

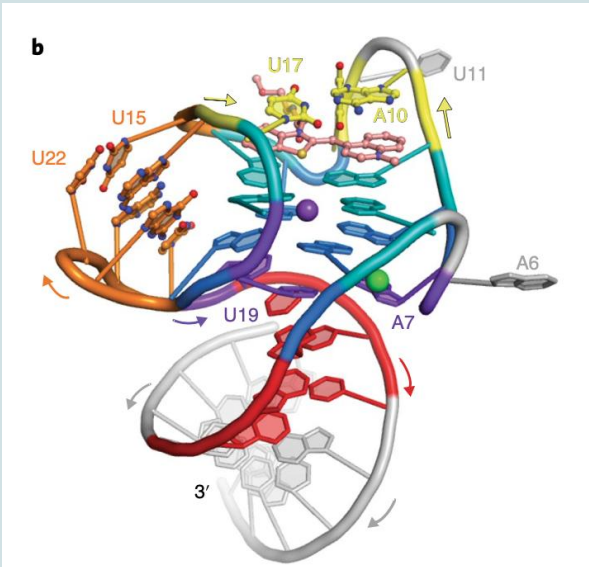
Selection of novel **fluorogenic RNA aptamers** by affinity- and fluorogenic-based method and their characterization

Tomotaka Tavama, Keisuke Ito, Sotaro Uemura, and Ryo Iizuka

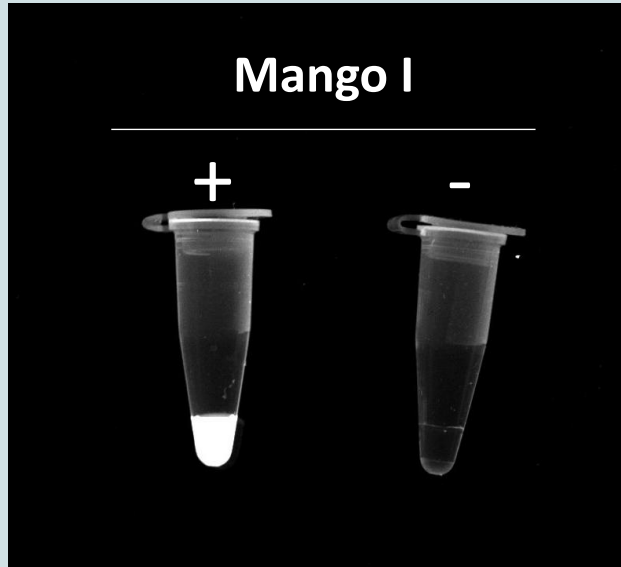
Dept. of Biological Sciences, Graduate School of Science, The University of Tokyo

Background

Fluorogenic RNA aptamers bind to fluorogenic molecules and dramatically enhance fluorescence. They are expected to be applied to RNA imaging in live cells, but the brightness is often insufficient. The demand for brighter fluorogenic RNA aptamers is increasing.



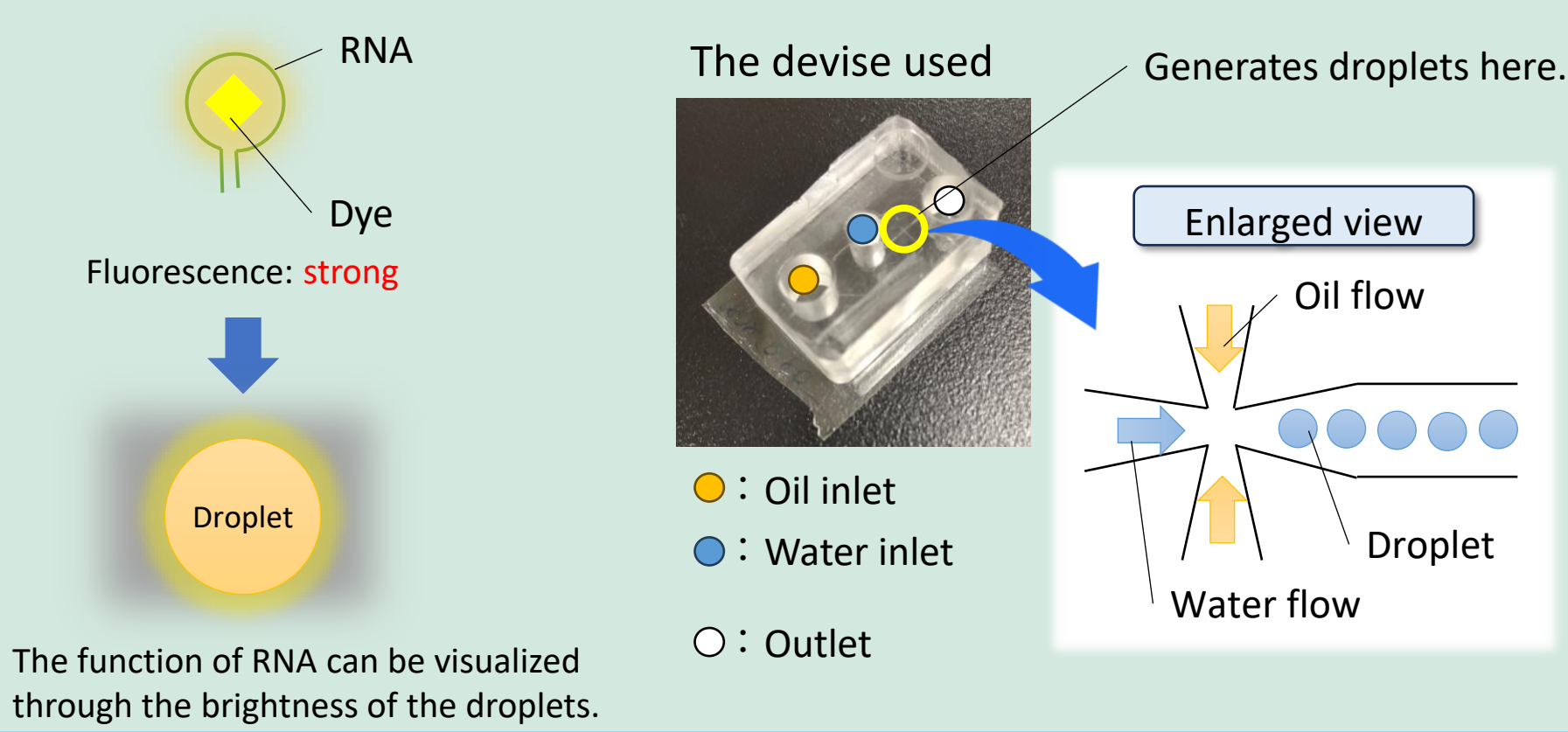
3D structure of Mango III
Trachman et al. (2019)
Nat. Chem. Biol. **15**, 473



