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Single-Walled Carbon Nanotubes Binding to Human Telomeric i-Motif DNA Under Molecular-Crowding Conditions: More Water Molecules Released

Chao Zhao, Jinsong Ren, and Xiaogang Qu*[a]

Abstract: The natural occurrence of the human telomeric G-quadruplex or i-motif in vivo has not been demonstrated and the biological effects of the induction of these structures need to be clarified. Intracellular environments are highly crowded with various biomolecules and in vitro studies under molecular-crowding conditions will provide important information on how biomolecules behave in cells. Here we report that cell-mimic crowding can increase i-motif stability at acid pH and cause dehydration. However, crowding can not induce i-motif formation at physiological pH. Intriguingly, single-walled carbon nanotubes (SWNTs) can drive i-motif formation under cell-mimic crowding conditions and cause more water to be released. To our knowledge, there is no report to show how SWNTs can influence DNA under cell-mimic crowding conditions. Our results indicate that SWNTs may have the potential to modulate the structure of human telomeric DNA in vivo, like DNA B–A transitions and B–Z changes on SWNTs in live cells, which demonstrates potential for drug design and cancer therapy.

Keywords: DNA · i-motif · ligand effects · molecular crowding · nanotubes

Introduction

Human telomeric DNA has received great attention in recent years.[1–6] The natural occurrence of the human telomeric G-quadruplex or i-motif in vivo has not been demonstrated and the biological effects of the induction of these structures need to be clarified. Intracellular environment is highly crowded with various biomolecules[7–13] and in vitro studies under molecular-crowding conditions will provide invaluable information on how biomolecules behave in cells.[7–13] Recent studies have shown that cell-mimic crowding can induce human telomeric G-quadruplex formation and cause an antiparallel to parallel transition.[10–17] However, the molecular-crowding effect on a human telomeric C-rich strand, which is complementary to the G-rich sequence, has not been reported.[10–17] C-rich strands may adopt i-motif structures with intercalated $C-C^+$ base pairs.[5,6] A large amount of effort has been made to investigate this structure and its involvement in human telomeric and centromeric DNA, and for its potential as a molecular motor[6] and anticancer drug target.[5] Therefore, it is important and of general interest to study the i-motif DNA under molecular-crowding conditions.

Herein we report that molecular crowding can cause i-motif DNA dehydration and increase the stability of DNA at acid pH. Investigations that use PEG1000 (polyethylene glycol with an average molecular weight of 1000) show the strongest effect on DNA stability by increasing T_m by 30 °C at a concentration of 30 wt %. Nevertheless, PEG can not induce i-motif formation at physiological pH, this demonstrates that molecular crowding is not an effective method for the induction of the formation of the i-motif. Single-walled carbon nanotubes (SWNTs), the leading nanodevice candidate,[18,19] have potential applications, ranging from gene therapy to novel drug delivery to membrane separation. In this report, we find that the i-motif structure is formed with the addition of single-walled carbon nanotubes (SWNTs). More water molecules can be released, indicating that SWNTs can induce i-motif formation under cell-mimic crowding conditions. SWNTs can also drive i-motif formation in dilute solutions, as shown in our recent reports.[18,19] SWNTs have been shown to modulate DNA B–A transitions[20] and B–Z changes on SWNTs in live cells,[21] and thus have potential to modulate the structure of human telomeric

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DNA in vivo, which, in turn, would have potential applications in drug design and cancer therapy. To our knowledge, there is no report to show how SWNTs can influence DNA under cell-mimic crowding conditions.

