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Synthesis and Analysis of Dendrimeric Molecular Gyroscopes

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Molecular gyroscopes are molecular machines which could be used for the construction of functional materials. The affects of different topologies on motion in the solid state are understood by studying the solid state-dynamics of molecular gyroscopes with such techniques as C-13 cross polarization/magic angle spinning (CPMAS) and deuterium quadrupolar echo NMR. Previous studies have shown that the dynamics of rotation of molecular gyroscopes become more favorable as the packing coefficient decreases. This was achieved by increasing the steric bulk of the stator, the static segment of the molecule. The purpose of this project is to design a new rotor with bulkier stators that will provide for more open space and minimize the barrier to rotation. This design involves dendrimers, molecules that repeatedly diverge outward similar to trees. A procedure was developed for the synthesis of the dendritic rotor consisting of a core para-phenylene rotator coupled to two axially positioned trityl acetylene repeating branches. Preparation of the target rotor utilized Palladium(0)-catalyzed cross coupling reactions of trityl acetylene to dihalogenated arenes.

Characterizations of surfaces for DNA quantum dot hybrid analysis

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Transcription is the synthesis of RNA from DNA by RNA polymerase. This complex process is regulated by various transcription factors and leads to gene expression and protein synthesis. Currently, we are using quantum dots as fluorescent tags to label DNA-binding proteins. Our objective is to map the locations of these proteins on linearly aligned DNA using single molecule detection techniques and atomic force microscopy to further understand intracellular processes such as transcription and gene expression. Because of the small diameter of DNA (~2nm) we must work with flat and hydrophobic surfaces to enable the alignment of DNA for observations under the AFM. Polymethylmethacrylate, polystyrene, and Zeonex polymers were prepared in three organic solvents (chloroform, toluene, and xylene) and analyzed for their optical properties, non-specific binding properties, and ability to align DNA protein complexes. Once a suitable substrate for combined optical and AFM measurements is found, we will use this surface for mapping protein binding sites on DNA. The method of mapping DNA-binding proteins at the single molecule level using quantum dots may be useful for in *vivo* applications such as disease diagnosis and therapy.

Encapsulation of Semiconducting Polymer Using Cowpea Chlorotic Mottle Virus

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Cowpea Chlorotic Mottle Virus is a single stranded RNA plant virus, whose protein cage has potential applications for delivery and detection of various molecules. The protein capsid of this virus can be purified and, depending on the pH and ionic strength of the buffer, the protein dimers can self-assemble into different structures and shapes due to charge interactions with the buffer. Our research focuses on using these protein dimers to encapsulate a fluorescent, semiconducting polymer, MPS-PPV (poly-2-methoxy-5-propyloxy sulfonate phenylene vinylene), in order to obtain optically active virus-like particles with long fluorescent lifetimes. The fluorescent quantum yield of MPS-PPV is related to the physical conformation of the molecule that can be altered by changing its solvent environment. After encapsulating MPS-PPV, fluorescence spectroscopy showed two different peaks, which suggests the polymer to be in two conformations. We conclude that the protein can alter the polymer's optical properties, and the polymer's different backbone conformation provides information about structural encapsulation. The structure and fluorescence properties of the biological cage may be developed into a new biological imaging tool.

Expression of Human Prolyl-4-Hydroxylase for the Production of Collagen III in *Saccharomyces cerevisiae*

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The extracellular matrix is an important material composed of proteins and biological molecules that anchors cells and regulates intercellular communication. Collagen, the most abundant protein in the extracellular matrix, plays a large role in relaying messages concerning cell function, growth, and division to the cells through binding sites found on the protein. For this reason, collagen is of great interest in medical applications such as tissue regeneration, drug delivery, and medical implants. The production of synthetic collagen in microbial systems poses challenges associated with hydroxylating the prolines that stabilize the triple helical structure of collagen. The enzyme prolyl-4-hydroxylase is responsible for the hydroxylation of specific prolines in collagen. Here we report the integration of a second copy of prolyl-4-hydroxylase beta subunit into the *Saccharomyces cerevisiae* genome. Western blot analysis reveals that expression of the beta subunit of prolyl-4-hydroxylase is increased through codon-optimization technology.

