AUTHOR CONTRIBUTIONS

P.S.C. designed the MNG amphiphiles, with contributions from S.G.F.R, B.K. and S.H.G.. P.S.C. synthesized the amphiphiles. P.S.C., S.G.F.R., R.R., K.G., R.C., M.A.G., A.C.K., S.N., Y.P. and D.P. designed and performed the research and interpreted the data. C.J.L., D.D., B.G.F., L.G., U.G., J.-L.P., B.B., B.K. and S.H.G. contributed to experimental design and data interpretation. P.S.C. and S.H.G. wrote the manuscript, with oversight from S.G.F.R., R.R., K.G., R.C., M.A.G., A.C.K., S.N., C.J.L., Y.P., D.D., J.-L.P., D.P., B.G.F., L.G., U.K., B.B., B.K..

COMPETING INTERESTS STATEMENT

P.S.C, S.G.F.R., B.K. and S.H.G. are co-inventors on a patent application that covers the MNG amphiphiles.

Online Methods

Detergents and amphiphiles We purchased conventional detergents (DM, UDM, DDM, TDM, LDAO, SDS, and OG) from Anatrace. We obtained the starting materials and reagents used for preparation of MNG amphiphiles from Sigma-Aldrich. We used all of these agents without purification. Details for the syntheses of MNG amphiphiles can be found in the **Supplementary Note**.

β_2AR stabilization and solubilization studies. The β_2AR-T4L was expressed in Sf9 insect cells, solubilized and purified in DDM as previously described[16]. Briefly, the receptor was purified by M1 FLAG antibody affinity chromatography prior to and following an alprenolol-Sepharose chromatography step. Carazolol was bound to the receptor on the second M1 resin following extensive washing in buffer (0.1% DDM, 100 mM NaCl, 20 mM HEPES, pH 7.5) containing 30 μM carazolol. The eluted and carazolol-bound receptor was dialyzed against buffer containing 1 μM carazolol to reduce free carazolol concentration. The β_2AR-T4L was spin concentrated to 7 mg/ml (~140 μM) using a 100 kDa molecular weight cut-off Vivaspin (Vivascience) concentrator. For stability measurements the carazolol-bound receptor was diluted below the CMC for DDM by adding 3 μL of the concentrated receptor in a quartz cuvette containing 600 μL buffer (100 mM NaCl, 20 mM HEPES, pH 7.5) with detergents and amphiphiles at various concentrations ranging from 1.5 × to 250 × CMC). The cuvette was placed in a Spex FluoroMax-3 spectrofluorometer (Jobin Yvon Inc.) under Peltier temperature control. Fluorescence emission from carazolol was obtained following 5 min incubations from 25 to 85 °C in twelve continuous 5 °C increments. Excitation was set at 325 nm, and emission was measured from 335 to 400 nm with an integration time of 0.3 s nm^{-1} using a bandpass of 1 nm for both excitation and emission. The 341:356 nm peak ratio was calculated and graphed using Microsoft Excel and GraphPad Prism software. For solubilization study from cell membranes Sf9 cells were grown to a density of approximately 4×10^6 per mL then infected with baculovirus expressing the wild-type β_2AR (truncated after residue 365). After 48 hours cells were harvested by centrifugation. No

exogenous ligand was present during protein expression. Cells were resuspended and incubated in ice-cold lysis buffer (1 mM EDTA, 10 mM Tris pH 8) containing protease inhibitors (5 μg/ml leupeptin and 320 μg/ml benzamidine) for 5 minutes, then pelleted by centrifugation. The pellet was resuspended in phosphate buffered saline and aliquoted into 1.5 mL tubes so that each tube contained 35 mg of wet pellet after centrifugation and removal of supernatant. Solubilization buffers contained 100 mM NaCl, 20 mM HEPES pH 7.5, 5 μg/ml leupeptin and 320 μg/ml benzamidine, and either 1 wt % or 2 wt % of the indicated detergent. 300 μL of solubilization buffer was added to each pellet, which was resuspended and homogenized by forcing the mixture through a 27 gauge syringe 30 times. Tubes rocked at 4 °C for 1.5 hours, then centrifuged at 16,000 × g to pellet insoluble material. Supernatant was then checked for total protein content by a Bio Rad D_C protein assay calibrated against a BSA standard. Measurements were performed in triplicate and then averaged. Active protein content was determined by incubation of soluble material with antagonist [^{3}H]-dihydroalprenolol at a single saturating concentration (10 nM) for 40 min, then separating bound radioligand from free by a G50 column. All binding reactions were performed in buffer containing 0.1% of the same detergent used in solubilization and G50 columns were likewise equilibrated and eluted in this buffer (100 mM NaCl, 20 mM HEPES pH 7.5, 0.1% detergent). Non-specific binding was determined in the same manner with 10 μM alprenolol in the binding reaction. All binding reactions were performed in triplicate and protein was diluted prior to binding such that bound radioligand never exceeded 10% of the total.

