

## **July 2025**

### **Week 1 | 7.25 – 7.31**

This week, we engaged in the groundwork for DNA origami assembly. We verified the staple sequences provided by the dry group and ordered all the staple sequences and M13mp18. We then assembled our DNA origami for the first time.

- 1) All the staple sequences and M13mp18 were received.
- 2) We prepared the stock solution for every staple and aliquoted the stock solution. We then diluted the aliquots to the desired concentration.
- 3) The Amicon® Ultra-0.5 Centrifugal Filter Devices (100kDa) were received.
- 4) We tested and optimized the centrifugation speed and duration suitable for removing redundant staples using Amicon® Ultra-0.5 Centrifugal Filter Devices (100kDa).
- 5) We prepared the TAE/Mg<sup>2+</sup> Buffer according to the literature.
- 6) We assembled our first DNA origami according to the concentration ratio of staples to M13mp18 and thermocycling conditions of PCR supplied by other literature.
- 7) We tried to visualize the structure of DNA origami using an atomic force microscope. Unfortunately, the DNA origami was not assembled as we expected.

## **August 2025**

### **Week 2 | 8.1 – 8.7**

This week, based on the suggestions from the advisor and the statements from other literature, we made some improvements on our experimental design of DNA origami assembly.

- 1) We changed to preserve DNA origami at 4 degrees Celsius to avoid repeated freezing and thawing.

- 2) We added a 10-minute initial denaturation process to the PCR program.
- 3) We measured the absorbance of DNA origami solution at 260 nm during the removal of redundant staples to roughly assess DNA origami assembly.
- 4) We also used DNA electrophoresis analysis to preliminarily evaluate DNA origami assembly, based on the relative positions of M13mp18 band and DNA origami band.
- 5) We then retried to visualize the structure of DNA origami using atomic force microscope. This time, the DNA origami was assembled as we expected.

### **Week 3 | 8.8 - 8.14**

This week, we conducted the in vitro transcription of sgRNA<sub>L</sub>, the extracellular targeted cleavage of *lacZ* gene, and the preparation of FITC-sgRNA<sub>L</sub>/Cas9 complex.

#### **In vitro transcription of sgRNA<sub>L</sub>**

- 1) The transcription template of sgRNA<sub>L</sub> and primers were received.
- 2) We amplified the transcription template by PCR and purified the PCR products.
- 3) We transcribed the purified PCR products and purified the transcribed products.
- 4) We then used DNA electrophoresis analysis to visualize the bands of the transcribed products. We also measured the concentration and purity of the transcribed products. As a result, the transcription template was successfully transcribed.

#### **Extracellular cleavage of *lacZ* gene**

- 1) We amplified *lacZ* gene by colony PCR and purified the PCR products.

- 2) We incubated *lacZ* gene with sgRNA<sub>L</sub>/ Cas9 complex.
- 3) We used DNA electrophoresis analysis to determine whether the sgRNA<sub>L</sub>/ Cas9 complex correctly cleaved *lacZ* gene. As a result, the sgRNA<sub>L</sub>/ Cas9 complex successfully cleaved *lacZ* gene as we expected.

### **Preparation of FITC-sgRNA<sub>L</sub>/Cas9 complex**

- 1) We tried to label Cas9 nuclease with FITC but failed.
- 2) We changed to label sgRNA<sub>L</sub> with FITC.
- 3) We used Thermo Fisher Scientific NanoDrop One 2.12.0 and python 3.13 to measure the absorbance of FITC-sgRNA<sub>L</sub> at 495nm. As a result, FITC-sgRNA<sub>L</sub> exhibited an absorbance peak at 495nm, indicating successful FITC labeling.

### **Week 4 | 8.15 - 8.21**

This week, we loaded FITC-sgRNA<sub>L</sub>/Cas9 complex onto our DNA origami. We then verified the successful loading of FITC-sgRNA<sub>L</sub>/Cas9 complex.

- 1) We loaded F-H onto a DNA origami through a PCR program to conduct a Cy5-labeled DNA origami.
- 2) We then loaded FITC-sgRNA<sub>L</sub>/Cas9 complex onto our Cy5-labeled DNA origami through a PCR program.
- 3) After removal of redundant FITC-sgRNA<sub>L</sub>/Cas9 complex, we tried to confirm the successful loading of FITC-sgRNA<sub>L</sub>/Cas9 complex using DNA electrophoresis analysis. Unfortunately, we failed to observe the merged fluorescent signal from the gel imaging.
- 4) We replaced the fluorescent gel imaging method with absorption spectrum analysis to verify the successful loading of FITC-sgRNA<sub>L</sub>/Cas9 complex.
- 5) We measured the absorbances of the purified PCR product at

650nm and 495nm. As a result, the purified PCR product exhibited absorbance peaks at 650nm and 495nm, indicating successful loading of FITC-sgRNA<sub>L</sub>/Cas9 complex.

