

## **-SUPPORTING INFORMATION-**

### **A ratiometric fluorescent probe for pH measurement over a wide range composed of three types of fluorophores assembled on a DNA scaffold**

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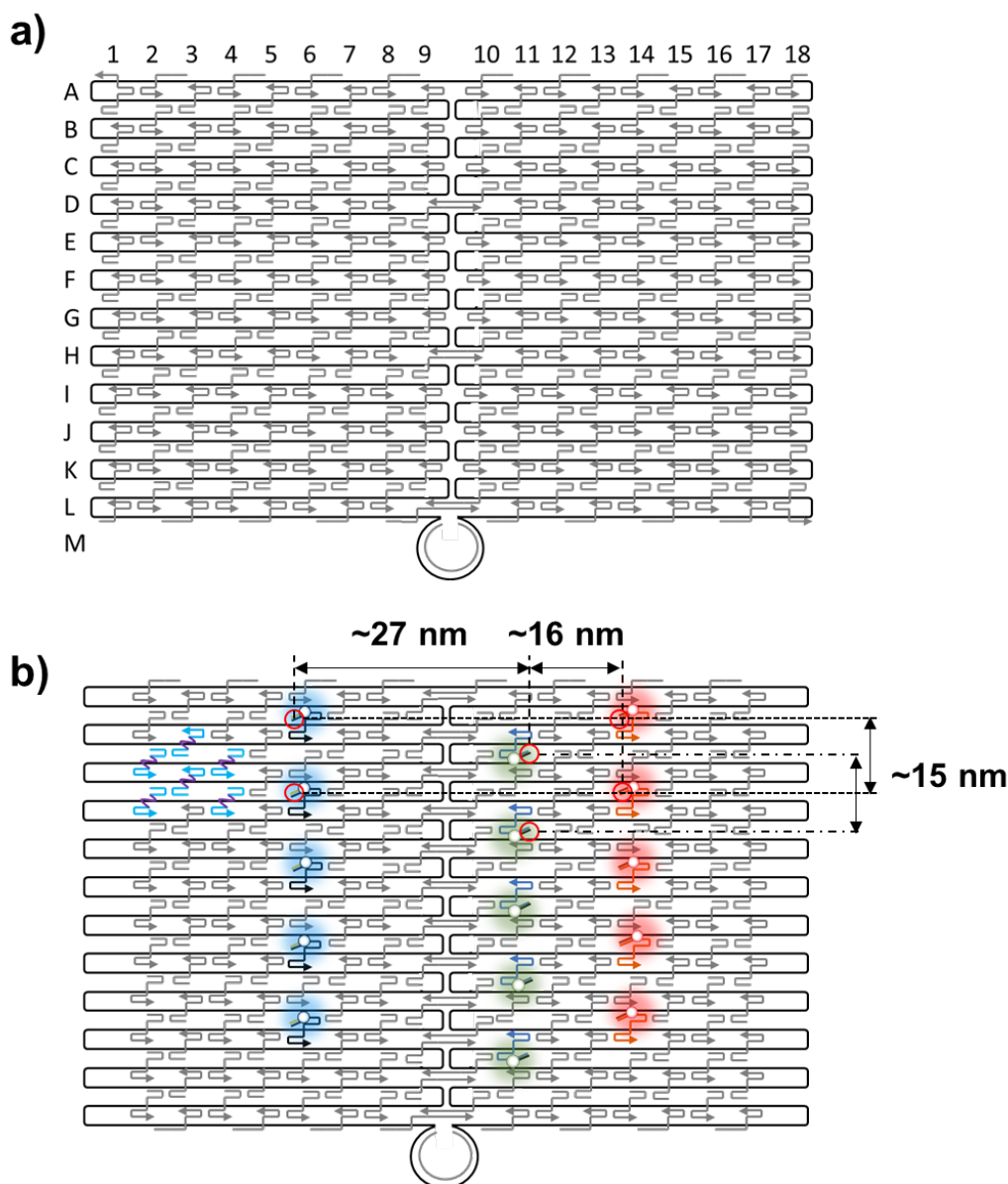
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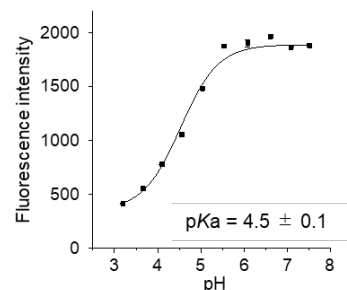
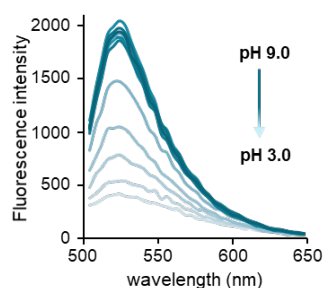
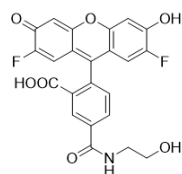
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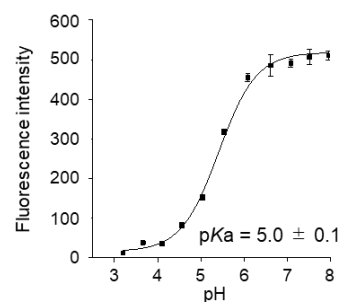
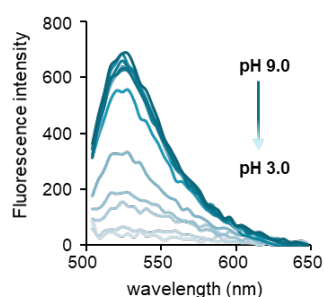


**Figure S1** Design of a DNA origami scaffold (rec-Ori). a) An illustration of the original design of rec-Ori [S1]. The ssDNA scaffold (M13mp18 ssDNA) and staple strands were shown in black lines and gray lines, respectively. b) An illustration of rec-CF5/OG5/CR5 shows the calculated distance between each loading position. The staple strands for both short sides (1A-1L and 18A-18L, 24 staple strands in total) in the original design (Figure S1a) were removed to prevent the intermolecular interaction between the folded rec-Ori. The staple strands of the middle position (9A-9L and 10A-10L, 24 staple strands in total) were hybridized with each other to increase the stability of rec-Ori. The position to load each fluorophore was at least 15 nm away from the loading position of the neighboring fluorophore.

