SUPPORTING INFORMATION FOR

User-loaded SlipChip for equipment-free multiplexed nanoliter-scale experiments

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Chemicals and Materials

All solvents and salts purchased from commercial sources were used as received unless otherwise stated. 1,2,3–Heptanetriol (high melting point isomer) was purchased from Fluka Biochemika (St. Louis, MO); FC–40 (a mixture of perfluoro–tri–n–butylamine and perfluoro–di–n–butylmethylamine) and FC–70 (perfluorotripentylamine) were obtained from 3M (St. Paul, MN). Food dyes were purchased from Ateco (Glen Cove, NY). Tridecafluoro–1,1,2,2–tetrahydrooctyl–1–trichlorosilane was purchased from United Chemical Technologies, Inc. (Bristol, PA). Alexa Fluor ® 488 dye (Alexa–488) was purchased from Invitrogen (Eugene, OR). Soda–lime glass plates with chromium and photoresist coating were purchased from Telic Company (Valencia, CA). Amorphous diamond coated drill bits were obtained from Harvey Tool (0.030 inch cutter diameter, Rowley, MA). Fluorescence reference slides were purchased from Microscopy/Microscopy Education (McKinney, TX). Binderclips (5/32’ inch capacity, 1/2’ inch size) were purchased from Officemax (Itasca, IL). Pipettors were obtained from Eppendorf Inc. (Westbury, NY). Fisherbrand pipettor tips were from Fisher Scientific (Hanover Park, IL).

Experimental Procedures

Chip Design and Fabrication. Soda-lime glass plates with chromium and photoresist coating (Telic Company, Valencia, CA) were used to fabricate devices. Microchannels and wells on the glass plates were made by using standard photolithographic and wet chemical etching techniques.[1] Briefly, the glass plate with photoresist coating was aligned with a photomask containing the design of the microchannels and wells and

exposed to UV light for 1 min. The photomask was removed, and the glass plate was developed by immersing it in 0.1 mol/L NaOH solution for 2 min. The exposed underlying chromium layer was removed using a chromium etchant (a solution of 0.6:0.365 M $HClO_4$ / $(NH_4)_2Ce(NO_3)_6$). The plate was rinsed with Millipore water and dried with nitrogen gas, and the back of the glass plate was taped with PVC sealing tape (McMaster-Carr) to protect the back side of glass. The taped glass plate was then carefully immersed in a plastic container with a glass etching solution (1:0.5:0.75 M $HF/NH_4F/HNO_3$) to etch the glass surface that was exposed after the chromium coating was removed. A 40 °C constant-temperature water bath shaker was used to control the etching speed. ~ 45 minutes of etching yielded a depth of ~ 60 μm. After etching, the tape was removed from the plates. The plate was then thoroughly rinsed with Millipore water and dried with nitrogen gas. Access holes were drilled with a diamond drill bit 0.030 inches in diameter. The surfaces of the etched glass plates were cleaned with Millipore water, followed by ethanol and subjected to an oxygen plasma treatment before silanization[2] or Fluorinated Ethylene Propylene (FEP) coating.

Spin Coating FEP. An aqueous emusion of FEP (TE-9568, Dupont) was first diluted 4 times with Millipore water before use. Following plasma cleaning the SlipChip device, the solution was evenly spread onto the device by using a plastic pipet. For spin coating, the spin speed was set at 1500 rpm and the process was executed for 30 seconds, or the spin speed was set at 2000 rpm and the process was executed for 30 seconds. Once the coating was finished, the SlipChip was transferred to a 120 °C oven and incubated for 10 minutes. After incubation, the SlipChip was baked at 250 °C on a hot plate for 10 minutes, followed by baking at increasing the temperature to 265 °C for another 10

minutes. After baking, the SlipChip was sintered at 340 °C on a hot plate for 1 minute. The sintered Chip was then cooled to room temperature.

