

Quantitative Evaluation of DNA Condensates Formation Dynamics

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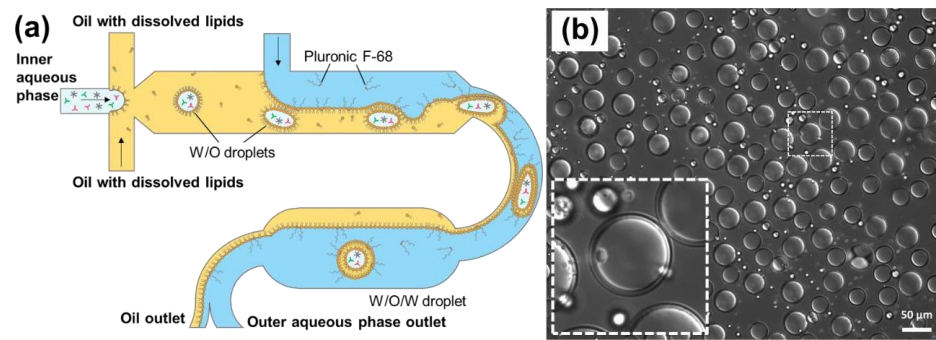


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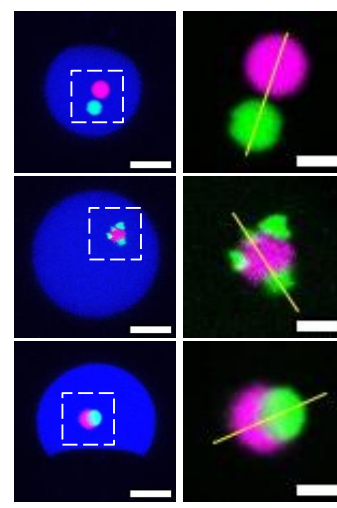
Background

Generation of uniformly-sized artificial cells using microfluidic channels



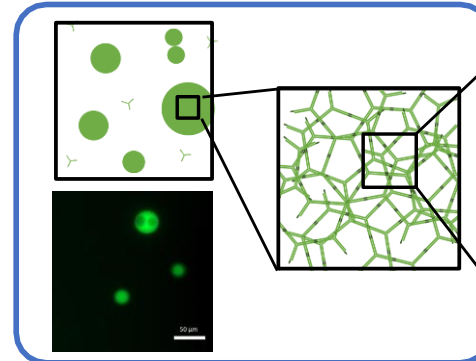
- Use a microfluidic channel and On-chip pressure pump to produce liposomes from uniform W/O droplets
- Encapsulation of cytoplasmic components of artificial cells in liposomes → Formation of DNA condensates as artificial cell nuclei

Formation of patchy and Janus-type condensates within artificial cells

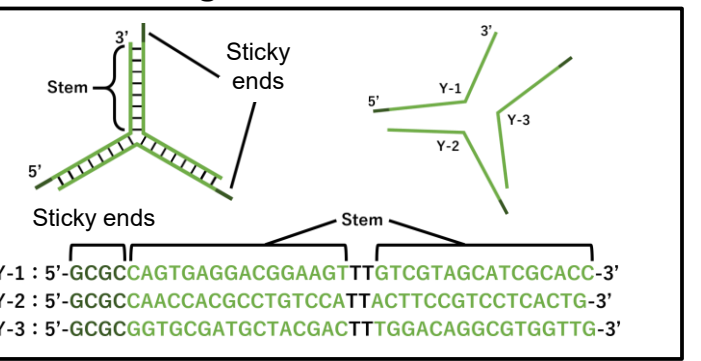


What are DNA condensates?

DNA condensates



Y-motifs are generated from single-stranded DNA

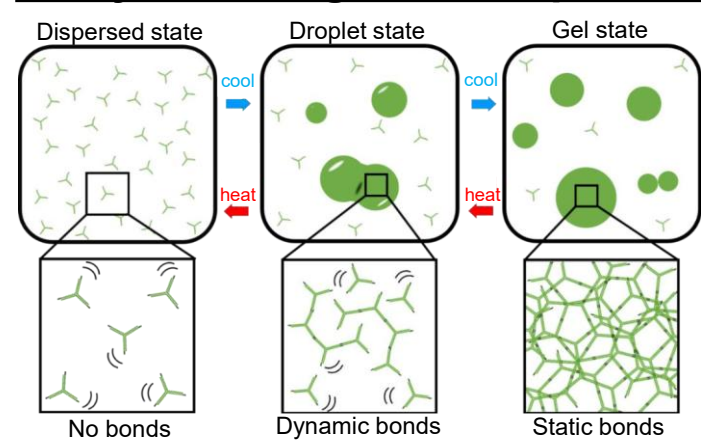


Y. Sato, T. Sakamoto, M. Takinoue, *Science. Advances*, 6.23 (2020)

- DNA condensates: Soft materials consisting of condensed DNA nanostructures (Y-motifs).
- Physical properties and interactions can be programmed by sequence design.

