Electronic Supplementary Information (ESI)

Formation of i-motifs from acyclic (L)-threoninol nucleic acid

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Mass of Oligonucleotides

All aTNA oligonucleotides were analyzed by liquid chromatography-mass spectrometry (LC-MS) or Matrix-Assisted Laser Desorption Ionization-Time-of-Flight-Mass Spectrometry (MALDI-TOF-MS) (Table S1). The 3-hydroxypicolinic acid (HPA) matrix was used for MALDI analysis.

Table S1: Mass of (L)-aTNA oligonucleotides

Entry	Oligonucleotide sequences [a]	Cal. Mass	Obs. Mass
ON-1	5'-ccccccccccT-3'	3740.9	3743.2
ON-2	5'-ccccccccT-3'	3104.7	3106.0
ON-3	5'-cccccccT-3'	2468.6	2469.8
ON-4	5'-cccccccttcccccccT-3'	5694.3	5696.9
ON-5	5'-ccccttcccccttcccccttcccT-3'	7329.7	7333.7
ON-6	5'-ccccttcccccttcccccttcccccttcccT-3'	8328.9	8332.5
ON-7	5'-cccccccT-3'	2150.5	2151.1
ON-8	5'-Pyccccccccccccc-3'	4178.0	4180.3
ON-9	5'-PyttttttttT-3'	3010.7	3011.7
ON-10	5'-PyctttttttT-3'	3328.8	3330.7
ON-11	5'-PyccccttcccccttcccccttcccccttcccT-3'	6494.5	6511.3
ON-12	5'-PyccccccccccPyC-3'	4282.1	4283.6
ON-13	5'-PyccccccPyT-3'	2706.7	2707.9
ON-14	5'-PycccPyT-3'	2070.6	2075.3
ON-15	5'-PyttttttttPyT-3'	3447.9	3455.4

[a]Capital and small letters are DNA and aTNA nucleotides respectively, Py = pyrene.

Thermal denaturation by UV: UV-melting experiments were carried out on a Thermo Scientific Evolution 260 Bio spectrophotometer. Quartz cuvettes (Hellma) were used with a path length of 1 cm in 10 mM sodium phosphate or sodium acetate buffer with 100 mM sodium chloride. Oligonucleotides of 4 µM concentration were scanned at rate of 1 ^{o}C/min (for both heating and cooling) at wavelength of 295 nm or 260 nm. Polycytosine strands were scanned at a rate of 0.5 ^{o}C/min at pH 7.4. Melting curves were applied for sigmoidal fit by Origin 2015 and melting temperatures (T_m) were determined by first derivative. All the thermal stability data were obtained from second melting curve. For i-motif structures melting transitions were reversible in nature and annealing temperatures were approximately 2 °C less than melting temperatures.

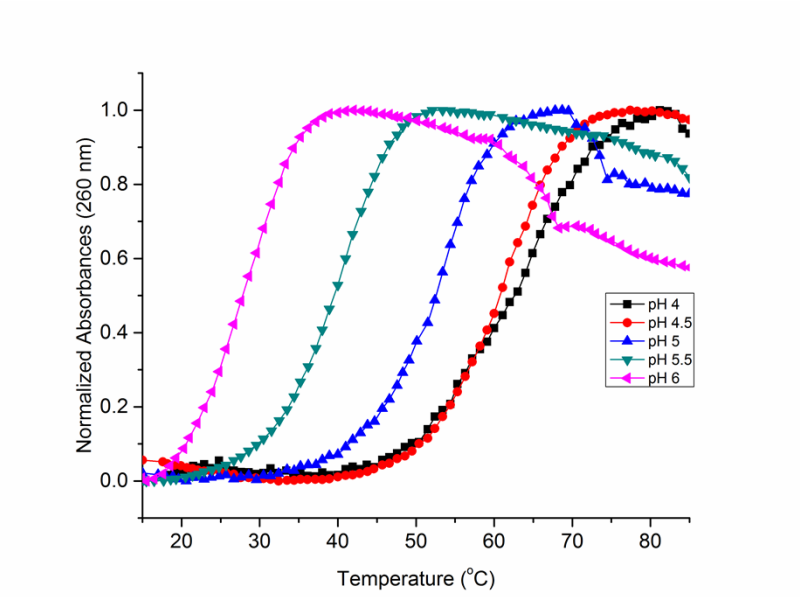

Figure S1: UV-melting profiles of ON-1 recorded at 260 nm at different pH values.

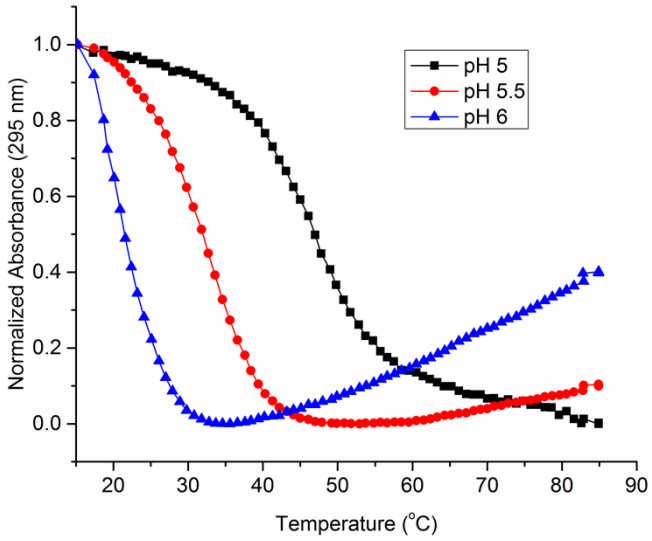

Figure S2: UV-melting profiles of ON-2 recorded at 295 nm at different pH values.

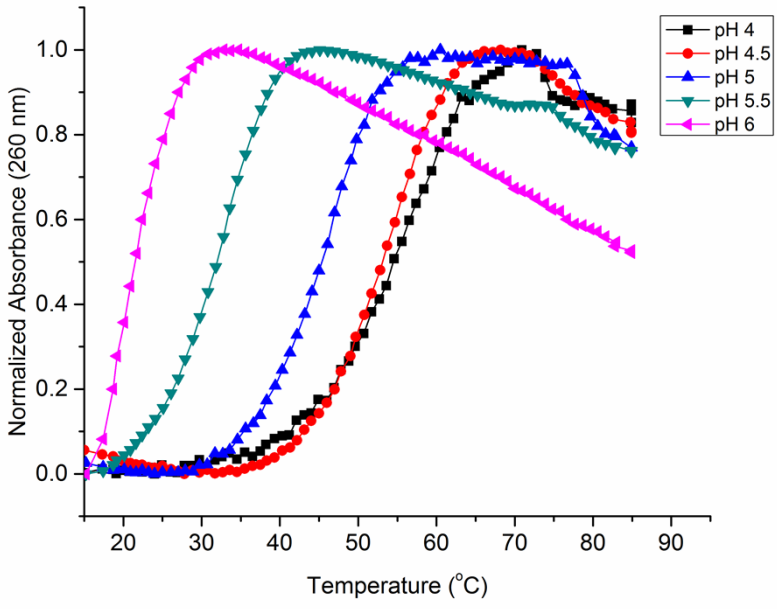

Figure S3: UV-melting profiles of ON-2 recorded at 260 nm at different pH values.

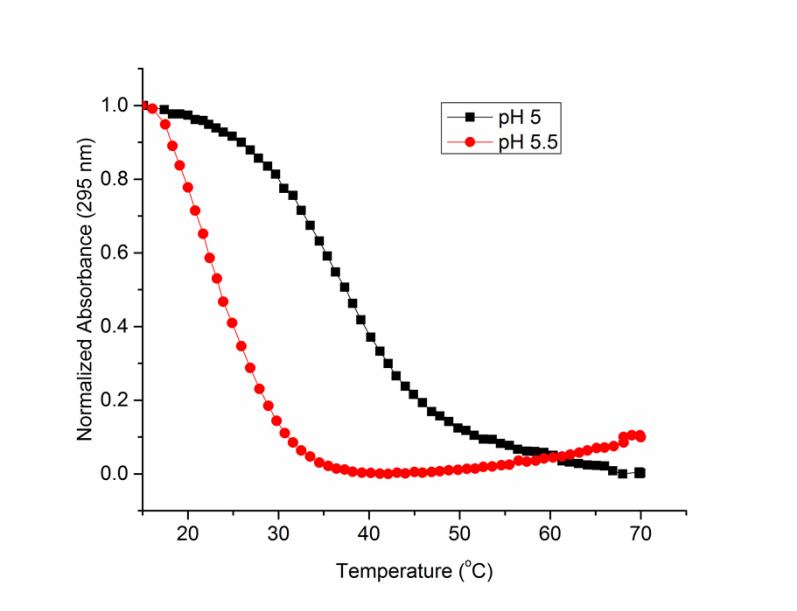

Figure S4: UV-melting profiles of ON-3 recorded at 295 nm at different pH values.

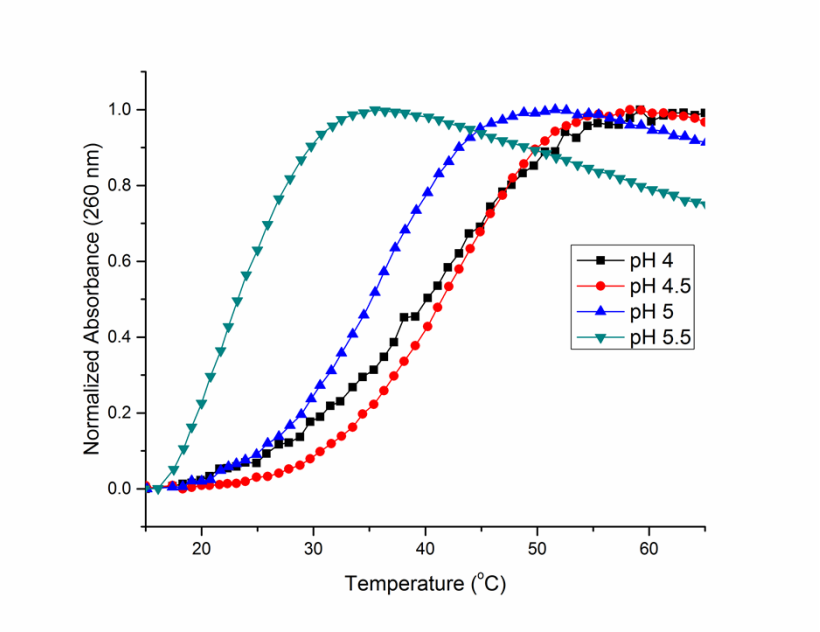

Figure S5: UV-melting profiles of ON-3 recorded at 260 nm at different pH values.

