Cross-platform comparison of nucleic acid hybridization: Toward quantitative reference standards

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Abstract

Measuring interactions between biological molecules is vitally important to both basic and applied research as well as development of pharmaceuticals. Although a wide and growing range of techniques is available to measure various kinetic and thermodynamic properties of interacting biomolecules, it can be difficult to compare data across techniques of different laboratories and personnel or even across different instruments using the same technique. Here we evaluate relevant biological interactions based on complementary DNA and RNA oligonucleotides that could be used as reference standards for many experimental systems. We measured thermodynamics of duplex formation using isothermal titration calorimetry, differential scanning calorimetry, and ultraviolet–visible (UV–vis) monitored denaturation/renaturation. These standards can be used to validate results, compare data from disparate techniques, act as a teaching tool for laboratory classes, or potentially to calibrate instruments. The RNA and DNA standards have many attractive features, including low cost, high purity, easily measurable concentrations, and minimal handling concerns, making them ideal for use as a reference material.

Characterizing interactions between and within biomolecules can be critical to understanding biology and to devising pharmaceuticals to intervene where normal biological pathways fail [1,2]. A small arsenal of techniques is available to measure various aspects of biomolecular interactions, including surface plasmon resonance [3], nuclear magnetic resonance [4], isothermal titration calorimetry (ITC) 1 and differential scanning calorimetry (DSC) [5], polyacrylamide gel electrophoresis [6], fluorescence [7], and the scintillation proximity assay [8]. Considering the wide range of techniques, it can be challenging to interpret different results across various platforms. This is especially true in quantifying nucleic acid interactions with other nucleic acids, proteins, and small molecules.

To this end, it would be useful to have a well-characterized cross-platform biological reference standard. Although many instruments use internal calibration standards (e.g., acid–base titrations in ITC [9,10]), these standards are often specific for a single instrument and not transferable to other techniques and may have little to no relevance to biological macromolecular interactions. We believe that nucleic acids could be an ideal cross-platform reference for several reasons. First, nucleic acid–base pairing interactions are “tunable” based on the base content and length. Second, oligonucleotides are readily available at low cost and high purity. Third, oligonucleotides can be readily modified with a wide array of labels. Finally, oligonucleotides can be stored and handled without degradation.

We designed and evaluated a set of nucleic acids to be used as references for measuring biological interactions. We designed complementary 11-mer oligonucleotides in both DNA and RNA containing negligible secondary structure as well as truncated versions consisting of seven nucleotides. We chose to evaluate both DNA and RNA because each has practical advantages depending on the usage, with DNA being cheaper and more stable and RNA being useful where RNase contamination is of concern. We measured the thermodynamic properties of the oligo duplexes with ITC, DSC, and ultraviolet–visible (UV–vis) monitored denaturation/renaturation (melting) experiments. Our results provide useful reference points for researchers measuring biomolecular interactions with these or other methods.

Materials and methods

Materials

Eight oligonucleotides were purchased from Integrated DNA Technologies with standard desalting and deprotection (Table 1).
The manufacturer claims an average coupling efficiency of 99.2% per reaction, giving approximate full-length yields of 92% for 11-mers and 95% for 7-mers. We verified the purity of the oligos using an ultra-performance liquid chromatography (UPLC) instrument (Waters) and gel electrophoresis and found a dominant single peak consistent with published purities. Phosphate-buffered saline (PBS) buffer was made from a 10× concentrated solution (Fisher Scientific, with the 1× solution consisting of 11.9 mM phosphates, 137 mM sodium chloride, and 2.7 mM potassium chloride at pH 7.4).

Extinction coefficient measurements

Extinction coefficients for the eight oligos were determined by monitoring UV absorbance of individual oligo solutions during digestion by phosphodiesterase I as described previously [11]. Disposable semi-mini UV transparent cuvettes (Brand Scientific) were filled with 1.6 ml of PBS and individually zeroed. Concentrated oligos were added to each cuvette except for one blank (20 μl for DNA and 30 μl for RNA to give a final absorbance at 260 nm between 0.1 and 0.25 OD

\[\text{OD}_{260}/\text{C}_{260}\]

mixed before measuring the absorbances again. Phosphodiesterase I (10 μl) from snake venom (Sigma) was added to each cuvette. The extinction coefficient at room temperature was calculated by applying the sum of nucleotide absorbances to the hydrolyzed oligos and extrapolating to the room temperature absorbances [11,12].

