Cumhuriyet Sci. J., 47(4) (2026) 701-705
DOI: 10.17776/csj.1830863

Cumhuriyet Science Journal

http://csj.cumhuriyet.edu.tr Founded: 2002 ISSN: 2587-2680 e-ISSN: 2587-246X Publisher: Sivas Cumhuriyet University

Effects of Detergent Selection on Stability of a Recombinant Integral Membrane Protein

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Research Article

History
Received: 27.11.2025
Accepted: 19.08.2026

ABSTRACT

Characterization and structural analysis of integral membrane proteins (IMPs) require detergents that can efficiently disrupt the lipid bilayer while maintaining the native conformation, solubility, and stability of the protein. Selecting an appropriate detergent therefore represents a critical bottleneck in membrane protein biochemistry. In this study, we systematically benchmarked six commonly used detergents—fos-choline-12 (FC-12), lauryldimethylamine-N-oxide (LDAO), n-dodecyl-β-D-maltoside (DDM), n-decyl-β-D-maltoside (DM), lauryl maltose neopentyl glycol (LMNG), and nonyl glucoside (NG)—to determine their suitability for the stabilization of a representative IMP. For each detergent, we assessed solubility, followed by an evaluation of oligomeric homogeneity using size-exclusion chromatography. Purity and yield were quantified to enable a comparative view of detergent-dependent protein recovery. To evaluate structural integrity and stability, we conducted circular dichroism spectroscopy and derived thermal unfolding profiles to assess melting temperatures. Our results reveal clear trade-offs between detergents that excel in membrane solubilization and those that better preserve conformational stability, with no single detergent outperforming across all metrics. While NG caused protein precipitation, other detergents maintained protein solubility but produced distinct SEC profiles and apparent thermal stabilities. LDAO and LMNG provided the highest apparent melting temperatures, while DM showed the lowest. These findings provide a practical framework for detergent selection and optimization, and offer broadly applicable insights for researchers pursuing structural, functional, or biophysical studies of other IMPs.

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Keywords: *Detergents, Homogeneity, Integral membrane proteins, Solubility, Thermal stability.*

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1. Introduction

Integral membrane proteins (IMPs) are embedded in lipid bilayers and perform essential transport, signaling, and energy-transduction functions [1]. Their extraction for biochemical and structural analysis almost always requires amphiphiles/detergents that both disrupt native membranes and maintain the native fold of hydrophobic transmembrane segments in aqueous solution [2]. Detergent choice therefore governs every downstream readout: solubility, oligomeric integrity, activity, chromatographic behavior, and thermal stability [3]. This is a major reason why IMP projects either succeed or stall [4]. Detergents' headgroup chemistry, alkyl chain length, critical micelle concentration (CMC), micelle size/shape, and exchange kinetics together determine whether a given detergent will stabilize or unravel a target membrane protein [5].

Maltoside and phosphocholine detergents are commonly used for IMP isolation. Nonionic maltosides, notably n-dodecyl-β-D-maltoside (DDM) and n-decyl-β-D-maltoside (DM), tend to form relatively large, gentle micelles that preserve activity and long-term stability for many multi-pass targets [6]. In contrast, zwitterionic fos-cholines (e.g., FC-12) and amine oxides (e.g., LDAO) give smaller, more dynamic micelles that can improve homogeneity and stability [7]. Recent research reinforces that detergent is not merely a buffer component but a design variable: across thousands of structures, specific detergents recur with distinct success patterns [8].

Target-specific optimization remains indispensable for the successful expression, purification, and structural/functional characterization of multi-pass membrane proteins. This is particularly so for polytopic (multi-pass) membrane proteins whose stability is intimately linked to the surrounding lipid composition and their oligomeric state [9]. Without such target-specific tuning, IMPs may misfold, aggregate, dissociate, or adopt non-native oligomeric states, leading to loss of stability, function or structural homogeneity [10]. Here, we used a comparative, side-by-side screens with six different detergents FC-12, LDAO, DDM, DM, LMNG, and NG for isolation of a multi-pass IMP to provide a process map for the extraction–stability trade-offs.