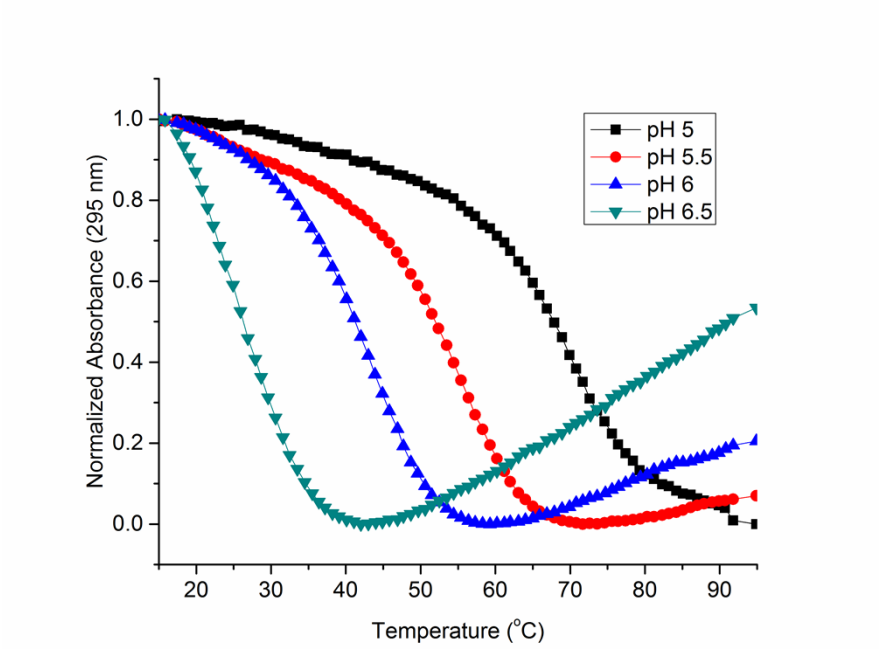

Figure S6: UV-melting profiles of ON-4 recorded at 295 nm at different pH values.

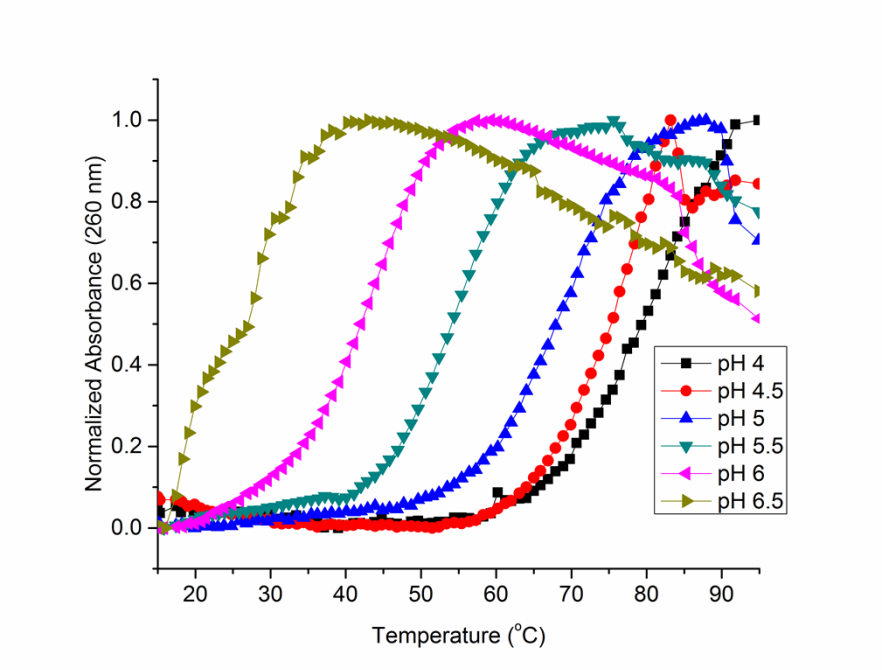

Figure S7: UV-melting profiles of ON-4 recorded at 260 nm at different pH values.

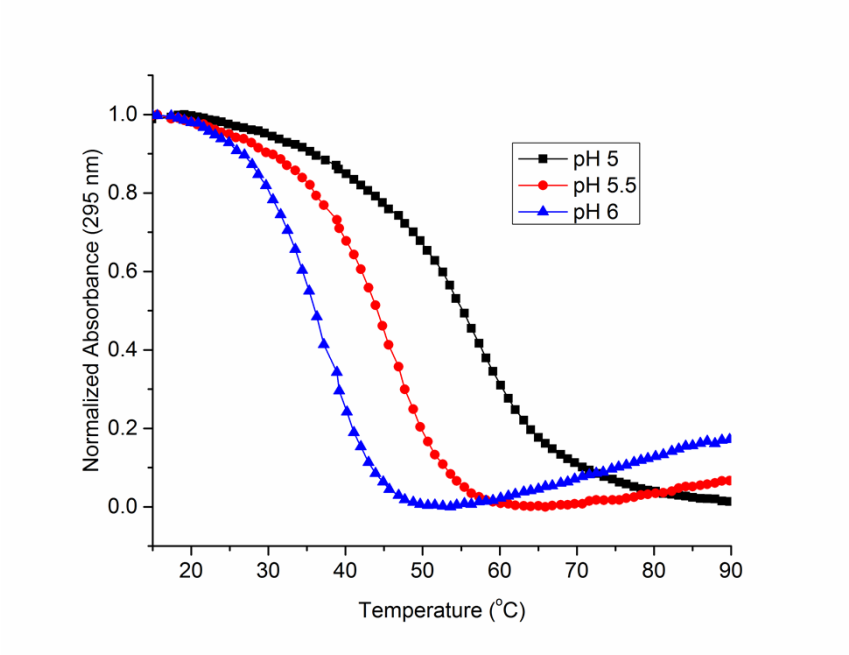

Figure S8: UV-melting profiles of ON-5 recorded at 295 nm at different pH values.

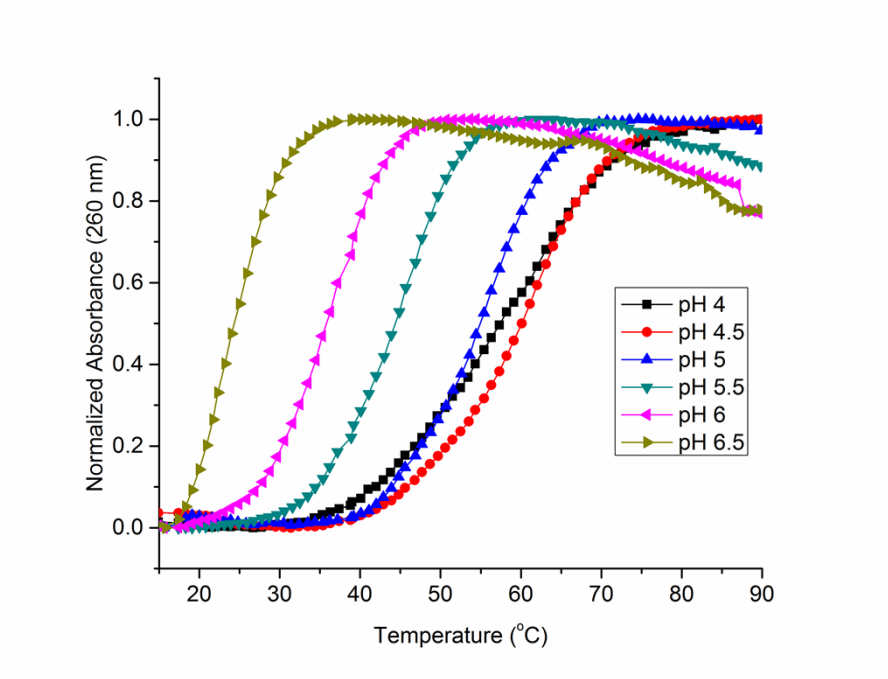

Figure S9: UV-melting profiles of ON-5 recorded at 260 nm at different pH values.

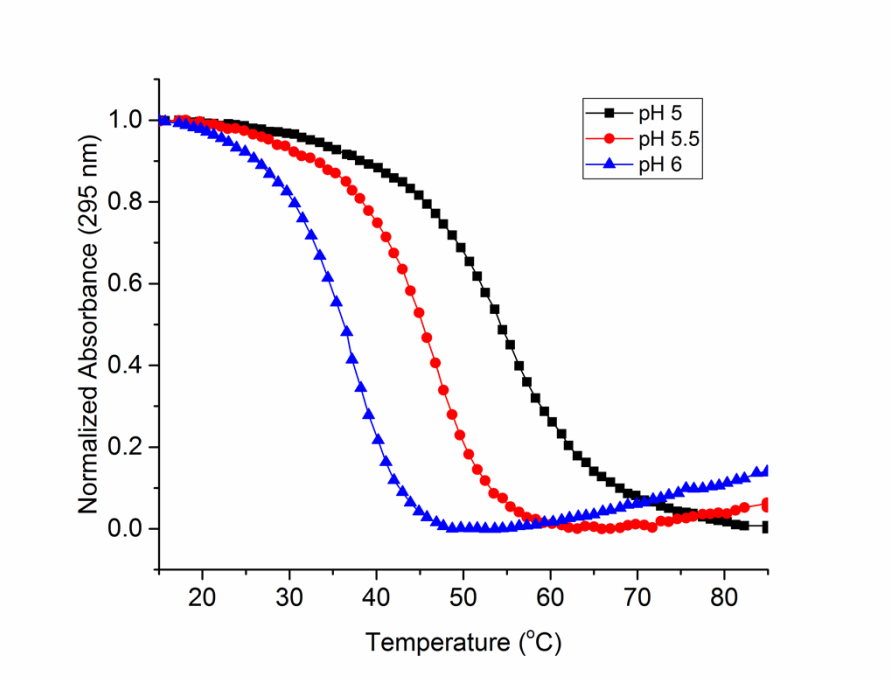

Figure S10: UV-melting profiles of ON-6 recorded at 295 nm at different pH values.

