Probing Changes in the Conformation of tRNA^{Phe}: An Integrated Biochemistry Laboratory Course

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Within the last decade or so, two new trends have arisen in the development of laboratory components for upper-level biochemistry courses: integrated laboratory experiences and inquiry-based laboratories that lead ultimately to discovery-driven independent research. Participation in undergraduate research is now widely recognized for its ability to facilitate critical thinking and expose students to the excitement (and heartbreak!) of scientific discovery, while providing preparation for careers in science *(1–5)*. At the same time, integrated laboratories that feature biochemical research centered around a single biomolecule afford a thematic cohesiveness that students find attractive *(6–9)*.

A search of the recent literature turned up semester-long "integrated" or "project-oriented" biochemistry laboratories centered around a number of different enzymes *(4, 5–9)*.[1] Only one integrated biochemistry laboratory course featured DNA *(10)*, and none featured RNA. In fact, whereas individual laboratory projects involving DNA are common in the literature *(9, 11, 12)* and in published biochemistry laboratory texts *(13, 14)*, we found only a few involving RNA *(15–17)*. In this article, we describe a unique integrated and project-oriented laboratory course[2] centered around tRNA^{Phe}.

Transfer RNA (tRNA) is an important polynucleotide that carries amino acids to the ribosome to be added to the growing polypeptide chain during protein synthesis. tRNA^{Phe} specifically carries the amino acid phenylalanine. Like all nucleotide chains, tRNA is composed of purine and pyrimidine nucleotide bases that are attached in a specific order via phosphodiester bonds. This base sequence comprises the molecule's primary structure. Polynucleotide chains maximize stabilizing interactions (e.g., purine–pyrimidine hydrogen bonding, aromatic ring π-stacking) by folding into well-established three-dimensional secondary and tertiary structures. Although the approximately 200 tRNA molecules have widely varying primary sequences, they all share a common secondary and tertiary structure. Thus the information gained from studying conformational changes in a specific tRNA molecule such as tRNA^{Phe} can often be extended to the entire family of molecules. Because tRNA is a crucial component in protein synthesis, the medical implications are clear.

Figure 1. Tertiary structure of tRNA^{Phe}, 6tna.pdb visualized in RasMol.

Ligands are known to bind to tRNA, altering its conformation. The crystal structure of tRNA^{Phe} has been solved (Figure 1), and binding sites for various metals and polyamine ligands are well-characterized. Three aspects of tRNA^{Phe} tertiary structure can easily be probed: (a) metal-catalyzed hydrolysis (cleavage) of a specific phosphodiester bond in tRNA^{Phe}; (b) thermal unfolding ("melting") of various tRNA^{Phe}–ligand complexes; and (c) ligand binding-induced changes in tRNA^{Phe} conformation. We have focused on these three experiments in designing a novel laboratory that explores a number of subfields of biochemistry, while exposing students to important experimental techniques (electrophoresis, UV–vis spectrophotometry, and fluorescence spectroscopy).

This laboratory component accompanies an upper-level biochemistry course that we created to serve as a final advanced course in the biochemistry track of our chemistry major *(18, 19)*. The course is offered every year, rotating among three faculty members whose foci include metalloenzymes and metal-based drugs (bioinorganic), nucleotide biochemistry and drug design (bioorganic), and bioenergetics or neurochemistry (biochemistry). We set out to devise a single laboratory component that would be useful for all three versions of the course.

In this laboratory we require students to explore the theoretical and thermodynamic underpinnings of macromolecular structure and apply this background to the appropriate analysis and presentation of experimental data. We also introduce students to a true research experience, from the initial literature search through reproducing published results to designing and carrying out novel experiments. Finally, we have designed the laboratory to encourage critical thinking and collaborative teamwork. Recently the American Chemical Society Committee on Professional Training (ACS CPT) proposed new Guidelines for Undergraduate Chemistry Programs. The guidelines call for upper-level "in-depth" coursework that emphasizes "process skills", such as "critical thinking, verbal and written communication, ... collaborative teamwork, and accessing and using scientific information *(5)*." The laboratory course we developed embodies all of these principles.

Course Description

A general schedule of laboratory activities is shown in Table 1. The goal of the first laboratory meeting is to provide each student with a solid understanding of the $tRNA^{Phe}$ molecular structure and its reactivity with Pb^{2+}. At the start of the lab we expect the students to have already read Chapter 15 of Saenger's *Principles of Nucleic Acid Structure (20),* providing background on the structure of the $tRNA^{Phe}$ molecule. While there are several pages in the in-house laboratory manual with general information on tRNA (see online supplement), students are referred to the primary literature *(21, 22)* to complete their overview. This introduces them to a key component of beginning a research program, namely, consulting the literature directly.

Students carry out a computer modeling exercise that uses Chime, a no-fee browser plug-in for molecular modeling, along with an online tutorial *(23)*. Students take about two hours in the computer lab to extend their knowledge interactively, while they visualize the three-dimensional features of $tRNA^{Phe}$ tertiary structure, including stems, loops, and bulges. The exercise also enables students to identify the specific sites within the $tRNA^{Phe}$ structure where spermine, spermidine, Mg^{2+}, and Pb^{2+} bind *(20, 23)* and also where Pb^{2+}-catalyzed cleavage occurs *(22, 23)*. A version of a student-prepared structural diagram can be seen in Figure 2.