In vitro-transcribed Mango I
enhances TO1-B fluorescence.

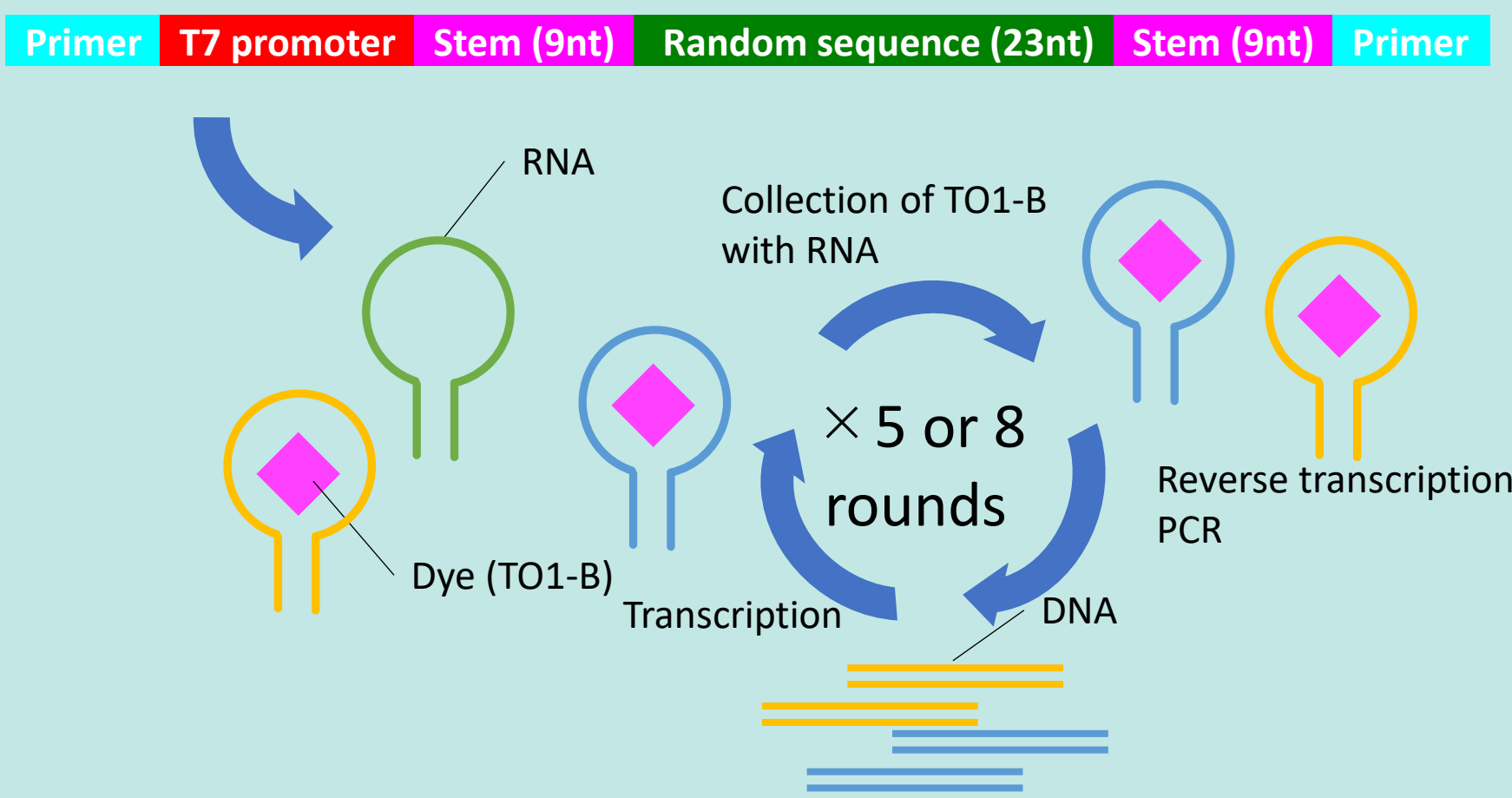
Outline of research

We performed affinity-based selection (systematic evolution of ligands by exponential enrichment: SELEX), followed by fluorescence-based selection using water-in-oil (w/o) droplets to obtain fluorogenic RNA aptamers.



