A LETTERS JOURNAL EXPLORING THE FRONTIERS OF PHYSICS

OFFPRINT

Short-fragment Na-DNA dilute aqueous solutions: Fundamental length scales and screening

S. Tomić, S. Dolanski Babić, T. Ivek, T. Vuletić, S. Krča, F. Livolant and R. Podgornik

EPL, **81** (2008) 68003

TAKE A LOOK AT THE NEW EPL

Europhysics Letters (EPL) has a new online home at
www.epljournal.org

Take a look for the latest journal news and information on:

- reading the latest articles, free!
- receiving free e-mail alerts
- submitting your work to EPL

www.epljournal.org

A Letters Journal Exploring the Frontiers of Physics

March 2008

EPL, **81** (2008) 68003 www.epljournal.org
doi: 10.1209/0295-5075/81/68003

Short-fragment Na-DNA dilute aqueous solutions: Fundamental length scales and screening

S. Tomić[1(a)], S. Dolanski Babić[1(b)], T. Ivek[1], T. Vuletić[1], S. Krča[2], F. Livolant[3] and R. Podgornik[4,5,6]

[1] *Institut za fiziku - HR-10001 Zagreb, Croatia*
[2] *Rudjer Bošković Institute - HR-10001 Zagreb, Croatia*
[3] *Laboratoire de Physique des Solides, Université Paris Sud - F-91405 Orsay, France*
[4] *Department of Physics, University of Ljubljana - SI-1000 Ljubljana, Slovenia*
[5] *J. Stefan Institute - SI-1000 Ljubljana, Slovenia*
[6] *Laboratory of Physical and Structural Biology, NICHD, National Institutes of Health - Bethesda, MD 20892, USA*

received 20 July 2007; accepted 28 January 2008
published online 25 February 2008

PACS 87.15.H- – Dynamics of biomolecules
PACS 82.39.Pj – Nucleic acids, DNA and RNA bases
PACS 77.22.Gm – Dielectric loss and relaxation

Abstract – Dielectric spectroscopy is used to investigate fundamental length scales of 146 bp short-fragment (nucleosomal) dilute Na-DNA solutions. Two relaxation modes are detected: the high- and the low-frequency mode. Dependence of the corresponding length scales on the DNA and on the (uni-valent) salt concentration is studied in detail, being different from the case of long, genomic DNA, investigated before. In low-added-salt regime, the length scale of the high-frequency mode scales as the average separation between DNAs, though it is smaller in absolute magnitude, whereas the length scale of the low-frequency mode is equal to the contour length of DNA. These fundamental length scales in low-added-salt regime do not depend on whether DNA is in a double-stranded or single-stranded form. On the other hand, with increasing added salt, the characteristic length scale of the low-frequency mode diminishes at low DNA concentrations probably due to dynamical formation of denaturation bubbles and/or fraying in the vicinity of DNA denaturation threshold.

Semiflexible polyelectrolytes are fundamental components of biological environment, ranging from charged polymers such as deoxyribonucleic acid (DNA) and proteins, all the way to molecular aggregates such as bacterial *fd* viruses and the tobacco mosaic virus [1]. DNA is in many respects a paradigm of a semiflexible highly charged polymer. In aqueous solutions it assumes a conformation of an extended statistical coil, whereas *in vivo* quite long genomic DNA is usually folded in dense and compact states to fit within the micron-sized nucleus of eukaryotic cells or even smaller viral capsids [2]. Such wide range of complex behaviors of DNA is due to its connectivity, stiffness and strong electrostatic interactions. To a large degree this behavior seen *in vivo* can be closely reproduced *in vitro* by tuning the DNA concentration, varying the amount of added salt, as well as valency of the counterions [2,3]. A full description and understanding of single DNA chain structure together with the structural organization of DNA chains in aqueous solutions are of fundamental importance in the study of living systems.

Let us first reiterate the results of a recent dielectric-spectroscopy study of polydisperse Na-DNA [4,5] in the case of semidilute aqueous solutions of long (2–20 kbp), genomic DNA. It revealed two relaxation modes that can be attributed to diffusive motion of DNA counterions, with fundamental length scales consistent with theoretical estimates [3,6,7]. Both relaxation modes are found to be strongly DNA concentration dependent, setting them apart from previously observed concentration-independent processes that are contingent on the degree of polymerization N (number of monomers in a given molecule) [8]. The measured fundamental length scale, probed by the low-frequency (LF) mode, characterizes single-chain properties, being equal to the size of the

(a) E-mail: stomic@ifs.hr
(b) Permanent address: Department of Physics and Biophysics, Medical School, University of Zagreb - Zagreb, Croatia.

