This journal is (C) The Royal Society of Chemistry 2009

ELECTRONIC SUPPLMENTARY INFORMATION for

SlipChip

Wenbin Du, Liang Li, Kevin P. Nichols, Rustem F. Ismagilov*

Department of Chemistry and Institute for Biophysical Dynamics, The University of Chicago, 929 East 57th Street, Chicago, Illinois 60637

* To whom correspondence should be addressed. E-mail: r-ismagilov@uchicago.edu

Supplementary Methods

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). Tridecafluoro−1,1,2,2−tetrahydrooctyl−1−trichlorosilane was purchased from United Chemical Technologies, Inc. (Bristol, PA). Alexa Fluor ® 488 dye (Alexa−488), and 8−methoxypyrene−1,3,6 trisulfonic acid (MPTS) were 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). Teflon tubing (O.D. 250 μm, I.D. 200 μm and O.D. 225 μm, I.D. 150 μm) was purchased from Zeus (Orangeburg, SC). Teflon tubing (I.D. 370 μm) was obtained from Weico Wire & Cable (Edgewood, NY). Standard wall glass tubing (O.D. 3 mm, I.D. 2 mm) was obtained from Chemglass (Vineland, NJ). Gastight syringes were obtained from Hamilton Company (Reno, NV). 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).

Preparing pre−loaded reagents for food dye experiment.

Blue, yellow, and red food dye solutions were made by diluting the stock solution 100 fold. Plugs of the three dye solutions were formed in a flow−focusing device in three separate experiments. In each experiment, a 10 cm long piece of Teflon tubing (O.D. 225 μm, I.D. 150 μm) was inserted flush with the junction of the flow focusing device and was then sealed with wax to be air−tight. FC−40 was used as the carrier fluid in this experiment. The flow rates for both the carrier fluid and the dye solutions were 1 μL/min. At these flow rates, the volume of the plugs was ~ 5 nL.

This journal is (C) The Royal Society of Chemistry 2009

Once the 10 cm long piece of Teflon tubing was fully filled with plugs of a particular dye solution, the experiment was stopped. The Teflon tubing was then sealed at both ends with wax and stored in glass tubing (O.D. 3 mm, I.D. 2 mm) prefilled with FC−70, Images of plugs were taken with a Leica MZ 16 Stereoscope with a Plan APO 0.63× objective (Fig. 2a-c).

Characterization of cross−contamination on the pre−loaded SlipChip.

Three arrays of plugs, containing solutions of 1) 44.8 μM Alexa−488 in Tris pH 7.8,2) 10 mM Tris pH 7.8 and 3) 400 μM MPTS in 1X PBS, respectively, were prepared in the same way as described in the dye experiment. The plugs of the three solutions were then deposited into the bottom plate of the SlipChip in the same manner as in the dye experiment to create a repeating pattern of Alexa−488, buffer, and MPTS. The whole SlipChip was then assembled as described above. To characterize possible cross−contamination, we took measurements of the fluorescent intensity of wells containing solutions. Fluorescence intensity was recorded using a Leica DMI6000 microscope (Leica Microsystems) with a 20 × 0.4 Leica objective and a Hamamatsu ORCAER camera. GFP and DAPI filters were used to collect Alexa−488 and MPTS fluorescence, respectively, and the exposure time was 10 ms for both Alexa−488 and MPTS. Measurements were taken at 6 different time points: 0 hours, 12 hours, 1 day, 2 days, 4 days, and 7 days. To calibrate the microscope, fluorescent intensity of two fluorescence reference slides, for GFP and DAPI filters respectively, were recorded and used for background correction at every time point. Images were acquired and analyzed by using Metamorph imaging system version 6.3r1 (Universal Imaging) (Fig. 4).

Characterization of stability of reagents on the pre−loaded SlipChip.

To check the stability of reagents stored in the SlipChip, we loaded two solutons, 22 μM Alexa−488 in 10 mM Tris pH 7.8 and 44.8 μM Alexa−488 in 10 mM Tris pH 7.8, into two wells. 6 nL of each solution was loaded into each well. The wells were covered by a silanized glass slide (500 μm thick). The fluorescent intensity was measured at 9 time points: 1 hour, 7 hours, 24 hours, 50 hours, 99 hours, 169 hours, 270 hours, 435 hours and 772 hours. Fluorescence intensity was recorded using a Leica DMI6000 microscope (Leica Microsystems) with a 10 ×0.4 Leica objective and a Hamamatsu ORCAER camera. A GFP filter with an exposure time of 4 ms was used to collect Alexa−488 fluorescence. To calibrate the microscope, fluorescent intensity of a fluorescence reference slide for GFP filter was recorded and used for background correction at every time point; here the exposure time was 1 ms. 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 200 pixels by 200 pixels was selected in the middle of both wells. The integrated intensity of the region was plotted over time (Fig. S1).

Characterization of dilution ratio on the SlipChip.

