Electronic Supplementary Information (ESI)

Enzyme Kinetics in Micrometre-to-Nanometre Scale Chambers at the Single-Enzyme Level

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Movie S1. Brownian motion of the nanoparticles inside the microchambers

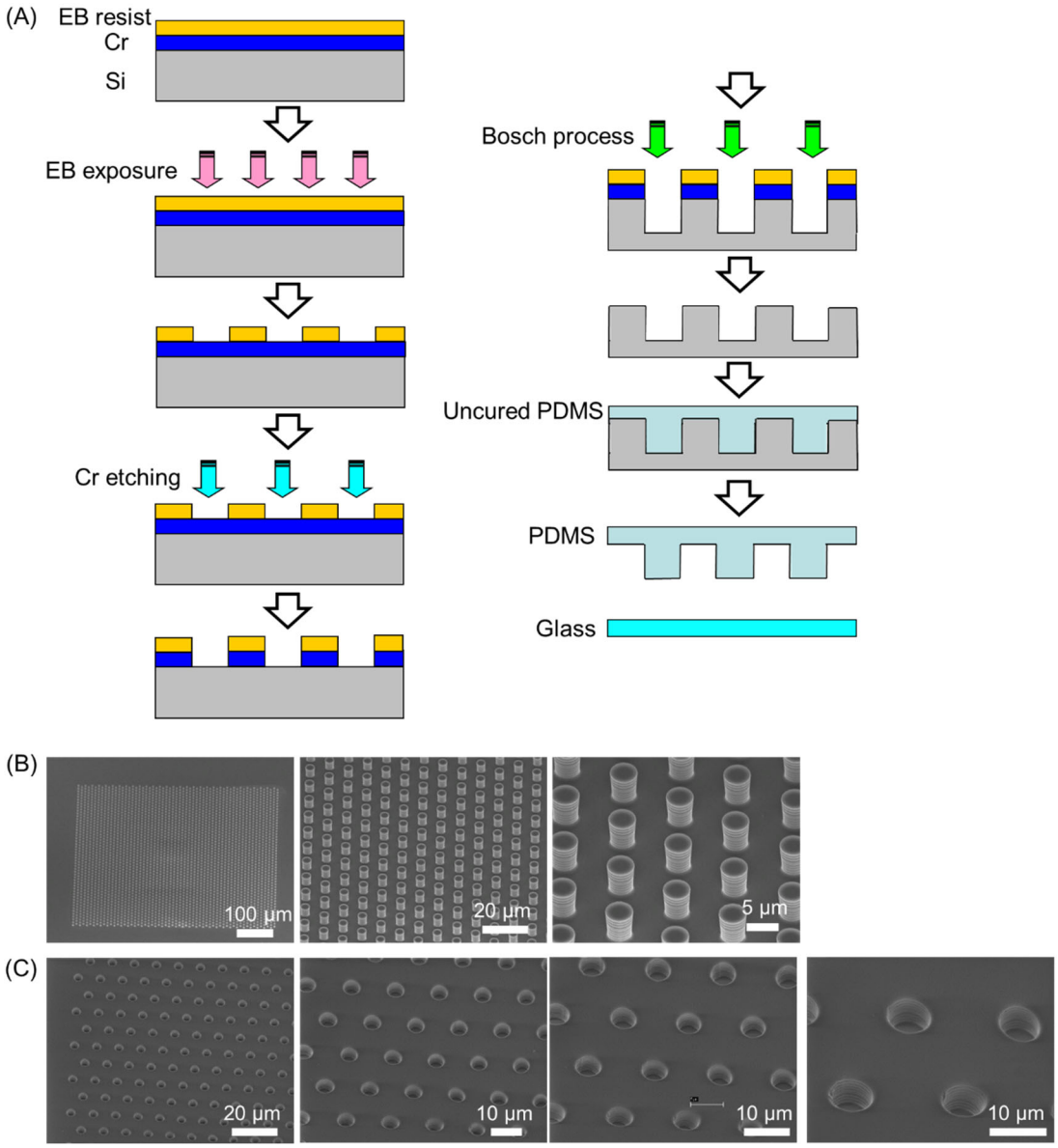

Figure S1. (A) Scheme of the fabrication of the microchamber mold and PDMS microchambers. (B) Fabricated silicon mold and the (C) PDMS replica of the 61-fL microchambers (diameter = 4.0 μm and height = 5.1 μm)

