

Alternating current cloud point extraction on a microchip for preconcentration of membrane-associated biomolecules

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Electronic Supplementary Information

1. Movie of extraction by ACPE visualized with fluorescent DHPE (ACPE.mpg)

Experimental details are as noted in the main text. The movie is speeded up 4 times.

2. Estimation of Clausius-Mossotti factor in our system

Clausius-Mossotti factor in the ACPE experiment is:

$$\frac{\tilde{\epsilon}_{SR} - \tilde{\epsilon}_{SP}}{\tilde{\epsilon}_{SR} + 2\tilde{\epsilon}_{SP}}$$

where $\tilde{\epsilon}_{SR}$ and $\tilde{\epsilon}_{SP}$ denote the complex permittivities of the SR and the SP phases, respectively.

In general, complex permittivity $\tilde{\epsilon}$ is written as:

$$\tilde{\epsilon} = \epsilon_r \epsilon_0 - i \frac{\sigma}{2\pi f}$$

where $i^2 = -1$, f is the frequency, ϵ_0 is the permittivity *in vacuo*, ϵ_r is the relative permittivity of the phase. and σ is the conductivity of the phase.

The values of ϵ_r and σ of the SR (or SP) phase depend on the concentrations of Triton X-114 and KCl in the SR (or SP) phase. To estimate these values, we carried out a set of experiment and calculation. First, 10 mL of a test solution containing 0.15 mol L⁻¹ KCl and 0.5 wt % Triton X-114 (cloud point = 22 °C) was incubated at 37 °C overnight. The solution separated into the SR and the SP phases, and the volumes of the two phases were measured to be 0.18 and 9.82 mL, respectively. The concentration of Triton X-114 in the SP phase was assumed to be equal to its critical micelle concentration: 0.012 wt %. Therefore, the concentration of Triton X-114 in the SR phase was calculated to be 27 wt %.

Next, we estimated ϵ_r and σ of the SR and SP phases. Since the SP phase contains only a small amount of Triton X-114, ϵ_r and σ of the SP phase were assumed to be the same as those of 0.15 mol L⁻¹ KCl solution, and estimated to be 80 and 1.82 S m⁻¹, respectively. We also estimated those of the SR phase by using reported values obtained from Triton X-100 solutions,¹ since the concentration dependence of ϵ_r and σ of Triton X-114 solution has never been reported. ϵ_r and σ of the SR phase were estimated to be 60 and 0.6 S m⁻¹, respectively. Using these ϵ_r and σ values, ϵ_0 of 8.854×10^{-12} F m⁻¹, f of 5 MHz and the equations shown above, we obtained -0.29 as the real part of Clausius-Mossotti factor.

3. Numerically calculated electric fields in the microchannel with a two-electrode geometry

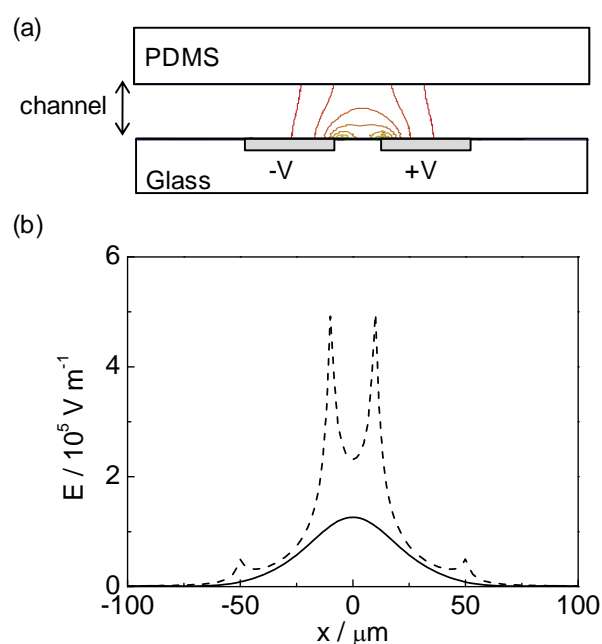


Fig. S1 Numerically calculated static electric fields in the microchannel with the two-electrode geometry, which was assumed to be infinitely wide in the y direction. The electrodes are symmetric in x -axis about the midpoint of the gap between the electrodes. The width and gap

between the electrodes are 40 μm and 20 μm , respectively. Channel depth: 25 μm . Applied voltage for each electrode was ± 10 V. (a) Contour plot of the electric field strength E . (b) E - x curves at $z = 0$ (the electrode surface, the dashed line) and at $z = 25$ μm (the PDMS surface, the solid line).

4. ACPE experiments with a two-electrode geometry

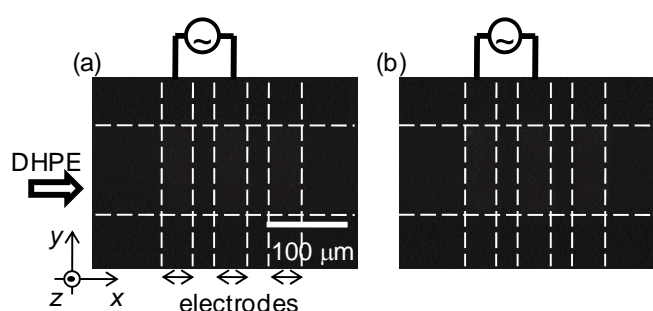


Fig. S2 Typical fluorescence micrographs of the ACPE experiments with the two-electrode geometry. AC voltages ($20 V_{p-p}$, 5 MHz) were applied between the left and the central electrode, and the right electrode was kept floated. The test solutions contained 0.5 wt % Triton X-114, and were flowing at a mean velocity around 0.1 mm s^{-1} . (a) DHPE solution at $t = 0$, (b) DHPE solution at $t = 60$ s. No increase in fluorescence was observed within the imaged area. We chose measurement areas on the left and the central electrodes with the same area size as used for the three-electrode geometry. CI at $t = 60$ s on the left and central electrodes were 0.93 ± 0.05 and 0.96 ± 0.08 , respectively.

Reference

1. K. Asami, *J. Phys.-Condes. Matter*, 2007, **19**, 376102.