Detecting Dimerisation

Materials

Material needed for DNA origami (see preparation)
*Trans*5K DNA marker and Gelstain were obtained from TransGen Biotech (Beijing, China).
6×DNA loading buffer was obtained from Toroivd (Shanghai, China).

Procedures

1. Loading helper strands
   a. Prepare 2 microcentrifuge tubes on ice, labeling them as group 1 and group 2. For group 1, add M13, S-x, and S-PAM-cap-x in the indicated amounts. For group 2, add M13, S-G4-x, S-x, and S-PAM-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 S-PAM-cap-x)

Reagents	Volume
TAE/Mg^{2+} buffer	47μl
S-x	0.25ul × 186 = 46.5μl
S-PAM-cap-x	0.25ul × 6 = 1.5μl
M13	5μl

S-13	**S-53**	**S-93**	**S-139**	**S-179**
S-14	**S-54**	**S-94**	**S-140**	**S-180**
S-15	**S-55**	**S-95**	**S-141**	**S-181**
S-16	**S-56**	**S-96**	**S-142**	**S-182**
S-17	**S-57**	**S-97**	**S-143**	**S-183**
S-18	**S-58**	**S-98**	**S-144**	**S-184**
S-19	**S-59**	**S-99**	**S-145**	**S-185**
S-20	**S-60**	**S-100**	**S-146**	**S-186**
S-21	**S-61**	**S-101**	**S-147**	**S-187**
S-22	**S-62**	**S-105**	**S-148**	**S-188**
S-23	**S-63**	**S-106**	**S-149**	**S-189**
S-24	**S-64**	**S-107**	**S-150**	**S-190**
S-25	**S-65**	**S-108**	**S-151**	**S-191**
S-26	**S-66**	**S-109**	**S-152**	**S-192**
S-27	**S-67**	**S-110**	**S-153**	**S-193**
S-28	**S-68**	**S-111**	**S-154**	**S-194**
S-29	**S-69**	**S-112**	**S-155**	**S-195**
S-30	**S-70**	**S-116**	**S-156**	**S-196**
S-31	**S-71**	**S-117**	**S-157**	**S-197**

S-32	**S-72**	**S-118**	**S-158**	**S-198**
S-33	**S-73**	**S-119**	**S-159**	**S-199**
S-34	**S-74**	**S-120**	**S-160**	**S-200**
S-35	**S-75**	**S-121**	**S-161**	**S-201**
S-36	**S-76**	**S-122**	**S-162**	**S-202**
S-37	**S-77**	**S-123**	**S-163**	**S-203**
S-38	**S-78**	**S-124**	**S-164**	**S-204**
S-39	**S-79**	**S-125**	**S-165**	**S-PAM-Cap102**
S-40	**S-80**	**S-126**	**S-166**	**S-PAM-Cap103**
S-41	**S-81**	**S-127**	**S-167**	**S-PAM-Cap104**
S-42	**S-82**	**S-128**	**S-168**	**S-PAM-Cap113**
S-43	**S-83**	**S-129**	**S-169**	**S-PAM-Cap114**
S-44	**S-84**	**S-130**	**S-170**	**S-PAM-Cap115**
S-45	**S-85**	**S-131**	**S-171**	
S-46	**S-86**	**S-132**	**S-172**	
S-47	**S-87**	**S-133**	**S-173**	
S-48	**S-88**	**S-134**	**S-174**	
S-49	**S-89**	**S-135**	**S-175**	
S-50	**S-90**	**S-136**	**S-176**	
S-51	**S-91**	**S-137**	**S-177**	
S-52	**S-92**	**S-138**	**S-178**	

Group 2: DNA origami G4 (DO G) (with S-PAM-cap-x)

Reagents	Volume
TAE/Mg^{2+} buffer	47μl
S-G4-x	0.25ul × 136 = 34μl
S-x	0.25ul × 50 = 12.5μl
S-PAM-cap-x	0.25ul × 6 = 1.5μl
M13	5μl

