Pendant-bearing detergents: strategies and membrane protein stabilization

Yewon Lee,[a] Hong Sik Yun,[a] Muhammad Ehsan,[a] Bernadette Byrne,[b] Pil Seok Chae[a] *

[a] Major in Bionano Engineering, School of Bio-Pharmaceutical Convergence, Hanyang University, Ansan, 155-88, Republic of Korea

[b] Department of Life Sciences, Imperial College London, London SW7 2AZ, U.K.

Correspondence: pchae@hanyang.ac.kr (P.S. Chae)

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Abstract

Membrane proteins (MPs) are vital to many biological processes, making their atomic-level structures essential for drug discovery. Detergent micelles are commonly employed in MP research, from extraction, purification, and structure determination. Recently, detergents featuring small pendant groups positioned at the hydrophilic-hydrophobic interface of the micelle have emerged as promising membrane mimetics. This review highlights recent developments in pendant-bearing detergents and explores the roles of pendant groups in MP stabilization. Hydrophobic or hydrophilic pendants enhance alkyl chain packing within micelle interiors through distinct mechanisms. Additionally, reactive pendants enable *in situ* structural modification of detergents. Pendant-bearing detergents represent valuable tools for MP structural studies, and their rational design would pave the way for next-generation detergents to advance MP research.

Membrane mimetic systems for membrane protein structural studies

Membrane proteins (MPs) are essential for regulating a wide range of cellular functions [1], including signal transduction [2], molecular transport [3], and intercellular communication [4]. These bio-macromolecules are implicated in numerous diseases, such as cancers, asthma, and Alzheimer disease, making them major targets for pharmaceutical drugs [5-9]. Elucidating their atomic structures enables structure-based drug design (SBDD), critical for development of effective therapeutics [10-12]. However, MP structural studies remain challenging due to their tendency to denature or aggregate in aqueous environments, explaining the significantly smaller number of MPs with known structures compared to their soluble counterparts [13,14] (**Figure 1**). To address this, a variety of amphiphilic systems have been developed to stabilize MPs outside their native membranes. These include polymer-based amphiphiles such as amphipathic polymers (APols) [15,16] and styrene-maleic acid copolymers (SMAs) [17,18], as well as peptide/protein-based systems such as lipopeptide detergents (LPDs) [19], beta-peptides (BPs) [20,21], bihelical peptides [22], saposin A [23,24] and membrane scaffold proteins (MSPs) [25] (**Figure 1**). Some of these amphiphiles form discoidal self-assemblies with embedded MPs, resulting in nano-structures such as lipoparticles (SMA), peptidiscs (bihelical peptides), salipro (saposin A), and nanodiscs (MSPs). These **membrane-mimetic systems** (see Glossary) are particularly useful for structural studies via single particle **cryo-electron microscopy** (cryo-EM) [26-31]. They have also been applied to study protein conformational dynamics using nuclear magnetic resonance (NMR) and mass spectrometry (MS) [32]. However, their utility in MP extraction and crystallography remains limited [33,34]. In contrast, detergents are widely used throughout all stages of MP studies, including extraction, purification and structural characterization [35-38]. Detergents are compatible with the three major structure determination techniques: X-ray crystallography, NMR spectroscopy and cryo-EM [39-41]. Despite this versatility, conventional detergent micelles often offer suboptimal protein stabilization [42,43]. Over the past two decades, many novel detergents have been developed to improve this limitation. Representatives include lauryl maltose neopentyl glycol (LMNG) [44], octyl glucose neopentyl glycol (OGNG) [45], and glyco-diosgenin (GDN) [46], which have gained wide usage in MP structural studies. For instance, LMNG was used in structural studies of 302 MPs from 2011 to 2021, accounting for approximately 14% of MP structures published in that period – highlighting the significant impact of novel detergents in

this field [40].

Although numerous novel detergent studies have been reported, few studies have discussed general strategies and concepts for rational detergent design. This gap limits the ability to develop effective next-generation detergents. In this review, we first describe key detergent properties critical for MP stabilization and then introduce several classes of recently developed pendant-bearing detergents. We discuss how their structural features contribute to improved protein stabilization based on detergent structure-property-efficacy relationships (**Figure 1**). The pendant chains in these detergents are incorporated at the hydrophilic-lipophilic interface and can be categorized into three types: hydrophobic, hydrophilic, and reactive. Hydrophobic pendants help fill voids at the interface, increasing hydrophobic density and enhancing protein stability. Hydrophilic pendants, when attached to a flexible detergent core, can improve protein stabilization through pendant polarity-dependent detergent conformational change. Reactive pendants are especially promising, as they allow for detergent modification via chemical reactions in the presence of MPs. These pendant-bearing detergents are strong candidates for advancing MP structural studies, and the underlying design strategies are essential for future detergent development in this area.

Detergent and micellar properties critical for MP stability

Several physical properties of detergents and their micelles are crucial for maintaining MP stability. One key factor is the **hydrophilic-lipophilic balance** (HLB), which influences how strongly detergents encapsulate MPs [47,48]. Strong *protein-detergent* interactions help minimize protein-protein contacts, thereby reducing the risk of protein aggregation [49,50]. For optimal protein stabilization, the HLB should typically fall within the range of 11to 14, as calculated by **Griffin's method** [51,52]. Alternatively, detergent hydrophobicity or hydrophilicity can be experimentally assessed using high-performance liquid chromatography (HPLC), which could provide more relevant insights than theoretically calculated HLB values [53,54]. Detergent hydrophobic length is another important factor, given that the transmembrane domains of most MPs span a narrow hydrophobic width of ~28–32 Å. Detergents with alkyl chain lengths between C11 and C15 are generally optimal for matching this range [55,56]. Interestingly, this optimal range differs from that of native membrane lipids (C14–C18), likely due to their different self-assembly structures – globular micelles versus

planar bilayers [57,58]. The **critical micelle concentration** (CMC), a measure of micelle stability, is also an essential parameter for MP stability [47,48,59]. While CMC is primarily determined by the hydrophobicity of detergent lipophilic group rather than the hydrophilicity of the head group, the nature of the hydrophilic group – especially its charge – can strongly affect MP stabilization [60,61]. Thus, CMC comparisons are only meaningful when detergents have identical or closely related hydrophilic groups. One of most important contributors to protein stabilization is the packing density of alkyl chains within the micelle interior. Higher hydrophobic density promotes micelle integrity and, in turn, improves MP stability [40]. This can be achieved by several methods, for example, by positioning multiple alkyl chains in close proximity within the detergent scaffold. Micelle size, determined by the relative volumes of the detergent head and tail groups and quantified by the **critical packing parameter** (CPP), is often considered to be important for MP stability [62,63]. However, its correlation with protein stability is neither direct nor strong, as reflected by the fact that micelle size is not tightly linked to detergent HLB, hydrophobic length, or micelle alkyl chain packing. Nevertheless, micelle size often correlates with the size of protein-detergent complexes (PDCs), which is important in structural studies [63-65]. Small PDCs are generally preferable, as they facilitate high-quality crystal formation, yield clearer NMR signals, and enhance the visibility of hydrophilic protein surfaces in EM [66]. Importantly, the physical properties of detergents are highly interrelated. For instance, within a set of detergents with the same head group, increasing alkyl chain length generally lowers both HLB and CMC, while increasing detergent hydrophobic length and micelle size [67]. Furthermore, a single deviation from the optimal range of any of these parameters could be detrimental for MP integrity. Therefore, a comprehensive understanding and balanced consideration of these properties is essential when designing new detergents or modifying existing ones to enhance protein stability.

