Targeting

Materials

E. coli ATCC25922 was obtained from hopebio® (Qingdao, China).
Materials needed for origami (see preparation)
Zeiss confocal laser scanning microscope (LSM) 880 (Zeiss, Germany)

Procedures

DNA Origami Assembly

1. Loading of helper strands
    a. Prepare two microcentrifuge tubes on ice, labeling them as group 1 and group 2. For group 1, add M13, S-x, F-cap-x, and S-PAM-cap-x in the indicated amounts. For group 2, add M13, 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) (with F-cap-x and S-PAM-cap-x)

Reagents	Volume
TAE/Mg^{2+} buffer	47μl
S-x	0.25ul × 150 = 37.5μl
F-cap-x	0.25ul × 36 = 9μl
S-PAM-cap-x	0.25ul × 6 = 1.5μl
M13	5μl

S-13	**S-62**	**S-117**	**S-166**	**F-Cap76**
S-14	**S-63**	**S-118**	**S-167**	**F-Cap77**
S-15	**S-64**	**S-119**	**S-168**	**F-Cap78**
S-16	**S-65**	**S-120**	**S-169**	**F-Cap79**
S-17	**S-66**	**S-121**	**S-170**	**F-Cap80**
S-18	**S-67**	**S-122**	**S-180**	**F-Cap81**
S-19	**S-68**	**S-132**	**S-181**	**F-Cap82**
S-20	**S-69**	**S-133**	**S-182**	**F-Cap83**
S-21	**S-70**	**S-134**	**S-183**	**F-Cap123**
S-22	**S-71**	**S-135**	**S-184**	**F-Cap124**
S-23	**S-72**	**S-136**	**S-185**	**F-Cap129**
S-24	**S-73**	**S-137**	**S-186**	**F-Cap130**
S-25	**S-74**	**S-138**	**S-187**	**F-Cap131**
S-26	**S-84**	**S-139**	**S-188**	**F-Cap171**
S-36	**S-85**	**S-140**	**S-189**	**F-Cap172**
S-37	**S-86**	**S-141**	**S-190**	**F-Cap173**
S-38	**S-87**	**S-142**	**S-191**	**F-Cap174**

S-39	**S-88**	**S-143**	**S-192**	**F-Cap175**
S-40	**S-89**	**S-144**	**S-193**	**F-Cap176**
S-41	**S-90**	**S-145**	**S-194**	**F-Cap177**
S-42	**S-91**	**S-146**	**S-195**	**F-Cap178**
S-43	**S-92**	**S-147**	**S-196**	**F-Cap179**
S-44	**S-93**	**S-148**	**S-197**	**F-Cap125**
S-45	**S-94**	**S-149**	**S-198**	**F-Cap126**
S-46	**S-95**	**S-150**	**S-199**	**F-Cap127**
S-47	**S-96**	**S-151**	**S-200**	**F-Cap128**
S-48	**S-97**	**S-152**	**S-201**	**S-PAM-Cap102**
S-49	**S-98**	**S-153**	**S-202**	**S-PAM-Cap103**
S-50	**S-99**	**S-154**	**S-203**	**S-PAM-Cap104**
S-51	**S-100**	**S-155**	**S-204**	**S-PAM-Cap113**
S-52	**S-101**	**S-156**	**F-Cap27**	**S-PAM-Cap114**
S-53	**S-105**	**S-157**	**F-Cap28**	**S-PAM-Cap115**
S-54	**S-106**	**S-158**	**F-Cap29**	
S-55	**S-107**	**S-159**	**F-Cap30**	
S-56	**S-108**	**S-160**	**F-Cap31**	
S-57	**S-109**	**S-161**	**F-Cap32**	
S-58	**S-110**	**S-162**	**F-Cap33**	
S-59	**S-111**	**S-163**	**F-Cap34**	
S-60	**S-112**	**S-164**	**F-Cap35**	
S-61	**S-116**	**S-165**	**F-Cap75**	

