Li *et al.* 10.1073/pnas.0607502103.

Supporting Information

Files in this Data Supplement:

Supporting Figure 5
Supporting Figure 6
Supporting Figure 7
Supporting Table 1
Supporting Table 2
Supporting Table 3
Supporting Text
Supporting Figure 8
Supporting Figure 9
Supporting Figure 10
Supporting Figure 11
Supporting Table 4

Supporting Figure 5

Fig. 5. Graph depicting the cycling of the flow rates of the reagent and buffer streams to vary the concentration of the reagent in each plug. As the reagent stream flow rate is increased in 1.7-nl/s steps, the buffer stream is cycled out of phase to correspondingly decrease in 1.7-nl/s steps. This method keeps the total flow rate of the aqueous streams constant while changing the reagent concentration in each plug.

Supporting Figure 6

Fig. 6. Relative plug size (plug length versus Teflon capillary inner diameter) as a function of water fraction (aqueous flow rate versus total flow rate). Three different viscosity solutions (~1 mPa s to ~6 mPa s to ~36 mPa s) were tested, and all three showed linear dependence between relative plug sizes with changing water fraction.

Supporting Figure 7

Fig. 7. Standard dilution curves for (*a*) fluorscein in 10 mM Tris (pH 7.8) and 0.05% (wt/vol) LDAO; (*b*) rhodamine B in 25% (wt/vol) PEG 8000 in 10 mM Tris (pH 7.8) and 0.05% (wt/vol) LDAO; (*c*) PSS in 50% (wt/wt) glycerol in 10 mM Tris (pH 7.8) and 0.05% (wt/vol) LDAO.

Supporting Figure 8

Fig. 8. Channel design for the hybrid device. (*a*) The AutoCAD mask design used to generate silicon masters is shown. The AutoCAD file is also provided as part of the supporting information. The dotted square is drawn around one of the channel designs, and a schematic of the channel with dimensions is shown in *b*, where dimensions of the different junctions of the device are provided.

Supporting Figure 9

Fig. 9. Dispensing submicroliter volumes of membrane protein solutions with no losses. (*a*) A schematic of protein solution in Teflon capillary filled with fluorinated carrier fluid and connected to a syringe. (*b*) A microphotograph of 0.8 μl of rhodopsin with 0.1% LDAO as the detergent in Teflon capillary (350 μm, i.d.) shown next to a 10-μl syringe.

Supporting Figure 10

Fig. 10. ΣA-weighted $2F_o-F_c$ electron-density map depicted at 1.4 σ level around the selected regions of the RC structure refined at 1.96-Å resolution. (*a*) HEME C, residue 401 C. (*b*) Ubiquinone-1, second binding site, residue 503 L. (*c*) 1,2,3-heptanetriol, residue 705 U, bound to subunit H residues 124-126.

Supporting Figure 11

Fig. 11. Flow chart illustrating the procedures of the hybrid method.

Table 1. Solubility of solutes in fluorinated amines

Chemical name	F.w.	Solubility of solutes in fluorinated amines, mol/liter)*	
		FC40	FC70
LDAO	229.4	None	None
OG	292.4	None	None
DDM	510.6	None	None
Deriphat 160C	351.5	None	None
1,2,3 heptanetriol	148.2	None	None
1,2,3-hexanetriol	134.2	None	None
Myo-inositol	180.2	None	None
Ethylene glycol	62.07	None	None
2R,3R butanediol	90.12	3.5 ?10^{-4}	3.90 ?10^{-4}
Ethanol	46.06	8.50 ?10^{-3}	4.70 ?10^{-3}

Isopropanol	60.10	7.05 ?10^{-3}	7.60 ?10^{-3}
MPD	118.2	6.50 ?10^{-3}	2.30 ?10^{-3}

*None indicates the solute was not detectable by NMR, suggesting a solubility well below 10^{-4} mol/liter.

