Electronic Supplementary Information for
Kinetic description of water transport during spontaneous emulsification induced by Span 80.

Mao Fukuyama*, Tomoko Mizuguchi*, Piangrawee Santivongskul, Yuri Ono, Motohiro Kasuya, Arinori Inagawa, and Akihide Hibara*

Corresponding authors:
Mao Fukuyama, Tomoko Mizuguchi, and Akihide Hibara
Email: maofukuyama@tohoku.ac.jp, mizuguti@kit.ac.jp, and hibara.a.aa@m.titech.ac.jp

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Section 1. Image analysis of microdroplets in the microfluidic device.

The water flux from microdroplets to nanodroplets was calculated from the micrographs of microdroplets. The volume and surface area of the microdroplets were calculated according to the previous report.[1] Briefly, the shape of the microdroplet was expressed as the combination of the outer half of a torus and a cylinder as shown in Figure S1. The height and the radius of the microdroplet were defined as r and h, respectively. The volume, V, and surface area, S, of the microdroplets were calculated using the following equation.

$$V = \pi \int_{-\frac{h}{2}}^{\frac{h}{2}} \left\{ \left(r - \frac{h}{2} \right) + \sqrt{\left(\frac{h}{2} \right)^2 - x^2} \right\}^2 dx$$

$$= \frac{\pi h}{2} \left\{ 2 \left(r - \frac{h}{2} \right)^2 + \frac{\pi h}{2} \left(r - \frac{h}{2} \right) + \frac{4}{3} \left(\frac{h}{2} \right)^2 \right\} \quad \text{(S1)}$$

$$S = 2\pi (r - h/2)^2 + \int_{-\frac{h}{2}}^{\frac{h}{2}} \left\{ \left(r - \frac{h}{2} \right) + \sqrt{\left(\frac{h}{2} \right)^2 - x^2} \right\} \sqrt{1 + \frac{x}{h^2 - x^2}} dx$$

$$= 2\pi \left(r - \frac{h}{2} \right)^2 + \pi h \left(h + \pi \left(r - \frac{h}{2} \right) \right) \quad \text{(S2)}$$

