# Native dynamics of single ssDNA molecule hybridization in solution revealed by single-molecule regulated chemical (SMRC) process

## 1分子制御化学（SMRC）プロセスを用いた溶液中単一ssDNA分子ハイブリダイゼーション動態の解明


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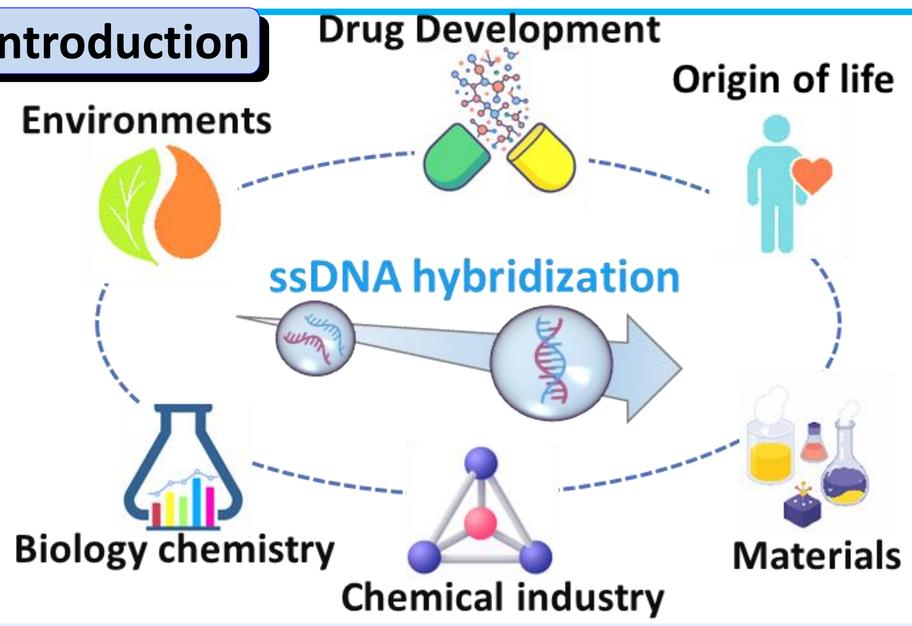
## 1. Introduction

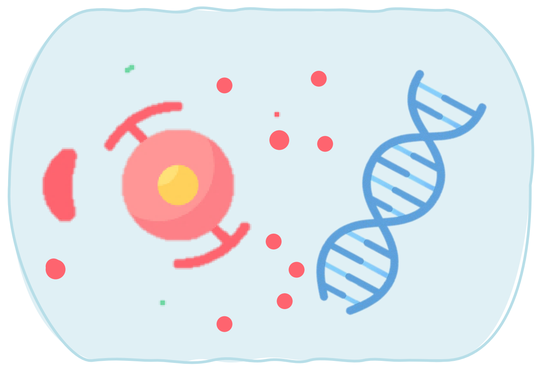



**DNA hybridization** is important in biology, medicine, and materials science.

## 2. Background

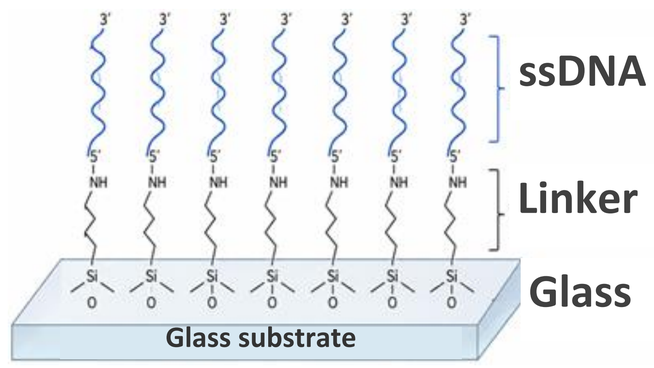
**Critical issue:**
Surface immobilization limited native ssDNA dynamics.

