Supplementary Information of "Charge Evolution during the Unfolding of a Single i-motif DNA"

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1. Materials

Two types of fluorescent molecule for DNA labelling were from a commercial source (Invitrogen, USA), including the pH-responsive fluorescent molecule, Oregon Green 514 carboxylic acid, succinimidyl ester (OG514), and fluorescent molecule without pH response, Alexa Fluor 488 carboxylic Acid, succinimidyl ester, mixed isomers (Alexa488). Single-stranded DNAs (ssDNA, Sangon Biotechnique, China) was modified with -$(CH_2)_6NH_2$ at their 5'-end, through which fluorescent molecules are attached. Three DNA samples were used: 1) i-motif, 5'-NH_2-$(CH_2)_6$-CCC-TAACCC-TAACCC-TAACCC-3'); 2) T21, 5'-OG514-$(CH_2)_6$-$(T)_{21}$-3'; 3) C12, 5'-OG514-$(CH_2)_6$-CCCTCTCTCTCTCTCT-CTCTC-3'. Polyacrylamide gel (Bio-Gel P-6 media; Bio-Rad, USA) and centrifugal ultrafiltration (MWCO with the cutoff molecular weight of 2,000 $g \cdot mol^{-1}$, Sartorius, Germany) were utilized to purify the labeled DNA. Rhodamine 6G (R6G; standard purity, Fluka) was used to calibrate the excitation-detection volume of fluorescence correlation spectroscopy (FCS). De-ionized water was purified with a Millipore system (18.2 MΩ·cm). Other reagents were from commercial sources and used as received without further purification.

2. Preparation of fluorescent labeled DNA

Taking the OG514-i-motif as an example, the process of DNA labelling is as the following. 215.3 nmol of DNA (40 OD) was dissolved in 350 μL buffer solution of 0.1 M Na_2CO_3-$NaHCO_3$ (pH=8.3) and 820.56 nmol of OG514 was dissolved in 50 μL of DMSO. After mixing these two solutions, the mixture was stirred and incubated under the lucifugal condition at ambient temperature overnight. The solution was then purified by passing through polyacrylamide gel column using 0.1 M Na_2CO_3-$NaHCO_3$ buffer (pH=8.3) as eluent media. Afterwards, it was further purified by the centrifugal ultrafiltration for more than 50 times until no free fluorescent dye in the ultra-filtrate was detected by single molecule fluorescence detection. At the final step, the samples were further centrifuged with ultrafiltration for more than 10 times with de-ionized water so that buffer salts were removed.

The labeling efficiency was determined using the concentration of stock DNA solution measured by UV-Visible spectroscopy at 260 nm and the concentration of the labeled DNA solution measured by FCS. The molar extinction coefficient of i-motif DNA sequence is taken as 185,900 $L \cdot mole^{-1} \cdot cm^{-1}$ at 260 nm, according to a well-known data source, http://www.idtdna.com/calc/analyzer. The labeling efficient is determined as 75.3%.

