Supplementary information

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Table S1A Sieving coefficients, pressures, and volume reduction factors (VRF) for DM at different concentrations in flat versus commercial filter systems

	Flat Membrane				Commercial Membrane			
% DM	Concentration Factor	Sieving Coefficient	Pressure (psi)	Material Balance (%)	Concentration Factor	Sieving Coefficient	Pressure (psi)	Material Balance (%)
0.01087	2.7	1.0	30	0.0	2.7	1.0	22	0.0
0.0174	2.8	1.0	22	0.0	2.7	1.0	21	0.0
0.087	2.7	0.70	21	12.6	2.7	0.41	21	11.2
0.435	2.8	0.85	22	-0.5	2.8	0.15	21	-17.5
0.696	2.7	0.91	29	-3.8	2.8	0.15	22	-12.5

Table S1B Sieving coefficients, pressures, and volume reduction factors (VRF) for OG at different concentrations in flat versus commercial filter systems

	Flat Membrane				Commercial Membrane			
% OG	Concentration Factor	Sieving Coefficient	Pressure (psi)	Material Balance (%)	Concentration Factor	Sieving Coefficient	Pressure (psi)	Material Balance (%)
0.175	2.5	1.0	20	0.0	2.4	1.0	23	0.0
0.35	2.5	1.0	20	0.0	2.5	1.0	23	0.0
0.7	2.5	0.87	20	1.2	2.4	0.83	23	4.0
2.8	2.5	0.90	21	4.2	2.4	0.70	23	2.5
5.6	2.5	0.89	21	1.5	2.4	0.63	23	2.6

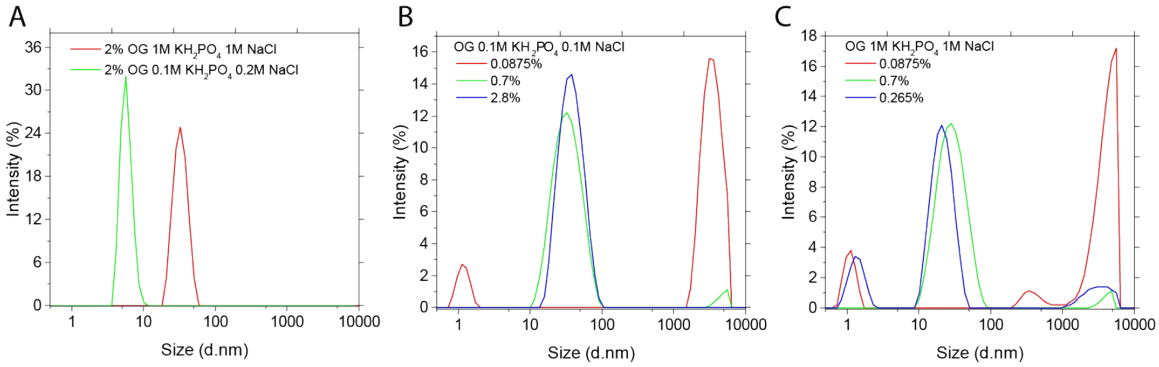


Figure S1. Influence of salt concentrations on OG micelle properties. A. The micelle size of 2 % OG in 1 M salt is significantly larger compared to that at 0.1 M salt; Z-average size and PDI being 33 nm and 0.15 for 1 M compared to 9.5 nm and 0.03 for 0.1 M salt, respectively, as seen from dynamic light scattering measurements. **B and C.** The transition from unimeric to micellar structure for OG occurs at a lower detergent concentration of 0.265% at higher salt concentration (**B**) compared to 0.7% OG in the low salinity buffer (**A**). This is consistent with the salting out effect on alkyl chains leading to lowering of CMC.

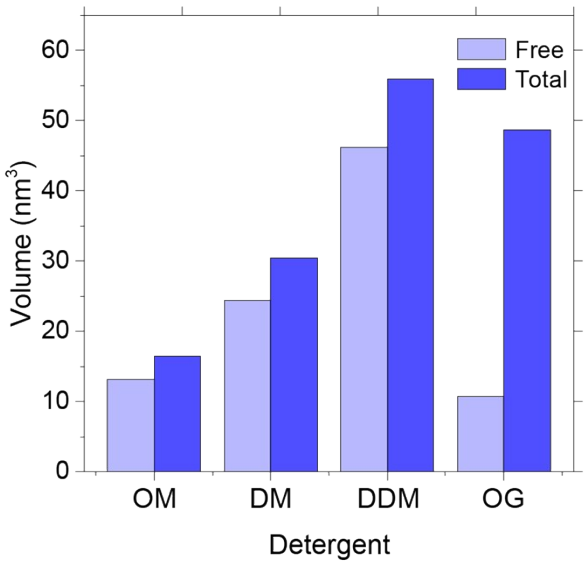


Figure S2. Summarized properties of detergent micelles of varying chain length and head groups. Calculated values of total and accessible/free micellar volume based on micellar[1, 2] dimensions increase with increasing alkyl chain length.

