

Electronic Supplementary Information for: “Solubilization of Membrane Proteins with Novel N-Acylamino Acid Detergents.”

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Experimental section.

Materials — N-dodecylamino acid detergents C12-Gly, C12-Ala, C12-Val, C12-Ile, C12-Phe, C12-Ser, and C12-Glu were a gift from Ajinomoto Inc., Japan. Other detergents employed in this study were obtained from commercial sources: dodecyl-maltoside (DDM), octyl-glucoside (OG), TritonX-100, and dodecyl-N-sulfobetaine (Z3-12) from Dojindo, Japan; lauroyldimethylamine-N-oxide (LDAO) from Anatrace, USA; and sodium dodecyl-sulphate (SDS) from Wako, Japan.

Determination of Critical Micellar Concentration (CMC) — 10 mg fluorescein (Wako, Japan) was added to 1 ml detergent solution in the presence of 300 mM NaCl at neutral pH and incubated overnight, followed by fluorescence measurements in a F2500 model fluorimeter (Hitachi, Japan) at room temperature. The fluorescence intensity of fluorescein was recorded with excitation and emission wavelengths set at 386 nm and 342 nm, respectively. The detergent concentration at which fluorescence intensity of fluorescein appeared was regarded as the CMC value.¹

DLS measurements—1% detergent solutions were analyzed with an 802DLS instrument (Viscotek, UK). Measurements were performed in the presence of 300 mM NaCl at room temperature.

Preparation of membrane fractions—Expression of recombinant HR labeled with a hexa-histidine tag at the C-terminus was carried out in *Escherichia coli* strain BL21 (DE3) as previously described.² The genes encoding MsbA and YidC from *Escherichia coli* were cloned into pET26b(+) vectors (Novagen, USA) displaying a hexa-histidine tag at the protein C-terminal region. *Escherichia coli* strain C43 (DE3) was transformed with the expression vector containing the desired protein and grown at 37°C in LB medium supplemented with 50 $\mu\text{g mL}^{-1}$ kanamycin until the OD at 600 nm reached a value of 0.6-0.7. To induce the expression of the proteins, isopropyl β -D-1-thiogalactopyranoside was added to a final concentration of 0.5 mM, and the culture grown for an additional 4 hours at 37°C. Cells were harvested by centrifugation at $7000 \times g$ for 10 min at 4 °C, and suspended in a buffer composed of 50 mM Tris-HCl and 300 mM NaCl at pH 7.0. Cells were broken with a French press instrument (Emulsiflex C-5, Avestin, Canada), followed by centrifugation at $40,000 \times g$ for 30 min at 4°C. The soluble fraction was further centrifuged at $100,000 \times g$ for 1 hour at 4°C to precipitate the membrane fraction. The collected membrane fractions were frozen in liquid nitrogen and stored at -80 °C.

Solubilization of membrane proteins — Membrane fractions of each protein were treated with solutions containing 1% detergent in a buffer consisting of 50 mM Tris-HCl (pH 8.0) and 300 mM NaCl at room temperature for 1 hour. The solubilized proteins were separated from insoluble material by centrifugation at $100,000 \times g$ for 1 hour at 25°C. Aliquots thus obtained were further analyzed by SDS-PAGE and immunoblotting. Identification of membrane proteins was performed using a murine anti-His tag conjugated antibody (Santa Cruz Biotechnology, USA). Images were recorded in a ChemiDoc XRS instrument (BioRad, USA) and the band intensities quantified with the ImageJ software program (NIH, USA). MsbA appeared in two separate bands, corresponding to a monomeric and an oligomeric form. All experiments were performed in duplicate.

UV-Visible Spectroscopy of HR—Membrane fractions containing HR were treated with detergents as above, and their absorbance spectra in the 450-700 nm region recorded with a V660 spectrophotometer (Jasco, Japan) at 25°C.

Supplementary Table 1. Properties of the detergents used in this work.

