ABTS assay

Materials

ABTS was obtained from Mecklin (Shanghai, China).
Hemin was obtained from MCE (Shanghai, China).
Concentrated $DO_{PAM}GH$ solution
KCl
H_2O_2
methyl alcohol
PBS (pH 5.0, 50mM)
ddH_2O
Materials needed for origami (see preparation)

Procedures

1. Preparation of stock solution:
   a. ABTS stock solution (20 mM, 10 mL, store at 4 °C in a dark place)
      Dissolve 0.11g of ABTS in 200 μL of methyl alcohol and make up to 10 mL with PBS (pH 5.0, 50mM).
   b. H_2O_2 stock solution (1M, store at RT)
   c. TAE/Mg^{2+}/K^+ buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA, 12.5 mM magnesium acetate, 25 mM potassium chloride, pH = 8.3, store at RT)
   d. Hemin stock solution (2.55μM, 100ul, store at -20 °C in a dark place)
      Dissolve 0.016g hemin in 100ml DMSO to prepare a 255 μM hemin solution. Use a magnetic stirrer at 45°C to ensure complete dissolution, with the stirring bar rotating at a speed that forms a distinct vortex. Add 1μL hemin to 99 μL TAE/Mg^{2+}/K^+ buffer.
   e. Free G4/hemin stock solution
      Add 0.34μl S-G4-x stock solution and 1μl hemin stock solution to 98.66μl TAE/Mg^{2+}/K^+ buffer.

2. Loading helper strands
   a. Prepare the M13, S-G4-x, S-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 G4 (DO G) (with S-PAM-cap-x)

Reagents	Volume
TAE/Mg^{2+}/K^+ 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 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.

3. Loading functional strands
   a. Add 1.5 μL of the PAM-rich stock solution to the purified solution. Then, restore the volume to 100ul with TAE/Mg^{2+}/K^{+} 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 purified $DO_{PAM}G$ solution is obtained.

4. Loading of hemin
   a. Add 1μl of hemin stock solution to the purified $DO_{PAM}G$ solution obtained in the last step. Then, restore the volume to 100ul with TAE/Mg^{2+}/K^{+} buffer.
   b. Gently resuspend the solution by pipetting three times using a wide-bore pipette tip.
   c. Briefly centrifuge at 500 × g for 3 seconds to sediment droplets.
   d. Incubate at room temperature for 1 hour.
   e. The Amicon® Ultra-0.5 Centrifugal Filter Devices (100 kDa) are used to remove redundant hemin. The purified $DO_{PAM}GH$ solution is obtained. Then, restore the volume to 100ul with TAE/Mg^{2+}/K^{+} buffer.

5. ABTS assay
   a. Prepare a 96-well plate and add the components of each group according to the table below. Each group repeats three times.

Group	Components
1	89ul TAE/Mg^{2+}/K^{+} buffer + 10ul ABTS + 1ul H_2O_2
2	89ul Hemin + 10ul ABTS + 1ul H_2O_2
3	89ul Free G4/hemin stock solution + 10ul ABTS + 1ul H_2O_2
4	89ul $DO_{PAM}GH$+ 10ul ABTS + 1ul H_2O_2

   b. Immediately after adding H_2O_2, the absorbance spectrum at 415 nm was recorded using a microplate reader.