2. Materials and Methods

Molecular cloning: IMP is an ER-resident intramembrane aspartyl protease with nine-transmembrane helices (Uniprot: Q8TCT9). IMP expression gene, pelB signal sequence and C-term hexahistine tag were synthesized by GenScript and cloned into pET-22b(+) vector (Novagen) via restriction sites NcoI and SalI.

Recombinant expression and production: Chemically competent BL21(DE3) pLysS cells were transformed with the construct by heat shock and plated on LB–agar containing ampicillin (60 µg/mL) and chloramphenicol (34 µg/mL). After 16 -18 h at 37 °C, a single colony was used to inoculate a 5 mL LB starter culture. It was grown at 37 °C, 225 rpm for 8 h and used to inoculate 200 mL LB, which was grown overnight (16 - 18 h) at 37 °C, 225 rpm. The next morning, 1 L LB flasks (with antibiotics) were each inoculated with the 200 mL culture (starting OD600 < 0.1) and grown at 37 °C, 225 rpm to OD600 0.6 - 0.8. Cultures were cooled to 18 °C for 1 h and induced with 0.5 mM IPTG; expression proceeded at 18 °C, 225 rpm for 16 - 20 h. Cells were harvested by centrifugation (4,200×g, 10 min), pellets were flash-frozen in liquid nitrogen, and stored at −80°C until use.

Purification: Cell pellets were resuspended in a chilled, detergent-free lysis buffer (50 mM HEPES pH 7.5, 200 mM NaCl, Roche complete EDTA Free Protease Inhibitor - 1 tablet to 50 mL buffer). Cells were disrupted by sonication, and lysates were clarified. Crude membranes were enriched by ultracentrifugation, washed once in the same buffer, and resuspended in purification buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 20 mM imidazole). n-dodecyl β-D-maltoside (DDM) was weighed out equal to the mass of membrane. The detergent was dissolved in enough membrane resuspension buffer to make a final 1% solution. Solubilized membrane was centrifuged at 120,000 x g at 4°C for 45 minutes to remove any unsolubilized material. The pellet was discarded and the supernatant was used for purification. Detergent-solubilized protein was captured on Ni-affinity column (Cytivia). Protein was eluted by elution buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 500 mM imidazole, 0.1% DDM).

Size-exclusion chromatography (SEC): Affinity-purified samples were buffer-exchanged on a SEC column (Superose 12 10/300 GL) equilibrated in each detergent. Each buffer contained 1X PBS with approximately 2x the critical micelle concentration (CMC) of corresponding detergent. Used detergents include 0.1% Fos-Choline-12 (FC-12), 0.1% N,N-dimethyldodecylamine-N-oxide (LDAO), 0.001% Lauryl Maltose Neopentyl Glycol (LMNG), 0.5% n-Nonyl-β-D-Glucopyranoside (NG), 0.15% n-decyl-β-D-maltopyranoside (DM) and 0.015% n-dodecyl-β-D-maltopyranoside (DDM). UV traces were analyzed for elution volume and peak symmetry for apparent size and homogeneity.

SDS-PAGE analysis: SEC-fractionated samples were mixed with Laemmli sample buffer and denatured prior to loading. Proteins were resolved on a 12% Tris-glycine polyacrylamide gels. Gel was Coomassie stained and gently destained in aqueous buffer until bands were clearly resolved. Gels were imaged on a BioRad imager.

Thermal melt by Circular Dichroism (CD): Spectra were collected in quartz cuvettes using a CD spectropolarimeter (Jasco J-1500) equipped with Peltier temperature control and nitrogen purge. Reference baselines (buffer + detergent) were recorded under identical conditions and subtracted from sample traces. For secondary-structure scans, far-UV spectra were acquired (190–260 nm), with bandwidth and scan rate set to maintain sufficient signal-to-noise without exceeding HT/absorbance limits. Multiple accumulations were averaged per condition. For thermal denaturation, ellipticity was monitored as single-wavelength traces (222 nm for the α-helical content of used IMP) while ramping temperature under controlled equilibration. Raw ellipticity (θ, mdeg) was recorded and normalized for each. For each condition, repeated CD acquisitions were collected from the same sample and averaged to improve the signal-to-noise ratio. Thermal melt (T_m) was obtained by fitting the temperature-dependent signal using Hill equation.

