Supporting Information

Title: Switchable Redox Activity by Proton fuelled DNA Nano-machines

Experimental procedure

The SWNT were purchased from CNI Inc. DNA with the sequence, $H_2NC_6H_{12}$–5′–CCCTAACCCTAACCCTAACCCTAA–3′–$C_6H_{12}(CH_2OH)NH_2$, designated as were purchased from IDT. The SWNT was mixed with a solution of sulfuric (H_2SO_4) and nitric (HNO_3) acids in a 3:1 ratio, and was sonicated for 1 h. The functionalized SWNT was filtered and washed with deionized water until neutral. The functionalized SWNT molecules were sonicated in the presence of ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) for 30 min to form a labile intermediate. Then, amino modified oligonucleotides were added, and the pH was raised to 6.5. The reaction mixture was stirred for 24 h at room temperature. The reaction mixture was centrifuged for 20 min at 4,400 rpm. This solution was purified by dialysis for 6 d at room temperature to remove any unreacted i-motif DNA (molecular weight cutoff (MWCO) of the membrane = 12,000–14,000).

Cryo-TEM images (Tecnai-12; FEI, USA) were acquired using a charge coupled detector (CCD) camera (Multiscan 600W; Gatan) at −170 ^{o}C. Samples were loaded onto holey-carbon film-supported grids. A thin aqueous film was produced by blotting with filter paper. The grids were immediately plunged into liquid ethane before the thin samples began to evaporate. The frozen grids were stored in liquid nitrogen and transferred to a cryotransfer (Gatan model 630; Gatan, USA) under liquid nitrogen at approximately –190 ^{o}C. Raman spectroscopy measurements were performed using a Jobin Yvon LabRam Model HR800 Raman microscope equipped with an Ar-ion excitation laser (λ = 514.532 nm). All CD spectra were recorded on a Jasco–810 spectropolarimeter equipped with a programmable temperature control unit. The CD melting curves were obtained at 287 nm using a heating rate of 1 °C/min. UV spectroscopic studies were conducted using a Varian UV/VIS/NIR spectrometer. A three-electrode electrochemical cell coupled to a CHI 600B potentiostat (USA) was used for the cyclic voltammetry. A SWNT/DNA hybrid electrode was prepared drop casting of SWNT/DNA dispersion solution on glassy carbon electrode (diameter: 3mm) and then incubated for 3 days to avoid fall off on glassy carbon electrode. The SWNT/DNA hybrid electrode was used as the working electrode with a Ag/AgCl reference electrode and a Pt wire counter electrode. The specific capacitance, C_{mass}, was derived from the current plateau (at -0.27V), I, at a given scan rate, v, and the weight of the SWNT/DNA hybrid, m, (where $C_{mass} = I / (v \times m)$).