The flux of water (J) is described as

$$J(t) = -\frac{\Delta V(t)/\Delta t}{S(t)} \quad \text{(S3)}$$

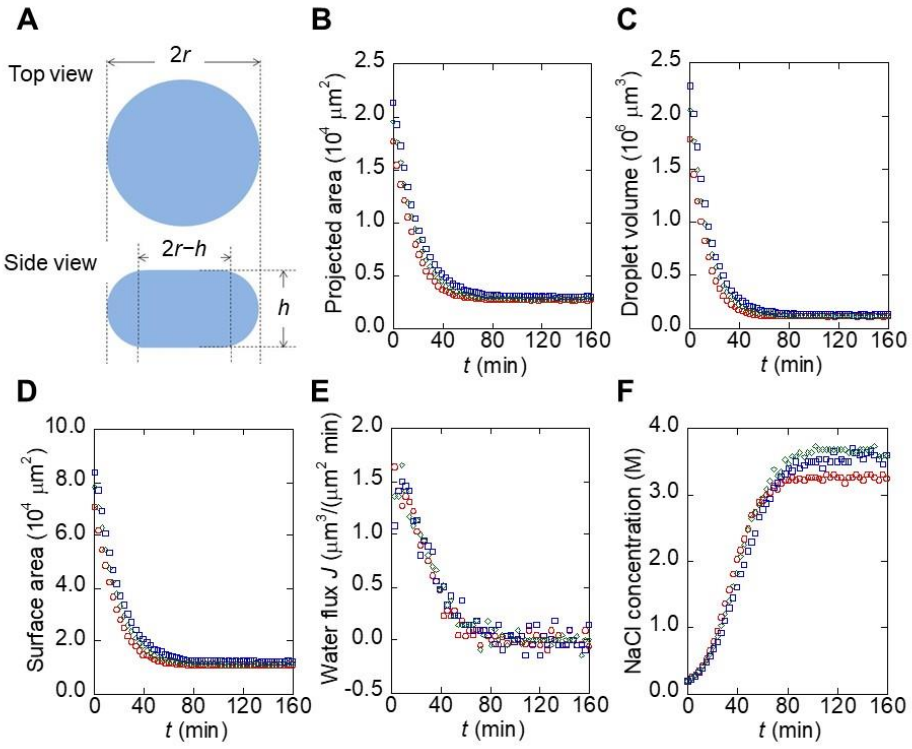

Figure S1. Image analysis of microdroplets in microfluidic devices during spontaneous emulsification. (A) Microdroplet shape. Time evolution of the projected area of microdroplets (B), droplet volume (C), surface area (D), water flux (E), and NaCl concentration (F). The initial NaCl concentration in the microdroplet and the NaCl concentration for the pretreatment of organic phase were 0.2 M and 3 M, respectively.

Section 2. Calculations of the activity of water in the aqueous phase.

The activity and chemical potential of water in NaCl aqueous solution was calculated using following equation.[2]

$$\mu_{w,aq} = \mu_w^\circ + \Delta\mu_{w,aq} \quad \text{(S4)}$$

$$= \mu_w^\circ + k_B T \, ln a_{w,aq} \quad \text{(S5)}$$

$$= \mu^\circ + k_B T \, (-\nu m W_w \varphi / 1000) \quad \text{(S6)}$$

where $\mu_{w}{}^\circ$, k_B, T, and $a_{w,aq}$ are the standard chemical potential of water, Boltzmann constant, temperature, and water activity in the aqueous phase, respectively. The activity term is described using the number of ions in the salt, ν, molarity, m, molecular weight of water, W_w, and the osmotic coefficient, φ. The value of $a_{w,aq}$ is obtained by calculating the molarity of NaCl [3,4] and the osmotic coefficient of water[5] from molar concentration of NaCl in aqueous phase ($C_{NaCl,aq}$).

Section 3. MD simulation of water behavior in Span 80 nanodroplets with different water content.

MD simulations of the nanodroplets consisting of water and Span 80 with ratios of 70/70 molecules and 70/140 molecules were conducted (Figure S2A). It should be noted that although the 70/70 molecules nanodroplet looks smaller than 70/140 nanodroplet, the actual size are similar. As shown in Figure 2SB the distribution of the hydrophobic moiety of Span 80 is almost the same. The hydrophilic moiety of Span 80 is distributed more widely in 70/70 molecules (Figure S2C). It is because that sometimes the 70/70 nanodroplet distorted. Figure S2D shows the Gibbs free energy profile for the insertion of a water molecule from the hexadecane phase into the reverse micelle. The potential of mean force is a free energy surface along the chosen reaction coordinates. Here, the distance between the center of mass of the reverse micelle and the water molecule was chosen as the reaction coordinate. The position at $R = 0$ is the center of micelle, and the positions at $R > 2.5$ nm are in the hydrocarbon phase. The value of the free energy when the water molecule is at $R = 4.0$ nm in the hexadecane phase is set to zero. In the regions at $R > 1.5$ nm, the free energies in both systems are almost same. At $R < 1.5$ nm, the system s70/w70 has smaller values than the system s70/w140, and in the center of micelle, the free energy difference between two systems is about 1 kcal/mol. Therefore, a water molecule is more stabilized inside the micelle of s70/w70 system. This result is consistent with the experimental result, in which the chemical potential of a water molecule is smaller in the nanodroplets with less water.

In addition, the hydrogen bonds between the water-water, water-Span 80, and Span 80-Span 80 molecules were stronger in the s70/w70 nanodroplet compared to those in the s70/w140 nanodroplet (Table S1). This result is consistent with previous studies regarding the stronger hydrogen bonds of water molecules at the interface of the Span 80 [6] and AOT [7] nanodroplets.