To confirm that the fluorescent intensity of Alexa–488 is linearly correlated to the concentration in the working range of the fluorescent microscope (between 20 to 45 μM), we made a dilution curve on a SlipChip. First, 4 solutions, at strengths of 1/8, 1/4, 1/2, and 1 times the strength of the original Alexa–488 solution (44.8 μM in 10 mM Tris pH 7.8), were deposited into wells in the bottom plate of a SlipChip. 5 nLof solution were deposited into each well. The deposition was repeated twice more. The two plates were then assembled, as described in the dye experiment. The fluorescent intensity of the loaded wells was then measured by using a Leica DMI6000 microscope (Leica Microsystems) with a 20 × 0.4 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 200 pixels by 200 pixels was selected in the middle of every well of interest. The average of the integrated intensity of the regions belonging to the wells with the same Alexa–488 concentration was plotted against the corresponding concentration. (Fig. S2)

An array of fluorescent plugs with 45 μM Alexa–488 in 10 mM Tris pH 7.8, and an array of plugs with 10 mM Tris, were both prepared in the same way as in the dye experiment. On the bottom plate of a SlipChip, the two rows of wells in the bottom plate were loaded using the array of fluorescent plugs and two rows of wells at the bottom of the plate were loaded using the array of buffer plugs. The loading process was the same as in the dye experiment. The SlipChip was then assembly as described above. A sample solution containing 45 μM Alexa–488 in 10 mM Tris pH 7.8 was injected through the continuous fluidic path, followed by slipping to mix the sample fluorescent solution with either the other fluorescent solution or the buffer solution in the preloaded wells on the bottom plate. After ~ 10 minutes (to make sure that the sample and the reagents had fully mixed), we slipped the SlipChip back to its original position (i.e. the wells in the top plate were not directly in line with the dye–containing wells in the bottom plate). We then measured the fluorescent intensity of the wells in the bottom plate. By doing that, we could make the working parameters for measuring fluorescent intensity 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 (Fig. 5).

Protein samples for crystallization experiments on the SlipChip.

We obtained a sample of the photosynthetic reaction center (RC) from *B. viridis* from Professor James Norris of the University of Chicago. The sample arrived as 43 mg/mL in 0.08% (w/v) LDAO and 20 mM Na_2HPO_4/NaH_2PO_4 pH 6.0. We further modified the sample for crystallization. The sample ultimately consisted of 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.

Microphotographs under dim light were taken with a Leica MZ 16 Stereoscope with a Plan APO 0.63× objective **(**Fig. 6**)**.

Supplementary Table and Figures

Table S1: Screening Conditions for Crystallizing RC from *B.viridis*

No.	Precipitant	Salt	Buffer (pH)
1	30% PEG−8000		0.1 M CHES, pH 9.5
2	30% PEG−3000		0.1 M sodium citrate, pH 5.5
3	20% PEG−8000	0.2 M $Zn(OAc)_2$	0.1 M MES pH 6.0
4	3.2 M $(NH_4)_2SO_4$		40 mM Na_2HPO_4 / NaH_2PO_4,pH 6.0
5	3.6 M $(NH_4)_2SO_4$		45 mM Na_2HPO_4 / NaH_2PO_4,pH 6.0
6	4 M $(NH_4)_2SO_4$		50 mM Na_2HPO_4 / NaH_2PO_4,pH 6.0
7	2.8 M $(NH_4)_2SO_4$		0.1 M sodium citrate, pH 5.5
8	1.5 M $(NH_4)_2HPO_4$		0.1 M sodium acetate, pH 4.5
9	30% PEG−2000 MME		0.1 M Tris, pH 7.0
10	32% PEG−2000 MME		40 mM sodium citrate, pH 6.0
11	36% PEG−2000 MME		45 mM sodium citrate, pH 6.0
12	40% PEG−2000 MME		50 mM sodium citrate, pH 6.0
13	30% PEG−1000	0.2 M $Ca(OAc)_2$	0.1 M imidazole, pH 8.0
14	2.52 M $(NH_4)_2SO_4$		0.1 M sodium cacodylate, pH 6.5
15	1.4 M sodium citrate		0.1 M sodium cacodylate, pH 6.5
16	40 % PEG−4000	0.88 M NaCl	40 mM Tris, pH 7.8
17	45% PEG−4000	0.99 M NaCl	45 mM Tris, pH 7.8
18	50% PEG−4000	1.1 M NaCl	50 mM Tris, pH 7.8
19	3.75 M NaCl		0.1 M sodium potassium phosphate, pH 6.2
20	40% PEG−8000	0.2 M Li_2SO_4	0.1 M sodium acetate, pH 4.5
21	1.5 M sodium potassium tartrate	0.2 M NaCl	0.1 M imidazole, pH 8.0
22	30% PEG−1000		0.1 M Tris, pH 7.0
23	0.6 M NaH_2PO_4/1.6 M K_2HPO_4	0.2 M NaCl	0.1 M imidazole, pH 8.0
24	30% PEG−5000 MME	1 M NaCl	0.1 M sodium acetate, pH 4.8