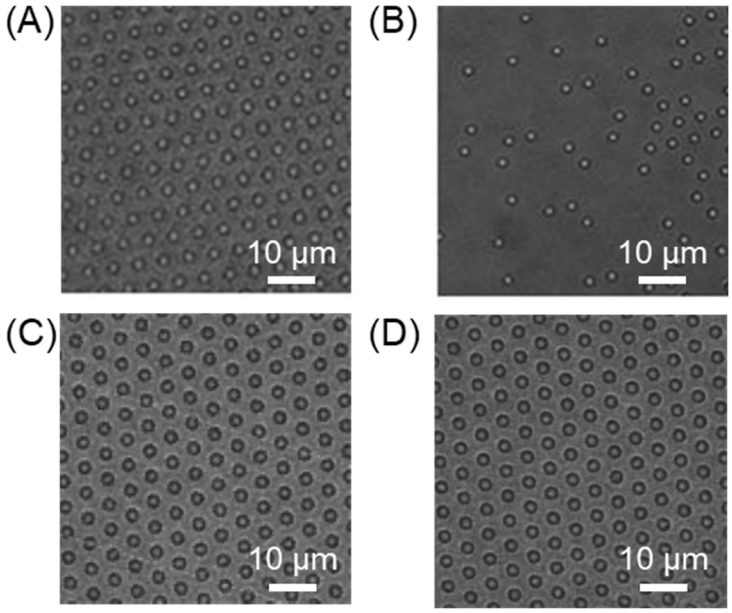

Figure S2. Liquid-evaporation test from the spaces of the buffer-enclosed 7.2-fL microchambers. The bright field images of the microchambers without any pretreatment at (A) 0 and (B) 5 min. (C) and (D) show the presoaked microchambers in a buffer solution for 0 and 5 min, respectively.

