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Comment

# The elusive keys to nucleic acid stability
## Comment on "DNA melting and energetics of the double helix" by Alexander Vologodskii and Maxim D. Frank-Kamenetskii

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Although nucleic acid structure and DNA melting have been studied for sixty years, Maxim Frank-Kamenetskii and Alexander Vologodskii demonstrate through careful analysis of the literature that many aspects of the field are still surprisingly poorly understood [1]. Decades of experiments using a variety of techniques have refined energy estimates of various sequences and even led to predictive models for a wide variety of solution conditions [2–4]. The greatest strength of the current review is the willingness of the authors to analyze existing models in detail while quantitatively comparing each model's prediction with available measurements. These comparisons demonstrate that current models cannot predict DNA stability with an accuracy greater than a few degrees for even the simple case of short DNA oligomers. This work therefore highlights the need for further measurements while also providing a definitive statement about the reliability of current models, allowing workers to determine an uncertainty in predictions made by these models.

The authors begin with standard models for DNA stability that can be found in classic reference texts [5–7] in addition to a more recent treatment [8]. However, none of these texts provides a detailed comparison of available data with the results of these standard models, as is provided here for the first time. The authors also discard major competing models such as coarse-grained microstate-based models as well as predictions from molecular dynamics simulations. While detailed, quantitative justifications for discarding the models are not presented, it is true that these models are not typically used by workers in the field when trying to predict DNA stability. The microstate-based models were also reviewed in detail in an earlier work in this journal [9]. Overall, the treatment of the simplest phenomenological models for DNA stability is the most recent and authoritative one available, providing a good basis for evaluating how well we understand the fundamental nature of sequence-dependent DNA stability. The model for long DNA molecules works particularly well, indicating that the overall features and even some details of the stability of long DNA molecules is reproduced well by a relatively simple model for DNA stability.

The most surprising difficulty comes when the authors attempt to predict the stability of short DNA sequences. The ability to make such predictions is critical for DNA biotechnology applications. To analyze the stability of short duplexes, the authors determine the free energy of DNA melting from the constituent enthalpy and entropy ($\Delta G^{helix} = \Delta H^{helix} - T\Delta S^{helix}$) using values for these parameters for all possible base pair contacts obtained from a variety of

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previous studies. Parameters are determined from measurements using several different techniques, including thermal melting experiments on DNA restriction fragments [10–12], oligonucleotides [13] and DNA dumbbells [14,15], as well as force unzipping experiments [16]. They then obtain variations in the expected melting temperature ($\delta T_m$) with overall G-C content ($x_{G\text{-}C}$) as well as the $\delta T_m$ for an array of 92 duplexes previously measured. The authors conclude that, while many of these experiments adequately capture the melting temperature to within a few degrees, there is significant disagreement over the underlying energy and enthalpy, and some experiments did not result in a set of parameters that matched the consensus from the other experiments, as discussed previously [17]. Here the authors do verify that the selected parameter sets all give reasonably linear dependence on GC content. Surprisingly, even the consensus experimental parameters found above do not improve estimates of the melting temperature over the simplest theoretical models, which exclude stacking heterogeneity. Thus, although comprehensive sets of experiments on multiple DNA constructs exist, further studies are needed to obtain accurate and predictive models for the stability of short DNA duplexes.

The second major contribution of this work is to revisit the contributions of base stacking and pairing to duplex stability. Earlier work featured a clever experiment that utilized short strands nicked in specific sites, measuring oligomer energies in the absence of selected stacking interactions [18]. The results convincingly demonstrated that the stability of the double helix is almost entirely due to base stacking interactions, while base pairing is either weak or even detrimental to the overall DNA structure. However, it is still very common to assume the opposite – that the three hydrogen bonds of G-C pairs are responsible for higher stability relative to A-T pairs, which contain two hydrogen bonds. The authors discuss the reasons behind this common assumption, the linear dependence of duplex stability on GC content, as well as the more intuitive structural picture that arises from characterizing base pairs as primarily due to hydrogen bonds. This is an important discussion, as reconciling the intuitive picture with existing measurements is critical for workers to gain a useful understanding of DNA stability [19,20].

The authors compare their previous measurements of stacking parameters using nicked strands with recent single molecule experiments that measured the stacking interactions directly [21]. Because these two measurements obtain significantly different stacking parameters, it is important to compare the results. To test the results, they compare the calculated dependence of the melting temperature on GC base-pair content, which is known to be linear. Because the results for the earlier studies are quite linear with GC content, while the single molecule parameters are not, the authors conclude that the single molecule parameters are not accurate. However, these parameters were determined by direct unstacking measurements, so it is not clear why the results are not linear with G-C content. As the authors state, it could just be that the signal to noise is too low. Indeed, one must be careful about extrapolation of force-dependent measurements to zero force because the extrapolation is along an exponentially varying parameter, which amplifies uncertainty. On the other hand, the uncertainties in force dependence should be reflected in the resulting parameters, and single molecule measurements of DNA and RNA hairpins are able to accurately determine their stability when compared to mfold predictions, which contains parameters determined from melting experiments [22–24]. In any case, the single molecule measurements of DNA stacking parameters should be revisited to determine the reason that the resulting parameters do not produce a linear dependence of the melting temperature on G-C content.

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