DNA-DNA Interaction Analysis

Application Note NT018

Thermodynamic characterization of DNA hybridization

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Abstract

Here we report a thermodynamic analysis of biomolecular interactions using MicroScale Thermophoresis (MST). Using DNA hybridization with fluorescently labeled oligonucleotides as a well-characterized model system, we monitor the shift in the dissociation (K_d) constant of the interaction over a range of temperatures and calculate the free enthalpy (ΔH) and entropy (ΔS) for the hybridization reaction. The obtained values were in excellent agreement with theoretically calculated values, demonstrating that MST is feasible to perform thermodynamic analysis of biomolecules.

Introduction

Biomolecular interactions display a remarkable degree of specificity, which is determined by distinct molecular recognition events. Binding between two interacting partners has enthalpic (ΔH) and entropic (TΔS) components, corresponding to changes in both, structure and dynamics of each counterpart. In general, binding occurs only when the associated Gibbs free energy (ΔG) is negative (see below), which can be achieved by an entropy- and/or an enthalpy-driven process.

The information content from thermodynamic parameters is vast. The parameters are instrumental to elucidate the molecular mechanism of an interaction. Moreover, this knowledge can be used for the rational design of interactions, paving the way for novel pharmaceuticals or biomaterials.

Importantly, thermodynamic parameters are not only accessible through the direct measurement of reaction temperatures, but can also be extracted from the temperature dependence of the dissociation constant as shown by the following relations:

Given a reversible, bimolecular reaction

$$L + M \leftrightarrow LM$$

the dissociation constant K_d that is defined as

$$K_d = [L]\frac{[M]}{[LM]} \quad (1)$$

which in turn directly depends on the Gibbs free energy change:

$$\Delta G = RT \ln(K_d) \quad (2.1)$$

Thus, by measuring K_ds over a temperature range, ΔG, ΔH and ΔS can be calculated. Here we demonstrate that MicroScale Thermophoresis (MST) can precisely recapitulate thermodynamic parameters of DNA hybridization reactions.

Results

In the initial experiments, a perfect-match oligo nucleotide was titrated against Cy5-labeled template (Figure 1A), and the dissociation constant was calculated from the differing MST signals for single-stranded (ssDNA) and double-stranded DNA (dsDNA) (Figure 1B). The binding curves showed a typical sigmoidal shape, with the ssDNA displaying a stronger MST response than the dsDNA (Figure 1C). Increasing the temperature resulted in a clear shift of the K_d towards higher values. We moreover note that the Cy5-fluorescence intensity dropped significantly with increasing temperature (Figure 1D), which is due to shorter fluorescence lifetimes and reduced quantum yields at higher temperature. This

phenomenon however does not compromise the MST experiments.

After calculating the K_d of DNA hybridization for each temperature, a van´t Hoff plot (Figure 2A) was used to deduce the thermodynamic parameters ΔH and ΔS as described in detail in the Material and Methods section (see below). In addition, we performed experiments with two oligonucleotides carrying one or two mismatches, respectively (Figure 1A). As expected, the hybridization affinity was greatly reduced for the mismatch-nucleotides, and the free enthalpy and entropy where higher compared to the perfect match hybridization reaction (Figure 2B).

One of the big benefits of using DNA hybridization as a model system is that all interactions can be simulated in detail. We compared the experimentally determined thermodynamic parameters to calculated ones (using the IDT Biophysics tool http://biophysics.idtdna.com/). The comparison showed an excellent agreement of the parameters for the perfect match- as well as the mismatch hybridizations. We note that the parameters obtained for the mismatch construct with two mismatches shows the biggest divergence from the calculated parameters, which could however be due to limitations of the simulation software rather than due to a larger experimental error.