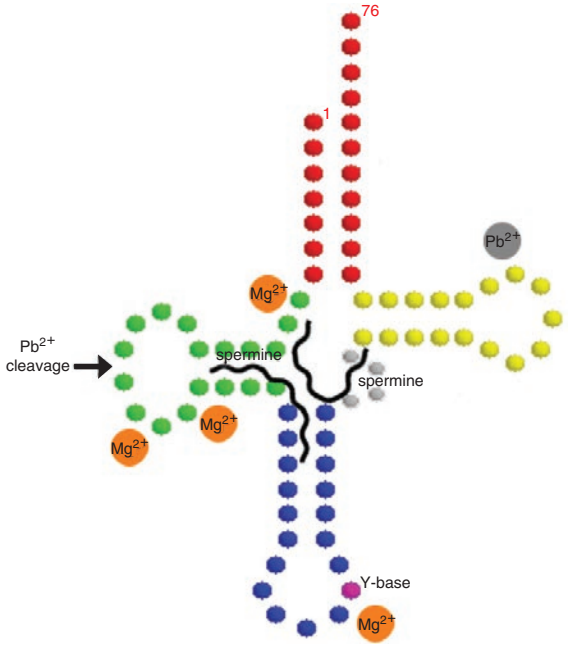


Figure 2. Secondary structure of $tRNA^{Phe}$, showing sites for the Y base, Pb^{2+}-catalyzed cleavage, and bound Mg^{2+}, Pb^{2+}, and spermine.

Following the computer-aided visualization exercise, the students embark on a semester of "guided inquiry"-based laboratory work. Prelaboratory questions serve as a springboard into a deeper set of questions that students frame and answer on their own. At the end of each week, student pairs reflect on the meaning of their results and prepare for their next set of experiments. Rather than moving to the next experiment and writing a synopsis of "what went wrong and why", as is usually done in lower-level laboratory courses, the students are expected to use negative or incomplete results to repeat an experiment or adjust the experimental conditions.[3] In other words, this mirrors aspects of a true research experience.

To give students the opportunity to learn more about performing research firsthand, we deliberately walk a fine line between providing guidance and allowing them to work independently. This means that while students are given ample room to succeed, they are also given ample room to fail, but not with-

Table 1. Weekly Activities in the Laboratory

Week	Stage	Laboratory Activities
1	Common experience	$tRNA^{Phe}$ lecture and structure tutorial; standardize pipets
2		Polyacrylamide gel electrophoresis; **lead(II)-catalyzed cleavage of $tRNA^{Phe}$**
3	Rotating experiments	Run lead cleavage gel; lecture on UV–vis and fluorescence spectroscopy
4		Lead(II)-catalyzed cleavage of $tRNA^{Phe}$ in the presence of a small molecule ligand; start UV–vis and fluorescence spectroscopy calibration curves
5		Run small molecule ligand gel; finish UV–vis and fluorescence spectroscopy calibration curves
6		**Fluorescence spectroscopy of $tRNA^{Phe}$ Y base** in the presence of Mg^{2+} and a small molecule ligand
7		Fluorescence spectroscopy of $tRNA^{Phe}$ Y base in the presence of ethidium bromide and a small molecule ligand
8		Metal ion catalyzed cleavage of $tRNA^{Phe}$
9		Run metal ion cleavage gel; **UV thermal melting curves of $tRNA^{Phe}$** in the presence of ligands
10	Research experience	Design novel gel experiment
11		Run gel; UV thermal melting curves of $tRNA^{Phe}$ in the presence of increasing concentrations of a small molecule ligand
12		Repeat necessary experiments; further data analysis
13		Finish data analysis and discussion of results
14		Formal presentations

NOTE: The bolded phrases indicate the three major projects.

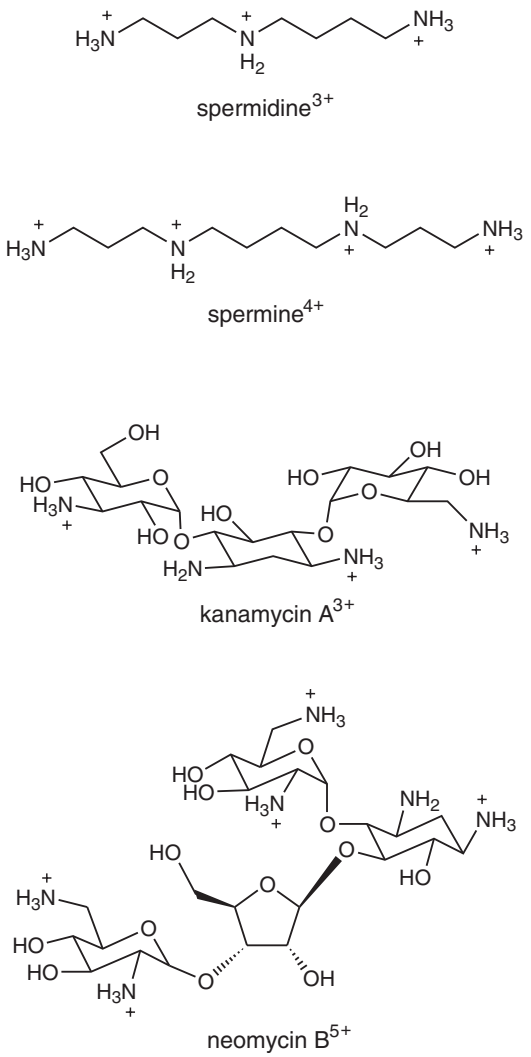


Figure 3. Structures of polyamine ligands in their predominant protonation states at pH 7.