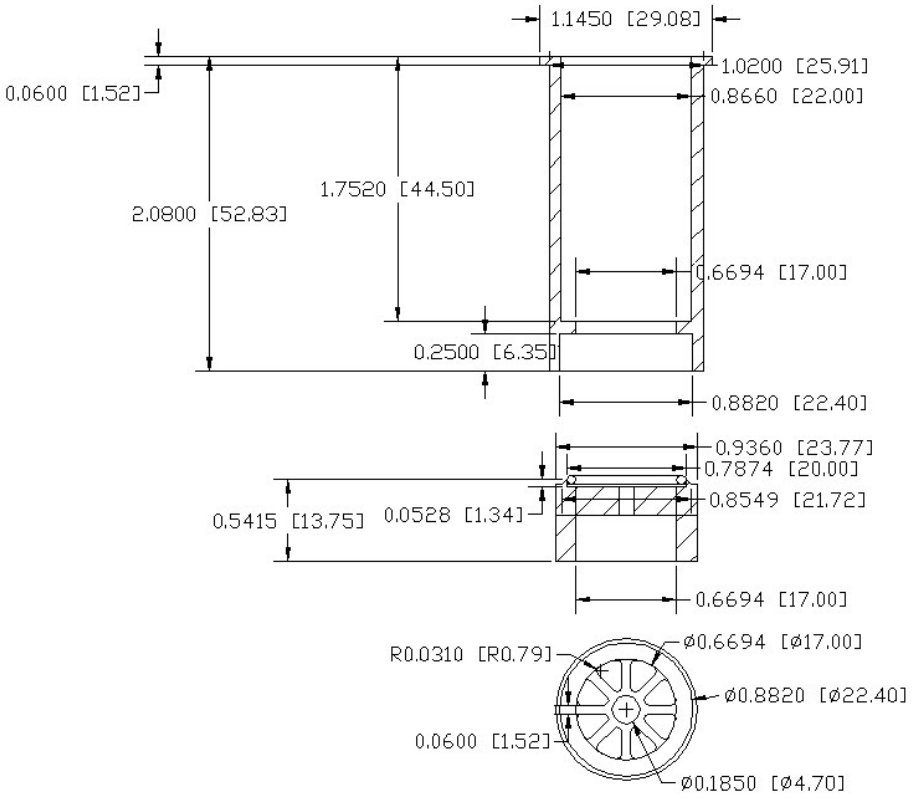


Figure S3. Detailed design of flat-bottomed membrane protein concentration filter; dimensions in inches [mm].

Determination of the sieving characteristics of the commercial versus flat membrane centrifugal filter.

Experiments were conducted with dextran standards with molecular weights of 11, 21, 25, 33 and 41 kDa in 50 mM PBS buffer of composition of 0.017 M NaOH, 0.03 M KH_2PO_4, 0.03 M Na_2HPO_4, pH 7. Each standard was used at a concentration of 0.25 g/L giving an overall dextran concentration of 1.25 g/L. Experiments were conducted with 4 ml sample volume in the flat and commercial centrifugal systems – waste was collected after a one-minute 1500 rpm spin followed by actual sample collection after an additional one-minute spin at 3000 rpm. The stirred cell experiments were conducted with 10 ml

solutions using a membrane from the same lot as used for the flat centrifugal filters using a previously reported procedure.[3] Up to 500 µL of waste was collected to wash out the dead volume before collecting 500 µL of sample at constant pressure. The water permeability of all three systems was also measured before and after the dextran filtration experiments. Differences in water permeability were less than 20 % indicating that there was no irreversible fouling of the membrane during the dextran filtration and that the membranes in all three systems had similar hydraulic permeability. Dextran concentrations in the retentate and permeate samples were determined using a Superdex 200 G/L (GE Healthcare, Uppsala, Sweden) at a flow rate of 0.5 ml/min. The column was equilibrated with phosphate buffer for 2 h. Next, 80 µL of dextran samples were injected by an autosampler with the dextran concentrations evaluated by an Agilent 1100 refractive index detector. A calibration curve was constructed using dextran standards of known molecular weights (American Polymer Standards Corporation, Mentor, OH). Data were analyzed using Agilent Chestation software (Agilent Technologies, Santa Clara, CA).[4, 5]

