Supporting Information

Photophysical and structural investigation of ^{Py}A-modified adenine cluster: its potential use for fluorescent DNA probes exhibiting distinct emission color changes

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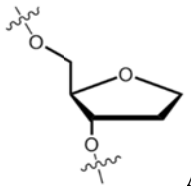
Experimental methods

Materials and general methods

All chemical reagents and solvents for organic synthesis were purchased from Sigma–Aldrich, Alfa Aesar, Tokyo Chemical Industry, and Junsei Chemical. For oligonucleotide synthesis, reagents were obtained from Glen Research and Proligo. Dichloromethane, THF, and DMF were purified in a distillation tower. All natural DNA and RNA samples were purchased from Integrated DNA Technologies (IDT). The phosphoramidite for preparing abasic sites was purchased from Glen Research. The structure of the abasic site is shown below. All fluorescent nucleosides and phosphoramidites, including ^{Py}**A**, were synthesized using previously reported methods.[1] Synthesis, purification (HPLC), identification (MALDI-TOF mass spectrometry), and quantification of oligonucleotides and measurement of UV spectra, and circular dichroism (CD) spectra were performed according to general methods reported prevously.[2] Fluorescence emission/excitation spectra were also recorded using the same conditions as those described previously,[2] except for the excitation/emission slit widths.



Abasic site

Preparation of samples for measurements

For UV and fluorescence emission/excitation spectra, melting curves, and anisotropy spectra, 1.5 nmol of each A-Cluster–forming sequence, 500 μL of 100 mM Trizma buffer (pH 7.2) containing 200 mM NaCl, and 20 mM $MgCl_2$, and additional nuclease-free water were mixed in a 1.5-mL microtube to a total volume of 1 mL. After vortexing for 1 min, the sample for annealing was maintained at 95 °C for 3 min, then under ambient conditions for 4 h, followed by cooling at 10 °C for 3 h, prior to any measurements. In case of miR-21 detection, all UV and fluorescence emission/excitation spectra were obtained without the annealing.

Fluorescence excitation and emission anisotropy

Fluorescence anisotropy was monitored using Eclipse spectrometers (Varian) and the “Automated

Polarization Measurements in Scan" method, with the following parameters: scanning ranges, 420–600 nm for emission and 300–440 nm for excitation; data interval, 1.0 nm; scan rate, 120 nm/min; excitation and emission slit width, 2.5 nm; PMT voltage, Auto; temperature, 25 °C; measure G factor once, vertical, 1, horizontal, 3, for excitation and emission.

Time-resolved fluorescence (TRF) spectra

Time-correlated single photon counting (TCSPC) technique was used for TRF experiments. The light source was a home-built cavity-dumped Ti:sapphire oscillator pumped by a frequency-doubled Nd:YVO_4 laser (Verdi, Coherent). The center wavelength of the oscillator output pulse was adjusted to 800 nm and its frequency was doubled. Cavity-dumping provided 30 nJ pulses at 380 kHz. Pump pulses were generated by the second harmonic generation in a 300-μm-thick β-barium borate crystal. In the TCSPC experiment, fluorescence was collected and detected by a parabolic mirror and a silicon avalanche photodiode (id100-50, ID Quantique), respectively. The instrument response function was measured to be 60 ps (full width at half maximum). For TRF spectra, a spectral reconstruction method was used in conjunction with their steady-state emission spectra. Details of the TCSPC setup have been described elsewhere.[3]

T_m measurement of oligonucleotides

For T_m determination, we utilized Cary 100 UV-visible spectrophotometer and Cary temperature controller to record the hyperchromicity; wavelength: 260nm, SBW: 1.0 nm, start: 10 °C, end: 98 °C, data interval: 1 °C, rate: 2 °C/min. All samples were recorded three times. The T_m curves were analyzed by general method for T_m determination (temperature at which half of the sample is folded, and half is unfolded). T_m determination was done by finding middle point of UV absorption corrected by upper and lower baselines.[4a]

Calculation of thermodynamic parameters

The van't Hoff equation was used to calculate thermodynamic parameters ($\Delta H°$, $\Delta S°$, $\Delta G°$) according to previous reports.[4] To obtain these thermodynamic parameters from UV melting curves, the following equations, derived by Puglisi and Tinoco[4b] for bimolecular equilibrium, were employed:

$$\Delta H° = 6\ R\ T_m^2\ \mathrm{d}(\theta)/\mathrm{d}(T)_{\mathrm{at}\ T=T\mathrm{m}}$$

$$\Delta S° = -R \ln(C_0/2) + \Delta H°/T_m$$

$\Delta G° = \Delta H° - T\,\Delta S°$

where C_0 is the initial strand concentration. The fraction folded (θ) was obtained from UV melting curves. The slopes were obtained by differentiation of the fraction folded (θ) as a function of the temperature (T).

Calculation of photophysical properties

To calculate the ratio of the fluorescence intensities in the short- and long-wavelength regions, two separate emission spectra having Gaussian curves were obtained by using the Fit Multi-peaks function in the Origin program. Each area was used to calculate the quantum yield (QY) of two different types of fluorescence emissions. Quinine sulfate in 0.5 M H_2SO_4 (1– 10 μM; φ_F = 0.546; excitation: 310 nm) was used as the reference material;[5] the QY was calculated using equations described previously.[2,6] The absorbance was monitored at 385 nm; this wavelength was also used for excitation of the ^{Py}**A** residues.

Polyacrylamide gel electrophoresis (PAGE)

A non-denaturing polyacrylamide gel was prepared using a previously reported technique.[2] Aliquots (200 pmol) of samples having the multi-A-Cluster sequence were used for PAGE. Aliquots (133 μL) of samples (containing 200 pmol of the sample, 6.70 μmol of Trizma buffer, 13.3 μmol of NaCl, 1.30 μmol of $MgCl_2$) for measurement of fluorescence emission spectra were dried and then dissolved in water/formamide mixture (6 μL) for loading. PAGE was performed at 90 V, 260 mA, 22 W, and 25 °C for 4 h. After running, the gels were stained using Stains All (Sigma–Aldrich) in formamide for 30 min and exposed to sunlight until bands were clear.

Molecular modeling of A-Cluster

Adenine-pentad duplex for the A-Cluster was built based on the zipper-like structure observed previously through X-ray crystallography.[7] The initially built structure was optimized geometrically to perform molecular dynamics simulations by using the AMBER94 force field[8] through Hyperchem (v. 8.0). Firstly, zipper-like structures for the adenine-pentad duplexes featuring one or two ^{Py}**A** residues were built (Fig. S5A and B). For optimized structure of part of **A3+A4**, one of the ^{Py}**A** residues has a *syn* conformation to construct the stacked pyrenes, generating the large stabilization effect for the DNA duplexes[9-11] as observed experimentally. Likewise, was built a part of **A3+A4** containing the A-Cluster and 3-mer Watson–Crick duplexes at the end of the sequence (Fig. S5C and D).

Table S1 Photophysical properties of oligonucleotides

ODNs	λ_{max}	Abs. λ_{385}	Abs. λ_{415}	Abs. ($\lambda_{385}/\lambda_{415}$)	λ_{emi}	Fl. area ($\lambda_{short}/\lambda_{long}$)	short area	long area	Q.Y. λ_{short}	Q.Y. λ_{long}
A1	387, 417	0.04	0.05	0.82	456	n. d.	15563	n. d.	0.54	n. d.
A2	385, 415	0.04	0.04	0.91	453	n. d.	13548	n. d.	0.48	n. d.
A1+N2	385, 415	0.04	0.04	0.95	453	n. d.	12131	n. d.	0.46	n. d.
A2+N1	386, 416	0.04	0.04	0.93	455	n. d.	13884	n. d.	0.50	n. d.
A1+A2	384, 416	0.08	0.06	1.22	466	n. d.	15864	n. d.	0.30	n. d.
A3	385, 416	0.05	0.05	0.96	453	n. d.	19727	n. d.	0.57	n. d.
A4	386, 415	0.06	0.07	0.88	454	n. d.	22909	n. d.	0.54	n. d.
A3+N4	385, 415	0.05	0.06	0.82	454	n. d.	21785	n. d.	0.63	n. d.
A4+N3	387, 415	0.06	0.07	0.83	455	n. d.	26925	n. d.	0.61	n. d.
A3+A4	385, 413	0.11	0.09	1.26	456, 580	28:72	3753	9651	0.05	0.12
A3+A5	385, 415	0.11	0.11	1.01	460	n. d.	14222	n. d.	0.19	n. d.
A3+A6	385, 417	0.11	0.08	1.34	465, 546	29:71	5351	13100	0.07	0.17
A3+A7	385, 415	0.11	0.12	0.87	458	n. d.	39926	n. d.	0.52	n. d.
A3+A8	386, 416	0.11	0.12	0.87	461	n. d.	12279	n. d.	0.16	n. d.
A3+A9	385, 415	0.12	0.10	1.19	463	n. d.	24770	n. d.	0.30	n. d.
A3+A10	386, 415	0.10	0.08	1.27	465, 549	24:76	3422	10837	0.05	0.15
A3+A11	386, 417	0.10	0.09	1.23	466, 538	28:72	5486	14107	0.07	0.19
A3+A12	385, 414	0.11	0.08	1.28	459, 563	35:65	5345	9927	0.07	0.13
A3+A13	385, 414	0.11	0.08	1.32	465, 542	45:55	9091	11111	0.12	0.14
A3+A14	385, 414	0.10	0.08	1.26	461, 552	40:60	6895	10342	0.09	0.14
A4+A15	385, 415	0.11	0.09	1.20	463	n. d.	26958	n. d.	0.35	n. d.
A3+A16	385, 415	0.10	0.08	1.20	463	n. d.	25693	n. d.	0.36	n. d.
A12 +A15	385, 415	0.11	0.09	1.25	464	n. d.	22879	n. d.	0.30	n. d.
A13+A15	384, 415	0.10	0.08	1.35	467	n. d.	24405	n. d.	0.33	n. d.
A9+A15	385, 414	0.11	0.11	1.02	460	n. d.	36439	n. d.	0.45	n. d.
A14+A15	385, 416	0.10	0.09	1.11	461	n. d.	28940	n. d.	0.39	n. d.
A15+A16	385, 415	0.10	0.09	1.21	463	n. d.	25844	n. d.	0.34	n. d.
A29+A30	384, 417	0.18	0.12	1.47	480, 549	25:75	5980	17939	0.05	0.14
A31+A32	385, 418	0.20	0.15	1.34	476, 544	25:75	8033	24099	0.06	0.17
A33+A34	385, 414	0.23	0.16	1.40	461, 577	6:94	1133	17760	0.01	0.11
A33+A35	385, 415	0.22	0.17	1.33	463, 570	12:88	2460	18042	0.02	0.11
A33+A36	385, 414	0.22	0.17	1.32	462, 564	19:81	4414	18817	0.03	0.12
A33+A37	385, 415	0.23	0.20	1.17	467, 541	35:65	12026	22334	0.07	0.13
A33+A38	385, 414	0.21	0.15	1.38	463, 571	12:88	2447	17941	0.02	0.12
A33+A39	385, 415	0.22	0.16	1.40	461, 574	8:92	1575	18113	0.01	0.12
A33+A40	385, 415	0.21	0.16	1.36	464, 563	14:86	3135	19258	0.02	0.13
A41	386, 416	0.21	0.16	1.29	460, 574	5:95	976	18548	0.01	0.12
A42	385, 415	0.17	0.13	1.24	467, 552	23:77	5098	17066	0.04	0.14
A43	384, 415	0.22	0.16	1.38	477, 543	36:64	13594	24166	0.09	0.15
A44	383, 415	0.19	0.13	1.48	478	n. d.	40938	n. d.	0.30	n. d.
A45	383, 416	0.18	0.12	1.46	490	n. d.	36480	n. d.	0.28	n. d.