Isothermal calorimetry

All ITC experiments were performed using the low-volume Nano ITC (TA Instruments) at 25 and 37 °C. The instrument was factory calibrated using KHCO$_3$–HCl titrations to determine the effective cell volume. The injection syringe was calibrated by mass of water [9,13]. Experiments consisted of a solution of one oligo (the “B” strand) being titrated into a solution of its complement in the cell (the “A” strand) using 20 2.5-μl injections spaced 200 s apart with 350-rpm stirring. The burette was driven to 99% volume before the syringe was loaded to minimize the effects of mechanical backlash [14]. Experiments were performed in triplicate at various temperatures using the same stock solutions where possible. Integration of the heat curves was performed using NanoAnalyze software (TA Instruments), with automated baseline correction at all temperatures. Averaged curves were converted to curves of fraction folded as described elsewhere [15]. This constant baseline was taken as a sin-

\[a\]

for enthalpy, stoichiometry, and

\[K_a\]

were determined by using the mean and standard deviation of the fitted results for each of the triplicate experiments.

UV–vis experiments

All UV melting experiments were performed in an Agilent Cary 100 system. Semi-micro quartz cuvettes held 1 ml of DNA or RNA solution at various concentrations. The concentrations used were serially diluted by 2× from stocks with absorbances near 2.0, such that the range extended to absorbances as low as 0.05. The temperature was ramped between 10 and 90 °C at 2 °C/min for duplex melting experiments, and these conditions varied slightly for individual strand melting experiments (melting temperatures were unaffected by changing ramp speeds from 1 to 2 °C/min). The temperature was monitored in a dummy cuvette at the height of the light path. Experiments were performed in triplicate, and curves were averaged. Normalizing raw data traces by the high temperature absorbance collapsed all three curves together, suggesting that variation between curves is mostly due to evaporation. Forward and reverse curves were also superimposable in this way, suggesting that our ramp rate was slow enough to allow for equilibration at all temperatures. Averaged curves were converted to curves of fraction folded as described elsewhere [16]. Regions of full association and dissociation were chosen differently for each of the four duplexes (dependent on the melting temperature) but the same for various concentrations of each duplex. Two types of melting analysis were performed: (i) van’t Hoff analysis and (ii) analysis of the concentration dependence of the melting temperature. For van’t Hoff analysis, the highest concentration curve was used to determine the derivative of ln(K$_A$) with respect to (1/T) at the melting temperature. This value was multiplied by the gas constant R to determine the ΔH$_{vis}$ for the interaction. Error was taken as 2% based on the magnitude of local variations in the value of the derivative. For the concentration dependence of melting temperature, 1/T$_{m}$ was plotted against ln(C), where C is the concentration of oligo. These data, with error bars representing 0.5 °C in temperature and 10% in concentration, were fit with a line. Dividing the gas constant R by the slope of the line was used to determine ΔH$_{conc}$. These analysis methods are presented in detail elsewhere [16].

Differential scanning calorimetry

All DSC experiments were performed on a Nano DSC instrument (TA Instruments) with a 300-μl cell volume. Duplexes were formed by mixing the complementary DNA or RNA strands in equimolar concentrations from various stock concentrations (95–142 μM). The duplexes were dialyzed against 500 ml of PBS buffer for 18 h at 4 °C with a 1-kDa cutoff to ensure matching conditions between sample and reference cells. The 260-nm absorbances of the dialyzed duplexes were measured before the experiment. Final duplex concentrations were as follows: 47.6 μM for RNA 11-mer, 65.5 μM for RNA 7-mer, and 65.8 μM for DNA 7-mer. Both the sample and matching reference buffer were extensively degassed under vacuum for at least 20 min to prevent bubbles. Scans were performed from 10 to 100 °C at 2 °C/min at a pressure of 3 atm. The first heating and cooling cycle of each experiment was discarded due to its unique thermal history. Three additional heating and cooling scans were performed to ensure reproducibility. A reference scan was made under the same conditions using reference buffer in both cells. Data analysis was performed using NanoAnalyze software (TA Instruments) and Origin...

9.1 (OriginLabs). Two representative reference scans (one each for heating and cooling) were subtracted from all duplex scans, and a partial specific volume for RNA was assumed to be 0.5 cm²/g based on previous reports [17]. We used spline interpolation between pre-transition and post-transition regions to define the baseline that was used for integration and determination of $\Delta H$ and $\Delta S$ [18]. Specifically, a spline was fit to six data points consisting of three low-temperature points and three high-temperature points. These temperatures were chosen consistently for each duplex but varied slightly between the 7- and 11-mers to accommodate shifting melting temperatures. For each duplex, the three heating scans and three cooling scans were individually analyzed and the results were averaged.