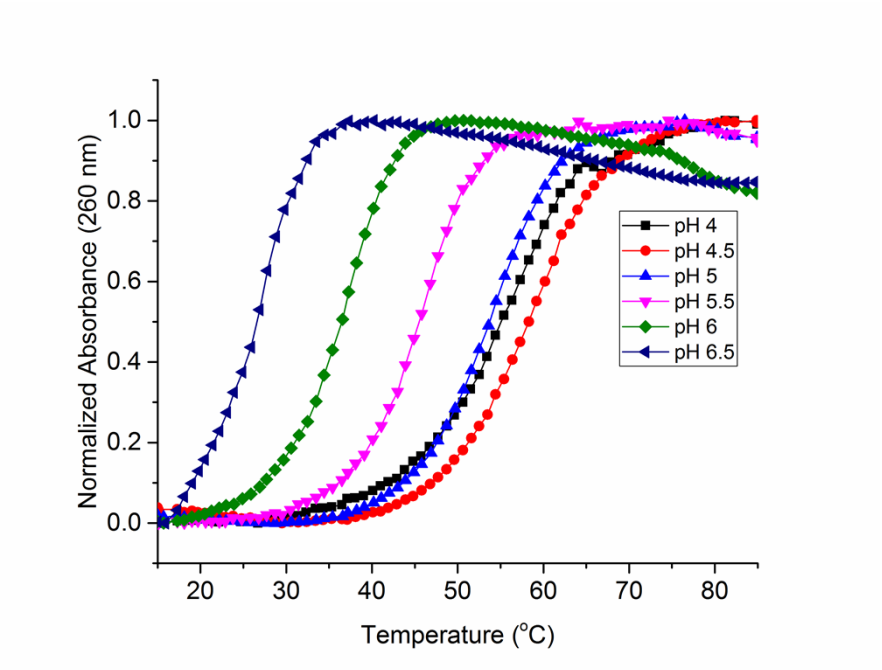

Figure S11: UV-melting profiles of ON-6 recorded at 260 nm at different pH values.

Circular dichroism (CD): CD experiments were carried out on a Jasco model J-810 instrument. Quartz cuvette (Hellma) was used for scanning the samples with path length of 1 mm at 22 ºC. The concentration of oligonucleotides was 20 µM in 10 mM sodium acetate buffer containing 100 mM sodium chloride at pH 4.5 or 10 mM sodium phosphate buffer containing 100 mM sodium chloride at pH 7.4. Samples were heated at 90 °C, cooled at room temperature, and scanned at 100 nm min^{-1} with a data pitch of 0.2 nm. Each CD spectra obtained by average is three scans.

Acid-base titration by UV: Phosphoric acid was added to obtain the pH 3.5 of Milli-Q water containing 50 mM sodium chloride. Oligonucleotides were added and heated to 90 °C for 2 minutes and then slowly cooled at room temperature followed by the overnight incubation at room temperature. 50 mM sodium hydroxide was added in aliquots to increase the pH of the solution. The absorbance was recorded after each addition and the pH of the solution was monitored by a pH meter. The pH meter was calibrated before each experiment. Titration curves were applied for sigmoidal fit by Origin 2015 and the apparent pKa values were determined by first derivative.

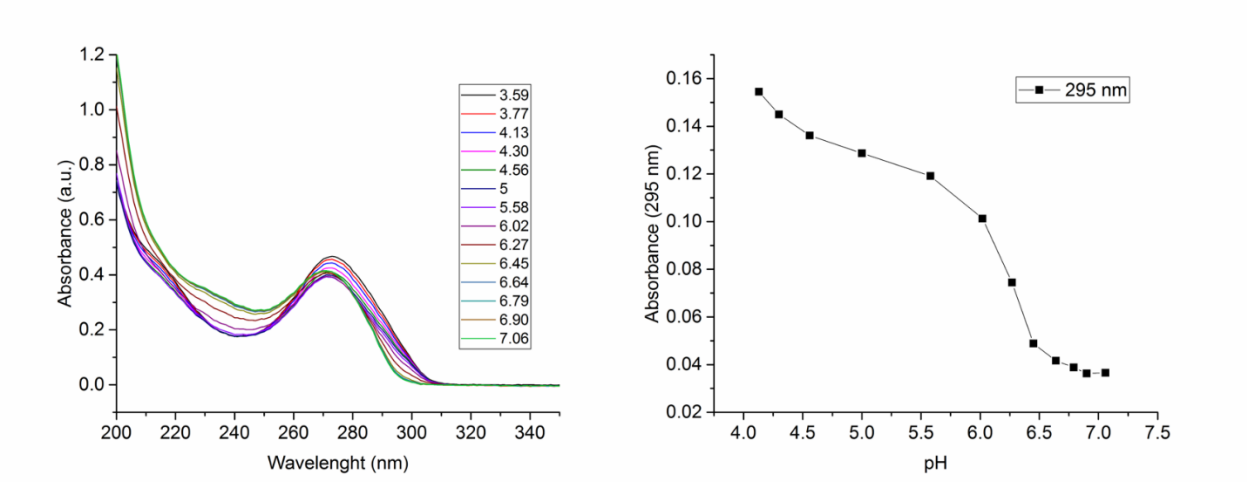

Figure S12: Acid-base titration of ON-1 at room temperature.

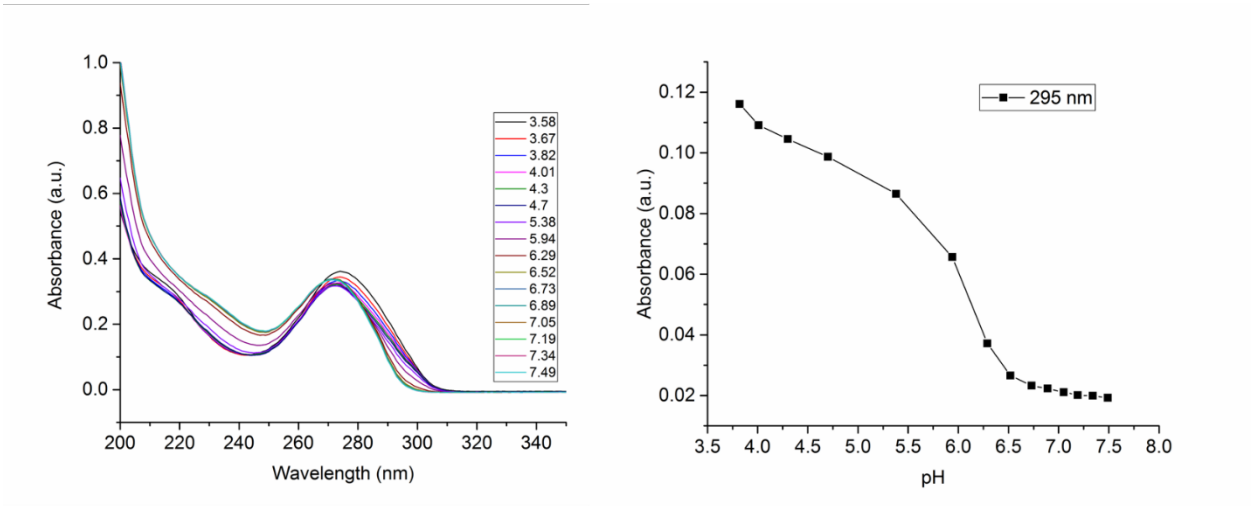

Figure S13: Acid-base titration of ON-2 at room temperature.

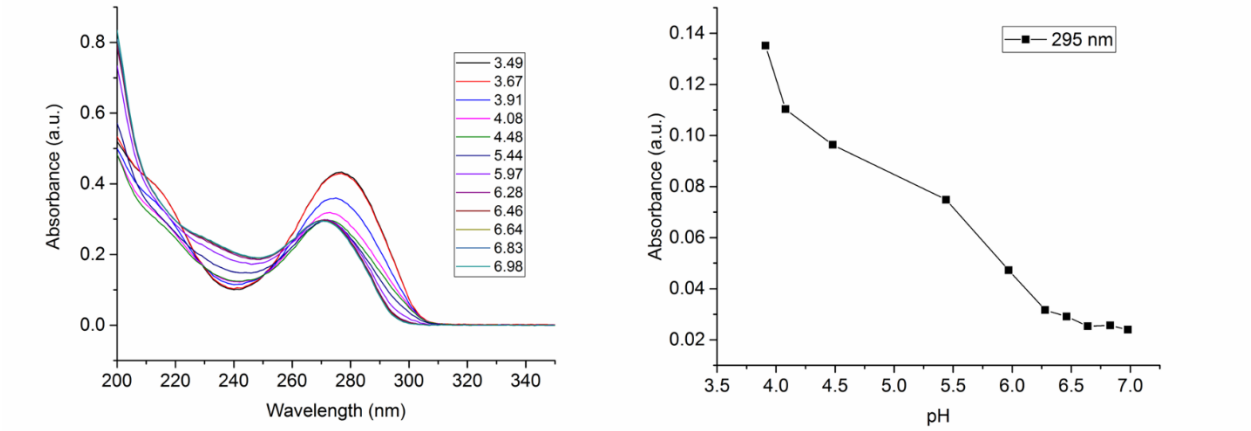

Figure S14: Acid-base titration of ON-3 at room temperature.

