$ present in untreated membranes. Error bars, SEM, n = 3.

We first evaluated the effects of these agents on the leucine transporter (LeuT), a prokaryotic homologue of the mammalian neurotransmitter/sodium symporters (NSSs family) from *Aquifex aeolicus*.[7] This transporter was extracted and purified using 1.0 wt% and 0.05 wt% DDM, respectively. DDM-purified LeuT was diluted into buffer solutions containing individual new agents, DDM or TFA-1 to give final detergent concentrations of CMCs + 0.04 wt% or CMCs + 0.2 wt%. We monitored protein stability assessing radiolabeled leucine ([^{3}H]-Leu) binding by scintillation proximity assay (SPA)[8] at regular intervals during a 12-day incubation at room temperature. As can be seen in Fig. 3, TFA-1 was worse than DDM at CMC + 0.04 wt%, but was a little better than this conventional detergent in preserving transporter activity long term at CMC + 0.2 wt%. Of the new agents, TFM-C5E with the longest lipophilic group was the worst while the amphiphiles with the shortest lipophilic group such as TFM-C0E and TFG-C0E were overall superior to DDM. TFM-C3E with a medium lipophilic length showed intermediate detergent efficacy. This result indicates that a facial amphiphile with a relatively short lipophilic group is favorable for stabilizing the transporter long term. Exceptionally, TFM-PXE with a lipophilic length comparable to that of TFM-C5E was one of the best LeuT-stabilizing agents, indicating that the ring-bearing spacer of *para*-xylene has a favorable effect on LeuT. The key difference of this TFM compared to TFM-C5E is the spacer structure. Importantly, the amide control agent (*i.e.,* TFM-C3Am) was less effective than TFM-C3E at maintaining transporter activity at both detergent concentrations tested, strongly supporting our hypothesis that detergent faciality is key for protein stability. Finally, deoxycholate-*bis*-maltoside seems more suitable as a building block than the cholate-*tris*-maltoside, as TFA-1 was superior to TFM-C3Am in stabilizing the transporter.

We next turned to the melibiose permease of *Salmonella typhimurium* (MelB$_{St}$)[9] for further evaluation of the TFMs/TFG. *E. coli* membranes expressing MelB$_{St}$ at ~10 mg/mL were treated with 1.5 wt% DDM, TFA-1, or individual TFMs/TFG at four different temperatures (25, 45, 55 and 65 °C) for 90 mins and then subjected to ultracentrifugation to remove insoluble material. The amounts of soluble MelB$_{St}$ in the individual conditions were estimated by SDS-PAGE and Western blotting (Fig. S9) and represented as percentages in a histogram (Fig. 4). DDM yielded almost quantitative solubilization of MelB$_{St}$ at 25 °C while the TFMs/TFG gave only 25 to 50% solubilization yields, indicating that the new agents were rather poor or moderate at extracting MelB$_{St}$ from the membranes. When the same assay was conducted at an elevated temperature of 45 °C, the amount of MelB$_{St}$ solubilized by each detergent generally increased compared to that obtained at 25 °C. This is likely due to increased membrane dynamics at the elevated temperature. Of the new agents, TFM-C3E with an intermediate hydrophobic length was most effective at yielding soluble MelB$_{St}$, but its extraction efficiency was still worse than DDM. When the incubation temperature was further increased to 55 °C, superior efficacies of the new agents were observed compared to DDM/TFA-1. At this temperature, DDM/TFA-1 yielded very small amounts (10~20% of control) of soluble MelB$_{St}$ while much higher amounts (~40% of control) obtained for the TFMs, with the best performance achieved by TFM-C3E and TFM-C0E. Ether-based TFM-C3E appeared to be a little better than amide-based TFM-C3Am in this respect. Some new agents (TFM-C3Am, TFM-C3E, and TFM-C5E) yielded detectable amounts of soluble MelB$_{St}$ even at 65 °C, but no clear difference was observed between amide and ether-based TFMs (TFM-C3Am *vs* TFM-C3E) at this high temperature. Combined together, although these agents were inferior to the conventional agent at extraction from the membranes, we identified some TFMs (TFM-C0E, TFM-C3E, TFM-C3Am and TFM-C5E) that were more effective at maintaining MelB$_{St}$ in a soluble state than DDM.