The dextran sieving coefficients evaluated in the stirred-cell membrane system are similar to the values in the commercial filter even though the average filtrate flux in the stirred cell (19 µm/s) was less than half that in the Amicon Ultra-4 (45 µm/s), which is consistent with the very high mass transfer coefficient in the commercial centrifugal filter. The dextran sieving coefficients in the flat membrane system are much higher than those in the stirred cell and Amicon Ultra-4 device, especially for the higher molecular weight dextrans. This finding is consistent with the much greater extent of CP in the flat system where there is no agitation. As further verification, we evaluated the dextran sieving coefficients for the Ultracel 30 kD membrane in the stirred cell but in absence of any stirring, with the sieving profile being very similar to that obtained in our newly designed flat membrane device (**Figure S4**). Thus we conclude that the differences in dextran (and detergent) transmission in the different experimental systems are due to differences in CP rather than due to differences in the membranes (all of which are rated as 30 kDa nominal molecular weight cut-off).

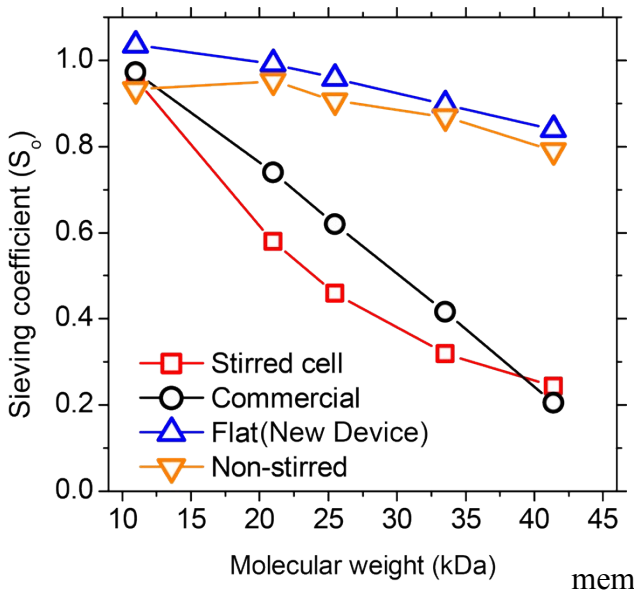


Figure S4. Molecular weight cut-off determination under conditions of low concentration polarization (CP) reveal similar sieving properties for the stirred cell (red) and centrifugal (black) devices (using Ultracel 30 kD membrane from Millipore). The high shear induced by natural convection in the commercial device (at time-averaged flux of 45 µm/s), and by stirring at 700 rpm in the stirred cell (at time-averaged flux of 19 µm/s) results in low CP and thus similar retention profiles. However, for 30kDa membranes from the same lot drastically higher sieving coefficients are observed under high CP conditions in the new device (blue, time-averaged flux of 63 µm/s) and in the stirred cell in the absence of stirring (orange, time-averaged flux of 82 µm/s). Thus the observed difference in detergent sieving of the flat and commercial systems is due to operating conditions that induce concentration polarization rather than differences in intrinsic membrane properties.

Mass transfer calculations for DM and OG

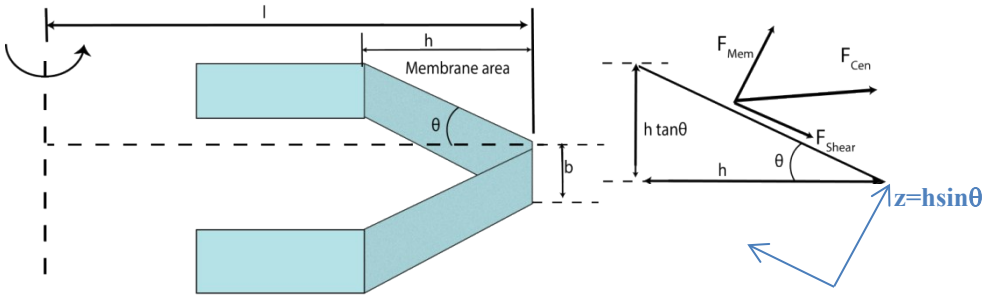


Figure S5. Detailed geometry of commercial filter for mass transfer coefficient calculation