ODNs	λ_{max}	Abs. λ_{385}	Abs. λ_{415}	Abs. ($\lambda_{385}/\lambda_{415}$)	λ_{emi}	Fl. area ($\lambda_{short}/\lambda_{long}$)	short area	long area	Q.Y. λ_{short}	Q.Y. λ_{long}
A46	386, 416	0.21	0.17	1.22	460, 569	14:86	3056	18770	0.02	0.13
A47	385, 416	0.22	0.18	1.25	464, 558	15:85	3806	21567	0.02	0.14
A48	385, 416	0.21	0.16	1.36	468, 536	28:72	10001	25716	0.07	0.17
A49	384, 417	0.19	0.14	1.35	467, 527	28:72	11120	28593	0.08	0.21
A50	385, 416	0.23	0.18	1.23	466, 557	18:82	4549	20724	0.03	0.13
A51	385, 415	0.23	0.17	1.40	461, 567	12:88	2683	19675	0.02	0.12

Table S2 Time constants determined from TCSPC experiments

A3+A10 (-AAFGA-)					**A3+A4** (-AAFAA-)			
450 nm	0.35	0.61	0.04		450 nm	0.3	0.67	0.03
	440 ps	2.0 ns	11 ns			390 ps	2.2 ns	6.8 ns
580 nm	0.35	0.28	0.37		580 nm	0.58	0.42	
	2.4 ns	7.6 ns	34 ns			3.4 ns	31 ns	
A3+A11 (-AAFTA-)					**A3+A12** (-CAFAA-)			
450 nm	0.28	0.67	0.05		450 nm	0.3	0.62	0.08
	360 ps	2.2 ns	10 ns			330 ps	2.0 ns	6.1 ns
580 nm	0.5	0.23	0.27		580 nm	0.57	0.43	
	2.8 ns	8.2 ns	33 ns			3.6 ns	31 ns	
A1+A2 (-AFA-)					**A3+A13** (-ACFAA-)			
450 nm	0.3	0.65	0.05		450 nm	0.33	0.57	0.1
	440 ps	2.3 ns	12 ns			330 ps	1.9 ns	6.7 ns
580 nm	0.49	0.27	0.24		580 nm	0.74	0.26	
	2.4 ns	7.2 ns	35 ns			3.4 ns	30 ns	
A+A0					**A3+A9** (-AAFCA-)			
450 nm	0.63	0.29	0.08		450 nm	0.4	0.51	0.09
	90 ps	460 ps	2.0 ns			280 ps	1.7 ns	5.5 ns
580 nm	0.37	0.24	0.27	0.12	580 nm	0.69	0.31	
	150 ps	740 ps	3.1 ns	18 ns		3.4 ns	27 ns	
A3 (Single strand)					**A3+A14** (-AAFAC-)			
450 nm	0.45	0.55			450 nm	0.37	0.56	0.07
	950 ps	2.4 ns				330 ps	1.8 ns	6.3 ns
					580 nm	0.7	0.3	
						3.1 ns	31 ns	

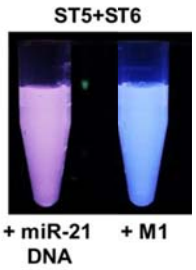
Table S3 Experimental Thermodynamic Parameters. Thermodynamic parameters were calculated from the UV melting curves according to general methods.

ODNs	T_m	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol K)	$\Delta G°$(25 °C) (kcal/mol)
A1+N2	55.6	–74.8	–200	–15.3
A2+N1	55.6	–72.9	–194	–15.1
A1+A2	60.0	–69.3	–180	–15.6
N1+N2	54.8	–84.5	–230	–16.0
A3+N4	55.1	–88.1	–240	–16.4
A4+N3	55.4	–86.0	–234	–16.3
A3+A4	61.4	–90.7	–243	–18.2
N3+N4	52.2	–80.4	–219	–15.1
A3+A5	58.9	–76.5	–202	–16.2
A3+A6	60.6	–87.6	–234	–17.7
A3+A7	56.3	–59.6	–153	–14.0
A3+A8	56.0	–78.2	–210	–15.7
A3+A9	58.7	–76.3	–202	–16.2
A3+A10	58.8	–77.2	–204	–16.2
A3+A11	59.1	–79.8	–212	–16.5
A3+A12	59.3	–84.6	–226	–17.1
A3+A13	58.0	–77.3	–205	–16.1
A3+A14	58.6	–83.5	–226	–17.1
A4+A15	57.0	–73.3	–194	–15.5
A3+A16	56.6	–72.8	–193	–15.3
A12 +A15	57.2	–69.8	–183	–15.2
A13+A15	56.7	–73.8	–196	–15.4
A9+A15	56.2	–68.0	–179	–14.8
A14+A15	55.5	–69.3	–183	–14.8
A15+A16	56.1	–70.7	–187	–15.0
A3+A21	56.8	–67.5	–177	–14.9
A3+A22	58.2	–68.2	–178	–15.2
A23+A24	55.8	–70.0	–185	–14.9
A25+A26	62.9	–73.0	–189	–16.6
A27+A28	57.5	–70.4	–185	–15.3
A29+A30	59.1	–65.1	–168	–15.0

ODNs	T_m	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol K)	$\Delta G°$(25 °C) (kcal/mol)
A31+A32	62.2	–66.7	–171	–15.8
A33+A34	64.3	–90.5	–240	–18.9
A33+A35	61.4	–81.1	–214	–17.2
A33+A36	58.0	–80.0	–213	–16.3
A33+A37	55.9	–67.6	–177	–14.7
A33+A38	58.5	–77.9	–207	–16.2
A33+A39	61.5	–80.2	–212	–17.1
A33+A40	56.4	–68.1	–179	–14.8

Table S4 Evaluation of Three-way junctinon type molecular beacons based on the A-Cluster. Discrimination factor was calculated by the equation, $(I_{455}/ I_{600})_{MB}$ / $(I_{455}/ I_{600})_{MB+Target}$. I_{455} : Fluorescence intensity at the 455 nm; I_{600} : Fluorescence intensity at the 600 nm. In the presence of miR-21, the **ST5+ST6** exhibited the highest I_{600} and lowest I_{455} among the molecular beacons.

miR-21	5′-r(UAG CUU AUC AG A CUG AUG UUG A)-3′
miR-21 DNA	5′-d(TAG CTT ATC AG A CTG ATG TTG A)-3′
M1	5′-d(TAG CTT ATC AG T CTG ATG TTG A)-3′
M2	5′-d(TAG CTT ATC AG G CTG ATG TTG A)-3′
M3	5′-d(TAG CTT ATC AG C CTG ATG TTG A)-3′
M4	5′-d(TAG CTT ATC AA A CTG ATG TTG A)-3′
M5	5′-d(TAG CTT ATC A T A CTG ATG TTG A)-3′
M6	5′-d(TAG CTT ATC AC A CTG ATG TTG A)-3′



Samples	I_{455}	I_{600}	I_{455}/ I_{600}	Discrimination factor
ST1+ST2	312	37	8.5	
ST1+ST2+miR-21 DNA	208	52	4.0	2.1
ST1+ST2+ miR-21	245	47	5.5	1.5
ST3+ST4	608	13	45.2	
ST3+ST4+ miR21 DNA	324	42	7.8	5.8
ST3+ST4+ miR-21	469	30	15.6	2.9
ST5+ST6	570	12	49.4	
ST5+ST6+ miR21 DNA	211	45	4.7	10.5
ST5+ST6+ miR-21	231	44	5.2	9.4
ST7+ST8	441	16	27.4	
ST7+ST8+ miR21 DNA	389	24	16.5	1.7
ST7+ST8+ miR-21	353	34	10.3	2.7
ST5+ST6+M1	416	23	18.3	2.7
ST5+ST6+M2	323	29	11.3	4.4
ST5+ST6+M3	281	34	8.2	6.0
ST5+ST6+M4	333	32	10.5	4.7
ST5+ST6+M5	333	33	10.0	5.0
ST5+ST6+M6	328	34	9.6	5.1

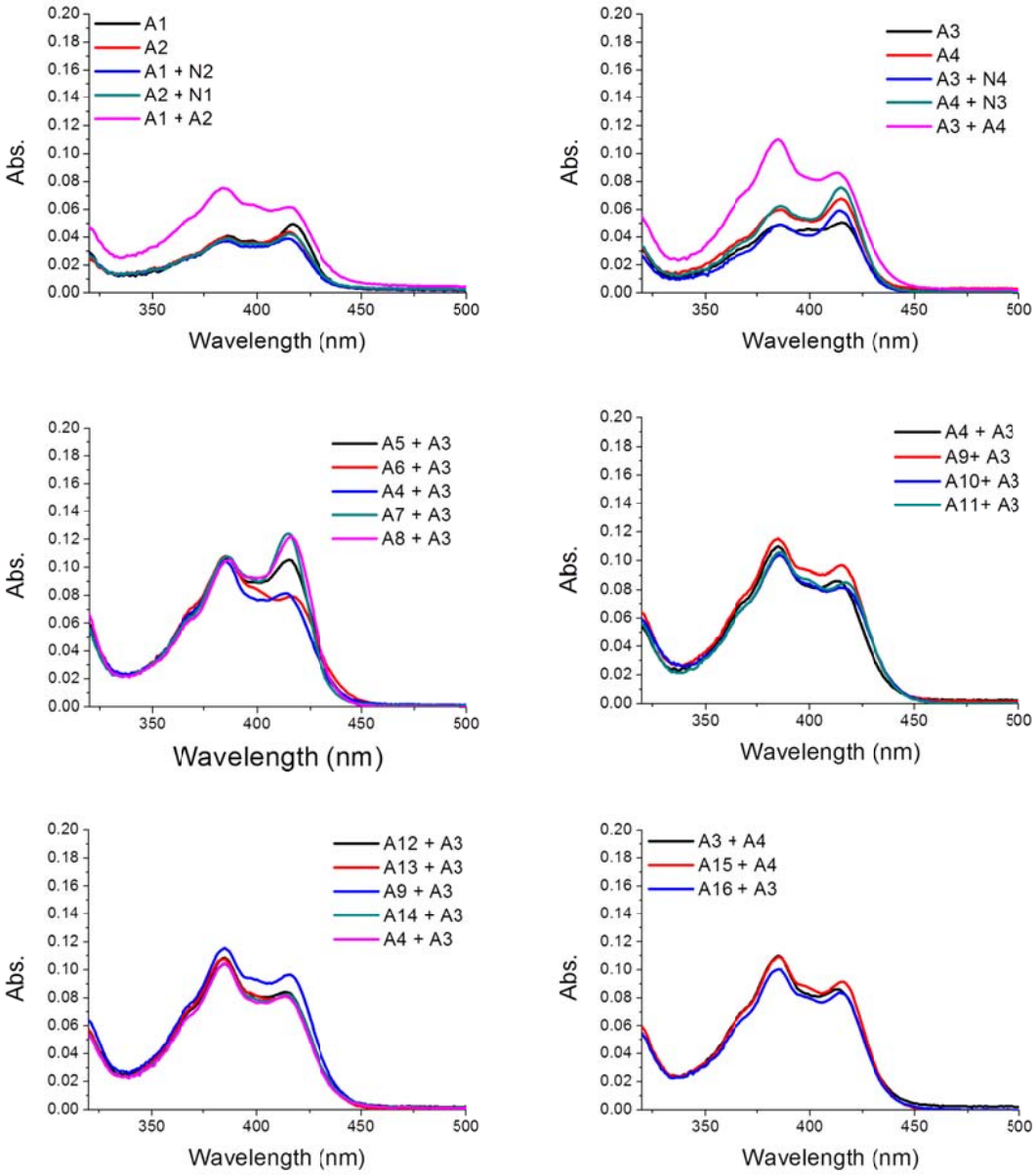
Table S5 MALDI-TOF MS data for synthesized oligonucleotides.

