COVER SHEET

TITLE: Interpretation of Effects of Hydroxylated Solutes on DNA Hairpin Stability

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YEAR: 2012

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Abstract

The identification and quantification of the interactions between hydroxylated solutes and biological macromolecules will aid in the development of solutes, as tools, in a number of biochemical contexts including protein stabilization and crystallization. Little information is known regarding these non-covalent interactions, thus, this study used solubility analysis to quantify the interactions between the surfaces on hydroxylated solutes and purine/pyrimidine derivatives. Subsequently, we utilized DNA melting studies to quantify the effects of the solutes on the overall stability of DNA hairpins. Interpretation of this data using the solute partitioning model allowed for the identification of these specific interactions and the development of the ability to predict the effect of any hydroxylated solute on DNA stability. Glycerol was found to have a favorable preferential interaction with the sp²N, sp³N, and sp³C of DNA bases/analogues and an unfavorable preferential interaction with sp²O (other solutes exhibited similar trends). The effect of these hydroxylated solutes on 12nt and 16nt DNA hairpin stability has also been quantified, and the data demonstrates a positive correlation between solute surface area and the destabilization of DNA.

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Interpretation of Effects of Hydroxylated Solutes on DNA Hairpin Stability

Senior Honors Thesis
Friday, May 11th, 2012

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"Interpretation of Effects of Hydroxylated Solutes on DNA Hairpin Stability"

By

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Senior Honors Thesis

Friday, May 11th, 2012

I have supervised this work, read this thesis and certify that it has my approval.

[Signature]

Date

Thesis Advisor’s Signature
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Introduction

Solutes affect protein and DNA processes such as folding and association through interactions with chemical functional groups present on these biological macromolecules (Record et al., 1998). For example, model compound studies show that urea denatures protein primarily through favorable interactions with both amide O and hydrocarbon C surfaces exposed in unfolding, and DNA through favorable interactions with all of the functional groups and ring surfaces present on the bases exposed upon denaturation (Guinn et al., 2011). Early studies showed that mono and poly-hydroxylated compounds destabilize DNA, but underlying interactions with the nucleobases were not characterized (Herskovits et al., 1972).

At present, it is not possible to predict the effect (stabilizing or destabilizing) of an arbitrary solute on an arbitrary protein or nucleic acid because the chemical interactions between most biologically relevant solutes and the functional groups that make up biopolymers have yet to be measured. Characterizing solute-biopolymer interactions will allow us to develop a framework for predicting and interpreting the molecular basis of these effects. This will lead to the rational development of solutes as tools, for example, to stabilize proteins used as industrial catalysts, for crystallization of proteins and nucleic acids, and as probes for protein conformational changes (Gries et al., 2010).

Recent research in the Record lab at UW-Madison has focused on the development of a model and molecular thermodynamic analysis to interpret and predict solute effects on biopolymer processes quantitatively in terms of structural information. Specifically, our approach is based upon the assumption that the non-covalent interactions between the various surfaces found on the solute and the biopolymer can be quantified in an additive manner to determine the composite effect of a solute on a process. Though this approach has not been applied to the study of hydroxylated solutes on DNA hairpin stability, this surface area breakdown methodology has been previously applied to the study of osmolyte effects on protein stability (Auton et al., 2011). Additionally, the Record lab has previously used protein model compound data to characterize the effects of Hofmeister salts on DNA stability (Pegram et al., 2011). Our study investigates the interactions between the hydroxylated solutes methanol, ethanol, glycerol, xylitol,
mannitol, sorbitol, 1-propanol, 2-propanol, 1,2-propanediol, 1,3-propanediol, 1,3-butanediol, and 1,4-butanediol and the different chemical groups present on the nucleic acid bases via solubility (Figures 1, 2, and 3). Subsequently, the effects of these solutes on the stability of DNA hairpins were determined via melting studies. Our hypothesis is that these solutes will destabilize DNA, and that knowledge of interactions between the solutes and the bases will allow us to interpret and ultimately predict effects of mono and poly-hydroxylated solutes on nucleic acid conformational stability.

