Molecular conductance of double-stranded DNA evaluated by electrochemical capacitance spectroscopy

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Electronic Supporting Information

Section 1 – Theoretical Background

One of the major themes involving molecular electronics is the measurements of current-voltage pattern of molecular entities acting as charge conductive pathways. Generally, molecular junctions are structures in which single or small group of molecules transmit electrical current (or charge changing in time is quantified) between two electrodes with different potential energies or in between electrodes and chemical redox (or electrochemical) states embedded on an electrolyte solution, i.e. wherein the driving force to impose charge dynamics is made by some electrochemical potential differences (see Figure 1 of the main text for more information).

In Electrochemical Capacitance Spectroscopy (ECS) approach as proposed to be used in measuring molecular conductance in the present work, the measurements are performed from an alternate voltage perturbation of the system in which the response is thus an alternate current from wherein density of states or electron particle number is obtained (more details in Figure 1 and references[1, 2]). In the proposed M-B-R (metal-bridge-redox) structure of Figure 2 (main text) the ECS pattern and information comimg from measured impedance spectra can be converted into admittance or complex capacitance[3]. The states in the electrode (M) and that of redox centres (R) are allowed to communicate throught an electron dynamic transmittance/reflectance under an oscillatory perturbation in a way that the maximum stationary current (obtained at low frequency) is possible to be measured when the potential energy of the electrode is poised at the same energy levels of those of redox states in the molecular layer ($\bar{\mu} = E_r$) which in electrochemistry nomenclature is called/known (for reversible processes) as the energy associated with the half-wave potential.

The average transit time[3] of the electrons in the molecular layers, that is τ_d (either referred as the time scale of the electrochemical process), can be measured independently of the operating transport mechanisms, i.e. either to hopping or tunnelling electron transport. The metallic (M) states in communication with the redox (R) states depends on the electronic structure of the bridge[3] and is contained in the measurement of τ_d and the EDOS (as obtained through the measurement of the electrochemical capacitance). Itself τ_d is easily (and directly) obtained from the Nyquist capacitive diagram as shown in Figure

ESI 1 below, following ECS methodology[2]. It was herein applied in both ds-DNAc and DNAi layers as chemically obtained according to the procedure given in ESI section 2.

The theory and methodologies related to ECS approach is not herein our primary focus since it was already treated in other references[1-6]. The main purpose of the present text/work is to demonstrate that ECS either allows an additional alternative and powerful means to experimentally measure molecular conductance and so constitutes a suitable alternative in studying the fundaments involving with molecular electronics, molecular electrochemistry as well as more deeply allowing electrochemistry mechanisms associated with biological systems to be resolved.