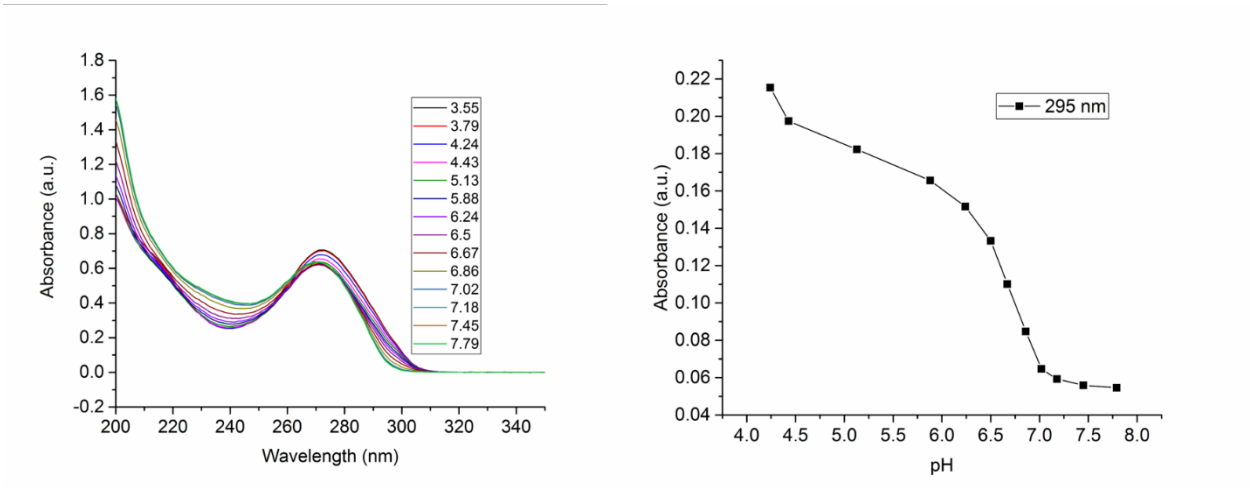

Figure S15: Acid-base titration of ON-4 at room temperature.

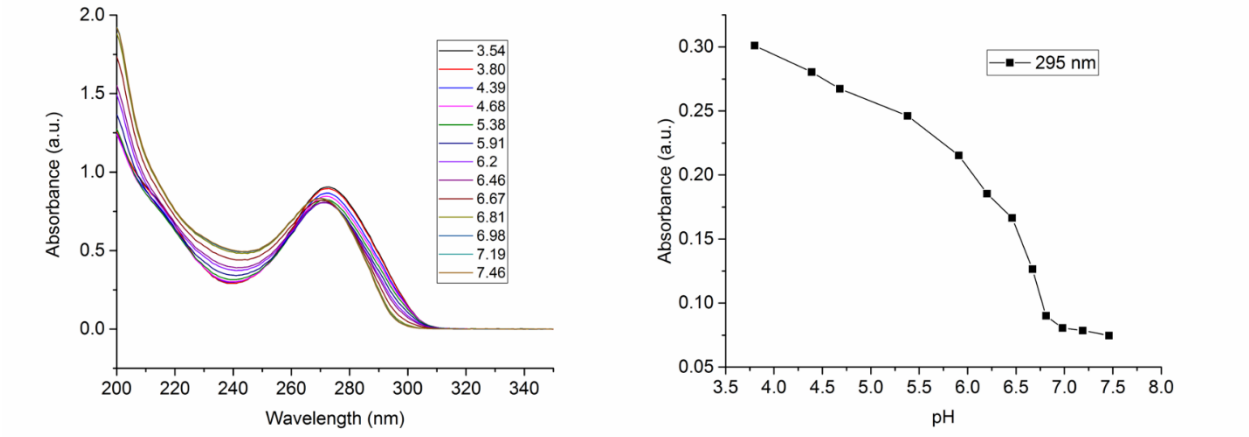

Figure S16: Acid-base titration of ON-5 at room temperature.

ESI-MS: To determine the molecularity of structures, samples were characterized by mass spectrometry (MS) analysis on a Q Exactive HF-X instrument (Thermo Scientific, Bremen, Germany) operated in negative mode. aTNA strands were obtained in LC-MS grade water. Before

injection, samples were diluted in a 1:1 water acetonitrile mixture (20 µM final concentration) and analyzed by direct infusion at 5 µL/min. The electrospray voltage was 3000 V, the transfer tubing had 200 °C, and the gas flow rate settings of the ion source were 8, 2 and 1 L/min for sheath, auxiliary and sweep gas, respectively. Funnel radiofrequency (RF) level was 70, automatic gain control (AGC) target 1×106, trapping gas pressure was 1.0, mass resolution was 30,000 (at 200 m/z) and scan range 700-3000 m/z. Data were acquired for at least ten seconds, and subsequent data treatment was performed in Xcalibur Qual Browser 4.2.28 (Thermo Scientific).

LC-MS conditions: To determine the molecularity of ON-3 i-motif structure, sample was analyzed by LC-MS (Shimadzu). Interface temp 300 °C, DL-temp 300 ºC, Nebulizing gas flow 1.5 ml per min, Heat Block 300 °C, Drying gas flow 15 l per min, Column temperature 40 °C, LC flow 0.25 ml per min, 20 min (0 to 50 ml methanol). Buffer: TEA (Triethylamine)–HFIP (Hexafluoroisopropanol).

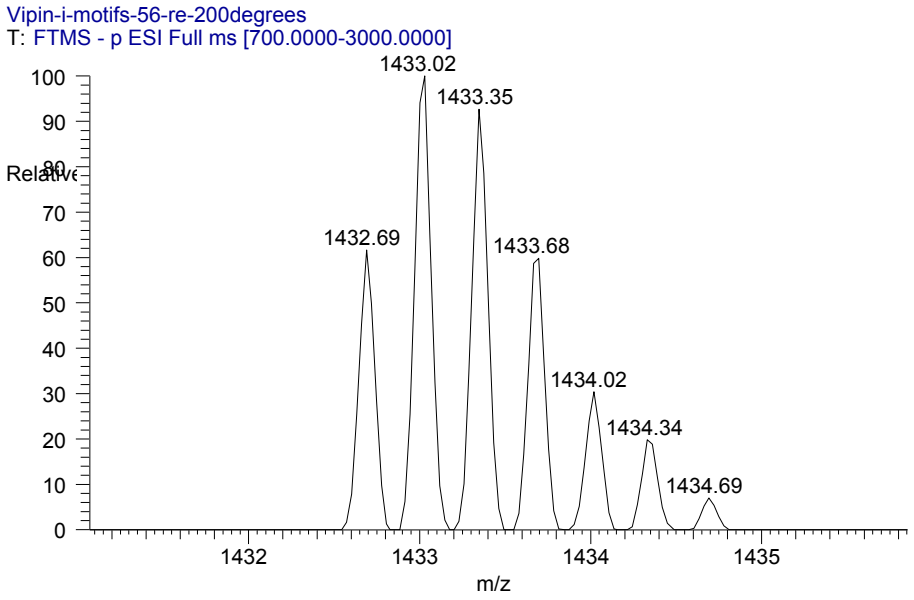

Figure S17: ESI-MS spectrum of ON-7 $[2M - 3H]^{3-}$

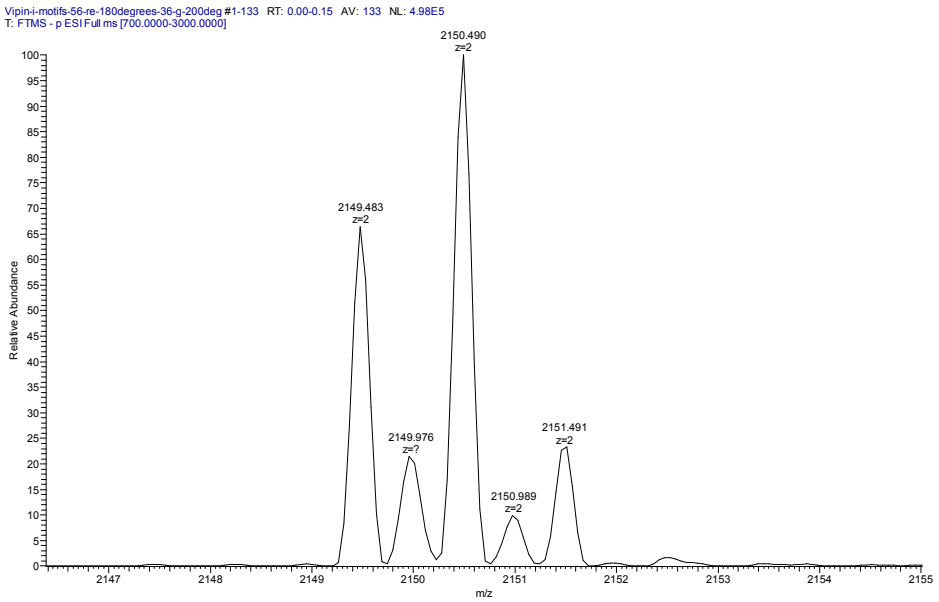

Figure S18: ESI-MS spectrum of ON-7 $[2M - 2H]^{2-}$. Fig S5.1 above shows that the dimer, 2M, is present and this spectrum confirms it through the correct monoisotopic mass and with charge = 2 assignment. This spectrum also has contribution from $[1M-H]^{-1}$ which has two peaks with same m/z value and thus elevates the, from the left, first (monoisotopic) peak and the third peak.

