PI Name	Institution	Title	Project	Years Awarded	Funding Institute
Collier, Charles P	California Institute Of Technology	"Nanowiring" Biomolecules to Carbon Nanotube Probes	R21GM071702	3 years	NIGMS

Abstract: The observation of spectroscopic signals in response to mechanically induced changes in biological macromolecules can be enabled at an unprecedented level of resolution by coupling single-molecule manipulation/sensing using carbon nanotubes with single-molecule fluorescence imaging. Proteins, DNA and other biomolecules can be attached to nanotubes to give highly specific single-molecule probes for the investigation of intermolecular dynamics, the assembly of hybrid biological and nanoscale materials and the development of molecular electronics. Recent advances in nanotube fabrication and Atomic Force Microscope (AFM) imaging with nanotube tips have demonstrated the potential of these tools to achieve high-resolution images of single molecules. In addition, proof-of-principle demonstrations of nanotube functionalization and attachment of single molecules to these probes have been successfully made. Improved techniques for the growth and attachment of single wall carbon nanotubes as robust and well-characterized tools for AFM imaging are being developed. This work serves as a foundation toward development of single-molecule sensors and manipulators on nanotube AFM tips for a hybrid atomic force microscope that also has single-molecule fluorescence imaging capability. An individual single wall carbon nanotube (SWNT) attached to an AFM tip can function as a structural scaffold for nanoscale device fabrication on a scanning probe. Such a probe can have a novel role, to trigger specific biochemical reactions or conformational changes in a biological system with nanometer precision. The consequences of these perturbations can be read out in real time by single-molecule fluorescence and/or AFM sensing. Of particular interest is the possibility of electrical wiring of single redox enzymes to carbon nanotube scanning probes, which will allow for observation and electrochemical control of single enzymatic reactions, by monitoring fluorescence from a redox-active cofactor or the formation of fluorescent products. Enzymes "nanowired" to carbon nanotube tips may enable extremely sensitive probing of biological stimulus-response with high spatial resolution, including product-induced signal transduction.

Gersappe, Dilip	State University New York Stony Brook	DNA Electrophoresis On Nanostructured Surfaces	R21HG003064	3 years	NHGRI

Abstract: We propose to develop a novel methodology to separate DNA and related biomolecules using nanostructured surfaces. We will use a combination of theoretical and experimental methods to study electrophoresis of charged biological molecules on patterned surfaces. The goal is to understand the fundamental mechanisms which control the dynamics near surfaces and to formulate predictive models which will allow the engineering of high resolution separation devices with optimum throughput and chemical selectivity. Nanoscale patterns will be imprinted using polymer self assembly, while more complicated micron scale structures with a combination of topological and chemical patterns will be manufactured by micro-contact printing. Electrophoresis will be performed and the mobility of DNA chains on these various surfaces will be observed either by confocal, near field microscopy, or CCD coupled video imaging. Fluorescence recovery after photobleaching (FRAP) coupled with Linear Dichroism detection (FDLD) will be used to measure surface relaxation times and diffusivity. The measurements will be performed as a function of pattern morphology, buffer concentration, chemical interactions, and chain structure. From these measurements we should be able to elucidate the relative importance of surface interactions, surface charges, electroosmotioc flow, and topological confinement in the surface dynamics of charged molecules. Due to the complexity of the problem, a variety of complementary theoretical treatments will be employed in order to obtain a quantitative model. Coarse grained models will be used to focus the application of more computationally intensive molecular models into those regions of phase space which control the behavior of the system. Theoretical methods used will range from Molecular dynamics simulations, to scaling analysis, to studies of flow on patterned media. The results should have broad applicability to a variety of devices and molecules including microfluidic channels, microarrays, complexed proteins, and cellular materials.