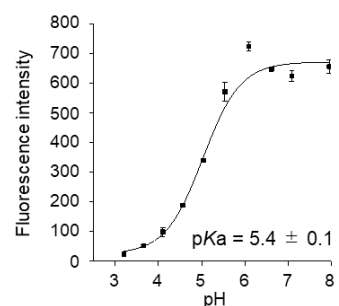
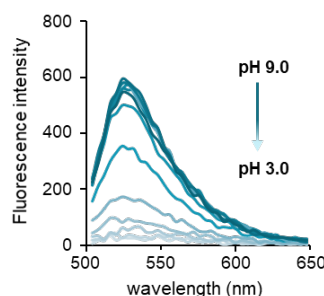
**a) OG-AE**



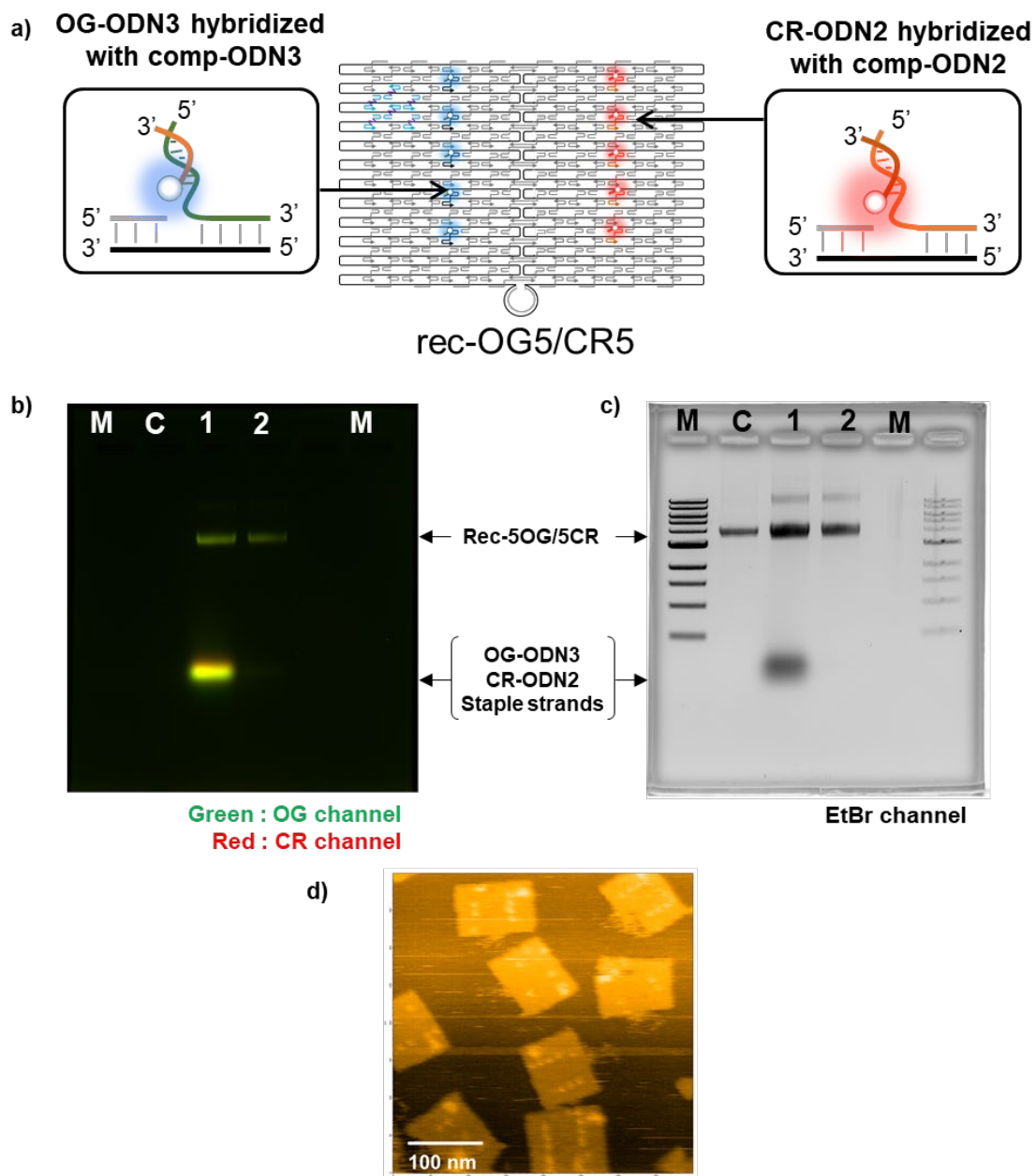
**b) OG-ODN3**



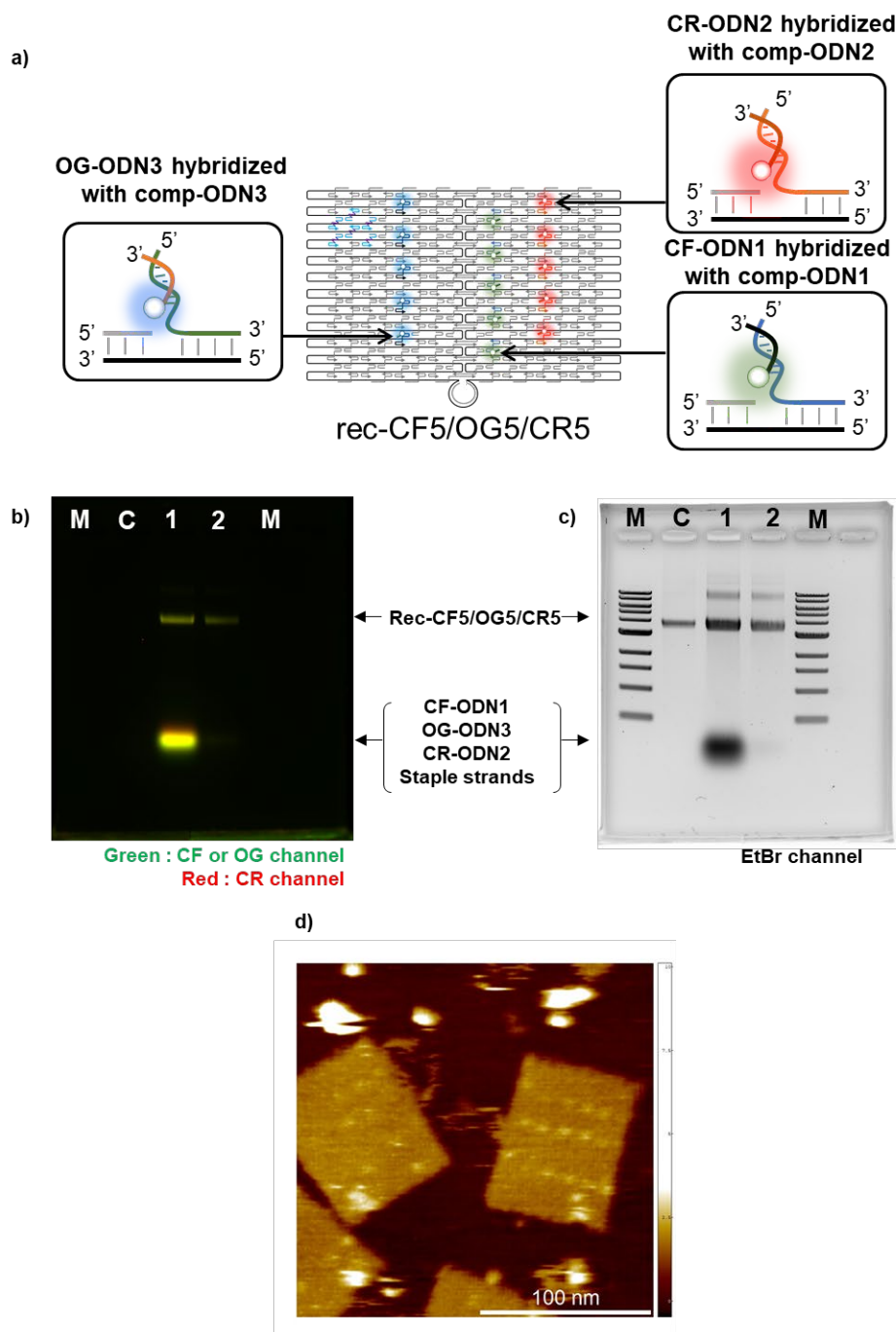
**c) OG-dODN3**



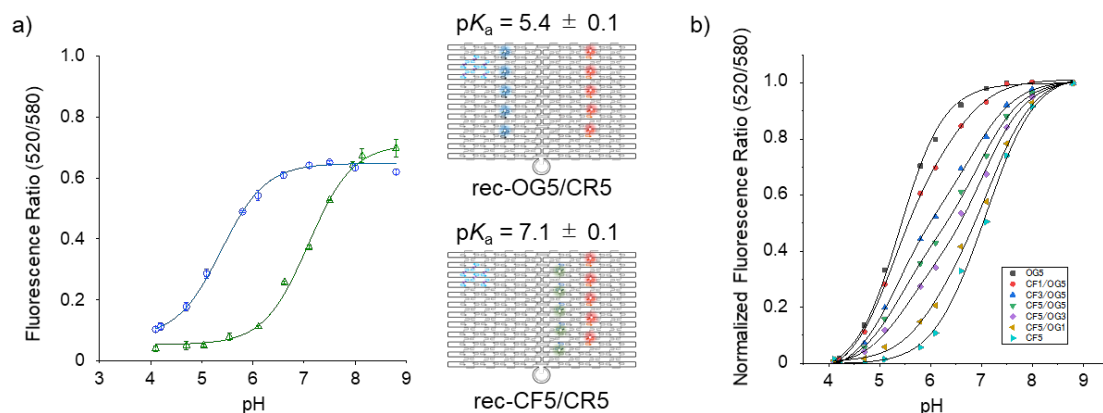
**Figure S2** Fluorescence spectral changes of OG derivatives as the function of pH. a) OG-AE (2-aminoethanol) at different pH (3.2, 3.7, 4.1, 4.6, 5.1, 5.6, 6.1, 6.6, 7.1, 7.5) b) OG modified with a single-stranded DNA (OG-ODN3) at different pH (3.2, 3.7, 4.1, 4.6, 5.1, 5.6, 6.1, 6.6, 7.1, 7.5, 8.0). c) OG-ODN3 hybridized with comp-ODN3 (OG-dODN3) at different pH (3.2, 3.7, 4.1, 4.6, 5.1, 5.6, 6.1, 6.6, 7.1, 8.0). The details of the buffer used for each pH condition were shown in Materials and Methods.