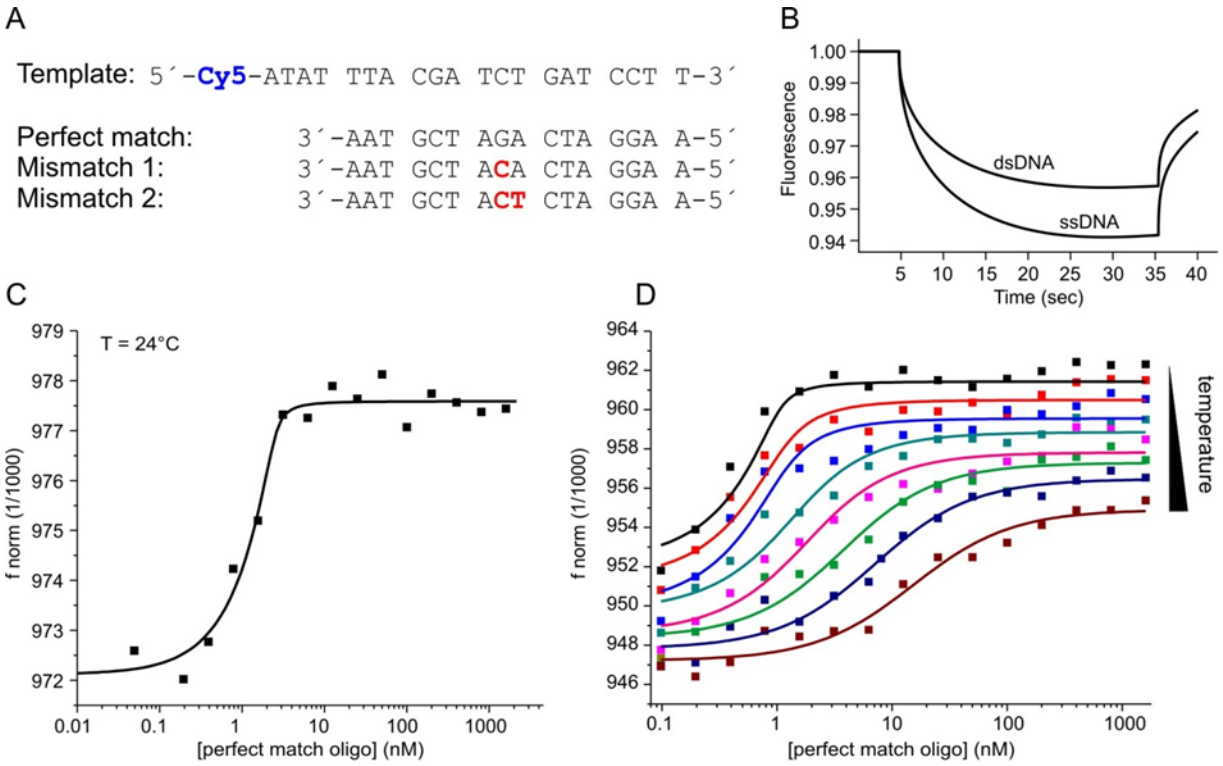
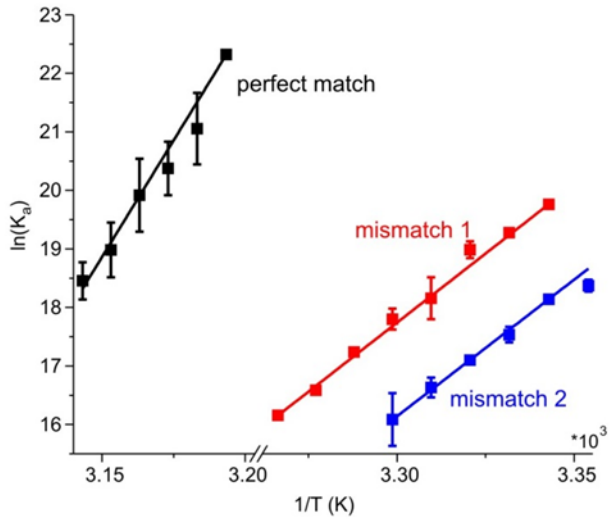


Figure 1: A) Sequences of the DNA oligomers. Single base substitutions of the mismatches are highlighted in red, 5'-terminal Cy5 in blue. B) Exemplary MST timetraces for a single-stranded (ssDNA) and a double-stranded DNA (dsDNA). ssDNA shows a stronger MST response then dsDNA. C) Fitted sigmoidal binding curve of a temperature-jump MST signal for a template-PM interaction at 24 °C. D) Temperature-jump binding curves over a temperature range from 38 °C to 45 °C; increments 1 °C.

A



B

	experimental		calculated (IDT biophysics tool)	
	ΔH (kcal/mol)	ΔS (cal/K*mol)	ΔH (kcal/mol)	ΔS (cal/K*mol)
PM	-158.1	-460.6	-146.2	-421.9
MM1	-93.8	-274.1	-94.9	-276.4
MM2	-92.8	-274.3	-116.7	-333.4

Figure 2: A) Van´t Hoff plots of the DNA-DNA hybridization reactions. The template-PM, template-MM1 and template-MM2 interactions are shown in black, red and blue, respectively (n=2). B) Comparison between experimentally determined and calculated ΔH and ΔS values (PM= perfect match, MM=mismatch).

Conclusion

Using MST, we determined ΔH and ΔS for the hybridization reaction of a DNA template with three different complementary oligonucleotides. All values were well within the margin of error compared to the values calculated by the IDT Biophysics tool. Thus MST is a feasible method to determine thermodynamic parameters of biomolecular interactions with minimal sample consumption.
This app note thus provides a guideline of how to perform thermodynamic measurements - be it for DNA and/or protein interactions.

