Synthesis and characterization of DNA three-way junction I (DNA TJ-I)

May 20th

◆ Preparation of DNA TJ-I

1. Add the reagents listed in the table below into a 250 μL PCR tube.

100 μM DNA1	100 μM DNA2	100 μM DNA3	10×PCR Buffer	H_2O
10 μL	10 μL	10 μL	5 μL	15 μL

2. Anneal the mixed solution in Veriti™ 96 Well Thermal Cycler under the temperature gradient below.

Temperature (℃)	95	80	65	50	35	20
Time (min/step)	10	10	10	10	10	10

May 21st

◆ Purification of DNA TJ-I with PCR product purification kits

1. Add 50 μL DNA TJ-I, 75 μL Buffer MP and 10 μL MagicMag Beads into a 1.5 mL EP tube.
2. Let it stay at room temperature for 1 min.
3. Place the tube on a magnetic separation rack. Discard the supernatant liquid after the MagicMag Beads stick to the tube wall.
4. Remove the tube from the magnetic separation rack. Add 500 μL 80% ethanol and agitate the tube. Place the tube on the magnetic separation rack. Discard the supernatant liquid after the MagicMag Beads stick to the tube wall.
5. Repeat step 4.
6. Remove the tube from the magnetic separation rack. Dry the tube for 20 min at room temperature.
7. Add 50 μL TE Buffer (pH 8.0) and heat the tube in 65 ℃ water bath for 5 min.
8. Place the tube on the magnetic separation rack. Pipet the supernatant liquid after the MagicMag Beads stick to the tube wall.

◆ Characterization of DNA TJ-I by Electrophoresis

1. Prepare the gel with 0.4 g agarose, 25 mL 1×TAE Buffer and 2.5 μL EB solution.
2. Mix the solutions (solvents) in 250 μL EP tubes according to the table below.

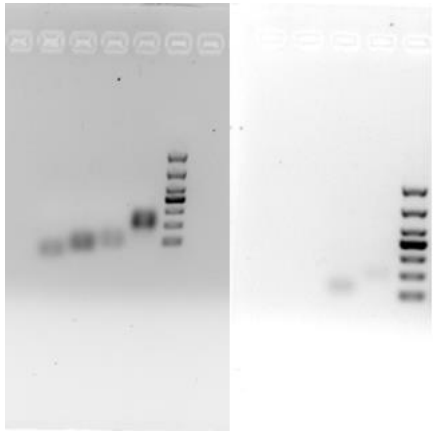
	Sample	H_2O	10×Loading Buffer
①	2 μL DNA1	4 μL	1 μL
②	2 μL DNA2		
③	2 μL DNA3		
④	2 μL Unpurified DNA TJ-I		
⑤	2 μL Purified DNA TJ-I		
⑥	2 μL DNA2		

3. Add sample solutions of 5 μL each into the sample holes, according to the table below. Set the voltage to 80 V and run the electrophoresis for 17 min.

Lane	1	2	3	4	5
Sample	DNA1	DNA2	DNA3	Unpurified DNA TJ-I	DL1,000 DNA Marker

Lane	1	2	3
Sample	DNA2	Purified DNA TJ-I	DL1,000 DNA Marker

4. Obtain the electrophoretogram with GenoSens 1800 gel imaging system.



May 22nd

◆ Preparation of silicon wafers used for atomic force microscope (AFM)

1. Cut a large silicon wafer into small squares.
2. Soak the silicon wafers in chromic acid and let them stay for 8 hours.
3. Soak the silicon wafers in the solvents listed below and treat them with ultrasound.

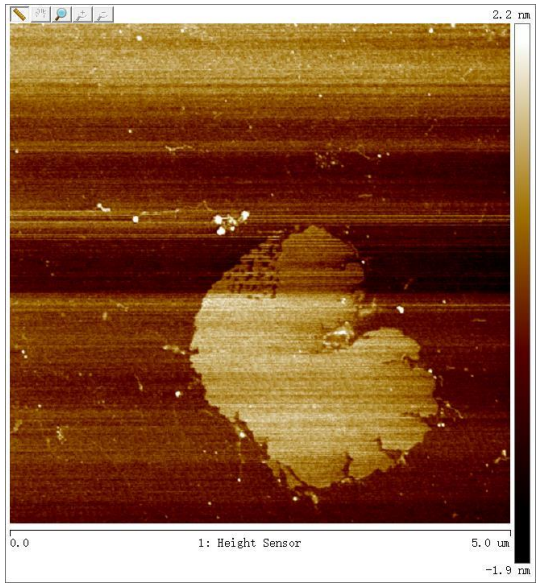
Solvent	Isopropanol	Methylbenzene	Methanol	Water
Time	7 min	7 min	7 min	7 min
Number of times	1	1	1	3

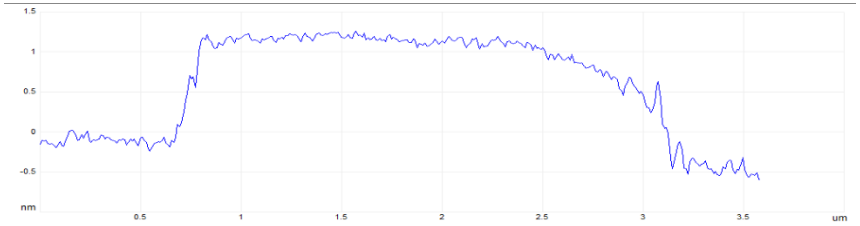
4. Soak the silicon wafers in a mixture of 15 mL H_2O_2 and 35 mL concentrated sulfuric acid. Heat the solution for about 30 min until bubbling stops.
5. Let it cool to room temperature. Discard the acid solution and rinse the wafers with water. Then soak them in water and treat them with ultrasound for 7 min three times.
6. Transfer the silicon wafers to a clean beaker and store them in water.

May 23rd

◆ Characterization of DNA TJ-I by AFM

1. Prepare an AFM sample.
   (1) Obtain a silicon wafer and dry it with nitrogen. Place a piece of filter paper in a watch glass and place the wafer on the filter paper.
   (2) Dilute DNA TJ-I sample by a factor of 10 with water and drop it onto the silicon wafer. Let it stay for 5 min. Then discard excess liquid.
   (3) Rinse the wafer with a drop of water.
   (4) Dry the wafer at room temperature.
2. Obtain the image with Dimension Fastscan atomic force microscope.





May 24th

- Preparation of more DNA TJ-I for further experiments

  The method of assembly is the same as the method on May 20th.

- Characterization of DNA TJ-I by Electrophoresis

  The method of characterization is the same as the method on May 21st.

May 25th

◆ Purification of DNA TJ-I by PCR product purification kits

The method of purification is the same as the method on May 21st.

◆ Measurement of the concentration of purified DNA TJ-I

Measure the concentration by BioPhotometer nucleic acid protein quantitative detector (Eppendorf) using the method for the determination of dsDNA. The concentration is 1.9 μM.