Gaussian chain composed of correlation blobs that scales as $c_{\rm DNA}^{-0.25}$ in the low-added-salt limit [3]. In the high-added-salt limit the LF mode length scale equals the persistence length $L_{\rm p}$ and scales as $L_{\rm p} = L_0 + aI_{\rm s}^{-1}$ (in Å) which is nothing but the well-known Odijk-Skolnick-Fixman (OSF) result. Here L_0 is close to the structural persistence length of 500 Å and $I_{\rm s}$ is the ionic strength of the added salt (in M). On the other hand, the high-frequency (HF) mode is probing the collective properties of the DNA solution which are characterized by the de Gennes-Pfeuty-Dobrynin (GPD) correlation length or the mesh size, that scales as $c_{\rm DNA}^{-0.5}$. At low DNA concentrations and in the low-added-salt limit the LF mode length scale reflects the locally fluctuating DNA regions with partially exposed hydrophobic core that yields the scaling form $c_{\rm DNA}^{-0.33}$. Even when the denaturation protocol is applied, unzipping of the two DNA strands appears to be at most local and complete separation of the strands in semidilute solutions is never really accomplished. For both modes the high and the low salt are characterized by the competition between added salt and intrinsic DNA counterions.

Due to the size of DNA used in these experiments, all DNA length dependence was already saturated and none of the two modes show any. For shorter chains other experiments show that even N-dependent modes can often be concentration dependent [9], leading to a complicated interdependence of the two effects. Another intriguing issue is if and how the screening and fundamental length scales will change in the limit of very low DNA density, that is, below the semidilute-dilute crossover. Contrary to the semidilute regime, where polyelectrolyte chains are in general entangled with each other, the dilute regime with no or low added-salt is characterized by extended DNA conformations where each chain is well separated from all other ones, leading to an average separation between chains that scales as $c_{\rm DNA}^{-0.33}$ [3]. In addition, the low concentration of chains also affects the characteristics of the Manning-Oosawa counterion condensation which in fact takes place in two separate zones of volume associated with each chain [10].

In this letter we address some of these issues while trying to characterize the structure of dilute DNA solutions composed of short-fragment DNA. To this effect we used nucleosomal DNA (~ 146 bp) chains prepared as described previously [11]. The low protein content was verified and DNA concentration was determined by UV spectrophotometry. We perform a systematic investigation of how the dielectric properties of these monodisperse short-fragment DNA aqueous solutions evolve upon change of DNA concentration and added salt over a range of two to three orders of magnitude. DNA solutions were prepared as described previously (see *Materials and Methods* in ref. [5], preparation protocols I and II.3). Since the contour length $L_{\rm c}$ of DNA chains is on the order of 500 Å, the concentration of polyelectrolyte solutions that we deal with here is always below the chain overlap concentration c^* [3]. A very crude estimate of c^* based on de Gennes arguments [7] yields c^* of the order of 1 mg/mL, which is close to the upper concentration bound in our experiments[1]. This means that we are effectively always in dilute regime which, in contrast to the semidilute one, has not been much studied experimentally. Concurrently we have also considered to what extent the interpretation of our results depends on DNA conformation in low salt and in pure water solutions, more specifically on whether DNA is in a single-stranded or double-stranded form. Denaturation studies (performed by dielectric spectroscopy as described previously [5]) indicate that for these dilute conditions DNA double helix is denatured in pure water solutions for DNA concentrations below about 0.4 mg/mL, which corresponds to 1 mM of intrinsic DNA counterions[2]. This value is also in accord with results obtained by UV spectrophotometry on solutions of varying DNA and added-salt concentrations in that they gave a limit on the order of 1 mM of total counterions (intrinsic and added salt) below which double-stranded DNA denatures. We detected no change in the scaling behavior of fundamental lengths of the two dielectric modes as we cross these solution conditions; nevertheless, a decrease in magnitude of the LF length scale is observed in the presence of added salt in the vicinity of the denaturation threshold.