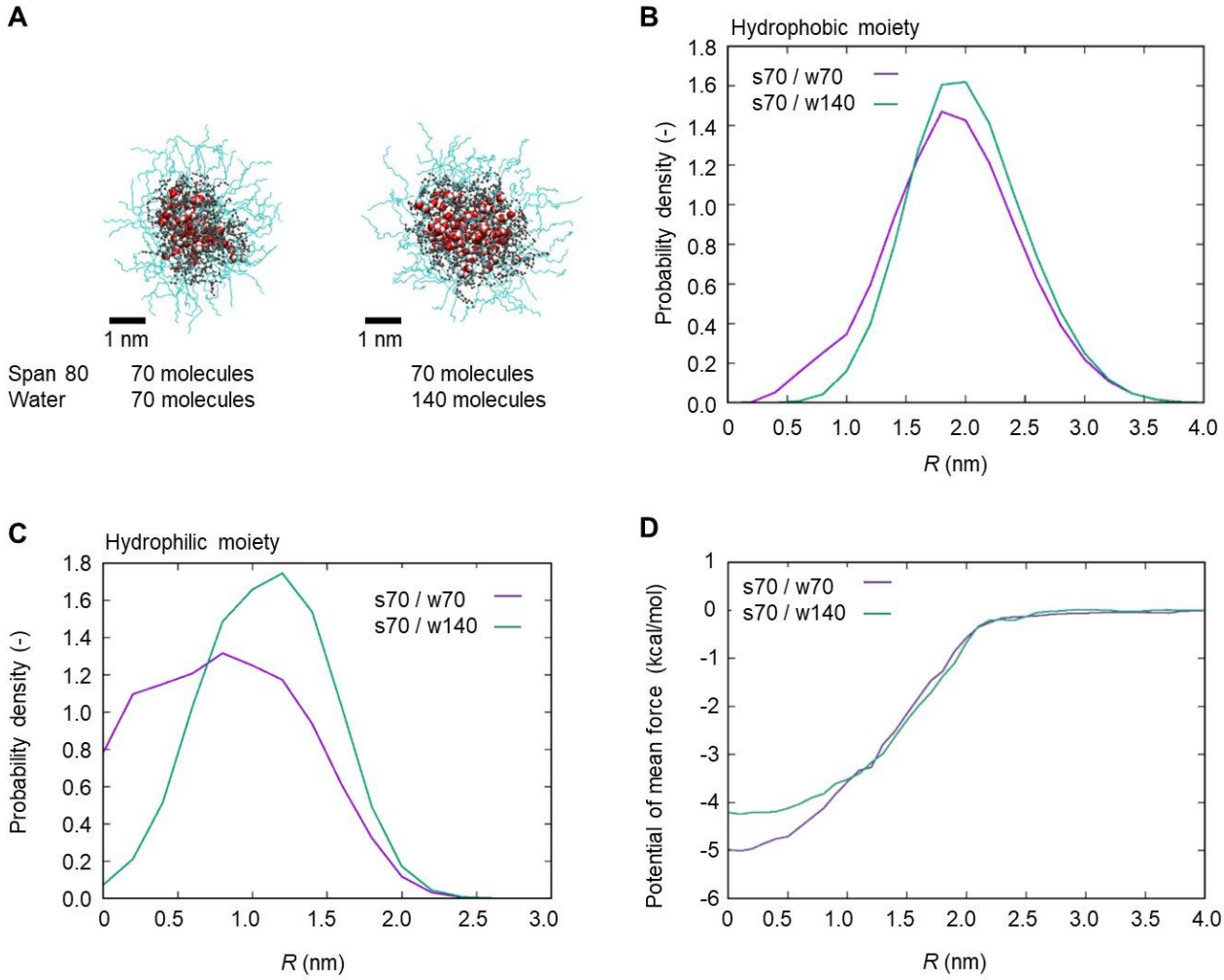

Figure S2. MD simulation of water behavior in Span 80 nanodroplets with different water content. (A) Snapshots of the final configurations of Span 80 with different numbers of water molecules. (B) and (C) The distribution of the probability densities of hydrophobic moiety (B) and hydrophilic moiety (C) of Span 80 in the nanodroplets. (D) The distribution of the potential of the mean force over the distance from the center of the micelle (R).