Recent advances in pendant-bearing detergents

Hydrophobic pendant-bearing detergents

The introduction of hydrophobic pendant chains into detergent molecules has proven to be a valuable strategy for improving MP stabilization. This approach was first applied to maltoside detergents by incorporating short alkyl pendants – methyl (C1), ethyl (C2) and propyl (C3) – at the carbon adjacent to the maltoside head group, producing a series of branch-chained

maltoside detergents: Mal 10_1/11_1/12_1, Mal 10_2/11_2/12_2 and Mal 10_3/11_3 (**Figure 2b**) [68]. The micelle size of these detergents was found to be highly dependent on pendant length. While the methyl pendant had little effect, the ethyl and propyl pendants resulted in modest and substantial increases in micelle size, respectively. When evaluated with the human gap junction protein Cx26, a few detergents such as Mal 10_2 and Mal 11_1 showed membrane protein extraction efficiency comparable to that of *n*-dodecyl-β-D-maltoside (DDM) (**Table 1**). Furthermore, thermal denaturation assays revealed that these branch-chained detergents provided a similar level of protein stabilization as DDM. These results indicate no clear improvement in protein extraction and stability compared to a standard detergent. Although addition of methyl or ethyl pendant was expected to increase alkyl chain packing density at the hydrophilic-hydrophobic interfaces, this potential benefit appears to be offset by drawbacks such as shorter alkyl chain lengths and higher CMCs. However, Mal 11_1 facilitated the crystallization of Cx26 in a different **space group** (hexagonal P3 *vs* monoclinic C2 for DDM) and yielded improved diffraction compared to DDM (3.5 Å vs 4.5 Å). This result demonstrated that even subtle changes in detergent structure can significantly affect crystallization outcomes.

The large increase of the self-assembly sizes of branch-chained maltosides associated with a pendant length increase from methyl to ethyl suggests that conventional micelles formed by *n*-undecyl-β-D-maltoside (UDM) or DDM contain small voids at their hydrophilic-hydrophobic interfaces. The inability of these micelles to accommodate larger pendants without rearrangement results in expansion of the micelle size. The favorable effect of pendants on protein stabilization became evident with the evaluation of pendant-bearing GNG detergents (P-GNGs) [69,70]. In these molecules, an alkyl pendant (C1–C6) was introduced at the hydrophilic-hydrophobic interfaces of the OGNG scaffold, yielding amphiphiles with a long main chain (C11–C15) and a short pendant chain (**Figure 2b**). Unlike the branch-chained maltoside detergents, the P-GNGs exhibited little change in self-assembly size with increasing pendant length up to C4. This result suggests the presence of sufficient internal voids to accommodate these pendants due to their bulky branched diglucoside group (**Figure 3a** and Table 1). However, further increases to C5 resulted in significant micelle enlargement, indicating maximal hydrophobic packing density for the butyl pendant. Consistent with this, the best protein stabilization performance was observed with the butyl pendant-bearing GNGs (e.g., GNG-4,14 and GNG-4,15), across various MPs, including the leucine transporter (LeuT),

melibiose permease (MelB), human beta2 adrenergic receptor (β_2AR), and mouse μ-opioid receptor (MOR). Protein stability was evaluated based on substrate binding (LeuT), ligand binding (β_2AR and MOR), or thermal stability (MelB). Pentyl pendant-bearing GNGs such as GNG-5,13 and GNG-5,14 also showed excellent stabilization efficacy despite forming large self-assemblies. These findings confirm that micelle size is not necessarily correlated with protein stability. Similar to LMNG, these pentyl pendant-bearing GNGs likely form non-globular self-assemblies (e.g., rod-like aggregates) while maintaining high internal alkyl chain density. Notably, many P-GNGs demonstrated efficient extraction of MelB from membranes, performing similar to or better than DDM (**Table 1**). These results suggest that this class of detergents may serve as an effective alternative to DDM and LMNG for MP structural studies.

Table 1. Molecular weights (MWs), critical micelle concentrations (CMCs), water-solubility, hydrophilic-lipophilic balance (HLB) values, membrane protein (MP) extraction efficiency of detergents and the hydrodynamic radii (R_h) of their micelles in water at room temperature.