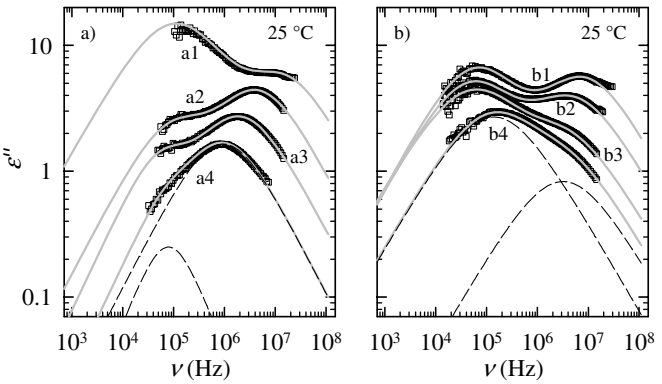

Fig. 1: Double logarithmic plot of the frequency dependence of the imaginary part of the dielectric function (ε'') at $T = 25\,^{\circ}$C of a) pure water 146 bp DNA solutions (for DNA concentrations a1–a4 (5, 0.5, 0.15, 0.05 mg/mL)) and b) 146 bp DNA solutions with added salt $I_{\rm s} = 1$ mM (for DNA concentrations b1–b4 (1.5, 0.8, 0.4, 0.3 mg/mL)). The full lines are fits to the sum of two Cole-Cole forms (see text); the dashed lines represent the single form.

Figure 1 shows the frequency-dependent imaginary part of the dielectric function for solutions with selected DNA concentrations. The results for pure water short-fragment DNA solutions are shown in panel a), while results for DNA solutions with added salt of ionic strength $I_{\rm s} = 1$ mM

[1] c^* is given by the concentration where there is only one polymer molecule in the volume of a polymer globule $c^* =$ molecule mass$/V_{\rm c}$, where the molecule mass is $N \cdot m_{\rm bp}$, and $V_{\rm c} \approx L_{\rm c}^3 = N^3 \cdot a^3$; $m_{\rm bp}$ is a mass of a base pair $\approx 10^{-18}$ mg, $N = 146$, $L_{\rm c} = N \cdot a$; $a = 3.4$ Å; a is the monomer size.

[2] The concentration of intrinsic counterions is given by $c_{\rm in}$ (mM) = $c_{\rm DNA}$ (mg/mL) $\cdot\, 3\,\mu$mol/mg.

are shown in panel b). The observed dielectric response is complex [12] and the data can only be successfully fitted to a formula representing the sum of two Cole-Cole forms $(\varepsilon(\omega) - \varepsilon_{\rm HF} = (\varepsilon_0 - \varepsilon_{\rm HF})/(1 + (i\omega\tau_0)^{1-\alpha}))$, equivalent to the Havriliak-Negami type with skewness parameter $\beta = 1$ [13]. The spectra consist of two broad modes that show a symmetrical broadening of the relaxation time distribution function described by the parameter $1 - \alpha \approx 0.8$. The mode in the high-frequency region (HF mode) has a strength $2 < \Delta\varepsilon_{\rm HF} < 15$ and is centered in the range $0.3\,{\rm MHz} < \nu_{\rm HF} < 15\,{\rm MHz}$. The mode in the low-frequency region (LF mode) has a strength $0.5 < \Delta\varepsilon_{\rm LF} < 50$ and for pure water solutions it does not move much in frequency remaining centered around 80 kHz ($60\,{\rm kHz} < \nu_{\rm LF} < 110\,{\rm kHz}$).

The polarization response of DNA solutions in the kHz–MHz range is due to an oscillating flow of net charge associated with DNA counterions induced by an applied ac field. Since the counterion displacement is facilitated through diffusion, the dielectric response is basically characterized by the mean relaxation time $\tau_0 \propto L^2/D_{\rm in}$, where L is the associated length scale, and $D_{\rm in}$ is the diffusion constant of counterions. This constant is sufficiently well approximated by the diffusion constant of bulk ions [12,14] leading to a value of $D_{\rm in} = 1.33 \cdot 10^{-9}\,{\rm m}^2/{\rm s}$. In other words, the fit to the Cole-Cole functions allows us to extract the characteristic time τ_0 and calculate the corresponding length scale for each of the relaxation modes. Similarly complex spectra have also been observed for long polydisperse DNA semidilute solutions [4,5]. However, the DNA concentration and added-salt dependence measured for short-fragment DNA are rather distinct, indicating that mechanisms of counterion relaxation for short-fragment as opposed to long Na-DNA solutions are not identical.

