

## Proposal for Senior Honors Thesis

HONS 497 Senior Honors Thesis Credits 4 (2 minimum required)

Directions: Please return signed proposal to the Honors Office **at least one week prior to your scheduled meeting with the Honors Council**. This proposal must be accepted by Honors Council the semester before presentation.

Student's Name: Erica Evans

Primary Advisor: Dr. Ryan Hayes

Secondary Advisor: Dr. Desmond Murray

Thesis Title: Fluorescent Copper Sensing using Dimethylaminocyanostilbene

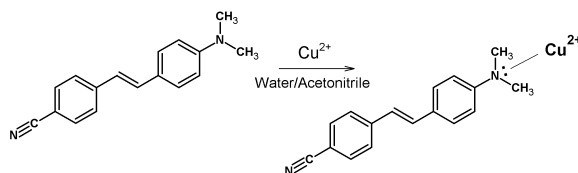
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Expected date of Graduation: May 5, 2013

### I. Provide goals and brief description of your project or research.

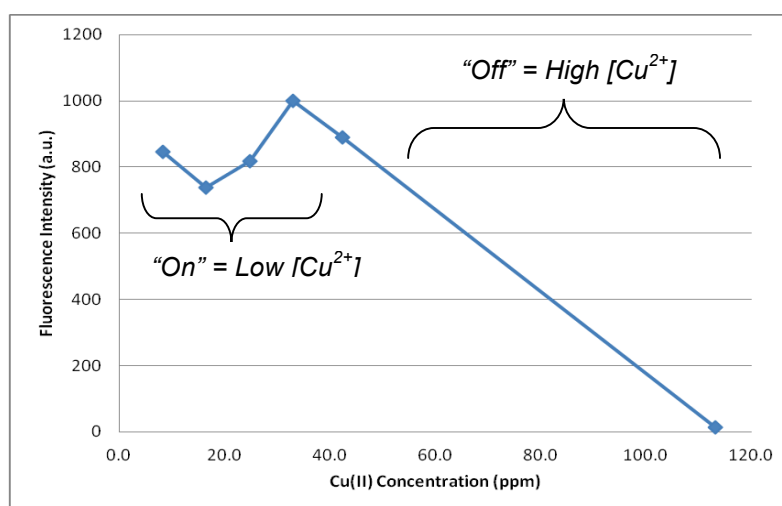
Copper ions are important biologically and environmentally— $\text{Cu}^{2+}$  is the third most abundant essential transition element in the body. However, too much copper has been connected to several diseases, and is also a significant environmental metal pollutant (Liu and Wu, 2011). Detection of copper has been studied in the field of analytical chemistry because of its toxicity to humans, bacteria, and aquatic environments at high concentrations (Zhao et al., 2009). The goal of my project is to study the optical activity of the donor-acceptor stilbene dimethylaminocyanostilbene (DCS) with  $\text{Cu}^{2+}$  (see **Figure 1**). In a rapid, high concentration screen, DCS showed significant and selective fluorescent quenching in the presence of high  $\text{Cu}^{2+}$  concentrations indicating its potential as a copper ion sensor.



**Figure 1)** Proposed mechanism of dimethylaminocyanostilbene (DCS) in the presence of  $\text{Cu}^{2+}$ .

While the synthesis of various fluorescent  $\text{Cu}^{2+}$  probes have been presented in peer-review literature in the last two years, the DCS molecule has not yet, to date, been studied and reported on in any chemical literature for its high selectivity for  $\text{Cu}^{2+}$  ions (Hrishikesan et al., 2011, You et al., 2011). One of the main reasons the DCS molecule has not been studied in the past is because of its high cost. Dr. Murray has pioneered a method to synthesize DCS in large batches and high purity with a one-step process which greatly reduces the costs associated with the manufacturing DCS.

My research involves the characterization of the DCS molecule's optical properties in the presence and absence of copper to determine if DCS can be used to detect copper ions. The main goal of my project is to determine the extent of specificity of the  $\text{Cu}^{2+}$  sensing and the detection range of this interaction utilizing UV-VIS absorbance and fluorescence emission spectroscopy (Badugu et al., 2009). Initial results show that this interaction is in the visible range so it may be possible to have a sensor that could be implemented with just the human eye. Based on data that I collected and presented at the Andrews Undergraduate Research Poster Symposium last spring, DCS revealed no linear correlation between fluorescence intensity and  $\text{Cu}^{2+}$  concentration. This behavior was different than expected but, at certain copper concentrations such as 0.001 M (100 ppm) and higher, the fluorescence emission of 180 ppm DCS was quenched. The "turning-off" point of the DCS fluorescent appeared to occur over a very narrow range of copper ion concentration which led us to consider that a different emission quenching process might be involved than what was observed for previously published optical copper sensors (Liu and Wu, 2011). DCS appears to work as an "on-off" sensor rather than a quantitative sensor measuring exact concentrations of copper ions (see **Figure 2**).