Background

The chemical interactions between most biologically relevant solutes have yet to be measured. For two solutes to interact in aqueous solution to form, for example, an intermolecular hydrogen bond or a Van der Waals contact, they must out-compete water of hydration (solvation). A favorable interaction between two solutes arises from their ability to out-compete water of solvation to interact with each other (Record et al., 1998). The effect of a solute on the conformational stability of DNA is determined by non-covalent interactions of the solute with the surfaces exposed in the melted state (Record et al., 1998). If these interactions are more favorable than those with the surface exposed in the native state, then the addition of solute will drive unfolding and exposure of those surfaces with which it interacts favorably (Figure 4).

Interactions between solutes can be quantified via solubility experiments. By measuring the solubility of one solute in a saturated solution of the other, interaction favorability may be determined (Cannon et al., 2007). An increase in solubility is indicative of a favorable preferential interaction (relative to interactions with water), while a decrease in solubility is indicative of an unfavorable preferential interaction. At a molecular level, the solute partitioning model can be applied to explain the thermodynamic data obtained from solubility measurements (Figure 5). A favorable interaction arises from a particular solute's ability to out-compete water of solvation to interact with another solute (Jencks, 1969). The water of solvation is comprised of two layers of molecular water and is designated the local domain, while the remaining solution is designated the bulk domain. The microscopic partition coefficient, $K_p$, which is a ratio of local to bulk solute concentration, can then be determined. A $K_p$ value
greater than one is indicative of a favorable preferential interaction with a particular solute and a $K_p$ value less than one is indicative of an unfavorable interaction.

A solute’s effect on the stability of DNA can be quantified via melting studies. The effect of a particular solute on the transition temperature of a DNA hairpin is measured and used to analyze the solute’s effect on the hairpin’s stability quantitatively. An increase in the transition temperature represents an increase in the thermodynamic stability of the molecule and vice versa. This change in stability arises from the cumulative preferential interactions that exist between the various functional groups on the solute and the biochemical surfaces on the DNA that are buried in the native state (duplex, hairpin etc.) and exposed in unfolding.

**Materials and Methods**

**Solubility Measurements**

All compounds were obtained from Sigma Aldrich and stored at 4°C. Saturated solutions of a particular base in varying molal concentrations of a solute (0.0m-5.0m in 0.5m increments) were prepared by mass and allowed to incubate for 7-14 days in a shaking water bath maintained at 25°C (Herskovits and Harrington, 1972). 1 mL aliquots were then taken from the supernatant of these solutions and clarified by centrifugation (for 5 minutes at 10,000 rpm) or syringe filtration (with a 0.02 micron syringe filter) in order to remove insoluble particulate matter. These samples were then serially diluted into water and their absorbances measured at 0.1 nm steps between 300 nm and 200 nm on a Varian Cary 1 UV/VIS spectrophotometer.

Using the diluted solution absorbances and Beer’s Law, the absorbances of the original solutions were back calculated via dilution factors. An increase in solubility of the base (as indicated by a higher absorbance on the spectrophotometer) with increasing solute concentration is indicative of a favorable preferential interaction between the solute and a particular base. Molar concentrations were converted to molal concentrations and the $-\ln(m_2/m_1)$ were plotted against $m_1$ (solute molal concentration), $m_2$ being the molal base concentration in solute solution (at concentration $m_1$) and $m_2^+$ being the molal base concentration in the absence of the solute. The solubility graphs were then used to obtain the Setschenow
constants \((K_s)\):

\[
\frac{-\partial \ln \left( \frac{m_2}{m_3^*} \right)}{\partial m_3} \equiv K_s \approx \frac{\mu_{21}}{RT} \quad (eq. \ 1)
\]