Guo, Peixuan | Purdue University West Lafayette | Nanotube and nanogold to gear phi29 DNA packaging motor | R01EB003730 | 4 years | NIBIB

Abstract: Nanotechniques involve the creation, characterization, and modification of organized nanomaterials to serve as building blocks for the construction of large-scale devices and systems. Living systems contain a wide variety of nanomachines and highly ordered structures, including motors, arrays, pumps, membrane cores, and valves. The novelty and ingenious design of bacterial virus phi29 DNA packaging motor, the strongest biomotor studied to date, have inspired the synthesis of this motor and its components as biomemitics for nanodevices. This 30 nm nanomotor uses six ATP-binding pRNA molecules to gear the motor. An imitative motor has been successfully constructed using purified recombinant proteins and artificially synthesized RNA. It can be turned on and off at will. The formation of ordered structural arrays of the motor complex and its components, the retention of motor function after 3'-end extension of the pRNA, and the ease with which pRNA dimers and trimers can be manipulated combine to make the RNA-containing motor a viable option as mechanical parts in nanotechnology. The long-term objective is to utilize this synthesized motor, together with arrays of its components, as parts in nanodevices, with several major applications: 1) To use this artificial motor as parts for nanodevices, such as apparatuses for in vivo drug delivery. 2) To use the ordered arrays of motor components as building facades for large-scale supramolecular structures to serve as molecular sieves, chips for the diagnosis of diseases, or as ultrahigh density data storage systems. The presence of six pRNA subunits in the hexameric building blocks will allow for the construction of arrays with multiple functions, for example to allow for the separate identification of multiple pathogens. 3) To develop this nanomotor into a DNA-sequencing apparatus, since the DNA-packaging process involves movement of the DNA through a 3.6-nm pore surrounded by six RNA that can be modified to accept chemical signals. Though all of these long-term applications are becoming more and more realizable, practical nanotechnology is still in its infancy. It would be unrealistic to propose applying the phi29 motor system directly to nanodevices in only three or four years. Thus, the short-term objective of this proposal is to construct pRNA or protein arrays that will serve as templates for the construction of patterned supramolecular structures, and to find the best routes through which to link nanotubes and nanogolds as well as to attach biological moieties and chemical groups to the motor or the constructed arrays, pRNA dimers, trimers and hexamers will be used for array construction. Chief among early concerns will be avoiding disruption of the assembly and functioning of the motor and/or arrays due to the incorporation of foreign components. This phase of the study will pave the way toward direct and practical technological applications of the motor and its arrays in nanodevices.

Khan, Mohamed K | Roswell Park Cancer Institute Corp | Imaging Nanocomposites Targeting Tumor Microvasculature | R01CA104479 | 5 years | NCI

Abstract: Objectives: This proposal will develop novel agents for imaging the tumor microvasculature present in all cancers. It proposes to design, synthesize, and characterize novel radioisotope containing nanoparticles (nanocomposite devices or NCDs) made from dendrimer templates and targeted at the angiogenic tumor microvasculature. This technology will encapsulate different radioisotopes within devices of defined size and targeting surface properties. The radioactivity delivered to a tumor is increased by increasing the particle size, or the number or specific activity of the guest atoms, without destroying the targeting ability of the NCD. By varying the particular guest atoms (metals/isotopes), different forms of imaging will be permitted (eg. film, SPECT, PET). These NCDs can deliver at least a log fold more radioactivity to tumors than possible with current antibody technologies, giving the added potential for imaging microscopic tumor burdens, and even molecular imaging. NCDs could permit combined imaging and therapy of all cancers. Specific Aims: (1) To chemically link the angiogenic microvascular targeting peptide, RGD, onto the surface of gold or copper containing NCDs. (2) To carry out biodistribution and biosafety studies with RGD-surfaced 5nm {Au} nanocomposites and RGD-surfaced 5 nm {Cu} nanocomposites to determine whether we can exploit the targeting ability of RGD to deliver nanocomposites to the tumor microvasculature (3) To carry out imaging of the RGD-surfaced NCDs a) lntracellularly, b) intratumorally, and c) in the whole animal. Design and Methods: (1) The NCDs will be made from PAMAM polymer templates, and then linked to RGD or, alternatively they may be made from RGD pre-substituted templates with the later introduction of gold (or copper) based on the principle of reactive encapsulation. Next, {198Au} and {64Cu} NCDs will be developed by direct activation or by using conventional radiochemistry. (2) Specificity of binding studies will be carried out by intravenous injection of the NCDs into B16 melanoma or MatLyLu prostate tumor bearing mice, and isolation of the organs/tissues for analysis of NCD content, and competitive injection of excess RGD. Biosafety studies include daily weights and a detailed toxicity table. (3) intracellular, intratumoral, and whole animal imaging will be accomplished via Transmission Electron Micrography (TEM), an autoradiography and immunohistochemical method, and SPECT imaging respectively.