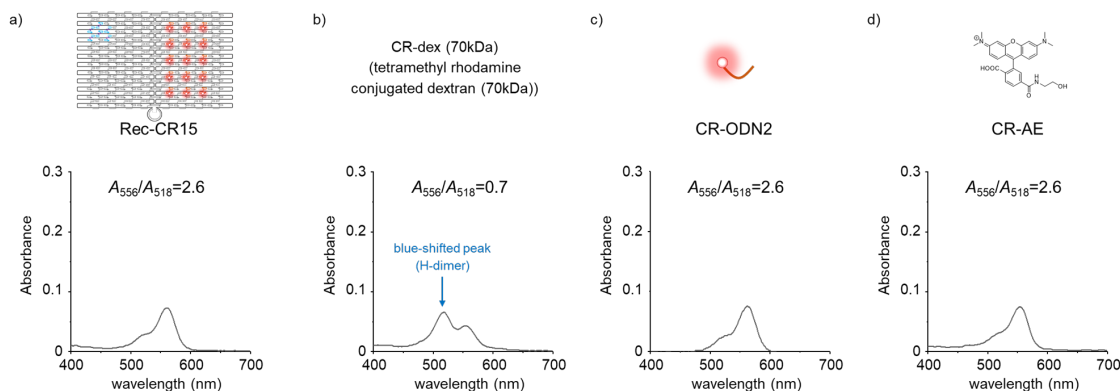
**Figure S3** Characterization of rec-OG5/CR5 by gel electrophoresis and AFM imaging. a) Design of rec-OG5/CR5. b-c) Images for gel electrophoresis analysis of rec-OG5/CR5. b) Visualized by the emission of OG (green) and CR (red). c) Visualized by ethidium bromide (EtBr) staining. Lane M: 1 kbp DNA ladder as marker; Lane C: M13mp18; Lane 1: Rec-OG5/CR5 before purification; Lane 2: Rec-OG5/CR5 after purification. d) AFM image of rec-OG5/CR5.



**Figure S4** Characterization of rec-CF5/OG5/CR5 by gel electrophoresis and AFM imaging. a) Design of rec-CF5/OG5/CR5. b-c) Images for gel electrophoresis analysis of rec-CF5/OG5/CR5. b) Visualized by the emission of CF and OG (green), and CR (red). c) Visualized by ethidium bromide (EtBr) staining. Lane M: 1 kbp DNA ladder as marker; Lane C: M13mp18; Lane 1: Rec-CF5/OG5/CR5 before purification; Lane 2: Rec-CF5/OG5/CR5 after purification. d) AFM image of rec-CF5/OG5/CR5.



**Figure S5** Estimation of the ratio of fluorescence emission intensities of CF, OG, and CR assembled on rec-Ori with different numbers of CF and OG. a) The plot of the ratio of fluorescence emission intensity of OG or CF to CR of rec-OG5/CR5 or rec-CF5/CR5 at different pH values ranging from 4 to 9 (4.1, 4.2, 4.7, 5.1, 5.8, 6.1, 6.6, 7.1, 7.5, 8.0, and 8.8). b) The simulation plots of the normalized fluorescence emission ratio of rec-CF1/OG5/CR5 (CF1/OG5), rec-CF3/OG5/CR5 (CF3/OG5), rec-CF5/OG5/CR5 (CF5/OG5), rec-CF5/OG3/CR5 (CF5/OG3), and rec-CF5/OG1/CR5 (CF5/OG1) based on the actual ratio data of rec-CF5/CR5 (CF5) and rec-OG5/CR5 (OG5) at different pH values ranging from 4 to 9 (4.1, 4.2, 4.7, 5.1, 5.8, 6.1, 6.6, 7.1, 7.5, 8.0, and 8.8).



**Figure S6** Absorbance spectra of tetramethylrhodamine (CR) modified scaffold (rec-CR15 or CR-dex (70kDa)) and monomeric CR (CR-ODN and CR-AE) in 40 mM MES buffer (pH7.0). a) rec-CR15 (0.8  $\mu$ M), b) CR-dex (70kDa) (0.5  $\mu$ M), c) CR-ODN (0.7  $\mu$ M), and d) CR-AE (0.8  $\mu$ M).

Note: As shown in Figure S6(b), a blue-shifted peak at 518 nm, indicating H-dimer of CR [S2-S4]. To demonstrate the existence of H-dimer, the ratio of absorbance at 556 nm ( $\lambda_{\text{max}}$  of CR) and 518 nm ( $\lambda_{\text{max}}$  of H-dimer) ( $A_{556}/A_{518}$ ) was compared. CR-dex (70kDa) showed a lower value ( $A_{556}/A_{518} = 0.7$ ) than monomeric CR (CR-ODN2 and CR-AE:  $A_{556}/A_{518} = 2.6$ ). The result indicated that a portion of approximately 8 molecules of CR on 70kDa dextran formed H-dimer, resulting in the appearance of the blue-shifted peak, which is known to cause fluorescence quenching. On the other hand, rec-CR15 had a similar value ( $A_{556}/A_{518} = 2.6$ ) with CR-ODN2 and CR-AE. The result strongly indicated that 15 molecules of CR were assembled on the DNA scaffold without interference between them.