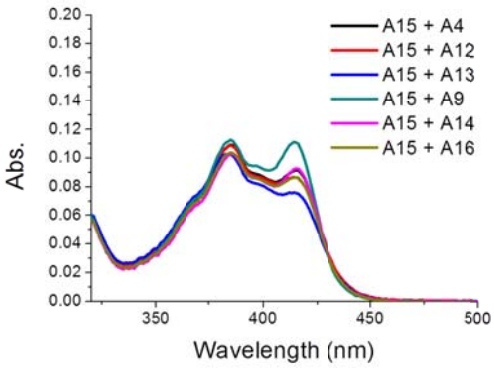
ODN	Formula	calcd. Mass (m/z)	Obs. Mass (m/z)
A	$C_{181}H_{218}N_{56}O_{103}P_{16}$	**5318.9431**	**5318.3958**
A0	$C_{185}H_{214}N_{76}O_{95}P_{16}$	**5515.0139**	**5515.2605**
A1	$C_{201}H_{242}N_{66}O_{113}P_{18}$	**5945.0593**	**5945.4042**
A2	$C_{205}H_{238}N_{86}O_{105}P_{18}$	**6141.1301**	**6141.6017**
A3	$C_{221}H_{266}N_{76}O_{123}P_{20}$	**6571.1755**	**6571.2323**
A4	$C_{225}H_{262}N_{96}O_{115}P_{20}$	**6767.2463**	**6768.5527**
A5	$C_{225}H_{262}N_{96}O_{115}P_{20}$	**6767.2463**	**6767.2625**
A6	$C_{225}H_{262}N_{96}O_{115}P_{20}$	**6767.2463**	**6767.5043**
A7	$C_{225}H_{262}N_{96}O_{115}P_{20}$	**6767.2463**	**6767.6594**
A8	$C_{225}H_{262}N_{96}O_{115}P_{20}$	**6767.2463**	**6767.1655**
A9	$C_{224}H_{262}N_{94}O_{116}P_{20}$	**6743.2351**	**6743.2579**
A10	$C_{225}H_{262}N_{96}O_{116}P_{20}$	**6783.2413**	**6783.0471**
A11	$C_{225}H_{263}N_{93}O_{117}P_{20}$	**6758.2348**	**6758.7629**
A12	$C_{224}H_{262}N_{94}O_{116}P_{20}$	**6743.2351**	**6743.2846**
A13	$C_{224}H_{262}N_{94}O_{116}P_{20}$	**6743.2351**	**6743.7525**
A14	$C_{224}H_{262}N_{94}O_{116}P_{20}$	**6743.2351**	**6743.4408**
A15	$C_{219}H_{266}N_{72}O_{125}P_{20}$	**6523.1531**	**6522.9358**
A16	$C_{223}H_{262}N_{92}O_{117}P_{20}$	**6719.2239**	**6718.9619**
A17	$C_{214}H_{290}N_{86}O_{133}P_{22}$	**7197.2917**	**7197.4826**
A18	$C_{245}H_{286}N_{106}O_{125}P_{22}$	**7393.3625**	**7393.4030**
A19	$C_{261}H_{314}N_{96}O_{143}P_{24}$	**7823.4079**	**7823.2996**
A20	$C_{265}H_{310}N_{116}O_{135}P_{24}$	**8019.4787**	**8019.8555**
A21	$C_{220}H_{259}N_{91}O_{115}P_{20}$	**6634.2075**	**6634.2938**
A22	$C_{220}H_{259}N_{91}O_{115}P_{20}$	**6634.2075**	**6634.5782**
A23	$C_{217}H_{266}N_{68}O_{127}P_{20}$	**6475.1307**	**6474.9708**
A24	$C_{221}H_{262}N_{88}O_{119}P_{20}$	**6671.2015**	**6671.7978**
A25	$C_{221}H_{266}N_{76}O_{127}P_{20}$	**6635.1555**	**6635.0037**
A26	$C_{225}H_{262}N_{96}O_{119}P_{20}$	**6831.2263**	**6831.1960**
A27	$C_{221}H_{270}N_{64}O_{131}P_{20}$	**6535.1295**	**6535.3347**
A28	$C_{225}H_{266}N_{84}O_{123}P_{20}$	**6731.2003**	**6731.9409**

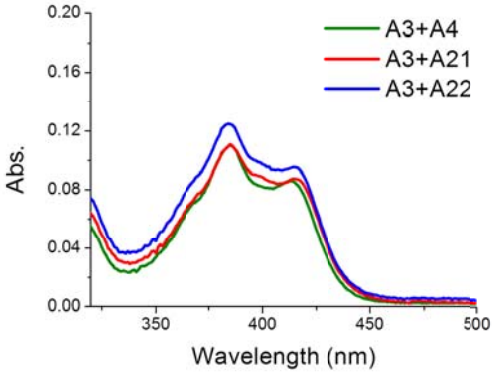
A29	**$C_{249}H_{286}N_{81}O_{128}P_{21}$**	**7108.2962**	**7107.5284**
A30	**$C_{253}H_{282}N_{101}O_{120}P_{21}$**	**7304.3670**	**7304.8779**
A31	**$C_{259}H_{298}N_{86}O_{133}P_{22}$**	**7421.3543**	**7421.6788**
A32	**$C_{263}H_{294}N_{106}O_{125}P_{22}$**	**7617.4251**	**7617.0038**
A33	**$C_{269}H_{310}N_{91}O_{138}P_{23}$**	**7734.4124**	**7734.4102**
A34	**$C_{273}H_{306}N_{111}O_{130}P_{23}$**	**7930.4832**	**7930.8312**
A35	**$C_{272}H_{306}N_{109}O_{131}P_{23}$**	**7906.4720**	**7906.7129**
A36	**$C_{272}H_{306}N_{109}O_{131}P_{23}$**	**7906.4720**	**7906.9807**
A37	**$C_{271}H_{306}N_{107}O_{132}P_{23}$**	**7882.4608**	**7882.3640**
A38	**$C_{272}H_{306}N_{109}O_{131}P_{23}$**	**7906.4720**	**7906.3745**
A39	**$C_{272}H_{306}N_{109}O_{131}P_{23}$**	**7906.4720**	**7906.3535**
A40	**$C_{271}H_{306}N_{107}O_{132}P_{23}$**	**7882.4608**	**7882.3710**
A41	**$C_{212}H_{201}N_{70}O_{68}P_{13}$**	**5217.1075**	**5216.7735**
A42	**$C_{211}H_{201}N_{68}O_{69}P_{13}$**	**5193.0963**	**5193.7430**
A43	**$C_{210}H_{201}N_{66}O_{70}P_{13}$**	**5169.0851**	**5169.4843**
A44	**$C_{209}H_{201}N_{64}O_{71}P_{13}$**	**5145.0739**	**5144.8105**
A45	**$C_{208}H_{201}N_{62}O_{72}P_{13}$**	**5121.0627**	**5121.5088**
A46	**$C_{208}H_{201}N_{62}O_{72}P_{13}$**	**5193.0963**	**5192.8749**
A47	**$C_{210}H_{201}N_{66}O_{70}P_{13}$**	**5169.0851**	**5168.6377**
A48	**$C_{209}H_{201}N_{64}O_{71}P_{13}$**	**5145.0739**	**5145.8321**
A49	**$C_{208}H_{201}N_{62}O_{72}P_{13}$**	**5121.0627**	**5120.2982**
A50	**$C_{208}H_{201}N_{62}O_{72}P_{13}$**	**5193.0963**	**5193.0353**
A51	**$C_{208}H_{201}N_{62}O_{72}P_{13}$**	**5193.0963**	**5193.1187**
N1	**$C_{183}H_{234}N_{66}O_{113}P_{18}$**	**5720.9967**	**5720.0030**
N2	**$C_{187}H_{230}N_{86}O_{105}P_{18}$**	**5917.0675**	**5917.1224**
N3	**$C_{203}H_{258}N_{76}O_{123}P_{20}$**	**6347.1192**	**6347.0610**
N4	**$C_{207}H_{254}N_{96}O_{115}P_{20}$**	**6543.1837**	**6543.6756**
ST1	**$C_{242}H_{289}N_{91}O_{130}P_{20}$**	**7230.3144**	**7230.8019**
ST2	**$C_{244}H_{289}N_{95}O_{130}P_{22}$**	**7310.3268**	**7311.2026**
ST3	**$C_{233}H_{277}N_{88}O_{124}P_{21}$**	**6941.2675**	**6941.8640**
ST4	**$C_{234}H_{277}N_{90}O_{124}P_{21}$**	**6981.2737**	**6981.7523**
ST5	**$C_{223}H_{265}N_{83}O_{118}P_{20}$**	**6612.2144**	**6612.7059**
ST6	**$C_{225}H_{265}N_{87}O_{118}P_{20}$**	**6692.2268**	**6692.6375**

