

# Internalization

## Material

*E. coli* ATCC25922 was obtained from hopebio® (Qingdao, China).

Materials needed for origami (see preparation)

Zeiss confocal laser scanning microscope (LSM) 880 (Zeiss, Germany)

## Procedure

### DNA Origami Assembly

1. Loading helper strands
  - a. Prepare two microcentrifuge tubes on ice, labeling them as group 1 and group 2. For group 1, add M13, S-G4-x, S-x, F-cap-x, and S-PAM-cap-x in the indicated amounts. For group 2, add M13, S-G4-x, S-x, F-cap-x, S-PAM-cap-x, and Apt-cap-x in the indicated amounts. The molar ratio of M13 (5nM) and helper strands (50 nM) is 1:10.

Group 1: DNA origami (DO G) (with F-cap-x, and S-PAM-cap-x)

Reagents	Volume
TAE/Mg <sup>2+</sup> /K <sup>+</sup> buffer	47μl
S-G4-x	0.25ul × 104 = 26μl
S-x	0.25ul × 46 = 11.5μl
F-cap-x	0.25ul × 36 = 9μl
S-PAM-cap-x	0.25ul × 6 = 1.5μl
M13	5μl

S-G4-15	S-G4-93	S-G4-163	S-132	F-Cap80
S-G4-16	S-G4-94	S-G4-164	S-133	F-Cap81
S-G4-17	S-G4-95	S-G4-165	S-156	F-Cap82
S-G4-20	S-G4-98	S-G4-166	S-157	F-Cap83
S-G4-21	S-G4-99	S-G4-167	S-180	F-Cap123
S-G4-22	S-G4-100	S-G4-170	S-181	F-Cap124
S-G4-26	S-G4-105	S-G4-182	S-194	F-Cap129
S-G4-38	S-G4-106	S-G4-183	S-198	F-Cap130
S-G4-39	S-G4-107	S-G4-184	S-199	F-Cap131
S-G4-40	S-G4-110	S-G4-185	S-203	F-Cap171
S-G4-41	S-G4-111	S-G4-186	S-204	F-Cap172
S-G4-42	S-G4-112	S-G4-187	S-24	F-Cap173
S-G4-43	S-G4-117	S-G4-188	S-25	F-Cap174
S-G4-44	S-G4-118	S-G4-189	S-48	F-Cap175
S-G4-45	S-G4-119	S-G4-190	S-49	F-Cap176
S-G4-46	S-G4-122	S-G4-191	S-72	F-Cap177
S-G4-47	S-G4-134	S-G4-195	S-73	F-Cap178

S-G4-50	S-G4-135	S-G4-196	S-96	F-Cap179
S-G4-51	S-G4-136	S-G4-197	S-97	F-Cap125
S-G4-52	S-G4-137	S-G4-200	S-120	F-Cap126
S-G4-53	S-G4-138	S-G4-201	S-121	F-Cap127
S-G4-54	S-G4-139	S-G4-202	S-144	F-Cap128
S-G4-55	S-G4-140	S-13	S-145	S-PAM-Cap102
S-G4-56	S-G4-141	S-14	S-168	S-PAM-Cap103
S-G4-57	S-G4-142	S-18	S-169	S-PAM-Cap104
S-G4-58	S-G4-143	S-19	S-192	S-PAM-Cap113
S-G4-59	S-G4-146	S-23	S-193	S-PAM-Cap114
S-G4-62	S-G4-147	S-36	F-Cap27	S-PAM-Cap115
S-G4-63	S-G4-148	S-37	F-Cap28	
S-G4-64	S-G4-149	S-60	F-Cap29	
S-G4-65	S-G4-150	S-61	F-Cap30	
S-G4-66	S-G4-151	S-84	F-Cap31	
S-G4-67	S-G4-152	S-85	F-Cap32	
S-G4-68	S-G4-153	S-89	F-Cap33	
S-G4-69	S-G4-154	S-90	F-Cap34	
S-G4-70	S-G4-155	S-91	F-Cap35	
S-G4-71	S-G4-158	S-92	F-Cap75	
S-G4-74	S-G4-159	S-101	F-Cap76	
S-G4-86	S-G4-160	S-108	F-Cap77	
S-G4-87	S-G4-161	S-109	F-Cap78	
S-G4-88	S-G4-162	S-116	F-Cap79	