**Figure 1)** Fluorescence Quenching of DCS in Acetonitrile in Various  $\text{Cu}^{2+}$  Concentrations (peak wavelength at 522 nm).

This semester, the focus of my research is to determine the necessary concentrations (mole ratios) of DCS and  $\text{Cu}^{2+}$  for DCS to work as an "on-off" sensor and at various levels of copper concentrations. To accurately measure the fluorescence properties of DCS in the presence of  $\text{Cu}^{2+}$ , my plan is to initially run a kinetics study to determine the rate of the reaction to establish at what point time to run the spectroscopic tests after adding  $\text{Cu}^{2+}$ . Furthermore, an absorption spectrum analysis for the DCS and  $\text{Cu}^{2+}$  titration will take place to accurately determine how the ground state absorption processes are changing upon the addition of  $\text{Cu}^{2+}$ . Once the kinetics and absorption studies are complete, I will be able to properly evaluate the relative concentrations of DCS and  $\text{Cu}^{2+}$  in the fluorescence spectrophotometer to see the "on" and "off" effects of DCS in the presence of  $\text{Cu}^{2+}$ . In addition, I will also check to see if the fluorescence quenching process is reversible by changing the pH of the solution or adding a metal chelator, such as EDTA, to see if the DCS fluorescence will return. Lastly, I will check for DCS's fluorescence levels by mixing it with potentially interfering metals to verify the molecule's selectivity for  $\text{Cu}^{2+}$ .

## II. Outline your methodology. Please be specific. How does this achieve your goals and how reliable is it?

### SOLUTION PREPARATION

The molecule of interest, dimethylaminocyanostilbene (DCS) was synthesized by Dr. Desmond Murray and his research team from the Department of Chemistry and Biochemistry. All other solvents and reagents that will be used for my research project will be obtained from commercial sources and used without further purification. A stock solution of DCS will be prepared by dissolving DCS in acetonitrile, a polar organic solvent, which has been chosen as the carrier solvent based upon its miscibility with DCS and an aqueous solution of  $\text{Cu}^{2+}$ . A standard stock solution of concentrated  $\text{Cu}^{2+}$  will be prepared in a volumetric flask by dissolving the appropriate amount of copper (II) triflate in water and adjusting the volume. The triflate counter ion of copper aids in solubility of  $\text{Cu}^{2+}$  in organic solvents, such as acetonitrile.

To measure the effects of fluorescence quenching of DCS in the presence of  $\text{Cu}^{2+}$  ions, I will carry out  $\text{Cu}^{2+}$  titrations with DCS using two different methods. In **Method 1**, I will perform out serial dilutions of the original  $\text{Cu}^{2+}$  stock solution with water in volumetric flasks to make the appropriate concentrations necessary for titrations. In cases where I may need to make several varying concentrations of  $\text{Cu}^{2+}$ , I will utilize a micropipetting technique to speed up the process of dilution. This alternative method, **Method 2**, will allow me to insert microliter amounts of high concentration  $\text{Cu}^{2+}$  solution into the DCS solution. The addition of water into the sample with each microliter increment of  $\text{Cu}^{2+}$  will be so minute that the additions will not affect the significant figures in my calculations of volume—all the data points collected will be from the same cuvette or flask. This method will allow for the analysis of more concentrations to provide a more detailed picture of the relationship between copper concentration and optical properties. Thus, the total volume will be considered constant, and the  $\text{Cu}^{2+}$  can be added directly into the sample. All solutions will be kept at room temperature, around  $20^\circ\text{C}$ .

