Unsymmetric or hybrid detergents for membrane protein structural study

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Abstract

Detergent micelles are widely used as a membrane-mimetic system for membrane protein extraction, isolation and structural study. Many recently developed detergents feature multiple tail and head groups, with architectures that are symmetric (i.e., the same alkyl chain) and non-hybrid (single head group type). Further diversification has involved incorporating structural differences in the tail groups (unsymmetric), head groups (hybrid), or both head and tail groups (unsymmetric hybrid). In this mini-review, we introduce these novel detergents, focusing on the relationships between their structural features, physical properties and performance in MP applications. The detergent design strategy utilizing unsymmetric/hybrid structures expands the detergent repertoire and the detergent structure-property-efficacy relationships presented offer valuable design guidelines, collectively advancing membrane protein research.

Introduction

Unveiling the structures of membrane proteins (MPs) is crucial for understanding their mechanisms of action and for developing effective protein specific therapeutic agents and [1,2]. However, MPs are inherently amphiphilic,containing both hydrophilic and hydrophobic groups. This amphiphilicity causes MPs to denature or aggregate when exposed to aqueous environments, making the investigation of MP structures challenging[3,4]. This challenge is particularly pronounced when studying eukaryotic MPs with multiple subunits or oligomeric architectures[5-7]. Consequently, the number of known MP structures is only a small fraction of that of total protein structures determined, although has recently increased dramatically in the last ten years, reaching approximately 1,800 unique structures as of February 20, 2025 (Figure 1a)[8]. This surge is largely due to the development of cutting-edge technologies, particularly single-particle cryo-electron microscopy (cryo-EM) [cryo-EM ref]. Additionally, recent advances in membrane-mimetic systems, including amphipathic polymers (APols), bicelles, and membrane scaffold protein (MSP)-, styrene-maleic acid copolymer (SMA)-, and saposin-based nanoassembly (nanodiscs (NDs), SMA lipid particles (SMALPs), and Salipro, respectively), have significantly contributed to MP structure determination (Figure 1b)][9-17]. Detergent micelles, as a

membrane-mimetic system, have been widely used for MP structural studies since the first crystal structure of *Rhodopseudomonas viridis* photosynthetic assembly was reported using lauryldimethylamine-*N*-oxide (LDAO) in 1985[18-20]. Unlike most other membrane mimetic systems, detergent micelles can be used throughout the entire course of MP manipulation, including protein extraction, purification and structural study[21,22]. However, one main issue associated with detergent micelles is their limited ability to stabilize MPs in aqueous media, likely due to their dynamic nature[23,24]. To increase the stability of MPs in detergent-based solution, various novel detergents have been developed over the past two decades[25-32]. In this review, we introduce recently developed detergents containing multiple tail and/or head groups, which we have categorized into four classes, based on the tail group symmetry and head group identity: detergents with the same or different alkyl chains (symmetric/unsymmetric) and detergents with identical or different head groups (non-hybrid/hybrid) (Figure 1c). We focus on the structural distinctions among these four detergent classes and their effects on physical properties, such as critical micellar concentrations (CMCs), micelle size, and hydrophilic-lipophilic balance (HLB). Importantly, this review also explores the relationship between the physical properties of these detergents and their protein stabilization efficacy. Overall, the detergent structure-property-efficacy relationships introduced in this review provide valuable insights for future detergent design.