Synthesis and characterization of Vesicle I

May 26th

➢ Study of the effects of the ratio of DNA TJ-I and glutaraldehyde on the self-assembly of DNA TJ-I

◆ Self-assembly of DNA TJ-I and glutaraldehyde at different ratios of DNA TJ-I and glutaraldehyde

1. Prepare aqueous solutions of glutaraldehyde.
   (1) Dilute 5 μL 50 wt. % glutaraldehyde solution (4.73 M) with 5 mL H_2O. Then dilute 5 μL this solution with 88.7 μL H_2O to obtain 300 μM glutaraldehyde solution.
   (2) Dilute 28.5 μL 300 μM glutaraldehyde solution with 271.5 μL H_2O to obtain 28.5 μM glutaraldehyde solution.
   (3) Dilute 19 μL 28.5 μM glutaraldehyde solution with 9.5 μL H_2O to obtain 19 μM glutaraldehyde solution.
   (4) Dilute 10 μL 28.5 μM glutaraldehyde solution with 20 μL H_2O to obtain 9.5 μM glutaraldehyde solution.
2. Prepare 0.2 M pH 8.0 phosphate buffer (PB).
   (1) Dissolve 0.361 g $NaH_2PO_4 \cdot 2H_2O$ in 10 mL H_2O to obtain solution A.
   (2) Dissolve 0.717 g $Na_2HPO_4 \cdot 12H_2O$ in 10 mL H_2O to obtain solution B.
   (3) Mix 53 μL solution A with 947 μL solution B to obtain 0.2 M pH 8.0 PB.
3. Mix the solutions in vials according to the table below.

	Ratio	1.9 μM DNA TJ-I	Glutaraldehyde solution	0.2M pH 8.0 PB
0526-1	1:5	5 μL	28.5 μM, 5 μL	40 μL
0526-2	1:10		19.0 μM, 5 μL	
0526-3	1:15		9.5 μM, 5 μL	

4. Stir the solutions at room temperature for 72 h.
5. Obtain the assemblies on May 29th and store them in EP tubes at -20 ℃.

May 29th

➢ Study of the effects of concentration of DNA TJ-I and glutaraldehyde on the self-assembly of DNA TJ-I

◆ Self-assembly of DNA TJ-I and glutaraldehyde at different concentrations of DNA TJ-I and glutaraldehyde

1. Mix the solutions in vials according to the table below.

	1.9 μM DNA TJ-I	28.5 μM Glutaraldehyde solution	0.2 M pH 8.0 PB	H_2O
0529-1	2 μL	2 μL	80 μL	16 μL
0529-2	4 μL	4 μL		12 μL
0529-3	6 μL	6 μL		8 μL
0529-4	8 μL	8 μL		4 μL
0529-5	10 μL	10 μL		0

2. Stir the solutions at room temperature for 72 h.
3. Obtain the assemblies on June 1st and store them in EP tubes at -20 ℃.

June 1st

◆ Self-assembly of DNA TJ-I and glutaraldehyde in pH 8.0 PB

1. Prepare 28.5 μM aqueous solution of glutaraldehyde.

   The method is the same as the method on May 26th.

2. Prepare 0.2 M pH 8.0 PB.

   The method is the same as the method on May 26th.

3. Mix the solutions in a vial according to the table below.

	1.9 μM DNA TJ-I	28.5 μM Glutaraldehyde solution	0.2 M pH 8.0 PB
0601-1	10 μL	10 μL	80 μL

4. Stir the solution at room temperature for 72 h.

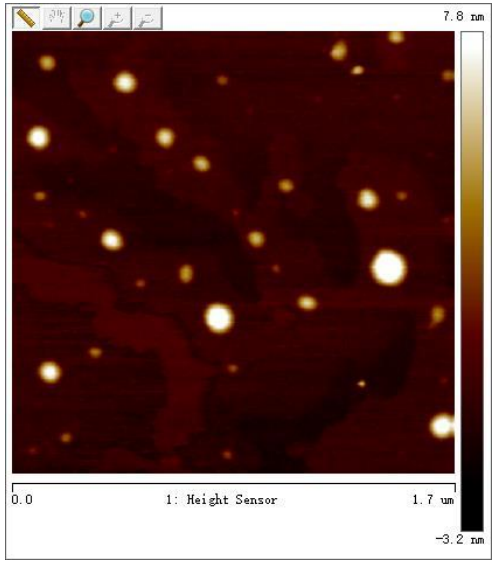
5. Obtain the assembly on June 7th and store it in an EP tube at -20 ℃.

June 5th

◆ Characterization of the assemblies by AFM

Obtain the images of the assemblies by Dimension Fastscan atomic force microscope. The method of characterization is the same as the method on May 23rd.

The AFM image of 0601-1 is shown below.



June 6th

◆ Characterization of the assemblies by transmission electron microscope (TEM)

1. Prepare 1.0% uranyl acetate aqueous solution.

   Dissolve 4 mg uranyl acetate in 400 μL H_2O.

2. Prepare TEM samples.

   (1) Obtain 300-mesh carbon support films. Place a piece of filter paper in a watch glass and place the 300-mesh carbon support films on the filter paper.

   (2) For each sample, drop 1.5 μL sample solution onto a 300-mesh carbon support film three times. Let it stay for 5 min.

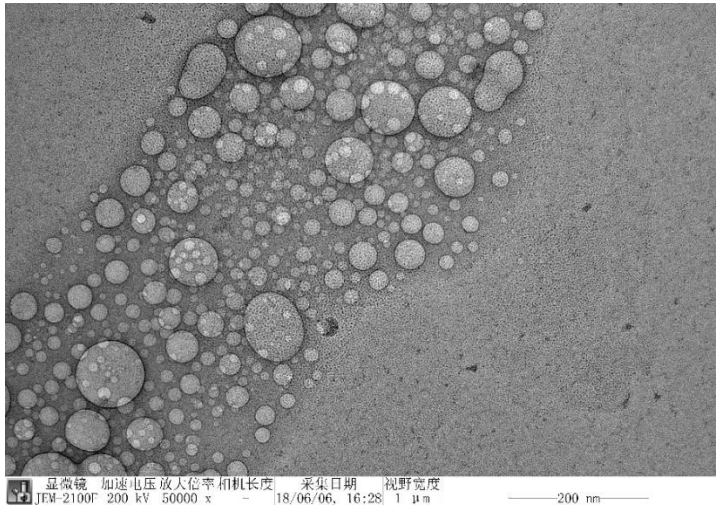
   (3) Rinse the 300-mesh carbon support films with 5.0 μL water.

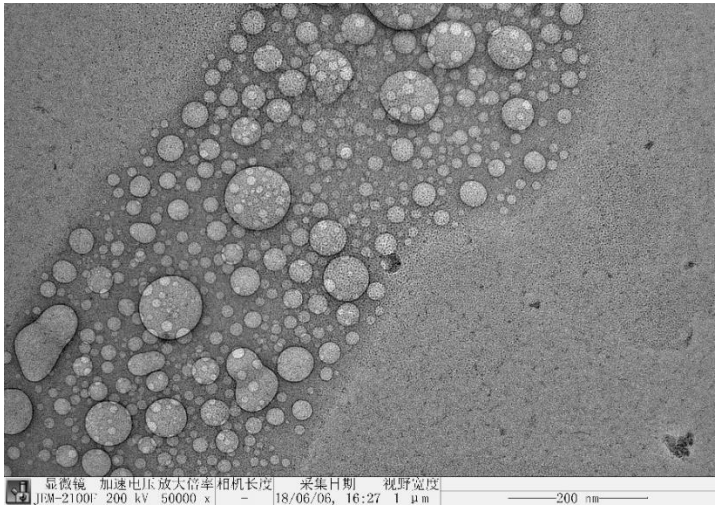
   (4) Drop 5.0 μL uranyl acetate solution (1.0%) onto the 300-mesh carbon support films. Remove excess liquid with filter paper after 5 min.