Li, Jiali University Of Arkansas At Fayetteville Solid-State Nanopore Identification For Proteins R21HG003290 3 years NHGRI

Abstract: This proposed research is to draw single molecules of denatured proteins through a small pore, a solid state nanopore in silicon nitride membrane, that is integral to a sensitive detector. The solid state nanopore detector is designed to guarantee that driven by electric field, each polypeptide traverses the nanopore in sequencial, single file order. The translocation of each polypeptide will induce a transient electronic signal, a current blockade in the detector. The research goal is to develop the nanopore technique to record single polypeptide translocations by measuring the current blockades, probe the peptide's fundamental properties including their length, diameter, secondary structure, charge, and eventually the amino acid sequence at high speed, high resolution, and low cost. The specific aims are: 1. Develop single channel recordings of polypeptide chains translocating through solid state nanopores driven by electric field in aqueous ionic solution. Such recordings will be used to achieve an electronic read-out of the fundamental properties of polypeptide chains. Study how the electronic signals are related to these fundamental molecular properties of interest. 2. Develop reliable control over the thickness, chemical activity, electrical conduction and noise properties of solid state nanopores compatible with the requirements of single protein sensing. 3. Study and test the appropriate temperature, pH, bias voltage and nanopore size conditions for optimize the process of amino acid chain translocation and identification through solid state nanopore sensors. 4. Develop data analysis software and statistical models to interpret nanopore electronic signals. If the research goals proposed here are reached, a high-throughput device that can probe and directly "read" electronically, at the single molecule level, the size, charge, folding, and sequence of proteins, will dramatically alter the pace of biology and medical science. The development of high throughput nanopore probes of the molecular and atomic characteristics of single peptide chains, and the fundamental understanding that will make development of such probes possible, would revolutionize functional genomics, and proteomics.

Lindsay, Stuart M Arizona State University Molecular Reading Head For Single-Molecule DNA Sequencing R21HG003061 3 years NHGRI

Abstract: The goal of the proposed study is to evaluate a novel single-molecule DNA sequencing technology that has the potential to sequence a molecule of genomic dimension in hours. The DNA is attached to a rotaxane complex consisting of a molecular ring (cyclodextrin) that self-threads onto a propylene oligomer. The far end of the propylene oligomer is attached to a fixed surface, and the cyclodextrin ring is covalently attached to an AFM probe. As the AFM probe is pulled away from the fixed surface, the DNA passes through the cyclodextrin ring, one base at a time. Fluctuations in molecular friction as the ring passes each base are recorded as deflections of the AFM cantilever. If these data can be interpreted in terms of the base sequence of long DNA molecules, then single DNA molecules can be sequenced rapidly with this new technology. Preliminary studies appear to show that the DNA can be pulled through a cyclodextrin ring. They also indicate that the cantilever deflection during retraction depends on the DNA sequence. An unanticipated discovery is that double stranded DNA appears to pass the ring more easily than single stranded DNA, and does so with less random fluctuation than is the case for single stranded DNA. Our first goal is to put the 'ring sliding' model to further test. Does the ring really slide over one strand of double-stranded DNA, peeling the complementary strand off? Does sequence-specifc adhesion between the DNA and the fixed surface contribute to the sequence-related signal? If these experiments unearth a problem with our system, we will modify the chemistry appropriately. Once the operation of the system is verified, we will carry out a program of theory and experiment aimed at understanding these initial observations and establishing the limitations of the technology as developed thus far. Guided with information from these studies, improved molecular 'reading heads' will be designed, sequencing parameters will be optimized and hardware will be improved with the goal of reliable sequencing of oligomers, a pre-requisite for subsequent attempts at large-scale sequencing.