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Symmetric detergents

Conventional detergents widely used for MP studies, as exemplified by *n*-dodecyl-β-D-maltoside (DDM), *n*-octyl-β-D-glucoside (OG), LDAO and tetraethylene glycol monooctyl ether (C_8E_4), have a canonical architecture consisting of a single tail and single head group (Figure 2a). In this architecture, straight alkyl chains with various lengths serve as lipophilic groups, while polar groups, such as amine-*N*-oxide (LDAO), glucoside (OG), maltoside (DDM), and oligoethylene glycol (C8E4), act as hydrophilic groups. While the dynamic nature of micelles formed by conventional detergents may be favorable for protein extraction, many MPs in these micelles exhibit limited stability, posing a challenge for their structure determination[33,34]. To address this limitation, various novel detergents have been developed, many of which contain multiple alkyl chains (*ranging from two to four*) and multiple head groups (*ranging from two to eight*). Examples include maltose neopentyl glycols (MNGs), glucose neopentyl glycols (GNGs), TRIS linker-bearing triazine-based triglucosides (TTGs) and tandem malonate-derived glucosides (TMGs) (Figure 2b) [35-38]. The presence of multiple tail groups enhances MP stability by increasing *detergent-detergent* and *protein-detergent* interactions, as supported by MD simulations of LMNG[39]. As seen with these detergents, the presence of multiple tail groups necessitates inclusion of multiple head groups to achieve an optimal HLB in the range of 11 to 14 for MP studies [40]. All of these detergents feature identical tail and head groups, and are therefore classified as symmetric non-hybrid detergents. Due to the presence of multiple alkyl chains, these symmetric non-hybrid detergents have significantly lower CMCs than single alkyl chain detergents like DDM (Table 1) and are significantly more effective at stabilizing MPs.

Unsymmetric detergents

Despite their enhanced protein stabilization efficacy, symmetric detergents are often associated with issues related to MP studies. As exemplified by LMNG (also known as MNG-10,10), these symmetric

detergents tend to form larger micelles (hydrodynamic diameter (D_h) =19.6 nm at 1.0 wt%). Small micelle-forming detergents are preferred for MP structural study via x-ray crystallography and cryo-EM[19,41]. In relation to large micelle formation, symmetric detergents often exhibit limited water-solubility when long alkyl chains are introduced to match the hydrophobic thickness of MPs (28–32 Å)[24,42]. For example, one-carbon extension versions of OGNG (GNG-6,6) and LMNG—GNG-7,7 and MNG-11,11, respectively—showed limited water-solubility (~1.0 wt%) (Table 1). Introducing unsymmetric hydrophobic groups effectively resolved these issues while retaining the favorable properties of the multiple alkyl chains. For the GNGs, the main chain length could be extended to C13/C14 without having water-solubility problems, and their micelle sizes decreased from 4.5 nm (GNG-6,6) to 3.2 nm upon introducing unsymmetric hydrophobic groups (Figure 2c & Table 1)[43]. Similar behaviors were observed for unsymmetric MNGs and TTGs, designated U-MNGs and U-TTGs, respectively (Figure 2c)[44,45]. The U-MNGs, which contain different alkyl chains (C9-C11, C8-C12, C6-C14, or C4-C16), form substantially smaller micelles and exhibit slightly lower CMCs compared to the symmetric LMNG (Table 1), potentially benefiting MP studies. The differences in micelle sizes and CMCs between LMNG and U-MNGs tend to increase as the difference in alkyl chain length (i.e. unsymmetry) increases. When evaluated with several model MPs (leucine transporter (LeuT), melibiose permease (MelB), human beta2 adrenergic receptor (β_2AR), mouse μ-opioid receptor (MOR)), the U-MNGs outperformed LMNG in terms of protein stability, with the best performance observed for MNG-8,12[44]. These results demonstrate that the introduction of unsymmetric hydrophobic groups can improve not only detergent properties (water solubility, CMC and micelle size) but also enhance detergent efficacy for MP stabilization. Interestingly, the high efficiency of LMNG for MP extraction was well maintained even with their unsymmetric architecture[44]. Similar to the U-MNGs, the U-TTGs (e.g., TTG-9,11 and TTG-8,12) exhibited lower CMCs than TTG-10,10 (Table 1) and demonstrated improved MP stabilization compared to the latter when evaluated with the same set of MPs[45]. However, unlike the U-MNGs, the decrease in micelle size with increasing detergent unsymmetry was not as pronounced for the U-TTGs (Table 1), which may be due to the different ways the two alkyl chains are introduced into the detergent cores. Given the availability of many symmetric detergents[35-38,46,47], studies on their unsymmetric version will further clarify the advantages and disadvantages of unsymmetric detergents for MP studies.

