Supplementary Information

Microfluidic Generation of ATPS Droplets by Transient Double Emulsion Technique

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I Supplementary Figures

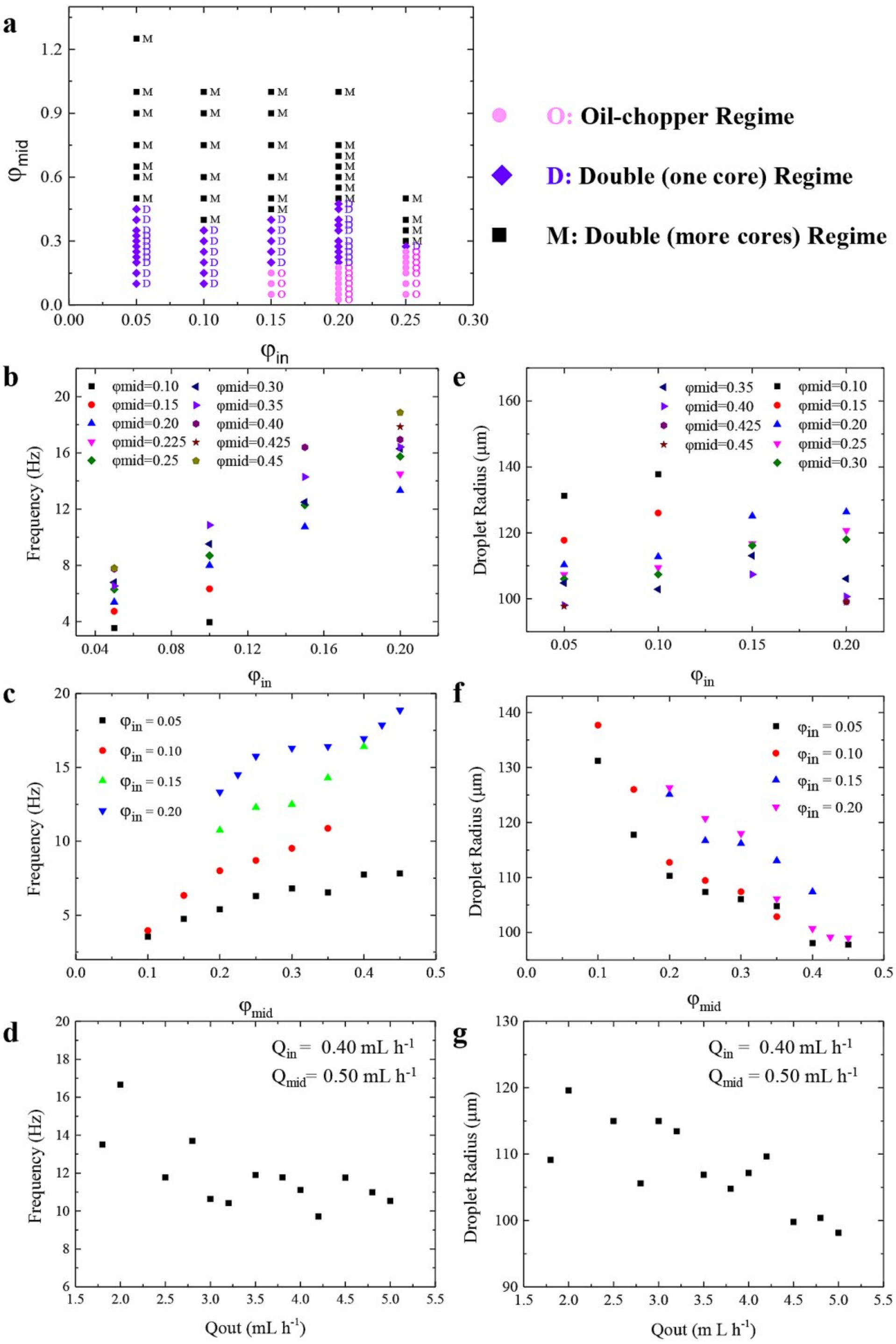

Fig. S1 ATPS droplets generated by using System II (see **Table S1**). (a) Phase diagram for dextran-in-PEG ATPS droplet generation by changing the flow rate ratio of the inner ($\varphi_{in} = Q_{in}/Q_{out}$) and middle oil phases ($\varphi_{mid} = Q_{mid}/Q_{out}$), while keeping the flow rate of outer PEG-rich phase constant at Q_{out} = 2.00 mL h^{-1}. (b-d) Controlling the generation frequency of ATPS droplets by tuning the inner phase flow rate ratio $\varphi_{in}= Q_{in}/Q_{out}$ (b), the middle phase flow rate ratio $\varphi_{mid}= Q_{mid}/Q_{out}$ (c), and the outer phase flow rate Q_{out} (d). (e-g) Controlling the radius of ATPS droplets by tuning φ_{in} (e), φ_{mid} (f), and Q_{out} (g). In (b), (c), (e), and (f), Q_{out} = 2.00 mL h^{-1}. In (d) and (g), Q_{in}= 0.40, Q_{mid}= 0.50 mL h^{-1}.