Marohn, John A Cornell University Ithaca Cantilever Magnetic Resonance Microscopy of Biomolecules R01GM070012 5 years NIGMS

Abstract: This proposal aims to develop technology for dramatically increasing the sensitivity of magnetic resonance imaging. The goal is to develop a "molecular microscope" to detect, analyze, and image nanoscale entities of relevance to biomedicine. Tools for determining protein structure are central to biological research. Improved tools for determining the three dimensional structure of large macromolecules and aggregate structures are urgently needed. A majority of proteins are not well suited for analysis by current methods because they cannot be isolated in large enough quantities or because they do not form crystals. In this proposal we present plans for developing a cantilever-based molecular microscope that can determine the structure of a single copy of a protein, a task which no current technology can achieve. Such an instrument would revolutionize structural biology, dramatically impacting a broad spectrum of biological processes, disorders, and diseases. In this proposal we detail a stepwise approach to developing a molecular microscope for imaging single biomolecules based on a marriage of atomic force microscope and magnetic resonance imaging technologies. Our specific aims are: (1) To detect nuclear magnetic resonance in a new way, as a change in the spring constant of a magnetically tipped microcantilever, (2) Fabricate and characterize nanomagnets suitable for single-proton cantilever detected magnetic resonance. Explore experimentally the minimum forces and spring constant changes that can be detected when a thin, ultrasensitive, silicon microcantilever is brought close to a surface, and (3) Develop and test magnetic resonance imaging protocols suited to small ensembles of nuclear spins.

Marszalek, Piotr Duke University Nanoscale DNA Diagnostics By Single Molecule AFM R21GM071197 3 years NIGMS

Abstract: DNA damage is the first step in the initiation of cancer. Every human cell experiences approximately 50,000 base damage/removal events and ~1000-2000 base mispair errors per day, Since DNA alterations vary from one DNA molecule to another it is important to detect such lesions in individual molecules. The primary objective of this R21 proposal is to develop a methodology for detecting DNA lesions at the single molecule level using Atomic Force Microscopy (AFM) techniques. Lesions will be detected either directly, by force spectroscopy and/or by imaging with carbon nanotube (SWNT) AFM tips, or indirectly, by detecting the secondary changes induced in DNA by the repair proteins. These secondary changes include e.g. strand incision, unwinding, degradation and re-synthesis. They are co-localized with the primary lesion and should be easily detected by our AFM assay. To achieve our objective we need to optimize the AFM platform to achieve: Angstrom level precision in the x, y, and z-axis and force errors below 10 pN. The platform must be able to align a molecule with the pulling direction and an ability to set a limit on the pulling force in order to preserve the coupling of the molecule to the instrument. We will construct various DNA molecules with model lesions and use AFM imaging and force spectroscopy to determine their fingerprints. We will study the effect of UV radiation on DNA elasticity and the course of its photoreactivation. A second objective is to explore the use of AFM spectroscopy and imaging to study the mismatch repair (MMR) in E. coil and in humans. We will determine force spectrograms of DNA molecules containing a mismatched base in the presence of bacterial and human repair activities. We will record the changes of DNA elasticity during the course of repair. We will also visualize primary lesions, repair-induced secondary alterations and DNA/proteins complexes. These measurements will allow us to examine key intermediates in the repair reaction and to further clarify molecular functions of the repair proteins and their assemblies. The proposed experiments will use for the first time, AFM-based single-molecule force spectroscopy to detect DNA lesions and to examine the course of a repair reaction. The findings should be of great significance for nanoscale DNA diagnostics and DNA damage and repair research.

Marziali, Andre | University Of British Columbia | Nanopores For Trans-Membrane Bio-Molecule Detection | R01HG003248 | 3 years | NHGRI

