An *in Silico* Design for a DNA Nanomechanical Switch

David Řeha,[†] Alexander A. Voityuk,*[,‡] and Sarah A. Harris*[,§]

[†]Department of Biological Sciences, University of Essex, United Kingdom, [‡]. Institució Catalana de Recerca i Estudis Avançats, Barcelona and Institut de Química Computational, Universitat de Girona, 17071 Girona, Spain, and [§]School of Physics and Astronomy, University of Leeds, United Kingdom

ABSTRACT We have calculated how the charge transfer properties of DNA change in response to the application of an external stretching force. Since charge transfer occurs through the DNA π-stack, any disruption to this stacking causes dramatic changes in the transport properties of the biomolecule, as our calculations demonstrate. We therefore propose that the mechanical response of DNA to an applied stretching force might be used in the design of a nanomechanical switch.

KEYWORDS: DNA conduction · semi-empirical quantum mechanics · steered molecular dynamics

The ability of the π-stack of DNA to conduct electrons and holes has generated much interest in the nanotechnology community due to the potential for single DNA strands to act as molecular wires. This is in spite of the continuing controversy as to the interpretation of the experimental results in terms of the conducting properties of DNA. The development of techniques to stretch, twist and unzip DNA has enabled experimentalists to probe the mechanical properties of the duplex at the single molecule level. Pioneering experimental studies have combined DNA manipulation experiments with charge transfer measurements.[1] In this study, we use atomistic molecular dynamics (MD) simulations to mimic experiments that stretch single molecules of duplex DNA and semiempirical quantum mechanical methods to calculate the change in the charge transfer properties of the biomolecule when an external force is applied.

The interpretation of force−extension curves for stretched DNA in terms of structural changes at the atomic level has proven controversial. Single strands of polymeric double-stranded (ds) DNA respond approximately elastically up to forces of 65pN, at which an abrupt transition occurs, and then become stiff again until the molecule disassociates into two single strands at forces of around 150pN (depending upon the precise experimental conditions). Initially, this transition at 65pN was hypothesized to result from a conformational change into a novel elongated DNA structure known as S-DNA. However, a detailed thermodynamic analysis of the stretching curves suggested rather that the DNA becomes unstable and starts to melt into two separate strands.[2] The ability of the DNA to resist higher forces was then ascribed to nonequilibrium effects, particularly as the transition was found to be reversible up until the center of the plateau, but to exhibit hysteresis beyond this point.[3] Striking evidence for the force-induced melting model was subsequently obtained using biochemical experiments, which detect the interaction between a stretched duplex and the small molecule glycol,[4] which preferentially binds single stranded DNA. More recently, using a unique combination of optical tweezers, microfluidics and a complementary pair of fluorescent dyes (one recognizing dsDNA and the other ssDNA), van Mameren et al. directly visualized the single stranded (ss) regions forming within stretched DNA.[5] Although the first pioneering computer modeling studies suggested that DNA can indeed adopt a novel stretched structure when subjected to force, later simulations showed that this structure does not remain intact when subjected to long time scale MD.[6]

Charge transfer has been already studied in GC-rich stretched DNA using *ab initio* density function theory and semiempirical electronic structure calculations,[7] but not through conformations containing the denatured DNA regions expected to form in overstretched DNA. For these calculations, we have considered hole transfer through the DNA sequence 5′-CAAAAAAAAAGC-3′.

*Address correspondence to alexander.voityuk@icrea.es, s.a.harris@leeds.ac.uk.

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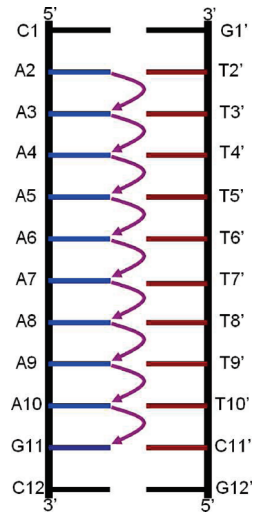

Figure 1. Schematic labeling of DNA bases indicating the movement of the charge in the 5′-CAAAAAAAAAGC-3′ duplex.