Group 2: DNA origami (DO G) (with F-cap-x, Apt-cap-x, and S-PAM-cap-x)

Reagents	Volume
TAE/Mg <sup>2+</sup> /K <sup>+</sup> buffer	44μl
S-G4-x	0.25ul × 104 = 26μl
S-x	0.25ul × 46 = 11.5μl
F-cap-x	0.25ul × 36 = 9μl
Apt-cap-x	0.25ul × 12 = 3μl
S-PAM-cap-x	0.25ul × 6 = 1.5μl
M13	5μl

S-G4-15	S-G4-93	S-G4-163	S-132	F-Cap80
S-G4-16	S-G4-94	S-G4-164	S-133	F-Cap81
S-G4-17	S-G4-95	S-G4-165	S-156	F-Cap82
S-G4-20	S-G4-98	S-G4-166	S-157	F-Cap83
S-G4-21	S-G4-99	S-G4-167	S-180	F-Cap123
S-G4-22	S-G4-100	S-G4-170	S-181	F-Cap124
S-G4-26	S-G4-105	S-G4-182	S-194	F-Cap129

S-G4-38	S-G4-106	S-G4-183	S-198	F-Cap130
S-G4-39	S-G4-107	S-G4-184	S-199	F-Cap131
S-G4-40	S-G4-110	S-G4-185	S-203	F-Cap171
S-G4-41	S-G4-111	S-G4-186	S-204	F-Cap172
S-G4-42	S-G4-112	S-G4-187	S-24	F-Cap173
S-G4-43	S-G4-117	S-G4-188	S-25	F-Cap174
S-G4-44	S-G4-118	S-G4-189	S-48	F-Cap175
S-G4-45	S-G4-119	S-G4-190	S-49	F-Cap176
S-G4-46	S-G4-122	S-G4-191	S-72	F-Cap177
S-G4-47	S-G4-134	S-G4-195	S-73	F-Cap178
S-G4-50	S-G4-135	S-G4-196	S-96	F-Cap179
S-G4-51	S-G4-136	S-G4-197	S-97	F-Cap125
S-G4-52	S-G4-137	S-G4-200	S-120	F-Cap126
S-G4-53	S-G4-138	S-G4-201	S-121	F-Cap127
S-G4-54	S-G4-139	S-G4-202	S-144	F-Cap128
S-G4-55	S-G4-140	S-13	S-145	S-PAM-Cap102
S-G4-56	S-G4-141	S-14	S-168	S-PAM-Cap103
S-G4-57	S-G4-142	S-18	S-169	S-PAM-Cap104
S-G4-58	S-G4-143	S-19	S-192	S-PAM-Cap113
S-G4-59	S-G4-146	S-23	S-193	S-PAM-Cap114
S-G4-62	S-G4-147	S-36	F-Cap27	S-PAM-Cap115
S-G4-63	S-G4-148	S-37	F-Cap28	Apt-Cap1
S-G4-64	S-G4-149	S-60	F-Cap29	Apt-Cap2
S-G4-65	S-G4-150	S-61	F-Cap30	Apt-Cap6
S-G4-66	S-G4-151	S-84	F-Cap31	Apt-Cap7
S-G4-67	S-G4-152	S-85	F-Cap32	Apt-Cap11
S-G4-68	S-G4-153	S-89	F-Cap33	Apt-Cap12
S-G4-69	S-G4-154	S-90	F-Cap34	Apt-Cap205
S-G4-70	S-G4-155	S-91	F-Cap35	Apt-Cap206
S-G4-71	S-G4-158	S-92	F-Cap75	Apt-Cap210
S-G4-74	S-G4-159	S-101	F-Cap76	Apt-Cap211
S-G4-86	S-G4-160	S-108	F-Cap77	Apt-Cap215
S-G4-87	S-G4-161	S-109	F-Cap78	Apt-Cap216
S-G4-88	S-G4-162	S-116	F-Cap79	

- b. Mix the above reagents and briefly centrifuge them. The mix is first heated at 95 °C for 10 minutes. Subsequently, annealing is performed by slowly cooling the mixture from 95 °C to 20 °C at a rate of 1 °C/min.
  - c. The Amicon® Ultra-0.5 Centrifugal Filter Devices (100kDa) are used to remove redundant helper strands three times. The purified DO G solution is obtained.
2. Loading functional strands
  - a. Add 1.5 µL PAM-rich and 9µl F-H stock solution to group 1. Add 1.5µl