### KINETICS

The Cary Eclipse Spectrophotometer will be used to perform the kinetics studies which measure the change in fluorescence intensity over time. Using the preparation methods stated above, I will combine  $\text{Cu}^{2+}$  solutions with a fixed concentration of DCS, and then scan the fluorescence intensity after each increment of  $\text{Cu}^{2+}$  added. Because of the volatility of acetonitrile, cuvettes will be capped after the addition of  $\text{Cu}^{2+}$  and additionally wrapped with parafilm to form a tight seal before being placed in the spectrophotometer. For consistency, I have set up a protocol for time measurement that will allow time for mixing reagents and preparing the instrument for recordings. I will give myself exactly one minute from the moment the reagents are combined to the time when the scan begins. Time will be recorded as soon as the  $\text{Cu}^{2+}$  is added into the DCS solution. Once the scan begins, I will wait to observe the fluorescence intensity of the sample until it decreases to a specific intensity which will be established as the fixed “quenched” intensity. These scans will be repeated at different concentrations of  $\text{Cu}^{2+}$  and the amount of time,  $t$ , it takes to reach the fixed quenched intensity will be recorded.

Studying the kinetics of the reaction between DCS and  $\text{Cu}^{2+}$  is useful for determining the amount of time it takes for  $\text{Cu}^{2+}$  to quench DCS fluorescence to a certain level of intensity in various concentrations of  $\text{Cu}^{2+}$ . After obtaining many data points, approximately 10 points, I will plot a graph with the concentration of  $\text{Cu}^{2+}$  versus time in an attempt to linearly fit one of the integrated rate laws to the points to determine the order of the reaction. These 10 data points can be repeated three times to create an error analysis of each data. If the error of each

data point appears large, greater than 5% error, than more data points will be collected per time point to establish a relative standard deviation for each.

Order of Reaction	Integrated Rate Law	Characteristic Kinetic Plot	Slope of Kinetic Plot
0	$[Cu^{2+}] = [Cu^{2+}]_0 - k t$	$[Cu^{2+}]$ vs $t$	$-k$
1	$[Cu^{2+}] = [Cu^{2+}]_0 e^{-k t}$	$\ln [Cu^{2+}]$ vs $t$	$-k$
2	$[Cu^{2+}] = [Cu^{2+}]_0 / (1 + k t [Cu^{2+}]_0)$	$1/[Cu^{2+}]$ vs $t$	$k$

The data will be analyzed to select an appropriate time at which the best consistent quenching behaviour for a certain range of sample concentrations will take place. This time will then be used as the time of measurement for the absorbance and fluorescence studies. The rate law will help me to make a better prediction regarding the fluorescence quenching time related to copper ion concentration, especially at low concentrations.

## ABSORPTION SPECTRUM ANALYSIS

All absorption spectra will be scanned in the Cary 5000 UV-Vis-NIR Spectrophotometer which has a recording range of 200-3500 nm. I will be collecting data from the UV and visible range (200-800 nm). Quartz cuvettes will be used because of their minimal interference with light absorption. A reference sample consisting of the cuvette and the solvent will be read first and stored, followed by variable concentrations of DCS with  $Cu^{2+}$ . Very dilute concentrations of DCS and  $Cu^{2+}$  will be transferred into cuvettes to avoid saturating the detector leading to non-linear results which can occur at high concentrations. Using the protocol mentioned in the kinetics study, the DCS and  $Cu^{2+}$  mixture will be scanned in the absorption spectrometer after each microliter addition of  $Cu^{2+}$ . I will then measure the peak absorbance for each  $Cu^{2+}$  titration and create a plot of absorbance versus  $Cu^{2+}$  concentration. The kinetics of any changes in absorbance spectrum can also be analyzed as stated above. In general, I will try to be consistent relative to time regarding the taking of absorbance spectra.

Ideal results would indicate the absorption intensity versus  $Cu^{2+}$  concentration graph to be linear, and the wavelength producing the peak absorbance would be the fluorescence excitation wavelength. However, from previous observations in the research performed last year, I noticed that the absorption spectrum of DCS changed with the addition of  $Cu^{2+}$ . Therefore, I will not only be looking at the absorbance peaks, but I will also check to see how the peak absorbance is shifting to most accurately select the excitation wavelength that will produce the best results for the fluorescence studies. Finally, I will calculate the mole ratio of DCS and  $Cu^{2+}$  at which changes occur in the absorption spectra. This can be best done with a Job plot which has been previously used with copper-ion sensing studies.

The solutions will also be evaluated by sight to determine if there is a noticeable and visible means to detect the "off" point in this sensor. My previous work suggests that there is a noticeable color change. These absorption experiments will help quantify this effect and demonstrate the nature of the absorption changes.