These constants may be approximated as the chemical potential derivative characterizing the effect of changes in solute concentration on the chemical potential of the base. Setschenow constants were interpreted as arising from the additive contributions of the interaction of a particular "chemical surface" on the solute with each type of "chemical surface" on the base:

\[
K_s = \sum_i \alpha_i \text{ASA}_i^{\text{Base}} \quad (eq. \ 2)
\]

where the subscript "i" denotes each of the different types of surface interaction combinations based upon a two-way breakdown. The solute is broken down into three types of surfaces: hydroxyl O, aliphatic C, and ether O; whereas the bases are broken down into four types of surfaces: ring, methyl, carbonyl O, and amino N. \(\text{ASA}_i^{\text{Base}}\) is the amount of the corresponding surface type on the base. This two-component breakdown methodology was applied to all of the tested solutes except for glycerol, which was analyzed via a one-way breakdown analysis based upon the interactions between glycerol as a whole and the following surface types on the bases: \(\text{sp}^2\text{C}, \text{sp}^2\text{C}, \text{sp}^3\text{N}, \text{sp}^3\text{N}, \text{sp}^2\text{O}\). All of the surface areas were calculated using the Surface Racer 5.0 program (Tsodikov et al., 2002). The \(\alpha_i\) values were determined via a least squares regression analysis in order to find which values will best fit all of the observed slopes. Then, these \(\alpha_i\) values were converted to \(K_p\) values (partition coefficients):

\[
K_p = \frac{m_{\text{local}}}{m_{\text{bulk}}} \quad (eq. \ 3)
\]

in accordance with the solute partitioning model which allows us to interpret the results based on relative concentrations between the local and bulk solution.

**DNA Melting**

Solid DNA obtained from IDT was dissolved in melting buffer to an approximate concentration of 3\(\mu\)M in separate aliquots and frozen at -80° C. A Varian Cary 1 or 400 UV/VIS spectrophotometer equipped with a 12-sample peltier block was used to obtain melting profiles between 7 and 95° C of both 12nt DNA hairpin (5'-CGTAGCTATACG-3') and 16nt DNA hairpin (5'-CGTGTAGCTATACACG-3')
in varying concentrations of added polyol or alcohol.

Observed equilibrium constants ($K_{obs} = \frac{[\text{folded}]}{[\text{unfolded}]}$) for two-state hairpin folding obtained from DNA melting curves were used to calculate solute $m$-values, the derivative of the standard free energy change for hairpin formation with respect to solute concentration:

$$\frac{\partial \ln(K_{obs})}{\partial m_S} = m - value = \frac{\Delta H^\circ_{298}}{RT} \quad (eq. \ 4)$$

An $m$-value quantifies the effect of a solute on DNA hairpin stability. These $m$-values were measured for folding of both a 12nt and 16nt DNA hairpin in solutions of different hydroxylated solutes and interpreted in terms of interactions between the solute and the surfaces exposed on the DNA upon melting:

$$m - value = \sum_i \alpha_i \Delta ASA_i^{DNA} \quad (eq. \ 5)$$

where the subscript “$i$” denotes the five different types of surface on the DNA molecule and $\Delta ASA_i$ is the change in the amount of that type of surface upon melting (Tsodikov et al, 2002). The $\alpha_i$ values are a quantification of the interactions between the solute and chemical surface type “$i$” on the DNA, and were determined from analysis of solubility experiments described above.

**Results**

**Solubility**

The interactions between glycerol and the DNA bases were investigated and it was found that glycerol interacted favorably with the DNA bases, increasing their solubility in aqueous solution. Figure 6 plots the log of the solubility of various purine/pyrimidine derivatives as a function of glycerol molality. A set of “interaction potential” terms ($\alpha_i$) and partition coefficients ($K_p$) characterizing the interactions of glycerol with different base surfaces was subsequently determined by a least squares analysis of the data set using equation 2 (Table 1). Data for all of the other solutes was gathered and analyzed in a similar fashion (data not included here).

**DNA Melting**

The effects of various hydroxylated solutes on DNA stability were investigated and it was found
that every solute was hairpin destabilizing as demonstrated by their negative m-values. A summary of these m-values and their corresponding errors, determined from a standard least squares analysis of each plot, are provided (Table 2). In comparing the various subsets of solutes (alcohol, diol, and polyol), the destabilization effect of a group of solutes appears to decrease as the total amount of surface area characteristic of that group of solutes increases (i.e., alcohols demonstrate more destabilization than diols, which in turn demonstrate more destabilization than polyols).

