Available online at www.sciencedirect.com

ScienceDirect

Physics of Life Reviews ••• (••••) •••–•••

PHYSICS of LIFE reviews

www.elsevier.com/locate/plrev

Comment

Open questions about DNA melting Comment on "DNA melting and energetics of the double helix" by Maxim Frank-Kamenetskii et al.

Felix Ritort [a,b,*]

[a] *Physics School, Condensed Matter Physics Department, University of Barcelona, C/ Marti i Franques s/n, 08028 Barcelona, Spain*
[b] *Ciber-BBN Center for Bioengineering, Biomaterials and Nanomedicine, Instituto de Salud Carlos III, Madrid, Spain*

Received 1 March 2018; accepted 5 March 2018

Communicated by E. Shakhnovich

In this review paper [1] the authors have presented a synthetic and brief account of our current understanding of the energetics of the melting process in DNA molecules. The authors highlight open questions related to the thermodynamics of the nearest neighbor model comparing performances of a few selected currently available thermodynamic datasets. They also discuss the kinetics of the melting process emphasizing its relevance for practical applications where melting is often carried out under irreversible conditions. DNA melting is the process by which the two complementary strands of a DNA molecule dissociate into two single strands [2–4]. DNA melting can be achieved by a series of methods such as heating, adding denaturant to the solvent or mechanically pulling the two ends. Despite of its fundamental importance in biology (local DNA melting is a essential process in gene regulation) and biotechnology (DNA melting is a step of PCR amplification reactions) the fundamental understanding of this process is not fully understood.

The dissociation of DNA into two strands shares some of the characteristics of a first order phase transition (such as the melting of a solid or the evaporation of a liquid) but with marked differences. Whereas ordinary first-order phase transitions occur at well-defined conditions of temperature and pressure, DNA melting occurs gradually in a series of steps. The resulting melting curve depends strongly on the DNA sequence exhibiting multiple transition events rather than a single sharp transition. Half a century ago, Devoe and Tinoco [5] and Crothers and Zimm [6] proposed a simple model for the energetic stability of double helical structures. The nearest neighbor (NN) model for nucleic acids predicts the free energy of formation of a duplex from its dissociated strands by summing all energy contributions of adjacent nearest neighbor stacks along the sequence. The motivation behind the NN model originates from the fact that hydrogen bonding is not enough to explain the stability of the double helix. With only hydrogen bonding DNA might form a ladder rather than a helix. However the dipole-dipole induced attraction between adjacent bases makes the two strands twist around each other making DNA collapse into a double helix. In principle the total number of NN energies equals 16 (4×4), however there are only 10 due to the complementary strand symmetry. The additional circular symmetry (the total number of A and G equals to the total number of T and C, respectively) reduces the total

DOI of original article: https://doi.org/10.1016/j.plrev.2017.11.012.
* Correspondence to: Physics School, Condensed Matter Physics Department, University of Barcelona, C/ Marti i Franques s/n, 08028 Barcelona, Spain.
E-mail address: ritort@ub.edu.

https://doi.org/10.1016/j.plrev.2018.03.009

number of free parameters from 10 to 8. The actual number of parameters is however larger: to the total number of entropies and enthalpies of each NN motif ($8 \times 2 = 16$) one should also add the so-called initiation factors (at least 2, accounting for GC and AT free ends) resulting in a total of, at least, 18 parameters making the NN model sufficiently complex. During the past 30 years a large number of laboratories were committed into deriving the different energy parameters from large datasets of melting temperatures of short duplexes and long polymers in varied conditions such as GC content, oligo concentration and length, monovalent and divalent salt, etc. However the different experimental methods used (calorimetry, optical, etc.), the specific set of sequences analyzed, the precise definition of the melting temperature and the various approximation schemes used for the NN parameters lead to datasets which consistency is difficult to evaluate. In 1998 John Santalucia proposed [7] a unified dataset of energy values and initiation factors melding together his own results with those derived by several other labs worldwide. This has led to the so-called unified oligonucleotide (UO) dataset [8] widely used by several prediction tool web servers. The UO energy values have been recently validated by single molecule force methods by unzipping long DNA sequences [9,10]. This is remarkable given the fact that the dissociated state is completely different in both cases (a random coil upon melting and a stretched single stranded DNA upon unzipping) and that melting experiments report on melting temperatures whereas unzipping experiments measure free energy differences. Therefore there is a pretty large consensus on the reliability of the NN model and its energy parameters.

However, there still remain questions to be understood. One is the different performance of the currently available NN energies in long and short duplexes. While the prediction of differential melting curves is reasonably good in the case of long polymers, the same cannot be said of short DNA duplexes where the nearest neighbor model systematically underestimates the melting temperature values. The reason of such deviation is not known. Single molecule unzipping studies showed the same effect upon implementation of the nearest neighbor model with 10 parameters and the UO initiation factors [9]. Recent single molecule studies that include the circular symmetry of the NN model and that directly extract initiation factors from unzipping force data show however a better agreement between predicted and measured melting temperatures [10]. This suggests the importance of accurately determining initiation factors for melting temperature prediction of short duplexes where end effects are expected to be stronger. The question remains open and requires further research.