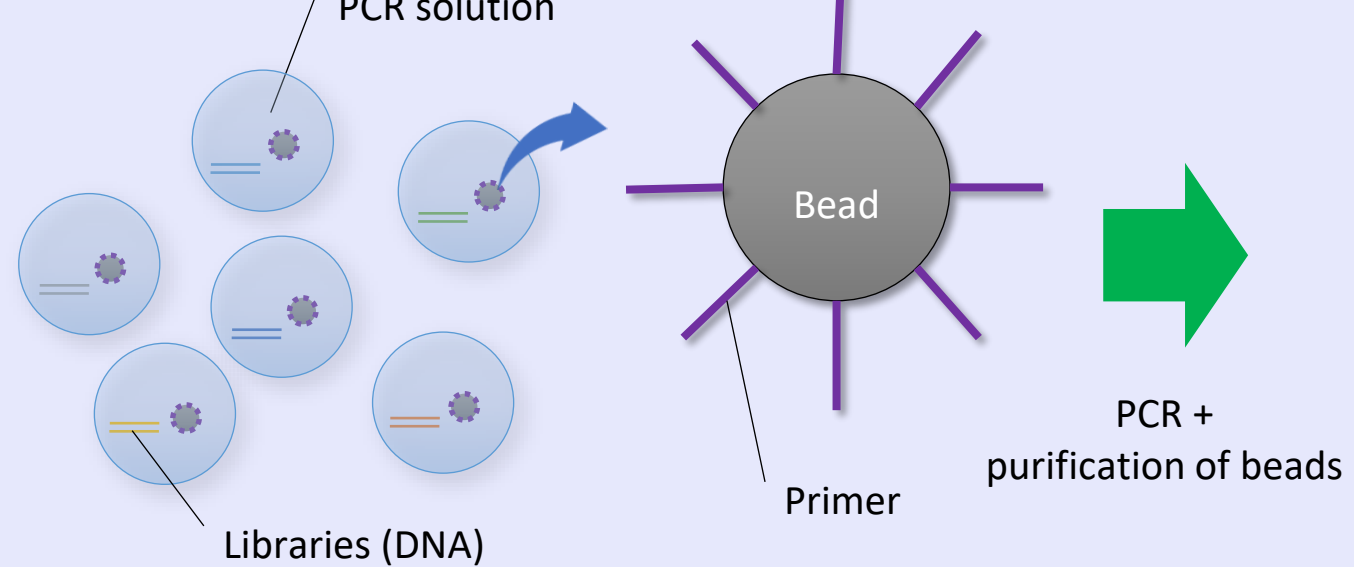
Procedures for affinity-based selection

Based on the structure of Mango I, a DNA library containing a 23-nucleotide randomized region flanked by constant stem-loop-forming sequences and primer-binding sites was prepared. SELEX was performed to enrich RNAs with binding affinity for the dye (TO1-B).