Assembling the SlipChip. The SlipChip was assembled under FC-40. The bottom plate (Figure 2B) was first immersed into FC-40 in a Petri dish, with the patterns facing up. The top plate was then laid on top of the bottom plate, with the patterns facing down. The two plates were aligned into the position, shown in Figure 2C, by moving them relative to each other and then fixed by using four micro binder clips. The SlipChip was ready for use after the extra FC-40 on the surface was removed.

Measuring Contact Angles. The plate of the SlipChip was first immersed into fluorocarbon in a tank. The plate, facing down, was clamped by two micro binderclips on each end to create a gap between the plate and the bottom of the tank. 5 μL of the measured aqueous solution was pipetted into the gap, and the aqueous droplet contacted the plate due to its buoyancy in the surrounding fluorocarbon. The contact angle of the droplet on the substrate was then measured by using an optical contact angle meter (Ramé-Hart Instrument Co., Model 500).

Food Dye Experiments. All the solutions used for food dye experiments were filtered with a 0.45 μm PVDF syringe filter before use. Four food dyes (brown, pink, red, and blue, Ateco, Glen Cove, NY) were diluted ~10 times from their stock solutions and were pipet-loaded into 16 reagent channels, resulting in the pattern shown in Figure 1B. To load each channel, 4 μL of dye was first pushed through the inlet using a pipette until the dye solution emerged from the outlet (Figure 2A, D). After loading reagents, the Chip was slipped to form a continuous fluidic path for the sample (Figure 2E). A green dye was diluted 20 times and then loaded through the sample inlet (Figure 2A, 2E). Using a

pipette 4 μL of dye was loaded into the Chip until all the sample channels were fully filled. Once the sample was loaded, the SlipChip was slipped again to mix the solutions by diffusion (Figure 2F).

Quantifying Mixing Ratio: Experimental Setup. The loading procedure was similar to that for the food dye experiments. Two solutions, the fluorescent solution (44.8 μM Alexa-488 in 10 mM Tris, pH 7.8) and the buffer (10 mM Tris, pH 7.8), were used. The outermost four fluidic paths, each path containing 11 wells, were loaded with the fluorescent solution, and the remaining 12 fluidic paths were loaded with the buffer. The fluorescent solution was also used as the sample. After the wells for the reagent and wells for the sample were combined, the SlipChip was incubated for one hour in the dark to allow complete mixing. The SlipChip was then slipped a second time to separate the wells for the reagent from those for the sample. The outermost four fluidic paths containing the fluorescent solution were not diluted, providing a control experiment for calibrating intensity measurements.

Quantifying Mixing Ratio: Measuring fluorescence. To confirm that the fluorescent intensity of Alexa−488 is linearly correlated with the concentration in the working range of the fluorescent microscope, we made a dilution curve on a SlipChip. First, four solutions, including one buffer (10 mM Tris, pH 7.8) and three solutions at concentrations of 1/4, 1/2, and 1 times the concentrations of the original Alexa−488 solution (44.8 μM in 10 mM Tris pH 7.8), were loaded into four separated fluidic paths in a pre-assembled user-loaded SlipChip. The top plate was slipped relative to the bottom plate so that all the wells were separated. The fluorescent intensity of the loaded wells on the bottom plate was then measured by using a Leica DMI6000 microscope (Leica

Microsystems) with a 10 × 0.4NA Leica objective and a Hamamatsu ORCAER camera. A GFP filter was used to collect Alexa−488 fluorescence. An exposure time of 4 ms was used. Images were acquired and analyzed by using Metamorph imaging system version 6.3r1 (Universal Imaging). To extract the intensity of the fluorescent signal, a region of 100 pixels by 100 pixels was selected in the middle of every well of interest. The average integrated intensity of the regions belonging to wells with the same Alexa−488 concentration (five wells for each concentration) was plotted against the corresponding concentration to obtain a calibration curve. (Figure S1)

The fluorescent measurement was then performed by using the sample wells. We measured the fluorescent intensity of the wells in the bottom plate. This ensured that the working parameters for measuring fluorescent intensity were consistent. The same setup for the fluorescent microscope was used in this experiment as was used in making the dilution curve. The intensity from the measurements was then converted to concentration based on the dilution curve. To calibrate the microscope, the fluorescent intensity of a fluorescence reference slide for GFP was recorded and used for background correction. Images were acquired and analyzed by using Metamorph imaging system version 6.3r1 (Universal Imaging). Any systematic errors in measurements could be caused by the drift of the intensity of the lamp between calibration and measurement).