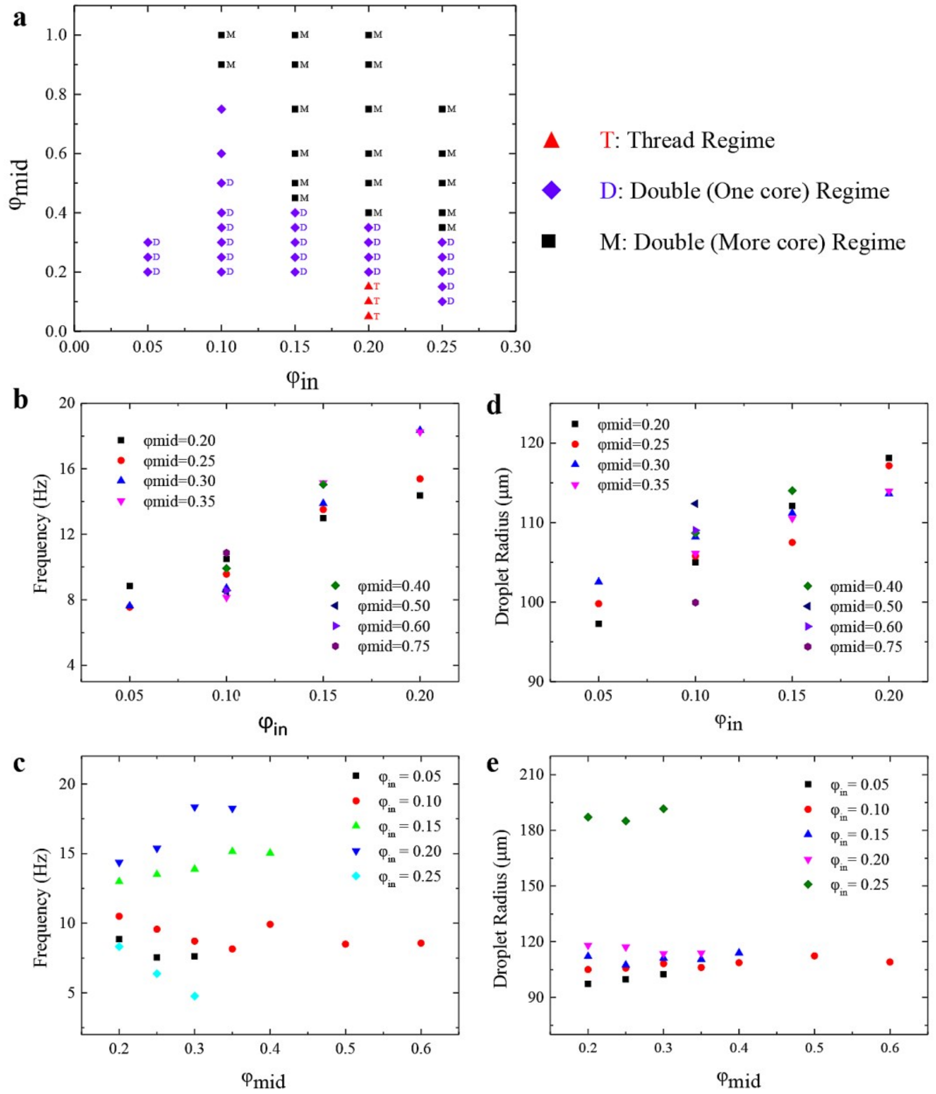

Fig. S2 ATPS droplets generated by using System III (see **Table S1**). (a) Phase diagram for dextran-in-PEG ATPS droplet generation by changing the flow rate ratio of the inner ($\varphi_{in} = Q_{in}/Q_{out}$) and middle oil phases ($\varphi_{mid} = Q_{mid}/Q_{out}$), while keeping the flow rate of outer PEG-rich phase constant at Q_{out} = 2.00 mL h^{-1}. (b-c) Controlling the generation frequency of ATPS droplets by tuning the inner phase flow rate ratio $\varphi_{in}= Q_{in}/Q_{out}$ (b), and the middle phase flow rate ratio $\varphi_{mid}= Q_{mid}/Q_{out}$ (c). (d-e) Controlling the radius of ATPS droplets by tuning φ_{in} (d), and φ_{mid} (e). In (b)-(e), Q_{out} = 2.00 mL h^{-1}.

