SUPPORTING INFORMATION

to

"**On the hydrodynamic nature of DNA acoustic sensing**"

Achilleas Tsortos,[1,*] George Papadakis,[1] Electra Gizeli[1,2]

[1] Institute of Molecular Biology & Biotechnology, FO.R.T.H, Vassilika Vouton, 70013, Heraklion
[2] Department of Biology, University of Crete, Vassilika Vouton, 71409, Heraklion, Greece

A)
EXPERIMENTAL

Materials
Phosphate buffered saline (PBS) (pH 7.5, 150 mM NaCl) and Tris (50 mM, pH 7.5, 10 mM KCl) tablets were purchased from Sigma, Neutravidin was obtained from Pierce (Germany) and Invitrogen. Primers and oligos of HPLC purity were from Metabion (Germany), IDT (Belgium), Microchemistry (Heraklion), FRIZ Biochem (Germany) AND IDT (USA).

DNA design and preparation
A total of 23 dsDNA molecules were measured with the QCM sensor, i.e., DNAs of *20*, *30*, *50*, 60, *75*, *90*, *110*, *132*, *157*, *167*, *198*, 249, *270*, *297*, 361, 395, *422*, *524*, *689*, *852*, *1011*, *1294* and *1724* base pairs. Nineteen of the above molecules (shown in italics) were also measured with the Love wave sensor. Double stranded DNA molecules were produced by two methods:
a) Short fragments ranging from 20 up to 75 bp were produced by annealing a biotinylated oligonucleotide with a 10-fold excess of its non-biotinylated complementary strand either in PBS or in a buffer containing 10 mM KCl, 10 mM $MgCl_2$ and 50 mM Tris-HCl pH 7.5. More information on their sequence and hybridization conditions can be found elsewhere[1].
b) Longer DNA fragments up to 1724 bp were produced by standard PCR reactions following the instructions of the Taq polymerase supplier, either KAPA Biosystems (UK) or Minotech (Greece). PCR products were purified using a nucleospin kit (Macherey-Nagel, Germany). The production of longer DNAs was achieved using 6 different templates based on protocols described before: Human genomic DNA (636401, Clontech, USA)[2], pBR322 plasmid (Minotech, Greece)[3], mosquito genomic DNA extracted using DNAzol reagent from Kisumu laboratory colonies [4] and finally, extracted bacterial genomic DNA from *Pseudomonas syringae*, Xanthomonas *campestris* pv. *vesicatoria* and *Salmonella* Typhimurium cultures[5]. For acoustic measurements, one of the two PCR primers was biotinylated at its 5' end. Specific information on the sequences of the tested molecules can be found in previous publications [3-6]. In addition to the above, the 852bp DNA was produced from *Salmonella* genomic DNA using the following two primers: forward 5'-Biotin-GTC ACG GTG ATC GAT CCG GT-3' and reverse

5'-CAC GAT ATT GAT ATT AGC CCG-3' and under PCR conditions described in Ref.[7] for the purE *Salmonella* gene. The 1011 and 1294 bp fragments were produced from pBR322 plasmid using the forward primer 5'-TCT TGC TGG CGT TCG CGA CG-3' combined either with the reverse 5'-GTC TGG CTT CTG ATA AAG CGG GCC-3' (1011bp) or the 5'-GCC AGT ATAC ACT CCG CTA TCG CTA CG-3' (1294bp).

The ssDNA measured with the QCM had 2, 5, 10, 21, 50, 60, 75, 86 [1,8] and 110 nt of random sequence. The sequences of the 2, 5 and 10 ssDNAs were as follows:
2nt: 5' – GC – 3'
5nt: 5' - GTTGT – 3' or 5' - TCGAC – 3'
10nt: 5' - TAGAGCTCCC - 3'

Information on the sequences and preparation procedure for the triple stranded (ts) and Holliday junction (H) DNA molecules can be found in Refs [9,10].