Abstract: Single-molecule approaches to the collection of biological data can reveal temporal dynamics of processes that would otherwise be unavailable through measurements of ensembles of molecules or cells. The complete elucidation of regulatory networks in cells will require time-resolved gene expression data obtained from a single cell to determine the time constants of the network feedback loops. It has been shown that there is a strong analogy between networks in cell biology and electronic circuits - present tools available to cell biologist are the equivalent of a voltmeter in electronics, yielding information only on slowly varying averages. Cell biologists will eventually need the biological equivalent of an oscilloscope to perform minimally invasive measurements of bio-molecule levels in live cells in real time. Single molecule techniques are the most promising candidate at this time for such a tool. Furthermore, single molecule approaches may lead to highly sensitive assays with broad applications including genotyping, gene expression studies, and protein detection. It is conceivable that arrays of single-molecule nanosensors would provide data similar to microarrays for gene expression or SNP determination, but with increased data quality and higher sensitivity. In preliminary work, we have developed an organic nanosensor capable of detecting and distinguishing between similar nucleic acid strands across a lipid membrane. The sensor is based on a 2 nm wide protein channel that self-assembles into a lipid membrane, with an engineered nucleic acid and protein construct inserted into the pore under an applied electric field. This nanosensor assembly results in a nucleic acid tail protruding through the lipid bilayer the pore is inserted in. This tail is engineered to bind to specific analytes, such that when an analyte is bound and an attempt is made to withdraw the tail from the pore, resistance is encountered - the whole operation resulting in something analogous to ice-fishing. We have successfully used this nanosensor to detect and characterize binding of single DNA strands. In this application, we propose an expansion of this work to determine the operating limitations of this prototype nanosensor, and to develop additional nanosensor prototypes for improved detection of both nucleic acids and other bio-molecules. Though beyond the scope of this initial application, this research is intended to eventually provide a powerful tool for in-vivo sensing of bio-molecules for the study of cellular function and complex cellular diseases (such as cancer), as well as novel synthetic nanosensor arrays for highly accurate quantitation of gene expression and improved, low cost genotyping.

Rosenthal, Sandra J | Vanderbilt University | Quantum Dot Nanoconjugate Imaging Of Neural Receptors | R01EB003728 | 4 years | NIBIB

Abstract: Receptor driven cellular behavior, ranging from signaling and excitability in neural activity to immune system response to disease invasion, represents an important class of functional nanobiostructures in biological systems. Receptors are uniquely suited to direct such processes due to their ability to sense the environment, through ligand binding, and their ability to transmit this signal to the cell interior via signal cascades. The goal of this proposal is the development of novel imaging tools and methods to establish a quantitative molecular level understanding of the function of biological receptors. Unfortunately, the study of these fundamental biological components is limited by currently available imaging tools such as radiolabeled ligands or indirect detection via antibodies. These approaches suffer due to the poor spatial resolution of radiotracer studies, the limited availability of surface-domain antibody probes for membrane proteins, the broad emission spectra of available fluorophores and their photochemical degradation. In this proposal, we will continue to develop our novel, non-isotopic, labeling strategy involving ligand-conjugated fluorescent nanocrystals (nanoconjugates). Specifically, we will: 1. Design, synthesize, and characterize novel nanoconjugate probes for imaging neural receptors. 2. Develop a molecular level understanding of nanoconjugate-receptor interactions. 3. Demonstrate dynamic imaging of neurotransmitter receptors in order to map their regulation and function. To accomplish these specific aims, we have assembled a multidisciplinary team including chemists, physicists, pharmacologists and neuroscientists. Completion of this grant will result in the development of a novel class of nanoconjugates based on highly fluorescent small-molecule and peptide conjugated CdSe/ZnS core shell nanocrystals. These probes will enable investigators in neuroscience and membrane biology to answer questions previously unanswerable due to the limitations of current methods. The nanoconjugates described herein present the opportunity for dynamic imaging of fundamental processes involving neural G protein-coupled receptors and transporter proteins. Such "real time" experiments will provide new insight into questions concerning the molecular details of these neuroreceptors, their trafficking and localization in response to external stimuli. Such results will provide new molecular level insights into neural processes such as depression, addiction and learning. Additionally, the nanoconjugates may serve as the basis for new drug discovery methods to identify unique drugs that target specific receptors previously implicated in neurological disorder.

Rout, Michael P. Rockefeller University Virtual Gating Machines For Protein Purification R01GM071329 4 years NIGMS

Abstract: Most commercial large-scale production of proteins uses column chromatography and synthetic membranes to fractionate and concentrate proteins. Though the requisite multiple recovery steps increase purity, yields drop quickly and expenses rise. Improving the performance of these processes is therefore a high priority. Nature has already solved this kind of protein enrichment problem with the nuclear pore complex (NPC), the macromolecular machine that efficiently segregates proteins between the nucleus and cytoplasm of all eukaryotic cells. Since we now have an understanding of the mechanism by which the NPC transports proteins, our goal is to mimic the molecular machinery of the NPC, with its exquisite selectivity and high throughput, in a robust synthetic platform. Our approach is one of reverse engineering, in which we will dissect the nuclear transport system to elucidate its key elements in order to duplicate them. We will use the yeast NPC, the best understood system, as our starting point. Our approach is one of reverse engineering, in which we will dissect the nuclear transport system to elucidate its key elements in order to duplicate them. We will use the yeast NPC, the best understood system, as our starting point. Specific Aim 1 and Specific Aim 2 seek to detail how the NPC is configured to function as a transporter. For this, we must separate those components that are essential for transport from those needed for other NPC functions, such as self-assembly and NE maintenance, and learn how those components function. Following this, we will study in detail the behavior of the key components needed for nuclear transport, to understand why they make the NPC function so efficiently in vivo. In Specific Aim 3 we will develop a computational simulation of the NPC to explore how to translate these optimal parameters into an artificial machine, and in Specific Aim 4 we will explore several avenues to build such machines at various scales.