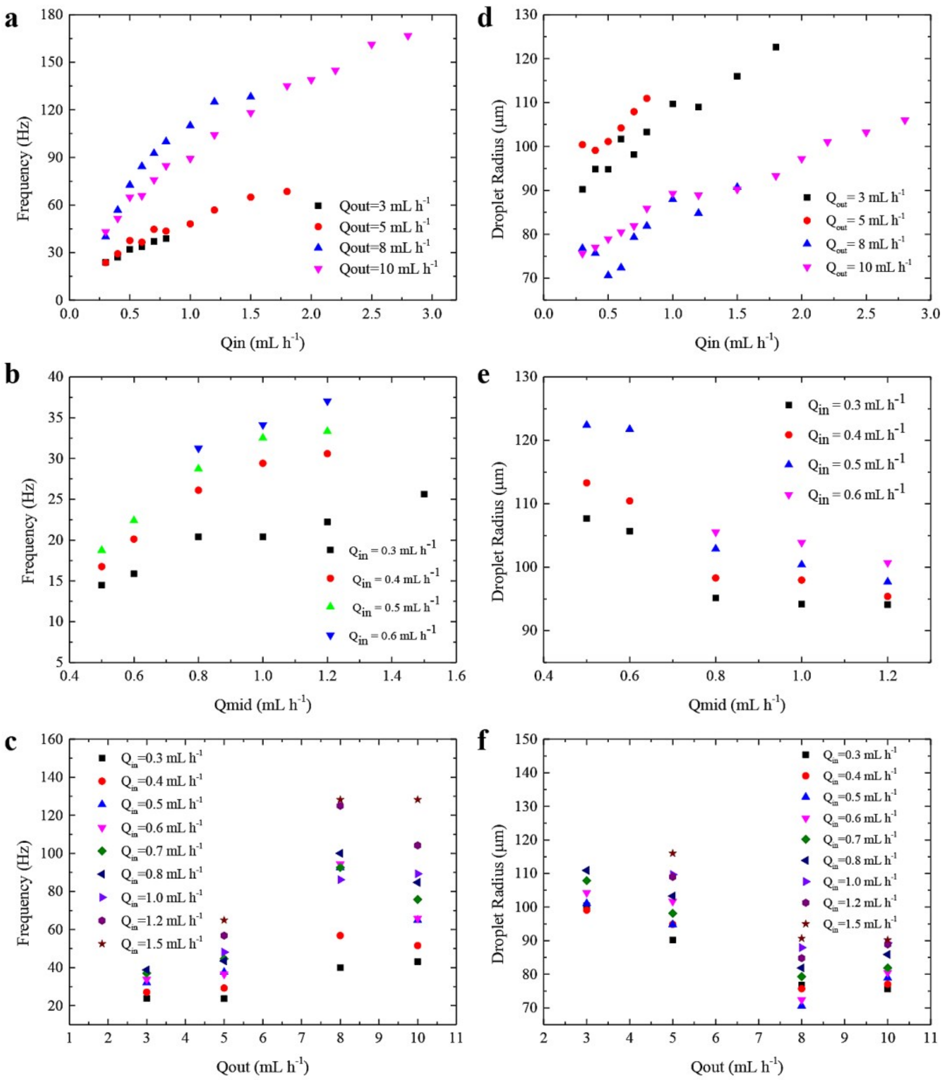

Fig. S3 The generation frequency and radius of PEG-in-dextran ATPS droplets by using System IV (see **Table S1).** (a-c) Controlling the generation frequency of PEG-in-dextran ATPS droplets by tuning the inner phase flow rate Q_{in} (a), the middle phase flow rate Q_{mid} (b), and the outer phase flow rate Q_{out} (c). (d-f) Controlling the radius of PEG-in-dextran ATPS droplets by tuning Q_{in} (d), Q_{mid} (e), and Q_{out} (f). In (a) and (d), Q_{mid} = 1.00 mL h^{-1}. In (b) and (e), Q_{out}= 3.00 mL h^{-1}. In (c) and (f), Q_{mid} = 1.00 mL h^{-1}.