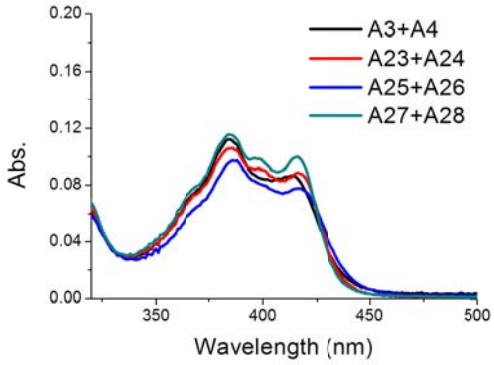
ST7	**$C_{242}H_{289}N_{91}O_{129}P_{22}$**	**7214.3194**	**7213.9168**
ST8	**$C_{245}H_{290}N_{94}O_{131}P_{22}$**	**7325.3265**	**7324.9519**
miR-21	**$C_{209}H_{257}N_{80}O_{154}P_{21}$**	**7001.1021**	**7001.1443**
miR-21 DNA	**$C_{217}H_{273}N_{80}O_{132}P_{21}$**	**6761.1713**	**6761.6657**
Na	**$C_{215}H_{264}N_{76}O_{123}P_{20}$**	**6497.1599**	**6497.3065**
Na1	**$C_{219}H_{260}N_{96}O_{115}P_{20}$**	**6693.2307**	**6693.3576**
Na2	**$C_{219}H_{262}N_{90}O_{119}P_{20}$**	**6675.2077**	**6675.3443**
Na3	**$C_{219}H_{264}N_{84}O_{123}P_{20}$**	**6657.1847**	**6657.5889**
An	**$C_{219}H_{266}N_{76}O_{123}P_{20}$**	**6547.1755**	**6546.9872**
An1	**$C_{223}H_{262}N_{96}O_{115}P_{20}$**	**6743.2463**	**6743.4978**
An2	**$C_{223}H_{264}N_{90}O_{119}P_{20}$**	**6725.2233**	**6725.2257**
An3	**$C_{223}H_{266}N_{84}O_{123}P_{20}$**	**6707.2003**	**6707.3223**
Py2	**$C_{225}H_{264}N_{90}O_{119}P_{20}$**	**6749.2233**	**6749.2117**
Py3	**$C_{225}H_{266}N_{84}O_{123}P_{20}$**	**6731.2003**	**6731.3010**
Pe	**$C_{225}H_{268}N_{76}O_{123}P_{20}$**	**6621.1912**	**6621.5690**
Pe1	**$C_{229}H_{264}N_{96}O_{115}P_{20}$**	**6817.2620**	**6817.5094**
Pe2	**$C_{229}H_{266}N_{90}O_{119}P_{20}$**	**6799.2390**	**6799.2795**
Pe3	**$C_{229}H_{268}N_{84}O_{123}P_{20}$**	**6781.2390**	**6781.0075**

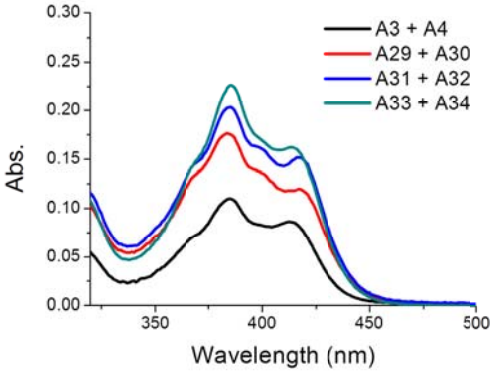
Fig. S1 UV/Vis absorption spectra of synthesized ODNs

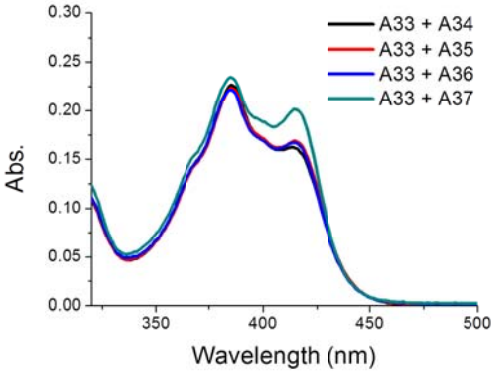


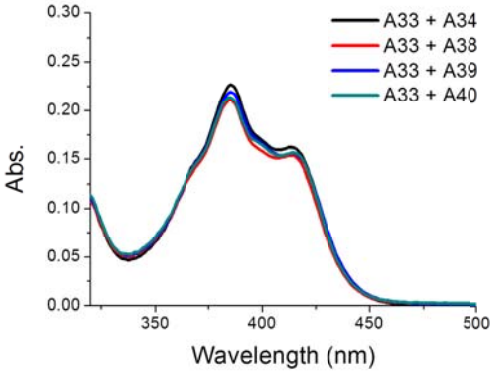

A15 + A4
A15 + A12
A15 + A13
A15 + A9
A15 + A14
A15 + A16
Abs.
Wavelength (nm)


A3+A4
A3+A21
A3+A22
Abs.
Wavelength (nm)


A3+A4
A23+A24
A25+A26
A27+A28
Abs.
Wavelength (nm)


A3 + A4
A29 + A30
A31 + A32
A33 + A34
Abs.
Wavelength (nm)


A33 + A34
A33 + A35
A33 + A36
A33 + A37
Abs.
Wavelength (nm)


A33 + A34
A33 + A38
A33 + A39
A33 + A40
Abs.
Wavelength (nm)

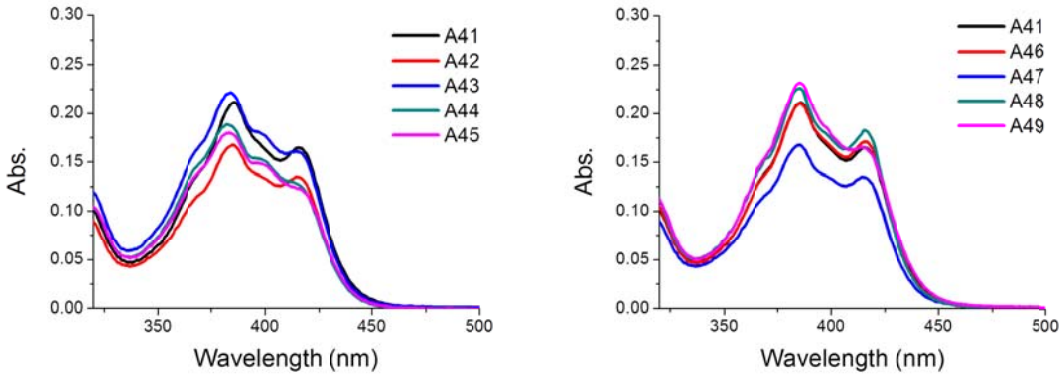


Fig. S2 Fluorescence emission spectra of triply and quadruply modified A-Clusters.

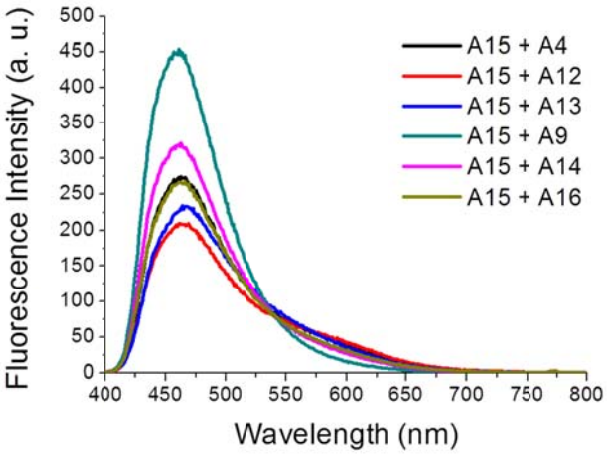


Fig. S3 Fluorescence emission/excitation anisotropy of synthesized ODNs

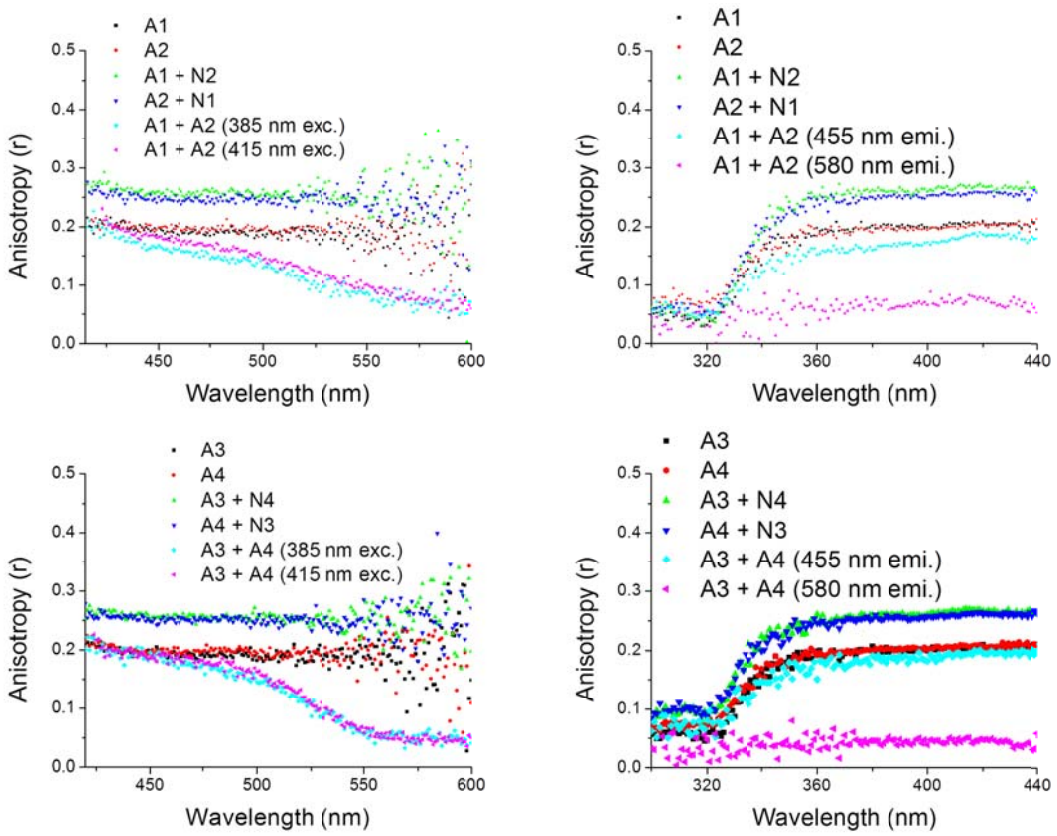


Fig. S4 Fluorescence emission/excitation spectra of (A) **A1+A2**, (B) **A3+A4**, (C) **A17+A18**, and (D) **A19+A20**. The numbers on spectra indicate the ratio of the fluorescence intensity under 260 nm to 385 nm. Conditions: 1.5 μM of duplexes; total volume, 1 mL; 50 mM trizma buffer (pH 7.2), 100 mM NaCl, 10 mM $MgCl_2$; 25 °C.