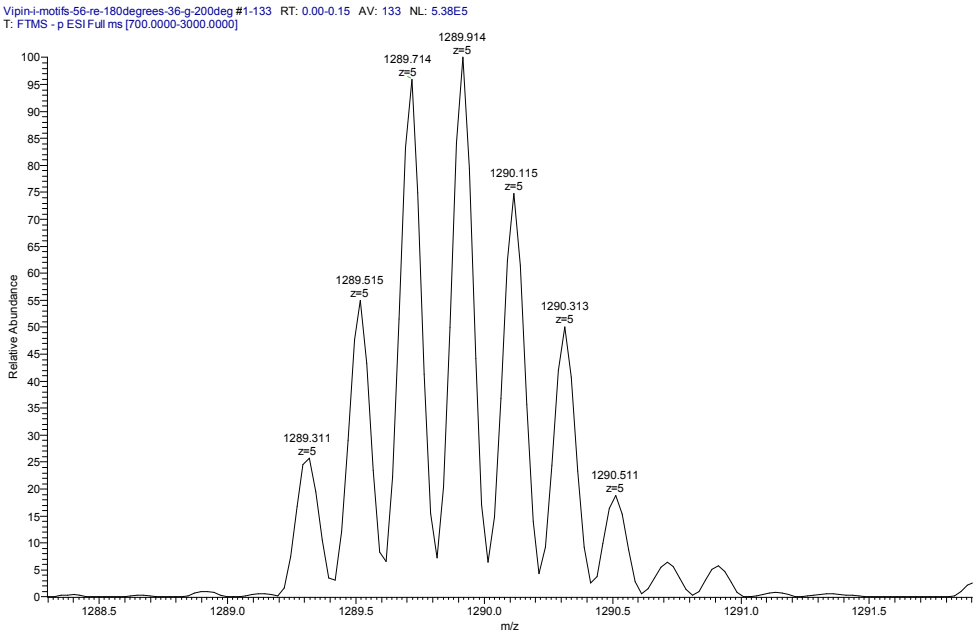

Figure S19: ESI-MS spectrum of ON-7 $[3M - 5H]^{5-}$

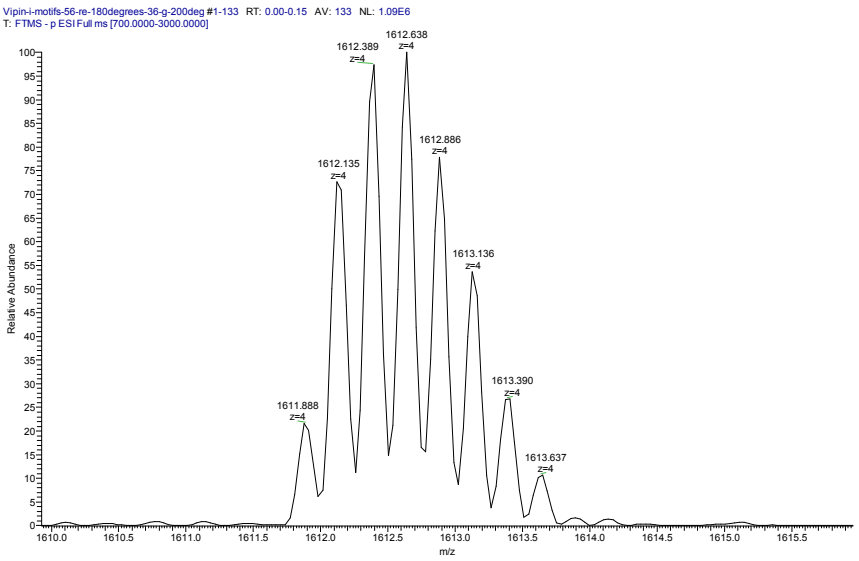

Figure S20: ESI-MS spectrum of ON-7 $[3M - 4H]^{4-}$

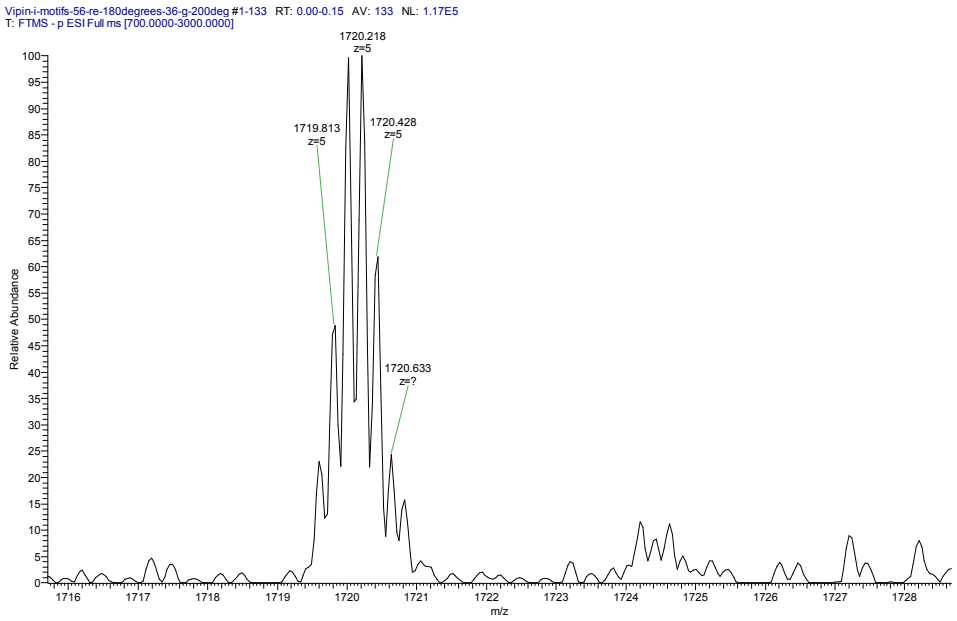

Figure S21: ESI-MS spectrum of ON-7 $[4M - 5H]^{5-}$

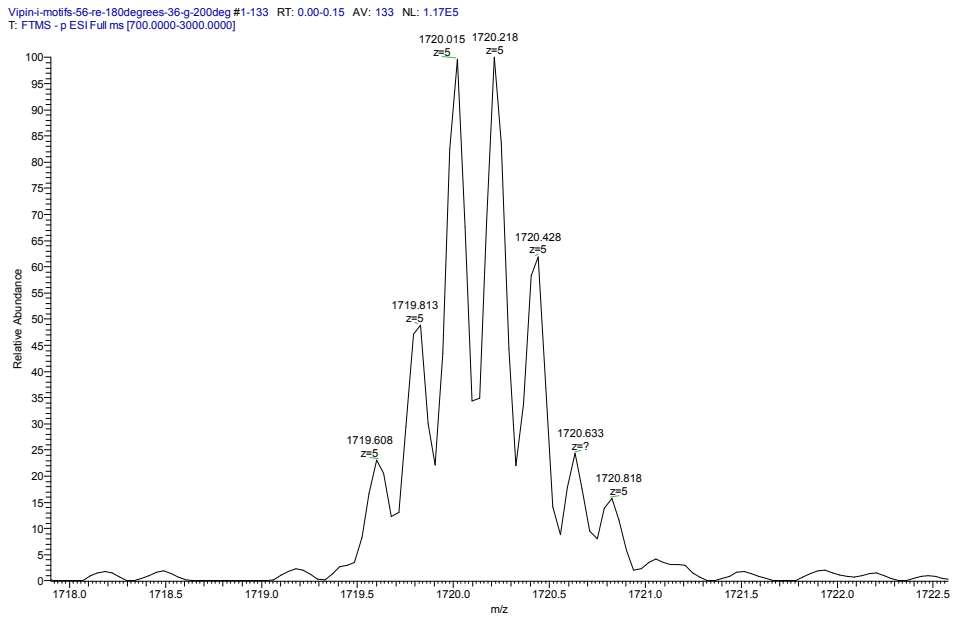

Figure S22: ESI-MS spectrum of ON-7 $[4M - 5H]^{5-}$

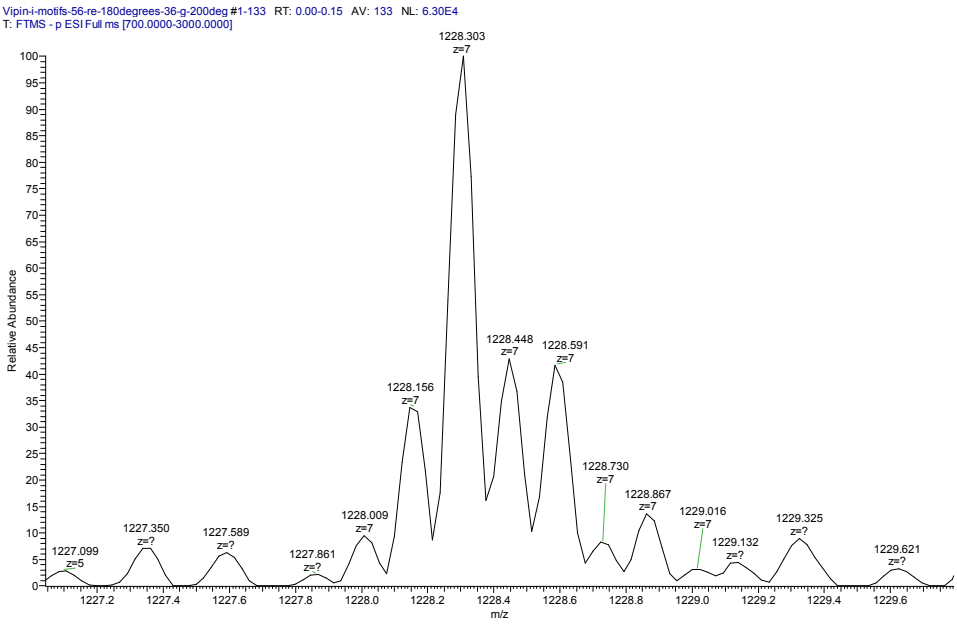

Figure S23: ESI-MS spectrum of ON-7 $[4M - 7H]^{7-}$

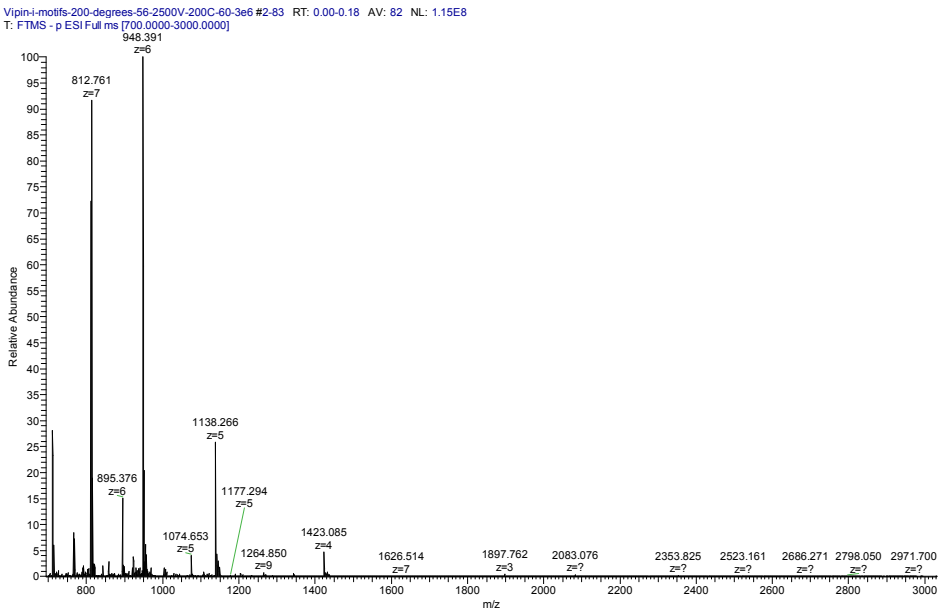

Figure S24: ESI-MS spectrum of ON-4.

