Electronic Supplementary Information (ESI)

Liquid–liquid phase separation-assisted Raman microscopy for sensitive and label-free analysis of enzymatic reactions and protein–small molecule interactions

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1. Figures

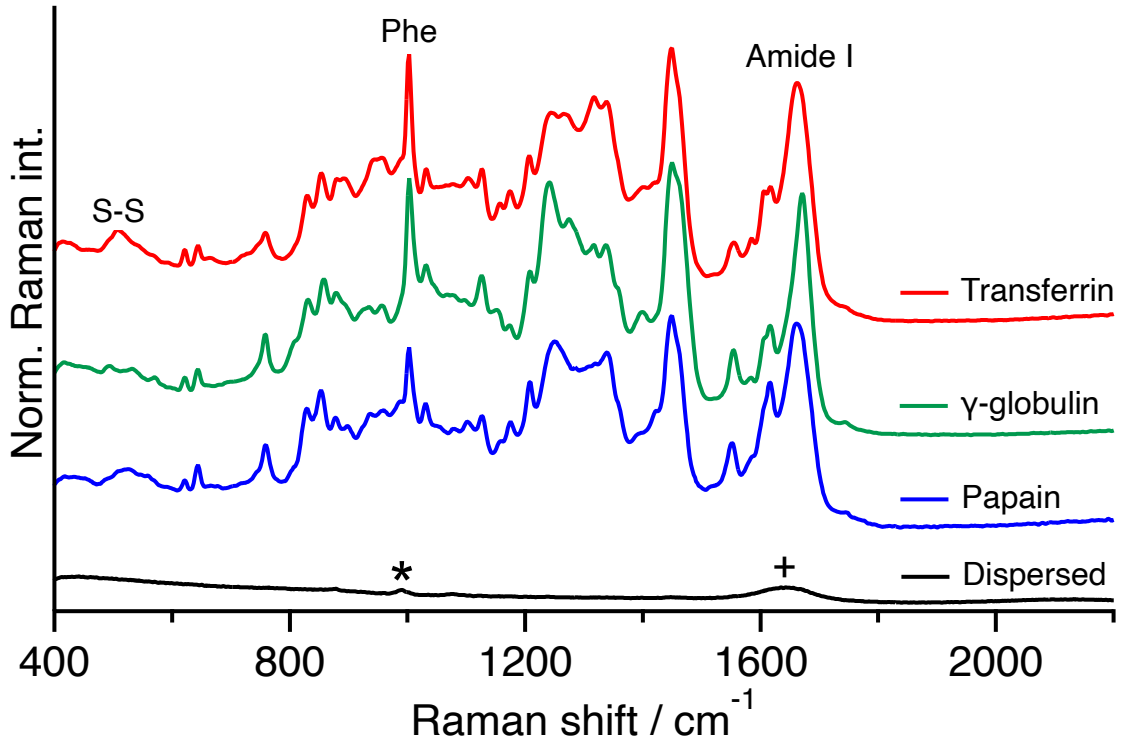

Fig. S1 Examples of Raman spectra of the droplets formed from 50 μM protein solutions (transferrin (red), γ-globulin (green) and papain (blue)) using the LLPS method, together with that of the dispersed solution of 50 μM γ-globulin (black). Polyethylene glycol (PEG 6000) powder was dissolved in a buffer solution (50 mM phosphate, 300 mM NaCl, pH 7) containing a protein with 50 μM until a concentration of 50% was reached, and protein droplets were created. All the spectra were normalized to the intensity of the O–H stretching band in the 3500–3600 cm^{-1} region. Almost no protein Raman bands were detected in the Raman spectra of dispersed solutions due to low concentrations. In contrast, Raman bands of proteins were clearly observed using the LLPS method. The bands labeled with * and + in the dispersed spectrum are due to phosphate ions and water, respectively.