   (5) Dry the 300-mesh carbon support films at room temperature.

3. Obtain the TEM images of the assemblies by JEM-2100F field emission transmission electron microscope.

   The TEM images of 0601-1 are shown below.



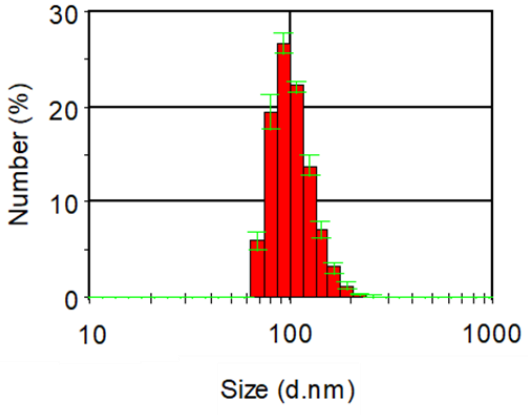


June 11th

◆ Characterization of the assemblies by dynamic light scattering (DLS)

1. For each sample, dilute 20 μL sample solution by a factor of 5 with H_2O.
2. Set the solvent viscosity to 0.82 cP, the dielectric constant to 78.3 $V \cdot m^{-1}$, and the refractive index to 1.330. Set the balance time to 120 s.
3. Obtain the particle size distribution of the assemblies with Zetasizer Nano ZS90.

   The DLS result of 0601-1 is shown below.



June 12th

➢ Study of the effects of pH on the self-assembly of DNA TJ-I

◆ Self-assembly of DNA TJ-I and glutaraldehyde at different pH

1. Prepare 0.2 M pH 6.0 PB (0.2 M).

   Mix 877 μL solution A with 123 μL solution B to obtain 0.2 M pH 6.0 PB. (Solution A and solution B were prepared on May 26th)

2. Prepare 0.2 M pH 5.0 acetate buffer (AB).

   (1) Dilute 0.1155 mL glacial acetic acid with 10 mL H_2O to obtain solution C.

   (2) Dissolve 0.272 g $NaAc·3H_2O$ in 10 mL water to obtain solution D.

   (3) Mix 300 μL solution C with 700 μL solution D to obtain 0.2 M pH 5.0 acetate buffer.

3. Mix the solutions in vials according to the table below.

	1.9 μM DNA TJ-I	28.5 μM Glutaraldehyde solution	Buffer
0612-1	10 μL	10 μL	0.2 M pH 8.0 PB 80 μL
0612-2	10 μL	10 μL	0.2 M pH 6.0 PB 80 μL
0612-3	10 μL	10 μL	0.2 M pH 5.0 AB 80 μL

4. Stir the solutions at room temperature for 72 h.

5. Obtain the assemblies on June 15th and store them in EP tubes at -20 ℃.

➢ Study of the effects of linker on the self-assembly of DNA TJ-I

◆ Self-assembly of DNA TJ-I and glyoxal at pH 8

1. Prepare the aqueous solution of glyoxal (28.5 μM).

   (1) Dilute 5 μL 40 wt. % glyoxal solution (10.16 M) with 5 mL H_2O. Then dilute 3 μL this solution with 98.6 μL H_2O to obtain 300 μM glyoxal solution.

   (2) Dilute 28.5 μL 300 μM glyoxal solution with 271.5 μL H_2O to obtain 28.5 μM glyoxal solution.

2. Mix the solutions in a vial according to the table below.

	1.9 μM DNA TJ-I	28.5 μM Glyoxal solution	0.2M pH 8.0 PB
0612-4	10 μL	10 μL	80 μL

3. Stir the solution at room temperature for 72 h.

4. Obtain the assembly on June 15th and store it in an EP tube at -20 ℃.

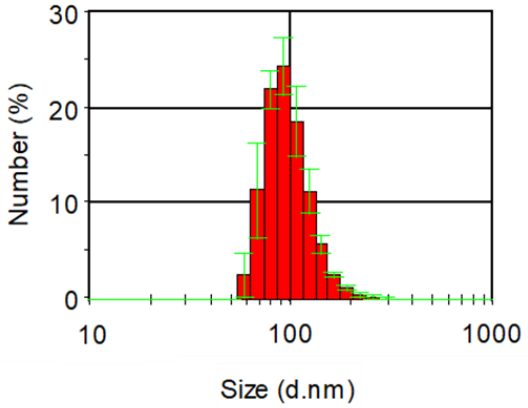
June 16th

◆ Characterization of 0612-2 and 0612-3 by DLS

Obtain DLS results by Zetasizer Nano ZS90.

The method is the same as the method on June 11th.

The DLS result of 0612-2 is shown below.



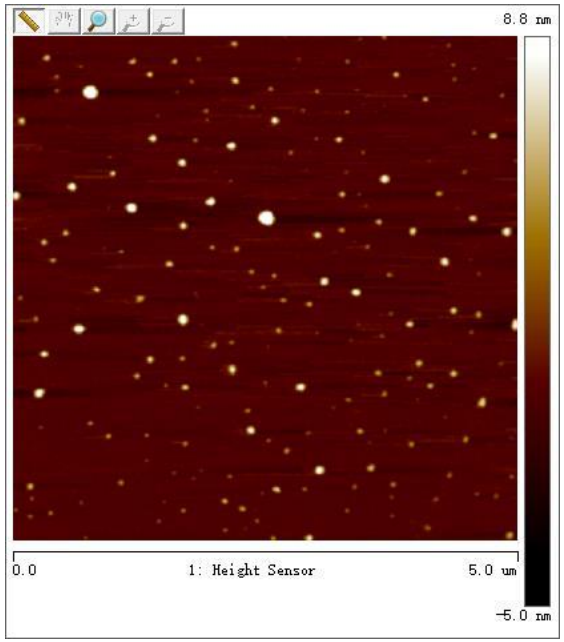
The DLS result of 0612-3 showed that no nanoparticle was detected.

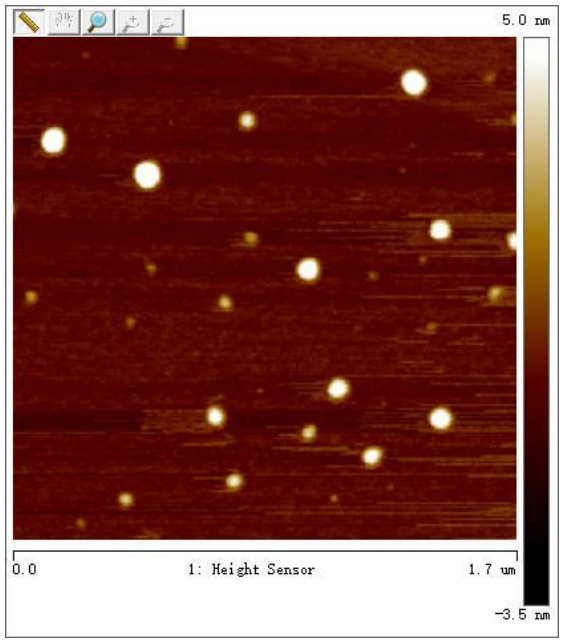
June 19th

◆ Characterization of 0612-4 by AFM

Obtain the images by Dimension Fastscan atomic force microscope. The method is the same as the method on May 25th.