3. Conformational transitions characterized by CD spectroscopy

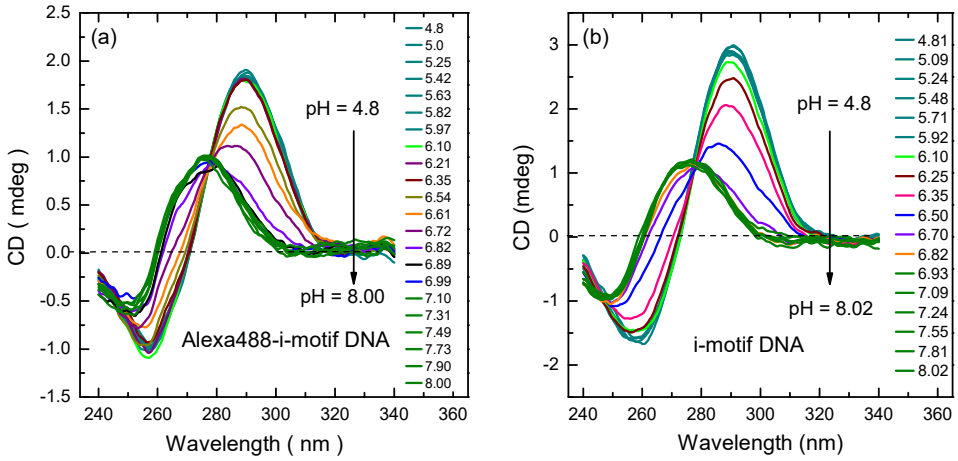

Figure S1 CD spectra of Alexa 488-labeled i-motif DNA (a) and i-motif DNA (b) under different pH in 50 mM NaCl aqueous solution. The concentration of un-labelled DNA and that labelled with Alexa 488 is ~300 nM. All measurements were conducted at room temperature.

Conformation transition of DNA strands driven by pH variation with and without fluorescence labelling was compared by CD spectroscopy (data provided in Figure S1). For both un-labelled DNA and that labelled with Alexa 488 (a bright and stable fluorophore without pH-response), characteristic peaks (positive peak at ~290 nm, negative peak at ~260 nm and crossover at ~270 nm) were observed under slightly acidic conditions (pH<6), indicating the formation of ordered i-motif structure. At alkali pH value (pH>7), characteristic peaks of random coil conformation (positive peak at ~280 nm, negative peak at ~250 nm and crossover at ~260 nm) were observed. The above data are similar to the DNA strand labelled with OG514, showing that the fluorescence labelling does not prevent the formation of the ordered i-motif. However, slight difference in pH values still exists, showing the possible end effect from the fluorescence labelling.

4. Typical data of fluorescence correlation spectroscopy and the numerical fitting

Typical FCS curves of free OG514, OG514-labeled DNA in salt-free aqueous solution and solution with salt (50 mM and 100 mM NaCl) are shown in Figure S2. The fittings (denoted as the solid lines in the figures) are by the three-dimension Gaussian model. Satisfactory fittings with the experimental data are found, demonstrated by the minor values of the residuals.