Acoustic wave devices and instrumentation
Quartz Crystal Microbalance devices, purchased from Biolin (Sweden), were based on a standard Thickness Shear Mode (TSM) resonator configuration with a fundamental frequency of 5 MHz and a working range of 5-65 MHz. These devices were used with the Q-Sense D300 or E4 instruments (Biolin, Sweden) to measure insertion loss (D) and frequency (F) in real time. The Love wave acoustic device was prepared by photolithography using single-crystal Y-cut z-propagating 0.5 mm-thick quartz, with a 100nm gold overlayer and a 20nm chromium adhesion layer. The operating frequency of the SAW devices was 155MHz. A PMMA waveguide layer was deposited on the surface as described in previous publications[3]. A network analyzer (E5061A Agilent, Santa Clara, CA) was used to measure wave amplitude (A) and phase (Ph); data were collected using LabVIEW interface software (National Instruments, Austin, TX). A perspex flow cell and a silicone rubber gasket were used to hold the solution in place over the region between the IDTs. In both cases, i.e., the QCM and Love wave device, a peristaltic pump (Gilson, USA) was used to provide a constant flow of 20 μl/min.

Acoustic measurements
Prior to any experiment, the device surface was cleaned with Hellmanex (2%) and water, followed by air plasma etching in a Harrick (USA) chamber. A typical experiment involved initially addition of PBS buffer until a stable baseline was obtained; then a solution of 0.2 mg/ml of neutravidin in PBS was injected followed by buffer rinse. Biotinylated DNA molecules were then applied at various concentrations within the range of nM-μM depending on the DNA size; dsDNA was dissolved in PBS or Tris buffer while ssDNA was always applied in PBS. Results were not influenced by the buffer choice for dsDNA, not even by the addition of 10 mM $MgCl_2$; for ssDNA salt content was very important The triple stranded and the Holiday junction molecules were measured in Mes buffer[9,10] . In the case of the QCM device, energy dissipation and frequency shifts (F) were recorded for the 7th harmonic (i.e., 35 MHz) with the frequency data not divided by the harmonic. For determining the acoustic ratio, the frequency and dissipation changes were recorded and divided to produce a $\Delta D/\Delta F$ ratio. This ratio was shown for all DNA used in this work to be independent of their concentration[3], since the $\Delta D/\Delta F$ plot versus ΔF is a straight line parallel to the x axis.

Viscosity measurements and intrinsic viscosity determination
The intrinsic viscosity of a solute molecule dissolved in a solvent is determined from a graph depicting the viscosity η of the solution as a function of its concentration, and is defined as the value of η when the concentration is extrapolated to zero. For this reason, dsDNA samples were prepared at various initial

concentrations (~ 1.2-5.5 mg/ml) as determined with a spectrophotometer (NanoDrop, ND-1000). An automated microviscometer (AMVn-Anton Paar) was used to perform measurements at a fixed angle of 30 deg, with a 0.9 mm diameter glass capillary and a volume of 150 µl. Calibration was performed according to the supplier's instructions; each measurement was repeated at least three times. A similar protocol was followed for the measurement of ssDNAs, and at a 30 or 50 degrees angle. The temperature was 25ºC in all types of experiments. Information on the measurement of the Holliday junction 'open' and 'close' viscosities can be found in Ref. [9].

B)

The $[\eta]$ results for ssDNA (5, 21 and 86 bases) in PBS buffer are shown below (Figure S1). The linear fit gives