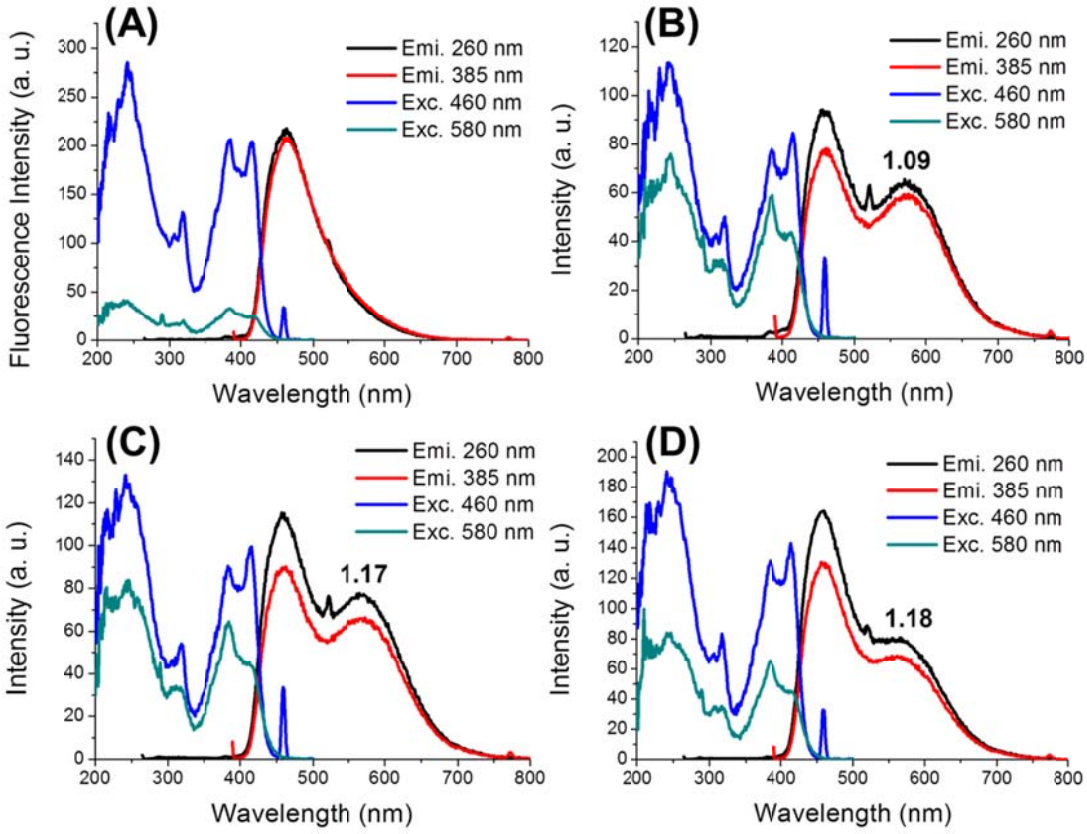
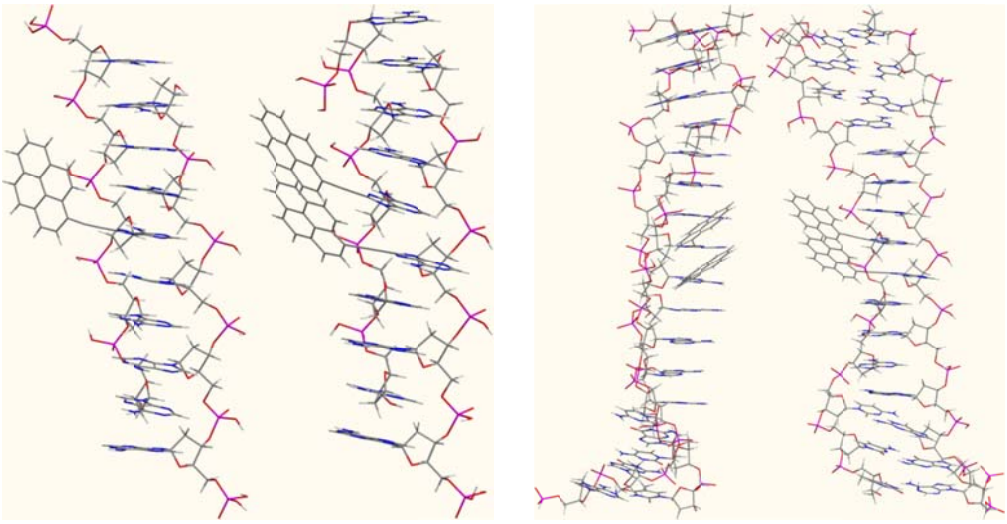
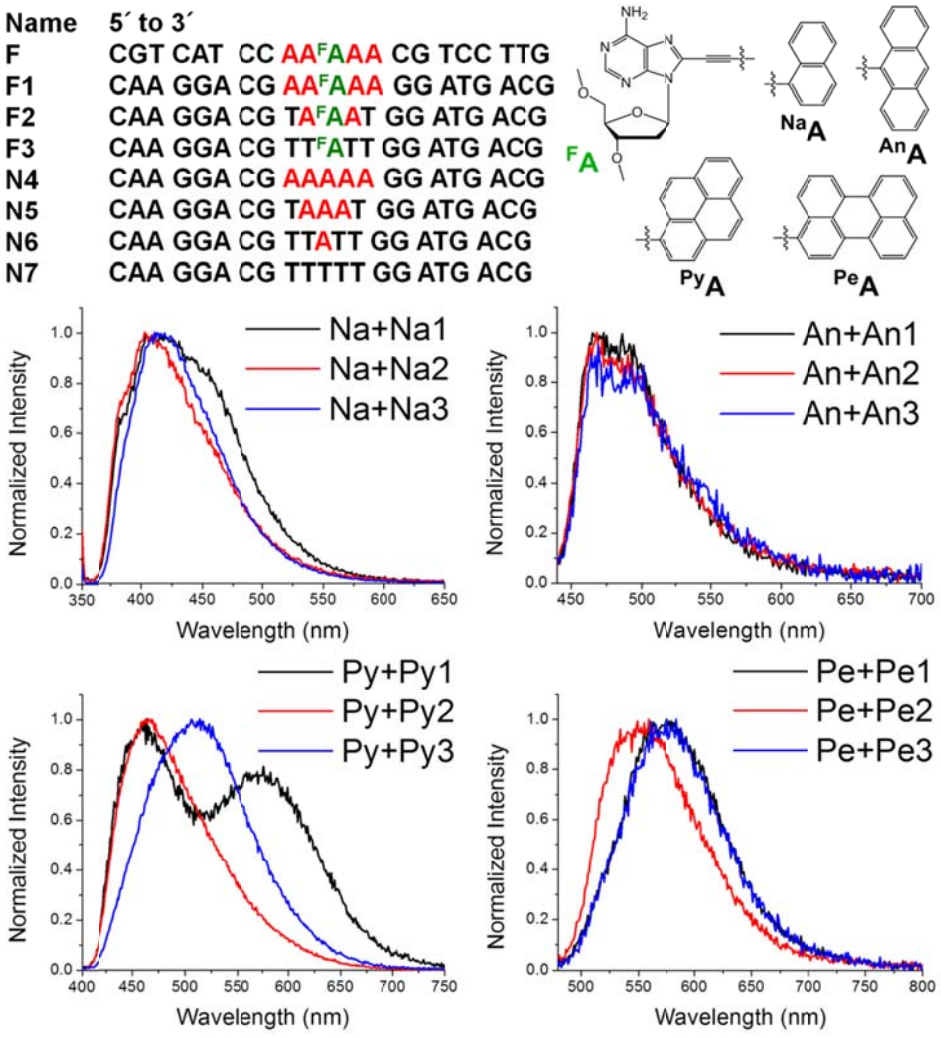


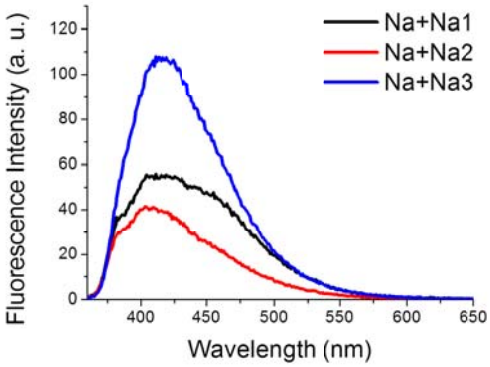
Fig. S5 Energy-minimized structure of the A-Cluster (part of **A3+A4**) on the zipper-like structure, determined using the AMBER force field (Hyperchem 8.0). Side view of (A) singly and (B) doubly ^{Py}**A**-modified adenine-pentad zipper-like structure. (C) Side and (D) front views of the zipper-like structure containing two stacked ^{Py}**A** residues with a 3-mer Watson–Crick base pair part. Well-stacked adenine residues and two pyrene moieties in the middle of the A-Cluster were observed. Red: oxygen; magenta: phosphorus; gray: carbon; blue: nitrogen.

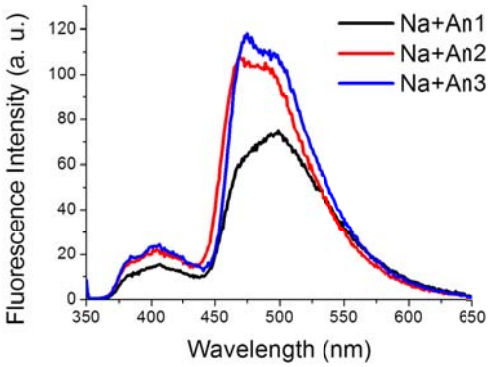


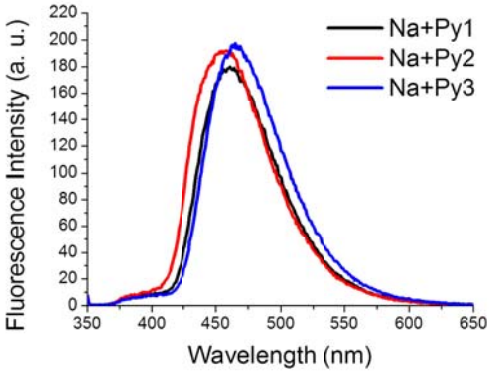
(A) **(B)** **(C)** **(D)**

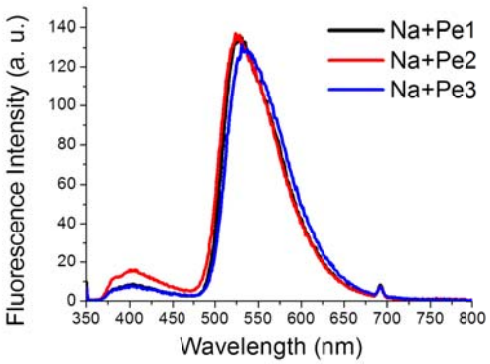
Fig. S6 Fluorescence emission spectra of A-Clusters containing a variety of PAH-modified 2′-deoxyadenosines. **F** can be **Na**, **An**, **Py**, or **Pe** when **F** contains ^{Na}**A**, ^{An}**A**, ^{Py}**A**, or ^{Pe}**A**, respectively. The **F** sequence is fully or partially complementary to **F1**, **F2**, and **F3** and forms either an original A-Cluster or a modified A-Cluster. With the exception of **An+An1**, the A-clusters containing the anthracene moiety on one of the sides (**An+Py1**, **An+Pe1**) exhibited small Stokes shift; nevertheless, most cases, including singly modified-duplexes (**F**+**N4-7**; data not shown), resulted in no dramatic changes in Stokes shift.