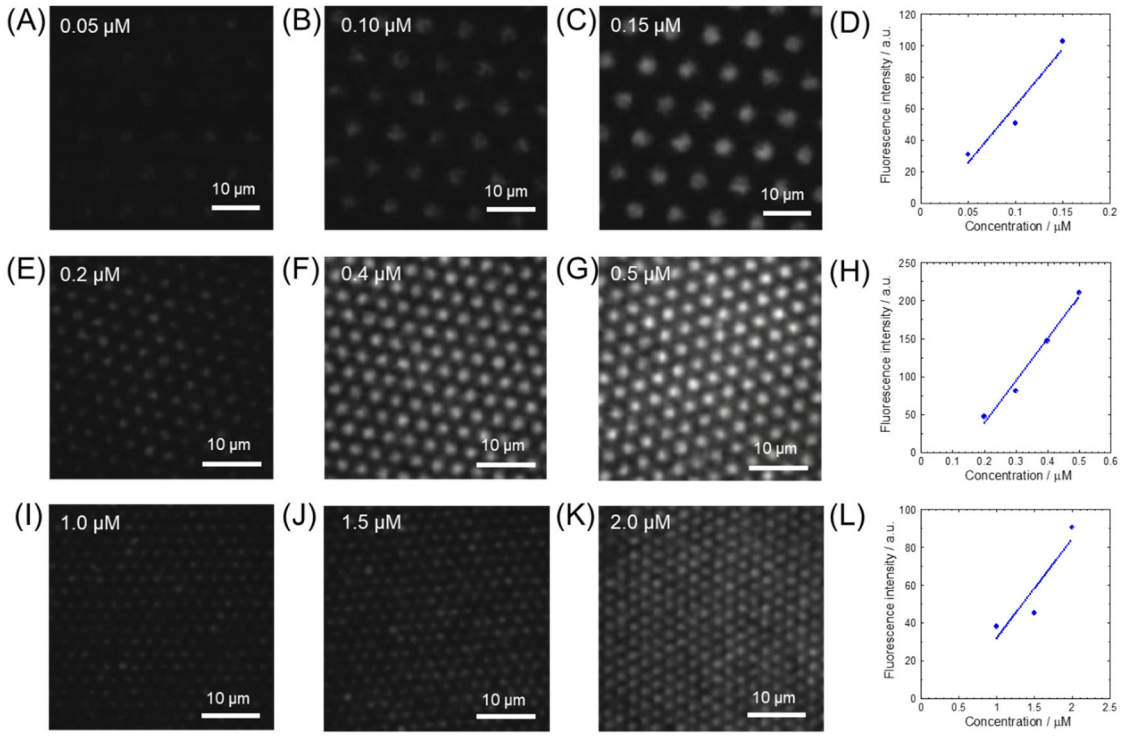

Figure S3. Generation of the standard curves of the fluorescence intensity of fluorescein in the microchambers for the quantification of the hydrolysis rate of the enzyme. Fluorescent microscopy images of the (A–C) 61 fL, (E–G) 7.2 fL, and (I–K) 510 aL microchambers containing fluorescein at the concentration indicated in the images, as well as their standard curves, (D) 61 fL, (H) 7.2 fL, and (L) 510 aL