Detergent	MW	CMC (mM)	R_h (nm)[a]	Solubility (%)	HLB[b]	MP extraction efficiency (%)[c]	Ref.
UDM	496.59	0.59	3.1 ± 0.07	> 10	13.7	-	[68]
DDM	510.6	0.17	3.5 ± 0.1	> 10	13.4	100	[40]
OGNG	568.7	~ 1.0	4.4 ± 0.3	> 10	12.6	~100	[40]
LMNG	1005.2	~ 0.010	10.6 ± 0.2	~ 10	13.6	~100	[40]
GDN	1165.3	~ 0.018	4.4 ± 0.1	~ 10	11.7[d]	~50	[40]
Mal 10_1	496.6	1.2	2.7 ± 0.1	> 10	13.4	-	[68]
Mal 10_2	510.6	0.63	-	> 10	13.0	~100	[68]
Mal 11_1	510.6	0.37	3.0 ± 0.07	> 10	13.0	~100	[68]
GNG-5,13	652.9	~ 0.015	24 ± 0.6	~ 5.0	11.0	~100	[69]
GNG-3,14	638.8	~ 0.025	3.2 ± 0.0	~ 10	11.2	~80	[70]
GNG-4,14	652.9	~ 0.025	3.4 ± 0.0	~ 10	11.0	~100	[69]
GNG-5,14	666.9	~ 0.020	27 ± 1.5	~ 5.0	10.7	~95	[69]
GNG-3,15	652.9	~ 0.015	3.7 ± 0.1	~ 10	11.0	~80	[69]
GNG-4,15	666.9	~ 0.010	3.7 ± 0.1	~ 10	10.7	~80	[69]
GNG-5,15	680.9	~ 0.010	73 ± 6.0	~ 5.0	10.5	~90	[69]
GNG-F1,2,15	706.8	~ 0.020	3.8 ± 0.2	~ 10	9.6[e]	~130	[69]
GNG-F1,3,14	706.8	~ 0.020	3.3 ± 0.1	~ 10	9.6[e]	~120	[69]
MPG-D2,12	905.1	~ 0.045	3.5 ± 0.1	> 10	13.8	~50	[71]
MPG-D3,12	933.1	~ 0.020	3.5 ± 0.1	> 10	13.3	~50	[71]
MPG-D4,12	961.2	~ 0.018	3.5 ± 0.1	> 10	13.0	~90	[71]
MPG-T2,11	961.2	~ 0.025	3.5 ± 0.1	> 10	13.0	~50	[71]
MPG-T3,11	1017.3	~ 0.020	3.6 ± 0.1	> 10	12.2	~70	[71]
MPG-T4,11	1073.5	~ 0.008	23.9 ± 0.1	~ 7.0	11.6	~5	[71]
LBA-C0	1393.5	~ 0.11	2.6 ± 0.0	> 5.0	14.7	-	[72]
LFA-C4	1449.6	~ 0.018	2.6 ± 0.0	5.0	14.1	-	[72]
LFA-C5	1463.6	~ 0.015	3.3 ± 0.1	2.5	14.0	-	[72]
E-GTM-C10	1750.1	~ 0.005	23.5 ± 0.2	~ 10	11.7	~40	[73]
E-GTM-C11	1792.1	~ 0.002	38.9 ± 0.1	~ 5.0	11.4	-	[73]

E-GTM-C12	1834.2	~ 0.0015	42.8 ± 0.7	~ 1.0	11.2	-	[73]
M-GTM-C10	1810.1	~0.005	7.6 ± 0.2	~ 10	11.3	~80	[83]
M-GTM-C11	1852.2	~ 0.004	6.3 ± 0.1	~ 10	11.1	~80	[73]
M-GTM-C12	1894.3	~ 0.003	16.8 ± 0.3	~ 10	10.9	~15	[73]
AM-C8	638.7	~ 3.74	-	> 1.0	14.7[f]	~45	[74]
AM-C10	666.8	~ 0.47	2.9 ± 0.7	> 0.4	14.1[f]	~45	[74]
ADG-C10	682.8	~ 1.37	2.4 ± 0.6	> 0.7	14.3[f]	~65	[74]
ADG-C11	696.8	~ 0.51	-	-	14.0[f]	~65	[74]
CatCD-1	534.6	~ 0.10	4.0 ± 0.2	> 10	12.8[f]	~100[g]	[75]
LD1	542.7	0.08	6.3 ± 0.3	> 10	12.6[f]	-	[76]
LD2	556.7	0.08	16.0 ± 0.4	> 10	12.3[f]	-	[76]
LD1-2	580.7	0.08	15.6 ± 1.6	> 10	11.8[f]	-	[76]
LD1-3	594.8	0.08	30.8 ± 0.5	> 10	11.5[f]	-	[76]
LD1-7	613.8	0.40	3.2 ± 0.7	> 10	12.5[f]	-	[76]
LD1-8	627.8	0.06	12.1 ± 0.3	> 10	12.2[f]	-	[76]
LD2-1	570.7	0.10	2.7 ± 0.1	> 10	12.0[f]	-	[76]
LD2-2	594.8	0.25	3.1 ± 0.0	> 10	11.5[f]	-	[76]
LD2-7	627.8	0.08	6.3 ± 0.3	> 10	12.2[f]	-	[76]
LD2-8	641.8	0.08	16.0 ± 0.4	> 10	12.0[f]	-	[76]

[a] Hydrodynamic radius of micelles measured at a detergent concentration of 1.0 wt% using dynamic light scattering (DLS). [b] Calculated by Griffin's method [52]. [c] MP extraction efficiency normalized relative to that obtained with DDM based on extraction of Cx26, MsbA, GHSR and MelB using branch-chained detergents, CatCD-1, AMs/ADGs, and the other detergent classes, respectively. [d]Calculated by classifying three ether bonds in the lipophilic portion as hydrophobic groups. [e] Calculated by Davies' Method [77]. [f] Calculated by classifying the acrylamide, carbamate, and amide groups located at the hydrophilic-hydrophobic interfaces as hydrophilic, while the alkyne, thiol, and thioether groups at the same interfaces were treated as hydrophobic. [g] MP extraction efficiency normalized relative to UDM instead of DDM.

A further advancement involved development of multi-pendants-bearing glucosides (MPGs) with a branched triglucoside head group and a single main chain [71]. By attaching two or four pendants to the linker between the head and tail groups, di-pendant (MPG-Ds) and tetra-pendant (MPG-Ts) detergents were produced (**Figure 2b**). As pendant length increased from ethyl (C2) to propyl (C3) to isobutyl (C4), the CMC values decreased, indicating enhanced hydrophobicity (Table 1). Self-assembly sizes exhibited minimal dependency on pendant length, except for a notable size increase in the MPG-Ts with the C4 pendant. This result suggests that self-assemblies have voids large enough to accommodate four propyl pendants (4xC3), are too small to effectively accommodate four isobutyl chains (4xC4). Overall, the MPGs exhibited lower MelB extraction efficiency compared to DDM. However, certain MPGs such as MPG-D4,12 and MPG-T3,11 achieved as high as 70-90% of DDM extraction efficiency. Protein stabilization efficacy of the MPG-Ds increased with longer pendant length, with MPG-D4s outperforming others in stabilizing LeuT, MelB and β_2AR. A further stability enhancement was observed for the series of MPG-T3s, with the best performance obtained with MPG-T3,11. Due to the presence of four pendant chains, MPG-T3,11 has a high hydrophobic density at the

interfaces, supporting its outstanding performance. This high hydrophobic density is effective at minimizing water penetration into the micelle interiors. **Molecular dynamic (MD) simulations** confirmed that MPG-T3,11 exhibited significantly lower hydration in the alkyl tail region, both as pure micelles and in complexes with β_2AR, compared to di-pendant MPG-D4s and DDM. The four propyl pendants strengthen *detergent-detergent* interactions, forming a water-impermeable layer at the hydrophilic-hydrophobic interfaces (**Figure 3b**)— an attribute that reduces alkyl chain hydration and enhances MP stability [78]. This result is meaningful considering the effect of water-penetration into micelle interiors and biological membranes on MP stability and conformational dynamics. Collectively, systematic changes in head group bulk - from single maltoside to branched diglucoside to branched triglucoside – result in increasingly spacious micelle interiors available for pendant chains. This progress facilitates the incorporation of larger and/or more pendant groups at the hydrophilic-hydrophobic interfaces. Furthermore, trends in micelle size variation allow estimation of the maximum allowable pendant size for each detergent scaffold, which correlates well with protein stabilization performance. Considering the favorable impact of small micelles on MP structural studies, the pendant length that produces minimal changes in micelle size, as observed with Mal 11_1, GNG-4,14, and MPG-T3,11, can be considered optimal for protein structural study.