3. Results and Discussion

Because initial solubilization was performed in DDM, this study evaluates detergent compatibility and stability after purification and buffer exchange rather than membrane extraction efficiency. After affinity purification, our protein was buffer-exchanged into 1X PBS with each of six different detergents (Table 1). While 4 of those detergents are traditional nonionic amphiphiles, 2 of them are newly explored zwitterionic detergents.

Table 1. Detergents used for IMP screening.

Detergent	Full name	Type / Headgroup	CMC (% w/v)	Micelle traits	Typical use levels
FC-12	n-Dodecylphosphocholine	Zwitter-ionic / phosphocholine	~ 0.04 – 0.07	Small - mid	0.05 – 1%
LDAO	n-Decyl-N,N-Dimethylamine-N-Oxide	Zwitter-ionic / amine oxide	~ 0.03 – 0.06	Small, tight	0.05 – 1%
DDM	n-Dodecyl-β-D- Maltopyranoside	Non-ionic /maltoside	~ 0.007 – 0.01	Large, gentle	0.05 – 1%
DM	n-Decyl-β-D-Maltopyranoside	Non-ionic / maltoside	~ 0.07 – 0.10	Smaller than DDM	0.05 – 1%
LMNG	Lauryl Maltose Neopentyl Glycol	Non-ionic / maltoside	~ 0.0006 – 0.01	Large, very stable	0.005 – 0.05%
NG	n-Nonyl-β-D-Glucoside	Non-ionic / glucoside	~ 0.25 – 0.35	Small, dynamic	0.1 – 1%

FPLC-SEC was used for the buffer exchange and all detergents except NG yielded analyzable SEC profiles (Figure 1). Detergent-dependent peak positions and asymmetry indicated changes in micelle-protein assembly and apparent hydrodynamic size. Such detergent-dependent shifts and peak shapes are expected because headgroup chemistry, chain length, and micelle properties control the size and dynamics of the micelle-protein assembly [11]. NG triggered precipitation upon exchange and was excluded from further analysis. Although NG (a short-chain glucoside) is often useful with its small, dynamic micelles, it can be comparatively harsh reducing solubility consistent with the precipitation observed in this study [12].