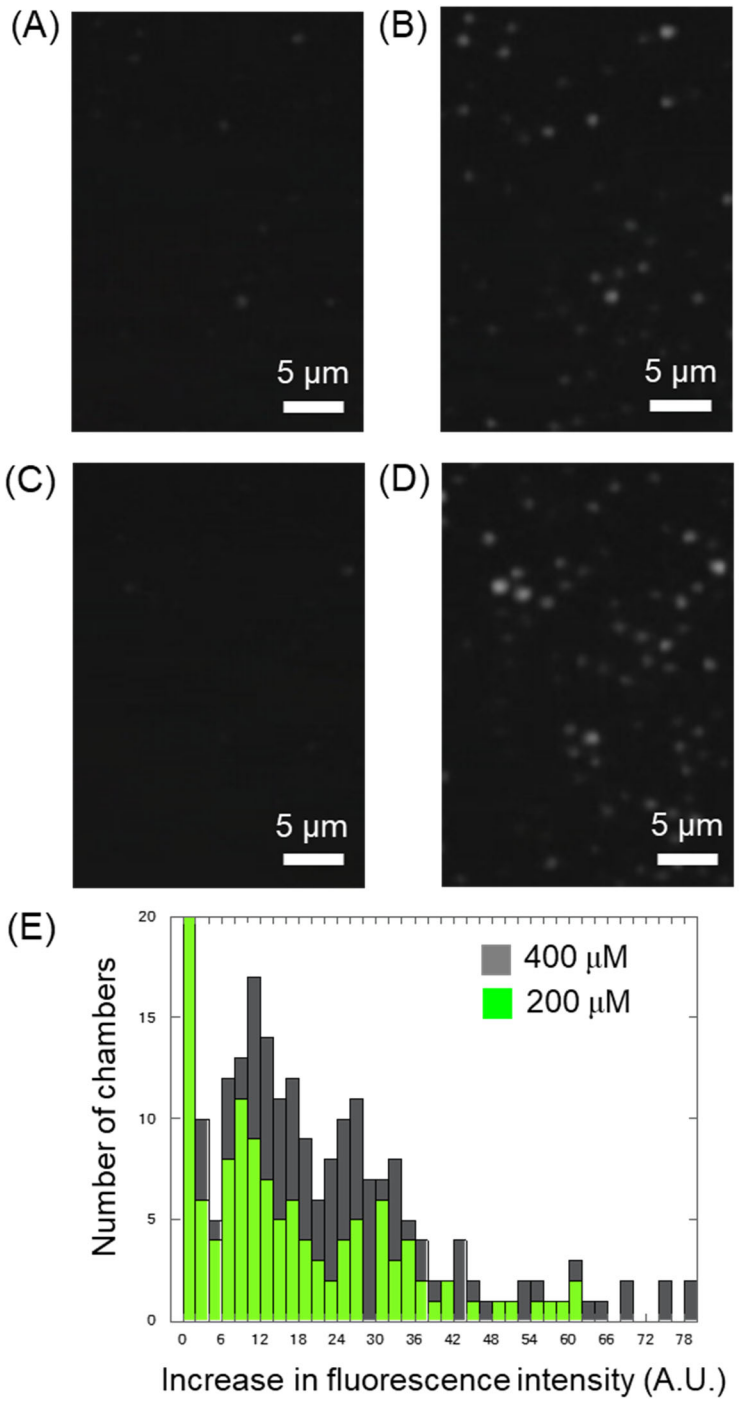

Figure S4. Single-β-gal assays in the 510-aL chambers. Fluorescent images of the chambers at (A) 0 and (B) 1 min when the concentrations of β-Gal and FDG were 6.25 ng/mL and 200 μM, respectively. (C) and (D) show the same concentration of β-Gal but 400 μM FDG at 0 and 1 min, respectively. (E) Histogram of the changes in the fluorescent intensity for 1 min at different FDG concentrations

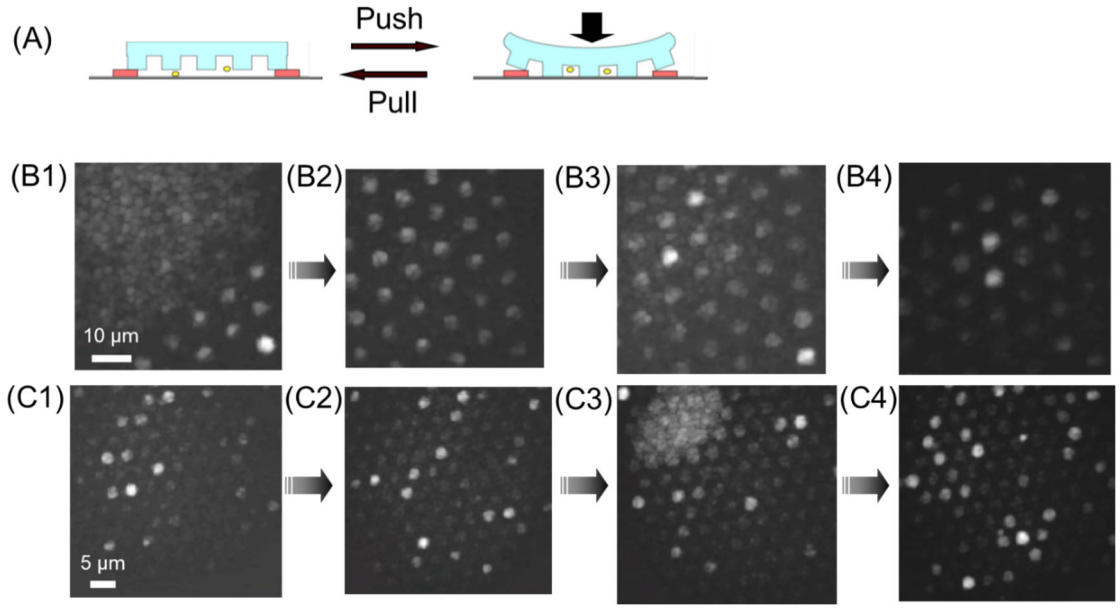

Figure S5. Push and pull of the microchamber experiments. (A) Schematic diagram of the push and pull operation. Consecutive fluorescent images of the (B1–4) 61-fL and (C1-4) 7.2-fL microchambers in the closed (pushing the chambers) state by repeating the push and pull operations. Location of the microchambers exhibiting high changes in the fluorescence intensity by repeating the push and pull operations, which indicate that β-gal, FDG, and fluorescein were not adsorbed on the surfaces of the chambers