Quantifying Mixing Ratio: Characterization of well sizes. The wet etching of glass is isotropic, and the speed of etching is the same in all directions. The size of the wells after etching was measured by using a Leica MZ 16 Stereoscope calibrated by a micro-ruler (Figure S2) and the volume of the wells were calculated as follows:

The volume of the well is expressed in Equation 1, where W_1 is the original width of the well, L is the original length of the well, r is the expanding distance, and d is the depth of the well after etching. The parameters used to calculate the volume of the well are shown in Figure S2.

Eq. 1 $$Volume = W_1 Ld + 0.5W_1^2 d + 0.707\pi rdW_1 + 0.666\pi dr^2 + 0.5\pi rdL$$

The size of the wells after etching was measured by using a Leica MZ 16 Stereoscope calibrated by a micro-ruler. The expanding distance r was then calculated using Equation 2, where W_2 is the width of the well after etching.

Eq. 2 $$r = 0.5(W_2 - W_1)$$

We assumed that the etching speed was the same in all directions, so the original pattern of the well expanded the same distance in all directions. The expanding distance, r (Figure S2, A), was assumed to be the same as the depth, d (Figure S2, B). Therefore, the volume of the wells can be calculated by combining Equations 1 and 2.

Eq. 3

$$Volume = W_1 L\frac{W_2 - W_1}{2} + 0.5W_1^2\frac{W_2 - W_1}{2} + 0.707\pi\frac{(W_2 - W_1)^2}{4}W_1 + 0.666\pi\frac{(W_2 - W_1)^3}{8} + 0.5\pi\frac{(W_2 - W_1)^2}{4}L$$

In this paper, we designed the wells such that W_1 was always 236 μm and L was varied to be 0, 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 μm. By etching the wells to be 60 μm deep, we made wells with volumes of 4.0, 4.4, 4.8, 5.2, 5.6, 6.0, 6.4, 6.8, 7.2, 7.6 and 8.0 nL, respectively. We did not measure the sizes of wells directly, and variation in these sizes could cause some errors in measurements of concentrations.

Quantifying Mixing Ratio: Data analysis. To calibrate the intensity measurements, the background intensity was first subtracted from all the fluorescent images. The intensity of

each well was then extracted from the integrated intensity of a 100 pixel by 100 pixel region located at the center of each well. The dilution ratio for each well was obtained by dividing the intensity of that well by the intensity of a well of the same size that did not get diluted.

RC crystallization. A sample of the photosynthetic reaction center (RC) from *Blastochloris viridis* was generously provided by Professor James Norris of the University of Chicago. The loading procedure was similar to that for the food dye experiments. The precipitant (3.2 M $(NH_4)_2SO_4$ in 40 mM NaH_2PO_4/Na_2HPO_4, pH 6.0) was loaded into seven reagent channels and the protein sample (36 mg/mL RC in 0.07% (w/v) LDAO, 7% (w/v) 1,2,3–heptanetriol, 4.5% (w/v) triethylamine phosphate (TEAP), 17 mM Na_2HPO_4/NaH_2PO_4, pH 6.0) was loaded into the sample channel. The SlipChip containing the trials was then stored in FC-70 in a petri dish[3] at room temperature in the dark. The trials were monitored over 10 days to check for the formation of crystals.