Runs of purine (A and G) bases located on a single DNA strand are well-known to be efficient conduits for hole transfer. As pyrimidine bases (C and T) have larger ionization potentials than purines, hole transfer through 5′-CAAAAAAAAAGC-3′ is expected to occur predominantly along one single DNA strand, as shown in Figure 1. Charge injection into dsDNA can be achieved experimentally either through application of an electric field across a single DNA molecule, by injection of a charge by photoexcitation of a synthetic dye, which is chemically incorporated into the π-stack, by the locality of a highly oxidative enol-ether radical cation species or through chemical attack by other oxidizing free radicals in the environment.[8] In our calculations, we have assumed that sufficient experimental control can be achieved to inject a hole at the topmost adenine base (A2 in Figure 1) within the DNA and to collect it at the final guanine (G11). This sequence has been specifically designed to trap the hole between the insulating C1 and C12 bases. The choice of soft AT rich DNA ensures that this sequence will be highly responsive to an applied force, whereas incorporation of a more stable GC base pair at the ends of the sequence is desirable to prevent fraying of the duplex.

Elongated DNA structures were generated for the DNA duplex 5′-CAAAAAAAAAGC-3′ by applying harmonic restraints to the 5′ ends of the molecule, and gradually forcing the distance between the 5′ ends of the molecule to increase over a 4 ns time period. The duplexes were stretched by 0.5 Å and were then allowed 50 ps structural relaxation before the next elongation step. The duplexes were thereby extended by 40 Å during the course of the steered MD simulation.

To observe the formation of denaturation bubbles in the DNA over longer MD time scales, we performed three 10 ns MD simulations of the DNA duplex, which are restrained at various extensions using conformations sampled from the simulation in which the DNA was progressively elongated, as in Figure 2.

The charge transfer rate between individual nucleobases was calculated using the Marcus equation:

$$k = \frac{2\pi}{\hbar} < J^2 > \frac{1}{\sqrt{4\pi\lambda k_B T}} \exp\left[-\frac{(\Delta G + \lambda)^2}{4\lambda k_B T}\right]$$

where J is the electronic coupling, ΔG is the driving force, λ is the reorganization energy, T is the temperature and k_B is Boltzmann's constant. As has been shown in structurally flexible donor−acceptor systems where the electronic coupling changes are much faster than the charge transfer process,[9] the coupling squared J^2 must be averaged over an ensemble of thermally accessible configurations.[10] We assume that the dependence of the hole hopping rate on DNA conformation is primarily due to the change in electronic coupling J due to thermal fluctuations, and that the reorganization energy and the driving force ΔG remain constant. Consequently, the charge transfer rate between two individual nucleo-bases is proportional to J^2. The values of the electronic coupling were calculated at 1 ps intervals from the molecular structures sampled from atomistic MD using a two-state Mulliken-Hush model[11] with the ZINDO Hamiltonian.[12] This semiempirical method has been shown to provide accurate estimates of the electronic coupling between stacked nucleobases.[13]

Representative structures from the stretching simulation of 5′-CAAAAAAAAAGC-3′ are shown in Figure 2 (top). At small elongations (<13 Å), the planes of the base pairs tilt in response to the applied force, but there is little disruption to either the hydrogen bonding interactions between complementary base pairs, or to the π-stacking interactions between bases on the same DNA strand. However, at extensions greater than 14 Å, the hydrogen bonds between a single pair of bases break at the center of the duplex, creating a "denaturation bubble" that disrupts the π-stack; a second denaturation bubble is created when the DNA is extended by 30.0 Å. The integrity of the π-stack for a given DNA structure can be determined from the angle between the planes of the nucleobases. This angle fluctuates around 20° for intact bases, but abruptly increases to values >90° on formation of a denaturation bubble. Figure 3 shows these angles as a function of extension for two representative base steps alongside the magnitude of the electronic coupling (J^2); for A4−A5 (which remain intact throughout the stretching procedure) and for A5−A6, which form part of a denaturation bubble at an extension of 13.5 Å. Data for the remaining base steps are provided as Supporting Information. The electronic coupling for the intact A4−A5 base pair exhibits large fluctuations, but remains above zero. When base pair A5−A6 forms a denaturation bubble after ~1.5 ns, there is a sudden reduction in the electronic coupling from an average of 0.09 eV, which is sufficiently high for