S-G4-15	**S-G4-66**	**S-G4-134**	**S-G4-182**	**S-133**
S-G4-16	**S-G4-67**	**S-G4-135**	**S-G4-183**	**S-156**
S-G4-17	**S-G4-68**	**S-G4-136**	**S-G4-184**	**S-157**
S-G4-20	**S-G4-69**	**S-G4-137**	**S-G4-185**	**S-180**
S-G4-21	**S-G4-70**	**S-G4-138**	**S-G4-186**	**S-181**
S-G4-22	**S-G4-71**	**S-G4-139**	**S-G4-187**	**S-194**
S-G4-26	**S-G4-74**	**S-G4-140**	**S-G4-188**	**S-198**
S-G4-27	**S-G4-75**	**S-G4-141**	**S-G4-189**	**S-199**
S-G4-28	**S-G4-76**	**S-G4-142**	**S-G4-190**	**S-203**
S-G4-29	**S-G4-77**	**S-G4-143**	**S-G4-191**	**S-204**
S-G4-30	**S-G4-78**	**S-G4-146**	**S-G4-195**	**S-24**
S-G4-31	**S-G4-79**	**S-G4-147**	**S-G4-196**	**S-25**
S-G4-32	**S-G4-80**	**S-G4-148**	**S-G4-197**	**S-48**

S-G4-33	S-G4-81	S-G4-149	S-G4-200	S-49
S-G4-34	S-G4-82	S-G4-150	S-G4-201	S-72
S-G4-35	S-G4-83	S-G4-151	S-G4-202	S-73
S-G4-38	S-G4-86	S-G4-152	S-13	S-96
S-G4-39	S-G4-87	S-G4-153	S-14	S-97
S-G4-40	S-G4-88	S-G4-154	S-18	S-120
S-G4-41	S-G4-93	S-G4-155	S-19	S-121
S-G4-42	S-G4-94	S-G4-158	S-23	S-144
S-G4-43	S-G4-95	S-G4-159	S-36	S-145
S-G4-44	S-G4-98	S-G4-160	S-37	S-168
S-G4-45	S-G4-99	S-G4-161	S-60	S-169
S-G4-46	S-G4-100	S-G4-162	S-61	S-192
S-G4-47	S-G4-105	S-G4-163	S-84	S-193
S-G4-50	S-G4-106	S-G4-164	S-85	S-PAM-Cap102
S-G4-51	S-G4-107	S-G4-165	S-89	S-PAM-Cap103
S-G4-52	S-G4-110	S-G4-166	S-90	S-PAM-Cap104
S-G4-53	S-G4-111	S-G4-167	S-91	S-PAM-Cap113
S-G4-54	S-G4-112	S-G4-170	S-92	S-PAM-Cap114
S-G4-55	S-G4-117	S-G4-171	S-101	S-PAM-Cap115
S-G4-56	S-G4-118	S-G4-172	S-108	
S-G4-57	S-G4-119	S-G4-173	S-109	
S-G4-58	S-G4-122	S-G4-174	S-116	
S-G4-59	S-G4-123	S-G4-175	S-125	
S-G4-62	S-G4-124	S-G4-176	S-126	
S-G4-63	S-G4-129	S-G4-177	S-127	
S-G4-64	S-G4-130	S-G4-178	S-128	
S-G4-65	S-G4-131	S-G4-179	S-132	

b. Mix the above reagents and briefly centrifuge them. The 2 groups are 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 and DO G solutions are obtained.

2. Loading functional strands
   a. Add 1.5 μL of the PAM-rich stock solution to Groups 1 and 2. Then, restore the volume 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 PAM-rich three times. The concentrated DO_{PAM} and

$DO_{PAM}G$ solutions are obtained.

d. Prepare a new tube, place it on ice, add M13, and fill it up with TAE/Mg^{2+} buffer to prepare the M13 dilution solution.

3. Agarose Gel Electrophoresis
   a. Prepare a 1.5% agarose gel
   b. Load 5 μl DNA marker, 10μl M13 dilution solution, 10μl concentrated DO_{PAM} and 10μl concentrated $DO_{PAM}G$. Start the electrophoresis with 120V for 25 min.
   c. After electrophoresis, put the gel under UV light to observe the DNA band of each group.