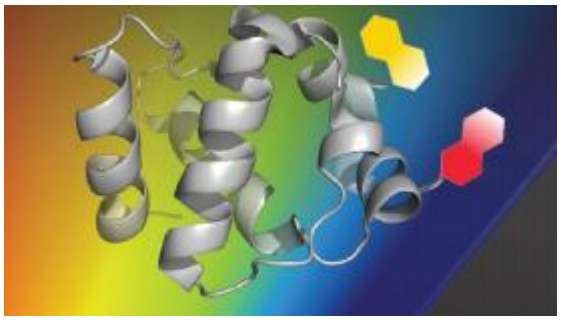
Undergraduate Research Grant
Application Florida Atlantic University

Project: ***2-Hydroxychalcone as a Luminescent Probe and Tag for Small Peptides***

<student name> - Florida Atlantic University

1. Project Description

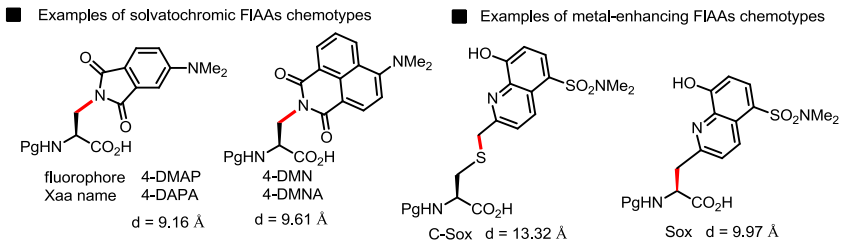
1.1. Objective of Research



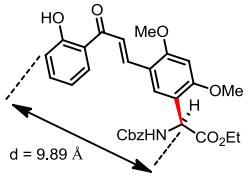
The aim of the project is to develop a synthetic method for a facile introduction of a fluorescent probe directly onto a fully functional peptide (i.e. drug) at a late stage of synthesis. We hypothesized that 2-hydroxychalcone (2HC) would be a novel and ideal fluorescent probe as it could function as both a solvatochromic and metallochromic sensitive tag. Other fluorophores functioning as tags examine either environment (hyrdophobic or aqueous) or proximity metal binding (metalloprotease) or phosphorylation sites. After analyzing the photochemical properties of 2-hydroxychalcone, this fluorophore will be attached to an α-amino acid residue and several small peptides. As chemistry advances, this tag will be useful in analyzing the folding of proteins and enzyme active sites as well as proximity of certain metals and functional groups in a dynamic and real-time manner.

1.2. Background

Peptides and proteins with photochemical sensors are valuable tools when analyzing biochemical processes and peptide properties.[1] When fluorescent tags are appropriately attached to proteins they allow for the detection of their environment and changes therein. Several challenges face the topic of selectively excitable fluorescent probes exist. These include limits on the size and lifetime of synthesized proteins and enzymes, attaching the tag in a location on a peptide chain which will take advantage of the photochemical properties of the tag, and developing molecules that will readily exhibit environment-sensitive fluorescence. Recent work on fluorescent α-amino acids (FlAAs) proved extremely useful in studying protein folding, conformational changes and reactivity (**Scheme 1**).[2-3] 2-Hydroxychalcone (2-HC) exhibits environmentally specific light emission due to keto-enol tautomerism resulting in excited state intramolecular proton transfer (ESIPT). This proton rearrangement conveys whether the environment is aqueous or hydrophobic. The chalcone can also experience metal-binding affinity, which can give insight to the proximity and function of active sites to the tags. 2-Hydroxychalcone is relatively unexplored and has potential for a number of interesting functions for mapping proteins. The goal of this project is collect and develop standards for the photochemistry of a 2-HC-modified α-amino acid, and then to attach it as a probe on fully functional peptide (**Scheme 2**)



Scheme 1. Representative examples of successful fluorescent α-amino acids



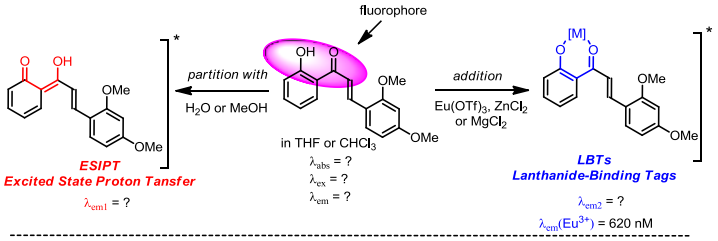
Scheme 2. Novel FlAA based on the 2-hydroxychalcone fluorophore.