Crystallization of glutaryl-CoA dehydrogenase from Burkholderia pseudomallei in SlipChip. The protein sample was obtained from the Seattle Structural Genomics Center for Infectious Disease (SSGCID). 48 precipitants from a home-made screening kit based on the Wizard screen (Table S2) were loaded into three SlipChips, 16 precipitants in each Chip; the same loading procedure was the same as in the food dye experiments. Each SlipChip was then immersed into FC-70 in separate petri dishes. The petri dishes were incubated at room temperature and the results were monitored for two weeks. Images of wells containing crystals were taken by a SPOT Insight camera (Diagnostic Instruments, Inc., Sterling Heights, MI) coupled to a Leica MZ 16 Stereoscope.

Crystallization of glutaryl-CoA dehydrogenase from Burkholderia pseudomallei in well plates. Once a crystallization condition for glutaryl-CoA dehydrogenase was identified, the experiment was scaled up in a sitting-drop well plate (Hampton research) using microbatch method (Figure S3). At the same mixing ratio identified by the screening experiments on the SlipChip, the protein sample was mixed with the precipitant to obtain a final volume of 3 μL in the well. In the reservoir, Millipore water was mixed with the precipitant to give the same precipitant concentration as in the well; the final volume was 600 μL. Each condition had one duplicate. The plate was then sealed with sealing tape (Hampton research) and incubated at room temperature. Images of crystals were taken by a SPOT Insight camera (Diagnostic Instruments, Inc., Sterling Heights, MI) coupled to a Leica MZ 16 Stereoscope.

X-ray diffraction and data processing. Crystals for x-ray diffraction were obtained from the well plate experiments. For precipitants that contained PEG-400, the mother liquor was used as a cryo-protectant, and the concentration of PEG-400 was changed to be 25% (w/v). For other precipitants, the mother liquor plus 20% (v/v) glycerol was used as a cryo-protectant. Briefly, a crystal was first transferred from the original well to the well containing the cryo-protectant by using a nylon loop. Then the crystal was 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 using a wavelength of 1.0332 Å.

The data were processed and analyzed by using HKL-2000.[4] The statistics of the data are listed in Table S4.

X-ray structure determination of glutaryl-CoA dehydrogenase. The structure of glutaryl-CoA dehydrogenase was solved by molecular replacement using the PDBid 3D6B structure as a starting model and the MOLREP program[5] in CCP4 suite.[6] The data collected from crystals grown in the condition containing PEG-400 was used. The rigid-body, positional, and temperature factor refinement was performed using maximum likelihood target with the program REFMAC5.[7] The SigmaA-weighted 2Fobs-Fcalc and Fobs-Fcalc Fourier maps were calculated using CCP4. The Fourier maps were displayed and examined in COOT.[8] The search for new solvent molecules was performed with help of COOT. The refinement statistics are summarized in Supporting Table S4. The coordinates and structure factors have been deposited in the Protein Data Bank with entry code 3II9 (pending).

References:

(1) He, Q. H.; Chen, S.; Su, Y.; Fang, Q.; Chen, H. W., *Anal. Chim. Acta* **2008**, *628*, 1-8.
(2) Roach, L. S.; Song, H.; Ismagilov, R. F., *Anal. Chem.* **2005**, *77*, 785-796.
(3) Du, W. B.; Li, L.; Nichols, K. P.; Ismagilov, R. F., *Lab Chip* **2009**, *9*, 2286-2292.
(4) Otwinowski, Z.; Minor, W. In *Macromolecular Crystallography, Pt A*; Academic Press, Inc: San Diego, 1997; Vol. 276, pp 307-326.
(5) Vagin, A.; Teplyakov, A., *J. Appl. Crystallogr.* **1997**, *30*, 1022-1025.
(6) Bailey, S., *Acta Crystallogr. D, Biol. Crystallogr. (Denmark)* **1994**, *D50*, 760-763.
(7) Murshudov, G. N.; Vagin, A. A.; Dodson, E. J., *Acta Crystallogr. Sect. D-Biol. Crystallogr.* **1997**, *53*, 240-255.
(8) Emsley, P.; Cowtan, K., *Acta Crystallogr. Sect. D-Biol. Crystallogr.* **2004**, *60*, 2126-2132.