The unsymmetric nature introduced into the TMG-Ps somewhat differs from that in the U-MNGs/U-TTGs, as an additional alkyl chain was inserted into the hydrophobic region between two alkyl chains of symmetrical TMGs (Figure 2c)[48]. Consequently, the TMG-Ps have higher hydrophobic density than the symmetrical counterparts, leading to decreased HLB values and enhanced MP stability. For example, TMG-P10,8 has a lower HLB value than TMG-A13 (11.3 *vs.* 11.9) (Table 1) and demonstrates enhanced efficacy in stabilizing LeuT, MelB and β_2AR. Interestingly, this unsymmetric detergent exhibited higher MelB extraction efficiency compared to DDM and symmetric. TMG-A13. However, the increase in the number of alkyl chains resulted in larger micelles (R_h = 8.7 nm for TMG-P10,8 *vs.* 3.6 nm for TMG-A13), explained by the increased hydrophobic volume (Table 1). This unfavorable property could be mitigated by making the detergent more unsymmetric. The unsymmetric strategy used for the TMG-Ps is likely to be useful in improving the physical properties and MP stabilization efficacy of symmetric detergents with a large inter-alkyl chain distance, due to enhanced hydrophobicity

and alkyl chain density.

Hybrid detergents

Two different types of head groups can be combined to generate hybrid detergents. The detergent's hydrophilic group is a key factor in determining overall molecular geometry and hydrophilicity, which in turn influence detergent packing parameter (i.e., micelle size) and HLB values, respectively[49]. By selecting and combining two head groups from glycerol (G0), tetraethylene glycol (E4), and glucoside (Glu), several non-hybrid or hybrid detergents were created, including G0-G0 ([G1]OGD), E4-G0, E4-E4, E4-Glu, Glu-Glu, and Gl-G1 (G2[OGD]) (Figure 2d)[50]. These non-hybrid/hybrid detergents provide a method to fine-tune both the HLB and packing parameter (Table 1). When tested with the outer membrane protease T (OmpT) from *E. coli*, a hybrid detergent (E4-Glu) was more effective than its non-hybrid counterparts (C8E4 and OG) at maintaining the protease's activity. The lower CMC of E4-Glu compared to the non-hybrid counterparts [0.5 mM *vs.* C8E4 (8 mM) and OG (23 mM)] allowed for the use of a smaller amount of detergent to maintain OmpT function (Table 1). A hybrid detergent containing maltoside (Mal) and E4 (i.e., Mal-E4) was used to investigate which factor, detergent concentration or detergent properties, most influences protein-lipid interaction[51,52]. When the amount of lipid molecules co-purified with the tetrameric aquaporin channel (AqpZ) was quantified by native mass spectrometry (MS), two low-CMC detergents, DDM (0.17 mM) and Mal-E4 (0.21 mM), showed increased retention of lipid molecules around tetrameric AqpZ than the high-CMC C8E4 (8 mM) (Table 1). This result demonstrates that protein-lipid interaction is primarily influenced by detergent concentration in purification buffers. As detergents are commonly used as multiples of their CMCs in purification buffers, low-CMC detergents (e.g., Mal-E4) can be favorably used for maintaining protein-lipid interaction.

Different head groups can also be combined to utilize their respective merits for MP studies. For example, dicarboxylate-oxide detergents (DCODs) exploit the ability of a branched dicarboxylate group to form salt-bridges with basic residues present at the membrane-cytoplasm interfaces of MPs (Figure 2d)[53]. By combining the dicarboxylate group and a MP-stabilizing glucoside/maltoside group, the DCOD hybrid detergents (8b and 9b) preserved the function of BmrA, an ATP-binding cassette (ABC) pump, and achieved a high melting temperature for A2A adenosine receptor (A_{2A}AR). DCOD-9b was successfully applied to the purification and characterization of Cdr1, a protein responsible for azole resistance in *Candida glabrata*[54]. Furthermore, a related compound (3d) was used as an additive for crystal structure determination of BmrA [55]. These results suggest that hybrid detergent structures can be applied to a wide range of MP studies. In this context, the use of spermine as detergent head group is particularly interesting, as this polyamine group has been reported to effectively reduce protein surface charge, a useful feature for native MS (Figure 2d)[56]. Hypothetical hybrid detergents containing both spermine and maltoside groups are expected to not only stabilize MPs but also facilitate the mass analysis of MPs [Figure 3b].