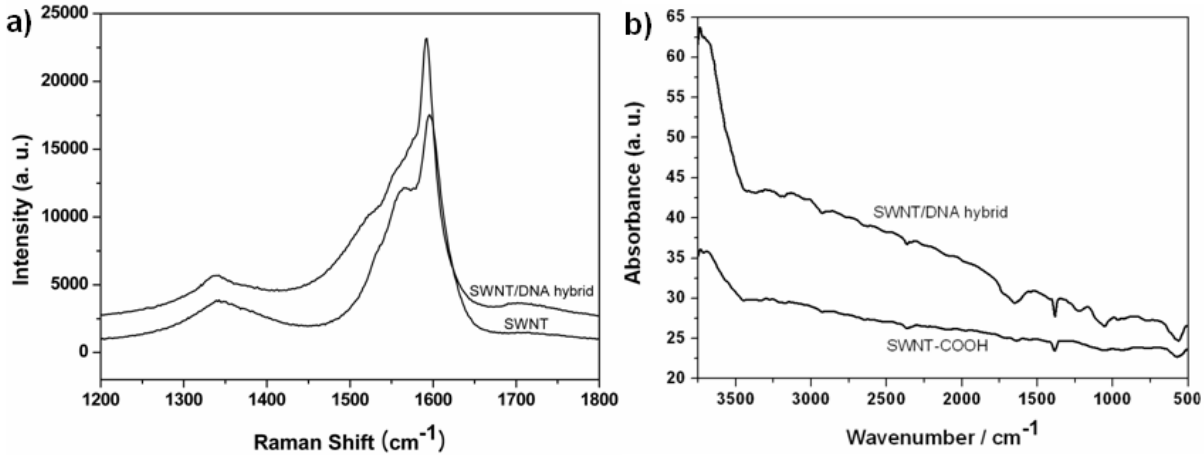

Figure S1. (a) Raman spectra and (b) FT-IR spectra of carboxylic functionalized SWNT (f-SWNT) and an SWNT/DNA hybrid.

Both Raman and FT–IR spectra were collected for analytical characterization of the SWNT/DNA hybrids. In the Raman spectrum, the band at 1594 cm^{-1} was assigned to the G band of the f-SWNT. The band at 1361 cm^{-1} was due to the D band of the f-SWNT. Covalent functionalization, which converts the sp^2 carbon atoms to sp^3 hybridization and disrupts the extended π-conjugation system, leads to the a of structure in the absorption spectrum, and causes a significant increase in the disorder-induced D-band intensity in the Raman spectrum.

We used FT–IR spectroscopy to confirm that cross-linkage bonding had occurred in the SWNT/DNA hybrids. The absorbance band of the DNA backbone was located at 964 cm^{-1}, and the bands at 1053 and 1220 cm^{-1} were a result of the symmetric PO_2 and antisymmetric PO_2 vibration modes, respectively. The appearance of a weak, broad band at ~3440 cm^{-1} was attributed to the presence of O–H groups on the surface of the f-SWNT, and is believed to be the result of ambient atmospheric moisture that was tightly bound to the f-SWNT molecules. It was found that three new peaks around 1637, and 1714 cm^{-1} appeared after the oxidation treatment. These peaks were assigned to the C=O, and the COOH stretching vibrations, respectively. The new absorption band at 1650 cm^{-1} correlated with the carbonyl stretching frequency of the amide group formed between f-SWNT and the i-motif DNA. The presence of these two distinctive absorption bands suggests covalent bonding of the i-motif DNA to the f-SWNT.

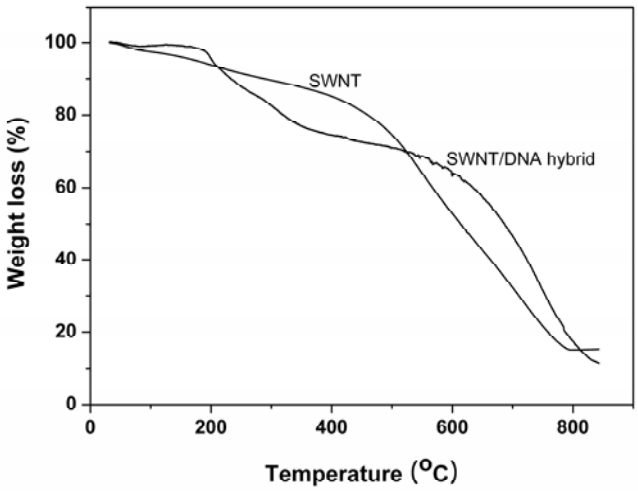

Figure S2. TGA using a ramp rate of 10 °C/ min starting at 33 °C and finishing at 850 °C for f-SWNT and SWNT/DNA hybrid under flowing N_2 (30 ml/min).

We also attempted to quantify the weight fraction of f-SWNT in the hybrids using thermogravimetric analysis (TGA). Figure S2 shows the TGA profile of f-SWNT, showing that a 15% residue remained after heating to 800 °C because of nondegradable impurities, such as the iron catalyst. The SWNT/DNA hybrid had an increase in residue of about 8% compared to raw SWNT, resulting from DNA coating on the f-SWNT surface. In the TGA profiles, it was difficult to obtain an accurate quantitative measure of the TGA data, as a satisfactory fit to the peaks was disrupted by unresolved multiple components over a wide degradation temperature range. However, we estimated the weight ratio of the i-motif DNA to the f-SWNT to be1:2.07.