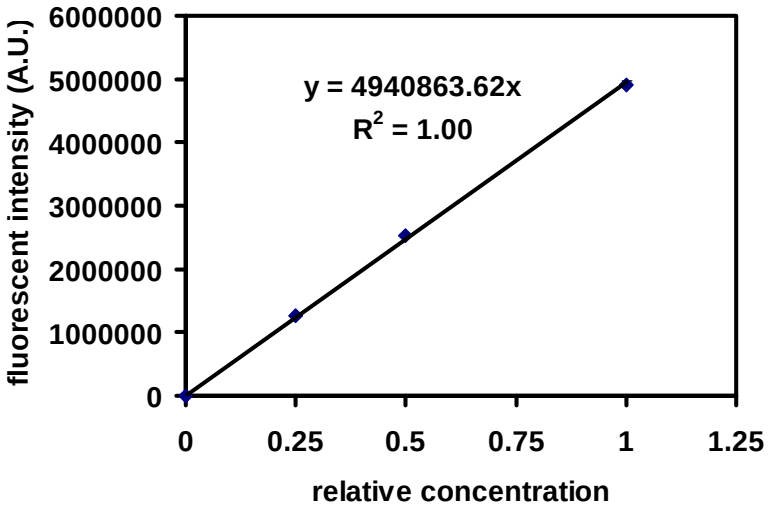

Figure S1: A dilution curve confirms the linear relationship between fluorescent intensity of Alexa-488 and concentration. The average intensity was plotted against the corresponding concentration (5 data sets for each concentration). The fluorescent intensity was collected from wells with the same configurations as the wells used to quantify mixing ratios. The error for each point was less than 1.5%.

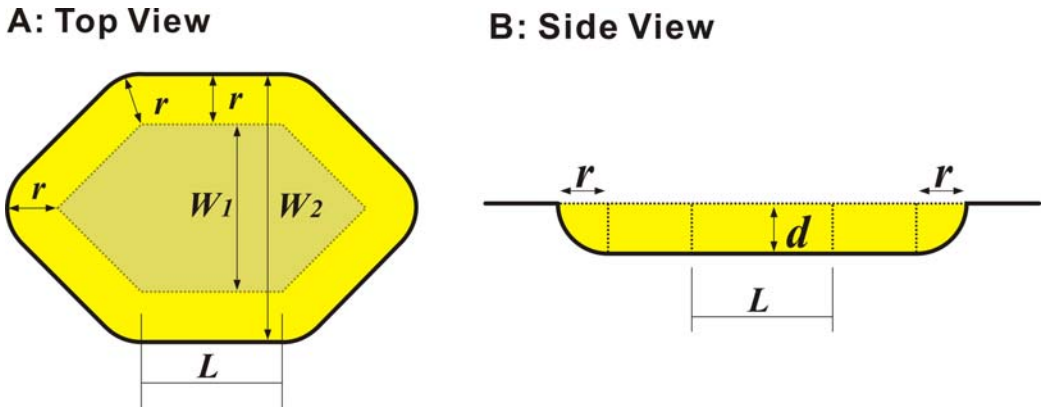

Figure S2: A schematic of an etched well illustrates the parameters used to calculate the volume of an etched well. The original pattern before etching is shaded and the etched area is shown in yellow. W_1 and L are the original well width and length, respectively. W_2 is the width of the etched well, r is the expanding distance (assumed to be the same in all directions), and d is the well depth. A) The top view of the etched well. B) The side view of the etched well.