**Gel electrophoresis**

Single- and double-stranded oligomers were analyzed with a 15% polyacrylamide gel (Bio-Rad Mini-Protein precast gel) in 0.5× Tris–borate ethylenediaminetetraacetic acid (EDTA) (TBE: 45 mM Tris base, 45 mM boric acid, and 1 mM EDTA) buffer. A Ficoll-based loading buffer was used for the samples. The gel was run at 150 V for 60 min and was post-stained for 20 min in a 1× SYBR Gold solution (Life Technologies). The stained gel was imaged using a Bio-Rad Gel Doc XR+ system.

**Results**

We designed and evaluated a set of RNA and DNA oligos to be used as reference standards for measurements of biomolecular interactions. An 11-mer RNA duplex was designed to have properties similar to the Dickerson dodecamer [19,20]. The length was chosen to be one full A-form helical turn. The sequences of the two individual 11-mer strands were designed to avoid all possible self-complementariness so that they would not self-interact or fold on themselves (Table 1). Predictions in Mfold [21] show that the sequences exhibit no secondary structure, and a search in BLAST [22] revealed no such genomic sequence. The shorter sequences are taken from the 11-mers (Table 1). Electrophoretic gel migrations of the individual strands and their duplexes were consistent with their lengths and whether or not they were duplexes. Varying the ratio of RNA 11-mer A strand with its complementary B strand demonstrated stoichiometric 1:1 duplex formation when the constructs were run on a gel (Fig. 1). In all cases, the concentration-limited strand appears to be present only in the duplex, indicating a dissociation constant significantly smaller than the micromolar-level concentrations used in the gel.

Room temperature extinction coefficients of the oligos at 260 nm were determined to ensure accurate concentrations requisite for extracting thermodynamic parameters. DNA and RNA oligos were fully hydrolyzed using phosphodiesterase I from snake venom, and room temperature extinction coefficients were calculated using base extinction coefficients and the ratio of intact versus hydrolyzed oligos (Table 1) [11,12]. The errors are estimated to be less than 2% based on the primary source of measurement error—concentration changes due to evaporation from the cuvettes. Consistent with previous work [11], we found that our measured values were significantly lower than calculated extinction coefficients for these sequences. For example, the manufacturer (Integrated DNA Technologies) reported extinction coefficients for the RNA 11-mers of 113,000 L/mol cm and 96,700 L/mol cm for strands A and B, overestimating the measured values by 11 and 10%, respectively. We also measured the change in absorbance of the individual strands for a temperature ramp between 20 and 90 °C (see Supplemental Fig. 1 in online supplementary material). We found that the RNA A strands have changes in absorbance near room temperature, but none of the other strands show this behavior. We attribute this behavior to a tendency of the A strands to form homodimers, which is supported by predictions in Mfold and supported by other experimental evidence from ITC data at different temperatures (see below). It is likely not seen in the DNA A strands due to the lower stability of the homodimers in DNA than in RNA. The changes in absorbance for the individual strands during temperature ramping do not recapitulate the changes seen from the digestion. This suggests that high temperature should not be substituted for digestion in determining accurate extinction coefficients for oligos, as is sometimes done [23,24].

One of the most common and useful measures of biomolecular interactions is the association constant $K_A$ (or its inverse $K_D$), which reports the affinity of the two molecules at equilibrium. ITC is becoming a useful tool by the biophysical community of scientists as a means to assess oligonucleotide interactions as well as to understand the thermodynamics of these interactions [25–27]. Using ITC, we measured the exothermic heat of reacting one strand with the other as the duplex was formed (Fig. 2A and B). Experiments were performed in triplicate from a single set of working solutions whose concentrations were determined by a NanoDrop spectrophotometer using the extinction coefficients determined previously. The data from experiments in PBS at 25 and 37 °C were analyzed with and fit a two-state model (Table 2 and Fig. 2C).

