Supporting Information

Laser-induced shockwave chromatography: a separation and analysis method for nanometer-sized particles and molecules

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SI-1. Sample preparation

I. Water solutions in the capillary:

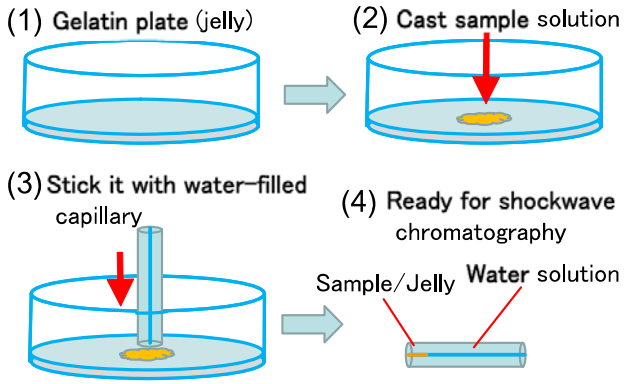
Water solutions were prepared from the buffers used for the protein solutions (see below) with glycerol (40% v/v) to obtain almost equal viscosity of the jelly in sol phase at 35 °C. The solutions were degassed in an ultrasonic bath before use and were filled in the glass capillary (0.5 μL, Minicaps, Hirschmann; 140-μm inner diameter, 3-cm long) before application of the sample.

II. Jelly (Gelatin plate):

Fish gelatin (0.4 g, P-5802, Nitta Gelatin) was added under stirring to the buffer solutions (10 mL) used for preparation of the protein solution (see below). The solution was kept at 60 °C for 20 min under stirring. Deionized water was used instead of the buffer for Qdot experiments. The gelatin solution (4 wt %) was cast on a dish and was cooled in the refrigerator to obtain a ~300 μm thick gelatin plate stable at room temperature (22 °C). The gel-sol transition temperature of the jelly was slightly below 35 °C.

III. Introduction of the sample/jelly into the capillary

The sample solution [0.5 μL, see below (**IV. Sample solutions**)], with an exception of bovine collagen, was cast on the gelatin plate (1 cm^2). The sample-gelatin mixture was stuck with the water filled capillary to introduce the sample at one end of the capillary. See steps (1)-(4) in the scheme shown below.



FITC-I (fluorescein isothiocyanate isomer-I) labeled bovine collagen:

FITC-I (6.5 mg, Wako Pure Chemicals) was added to borate buffer (50 mL, pH 9.18) to obtain 0.3 mM solution. The bovine gelatin (P-6393, Nitta Gelatin) used in our experiments is known to be a mixture of monomer, dimer and trimer of collagen according to the manufacturer's information (measured by GPC). Bovine gelatin (1.25 g) was added to the solution (50 mL), and the solution was stirred at 40 °C for 3 h under dark condition to obtain a fluorescent labeled gelatin solution (2.5 wt %). The gel-sol transition temperature of the jelly was slightly below 35 °C. The fluorescent gelatin solution was cast on a dish and cooled to obtain a ~300 μm thick fluorescent gelatin plate which was stuck with the glycerol/borate buffer filled capillary. In this case, step (2) in the scheme shown above is not necessary because the labeled collagen in the jelly is used as the sample.

IV. Sample solutions (FITC-I labeled proteins, Qdots and EtBr labeled DNA)

DNase I from bovine pancreas:

DNase I (3 mg, Wako Pure Chemicals) was added to borate buffer (10 mL, pH 9.18) to obtain 1×10^{-5} M solution. FITC-I/buffer (0.5 mL, 0.2 mM) was added under stirring to the solution. The reaction was kept in dark at 23 °C for 30 min under stirring. EDTA (Wako Pure Chemicals) and Dithiothreitol (DTT, Wako Pure Chemicals) was added to the solution ([EDTA] = 0.3 mM, [DTT] = 1 mM).

Ovalbumin from chicken egg and bovine serum albumin (BSA):

Albumin (4.4 mg of ovalbumin or 6.7 mg of BSA, Wako Pure Chemicals) was added to borate buffer (pH 9.18, 10 mL) to obtain a mixed solution of monomer and dimer ([albumin] = 1×10^{-5} M). DTT was added to the solution before fluorescent labeling to obtain monomer solution ([DTT] = 1 mM). FITC-I/buffer (0.5 mL, 0.2 mM) was added under stirring to the protein solution. The reaction was kept in dark at 36 °C for 4 h under stirring.

