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BIO-MEDIATED GOLD NANOROD CHAIN ASSEMBLY

Yao-Jen Chang, Raymond R. Unocic, Nicholas A. Merill, Marc R. Knecht and Beth S. Guiton

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

Extensive studies have focused on exploiting the optical properties of gold nanorods, but few have studied the surfactant layer. The surface passivant plays a critical role by both stabilizing the nanorod, and its exposure to solution. In this project we investigate the assembly dynamics of gold particle chains which assemble via biological linking molecules. We image the assembly mechanism directly using a liquid flow cell holder in the transmission electron microscope, varying solution conditions and ligand structure. In this way we elucidate the dominant factors controlling nanoparticle assembly to produce desired structures and plasmonic behaviors.

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BIOMIMETIC ORAL MUCIN USING POLYMERIC FILOMICELLE NETWORKS

Sundar P. Authimoolam, Nihar M. Shah, David A. Puleo and Thomas D. Dziubla

Department of Chemical and Materials Engineering, University of Kentucky, Lexington, KY, 40506, USA
Department of Biomedical Engineering, University of Kentucky, Lexington, KY, 40506, USA
Bluegrass Advanced Materials, LLC, A268 ASTeCC, Lexington, KY, 40506, USA

In the oral cavity, mucin networks provide a protective, lubricating barrier to soft buccal tissues. These networks typically resemble a nano-porous mesh, formed through a continuous self-assembly of mucin glycoprotein complexes. In order to elucidate their functional behavior, it is important to decouple their chemical and physical effects. To this end, we developed a synthetic biomimetic mucin network using a layer-by-layer (LBL) approach. Unlike conventional polyelectrolyte-based LBL methods, we utilized biotinylated-filamentous micelles as the network building blocks, which are cross-linked together into mucin-like networks using streptavidin. The physicochemical properties of the network were studied by evaluating its structural and bio-functional properties.

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COTININE INDUCED UPREGULATION OF NICOTINIC ACETYLCHOLINE RECEPTORS: A POTENTIAL ROLE IN NICOTINE ADDICTION

Ashley M. Fox, and Christopher I. Richards

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

Nicotinic acetylcholine receptors (nAChRs) are pentameric cation-selective membrane receptors composed of a combination of alpha (α1-α10) and beta (β2-β4) subunits. Traditionally, changes in composition and stoichiometry of receptors exposed to nicotine have been linked to nicotine addiction. Cotinine, the primary metabolite of nicotine, may also play a potential role in nicotine addiction due its longer half-life. A pH sensitive fluorophore, super ecliptic phluorin, can differentiate between intracellular and plasma membrane nAChRs to investigate cotinine induced changes. Subtypes α4β2, α4β2α5, and α4β2α5D398N show clear differences in response to nicotine and cotinine, with an overall upregulation of α4β2 in total and percentage on the membrane.

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DETERMINING THE PHOTOSUBSTITUTION MECHANISM OF RU(BPY)2DMDPPZ, A PHOTOCHEMICAL "LIGHT SWITCH" AND DNA METALATING AGENT

Erin Wachter, Sean Parkin, and Edith C. Glazer

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

Ru(bpy)2dmdppz, a strained Ru(II) polypyridyl complex, behaves as an unusual photochemical "light switch", similar to the behavior of its unstrained analog, Ru(bpy)2dppz, a well known photophysical "light switch". A detailed photochemical study was completed to determine the photosubstitution mechanism, environmental sensitivity, and selectivity for DNA tertiary structures where reactions were monitored using UV/Vis spectroscopy. Photosubstitution was shown to be dependent on the solvent, temperature, binding to biomolecules, and the identity and concentration of the incoming ligand, suggesting an associative or interchange associative mechanism. The photochemical reaction was promoted in aprotic solvents and when the complex was bound to specific biomolecules. Ligand loss led to DNA metalation when the complex was intercalated into the base stack. Ejection rates were faster in the presence of G-quadruplex DNA, a medically significant target, showing sequence and/or structure selectivity.

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EFFECT OF CHARGE AND HYDROPHILICITY ON BIOLOGICAL ACTIVITIES OF SIMPLE RU(II) POLYPYRIDYL COMPLEXES

Yang Sun, Matt Dickerson, Brock Howerton and Edith Glazer

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

Ruthenium(II) polypyridyl complexes have gained increasing interest as potential anti-cancer agents in photodynamic therapy (PDT) to eliminate adverse effects of traditional chemotherapeutic agents. Simple ligand modifications in two structurally similar Ru(II) polypyridyl complexes with almost identical photophysical properties result in distinct physical properties including charge state and hydrophilicity. The two complexes also showed radically different biological activities in human cancer cell lines including cytotoxicity, cellular uptake, subcellular localization, biological targets, mechanism of action, and mechanism of cell death. This study reveals that modifying charge and hydrophilicity can have dramatic effects on physiochemical and biological properties of structurally simple Ru(II) complexes.