Let us first describe the characteristics of the HF mode. For pure water short-fragment DNA solutions the characteristic length $L_{\rm HF}$ increases with decreasing DNA concentration in almost three-decades-wide concentration range (main panel of fig. 2) following the power law $L_{\rm HF} \propto c_{\rm DNA}^{-0.33}$ as a function of the DNA concentration. In dilute solutions this scaling form is typical for the average distance between chains [3]. This result also confirms our claim in the case of long, genomic DNA that the HF relaxation process describes the collective structural properties of solution composed of many chains [4,5]. It is noteworthy that although the DNA double-helix appears to be denatured for $c_{\rm DNA} < 0.4\,{\rm mg/mL}$, the overall change in the prefactor of the scaling law is small and is within the error bar of the experiment. This result is not too surprising. First, the same scaling law is expected to be valid also for single-stranded DNA, and second, such a solution should contain twice the number of chains corresponding to only about 20% decrease in the average distance between chains, which is at the resolution limit of our measurement. Also, the two chains that partake in the organization of the common counterion cloud should

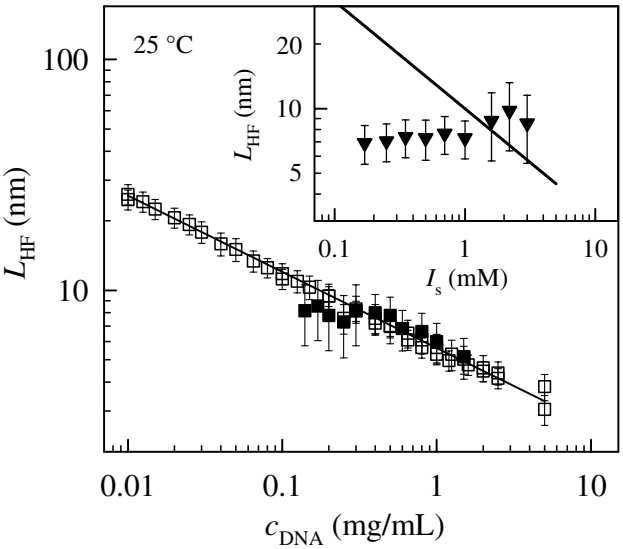

Fig. 2: Main panel: characteristic length of the HF mode ($L_{\rm HF}$) for pure water short-fragment DNA solutions (open squares) and for short-fragment DNA solutions with added salt $I_{\rm s} = 1\,{\rm mM}$ (full squares) as a function of DNA concentration ($c_{\rm DNA}$). The full line is a fit to the power law $L_{\rm HF} \propto c_{\rm DNA}^{-0.33}$. Inset: $L_{\rm HF}$ for short-fragment DNA solutions *vs.* added salt ($I_{\rm s}$) for $c_{\rm DNA} = 0.5\,{\rm mg/mL}$ (full triangles). The full line denotes the Debye screening length for the investigated range of added salt.

probably remain in relatively close proximity even after they are nominally dissociated. Furthermore, even at high DNA concentrations there is still no sign of a dilute-semidilute crossover, confirming our estimate that c^* is on the order of 1 mg/mL. Finally, we remark that in semidilute solutions, but only in semidilute solutions, this scaling form would be typical for charged chains with partially exposed hydrophobic cores [3].

With added 1 mM salt, the behavior of $L_{\rm HF}$ remains unchanged (main panel of fig. 2), thus $L_{\rm HF} \propto c_{\rm DNA}^{-0.33}$, as long as the concentration of intrinsic counterions $c_{\rm in}$ (proportional to $c_{\rm DNA}$) is larger than the concentration of added-salt ions. At lower DNA concentrations, the $L_{\rm HF}$ apparently shows a leveling-off, with a limiting value close to the Debye length appropriate for this salt concentration. A set of additional data (inset of fig. 2) for $c_{\rm DNA} = 0.5\,{\rm mg/mL}$ with varying added-salt concentration shows that $L_{\rm HF}$ does not change with $I_{\rm s}$ in most of the measured range. However, this behavior seems to remain even in the limit when the added-salt concentration is larger than the concentration of DNA intrinsic counterions, showing $L_{\rm HF}$ values apparently above the corresponding Debye length. This is in contrast to 1 mM data (shown in the main panel of fig. 2). We are tempted to believe that this apparent contradiction is related to a poorer accuracy of these data, in comparison to 1 mM ones, due to the progressive merging of the HF and LF modes when one approaches the regime of high added salt.