In all cases, the hydrophobic pendants improved MP stability by increasing hydrophobic density of micelles at the hydrophilic-hydrophobic interfaces. However, additional mechanisms may contribute to protein stability, as shown by pendant-bearing lithocholate-based **facial amphiphiles** (LFAs) [72,79,80]. In these amphiphiles, butyl (C4) or pentyl (C5) pendants were introduced into a lithocholate-based scaffold containing two hydrophilic groups (maltoside and branched dimaltoside) (**Figure 2b**). These LFAs formed smaller micelles with a hydrodynamic radii of 2.6 nm (LFA-C4) and 3.3 nm (LFA-C5) than DDM (3.4 nm) and the C4 variant significantly outperformed its non-pendant **bola-amphiphile** (LBA-C0) [81,82] in stabilizing LeuT, bacteriorhodopsin (bR) and β_2AR (Table 1). The enhanced stability is partly due to increased hydrophobic density within the micelle interior but primarily attributed to the ability of LFA-C4 to bind the MPs in a facial manner. Upon interaction with MPs, two detergent molecules align antiparallel in a bilayer-like arrangement, with overlapping butyl pendants and effectively covering the protein hydrophobic region (**Figure 3c**). Depending on the degree of

chain overlap, the combined hydrophobic length is calculated to range from 29.2 Å (maximum overlap) to 35.8 Å (minimum overlap) – well-matched to the hydrophobic widths (29.5-32.0 Å) of the tested MPs. Thus, the presence of a small alkyl pendant transforms the detergent from a classical bola-type to a facial amphiphile, along with adaptable properties suited for MPs with varying hydrophobic spans.

Partially fluorinated or hydrophilic pendant-bearing detergents

Fluorinated carbon chains, due to their unique hydrophobic yet lipophobic nature, have been employed as detergent tail groups to generate fluorinated surfactants (FSs) [83,84]. This class of detergents offers distinct advantages for MP stability, such as enhanced retention of cofactors and specific membrane lipids. Preserving cofactor binding within active sites and maintaining endogenous lipid interactions at protein surfaces are essential for supporting proper protein function [85,86]. However, fully fluorinated surfactants tend to exhibit weak interactions with MPs, often leading to protein aggregation [87]. To address this limitation, partially fluorinated surfactants – hemi-fluorinated surfactants (HFSs) – have been developed as alternatives [88,89]. Nonetheless, most HFSs reported to date have shown suboptimal performance, being neither efficient at extracting MPs from the membranes nor effective in stabilizing G protein-coupled receptors (GPCRs) [90,91]. Moreover, the inclusion of fluorinated segments along the main hydrocarbon chain, renders their synthesis challenging. To overcome both the synthetic difficulties and limited biological performance, partially fluorinated pendants were introduced into the P-GNG scaffold (**Figure 2c**) [69]. The resulting fluorinated pendant-bearing GNGs (FP-GNGs) incorporate a trifluoromethyl (CF_3)-terminated propyl (GNG-F1,2,15) or butyl pendant (GNG-F1,3,14). These FP-GNGs formed small micelles comparable to the parent P-GNGs and exhibited superior performance compared to DDM, both in extracting MelB and in stabilizing β_2AR than DDM (**Table 1**). Notably, GNG-F1,2,15 provided β_2AR stabilization comparable to that of LMNG, underscoring its promise for GPCR structural studies [92].

Hydrophilic pendants can also contribute positively to MP stabilization, although through mechanisms distinct from hydrophobic pendants. This concept was demonstrated using glycerol-decorated tris(hydroxymethyl)methane-cored maltosides (GTMs) [73]. In this detergent class, a flexible core, glycerol-decorated tris(hydroxymethyl)methane, was used to introduce either a hydrophobic ethyl or a hydrophilic methoxyethoxymethyl (MEM) pendant,

along with the maltoside head and alkyl tail groups, resulting in E-GTMs and M-GTMs, respectively (**Figure 2c**). All M-GTMs were sufficiently water-soluble (10%), while the E-GTMs exhibited limited solubility. Although overall MelB extraction by the GTMs was lower than that achieved with DDM, certain M-GTMs such as M-GTM-O10 and M-GTM-O11 extracted soluble MelB at approximately 80% efficiency relative to DDM. Due to the presence of ethyl pendants, the E-GTMs are expected to have high hydrophobic density within the micelle interior compared to the M-GTMs, and thus were anticipated to offer better protein stabilization. However, experimental evaluation with several MPs (LeuT, MelB and β_2AR) revealed that the M-GTMs generally outperformed the E-GTMs in stabilizing these target proteins. This unexpected result is attributed to polarity-dependent inversion of pendant orientation within the GTM scaffold. MD simulations indicated that in micelle environments, the hydrophobic ethyl pendant aligns with the detergent's alkyl chains, whereas the hydrophilic MEM pendant orients in the same direction as the maltoside group (**Figure 3d**). This polarity-driven pendant inversion dramatically increases the hydrophilic volumes and resultantly reduces the hydrophobic volumes of the M-GTM molecules. This pendant polarity-dependent conformational change results in enhanced alkyl chain density in the micelle interiors, which contributes favorably to protein stabilization. This difference in detergent conformation was further reflected in smaller self-assembly sizes formed by the M-GTMs compared to the E-GTMs (**Table 1**). Thus, modifying pendant polarity can induce substantial differences in micelle properties and significantly impact detergent performance in solubilizing and stabilizing MPs. While hydrophobic pendants are commonly used to promote protein stabilization, this study highlights that hydrophilic pendants – when integrated into a flexible core – can also be highly effective.