(a) *In vivo ssDNA*



☑ Native ☑ Free
☑ Dynamic ☒ Difficult control

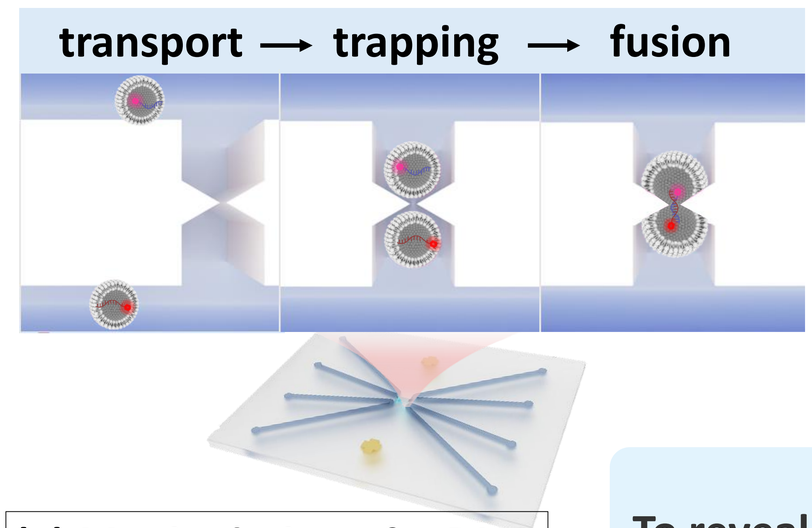
(b) *In vitro ssDNA*



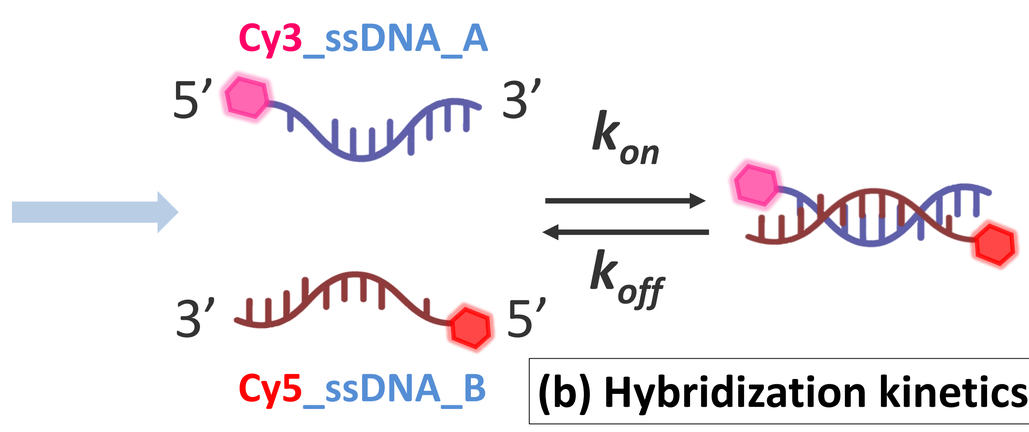

☑ Observable
☒ Immobilization ☒ Flexibility

## 3.Concept

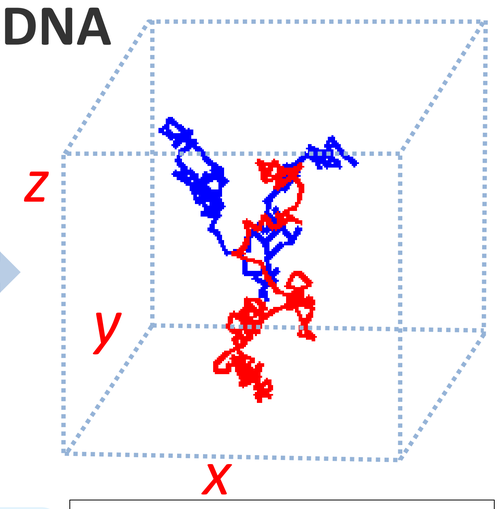
### Single-molecule regulated chemical (SMRC) process of ssDNA