Second, we address the LF mode. For pure water short-fragment DNA solutions, the characteristic length

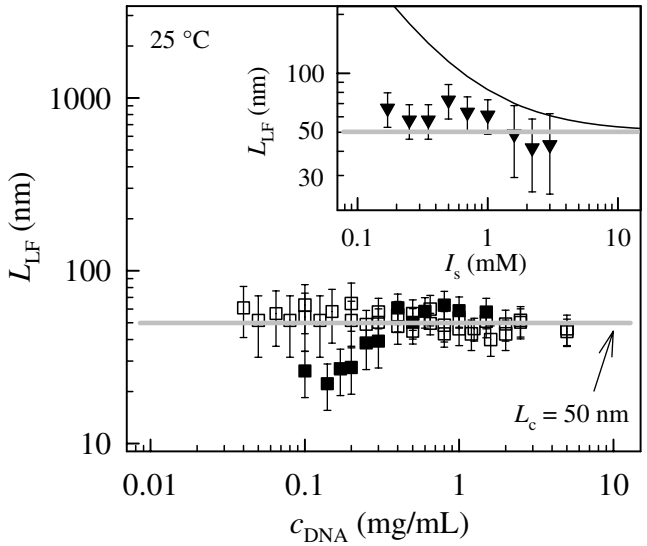

Fig. 3: Main panel: characteristic length of the LF mode ($L_{\rm LF}$) for pure water short-fragment DNA solutions (open squares) and for short-fragment DNA solutions with added salt $I_{\rm s} = 1\,$mM (full squares) as a function of the DNA concentration ($c_{\rm DNA}$). The full line denotes the contour length $L_{\rm c} \approx 500\,$Å. Inset: $L_{\rm LF}$ for short-fragment DNA solutions with varying ionic strength of added salt for $c_{\rm DNA} = 0.5\,$mg/mL (full triangles). The grey and full lines denote the contour length of studied short-fragment DNA and the persistence length as predicted by the OSF theory, respectively (ref. [6]).

$L_{\rm LF}$ remains approximately constant in more than two-decades-wide concentration range (open squares in fig. 3) at the level of $L_{\rm c} \approx 500\,$Å. The latter value suggests that in this regime $L_{\rm LF}$ is proportional to the contour length of the polyelectrolyte chain[3]. This result confirms that the LF relaxation represents a single-chain property [4,5]. For DNA solutions with added salt $I_{\rm s} = 1\,$mM (full squares in fig. 3) $L_{\rm LF}$ is the same as for pure water DNA solutions at high DNA concentrations. At the concentrations of intrinsic counterions $c_{\rm in}$ (proportional to $c_{\rm DNA}$) smaller than the concentration of added-salt ions, $L_{\rm LF}$ starts to deviate from the $L_{\rm LF} \propto L_{\rm c}$ behavior and decreases to attain a value of about 250 Å. Additional data (inset of fig. 3) for $c_{\rm DNA} = 0.5\,$mg/mL with varying added salt show that $L_{\rm HF}$ does not vary with $I_{\rm s}$ in most of the measured range of added salt. When the added-salt concentration is larger than the DNA concentration, the behavior of $L_{\rm LF}$ indicates only a minor decrease, in contrast to a more substantial one shown by 1 mM data (main panel of fig. 2). This discrepancy might be ascribed, similarly as for the HF process, to a poor accuracy of former data, which are severely influenced by high-salt environment. The added-salt–independent behavior in the limit of low-added-salt is not surprising since the contour length of the short-fragment DNA chains is on the order of the DNA intrinsic persistence length. This fact immediately excludes the effects of electrostatic interactions on the persistence length as predicted by the OSF theory [6] and shown for comparison in the inset of fig. 3.