The AFM images of 0612-4 are shown below.





➢ Study of the effects of Tris and solvent polarity on the self-assembly of DNA TJ-I

July 15th

- Self-assembly of DNA TJ-I and glutaraldehyde in an aqueous pH 8.0 solution

1. The method is the same as the method on June 4th.
2. Obtain the assembly on July 18th and store it in an EP tube at -20 ℃. The sample is named 0715-1.

July 23rd

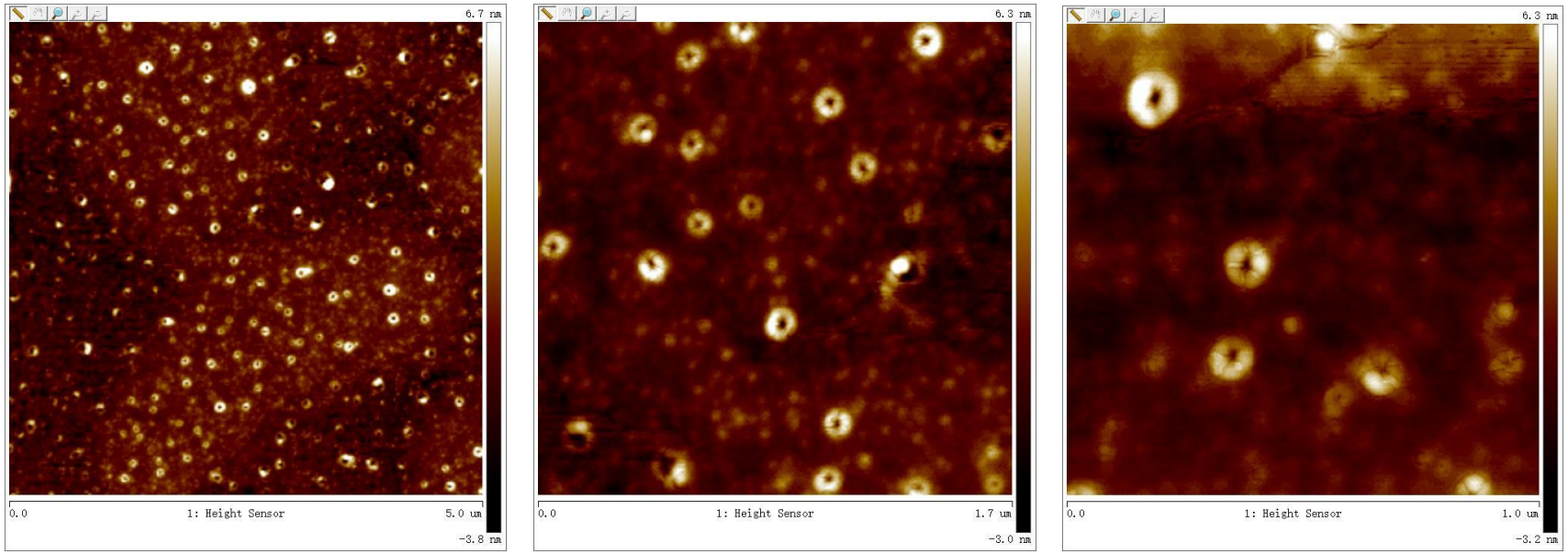
◆ Etching of 0715-1 by Tris

1. Dilute 1 μL sample solution by a factor of 10 with H_2O.
2. Mix 10 μL diluted sample with 1.0 μL EB solution containing 100 μM Tris. The new sample is named 0723-1.

◆ Characterization of 0723-1 by AFM

Obtain the images by Dimension Fastscan atomic force microscope. The method is the same as the method on May 25th.

The AFM images of 0723-1 are shown below.



◆ Self-assembly of DNA TJ-I and glutaraldehyde in mixed solvent of water and tetrahydrofuran (THF)

1. Mix the solutions in vials according to the table below.

	1.9 μM DNA TJ-I	28.5 μM glutaraldehyde solution	Buffer	THF
0723-2	10 μL	10 μL	0.2M pH 8.0 PB 30 μL	50 μL
0723-3	10 μL	10 μL	0.2M pH 6.0 PB 30 μL	50 μL

2. Stir the solutions at room temperature for 72 h.
3. Obtain the assemblies on June 26th and store them in EP tubes at -20 ℃.

July 30th

◆ Etching of 0723-2 and 0723-3 by Tris

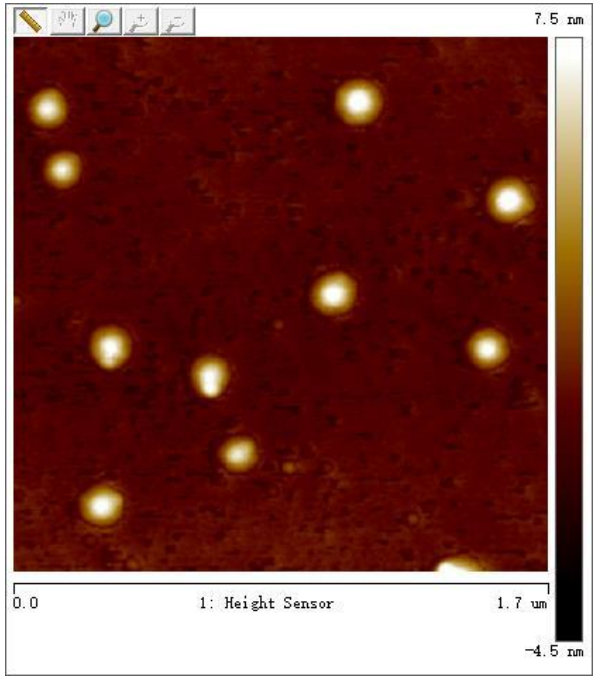
1. For each sample, dilute 1 μL sample solution by a factor of 10 with H_2O/THF (1:1).
2. For each sample, mix 10 μL diluted sample with 1.0 μL EB solution containing 100 μM Tris. The new samples are named 0730-1 and 0730-2 respectively.

◆ Characterization of 0723-2 and 0723-3 by AFM

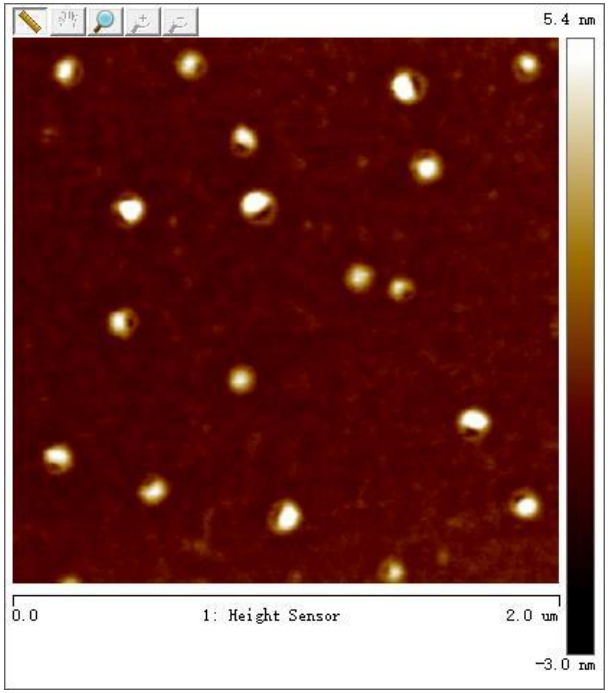
Obtain the images by Dimension Fastscan atomic force microscope.