**Table S1** Nucleotide sequences for the staple strands

	Position (see Fig.S1a)	Sequence (from 5' to 3')
rec-1	2A	ACGTTAGTAAATGAATTTTCTGTAAGCGGAGT
rec-2	2B	GAGAATAGCTTTTGCGGGATCGTCGGGTAGCA
rec-3	2C-I-1	ACGGCTACTTACTTAG <b><u>TCCTCTTTTGAGGAACAAGTTTCTTGT</u></b> CCGGAACGCTGACCAA
rec-4	2D-I-1	CTTTGAAAAGAACTGG <b><u>TCCTCTTTTGAGGAACAAGTTTCTTGT</u></b> CTCATTATTTAATAAA
rec-5	2E	ACGAACTAGCGTCCAATACTGCGGAATGCTTT
rec-6	2F	AAACAGTTGATGGCTTAGAGCTTATTTAAATA
rec-7	2G	TGCAACTAAGCAATAAAGCCTCAGTTATGACC
rec-8	2H	CTGTAATATTGCCTGAGAGTCTGGAAGAACTAG
rec-9	2I	CATGTCAAGATTCTCCGTGGGAACCGTTGGTG
rec-10	2J	TAGATGGGGGTAACGCCAGGGTGTGCCAAG
rec-11	2K	CTTGCATGCATTAATGAATCGGCCGCCAGGG
rec-12	2L	TGGTTTTTAACGTCAAAGGGCGAAGAACCATC
rec-13	3A	AAAGGCCGAAAGGAACAATAAGCTTTCCAG
rec-14	3B-I-1	GCTCCATGAGAGGCTT <b><u>TCCTCTTTTGAGGAACAAGTTTCTTGT</u></b> TGAGGACTAGGGAGTT
rec-15	3C-I-1	CGATTTTAGAGGACAG <b><u>TCCTCTTTTGAGGAACAAGTTTCTTGT</u></b> ATGAACGGCGCGACCT
rec-16	3D	ACTGGATAACGGAACAACATTATTACCTTATG
rec-17	3E	TTTTTGCGCAGAAAACGAGAATGAATGTTTAG
rec-18	3F	CAAAATTAAAGTACGGTGTCTGGAAGAGGTCA
rec-19	3G	TCAGGTCACTTTTGCGGGAGAAGCAGAATTAG
rec-20	3H	ACCCGTCGTCATATGTACCCCGGTAAAGGCTA
rec-21	3I	ATTAAGTTCGCATCGTAACCGTGCAGTAACA
rec-22	3J	GCCAGCTGCCTGCAGGTCGACTCTGCAAGGCG
rec-23	3K	TGGACTCCCTTTTCACCACTGAGACCTGTCTGT
rec-24	3L	ACCCAAATCAAGTTTTTTGGGGTCAAAGAACG
rec-25	4A	CGTAACGATCTAAAGTTTTGTCTGTAATTGCG
rec-26	4B	AATAATAAGGTCGCTGAGGCTTGCAAAGACTT
rec-27	4C-I-1	TTTCATGAAAATTGTG <b><u>TCCTCTTTTGAGGAACAAGTTTCTTGT</u></b> TCGAAATCTGTACAGA
rec-28	4D-I-1	CCAGGCGCTTAATCAT <b><u>TCCTCTTTTGAGGAACAAGTTTCTTGT</u></b> TGTGAATTACAGGTAG
rec-29	4E	AAAGATTCAGGGGGTAATAGTAAACCATAAAT
rec-30	4F	CAAAAATCATTGCTCCTTTTGATAAGTTTCAT
rec-31	4G	TCCATATACATACAGGCAAGGCAACTTTATTT
rec-32	4H	CAACGCAATTTTTGAGAGATCTACTGATAATC
rec-33	4I	AGAAAAGCAACATTAAATGTGAGCATCTGCCA



rec-34	4J	GTTTGAGGGAAAGGGGGATGTGCTAGAGGATC
rec-35	4K	CCCGGGTACTTTCCAGTCGGGAAACGGGCAAC
rec-36	4L	AGCTGATTACAAGAGTCCACTATTGAGGTGCC
rec-37	5A	ATATATTCTTTTTTCACGTTGAAAATAGTTAG
rec-38	5B	CGCCTGATGGAAGTTCCATTAAACATAACCG
rec-39	5C	TTTCAACTATAGGCTGGCTGACCTTGTATCAT
rec-40	5D	TTTGCCAGATCAGTTGAGATTTAGTGGTTTAA
rec-41	5E	TACCTTTAAGGTCTTACCCTGACAAAGAAGT
rec-42	5F	CAATAAATACAGTTGATTCCCAATTTAGAGAG
rec-43	5G	GGTAGCTAGGATAAAAATTTTAGTTAACATC
rec-44	5H	CTTTCATCCCCAAAAACAGGAAGACCGGAGAG
rec-45	5I	CAGCTGGCGGACGACGACAGTATCGTAGCCAG
rec-46	5J	ACTGCCCGCCGAGCTCGAATTCGTTATTACGC
rec-47	5K	AGTTTGGAGCCCTTCACCGCCTGGTTGCGCTC
rec-48	5L	GTAAAGCACTAAATCGGAACCCTAGTTGTTCC
rec-49	6A	TGTAGCATTCCACAGACAGCCCTCATCTCAA
rec-50	6B	AAAAAAGGACAACCATCGCCACGCGGTAAA
rec-51	6C	ATACGTAAAAGTACAACGGAGATTTTCATCAAG
rec-52	6D	AGTAATCTTAAATTGGGCTTGAGAGAATACCA
rec-53	6E	CATTCAACGCGAGAGGCTTTTGCATATTATAG
rec-54	6F	TCAGAAGCCTCCAACAGGTCAGGATCTGCGAA
rec-55	6G	CGAGTAGAACTAATAGTAGTACAAACCCTCA
rec-56	6H	TATATTTTAGCTGATAAATTAATGTTGTATAA
rec-57	6I	GCAAAATATCGCGTCTGGCCTTCCTGGCCTCAG
rec-58	6J	GAAGATCGGTGCGGGCCTCTTCGCAATCATGG
rec-59	6K	TCATAGCTACTCACATTAATTGCGCCCTGAGA
rec-60	6L	GAGTTGCACGAGATAGGGTTGAGTAAGGGAGC
rec-61	7A	CAATGACACTCCAAAAGGAGCCTTACAACGCC
rec-62	7B	GCGAAACATGCCACTACGAAGGCATGCGCCGA
rec-63	7C	ACGAGTAGTGACAAGAACCGGATATACCAAGC
rec-64	7D	CCAAAATATAATGCAGATACATAAACACCAGA
rec-65	7E	GAAGCAAAAAAGCGGATTGCATCAGATAAAAA
rec-66	7F	TCAATTCTTTTAGTTTGACCATTACCAGACCG
rec-67	7G	ACCGTTCTAAATGCAATGCCTGAGAGGTGGCA
rec-68	7H	AAATAATTTTAAATTGTAAACGTTGATATTCA