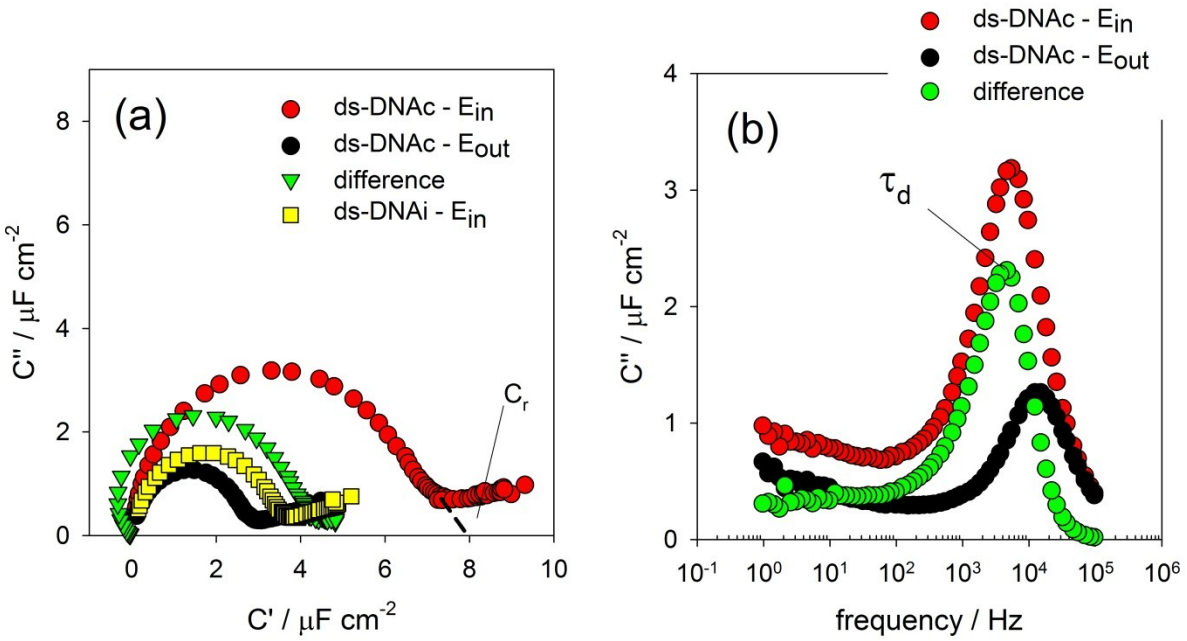

Figure ESI 1 – (a) Nyquist capacitive plots of ds-DNAc and DNAi compared in E_{in} (herein equivalent to E_r) steady state potentials. A comparison is also shown for ds-DNAc at E_{in} and E_{out} potentials. The differences between E_{in} and E_{out} for ds-DNAc (in green) reflect the response in the absence of parasitic non-faradaic terms as discussed in previous references[1, 2, 4]. The diameter of the semicircle (after corrections using parasitic terms) provides the value of the electrochemical (redox) capacitance value, C_r. (b) Bode diagram of the imaginary component of complex capacitance. The observed peak frequency indicates an electronic/electrochemical transit time of about 0.167 ms (a slightly lower value is obtained by ds-DNAi, data not shown). The data were recorded in the range of frequency between 100 kHz to 1 Hz.

Section 2 – Experimental procedure

Part I – Single stranded DNA features

Lyophilized single stranded modified DNA (ss-DNA) fragments were purchased from TIB MOLBIOL Syntheselabor (Berlin, Germany). The quantity purchased was 1.0 OD of the following items:

A) 5'-NH_2-AAAAAAAAAAAAAAAAAAG
3.3 nmol, annealing temperature 53.9 °C

B) 5’-SH-$(CH_2)_7$-CTTTTTTTTTTTTTTTTTTT
6.0 nmol, annealing temperature 55.4 °C

C) 5’-NH_2-GGGGGGGGGGGGGGGGGGGG
4.2 nmol, annealing temperature 72.1 °C

D) 5’-SH-$(CH_2)_7$-CCCCCCCCCCCCCCCCCCCC
7.1 nmol, annealing temperature 72.1 °C

Ultra-pure DNase free water (resistivity not less than 18.2 MΩ cm at 298 K) was added to obtain a final a stock solution of 10 µM. The solution was divided into 50 µL aliquots and suitably stored at -20 °C.