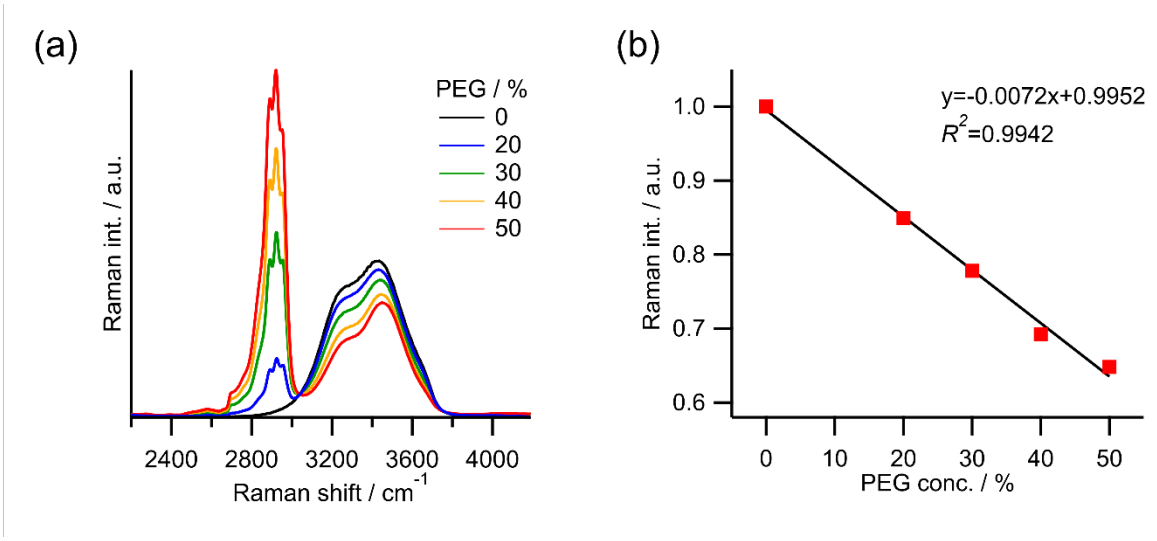

Fig. S2 (a) Raman spectra of 0-50 wt% PEG aqueous solutions in buffer. (b) Ratio of the integrated Raman intensity of the buffer solution containing PEG to the buffer solution in the region of the O-H stretching band (3500-3600 cm^{-1}). The integrated relative Raman intensity was 0.68 in the presence of 50 wt% PEG, due to the volume-exclusion effect. Therefore, protein concentrations evaluated by calibration lines need to be multiplied by a factor of 0.68 to obtain absolute concentrations.

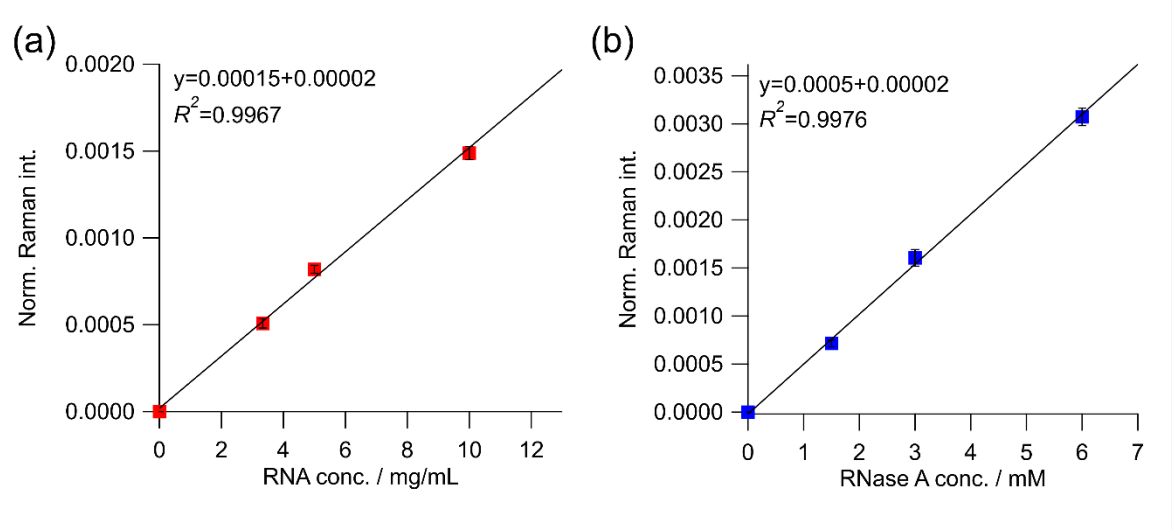

Fig. S3 Calibration lines for the quantification of (a) RNase A and (b) RNA concentrations. The Raman spectra of RNase A and RNA in homogeneous solutions were normalized to the integrated intensity of the O–H stretching band of water in the region of 3500–3600 cm^{-1}. The average intensity of the band at 1001±5 cm^{-1} of RNase A was plotted against the RNase A concentration in (a). The average intensity of the band at 1480±5 cm^{-1} was plotted against the RNA concentration in (b). Error bar is SE (n = 3).