rec-69	7I	GGCGATCGCACTCCAGCCAGCTTTGCCATCAA
rec-70	7J	GTGAGCTAGTTTCCTGTGTGAAATTTGGGAAG
rec-71	7K	GAATAGCCGCAAGCGGTCCACGCTCCTAATGA
rec-72	7L	CCCCGATTTAGAGCTTGACGGGGAAATCAAAA
rec-73	8A	TGAGTTTCGTACCAGTACAACTTAATTGTA
rec-74	8B	TCGGTTTAGCTTGATACCGATAGTCCAACCTA
rec-75	8C	AAACGAAATGACCCCAGCGATTATTCATTAC
rec-76	8D	CCAAATCACTTGCCCTGACGAGAACGCCAAAA
rec-77	8E	GGAATTACTCGTTTACCAGACGACAAAAGATT
rec-78	8F	AAGAGGAACGAGCTTCAAAGCGAAGATACATT
rec-79	8G	TCGCAAATGGGGCGCGAGCTGAAATAATGTGT
rec-80	8H	AGGTAAAGAAATCACCATCAATATAATATTTT
rec-81	8I	GTTAAATTTTAACCAATAGGAACCCGGCACC
rec-82	8J	GCTTCTGGTCAGGCTGCGCAACTGTGTTATCC
rec-83	8K	GCTCACAATGTAAAGCCTGGGGTGGGTTTGCC
rec-84	8L	CCAGCAGGGGCAAAATCCCTTATAAAGCCGGC
rec-85	9A-connect	CTTAAACATCAGCTTGCTTTCGAGAAACAGTT
rec-86	9B-connect	CTCATCTTGAGGCAAAAGAATACACTCCCTCA
rec-87	9C-connect	GAATAAGGACGTAACAAAGCTGCTGACGGAAA
rec-88	9D-connect	CATAACCCGAGGCATAGTAAGAGCTTTTAAAG
rec-89	9E-connect	TTTAAATTGCCCGAAAGACTTCAATTCCAGAG
rec-90	9F-connect	TTTCATTTGGTCAATAACCTGTTTAATCAATA
rec-91	9G-connect	AGACAGTCATTCAAAGGGTGAGATATCATAT
rec-92	9H-connect	GCTCATTTTCGCATTAAATTTTGTAGCTTAGA
rec-93	9I-connect	TTCGCCATTGCCGAAACCAGGCAACAGTAC
rec-94	9J-connect	GCATAAAGTTCACACAACATACGAAACAATT
rec-95	9K-connect	CCGAAATCCGAAATCCTGTTTGAAATACCGA
rec-96	9L-connect	GAACGTGGCGAGAAAGGAAGGGAACAACTAT
rec-97	10A-connect	CAAGCCCAATAGGAACCCATGTACCGTAACAC
rec-98	10B-connect	AATGCCCCGTAACAGTGCCCGTATGTGAATTT
rec-99	10C-connect	GAGCCGCCCCACCACCGGAACCGCTAAAACA
rec-100	10D-connect	TTATTCATAGGGAAGGTAAATATTCATTAGT
rec-101	10E-connect	AAAAGTAATATCTTACCGAAGCCCAACACTAT
rec-102	10F-connect	CCTAATTTACGCTAACGAGCGTCTATATCGCG
rec-103	10G-connect	ATCGGCTGCGAGCATGTAGAAACAGCTATAT