Myosin HC from rabbit muscle:

Myosin HC solution (50 μL, 4.3 mg/mL, Sigma-Aldrich) was diluted with phosphate buffer (50 μL, pH 7.7). DTT was added to the solution ([DTT] = 1 mM). FITC-I/buffer (5 μL, 0.2 mM) was added to the solution. The reaction was kept in dark at 4 °C (to avoid denaturation) for 3 h.

L-Lactic dehydrogenase (LDH) from bovine heart:

LDH solution (10 μL, 9×10^{-5} M, Sigma-Aldrich) was diluted by phosphate buffer (80 μL, pH 7.7). FITC-I/buffer solution (5 μL, 0.2 mM) was added to the solution. The reaction was kept in dark at 36 °C for 3 h.

Mixture of myosin HC and LDH:

FITC-I labeled Myosin (50 μL) and LDH (50 μL) solutions in phosphate buffer were mixed and diluted by the buffer (100 μL, pH 7.7).

Ferritin from equine spleen:

Ferritin solution (20 μL, 6×10^{-5} M, Sigma-Aldrich) was diluted by Tris-HCl buffer (100 μL, pH 8) to obtain ferritin/Tris-HCl ([ferritin] = 1×10^{-5} M) solution. FITC-I/buffer solution (5 μL, 0.2 mM) was added to the solution. The reaction was kept in dark at 36 °C for 3 h.

Urease from jack bean:

Urease (5 mg, Tokyo Chemical Industry) was added to Tris-HCl buffer (1 mL, pH7.5). FITC-I/buffer solution (50 μL, 0.2 mM) was added under stirring to the solution. The reaction was kept in dark at 36 °C for 4 h under stirring.

CdSe quantum dots (Qdots):

The mass of quantum dots, Qdot565 (CdSe, sphere, r = 2.3 nm) and Qdot655 (CdSe, prolate

spheroid, r_1 = 3, r_2 = 6 nm), were estimated to be ~180000 and ~790000, respectively, from the radius (*r*s, according to the manufacturer's information) and density of CdSe (5.8 g/cm^3).

Qdot565 and Qdot655 solutions (5 μL, 8 μM, Invitrogen) were diluted by deionized water (20 μL for Qdot565 and 24 μL for Qdot655) to obtain 1.6 μM and 2.0 μM solutions, respectively.

Mixture of Qdots:

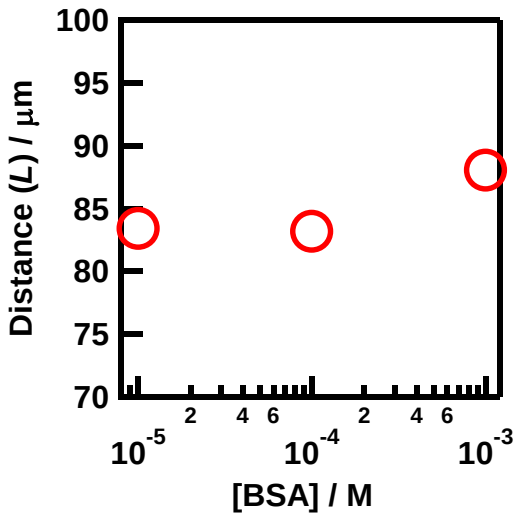
Qdot565 (3 μL) and Qdot655 (4 μL), shown above, were mixed and diluted by deionized water (7 μL) ([Qdot565] = 1.7 μM, [Qdot655] = 2.2 μM).