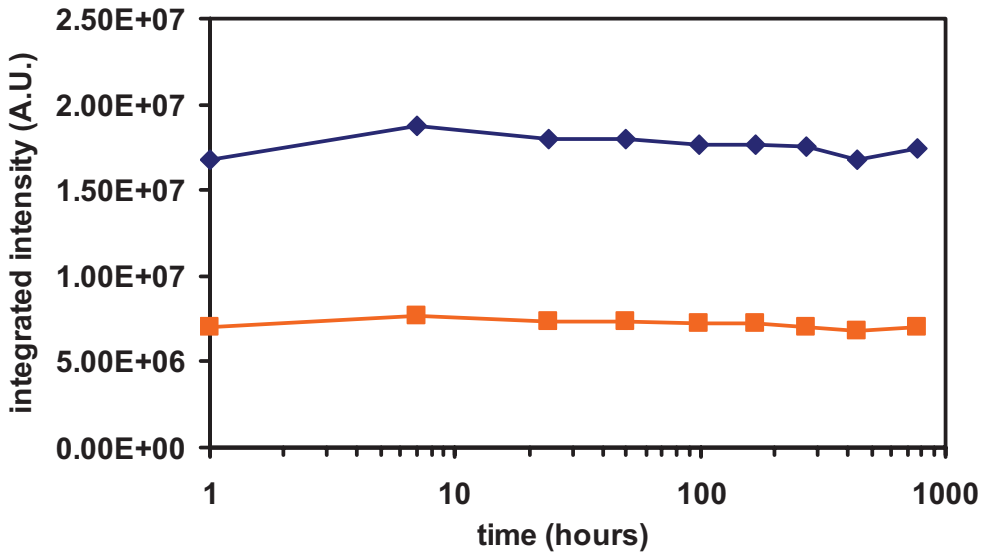

Fig. S1. Alexa−488 was used to characterize long term storage of reagent solutions in the SlipChip. Tracking the intensity (A.U.) of 2 individual wells over time indicated that the solutions in the wells were stable over 750 hours. In this experiment, the wells were covered by a silanized glass slide (500 μm thick), and the whole chip was immersed in FC−70 for storage in the dark.

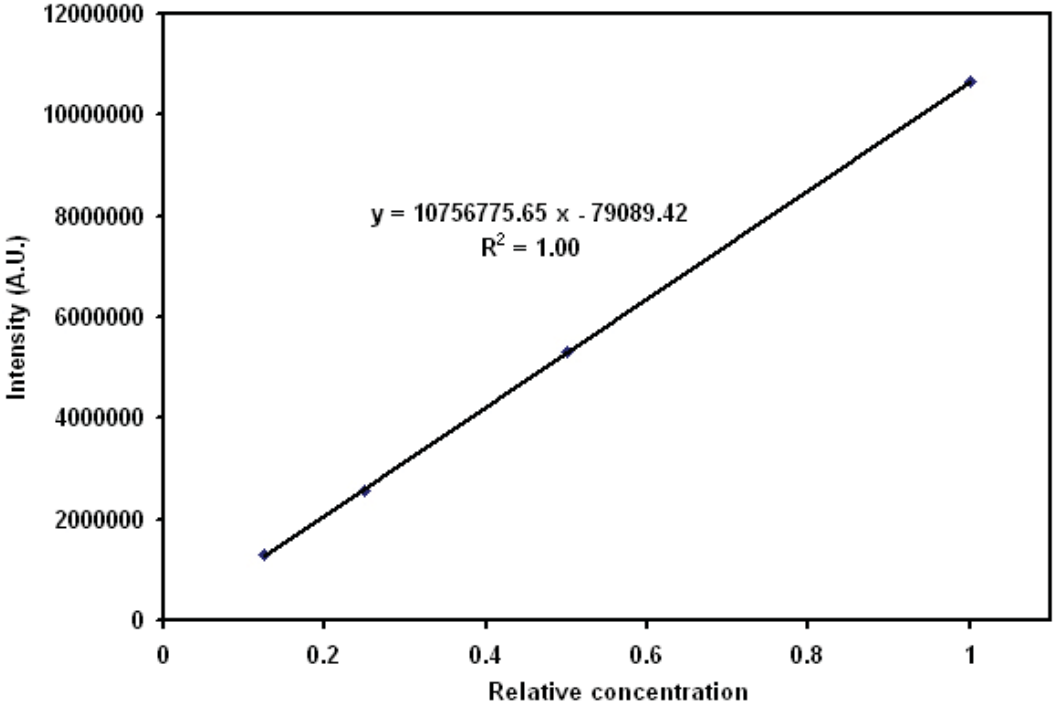

Fig. S2 Alexa−488 was used to quantify the mixing ratio in the SlipChip. A dilution curve (3 data sets) with 4 points (1/8, 1/4, 1/2, and 1 times the original solution) was plotted to confirm the linear relationship between intensity (A.U.) and concentration. In this experiment, 5 nL of each solution was deposited into the wells in the bottom plate of a SlipChip. The wells were then covered by the top plate of the SlipChip and the fluorescent intensity of each well was measured.