Electronic Supplementary Material (ESI) for Chemical Communications.

SUPPLEMENTARY INFORMATION

Iron gall ink revisited: hierarchical formation of Fe(III)–tannic acid coacervate particles in microdroplets for protein condensation

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Contents

Experimental Details

Materials. Tannic acid (TA, Sigma), iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, 98%, Sigma), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, ≥98.0%, Sigma), dextran (500 kDa, Spectrum Chemical), poly(ethylene glycol) (PEG, 20 kDa, Sigma), hexadecane (99%, Sigma), sorbitan monooleate (Span 80, Sigma), fluorescein isothiocyanate-dextran (dextran-FITC, 500 kDa, Sigma), rhodamine B-labeled polyethylene glycol (PEG-RhoB, 10 kDa, Nanocs Inc.), Alexa Fluor 647-conjugated albumin from bovine serum (BSA-Alexa Fluor 647, Life Technologies) were used as received. Deionized (DI) water (18.3 MΩ·cm) from Milli-Q Direct 8 (Millipore) was used.

Formation of PEG/Dextran LLPS. The iron (Fe) solution was prepared by dissolving $FeCl_2$ (or $FeCl_3$) in the aqueous PEG solution (14% w/v) to the final concentration of 1 mM, and the TA solution by dissolving TA in the aqueous dextran solution (15% w/v) to the final concentration of 10 mM. For visualization of the PEG/dextran LLPS, PEG-RhoB or dextran-FITC was used with the concentration of 1% (w/w). Hexadecane containing 5 wt% Span® 80 was used as the continuous phase in the microfluidic channel. Microdroplets were generated by flowing the PEG and dextran solutions at the speed of 1 μL/min into two middle channels of a flow-focusing microfluidic device (Dolomite) with the continuous phase of hexadecane (6 μL/min). After being collected to a microtube containing hexadecane, the microdroplets were incubated at room temperature overnight. For protein condensation, BSA-Alexa Fluor 647, as a protein model, was added to a PEG solution at the concentration of 1.0 mg/mL.

Characterizations. Optical micrographs were taken with a DMI3000B inverted microscope (Leica). DIC and CLSM imaging was performed with a LSM 700 confocal microscope (Carl Zeiss). The data on average diameters and fluorescence intensity profiles were obtained by Image J (NIH).

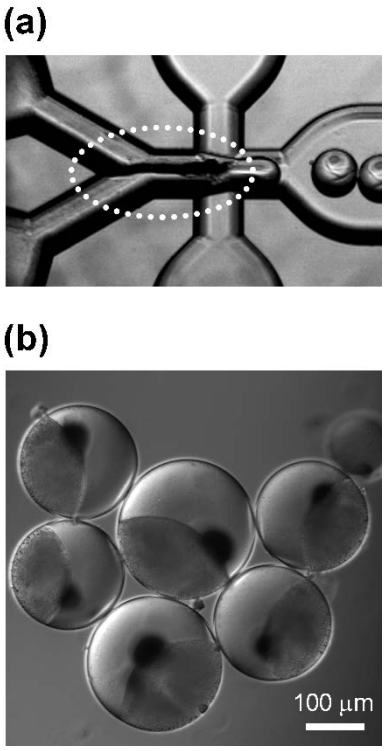

Fig. S1 (a) Optical image of a microfluidic junction clogged by uncontrollable formation of Fe(III)-TA complexes, when $FeCl_3$ was used. The dotted white ellipsoid shows that the Fe(III)-TA complex was formed uncontrollably in the channel. (b) DIC image of polydisperse microdroplets, when $FeCl_3$ was used.

Fig. S2 Optical image of aqueous PEG and dextran biphasic solutions. The purple color of the PEG phase indicates that TA in the dextran phase moved into the PEG phase.

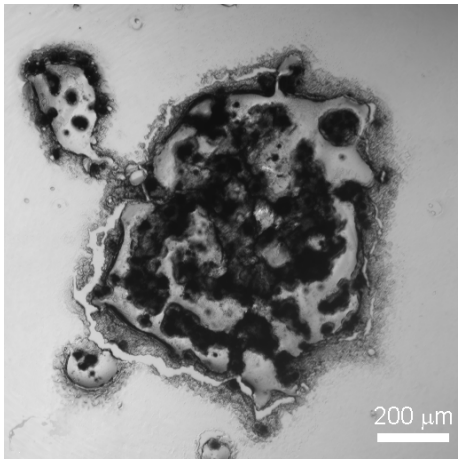

Fig. S3 DIC image of the Fe(III)-TA particles in the dried microdroplets. The non-spherical shapes of the particles imply that the particles are liquid-like.