Unsymmetric and hybrid detergents

As a logical extension, unsymmetric *and* hybrid detergents have been developed, such as triazole-linked pre-assembled detergents (T-PADs)[57]. Designed via cross-coupling reactions of two monomeric

detergents (OG and DDM analogs), the T-PAD architecture can be symmetric, unsymmetric, hybrid, or a combination of unsymmetric and hybrid (Figure 2e). The hybrid T-PADs generally exhibit lower CMCs than their non-hybrid counterparts, a trend similar to that observed for other hybrid detergents (Table 1). Micelle sizes formed by the T-PADs tend to decrease with increasing detergent unsymmetry, though no clear relationship was observed between the hybrid structure and micelle size in this system. Among the T-PADs, unsymmetric dimaltoside detergents (e.g., 13M9M and 13M10M) were most effective at maintaining the ATPase activity of an ABC transporter (MsbA), followed by the unsymmetric hybrid detergents (e.g., 14M8G) and the symmetric detergents (e.g., 12M12M and LMNG), confirming the beneficial effect of unsymmetric architecture on MP stability. Additionally, the unsymmetric non-hybrid (e.g., 10M14M and 13M11M) and unsymmetric hybrid detergents (14M8G and 14M9G) outperformed symmetric LMNG in retaining ligand binding of the A_{2A}AR. Notably, 14M8G and 14M9G were favorably used in EM studies of MsbA and NMR studies of A_{2A}R, respectively, highlighting the potential of unsymmetric hybrid detergents for MP structural studies.

Various (pseudo) unsymmetric and/or hybrid architecture have also been generated by the Ugi four-component reaction, which requires amine, carboxylic acid, aldehyde and isocyanate as reactants (Figure 2e)[58]. When the resulting dipeptide-based U-PADs were tested with MsbA and four GPCRs [A_{2A}AR, the cannabinoid receptor type 2 (CB2R), the smoothened receptor (SMO), the glucagon-like peptide-1 receptor (GLP-1R)], the symmetric detergents, M-23-M and 12-M-12M, were generally more effective at stabilizing these MPs compared to the unsymmetric hybrid detergents (8-12M-G and 8-12M-E). One representative (M-23-M) was particularly effective for maintaining the homogeneity of GLP-1R and showed narrow signals in ^{19}F NMR spectra of 2,2,2-trifluoroethanethiol-modified receptor, comparable to LMNG. This stands in contrast to the trends observed for other unsymmetric and/or hybrid detergents, such as the U-MNGs, Mal-E4, and T-PADs. The rigid dipeptide scaffold of the U-PADs likely accounts for this different trend, as it limits the flexibility of the detergent head and tail groups, making it difficult to achieve their optimal conformations for MP stability. Thus, the detergent scaffold is a key variable in detergent design for MP studies.

Beta-peptides (BPs) are another example of unsymmetric hybrid detergents, containing four head groups (two hydroxyl and two carboxylate groups) and four tail groups (two isobutyl and two octyl chains) (Figure 2e)[59]. BP-1 was highly effective at stabilizing various MPs; MsbA, bacteriorhodopsin, the tetrameric voltage-gated potassium channel, KcsA, and full-length glucagon receptor. This peptide-based detergent was also successfully applied to visualization of the dynamic conformation of MsbA through EM studies. These favorable properties are attributed to the formation of a hydrogen-bond network between the beta-peptide backbones. Recently, hybrid versions of the BPs with conventional detergents, designated AXBX detergents, were generated by conjugating OG/DDM to both ends of the BP-based scaffold (Figure 2e)[60]. Among the various AXBX detergents, A4B2, containing a head group of two maltosides and two carboxylic acids and a tail group of two C12 and two isobutyl chains, was shown to be most effective at stabilizing the three GPCRs (A_{2A}R, SMO and GLP-1R) and demonstrated promising behavior for EM studies of A_{2A}R. Furthermore, A4B2 outperformed BP-1 in extracting A_{2A}R, indicating that well-designed unsymmetric hybrid detergents can be useful for MP extraction. However, due to their peptide scaffolds, these peptide-based detergents (BPs and AXBX)

may have issues with synthetic accessibility.