1.3. Methodology

The research design encompasses exploratory research and analysis to accomplish the three aims of the project.

Aim 1: Synthesis of 2-Hydroxychalcone.

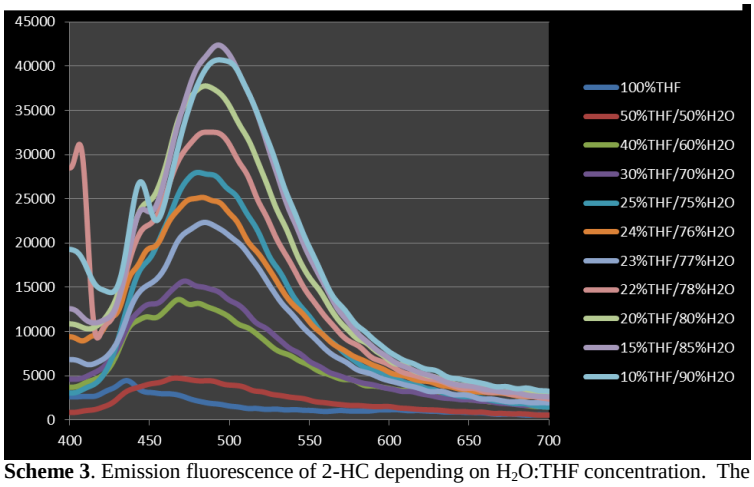
The initial stage of the project involves synthesizing 2-HC. This is accomplished by an aldol reaction using 2-hydroxyacetophenone and 2,4-dimethoxybenzaldehyde in the presence of potassium hydroxide with methanol as the solvent yielding the desired 2-HC in 84%.



Left Arrow: Tautomer of 2-hydroxychalcone present in water or methanol. **Right arrow:** Metalochromic fluorophore enhancement.

Aim 2: Analyzing the photochemical properties of 2-hydroxychalcone.

After synthesis, the next step is to record the absorption and emission spectra of 2-HC to determine the maximum absorption wavelength (λ_{max}) of 2-HC. This was accomplished using a photospectrometer and determined to be 385nm. Excitation of 2-HC at λ_{max} = 385nm leads to an emission wavelength around 500nm. Next, the ratio of water to solvent which optimizes the fluorescence intensity of the chalcone in the aqueous environment is found, demonstrating the ESPIT process. Tetrahydrofuran (THF) is used as the solvent for the initial fluorescence test because of its mid-level polarity. The maximized fluorescence occurs in an 85:15 water to THF solution. The second experiment was to determine the minimum concentration to which the probe is effective in aqueous conditions. We found that 3 µM solution of 2-HC in THF/H_2O (85:15) was the minimum for a detectable signal and 100 µM is ideal. Using the optimal solvent concentration and water ratio for the 2-HC ESIPT process, other solvents will be tested for the fluorescence: toluene, acetonitrile, chloroform and trifluoro-ethanol.



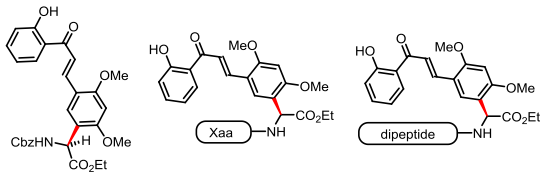
Scheme 3. Emission fluorescence of 2-HC depending on H_2O:THF concentration. The 100% THF does not show any ESIPT shift.

Next, the absorption and emission changes related to metallochromic fluorophore enhancement will be analyzed. The metals studied will be common to biochemical reactions including: Zn^{2+}, Cu^{2+}, Ca^{2+}, and Mg^{2+}; as well as Eu^{3+}, Tb^{3+}, and La^{3+}.

After separately studying the effects of solvatochromic and metal-binding enhancement changes in fluorescence, both will be studied simultaneously, and interactions or competition between the two will be noted.

Aim 3: Attachment of 2-hydroxychalcone to an α-amino acid and peptides. Photochemical analysis of peptides with 2-hydroxychalcone tag.