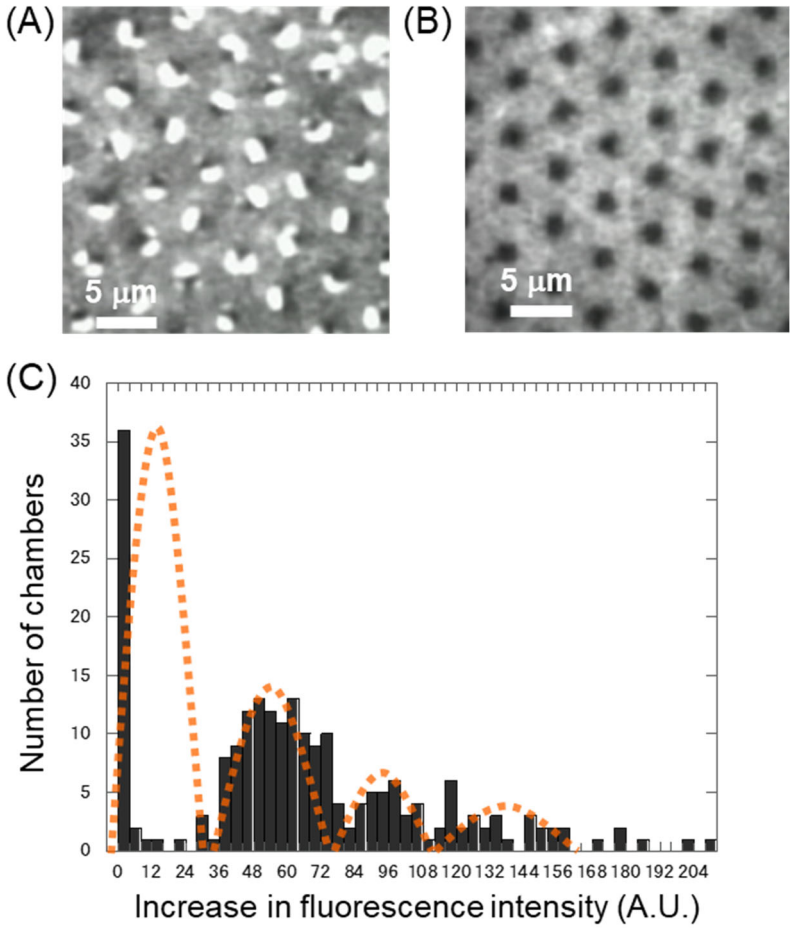

Figure S6. Effect of the dynamic coating of PC to prevent the nonspecific adsorptions of β-gal, FDG, and fluorescein onto the surface of the PDMS. Fluorescence images of the encapsulation of 1 mg/mL FITC–BSA in a 100-nM phosphate buffer (pH = 7.5) by the 7.2-fL chambers (A) without and (B) with the PC coating. (C) Calculated hydrolysis rate of β-gal was 7.8 s^{-1}, which was almost the same as that in the noncoated chambers (5.7 s^{-1})