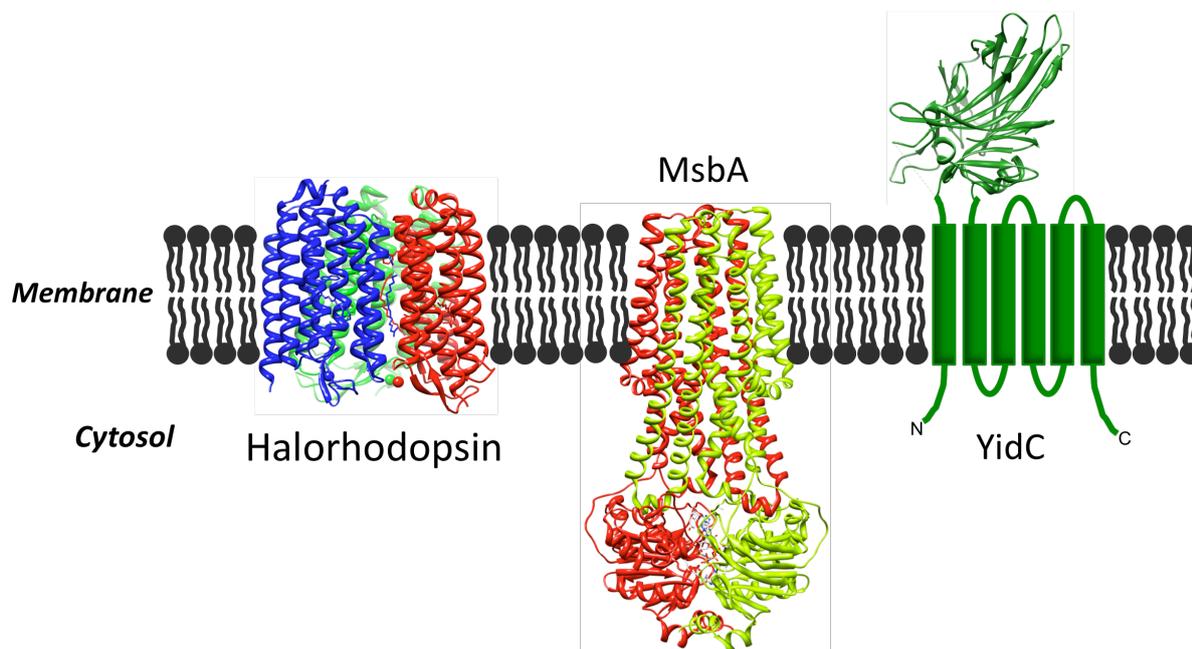
	Mw (Da)	CMC (mM)	Size (nm)	Reference
C12-Gly	239	1.2 ^a	2.6 ^a	This work
C12-Ala	254	0.79 ^a	2.4 ^a	This work
C12-Val	282	0.35 ^a	2.4 ^a	This work
C12-Ile	296	2.0 ^a	2.4 ^a	This work
C12-Phe	330	0.15 ^a	20.7 ^{a, c}	This work
C12-Ser	270	1.9 ^a	2.5 ^a	This work
C12-Glu	298	0.67 ^a	75.0 ^{a, c}	This work
SDS	288	1.2-7.1 ^b	2.7 ^a	[3-5]
DDM	511	0.18	3.4 ^a	[4, 6-9]
OG	292	19-25	-	[4, 7, 9-11]
TX-100	625	0.25	-	[6, 12-14]
LDAO	229	2.2	-	[6, 15-17]
Z3-12	336	1.4-4.0 ^b	-	[4, 18]

^a Experimental data were obtained at 300 mM NaCl.

^b Lower range values correspond to Na⁺ concentrations of 0.1-0.2M; higher range values corresponds to 0-0.05 M Na⁺.

^c A suitable fitting curve could not be acquired.

Supplementary Figure 1



Supplementary Figure 1. Structure of membrane proteins used in this study: Halorhodopsin (GeneBank ID 3702828), MsbA (GeneBank ID 945530), and YidC (GeneBank ID 948214). The models depicted correspond to the crystal structures of the Halorhodopsin trimer (Protein Data Bank accession code 1E12),¹⁹ the MsbA dimer (Protein Data Bank accession code 3B60),²⁰ and the periplasmic domain of Yidc (Protein Data Bank accession code 3BS6).²¹ The arrangement of alpha-helices of YidC embedded in the membrane is based on the known topology of its transmembrane region.²² Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR-01081).²³

Supplementary References.

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