Using Thioflavin T as a Biosensor for the Detection of Nucleic Acid Secondary Structures

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Abstract

This is a Freshman Fellowship research project undertaken in the Chemistry at NMU with the main goal of training on general laboratory experience on chemical and biochemical analysis. To begin to develop some of the basic skills in biochemical analysis we choose to study a very important molecule called Thioflavin T which can be used to develop a versatile system to target and detect other biomolecules. The purpose of this ongoing research project is to learn more about Thioflavin T by developing methods using a UV/Vis spectrophotometer to investigate how this molecule interacts with other biomolecules. Thioflavin T (ThT), is a fluorescent dye that can bind proteins and nucleic acids and can be used to probe protein aggregation and unique nucleic acid secondary structures. It can be used as a biosensor to detect specific abnormal proteins called amyloid fibrils in biological samples. In this project, we will investigate how thioflavin T interacts with different nucleic acid secondary structures with the goal of developing a ThT-based biosensor for nucleic acid structures.

Background

Thioflavin T, ThT, is a chemical compound with a specific structure that allows it to absorb UV light and also fluoresces under certain conditions. It is commonly used as a staining dye to visualize specific misfolded proteins. Thioflavin T can be used as a fluorescent marker to identify the presence of amyloids. This is important because misfolded proteins can lead to diseases including Alzheimer's, Parkinson's disease, type 2 diabetes, and several types of amyloidosis, which can lead to other diseases[1].

Not only does Thioflavin T target proteins, but it can also selectively target certain nucleic acid secondary structures[2,3] such as specific base pairing in the Watson-Crick helices, hairpins, and G-quadruplex structures. It is therefore a great biomarker[4] for probing these unique biologically relevant structures.

A UV-Vis spectrophotometer is an analytical instrument routinely used for the quantitative analysis of UV-active organic compounds, metal ions, and biological macromolecules where the absorbance of UV or visible light correlates with the concentration of the absorbing molecules in solution. Thioflavin T absorbs at wavelength range 412-416 nm with a molar absorptivity of 3600 $M^{-1}cm^{-1}$. In these studies, we will use a UV/Vis spectrophotometer to train on analytical samples preparation and experimentation skills by measuring the absorbance of varying ThT standards concentrations. Next, we will investigate the binding of ThT to nucleic acid structures by monitoring the absorbance of free Thioflavin T in solution and in the presence of nucleic acid structures. These studies will give insights on the use of ThT in developing a biosensor for nucleic acid secondary structures.
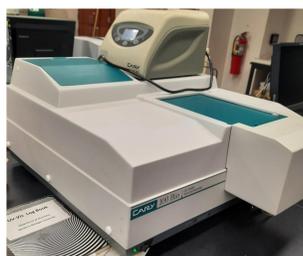
A Varian Cary 100 Bio UV-Visible Spectrophotometer used in performing these experiments

Methods

MOPS Buffer Preparation

Most chemical and biochemical reactions occur at controlled pHs in buffer solutions. A solution of 3-morpholinopropane-1-sulfonic acid (MOPS) provides an excellent buffer at near neutral pH for most biological systems. The first step in making the MOPS buffer involves making a high pH buffer and a low pH buffer. The recipe for the MOPS buffer is two parts – low pH MOPS solution consisting of 10 X 0.50 M MOPS and 0.05 M NaOH and high pH MOPS solution consisting of 10 X 0.50 M MOPS and 0.45 M NaOH. The high pH MOPS is then titrated against the low pH MOPS to a desired pH shown in Figure 1. This is an important and robust recipe because using the linear regression line of the pH versus percent of high pH plot, any buffer of desired pH can easily be made by simply mixing the correct fraction of low and high pH MOPS stock solutions.