Table 2. Screening Conditions for Crystallizing Porin from *R. Capsulatus*

Number	Precipitant	Salt	Buffer/pH
3	10% wt/vol PEG 4000	0.2 M ammonium sulfate	0.1 M sodium acetate trihydrate pH 4.6
6	1.0 M ammonium sulfate		0.1 M sodium acetate trihydrate pH 4.6
10	12% wt/vol PEG 6000	0.1 M sodium chloride	0.1 M sodium acetate trihydrate pH 4.6
17	12% wt/vol PEG 4000	0.1 M sodium chloride	0.1 M tri-sodium citrate dihydrate pH 5.6
21	4% vt/vol PEG 400	0.1 M lithium sulfate monohydrate	0.1 M tri-sodium citrate dihydrate pH 5.6
31	18% vol/vol PEG 400	0.1 M magnesium chloride hexahydrate	0.1 M Hepes-sodium pH 7.5
39	0.1 M potassium sodium tartrate	0.1 M lithium sulfate monohydrate	0.1 M Hepes-sodium pH 7.5
*	70.6% PEG 550MME	3 M LiCl	150 mM Tris pH 8.0

Condition numbers are from Membfac screening kit from Hampton Research.

*Condition prepared from known condition (6).

Table 3. Screening conditions for crystallizing RC condition numbers are from a custom-made screening kit from deCode Biostructures

Number	Buffer/pH	Precipitant	Salt
1	125.000 mM NaAc/acetic acid pH 4.5	22.500% wt/vol PEG 6000	187.500 mM NaCl
2	125.000 mM NaAc/acetic acid pH 4.5	22.500% wt/vol PEG 6000	187.500 mM $MgCl_2$
3	125.000 mM tri-sodium citrate/citric acid pH 5.5	22.500% wt/vol PEG 6000	187.500 mM lithium sulfate
4	125.000 mM ADA/NaOH pH 6.5	22.500% wt/vol PEG 6000	187.500 mM $MgCl_2$
5	125.000 mM Tris稿 Cl/HCl pH 8.5	22.500% wt/vol PEG 6000	187.500 mM di-ammonium phosphate
6	125.000 mM NaAc/acetic acid pH 4.5	22.500% wt/vol PEG 4000	187.500 mM $ZnAc_2$
7	125.000 mM NaAc/acetic acid pH 4.5	18.750% wt/vol PEG 4000	375.000 mM di-ammonium sulfate
8	125.000 mM NaAc/acetic acid pH 4.5	22.500% wt/vol PEG 4000	
9	125.000 mM Tri-sodium citrate/citric acid pH 5.5	22.500% wt/vol PEG 4000	187.500 mM lithium sulfate
10	125.000 mM Tri-sodium citrate/citric acid	22.500% wt/vol PEG 4000	187.500 mM NaCl

	pH 5.5		
13	125.000 mM Hepes sodium salt/HCl pH 7.5	18.750% wt/vol PEG 4000	187.500 mM ammonium sulfate
15	125.000 mM Tri-sodium citrate/citric acid pH 5.5	33.750% vol/vol PEG 400	187.500 mM NaCl
18	125.000 mM Tris HCl/HCl pH 8.5		2500.000 mM ammonium sulfate
29	125.000 mM NaAc/acetic acid pH 4.5		1875.000 mM magnesium sulfate
30	125.000 mM Tri-sodium citrate/citric acid pH 5.5		1875.000 mM magnesium sulfate
31	125.000 mM ADA/NaOH pH 6.5		187.500 mM lithium sulfate 1875.000 mM magnesium sulfate
*	1.0 M di-sodium phosphate pH 6.0		4 M ammonium sulfate
?	50mM Tris pH 7.8		3.6 M ammonium sulfate

*Condition prepared from known condition (4, 5).

?/SUP>Modified condition at higher pH.

Table 4. Data collection and refinement statistics

Data collection	Microfluidic hybrid method
Space group	$P4_32_12$
Cell dimensions	
a, *b*, *c*, Å	220.4, 220.4, 113.0
α, β, γ, ?	90, 90, 90

Resolution, Å	50–1.96 (2.0–1.96)
R_{merge}	0.082 (0.48)
$I/\sigma I$	17.5 (2.3)
Completeness, %	94.5 (67.4)
Redundancy	5.9 (4.1)
Refinement	
Resolution, Å	20–1.96
No. of reflections	189,189
R_{work}/R_{free}	0.172/0.186
No. of atoms	
Protein	9,293
Ligand	790
Fe/Mg/SO_4 ions	5/4/13
Water	771
B-factors	
Protein	24.5
Ligand	27.1
Fe/Mg/SO_4 ions	20.1/15.9/43.4
Water	33.1
rmsds	
Bond lengths, Å	0.008
Bond angles, ?	1.330

