Development of a Simple, Efficient, and High Yield Quantum Dot Bioconjugate Reaction

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Abstract

Fluorescence staining through the use of organic fluorophores has been the standard technique employed by microbiologists for the study of cellular and biological mechanisms and behavior for the past half a century. As a result of their high sensitivity, broad absorption and tight emission spectra, high photostability, and non-toxicity, semi conductor nanocrystals, or quantum dots, have been considered for use as a new and superior class of biological labels – a function of their remarkable optical properties.

At present, the use of quantum dot fluorophores has been limited, in a large part, due to the lack of efficient and straight forward protocols that describe the important parameters and steps required for developing quantum dot biobrobe conjugates.

In this paper I describe my experience and preliminary results using the zero length cross linkers 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysulfosuc-
cinimide (S-NHS) to covalently bind primary amine containing proteins to carboxy functionalized quantum dots. Through optimization of the parameters that effect conjugation performance and overall yield, a simple, efficient, straight forward, and high yield protocol for the conjugation of these proteins to quantum dots has been developed for use in immunohistochemistry studies.

The Effects of Chiral Auxiliaries on the Asymmetric Synthesis of Pyrrolidines

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Abstract

Pyrrolidine systems are a common theme in many biologically active molecules. Catalytic hydrogenation of unsaturated molecules to yield their optically active counterparts is a well known process. The purpose of this project in general is to explore the directing effects that lead to diasteriomeric and eventually enantiomeric selectivity during heterogeneous reduction of pyrrole systems. Previous work in this area here at U of R has lead to a greater understanding of the required substitution patterns surrounding the pyrrole ring that lead to enhanced diasterioselectivity. The focus of this paper is to study the effects that the chiral auxiliary (S)-1-amino-2-methoxymethylpyrrolidine, also known as SAMP, has when condensed with an aldehyde moiety on a bicyclic pyrrole system. The first steps in this process involve synthesis of a simple bicyclic pyrrole system with an aldehyde functional group. Subsequent steps will involve the synthesis of the SAMP auxiliary followed by condensation with the aldehyde and eventual reduction of the entire system with a 5% Rh/Al_2O_3 catalyst. It is our hope that this set of experiments will lead to a better understanding of the requirements for enantiomeric selectivity in similar systems.

Discovery of noncoding RNA genes in *S. mutans* by comparative sequence analysis

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Abstract

We examined the genome for *S. mutans* UA159 serotype C strain, Genbank accession number AE014133, with the objective to find a novel candidate noncoding RNA (ncRNA) for further wet-lab experimentation. There is no current accurate computational method to predicting non-coding RNA genes; however, comparative sequence analysis can be used to test for conserved RNA secondary structure over homologous sequences with a significant number of compensatory base pair changes. We aligned three homologous genomes of oral pathogens with *S. mutans* and determined candidate ncRNA genes in each alignment using free energy minimization of 150 nucleotide windows. We narrowed our selection to those gene segments with low z score and high alignment overlap across the *S. mutans* genome. Using a z score cutoff of -9.5, we found 7 candidate ncRNA genes having overlap, 4 of which had no coding sequence annotation and 3 found to have coding sequence annotation. We performed a fold and align analysis of the most frequent ncRNA candidate without annotation in order to find a common secondary structure for all sequences.

Investigations of the Transformation, Purification and Spectroscopic Analysis of *Hydrogenobacter Thermophilus* (Ht) cytochrome *c* Mutations

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Abstract

Hydrogenobacter Thermophilus (Ht) cytochrome *c*-522 is a thermophilic electron transfer protein being studied according to its stability and protein folding dynamics. Its role as an electron transfer protein is investigated through the relationship between the heme-containing protein and mutations of its polypeptide backbone. Mutations in the Cis-X-X-Cis-His motif of the protein are being studied in order to better understand their relationship to the ruffling of the covalently bonded heme and the overall stability of the protein. These mutations, located at Ht L18I, Ht D17A/K22M, and Ht M13V/D17A/K22M, were incorporated into the wild type strains of Ht cytochrome *c* and the proteins were transformed into Pet-17B expression cells. Analysis of the transformed proteins was done using circular dichroism (CD) spectroscopy in the form of both thermal and chemical denaturations. The denaturation curves and midpoints of each the mutated systems are consistent with the stability of the wild type protein and provide insight into its thermodynamic and chemical stability.