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

Reagents	Volume
TAE/Mg^{2+} buffer	44μl
S-x	0.25ul × 150 = 37.5μl
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-13	**S-63**	**S-119**	**S-169**	**F-Cap80**
S-14	**S-64**	**S-120**	**S-170**	**F-Cap81**
S-15	**S-65**	**S-121**	**S-180**	**F-Cap82**
S-16	**S-66**	**S-122**	**S-181**	**F-Cap83**
S-17	**S-67**	**S-132**	**S-182**	**F-Cap123**
S-18	**S-68**	**S-133**	**S-183**	**F-Cap124**

S-19	S-69	S-134	S-184	F-Cap129
S-20	S-70	S-135	S-185	F-Cap130
S-21	S-71	S-136	S-186	F-Cap131
S-22	S-72	S-137	S-187	F-Cap171
S-23	S-73	S-138	S-188	F-Cap172
S-24	S-74	S-139	S-189	F-Cap173
S-25	S-84	S-140	S-190	F-Cap174
S-26	S-85	S-141	S-191	F-Cap175
S-36	S-86	S-142	S-192	F-Cap176
S-37	S-87	S-143	S-193	F-Cap177
S-38	S-88	S-144	S-194	F-Cap178
S-39	S-89	S-145	S-195	F-Cap179
S-40	S-90	S-146	S-196	F-Cap125
S-41	S-91	S-147	S-197	F-Cap126
S-42	S-92	S-148	S-198	F-Cap127
S-43	S-93	S-149	S-199	F-Cap128
S-44	S-94	S-150	S-200	S-PAM-Cap102
S-45	S-95	S-151	S-201	S-PAM-Cap103
S-46	S-96	S-152	S-202	S-PAM-Cap104
S-47	S-97	S-153	S-203	S-PAM-Cap113
S-48	S-98	S-154	S-204	S-PAM-Cap114
S-49	S-99	S-155	F-Cap27	S-PAM-Cap115
S-50	S-100	S-156	F-Cap28	Apt-Cap1
S-51	S-101	S-157	F-Cap29	Apt-Cap2
S-52	S-105	S-158	F-Cap30	Apt-Cap6
S-53	S-106	S-159	F-Cap31	Apt-Cap7
S-54	S-107	S-160	F-Cap32	Apt-Cap11
S-55	S-108	S-161	F-Cap33	Apt-Cap12
S-56	S-109	S-162	F-Cap34	Apt-Cap205
S-57	S-110	S-163	F-Cap35	Apt-Cap206
S-58	S-111	S-164	F-Cap75	Apt-Cap210
S-59	S-112	S-165	F-Cap76	Apt-Cap211
S-60	S-116	S-166	F-Cap77	Apt-Cap215
S-61	S-117	S-167	F-Cap78	Apt-Cap216
S-62	S-118	S-168	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 solution is obtained.

2. Loading functional strands
    a. Add 1.5 μL PAM-rich stock solution and 9μl F-H stock solution to group 1. Add 1.5 μL PAM-rich stock solution, 9μl F-H, and 1.5μl aptamer stock solution to group 2. Then, restore the volumes to 100ul with TAE/Mg^{2+} buffer.
    b. 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.
    c. The Amicon® Ultra-0.5 Centrifugal Filter Devices (100kDa) are used to remove redundant functional strands three times. The purified Cy5-labeled DO_{PAM} and Cy5-labeled DO^{A}_{PAM} solution are obtained.

LSM sample preparation

1. 100 μL of *E. coli* solution (OD600=1.0) is added into 900 μL of PBS solution with 200μl Cy5-labeled DNA origami (DO_{PAM} or DO^{A}_{PAM}) to get the final concentration of 1 nM DNA origami.
2. The mixture is incubated at 37 °C for 15 min.
3. Bacteria are then collected by centrifuging (4000 rpm, 1 min) and washed with PBS.
4. The bacteria are added to a clean glass slide and immobilized with a glass coverslip for LSM 880 (Zeiss, Germany) observation.

LSM imaging

1. 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.
2. 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

1. All pictures are processed and analyzed by Fijj ImageJ x86-64, Python 3.13, and Pycharm 2025.2.2.1. 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 DO_{PAM}, and the other group of +aptamer: Cy5-labeled DO^{A}_{PAM}) conduct statistical analysis. Data represent the mean ± 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.