Bio322- Lab1: Fitting Melting Curve Data to Thermodynamic Parameters

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1 Evaluating the Melting Curve of Salmon Sperm DNA

1. You are provided a text file which has temperature, mean absorbance at that temperature and standard deviation of the absorbance.

2. The concentration of DNA used was: **20** $\mu g/ml$**.**

3. Open a terminal of MATLAB or Octave or Python (Pylab).

4. Read in the temperature vs. absorbance at 260 nm measurement values into a matrix.

5. The primary data is first scaled by calculating the normalized absorbance at 260 nm using the expression

$$A = \frac{A_{ss} - A_T}{A_{ss} - A_h} \tag{1}$$

where A_{ss}: absorbance of single stranded DNA (highest temperature), A_T: absorbance at a given temperature T, A_h: absorbance of helical DNA (lowest temperature).

6. Melting temperatures are determined from the first derivative of this absorbance and superimpose it on the graph of normalized absorbance. Use the numerical approximation:

$$\frac{dA}{dT} = A'(n) = A(n+1) - A(n)\Delta T \tag{2}$$

to calculate the first derivative graph of absorbance (A) as a function of temperature (T), where n is the index of the measurement and ΔT is the temperature interval.

7. Find the X-intercept of the maximal value in the derivative graph of relative absorbance to determine the melting temperature (T_m).

8. If the data is very sparsely sampled only qualitative judgements can be made about the melting. To improve density of sampling use an interpolation function (for e.g. in MATLAB *interp(XX, factor)*) and then try calculating the discrete derivative.

2 Thermodynamics of DNA Melting

Based on the theory explained in class and previous work on the thermodynamics of DNA[1] the function to fit a melting curve is based on the fraction of dsDNA (f) is given by:

$$f = \frac{1 + C_t \cdot e^A - \sqrt{1 + 2 \cdot C_t \cdot e^A}}{C_t \cdot e^A} \tag{3}$$

where C_t is the total concentration of DNA in Molar terms and the term in the exponent A is:

$$A = \left[\frac{\Delta S^o}{R} - \frac{\Delta H^o}{R \cdot T} \right] \tag{4}$$

where R is the universal gas constant, ΔS^o and ΔH^o are the change in entropy and enthalpy associated with the melting of the given DNA.

Use a non-linear optimization routine to fit ΔS^o and ΔH^o.

3 Reporting

Report the program/script/method used to analyze the data.

Include a Table to explain the values of T_m, ΔS^o and ΔH^o. Compare to the literature for Salmon sperm DNA.

Add an explanatory note for the multiple, smaller peaks seen.

Please ensure you add your full name and roll number to your submission. Submit to compcelllab@gmail.com with the title of the email "Lab 1: DNA melting analysis".

References

[1] Naoki Sugimoto et al. (1996) Improved Thermodynamic Parameters and Helix Initiation Factor to Predict Stability of DNA Duplexes. Nucl. Acid Res.