Supporting Text

Chemicals and Reagents. All solvents and salts used in buffers purchased from commercial sources were used as received unless otherwise stated. Tris(hydroxymethyl)aminomethane (Tris base) and fluorscein (disodium salt) were obtained from Fisher Scientific. Rhodamine B and PSS (1,3,6,8-pyrenetetrasulfonic acid, tetra sodium) were obtained from Acros Organics. PEG 8000 was obtained from Fluka Biochemika. Lauryldimethylamine oxide (LDAO), *n*-dodecyl-β-D-maltopyranoside, and octyl-β-D-glucopyranoside detergents were obtained from Anatrace. PEG 550MME and paraffin oil were obtained from Hampton Research. (tridecafluoro-1,1,2,2,-tetrahydrooctyl)-1 trichlorosilane was obtained from United Chemical Technologies. Poly(dimethylsiloxane) (PDMS, Sylgard 184 Silicone Elastomer kit) was obtained from Dow Corning. FC-40 (a mixture of perfluoro-tri-*n*-butylamine and perfluoro-di-nbutylmethylamine) and FC-70 (perfluorotripentylamine) were obtained from 3M. The absorption inks were blue, red, and green ink from Conway Stewart. Teflon capillaries (OD 250 μm, i.d. 200 μm) were received from Zeus. Thirty-gauge Teflon tubing was obtained from Weico Wire & Cable. The standard wall glass tubing was obtained from Chemglass. Gastight syringes were obtained from Hamilton Company.

PDMS Device Fabrication. PDMS was used to fabricate all microfluidic devices. Microchannels with rectangular cross-sections were fabricated with rapid prototyping (1). The hybrid microfluidic devices consisted of PDMS microchannels (cross-section: 250 μm by 250 μm) with three tapered inlets (100 μm by 250 μm) for aqueous phases and one orthogonal inlet for carrier fluid to form nanoliter plugs. The channel walls were functionalized with (tridecafluoro1,1,2,2-tetrahydrooctyl)-1-trichlorosilane to render them hydrophobic and fluorophilic (2).

Preparing Preformed Arrays. All input arrays were aspirated by using a manual microsyringe pump with glass syringes 10 or 50 μl in volume (3). Arrays were aspirated into Teflon capillary (O.D. 250 μm, i.d. 200 μm, length ~10 cm), which was sealed to 30-gauge Teflon tubing by using capillary wax. Thirty-gauge Teflon tubing was connected to the syringe with a removable 27-gauge needle. The syringe and Teflon tubing were filled with FC-40. The input array was generated by aspirating ~120-140 nl of a reagent in alternation with ~40 nl of air as the gas spacer. The aspirated cartridge was interfaced with the hybrid devices and sealed to the inlet with capillary wax. Preformed arrays were used within 30 min of aspiration.

Standardization of Hybrid Concentration Gradient. To quantify on-chip dilution, fluorescence of 0.10 mM fluorscein in 10 mM Tris (pH 7.8) and 0.05% (wt/vol) LDAO, 0.10 mM rhodamine B, 25% (wt/vol) PEG 8000 in 10 mM Tris (pH 7.8) and 0.05% (wt/vol) LDAO, and 0.10 mM PSS, 50% (wt/wt) glycerol in 10 mM Tris (pH 7.8) and 0.05% (wt/vol) LDAO at six dilutions was measured (100%, 80%, 60%, 40%, 20%, and 0% fluorescent solution). The dilutions were made by using a stock solution of 10 mM Tris (pH 7.8) and 0.05% LDAO (wt/vol). Plugs of these six solutions were aspirated into Teflon capillary (O.D. 250 μm, i.d. 200 μm, length ~10 cm) that was sealed to 30-gauge Teflon tubing by using a manual microsyringe pump with a glass syringe 50 μl in volume. Fluorescent solutions (~25-nl plugs) were aspirated in duplicate, alternating with FC-40 (~25-nl plugs), for a total of 12 fluorescent plugs in the array. Once the array was complete, the Teflon capillary was detached from the syringe, sealed at both ends with capillary wax, and placed in a Petri dish filled with water to facilitate fluorescence measurements. Fluorescence intensity of plugs was recorded by using a Leica DMI6000 microscope (Leica Microsystems) with 10 × 0.4 Leica objective and Hamamatsu ORCAER camera. GFP, Texas red, and DAPI filters were used to collect fluorscein, rhodamine B, and PSS fluorescence, respectively. Images were acquired and analyzed with Metamorph imaging system version 6.3r1 (Universal Imaging). Exposure times of 2, 3, and 150 ms were used for fluorscein, rhodamine B, and PSS. The baseline fluorescence intensity was taken of the buffer plugs (0% fluorescent solution) in each aspirated array. This baseline value was subtracted from the intensity of the fluorescent plugs to obtain the true intensity of each fluorescent dilution.