Results and Discussion

The melting profiles of i-motif in the absence or presence of PEG1000 were shown in Figure 1. Molecular crowding can significantly stabilize the i-motif structure at pH 5.5 (Figure 1A) by increasing T_m from 40 to 70°C at a PEG1000 concentration of 30 wt%. In the presence of SWNTs (5 μg mL^{-1}) (Figure 1B, ●), the T_m of the i-motif was raised even higher than the use of PEG1000 alone (Figure 1B, ■) to show that the binding of SWNTs further stabilizes the i-motif DNA under crowding conditions. Similar results were obtained for other PEG molecules with the molecular weight of 200 (Figure S1 in the Supporting Information), 3400, 6000 and 8000 (Table S1 in the Supporting Information). It should be noted that PEG1000 has the strongest effect on the stability of the i-motif, and was superior to lower or higher molecular weight PEGs. Previous reports have shown that although PEGs do not favorably interact with nucleotides[13] they can decrease water activity and disturb DNA hydration, and influence duplex, triplex, and G-quadruplex DNA stability.[9–17] These effects depend on PEG molecular weight,[9–13] in our case, PEG1000, the size and appropriate chain length may be the most effective cosolute to stabilize human telomeric i-motif DNA. CD studies confirm that DNA remains in the i-motif structure in the presence of different molecular weight PEGs and SWNTs (Figure 2), which excludes the possibility that the increased stability was caused by the structural transition.[18,19] Because direct interaction between PEG and DNA strands is thermodynamically unfavorable,[13] the enhanced stability of the i-motif is expected to be a result of the molecular-crowding effect. Typical CD spectra of the i-motif at different temperatures in the absence or presence of PEG 200, and PEG 200 with SWNTs are shown in Figure 3. Analysis of the two obvious iso-dichroic points around $\lambda = 242$ and 275 nm shows that the i-motif formation in these conditions follows a two-state transition model. We also estimated[13,18] the thermodynamic parameters of the i-motif in the presence of various cosolutes and SWNTs. These parameters were summarized in Table S1. If the PEG1000 concentration was increased from 0 to 30 wt%, the values of ΔH^o, and ΔG^o_{25} decreased from -62.3 ± 3.5 to -63.6 ± 4.2 kcal mol^{-1}, -4.6 ± 0.3 to -8.3 ± 0.6 kcal mol^{-1}, respectively, and $T\Delta S^o$ increased from

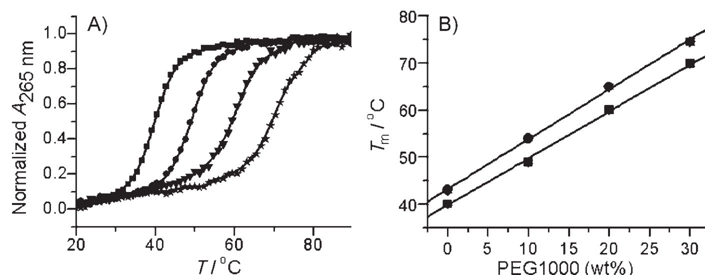

Figure 1. A) Normalized UV melting profiles of i-motif DNA in NaCl (100 mM) and sodium cacodylate buffer (10 mM, pH 5.5) containing various concentrations of PEG1000: 0 (■), 10 (●), 20 (▲), 30 wt% (⋆); [DNA] = 9.2 μg mL^{-1}. B) Plots of DNA melting temperature T_m versus PEG1000 concentration in the absence (■) or presence (●) of SWNTs (5 μg mL^{-1}) in NaCl (100 mM) and sodium cacodylate buffer (10 mM, pH 5.5).

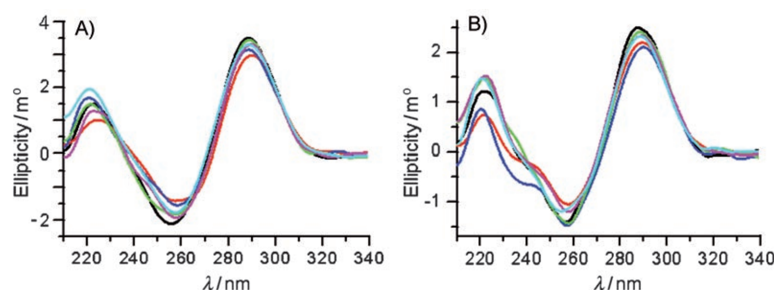

Figure 2. CD spectra of i-motif in NaCl (100 mM) and sodium cacodylate buffer (10 mM, pH 5.5) in the absence (——) or presence of different PEGs: 50 wt% PEG200 (——), 40 wt% PEG1000 (——), 30 wt% PEG3400 (——), 20 wt% PEG6000 (——), and 20 wt% PEG8000 (——). A) i-motif with different PEGs alone; B) i-motif with different PEGs and SWNTs (10 μg mL^{-1}) in the buffer. [DNA] = 9.2 μg mL^{-1}.