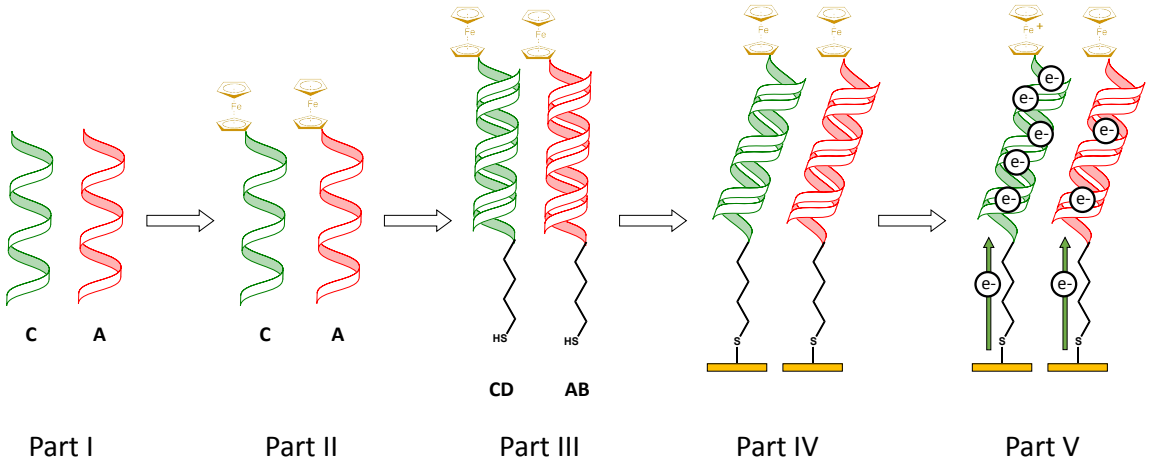

Figure ESI 2 – Scheme of the experimental setup. Part I – Single stranded DNA features, Part II – Modification with ferrocene, Part III – hybridization reaction, Part IV – Electrode modification, and Part V – Electrochemical measurement.

Part II – Modification with ferrocene

A and C ss-DNA were modified with a ferrocene group by a reaction with ferrocene carboxylic N-hydroxysuccinimide ester (Fc-NHS, purity above 98%, FIVEphoton Biochemicals, San Diego, USA). 100 µL of Fc-NHS (0.175 mg/mL) in dimethylformamide was reacted overnight with 350 µL of ss-DNA (10 µM) and 50 µL of sodium hydrogen carbonate (0.2 M, pH 9.5) at 4 °C in the dark.

After modification of the DNA with ferrocene, the solution was purified to remove all the non-reacted free ferrocene molecules in order to avoid unwanted electrochemical signals. This was performed by molecular exclusion chromatography, using a dextran desalting column from Thermo Fisher Scientific (Waltham, USA) with a 5000 Da cut-off. Separation was driven by gravity at a flow rate of approximately 60 mL h^{-1}. The column was first conditioned with sodium hydrogen carbonate (0.2 M, pH 9.5), 30 x 1 mL, and then with 5 x 1 mL of ultra-pure water. The 500 µL of ss-DNA modified with ferrocene was then injected into the column followed by 20 x 0.5 mL of ultra-pure water. 500 µL aliquots were promptly collected and analyzed by UV-Vis (with a NanoDrop 3300 Fluorospectrometer, also from Thermo Fisher Scientific) at a wavelength of 260 nm. The two aliquots with the highest absorbance, shown in Figure 2, were collected, mixed together and lyophilized prior to storage.

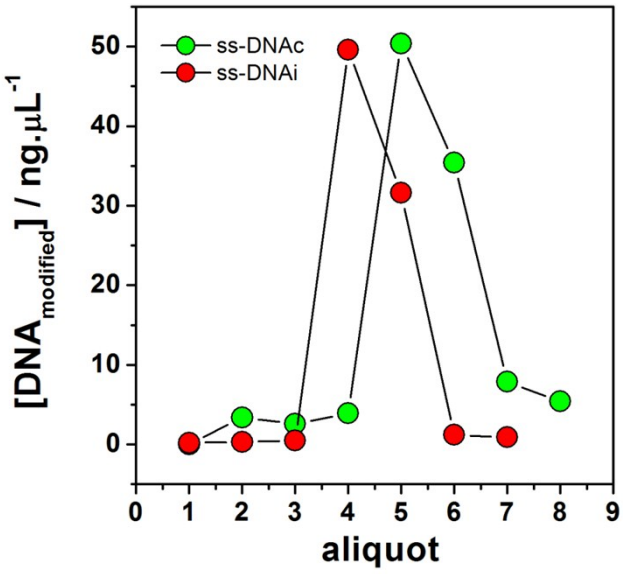

Figure ESI 3 – Concentration of the modified conductor and insulator single stranded DNA (ss-DNAc and ss-DNAi, respectively) with ferrocene in the several purified aliquots.

Part III – hybridization reaction

The ferrocene-modified ss-DNA (10 µM) and the complementary single strand DNA solution (10 µM) were both diluted with a mixture of saline phosphate buffer solution (0.1 M) and magnesium chloride solution (0.5 M) to reach a concentration of double stranded DNA (ds-DNA) equal to 300 nM in a final volume of approx. 0.7 mL. The hybridization process involves chain A with chain B (conductor), and chain C with chain D (insulator).