The method is the same as the method on May 25th.

The AFM image of 0723-2 is shown below.



The AFM image of 0723-3 is shown below.



Functionalization of Vesicle I by loading streptavidin

July 31st

◆ Preparation of more DNA TJ-I for further experiments

The method of assembly is the same as the method on May 20th.

◆ Self-assembly of impurified DNA TJ-I and glutaraldehyde in pH 8.0 PB (H_2O: THF=3:1)

1. Mix the solutions in a vial according to the table below.

	DNA TJ-I (20 μM)	Glutaraldehyde solution (300 μM)	0.2M pH 8.0 PB	THF
0731-1	10 μL	10 μL	10 μL	10 μL

2. Stir the solution at room temperature for 72 h.
3. Obtain the assembly on August 3rd and store it in an EP tube at -20 ℃.

August 4th

◆ Characterization of 0731-1 by optical microscope and fluorescence microscope

1. Mix 5.0 μL sample solution with 0.5 μL ethidium bromide and drop the mixture onto a glass slide. Remove excess solution after 5 min.
2. Rinse the glass slide with 5.0 μL water.
3. Dry the glass slide at room temperature.
4. Obtain optical microscope and fluorescence microscope images with the digital imaging system Olympus DP72.

◆ Loading streptavidin onto 0731-1

1. Mix 10 μL 0731-1 with 2 μL 0.26 g/L aqueous solution of streptavidin.
2. Let it stay at room temperature for 6 min. The new sample is named 0804-1.

◆ Characterization of 0804-1 by optical microscope and fluorescence microscope

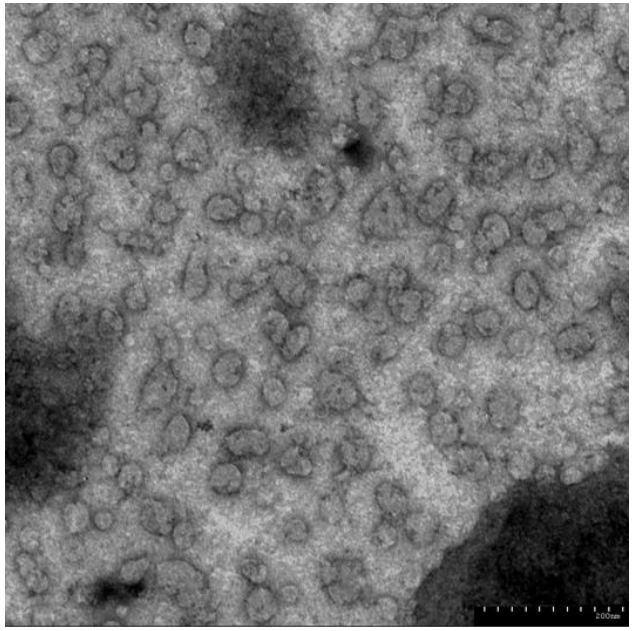
Obtain optical microscope and fluorescence microscope images with the digital imaging system Olympus DP72.

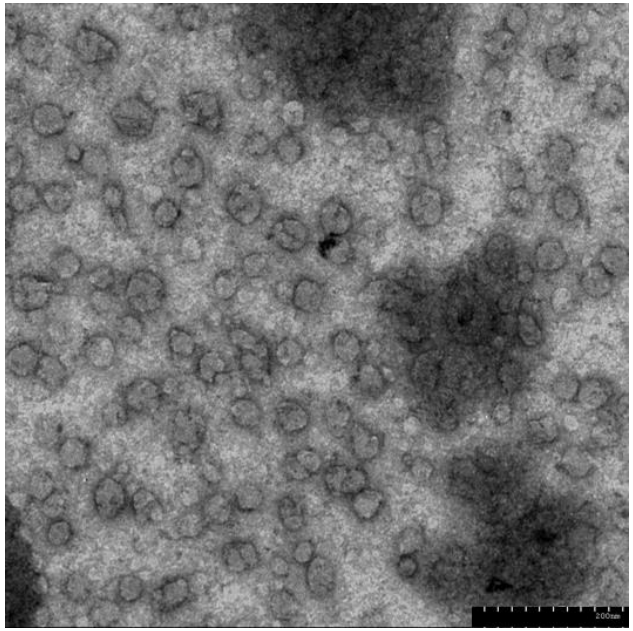
The method is the same as the method used above.

◆ Characterization of 0612-2 by TEM

Obtain the TEM image of the assemblies by JEM-2100F field emission transmission electron microscope. The method is the same as the method on June 6th.

The TEM images of 0612-2 are shown below.


200nm


200nm

Synthesis and characterization of DNA three-way junction II (DNA TJ-II)

August 20th

➢ Study of the effects of salt concentration on the self-assembly of ssDNAs

◆ Preparation of DNA TJ-II in NaCl solution

1. Prepare 0.5 M NaCl aqueous solution.

   Dissolve 0.0293 g NaCl in 1 mL H_2O to obtain 0.5 M NaCl aqueous solution.

2. Add the reagents listed in the table below into 250 μL PCR tubes respectively.

	100 μM DNA-tif-1	100 μM DNA-tif-2	100 μM DNA-tif-3	H_2O	0.5 M NaCl
0820-1	10 μL	10 μL	10 μL	20 μL	0
0820-2	10 μL	10 μL	10 μL	15 μL	5 μL

3. Anneal the mixed solutions in Veriti™ 96 Well Thermal Cycler under the temperature gradient below.

Temperature (℃)	95	80	65	50	35	20
Time (min/step)	10	10	10	10	10	10

◆ Characterization of 0820-1 and 0820-2 by Electrophoresis

The method is the same as the method on May 21st.

August 21st

◆ Preparation of DNA TJ-II at different NaCl concentration

1. Prepare NaCl aqueous solutions.

   (1) Dissolve 0.117 g NaCl in 1 mL H_2O to obtain 2.0 M NaCl aqueous solution.

   (2) Dilute 0.5 mL 2.0 M NaCl with 0.5 mL H_2O to obtain 1.0 M NaCl aqueous solution.

   (3) Dilute 0.5 mL 1.0 M NaCl with 0.5 mL H_2O to obtain 0.5 M NaCl aqueous solution.

