Materials

Material needed for DNA origami (see preparation)

Procedures

1. Loading of helper strands
   a. Prepare the M13, S-x, F-cap-x and S-PAM-cap-x mix in a microcentrifuge tube on ice by adding the components indicated below. The molar ratio of M13 (5nM) and helper strands (50 nM) is 1:10.

DNA origami (DO) (with S-PAM-cap-x and F-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**	

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 concentrated DO solution is obtained.

2. Loading functional strands
   a. Add 1.5 μL of the PAM-rich stock solution and 9μl F-H stock solution to the concentrated solution. Then, restore the volume to 100μl 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 PAM-rich and F-H three times. The concentrated Cy5-labeled DO_{PAM} solution is obtained.

3. Loading FITC-$sgRNA_L$/Cas9
   a. Prepare the FITC-$sgRNA_L$ and Cas9 mix in a single microcentrifuge tube on ice by adding the components indicated below. The molar ratio of FITC-$sgRNA_L$ and Cas9 is 1:1.

Reagents	Volume
PBS (PH=7.4, free of RNase H)	94 μl
FITC-$sgRNA_L$ (5 μM)	3μl
Cas9 (5 μM)	3μl

   b. Mix the above reagents and briefly centrifuge them. Incubate at 37 °C

for 10 minutes to get FITC-sgRNA$_L$/Cas9 complex.

c. Add 10 μl of FITC-sgRNA$_L$/Cas9 mixture to the concentrated Cy5-labeled DO$_{PAM}$ solution, and make up to 100 μl with TAE/Mg^{2+} Buffer. The molar ratio of FITC-sgRNA$_L$/Cas9 complex and Cy5-labeled DO$_{PAM}$ is 3:1.
d. Mix the above reagents and briefly centrifuge them. The mix is annealed from 37 °C to 4 °C at a rate of 1°C/min.
e. The Amicon® Ultra-0.5 Centrifugal Filter Devices (100 kDa) are used to remove the redundant FITC-sgRNA$_L$/Cas9. The concentrated Cy5-labeled DO$_{PAM}$R^{FITC}C solution is obtained.

4. Verifying the loading of FITC-sgRNA$_L$/Cas9 complex
   a. Measure the absorbance of Cy5-labeled DO$_{PAM}$R^{FITC}C solution at 495nm and 650nm by using Thermo Fisher Scientific NanoDrop One 2.12.0 and python 3.13.
   b. Plot the absorption spectrum of the Cy5-labeled DO$_{PAM}$R^{FITC}C solution.