Another interesting question raised by the authors is the relative strength of the base stacking and base pairing interaction for the helix elongation parameters. A main feature of melting temperature prediction is the so-called Marmur–Doty plot, i.e. the observed linear dependence between the melting temperature of duplexes and the fraction of GC base pairs in the sequence [11]. Such linearity suggests hydrogen bonding (rather than base stacking) as the main contribution to duplex stability. Similar dependences have been studied for the dependence of melting temperature as a function of monovalent and divalent salt concentration [12,13]. In the NN model the free energy of formation and the melting temperature of a DNA molecule is expected to exhibit a quadratic dependence on the fraction of GC content, however such dependence has not been observed suggesting that either the contribution between stacking and hydrogen bonding is not purely additive or that enthalpy-entropy compensations occur that make the quadratic term negligible [14]. The question of the relative weight between the base pairing and the stacking terms has been addressed in two studies. One is based on the electrophoretic motion of nicked DNA molecules across polyacrylamide gels at different denaturing conditions [15], whereas the other is based on single molecule studies of DNA origami thick fibers containing blunt ends pulled by mechanical forces [16]. The results of Ref. [15] suggest that the main contribution to duplex stability is stacking of neighboring bases, the enthalpy of hydrogen bonding being negligible (one tenth approximately) as compared to stacking. However the free energies obtained from both approaches are in disagreement possibly because the different experimental assays (which require extrapolating results to zero denaturant concentration in one case and zero force in the other case) measure free energies along different pathways requiring different entropy correction terms. The reconciliation of these two studies [15,16] and the understanding of the linearity of the Marmur–Doty plots remains an open question.

Another important topic discussed in the paper is the question of the kinetics of melting and hybridization that shows strong hysteresis in denaturation–renaturation cycles. Nucleation of a few base pairs is often required for renaturation to occur meaning that the transition state is close to the dissociated state. The long timescales expected for thermalization (about several minutes for a few hundred base pairs DNA) make experiments difficult to interpret. Force unzipping experiments with temperature controlled optical tweezers may offer a new experimental window to accurately quantify such kinetic phenomena. Moreover the denaturation–renaturation timescales might be tuned by force making possible to observe bubble formation and strand fraying under reversible conditions [17].

Please cite this article in press as: Ritort F. Open questions about DNA melting. Phys Life Rev (2018), https://doi.org/10.1016/j.plrev.2018.03.009

DNA melting is one of the most essential and fascinating molecular transformations in biophysics that still hides secrets for the scientists. The paper by Vologodskii and Kamenteskii offers a fresh account of some of these problems still awaiting for new theoretical and experimental developments.

References

[1] Vogolodskii A, Frank-Kamenetskii M. DNA melting and energetics of the double helix. Phys Life Rev 2018. https://doi.org/10.1016/j.plrev.2017.11.012 [in this issue].
[2] Frank-Kamenetskii M. Unraveling DNA. Cambridge University Press; 1997.
[3] Calladine C, Drew H, Luisi B, Travers A. Understanding DNA: The molecule and how it works. 3rd ed. Amsterdam: Elsevier Academic Press; 2004.
[4] Vogolodskii A. Biophysics of DNA. Cambridge, UK: Cambridge University Press; 2015.
[5] Devoe H, Tinoco IJ. The stability of helical polynucleotides: base contributions. J Mol Biol 1962;4:500–17.
[6] Crothers DM, Zimm BH. Theory of the melting transition of synthetic polynucleotides: evaluation of the stacking free energy. J Mol Biol 1964;9:1–9.
[7] SantaLucia JJ. A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. Proc Natl Acad Sci USA 1998;95:1460–5.
[8] SantaLucia J Jr, Hicks D. The thermodynamics of DNA structural motifs. Annu Rev Biophys Biomol Struct 2004;33:415–40.
[9] Huguet JM, Bizarro CV, Forns N, Smith SB, Bustamante C, Ritort F. Single-molecule derivation of salt dependent base-pair free energies in DNA. Proc Natl Acad Sci USA 2010;107:15431–6.
[10] Huguet JM, Ribezzi-Crivellari M, Bizarro CV, Ritort F. Derivation of nearest-neighbor DNA parameters in magnesium from single molecule experiments. Nucleic Acids Res 2017;45:12921–31.
[11] Marmur J, Doty P. Heterogenity in deoxyribonucleic acids: I. Dependence on composition of the configurational stability of deoxyribonucleic acids. Nature 1959;183:1427–9.
[12] Owczarzy R, Moreira B, You Y, Manthey JA, Huang L, Behlke MA, et al. Effects of sodium ions on DNA duplexoligomers: improved predictions of melting temperatures. Biochemistry 2004;43:3537–54.
[13] Owczarzy R, You Y, Moreira B, Manthey JA, Huang L, Behlke MA, et al. Predicting stability of DNA duplexes in solutions containing magnesium and monovalent cations. Biochemistry 2008;47:5336–53.
[14] Protozanova E, Yakovchuk P, Frank-Kamenetskii MD. Stacked–unstacked equilibrium at the nick site of DNA. J Mol Biol 2004;342:775–85.
[15] Yakovchuk P, Protozanova E, Frank-Kamenetskii MD. Base-stacking and base-pairing contributions into thermal stability of the DNA double helix. Nucleic Acids Res 2006;34:564–74.
[16] Kilchherr F, Wachauf C, Pelz B, Rief M, Zacharias M, Dietz H. Single-molecule dissection of stacking forces in DNA. Science 2016;353:aaf5508.
[17] De Lorenzo S, Ribezzi-Crivellari M, Arias-Gonzalez JR, Smith SB, Ritort F. A temperature-jump optical trap for single-molecule manipulation. Biophys J 2015;108:2854–64.

Please cite this article in press as: Ritort F. Open questions about DNA melting. Phys Life Rev (2018), https://doi.org/10.1016/j.plrev.2018.03.009