Expected to be applied in fields such as biomimetics and artificial molecular systems.

DNA nanostructures change into three states depending on sticky end design and temperature

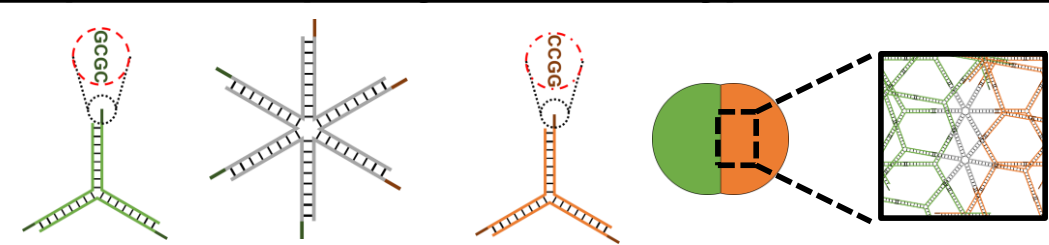


Dispersed state: Nanostructures are unbound and dispersed.
Droplet state: Nanostructures dynamically associate and disassociate.
Fluidized state (droplets)
Gel state: Gel state with stable bonds.

Y. Sato, T. Sakamoto, M. Takinoue, *Science. Advances*, 6.23 (2020)

- A phase diagram of sequence space and condensation temperature is created. → For design guidelines for patchy and Janus-type DNA condensates

Preparation of patchy and Janus-type condensates



Schematic of Janus-type condensate formation from DNA Y-motifs

- The Binding of different DNA condensates can be controlled by introducing linker motifs.
- The condensation speed of the Y-motifs is important in influencing the final shape of the condensates.
- The condensation speed depends on the sequences of the Y-motifs, but there is no unified understanding of it.

Objective

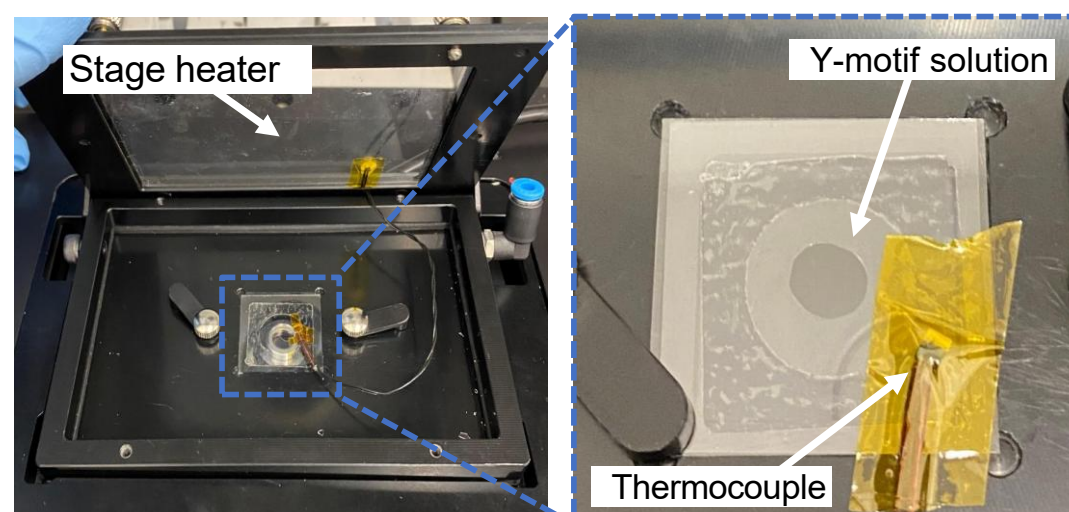
Measurement of the condensation temperatures of Y-motifs with different sequences