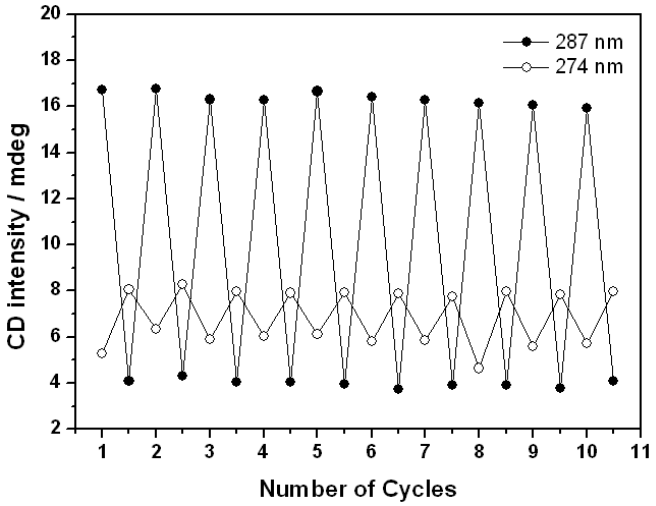

Figure S3. Cycling between pH 8 and 5 with a SWNT/DNA hybrid, as observed by the CD spectra at 287 nm (●) and 274 nm (○). Interconversion of the closed and open states of the machine was mediated reversibly by alternating the addition of 2M HCl and NaOH. The average cycling efficiency was defined as $\eta = (F_n/F_0)^{1/n}$, where n is the number of cycles, and F_n and F_0 are the change in CD intensity after n cycles and after the initial cycle, respectively.

An important part of this study regarding the reversible conformational change of DNA was to determine whether the SWNT molecules of a hybrid machine could disturb the preformed random coil conformation of i-motif DNA in the extended state (at pH 8) and reduce the cycling efficiency after a given number of cycles. To achieve a cyclical machine, the SWNT/DNA hybrid was required to continuously cycle when the environmental pH value oscillated between pH 5.0 and 8.0 in the absence of a fuel strand. We monitored the CD signal at 287 nm and 257 nm, the maximum positive band wavelength of the contracted and expanded state, respectively, while changing the solution pH. The average cycling efficiency (η) of a switch, estimated from the change in CD intensity at 287 nm, was approximately 100%, indicating no loss per cycle over 10 cycles. This high cycling efficiency suggests that an irreversible aggregation of DNA was not induced by the SWNT bonding to the base sequence.

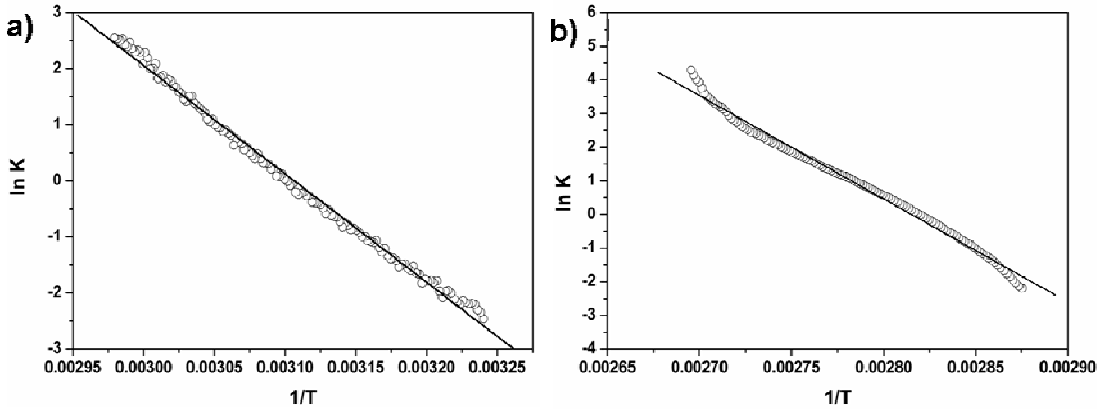

Figure S4. Van't Hoff plots (1/T versus ln*K*) for: (a) i-motif DNA, and (b) SWNT/DNA hybrids at pH 5 from the CD melting profiles at 287 nm. The standard deviation of the plots was > 0.99.