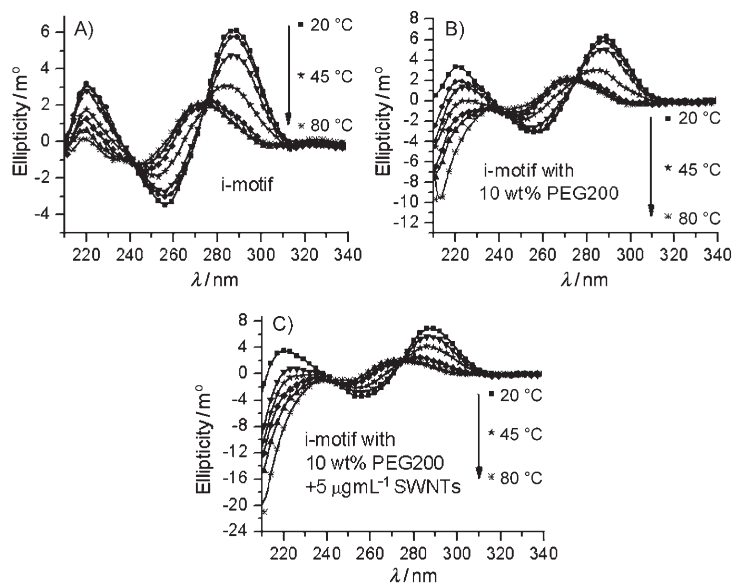

Figure 3. CD spectra of the i-motif at different temperatures in the absence (A) or presence (B) of 10 wt% PEG 200, and C) 10 wt% PEG 200 with SWNTs (5 μg mL^{-1}) in NaCl (100 mM) and sodium cacodylate buffer (10 mM, pH 5.5). [DNA] = 9.2 μg mL^{-1}. T =: 20°C (■), 35°C (●), 40°C (▼), 45°C (⋆), 50°C (◆), 60°C (▲), 80°C (✱).

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−57.7 ± 3.2 to −55.2 ± 3.6 kcal mol^{-1}. These data indicate that the i-motif stabilization by PEG is enthalpy favorable, and the same trend was held for other PEG molecules (Table S1).

Water has long been considered as an integral part of the structure of DNA.[22–24] Next we studied further how the i-motif DNA hydration changes under molecular-crowding conditions.[13,22] Figure 4 shows molecular-crowding effect on the thermodynamic equilibrium constants of i-motif formation in the absence (Figure 4A) or presence of SWNTs (Figure 4B). As the cosolute concentration is increased, so does the equilibrium constant, whereas the water activity decreases[9–13,22]. The positive slope indicates that water molecules were released when i-motif structure was formed.

Table 1. Summary of hydration changes for the intramolecular i-motif formation containing various cosolutes in the absence or presence of SWNTs in NaCl (100 mM), sodium cacodylate buffer (10 mM, pH 5.5).

Osmolyte	Δn_w[a]	Δn_w/nucleotide
PEG200	−68.7 ± 4.2	−3.1 ± 0.2
PEG1000	−369.5 ± 34.0	−16.8 ± 1.5
PEG3400	−282.6 ± 35.1	−12.8 ± 1.6
PEG6000	−322.0 ± 14.0	−14.6 ± 0.6
PEG8000	−332.7 ± 68.2	−15.1 ± 3.1
osmolyte + 5 μg mL^{-1} SWNT		
PEG200	−71.4 ± 1.7	−3.2 ± 0.1
PEG1000	−384.8 ± 48.3	−17.5 ± 2.2
PEG3400	−300.3 ± 23.2	−13.7 ± 1.1
PEG6000	−341.6 ± 32.3	−15.5 ± 1.5
PEG8000	−358.6 ± 52.8	−16.3 ± 2.4

[a] Δn_w was calculated according to reference [13].

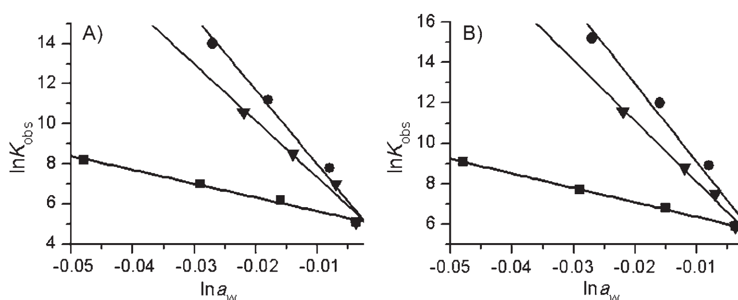

Figure 4. Dependence of i-motif DNA thermodynamic equilibrium constants on water activity in the absence (A) or presence (B) of SWNTs (5 μg mL^{-1}) at 25 °C in NaCl (100 mM) and sodium cacodylate buffer (10 mM, pH 5.5) containing different molecular weight PEG: PEG200 (■), PEG1000 (●), or PEG3400 (▼). Details as described in experimental section.