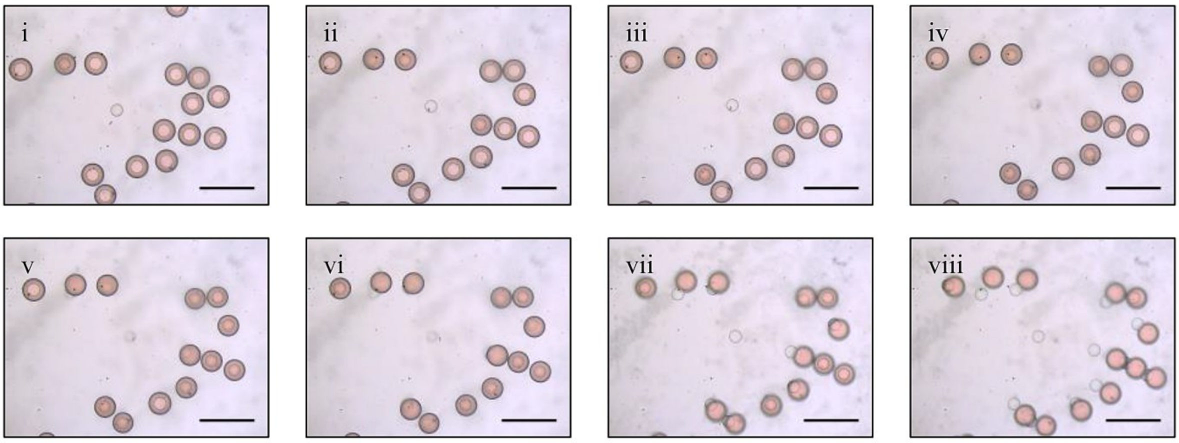

Fig. S4 Off-chip releasing process for double emulsion droplets with an average density smaller than the density of the continuous phase ($\rho_{Double} < \rho_{PEG}$), the lighter double emulsion droplets rise up to the air-liquid surface and then separate into oil-in-water (O/W2) droplets and ATPS (W1/W2) droplets (Q_{in}= 0.20 mL h^{-1}, Q_{mid}= 1.00 mL h^{-1}, and Q_{out}= 3.00 mL h^{-1}). Scale bars, 500 µm.