My mentor < name> developed a method to attach 2-HC moiety as the side chain of the α-amino acid. I followed this method and had a 40% yield of 2-HC tagged α-amino acid. The next step is to develop a method for synthesis of a dipeptide containing the tagged amino acid. Once a tagged dipeptide has been produced, the photochemical properties of the new dipeptide will be studied. The results, hopefully, will yield data confirming 2-HC's value as a multifunctional fluorescent tag.



1.4. Anticipated Outcomes

Since beginning research on 2-HC in January 2015, several of the aims of the project have yielded promising results. An efficient method for synthesis of 2-HC has been achieved. The analysis of 2-HC's solvatochromic properties is yielding results supporting its' potential as a fluorescent tag for the discrimination of hydrophilic vs hydrophobic environment (**aim 1**).

Then we will study the effects of metal cations on absorption and emission in 2-HC. A change due to metal-binding fluorescence enhancement is expected to be observed. Once the data is recorded, relationships between specific shifts in absorption or emission peaks and presence of a metal will be analyzed (**aim 2**). These standards should allow for the use of 2-hydroxychalcone as an indicator of metallic presence in the future.

Attaching 2-HC to an α-amino acid was achieved and will be reproduced on a dipeptide, and possibly longer peptides, to analyze the fluorescence properties. These results will be useful to analyze the structure of small peptides, proteins and enzymes (**aim 3**).

2. Timeline

Action	Expected Start Date
Commence compiling published research papers on fluorescence and tagging peptides	In Process
Synthesize 2-Hydroxychalcone for use in photochemical analysis	Complete
Analyze and compile information on photochemical properties of 2-HC	In process
Develop effective method for attaching 2-HC	4/1/2015
Finalizing key reaction and library of compounds	**Date when funds are available**
Project end & possible publications	8/1/2015
Expected graduation	5/1/2016
Present at UG Research Symposium	Fall 2015

3. Budget & Justification

Line-item Budget

Item description	Supplier	Price	Quantity needed	Total list price
$ZnCl_2$, anhydrous, 99.99%	Sigma-Aldrich	$61.20/5g	5g	$61.20
Water, Molecular Biology Reagent	Sigma-Aldrich	$48.90/L	1L	$48.90
$CaCl_2$, anhydrous, beads, 99.9%	Sigma-Aldrich	$159.50/10g	10g	$159.50
$MgCl_2$, anhydrous, beads, 99.9%	Sigma-Aldrich	$71.90/10g	10g	$71.90
Toluene	Sigma-Aldrich	$64.70/L	1L	$64.70
EuF_3, anhydrous, powder, 99.99%	Sigma-Aldrich	$104.00/g	1g	$104.00
Total Cost				**$510.20**

***All chemicals are listed with minimum quantities/prices for purchase**

Budget Justification

1) Pure $ZnCl_2$, $CaCl_2$, $MgCl_2$, and EuF_3 are required for the metal-binding enhancement study due to high sensitivity of photochemical analysis apparatus.

2) Ultra-pure H_2O, beyond that of distilled water is required for accurate fluorescence emission readings.

3) Pure toluene is required for accurate photospectroscopy.

4. References

1. Strizhak, A. V., Postupalenko, V.Y., Shvadchak, V.V, Morellet, N., Guittet, E., Pivovarenko, V.G., Klymchenko, A.S., Mely, Y. *Bioconj. Chem*. **2012**, *23*, 2434-2443
2. Krueger, A. T.; Imperiali, B., *Chem. Bio. Chem.* **2013**, 14, 788-799.
3. Goldberg, J.M., Speight, L.C., Fegley, M. W., Petersson, E. J. *J. Am. Chem. Soc.* **2012**, *134*, 6088-6091
4. Sun, Y., Chen, H., Cao, D., Liu, Z., Chen, H., Deng, Y., Fang, Q. *J. Photochem. Photobio*. **2012,** *244*, 65-70
5. Juskowiak, G. L., Stachel, S. J., Tivitmahaisoon, P., Van Vranken, D. L. *J. Am. Chem. Soc.* **2004**, *126*, 550-556.
6. Bark, S. J., Schmid, S., Hahn, K. M. *J. Am. Chem. Soc.* **2000**, *122*, 3567-3573
7. Roche, S. P., Samanta, S. S., Gosselin, M. M. J. *Chem. Commun.* **2014**, *50*, 2632-2634
8. Goldberg, J. M., Chen, X., Meinhardt, N., Greenbaum, D. C., Petersson, E. J. *J. Am. Chem. Soc.* **2014**, *136*, 2086-2093