As described previously,[13,14,22] the number of water molecules released upon formation of an intramolecular structure by a DNA strand in an aqueous solution containing a cosolute (PEG) and a cation (such as Na$^+$) can be obtained by Equation (1):

$$\frac{d\ln K_{obs}}{d\ln a_w} = -\left[\Delta n_w + \Delta n_{cs}\left(\frac{d\ln a_{cs}}{d\ln a_w}\right) + \Delta n_{M^+}\left(\frac{d\ln a_{M^+}}{d\ln a_w}\right)\right] \quad (1)$$

In which K_{obs} is the observed equilibrium constant; a_w, a_{cs}, and a_{M+} are the activities of water, cosolute, and cation, respectively; Δn_w, Δn_{cs}, and Δn_{M+} are the numbers of water, cosolute, and Na$^+$, released, respectively.[13,14] The linear relationship between $\ln K_{obs}$ and $\ln a_w$ (Figure 4), which was measured by osmotic pressure measurements at 25 °C, clearly shows that the contributions from the other two variable terms, which are responsible for the cosolute and cation binding, are insignificant and the slope approximately equals the constant term, Δn_w,[13,14,22] in a way similar to the G-quadruplex.[13] The i-motif hydration changes with various cosolutes is summarized in Table 1.

Previous studies on the G-quadruplex have shown that crowding can alter DNA hydration, release of water molecules, and stabilization of DNA structures that have Hoogsteen base pairs.[13] These examples, which show that molecular crowding causes dehydration and increases i-motif stability, are consistent with our results. PEG1000 causes the i-motif to release the largest number of water molecules (Figure 4 and Table 1) and PEG 200 releases the smallest number of water molecules. This relationship shows that the number of oxyethylene units contained in different molecular weight PEGs directly influences the number of water molecules released. We carried out more DNA melting experiments and repeated the melting experiments for each sample at least three times. About 16.8 ± 1.5 water molecules per nucleotide were released upon formation of the i-motif. For i-motif DNA, the size and chain length of PEG 1000 makes it the most effective cosolute. SWNTs have been shown to bind to the major groove of i-motif at low concentrations (5 μg mL^{-1}).[18–20] In addition to the various interactions of DNA bases and backbone with SWNTs, such as hydrophobic and van der Waals interactions,[18–20] the favorable electrostatic interactions between the positively charged C–C$^+$ base pairs and the carboxyl groups on SWNTs can increase i-motif stability.[18,19] Here we found that the binding of the SWNTs causes even more water molecules (17.5 ± 2.2) to be released under molecular-crowding conditions (Table 1), which can further stabilize i-motif structure. For example, at 20 wt % PEG1000 in the absence of SWNTs, i-motif T_m = 60.0 °C (Table S1), in the presence of SWNTs, the value increased to T_m = 65.0 °C (Table S1).

It is well known that the i-motif structure of human telomeric C-rich DNA is pH dependent[18] in dilute solutions (Figure 5); it can be formed in acidic conditions and deformed at neutral and alkaline conditions. CD studies of a PEG1000 solution (40 wt %) at pH 7.0 showed that C-rich DNA did not form the i-motif structure (Figure 6A, ■), to indicate that molecular crowding could not effectively induce i-motif formation. However, in the presence of SWNTs (10 μg mL^{-1}), a typical i-motif CD spectrum (Figure 6A, ⋆), which has a positive band at λ = 288 nm and a negative band at λ = 260 nm,[18] was observed. This evidence that the i-motif was formed was supported by melting studies (Figure 6B). Under molecular-crowding conditions, C-rich DNA alone did not have a clear transition (Figure 6B, ■) at pH 7.0. In the presence of SWNTs (10 μg mL^{-1}), there