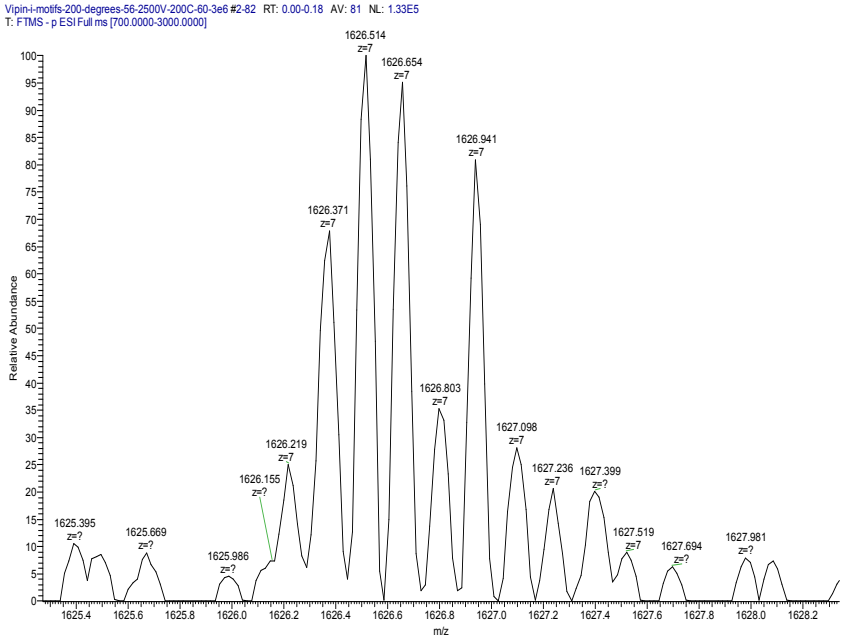

Figure S25: ESI-MS spectrum of ON-4 $[2M - 7H]^{7-}$

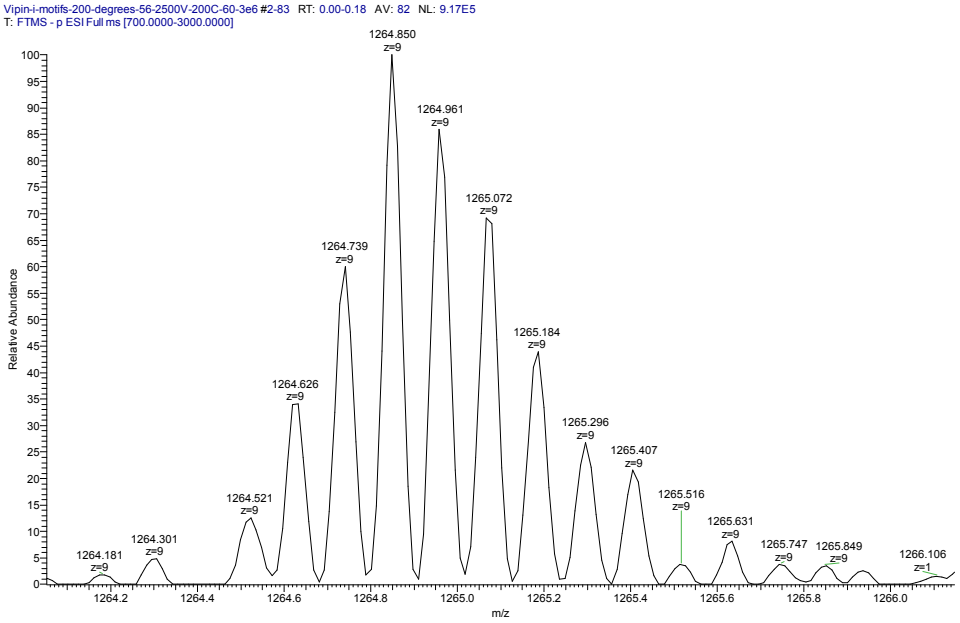

Figure S26: ESI-MS spectrum of ON-4 $[2M - 9H]^{9-}$

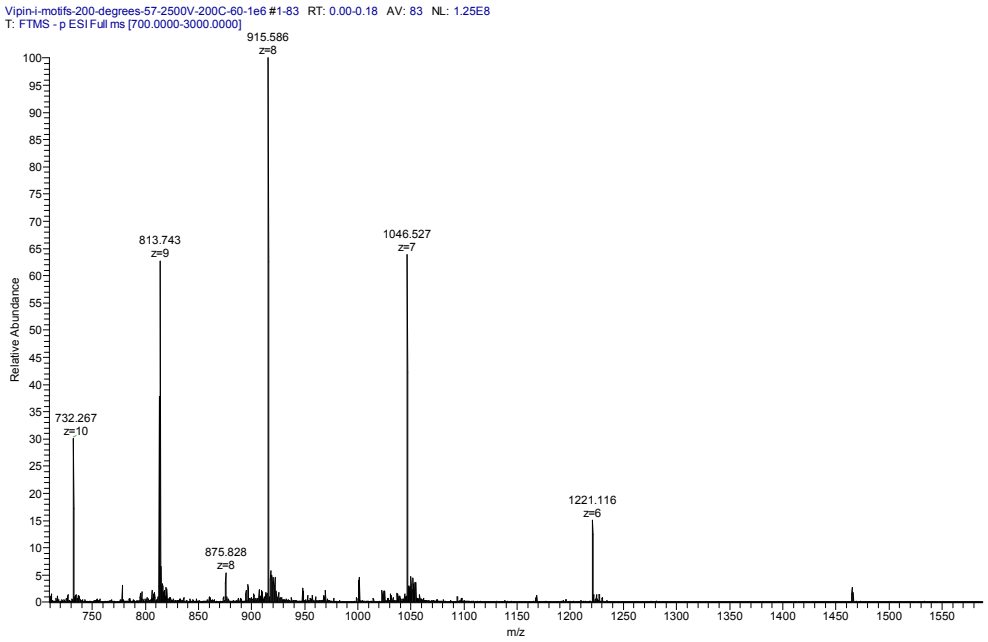

Figure S27: ESI-MS spectrum of ON-5.

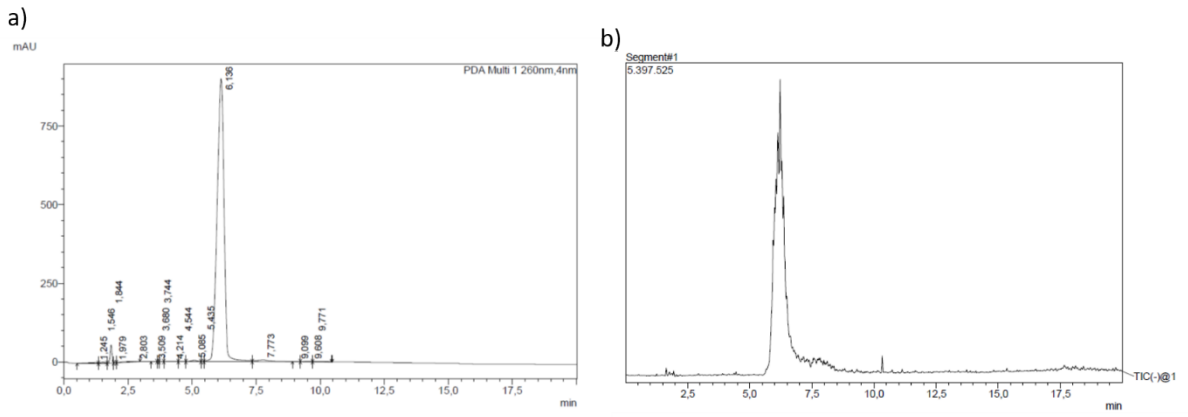

Figure S28: a) HPLC chromatogram of ON-3; b) MS chromatogram of ON-3.

Fluorescence studies: The studies were performed by using Horiba Scientific instrument. High precision cell (Quartz cuvette, Hellma) was used at light path 3×3 mm. Samples were excited at 340 nm and the emission recorded from 350 nm to 670 nm at 22 °C. Slits for excitation and emission were 5 nm. A concentration of 0.5 µM of each oligo was used for all the experiments. Ortho phosphoric acid was added to obtain the pH 3.5 of Milli-Q water containing 50 mM sodium chloride. Oligonucleotides were heated at 90 °C for 2 minutes and then slowly allowed to cool at room temperature followed by incubation at room temperature. The pH of the solution was increased using 50 mM sodium hydroxide aliquots and after each addition fluorescence was recorded. The pH of the solution was monitored by pH meter and pH meter was calibrated before each experiment. Titration curves were applied for sigmoidal fit by Origin 2015 and then apparent pKa were determined by first derivative.

Switching experiment: First, the fluorescence of a 0.5 µM solution of oligo in Milli-Q water was measured. The pH of the solution was increased (≈ 8.8) and decreased (≈ 4.1) by sodium hydroxide and hydrochloric acid solutions respectively in alternate fashion. The pH was monitored by a pH meter with a microelectrode.

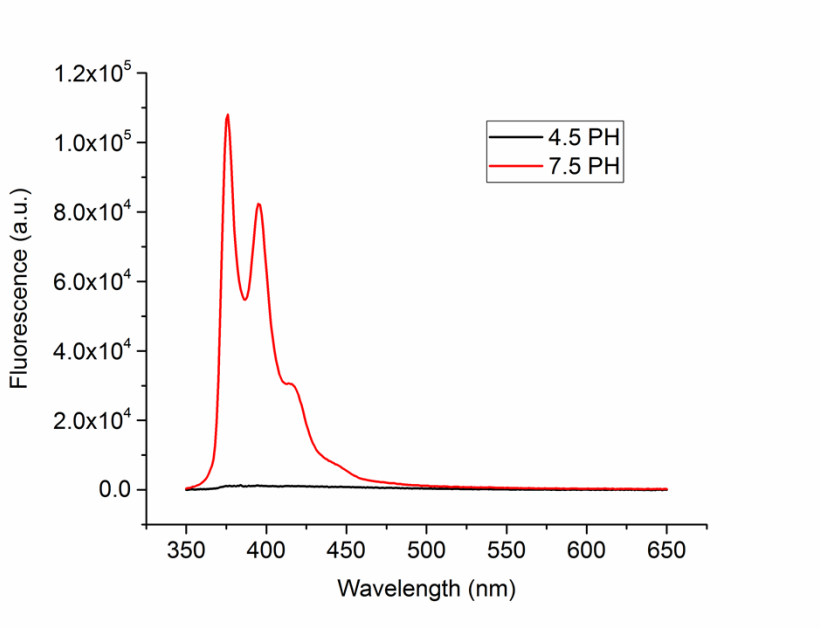

Figure S29: Fluorescence spectra of ON-8 in 10 mM sodium phosphate at pH 7.5 or sodium acetate buffer at pH 4.5 with 100 mM sodium chloride.