Effects of Estrogen Receptor (ER) Ligands on Intracellular Levels of ERα

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Abstract

Estrogen hormones, particularly the main circulating estrogen, 17β-estradiol (E2), play critical roles in many tissue functions. The effects of E2 are mediated by the transcription factors estrogen receptors (ERs) α and β. The binding of E2 to ERs augments the interaction of E2-ER with specific DNA sequences known as estrogen responsive elements (ERE). Once the E2-ER-ERE complex is formed, the complex recruits many co-regulatory proteins to DNA to initiate transcription. In addition to estrogens, there are many compounds capable of binding to ERs. These compounds are called ER ligands and exhibit various degrees of estrogenic (agonist) or anti-estrogenic (antagonist) properties. ER antagonists are being used in the treatment of estrogen target tissue malignancies, particularly in breast cancer. Recent studies using chromatin-immunoprecipitation (ChIP) assay that assess ER-ERE interactions in cells suggest that pure antagonist Imperial Chemical Industries 182,780 (ICI) completely blocks the ERα-ERE interaction in contrast to E2 or partial antagonist 4-hyroxyltamoxifen (4-OHT) which augment ERα-ERE interactions. The underlying mechanism by which ICI differentially alter ERα-ERE interaction remains unknown. However, ICI effect on ERα-ERE interaction could be due to ICI-mediated rapid degradation of ERα or sequestration of ERα away from DNA. To address this issue, I initially asked whether the binding of ICI to ERα leads to a rapid degradation of the receptor such a way that there would not be sufficient ERα in cells to interact with DNA. To accomplish this, I used transiently transfected cells that synthesize ERα. I then treated these cells with various ER ligands and examined the intracellular levels by Western blotting. I found that the treatment of transiently transfected cells with ICI, but not other compounds, depleted the detergent-extractable ERα fraction without altering ERα levels in non-detergent extractable fraction. Although it appears that the ICI blocks the ERα-ERE interaction by inducing a rapid degradation of ERα in cells, a definite conclusion awaits experiments directed to assess the effects of proteasomal inhibition on ERα levels when treated with a ligand.

Theoretical Study of Dielectric Response of 2-D Gold Clusters

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Abstract

We investigate the morphological dependence of surface plasmon resonance phenomena in 2D Gold clusters. This is done by calculating the dynamic polarizabilities of small gold clusters using Time-Dependent Density Functional Theory (ABINIT package). The initial geometry optimization is completed using Time-Independent Density Functional Theory. The DFT calculations were performed using the Trouiller-Martins Pseudopotential and planewave basis set provided within the opensource ABINIT package. Due to the memory insufficiency of available computers, calculations couldn't be carried out for clusters bigger than Au_6.

Exploring Cobaloximes as Molecular Catalysts in a Multi-Component System for the Photo-Catalytic Production of Hydrogen from Water

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Abstract

Cobaloxime complexes such as $[Co(dmgBF_2)_2(H_2O)_2]$ ($dmgBF_2$ = difluoroboryl-dimethylglyoxime), $[Co(dmgB(OMe)_2(H_2O)_2]$ ($dmgB(OMe)_2$ = dimethoxyboryl-dimethylglyoxime), and $[Co(4\text{-}COOMe\text{-}dpgBF_2)_2(H_2O)_2]$ (4-COOMe-$dpgBF_2$ = difluoroboryl-di(4-methylbenzoate)glyoxime) were investigated for their ability to serve as molecular catalysts in a multi-component system for the photo-catalytic production of hydrogen from water. While $[Co(dmgBF_2)_2(H_2O)_2]$ and $[Co(dmgH)_2(py)(Cl)]$ (dmgH = dimethylglyoxime) have previously been shown to electro-catalyze the production of hydrogen, the use of these types of cobaloxime complexes in photo-catalytic systems for the reduction of aqueous protons has not been previously investigated. The ability of the cobalt complexes to bind to TiO_2 was of particular interest since anchoring catalytic components, such as platinum colloids, to TiO_2 can facilitate electron transfer and thus generate hydrogen. Of the cobaloxime complexes that were successfully synthesized, only $[Co(4\text{-}COOMe\text{-}dpgBF_2)_2(H_2O)_2]$ was observed to sufficiently bind to TiO_2. When $[Co(4\text{-}COOMe\text{-}dpgBF_2)_2(H_2O)_2]$ was stirred in solution with a sacrificial donor (triethanolamine), a platinum chromophore, and a diquat as an electron transfer agent, no hydrogen was produced under $\lambda > 410$ nm radiation. However, when anchored to TiO_2, $[Co(4\text{-}COOMe\text{-}dpgBF_2)_2(H_2O)_2]$ was observed to generate hydrogen under $\lambda > 410$ nm radiation as part of a system involving triethanolamine and a platinum chromophore.