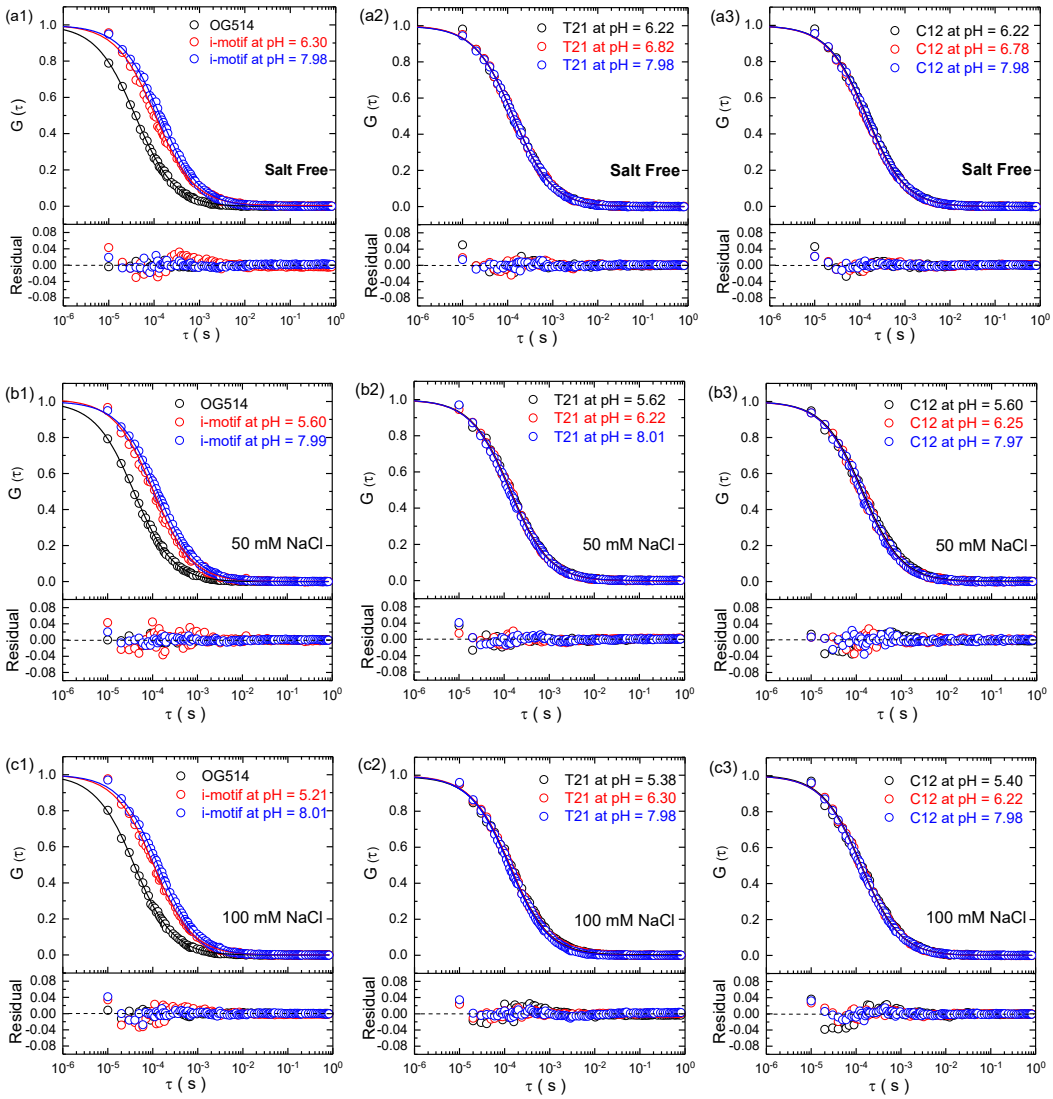

Figure S2 Typical FCS data of DNA samples under investigation in salt free solution (a1-a3), in 50 mM NaCl solution (b1-b3) and in 100 mM NaCl solution (c1-c3). The solid lines denote the numerical fitting using 3D Gaussian model. The concentration of DNA samples is ~5.0 nM. All measurements were performed at 25 °C.

5. *Typical data of photon counting histogram (PCH) and the numerical fitting*

Typical data of fluorescence photon counts histogram of OG514-i-motif under different pH values in salt-free, 50 mM and 100 mM NaCl aqueous solution are provided in Figure S3. The minor value of the residual after fitting indicates the satisfactory fitting using PCH one-photon correlation model.[1-4]

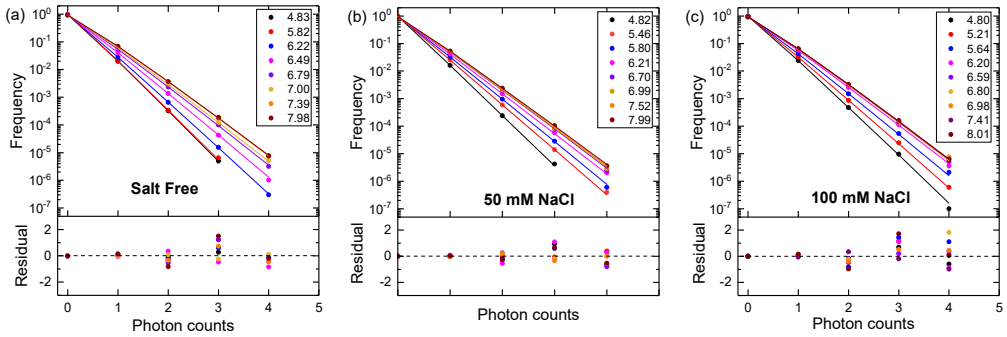

Figure S3 Typical data of photon counts histogram of OG514-i-motif under different pH values in salt-free aqueous solution (a), 50 mM NaCl aqueous solution (b) and 100 mM NaCl aqueous solution (c). The solid lines denote the fittings using one photon PCH model. The concentration of DNA samples is ~5 nM. All measurements were performed at 25 °C.

Molecular brightness of OG514-i-motif DNA, OG514-T21 and OG514-C12 in salt-free aqueous solution and in 50 mM and 100 mM NaCl solution as a function of pH value were presented in Figure S4. Using the data of free OG514 as the master curve, the local pH value at the vicinity of DNA strand is determined.