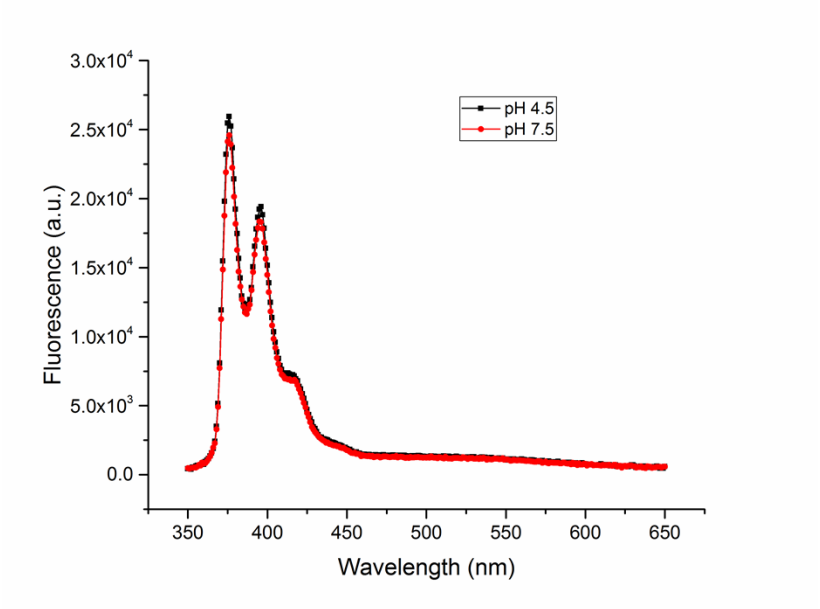

Figure S30: Fluorescence spectra of ON-9 in 10 mM sodium phosphate at pH 7.5 or sodium acetate buffer at pH 4.5 with 100 mM sodium chloride.

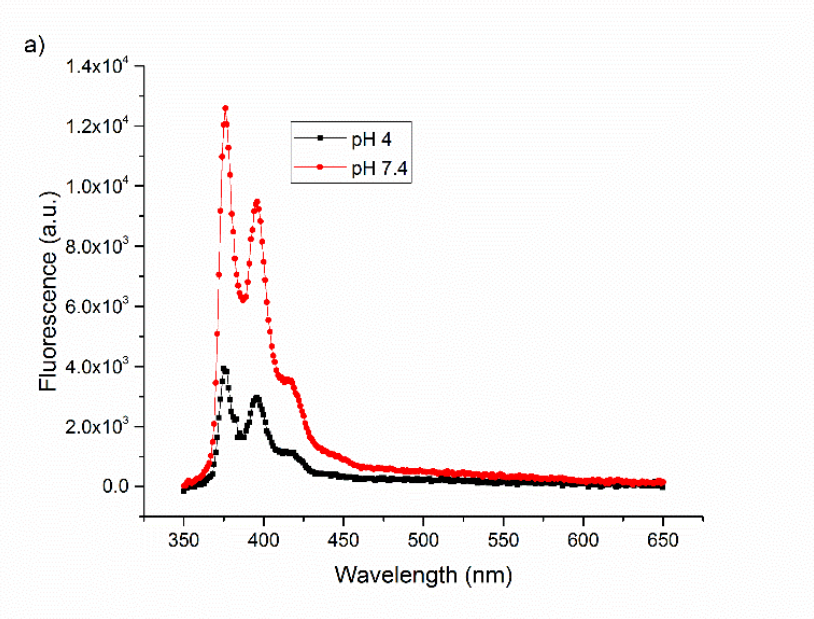

Figure S31 Fluorescence spectra of ON-10 (5'-PyctttttttT-3') under acid and basic conditions.10 mM sodium phosphate at pH 7.5 or sodium acetate buffer at pH 4.5 with 100 mM sodium chloride.

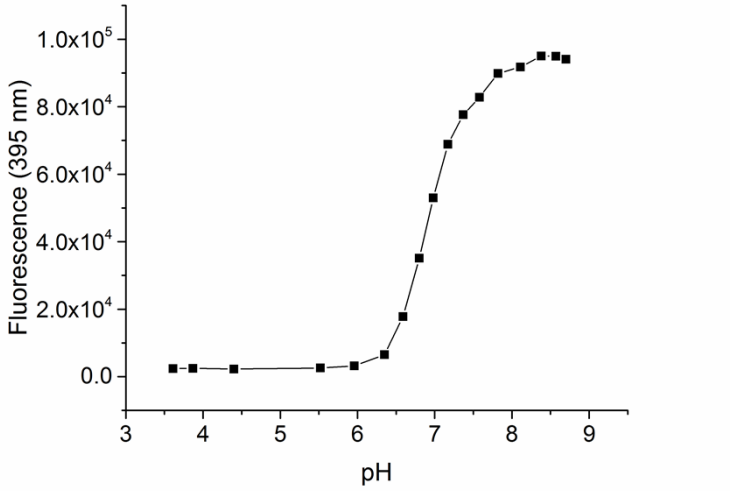

Figure S32: Fluorescence at wavelength 395 nm of ON-8 under acid-base titration.

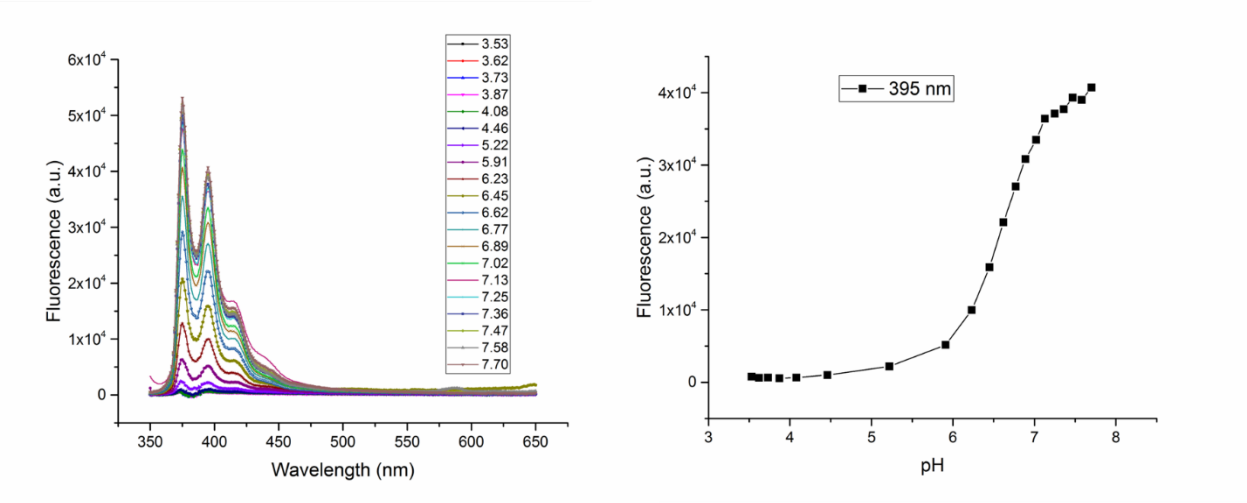

Figure S33: Fluorescence spectra of acid-base titration of ON-11.

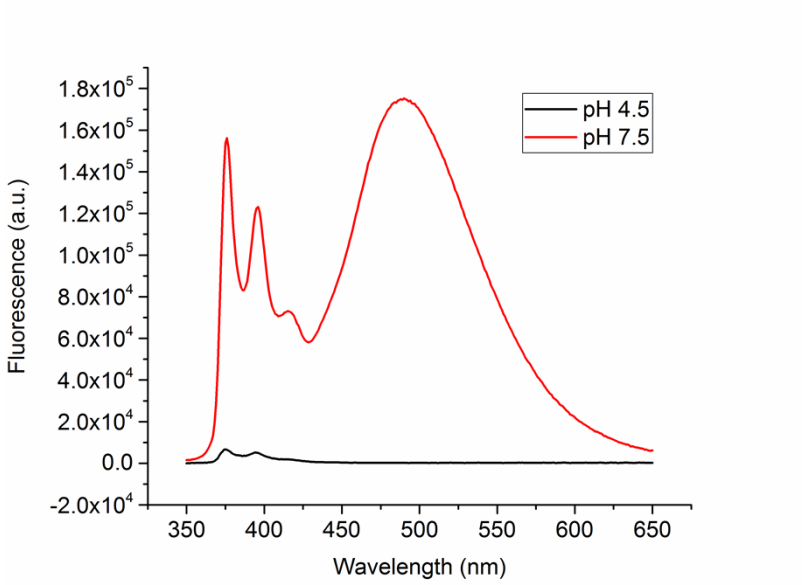

Figure S34: Fluorescence spectra of ON-12 in 10 mM sodium phosphate at pH 7.5 or sodium acetate buffer at pH 4.5 with 100 mM sodium chloride.

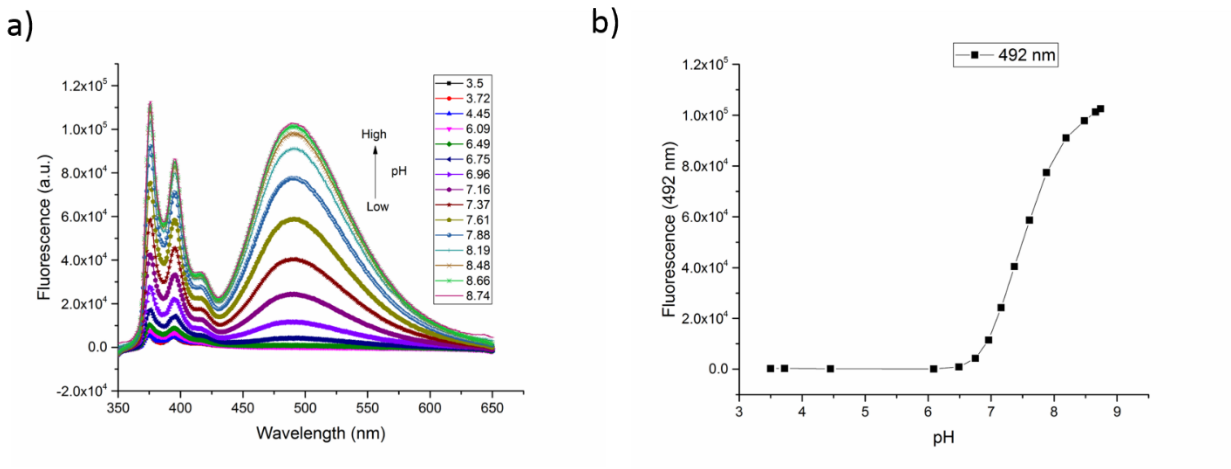

Figure S35: Fluorescence spectra of acid-base titration of ON-12.

