Supplementary Material (ESI) for Lab on a Chip
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Supplementary information

1. Cell lysis evaluation by SDS-PAGE

The cell lysis condition was evaluated quantitatively by absorptiometry at 280 nm (OD_{280}), as described in the main text. However, the measured absorbance is attributed to all proteins, including contaminants. We additionally electrophoresed several protein solutions used in our experiment, as shown in Fig. S1. For comparative purposes, a solution flowed in the cell lysis chamber with no applied voltage in lane 1. Lysates for lanes 2 and 3 were prepared by the on-chip cell lysis, with applied voltages of 150 V (60 kV/cm) and 250 V (100 kV/cm), respectively. In the cell lysis, other parameters were as follows: flow rate for the cell lysis chamber (3 μL/min), duty (0.01%), and pulse width (50 μs), which was used to examine the diffusion phenomenon in the assay channel. Lanes 4 to 9 were obtained by diluting an F_1 solution purified off-chip conventionally. The initial F_1 concentration of 2.5 μM was adjusted to 10~100 nM. The result indicates that F_1 molecules consisting of α, β, γ-subunits were predominantly detected in lanes 2 and 3, even though other contaminated proteins were slightly observed in the same lanes. Focusing on these F_1 components, F_1 concentrations in lysates can be approximately estimated as 30 nM and 70 nM for lanes 2 and 3, respectively.

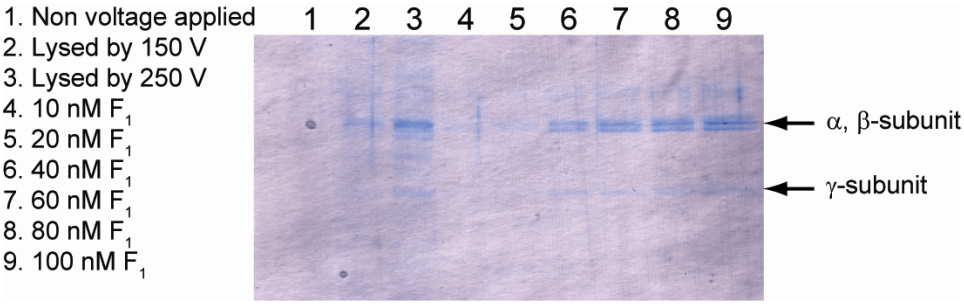

Fig. S1 Proteins separated by SDS-PAGE. The inset indicates the sample preparation for each lane.

2. The on-chip rotation assay using pseudo lysates

Unstable flow caused by missed operation of the SMM may disturb the concentration gradients of lysates, resulting in the failure to optimize the single molecule assay condition. To confirm the diffusion phenomena discussed in the main text, we imitated the diffusion using pseudo lysates in a Y-shaped channel. Lysates were prepared by mixing F_1 purified off-chip and contaminated proteins obtained by the sonication of *E. coli* without F_1 expression. Then the pseudo lysates were diluted to have the same OD_{280} as obtained in Fig. 3b: OD_{280}=0.16 for 150 V (60 kV/cm), 0.32 for 250 V (100 kV/cm), and 11.5 for sonication. The same assay protocol shown in Table 1 was performed by replacing the "cell solution" by the pseudo lysate. Densities of the rotating microspheres were plotted, as shown in Fig. S2, in which the same coloured symbols as used in Fig. 6 were used for comparison. The density was maximized at the similar position in the *y*-direction, as measured in the sequential assay in the SMM (Fig. 6b). The results support that lysate concentration is optimized for the single molecule assay.

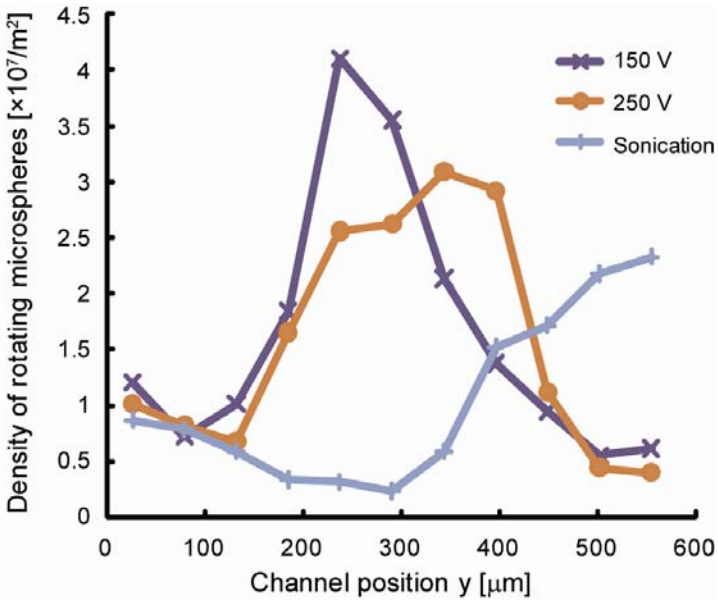

Fig. S2 Density distribution of rotating microspheres in the *y*-direction measured by assays using pseudo lysates.