Rubinstein, Israel University Of Illinois At Chicago Micellar VIP Nanoparticles For Rheumatoid Arthritis R01AG024026 4 years NIA

Abstract: Despite remarkable recent advances in therapeutics, rheumatoid arthritis still represents an unmet medical need. Hence, there is an urgent need to develop and test new biocompatible, long-acting and safe biological response modifiers for patients with this condition. To this end, the focus of this exploratory/development research project is on determining the efficacy and safety of an innovative strategy developed in our laboratory consisting of homing low dose VIP to injured joints in rheumatoid arthritis. Our approach exploits the endowed biophysical properties of VIP to self-associate with biocompatible and biodegradable phospholipid nanoparticles (average size, approximately 17 rim) composed of distearoylphosphatidylethanolamine- poly-(ethylene)glycol (PEG; Mr, 2000 (DSPE-PEG2000) that form sterically stabilized micelles. These interactions lead to conformational transition of the VIP molecule from a predominantly random coil in aqueous solution to alpha-helix, the preferred and most stable conformation for ligand-receptor interactions, in the presence of micelles. This process protects VIP from degradation and inactivation in biological fluids and prolongs its circulation time because the PEG molecules grafted on the surface of micelles confer steric hindrance thereby evading uptake by the reticuloendothelial system. Consequently, the dose of VIP required to achieve its intended biological effect is reduced appreciably as are adverse events relative to a similar nominal dose of the unstable VIP monomers. Importantly, the salutary effects of VIP-containing nanoparticles are amplified because they selectively extravasate from the leaky microcirculation of injured tissues into the interstitial space and subsequently bind to VIP receptors overexpressed on the surface of immune and inflammatory effectors cells in these tissues. This active targeting process is amplified by the absence of VIP receptors on the luminal side of microvascular endothelial cells. On aggregate, these attributes indicate that micellar VIP could represent a novel, biocompatible, long-acting and safe targeted disease-modifying drug for patients with rheumatoid arthritis. The purpose this study is to determine whether intravenous or subcutaneous administration of low dose micellar VIP abates collagen-induced arthritis in mice without affecting systemic arterial pressure. The basic tenet of this proposal is that low dose micellar VIP is actively targeted to injured joints where it downregulates certain key tissue injury-promoting cytokines and matrix proteinases while up-regulating certain key tissue repair-promoting cytokines elaborated by activated effector cells in injured joints of mice with collagen-induced arthritis. This, in turn, will shift the balance of the immune and inflammatory cascades toward tissue repair. The specific aims are: 1) Optimize the formulation of micellar VIP for in vivo administration; 2) Determine the efficacy and safety of intravenous or subcutaneous micellar VIP in mice with collagen-induced arthritis; 3) Determine the effects of intravenous or subcutaneous micellar VIP on circulating biomarkers of tissue injury and repair in mice with collagen-induced arthritis; and 4) Determine the pharmacokinetics and biodistribution of intravenous or subcutaneous micellar 125I-VIP in mice with collagen-induced arthritis. The anticipated results of the proposed studies will provide proof of principle and set the stage for testing micellar VIP as a novel, safe, long-acting and efficacious disease modifying drug in patients with rheumatoid arthritis.