Table S1. Melting Temperatures and van't Hoff Thermodynamic parameters of i-motif DNA and SWNT/DNA hybrid at pH 5.0.

Sample (pH 5.0)	T_m[a] (^oC)	ΔH (kJ/mol)	ΔS (J/mol·K)	ΔG[b] (kJ/mol)
i-motif DNA	49.8	-153.4	-477.4	-11.1
SWNT/DNA hybrid	80.4	-254.9	-716.8	-41.3

[a] The melting temperature of i-motif DNA and SWNT/DNA hybrid at ~15 μM.

[b] Calculated at 25 ^oC

The thermodynamic parameters were derived from the melting curves generated by monitoring the 287 nm CD band as a characteristic of the i-motif structure, and at this wavelength, the contribution from the random coil structure had its lowest value. The recorded spectra (in millidegrees of ellipticity (Θ) were converted to mean residue ellipticity, [Θ], in deg.cm^2.dmol^{-1} using the following equation, $[\Theta] = \Theta \times 100 \times M_r / c \times l \times N_A$, where c is the concentration of DNA in mg/mL, *l* is the path length in cm, M_r is the molecular weight of DNA, and N_A is the number of DNA molecules. The equilibrium constant, *K*, is expressed by, $K = ([\Theta]_c - [\Theta])/([\Theta] - [\Theta]_e)$, where $[\Theta]_c$ is the molecular ellipticity of the contracted state and $[\Theta]_e$ is the molecular ellipticity of the expanded state. The change in enthalpy, ΔH, was determined from the temperature dependence of the equilibrium constant, according to the van't Hoff plots, $\ln K = \Delta H_{vH}/RT + \Delta S_{vH}/R$, where ΔS is the change in entropy.

Figure S4 shows the van't Hoff plots for i-motif DNA and an SWNT/DNA hybrid at pH = 5. The change in free energy (ΔG) at 25 °C was calculated from the standard Gibbs's equation, $\Delta G = \Delta H - T\Delta S$. Table S1 contains the calculated thermodynamic parameters.

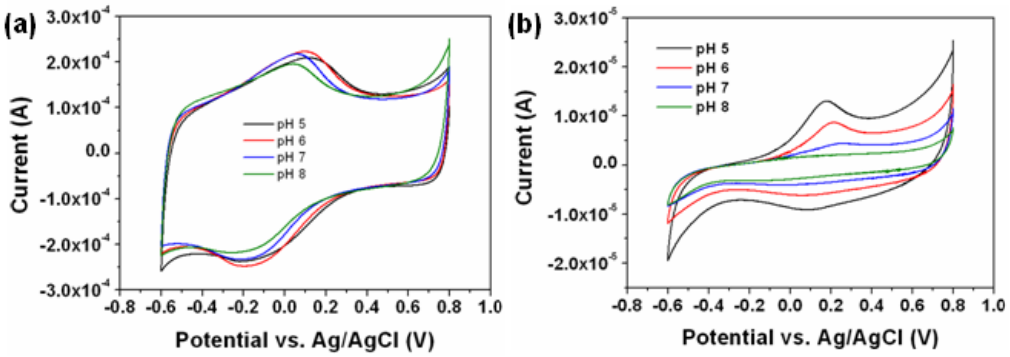

Figure S5. Cyclic voltammogram of (a) f-SWNT and (b) SWNT/DNA hybrid (Scan rate = 100 mV/s) during potential cycling. The change in pH of 0.15 M PBS was produced by the addition of 1M HCl and NaOH.

Figure S5 shows cyclic voltammogram of SWNT-COOH and SWNT/DNA hybrid at various pH conditions. In figure S5 (a), the SWNT-COOH exhibits redox responses which have been ascribed in the case of the electroactive functional groups (carboxyl group) on the surface of the nanotubes. (Ref:) In contrast, the SWNT/DNA hybrid exhibits redox responses which have been ascribed in the case of the electroactive functional groups (stacking DNA base pairs) through π stacking charges on the surface of the nanotubes.