$$[\eta]_{ss} = 0.84\, M^{0.334}\ (ml\ /\ g) \qquad \text{(S1)}$$

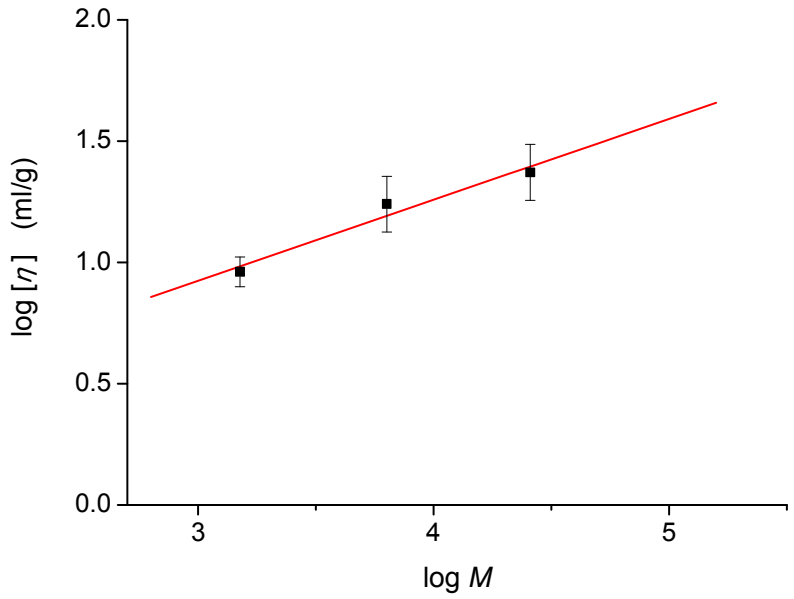

Figure S1. *The log-log plot of the Mark-Houwink scaling law for ssDNA*

C)

For ssDNA the reported l (distance between repeating units) is in the range 0.3-0.7 nm [11-14]. For the persistence length P we calculated the value ~ 2 nm for our buffer conditions[15]. The following Figure (S2) shows our attempt to obtain these (reliable) values for the persistence length and distance between repeating units in the ssDNA. Few data are available in the literature where the buffer conditions (pH and salt) are identical or close to ours, something very important for single stranded chains.

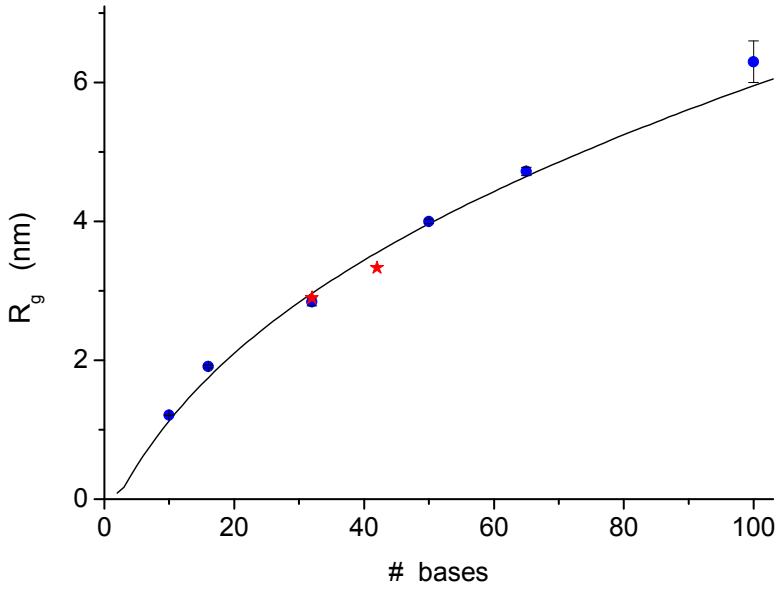

Figure S2. *The radius of gyration dependence on the number of bases in the ssDNA chain*

The calculation using the worm-like-chain model shown in Fig. S2 as a black line gives a good fit for the experimental data from *Ref.* [11] (blue dots) and *Refs.* [16,17] (red stars) using for (P, l) the values (2, 0.6) nm. It has to be noted that the drawn R_g line fits poly(dT) data [11,16,17] where base stacking is at a minimum; for a random sequence it is expected to be higher and thus a higher value for P could be used.