Shea, Thomas B University Of Massachusetts Lowell Nanospheres As Vehicles For Treatment Of Neuroblastoma R21CA104468 1 year NCI

Abstract: Neuroblastoma, the most common solid tumor of children, arises from a block of differentiation and a resultant continuation of the proliferative state. Neuroblastomas often spontaneously revert by undergoing partial differentiation, which leads to their ultimate degeneration. A useful therapeutic approach for clinical neuroblastoma may therefore encompass strategies to force differentiation. In our ongoing studies we have developed an anti-oxidant synergistic formulation ("ASF"), comprised of alpha-tocopherol (vitamin E), sodium pyruvate and phosphatidyl choline, that supports axonal elaboration in cultured neurons. We demonstrate herein that ASF prevents neuroblastoma proliferation and promotes their differentiation in culture, even in the presence of serum (which otherwise induces rapid neuroblastoma proliferation), This holds the promise that ASF, with proper administration, may foster differentiation (and therefore ultimate degeneration) of neuroblastoma in situ and may therefore represent a novel approach towards suppression of clinical neuroblastoma. However, ASF was unable to prevent continued increase in size of tumors generated following injection of neuroblastoma into nude mice, despite injection directly into the tumor. Since ASF is effective on these cells in culture in the presence of serum, one likely interpretation is that we were unable to maintain a sufficient concentration of ASF at the tumor site. To accomplish this, we propose administration of ASF within our novel non-toxic nanospheres, which foster intracellular delivery of their contents Encapsulation within nanospheres dramatically improves the efficacy of known anti-cancer drugs, as well as that of ASF, in culture. We propose to determine the efficacy of nanosphere-mediated delivery of ASF and known anti-cancer agents on neuroblastoma tumors in nude mice by injection into the tumor site, transdermal application (our data indicate that our nanospheres foster transdermal delivery of their contents), inclusion within their diet (our data indicate that dietary administration of ASF is physiologically effective), and combinations of these methods. Controls will include injection and transdermal application at sites distal to the tumor, administration of non-encapsulated compounds, and administration of "empty" nanospheres. Resulting data will indicate whether or not nanospheres represent useful delivery agents for cancer therapy.

Simpson, David G Virginia Commonwealth University Nanofabrication Of A Dermal Equivalent By Electrospinning R01EB003087 4 years NIBIB

Abstract: We propose to use electrospinning to fabricate a dermal equivalent composed of nano and micron scale diameter fibrils of collagen and elastin. Electrospinning is a rapid, and efficient, nanotechnology that uses an electric field to process synthetic and natural protein polymers into tissue-engineering scaffolds Tissue-engineering scaffolds composed of electrospun collagen are resilient, non-immunogenic and fully bioresorbable. When implanted as a dermal equivalent, this material is rapidly infiltrated by dermal fibroblasts, microvascular endothelial cells and epithelial cells. We attribute the "stealthy nature" and biological activity of electrospun collagen to the chemical composition of the polymer, the near physiological diameter of the fibers (100-200nm) and the 67 nm repeat feature that is observed at the ultrastructural level on these filaments. This 67 nm repeat is present on native collagen and is associated with specific binding sites that promote the migration of dermal and endothelial cells. From a commercial and clinical prospective, scaffolds of electrospun collagen have several distinct advantages: this material can be stored in a dry, sterile state to increase shelf-life, are easy to deploy and highly hemostatic. In addition, electrospun scaffolds can be supplemented with anti-bacterial agents, other pharmaceuticals like topical anesthetics and peptide growth factors during the fabrication process, providing enormous flexibility in the design of a tissue engineering scaffold. The Specific Aims of this Project are: Aim 1. Tailor an electrospinning device for the fabrication of a dermal equivalent composed of nano-scale to micron-scale diameter fibrils of native ECM constituents. Aim 2. Evaluate the mechanical and biological properties of a dermal equivalent as a function of composition and fiber diameter. Aim 3. Use the structure of a nanofabricated dermal equivalent as a novel solid-phase delivery platform for anti-bacterial agents. Aim 4. Evaluate candidate dermal equivalents in a full thickness dermal injury in the guinea pig.