Determination of Thioflavin T Standards Concentration by UV-Vis

A stock solution of Thioflavin T was taken and diluted into 50 mM MOPS, pH 7.0 buffer to make five standards of varying known concentrations. The UV-Vis Spectrophotometer was then used to measure the absorbance of each of the five standards by scanning from 300 – 500 nm wavelength (Figure 2). The maximum absorbance was obtained at 414 nm. These measurements were repeated three different times with freshly prepared standards each time so as to determine the reproducibility of the measurements by plotting the absorbance at 414 nm against the known concentrations of ThT as shown in Figure 3.

We then calculated the concentration of the five Thioflavin T standards from the UV-Vis absorbance measurements using Beer's law (A = ƐbC). A graph comparing the values of the known concentrations of the ThT standards was plotted against the concentrations calculated from the absorbances from the UV-Vis spectrophotometer (Figure 4). These measurements were done to confirm the concentrations of the ThT standards and also to determine the reproducibility and robustness of the instrument in performing these experiments. As shown in Figure 4, the ThT concentrations calculated from the absorbance measurements correlate very well with the concentrations of the standards.

Conclusion

These initial experiments show reproducible measurements indicating great skills training in pipetting and in making consistent measurements. By producing consistent measurements, these data also indicate the feasibility of our proposed method in using the UV-Vis instrument to carry out this research project in developing a ThT-based biosensor for nucleic acid structures. With these encouraging results we are set to begin performing experiments on nucleic acid secondary structures to probe how Thioflavin T interacts with and recognizes different nucleic acid structures.

References

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Acknowledgments

Results
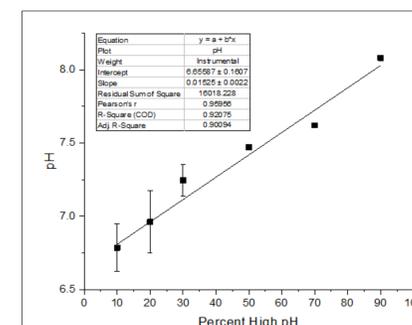
Figure 1. MOPS buffer preparation graph showing the relationship between the percentage of high pH solution required to make a new buffer solution of a known pH. The equation of the line can be used to make specific pH buffers.
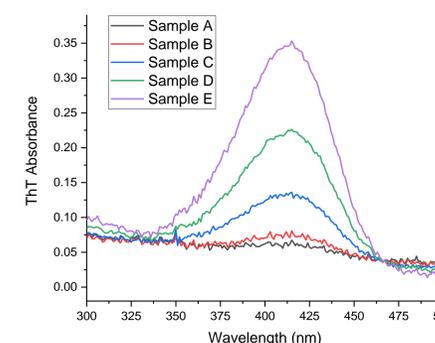
Figure 2. Raw data taken from the UV-Vis spectrophotometer. The most prominent wavelength was 414, and those were the values of the absorbance used to calculate the concentrations of the ThT standards.
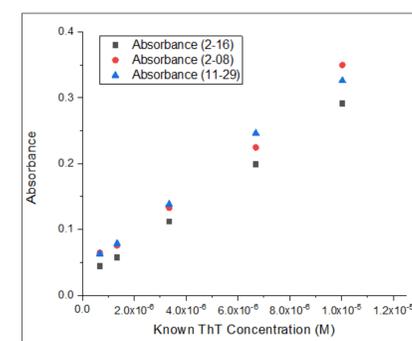
Figure 3. Graph showing the known concentrations of the ThT standards plotted against the absorbances taken from the UV-Vis data
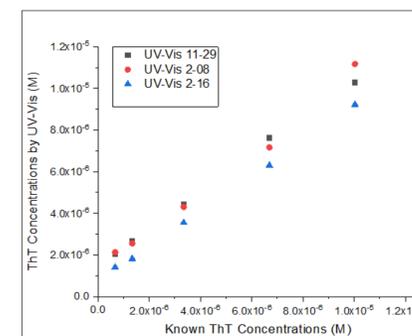
Figure 4. A plot of known ThT concentrations versus calculated ThT concentrations from the UV-Vis absorbance measurements.