The reaction mixture was incubated for one hour at 42 °C with slow homogenization of 700 rpm in an Eppendorf (Hamburg, Germany) 5436 Thermomixer. The incubation mix was placed in an ice bath until it was used. The solution was divided into 50 µL aliquots and suitably stored at -20°C.

Part IV – Electrode modification

Before immobilizing the modified DNA on the surface of the gold electrode discs (2.0 mm diameter, Metrohm), they were polished by hand with aluminum oxide suspensions of decreasing particle size (1, 0.3 and 0.05 µm) and sonicated in ultra-pure water. The electrodes were then treated electrochemically in sodium hydroxide 0.5 M (-1.5 and 0 V vs Ag|AgCl at a scan rate of 100 mV/s, 200 cycles) and in sulfuric acid 0.5 M (-0.2 V and 1.5 V at a scan rate of 100 mV/s, 25 cycles).

After electrochemical polishing, the electroactive areas were evaluated by integrating the cathodic peak in gold electropolishing voltammograms and converting them into the real surface area using a conversion factor of 400 µC cm^{-2}.[7]

Figure ESI 4 illustrates a typical voltammetric profile pattern of the electrochemical cleaning process. The surface quality was controlled considering a roughness factor of less than 1.4.

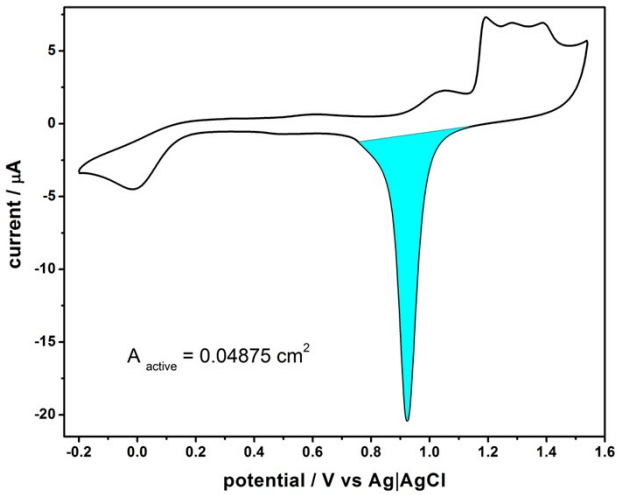

Figure ESI 4 – Voltammetric profile of the electrochemical cleaned surface. The shaded area was integrated to determine the electrode's active area.[7]

To immobilize the DNA on the electrode, a drop (approx. 5-10 µL) of 300 nM of the modified DNA was placed on the electrode surface and allowed to interact for 12 h in the dark. The electrode was then washed in ultrapure water and dried with nitrogen, after which about 50 µL of 6-mercapto hexan-1-ol, 20 mM, ethanol solution, was used to block the surface. After 12 h, the electrode was washed with absolute ethanol (with low metal content) and ultra-pure water and dried with nitrogen.

Part V – Electrochemical measurement

Electrochemical measurements were performed in an electrochemical workstation 650C from CH instruments (Austin, USA) with a 3-electrode system: DNA functionalized gold electrode discs (electroactive area of 0.048 to 0.057 cm^2 and a roughness factor of 1.5 to 1.8) as the working electrode; platinum spiral wire (area of 1.885 cm^2) as the counter electrode; and a Ag|AgCl (KCl 3 M) electrode as the reference electrode. All the results described in the main text were based on the average data of triplicate measurements over three different electrodes.

All the solutions were deoxygenated for 10 min with bubbling argon. Cyclic voltammetry measures were obtained between -0.2 and +0.7 V, 500 mV/s and 3 cycles. The complex Z^* (impedance) function was converted into C^* (capacitance) through $C^* = 1/j\omega Z^*$, where ω is the angular frequency and j is the imaginary number $j = \sqrt{-1}$.[2] All the experiments were conducted at room temperature (in a controlled range of 22 to 26 degrees Celsius). Ac power frequencies ranging from 100 kHz to 10 mHz, with an oscillation potential of 10 mV (peak to peak, or about 3 mV RMS), a waiting time of 300 s (for stabilization of the the steady state potential), and dc potential of 0.1 to 0.5 V (with a step size of 50 mV) were employed in the impedance spectroscopy analysis, where those mentioned as E_{in} and E_{out} as electrochemical potential energies are included. A solution of $NaClO_4$ 0.5 M was used for characterization of the ferrocene-modified ds-DNA.