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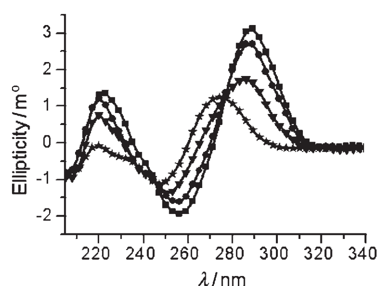


Figure 5. CD spectra of i-motif at pH 5.5 (■), pH 6.0 (●), pH 6.2 (▼), and pH 7.0 (⋆) in NaCl (100 mм) and sodium cacodylate buffer (10 mм). [DNA] = 9.2 μg mL^{-1}.

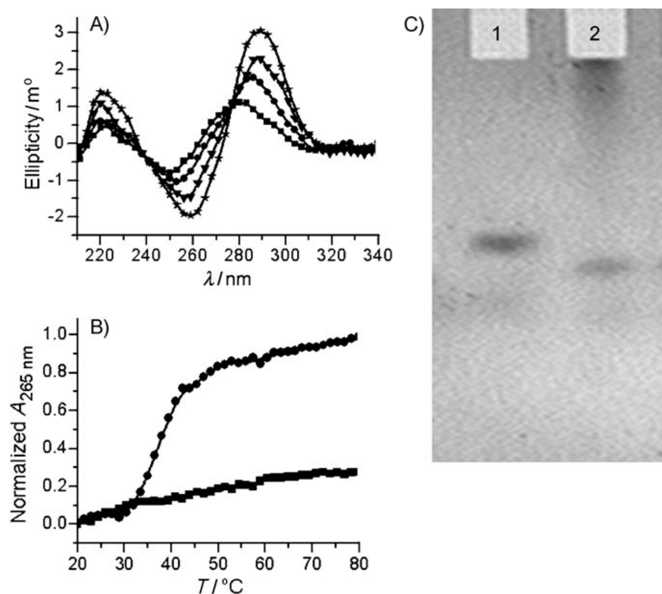
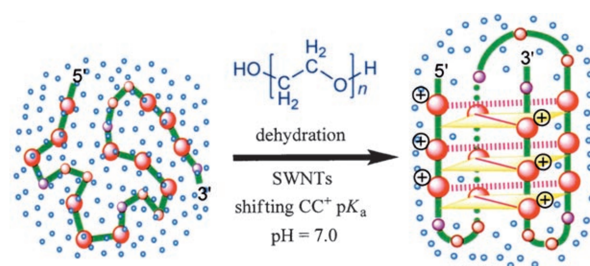


Figure 6. A) CD spectra of i-motif at pH 7.0 in NaCl (100 mм) and sodium cacodylate buffer (10 mм) containing 40 wt% PEG1000 in the absence (■) or presence of SWNTs: 2 (●), 5 (▼), and 10 μg mL^{-1} (⋆). B) Normalized UV melting profiles of i-motif at pH 7.0 in the absence (■) or presence of SWNTs (10 μg mL^{-1}) (●) in NaCl (100 mм) and sodium cacodylate buffer (10 mм) containing 40 wt% PEG1000. [DNA] = 9.2 μg mL^{-1}. C) Native PAGE images of i-motif at pH 7.0. C1) i-motif with 40 wt% PEG200; C2) i-motif with 40 wt% PEG200 and SWNTs (5 μg mL^{-1}). The experiments were run at 4 °C in TBE buffer (Tris/borate/EDTA) at 100 V. The gel was stained by using stains-all.

was an unambiguous transition at 37 °C (Figure 6B,●), which indicates that SWNTs can induce i-motif formation under molecular-crowding conditions.[18,19] A gel-mobility shift study (Figure 6C) provides independent evidence that the i-motif structure is formed in the presence of PEG with SWNTs. The structural transition of the i-motif DNA and water release under crowding conditions can be seen in Scheme 1. The molecular-crowding effect and the SWNT effect on the i-motif stability are different mechanisms. The former effect is a result of the alteration of the activity of water molecules, which is critical for the water bound DNA molecules. The latter effect is a result of the pK_a shift, which is critical for the C−C$^+$ base pair formation.



Scheme 1. Schematic illustrations of the i-motif DNA structural transitions under molecular-crowding conditions. Small blue circles represent water molecules.