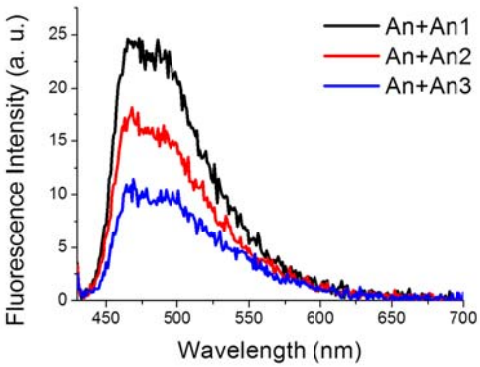


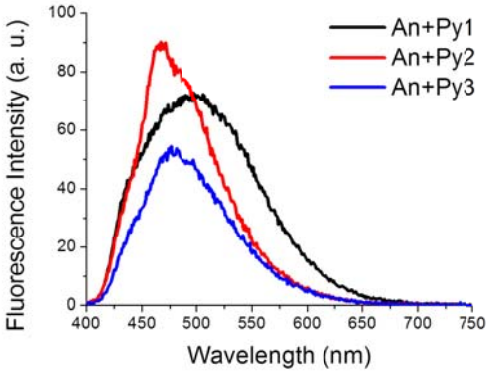

Na+Na1
Na+Na2
Na+Na3
Fluorescence Intensity (a. u.)
Wavelength (nm)

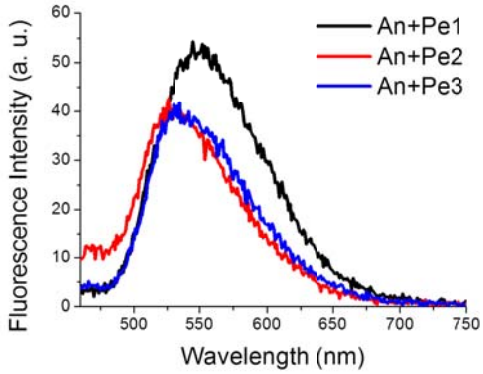

Na+An1
Na+An2
Na+An3
Fluorescence Intensity (a. u.)
Wavelength (nm)

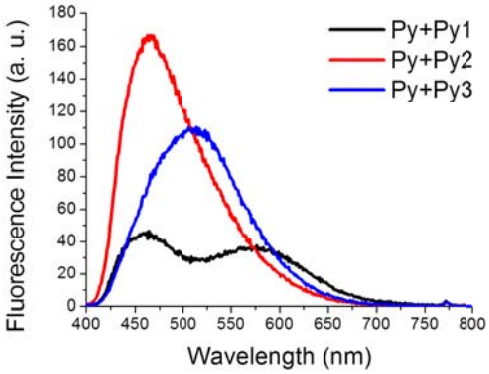

Na+Py1
Na+Py2
Na+Py3
Fluorescence Intensity (a. u.)
Wavelength (nm)

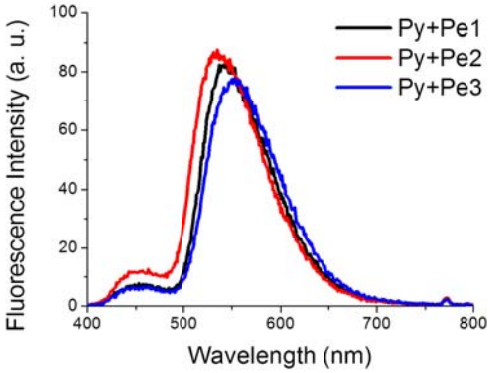

Na+Pe1
Na+Pe2
Na+Pe3
Fluorescence Intensity (a. u.)
Wavelength (nm)


An+An1
An+An2
An+An3
Fluorescence Intensity (a. u.)
Wavelength (nm)


An+Py1
An+Py2
An+Py3
Fluorescence Intensity (a. u.)
Wavelength (nm)


An+Pe1
An+Pe2
An+Pe3
Fluorescence Intensity (a. u.)
Wavelength (nm)


Py+Py1
Py+Py2
Py+Py3
Fluorescence Intensity (a. u.)
Wavelength (nm)


Py+Pe1
Py+Pe2
Py+Pe3
Fluorescence Intensity (a. u.)
Wavelength (nm)

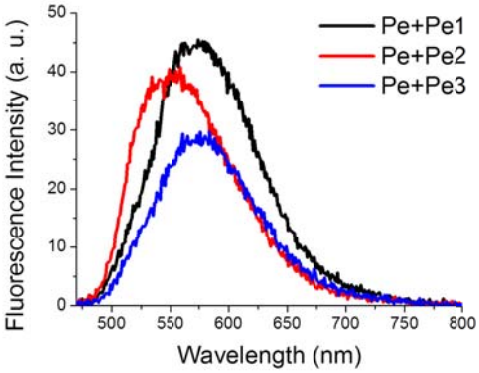

Pe+Pe1
Pe+Pe2
Pe+Pe3
Fluorescence Intensity (a. u.)
Wavelength (nm)

Fig. S7 (Top) Sequences of tested multi–A-Cluster systems. (A) Fluorescence spectra of two–A-Cluster systems with single or double C-variance at the 5′ positions of the ^{Py}**A** residue. (B, C) Fluorescence spectra of four–A-Cluster systems without anti-parallel inducing sequences having various C-variances at the (B) 3′ and (C) at 5′ positions of the ^{Py}**A** residues. Conditions for fluorescence spectra: 1.5 μM of duplexes; total volume, 1 mL; 50 mM trizma buffer (pH 7.2), 100 mM NaCl, 10 mM $MgCl_2$; 25 °C; λ_{ex} = 385 nm; $slit_{ex}$ = 2.5 nm; $slit_{em}$ = 5 nm. (D) 20% PAGE result of four–A-Cluster–forming sequences under UV irradiation. Surprisingly, part of **A46** and **A51** formed self-duplex by stacking interaction (upper bands), while **A42** and **A50** remained as the single strands (lower bands), even though they featured only a single C residue in different positions. This suggested that an internal C residue (**A42** and **A50**) has a negative effect on two adjacent A-Clusters.

Name	5′ to 3′
A33	CGT CAT CC AA^{Py}AAA^{Py}AAA CG TCC TTG
A34	CAA GGA CG AA^{Py}AAA^{Py}AAA GG ATG ACG
A38	CAA GGA CG AA^{Py}AAC^{Py}AAA GG ATG ACG
A39	CAA GGA CG AC^{Py}AAA^{Py}AAA GG ATG ACG
A40	CAA GGA CG AC^{Py}AAC^{Py}AAA GG ATG ACG
A41	AA^{Py}AAA^{Py}AAA^{Py}AAA^{Py}AAA
A42	AA^{Py}ACA^{Py}AAA^{Py}AAA^{Py}AAA
A43	AA^{Py}ACA^{Py}ACA^{Py}AAA^{Py}AAA
A44	AA^{Py}ACA^{Py}ACA^{Py}ACA^{Py}AAA
A45	AA^{Py}ACA^{Py}ACA^{Py}ACA^{Py}ACA
A46	AC^{Py}AAA^{Py}AAA^{Py}AAA^{Py}AAA
A47	AC^{Py}AAC^{Py}AAA^{Py}AAA^{Py}AAA
A48	AC^{Py}AAC^{Py}AAC^{Py}AAA^{Py}AAA
A49	AC^{Py}AAC^{Py}AAC^{Py}AAC^{Py}AAA
A50	AA^{Py}AAA^{Py}AAA^{Py}AAC^{Py}AAA
A51	AA^{Py}AAA^{Py}AAA^{Py}AAA^{Py}ACA

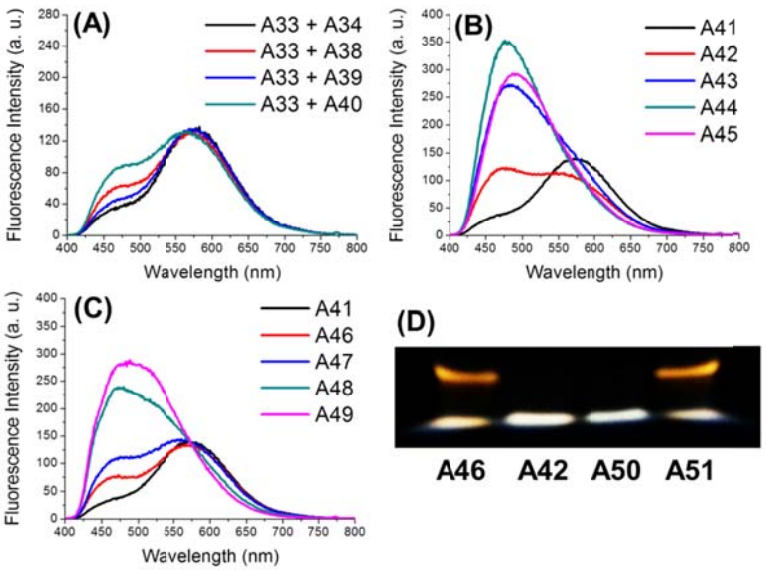
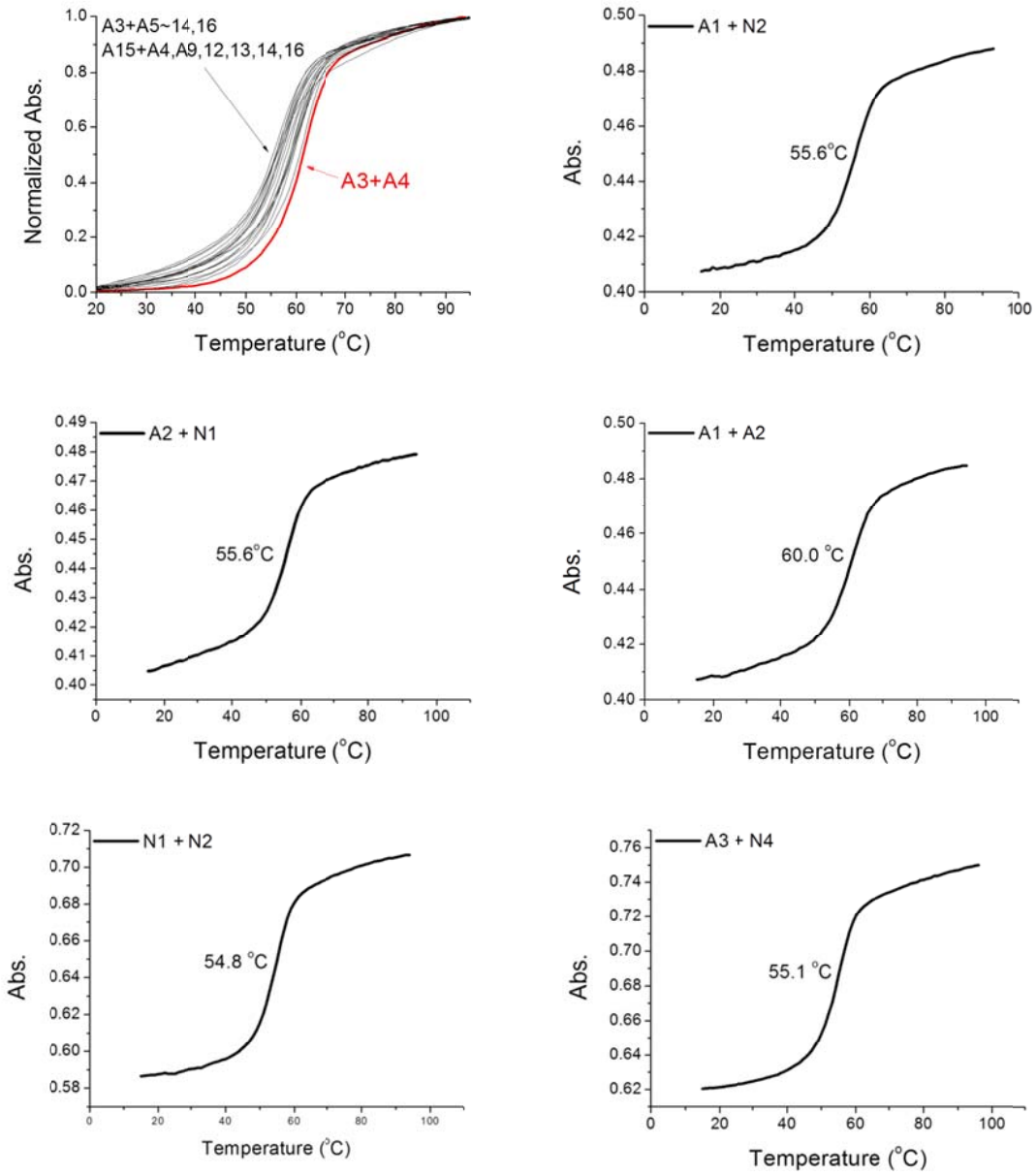
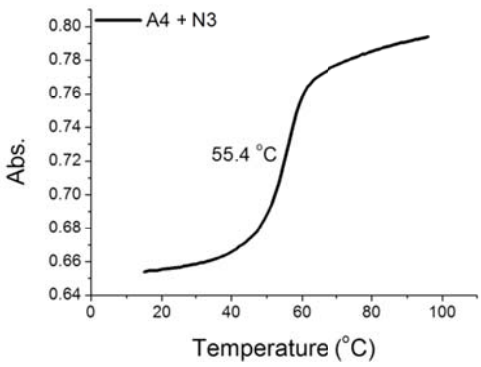
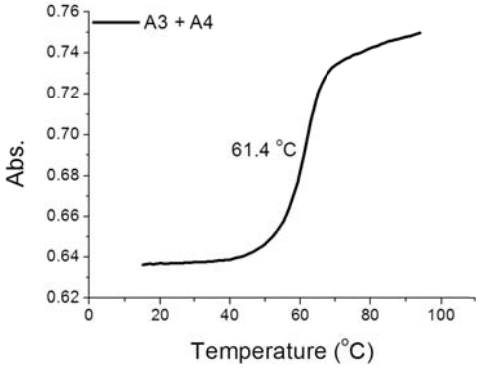