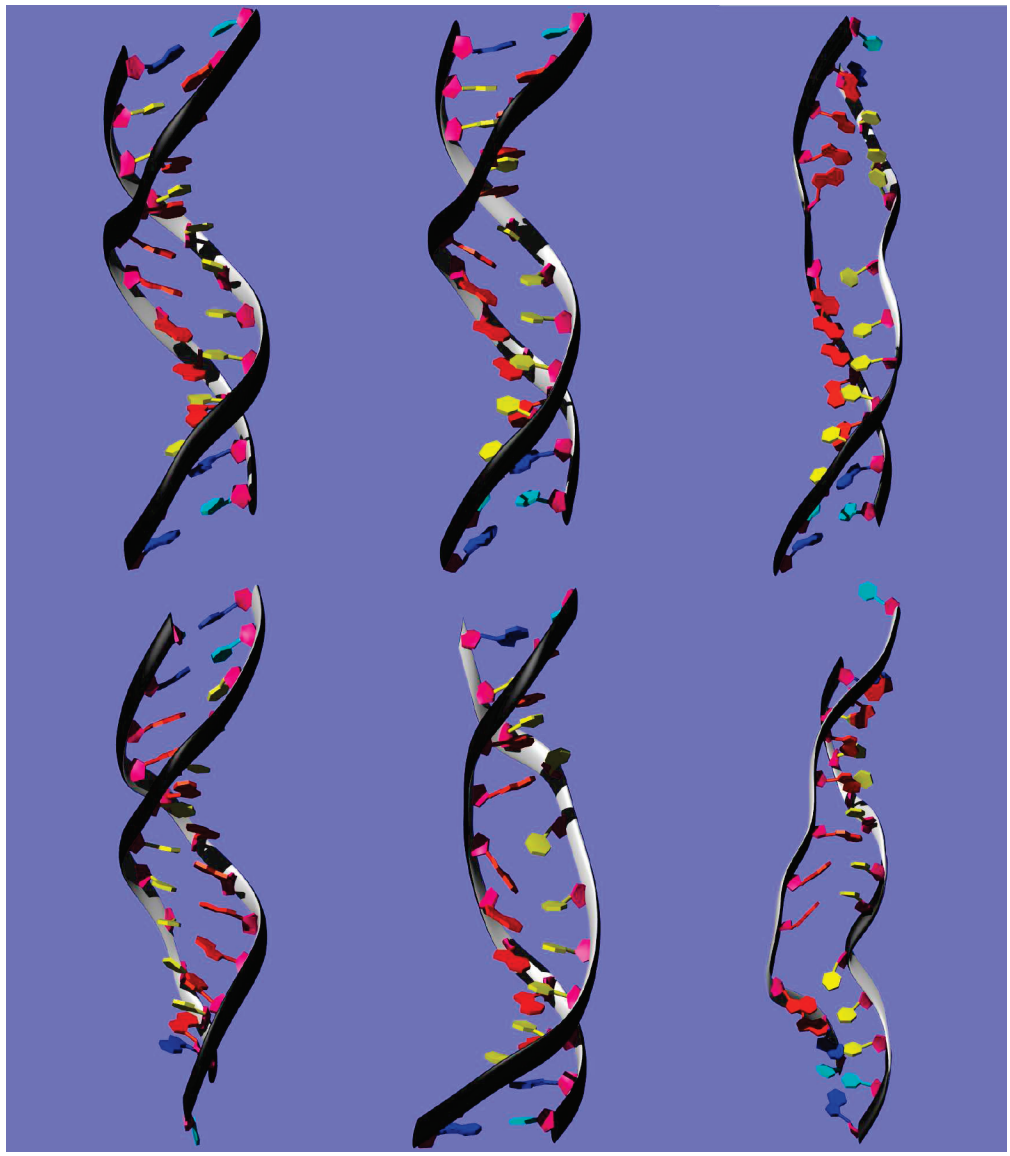

Figure 2. Starting geometry (top) and final geometry after 10 ns (bottom) for simulations of the duplex 5′-CAAAAAAAAAGC-3′ restrained at extensions of 13 Å (left), 14 Å (center) and 25 Å (right).

charge transport through the π-stack, to less than 10^{-5} eV, which indicates that the probability of charge transfer will significantly