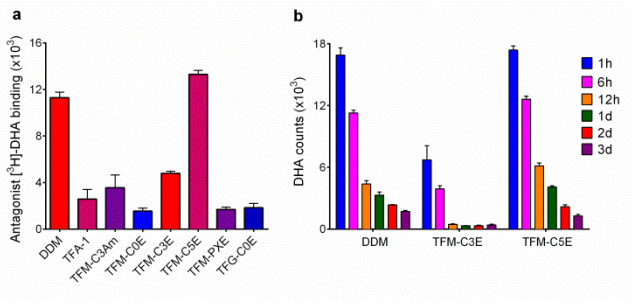

Fig. 5. (a) Initial and (b) long-term stability of β_2AR solubilized in a new agent (TFM-C3Am, TFM-C0E, TFM-C3E, TFM-C5E, TFM-PXE, or TFG-C0E). DDM and TFA-1 were used as positive controls. DDM-purified β_2AR was mixed into individual detergent-containing buffer solutions to give final detergent concentrations of CMC + 0.2 wt%. β_2AR stability was assessed by measuring the ability of the receptor to bind the radio-labeled antagonist ([^{3}H]-dihydroalprenolol (DHA)). Receptor activity was measured after 30-min dilution (a) or at regular intervals during a 3-day incubation at room temperature (b). Error bars, SEM, n = 3.

Finally, we assessed the new facial agents using a G-protein coupled receptor (GPCR), the human β_2 adrenergic receptor (β_2AR).[10] The receptor was isolated in 0.1% DDM and then individual agents were introduced into the samples by a dilution method, giving final detergent concentrations of CMCs + 0.2 wt%. As for a direct assessment of receptor stability in the individual agents, the ability of the receptor to bind the radioactive antagonist ([^{3}H]-dihydroalprenolol (DHA)) was measured.[11] A preliminary result was obtained by measuring the initial ligand binding ability of the receptor following the 30-min sample dilution. Of the ether-based TFMs, TFM-C5E was the best, followed by TFM-C3E and TFM-C0E (Fig. 5a), indicating that β_2AR stability is enhanced by increasing length of the TFM lipophilic group. TFM-PXE was worse than TFM-C3E/C5E in this respect, indicating that the *p*-xylene spacer is detrimental for receptor stability, in contrast to the result observed for LeuT stability. Importantly, ether-based TFM-C3E was better than the amide-based analog (TFM-C3Am), further supporting the favorable role of detergent faciality in protein stability. In order to further differentiate detergent efficacy, receptor activity in DDM, TFM-C3E, and TFM-C5E, was monitored at regular intervals over a 3-day incubation at room temperature (Fig. 5b). TFM-C5E was better than TFM-C3E and similar to DDM in terms of stabilizing β_2AR long-term.

Detergent efficacy tends to be strongly dependent on the target membrane protein. In this study, TFM/G-C0E, TFM-C3E and TFM-C5E were most effective at stabilizing LeuT, MelB$_{St}$ and β_2AR, respectively. This protein-specific nature could be explained by compatibility of detergent hydrophobic length with the dimensions of the protein hydrophobic surface. LeuT with the shortest hydrophobic width (~29.8 Å) of the tested proteins was most stabilized in TFM/G-C0E with the shortest lipophilic unit (26.5 Å) of the new agents while β_2AR with the longest hydrophobic width (~31.8 Å) retained its stability most effectively when solubilized in TFM-C5E with the longest lipophilic group (33.1 Å) (Table S2). TFM-C3E with an intermediate hydrophobic length (30.2 Å) was most suitable for thermo-stabilization of MelB$_{St}$ with the intermediate hydrophobic thickness (30.4 Å). Because of a non-zero tilt angle (11°), the actual hydrophobic length of MelB$_{St}$ is calculated to be 31.0 Å (Table S2). Thus, the lipophilic group length of the most effective TFM correlates with the hydrophobic thickness of the specific membrane protein in each case. The effect of the TFM agents on LeuT stability decreased with increasing lipophilic group length (from TFM-C0E to TFM-C3E to TFM-C5E), while an opposite trend was observed for amphiphile efficacy for β_2AR stabilization, further supporting the importance of having compatible hydrophobic dimensions between the TFMs and the target proteins. While the protein-specific nature of TFM efficacy can be reasonably explained in terms of the hydrophobic length compatibility as described above, it is unclear why the best detergents have slightly different hydrophobic lengths compared to the specific target protein (TFM-C0E (26.5 Å) − LeuT (29.8 Å) or TFM-C5E (33.1 Å) − β_2AR (31.8 Å)) (Table S2). We conceive that the TFMs could vary their hydrophobic lengths by changing conformation depending on the hydrophobic dimensions of a target protein, resulting in a small yet substantial increase/decrease in detergent hydrophobic length. For instance, the hydrophobic length of TFM-C0E can vary from 26.5 nm (energy-minimized conformation) to 29.5 nm (an extended conformation, Table S2). This extended hydrophobic length of TFM-C0E matches well the hydrophobic width of LeuT (29.5 and 29.8 Å). Collectively, the compatibility of hydrophobic dimensions between a facial amphiphile and its target protein is of prime importance for effective protein stabilization.