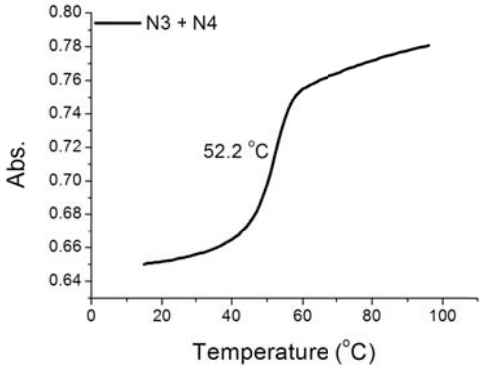
Fig. S8 20% Polyacrylamide gel electrophoresis of four–A-Clusters. (20% PAGE; 200 pmol samples; 6 µmol Trizma buffer (pH 7.2), 13 µmol NaCl, 1 µmol $MgCl_2$; L: $(dA)_{14}$ + $(dA)_{24}$; 1: **A41**; 2: **A46**; 3: **A42**; 4: **A43**; 5: **A44**; 6: **A45**; 7: **A47**; 8: **A48**; 9: **A49**). ^{Py}**A**-modified polyadenylates containing two or more C residues exhibited bands for only their single strands due to structural destabilization.

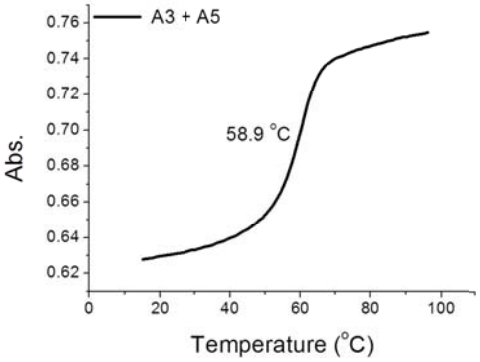
Fig. S9 Thermal stabilities of double-stranded oligonucleotides. Conditions: 1.5 μM of duplexes; total volume, 1 mL; 50 mM trizma buffer (pH 7.2), 100 mM NaCl, 10 mM $MgCl_2$. The **A3+A4** exhibited the highest T_m value in the adenine-pentad duplexes.

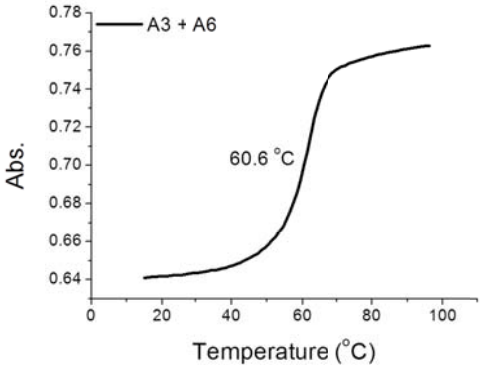


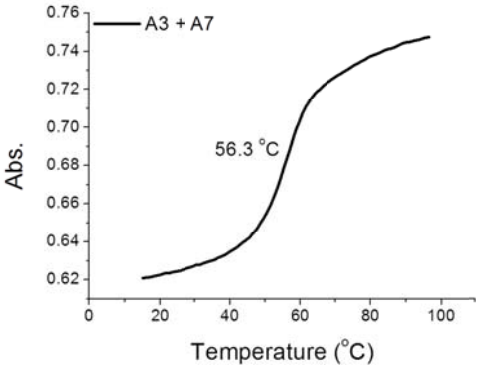

A4 + N3
55.4 °C
Abs.
Temperature (°C)

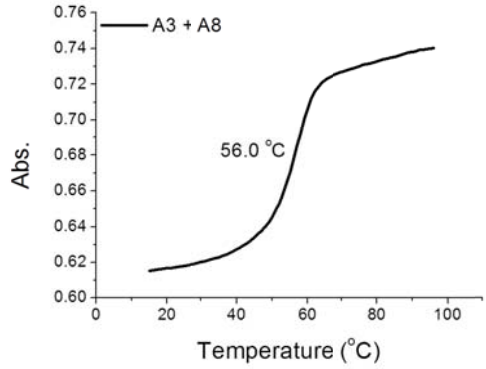

A3 + A4
61.4 °C
Abs.
Temperature (°C)

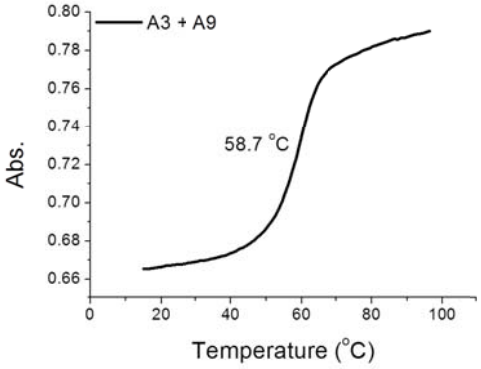

N3 + N4
52.2 °C
Abs.
Temperature (°C)

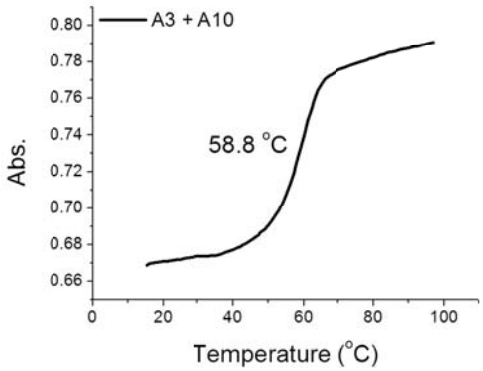

A3 + A5
58.9 °C
Abs.
Temperature (°C)

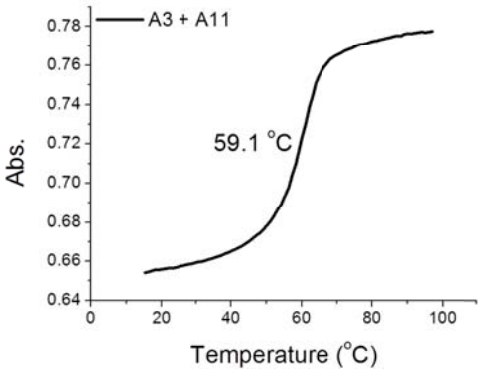

A3 + A6
60.6 °C
Abs.
Temperature (°C)

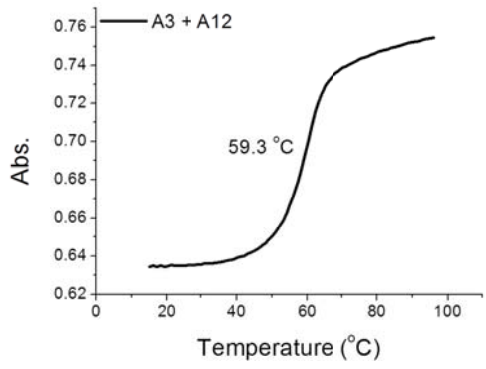

A3 + A7
56.3 °C
Abs.
Temperature (°C)

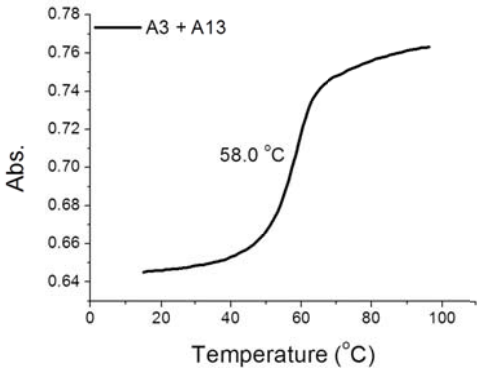

A3 + A8
56.0 °C
Abs.
Temperature (°C)

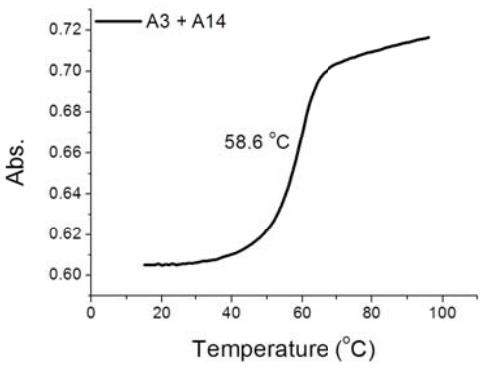

A3 + A9
58.7 °C
Abs.
Temperature (°C)