Experimental Procedures

DNA solution contents

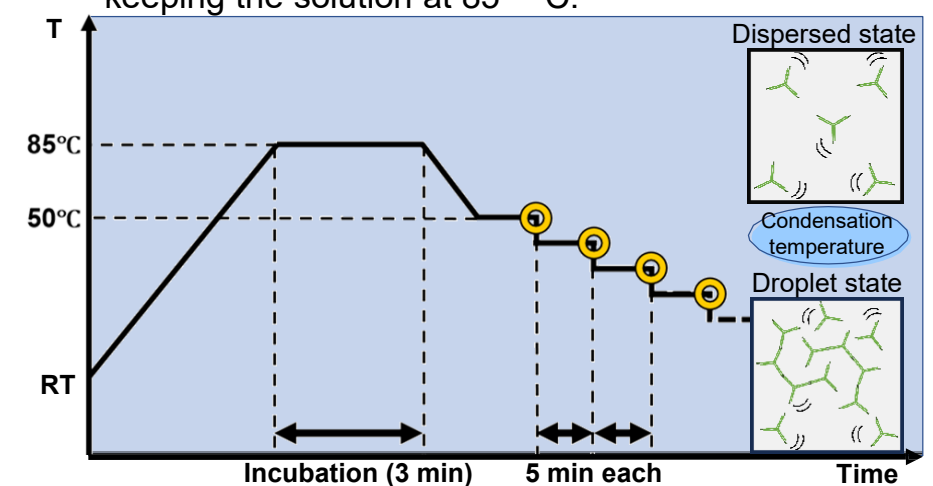
DNA Solution of Type.A	
Total	30 μL
Tris-HCL	40 mM
NaCl	100 mM
DNA-A1	10 μM
DNA-A2	9 μM
DNA-A3	10 μM
FAM	1 μM
MilliQ	-

Experimental system



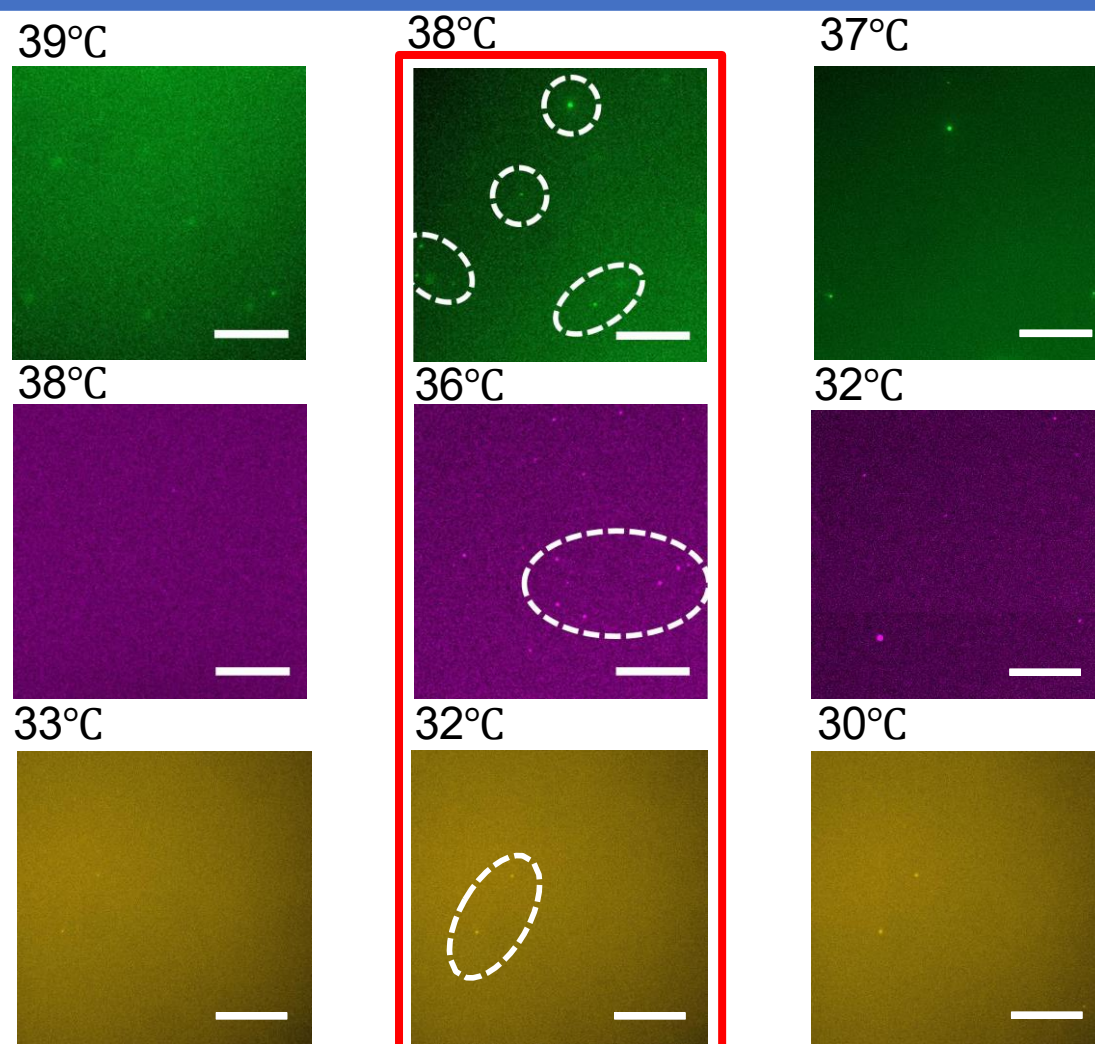
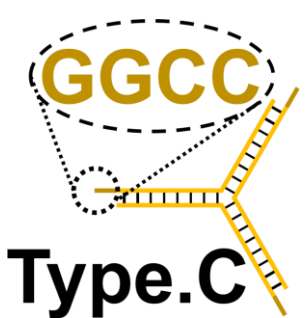
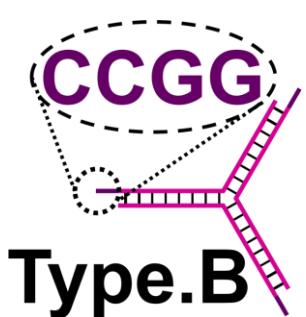
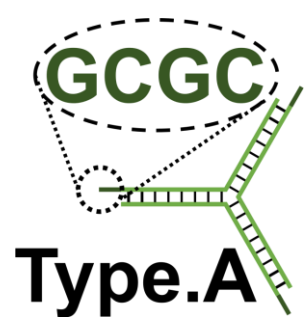
Temperature control