In the dilute regime it is expected that the average distance between chains ($R_{\rm cell}$) is larger than the contour length. Our data (see figs. 2 and 3, main panels) are at variance with this expectation: although $L_{\rm HF}$ scales as expected for the average distance between chains, it is smaller than $L_{\rm LF}$ in the whole range of DNA concentrations. In order to rationalize this finding, we deem it plausible that $L_{\rm HF}$ corresponds to a shorter scale inside the spherical cell zone of the size $R_{\rm cell}$ around the polymer. Indeed, theoretically the dilute regime is modelled by placing the polymer at the center of a cell of size $R_{\rm cell} \propto c^{-0.33}$ that is subdivided into two zones [3,10]: a smaller cylindrical one, inside which the electrostatic-interaction energy is large, and a larger spherical zone, where the electrostatic interactions are described by the low coupling Debye-Hückel approximation. As a result, the response of DNA counterions to an applied ac field would be mostly confined to a smaller cylindrical volume within the large spherical volume of radius equal to the average distance between chains. We remark that a similar relationship between $L_{\rm HF}$ and the contour length in dilute regime, although not discussed, can be discerned in the data reported by Ito *et al.* (ref. [15]) and by Katsumoto *et al.* (ref. [16]).

It is noteworthy that the contour length remains the relevant single-chain length scale as long as the concentration of intrinsic counterions is larger than the concentration of added salt, as can be clearly noticed from fig. 4. In other words, the single-chain length scale is independent of the DNA concentration due to the fact that in the dilute regime interchain interactions are negligible compared to intrachain interactions, in contrast with the behavior of long DNA chains in semidilute solutions [4,5]. The data for $L_{\rm LF}$ deviate from the $L_{\rm c}$ only in the high-salt limit, for $2I_{\rm s} > 2c_{\rm in}$, where we expect that the effects of added-salt become dominant. In this regime on addition of salt, (fig. 4), we note that the fundamental single-chain length scale shrinks in size, becoming smaller than the nominal contour length of the chain. Taking into account that the short fragments of DNA are almost exactly one persistence length long, it is difficult to envision any decrease in the rigidity, as quantified by the persistence length, that would lead to a smaller effective contour length. A possible speculative explanation for this strange behavior could be sought in the incipient dynamic dissociation of the two strands of DNA that is fully established for DNA concentrations below 0.4 mg/mL. Short bubbles of separated strands along DNA and/or pronounced fraying at the two ends could shorten its

[3]For $c_{\rm DNA} < 0.2\,$mg/mL the LF mode decreases in strength and merges into the larger HF mode. In the range $0.04\,{\rm mg/mL} < c_{\rm DNA} < 0.2\,{\rm mg/mL}$, two-modes fits with constraint on the relaxation time of LF mode were done in order to keep $L_{\rm LF}$ close to $L_{\rm c}$. The constraint did not influence the parameters obtained for the HF mode. For $c_{\rm DNA} < 0.04\,$mg/mL, the LF mode is of negligible strength and completely overwhelmed by the HF one, so that only single-mode fits to the HF mode were performed.

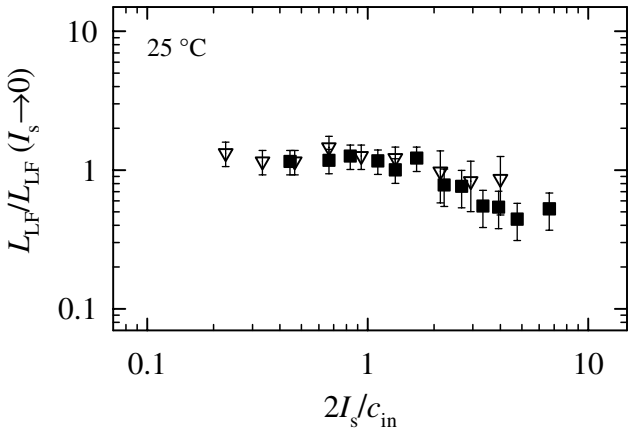

Fig. 4: Characteristic length of the LF mode ($L_{\rm LF}$) normalized with the value in pure water solutions $L_{\rm LF}(I_{\rm s}\to 0)\approx L_{\rm c}$ *vs.* added-salt concentration normalized by the concentration of intrinsic counterions ($2I_{\rm s}/c_{\rm in}$). Data are for one representative DNA concentration $c_{\rm DNA}=0.5\,$mg/mL and varying $I_{\rm s}$ (open triangles) and for $I_{\rm s}=1\,$mM and varying DNA concentrations (full squares).

effective contour length which would then correspond to the measured fundamental single-chain length scale.