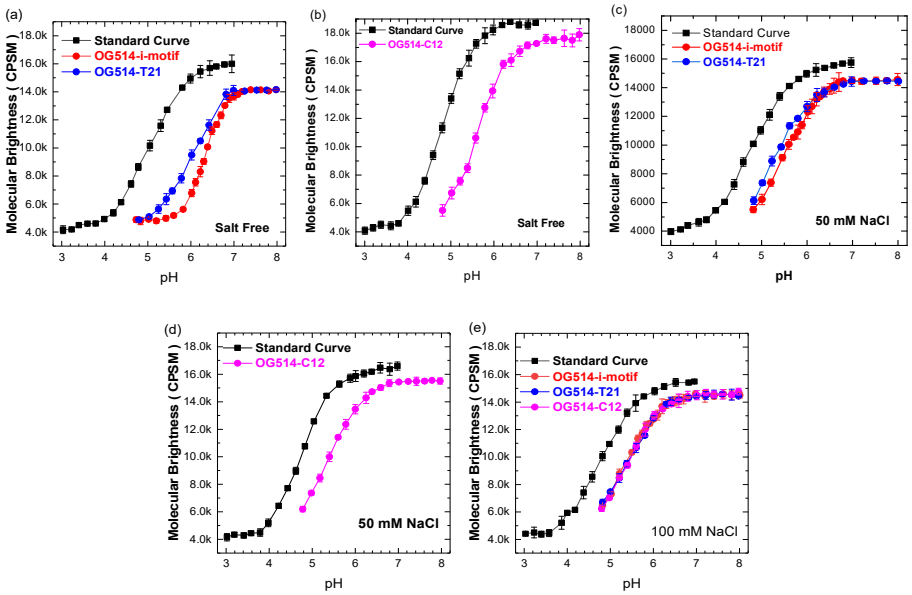

Figure S4 Molecular brightness as a function of pH OG514-i-motif DNA, OG514-T21 and OG514-C12 in salt-free aqueous solution (a and b), in 50 mM NaCl aqueous solution (c and d) and 100 mM NaCl solution. The concentration of DNA samples is ~5 nM. All measurements were performed at 25 °C.

6. *Single molecule fluorescence emission spectroscopy*

Single molecule fluorescence emission spectra of the OG514-i-motif were measured

using a separate home-built setup. The setup is based on an inverted optical microscope (IX71, Olympus) equipped with a spectrometer (SR-303i-A, Andor) and an EMCCD camera (DU970P-BVF, Andor). The excitation source is the 473 nm output of a solid laser, which is beam-expanded, collimated and introduced into the microscope. After reflected by a dichroic mirror, the excitation beam is focused into the sample by an objective lens (UPlanSApo, 60×, NA=1.2), through which the excited fluorescence was also collected. After passing the dichroic mirror, the fluorescence is guided into the spectrometer through its entrance slit of 50 μm in width.

The typical spectra of OG514-i-motif and free OG514 in salt-free and 50 mM NaCl aqueous solution are shown in Figure S5. The similarity of the emission spectra of the free OG514 and that attached to DNA strand but under different pH value demonstrates the validity of determination of local pH value using PCH method. For instance, in salt-free solution, the spectrum of OG514-i-motif at pH 4.85 is identical to that of free OG514 at pH =~3.41, while the spectrum of OG514-i-motif DNA at pH 4.83 corresponding to free OG514 at pH = ~3.95 in 50 mM NaCl aqueous solution.