## FLUORESCENCE STUDIES

I will scan the fluorescence emission of DCS and  $Cu^{2+}$  mixtures using the Cary Eclipse Spectrophotometer. A fixed excitation wavelength will be chosen from the absorption spectrum analysis, and slit widths that show the full range of fluorescence intensities will be consistently used to scan DCS in the presence of  $Cu^{2+}$  at a range of 400-800 nm.

To understand what occurs at the concentration at which self-quenching takes place for DCS, an analysis of fluorescence intensity versus DCS concentration will be made. High concentrations can cause the plot to exhibit non-linear correlation which means that molecules are quenching their own fluorescence. This comes from the overlap in absorbance and emission profiles that many organic chromophores exhibit. This study will take place in the absence of  $\text{Cu}^{2+}$ . These high concentrations, therefore, will be avoided in order to keep the DCS fluorescence emission in a linear regime.

To understand the effect of copper ions on the fluorescence intensity, various  $\text{Cu}^{2+}$  titrations of differing concentrations will be carried out using **Method 2**, and a plot of fluorescence intensity versus  $\text{Cu}^{2+}$  concentration will be graphed to calculate the mole ratio of DCS and  $\text{Cu}^{2+}$  ions at which fluorescence quenching takes place. In addition, a Job plot will show the stoichiometry of the binding phenomena. The Job plot is created by plotting the fluorescence intensity versus the mole fraction of one of the analytes (Hill and MacCarthy 1982). It is important to keep the total mole amounts of each component fixed.

## REVERSIBILITY

It is important to know under what conditions this sensor could be rejuvenated or what conditions could lead to spurious results. The pH of the solution will be changed and the fluorescent intensity of the DCS- $\text{Cu}^{2+}$  complex measured to determine any effects of pH. I anticipate that in a low pH environment, the amine group of DCS will be protonated which could break up the complex. However, this could also disturb the fluorescence intensity and may cause a shift in the emission spectrum. Previous work also suggests that ethylenediaminetetraacetic acid (EDTA) is an effective metal chelator which should effectively remove  $\text{Cu}^{2+}$  from the DCS- $\text{Cu}^{2+}$  complex and should restore the fluorescence of DCS. Various concentrations of EDTA will be made and mixed with the DCS- $\text{Cu}^{2+}$  complex to study and understand the binding properties  $\text{Cu}^{2+}$  to DCS.

## INTERFERING METALS

I will test the fluorescence levels of DCS in the presence of other metals like  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$  to make sure that DCS is indeed selective only for  $\text{Cu}^{2+}$ . While Dr. Murray and his research team have already tested a few other metals in a rapid, high concentration screen, I will be testing other these metal ions at lower concentrations using methods described in the Solution Preparation section to further ensure DCS's selectivity for  $\text{Cu}^{2+}$  ions only. This metal ion interfering experiment can be repeated in the presence of copper ions to see if the other metal ions change the binding characteristics of  $\text{Cu}^{2+}$  to DCS.

### III. Explain in what sense your project is original, unique, or beyond normal senior expectations. How does it relate to current knowledge in the discipline?

By doing research in chemistry, specifically analytical chemistry, I will be going beyond the normal chemistry coursework expectations for a senior biology student. Participating in research with Dr. Hayes will help improve my quantitative and laboratory skills, and give me the opportunity to work with absorption and emission spectroscopies, the basis of many medical laboratory tests. Developing these skills in the long term will help me become more aware as an analytical scientist and doctor as to the work involved in analytical testing. This will further increase my appreciation for the more detailed and technical aspects of medical technology and research. My research will help demonstrate an effective copper ion sensor using an affordable sensing element (DCS) as well as contributing further information regarding the nature of copper ions binding to nitrogen moieties within organic molecules.

#### IV. Include a substantive annotated bibliography of similar or related work.

Badugu, R.; Lakowicz, J. R.; Geddes, C. D. Cyanide-sensitive fluorescent probes. *Dyes and Pigments*. **2005**, *64*, 49-55.