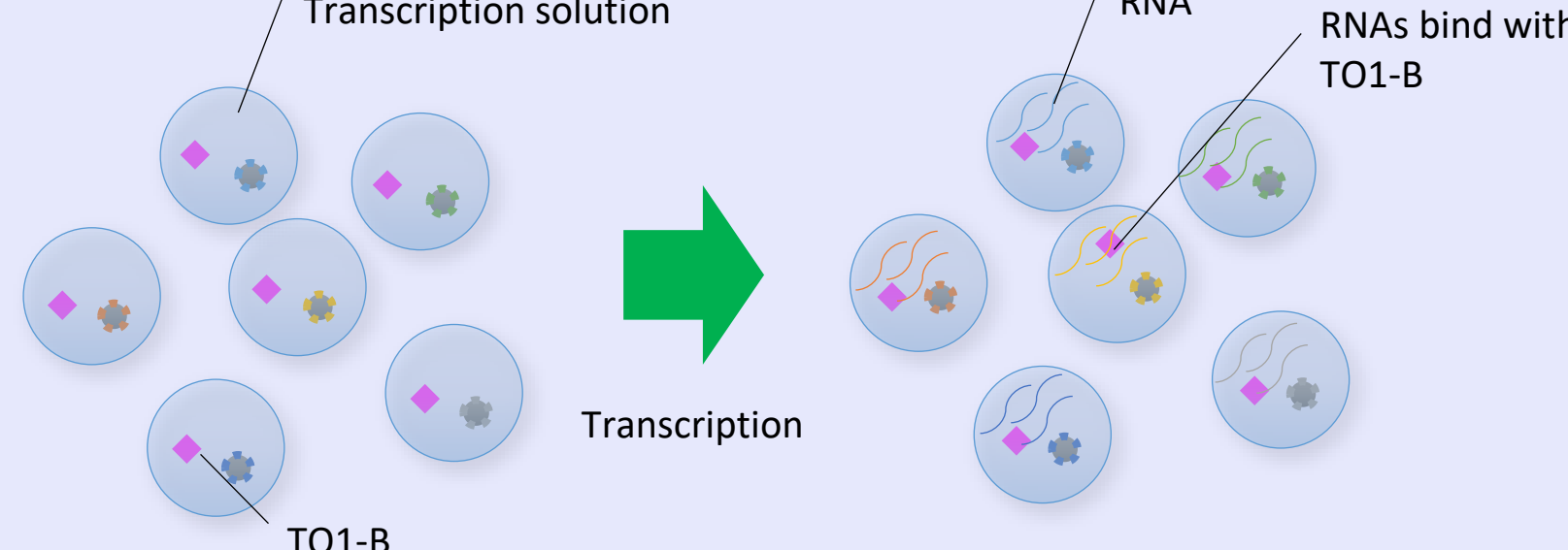


Procedures for fluorescence-based selection

1. Emulsion PCR

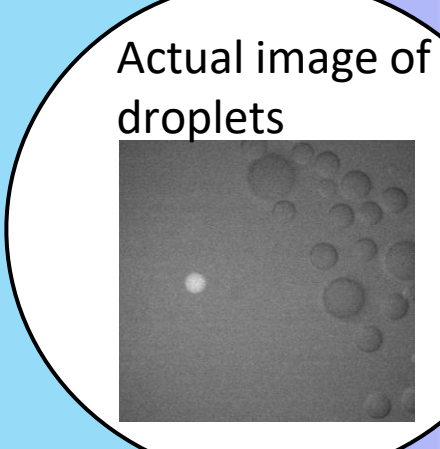


2. Transcription

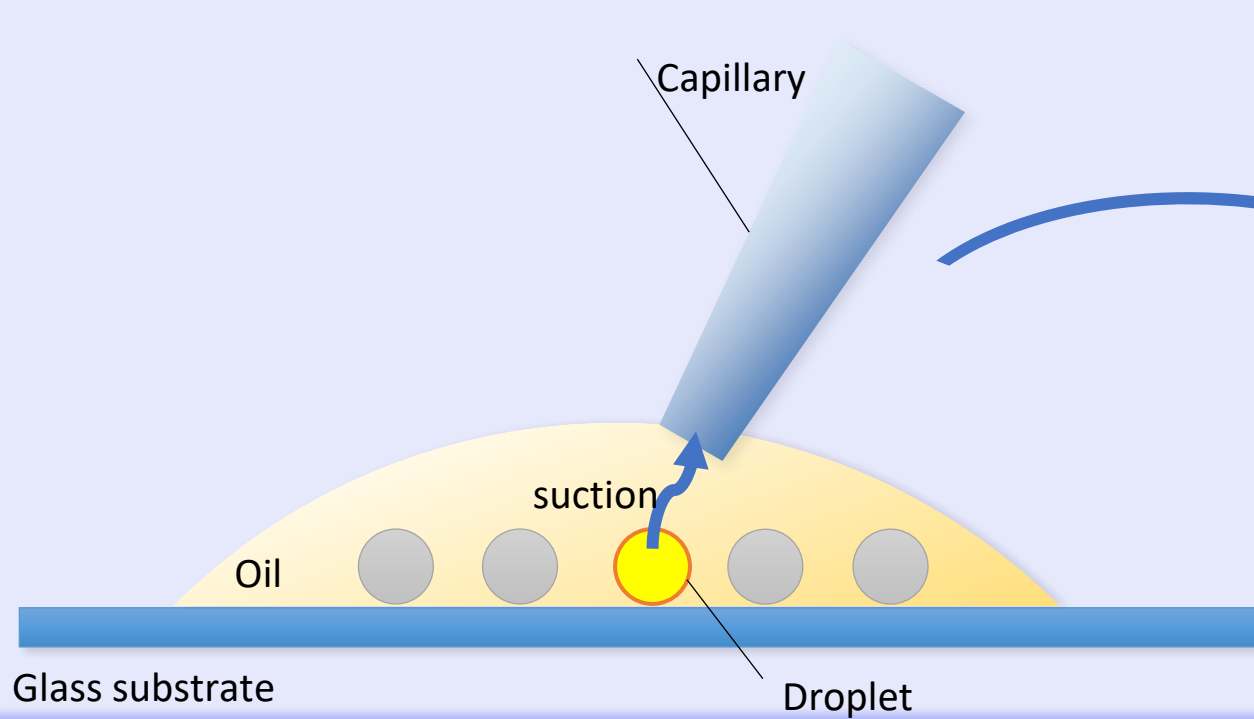


3. Screening

Observation under a fluorescence microscope

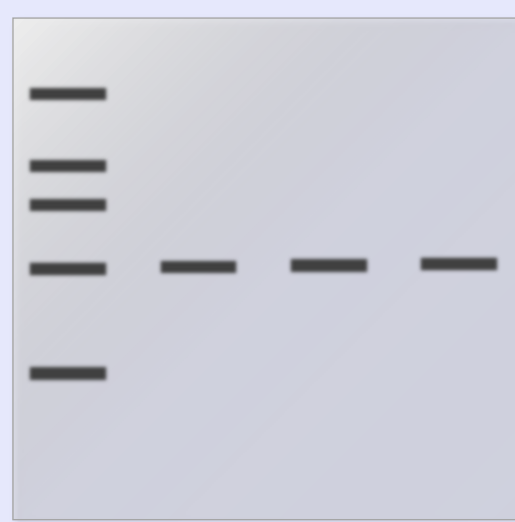


Selection

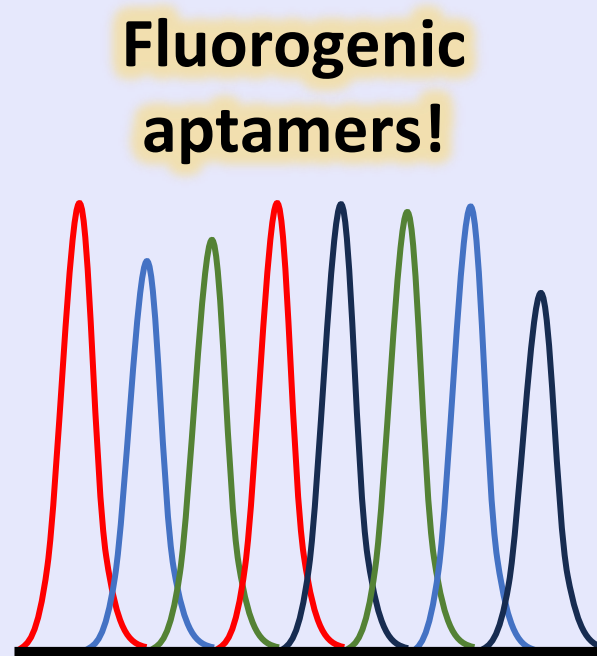


PCR tube

PCR

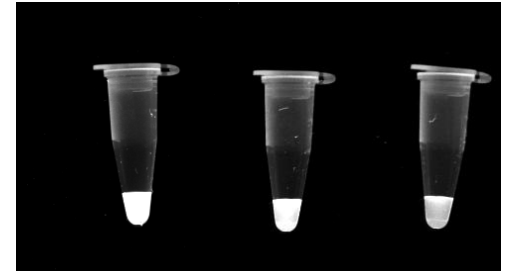