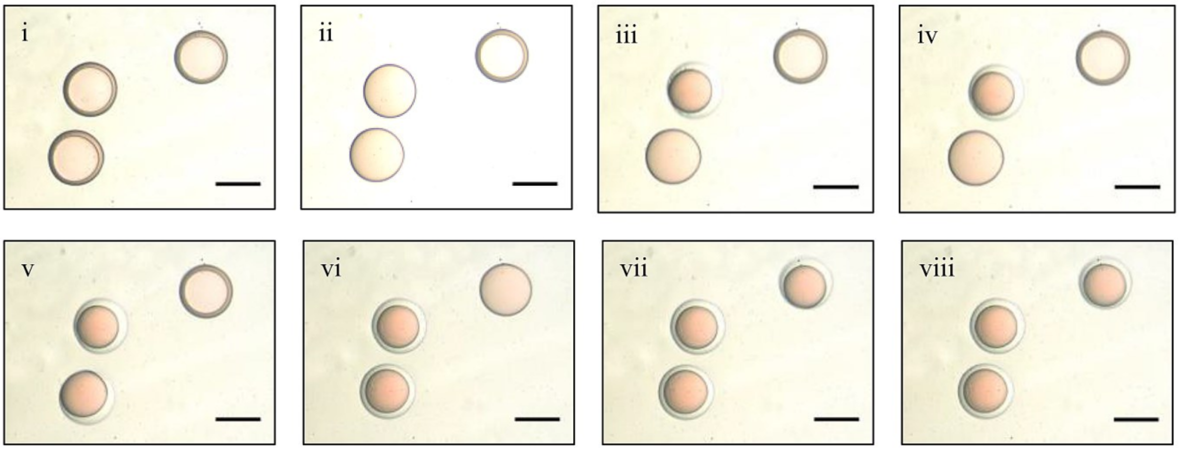

Fig. S5 Off-chip releasing process for double emulsion droplets with an average density equal to the density of the continuous phase ($\rho_{Double} = \rho_{PEG}$), the double emulsion droplets suspend in the continuous phase, keep stable for longer time and then separate (Q_{in}= 0.50 mL h^{-1}, Q_{mid}= 0.50 mL h^{-1}, and Q_{out}= 1.00 mL h^{-1}). Scale bars, 500 µm.