out the chance to learn from their mistakes. Mistakes often come to light in weekly laboratory meetings that are used as a forum to share and discuss results. For example, it has happened more than once that a student pair presenting results has been advised by their peers that their control experiment was either missing or done incorrectly. Insights gained from these meetings allow students to hone their experimental design and technique.

During the second week, the entire class, broken into pairs, performs a gel electrophoresis study of lead(II)-catalyzed cleavage of $tRNA^{Phe}$. Beginning with this common experience, students learn techniques, compare their results with one another and the literature, and sharpen their data analysis skills (including error and uncertainty) as a single class group. This experiment is far more than just an exercise, however. Each student pair cleaves $tRNA^{Phe}$ using overlapping ranges of lead(II) concentrations and incubation times. Based on their results, the class as a whole makes decisions to optimize subsequent experiments. The laboratory manual is written to guide students through experiments while still leaving room for adjustments to experimental parameters, such as concentrations and instrument settings. Finally, in this first experiment, students detect significant differences in samples run under "identical" conditions. This leads to a fruitful discussion of the importance of proper technique and error analysis.

The following week, the student pairs diverge and follow different but parallel trajectories for the remainder of the semester. Each student pair chooses a small organic polycationic ligand (spermidine^{3+}, spermine^{4+}, kanamycin A^{3+}, or neomycin B^{5+}, Figure 3) to study its effect on the rate of Pb^{2+}-catalyzed $tRNA^{Phe}$ cleavage. In addition, each pair studies $tRNA^{Phe}$ cleavage catalyzed by an assigned group of metal cations. The metals include main group, transition, and lanthanides, in various oxidation states. We have selected some whose effects on $tRNA^{Phe}$ have been reported *(24)* in the literature (e.g., Eu^{3+}, Mg^{2+}, Mn^{2+}) and some which are as yet unstudied (e.g., Gd^{3+}, Fe^{3+}, Zn^{2+}), in order to add to the research component of the course.

In addition to carrying out experiments on different organic ligands and metals, student pairs stagger their experiments, thus maximizing use of the various instruments.[4] While two student pairs continue on with polyacrylamide gel electrophoresis (PAGE) experiments, another pair begins fluorescence spectroscopy, and another collects thermal melting curve data using the UV–vis spectrophotometer.

By nine weeks into the semester, student pairs have completed all three major projects outlined in the laboratory manual (see the bolded items in Table 1) and they give two semi-formal talks describing the progress of their research. Here students and the professor ask each other questions and suggest changes to procedures. Students also make initial hypotheses as to the effects of their ligands and metal cations on $tRNA^{Phe}$ conformation.

At this point in the semester, the students have gained enough experience to design a novel independent project. This concluding phase of the laboratory provides a true research experience, where each student group has the opportunity to apply their skills to an uncharted area of related research. Student projects completed thus far have probed the cleavage of $tRNA^{Phe}$ catalyzed by metals other than lead(II), while examining the influence of pH, salt, or various small cationic ligands. Some of these experiments have led to full senior research projects.

During the last two weeks of class, our focus is on disseminating experimental results in a 30-minute oral presentation and a formal written report. The students are expected to interpret their own data, and also to incorporate the results of the other groups into their reports. Here we can see how imperative it is that there is continued communication among the students during the entire semester so that at the end they can carry out an in-depth discussion of the large quantity of data that has been collected and analyzed. Because this is a upper-level course, students are expected to think deeply about the implications of their results, using knowledge from other courses, most notably biochemistry, inorganic chemistry, and organic chemistry, but also physical chemistry and instrumental analysis where applicable. Our laboratory thus serves as an ideal integrative course in the major, as well as an excellent introduction to independent senior research.

Experimental Results

We present a sample of typical student results for the three major projects in the laboratory. Most of the data sets and figures

were contributed by our undergraduate coauthor Buck Taylor. Further experimental details for each of the three laboratory units are available (ref *25* and in the online supplement*)*.

Metal-Catalyzed Hydrolysis of tRNA^{Phe} Assayed by PAGE

The tRNA^{Phe} molecule undergoes phosphoester hydrolysis catalyzed by a bound lead(II)-aquo complex (Scheme I). Lead(II) binds between uracil-59 and cytosine-60, but cleaves between dihydrouracil-17 and guanine-18. Although these two sites are distant in the primary and secondary structures (Figure 2), they are adjacent in the folded tRNA^{Phe} molecule (Figure 1). Hence lead(II)-catalyzed cleavage only occurs in correctly folded tRNA^{Phe}, and therefore, cleavage is a sensitive assay for tRNA^{Phe} tertiary structure.