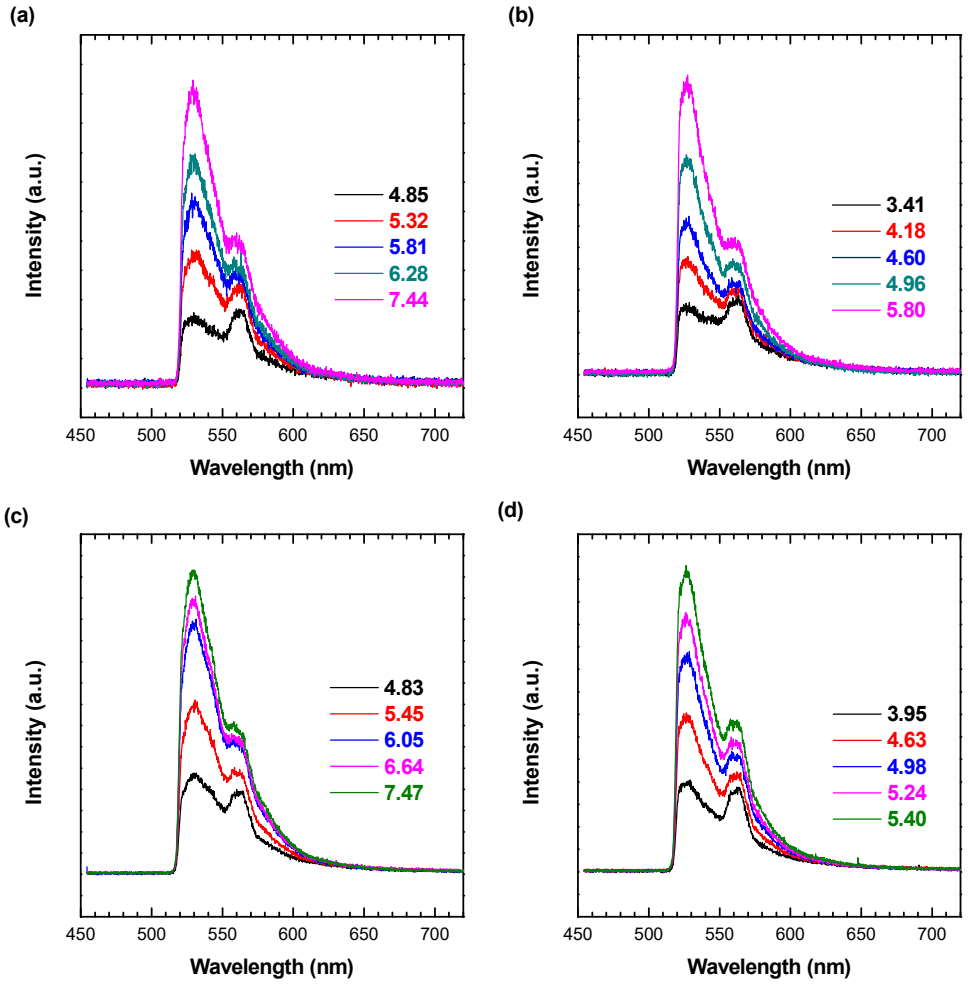

Figure S5 Fluorescence emission spectra of free OG514 and OG514-i-motif under different pH value in salt-free aqueous solution (a and b) and in 50 mM NaCl aqueous solution (c and d). The concentration of the samples are $c_{OG514\text{-}i\text{-}motif} = 2.6$ nM (a), $c_{free\ OG514} = 3.6$ nM (b), $c_{OG514\text{-}i\text{-}motif} = 3.5$ nM (c) and $c_{OG514} = 4.3$ nM (d). All measurements were performed at room temperature.

7. Comparison of electric potential of i-motif and the references

The amplitude of electric potential ($|\psi|$) of i-motif and the reference samples, T21 and C12, are compared in Figure S6. Detailed comparison of the data under salt-free condition shows that at low pH values (Figure S6a), the $|\psi|$ values of both C12 and T21 are lower than that of i-motif. C12 has an identical number of cytosine groups as i-motif but does not undergo noticeable dimension change within the pH range of the current study. Its $|\psi|$ value is lower than i-motif at low pH due to its much lower charge density as the chain keeps the random coil state. At low pH, its $|\psi|$ value is also lower than T21 because of its smaller number of net charges due to the protonation of cytosine group, which T21 does not have. When pH value is increased, the cytosine groups become deprotonated gradually, leading to an increase of the net charge and its $|\psi|$ value approaches the value of T21 and becomes identical beyond pH 7.0. Meanwhile, the unfolding of i-motif also finishes and its $|\psi|$ value becomes identical to T21 and C12.

Similar behaviors were observed under finite salt concentration (50 mM and 100 mM NaCl) and the |ψ| values were generally lower than salt-free condition (Figure S6b and c).