Section 3 – Analysis of the impedance data and conductance measurements

As discussed in the main text, conductance was measured by considering transport in DNA bridges or the electronic/electrochemical communications in between metallic and electrochemical states[3]. This communicative capability can be used to directly obtain the total density of electrochemical states, $dN_r/d\bar{\mu}$ (no matter the states are single or distributed levels), responsible for the electrochemical conductivity, at a given electrode potential energy. During a sinusoidal perturbation in potential, $\tilde{V} = \Delta V e^{j\omega t}$, imposed on the junction comprising metallic and electrochemical (redox) spatially coupled states, it is possible to obtain a current response as $\tilde{i} = \Delta i e^{j(\omega t - \phi)}$, where ΔV and Δi are the amplitude of the perturbation in potential and the amplitude in the current response, respectively. Note that ϕ is the phase and that the impedance is thus obtained as $Z^*(\omega) = \tilde{V}/\tilde{i} = |Z|e^{j\phi}$, where $|Z| = \Delta V/\Delta i$ is the modulus or the amplitude of the impedance. We are considering the linearity of the system, i.e. the ratio between the perturbation signal and its response is linear. This is assured if the magnitude of the perturbation is small, which is easy to be obtained experimentally during ECS experiments.

Now, to demonstrate how this can be used to calculate conductance, let us consider a sinusoidal current response associated with the transit time of the carriers, τ_d (as defined in the main text and reference[3]), as

$$\tilde{i} = \tilde{q}/\tau_d = \tilde{N}_r e/\tau_d \qquad \text{(ESI .1)}$$

where $\tilde{q} = \tilde{N}_r e$ is the sinusoidal charge response and $\tilde{N}_r$ is the number of electrochemical states (sinusoidal responding) that is varied under the sinusoidal potential perturbation $\tilde{V}$. Let us also assume that the occupation of these states follows the Fermi-Dirac statistics as $f = F(E_r, \bar{\mu}) = \{1 + exp[(\bar{\mu} - E_r)/k_B T]\}^{-1} = N_r/N_r^t$, where $\bar{\mu}$ is the electrochemical potential of the electrons in the electrode and E_r the the electrochemical potential of the electrons in the molecular layer. N_r^t is the total density of electrochemical or redox states which is a constant (for instance, in the single energy level mode N_r^t equates to the molecular coverage, i.e. $N_r^t = \Gamma$). Thus $N_r = fN_r^t$ and by considering an oscillatory perturbation it becomes $\tilde{N}_r = \tilde{f}N_r^t$.

From the uncertainty principle, in terms of energy and time, it is known that $\Delta E\, \Delta t = h/2$[8] (as a minimum uncertainty value, where h is Planck's constant). In other words, to calculate the conductance of DNA, we assume that the quantized probability of transmittance/reflectance is related to the uncertainly principle. In considering time/frequency perturbation the result for the uncertainty principle is $\tilde{E}\Delta t = e\tilde{V}\,\tau_d = h/2$. Note that $\tilde{E} = e\tilde{V}$ is the oscillatory perturbation of the electron energy by the pulse of the oscillatory potential perturbation in the electrode and $\Delta t = \tau_d$ is the average transit time of the charge between M and R states as defined in the main text and either previous work[3]. Finally we have

$$\tau_d = h/2e\tilde{V} \qquad \text{(ESI. 2)}$$

In substituting τ_d obtained in Eqn. (ESI. 1) we obtain

$$\tilde{q} = \tilde{i}(h/2e\tilde{V}) \qquad \text{(ESI. 3)}$$

and now assuming, in Eqn. (3), the low frequency limit $\omega \to 0$ for $\tilde{q} = \Delta q e^{j(\omega t - \phi)}$, $\tilde{i} = \Delta i e^{j(\omega t - \phi)}$ and $\tilde{V} = \Delta V e^{j\omega t}$ functions we have

$$\Delta q e^{-j\phi} = \Delta i e^{-j\phi}(h/2e\Delta V) \qquad \text{(ESI. 4)}$$