rec-104	10H-connect	GCGTTATAGAAAAAGCCTGTTTAGAAGGCCGG
rec-105	10I-connect	TTAAGACGTTGAAAACATAGCGATTAAATCA
rec-106	10J-connect	CTTTTACACAGATGAATATACAGTAAGCGCCA
rec-107	10K-connect	CGACAACCTAAGTATTAGACTTTACAGCCGGAA
rec-108	10L-connect	ACGAACCAAAACATCGCCATTAAATGGTGGTT
rec-109	11A	TGCCTTGACTGCCTATTTTCGGAACAGGATAG
rec-110	11B	AACCAGAGACCCTCAGAACCGCCAGGGTCAG
rec-111	11C	ATTGAGGGTAAAGGTGAATTATCAATCACCGG
rec-112	11D	GCAATAGCGCAGATAGCCGAACAATTCAACCG
rec-113	11E	TCTTACCAGCCAGTTACAAAATAAATGAAATA
rec-114	11F	CTAATTTATCTTTCCTTATCATTCATCTGAA
rec-115	11G	AATTACTACAAATCTTACCAGTAATCCCATC
rec-116	11H	TAGAATCCCTGAGAAGAGTCAATAGGAATCAT
rec-117	11I	TTTAACGTTCCGGGAGAAACAATAATTTCCCT
rec-118	11J	GGATTTAGCGTATTAAATCCTTTGTTTTCAGG
rec-119	11K	TAGCCCTACCAGCAGAAGATAAAAACATTTGA
rec-120	11L	CGGCCTTGCTGGTAATATCCAGAACGAACTGA
rec-121	12A	CTCAGAGCCACCACCCTCATTTTCCTATTATT
rec-122	12B	CTGAAACAGGTAATAAGTTTAAACCCCTCAGA
rec-123	12C	GCCACCACTCTTTTCATAATCAAACCGTCACC
rec-124	12D	GACTTGAGAGACAAAAGGCGACAAGTTACCA
rec-125	12E	GAAGGAAAATAAGAGCAAGAAACACAGCCAT
rec-126	12F	ATTATTTAACCAGCTACAATTTTCAAGAACG
rec-127	12G	GGTATTAAGAACAAGAAAAATAATTAAAGCCA
rec-128	12H	ACGCTCAAAATAAGAATAAACACCGTGAATTT
rec-129	12I	ATCAAAATCGTCGCTATTAATTAACGGATTCTG
rec-130	12J	CCTGATTGAAAGAAATTGCGTAGACCCGAACG
rec-131	12K	TTATTAATGCCGTCAATAGATAATCAGAGGTG
rec-132	12L	AGGCGGTCATTAGTCTTTAATGCGCAATATTA
rec-133	13A	AGTGTACTTGAAAGTATTAAGAGGCCGCCACC
rec-134	13B	GTTTGCCACCTCAGAGCCGCCACCGATACAGG
rec-135	13C	AGCGCCAACCATTGGGAATTAGATTATTAGC
rec-136	13D	GCCCAATACCGAGGAAACGCAATAGGTTTACC
rec-137	13E	TATTTTGCTCCCAATCAAATAAGTGAGTTAA
rec-138	13F	TAAGTCCTACCAAGTACCGCACTCTTAGTTGC

rec-139	13G	AGGCGTTACAGTAGGGCTTAATTGACAATAGA
rec-140	13H	CTGTAAATCATAGGTCTGAGAGACGATAAATA
rec-141	13I	ACAGAAATCTTTGAATACCAAGTTCCTTGCTT
rec-142	13J	AGATTAGATTTAAAAGTTTGAGTACACGTAAA
rec-143	13K	GAATGGCTAGTATTAAACCCGCCTCAACTAAT
rec-144	13L	CCGCCAGCCATTGCAACAGGAAAAATATTTT
rec-145	14A	CCCTCAGAACCGCCACCTCAGAACTGAGACT
rec-146	14B	CCTCAAGAATACATGGCTTTTGATAGAACCAC
rec-147	14C	CACCAGAGTTCGGTCATAGCCCCGCCAGCAA
rec-148	14D	AATCACCAAATAGAAAATTCATATATAACGGA
rec-149	14E	ATACCCAAGATAACCCACAAGAATAAACGATT
rec-150	14F	TTTGTGTTAAGCCTTAAATCAAGAATCGAGAA
rec-151	14G	CAAGCAAGACGCGCCTGTTTATCAAGAATCGC
rec-152	14H	CATATTTAGAAATACCGACCGTGTTACCTTTT
rec-153	14I	TAACCTCCATATGTGAGTGAATAAACAAAATC
rec-154	14J	GCGCAGAGATATCAAAATTATTTGACATTATC
rec-155	14K	ATTTTGCGTCTTTTAGGAGCACTAAGCAACAGT
rec-156	14L	GCCACGCTATACGTGGCACAGACAACGCTCAT
rec-157	15A	TAAGCGTCGAAGGATTAGGATTAGTACCGCCA
rec-158	15B	TCGGCATTCCGCCGCCAGCATTGACGTTCCAG
rec-159	15C	TCACAATCGTAGCACCATTACCATCGTTTTCA
rec-160	15D	ATCAGAGAAAGAACTGGCATGATTTTATTTTG
rec-161	15E	AGGTTTGAACGTCAAAAATGAAAGCGCTAAT
rec-162	15F	AATGCAGACCGTTTTTATTTTCATCTTGCGGG
rec-163	15G	AATGGTTTACAACGCCAACATGTAGTTCAGCT
rec-164	15H	AAATCAATGGCTTAGGTTGGGTTACTAAATTT
rec-165	15I	AACCTACCGCGAATTATTCATTTCCAGTACAT
rec-166	15J	CTAAAATAGAACAAAGAAACCACGAGGTAG
rec-167	15K	GCGTAAGAGAGAGCCAGCAGCAAAAAGGTTAT
rec-168	15L	GGAAATACCTACATTTTGACGCTCACCTGAAA
rec-169	16A	TATCACCGTACTCAGGAGGTTTAGCGGGGTTT
rec-170	16B	TGCTCAGTCAGTCTCTGAATTTACCAGGAGGT
rec-171	16C	TGAGGCAGGCGTCAGACTGTAGCGTAGCAAGG
rec-172	16D	CCGGAAACACACCACGGAATAAGTAAGACTCC
rec-173	16E	TTATTACGGTCAGAGGGTAATTGAATAGCAGC