Figures 7, 8, and 9 plot the ln (K_{obs}/K_0) for 12nt DNA hairpin unfolding as a function of alcohol, diol, and polyol concentration, respectively. In general, as the amount of total solute surface area increased within a subset of solutes, whether it be aliphatic C and/or hydroxyl O, the destabilization effect increased as well. The single exception to this trend, mannitol (a five carbon sugar alcohol), exhibited an m-value of smaller magnitude than that of glycerol (a three carbon sugar). Additionally, there were statistically significant discrepancies present in the destabilization effect of solutes with similar surface area composition: 1- vs. 2-propanol, 1,2- vs. 1,3-propanediol, and 1,3- vs. 1,4-butanediol.

Figures 10, 11, and 12 plot the ln (K_{obs}/K_0) for unfolding of the 16nt DNA hairpin as a function of alcohol, diol, and polyol concentration, respectively. As seen for the 12-mer DNA hairpin, the magnitude of the destabilization increased in proportion to the total solute surface area.

**Discussion and Conclusions**

**Solubility**

The data indicates that glycerol is able to out-compete water for interactions with sp^2N and sp^3N (as evidenced by partition coefficient values greater than one), which we interpret as hydrogen bonding interactions between the hydroxyl groups on glycerol and lone pair electrons or protons on the base. This apparent preference for hydrogen bonding between glycerol and the base may be explained by the large statistical likelihood of a hydrogen bond forming between one of hydroxyl moieties of glycerol and the base, as opposed to only two of these moieties being available on water. However, glycerol is not able to out-compete water for hydrogen bonding interactions with sp^3O (as evidenced by a partition coefficient.
value less than one). Glycerol was also shown to interact favorably with sp$^3$C, suggesting the presence of hydrophobic interactions between the carbon backbone of glycerol and the methyl groups found on the bases. Glycerol and water were also found to interact with sp$^3$C with nearly identical strength (interpreted from the near-one partition coefficient).

**DNA Melting**

All of these hydroxylated compounds display negative m-values indicating that they are hairpin destabilizing. This destabilization arises from favorable preferential interactions between the solute and the chemical surfaces exposed during melting. Based upon the solubility studies, we hypothesize that this general destabilization results from hydrophobic interactions between the aliphatic C surface on the solute and the methyl groups on the DNA hairpin molecules and hydrogen bonding interactions between the hydroxyl O of the solute and the carbonyl O and amino N moieties on the DNA hairpin. The solute hydroxyl groups are also capable of interacting with the ring surface area of the DNA molecules through dipole-dipole interactions.

Within the mono-hydroxylated alcohol subset of solutes, 1- and 2-PrOH have m-values of statistically larger magnitude than MeOH or EtOH, suggesting that the hydrophobic surface characteristic of the backbone of these solutes plays a large role in the effect of these solutes on DNA stability. Though this trend holds true for MeOH and EtOH in the H12 melting studies, it is reversed for these solutes when studied with H16. Given that the two hairpin molecules contain proportional amounts of the same nucleotides, and thus expose similar types and amounts of surface upon melting, this result is unexpected. 1- and 2-PrOH destabilize H16 DNA to the same extent; however, 1-PrOH is a statistically more effective destabilizer of H12. Though this may be explained by the relatively extended conformation of 1-PrOH, which confers the solute a larger amount of accessible hydroxyl and aliphatic C surface, this argument should be applicable to, and displayed by, the H16 studies.

The diol group of solutes exhibited similar trends. 1,3- and 1,4-BD were more effective destabilizers of both H12 and H16 DNA than 1,2- and 1,3-PD as quantified by their m-values. It should also be noted that 1,2-PD and 1,3-BD displayed statistically higher m-values than the other diols studied.
This suggests that the close proximity of the hydroxyl moieties on the solutes may facilitate hydrogen bonding and/or dipole-dipole interactions between the solute and the surfaces exposed on either DNA hairpin upon melting.

The poly-hydroxylated compounds display only a slight correlation between total solute surface area and DNA destabilization. For example, although mannitol and sorbitol have nearly identical surface areas both in composition and amount, they exhibit statistically different m-values for both hairpin structures. Additionally, sorbitol appears to be more destabilizing for H12, whereas mannitol is more destabilizing for H16. However, these sugar alcohols only differ by the stereochemistry around a single carbon.