Unlike the flat membrane centrifugal system, the tilted orientation of the commercial filters combined with the high speeds used during centrifugal ultrafiltration lead to the development of significant natural convection (buoyancy) induced flows. These vortices arise from the density gradient in the boundary layer near the membrane as the detergent

concentration increases near the membrane surface. This natural/free convection current minimizes concentration polarization in the commercial centrifugal system. The relative importance of buoyancy forces arising from the concentration difference versus those due to forced convection can be evaluated from the ratio of the Grashof number (Gr) to the square of the Reynolds number (Re) as discussed by Youm *et al.* [6],

$$Gr = \frac{(\omega^2 r \cos\theta)\alpha\beta(x)^4}{\upsilon^2}$$

$$\text{Re} = \frac{D_h v_x}{\upsilon}$$

where

$z = h\sin\theta$

$x = h\cos\theta$

$\alpha = \frac{1\partial\rho}{\rho\partial C} = 0.225\ g/cm_3$ for DM and 0.142 g/cm^3 for OG[7, 8]

$$\beta = \frac{1\partial C}{\rho\partial z} = 1 \times \frac{C_W - C_B}{\Delta X} = \frac{C_W - C_B}{Average(x_{before,} x_{after})}$$

$$C_B \approx \frac{C_{Permeate}}{S_0}$$

$$C_W \approx \frac{C_{Permeate}}{S_a}$$

$S_O = observed\ sieiving\ coefficient$ at time t=0

$S_a = actual\ sieiving\ coefficient$ which was evaluated from the sieving coefficient based on analysis of bulk mass tansfer

$$D_h, equivalent\ hydrodynamic\ diameter = 4 \times \frac{Area}{Wetted\ perimeter} = 4 \times \frac{2h\ tan\theta\ b}{(4h\ tan\theta + 2\ b)}$$

$v_x = \frac{dh}{dt}\cos\theta$, where $\frac{dh}{dt}, i.e., rate\ of\ change\ of\ liquid\ height$ is the velocity along the membrane obtained from retentate volume collected over different spin times divided by the area of membrane in contact with retentate at each time (**Figure S3**).

The Sherwood number for forced (Sh_F) and mixed (Sh_M) convection were determined as[6]

$$Sh_F = 0.303 Re^{0.465} Sc^{1/3}$$

$$\frac{Sh_M}{Sh_F} = [1 + 1.24 \times (\frac{Gr}{Sc^{0.143} Re^2})^{3/4}]^{1/3}$$

Mass transfer coefficient was determined from the correlation above,

$$Sh = \frac{k\, D_h}{D}$$

Thus $\frac{Gr}{Re^2}$ is far greater than the minimum value of 3 in centrifugal filter with angled walls[6] indicating significant role of free convection in generating vortices on the membrane surface with 2 orders of magnitude higher k_M higher than k_F.

The shear stress can be calculated from the Sherwood number using the correlation developed by Reiss and Hanratty [9-12]

$$\tau = 1.9 \frac{\mu D}{D_h} {Sh_M}^3$$

Thus τ=16 kPa and shear rate, γ=1.6 × 10^4 s^{-1}.

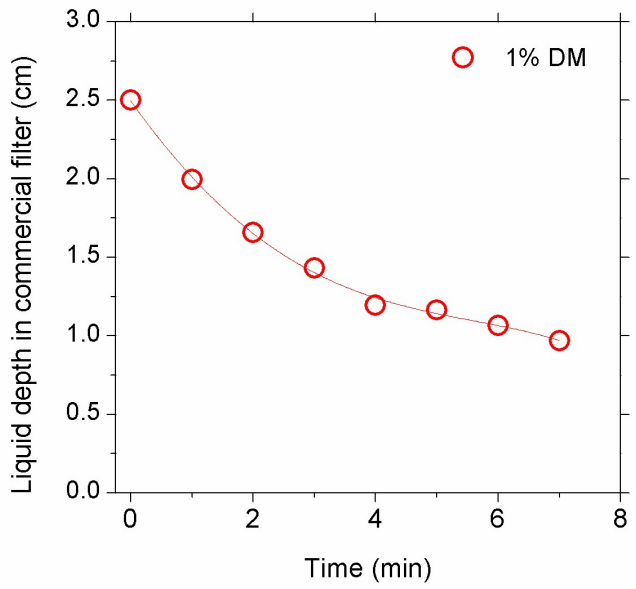


Figure S6. Flux determination for 1% DM in commercial centrifugal filter. Flux was calculated from change in permeate volume collected over time with respect to active membrane area for the commercial filter. Each flux point was obtained from individual experiments by changing the duration of spin from 1 minute to 7 minutes and differentiating the polynomial fit of height versus time.