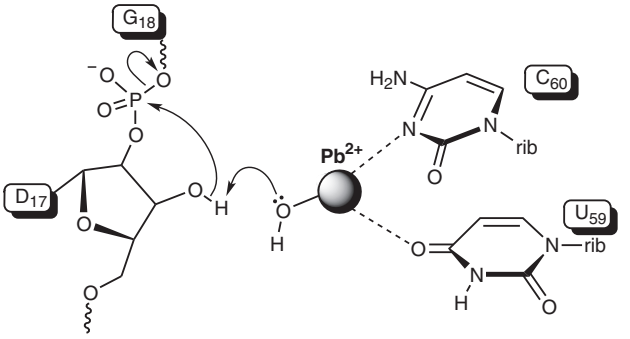
After incubating tRNA^{Phe} with Pb^{2+}, students run PAGE to separate the cleaved and uncleaved fragments of tRNA^{Phe}. They use software[5] to quantify the percentage of cleaved and uncleaved tRNA^{Phe} under various experimental conditions. Students then study the effects of small molecule ligands on the conformation of tRNA^{Phe} by measuring changes in the efficiency of lead(II)-catalyzed cleavage of tRNA^{Phe}. Typical student results are shown in Figure 4 where in each lane the top band contains uncleaved tRNA^{Phe} and the lower band is the largest cleaved fragment. Clearly, 400 μM Pb^{2+} catalyzes tRNA^{Phe} hydrolysis fairly efficiently (lanes 2 and 12), and hydrolysis is inhibited in a dose-dependent manner by 0.1 to 100 mM spermine (lanes 3–11).

Using Gel Doc[5] to quantify the bands in Figure 4, students find that spermine inhibition of lead(II)-catalyzed cleavage of tRNA^{Phe} saturates at high concentrations (Figure 5). The decline in cleavage can be fit to an equation for hyperbolic saturation,

$$y = y_f + \Delta y_{max} - \frac{\Delta y_{max}}{1 + \frac{K_d}{c}} = y_f + \frac{K_d\,\Delta y_{max}}{c + K_d} \quad (1)$$

where y is the percent of tRNA^{Phe} that is cleaved; y_f is the final, minimum y value; c is the ligand concentration; Δy_{max} is the maximum change in y, from zero to infinite concentration; and K_d is the ligand concentration giving a decline in y that is $\frac{1}{2}\Delta y_{max}$. The concentration of spermine that gives 50% inhibition, which can also be interpreted as the equilibrium dissociation constant (K_d) of the spermine–tRNA^{Phe} complex, is 14 ± 17 mmol/L. Note that owing to the difficulty of quantitating each gel band, uncertainties (error bars) are large for each data point in Figure 5, and the trend in the data is quite noisy (R^2 is only 0.67). Here students learn about the innate limitations of some experimental methods.

Besides studying the effects of lead(II), each pair of students is also assigned a set of metal cations to study. Students observe whether their assigned metals catalyze the hydrolysis of tRNA^{Phe} *(24)*. If cleavage occurs, students determine its dependence on metal cation concentration and also compare the resulting fragment sizes to those produced by lead(II). Typical student results are shown in Figure 6. We see that Gd^{3+} catalyzes tRNA^{Phe} hydrolysis, although not as well as Pb^{2+}, whereas Zn^{2+} is relatively ineffective. Even so, some cleavage is just discernible at ≥ 20 μM Zn^{2+} (lanes 12 and beyond); furthermore, at 2 mM Zn^{2+} (lane 18), a fragment smaller than that produced by Pb^{2+} and Gd^{3+} begins to appear as a very faint lower band.



Scheme I. Lead(II)-catalyzed hydrolysis of tRNA^{Phe}. Modified from ref *22*.

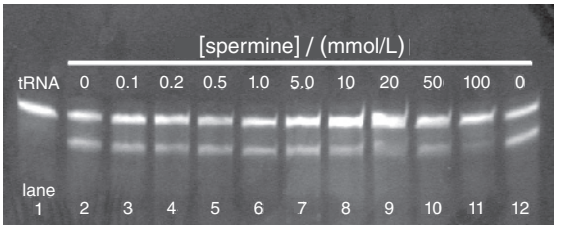


Figure 4. Cleavage of 16 μM tRNA^{Phe} catalyzed by 400 μM Pb^{2+} in the presence of increasing concentrations of spermine. Lane 1 shows tRNA with no lead. Incubation time was 40 minutes; buffer = 50 mM Tris·HCl, pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$.

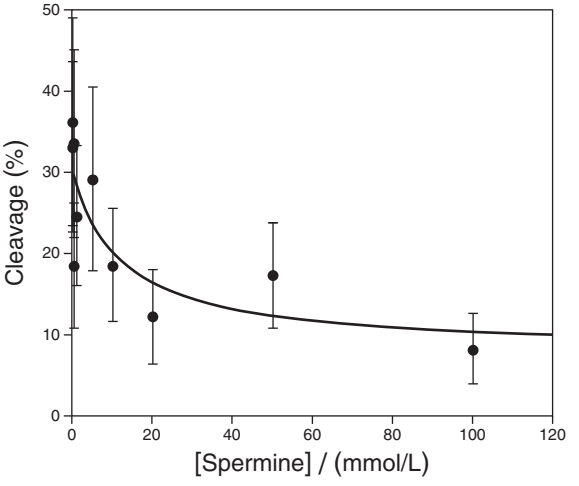


Figure 5. Percent of tRNA^{Phe} cleaved by 400 μM Pb^{2+} in the presence of increasing concentrations of spermine. Data derived from Figure 4. Solid curve is fit to eq 1 for hyperbolic saturation; K_d = 14 ± 17 mmol/L, R^2 = 0.67. Error bars derived from uncertainty values provided by Quantity One software.