In terms of data quality, a few experimental considerations should be noted. For example, different spectrophotometers were used throughout the course of this study. The Varian Cary 400 UV/VIS spectrophotometer demonstrated markedly lower amounts of scatter when compared to the Varian Cary 1, leading us to consider some of the m-values to be more precise than others. Additionally, the cuvettes utilized throughout the thermal spectrophotometry experiments conducted in this study were not of ideal transparency or clarity. In terms of the compounds tested, a few were not soluble in the standard 0.5 molal range: mannitol (≤1 molal), sorbitol (≤2 molal), and xylitol (≤4 molal). Thus, this discrepancy introduced another unaccounted variable into the analysis.

Analysis Quality

M-values were predicted for each solute utilizing the interaction potentials derived from the solubility studies performed for each solute via a two-way breakdown analysis. This analysis was based upon the assumption that the exposed surface area associated with the denatured state of the DNA hairpin molecule may be approximated as the total water accessible surface area of all of the component nucleosides contained within the hairpin DNA (100% un-stacked).

The H12 DNA comparisons (Figure 13) demonstrate systematic deviation in which the majority of solutes (75%) display significantly higher experimental m-values in comparison to their predicted values. Additionally, this observation is not confined to a particular subset of solutes. The only solute m-
values that were within statistical error of their predicted values were MeOH, 2-PrOH, 1,3-PD, and mannitol, the majority of which had relatively large error bars associated with their data points.

The H16 DNA comparisons (Figure 14) demonstrated more internal consistency; however, there was systematic deviation present among the polyol subset of solutes. Mannitol, sorbitol, xylitol and glycerol all demonstrated larger destabilization effects than what was predicted; a result that may be attributed to neglected excluded volume effects. All of the diols, 1-PrOH, and 2-PrOH aligned very closely with their predicted values, demonstrating the validity of the 100% un-stacked model for the denatured hairpin molecule for hydroxylated solutes within the 3-4 carbon range.

Future Directions

In conclusion, all of the studied hydroxylated solutes destabilized both H12 and H16 DNA through interactions with the surfaces exposed on the DNA molecules upon unfolding. These non-covalent interactions include dipole-dipole interactions, hydrophobic interactions, and hydrogen bonding.

In order to corroborate our solubility data, other techniques including Vapor Pressure Osmometry (VPO) and Critical Micelle Concentration (CMC) assays may be implemented. Additionally, more purine/pyrimidine derivatives and/or hydroxylated solutes can be studied to improve the accuracy of our interaction potential values. The DNA melting studies could also be compared to data obtained from single molecule methods.

Utilizing the methodology and analysis described in this study, the non-covalent interactions between any type of solute and biopolymer can be characterized. Solutes can range anywhere from simple alcohols to complex osmolytes, and biopolymers can include duplex (or triplex) DNA and proteins. A variety of functional groups can potentially be studied including: sulfhydryl (-SH), amino (-NH₂), carboxyl (-COOH) etc., as long as surface area data is available for the solutes and biopolymers being studied. The information gleaned from identifying and quantifying these non-covalent interactions can subsequently be applied to a variety of life science fields ranging from pharmacology to industrial chemistry.
References


Figures and Tables

**Figure 1.** Line structures of all of the alcohols tested in this study. From left to right: MeOH, EtOH, 1-PrOH, and 2-PrOH. Structures were adapted from [www.chemspider.com](http://www.chemspider.com).

**Figure 2.** Line structures of all of the diols tested in this study. From left to right: 1,2-PD, 1,3-PD, 1,3-BD, and 1,4-BD. Structures were adapted from [www.chemspider.com](http://www.chemspider.com).

**Figure 3.** Line structures of all of the polyols tested in this study. From left to right: glycerol, xylitol, mannitol, and sorbitol. Structures were adapted from [www.chemspider.com](http://www.chemspider.com).
Figure 4. The effects of small uncharged solutes on biopolymer processes are explained by solute preferential interactions. Biopolymer-solution interfaces are stabilized when a solute accumulates at the surface and destabilized when a solute is excluded from the surface. Accumulation and exclusion result from favorable and unfavorable interactions that different solutes experience with different chemical groups present on biopolymer surfaces; for example, in this depiction, urea hydrogen bonds with, and is accumulated at the DNA bases and so destabilizes duplex DNA.