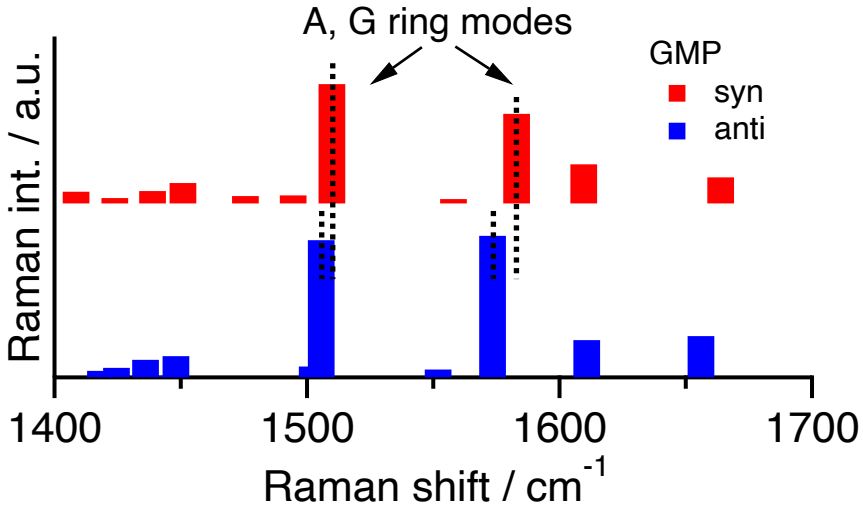

Fig. S4 Calculated Raman spectra of *syn* and *anti*-guanosine in vacuo using DFT calculations with B3LYP 6-311+G(2d, p). The bands at 1510 and 1583 cm^{-1} in red were attributed to the ring vibrations of *syn*-guanosine. The corresponding bands were calculated at 1506 and 1573 cm^{-1} for *anti*-guanosine (blue).

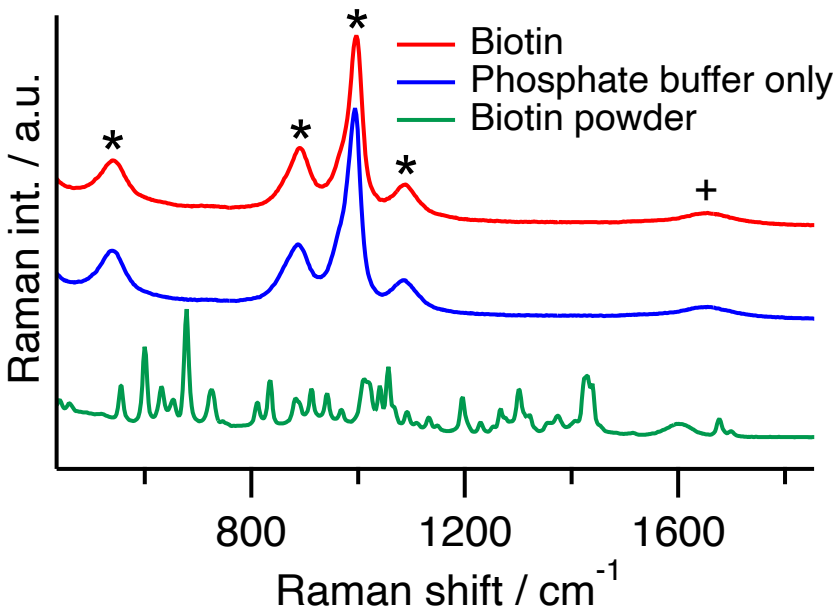

Fig. S5 Raman spectra inside droplets formed from a phosphate buffer including biotin (10 mM) (red) and a phosphate buffer only (blue), together with that of powdered biotin (green). The bands labeled with * and + are due to phosphate ions and water, respectively.

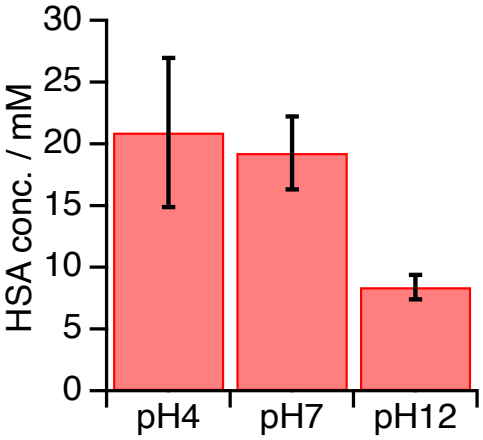

Fig. S6 pH dependence of the concentrations of HSA inside the droplets. The concentrations were determined by the method based on the O-H stretching band of water, as mentioned in the text. Error bar is SD (n = 3).