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EFFECTS OF CAVITY-FILLING MUTATION IN THE ENZYME CHOLINE ACETYLTRANSFERASE

Jyotica Batra, Corbin D. Ester, Yiying Cai, Jianhang Jia and David W. Rodgers

Department of Molecular and Cellular Biochemistry and Center for Structural Biology,
University of Kentucky, Lexington, KY, 40506, USA

Choline acetyltransferase (ChAT) synthesizes the neurotransmitter acetylcholine, and point mutations in the enzyme cause an often fatal neuromuscular disorder known as congenital myasthenic syndrome with episodic apnea (CMS-EA), the condition that results in severe muscular weakness and respiratory insufficiency. We hypothesize that the susceptibility of ChAT to point mutations at sites distributed over the enzyme is due to its unusually large number of core packing defects or cavities. Using site directed mutagenesis, we have tested our hypothesis by introducing single cavity filling mutation S106I near a known congenital mutation site L102. The cavity-filling mutation increases the thermal stability of the enzyme by almost 5 degrees Celsius, indicating that it has reduced cavity volume as expected. Importantly, filling the cavity largely restores the activity of the L102P CMS-EA mutation to wild type levels, providing a link between cavities and the effects of the congenital mutations. We are screening to identifying small ligands that bind in cavities and stabilize ChAT to a number of congenital mutations. These compounds will represent a new therapeutic approach for treating the disease, and we have identified a number of candidate molecules. We are also in the process of further testing the role of cavities in PC12 cells and a Drosophila model.
The ability to rescue the CMS mutants in an animal model will be an important step in validating the long-term goal of developing a therapy for the disorder by targeting ChAT packing defects.

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ELECTROCHEMICAL BIO-SENSING AND ELECTRO-CATALYTIC APPLICATIONS OF CARBON NANOONIONS

Yan Zhang, Juchan Yang, Doo Young Kim

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

Carbon nano-onions (CNOs) are emerging carbon-based materials with unique structural and electronic properties. Structure, physicochemical properties, and electrochemical performances of CNOs were studied, in comparison with other popular carbon electrodes such as multiwalled carbon nanotubes (MWCNTs), graphene nanoflakes (GNFs), and glassy carbon (GC). The biosensing performance of the above-mentioned carbon electrodes were tested with several redox-active molecules and neurotransmitters. The sensitivity, selectivity, and anti-fouling behavior were evaluated. The electrocatalytic activity of CNOs for oxygen reduction reaction was also studied, in comparison with other carbon nano-materials. Electrochemical behavior, kinetics, and detailed ORR mechanism were thoroughly studied.

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FUNDAMENTAL ELEMENTS OF THE CATALYTIC MECHANISM OF NITROREDUCTASE, A PROMISCUOUS ENZYME

Warintra Pitsawong and Anne-Frances Miller

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

The oxygen-insensitive nitroreductase from Enterobacter cloacae (NR) catalyzes two-electron reduction of nitroaromatics to the corresponding nitroso compounds and, subsequently, to hydroxylamine products. NR has an unusually broad substrate repertoire, which may be related to protein dynamics (flexibility) and/or a simple non-selective kinetic mechanism. To investigate the possible role of mechanism in NR's broad substrate repertoire, the kinetics of oxidation of NR by para-nitrobenzoic acid (p-NBA) were investigated using stopped-flow techniques at 4°C. Moreover, the measurement of primary, solvent, and multiple kinetic isotope effects (KIEs) have been combined with X-ray crystallography to gain insight into the source of protons participating in nitroaromatic reduction.

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INTERMOLECULAR FORCES IN DNA CONDENSATION WITH CATIONIC DENDRIMERS

Min An, and Dr. Jason DeRouchey

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

In order to compact DNA, linear cations are believed to bind in DNA grooves and to interact with the phosphate backbone of apposing helices. Hyperbranched polycations, such as polycationic dendrimers, presumably would not be able to bind DNA and correlate charges in the same manner as linear cations. We use osmotic stress coupled with x-ray scattering to examine the intermolecular forces in PAMAM-DNA complexes. The compaction, intermolecular forces, salt and pH dependence of dendrimer-DNA is compared to similarly charged linear arginine peptides. pH is shown to be an important parameter in controlling the packaging density in PAMAM-DNA.