Sequencing



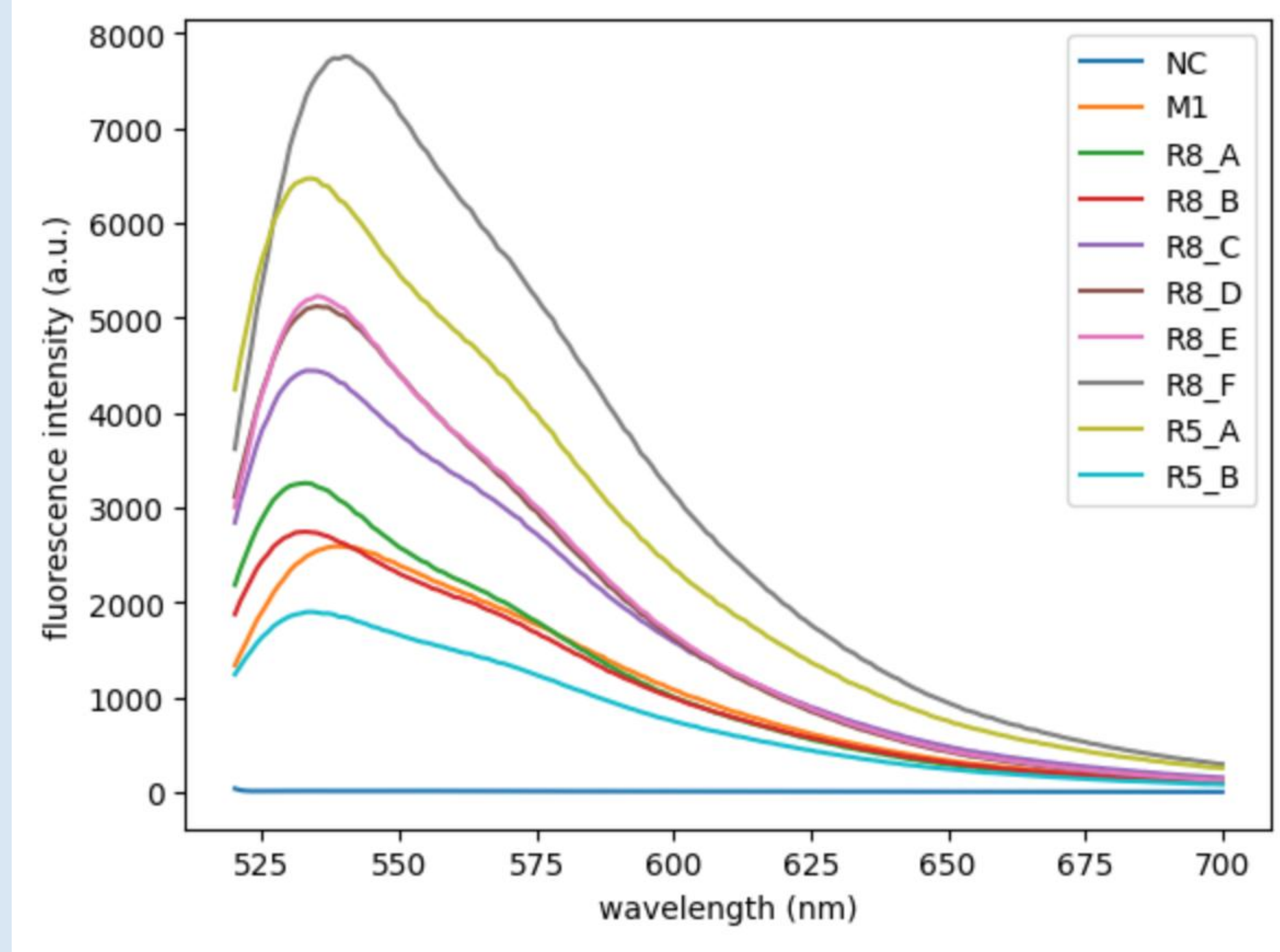
Fluorogenic aptamers!

Confirmation of fluorescence enhancement



Results

Fluorescence spectra with and without purified RNAs

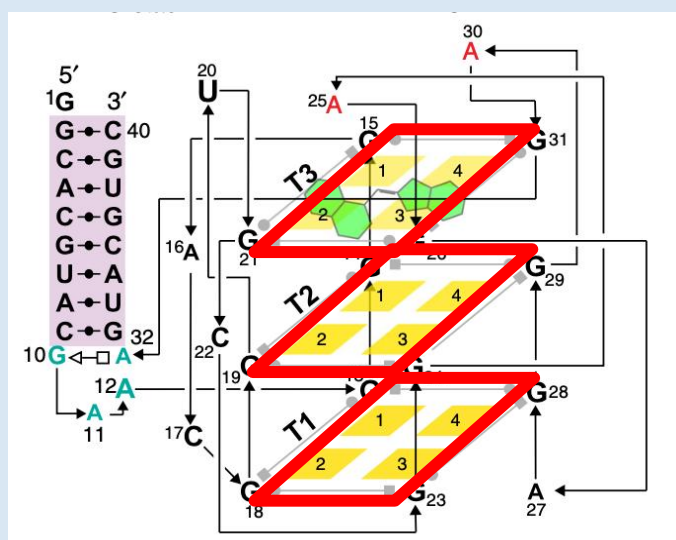


Sequences of obtained RNAs

Name	Sequence	Emission max wavelength (nm)	Relative fluorescence intensity
Mango I	G A A G G G A C G G T G C G G A G A G G A G A	539	1.00
8_A	A G A T T A G G T G G T G G T T G G T A G T	533	1.14
8_B	A G A T T G G A G G G T G G T T G G G T T T	533	1.06
8_C	G T G G A G A G G T A G G G A G G G A T T A T	534	2.11
8_D	A T C A G G A G A G G T G G T G G T T G G T T	535	1.8
8_E	G A G T G G G T T G G T G T G G A G T G G G T	535	1.31
8_F	A T T G G T G T G G A G T G G A G T G G G T C	540	2.56
5_A	T G G G C T G G G T G G G T C A A G G G A C	534	2.58
5_B	T T A G G G A G G T G A G G G A G G G T T T A	534	0.69