As expected, the stoichiometry for all experiments was near 1:1 and typically within a few percentage points of 1:0, indicating a 1:1 reaction between strands. For all duplexes, the stoichiometry decreased as the temperature was increased from 25 to 37 °C even when the same stock solutions were used for both temperatures. This suggests that at lower temperatures some fraction of strands is unavailable to form duplex during the injection due to self-interaction or dimerization. This deviation was especially dramatic for the shorter strands, which is likely due to higher uncertainty in concentration measurement at the higher concentrations used due to homodimer formation. It could also be partially attributed to the greater difficulty in accurately determining the stoichiometry as the association constant decreases.

At 37 °C, where self-interactions and homodimers are minimized, association constants and enthalpies behaved as expected, increasing with longer strands and increasing from DNA to RNA. Somewhat counterintuitively, the enthalpy of formation of all four duplexes becomes more favorable as the temperature increases from 25 to 37 °C. This again suggests that the oligos have some structure at 25 °C but that much of this structure is removed once the temperature is increased to 37 °C. This is consistent with the tendency of the A strands to form homodimers. The ITC data suggest that nearly all of the material is forming duplex at these conditions (within 150 s) but that there is a small enthalpic penalty for
Representative raw data of the power required to maintain a constant temperature using both DSC, which measures the power required to maintain the duplexes using temperature ramps. These were performed by ramping the temperature from 10 and 100 °C at 2 °C/min, with the pressure held at 3 atm, enough to prevent evaporation but not to significantly affect thermodynamics of duplex formation [28]. Data between heating and cooling cycles was repeatable, showing only a fraction of a degree shift in melting temperature in the most extreme cases (Fig. 3A). The integral between the molar heat capacity curve and baseline for all four duplexes is equal to ∆H between the folded and unfolded states (Fig. 3B and Table 3). These data follow the same trend as did the ITC data, and for three of the four duplexes the enthalpies are within approximately 10% of those determined from ITC at 37 °C. The errors listed in Table 3 are statistical errors from the automated integration routine, but we noticed that choice of baseline endpoints and shape (e.g., linear, cubic, spline) could result in changes up to 10% as well. The DSC results from the 7-mer DNA differed substantially from that from the ITC experiments, underestimating ∆H by nearly 40%. We believe that this is likely due to the difficulty in establishing a reliable baseline because this duplex uniquely shows a steep pre-transition phase and a low melting temperature. The entropy values were determined by dividing the curve by absolute temperature and integrating the area between the curves using the same spline parameters. We found entropies that were within error of RNA values calculated from ITC values at 37 °C and within 30% for those of DNA. It is worth noting that the DSC curves all exhibit some asymmetry with a more gradual rise at low temperatures than the fall at high temperatures. This behavior is most pronounced for the 11-mers, and for the RNA in particular, suggesting less than perfect two-state behavior.

For UV melt experiments, similar protocols were employed, where the 260-nm absorbance of duplexes measured the change in base stacking. Again we found repeatable data between subsequent heating and cooling cycles, with only slight shifts in absorbance at high temperatures due to evaporation (Fig. 4A). We measured the melting behavior at a variety of concentrations and found increasing melting temperatures as concentrations were increased (Fig. 4B). Enthalpies of formation were determined from the data by two methods: van’t Hoff analysis (Fig. 4C) and concentration dependence of melting temperature (Fig. 4D). Van’t Hoff analysis of the data (Table 3) shows some nonlinearity for all duplexes, whereas the concentration dependence of the melting temperature shows near perfect linearity over the concentrations probed. Enthalpies determined from these methods were typically more negative than those determined from the other methods, sometimes dramatically so. This likely arises because both analysis methods are valid only for two-state reactions, and we have already shown deviation from that behavior in the previous experiments.

### Table 2

Measurements of duplex formation by ITC.