Muscarinic M_3 acetylcholine receptor activity assay. M_3 muscarinic acetylcholine receptor in which the third intracellular loop had been replaced by T4 lysozyme and possessing an N-terminal FLAG epitope tag was expressed in the same manner as described above, but with the addition of 1 μM atropine to culture medium upon infection. Cells were lysed as described. Following centrifugation, the lysed cell pellet from 1 L culture was mixed with 200 mL M3 solubilization buffer (400 mM NaCl, 0.03% cholesterol hemisuccinate, 20 mM HEPES pH 7.5, 0.2% sodium cholate, 1% DDM, 5 μg/ml leupeptin, 320 μg/ml benzamidine). After douncing 20 times with a tight pestle the homogenized material was stirred at 4 °C for one hour. 400 mL Dilution buffer containing 0.1% DDM, 20 mM HEPES pH 7.5, 30 mM NaCl, 3 mM $CaCl_2$, 5 μg/ml leupeptin and 320 μg/ml benzamidine was added with stirring and the mix was then centrifuged to remove insoluble material. The resulting supernatant was split in half and flowed over FLAG affinity columns to bind the protein. Protein was then washed with either 50 mL of high salt DDM buffer (0.1% DDM, 20 mM HEPES pH 7.5, 500 mM NaCl, 0.01% CHS, 5 μg/ml leupeptin, 320 μg/ml benzamidine) or exchanged into MNG-3 buffer (0.1% MNG-3, 20 mM HEPES, 500 mM NaCl, < 0.01% CHS, 5 μg/ml leupeptin, 320 μg/ml benzamidine). The exchange was performed over the course of 1.5 hours by increasing MNG-3 and decreasing DDM concentrations in 0.005% increments until the final 0.1% concentration was reached. Columns were then washed with 50 mL of DDM buffer without CHS, or MNG-3 buffer without CHS (with MNG-3 decreased to 0.01%) over the course of 50 min. These and all other wash buffers contained 2 mM $CaCl_2$. Columns were eluted in washing buffer with 5 mM EDTA and no $CaCl_2$ with 0.2 mg/mL FLAG

peptide added. Elution volumes for MNG-3 and DDM were identical. Following elution, binding reactions were performed as described above but using saturating [^{3}H] N-methyl scopolamine (10 nM). Nonspecific binding was measured in the presence of 10 μM atropine. Protein assay was done as described above. All M3 binding reactions and G50 elution with MNG-3 were performed at 0.01% concentration. Binding was measured again after overnight incubation at 4 °C.

MelB thermo-stability assay. Vector pK95 ΔAHB/WT MelB/CH_6 encoding the wild-type MelB with a 6-His tag at the C terminus and *E. coli* DW2-R cells (Δ*melB* and Δ*lacZY*) are gifts from Gérard Leblanc. Overexpression of MelB was performed as described[34]. Cells were harvested, resuspended in a buffer containing 20 mM Tris, pH 7.5, 200 mM NaCl and 10% glycerol, broken by French press, and centrifuged at 20,000 g for 15 min to remove unbroken cells. Membranes were then harvested from the supernatant by ultracentrifugation at 43,000 rpm for 3 hr in the Beckman rotor, Ti 45. The pellets were resuspended in the same buffer, frozen in liquid N_2, and then stored at – 80 °C until use. A protein assay was performed with a BCA kit (Thermo Scientific). For solubilization and thermo-stability assay membrane samples containing MelB at a final protein concentration of 10 mg/mL with a solubilization buffer (20 mM Tris, 200 mM NaCl, 10% glycerol, 20 mM melibiose, pH, 7.5) were incubated with 1.5% (w/v) of a given amphiphile or detergent at 0 °C for 10 min and subsequently placed at a given temperature (0, 45, 55, or 65 °C) for 90 min. Samples were collected and then ultracentrifuged at 355,590 g in a Beckman OptimaTM MAX Ultracentrifuge using a TLA-100 rotor for 45 min at 4 °C. 10 μg protein, from untreated membrane or detergent extracts, and equal volume solutions after ultracentrifugation were analyzed by SDS-12% PAGE and immunoblotted with Penta-His-HRP antibody (Qiagen).