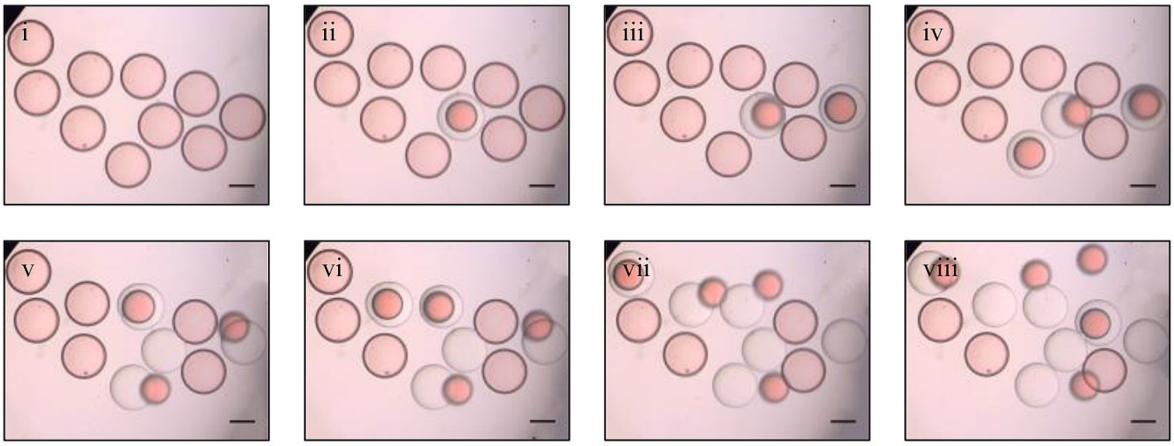

Fig. S6 Off-chip releasing process for double emulsion droplets with an average density higher than the density of the continuous phase, the heavier double emulsion droplets sink to the bottom of the collection container and then separate into oil-in-water (O/W2) droplets and ATPS (W1/W2) droplets at the bottom surface of the container (Q_{in}= 2.00 mL h^{-1}, Q_{mid}= 1.00 mL h^{-1}, and Q_{out}= 5.00 mL h^{-1}). Scale bars, 500 µm.

II Supplementary Tables

Table S1 Properties of the ATPS systems and middle oil phases used in the experiments.

System	Inner Phase	Middle Oil Phase	Outer Phase	Interfacial tension	Spreading Parameter
System I	Dextran-rich phase (25%) ρ= 1138.9 kg m^{-3} μ= 44.05 mPa s		PEG-rich phase (15%) ρ= 1062.6 kg m^{-3} μ= 11.043 mPa s	$\gamma_{W1/W2}$= 0.103 mN m^{-1} $\gamma_{W1/O}$= 26 mN m^{-1} $\gamma_{W2/O}$= 25.86 mN m^{-1}	S_O = -57.757 mN m^{-1} < 0
System II	Dextran-rich phase (25%) ρ= 1155 kg m^{-3} μ= 69.54 mPa s	Silicone oil ρ = ~ 960.2 kg m^{-3} μ = ~ 100 mPa s	PEG-rich phase (20%) ρ= 1059 kg m^{-3} μ= 13.29 mPa s	$\gamma_{W1/W2}$= 0.164 mN m^{-1} $\gamma_{W1/O}$ = 26.05 mN m^{-1} $\gamma_{W2/O}$ = 26.02 mN m^{-1}	S_O = -51.906 mN m^{-1} < 0
System III	Dextran-rich phase (20%) ρ= 1130.3 kg m^{-3} μ= 32.12 mPa s		PEG-rich phase (15%) ρ= 1057.1 kg m^{-3} μ= 8.2625 mPa s	$\gamma_{W1/W2}$ = 0.064 mN m^{-1} $\gamma_{W2/O}$ = 26.49 mN m^{-1} $\gamma_{W1/O}$ = 29.17 mN m^{-1}	S_O = -55.596 mN m^{-1} < 0
System IV	PEG-rich phase (15%) ρ= 1062.6 kg m^{-3} μ= 11.043 mPa s		Dextran-rich phase (25%) ρ= 1138.9 kg m^{-3} μ= 44.05 mPa s	$\gamma_{W1/W2}$ = 0.103 mN m^{-1} $\gamma_{W1/O}$ = 25.86 mN m^{-1} $\gamma_{W2/O}$ = 26 mN m^{-1}	S_O = -57.757 mN m^{-1} < 0
System V	Dextran-rich phase (25%) ρ= 1138.9 kg m^{-3} μ= 44.05 mPa s	Silicone oil ρ = ~ 960.2 kg m^{-3} μ = ~ 10 mPa s	PEG-rich phase (15%) ρ= 1062.6 kg m^{-3} μ= 11.043 mPa s	$\gamma_{W1/W2}$ = 0.103 mN m^{-1} $\gamma_{W2/O}$ = 25.18 mN m^{-1} $\gamma_{W1/O}$ = 26.98 mN m^{-1}	S_O = -52.057 mN m^{-1} < 0
System VI	Dextran-rich phase (25%) ρ= 1138.9 kg m^{-3} μ= 44.05 mPa s	PDMS oil (1:1) ρ = ~ 960.2 kg m^{-3} μ = ~ 275 m Pa s	PEG-rich phase (15%) ρ= 1062.6 kg m^{-3} μ= 11.043 mPa s	$\gamma_{W1/W2}$ = 0.103 mN m^{-1} $\gamma_{W2/O}$ = 22.75 mN m^{-1} $\gamma_{W1/O}$ = 21.19mN m^{-1}	S_O = -43.837 mN m^{-1} <0

III Supplementary Movies

Movie S1: ATPS thread without the middle oil phase.