2. Add the reagents listed in the table below into 250 μL PCR tubes respectively.

<table>
<tr><th></th><th>100 μM DNA-tif-1</th><th>100 μM DNA-tif-2</th><th>100 μM DNA-tif-3</th><th>Electrolyte</th><th>H_2O</th></tr>
<tr><td>0821-1</td><td colspan="3" rowspan="3">Each 4 μL</td><td>0.5 M NaCl 4 μL</td><td rowspan="3">4 μL</td></tr>
<tr><td>0821-2</td><td>1.0 M NaCl 4 μL</td></tr>
<tr><td>0821-3</td><td>2.0 M NaCl 4 μL</td></tr>
<tr><td>0821-4</td><td colspan="3">10×PCR Buffer 2 μL</td><td>6 μL</td></tr>
</table>

3. Anneal the mixed solution in Veriti™ 96 Well Thermal Cycler under the temperature gradient below.

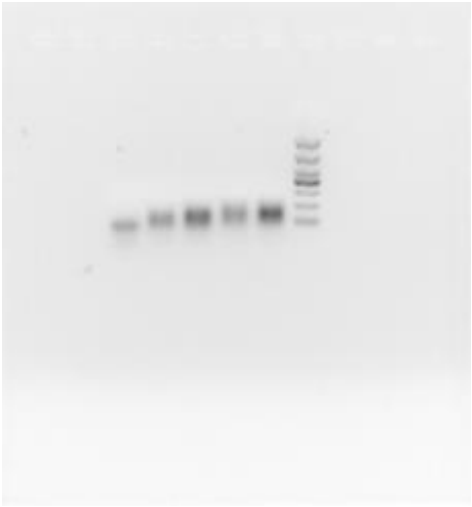
Temperature (°C)	95-4
Time (min/°C)	1

August 22nd

◆ Characterization of 0821-1, 0821-2, 0821-3, 0821-4 by Electrophoresis

Obtain the electrophoretogram with GenoSens 1800 gel imaging system. The method is the same as the method on May 21st.

Lane	1	2	3	4	5	6
Sample	DNA-tif-1	0821-1	0821-2	0821-3	0821-4	DL1,000 DNA Marker



➢ Study of the effects of temperature gradient on the self-assembly ssDNAs

◆ Preparation of DNA TJ-II under different temperature gradients

1. Add the reagents listed in the table below into 250 μL PCR tubes respectively.

	100 μM DNA-tif-1	100 μM DNA-tif-2	100 μM DNA-tif-3	Electrolyte	H_2O
0822-1	Each 4 μL			0.5 M NaCl 4 μL	4 μL
0822-2				1.0 M NaCl 4 μL	
0822-3				2.0 M NaCl 4 μL	
0822-4				10×PCR Buffer 2 μL	6 μL

2. Anneal the mixed solution in VeritiTM 96 Well Thermal Cycler under the temperature gradient below.

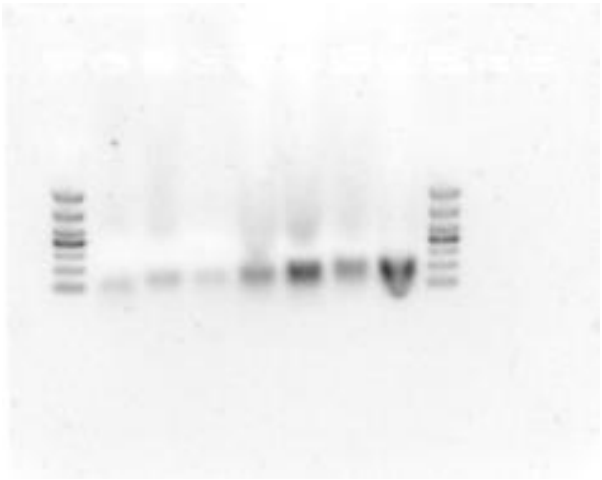
Temperature (℃)	95-71	70-35	34-4
Time (min/℃)	1	3	1

August 23rd

◆ Characterization of 0822-1, 0822-2, 0822-3, 0822-4 by Electrophoresis

Obtain the electrophoretogram with GenoSens 1800 gel imaging system. The method is the same as the method on May 21st.

Lane	1&9	2	3	4	5	6	7	8
Sample	DL1,000 DNA Marker	DNA-tif-1	DNA-tif-2	DNA-tif-3	0822-1	0822-2	0822-3	0822-4



➢ **Study of the effects of EDTA concentration on the self-assembly of ssDNAs**

◆ Preparation of DNA TJ-II at different EDTA concentration

1. Prepare EDTA aqueous solutions.

   (1) Dissolve 0.0749 g $Na_2EDTA{\cdot}2H_2O$ in 1 mL H_2O to obtain 0.2 M EDTA aqueous solution.

   (2) Dilute 0.1 mL 0.2M EDTA with 10 mL H_2O to obtain 2.0 mM EDTA aqueous solution.

   (3) Dilute 5 mL 2.0mM EDTA with 5 mL H_2O to obtain 1.0 mM EDTA aqueous solution.

   (4) Dilute 5 mL 1.0mM EDTA with 5 mL H_2O to obtain 0.5 mM EDTA aqueous solution.

2. Add the reagents listed in the table below into 250 uL PCR tubes respectively.

	100 μM DNA-tif-1	100 μM DNA-tif-2	100 μM DNA-tif-3	Electrolyte
0823-1	Each 4 μL			2.0 M NaCl 4 μL+ H_2O 4 μL
0823-2				2.0 M NaCl 4 μL+ 0.5 mM EDTA 4 μL
0823-3				2.0 M NaCl 4 μL +1.0 mM EDTA 4 μL
0823-4				2.0 M NaCl 4 μL +2.0 mM EDTA 4 μL
0823-5				10×PCR Buffer 2 μL+ H_2O 6 μL

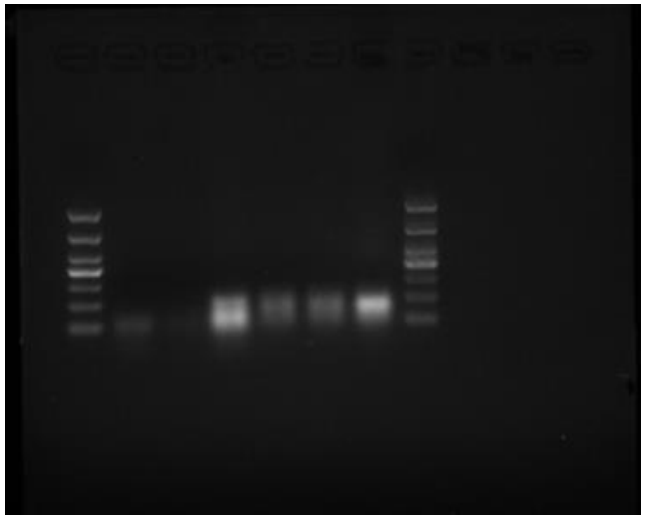
3. Anneal the mixed solution in Veriti^{TM} 96 Well Thermal Cycler under the temperature gradient below.

Temperature (℃)	95-71	70-35	34-4
Time (min/℃)	1	3	1

◆ Characterization of 0823-1, 0823-2, 0823-3, 0823-4, 0823-5 by Electrophoresis

Obtain the electrophoretogram with GenoSens 1800 gel imaging system. The method is the same as the method on May 21st.

Lane	1&8	2	3	4	5	6	7
Sample	DL1,000 DNA Marker	DNA-tif-3	0823-1	0823-2	0823-3	0823-4	0823-5



August 24th

◆ Preparation of DNA TJ-II

1. Prepare 20 mM EDTA solution.

   (1) Dissolve 0.0749 g $Na_2EDTA \cdot 2H_2O$ in 1 mL H_2O to obtain 0.2 M EDTA aqueous solution.