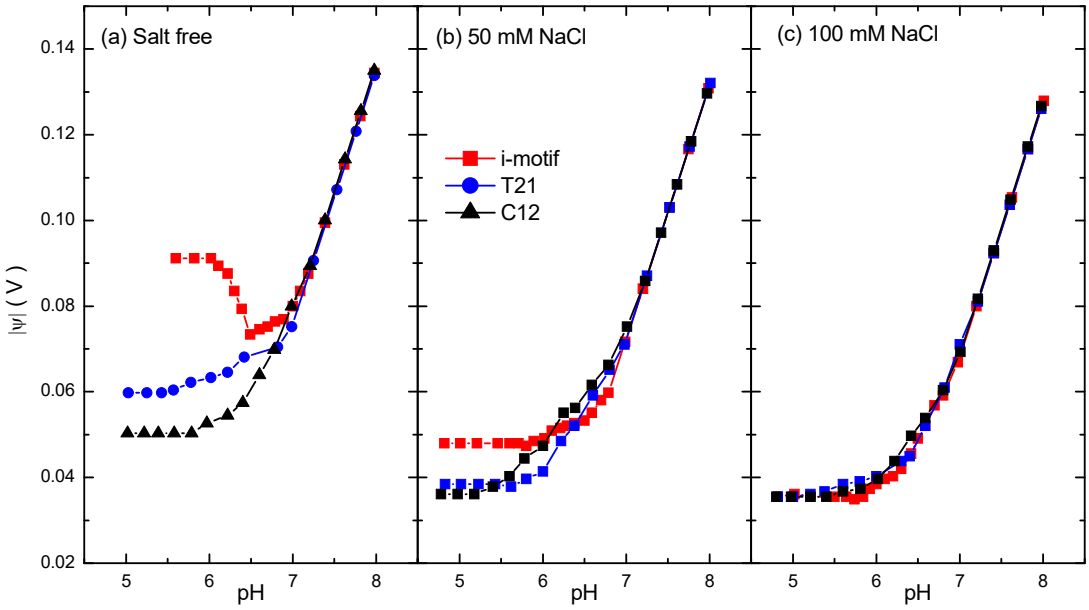

Figure S6 Amplitude of electric potential of i-motif, T21 and C12 as a function of the bulk pH value in salt free solution, 50 mM NaCl solution and 100 mM NaCl solution, respectively.

8. The absence of dimension change of reference samples

The reference samples, T21 and C12, do not exhibit noticeable dimension change within the pH range of the current study, as evidenced by the value of their hydrodynamic radius under different pH values (Figure S7).