Quantification of Hybrid Concentration Gradient. Concentration gradients of reagents in plugs were determined by measuring fluorescence intensity. The 0.10 mM fluorecein (Fisher Scientific), 0.10 mM 1,3,6,8pyrenetetrasulfonic acid, tetra sodium (PSS; Acros Organics), and 0.10 mM rhodamine B (Acros Organics) were added to 10 mM Tris, pH 7.8 (viscosity ~1 mPa s), 50% (wt/wt) glycerol in 10 mM Tris pH 7.8 (viscosity ~6 mPa s), and 25% (wt/vol) PEG 8000 in 10 mM Tris, pH 7.8 (viscosity ~36 mPa s), respectively. All solutions contained 0.05% (wt/vol) LDAO. The input array was generated by aspirating ~140 nl of the reagents in alternation with ~40 nl of air as the gas spacer. By using FC-40 as the carrier fluid, the array was merged with two streams of buffer [0.05% (wt/vol) LDAO in 10 mM Tris, pH 7.8]. The flow rates are given in the Fig. 1 legend and shown graphically in Fig. 5. After plugs were formed and flowed into a Teflon capillary, the flows were stopped, and the Teflon capillary was cut from the PDMS device and sealed with capillary wax on both ends. The Teflon capillary was placed in a Petri dish filled with water to facilitate fluorescence measurements. Fluorescence intensity of plugs was recorded by using a Leica DMI6000 microscope (Leica Microsystems) with 10 ×0.4 NA Leica objective and Hamamatsu ORCA-ER camera. GFP, Texas red, and DAPI filters were used to collect fluorescence of fluorscein, rhodamine B, and PSS, respectively. The size of each plug was measured under fluorescence. Images were acquired and analyzed with Metamorph imaging system version 6.3r1 (Universal Imaging).

To form plugs containing different concentrations of reagents, the relative flow rates of the three

aqueous streams were changed to combine streams in several ratios. To index these concentrations, the sizes of plugs were simultaneously changed by altering the flow rate of the carrier fluid.

The flow rates of both aqueous and carrier fluid streams were varied by using the Labview subroutine (available online at http://ismagilovlab.uchicago.edu/AutoCad_SI_2006_07502.dwg). The first buffer stream, made to mimic the substrate stream, was maintained at constant flow rate of 10 nl/s. The flow rate of the preformed plug array stream was cycled to first increase from 3.3 nl/s to 10 nl/s and then decrease from 10 nl/s to 3.3 nl/s with a step of 1.7 nl/s. Correspondingly, the second buffer stream was cycled to first decrease from 10 nl/s to 3.3 nl/s and then increase from 3.3 nl/s to 10 nl/s with a step of 1.7 nl/s. The flow rate of the carrier fluid was cycled to first increase from 23.3 nl/s to 50 nl/s and then decrease from 50 nl/s to 23.3 nl/sec with a step of 6.7 nl/s. Each flow rate step lasted for 1.5 s. The flow rate change of the carrier fluid was synchronous with the flow rate changes of the aqueous phases.