since $G = \Delta i/\Delta V$ is the magnitude of the conductance and $\Delta q = eN_r$, thus we can rewritten Eqn. (ESI. 4) as

$$G = (2e^2/h)N_r = G_0 N_r \qquad \text{(ESI. 5)}$$

where $G_0 = 2e^2/h$ (about 7.75 x $10^{-5}\,\Omega^{-1}$) is the quantum of conductance and G is, therefore, the conductance for a given N_r state of charge of the molecular layer. Now by the derivation of Eqn. (ESI. 5) as a function of $\bar{\mu}$ we have

$$dG/d\bar{\mu} = (2e^2/h)dN_r/d\bar{\mu} = G_0(dN_r/d\bar{\mu}) \qquad \text{(ESI. 6)}$$

and thus, independently of energy dispersion (or non-Nernstian distribution of electrochemical states), we have, by integration of Eqn. (ESI. 6) that the total conductance (over all the energy levels) is given by

$$G = G_0 N_r^t = G_0(1/e^2)\int C_r(\bar{\mu})d\bar{\mu} = G_0\int g_r(\bar{\mu})d\bar{\mu} \qquad \text{(ESI. 7)}$$

as measured over all the energies since $N_r^t = (1/e^2)\int C_r(\bar{\mu})d\bar{\mu}$ or $N_r^t = \int g_r(\bar{\mu})d\bar{\mu}$. Note that Eqn. (ESI. 7) is an easy equation proposed here to determine the conductance of a given electrochemical molecular layer. This is an obvious result since $N_r^t = 1$, i.e. in considering a single molecule (on the ensemble of the molecular layer) with an effective integer electron transfer (transfer of a single charge unit) and so that we obtain the expected quantum of conductance. Also obviously is that Eqn. (ESI. 5) resembles Landauer's formula,[2] i.e.,

$$G = G_0 T \qquad \text{(ESI. 8)}$$

where T is the electron transmittance of situation given in Figure 1*a* of the main text. In Eqn. (ESI. 7) it is implicit that for single channel and one single electron particle in assuming $T = 1$ we have again the value of the quantum of conductance as $G = G_0$. For a multiple quantum channels, Eqn. (ESI. 6) is equivalent to, $G(\mu) = G_0\sum T_n(\mu)$ where $\sum T_n(\mu)$ are transmission eigenvalues of individual channels, i.e. the conductance of a molecular scale conductor is given by the sum of all the transmission probabilities an electron has when propagating with an energy equal to the chemical potential, μ. Note that by using ECS approach we are not effectively measuring over one single molecule but over an ensemble of molecules thus giving us advantage in obtaining an average conductance value in a single experimental run. It is important to observe that in ECS experiments (at low frequency) it is not possible to mention about transmittance since we have only reflectance. This is to note that the comparisons with Landauer's formula should be taken very carefully (to be addressed in future works); in other words, in Eqn. (ESI. 8) we are only demonstrating the universality of the results in the sense it can predict quantized and mesoscopic effects as well as Landauer's formulation either does.

Note that in determining N_r^t (or the molecular coverage) the transmittance/reflectance (only reflectance for low frequency limit as obtained herein) probability is implicit within N_r^t values experimentally obtained [any thermal or electronic/structural dispersion of the potential or energies are contained on the integration involved in the Gaussian shape obtained in $C_r(\bar{\mu})$ which indeed contains $g_r(\bar{\mu})$]. Note that G can be normalized by the geometric characteristics of the molecular junctions, such as the length (L) of the molecular wire and the area (A) of the electrode, which in according to Ohm´s law provides the value of the conductivity $\sigma = G(L/A)$. In calculating L/A ratio the length of ds-DNA was estimated ~ 6.8 x 10^{-9} m (for both ds-DNAc and ds-DNAi) and the area was obtained as previously described above (see Figure ESI 4) for each type of ds-DNA molecular layer as an average over three different electrodes. In performing the geometric normalization, the values obtained experimentally for the conductivity (σ) reflect those average value expected for single molecule which are indeed in the same other of magnitude of those values obtained by others for similar ds-DNA nanowires[9, 10].

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