   (2) Dilute 0.1 mL 0.2 M EDTA with 0.9 mL H_2O to obtain 20 mM EDTA aqueous solution.

2. Add the reagents listed in the table below into 250 μL PCR tubes respectively.

	100 μM DNA-tif-1	100 μM DNA-tif-2	100 μM DNA-tif-3	Electrolyte
0824-1	Each 4 μL			2.0 M NaCl 4 μL+ 20 mM EDTA 4 μL
0824-2				2.0 M NaCl 8 μL

3. Anneal the mixed solution in Veriti™ 96 Well Thermal Cycler under the temperature gradient below.

Temperature (°C)	95-71	70-35	34-4
Time (min/°C)	1	5	1

◆ Characterization of 0824-1 and 0824-2 by Electrophoresis

Obtain the electrophoretogram with GenoSens 1800 gel imaging system. The method is the same as the method on May 21st.

Lane	1	2	3	4
Sample	DNA-tif-2	0824-1	0824-2	DL1,000 DNA Marker

◆ Preparation of more DNA TJ-II for further experiments

1. Add the reagents listed in the table below into a 250 μL PCR tube.

	100 μM DNA-tif-1	100 μM DNA-tif-2	100 μM DNA-tif-3	Electrolyte
0824-3	Each 10 μL			2.0 M NaCl 20 μL

2. Anneal the mixed solution in Veriti™ 96 Well Thermal Cycler under the temperature gradient below.

Temperature (°C)	95-71	70-35	34-4
Time (min/°C)	1	5	1

◆ Characterization of 0824-3 by Electrophoresis

Obtain the electrophoretogram with GenoSens 1800 gel imaging system. The method is the same as the method on May 21st.

Lane	1	2	3	4	5
Sample	DNA-tif-1	DNA-tif-2	DNA-tif-3	0824-3	DL1,000 DNA Marker

Synthesis and characterization of Vesicle II

August 25th

➢ Study of the effects of pH on the self-assembly of DNA TJ-II

◆ Self-assembly of DNA TJ-II and glutaraldehyde in H_2O at different pH

1. Prepare 0.2 M pH 7.0 PB.
   (1) Dissolve 0.361 g $NaH_2PO_4 \cdot 2H_2O$ in 10 mL H_2O to obtain solution A.
   (2) Dissolve 0.717 g $Na_2HPO_4 \cdot 12H_2O$ in 10 mL H_2O to obtain solution B.
   (3) Mix 390 μL solution A with 610 μL solution B to obtain 0.2 M pH 7.0 PB.
2. Mix the solutions in vials according to the table below.

	20 μM DNA TJ- II	300 μM Glutaraldehyde solution	0.2 M Buffer
0825-1	Each 10 μL		pH 5.0 AB 80 μL
0825-2			pH 6.0 PB 80 μL
0825-3			pH 7.0 PB 80 μL
0825-4			pH 6.0 PB 80 μL

3. Stir the solutions at room temperature for 72 h.
4. Obtain the assemblies on August 28th and store them in EP tubes at -20 ℃.

August 28th

◆ Characterization of 0825-1, 0825-2, 0825-3, 0825-4 by DLS

Obtain DLS results by Zetasizer Nano ZS90. The method is the same as the method on June 11th.

◆ Characterization of 0825-1, 0825-2, 0825-3, 0825-4 by fluorescence microscope

Obtain fluorescence microscope images by the digital imaging system Olympus DP72. The method is the same as the method on August 4th.

August 29th

➢ Study of the effects of temperature on the self-assembly of DNA TJ-II

◆ Preparation of more DNA TJ-II for further experiments

The method of assembly is the same as the method on August 24th.

◆ Self-assembly of DNA TJ-II and glutaraldehyde in H_2O at different pH and at different temperature

1. Mix the solutions in vials according to the table below.

	20 μM DNA TJ- II	300 μM Glutaraldehyde solution	0.2 M Buffer	Temperature (℃)
0829-1	Each 10 μL		pH 5.0 AB 80 μL	4
0829-2				25
0829-3			pH 6.0 PB 80 μL	4
0829-4				25
0829-5			pH 7.0 PB 80 μL	4
0829-6				25
0829-7			pH 6.0 PB 80 μL	4
0829-8				25

2. Stir the solutions for 72 h.
3. Obtain the assemblies on September 1st and store them at -20 ℃.

September 2nd – September 3rd

- Characterization of 0829-1, 0829-2, 0829-3, 0829-4, 0829-5, 0829-6, 0829-7, 0829-8 by DLS

  Obtain DLS results by Zetasizer Nano ZS90. The method is the same as the method on June 11th.

- Characterization of 0829-1, 0829-2, 0829-3, 0829-4, 0829-5, 0829-6, 0829-7, 0829-8 by fluorescence microscope

  Obtain fluorescence microscope images by the digital imaging system Olympus DP72. The method is the same as the method on August 4th.

September 4th

➢ Study of the effects of solvent polarity on the self-assembly of DNA TJ-II

◆ Self-assembly of DNA TJ-II and glutaraldehyde in solvents of different polarity

1. Mix the solutions in vials according to the table below.

	20 μM DNA TJ-II	300 μM Glutaraldehyde solution	0.2 M pH 6.0 PB	H_2O	THF
0904-1	Each 10 μL		30 μL	50 μL	0
0904-2				0	50 μL

2. Stir the solutions at 4 ℃ for 72 h.
3. Obtain the assemblies on September 7th and store them in EP tubes at -20 ℃.

◆ Characterization of 0904-1 and 0904-2 by DLS

Obtain DLS results by Zetasizer Nano ZS90. The method is the same as the method on June 11th.

◆ Characterization of 0904-1 and 0904-2 by fluorescence microscope

Obtain fluorescence microscope images by the digital imaging system Olympus DP72. The method is the same as the method on August 4th.

September 8th

➢ Study of the effects of concentration of DNA TJ-II on the self-assembly of DNA TJ-II

◆ Self-assembly of DNA TJ-II and glutaraldehyde at different concentrations of DNA TJ-II

1. Mix the solutions in vials according to the table below.

	20 μM DNA TJ-II	300 μM Glutaraldehyde solution	0.2 M pH 6.0 PB	H_2O	THF
0908-1	2 μL	2 μL	30 μL	16 μL	50 μL
0908-2	5 μL	5 μL		10 μL	
0908-3	8 μL	8 μL		4 μL	
0908-4	10 μL	10 μL		0	

2. Stir the solutions at 4 ℃ for 72 h.
3. Obtain the assemblies on September 11th and store them in EP tubes at -20 ℃.

September 12th – September 15th

- Characterization of 0908-1, 0908-2, 0908-3, 0908-4 by DLS

  Obtain DLS results by Zetasizer Nano ZS90. The method is the same as the method on June 11th.

- Characterization of 0908-1, 0908-2, 0908-3, 0908-4 by fluorescence microscope

  Obtain fluorescence microscope images by the digital imaging system Olympus DP72. The method is the same as the method on August 4th.

- Characterization of 0908-1, 0908-2, 0908-3, 0908-4 by TEM

  Obtain the TEM images of the assemblies by JEM-2100F field emission transmission electron microscope. The method is the same as the method on June 6th.