In the study conducted by Badugu et al., several boronic acid containing fluorophores, which have commonly been used for sugar determination, were characterized for their ability to sense aqueous cyanide. In one case, the fluorescence emission spectra of one of the stilbene derivative sensors showed an increase in fluorescence intensity as concentrations of cyanide increased—the formation of the anion cyanide bond caused the loss of the electron-withdrawing property of the boronic acid group. A different stilbene derivative probe revealed fluorescence quenching in the presence of cyanide, indicating that the boronic acid group can act as an electron-donating group. Their report demonstrates that the sensing mechanism is generic to all boronic acid moieties, providing many modalities for the synthesis of fluorescent based cyanide sensing. Since the copper sensor that I am characterizing is also a stilbene derivative, this article serves as a useful guide to help me study how fluorescence quenching occurs in the molecule, and which moieties participate in complexing with  $\text{Cu}^{2+}$ .

Hill, Z.; MacCarthy, P. Novel Approach to Job's Method: An Undergraduate Experiment. *J. Chem. Ed.* **1986**, *63*, 162-167.

The Job's method of continuous variation is frequently used in several fields of chemistry, from analytical chemistry and instrumental analysis to equilibrium studies and undergraduate research papers. Typically two equimolar stock solutions of a metal and ligand are mixed in such a way that the ligand-to-metal ratio varies in while the total volume in each mixture remains constant. The corrected absorbance is then plotted against the mole fraction and the Job's plot can be utilized to establish the stoichiometric ratio of the ligand to metal ratio. Hill and MacCarthy proposed a new technique by combing titrimetry and the Job's method, previously thought to have been distinct topics. This paper shows how data from titration experiments from a simple photometric titration assembly can be transformed directly or indirectly into Job's plots by using a special transformation equation. This is pertinent to my research because I will be using a Job's plot to analyze the stoichiometric binding coefficient of the complex formed by DCS with  $\text{Cu}^{2+}$ .

Hrishikesan, E; Saravanan, C; Kannan, P. Bis-Triazole-Appended Azobenzene Chromophore for Selective Sensing of Copper(II) Ion" *Ind. Eng. Chem. Res.* **2011**, *50*, 8225–8229.

Hrishikesan et al. designed and synthesized a  $\text{Cu}^{2+}$ -specific colorimetric sensor using bis-triazole-appended azobenzene receptors—chromogenic dyes commonly used as receptors in chemosensors. These sensors function on an intramolecular charge transfer (ICT) mechanism where the N-alkylated bis-triazole receptors act as the electron donor and the nitro group as the electron acceptor. Upon the addition of 0-3 equivalents of  $\text{Cu}^{2+}$  to the sensor, the absorption spectra revealed a gradual disappearance of the intensity band at 459 nm and a new, higher-energy band at 342 nm with a clear isosbestic point at 383 nm. The stoichiometry of the receptor/ $\text{Cu}^{2+}$  system was determined using Job plots: absorbance at 342 nm was plotted against molar fraction of the receptor. The maximum value was observed at 0.5, indicating a 1:1 complex between the sensor and  $\text{Cu}^{2+}$ . The  $\text{Cu}^{2+}$  causes a large change in the absorption spectra that is clearly visible to the naked eye. This makes it possible to for the molecule to detect  $\text{Cu}^{2+}$  ratiometrically using photophysical properties of the sensor. In a similar manner, I will be analyzing changes in the absorption spectra of DCS upon the addition of  $\text{Cu}^{2+}$  to look for shifts

in absorption and an isosbestic point.

Liu, S.; Wu, S. An NBD-based Sensitive and Selective Fluorescent Sensor for Copper(II) Ion. *J. Fluoresc.* **2011**, *21*, 1599–1605.

Liu and Wu synthesized a new 7-nitrobenz-2-oxa-1,3-diazole (NBD) derived fluorescent probe that exhibits high selectivity for  $\text{Cu}^{2+}$  metal. While previous methods provided good detection limits and concentration ranges, the development of new fluorescent chemosensors for  $\text{Cu}^{2+}$  has attracted much research because of its cost efficiency and ability to preserve samples. Metal ions  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$  were tested, and the  $\text{Cu}^{2+}$  metal ion was the only ion that caused fluorescence quenching when bound to the NBD chemosensor. Using a Job plot, the binding stoichiometry of the NBD sensor- $\text{Cu}^{2+}$  complex was determined to be a 1:1 ratio. Maximum fluorescence quenching took place at a 0.5 mole fraction, indicating a 1:1 ratio for NBD sensor- $\text{Cu}^{2+}$  complexes. At an optimal pH range of 6-10, the NBD-based  $\text{Cu}^{2+}$  sensor provides an effective and non-destructive method for  $\text{Cu}^{2+}$  sensing. I will be implementing many of the same methods for collecting absorbance and fluorescence recordings for my study and testing similar potential interfering metal ions used in Liu and Wu's paper.