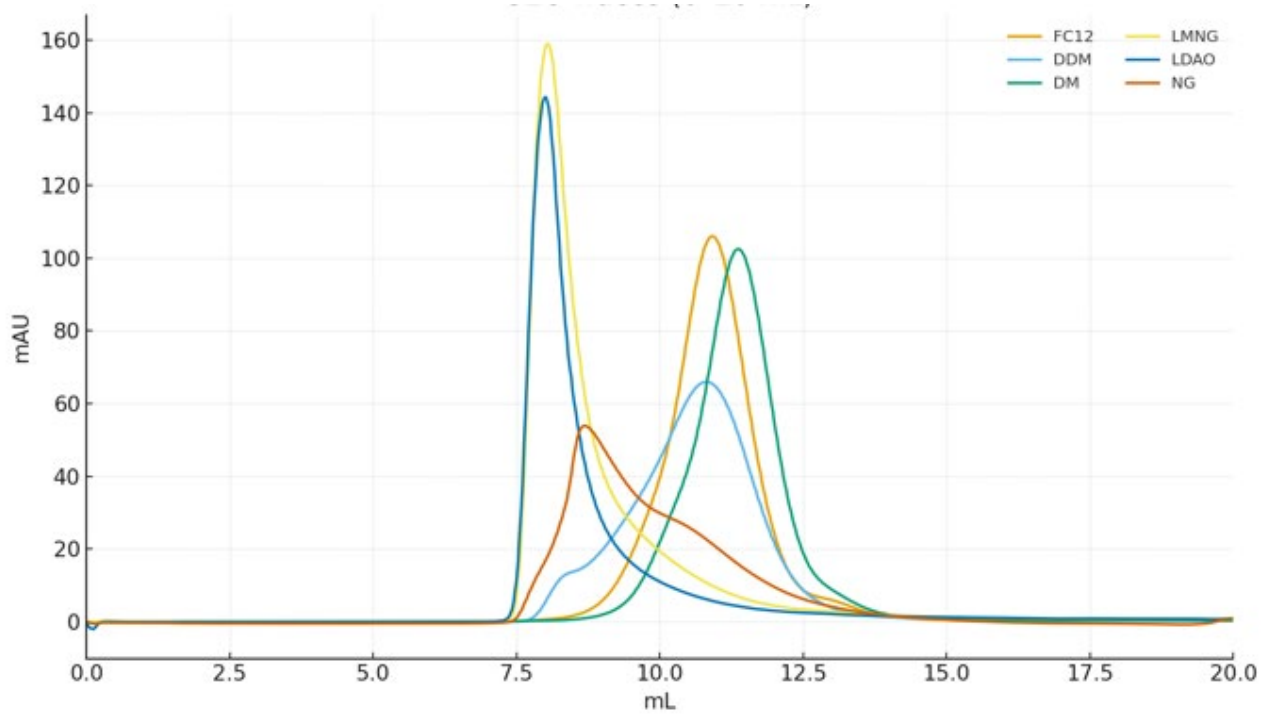


Figure 1. Size exclusion chromatography (SEC) profiles of IMP in different detergents.

Purity and homogeneity after SEC were also analyzed by SDS-PAGE (**Figure 2**). IMP with expected molecular weight (~40 kDa) were observed for FC-12, DDM, DM, LMNG, and LDAO, indicating comparable recovery and purity across the detergents. This outcome aligns with general experience that multiple detergents can deliver acceptable purity, while higher-order homogeneity (e.g., oligomeric state, lipid retention) is better discriminated by SEC and stability readouts [6, 13].

Detergent choice strongly affected thermostability. The relative thermal stability was measured by Circular Dichroism (CD) and representative normalized CD melt curves were plotted (**Figure 3**). Thermal melt (T_m) was calculated by fitting this data by Hill equation and T_m ordering for this IMP was: LDAO (72.8 °C) > LMNG (69.6 °C) > FC-12 (62.4 °C) ≈ DDM (62.3 °C) > DM (57.4 °C). LDAO conferred the highest T_m value, suggesting tighter zwitterionic micelles might best preserve the folded state during heating. LMNG gave the highest stability among nonionic detergents and DM was the least stabilizing. LMNG was the most stabilizing among the nonionics and maltosidases, consistent with extensive reports that MNG/LMNG micelles exhibit low CMCs and kinetically stable assemblies that enhance IMP stability relative to conventional maltosides like DDM/DM [14]. Practically, LDAO or LMNG could be strong defaults for storage and biophysical characterization; FC-12 or DDM are versatile for broader screens; and NG is not recommended under the present conditions.

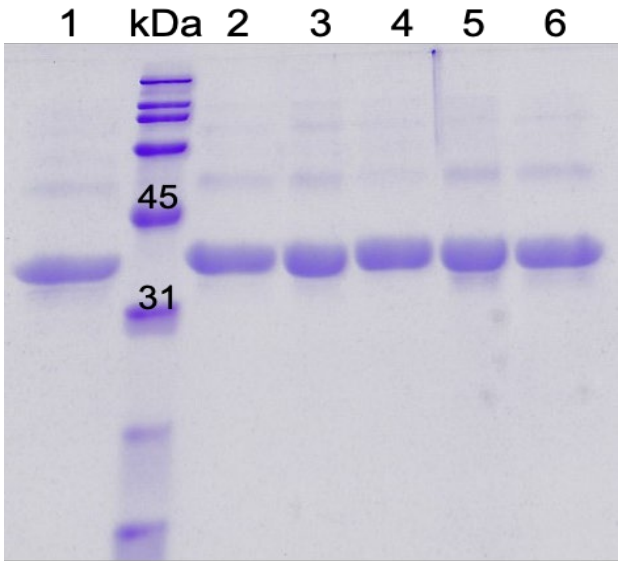


Figure 2. SDS-PAGE analysis of IMP after SEC. 1: After Ni-affinity purification, after SEC with 2: FC-12, 3: DDM, 4:DM, 5: LMNG, 6: LDAO. After buffer exchange with NG detergent, the protein precipitated, so no SDS-PAGE analysis was performed for NG.