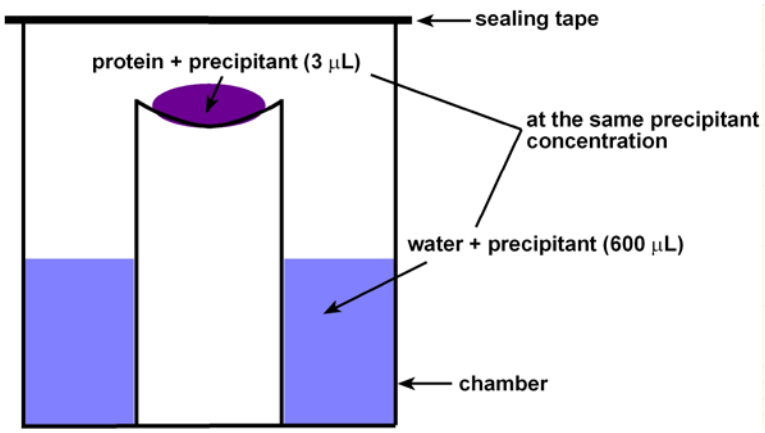

Figure S3: A schematic of the setup of the microbatch method used to scale up crystallization of proteins. The concentration of precipitant in the 600 μL reservoir was the same as the concentration of precipitant in the 3 μL well.

Table S1: Contact angle measurements of different aqueous solutions on two modified surfaces. All measurements were taken under FC-40.

	Water	0.1% (w/v) LDAO
Silanized glass	139.2	109.4
FEP coated glass	159.1	153.8

Table S2: Contents of the home-made screening kit

	precipitant	buffer (0.1 M)	salt (0.2 M)
1	30% (w/v) PEG-8000	CHES pH 9.5	
2	20% (v/v) 2-propanol	HEPES pH 7.5	NaCl
3	30% (v/v) ethanol	CHES pH 9.5	none
4	52.5% (v/v) 2-methyl-2,4-pentanediol	imidazole pH 8.0	$MgCl_2$
5	60% (v/v) PEG-400	CAPS pH 10.5	
6	30% (w/v) PEG-3000	citrate pH 5.5	
7	20% (w/v) PEG-8000	MES pH 6.0	$Zn(OAc)_2$
8	2.8 M $(NH_4)_2SO_4$	citrate pH 5.5	
9	1.5 M $(NH_4)_2HPO_4$	acetate pH 4.5	
10	30% (w/v) PEG-2000 MME	Tris pH 7.0	
11	40% (v/v) 1,4-butanediol	MES pH 6.0	Li_2SO_4
12	30% (w/v) PEG-1000	imidazole pH 8.0	$Ca(OAc)_2$
13	2.52 M $(NH_4)_2SO_4$	cacodylate pH 6.5	
14	1.4 M sodium citrate	cacodylate pH 6.5	
15	20% (w/v) PEG-3000	imidazole pH 8.0	Li_2SO_4
16	3.75 M NaCl	Na/K phosphate pH 6.2	
17	40% (w/v) PEG-8000	acetate pH 4.5	Li_2SO_4
18	1.5 M K/Na tartrate	imidazole pH 8.0	NaCl
19	30% (w/v) PEG-1000	Tris pH 7.0	
20	0.6 M NaH_2PO_4/1.6 M K_2HPO_4	imidazole pH 8.0	NaCl
21	30% (w/v) PEG-8000	HEPES pH 7.5	
22	20% (v/v) 2-propanol	Tris pH 8.5	
23	30% (v/v) ethanol	imidazole pH 8.0	$MgCl_2$
24	52.5% (v/v) 2-methyl-2,4-pentanediol	Tris pH 7.0	NaCl
25	45% (v/v) PEG-400	Tris pH 8.5	$MgCl_2$
26	20% (w/v) PEG-3000	CHES pH 9.5	
27	1.44 M NaH_2PO_4/0.64 M K_2HPO_4	CAPS pH 10.5	Li_2SO_4
28	30% (w/v) PEG-3000	HEPES pH 7.5	NaCl
29	20% (w/v) PEG-8000	CHES pH 9.5	NaCl
30	2.52 M $(NH_4)_2SO_4$	acetate pH 4.5	NaCl
31	30% (w/v) PEG-8000	phosphate-citrate pH 4.2	NaCl
32	20% (w/v) PEG-3000	Na/K phosphate pH 6.2	
33	2.0 M $(NH_4)_2SO_4$	CAPS pH 10.5	Li_2SO_4
34	1.5 M $(NH_4)_2HPO_4$	imidazole pH 8.0	
35	40% (v/v) 1,4-butanediol	acetate pH 4.5	
36	1.4 M sodium citrate	imidazole pH 8.0	
37	3.75 M NaCl	imidazole pH 8.0	
38	1.5 M K/Na tartrate	CHES pH 9.5	Li_2SO_4