Previous studies have shown that the pK_a values of conjugated polymers can be lowered by SWNTs to promote protonation.[25] We have reported that the charge stabilization between the positively charged C−C$^+$ base pairs and the SWNTs can lower the pK_a of the C−C$^+$ base pairs and induce i-motif formation.[18,19] Here we found that SWNTs can accelerate i-motif formation under cell-mimic crowding conditions and release more water molecules, this demonstrates that SWNTs have the potential to modulate human telomeric i-motif formation in cells. Detection of the DNA B–Z transition on SWNTs in live cells has been demonstrated recently.[21]

Conclusion

In summary, the natural occurrence of human telomeric G-quadruplex or i-motif in vivo has not been verified.[1–6] Molecular crowding can stabilize i-motif and cause water to be released. This effect is related to the size and chain length of the cosolute to which PEG1000 has been shown to have the strongest effect. Nevertheless, molecular crowding can not drive formation of the i-motif at pH 7.0. Intriguingly, SWNTs can accelerate i-motif formation at pH 7.0 under molecular-crowding conditions. This demonstrates the ability of SWNTs to drive the formation of the i-motif under cell-mimic crowding conditions and provides evidence to suggest that SWNTs may be able to modulate human telomeric DNA in vivo.

Experimental Section

General: SWNTs (ϕ = 1.1 nm, purity > 90%) were purchased from Aldrich (St. Louis, MO) and purified as described previously[18–20] by sonicating SWNTs in a 3:1 vol/vol solution of concentrated sulfuric acid (98%) and concentrated nitric acid (70%) for 24 h at 35–40 °C, and washed with water, leaving an open hole in the tube side and functionalizing the open end of SWNTs with carboxyl group to increase their solubility in aqueous solution. The stock solution of SWNTs (0.15 mg mL^{-1}) was obtained by sonicating the SWNTs for 8 h in a pH 7.0 aqueous solution.[18–20]

DNA oligomer 5′-CCCTAACCCTAACCCTAACCCT-3′ (i-motif), was purchased from Sangon (Shanghai, China) and used without further purification.[18,19] The DNA concentration was determined by measuring the absorbance at λ = 260 nm at high temperature (90 °C) by using a Carry

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300 Conc spectrophotometer connected to a thermal peltier controller. The extinction coefficient was calculated from mononucleotide and dinucleotide data by using the nearest-neighbor approximation.[18,19] All experiments were carried out in NaCl (100 mM), sodium cacodylate (10 mM, pH 5.5 or pH 7.0 buffer) unless stated otherwise. Polyethylene glycols were purchased from Sigma (St. Louis, MO) and had nominal molecular weights of 200, 1000, 3400, 6000, and 8000. All cosolutes were dissolved in the appropriate buffer for combination with DNA solutions.

Water activity measurements: Water activity[22,23] was determined by the osmotic stressing method through vapor phase osmometry by using a model 5520 pressure osmometer (Wescor, Utah, USA). The instrument was calibrated by using standard solutions before measurements.[22,23]

Bioassay: Absorbance measurements and melting experiments were carried out by using a Cary 300 UV/Vis spectrophotometer equipped with a Peltier temperature control accessory.[18–20] All UV/Vis spectra were measured in a 1.0 cm path-length cell with the same concentration of cosolutes and SWNT aqueous solution accordingly as the reference solution. Absorbance changes at $\lambda = 265$ nm versus temperature were collected at a heating rate of 1 °C min^{-1}. The melting measurements for each sample were repeated at least three times. Primary data were transferred to the graphics program Origin for plotting and analysis.[18] Native PAGE experiments[18] were carried out on acrylamide gel (12%) that contained PEG200 (40 wt%), and run at 4 °C in 1×TBE buffer (Tris/borate/EDTA, pH 7.0) at 100 V. The gel was stained by using stains-all.

CD experiments were measured by using a JASCO J-810 spectropolarimeter equipped with a temperature controlled water bath.[20] The optical chamber of CD spectrometer was deoxygenated with dry purified nitrogen (99.99%) for 5 min before use and kept the nitrogen atmosphere during experiments. The CD spectra were obtained by taking the average of at least three scans from $\lambda = 200$ to 340 nm. The SWNTs alone did not contribute to the CD signal between $\lambda = 200$ nm and 340 nm in our experimental conditions.[18,20]

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