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ISOLATION OF SINGLE RECEPTORS INTO NANOVESICLES TO CARRY OUT SINGLE MOLECULE STUDIES

Faruk H. Moonschi, Amy K. Effinger, William E. Martin, and Christopher I. Richards

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

Single molecule studies in biology are limited only to those proteins that can be purified and stabilized outside of cellular environment by means of surfactants. Many membrane proteins lose their structural and functional integrity in this type of environment. We have isolated single membrane receptors into nanovesicles in order to analyze them at the single molecule level. We have generated fluorescent labeled CFTR, and α3β4 nanovesicles and carried out single molecule studies. CFTR shows to have monomeric structure while α3β4 predominate presents as 2 (α3): 3 (β4) stoichiometry. FCS was carried out with fluorescent labeled EGFR nanovesicles and EFG, and found that EGF specifically binds with EGFR containing vesicles.

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MECHANISM STUDY OF PYROPHOSPHATASE FROM STAPHYLOCOCCUS AUREUS

Xinyi Zhang, Wei Lu, Yinan Wei

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

Family II inorganic pyrophosphatases (FIIP), catalyze the hydrolysis of inorganic phosphate, are essential enzymes in bacteria. In this study we characterized a novel FIIP, PpaC from Staphylococcus aureus. The catalytic activity of PpaC requires metal ions. FIIPs are dimers. In order to find out whether the enzyme also works as a monomer, we introduced 3 single point mutations at the dimer interface. The mutants decreased the dimer stability lost catalytic activity. Temperature melting experiments showed that the active PpaC are more stable than the inactive mutants. These results suggest that Family II inorganic pyrophosphatases, such as PpaC, function as dimers. Dimer dissociation leads to a decrease of stability and loss of activity.

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NANOPARTICLE TRANSPORT IN CHARGED AND UNCHARGED POLYMERIC GELS

Xiaolu Zhang and Dr. Jason DeRouchey

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

Controlled transport of nanoparticles (NPs) through biological gels, such as mucus or extracellular matrix (ECM), is critical to NP and drug delivery in cells. Using fluorescence correlation spectroscopy (FCS), we have studied transport and dynamics of different kinds of NPs, including probe molecules and cationic dendrimers, in polymeric gels. Diffusion of charged NPs in the presence of charged polymer solutions greatly influenced transport properties compared to uncharged polymer networks. The ability to control and exploit specific nanoparticle-gel interactions to develop better penetrating nanoparticles would be highly beneficial for many in vivo applications.

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NITROREDUCTASE REACTION PRODUCTS: AN ANAEROBIC METHOD FOR IN SITU DETECTION

John P. Hoben, Warintra Pitsawong, and Anne-Frances Miller

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

The unusually large substrate repertoire of the flavoenzyme nitroreductase (NR) from *Enterobacter cloacae* has led to an expanding body of work which seeks to advance both applications (e.g. - biodegradation, biosynthesis, prodrug therapy, etc.) as well as fundamental understanding of NR (e.g. - physiological relevance, flavin cofactor reactivity, enzyme dynamics, etc.). Amine formation by NR could provide a cost-effective route to valuable synthons. To evaluate the feasibility of using NR for aromatic amine biosynthesis, an anaerobic *in situ* method was developed. The method can be adapted for use as a screen to detect the production of amines by NR homologues and mutants.

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PHOTOCHEMICAL REDUCTION OF CO2 ON SPHALERITE

Ruixin Zhou, Marcelo I. Guzman

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

Photocatalytic reactions on semiconductor mineral surfaces may have played an important role in the synthesis of prebiotic molecules on early Earth. Particularly, sphalerite (ZnS) can photoreduce CO2 to formate in the presence of sodium sulfide hole scavenger. The rate of formate production (R) follows a dependence on wavelengths described by the equation R (uM/min) = 14.152 - 0.0410 * λ (nm), with a coefficient of correlation $r^2 = 0.995$. The apparent quantum yield of formate production (Φ) is studied under periodic illumination. The timescale for electron transfer and oxidizing hole loss are 3.3 ms, and 0.13 s, respectively.