SQR, Cyt *bo*$_3$ and CMP-Sia thermostability assay and SQR size exclusion chromatography (SEC) analysis and functional assay. The thermostability assay method was performed as described[18] with the following minor modifications. CPM (Invitrogen) dye, stored in DMSO (Sigma), was diluted in dye buffer [20 mM Tris (pH 7.5), 150 mM NaCl, 0.03% DDM, 5 mM EDTA]. All the detergents were used at 10 × CMC values in test buffer [20 mM Tris (pH 7.5), 150 mM NaCl]. The test proteins: SQR, Cyt *bo*$_3$, and CMP-Sia (10 mg/ml) were diluted (1:150) in test buffer in Greiner 96-well plates, and 3 μL of diluted CPM dye added to each test condition. The reaction was monitored for 130 min at 40 °C using a microplate spectrofluorometer set at excitation and emission wavelengths of 387 nm and 463 nm respectively. Relative maximum fluorescence was used to calculate percentage of relative folded protein remaining after 130 minutes at 40 °C. Relative unfolding profiles of proteins were plotted against time using GraphPad Prism. For SEC analysis, test protein samples of SQR were diluted (1:100) in SEC buffer [20 mM Tris (pH 7.5), 150 mM NaCl] containing either DDM or MNG-3 at 10 × CMC. Aliquots (500 μL) of the diluted protein were either applied directly onto the column or heated at 40 °C for 120 min prior to loading onto a Superdex 200

column (GE Healthcare) pre-equilibrated in the respective SEC buffers. For the functional assay of SQR, DDM and MNG-3 at 10 × or 50 × CMC were used in activation and assay buffers. SQR was activated in buffer [30 mM K_2PO_4, 0.2 mM EDTA, 10 mM malonate] to remove bound oxaloacetate from the active site. The sample was diluted to 1 mg/ml in activation buffer and then a further 50 times in the same buffer. Enzyme activation was performed at 30 °C for 20 min, aliquots were removed, and then the samples were heated at 40 °C for 60 min and 120 min. The amount of functional SQR was estimated based on the succinate-Q1-DCIP reductase activity of the samples. Functional activity was monitored at 600 nm as a decrease of absorbance of 2,6-dichloroindophenol (DCIP) (extinction coefficient = 21.8 mM^{-1} cm^{-1}) when 50 μM DCIP, 10 mM succinate, varied amounts of coenzyme Q1 (CoQ1) including blank and 0.6 μL of activated and/or heated SQR enzyme samples were added to assay buffers. The slopes of the absorbance curves (up to 20 min) were measured for a range of concentrations of coenzyme Q1 (0 to 30 μM) and converted to initial velocity (V_0) values using the Beer-Lambert law. The V_0 values were plotted against CoQ1 concentrations for each detergent condition to fit the Michaelis-Menten equation. The saturation concentration of CoQ1 was obtained for each condition as 20 μM. The Michaelis-Menten equation was used to calculate catalytic parameters for SQR-catalyzed succinate-Q1-DCIP reductase activity. The specific activity (k_{cat}) for SQR was calculated from 1 mL assay as follows. On addition of the enzyme, the change in absorbance over the initial 3 min of the reaction at 20 μM CoQ1 was converted to rate of the reaction using the Beer Lambert law. Since this rate of the reaction at 20 μM CoQ1 is also the V_{max}, the k_{cat} values were calculated as rate per amount of enzyme.