Table S2. Sieving coefficients, pressures, and volume reduction factors (VRF) for 0.27% OG in different buffer systems for both flat and commercial modules at 4500 rpm.

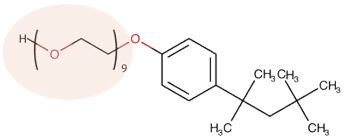
	Flat membrane				Commercial membrane			
Salt concentration (M)	S_o	Pressure (psi)	Concentration Factor	Duration (sec)	S_o	Pressure (psi)	Concentration Factor	Duration (sec)
0	1.0	10	2.4	40	1.0	22	2.4	40
0.1	1.0	9	2.5	40	1.0	22	2.5	40
1	0.59	10	2.4	80	0.49	23	2.4	150

HLB Calculation[13, 14]

1. Davies' method

$HLB = 7 + \Sigma(hydrophilic\ group\ numbers) - \Sigma(lipophilic\ group\ numbers)$

Triton x100



Group	Group number		
Hydroxyl (free)	1.9	x 1	1.9
-(CH2-CH2-O)-	0.33	x 9 or 10	2.97 or 3.3
Phenyl	-1.662	x 1	-1.662
-CH-,-CH2-,CH3-,=CH-	-0.475	x 6	-2.85
			+7
			= 7.358 or 7.688

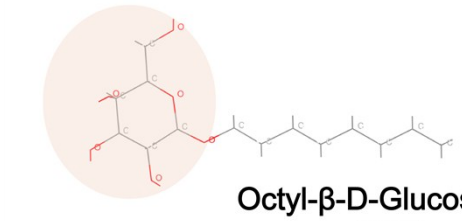
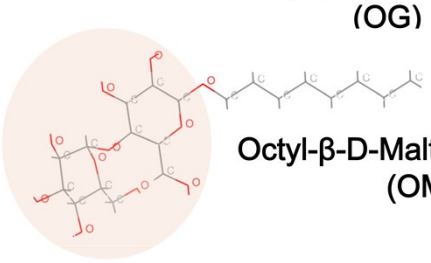
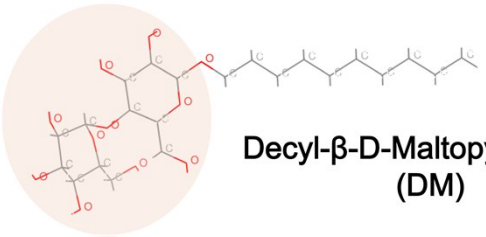
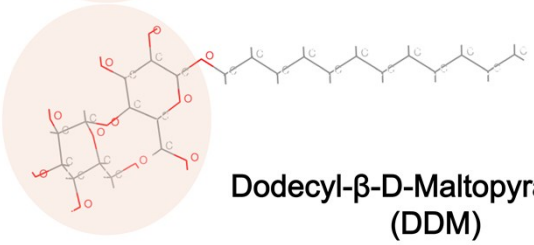
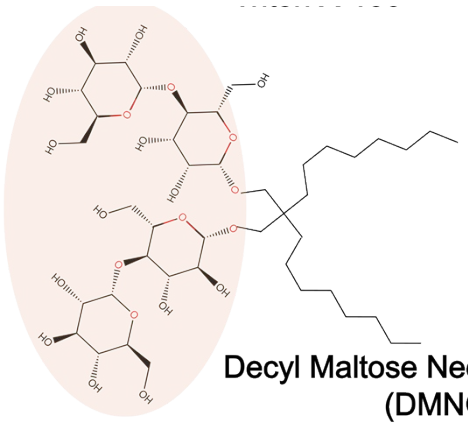
2. Griffin Method (OG,OM,DM,DDM)

$$HLB = 20\frac{M_h}{M}$$

where M_h is the molecular mass of the hydrophilic portion of the molecule, and M is the molecular mass of the whole molecule, giving a result on a scale of 0 to 20. An HLB value of 0 corresponds to a completely lipophilic/hydrophobic molecule, and a value of 20 corresponds to a completely hydrophilic/lipophobic molecule.