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REDUCING DNA INTERACTIONS WITH THE ADDITION OF NON-PLANAR LIGANDS TO RU(II) COMPLEXES

Brock Howerton, Erin Wachter, Achmad Hidayatullah, David Heidary, Sean Parkin, and Edith C. Glazer

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

The future of cancer photodynamic therapy (PDT) relies on the synthesis of compounds with low toxicity in the dark and high toxicity when exposed to light. Strained ruthenium (II) polypyridyl complexes are believed to induce cell death by damaging DNA, similar to the pathway by which the chemotherapy drug cisplatin induces cell death. Visible light activation leads to ligand loss and results in the Ru(II) center covalently modifying DNA; the compounds are designed to be inactive in the dark, and low toxicity in the absence of light-activation is a desired characteristic of Ru(II) complexes. It is known that intercalation of Ru(II)-coordinated ligands into DNA may account for some level of dark toxicity. When coordinate with Ru(II), flat phenanthroline derivatives with extended nitrogen-containing π systems, such as dipyrido[3,2-f:2′,3′-h]-quinoxaline (dpq) can intercalate DNA base pairs. Reduction of the intercalation activity of the complex by modification of coordinated ligands may be a viable method for decreasing dark toxicity of Ru (II) PDT agents. Dpq-ce, a non-planar cyclic ether-containing dpq derivative, was complexed with Ru(bpy)2 and Ru(dmphen)2 to give unstrained and strained complexes, respectively. The non-planar geometry of the dpq-ce ligand may reduce intercalation by preventing insertion of the ligand into the DNA base pair stacks.

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REPRESSOR MUTATIONS RESTORE FUNTION TO WEAKLY ASSOCIATED ACRB TRIMER

Zhaoshuai Wang, Meng Zhong, Wei Lu, Yinan Wei

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

AcrB is the inner membrane component of Resistance-Nodulation-Division family transporters in Gram-negative bacteria. In previous studies, we found the P223G mutation disrupted trimerization and lead to a drastic loss of function. To advance our understanding about interactions that stabilize AcrB trimer, random mutagenesis was conducted to identify suppressor mutations. The only one close to drug translocation pathway M662I was studied. We found the secondary structure and thermostability of WT-AcrB, AcrBP223G, AcrBP223G/M662I were comparable. Crosslinking experiment conducted showed the M662I mutation did not improve the interaction between AcrA and AcrB. Further investigation is underway to elucidate the potential role of substrate binding in the mechanism of functional recovery.

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SYNTHESIS AND CHARACTERIZATION OF CARBONIC ANHYDRASE ACTIVE-SITE MIMICS FOR CO2 HYDRATION

Deepshikha Gupta, Cameron A. Lippert, John P. Selegue, Joseph E. Remias and Kunlei Liu

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA
Center for Applied Energy Research, Lexington, KY 40511

The enhancement of CO2 absorption using a catalyst is a critical component to reduce the capital cost for CO2 capture. Our research focuses on exploring effective ways to minimize CO2 emission by developing a catalyst to enhance the rate of CO2 hydration. We focus our efforts on complexes of zinc(II) and similar metal ions with ligands such as 1,4,7,10-tetraazacyclododecane (cyclen), 5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane (teta and tetb), tris(benzimidazolylmethyl)amine (BIMA) and anionic tris (pyrazolylborate)s that mimic the enzyme, carbonic anhydrase. Several of these complexes reported so far contain the hazardous perchlorate ion. We are developing Cu, Co and Zn complexes with benign, non-coordinating counterions that avoid the potentially explosive perchlorate salts. [Zn(cyclen)H2O][SiF6] as well as a number of other catalysts have been synthesized and tested for their post-combustion CO2 capture enhancement capabilities in aqueous solvent mixtures. [Zn(cyclen)(H2O)][SiF6]•2H2O, which has an unreactive counteranion, has confirmed catalytic activity.

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SYNTHESIS OF INTERMEDIATES OF LOLINE BIOSYNTHESIS PATHWAY

Minakshi Bhardwaj, Juan Pan, Robert B. Grossman, and Christopher L. Schardl

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

Lolines are a class of alkaloids produced by fungi in the genus Neotyphodium that are in symbiotic relationships with cool-season grasses (Poaceae subfamily Poöideae). Lolines possess antifeedant and insecticidal properties that protect the plant from insect herbivory without any concomitant antimammalian activity; they also increase their hosts' phosphate uptake and tolerance towards drought and heat. Lolines are pyrrolizidines with an ether bridging C atom 2 and 7. The ether bridge is in an unusual context in biological compounds, which raises interesting questions about the lolines' biosynthesis. We have synthesized various proposed biosynthetic intermediates in isotopically labeled form and used them in feeding experiments, helping us to propose a biosynthetic pathway for the lolines.