To visualize the generation of the concentration gradient, the same setup as above was used, except instead of fluorscein, PSS, and rhodamine B, 50% (vol/vol) green ink (Conway Stewart, Plymouth, U.K.), 24% (vol/vol) blue ink (Conway Stewart), and 50% (vol/vol) red ink (Conway Stewart) were used, respectively. A diluting "buffer" stream of 0.05% LDAO in water and the membrane protein stream of ~7 mg/ml reaction center (RC) from *Rhodopseudomonas viridis* in 0.05% (wt/vol) LDAO and 10 mM Tris (pH 7.8) were merged with the stream of plugs from the preformed array. Images of plugs were taken with a Leica MZ 16 Stereoscope with a 1.0× objective. Images were collected with SPOT Advanced software (version 3.4.0 for windows).

Loading Protein-Detergent Solution with no Dead Volume into a Syringe. We used a simple technique to minimize the volume of protein solution required to set up the experiment and to eliminate losses of the solution when filling syringes (Fig. 9). A 10-μl syringe connected to a piece of Teflon capillary was filled with the carrier fluid, and a small volume of the membrane protein solution was aspirated into the capillary.

Crystallization of Reaction Center from *R. viridis* and Porin from *Rhodobacter capsulatus*

For crystallization of RC from *R. viridis*, 16 precipitants were selected from a custom crystallization screening kit obtained from deCode Biostructures. In addition, we used two additional precipitants, both in duplicate (Table 3). The two precipitants were 4M $(NH_4)_2SO_4$ in 50 mM Na_2HPO_4/NaH_2PO_4 buffer (pH 6.0) based on the published condition (4; 5) and a modified condition at higher pH, 3.6 M $(NH_4)_2SO_4$ in 50 mM Tris (pH 7.8). LDAO was added to precipitants from the kit at a concentration of 0.05% (wt/vol) and was added to the other two conditions at a concentration of 0.15% (wt/vol). The reagent plugs were combined with the buffer stream of 0.15% (wt/vol) LDAO in Millipore water and the RC stream of 22 mg/ml RC, 0.08% LDAO, 7% heptane-triol, 4.5% triethylammonium phosphate solution in 20 mM Na_2HPO_4/NaH_2PO_4 buffer, pH 6.0. The crystallizing concentration of $(NH_4)_2SO_4$ was 1.6-2.0 M for the pH 6.0 condition and 1.6-1.8 M for the pH 7.8 condition. The experiment was performed in a low-light environment, and the crystals were kept in dark. Trials were incubated at 18°C. Micrographs of crystals were taken on a Leica MZ 16 Stereoscope. The crystals that were photographed under bright-field illumination were not used for x-ray diffraction experiments.

For Porin from *R. capsulatus*, eight precipitants were tested under seven conditions from MembFac (Hampton Research), and conditions 3, 6, 10, 17, 21, 31, and 39 (Table 2) were chosen. In addition, 2% C_8E_4 (wt/vol), 3M LiCl, and 70.6% PEG 550MME in 150 mM Tris (pH 8.0) based on the published crystallization condition (6) was used. The reagent plugs were combined with the buffer stream of 5.1% C_8E_4 in 150 mM Tris (pH 8.0) and the protein stream of 29.8 mg/ml Porin in 0.6% (wt/vol) *n*-octyltetraoxyethylene (C_8E_4) and 20 mM Tris pH 7.8. The trials were incubated at 23°C. Crystals appeared only in the reported condition after 9 days of incubation at 23°C. Microphotographs of the

crystals were taken on a Leica MZ 16 Stereoscope with polarizer.

Scaling-Up Experiments for Porin from *R. capsulatus.* Scaling-up experiments were performed by increasing the dimensions of microchannels. Instead of interfacing a Teflon capillary to the hybrid device, we inserted a large silanized glass capillary (O.D. 600 μm with wall thickness of 10 μm; Hampton Research) into the plug-forming junction. Once plugs were flowed into the glass capillaries, the end was removed from the PDMS device, and both ends were sealed with capillary wax. The sealed glass capillaries were placed in Petri dishes and incubated at 23°C.

X-Ray Diffraction and Structure Determination. Crystals of RC grown by the hybrid microfluidic technique were extracted by attaching a syringe to one end of the Teflon capillaries and flowing the crystals slowly into a drop of paraffin oil (cryoprotectant) with the manual syringe driver. The plugs containing crystals could move >10 cm without sticking to the channel wall. This was accomplished in one of two ways. In both methods, the Teflon capillary with the crystals was attached to a 50 μl glass syringe. After that, the crystals could either be flowed out using a PHD 2000 infusion syringe pump from Harvard Apparatus or a manual microsyringe pump. The maximum flow rate used to flow out crystals with the PHD 2000 syringe pump was 17 nl/s. The manual syringe pump allowed for easier control as well as precise flow of only one plug at a time.