### **Week 5 | 8.22 - 8.28**

This week, we tested the presence of origami dimerization triggered by G-quadruplex and loaded hemin onto our DNA origami. We then verified the successful loading of hemin. We also drafted and refined the protocols of the ABTS assay of G4/hemin DNAzymes and NPN uptake assay.

- 1) We assembled our DNA origami and used DNA electrophoresis analysis to examine whether the number of G-quadruplexes in our design could induce origami dimerization. As a result, the G-quadruplexes did not trigger the dimerization of DNA origami.
- 2) Hemin was received.
- 3) We incubated hemin with our DNA origami at room temperature and removed redundant hemin by Amicon® Ultra-0.5 Centrifugal Filter Devices (100kDa).
- 4) We measured the absorbance of hemin-incubated DNA origami solution at 385nm. As a result, the hemin-incubated DNA origami solution exhibited an absorbance peak at 385nm, indicating the successful loading of hemin.

### **Week 6 | 8.29 - 9.4**

This week, we engaged in groundwork and preliminary tests of ABTS assay of G4/hemin DNAzymes and NPN uptake assay.

#### **ABTS assay of G4/hemin DNAzymes**

- 1) We conducted a preliminary test of ABTS assay of G4/hemin DNAzymes.
- 2) However, compared with the control group, G4/hemin DNAzymes on DNA origami did not generate an increase in the

absorbance at 415 nm.

### **NPN uptake assay**

- 1) We also conducted a preliminary test of NPN uptake assay. We prepared NPN stock solution using anhydrous ethanol and saline.
- 2) We then co-incubated bacterial cells with G4/hemin DNAzymes on DNA origami for various incubation periods to select the optimal incubation time.
- 3) After the incubation, we added all the components of each group into a 96-well plate.
- 4) Unfortunately, compared with the control group, G4/hemin DNAzymes on DNA origami did not produce an increase in the relative fluorescent unit.

## **September 2025**

### **Week 7 | 9.5 - 9.11**

This week, based on the suggestions from the advisor and the statements from other literature, we made some improvements on our experimental designs of ABTS assay of G4/hemin DNAzymes and NPN uptake assay.

### **ABTS assay of G4/hemin DNAzymes**

- 1) To mimic the concentration of H<sub>2</sub>O<sub>2</sub> in wounds, we added H<sub>2</sub>O<sub>2</sub> to a final concentration of 50 μM.
- 2) We retried ABTS assay based on the improvements. This time, compared with the control group, G4/hemin DNAzymes on DNA origami generated an increase in the absorbance at 415 nm.

### **NPN uptake assay**

- 1) To mimic the concentration of H<sub>2</sub>O<sub>2</sub> in wounds, we added H<sub>2</sub>O<sub>2</sub> to a final concentration of 50 μM.
- 2) We selected EDTA Na<sub>2</sub> as the positive control.

- 3) We changed the incubation period according to the literature.
  - 4) We prepared NPN stock solution using anhydrous ethanol and PBS.
- 3) We then retried NPN uptake assay. This time, G4/hemin DNAzymes on DNA origami still failed to produce an increase in the relative fluorescent unit.

## **Week 8 | 9.12 - 9.18**

This week, we refined our experimental design of NPN uptake assay. We also further verified the targeting of DNA origami using a laser scanning microscope.

### **NPN uptake assay**

- 1) We prepared NPN stock solution for the positive group using anhydrous ethanol and Tris-HCl, while the NPN stock solution for the other groups was prepared with anhydrous ethanol and PBS.
- 2) Based on the methods from other literature, we added all the components of each group directly into a black-bottomed 96-well plate without pre-incubation of bacterial cells and membrane permeabilizers.
- 3) We measured the relative fluorescent unit of each group immediately. This time, G4/hemin DNAzymes on DNA origami produced a significant increase in relative fluorescent unit. Moreover, G4/hemin DNAzymes on aptamer-loaded origami generated a higher relative fluorescent unit compared with G4/hemin DNAzymes on DNA origami.
- 4) We then calculated NPN uptake factor of each group based on the relative fluorescent unit of each group to make the results more intuitive.

## **Targeted fluorescence imaging of Cy5-labeled DNA origami**

- 1) We loaded F-H onto an aptamer-loaded DNA origami to conduct a Cy5-labeled DNA origami with attached aptamers.
- 2) We incubated *E. coli* MG1655 with the origami.
- 3) We then visualized the targeting of the origami using a laser scanning microscope. As a result, the origami successfully targeted the bacteria compared with the control group.