D)

For bent DNA Rivetti et al. [18] give the following formula regarding the end-to-end distance for a chain

$$R_{ee}^2 = 2PL\{1-\frac{P}{L}[(1-e^{\frac{-l}{P}})+(1-e^{\frac{-(L-l)}{P}})-\cos\beta(1-e^{\frac{-l}{P}})(1-e^{\frac{-(L-l)}{P}})]\} \qquad \text{(S2)}$$

where L is the total contour length and l the position of the bent (of angle β). In our case [3,6] the measured data are for (β, l) ≈ (62º, 1/3 L) and (75º, 1/2 L).

References

(1) Papadakis, G.; Tsortos, A.; Bender, F.; Ferapontova, E. E.; Gizeli, E. *Anal. Chem.* **2012**, *84*, 1854.

(2) Papadakis, G.; Gizeli, E. *Anal. Methods* **2014**, *6*, 363.

(3) Tsortos, A.; Papadakis, G.; Mitsakakis, K.; Melzak, K. A.; Gizeli, E. *Biophys. J.* **2008**, *94*, 2706.

(4) Papadakis, G.; Tsortos, A.; Kordas, A.; Tiniakou, I.; Morou, E.; Vontas, J.; Kardassis, D.; Gizeli, E. *Sci. Rep.* **2013**, *3*, 2033.

(5) Papadakis, G.; Skandalis, N.; Dimopoulou, A.; Glynos, P.; Gizeli, E. *PLoS One* **2015**, *10*, e0132773.

(6) Tsortos, A.; Papadakis, G.; Gizeli, E. *Biosens. Bioelectron.* **2008**, *24*, 836.

(7) Tsougeni, K.; Papadakis, G.; Gianneli, M.; Grammoustianou, A.; Constantoudis, V.; Dupuy, B.; Petrou, P. S.; Kakabakos, S. E.; Tserepi, A.; Gizeli, E.; Gogolides, E. *Lab Chip* **2016**, *16*, 120.

(8) Tsortos, A.; Grammoustianou, A.; Lymbouridou, R.; Papadakis, G.; Gizeli, E. *Chem. Commun.* **2015**, *51*, 11504.

(9) Papadakis, G.; Tsortos, A.; Gizeli, E. *Nano Lett.* **2010**, *10*, 5093.

(10) Papadakis, G.; Tsortos, A.; Gizeli, E. *Biosens. Bioelectron.* **2009**, *25*, 702.

(11) Sim, A. Y. L.; Lipfert, J.; Herschlag, D.; Doniach, S. *Phys. Rev. E* **2012**, *86*, 021901.

(12) Hansma, H. G.; Revenko, I.; Kim, K.; Laney, D. E. *Nucleic Acids Res.* **1996**, *24*, 713.

(13) Jing, T. W.; Jeffrey, A. M.; Derose, J. A.; Lyubchenko, Y. L.; Shlyakhtenko, L. S.; Harrington, R. E.; Appella, E.; Larsen, J.; Vaught, A.; Rekesh, D.; Lu, F. X.; Lindsay, S. M. *Proc. Natl. Acad. Sci. USA.* **1993**, *90*, 8934.

(14) Murphy, M. C.; Rasnik, I.; Cheng, W.; Lohman, T. M.; Ha, T. J. *Biophys. J.* **2004**, *86*, 2530.

(15) Tinland, B.; Pluen, A.; Sturm, J.; Weill, G. *Macromolecules* **1997**, *30*, 5763.

(16) Meisburger, S. P.; Sutton, J. L.; Chen, H. M.; Pabit, S. A.; Kirmizialtin, S.; Elber, R.; Pollack, L. *Biopolymers* **2013**, *99*, 1032.

(17) Chen, H.; Meisburger, S. P.; Pabit, S. A.; Sutton, J. L.; Webb, W. W.; Pollack, L. *Proc. Natl. Acad. Sci. USA.* **2012**, *109*, 799.

(18) Rivetti, C.; Walker, C.; Bustamante, C. *J. Mol. Biol.* **1998**, *280*, 41.