decrease. These calculations show that as soon as dsDNA is sufficiently extended for dena-

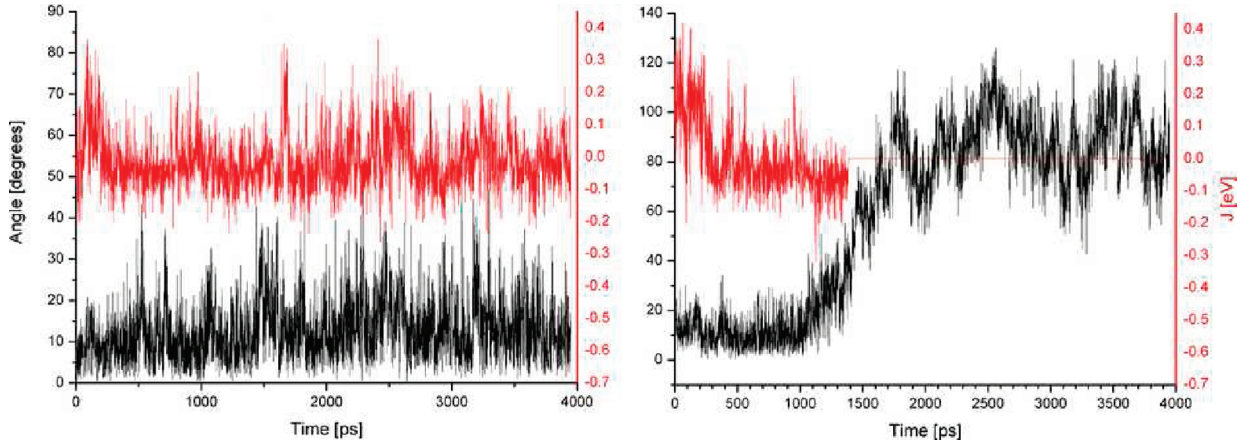

Figure 3. Angle between base planes (black) and the electronic coupling (red) during the stretching of 5′-CAAAAAAAAAGC-3′ showing the behavior of the representative intact base step A4−A5 (left) and the disrupted base step A5−A8 (right).