(a) Manipulation of ssDNA




(b) Hybridization kinetics



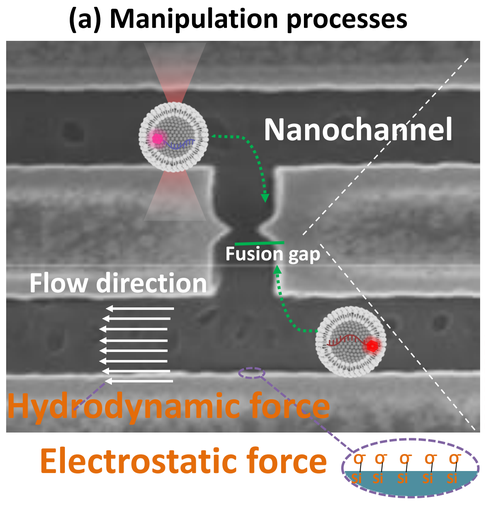

(c) Native dynamics

**Objective:**
To reveal native ssDNA hybridization dynamics using the SMRC process.

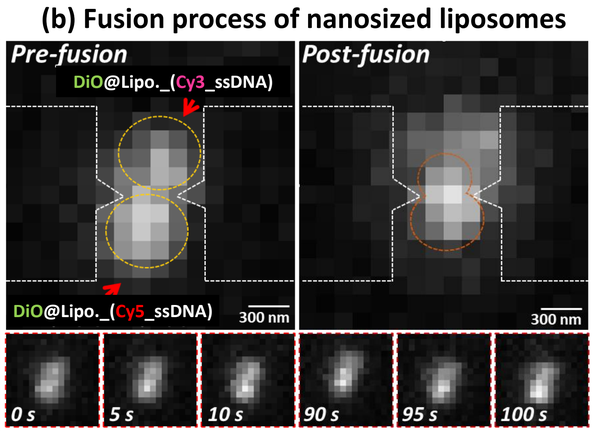
## 4.Methodology and Results

### Manipulation of nanosized liposomes in nanofluidic devices

(a) Manipulation processes

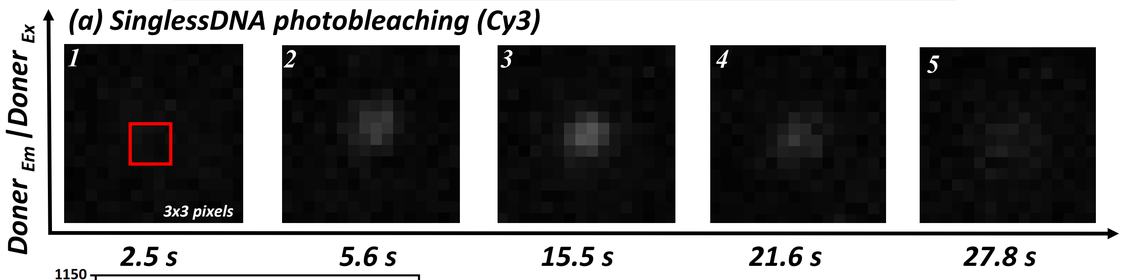



(b) Fusion process of nanosized liposomes

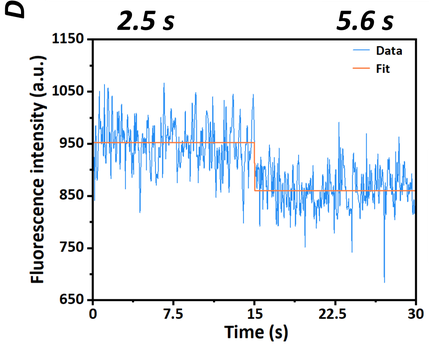



### Result 1: Confirmation of single-molecule ssDNA

(a) *SinglessDNA photobleaching (Cy3)*




(b) *Intensity trace of single ssDNA (Cy3)*

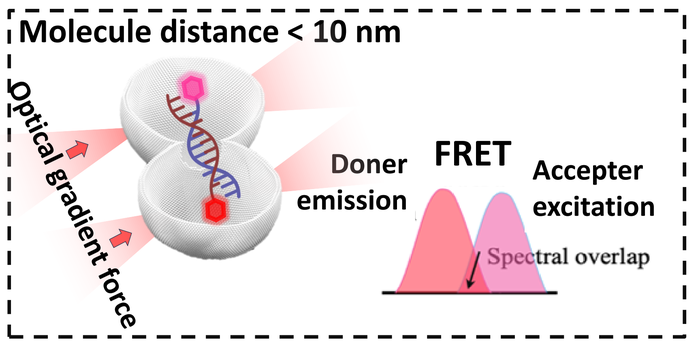



Single-step photobleaching confirmed that a single ssDNA was encapsulated in nanosized liposomes.