The video shows the formation of a stable ATPS thread without introducing the middle oil phase. The ATPS thread generates at the flow rates of Q_{in}=0.50, Q_{mid}=0.00 and Q_{out}= 5.00 mL h^{-1}. The Video is recorded at 500 frames per second (fps) and played at 10 fps.

Movie S2: The trasient W1/O/W2 double emulsion generation.

The video shows the transient W1/O/W2 double emulsion generation when introducing a middle oil phase. The W1/O/W2 double emulsion droplets are generated at the flow rates of Q_{in}=0.50, Q_{mid}=0.50 and Q_{out}= 5.00 mL h^{-1}, respectively. The Video is recorded at 500 fps and played at 10 fps.

Movie S3: The four generation regimes with transient double emulsion technique.

The video shows the four generation regimes: oil-chopper regime, thread regime, double emulsion (with one core) regime and double emulsion (with more cores) regime by varying the flow rate of the middle oil phase from 0.20 to 1.80 mL h^{-1} while keeping the inner and outer phases flow rates at Q_{in}=0.50 and Q_{out}= 3.00 mL h^{-1}, respectively. The Video is recorded at 500 fps and played at 10 fps.

Movie S4: ATPS droplets generation by on-chip separation of the transient W1/O/W2 double emulsion for low viscosu middle oil phase.

The video shows the on-chip dewetting process of the generated transient W1/O/W2 double emulsion droplets when using low viscous oil (~10 mPa s silicone oil) as the middle oil phase. The flow rates of the inner, middle and outer phases are Q_{in}=0.50, Q_{mid}=0.50 and Q_{out}= 3.00 mL h^{-1}, respectively. The Video is recorded at 1500 fps and played at 10 fps.

Movie S5: ATPS droplets generation by off-chip dewetting of the transient W1/O/W2 double emulsion (State 1: rising).

The video shows the off-chip dewetting process of the collected W1/O/W2 double emulsion for ~100 mPa s silicone oil system, where the W1/O/W2 double emulsion droplets are generated at the flow rates of Q_{in}=0.20, Q_{mid}=1.00 and Q_{out}= 3.00 mL h^{-1}, respectively. The Video is recorded at 24 fps and played at 96 fps.

Movie S6: ATPS droplets generation by off-chip dewetting of the transient W1/O/W2 double emulsion (State 2: suspending).

The video shows the off-chip dewetting process of the collected W1/O/W2 double emulsion for ~100 mPa s silicone oil system, where the W1/O/W2 double emulsion droplets are generated at the flow rates of Q_{in}=0.50, Q_{mid}=0.50 and Q_{out}= 1.00 mL h^{-1}, respectively. The Video is recorded at 24 fps and played at 96 fps.

Movie S7: ATPS droplets generation by off-chip dewetting of the transient W1/O/W2 double emulsion (State 3: sinking).

The video shows the off-chip dewetting process of the collected W1/O/W2 double emulsion for ~100 mPa s silicone oil system, where the W1/O/W2 double emulsion droplets are generated at the flow rates of Q_{in}=2.00, Q_{mid}=1.00 and Q_{out}= 15.00 mL h^{-1}, respectively. The Video is recorded at 24 fps and played at 96 fps.

Movie S8: Dewetting process of the transient W1/O/W2 double emulsion for high viscous PDMS oil system.

The video shows the dewetting process of the collected W1/O/W2 double emulsion for PDMS oil system. The Video is recorded and played at 25 fps.