CMP-Sia solubilization. Expression and solubilization of CMP-Sia was performed as described previously[35]. In brief, the CMP-Sia was expressed as a fusion protein with a C-terminal GFP in FGY217 *Saccharomyces cerevisiae* cells. Membranes, generated as described previously[31] were resuspended in 50 mM Tris–HCl (pH 7.5), 1 mM EDTA, 0.6 M sorbitol and the protein concentration measured. The membranes were adjusted to a concentration of 1 mg/ml and 1 ml aliquots solubilized individually in DDM and MNG-3 at final detergent concentrations of 0.5 wt %, 1.0 wt %, and 2.0 wt % for 1 hr at 4 °C. 100 μl aliquots were removed from each tube and a fluorescence reading taken for each sample before and after ultracentrifugation at 150,000 g for 1 hr to remove insoluble material. The solubilization efficiency (%) is the fluorescent reading of the soluble supernatant /fluorescent reading of the total sample times 100. The remaining soluble fraction for each condition was submitted to Fluorescent-SEC (FSEC) as described previously[36] using a Superose 6 column (GE Healthcare) equilibrated with buffer containing the appropriate agent (DDM or MNG-3).

LeuT solubilization, solubility and functionality assay. The wild type of the leucine transporter (LeuT) from *Aquifex aeolicus* in pET16b (pET16b-LeuT-8His) was expressed in *Escherichia coli* essentially as described[22]. The plasmid was kindly provided by Dr. Eric Gouaux. LeuT was purified from isolated cell membranes solubilized with 1 % DDM, followed by Ni^{2+}-affinity chromatography using

Chelating Sepharose Fast Flow (GE Healthcare), and elution in buffer composed of 20 mM Tris-HCl (pH 8.0), 1 mM NaCl, 199 mM KCl, 0.05% DDM and 300 mM imidazole. For comparison, in the solubilization assay, LeuT was extracted and eluted using MNG-3 in the final concentration of 1% and 0.042 %, respectively. In the solubility and functionality assay, selected LeuT fractions eluted in the presence of 0.05% DDM were pooled, divided into aliquots and diluted in the above-mentioned buffer without DDM, but containing MNG amphiphiles or conventional detergents to the final concentration of 10 × CMC or 0.026 wt % above their CMC values (OG was tested at 2 × CMC). After incubation at the room temperature, samples were centrifuged, and the protein concentration was determined at the indicated time points based on absorbance at 280 nm. Concomitantly, at the corresponding time points, [^{3}H]-Leu binding was assessed by scintillation proximity assay (SPA)[24]. Briefly, each SPA reaction mixture consisted of 6 μL from the respective samples, 33.3 nM [^{3}H]-Leu (PerkinElmer, USA) and copper chelate (His-Tag) YSi beads (GE Healthcare). SPA was performed in the presence or absence of 1×10^{-5} M Leu in 20 mM Tris-HCl (pH 8.0) supplemented with NaCl and the selected amphiphile or detergent to final concentrations of 200 mM and 3.3-fold dilution of the original concentrations, respectively. In the solubilization assay, the initial activity of LeuT was assessed by SPA performed in the presence of 0.05% DDM or 0.042% MNG-3, and Leu (-5-0 M, competition binding). SPA was performed with duplicate determination of all individual data points. Samples were counted in a MicroBeta liquid scintillation counter (PerkinElmer). Normalized results are expressed as mean ± s.e.m..

***R capsulatus* superassembly solubilization and stabilization assay**. Specialized photosynthetic membranes from an engineered strain of *Rhodobacter* (*R.*) *capsulatus*, U43[pUHTM86Bgl], lacking the LHII light-harvesting complex were used as the starting material[26]. The isolated flash frozen membrane aliquots from this strain were thawed, homogenized and equilibrated to 32 °C for 30 min. The amphiphiles/detergents were added at the designated concentration to 1 mL aliquots of the membranes. After incubation with the amphiphiles for 30 min at 32 °C, the solubilized material was separated from the membrane debris in an ultracentrifuge at 315,000 × *g* at 4 °C for 30 min. The supernatant was transferred into a new microcentrifuge tube containing Ni-NTA resin (Qiagen, Inc) and then the tube was incubated and inverted for 1 hr at 4 °C. Once binding was complete, samples were loaded onto resin-retaining spin columns (e.g., emptied His SpinTrap™ columns; GE Healthcare), which were then inserted into a 2 mL microcentrifuge tube. Samples were washed twice with 0.5 mL of amphiphile-containing binding buffer (a pH 7.8 Tris solution containing the amphiphile at 1 × CMC, 0.017 wt % above CMC, 0.2 wt % or 1.0 wt %). Finally, protein was eluted with three 0.2 mL elution buffer aliquots (this buffer was identical to binding buffer with the addition to 1 M of imidazole with same pH). Purified protein was diluted with 0.4 mL of binding buffer to reach the final sample volume to 1 mL. Small aliquots (0.2 mL) of the solutions were transferred to 0.8 mL binding buffer and UV-Vis spectra

of these solutions were measured as a function of time. Degradation of the material could be monitored with the 875 nm/680 nm absorbance ratio, which decreased with time.