- Observed the solution with a temperature drop after keeping the solution at 85 °C.



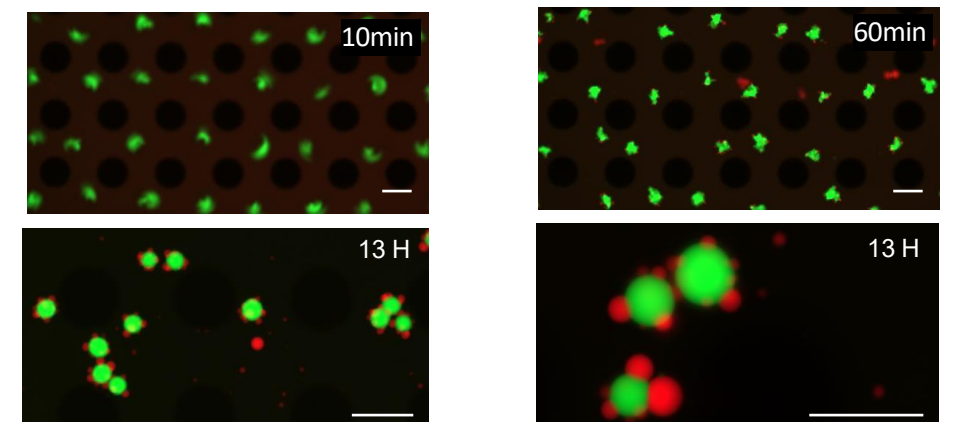
Measured the temperature at which the Y-motifs changed from the dispersed state to the droplet state.

Results

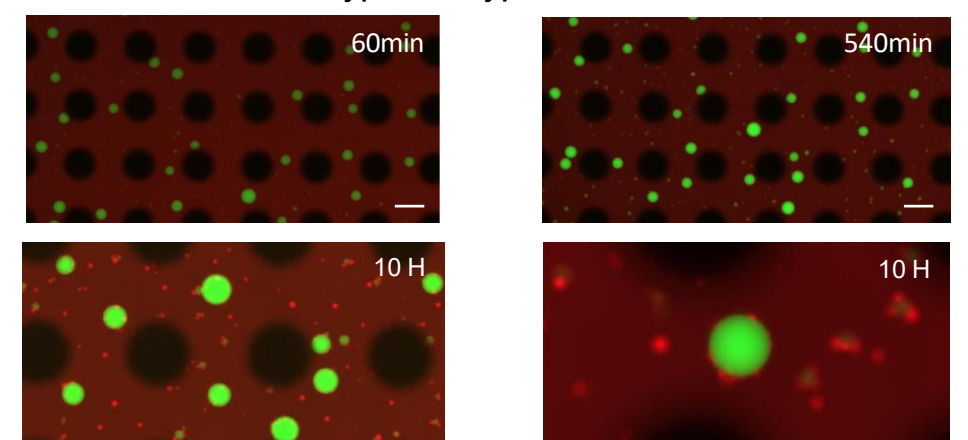


The Y-motifs with sticky ends of A (GCGC), B (CCGG), and C (GGCC) were measured to condense into droplets in this order as temperature dropped.

Type.A, Type.B, Linker



Type.A, Type.C, Linker



Z. HUANG and H. SUZUKI et al., *MicroTAS* 2024

Scale bar=100 μm

When pairs with different condensation temperatures were mixed, the type with the higher condensation temperature condensed first, and then condensates of the pairs adhered to the primarily-formed condensates to form patchy DNA condensates.

Conclusions

- It was found that the condensation temperature varies depending on the sequence of G and C at the sticky ends.
- For designing patchy DNA condensates, using pairs with distinct condensation temperatures is recommended.