Anharmonic Coupling to Low-Frequency Vibrational Modes in Ferrocene and Acetonitrile: Quantum Chemical Simulations using Density Functional Theory

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Abstract

I am working on quantum chemical simulations of vibrational coupling in acetonitrile and ferrocene. These calculations supplement the femtosecond stimulated Raman spectroscopy (FSRS) studies performed in Prof. David McCamant's laboratory. In particular, these techniques can be used to investigate anharmonic coupling by driving low-frequency vibrational modes into coherence and monitoring perturbations to the high-frequency modes at short time delays. We hoped to develop a simple methodology for predicting the magnitude of anharmonic coupling by observing relative frequency shifts of high-frequency modes along various displacements on a particular, low-frequency normal mode. We used the Gaussian03 software package to calculate Raman spectra of acetonitrile and ferrocene at several displacements along the low-frequency modes. Our calculations predict strong anharmonic coupling of the "sandwich" to the ring-breathing mode of ferrocene, of the C-C stretch of acetonitrile to the "umbrella" C-H bend and C≡N stretch, and of the C-C≡N bend to the C≡N stretch. In future studies, we hope to investigate anharmonic coupling using the technique of femtosecond stimulated Raman spectroscopy (FSRS), with guidance from our model calculations.

The Structure and Reactivity of Iron Tris(pyrazolyl)borate Complexes

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Abstract

Sodium 3-methyl 5- trifluoromethyl trispyrazolylborate reacted with Fe(OTf)2∙ 2MeCN. The 1H NMR spectra and an X-ray crystal structure indicate these reactions formed [TpCF3,CH3Fe(MeCN)¬3]OTf. 1H NMR experiments reveal that in solution the iron binds 1:1 with the ligand. A second equivalent of the ligand was added, and 1H NMR spectrum was obtained for the 2:1 Tp:Fe complex. Initial experiments were conducted on the reactivity of [TpCF3,CH3Fe(MeCN)¬3]OTf with salicylic acid, and 2-aminophenol to mimic the active site of known non-heme iron dioxygenases.

Label Free Biomolecular Sensing: Brewster's Angle Straddle Reflective Interferometry

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Abstract

Brewster's Angle Straddle Reflective Interferometry (BAS-RI) has many applications to biomolecular sensing and screening. BAS-RI can be used to screen and study the interactions of large libraries of molecules with an unlabeled biomolecule of interest with high throughput.

Amphiphilic Ligand Attachment to Cadmium Selenide Quantum Dots

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Abstract

Due to their broad absorption, narrow emission, and enhanced photostability, cadmium selenide (CdSe) quantum dots (QDs) have the potential to significantly advance many biological applications, such as fluorescent imaging. However, CdSe QDs, as synthesized, are insensitive to virtually all biological or chemical agents. In order to make them selectively target specific bacteria, viruses, pathogens, etc., the surface ligands of the QDs must be modified. To accomplish this, an amphiphilic ligand is attached to the QDs through hydrophobic interactions between the amphiphilic ligand and those on the surface, making the QDs soluble in DMF and allowing for further functionalization with organic dyes. When an appropriate dye is attached, the QD can be made soluble in water and allow for fluorescence resonance energy transfer (FRET) between the QD and dye to occur. This assay can be further modified to selectively target a species of interest. When the QD-dye assay encounters that species, the FRET will be halted, thus allowing the QD to fluoresce. The change in fluorescence would signal that the species of interest has been detected. A successful amphiphilic ligand synthesis and attachment procedure to CdSe QDs is reported here.

A Key Hydrozirconation Reaction And It's Application to the Design of a Novel Camphor-amine Organocatalyst

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Abstract

A first and second generation synthesis of a camphor based amine catalyst is described. Steps towards the optimization of a key one pot hydrozirconation reaction in the second generation synthesis is reported. Addition of Shwartz reagent to a bicyclic lacatm is followed by additon of various Grignard reagents allows functionalization in the last step of the synthesis sequence. The scope of the Zirconation is also expanded to include additions to various non-camphor based bicyclic lactams.