Stabilization and crystallization of cytochrome *b_6f*/MNG-3 complex. Purification of the complex cytochrome *b_6f* from *Chlamydomonas reinhardtii*[27] was carried out according to the literature with slight modifications to exchange DDM for MNG-3. Briefly, after solubilization of thylakoid membranes with DDM and a first ion exchange chromatography in 0.4 mM DDM, the protein was loaded on a 1ml Hitrap chelating Sepharose column (GE Healthcare), preloaded with nickel (2+); subsequently, the column was extensively washed with 50 ml of 0.5 mM MNG-3 in 20 mM Tris/HCl pH 8, 250 mM NaCl, and eluted with 300 mM imidazole in the same buffer. The sample was desalted on a Sephadex G25 column with 10 mM Tris/HCl pH 8, 0.5 mM MNG-3, and concentrated with a Vivaspin 500 (100,000 MWCO PES) concentrator (Sartorius) from 5 to 100 μM (i.e. from 0.5 to 10 mg/ml) prior to crystallization. All steps from purification until crystal handling were carried out at a temperature of 4 °C in a cold room. Crystallization trials used the protocol involving vapor diffusion with the hanging drop technique: the protein was mixed with an equal or half volume of reservoir solution devoid of surfactant[27]. The crystallization strategy was adapted from conditions suitable for crystallization with DDM. Crystals were flash frozen in liquid nitrogen, and diffraction experiments performed at the synchrotron SOLEIL (Saint-Aubin) on the beamline Proxima 1. Diffraction data were integrated with XDS[37] and analyzed with the CCP4 package[38]. The model was refined with PHENIX[39] and BUSTER[40]. Plastoquinone/plastocyanine oxidoreductase activity measurements were performed according to the literature[41].

Cell-Free expression of proteins in the presence of amphiphiles. Transcription and translation of genes cloned into the pEU-HIS cell-free expression vector was performed as previously reported[42]. Briefly, for each sample a 50 μL transcription reaction, containing 5 μg plasmid DNA, 80 mM HEPES, pH 7.5, 16 mM magnesium acetate, 2 mM spermidine, 10 mM dithiothreitol, 2.5 mM of each nucleotide triphosphate, 25 U RNasin (Promega), 30 U Sp6 RNA polymerase, was incubated at 37 °C for 3 hr. The resultant mRNA was purified by ethanol precipitation and air-dried. The pellet was re-suspended in 50 μL of the translation reaction solution which contained dialysis buffer (30 mM HEPES, pH 7.8, 100 mM potassium acetate, 2.7 mM magnesium acetate, 1.2 mM ATP, 0.25 mM GTP, 16 mM creatine phosphate, 0.4 mM spermidine, and 0.3 mM of each amino acid) supplemented with 35.6 μg creatine kinase, 24 U of RNAsin and 15 μL of WEPRO 2240 (Cell Free Sciences). The reaction was transferred into a 12 kDa MWCO dialysis cup (Cosmo Bio) suspended in a reservoir of dialysis buffer and incubated for 16 hr at 26 °C. Detergents and MNG amphiphiles were added when needed at 0.1 wt % or 0.2 wt % to both the translation reaction and dialysis buffer reservoir. After overnight incubation, the 50 μL translation reaction solution was transferred to a 1.7 mL centrifuge tube and spun at 15,000 rpm in an Allegra 21R centrifuge (Beckman Coulter) and F2402H rotor at 20 °C

for 10 min. The supernatant was removed and added to an equal volume of 2 × Sample Buffer, while the pellet was re-suspended in 100 μL 1 × sample buffer. After boiling for 5 min, the samples were loaded onto a SDS-PAGE gel, electrophoresed and visualized by Coomassie staining.