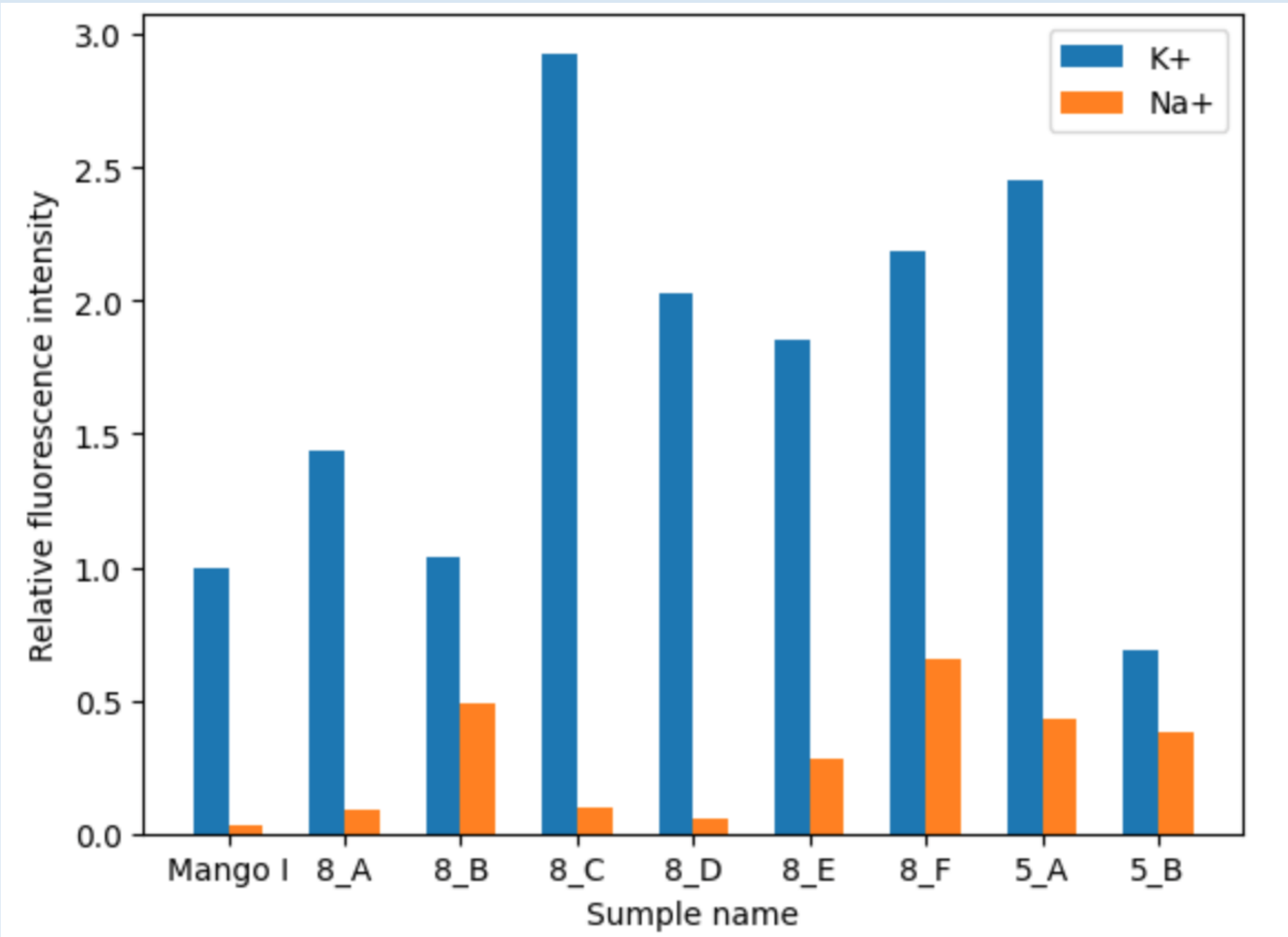
The fluorescence spectra of the obtained fluorogenic RNA aptamers were measured. Relative intensity refers to the comparison of fluorescence peak intensities to that observed in the presence of Mango I. R5 and R8 indicate the number of rounds of the SELEX selection. The presence of R8_F and R5_A resulted in about three times stronger fluorescence compared to that of Mango I. The results suggest that the combination of affinity- and fluorogenic-based selections enables the identification of fluorogenic RNA aptamers with a high fluorescence enhancement capacity.

The Mango aptamers form a guanine quadruplex structure (G4 structure), where four guanines are arranged in a planar configuration, as shown in the figure on the right. The structure provides an appropriate surface for the binding of TO1-B with Mango aptamers. The RNAs contained four islands with at least two adjacent guanines, suggesting that they form a G4 structure.