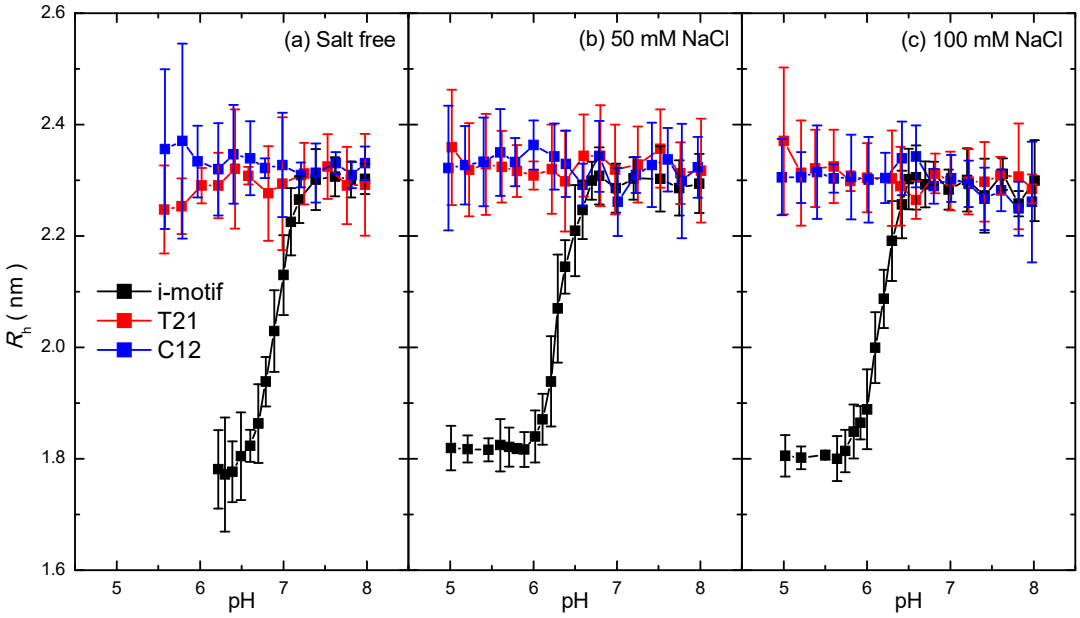

Figure S7 Values of hydrodynamics radius (R_h) of i-motif, T21 and C21 as a function of the pH value in salt free solution (a), in 50 mM NaCl solution (b) and in 100 mM NaCl solution (c).

CD spectra of these two reference sample are also measured (data shown in Figure S8. With the pH change, T21 does not undergo any formation of secondary structure while C12 does. It is beyond the scope of the current study what kind of secondary structure C12 are forming and it is emphasized that its chain dimension does not experience noticeable chain, indicating no change of charge density of them due to conformation change.

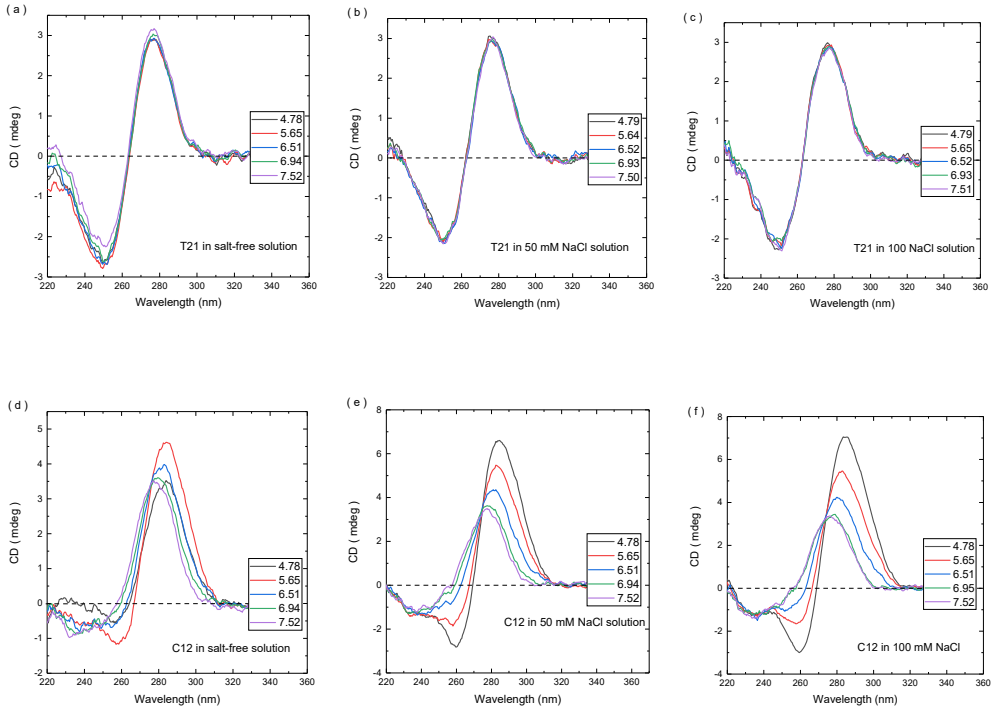

Figure S8 CD spectra of control samples T21 (a, b, c) and C12 (e, f, g) under different pH in salt-free, 50 mM and 100 mM NaCl solution, respectively. The concentration of T21 and C12 is 0.74×10^{-6} and 0.98×10^{-6} M, respectively. The pH values are displayed in the figures.

References

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2. Huang, B.; Perroud, T. D.; Zare, R. N. *ChemPhysChem* **2004**, *5*, 1523.
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4. Perroud, T. D.; Bokoch, M. P.; Zare, R. N. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 17570.