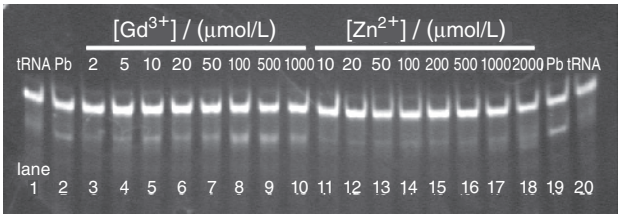


Figure 6. Cleavage of 16 μM tRNA^{Phe} catalyzed by Gd^{3+} and Zn^{2+}. Incubation time was 19.5 hours. Lanes 1 and 20 show tRNA with no metal ion. Lanes 2 and 19 are 400 μM Pb^{2+} controls. Buffer is the same as in Figure 4.

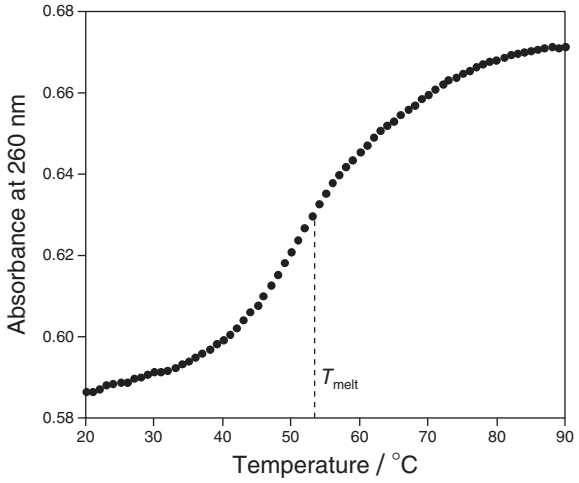


Figure 7. Absorbance (260 nm) vs temperature for 1 μM $tRNA^{Phe}$ in 50 mM Tris buffer, pH 7.5 with 100 mM NaCl, in the absence of ligands. The melting temperature occurs at T_{melt} = 53.3 ± 0.9 °C.

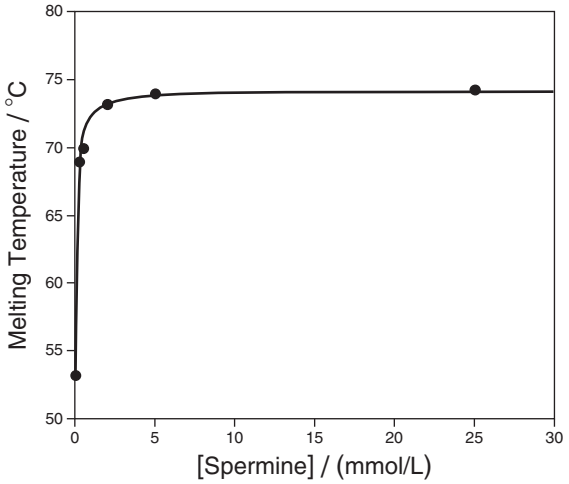


Figure 8. Plot of melting temperature of 1 μM $tRNA^{Phe}$ in the presence of increasing concentration of spermine. Data are fit to hyperbolic saturation curve (eq 2): K_d = 96 ± 16 μmol/L, R^2 = 0.996. Buffer is the same as in Figure 7.

Thermal Melting of $tRNA^{Phe}$ Probed by UV–Vis Spectrophotometry

The purine and pyrimidine bases of RNA may be used as endogenous reporters of conformation. These aromatic rings absorb UV light strongly when they are exposed to the external polar aqueous solution, and UV absorbance declines when the bases are internally π-stacked along with other nonpolar aromatic rings *(26)*. As RNA unfolds, whether due to chemical denaturants or heating (in a process called "melting"), the unstacked bases increase their exposure to water, causing UV absorbance to rise. On the other hand, RNA refolding causes absorbance to decline to its original level. By following absorbance as a function of temperature, students can determine T_{melt}, the temperature at which the folded and unfolded forms of RNA are in a 50/50 equilibrium. Typical student results for 1 μM $tRNA^{Phe}$ in 50 mM Tris buffer, pH 7.5 with 100 mM NaCl are shown in Figure 7. Under these conditions, T_{melt} = 53.3 ± 0.9 °C.