Figure 5. According to the solute partitioning model, glycerol must compete with two layers of water of solvation (as seen above) in order to interact with another solute. In this depiction, glycerol is preferentially interacting with thymine's aromatic N (sp^2N) via hydrogen bonding. There are also hydrophobic interactions present between the methyl group (sp^3C) of thymine and the carbons on glycerol.
Figure 6. A graph depicting the effect of glycerol on the solubility of various purine/pyrimidine derivatives in aqueous solution. Excluding thymine and uracil, glycerol increased the solubility of every derivative tested.

Figure 7. A graph depicting the effect of various mono-hydroxylated alcohols on the stability of 12nt DNA hairpin. Note: DMB = duplex melting buffer (0.1000 M KCl, 0.0256 M K$_2$HPO$_4$, 0.0001 M K$_3$EDTA; pH = 7.3).
Figure 8. A graph depicting the effect of various diols on the stability of 12nt DNA hairpin. Note: DMB = duplex melting buffer (0.1000 M KCl, 0.0256 M K$_2$HPO$_4$, 0.0001 M K$_2$EDTA; pH = 7.3).

Figure 9. A graph depicting the effect of various poly-hydroxylated solutes on the stability of 12nt DNA hairpin. Note: DMB = duplex melting buffer (0.1000 M KCl, 0.0256 M K$_2$HPO$_4$, 0.0001 M K$_2$EDTA; pH = 7.3).
Figure 10. A graph depicting the effect of various mono-hydroxylated solutes on the stability of 16nt DNA hairpin. Note: DMB = duplex melting buffer (0.1000 M KCl, 0.0256 M K$_2$HPO$_4$, 0.0001 M K$_3$EDTA; pH = 7.3).

Figure 11. A graph depicting the effect of various diols on the stability of 16nt DNA hairpin. Note: DMB = duplex melting buffer (0.1000 M KCl, 0.0256 M K$_2$HPO$_4$, 0.0001 M K$_3$EDTA; pH = 7.3).
Figure 12. A graph depicting the effect of various poly-hydroxylated solutes on the stability of 16nt DNA hairpin. Note: DMB = duplex melting buffer (0.1000 M KCl, 0.0256 M K$_2$HPO$_4$, 0.0001 M K$_3$EDTA; pH = 7.3).

Figure 13. A plot depicting the internal consistency of the interaction potential analysis as applied to 12nt DNA hairpin. Only four of the studied solutes exhibited m-values within statistical error of their predictions: MeOH, 2-PrOH, 1,3-Pro, and Mannitol.
Figure 14. A plot depicting the internal consistency of the interaction potential analysis as applied to 16nt DNA hairpin. Half of the studied solutes exhibited m-values within statistical error of their predictions: 1-PrOH, 2-PrOH, 1,2-PD, 1,3-PD, 1,3-BD, and 1,4-BD.

<table>
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<th>Surface Area</th>
<th>Interaction Potential (m$^{-1}$)</th>
<th>Partition Coefficient</th>
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<tbody>
<tr>
<td>5P$^2$N</td>
<td>-6.25E-04</td>
<td>1.193</td>
</tr>
<tr>
<td>5P$^3$N</td>
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<tr>
<td>5P$^3$C</td>
<td>-2.31E-04</td>
<td>1.071</td>
</tr>
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Table 1. A summary of interaction potential and solute partitioning model analysis data for glycerol with the purine/pyrimidine derivatives studied.
<table>
<thead>
<tr>
<th>Solute</th>
<th>m-Values</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H12</td>
<td>H16</td>
</tr>
<tr>
<td>MeOH</td>
<td>-0.088 ± 0.030</td>
<td></td>
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<td>EtOH</td>
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<td>Sorbitol</td>
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Table 2. A summary of the m-values determined for all of the hydroxylated solutes studied in the thermal spectrophotometry experiments. The error values were determined from a standard least squares analysis of the data and were multiplied by a coverage factor of 2.
Appendix