- PAM-rich, 9 $\mu$ l F-H, and 1.5 $\mu$ l aptamer stock solution to group 2. Then, restore the volume to 100 $\mu$ l with TAE/Mg<sup>2+</sup>/K<sup>+</sup> buffer.
- Mix the above reagents and briefly centrifuge them. The mix is annealed from 45 °C to 25 °C at a rate of 5 min/°C for six cycles.
  - The Amicon® Ultra-0.5 Centrifugal Filter Devices (100kDa) are used to remove redundant functional strands three times. The purified Cy5-labeled DO<sub>PAM</sub>G and Cy5-labeled DO<sup>A</sup><sub>PAM</sub>G solution are obtained.
3. Loading of hemin
- Add 1 $\mu$ l of hemin stock solution to the purified Cy5-labeled DO<sub>PAM</sub>G and Cy5-labeled DO<sup>A</sup><sub>PAM</sub>G solution obtained in the last step. Then, restore the volume to 100 $\mu$ l with TAE/Mg<sup>2+</sup>/K<sup>+</sup> buffer.
  - Gently resuspend the solution by pipetting three times using a wide-bore pipette tip.
  - Briefly centrifuge at 500 × g for 3 seconds to sediment droplets.
  - Incubate at room temperature for 1 hour.
  - The Amicon® Ultra-0.5 Centrifugal Filter Devices (100 kDa) are used to remove redundant hemin. The purified Cy5-labeled DO<sub>PAM</sub>GH and Cy5-labeled DO<sup>A</sup><sub>PAM</sub>GH solution are obtained. Then, restore the volume to 100 $\mu$ l with TAE/Mg<sup>2+</sup>/K<sup>+</sup> buffer.

### LSM sample preparation

- 100  $\mu$ L of *E. coli* solution (OD<sub>600</sub>=1.0) is added into 900  $\mu$ L of PBS solution with 200 $\mu$ l Cy5-labeled DNA origami (DO<sub>PAM</sub>GH or DO<sup>A</sup><sub>PAM</sub>GH) to get the final concentration of 1 nM DNA origami.
- The mixture was incubated at 37 °C for 15 min.
- Bacteria are then collected by centrifuging (4000 rpm, 1 min) and washed with PBS for three times.
- The bacteria are added to a clean glass slide and immobilized with a glass coverslip for LSM 880 (Zeiss, Germany) observation.

### LSM imaging

- First, locate the *E. coli* in the mode where the laser is turned off. After adjusting to the appropriate position, turn on the laser (for Cy5, HeNe 633nm laser is recommended) and first confirm whether there is a fluorescence signal at a smaller magnification.
- Then increase the magnification, adjust the observation, and continue until you obtain the fluorescence signal image of the required size area.

*Note: For bacteria, ~100x magnification is proposed. In our experiment, we successfully found the bacteria at 20x magnification (20x/1x) and finally chose the fluorescence image at 80x magnification (40x/2x).*

### Image processing and data analysis

All pictures are processed and analyzed by Fijj ImageJ x86-64, Python 3.13,

and Pycharm 2025.2.2.1.

1. The csv files exported from LSM are imported to Fijj ImageJ x86-64, conducting channels splitting or merging as demand, with brightness/contrast adjustment. The desired images are then exported after analysis and addition of scale bar.
2. Further, the quantitative and comparative analysis of fluorescence intensity is required. After altering the image type into 8-bit, the sites of target fluorescence signals are selected by threshold adjustment and mean fluorescence intensity is measured. The setting of measurement included that of area, mean gray value, standard deviation, min & max gray value, integrated density, limit to threshold and add to overlay.
3. Then, these two sets of values (one group of -aptamer: Cy5-labeled  $\text{DO}_{\text{PAMGH}}$ , and the other group of +aptamer: Cy5-labeled  $\text{DO}^{\text{A}}_{\text{PAMGH}}$ ) conduct statistical analysis. Data represent the mean  $\pm$  s.d. from indicated independent replicates, and statistical analysis is conducted using Pycharm 2025.2.2.1 (Python 3.13). For comparisons between two groups, means are compared using the unpaired two-tailed Student's t-test. A value of  $P < 0.05$  is considered statistically significant.