You, Y.; Han, Y.; Lee, Y.; Park, S.; Nam, W.; Lippard, S. Phosphorescent Sensor for Robust Quantification of Copper (II) Ion. *J. Am. Chem. Soc.* **2011**, *133*, 11488-11491.

You et al. synthesized and characterized a reversible and selective phosphorescent copper sensor that can detect  $\text{Cu}^{2+}$  in aqueous media. The sensor was based on a multichromophoric iridium (III) complex that exhibits simultaneous fluorescence intensity ratio and lifetime. A phosphorescence Job's plot revealed a 2:1 ratio for the  $\text{Cu}^{2+}$  and sensor, and the  $\text{Cu}^{2+}$  binding and change in phosphorescence intensity were shown to be reversible upon the addition of EDTA. Furthermore, the sensor was tested to detect intracellular copper by being extrinsically introduced to HeLa cells incubated in  $\text{Cu}^{2+}$ . In my reversibility study, I will apply the same technique of using EDTA to test for the reversibility of DCS as a copper sensor. The lab techniques utilized by You et al. will be implemented in my methodology and serve as a reference for my research.

Zhao, Y.; Zhang, X.; Han, Z.; Qiao, L.; Li, C.; Jian, L.; Shen, G.; Yu, R. Highly Sensitive and Selective Colorimetric and Off-On Fluorescent Chemosensor for  $\text{Cu}^{2+}$  in Aqueous Solution and Living Cells. *Anal. Chem.* **2009**, *81*, 7022-7030.

In this paper, Zhao et al describe the design and activity of a novel "off-on" fluorescent chemosensor (rhodamine spirolactam derivative) for  $\text{Cu}^{2+}$ . The sensor exhibits a highly sensitive "turn-on" fluorescent response that is both instantaneous and reversible. Upon addition of  $\text{Cu}^{2+}$ , a new absorption peak emerged at 529 nm and the solution simultaneously changed from a colorless to orange solution. The Job's method for absorbance was used to determine a 1:1 stoichiometry of the sensor and  $\text{Cu}^{2+}$ , and the fluorescence intensity increased in a linear fashion. The chemosensor has revealed remarkable specificity for  $\text{Cu}^{2+}$  in the presence of other heavy metals and transition ions, even at high concentrations, meeting the selective requirements for applications as a biomedical and environmental monitor of  $\text{Cu}^{2+}$ . While my copper sensor is an "on-off" sensor, the methods used and the analyses performed in this paper will be used in my research. I will be analyzing data from the absorption spectra and fitting it to a Job's plot and checking for reversibility and well as selectiveness for  $\text{Cu}^{2+}$  by testing other possible interfering metal ions.

**V. Provide a statement of progress to date and list the research methods coursework completed.**

I have completed the required research coursework for my biology major and chemistry minor (Research Methods II, General Chemistry, Organic Chemistry, and Biochemistry), and completed the required Honors credit (Research Pro-Seminar). I began my research during the fall semester of 2011 which was URS funded for both Fall 2011 and Spring 2012 semesters. I presented my findings at the Andrews Undergraduate Research Poster Symposium in March of 2012. I have worked on finding the proper solvent system for the DCS molecule and  $\text{Cu}^{2+}$  ions, gathering fluorescence intensity measurements of DCS in various  $\text{Cu}^{2+}$  concentrations, and graphing data points to look for a linear correlation. This semester, I have gathered fluorescence quenching data of DCS at 500 ppm in various  $\text{Cu}^{2+}$  concentrations, and my work so far appears to support the claim that DCS works as an “on-off” sensor in the presence of  $\text{Cu}^{2+}$ .

**Department Chair Approval**

- **This student’s performance in his/her major field is acceptable.**
- **He/she has completed the requisite research methods coursework for the research to be pursued.**
- **I understand that he/she plans to graduate with Honors.**

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Department Chair (signature)

**Research Advisor Approval**

**I have read and support this proposal:** \_\_\_\_\_  
 Primary Advisor (signature)

**I have read and support this proposal:** \_\_\_\_\_  
 Secondary Advisor (signature)

If human subjects or if live vertebrate animals are involved, evidence of approval from the Institutional Review Board or an Animal Use Committee is needed through the campus scholarly research offices (Ext. 6360).