<table>
<thead>
<tr>
<th></th>
<th>RNA 11-mer</th>
<th>DNA 11-mer</th>
<th>RNA 7-mer</th>
<th>DNA 7-mer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>25</td>
<td>37</td>
<td>25</td>
<td>37</td>
</tr>
<tr>
<td>[A strand] (µM)*</td>
<td>2.3</td>
<td>3.1</td>
<td>6.7</td>
<td>6.9</td>
</tr>
<tr>
<td>[B strand] (µM)*</td>
<td>18.2</td>
<td>20.1</td>
<td>34.9</td>
<td>52.4</td>
</tr>
<tr>
<td>Stoichiometry</td>
<td>1.02 ± 0.05</td>
<td>1.03 ± 0.01</td>
<td>1.01 ± 0.02</td>
<td>0.98 ± 0.02</td>
</tr>
<tr>
<td>Kc (1/µM)</td>
<td>205 ± 30</td>
<td>125 ± 41</td>
<td>268 ± 41</td>
<td>77 ± 20</td>
</tr>
<tr>
<td>ΔH (kcal/mol)</td>
<td>−68.0 ± 2.6</td>
<td>−87.1 ± 0.9</td>
<td>−56.1 ± 1.8</td>
<td>−64.1 ± 1.7</td>
</tr>
<tr>
<td>ΔS (cal/mol/K)*</td>
<td>−190.0 ± 8.4</td>
<td>−244.0 ± 0.5</td>
<td>−149.5 ± 6.4</td>
<td>−170.6 ± 5.3</td>
</tr>
<tr>
<td>ΔG (kcal/mol)*</td>
<td>−11.3 ± 0.1</td>
<td>−11.2 ± 0.2</td>
<td>−11.5 ± 0.1</td>
<td>−11.2 ± 0.2</td>
</tr>
</tbody>
</table>

Note. Errors represent only statistical variations between experiments.

* Merged cells signify a common solution for multiple experiments.

b Calculated values from other parameters.
**Fig. 3.** Differential scanning calorimetry of nucleic acid duplexes. (A) Heating and cooling curves for the RNA 11-bp duplex show minimal hysteresis (~0.5 °C) at 2 °C/min and near identical peak areas. (B) Representative heating traces for all four duplexes with arbitrary y offsets. Gray lines show baselines used for determining integrated area to get ∆H_{DSC}.

**Table 3**

<table>
<thead>
<tr>
<th></th>
<th>RNA 11-mer</th>
<th>DNA 11-mer</th>
<th>RNA 7-mer</th>
<th>DNA 7-mer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ΔH_{DSC} (kcal/mol)</strong></td>
<td>-81.9 ± 2.6</td>
<td>-70.9 ± 1.3</td>
<td>-45.4 ± 3.0</td>
<td>-28.7 ± 2.0</td>
</tr>
<tr>
<td><strong>ΔS_{DSC} (cal/mol/K)</strong></td>
<td>-240 ± 10</td>
<td>-212 ± 9</td>
<td>-139 ± 14</td>
<td>-87 ± 9</td>
</tr>
<tr>
<td><strong>ΔH_{cal} (kcal/mol)</strong></td>
<td>-85.9 ± 1.7</td>
<td>-79.3 ± 1.6</td>
<td>-63.4 ± 1.3</td>
<td>-51.5 ± 1.0</td>
</tr>
<tr>
<td><strong>ΔH_{cal} (kcal/mol)</strong></td>
<td>-121.6 ± 3.7</td>
<td>-86.1 ± 2.0</td>
<td>-74.3 ± 1.0</td>
<td>-70.2 ± 0.8</td>
</tr>
</tbody>
</table>

* Assuming a two-state system.

**Discussion**

Knowledge of thermodynamics, stoichiometries, and other biochemical parameters of RNA folding and its interactions with other RNAs, proteins, and small molecules is key to understanding RNA biology and RNA as target, tool, and possible therapeutic. However, the academic and biotechnology communities lack oligonucleotide standards for the many different types of instrumentation relevant to this research. RNA and DNA standards would be useful across various measurement technology platforms, especially for RNA, in order to maintain instrument reliability from day to day, and from week to week, and to compare one’s own data with published data. We have evaluated RNA and DNA oligonucleotide duplexes of different lengths using three methods to quantify thermodynamic properties. We found good agreement between calorimetric methods for most duplexes, but there were still some differences that warrant further discussion. One issue is the comparison of enthalpy among the various techniques. Several recent articles have commented on the difficulty in comparing results from these experimental methods, specifically in comparing van't Hoff and calorimetric enthalpies [29–33]. It is noted that enthalpies determined at low temperature using ITC are not predictable from