Validation of direct interaction between microRNA-9/-9* and their predicted targets

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MicroRNAs (miRNAs) are short RNA transcripts that regulate gene expression via interactions with the 3'UTRs of their target messenger RNAs (mRNAs). An obstacle in understanding miRNA function is the difficulty of target identification. While bioinformatics allows target prediction, experimental validation of miRNA-mRNA interactions is necessary. MiR-9 and miR-9* are brain-specific miRNAs whose functions are poorly understood. After conducting a database search, we hypothesize that CHMP2B and CNTNAP2 mRNAs are direct targets of miR-9/-9*. To validate this hypothesis, luciferase report constructs containing the predicted target's 3'UTR downstream of the reporter gene were cloned. Constructs of mutated predicted miRNA binding site were used as controls. HEK293 cells were cotransfected with the constructs and the miR-9/-9* precursor, and Luciferase activities were determined 48 hours following transfections. Preliminary results suggest that miR-9 does not regulate CNTNAP2, a gene implicated in autism. In contrast, expression of CHMP2B, a gene implicated in frontotemporal dementia, appears to be regulated by miR-9. After direct interactions are confirmed, future studies may further clarify miR-9/-9* role in regulation of their target mRNAs and how misregulation may lead to pathologies.

Analysis of Excited Jets in Crossflow

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This study explores the nature and behavior of acoustically pulsed jets in uniform crossflow, a flowfield extensively used in many propulsion and combustion systems. Prior findings suggest that by controlling the level of vorticity generated through acoustic pulsation, a greater level of penetration and mixing could be achieved, leading to better overall performance in several applications. We emphasized the impact of square wave excitation of the jet, where jet penetration has been found to be very efficient. The special relationship observed between stroke length and flow structure turned out to be practical when defining transition boundaries for different structural aspects of a pulsed transverse jet. Previous work has proposed a preliminary scaling for the flow structure of transverse jets based on stroke ratios, but these transition boundaries still needed clarification. Comparison with our experimental results suggests that the scaling previously proposed appears to be accurate, except for cases dealing with low jet-to-crossflow velocity ratio. This study has important implications in various applications dealing with transverse jets and allows for a more thorough understanding of optimization of transverse jets for enhanced jet penetration.

Selective Predation by *Pachygrapsus crassipes* and *Hemigrapsus oregonensis* on *Cerithidea californica* in the Laboratory

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Predation can greatly impact populations and communities. Studies showed that *Pachygrapsus crassipes* preferred to eat larger snails as its size increased but that snails were eaten at low rates that snail populations were not impacted. We investigated size specific predation by *P. crassipes* and *Hemigrapsus oregonensis* on *Cerithidea californica* and predation on *C. californica*'s egg mass. Investigating egg mass predation as a source of mortality in *C. californica* is necessary to assess potential impacts of predation on its populations. It was hypothesized that crabs would prefer egg masses over snails. Specimens were collected during the summer in Carpinteria Salt Marsh, Santa Barbara Co., California. We used 12 *P. crassipes* and 6 *H. oregonensis* of various carapace lengths. Each crab was offered four snails of various sizes and an egg mass. *Pachygrapsus crassipes* and *H. oregonensis* strongly preferred egg mass over snails regardless of carapace length. Predation on *C. californica* might be greater than previously thought since egg predation may have a significant impact on the population density of these snails.

MicroRNA Binds to the 3' Untranslated Region of Ataxia Telangiectasia Mutated (ATM), a Modulator of Cell Radiosensitivity

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MicroRNA (miRNA) are a class of endogenous small non-coding RNA, which bind with incomplete complementarity to the 3' untranslated region regulating gene expression. Using a luciferase reporter assay, a human miRNA has been shown to bind to the targeted 3' UTR of ATM, a central player in DNA damage repair. Immunoblot and quantitative real-time PCR confirmed that this miRNA suppresses endogenous ATM protein expression without causing degradation of ATM mRNA. Using the CONSITE transcription factor database six transcription factors c-FOS, Sox-5, ARNT, USF, n-MYC, and SOX-17 have been identified to putatively bind the promoter sequence of this miRNA. A luciferase reporter was constructed using a vector with this miRNA promoter insert and was co-transfected into HeLa cells with the transcription factor cDNA constructs. Results indicated that both c-Fos and n-Myc activated the miRNA promoter transcription. Determination of the transcriptional regulators of the miRNA may lead to lower dosage radiotherapy treatments for cancer patients through modulation of radiosensitivity in tumors by targeting ATM translation.