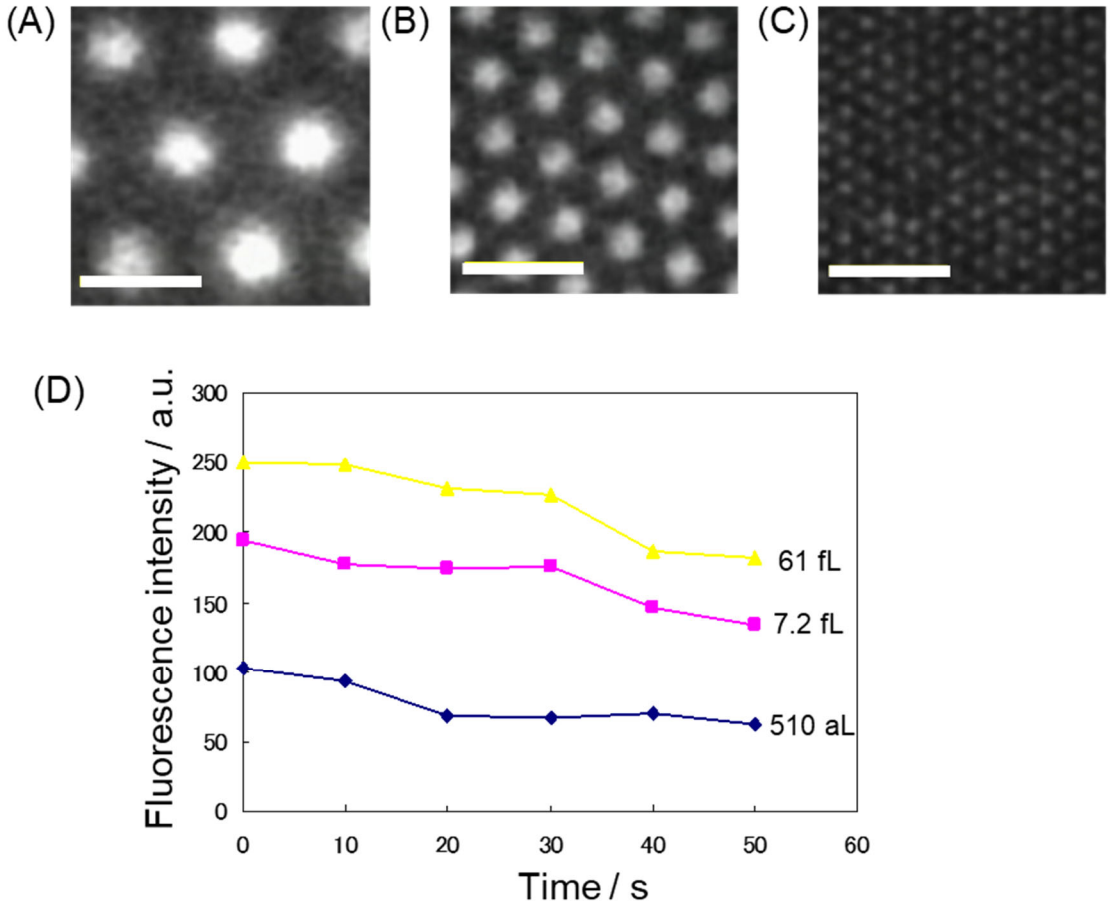

Figure S7. Photobleaching tolerance of fluorescein in the chambers. The excitation laser continued to irradiate for 50 s toward the (A) 61-fL, (B) 7.2-fL, and (C) 510-aL microchambers. (D) Time course of the fluorescence intensities under continuous illumination