After measuring T_{melt} for $tRNA^{Phe}$ in buffer, students characterize changes in T_{melt} in the presence of different concentrations of an assigned polycationic small molecule. Student results plotted in Figure 8 show that T_{melt} values for $tRNA^{Phe}$ increase with increasing concentration of spermine, indicating that spermine binding to $tRNA^{Phe}$ stabilizes the folded structure. Furthermore, the increase in T_{melt} shows a Michaelis–Menten-like hyperbolic saturation at high spermine concentrations. Fitting the data to the equation for hyperbolic saturation of equilibrium binding,

$$y = y_0 + \frac{\Delta y_{max}}{1 + \frac{K_d}{c}} = y_0 + \frac{c\,\Delta y_{max}}{c + K_d} \qquad (2)$$

where y is T_{melt} and y_0 is the initial y value for zero ligand concentration (the other variables are the same as defined in eq 1), gives a best-fit value of K_d = 96 ± 16 μmol/L for the equilibrium dissociation constant of the spermine–$tRNA^{Phe}$ complex. Students can measure similar changes in the T_{melt} of $tRNA^{Phe}$ in the presence of Mg^{2+} or ethidium bromide and draw conclusions regarding the effects of all of these cationic ligands on the conformational stability of $tRNA^{Phe}$.

$tRNA^{Phe}$ Conformational Changes Characterized by Fluorescence Spectroscopy

Many modified bases are present in $tRNA^{Phe}$, one of which is a Y base, located at position 37. Compared to a normal guanine base (Figure 9A), the Y base has an additional aromatic ring (Figure 9B) that gives it unique fluorescent properties. The Y base is normally found π-stacked in between nucleotide base pairs and shielded from the polar aqueous solvent. In this "stacked-in" nonpolar environment, the Y base fluoresces strongly. Under certain conditions, the conformation of $tRNA^{Phe}$ may change in such a way as to force the Y base out of the internal π-stack and increase its exposure to water; conversion to this "flipped-out" conformation results in a decrease in Y base fluorescence. On the other hand, most polycations bind to the major groove in $tRNA^{Phe}$ and stabilize the stacked-in conformation of the Y base, thus causing an increase in Y base fluorescence *(27)*.

Students titrate various small molecule cationic ligands into a $tRNA^{Phe}$ solution, and follow the increase in Y base fluorescence intensity (Figure A in the online supplement). The fluorescence increase saturates with ligand concentration, similar to the ligand effect on T_{melt} seen in Figure 8. Students fit their fluorescence versus ligand concentration titration data to eq 2, where y is the fluorescence intensity at λ_{max}; this allows them to determine K_d, the equilibrium dissociation constant

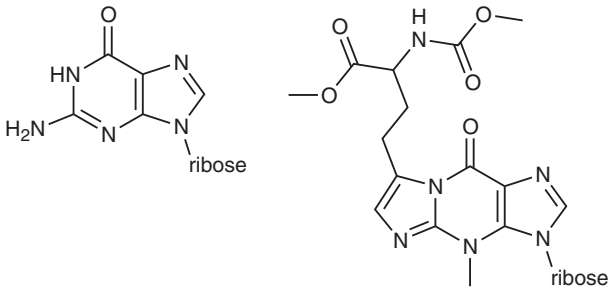


Figure 9. Structures of the (A) guanine nucleotide and (B) Y base nucleotide.

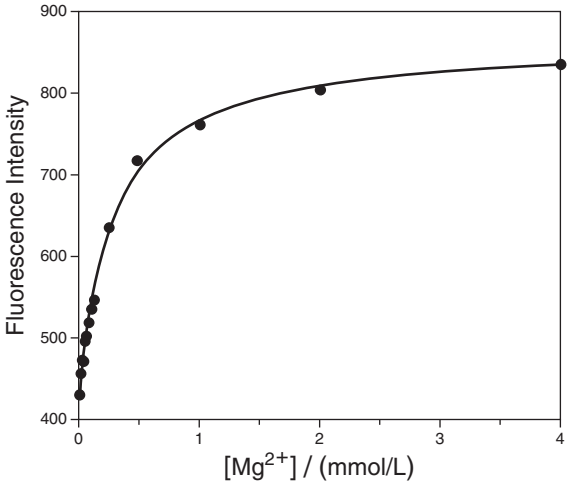


Figure 10. Plot of fluorescence intensity of $tRNA^{Phe}$ Y base (~430 nm) in the presence of increasing concentration of $MgCl_2$. Data are fit to hyperbolic curve (see eq 2): K_d = 0.296 ± 0.024 mmol/L, R^2 = 0.996.

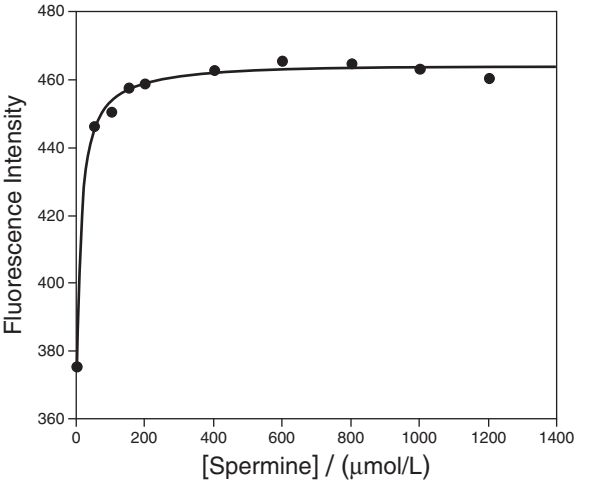


Figure 11. Plot of fluorescence intensity of $tRNA^{Phe}$ Y base (~430 nm) in the presence of increasing concentration of spermine. Data are fit to hyperbolic curve (see eq 2): K_d = 14.3 ± 2.1 µmol/L, R^2 = 0.990.