Conclusion

In summary, the emergence of LMNG has had a profound impact on membrane protein structural studies, leading to the development of various symmetric detergents with multiple head and tail groups. Over time, detergent architecture has evolved, incorporating variations in alkyl chain length or head group identity, which has spurred creation of unsymmetric and/or hybrid detergents. These unconventional detergents allow fine-tuning of key detergent properties, such as CMC, micelle size, and HLB values, all of which significantly influence MP stability. The introduction of unsymmetric hybrid detergents has dramatically expanded the detergent toolbox for MP research, but rational design and consideration of synthetic accessibility remain essential to ensure their practical application. Collectively, unsymmetric and/or hybrid detergents present vast potential for advancing MP structural studies.

Declaration of competing interest

The authors have declared no conflict of interest.

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Data availability

No data was used for the research described in the article.

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**This publication describes the design and synthesis of peptide-DDM hybrid detergents by conjugating DDM with amphiphilic β-peptide scaffolds via click chemistry. The resulting DDM-peptide conjugate exhibited superior membrane protein stabilizaiton efficacy compared to LMNG.

Commented [BB2]: In part a) the text next to the blue box at the top should should read "number". Also I don't think I would use the term "Stephen White Lab"-rather I would use "Membrane Proteins of Known 3D structure" and then maybe put the specific details of the website and the fact that this is curated by Stephen White in the figure legend.