Reactive pendant-bearing detergents

Unlike the hydrophobic and hydrophilic pendants discussed earlier, which are chemically inert, reactive pendant groups can be incorporated into detergent structures to enable functional modifications. This concept is exemplified by maltoside/diglucoside detergents (AMs/ADGs) bearing an acrylamide-functionalized pendant at the hydrophilic-hydrophobic interface [74] (**Figure 4a**). This polymerizable moiety allows detergents to undergo **free-radical telomerization**, resulting in the formation of AM- or ADG-derived polymers [93] (**Figure 4c**). Interestingly, the monomeric forms of these detergents (referred to as surfmers) and their

polymerized forms exhibit distinct effects on protein solubilization and stabilization. When the ghrelin receptor (GHSR) was used as a model MP, all tested monomeric surfmers (AM-C8, AM-C10, ADG-C10 and ADG-C11) extracted the ligand-competent receptor at levels ranging from 45-65% of that achieved by DDM. In contrast, the polymeric versions extracted only 10% of functional GHSR relative to DDM. However, the trend reversed in terms of receptor stability: while the surfmers retained only 10-40% of functional receptors after 24 hours, the polymers preserved 80-90% of the receptor activity, outperforming DDM, which retained only 50% activity under the same conditions. These findings indicate that, while monomeric detergents are more efficient for protein extraction, polymerized detergents are superior in long-term protein stabilization. This behavior is consistent with results of other amphipathic polymers, such as A8-35 and non-ionic APols [94,95], and highlights the difficulty in developing amphiphiles that perform well for both protein extraction and stabilization compared to DDM.

Reactive pendants can also be used to chemically alter detergent structure in the presence of MPs, a feature referred to as ***in-situ* structural modification**. For example, a DDM analog with an ethynyl pendant exhibited a lower CMC (0.1 *vs* 0.17 mM) and formed larger micelle sizes (3.4 *vs* 4.0 nm in R_h) than DDM (**Figure 4a** and Table 1) [75]. When evaluated using thermal denaturation assays, this detergent exhibited high protein stabilization efficacy for two MPs, the ATP-binding cassette transporter (MsbA) and the adenosine A2A receptor ($A_{2A}R$), comparable to DDM, though slightly inferior to LMNG. Additionally, it extracted MsbA as efficiently as UDM and LMNG. Importantly, due to the presence of alkyne functionality in the pendant region, this detergent underwent selective bond cleavage between the alkyl chain and the maltoside group upon treatment with a palladium (II) catalyst, and is therefore referred to as a catalytically cleavable detergent (CatCD-1) (**Figure 4c**). In phosphate-buffered saline, the addition of $[Pd(allylCl)]_2$ resulted in more than 95% cleavage of CatCD-1 within two hours, even in the presence of MPs. Thus, this **bio-orthogonal reaction** enabled detergent exchange surrounding MPs, allowing substitution of CatCD-1 with LMNG, UDM, or DDM — a widely used step to introduce detergent suitable for protein structural studies.

A similar approach was used in a set of thiol-functionalized DDM analogs, recently reported as 'living detergents' (LDs), which enables *in-situ* structural modifications through biocompatible chemistry (**Figure 4a**) [76]. These LDs exhibited lower CMCs (0.08 mM) and formed larger micelles (R_h: 6.3 nm for LD1 and 16.0 nm for LD2) than DDM, likely due to the

increased hydrophobicity and the added hydrophobic volume of the pendant thiol groups (Table 1). LD1 and LD2 underwent structural transformation via thiol-alkylation reactions with reagents such as methyl iodide (methylation), propargylic bromides (propargylation), or bromoacetamides (acetamidation), generating a series of modified LD1/LD2 derivatives (**Figure 4b, 4c**). Self-assembly sizes tend to vary depending on the polarity of any newly introduced group, a similar behavior to that observed for the E/M-GTMs. When evaluated using thermal denaturation assay with $A_{2A}R$, the LD variants modified with hydrophobic groups (e.g., LD1-2, LD1-3, and LD2-2) outperformed DDM in protein stabilization. In contrast, LD derivatives conjugated with relatively hydrophilic pendants (e.g., LD1-7, LD1-8, LD2-7, and LD2-8) were less effective, likely due to increased hydrophilicity (i.e. higher HLB values), which compromises protein stability. Notably, LD2-1, featuring a methylated pendant, reduced clustering of $A_{2A}R$-G protein complexes and improved image clarity in the negative-stain EM compared to DDM.

Concluding remarks

The incorporation of pendant chains at the hydrophilic-hydrophobic interface of detergents offers a versatile strategy to enhance MP stability through various mechanisms, depending on the nature of the pendant. Hydrophobic alkyl pendants, when integrated into scaffolds such as the GNG and MPG, increase alkyl chain density by effectively filling voids at the micellar interfaces. This leads to reduced water penetration into micelle interiors and enhanced stabilization of MPs. Additionally, conjugation of an alkyl pendant to one end of a bola amphiphile, as exemplified by LFA-C4, transforms it into a facial amphiphile with superior protein stabilization capabilities. The overlapping of pendants also enables adaptability of LFA-C4 to MPs with different hydrophobic widths, lending this system a customizable character. Partially fluorinated carbon chains used as pendants, such as in GNG-F1,3,15, have demonstrated a combination of efficient protein extraction and effective stabilization — a feature not typically observed with other fluorinated surfactants. When hydrophilic pendants are conjugated to flexible detergent cores, they can also promote protein stability by inducing conformational changes that enhance alkyl chain packing in micelle interiors.

Chemically reactive pendant groups further expand the functional scope of detergents in MP research. Acrylamide-functionalized detergents (surfmers) were polymerized via free radical

telomerization, yielding amphipathic polymers. A comparative study between surfmers and their polymeric forms revealed distinct advantages: monomers excel in protein extraction, while polymers outperform in long-term protein stabilization. Reactive pendants also enable the development of chemically cleavable detergents, such as the ethynyl-functionalized DDM analog (CatCD-1), which allows effective detergent exchange surrounding MPs — a critical step in protein structural studies. Furthermore, thiol-functionalized pendants have enabled *in-situ* structural modification of detergents. Through biocompatible reactions, diverse hydrophobic or hydrophilic groups can be introduced into these pendants in the presence of MPs, allowing fine-tuning of detergent properties to protein structural analysis.

Detergent efficacy in stabilizing MPs is often protein-specific. The pendant-bearing detergents described here have been evaluated with a limited set of model proteins including transporters and GPCRs. To generalize the structure-property-stabilization efficacy relationships discussed, broader evaluation across functionally distinct MP classes — such as β-barrel proteins and ion channels — is also necessary. Notably, OGNG (a P-GNG variant) has already been used in structural studies of nine channel proteins, including two ion channels: the two-pore domain potassium channel, K2P2.1, and a trimeric intracellular cation channel [40]. It is also important to note that a stable protein is not necessarily functional as overly stabilized proteins may lose activity. In the evaluation of pendant-bearing detergents, MP stability was generally assessed using substrate (LeuT) or ligand (MelB, β_2AR, MOR and GHSR) binding assays, indicating that these detergents are effective at maintaining protein function in addition to structural integrity.