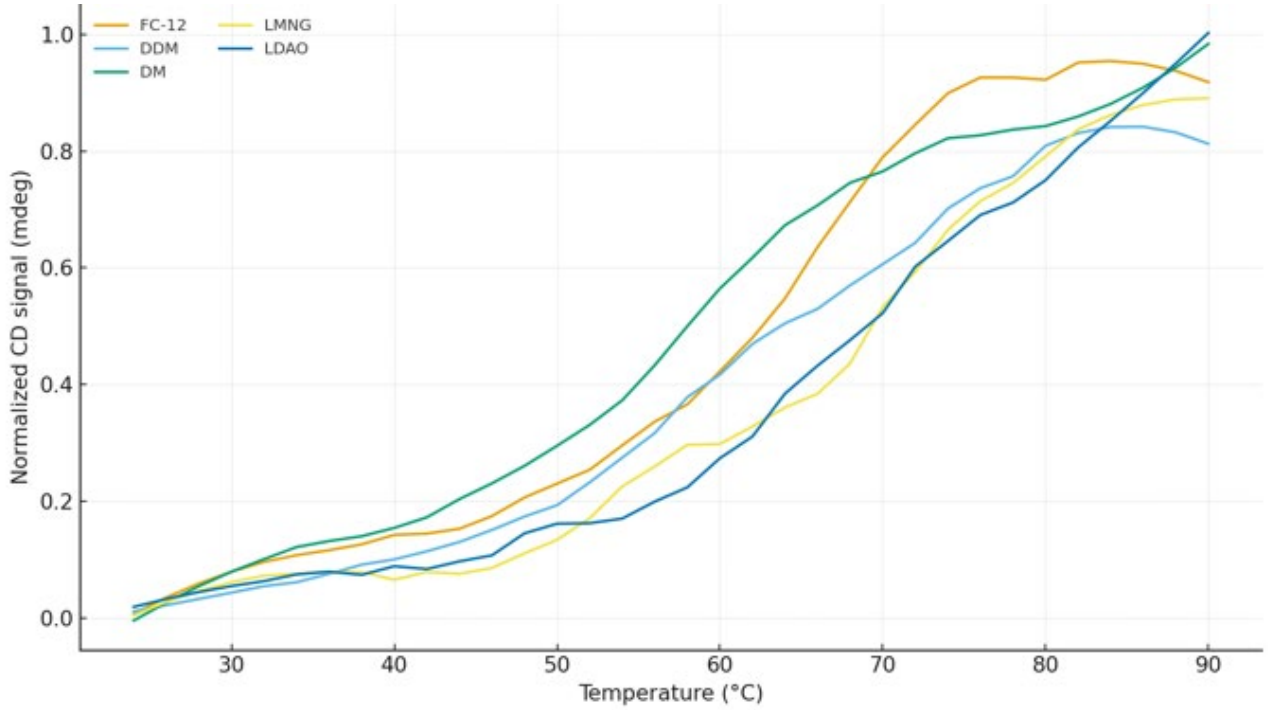


Figure 3. CD thermal melt analysis of IMP in different detergents. T_m values of IMP protein in LDAO, LMNG, FC-12, DDM and DM are 72.8°C, 69.6°C, 62.4°C, 62.3°C, 57.4°C, respectively. After buffer exchange with NG detergent, the protein precipitated, so no thermal melt analysis was performed for NG.

Overall, detergent exchange affected both the apparent hydrodynamic behaviour and thermal stability of the IMP. LDAO and LMNG provided the greatest thermal stabilization, whereas NG was incompatible with the protein under the tested conditions. Future improvements in IMP characterization will rely on advanced amphiphiles such as LDAO and LMNG, which offer superior stability and monodispersity compared to conventional detergents [14, 15]. Additionally, detergent-free systems such as nanodiscs might become popular for preserving native lipid interactions and oligomeric state [16]. Together, these advances will make membrane-protein workflows more stable, reproducible, and less empirical.

Conflict of Interest

There are no conflicts of interest in this work.

Declaration of Generative AI

The authors confirm that no generative AI technologies were used in the preparation, writing, or data analysis of this manuscript.

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