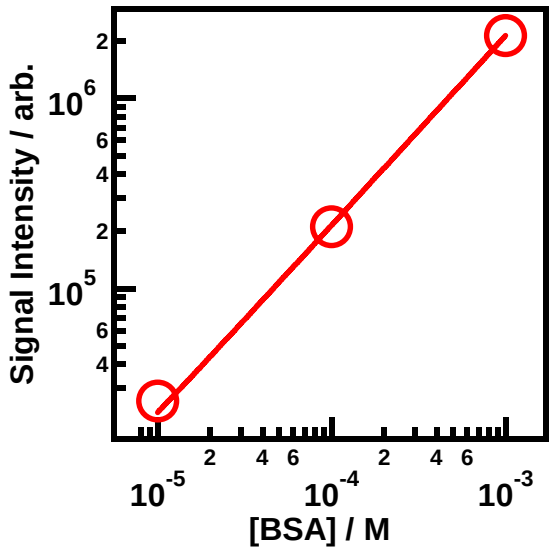
Calf thymus DNA:

Calf thymus DNA (8.6 mg, Worthington Biochemical) was added to Tris buffer (10 mL, [Tris] = 50mM, pH 7.5). Ethydium bromide/Tris buffer solution (5 μL, [EtBr] = 2×10^{-5} M, [Tris] = 50 mM) was added to the DNA solution (100 μL).

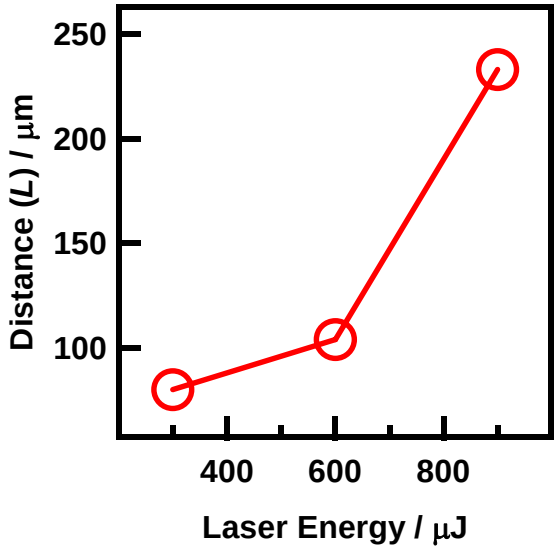
SI-2. Results for BSA obtained by the laser-induced shockwave chromatography with increased concentrations of the sample or with increased laser energies.

I. Distances (L / μm) and signal intensities were measured with increased concentrations of the BSA solution, and the data obtained were summarized in the figures shown below. The travel distances (Ls) were found to be almost independent of the concentrations (within the 2 pixels of CCD) while the signal intensities were almost proportional to the concentrations.

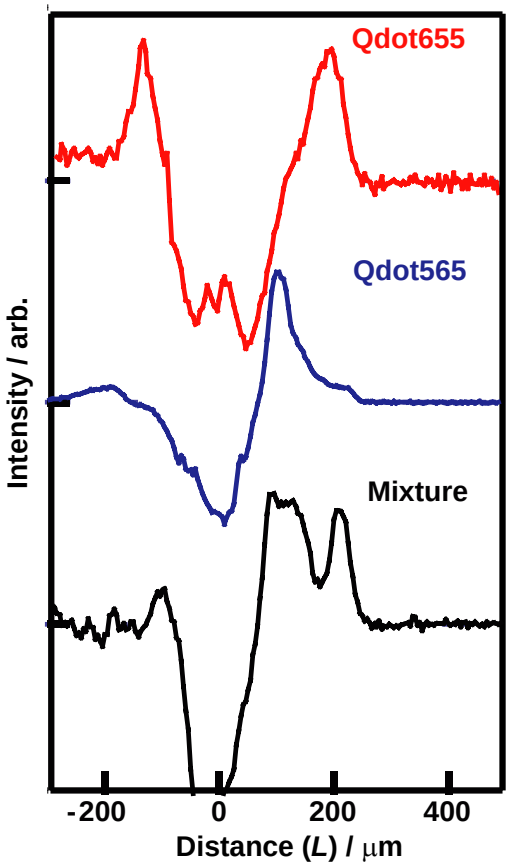




II. Distance (L / μm) of BSA was measured as a function of laser energy, and the data obtained were plotted in the figure shown below. The line in the figure is a guide for an eye. It was found that the travel distance increases as increasing laser energy.



SI-3. Chromatograms of inorganic nanoparticles (CdSe quantum dots) and their mixture.



Chromatograms of Qdot 655, Qdot 565 and their mixture. A mixture of different sized inorganic nanoparticles (CdSe quantum dot) was successfully separated.