for the ligand–tRNA complex. Typical student data, shown in Figures 10 and 11 provide for an experimental determination of K_d for the Mg^{2+}–$tRNA^{Phe}$ complex (296 ± 24 µmol/L) and the spermine–$tRNA^{Phe}$ complex (14.3 ± 2.1 µmol/L). Note that K_d (spermine–$tRNA^{Phe}$) from this experiment is almost seven times lower (i.e., tighter binding) than that determined from the change in T_{melt} (Figure 8, K_d = 96 µmol/L) and about 950 times lower than that determined from the inhibition of lead(II)-catalyzed cleavage (Figure 5, K_d = 14 mmol/L). This obvious discrepancy provides fertile ground for students to speculate on possible differences in the effects of spermine–$tRNA^{Phe}$ binding on T_{melt}, Y base fluorescence, and lead(II)-catalyzed cleavage.

Ethidium bromide (EtBr), a small fluorescent molecule (Figure 12), is another cationic ligand that binds to RNA. The fused aromatic rings of EtBr intercalate into the central π-stacked rings of RNA, and the EtBr quaternary ammonium cation salt bridges to the RNA anionic phosphate backbone. Similar to the above-mentioned Y base, EtBr fluorescence is also enhanced when it is π-stacked and bound to tRNA, as compared to its weakly fluorescent aqueous form *(28)*.

It turns out that although the formation of the EtBr–tRNA complex increases the fluorescence of the EtBr ligand (as described above), EtBr binding to $tRNA^{Phe}$ favors the Y base "flipped-out" conformation, thus causing a decrease in endogenous Y base fluorescence. Adding a small molecule cationic ligand to the EtBr–$tRNA^{Phe}$ complex displaces EtBr, causing $tRNA^{Phe}$ Y base fluorescence to increase, and EtBr fluorescence to decrease. Y base and EtBr fluorescence can be monitored separately at distinct excitation and emission wavelengths (Figure 13). In the spermine titration of the EtBr–$tRNA^{Phe}$ complex (Figure 13), both the increase in Y base fluorescence and the decrease in EtBr fluorescence yield a K_d of 240 µmol/L for spermine binding. Recall from Figure 11 that for the binding of spermine to $tRNA^{Phe}$ alone, Y base fluorescence increase yielded a K_d of only 14.3 µmol/L. Students clearly see that EtBr binding to $tRNA^{Phe}$ interferes with spermine binding, raising K_d almost seventeen-fold.

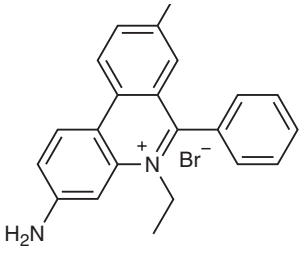


Figure 12. Structure of ethidium bromide (EtBr).

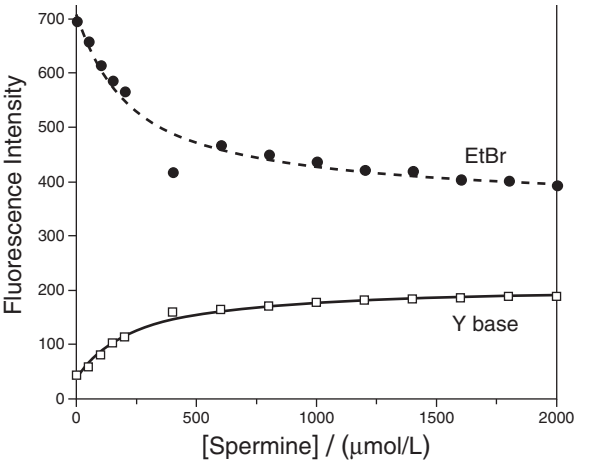


Figure 13. Plot of fluorescence intensity of EtBr (●, ~605 nm) and $tRNA^{Phe}$ Y base (□, ~430 nm) in the presence of increasing concentration of spermine. 2 µM $tRNA^{Phe}$ and 50 µM EtBr in 50 mM Tris buffer, pH 7.5 with 100 mM NaCl; excitation at 318 nm (Y base) and 546 nm (EtBr). K_d = 240 ± 70 µmol/L, R^2 = 0.955 from EtBr fluorescence decrease; K_d = 240 ± 30 µmol/L, R^2 = 0.989 from Y base fluorescence increase.

Table 2. Projects Probing tRNA^{Phe} Structure

Project	Assay Method	Biophysical Theories	Data Analysis
Metal-catalyzed hydrolysis	Gel electrophoresis	Catalysis; bioorganic and bioinorganic mechanisms of hydrolysis	Quantitation of gel bands; qualitative and quantitative assessment of metal-catalyzed hydrolysis and the influence of ligands
Thermal melting	UV–vis spectrophotometry	Ligand-binding equilibria; spectroscopy theory	Scatter plots fitted to equilibrium binding equations; determine K_d and compare to literature value
Ligand binding	Fluorescence spectroscopy	Same as thermal melting	Same as thermal melting

As noted above, the three major projects in this laboratory all probe conformational changes in the tertiary structure of tRNA^{Phe}. Table 2 summarizes the assay methods and biophysical and chemical theories that students use when analyzing their experimental results.