Table 1 Hydrogen bonds in nanodroplets composed of 70 Span 80 molecules in the MD simulation. t and ΔG indicate the lifetime of hydrogen bonds and activation free energy for hydrogen-bond breaking

	70 water molecules		140 water molecules	
	t (ps)	ΔG(kJ/mol)	t (ps)	ΔG(kJ/mol)
Water - water	187	17.5	22	12.2
Water - Span 80	506	20.0	132	16.6
Span 80 - Span 80	3559	24.7	2204	23.6

Section 4. Water content in the organic phase containing Brij S2 and bis(2-ethylhexyl) hydrogen phosphate (BEHP).

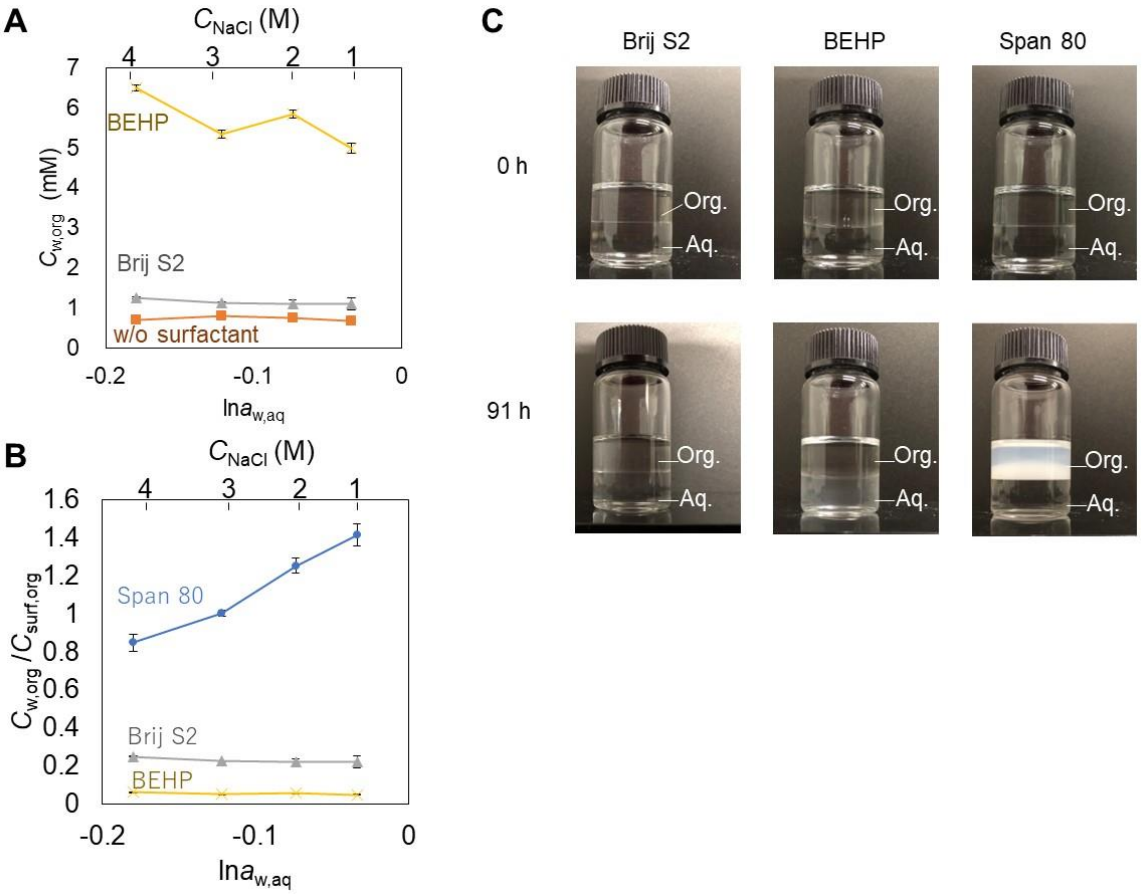

Figure S3. Water content in the organic phase containing Brij S2 and bis(2-ethylhexyl) hydrogen phosphate (BEHP). (A) The dependence of the water concentration in the organic phase ($C_{w,org}$) on ln$a_{w,aq}$. (B) The dependence of the ratio of water and surfactant concentration in the organic phase ($C_{w,org}/C_{surf,org}$) on ln $a_{w,aq}$. (C) Photographs of water-surfactant solutions at 0 h and 91 h. The organic phases are hexadecane solutions of 100 mM Span 80, 5 mM Brij S2, and 100 mM BEHP, respectively. The aqueous phases are water.

Section 5. Comparison of chemical potential of water in microdroplets and that in bulk

The major difference between microdroplets and bulk is the large interface-to-volume ratio. The chemical potential of water in microdroplets ($\mu_{w,micro}$) is described as[8]

$$\mu_{w,micro} = \mu_{w,bulk} + \frac{2\gamma v_w}{r} \quad (S7)$$

$$\mu_{w,micro} = \mu_w{}^o + k_B T ln a_{w,bulk} + \frac{2\gamma v_w}{r} \quad (S8)$$

Where, $\mu_{w,bulk}$ is the chemical potential of bulk phase, γ is interfacial tension, r is the radius of the microdroplet, v_w is the volume of one water molecule, $\mu_w{}^o$ is the standard chemical potential of water, k_B is Boltzmann constant, T is the temperature. If we assume 100 μm microdroplet, which is a typical size in our experiment, $2\gamma