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SYNTHESIS, FUNCTIONALIZATION, AND APPLICATION OF GRAPHENE QUANTUM DOTS

Yiyang Liu, Tim Little, Sinhea Yeom, Michael Ware, and Doo Young Kim

Department of Chemistry, University of Kentucky, Lexington, KY 40506-0055

Graphene quantum dots (GQDs), which are single layer or multiple layers of graphene confined in the dimension of 5-20 nm, are novel carbon nano-materials. In this poster, controlled synthesis and the surface functionalization of GQDs will be presented. In particular, the as-produced, oxidized GQDs were subsequently modified with different chemical functional groups: hydrogens, amines, and polyethylene glycols. The modified chemical structure of GQD edges showed tunable optical behaviors. The effect of synthetic conditions on fluorescence behavior was also thoroughly studied. We have tested the application of these GQDs in bio-imaging and heavy metal-ion sensing, and they showed very promising results.

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THE DIFFERENCES BETWEEN IRON AND IRON-SUBSTITUTED MANGANESE SUPEROXIDE DISMUTASE WITH RESPECT TO HYDROGEN PEROXIDE TREATMENT

Jianing Wang and Anne-Frances Miller

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

Iron-substituted manganese superoxide dismutase (Fe(Mn)SOD) was produced using an in vivo preparation method, whose properties upon H2O2 treatment were studied and compared to that of FeSOD. It's found that the responses to H2O2 treatment were different, including the changes of optical spectra, active site coordination and secondary structures. What's more, the activities of Fe(Mn)SOD and FeSOD were totally distinct based on peroxidase assay using ABTS or Amplex Red. These results indicated that the mechanism of peroxidase reaction of Fe(Mn)SOD is not identical to that of FeSOD.

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TOWARD THE TOTAL SYNTHESIS OF 7-EPI-CLUSIANONE

Shubhankar Dutta , Suresh Jayasekara and Robert B. Grossman

Department of Chemistry, University of Kentucky, Lexington, KY, 40506-0055, USA

Polycyclic polyprenylated acylphloroglucinols (PPAPs) are plant (Guttiferae) derived natural products. They have fascinating bicyclo[3.3.1]-2,4,9-trione or [3.2.1]-2,4,8-trione cores decorated with prenyl or geranyl groups. More than 100 PPAPs have been isolated, but only a few of them have been synthesized. We are trying to make the type B 7-endo PPAP, 7-epi-clusianone. This molecule shows very interesting biological properties such as antibacterial, anticancer, anti-HIV, and antimalarial activity. Encouraged by these findings, we started the synthesis of 7-epi-clusianone. The synthetic plan involves an alkynylation–aldol strategy to construct the bicyclic core. Having established the bicyclic core, the synthesis presents a new challenge, the oxidation of a very hindered 2-alkenone to the β-hydroxy 2-alkenone, and we are proposing methods for this oxidation.

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USING COLOR TO DECIPHER BASE COMPOSITION OF SHORT SINGLE-STRANDED DNA

Elizabeth M. Thomas and Stephen M. Testa

Department of Chemistry, University of Kentucky, Lexington, KY, 40506, USA

The analysis of oligonucleotides has become important in nucleic acids (DNA and RNA) therapeutics. The information encoded with the ordering of the nucleobases (e.g. adenine, A, guanine, G, cytosine, C, and thymine, T, or uracil, U in RNA) determine the structure and function of the nucleic acids within biological systems. These structures, especially the short single stranded nucleic acid oligonucleotides, are being exploited in many areas of biotechnology today. Single stranded DNA (ssDNA) is often used in diagnosis, molecular therapy, and research in molecular biology. Although many methods of preparing ssDNA exists, methods currently used for DNA sequencing in the first 40 bases still present difficulties. Moreover the current methods have many drawbacks such as: requiring expensive technology such as mass spectrophotometers, highly cumbersome resolution methods such as PAGE gels, HPLC, or TLC, as well as the need for high sensitivity fluorescent or radioactive markers.

Our laboratory's goal is to develop an inexpensive, non-cumbersome, simple-to-interpret methodology to determine base composition and ultimately gaining sequencing information of small ssDNA molecules. Herein we propose a simple approach to obtain nucleic acid sequence and structure information using color to visualize and quantify base composition within 5-mer oligonucleotides. This general method coined within our lab is CIENA: Colorimetric Identification of Exposed Nucleic Acids. Nucleobase specific colorimetric reactions have been developed that can be used in the CIENA protocols for the determination of base composition within short ssDNA.

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