In terms of MP extraction, the FP-GNGs outperformed other pendant-bearing detergents and even exceeded DDM in extracting MelB from membranes. Several P-GNGs as well as the cleavable detergent (CatCD-1) and branched-chain detergents showed comparable extraction efficiency to DDM. Although the MPGs and M-GTMs were generally less effective, the selected variants achieved 70-90% levels of DDM's extraction efficiency. These findings indicate that many pendant-bearing detergents are not only effective stabilizers but also efficient extractors of MPs from the membranes. Combined with their scalability and cost-effectiveness, this dual functionality highlights their broad potential for MP structural studies. In contrast, detergents such as the AMs and ADGs were less effective at stabilizing MPs and

showed lower GHSR extraction efficiency compared to other pendant-bearing systems.

Although pendant chains represent only a small portion of the detergent molecule and do not directly interact with MPs, they can profoundly influence the key physical properties of both detergent and micelle that are closely linked to protein stability. The use of chemically reactive pendants offers new opportunities for designing multifunctional detergents tailored for structural biology (see Outstanding questions). Nonetheless, their practical application remains challenging, as pendant modification must occur under mild, aqueous conditions compatible with MPs. Continued development of reactive pendant-bearing detergents is likely to play a pivotal role in advancing MP research.

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Declaration of interests

The authors declare no competing interests.

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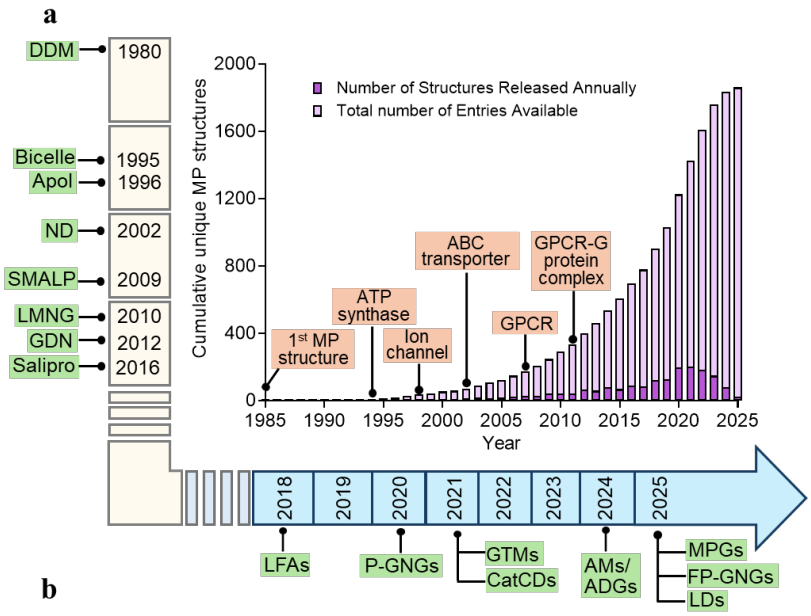
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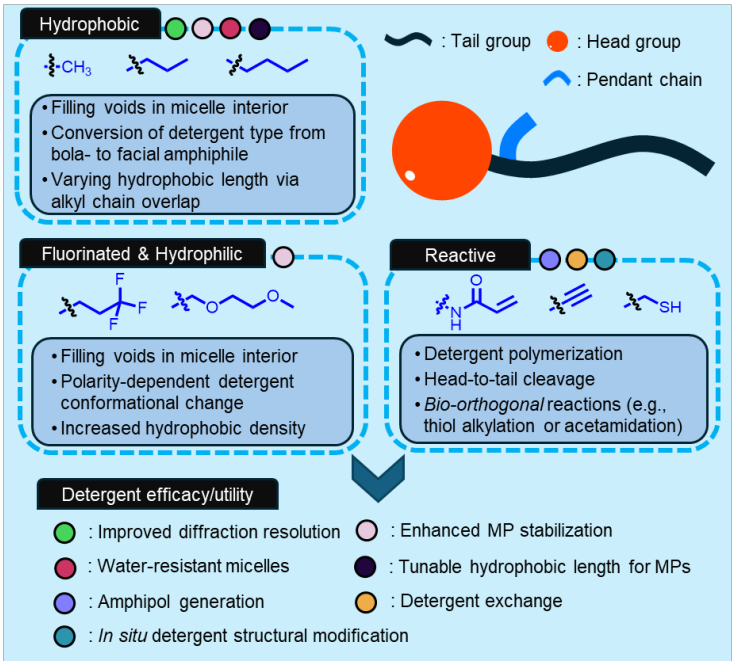
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a
DDM
1980
Bicelle
Apol
1995
1996
ND
2002
SMALP
2009
LMNG
GDN
Salipro
2010
2012
2016
Cumulative unique MP structures
2000
1600
1200
800
400
0
Number of Structures Released Annually
Total number of Entries Available
1st MP structure
ATP synthase
Ion channel
ABC transporter
GPCR
GPCR-G protein complex
1985
1990
1995
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2015
2020
2025
Year
2018
2019
2020
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2022
2023
2024
2025
LFAs
P-GNGs
GTMs
CatCDs
AMs/ ADGs
MPGs
FP-GNGs
LDs


b
Hydrophobic
: Tail group
: Head group
: Pendant chain
•Filling voids in micelle interior
•Conversion of detergent type from bola- to facial amphiphile
•Varying hydrophobic length via alkyl chain overlap
Fluorinated & Hydrophilic
•Filling voids in micelle interior
•Polarity-dependent detergent conformational change
•Increased hydrophobic density
Reactive
•Detergent polymerization
•Head-to-tail cleavage
•Bio-orthogonal reactions (e.g., thiol alkylation or acetamidation)
Detergent efficacy/utility
: Improved diffraction resolution
: Enhanced MP stabilization
: Water-resistant micelles
: Tunable hydrophobic length for MPs
: Amphipol generation
: Detergent exchange
: In situ detergent structural modification

Key figure : Emergence of pendant-bearing detergents in detergent history and the summarized effects of detergent pendants on micelle properties and efficacy/utility for membrane protein manipulation

Figure 1. (a) Annual and cumulative numbers of unique MP structures (data sourced from https://blanco.biomol.uci.edu/mpstruc/, with the data curated by the Stephen White laboratory), along with a timeline highlighting key developments in membrane mimetic systems. The years of the first reported structures of major MP classes are indicated. Abbreviations: ATP, adenosine triphosphate; ABC, ATP-binding cassette; GPCR, G protein-coupled receptor. Apol, amphipathic polymers; ND, nanodisc; SMALP, styrene maleic-acid lipid particle; LMNG, lauryl maltose neopentyl glycol; GDN, glyco-diosgenin; LFAs, lithocholate-based facial amphiphiles; P-GNGs, pendant-bearing glucose neopentyl glycol; GTMs, glycerol-decorated tris(hydroxymethyl)methane-cored maltosides; CatCDs, catalytically cleavable detergents; AMs/ADGs, acrylamide-functionalized maltosides/diglucosides; MPGs, multi-pendant-bearing glucosides; FP-GNGs, fluorinated pendant-bearing GNGs; LDs, living detergents. (b) Chemical structures and classification of pendant groups in pendant-bearing detergents, categorized as hydrophobic, fluorinated/hydrophilic, or reactive. The role and function of each pendant type are summarized in relation to micelle architecture and their impact on MP studies.