It is particularly important to note that the ether-based TFM (*i.e.*, TFM-C3E) was better than its amide-based counterpart (*i.e.*, TFM-C3Am) for the two tested proteins (LeuT and β_2AR). A similar trend was also observed for MelB$_{St}$ thermo-stability although the difference between these two agents was small. As described above, an ether-based facial amphiphile should be better than an amide-functionalized analog for protein stability because of the reduced polarity and enhanced faciality, strengthening hydrophobic interactions between the facial molecules and membrane protein. The significantly lower CMCs indicate increased hydrophobicity and/or enhanced faciality of the ether-based agents compared to the amide-based molecules (TFM-C3Am and TFA-1). The comparable efficacy of TFM-C3E and TFM-C3Am found for MelB$_{St}$ solubility at 65 °C could be explained by an accelerated rotamer interconversion at this high temperature, as detected in the temperature-dependent NMR study (Fig. S7). In this case, the facial *trans-trans* rotamer would be the dominant conformation of TFM-C3Am. This, in turn would make this amide-based TFM fully facial, conferring a high level of stability to the MelB$_{St}$ due to an increased interaction between the detergent and the cylindrical protein surface. Such stability gains are impossible for the other rotamers (*trans-cis and cis-cis* rotamers) as they are unable to efficiently interact with the membrane protein surface.

By preparing the ether-based facial amphiphiles, we identified a few agents (TFM-C0E/TFM-PXE, TFM-C3E and TFM-C5E) that were superior or comparable to DDM, a gold standard conventional detergent, for different target membrane proteins (LeuT, MelB$_{St}$ and β_2AR, respectively). The best ether-based agent was consistently better than the amide analogs (TFM-C3Am and TFA-1) for the three tested membrane proteins. This study not only demonstrated the potential detrimental effect of an amide-functionalized linker on membrane protein stability, but also highlights the importance of detergent hydrophobic length in protein stability. Thus, this study should facilitate the rational design and development of more facial amphiphiles in the future.

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Keywords: detergent design • facial amphiphiles • detergent faciality • protein stabilisation • protein structures

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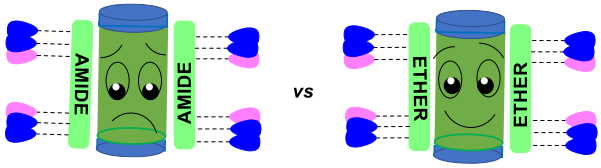
Tandem Facial Amphiphiles



Protein stabilization by tandem facial amphiphiles (amide-linked)

Protein stabilization by tandem facial maltosides (ether-linked)

The functionality and chain length variations of the hydrophobic linker in tandem facial amphiphiles led to a significant difference in detergent efficacy for membrane protein stabilization.

*Manabendra Das, Yang Du, Jonas S. Mortensen, Parameswaran Hariharan, Bernadette Byrne, Claus J. Loland, Lan Guan, Brian K. Kobilka, Pil Seok Chae**

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Rationally engineered tandem facial amphiphiles for improved membrane protein stabilization efficacy