The Formation and Evolution of Subtropical Mode Water

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Mode waters, thick layers of nearly homogeneous water, have been identified in every ocean basin. Eighteen Degree Water (EDW), the mode water of the North Atlantic, is

associated with the largest annual heat loss to the atmosphere and impacts the mid-latitude climate. Current mode water formation rate estimates are inaccurate because the formation mechanisms are poorly understood. The goal of this project is to better understand the annual renewal rate and the physical mechanisms responsible for EDW. Profiling float data provided a continuous time series of temperature, salinity and oxygen from the Gulf Stream region. Changes in mode waters and the mixed layer were documented through several winters. EDW layers were found to be thickest during and just after late-winter outcropping, indicating restratification of mode water. Oxygen saturation decreased in the EDW by 10% from late winter to late summer, indicating annual aging of the water mass. Vertical profiles also indicated mode water subduction crossing the Gulf Stream, as expected. Understanding the formation and evolution of EDW would greatly improve current climate models.

Identifying Interacting Partners of Human Small Timm Proteins

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Mohr-Tranebjaerg Syndrome (MTS) is a disease that causes post-lingual deafness, dystonia, mental deterioration, blindness, spasticity and psychiatric manifestations. MTS results from a mutation in hTimm8a transport protein found in the TIM22 import pathway. In yeast, Small Tim proteins Tim8p, Tim9p, Tim10, Tim12 and Tim13 behave as chaperone-like components that guide hydrophobic precursors across the mitochondrial intermembrane space. Research has shown an interaction between Tim8p and Tim13p as well as Tim9p and Tim10. Human homologues of these small Tims are known as hTimm8a, hTimm8b, hTimm9a, hTimm9b, hTimm10 and hTimm13. We aim to find all interacting partners of the human small Timms starting with hTimm8b (DDP2). Small Timm proteins will be purified in a native state from human stable cell lines that express His-PC tagged small Timms. Interacting partners were identified through western blotting and LC-MS/MS analysis. Preliminary studies show that hTimm8b interacts with hTimm13, hTimm22 and Timm23. Studies suggest that hTimm9a and hTimm10 may not complement yeast Tim9p and Tim10p. Research focused on Small Timms may help us develop treatments for MTS.

Cellular Localization of Nipah Virus Matrix Protein

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Nipah virus is a highly virulent, air-borne virus that causes acute encephalitis in humans. The matrix protein (M) underlies the viral envelope and is known to play important roles in the assembly and subsequent budding of viral particles. Interestingly, we have observed that Nipah M localizes to filapodia-like structures in transfected HeLa cells. Similar structures have previously been shown to be induced by retroviral infection and facilitate cell-to-cell spread of the virus. We performed immunofluorescence experiments

to determine whether actin filaments or microtubules co-localize with M on the intercellular structures. We observed that α -actin, but not β -tubulin, co-localized with M in the filamentous structures.

Furthermore, localization of M to these structures is disrupted in the presence of an actin polymerization inhibitor, cytochalasin D, but not microtubule inhibitor, nocodazole, confirming the actin-rich nature of these filaments. Additionally, mutation of the putative bipartite nuclear localization signal in M not only diminishes its translocation into the nucleus, but also abolishes its localization to the filaments, suggesting a link between the two processes.

Neutronic Design of the Control and Shutdown Rods Systems for the Modular PB-AHTR

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The Modular PB-AHTR (Pebble Bed-Advanced High Temperature Reactor) is a molten salt pebble bed reactor. The core consists of seven hexagonal graphite blocks pinched with channels through which pebbles—filled with TRISO fuel—circulate. The reactor features a buoyancy-driven passive shutdown rods system in the core and a control/shutdown rods system at the core's periphery. The cruciform-shaped control rods consist of a B_4C -graphite mix enclosed in a 1-cm graphite cladding and are neutrally buoyant with the salt. If coolant temperature rises (decreasing coolant density), the rods sink into the core. The systems must return the reactor to normal conditions during regular operation or shut it down in an accident. The neutronic efficiency of the control and shutdown rods systems were evaluated—independently. The core was modeled using MCNP5; which provided the reactor's effective multiplication factor for different levels of rods insertion. Both systems provide enough negative reactivity to shutdown the reactor. The inner system can shutdown the core even when 50% of the rods fail. Increasing ^{10}B concentration does not significantly increase rod worth.