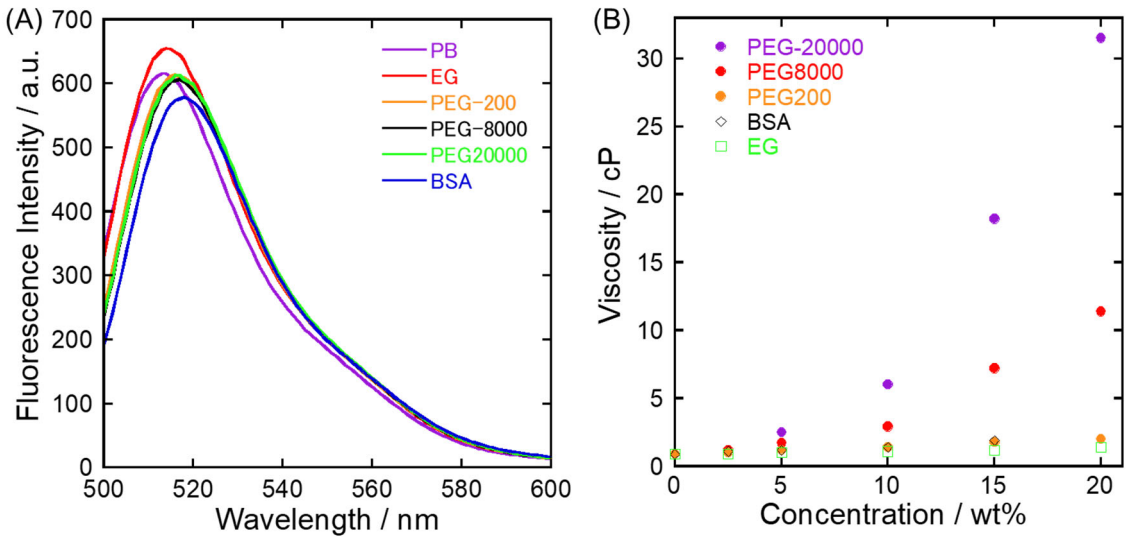

Figure S8. (A) Fluorescence spectra of the 0.5-μM fluorescein solution containing 10 wt% of the molecular crowders. Excitation wavelength: 488 nm. (B) Viscosities of the solution containing the molecular crowders at different concentrations

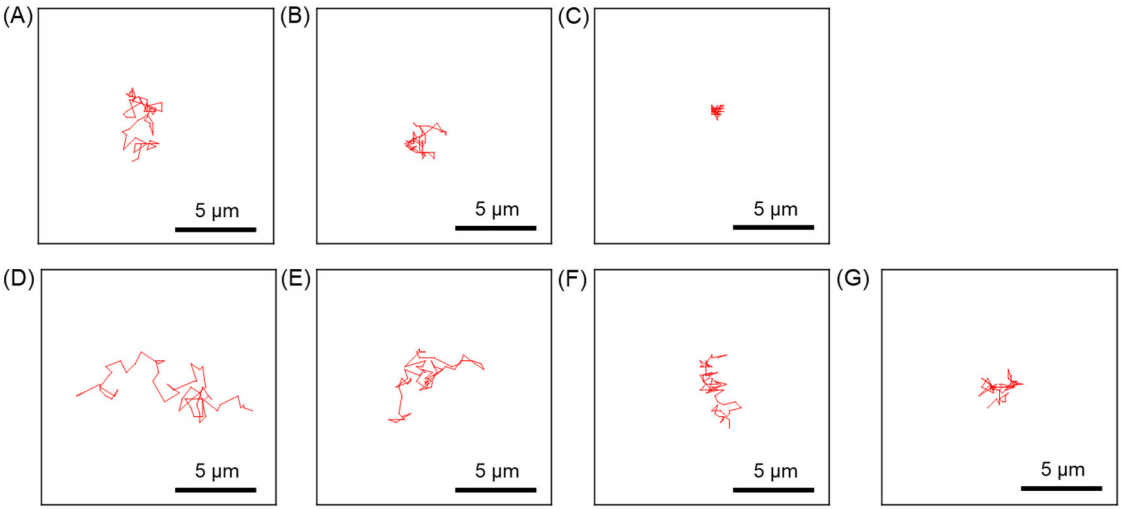

Figure S9. Trajectories of a fluorescent particle (diameter = 50 nm) in the (A) 61-fL, (B) 7.2-fL, and (C) 510-aL chambers, as well as in the (D) 5.0, (E) 10, (F) 20, and (G) 20 wt% PEG-8000 solution for 2 s