Once crystals were flowed into the cryoprotectant drop, the crystals were picked with a CryoLoop (Hampton Research) and flash-frozen in liquid nitrogen. The x-ray diffraction experiments were performed at GM/CA CAT station 23 ID-D of the Advanced Photon Source (Argonne National Laboratory). X-ray data were collected at 100 K by using a wavelength of 0.97943 Å, 2- or 3-second exposure times, and an oscillation width of 0.5°. The diffraction data were processed with HKL2000 (7). Because of small crystal size and substantial radiation damage, we had to merge x-ray data collected from two different crystals (25?oscillation range each) to obtain a complete (96.5%) data set with resolution of 1.96 Å. The RC crystals belong to space group $P4_32_12$ with cell dimensions $a = b =$ 220.4 Å, c = 113 Å and were isomorphous to crystals reported earlier with PDB codes 1PRC (2.3 Å resolution) (8) and 1DXR (His-L168-Phe mutant, 2.0 Å resolution) (9). We used the 1DXR structure with corrected L168 residue as a starting model in our refinement. The rigid-body positional and temperature factor refinement was performed by using maximum-likelihood target with the program REFMAC5 (10). R_{free} was monitored by setting aside 5% of the reflection as a test set. The ΣA-weighted $2F_{obs}-F_{calc}$ and $F_{obs}-F_{calc}$ Fourier maps were calculated by using CCP4 (11). The Fourier maps were displayed and examined in TURBO-FRODO (12). The search for new solvent molecules was performed with help of the ARP-WARP program (13). The crystal data, data collection, and refinement statistics are summarized in Table 4, and the example of electron-density maps are shown in Fig. 10. A Ramachandran plot calculated with PROCHECK (14) indicates that 99.7% of the non-Gly and non-Pro residues in the final models lie in the most-favored and additional-allowed regions. Our model contains 771 water molecules, 13 SO_4^{2-} ions (because we used phosphate buffer in the crystallization, some of the ions can indeed be PO_4^{3-} ions) and 3 molecules of 1,2,3-heptanetriol. In addition to the existing binding site reported in 1PCR, we have identified the second binding site of ubiquinone-1. As compared with 2.0-Å resolution 1DXR structure, we have identified 276 water molecules. Two thirds of the water molecules were found in the first hydration shell (cytochrome c: 87 H_2O, subunit H: 41 H_2O, subunit L: 16 H_2O, subunit M: 39 H_2O). The rmsds for main-chain atoms calculated for our final model and previously reported structures 1PRC and 1DXR are 0.39 Å and 0.31 Å, respectively. Figures showing the electron-density map and three-dimensional structures were prepared by using TURBO-FRODO (12) and RIBBONS (15). The coordinates and structure factors have been deposited in the Protein Data Bank with an entry code 2I5N.

X-ray diffraction experiments with Porin were carried out at 4°C by using the *in situ* method (16-18) at GM/CA CAT station 23 ID-D of the Advanced Photon Source (Argonne National Laboratories). Porin crystals in Teflon capillary were flowed into silanized glass capillaries (200 μm; Hampton Research) by using a manual syringe pump. Crystals that were grown from scaling-up studies were already in

glass capillaries for direct diffraction studies. The diffraction experiments were performed with 2-s exposure times. The first shots gave diffraction patterns at resolution at ~ 1.95 Å. The diffraction decayed rapidly in six frames, preventing the collection of a full data set. The Porin crystals grown by microfluidic technique belong to space group H3 with cell dimensions $a = b = 91.5$ Å, $c = 146.3$ Å and were isomorphous to ones reported earlier (6).

Additional Control Experiments. *Establishing plug size dependence on water fraction.* We used the "water fraction" (19), the ratio of the combined volumetric flow rates of the aqueous streams to the total flow rate of the carrier fluid and the aqueous streams, to index the concentration of the reagent in plugs generated by using the hybrid method. As the computer subroutine increased the relative flow rate of the reagent stream, it also decreased the flow rate of the buffer to keep the combined volumetric flow rates of the three aqueous streams constant (Fig. 5). In sync with this cycle, the subroutine increased the flow rate of the carrier fluid (FC-40), thereby decreasing the water faction and decreasing the size of the plug. We were able to show that the size of plugs changes approximately linearly with water fraction, and we found the dependence to be slightly sensitive to viscosity (Fig. 6).