Hazards

A number of chemicals used in this laboratory course are considered hazardous. The polyamines, metal salts ($MgCl_2$, $GdCl_3$, $EuCl_3$, $MnSO_4$, $ZnSO_4$), *N,N,N′,N′*-tetramethylethylenediamine, and ammonium persulfate are considered mild to moderate irritants to the skin and eyes. Exposure to $FeCl_3$ may cause problems in the kidney, liver, and central nervous system. Students are required to wear safety gloves and glasses at all times. To minimize student exposure to hazardous powders, stock solutions of ethidium bromide (a mutagen) and lead(II) acetate (a carcinogen) are prepared by our laboratory technician. Acrylamide (a carcinogen) is purchased as a 40% solution of 19:1 acrylamide:bisacrylamide. Our laboratory technician prepares the desired 10% acrylamide solution including the addition of urea and 1× Tris-borate-EDTA.

Keeping stock solutions to the lowest possible concentration minimizes both exposure and waste. Stained gels containing less than 0.1% w/w of ethidium bromide may be double bagged and disposed of in the laboratory trash *(29, 30).* Ethidium bromide staining solutions should be filtered over activated charcoal prior to disposal.

Assessment

We have taught our new laboratory three times, twice by SRK and one time by TPS. The laboratory has met with strong approval from students (for a compilation of student responses, see Table A in the online supplement). Common aspects that students enjoyed were (i) pursuing new knowledge and novel research; (ii) sharing data between class teams to build a broader understanding; (iii) the integrated nature of the laboratory; (iv) probing complex changes in a single biomolecule, tRNA^{Phe}; (v) the productive balance between student independence and professorial oversight; and (vi) learning many new laboratory skills, techniques, and instrumentation. Common concerns included unclear or unsuccessful laboratory protocols and lack of time and preparation for data analysis.

Based on student evaluations, there are two aspects of the laboratory we have continued to improve: (i) the accuracy of gel band quantitation[6] using Quantity One software and (ii) the sharing and pacing of data analysis. Accordingly, we have required four interim reports (two oral and two written) and had students develop a uniform spreadsheet format to share data. We have also adjusted the schedule so that the final dissemination of all shared data and the last oral presentation both occur at least a week before the final written report is due.

Besides the laboratory-related outcomes mentioned above, two other potential products are worth mentioning here: upper-level research projects and real-world applications. As mentioned previously, a number of the experiments carried out in this lab are novel; thus student results may eventually lead to a research publication. Furthermore, students have used the methods and experience gained in this laboratory to design novel research projects on such topics as investigating the possibility of a neomycin B–tRNA–rRNA ternary complex; using a fluorescent probe to explore small molecule–RNA binding; and studying ruthenium–tRNA complexes. Furthermore, studying tRNA structure offers students a view into the world of drug design. In order to design a drug rationally, the structure of the target must be known. In the last twenty years there has been a growing interest in nucleic acids as drug targets *(31–33).* This laboratory demonstrates how small molecule binding affects the structure of tRNA, which could in turn alter biological function in a therapeutic fashion.

Summary

In the laboratory described here, students use three important experimental techniques in the modern biochemistry laboratory: gel electrophoresis, UV–vis spectrophotometry, and fluorescence spectroscopy. The class as a whole explores how subtle changes in the structure of a small molecule ligand may affect its ability to bind to and alter the structure of tRNA^{Phe}. The binding constants derived for a given small molecule may vary depending on the assay used. Students must work to understand and explain how some assays probe long-range global structure, while others respond to specific (and perhaps distinct) local regions of the tRNA^{Phe} conformation.

Finally, the course gives students experience that can be applied to many aspects of basic biochemical research including experimental design, laboratory skills and instrumentation,

and data analysis and interpretation. In line with ACS CPT's proposed new chemistry curriculum guidelines *(5),* our course stresses critical thinking, scientific communication (written and oral), teamwork, accessing the literature, and novel independent research. This laboratory also allows students to combine knowledge gained from many of their previous chemistry courses, and in this way, our laboratory is an ideal capstone course.

Notes

1. Additional semester-long "integrated" or "project-oriented" biochemistry laboratories are listed in the online supplement.

2. The focus of this laboratory was inspired by the graduate dissertation work of coauthor SRK *(21).* The data sets featured in this article were collected by coauthor BLHT, an undergraduate who participated in the course first as a student and later as a teaching assistant.

3. Laboratory sessions meet one afternoon per week; however, motivated students are encouraged to come in during an open lab time to repeat experiments.

4. Our optimal class size so far has been six to eight students. If we had a second fluorimeter, or if we required two laboratory sessions per week, we could double that number to 12–16.

5. Biorad Gel Doc 1000 system with Quantity One software was used.

6. EtBr is an effective stain for tRNA gel bands; however, its toxicity gave us some pause. We therefore tried two other staining reagents, but neither Stains-all nor the non-toxic SYBR Safe *(34)* proved to be as reliable as EtBr.

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