**Glycerol Interaction Potential Analysis: Internal Consistency.** A plot depicting the internal consistency of the μ23 analysis as applied to glycerol and the following purine/pyrimidine derivatives: adenine, thymine, cytosine, caffeine, hypoxanthine, theobromine, and theophylline. Uncertainty values are not

**Melting Study Analysis: Step 1.** The figure displays a representative plot of the solvent expansion-corrected absorbance vs. temperature for a single thermal spectrophotometry run. This particular data set is for a "blank" solution with no solute and H16 DNA. The upper and lower baselines are approximated using standard least squares analyses.
Melting Study Analysis: Step 2. The figure displays a representative plot of the fraction hairpin vs. temperature for a single thermal spectrophotometry run. This particular data set is for a "blank" solution with no solute and H16 DNA. The $T_m$ is approximated using a standard least squares analysis circumventing the 50% denatured/50% matured data points.

$y = -0.05694x + 19.52128$

$R^2 = 0.99913$

Melting Study Analysis: Step 3. The figure displays a representative plot of $\ln(K_{obs})$ vs. $(\text{temperature})^{-1}$ for a single thermal spectrophotometry run. This particular data set is for a "blank" solution with no solute and H16 DNA. The observed equilibrium constant at 333 K is acquired from this plot.

$y = 25,288.25308x - 75.69821$

$R^2 = 0.99784$
Acknowledgements

I would like to extend an immense amount of gratitude and thanks to my mentor, Ben Knowles, my supervising professor, Thomas Record, and the entire Record Laboratory family at UW-Madison. I have experienced a large amount of personal and intellectual growth over the course of the last three years, and I am forever indebted to all of these individuals for their guidance and support. In fact, I owe a vast majority of my scholarly success to the accomplishments I have achieved as an undergraduate researcher.

As I now continue my academic career in medical school, I thank everyone in the Record Laboratory, once again, and wish all of them the best of luck of their future endeavors.
CALS Honors in Research
Thesis Presentation

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Date(s) of Presentation:
Wednesday, April 18th, 2012 (UW Undergraduate Symposium)
Wednesday, May 2nd, 2012 (Record Laboratory Meeting)

Location of Presentation:
   ___ CALS Undergraduate Symposium
   ___ UW Undergraduate Symposium
   ___ Lab/Research Group
   ___ Professional Conference (Please Specify) ________________________________
   ___ Other (Please Specify) ________________________________

Please attach a copy of the appropriate page from the program or other documentation indicating when and where your presentation took place.

Zeeshan Ul Haq                 M. Thomas Record, Jr.
Student Name                  Mentor Name

[Signature] 5/11/12           [Signature] 5/11/12
Student Signature/Date        Mentor Signature/Date

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Dear Honors Dean,

I am happy to write that I have successfully completed my senior honors thesis over the course of this last academic year.

In terms of coursework, I enrolled in 3 credits of 681 in the fall, and 3 credits of 682 in the spring. Additionally, I remained in Madison to do research on the weekdays during both winter and spring break. Though scheduling complications prevented me from enrolling in InterAg 488 (CALS Honors Interdisciplinary Senior Seminar), I frequented our laboratory’s weekly meetings as compensation. Additionally, I helped our laboratory in the moving process from the '85 wing into our new home in the Biochemical Sciences Building. The logistics and preparation required for the successful transplantation of all of our equipment, chemicals, and other items was fairly cumbersome. However, I have gained a lot more insight into, and appreciation for, the procedural and safety aspects of a laboratory.

In terms of bench work, I was successful in completing all of the thermal spectrophotometry studies that I outlined in my proposal from last year. There were a few hiccups in terms of faulty equipment and broken materials, but I was able to utilize the Biophysics Instrumentation Facility to wrap up my remaining experiments.

Upon acquiring all of the necessary data and working it up, I eventually presented in my lab’s weekly meeting and participated in a roundtable discussion at the Undergraduate Symposium.

Overall, I have thoroughly enjoyed my experience as an Honors in Research Candidate in CALS here at UW-Madison.

Sincerely,

Zeeshan Haq