Material and Methods

Experimental setup
Hybridization experiments were carried out in DNA buffer (20 mM HEPES, 100 mM NaCl, 0.05 % Tween-20). Standard enhanced gradient capillaries were used in all experiments because they reduce the risk of convection effects. Due to the strong thermophoresis of DNA, DNA oligomers are more prone to convection when compared to proteins. Also, the use of high MST power should be avoided to prevent convection. In order to minimize buffer evaporation during the experiment, the capillaries were sealed with NT Wax Tight Sealing. Additionally, the NT Anti Photobleaching Kit (A001) was used to prevent photobleaching. 5 mM EDTA was added to prevent enzymatic DNA decay during the measurement. The fluorescence intensities for the first measurements at low temperatures were adjusted to be well above the minimal fluorescence required for MST measurements, (the upper third of the detection range), since fluorescence decreased at higher temperatures.

DNA hybridization: MST measurements
All measurements were performed at LED power 90% and MST power 15% on a NT.115^{Pico} device. The concentration of the 5´Cy5-labelled DNA 20-mer was kept constant at 1 nM and its perfect match or one of two mismatches were diluted in a range from 1600 nM down to 0.05 nM, respectively. Measurements were performed over a temperature range of 25 °C to 45 °C increasing in increments of 1 °C. For each temperature, MST measurements were started 120 seconds after reaching the desired temperature.

The temperature-jump signals of the MST traces were fitted to obtain the K_d values.

DNA hybridization: thermodynamics
The thermodynamic parameters of the DNA-DNA hybridization were calculated by using the relationship between K_d and K_a (3)

$$K_d = \frac{1}{K_a} \quad (3)$$

$$\ln(K_a) = \frac{-\Delta G}{RT} \quad (2.2)$$

$$\Delta G = \Delta H - T\Delta H \quad (4)$$

and the equations (2.2) and (4), where R is the universal gas constant, T the reaction temperature, ΔG the change in Gibbs free energy, ΔH the change in enthalpy and ΔS the change entropy. Substituting (4) into (2.2) yields

$$\ln(K_a) = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} \quad (5)$$

Plotting ln (K_a) (in molar concentrations) against 1/T yields the so called van´t Hoff plot. ΔH can be obtained from the slope m of the linear fit (Fig.3 B) as

$$m = \frac{-\Delta H}{R}$$

Under the assumption that ΔH is constant in the relatively small linear range of the van´t Hoff plot (Figure 3) it is also possible to directly derive ΔS from the plot as

$$y(0) = \frac{\Delta S}{R}$$

The universal gas constant R was converted from SI units to [cal/mol *K]. An example of how to derive a linear fit of the van´t Hoff plot from MST measurements is shown in Figure 3.

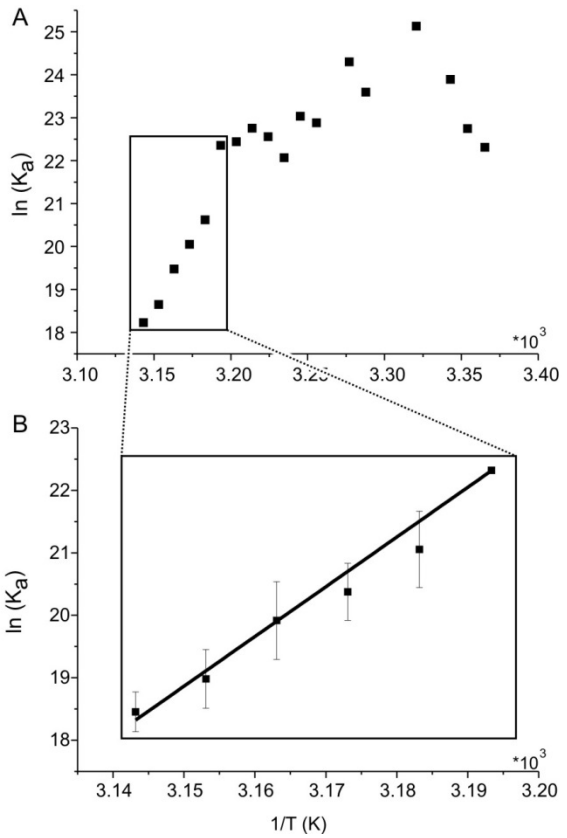


Figure 3: A) Van´t Hoff plot of a complete set of K_a values of the template-PM interaction for illustration of the data point distribution. Note that the divergence of the ln (K_a) values becomes high at low temperatures due to very high affinities (K_a>10^9) and thus a larger margin of error in K_d measurements. B) Linear range of the van´t Hoff plot and corresponding linear fit.

General comments on quality control

Once the entire temperature gradient was measured by MST, one additional experiment using the same capillaries at the starting temperature (24 °C) was performed as a quality control: Both fluorescence intensities and MST signal of this experiment was identical to the first experiment of the temperature gradient series, ensuring the validity of this approach. If this is not the case, the labeled biomolecules might degrade over time. In this case, measurement in overlapping intervals is recommended. For this, fresh solutions are prepared and measured in small intervals of 6 °C whereby 2 increments are overlapping. If the resulting timetraces still differ, the molecules might not tolerate the temperature gradient and/or acquisition times, which require optimization of buffer and experimental conditions to stabilize the molecules.

References

http://biophysics.idtdna.com/