Structure of Mango I
Autour et al. (2018) *Nat. Commun.* **9**, 656

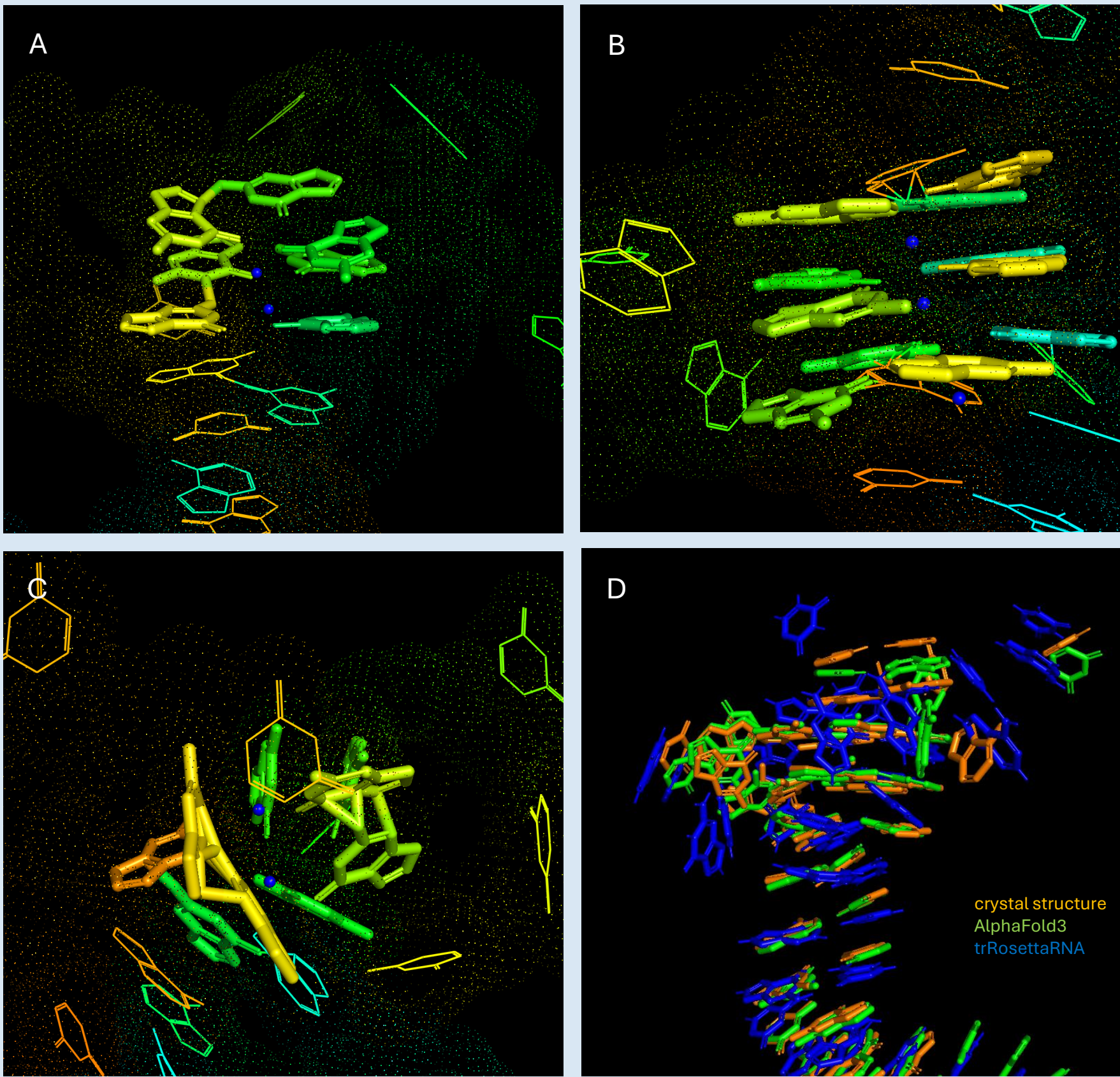
Potassium ion requirements for fluorescence enhancement



Potassium ions stabilize the G4 structures of Mango aptamers to enhance the fluorescence of TO1-B. The ion requirements of the fluorescent RNA aptamers were tested.

For all RNAs, TO1-B fluorescence was increased in the presence of potassium ions compared to that in the presence of sodium ions. The results suggest that potassium ions stabilize the structure of the obtained RNAs, enhancing TO1-B fluorescence.

Prediction of the 3D structures



The 3D structures of the RNAs were predicted using AlphaFold3. In the presence of potassium ions, some RNAs formed a G4 structure (A, B), while others did not (C).

D shows a comparison between the crystal and predicted structures of Mango III, a member of the Mango aptamers. The structure predicted by AlphaFold3 was in better agreement with the crystal structure, which was better than that predicted by trRosettaRNA.

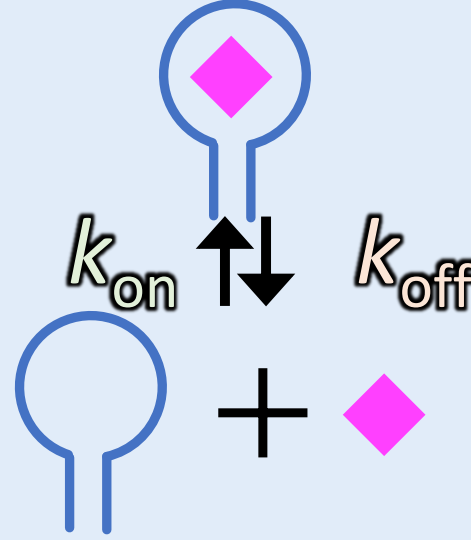
Future works

Determination of the 3D structure



Although the structure prediction suggests that the obtained RNAs form a G4 structure, it remains unclear whether these results accurately reflect the actual structure. Structure determination will be performed to confirm the actual structure.

Determination of kinetic parameters



The dissociation constants between the RNAs and TO1-B will be determined by titrating RNAs into solutions containing a fixed concentration of TO1-B. Additionally, the rate constants will be examined.

Design of new aptamers

Based on the structural information, the RNAs will be designed to have higher fluorogenic properties.