Using Griffin and Davies method, HLB of 6 detergents were calculated.

	Paper	Davies + Griffin (Calculated)
OG	12.4	12.24
Triton x100	-	7.358 (Davies)
DDM	14.4	13.37
DM	15.4	14.14
OM	16.3	15.02
DMNG	-	14.38

Detergent	HLB
 Octyl-β-D-Glucoside (OG)	OG $\frac{mass\ of\ hydrophilic\ portion}{molecular\ mass} = \frac{mass\ of\ glucose\ ring}{molecular\ mass} = \frac{179}{29}$
 Octyl-β-D-Maltopyranoside (OM)	OM $\frac{mass\ of\ hydrophilic\ portion}{molecular\ mass} = \frac{mass\ of\ maltose\ ring}{molecular\ mass} = \frac{341}{454}$
 Decyl-β-D-Maltopyranoside (DM)	DM $\frac{mass\ of\ hydrophilic\ portion}{molecular\ mass} = \frac{mass\ of\ maltose\ ring}{molecular\ mass} = \frac{341}{510}$
 Dodecyl-β-D-Maltopyranoside (DDM)	DDM $\frac{mass\ of\ hydrophilic\ portion}{molecular\ mass} = \frac{mass\ of\ maltose\ ring}{molecular\ mass} = \frac{341}{510}$
 Decyl Maltose Neopentyl Glycol (DMNG)	DMNG $\frac{mass\ of\ hydrophilic\ portion}{molecular\ mass} = \frac{mass\ of\ maltose\ ring}{molecular\ mass} = \frac{341}{510}$

Membrane protein purification

Halorhodopsin (pHR)

Expression and purification of pHR was adapted from the protocol of Sato, *et al.*[15] We expressed *Natromonas pharaonis* pHR by cloning an *E. coli* codon optimized gene with a C- terminal 6x histidine tag ([1]MAETLP.....TPADD[291]**LE**HHHHHH]). Using the T7 polymerase/pCDF Duet-1 enhanced co-expression system for *E. coli* BL21 (DE3) (Novagen), we obtained high expression of HR-LE-his from plasmid pCB8a.[15] The cells were grown at 37 ^{o}C in 2× YT medium supplemented with 50 μg/ml spectinomycin (Gold Biotechnology). At an optical density of OD_{600} = 0.6, 5 μg of all-*trans*-retinal was added per liter of culture and the cells were grown for an additional 4 hours before harvesting. The cell pellet was resuspended in lysis buffer (50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$) and lysed in a microfluidizer (M 110EH, Microfluidics Corporation). Cell debris was removed via low speed centrifugation (2000 × *g*) and the resulting supernatant was spun at 208,000 x g for 1 h at 4 ^{o}C to pellet the plasma membranes. The membrane pellets were suspended in 50 mM MES, pH 6, 300 mM NaCl, 1.5% DM (n-decyl-β-D maltopyranoside) (GLYCON Biochemicals, Germany) and solubilized overnight at 4 ^{o}C. Unsolubilized membranes were removed by ultracentrifugation at 208,000 x g for 1 hour at 4 ^{o}C. The supernatant fraction was incubated with Ni-NTA resin and the resin washed to remove non-specifically bound proteins [50 mM MES, pH 6.0, 300 mM NaCl, 45 mM imidazole, 0.2% DM]. The protein was eluted [50 mM MES, pH 6.0, 300 mM NaCl, 1 M imidazole, 0.2% DM] and then dialyzed for 48 hours against 0.2 % DM, 10 mM MES, 40 mM KCl, pH 6.0 before filtration experiments.

KR2

E. *coli* C41(DE3) strain expressing KR2 with six histidines at the C terminus was provided by Prof. Kandori, Nagoya Institute of Technology. The cells were grown at 37 ^{o}C in 2× YT medium supplemented with 50 μg/ml ampicillin. At an optical density of OD_{600} = 0.6, 1 mM IPTG and 10 μM all-*trans* retinal (dissolved in 200 proof ethanol) were added per liter of culture and the cells were grown for an additional 4 hours before harvesting. The procedures for solubilizing KR2 membrane fractions were the same as

that for pHR except the samples were split into three fractions to be treated with OM, DM and DDM. The conventional purification was carried out using the same procedure as that for pHR. The final extracted and purified protein concentration were determined by absorbance at 532 nm using the characteristic molar extinction coefficient of 51000 M^{-1} cm^{-1}.[16]

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