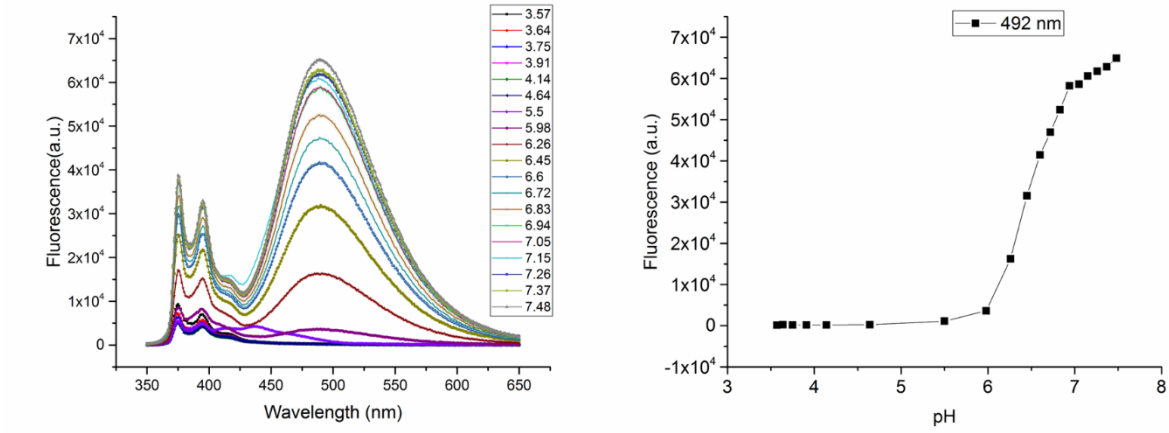

Figure S36: Fluorescence spectra of acid-base titration of ON-13.

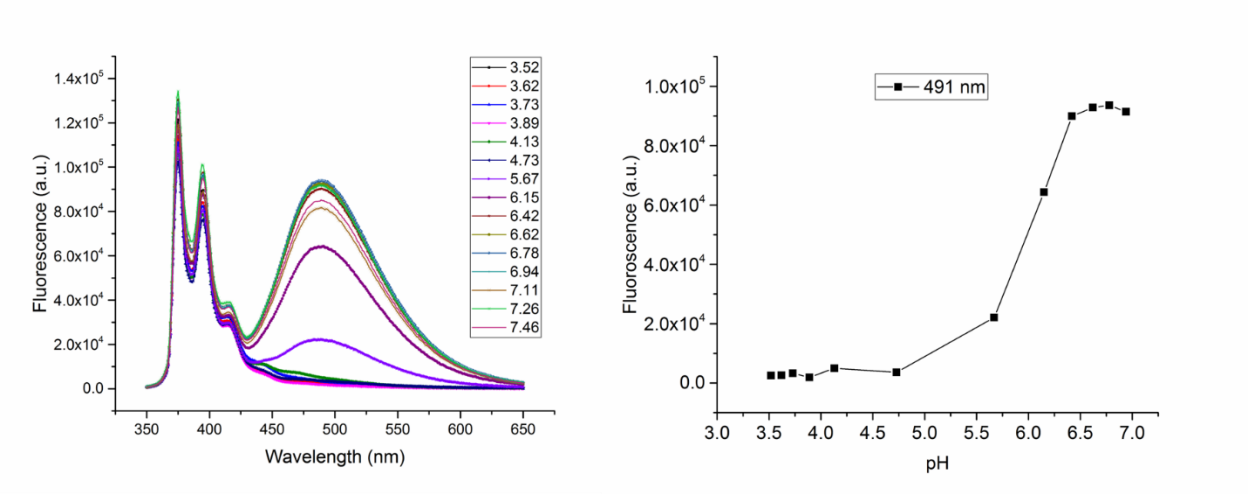

Figure S37: Fluorescence spectra of acid-base titration of ON-14.

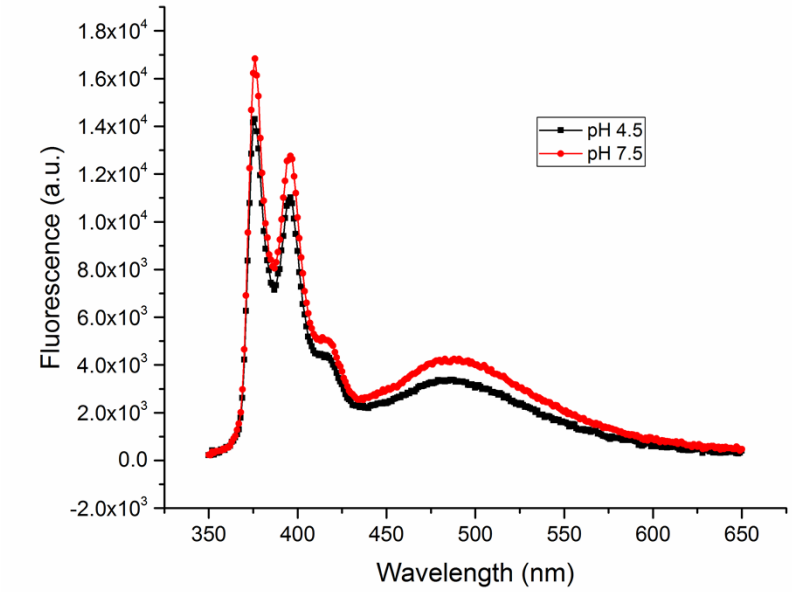

Figure S38: Fluorescence spectra of ON-15 in 10 mM sodium phosphate at pH 7.5 or sodium acetate buffer at pH 4.5 with 100 mM sodium chloride.

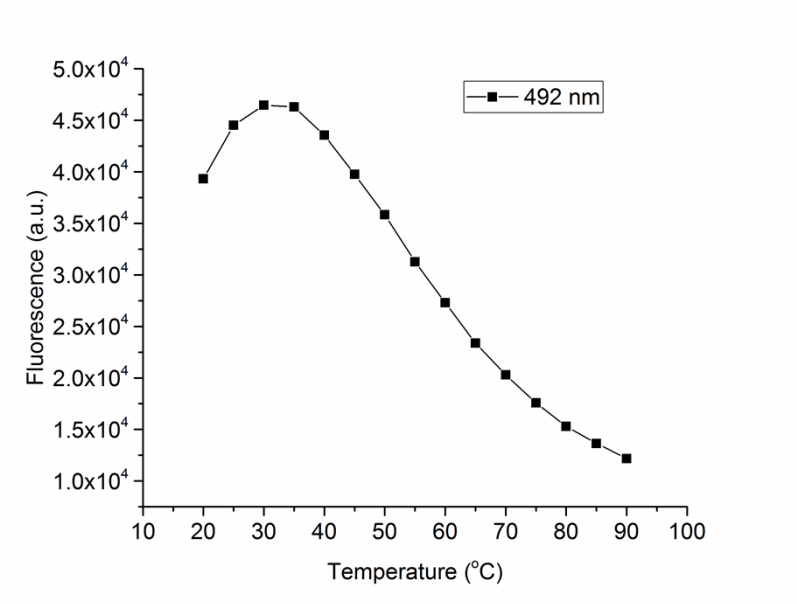

Figure S39: Temperature dependent fluorescence spectra of ON-12 in 10 mM phosphate buffer containing 100 mM sodium chloride at pH 7.5.

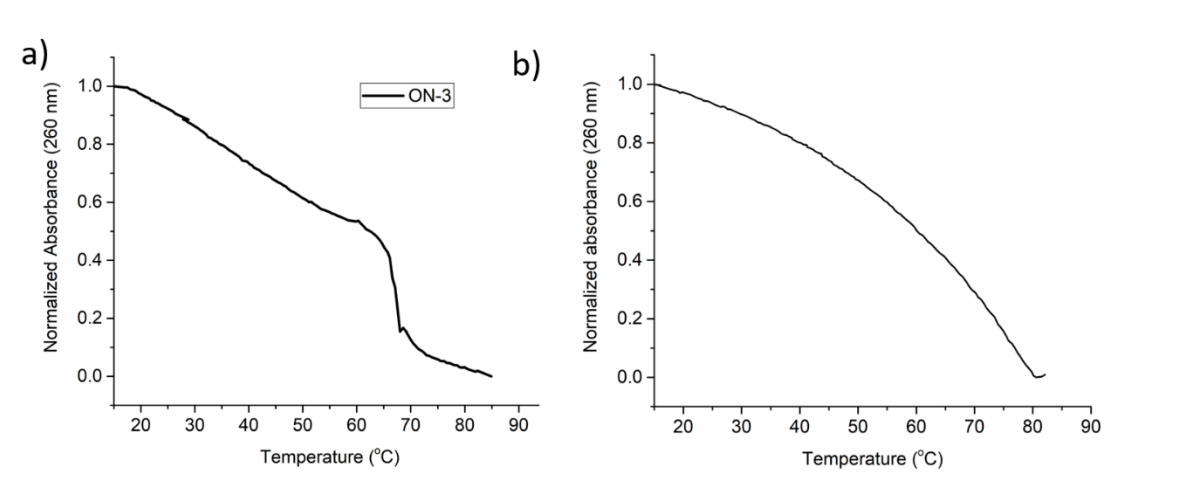

Figure S40 Second melting a) and First annealing b) curves recorded at 260 nm of ON-3 in 10 mM sodium phosphate buffer with 100 mM sodium chloride at pH 7.4.