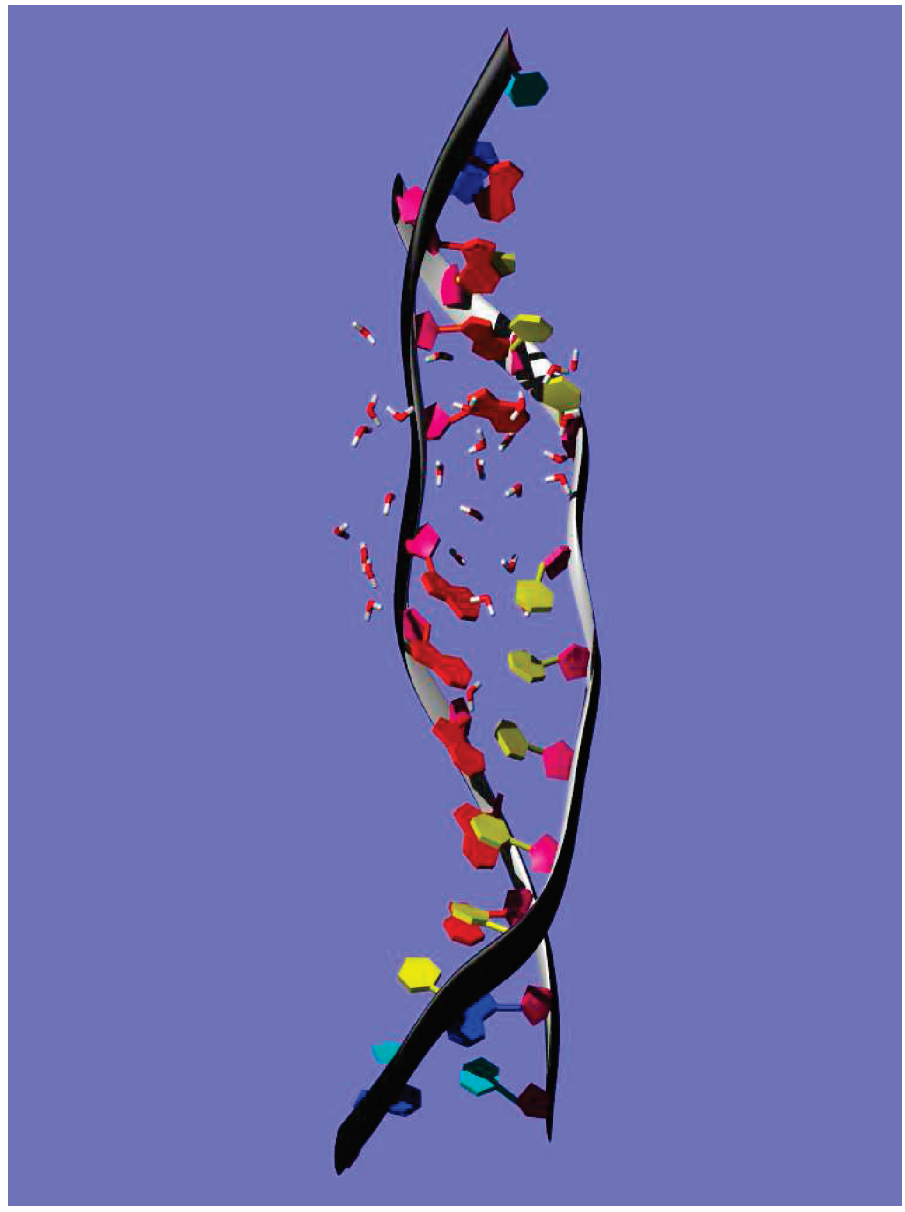

Figure 4. Denaturation bubble formed in 5′-CAAAAAAAAAGC-3′, showing the closest 20 water molecules which sit within the cavity.

turation bubbles to form within the duplex, hole hopping through the π-stack ceases. The effect of unpaired DNA bases can be particularly dramatic when charge transport takes place through stacks of guanines due to the possibility of deprotonating the hole-carrying guanine base to the neutral guanosyl radical, which is a far weaker oxidant.[8] Charge transport through DNA therefore depends critically on the integrity of the π-stack.

To prove conclusively that the formation of denaturation bubbles does indeed stop charge transfer, we also studied the possibility of charge transfer through the water molecules that sit inside the denaturation bubble. The 20 closest water molecules around A5 and A6 bases (see Figure 4) were extracted from the MD trajectories and the charge transfer couplings were calculated. On including the contribution from water, the coupling constant between A5 and A6 increased from 3×10^{-9} to 1.2×10^{-5} eV, still significantly less than the 0.09 eV for intact polyA base stack. Therefore, we conclude that the formation of the denaturation bubble is accompanied by a total disruption of charge transfer through the duplex.