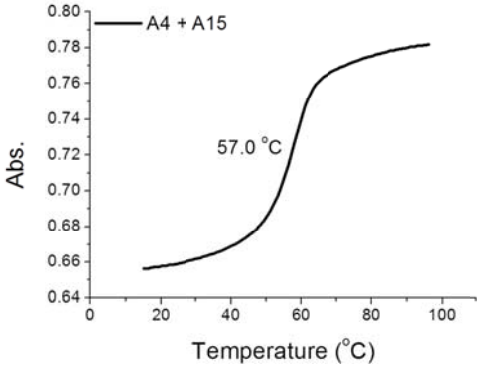

A3 + A10
58.8 °C
Abs.
Temperature (°C)

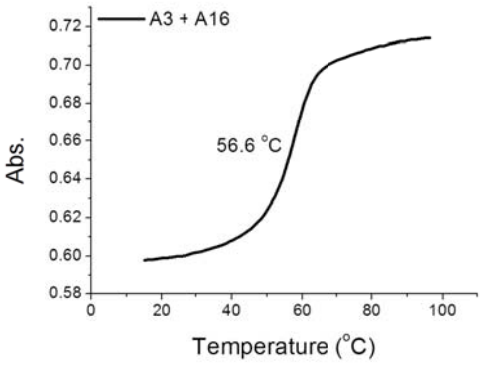

A3 + A11
59.1 °C
Abs.
Temperature (°C)

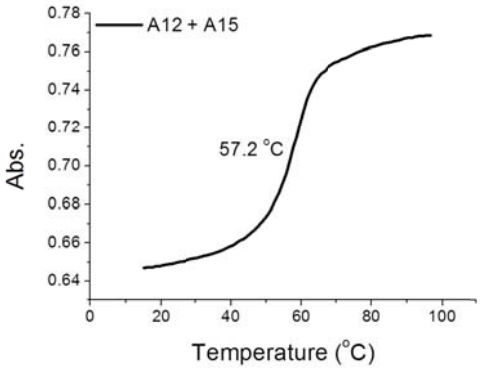

A3 + A12
59.3 °C
Abs.
Temperature (°C)

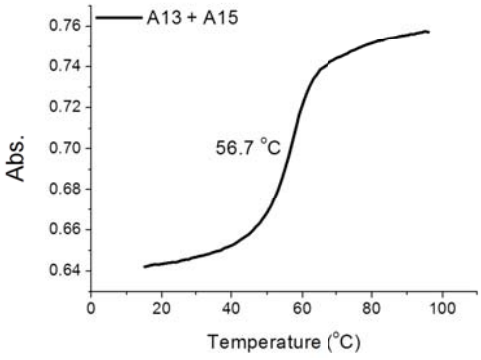

A3 + A13
58.0 °C
Abs.
Temperature (°C)

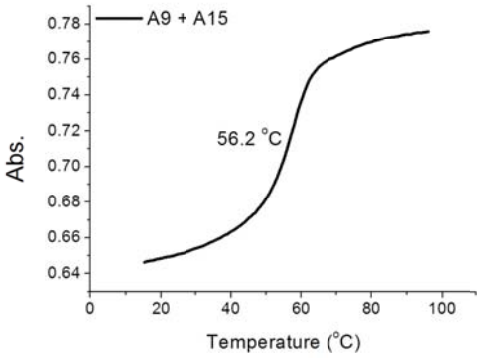

A3 + A14
58.6 °C
Abs.
Temperature (°C)

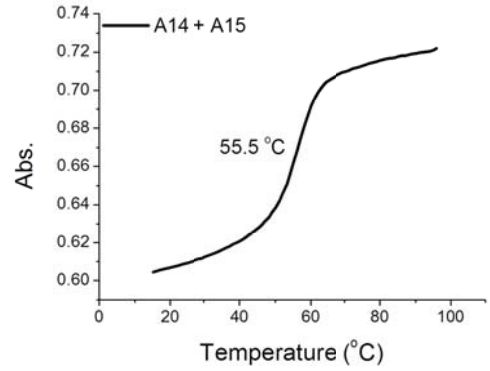

A4 + A15
57.0 °C
Abs.
Temperature (°C)

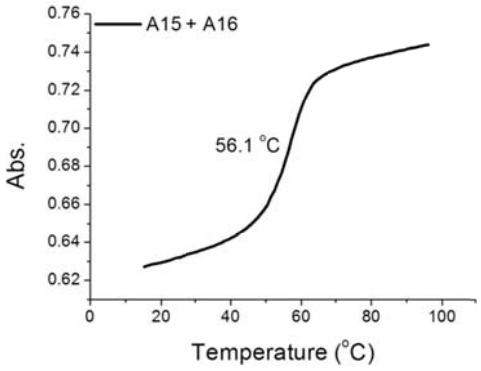

A3 + A16
56.6 °C
Abs.
Temperature (°C)

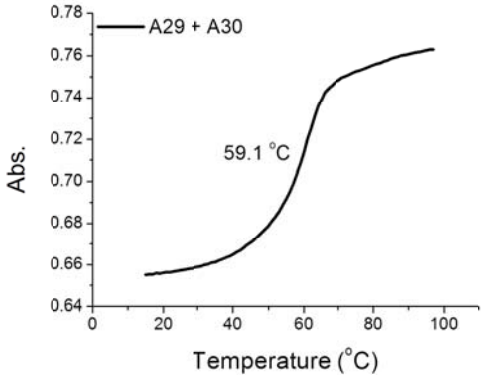

A12 + A15
57.2 °C
Abs.
Temperature (°C)

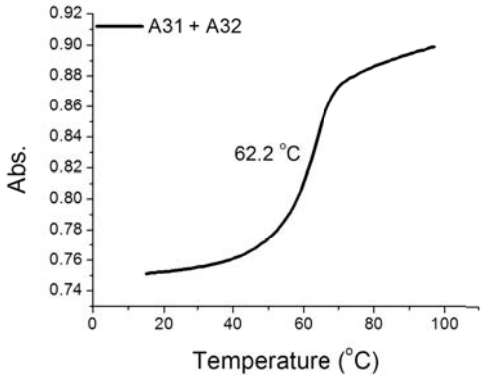

A13 + A15
56.7 °C
Abs.
Temperature (°C)

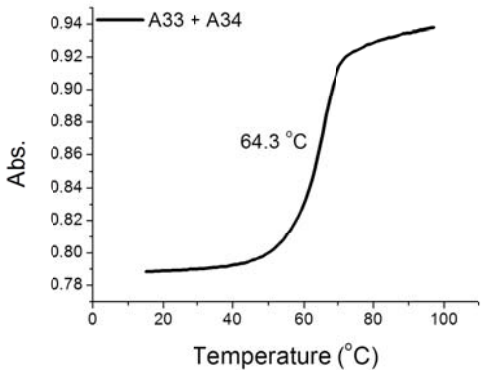

A9 + A15
56.2 °C
Abs.
Temperature (°C)

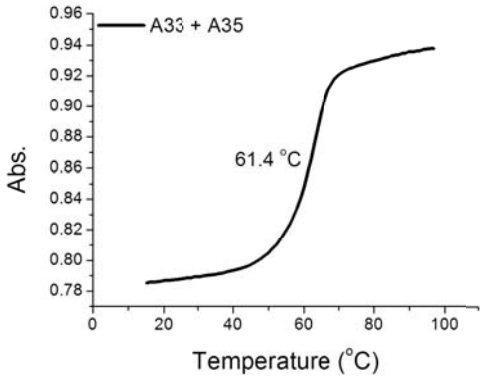

A14 + A15
55.5 °C
Abs.
Temperature (°C)

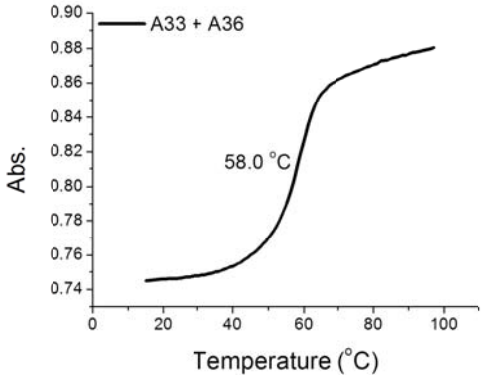

A15 + A16
56.1 °C
Abs.
Temperature (°C)

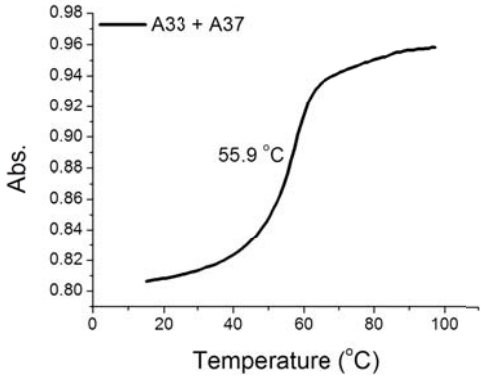

A29 + A30
59.1 °C
Abs.
Temperature (°C)

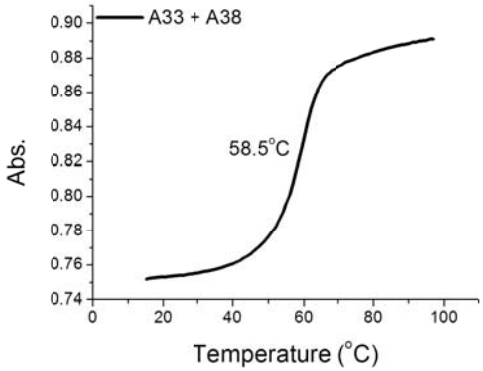

A31 + A32
62.2 °C
Abs.
Temperature (°C)

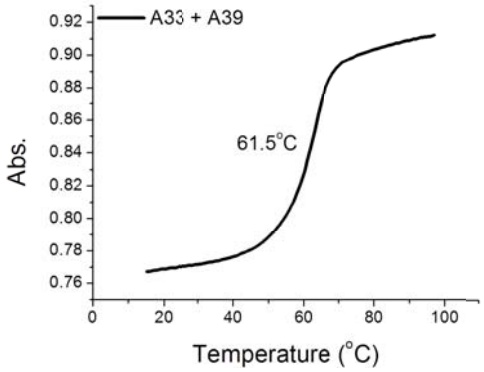

A33 + A34
64.3 °C
Abs.
Temperature (°C)

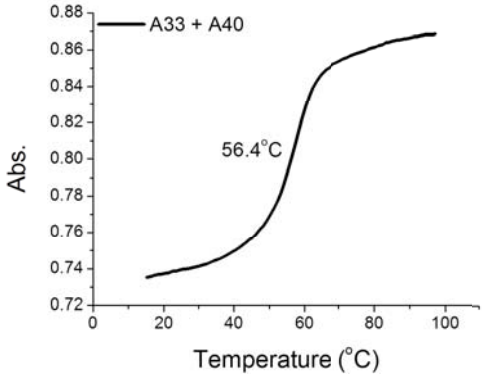

A33 + A35
61.4 °C
Abs.
Temperature (°C)


A33 + A36
58.0 °C
Abs.
Temperature (°C)


A33 + A37
55.9 °C
Abs.
Temperature (°C)


A33 + A38
58.5°C
Abs.
Temperature (°C)


A33 + A39
61.5°C
Abs.
Temperature (°C)


A33 + A40
56.4°C
Abs.
Temperature (°C)

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