39	30% (w/v) PEG-1000	phosphate-citrate pH 4.2	Li_2SO_4
40	20% (v/v) 2-propanol	MES pH 6.0	$Ca(OAc)_2$
41	45% (w/v) PEG-3000	CHES pH 9.5	
42	30% (v/v) ethanol	Tris pH 7.0	
43	52.5% (v/v) 2-methyl-2,4-pentanediol	Na/K phosphate pH 6.2	
44	30% (v/v) PEG-400	acetate pH 4.5	$Ca(OAc)_2$
45	30% (w/v) PEG-3000	acetate pH 4.5	
46	30% (w/v) PEG-8000	imidazole pH 8.0	$Ca(OAc)_2$
47	2.52 M $(NH_4)_2SO_4$	Tris pH 8.5	Li_2SO_4
48	30% (w/v) PEG-1000	acetate pH 4.5	$Zn(OAc)_2$

Table S3: Crystallization conditions for Glutaryl-CoA dehydrogenase. The reagent number correlates to the reagent numbers in Table S2. The specific conditions (mixing ratios) yielding crystals are highlighted in red. The same reagents yielded crystals in well plates.

Reagent No.	[ppt.]	0.33	0.37	0.4	0.43	0.47	0.5	0.53	0.57	0.6	0.63	0.67
	[pro.]	0.67	0.63	0.6	0.57	0.53	0.5	0.47	0.43	0.4	0.37	0.33
21												
25												

Table S4: Statistics for data collection and structural refinement on crystals from glutaryl-CoA dehydrogenase. Crystal form I was grown in the condition with the protein at a 0.57:0.43 mixing ratio with 45% (w/v) PEG-400, 0.2 M $MgCl_2$ and 0.1 M Tris, pH 7.8; And crystal form II was obtained in the condition the protein at a 0.67:0.33 mixing ratio with 30% (w/v) PEG-8000 and 0.1 M Hepes, pH 7.5.

Crystal form	I	II
Data collection		
Space group	$P2_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	81.0, 141.3,84.0	97.5, 109.9,148.2
. α, β, γ (°)	90, 112,90	90, 90, 90
Resolution (Å)	50-1.73 (1.79-1.73)	50-2.90 (3.00-2.90)
R_{merge} #	0.05 (0.97)	0.07 (0.74)
$I/\sigma I$	27.0 (1.7)	37.8 (4.8)
Unique observations	177485	35623
Total observations	673465	517984
Completeness (%)	99.3 (98.6)	100 (100)
Redundancy	3.8 (3.7)	14.5 (14.9)
Refinement		
Resolution (Å)	50-1.73	
No. reflections	179619	
R_{work}/ R_{free}	0.181/0.219	
B-factors		
Protein	29.606	
Ligand	55.371	
Water	49.673	
R.m.s deviations		
Bond lengths (Å)	0.012	
Bond angles (º)	1.475	

#: $R_{merge} = \sum_{hkl}\sum_i (I_i(hkl) - \langle I(hkl)\rangle)^2 / \sum_{hkl}\sum_I I_i(hkl)^2$