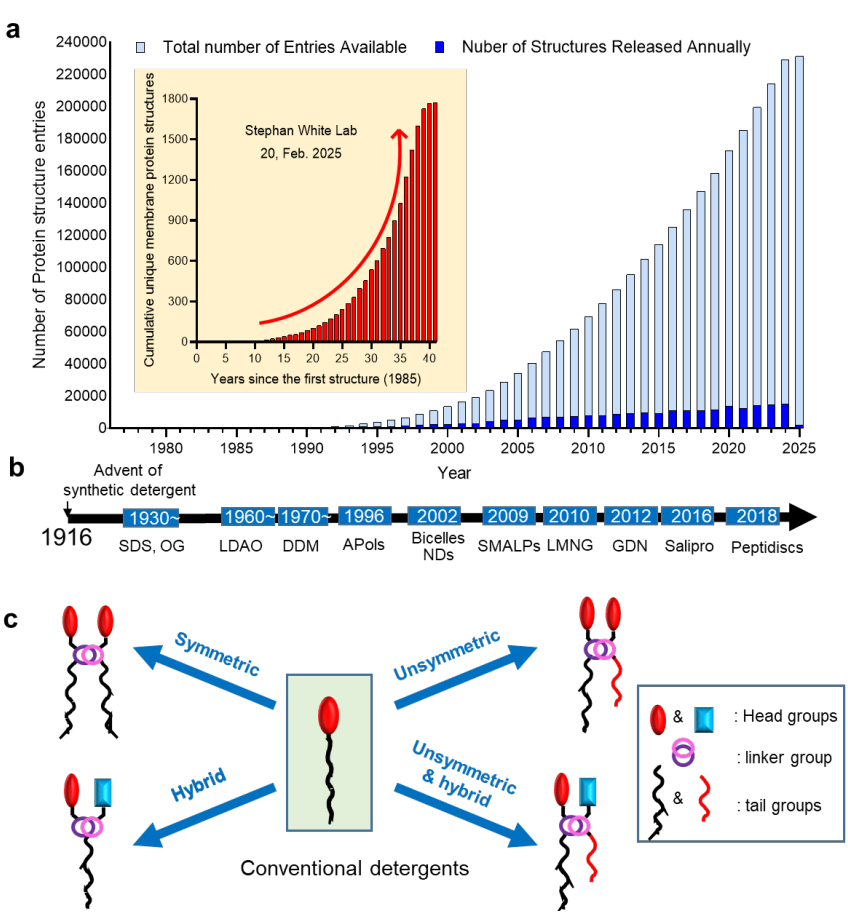

Figure 1. (a) Cumulative numbers of total protein structures (data obtained from the PDB; https://www.rcsb.org/). and unique membrane protein structures (inset, obtained from https://blanco.biomol.uci.edu/mpstruc/ with the data curated by the Stephen White laboratory). (b) Timeline for development of membrane-mimetic systems, including detergents, amphipols (APols), bicelles, nanodiscs (NDs), styrene-maleic acid-based lipid particles (SMALPs), Salipro and Peptidiscs. (c) Schematic representation of new detergent classes derived from conventional detergents (*center*): symmetric non-hybrid detergents (*top left*), unsymmetric detergents (*top right*), hybrid detergents (*bottom left*), and unsymmetric hybrid detergents (*bottom right*).

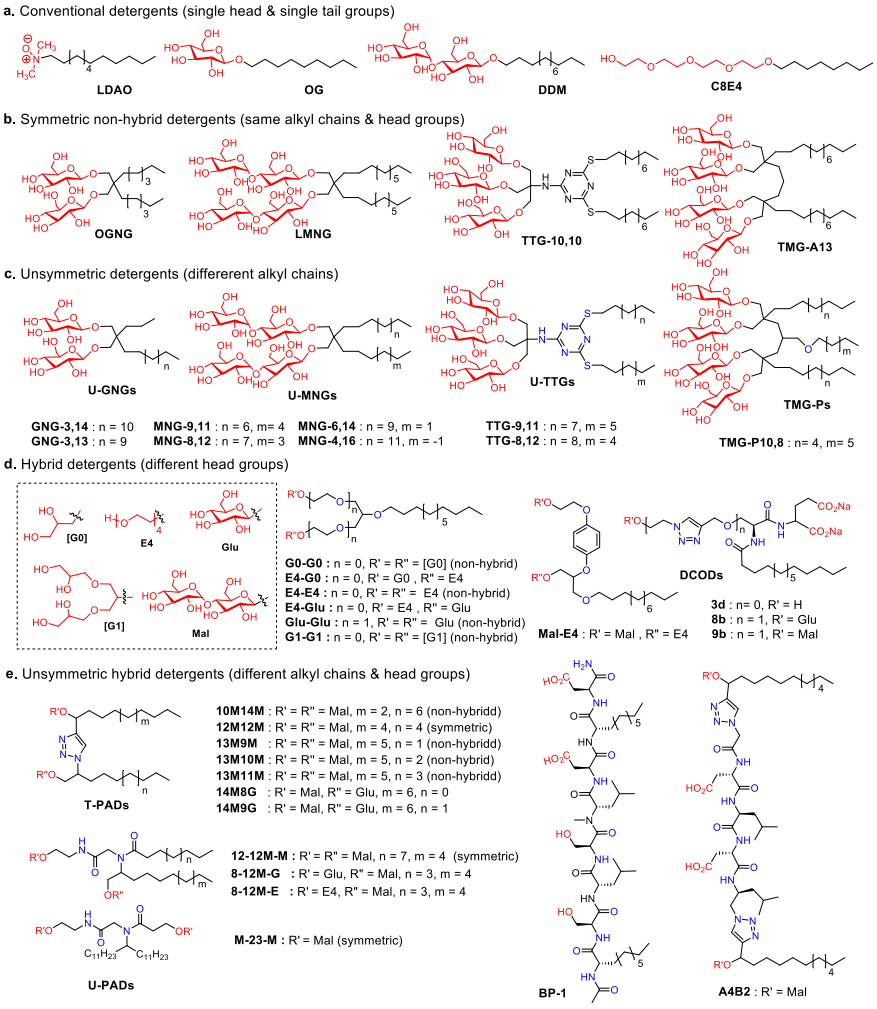

Figure 2. Chemical structures of (a) conventional detergents and (b-e) newly developed detergents with the symmetric alkyl chains and non-hybrid head groups (b), unsymmetric alkyl chains and non-hybrid head groups (c), hybrid head groups (d), and unsymmetric alkyl chains and hybrid head groups (e).