Identification of potential dimerization deficient mutants of Ste2p of *Saccharomyces cerevisiae*: a necessary step to understand the cause and effect of oligomerization in GPCRs

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Abstract

G protein coupled receptors (GPCRs) are a family of receptors that activate a wide range of different stimuli and signal transduction pathways. The mechanism and function of oligomerization in GPCRs is currently not completely understood. The yeast α-factor receptor (Ste2p) is a member of the GPCR family and is used in this study in order to determine the residues involved and the effects of oligomerization in GPCRs. Ste2p is particularly useful for collecting information due to yeast;s ability to grow easily and Ste2p's ability to be used as a model protein for homodimerized GPCRs. In this report two fluorophores, 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD) and Bodipy-TR, are attached to ligands of yeast α-factor receptor. These two fluorophores were chosen because of their overlap in excitation and emission spectra in order to analyze energy transfer. Energy transfer from one fluorophore to the other only occurs when the two fluorophores are in close proximity to each other necessarily implying oligomerization. In this study the isolation and screening for oligomerization deficient mutants of *ste2*, which codes for the yeast α-factor receptor, was performed in intact cells via a flow cytometer. These oligomerization deficient *ste2* mutants were isolated in order to study the cause and effects of oligomerization in GPCRs. The isolated mutants were analyzed by sequencing and ligand binding assays. Ligand binding was performed in order to compare the mutants binding as compared to wild type as well as measuring energy transfer in order to determine whether the desired mutants defective for oligomerization have been recovered.

Studies toward the Synthesis of (-)-Nakadomarin A

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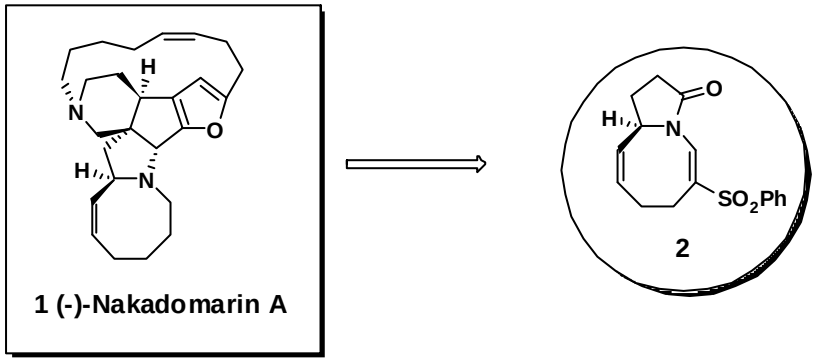
Abstract

The primary goal of this project is to synthesize alkaloid (-)-Nakadomarin A **1**. Nakadomarin A was first isolated from an Okinawan marine sponge, *Amphimedon* sp. by Kobayashi[1] in 1997. The molecule has been shown to have antitumor activity and other biological inhibitory activities[1]. The Nishida group has succeeded in both the total synthesis of natural (-)-Nakadomarin A with ~0.002% yield[2] and its enantiomer[3]. Recently, Young and Kerr have succeeded in making unnatural (+)-Nakadomarin as well[4]. There are also other studies towards the strategy to make the target. These successful syntheses confirmed the configuration of the bioactive enantiomer. Though it was a success, there was not a sufficient control of geometry of the molecule (Z/E 1:1.8). To solve this, for the first time, an approach using the aza- retroClaisen rearrangement is considered as the new strategy for the synthesis of the target. This approach has a potential to make the synthesis shorter and more stereoselective.

As shown below, in order for the planned synthesis of the target, key bicyclic intermediate **2** is necessary. Thus, the further optimizations towards the synthesis of intermediate **2** were carried.



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Implementation and Critical Analysis of a Generalized Born/Surface Area Solvent Model

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Abstract

Theoretical chemists have collectively developed and refined many aspects of the generalized Born/surface area (GB/SA) model since it was pioneered by Born, Onsager, and Kirkwood 60-80 years ago. The many incarnations of GB/SA techniques are applied in computational modeling to achieve the best results for the given constraints of a system. A derivation of a generalized Born solvation method for a fast protein-ligand binding model is described here. The surface area portion of the derived GB/SA method was implemented, and successful results were achieved for 4 ideal systems. An attempt was also made at replicating results found in Weiser et. al., J. Comp. Chem. 19, 797 (1998). A review of the prominent papers in this field is provided, including progress in explicit solvent models and numerical Poisson-Boltzmann treatments.

Synthesis of Potential Mitochondrial Targeting Antioxidant Fatty Acids

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Abstract

Recent scientific studies show that mitochondrial dysfunction is more commonplace than previously thought and that substantial mitochondrial involvement is present in many acute and chronic diseases. These dysfunctions can be attributed in some instances to reactive oxygen species (ROS) produced as a byproduct of oxidative phosphorylation in the mitochondria. ROS are known to interfere with a number of metabolic processes within the mitochondria. In order to better understand the role of RONS, the antioxidant molecules were designed so that they could accumulate specifically within the mitochondrial matrix. Fatty acids were chosen as a suitable vector for the antioxidants. The antioxidant domains of these compounds were limited to vitamin E analogs, cresol, and benzaldehyde oxime. These compounds underwent β-oxidation and those with more steric bulk near the fatty acid chain inhibited the rate of β-oxidation.