v_w/R = 6\times10^{-30}$ J/molecule, which is much smaller than $k_B T \ln a_{w,\,bulk}$ (-3×10^{-23} - -8×10^{-23}J/mol). Therefore, the contribution of the interface free energy is negligible. In conclusion, it is appropriate that the same model was used to describe the aqueous phase both in bulk and in microdroplets.

Section 6. Number of molecules in a Span 80 reverse micelle

We assumed that the number of molecules consisting of one Span 80 reverse micelle (N_{Span}) is described as

$$N_{\mathrm{Span\ 80}} = 4\pi r^2 / A_{\mathrm{Span\ 80}}$$

where, r is radius of the reverse micelle, $A_{\mathrm{Span\ 80}}$ is interface area which occupied by one Span 80 micelle at critical micellar concentration.
Since r = 2.5 nm, measured by dynamic light scattering, and $A_{\mathrm{Span\ 80}}$ is 29Å^2, [9] $N_{\mathrm{Span\ 80}}$ was calculated as 262.

References

1 M. Fukuyama, L. Zhou, T. Okada, K. V. Simonova, M. Proskurnin and A. Hibara, *Anal Chim Acta*, 2021, **1149**, 338212.
2 R. A. Robinson and R. H. Stokes, *Electrolyte solutions : the measurement and interpretation of conductance, chemical potential and diffusion in solutions of simple electrolytes*, Butterworths, 2nd ed., revised., 1965.
3 J. A. Gates and R. H. Wood, *J Chem Eng Data*, 1985, **30**, 44–49.
4 H.-L. Zhang and S.-J. Han, *J Chem Eng Data*, 1996, **41**, 516–520.
5 M. El Guendouzi, A. Dinane and A. Mounir, *Journal of Chemical Thermodynamics*, 2001, **33**, 1059–1072.
6 N. Smolentsev, W. J. Smit, H. J. Bakker and S. Roke, *Nature Communications 2017 8:1*, 2017, **8**, 1–6.
7 A. M. Dokter, S. Woutersen and H. J. Bakker, *Proc Natl Acad Sci U S A*, 2006, **103**, 15355–15358.
8 A. J. Webster and M. E. Cates, *Langmuir*, 1998, **14**, 2068–2077.
9 N. Politova, S. Tcholakova and N. D. Denkov, *Colloids Surf A Physicochem Eng Asp*, 2017, **522**, 608–620.