To confirm that disruption of the π-stack does indeed correspond to a severe reduction in the ability of polyA tracts to act as hole conduits, we have also performed a series of 10 ns simulations in which the DNA is held at increasing extensions with external restraints. These simulations provide an ensemble of denatured structures for analysis of the magnitude of *J*, and its dependence on the integrity of the π-stack. These correspond to extensions where the DNA is intact (an exten-

sion of 13 Å), where one denaturation bubble is in the process of formation (14 Å), and a larger extension of 25 Å. The start and end structures for each of these three simulations are shown in Figure 2 (top and bottom respectively).

No disruption of the π-stack was observed during the simulation restrained at an extension of 13 Å. Figure 3 (and Figure S1 in the Supporting Information) show that the increase in angle between planes of nucleobases corresponds to a minor decrease in the absolute value of the electronic coupling, but does not have a major effect on charge transfer through the DNA. For example, for A8-A9 the magnitude of the electronic coupling is reduced from 0.085 to 0.068 eV, but hole hopping can still occur (see Figure S2g, Supporting Information). The simulation performed at the larger extension of 14 Å, however, shows that the complete disruption of the π-stack between A5 and A6 (Figure S3d, Supporting Information), which is present during the whole simulation, leads to an electronic coupling constant of 6×10^{-7} eV, which is sufficiently small to prevent hole hopping and halt charge transfer. Once the denaturation bubble has formed, charge transfer is disrupted, and remains so. Moreover, in the simulation performed at an extension of 25 Å, a second denaturation bubble forms involving bases A8 and A9 after 500 ps (Figure S4g, Supporting Information), which reduces the magnitude of electronic coupling from an average of 0.08 eV to a value that is effectively zero. Again, this leads to a complete and permanent cessation of charge transport through the DNA. We conclude that the charge transport properties of the duplex are only marginally affected by stretching, up until the point where the duplex starts to disassociate, at which point charge transfer is halted abruptly. We therefore propose that charge transfer measurements performed as a single DNA molecule is stretched will provide new insight into the force-induced melting transition. Moreover, these observations have potential nanotechnological applications, as they show that a device based on stretching and relaxation of a single DNA strand will provide a reversible nanomechanical switch.

Our calculations show that hole transfer can occur by hopping along purine bases until denaturation bubbles form in the stretched duplex. The π-stack is then significantly perturbed, and the resulting drop in the hopping rate so dramatic that the duplex becomes insulating. Experimental studies have shown that DNA stretching is reversible, so long as the forces applied do not go beyond the center of the overstretching transition.[3] In principle, after denaturation through stretching, the duplex could be reannealed by relaxing the force and would again conduct holes. The precise range of forces required for an effective, reversible device would depend upon details such as the time scale of operation, the temperature, the length and sequence of the DNA and would need careful calibration.

Biochemical studies have shown that charge transfer can be disrupted by base mismatches, abasic sites, or by enzymes capable of extruding single bases from the π-stack.[8,14] These calculations also illustrate that charge transfer measurements can act as an intimate reporter of duplex structure, and can provide structural information about the response of single DNA molecules to force, for example stretching by AFM or twisting with magnetic tweezers. If experimental procedures continue to be developed that are capable of manipulating the duplex while the change in charge transfer properties are simultaneously monitored, then this will provide unprecedented insight into the relationship between the mechanical properties of the duplex and the manner in which its structure changes in response to external perturbations.

These observations suggest the possibility of using individual duplex DNA strands either as a miniscule stress detector, or as a nanoscale electrical switch as part of a more complex molecular electronics device. Experimental methods already exist that exploit the changing conductance of ion channels to construct a molecular detector.[15] Potentially, mechanical stress on DNA coupled to charge transfer could provide an analogous device capable of detecting the on/off rates of proteins that loop DNA, for example.[16] The unique self-assembly properties, if combined with the additional property of switchability, would make DNA an even more important tool in single molecule technologies.

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Supporting Information Available: Graphs showing the electronic coupling and the angle between base planes for all base pairs during both the steered and restrained MD simulations. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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