September 16th

- Preparation of more DNA TJ-II for further experiments

  The method of assembly is the same as the method on August 24th.

September 17th

◆ Self-assembly of DNA TJ-II and glutaraldehyde in pH 6.0 PB (H_2O: THF=1:1)

1. Mix the solutions in a vial according to the table below.

	20 μM DNA TJ-II	300 μM Glutaraldehyde solution	0.2 M pH 6.0 PB	H_2O	THF
0916-1	4 μL	4 μL	60 μL	100 μL	32 μL

2. Stir the solution at 4 ℃ for 72 h.
3. Obtain the assembly on September 20th and store it in an EP tube at -20 ℃.

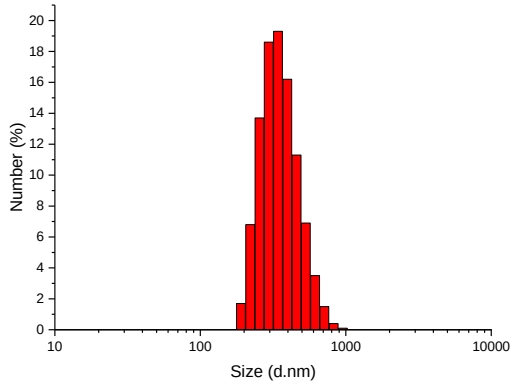
pH-responding size regulation of Vesicle II

September 20th

◆ Characterization of 0916-1 by DLS

Obtain DLS results by Zetasizer Nano ZS90. The method is the same as the method on June 11th.

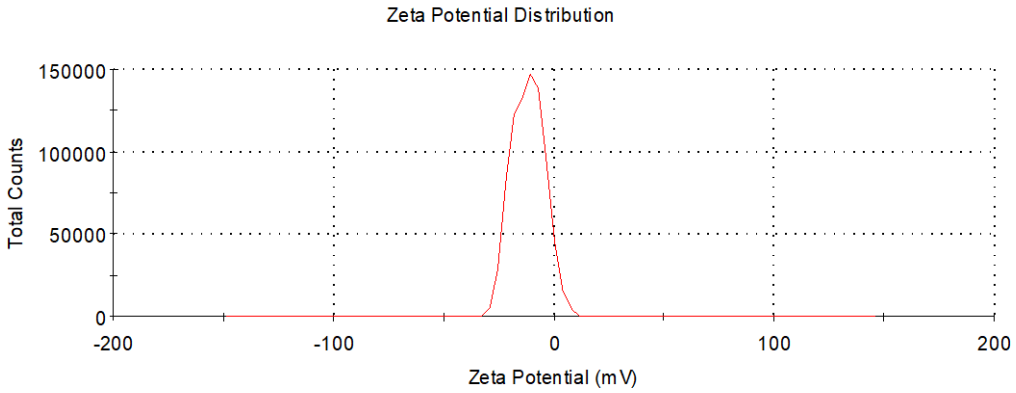
The DLS result of 0916-1 is shown below.



◆ Measurement of Zeta potential of 0916-1

Obtain Zeta potential results by Zetasizer Nano ZS90. The method is the same as the method on June 11th.

The Zeta potential result is shown below. The peak potential is -11 mV.



◆ pH-adjusting of 0916-1

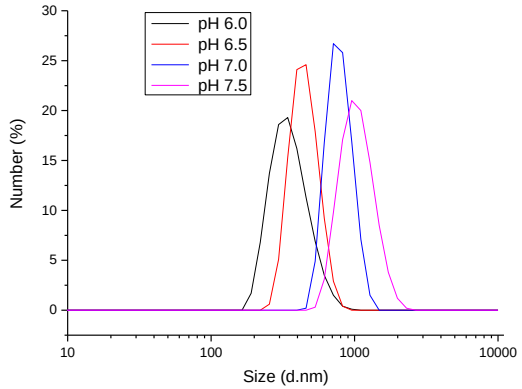
1. Prepare NaOH solutions.
    (1) Dissolve 0.00922 g NaOH in 5 mL H_2O. Mix 500 μL this solution with 500 μL THF to obtain 0.02304 M NaOH solution.
    (2) Dissolve 0.0234 g NaOH in 5 mL H_2O. Mix 500 μL this solution with 500 μL THF to obtain 0.05844 M NaOH solution.
    (3) Dissolve 0.00344 g NaOH in 0.5 mL H_2O. Add 500 μL THF to obtain 0.08604 M NaOH solution.
2. Slowly add 10 μL 0.02304 M NaOH solution to 20 μL 0916-1 to adjust its pH to 6.5. The new sample is named 0920-1.
3. Slowly add 10 μL 0.05844 M NaOH solution to 20 μL 0916-1 to adjust its pH to 7.0. The new sample is named 0920-2.
4. Slowly add 10 μL 0.08604 M NaOH solution to 20 μL 0916-1 to adjust its pH to 7.5. The new sample is named 0920-3.

September 21st

◆ Characterization of 0916-1, 0920-1, 0920-2, 0920-3 by DLS

Obtain DLS results by Zetasizer Nano ZS90. The method is the same as the method on June 11th.

The DLS results of 0916-1, 0920-1, 0920-2, 0920-3 are shown below.



◆ Self-assembly of DNA TJ-II and glutaraldehyde in diluted PB

1. Mix the solutions in a vial according to the table below.

	DNA TJ- II (20 μM)	Glutaraldehyde solution (300 μM)	pH 6.0 PB (0.2 M)	H_2O	THF
0921-1	4 μL	4 μL	10 μL	100 μL	82 μL

2. Stir the solution at 4 ℃ for 72 h.
3. Obtain the assembly on September 25th and store it in an EP tube at -20 ℃.

September 25th

◆ pH-adjusting of 0921-1

1. Prepare mixed solvent of H_2O and THF (1:1).

   Mix 1 mL H_2O with 1 mL THF.

2. Prepare NaOH solutions.

   (1) Dilute 0.02304 M NaOH solution by a factor of 6 with H_2O/THF (1:1) to obtain 0.00384 M NaOH.

   (2) Dilute 0.05844 M NaOH solution by a factor of 6 with H_2O/THF (1:1) to obtain 0.00974 M NaOH.

   (3) Dilute 0.08604 M NaOH solution by a factor of 6 with H_2O/THF (1:1) to obtain 0.01434 M NaOH.

3. Slowly add 10 μL 0.00384 M NaOH solution to 20 μL 0921-1 to adjust its pH to 6.5. The new sample is named 0925-1.
4. Slowly add 10 μL 0.00974 M NaOH solution to 20 μL 0921-1 to adjust its pH to 7.0. The new sample is named 0925-2.
5. Slowly add 10 μL 0.01434 M NaOH solution to 20 μL 0921-1 to adjust its pH to 7.5. The new sample is named 0925-3.

◆ Characterization of 0921-1, 0925-1, 0925-2, 0925-3 by DLS

Obtain DLS results by Zetasizer Nano ZS90. The method is the same as the method on June 11th.

September 26th – September 30th

- Characterization of the assemblies by AFM

  Obtain the images by Dimension Fastscan atomic force microscope. The method is the same as the method on May 25th.

- Characterization of the assemblies by TEM

  Obtain the TEM images of the assemblies by JEM-2100F field emission transmission electron microscope. The method is the same as the method on June 6th.