Quantification of solubility of common precipitants in perfluroamine carrier fluids. To assess if any chemicals used to crystallize membrane proteins were being dissolved in the perfluroamine carrier fluids, we quantified the solubilities of some typical detergents and precipitants with NMR experiments (Table 1).

In each case, the saturated solution was made first. We added 1% (wt/vol for solid and vol/vol for liquid) of the testing chemical to the carrier fluid (FC-40 or FC-70) in an Eppendorf tube. The tube was vortex-vibrated for one minute and then centrifuged (Eppendorf Centrifuge 5415D) at 10 krpm for 10 min. The FC-40 or FC-70 phase was collected with a pipette. The NMR sample was then made by taking 40 μl of 5% (vol/vol) 1H, 1H, 2H, 2H-Perfluorooctanol (PFO) in FC-40 or FC-70 and mixing it with 200 μl of the saturated solution and 260 μl of FC-40 or FC-70. Four blank experiments were also performed: (*i*) 40 μl of 5% PFO in FC-40 + 460 μl of FC-40, (*ii*) 40 μl of 5% PFO in FC-70 + 460 μl of FC-70, (*iii*) 500 μl of FC-40, and (*iv*) 500 μl of FC-70. PFO was used as an internal standard. The concentration (solubility) of the testing chemical was determined with the internal standard (Table 1).

1. Duffy DC, McDonald JC, Schueller OJA, Whitesides GM (1998) *Anal Chem* 70:4974-4984.

2. Roach LS, Song H, Ismagilov RF (2005) *Anal Chem* 77:785-796.

3. Zheng B, Ismagilov RF (2005) *Angew Chem Int Ed* 44:2520-2523.

4. Baxter RHG, Ponomarenko N, Srajer V, Pahl R, Moffat K, Norris JR (2004) *Proc Natl Acad Sci USA* 101:5982-5987.

5. Baxter RHG, Seagle BL, Ponomarenko N, Norris JR (2005) *Acta Crystallogr D* 61:605-612.

6. Kreusch A, Weiss MS, Welte W, Weckesser J, Schulz GE (1991) *J Mol Biol* 217:9-10.

7. Otwinowski Z, Minor W (1997) in *Macromolecular Crystallography* (Academic, San Diego) Pt A , Vol. 276, pp. 307-326.

8. Deisenhofer J, Epp O, Sinning I, Michel H (1995) *J Mol Biol* 246:429-457.

9. Lancaster CRD, Bibikova MV, Sabatino P, Oesterhelt D, Michel H (2000) *J Biol Chem* 275:39364-39368.

10. Murshudov GN, Vagin AA, Dodson EJ (1997) *Acta Crystallogr D* 53:240-255.

11. Collaboration (1994) *Acta Crystallogr D (Denmark)* D50:760-763.

12. Cambillau C, Roussel A (1997) Turbo Frodo, version OpenGL 1 (University Aix-Marseille II, Marseille, France).

13. Zwart P, Langer GG, Lamzin VS (2004) *Acta Crystallogr D* 60:2230-2239.

14. Laskowski RA, Macarthur MW, Moss DS, Thornton JM (1993) *J Appl Crystallogr* 26:283-291.

15. Carson M (1997) in *Macromolecular Crystallography* (Academic, San Diego) Pt B, Vol. 277, pp. 493-505.

16. McPherson A (2000) *J Appl Crystallogr* 33:397-400.

17. Yadav MK, Gerdts CJ, Sanishvili R, Smith WW, Roach LS, Ismagilov RF, Kuhn P, Stevens RC (2005) *J Appl Crystallogr* 38:900-905.

18. Zheng B, Tice JD, Roach LS, Ismagilov RF (2004) *Angew Chem Int Ed* 43:2508-2511.

19. Tice JD, Song H, Lyon AD, Ismagilov RF (2003) *Langmuir* 19:9127-9133.