rec-174	16F	CTTTACAGTTAGCGAACCTCCCGACGTAGGAA
rec-175	16G	TCATTACCCGACAATAAACACATATTTAGGC
rec-176	16H	AGAGGCATAATTTTCATCTTCTGACTATAACTA
rec-177	16I	TATGTAAACCTTTTTTAATGGAAAAATTACCT
rec-178	16J	GAGCAAAACTTCTGAATAATGGAAGAAGGAG
rec-179	16K	CGGAATTATTGAAAGGAATTGAGGTGAAAAAT
rec-180	16L	CTAAAGCAAGATAGAACCTTCTGAATCGTCT
rec-181	17A	GGAAAGCGACCAGGCGGATAAGTGAATAGGTG
rec-182	17B	TGCCTTTAGTCAGACGATTGGCCTGCCAGAAT
rec-183	17C	ACGCAAAGGTCACCAATGAAACCAATCAAGTT
rec-184	17D	TGAACAAACAGTATGTTAGCAAATAAAAGAA
rec-185	17E	GAGGCGTTAGAGAATAACATAAAAGAACACCC
rec-186	17F	CCAGACGAGCGCCCAATAGCAAGCAAGAACGC
rec-187	17G	TTTGTAGTTTTTCGAGCCAGTAATAAATTCTGT
rec-188	17H	TTGAATTATGCTGATGCAAATCCACAAATATA
rec-189	17I	TGGATTATGAAGATGATGAAACAAAATTCAT
rec-190	17J	ATCAACAGTCATCATATTCTGATTGATTGTT
rec-191	17K	GCCAACAGTCACCTTGCTGAACCTGTTGGCAA
rec-192	17L	GAAATGGATTATTACATTGGCAGACATTCTG
rec-193	910M1	AACATCACTTGCCCTGAGTAGAAGAACT
rec-194	910M2	TGTAGCAATACTTCTTTGATTAGTAAT
rec-195	910M3	AGTCTGTCCATCACGCAAATTAACCGT
rec-196	910M4	ATAATCAGTGAGGCCACCGAGTAAAG
rec-197	910M5	ACGCCAGAATCCTGAGAAGTGTTTTT
rec-198	910M6	TTAAAGGGATTTTAGACAGGAACGGT
rec-199	910M7	AGAGCGGGAGCTAAACAGGAGGCCGA
rec-200	910M8	TATAACGTGCTTTCCTCGTTAGAATC
rec-201	910M9	GTACTATGGTTGCTTTGACGAGCACG
rec-202	910M10	GCGCTTAATGCGCCGCTACAGGGCGC

**Table S2** Nucleotide sequences for the staple strands with comp-ODN

	Position (see Fig.S1a)	Sequence (from 5' to 3')
rec-203	11B-comp-ODN1	<u>TTCGCTGAAGAGCCGAATCC</u> AACCAGAGACCCTCAGAACC GCCAGGGGTCAG
rec-204	11D-comp-ODN1	<u>TTCGCTGAAGAGCCGAATCC</u> GCAATAGCGCAGATAGCCGAACAATTCAACCG
rec-205	11F-comp-ODN1	<u>TTCGCTGAAGAGCCGAATCC</u> CTAATTTATCTTTCCTTATCATTCATCCTGAA
rec-206	11H-comp-ODN1	<u>TTCGCTGAAGAGCCGAATCC</u> TAGAATCCCTGAGAAGAGTCAATAGGAATCAT
rec-207	11J-comp-ODN1	<u>TTCGCTGAAGAGCCGAATCC</u> GGATTTAGCGTATTAAATCCTTTGTTTTCAGG
rec-208	14B-comp-ODN2	<u>ACCACCACCACCACCACCAA</u> CCTCAAGAATACATGGCTTTTGATAGAACCAC
rec-209	14D-comp-ODN2	<u>ACCACCACCACCACCACCAA</u> AATCACCAAATAGAAAATTCATATATAACGGA
rec-210	14F-comp-ODN2	<u>ACCACCACCACCACCACCAA</u> TTTTGTTTAAGCCTTAAATCAAGAATCGAGAA
rec-211	14H-comp-ODN2	<u>ACCACCACCACCACCACCAA</u> CATATTTAGAAATACCGACCGTGTACCTTTT
rec-212	14J-comp-ODN2	<u>ACCACCACCACCACCACCAA</u> GCGCAGAGATATCAAAATTATTTGACATTATC
rec-213	6B-comp-ODN3	<u>CATAGGCCGAAGCCTTATCC</u> AAAAAAGGACAACCATCGCCACGCGGGTAAA
rec-214	6D-comp-ODN3	<u>CATAGGCCGAAGCCTTATCC</u> AGTAATCTTAAATTGGGCTTGAGAGAATACCA
rec-215	6F-comp-ODN3	<u>CATAGGCCGAAGCCTTATCC</u> TCAGAAGCCTCCAACAGGTGAGGATCTGCGAA
rec-216	6H-comp-ODN3	<u>CATAGGCCGAAGCCTTATCC</u> TATATTTTAGCTGATAAATTAATGTTGTATAA
rec-217	6J-comp-ODN3	<u>CATAGGCCGAAGCCTTATCC</u> GAAGATCGGTGCGGGCCTCTTCGCAATCATGG

## Reference for supporting information

- S1. P. W. K. Rothmund, Folding DNA to create nanoscale shapes and patterns. *Nature*, 2006, **440**, 297.
- S2. Ogawa M.; Kosaka N.; Choyke P.L.; Kobayashi H. H-type dimer formation of fluorophores: a mechanism for activatable, in vivo optical molecular imaging. *ACS Chem Biol.* **2009**, *4*, 535.
- S3. Valdes-Aguilera, O.; Neckers, D. C. Aggregation phenomena in xanthene dyes. *Acc. Chem. Res.* **1989**, *22*, 171.
- S4. Selwyn, J. E.; Steinfeld, J. I. Aggregation of equilibriums of xanthene dyes. *J. Phys. Chem.* **1972**, *76*, 762.