Commented [BB1]: This is a great graphic

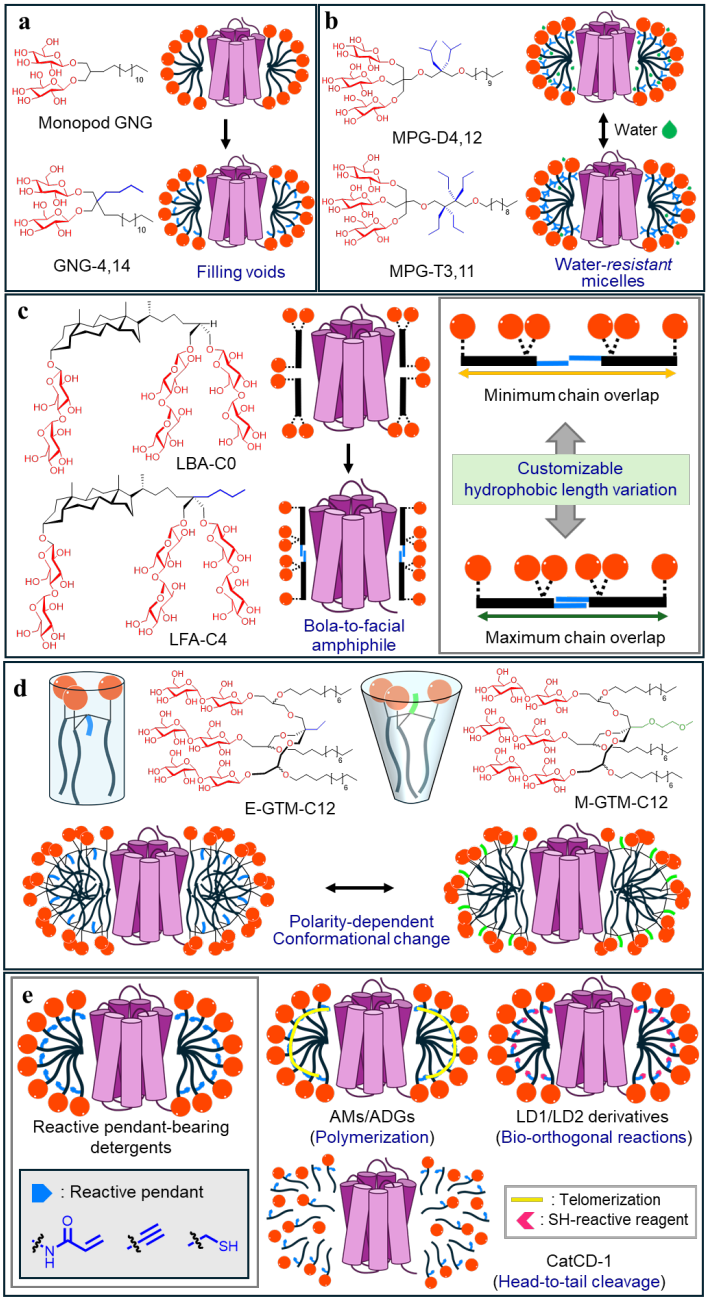

a
Monopod GNG
GNG-4,14
Filling voids
b
MPG-D4,12
Water
MPG-T3,11
Water-resistant micelles
c
LBA-C0
LFA-C4
Bola-to-facial amphiphile
Minimum chain overlap
Customizable hydrophobic length variation
Maximum chain overlap
d
E-GTM-C12
M-GTM-C12
Polarity-dependent Conformational change
e
Reactive pendant-bearing detergents
: Reactive pendant
AMs/ADGs (Polymerization)
LD1/LD2 derivatives (Bio-orthogonal reactions)
: Telomerization
: SH-reactive reagent
CatCD-1 (Head-to-tail cleavage)

Figure 2. Schematic representations of MP-detergent complex (PDC) structures for representative pendant-bearing detergents, illustrating the effects of pendant groups on micelle structure or properties. Panels show: (a) P-GNGs, (b) MPGs, (c) LFAs, (d) GTMs, and (e) reactive pendant-bearing detergents (AM/ADGs, LD1/LD2, and CatCD-1). The key functions of each pendant group are highlighted and summarized in the illustrations. Structures of corresponding control detergents are included for direct comparison where relevant.