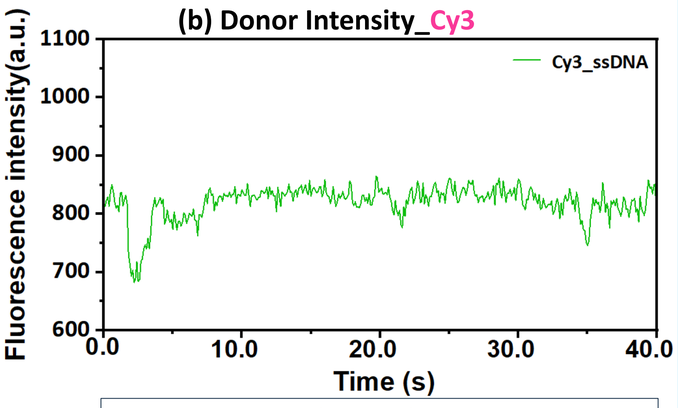
☑ Observable ☑ Native ☑ Dynamic ☑ Flexibility

### Result 2: FRET analysis of ssDNA hybridization dynamics in solution

(a) Schematic of ssDNA hybridization verified by FRET

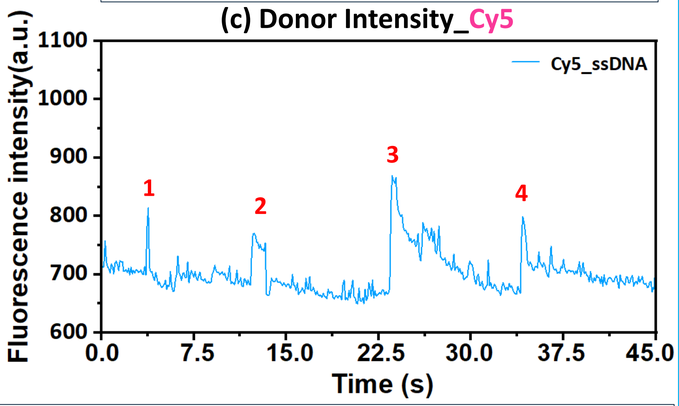



(b) Donor Intensity_Cy3




Donor intensity drop indicates FRET events.

(c) Donor Intensity_Cy5




Acceptor signal peaks confirmed ssDNA hybridization.

### Result 3: Dynamic ssDNA hybridization distance

(a) Native dynamic distance calculation of ssDNA

FRET efficiency

$$E = \frac{F_{A|D}}{F_{A|D} + F_{D|D}} \quad (1)$$

Distance of FRET molecules

$$r = R_0 \left( \frac{1}{E} - 1 \right)^{1/6} \quad (2)$$

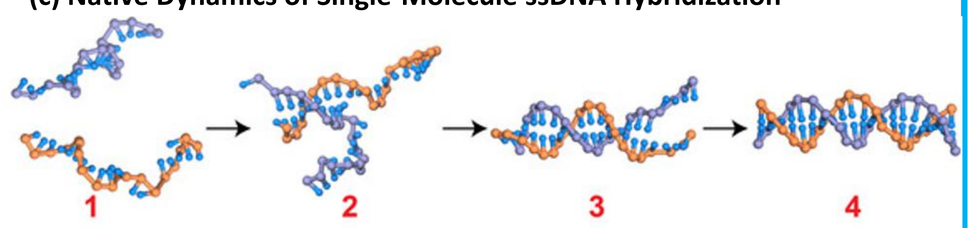
Förster radius $R_0$=5.0 nm for the Cy3_Cy5 pair.

(b) FRET analysis results

| No. | E | $R_0$ |
|---|---|---|
| 1 | 0.51 | 5.364 |
| 2 | 0.48 | 5.473 |
| 3 | 0.51 | 5.364 |
| 4 | 0.47 | 5.509 |

Stable FRET efficiency and distance (~5.4 nm) confirm ssDNA hybridization.

(c) Native Dynamics of Single-Molecule ssDNA Hybridization



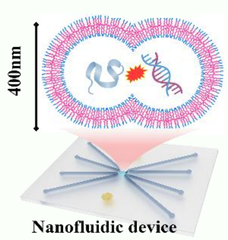

Two complementary ssDNA recognize, bind, and form a stable dsDNA structure.

## 5. Conclusions

- SMRC enables controllable single-molecule reactions in solution.
- Surface-free observation preserves native-like ssDNA dynamics.
- Nanoscale dynamic ssDNA hybridization at the single-molecule level.

## 6. Perspectives

- *Origin of life*
- *Artificial life*
- *Minimal cell*




## Acknowledgement


*This work was supported by JSPS KAKENHI Grant Numbers 21H05231, 21H04640, and Next Generation (SPRING).*