In summary, our results demonstrate that the LF and HF processes, detected by dielectric-spectroscopy measurements of short-fragment DNA solutions, are associated with structural properties of single chains as well as with collective properties of the solution composed of many chains, respectively. In dilute conditions and in the low-added-salt limit, the characteristic lengths of the two relaxation modes are given either by the contour length of a single DNA chain or by the reduced average distance between chains of the whole solution, consistent with the two-zone model of counterion condensation [3,10]. In the high-added-salt limit for $2I_{\rm s}>2c_{\rm in}$ our data indicate that the added-salt effects are relevant for both length scales. For the single-chain length scale, salt apparently facilitates the formation of denaturation bubbles and/or fraying at the two ends close to DNA concentrations where the strand separation is clearly seen. For the collective length scale, on addition of salt the Debye screening length seems to take the role of the fundamental length scale for the whole solution. The latter statement should be taken with caution, however, due to poor accuracy of the data at higher added salt. Finally, our results indicate that, at the dilute DNA conditions and in low-added-salt regime, the same fundamental length scales are obtained, even under the conditions where the dissociation of the DNA strands is likely to be expected.

* * *

We would like to thank D. Rau and A. Parsegian for valuable and illuminating discussions. This work was supported by the Croatian Ministry of Science, Education and Sports under grant 035-0000000-2836. RP would like to acknowledge the financial support of the Agency for Research and Development of Slovenia under grant P1-0055(C) and partial financial support by the Intramural Research Program of the NIH, National Institute of Child Health and Human Development.

REFERENCES

[1] Daune M., *Molecular Biophysics* (Oxford University Press, New York) 2003.
[2] Bloomfield V. A., Crothers D. M. and Tinocco I. jr., *Nucleic Acids* (University Science Books, Sausalito) 2000.
[3] Dobrynin A. V. and Rubinstein M., *Prog. Polym. Sci.*, **30** (2005) 1049; Dobrynin A. V., Colby R. H. and Rubinstein M., *Macromolecules*, **28** (1995) 1859.
[4] Tomić S., Vuletić T., Dolanski Babić S., Krča S., Ivanković D., Griparić L. and Podgornik R., *Phys. Rev. Lett.*, **97** (2006) 098303.
[5] Tomić S., Dolanski Babić S., Vuletić T., Krča S., Ivanković D., Griparić L. and Podgornik R., *Phys. Rev. E*, **75** (2007) 021905.
[6] Odijk T., *J. Polym. Sci. B: Polym. Phys.*, **15** (1977) 477; Skolnick J. and Fixman M., *Macromolecules*, **10** (1977) 944.
[7] de Gennes P. G., Pincus P., Velasco R. M. and Brochard F., *J. Phys. (Paris)*, **37** (1976) 1461; Pfeuty P., *J. Phys. (Paris), Colloq.*, **39** (1978) C2-149.
[8] Takashima S., *J. Phys. Chem.*, **70** (1966) 1372.
[9] Molinari R. J., Cole R. H. and Gibbs J. H., *Biopolymers*, **20** (1981) 977.
[10] Deshkovski A., Obukhov S. and Rubinstein M., *Phys. Rev. Lett.*, **86** (2001) 2341.
[11] Sikorav J. L., Pelta J. and Livolant F., *Biophys. J.*, **67** (1994) 1387.
[12] Bordi F., Cametti C. and Colby R. H., *J. Phys.: Condens. Matter*, **16** (2004) R1423.
[13] Havriliak S. and Negami S., *J. Polym. Sci. C*, **14** (1966) 99.
[14] Angelini T. E., Golestanian R., Coridan R. H., Butler J. C., Beraud A., Krisch M., Sinn H., Schweizer K. S. and Wong G. C. L., *Proc. Natl. Acad. Sci. U.S.A.*, **103** (2006) 7962.
[15] Ito K., Yagi A., Ookubo N. and Hayakawa R., *Macromolecules*, **23** (1990) 857.
[16] Katsumoto Y., Omori S., Yamamoto D., Yasuda A. and Asami K., *Phys. Rev. E*, **75** (2007) 011911.