**Fig. 4.** UV–vis spectrophotometry of the 11-mer RNA duplex melting. (A) Representative raw traces of absorbance as a function of temperature for heating and cooling ramps of 1 °C/min. Small divergence between curves occurs at higher temperatures as evaporation increases the concentration slightly. (B) Normalized melt curves at six different concentrations (11.7 μM, 5.9 μM, 3.0 μM, 1.5 μM, 670 nM, and 320 nM) show the increase in melting temperature with concentration. (C) Van’t Hoff plot of curves from panel B. (D) Linear plot showing concentration dependence of the melting temperature.
enthalpies measured over a narrow temperature range near $T_m$ in melting experiments unless $\Delta C_p$ is known. Because $\Delta C_p$ is not well known in this case, it adds additional uncertainty in comparison of the measured enthalpies. A second issue in comparing results is the analysis methods used. Both ITC and UV–vis data require fitting of a two-state model to extract thermodynamic data. For ITC, this is probably a valid approximation for the ensemble because there are vast energy differences between the single strands and the duplex at experimental temperatures. Any intermediate states that may exist are likely to be rapidly populated and unpopulated within the reaction time of 150 s. The ITC data also support the two-state model, with near perfect correspondence between model and data for all experiments. For UV–vis data, the assumption of a two-state model is probably not valid, as evidenced by nonlinear van't Hoff curves and asymmetrical curves from DSC experiments. The nonlinearity in the van't Hoff curves may also be due in part to heat capacity effects. However, the slope changes in our van't Hoff curves are more dramatic (up to 2.6 kcal/mol/K) than would be expected from heat capacity changes for duplex formation, which are typically smaller [26]. We suspect that intermediate states may be significantly populated at elevated temperatures but that these states are only transiently sampled in isothermal experiments. Analysis of UV–vis data can also be affected by subjectivity in choosing the baseline regions that define “fully associated” and “fully dissociated” states. DSC analysis is, in principle, model independent but requires defining a baseline to perform integration, which can again introduce subjectivity. These issues can contribute to uncertainties that go beyond the errors reported from statistical variations of multiple experiments.

Additional uncertainty arises if the concentration of oligos is not well known because the thermodynamic measures here all depend at least on relative concentrations (for UV–vis) and sometimes on absolute concentrations (for calorimetry). We have mostly addressed this issue by measuring the extinction coefficients. However, the noted propensity of some of the A strands to form homodimers may complicate the ability to determine concentrations accurately. The primary effects of homodimer formation as they relate to these experiments are (i) to introduce additional uncertainty in the measurement of concentration for those strands and (ii) to introduce a “reference” enthalpy for the unpaired duplex. The first effect arises because homodimer formation is concentration dependent and homomerized oligos absorb more UV light than free oligos due to hyperchromic shift. Practically speaking, this results in the extinction coefficient being concentration dependent. The second effect is due to the highly favorable enthalpy of duplex formation being slightly offset by the unfavorable enthalpy of dissociating homodimers. This effect is mainly evident with ITC, where both strands are mixed at constant temperature and the measured enthalpy for the reaction is less than the enthalpy for duplex formation from separated strands. This is further substantiated by the increase in measured enthalpy with increasing temperatures for all of our ITC experiments because higher temperatures cause fewer of the strands to be involved in homodimer formation. For melting experiments, the effect of homodimers is minimal if the experiments are done at equimolar concentrations or with an excess of B strands because full duplex formation is highly energetically favored over homodimer formation. In the future, we hope to optimize the oligo sequences to minimize homodimer formation without compromising the negligible secondary structure of individual oligos or the deep free energy minimum of full duplex formation. This would necessarily involve significant computational effort to develop in silico “screening” to find those sequences that give the closest approximation to true two-state behavior.

There is significant utility in developing short DNA and RNA duplexes into reference standards considering the lack of cross-platform standards for biomolecular nucleic acid interaction analysis by today’s highly sensitive instrumentation. One of the most notable features of using nucleic acids for this purpose is that their interactions are predictable and “tunable” depending on the number of interacting base pairs. Thus, they can be used either as absolute standards for quantitative instruments or as relative standards for qualitative or semi-quantitative instruments. Their tunable nature also enables their use for instruments that may be able to study only a limited range of kinetics or equilibrium constants. This is a common problem with other interactions that may be either “too weak” or “too strong” for certain instruments. For our experiments, we found data to be very repeatable, indicating that the DNA and RNA were stable for the duration of the experiments. Their tunable nature has been demonstrated in the use of various lengths as qualitative standards of size in gel electrophoresis. However, the compositions of these ladders differ from the various commercial sources and are not useful in standardizing other instruments. Characterized nucleic acids of tunable length could be useful in standardizing and calibrating separations from day to day, especially in the use of ultra-performance liquid chromatography. Nucleic acid standards are attractive because oligos are commercially available at low cost and high purity and their concentrations can be readily measured by spectrophotometry. Thus, DNA and RNA standards have the potential for use in a broad set of instrumentation and could also be used as a training tool for laboratory staff or students.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.jab.2014.08.001.

References