Table 1. Molecular weights (MW), critical micelle concentrations (CMC) 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
DDM	510.6	~ 0.17	3.4	> 10	13.4	[27]
C8E4	306.4	~ 8.0	2.4	> 10	12.6	[50][d]
OG	292.4	~ 25	2.6	> 10	12.3	[27]
LDAO	229.4	1-2	-	> 10	22.3[c]	[27] [Mdler et al., 1986]
LMNG	1005.2	~ 0.010	10.6	~ 10	13.6	[27]
MNG-11,11	1033.3	-	-	~ 1.0	13.2	-
OGNG	568.7	~ 1.0	4.4	> 10	12.6	[27]
GNG-7,7	596.8	-	-	~ 1.0	12.0	-
TTG-10,10	1031.3	~ 0.005	3.6	> 10	11.8	[44]
TMG-A13	1205.5	~ 0.006	3.6	> 10	11.9	[47]
GNG-3,13	624.8	~ 0.022	3.1	> 10	3.1	[42]
GNG-3,14	638.8	~ 0.023	3.2	> 10	3.2	[42]
MNG-9,11	1005.2	~ 0.008	8.3	> 10	13.6	[43]
MNG-8,12	1005.2	~ 0.008	6.0	> 10	13.6	[43]
MNG-6,14	1005.2	~ 0.006	3.8	> 10	13.6	[43]
MNG-4,16	1005.2	~ 0.004	3.5	> 10	13.6	[43]
TTG-9,11	1031.3	~ 0.004	3.7	> 10	11.8	[44]
TTG-8,12	1031.3	~ 0.003	3.6	> 10	11.8	[44]
TMG-P10,8	1263.6	~ 0.01	8.7	~ 10	11.3	[47]
G0-G0	408.5	~ 0.5	-	-	11.7	[49]
E4-G0	510.7	~ 0.4	-	-	13.3	[49]
E4-E4	612.8	~ 0.5	-	-	14.4	[49]
E4-Glu	642.8	~ 0.5	-	-	14.7	[49]
Glu-Glu	672.8	~ 0.5	-	-	14.9	[49]
G1-G1	704.5	~ 0.7	-	-	15.2	[49]
E4-Mal	880.5	~ 0.21	2.8~3.8	-	12.1	[50]
DCOD-8b	775.8	~ 1	0.3-1.0	-	-	[52]
DCOD-9b	1008.1	~ 2	0.5-1.8	-	-	[52]
10M14M	1086.3	~ 0.005	4.2	-	13.8	[56]
12M12M	1086.3	~ 0.004	4.7	-	13.8	[56]
13M9M	1058.2	~ 0.004	3.6	-	14.2	[56]
13M10M	1072.3	~ 0.004	3.7	-	14	[56]
13M11M	1086.3	~ 0.004	3.7	-	13.8	[56]
14M8G	896.1	~ 0.002	3.6	-	13.1	[56]
14M9G	910.1	~ 0.002	3.6		12.9	[56]
8-12M-G	928.5	~ 0.008	3.8	-	11.2	[57]
8-12M-E	942.6	~ 0.02	3.1	-	11.3	[57]
12-12M-M	1146.7	~ 0.002	3.7	-	11.9	[57]
M-23-M	1160.7	~ 0.003	13.6	-	11.8	[57]
BP-1	1042.3	< 0.001	3.0[d]	~ 0.1	-	[58]
A4B2	1313.6	0.01	2.6[e]	> 5	-	[59]

[a] Hydrodynamic radius of micelles measured at a detergent concentration [b] Calculated by Griffin's method. 'Calculated by Davies' Method. [d] Length of filamentous structure measured by electron microscopy. [e] Gyration radius (R_g) determined in 5.0 mM in phosphate buffer (20 mM, pH 6.2, 150 mM NaCl).