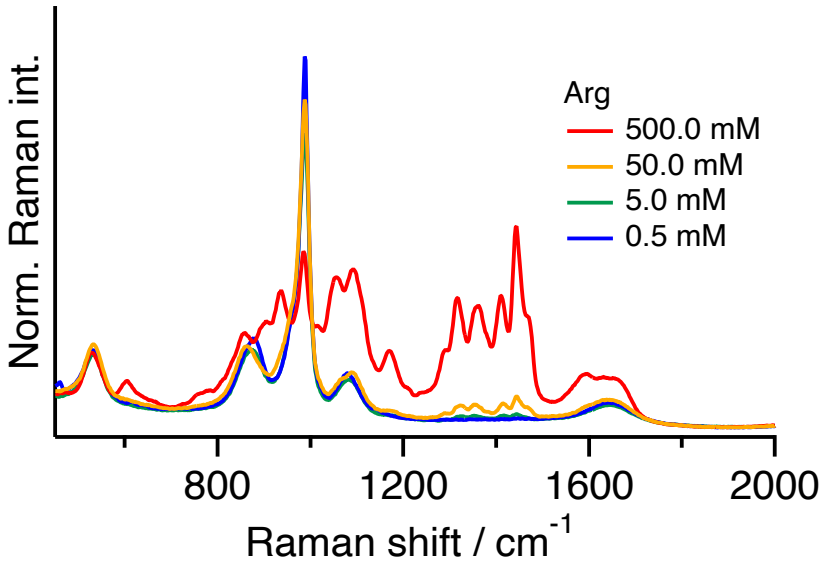

Fig. S7 Raman spectra of arginine droplets in a high-concentration PEG solution. The initial concentration of arginine in each spectrum is indicated in the upper right. The concentration efficiency of arginine depends on the initial arginine concentration. The Raman spectra were normalized to the O-H stretching band of water at around 3400 cm^{-1} outside the droplets.

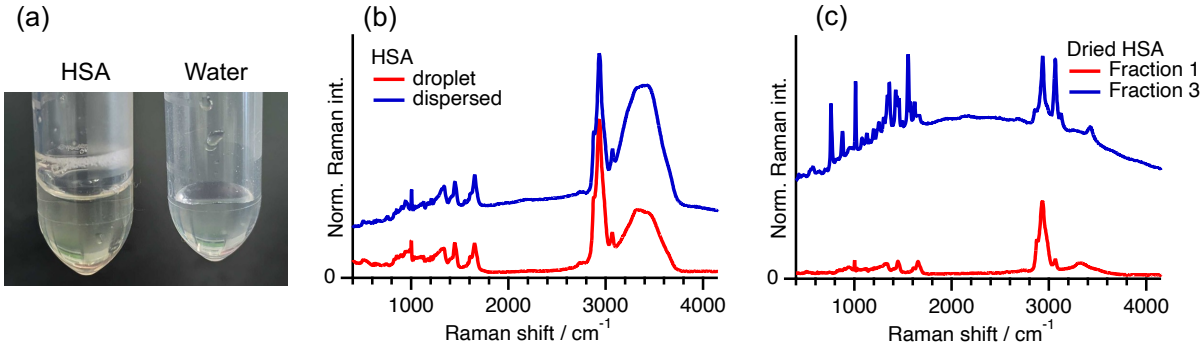

Fig. S8 (a) Photographs of buffer solutions with (left) and without (right) HSA. (b) Raman spectra of HSA in droplets (red) and in a dispersion solution (~ 10 mM) (blue). The Raman spectra are roughly normalized to the C-H stretching band at around 2900 cm^{-1}. (c) Raman spectra of Fraction 1 and Fraction 3 extracted from the gel filtration column. Fraction 1 contains extracted HSA, while Fraction 3 contains extracted impurities. To increase the concentration, the fractions were dried and then measured.

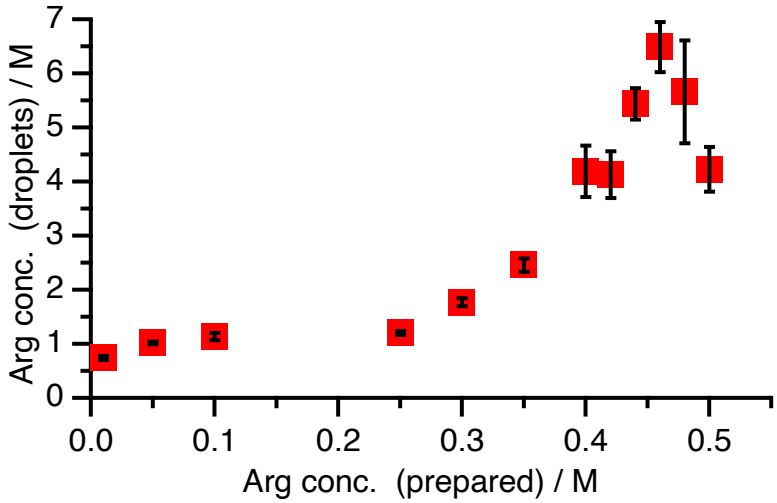

Fig. S9 Concentrations of arginine in droplets relative to initial arginine concentration. Error bar is SE (n = 5).