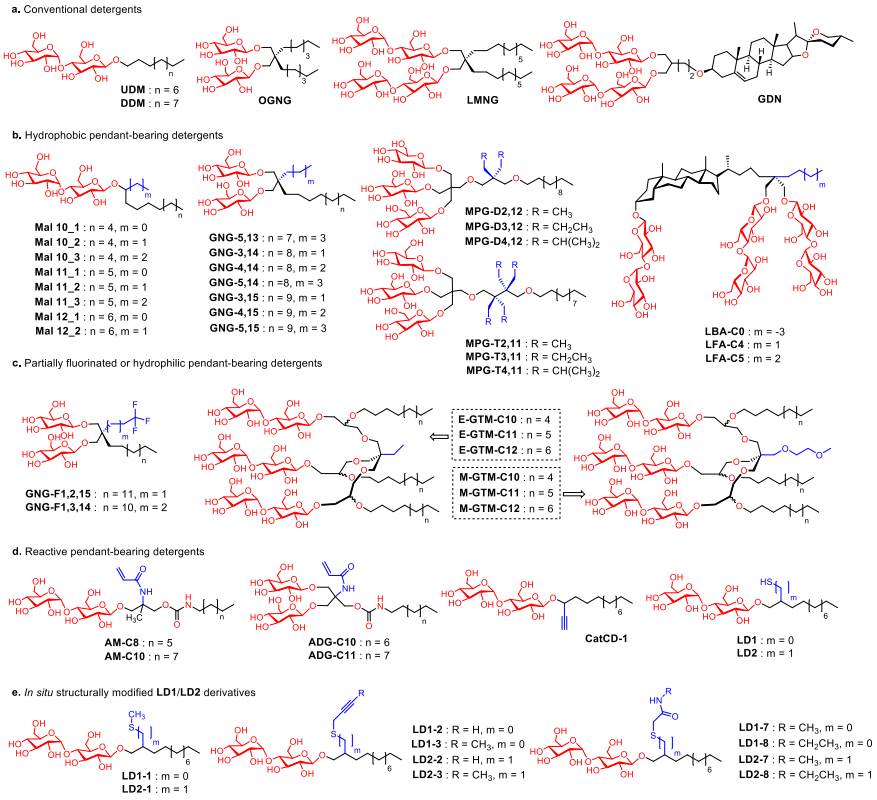


Figure 3. Chemical structures of various pendant-bearing detergents. (a) Conventional detergents (UDM/DDM, OGNG, LMNG and GDN), (b) hydrophobic pendant-bearing detergents (branch-chained maltosides, P-GNGs, MPGs, and LFAs), (c) partially fluorinated (FP-GNGs) and hydrophilic pendant-bearing detergents (GTMs), (d) reactive pendant-bearing detergents (AMs/ADGs, CatCD-1 and LDs), (e) *in situ* modified derivatives of LD1/LD2. Hydrophilic head and hydrophobic tail groups are colored red and black, respectively, while pendant chain groups are indicated in blue.

Table 1. Molecular weights (MWs), critical micelle concentrations (CMCs), water-solubility, hydrophilic-lipophilic balance (HLB) values of detergents and the hydrodynamic radii (R_h) of their micelles in water at room temperature.

Detergent	MW	CMC (mM)	R_h (nm)[a]	Solubility (%)	HLB[b]	Ref.
UDM	496.59	0.59	3.1 ± 0.07	> 10	13.7	[65]
DDM	510.6	0.17	3.5 ± 0.1	> 10	13.4	[40]
OGNG	568.7	~ 1.0	4.4 ± 0.3	> 10	12.6	[40]
LMNG	1005.2	~ 0.010	10.6 ± 0.2	~ 10	13.6	[40]
GDN	1165.3	~ 0.018	4.4 ± 0.1	~ 10	11.7[c]	[40]
Mal 10_1	496.59	1.2	2.7 ± 0.1	> 10	13.4	[65]
Mal 11_1	510.62	0.37	3.0 ± 0.07	> 10	13.0	[65]
GNG-5,13	652.9	~ 0.015	24 ± 0.6	~ 5.0	11.0	[66]
GNG-3,14	638.8	~ 0.025	3.2 ± 0.0	~ 10	11.2	[67]
GNG-4,14	652.9	~ 0.025	3.4 ± 0.0	~ 10	11.0	[66]
GNG-5,14	666.9	~ 0.020	27 ± 1.5	~ 5.0	10.7	[66]
GNG-3,15	652.9	~ 0.015	3.7 ± 0.1	~ 10	11.0	[66]
GNG-4,15	666.9	~ 0.010	3.7 ± 0.1	~ 10	10.7	[66]
GNG-5,15	680.9	~ 0.010	73 ± 6.0	~ 5.0	10.5	[66]
GNG-F1,2,15	706.8	~ 0.020	3.8 ± 0.2	~ 10	9.6[d]	[66]
GNG-F1,3,14	706.8	~ 0.020	3.3 ± 0.1	~ 10	9.6[d]	[66]
MPG-D2,12	905.1	~ 0.045	3.5 ± 0.1	> 10	13.8	[68]
MPG-D3,12	933.1	~ 0.020	3.5 ± 0.1	> 10	13.3	[68]
MPG-D4,12	961.2	~ 0.018	3.5 ± 0.1	> 10	13.0	[68]
MPG-T2,11	961.2	~ 0.025	3.5 ± 0.1	> 10	13.0	[68]
MPG-T3,11	1017.3	~ 0.020	3.6 ± 0.1	> 10	12.2	[68]
MPG-T4,11	1073.5	~ 0.008	23.9 ± 0.1	~ 7.0	11.6	[68]
LBA-C0	1393.5	~ 0.11	2.6 ± 0.0	> 5.0	14.7	[70]
LFA-C4	1449.6	~ 0.018	2.6 ± 0.0	5.0	14.1	[70]
LFA-C5	1463.6	~ 0.015	3.3 ± 0.1	2.5	14.0	[70]
E-GTM-C10	1750.1	~ 0.005	23.5 ± 0.2	~ 10	11.7	[83]
E-GTM-C11	1792.1	~ 0.002	38.9 ± 0.1	~ 5.0	11.4	[83]
E-GTM-C12	1834.2	~ 0.0015	42.8 ± 0.7	~ 1.0	11.2	[83]
M-GTM-C11	1852.2	~ 0.004	6.3 ± 0.1	~ 10	11.1	[83]
M-GTM-C12	1894.3	~ 0.003	16.8 ± 0.3	~ 10	10.9	[83]
AM-C8	638.7	~ 3.74	-	> 1.0	14.7[e]	[84]
AM-C10	666.8	~ 0.47	2.9 ± 0.7	> 0.4	14.1[e]	[84]
ADG-C10	682.8	~ 1.37	2.4 ± 0.6	> 0.7	14.3[e]	[84]
ADG-C11	696.8	~ 0.51	-	-	14.0[e]	[84]
CatCD-1	534.6	~ 0.10	4.0 ± 0.2	> 10	12.8[e]	[88]
LD1	542.7	0.08	6.3 ± 0.3	> 10	12.6[e]	[89]
LD2	556.7	0.08	16.0 ± 0.4	> 10	12.3[e]	[89]
LD1-2	580.7	0.08	15.6 ± 1.6	> 10	11.8[e]	[89]
LD1-3	594.8	0.08	30.8 ± 0.5	> 10	11.5[e]	[89]
LD1-7	613.8	0.40	3.2 ± 0.7	> 10	12.5[e]	[89]
LD1-8	627.8	0.06	12.1 ± 0.3	> 10	12.2[e]	[89]
LD2-1	570.7	0.10	2.7 ± 0.1	> 10	12.0[e]	[89]
LD2-2	594.8	0.25	3.1 ± 0.0	> 10	11.5[e]	[89]
LD2-7	627.8	0.08	6.3 ± 0.3	> 10	12.2[e]	[89]
LD2-8	641.8	0.08	16.0 ± 0.4	> 10	12.0[e]	[89]

[a] Hydrodynamic radius of micelles measured at a detergent concentration of 1.0 wt%. [b] Calculated by Griffin's method [52]. [c] Calculated by classifying three ether bonds in the lipophilic portion as hydrophobic groups. [d] Calculated by Davies' Method [90]. [e] Calculated by classifying the acrylamide, carbamate, and amide groups located at the hydrophilic-hydrophobic interfaces as hydrophilic, while the alkyne, thiol, and thioether groups at the same interfaces were treated as hydrophobic.

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