Takayama, Shuichi University Of Michigan At Ann Arbor Reconfigurable Nanoengineered Extracellular Matrices R21EB003793 3 years NIBIB

Abstract: Two of the most important problems in cell-based treatments of muscular and neurological diseases and injuries are: i) the regulation of cell proliferation and differentiation, and ii) the control of cell migration. For example, in human myoblast transplant trials for treatment of muscular dystrophies, major problems were the lack of proliferation, migration, and fusion of transplanted cells with existing muscle tissue. In repair of nerve damage, suppressing astrocyte proliferation and promoting neuroblast proliferation, along with guidance of neurite extensions are key issues. Although these cellular behaviors are critically depend on nanoscale adhesive cues in the extracellular matrix (ECM), there is currently a lack of understanding of what these crucial nanostructures are and how dynamic changes in those structures determining cell function. This lack of understanding is due, at least in part, to the lack of efficient, versatile, and convenient methods to engineer the ECM at the nanoscale across biologically relevant areas of micrometers, millimeters, and larger. The specific aims of this proposal are: i) understand and optimize a technology, called nanocrack patterning, to generate nanoengineered substrates with ECM molecule nanolines of defined widths, lengths, spacings, and orientations, ii) test the hypothesis that stretch-induced, nanoscale substrate reconfiguration can contribute to proliferation and lineage determination of myoblasts and neuroblasts, and iii) perform feasibility studies for discovery-driven research on cellular pathfinding where a microarray of criss-crossing nanopatterns of ECM molecules will be fabricated to rapidly profile cellular spreading/migrating preferences. The initial proof-of-concept studies will use C2C12 myoblasts and N27 neuronal precursor cells. Mesenchymal stems cells and primary myoblasts will be studied in the future. Although the biological problem to be addressed in this proposal is limited to myocyte and neuron behavior, the nanobiomaterials developed will be useful for addressing a much broader range of biological questions. The nanocrack patterning technique that will be developed uses nanoscale fracture mechanics and has the advantages of: (i) rapid nanopatterning over large areas (up to square centimeters and larger), (ii) nanopatterning over 3D substrates and inside microfluidic channels, (iii) generation of nanopattems consisting of multiple types of molecules on the same substrate, and (iv) stretch-induced in situ adjustment of the widths of ECM molecule nanolines generated.

Taton, Thomas A University Of Minnesota Twin Cities Magnetomicelle Nanoparticles For Triggered Drug Delivery R21EB003809 3 years NIBIB

Abstract: We propose to develop 'magnetomicelles' for the triggered release of hydrophobic pharmaceuticals either from the bloodstream or an injected gel at a particular location in the body. Monodisperse magnetic nanoparticles with high magnetocrystalline anisotropies will be encapsulated within crosslinked polyacrylate-PEO 'stealth' micelles, along with hydrophobic drugs. The encapsulated pharmaceuticals will then be released from these micelles in response to an external radiofrequency (RF) magnetic field supplied by an induction coil. The applied field will induce oscillating relaxation of the particles' magnetic moments, which will locally heat the magnetic particles. Thermal energy will be transported to the micellar shell, which will accelerate the diffusion of co-encapsulated, hydrophobic molecules from the glassy micellar core. Because subdomain magnetic particles can be excited with kHz frequencies and low field amplitudes, this technique will specifically heat the particles and not surrounding tissue. In principle, both the release period and amount of drug released could be controlled by the intensity and duration of the applied RF field. We anticipate that these composite micelles can act as biocompatible, latent reservoirs that can stably retain drugs in biological hosts for long periods of time until the moment the drugs are needed, when they can be released almost instantaneously. In the proposed R21 research, we will demonstrate the principle of magnetomicelle drug carriers; we hope that successful proof of principle will lead to further (R01- funded) research on the use of these vehicles in vivo.

Vinogradov, Sergei University Of Pennsylvania Dendritic O2 Sensor With Two-Photon Absorbing Antenna R21EB003663 2 years NIBIB

Abstract: We propose to construct a nano-scale molecular sensor for oxygen, which will take advantage of the two-photon (2P) absorption phenomenon, permitting high-resolution O2 measurements in a variety of biological objects, including oxygen imaging in brain neurons in vivo, using two photon laser scanning microscopy (2P LSM). The design of the nano-sensor will combine several principles and elements, currently under scrutiny in nano-science and nano-technology applications. These will include: two-photon absorption (2PA); an antenna-array, consisting of multiple chromophors coupled to the same functional core; directional intramolecular energy transfer (ET) from the antenna to the core; dendritic encapsulation of the core function to provide its protection and control of its local environment. In brief, 1) the central component of the device, serving as the terminal acceptor of the excitation energy, will be a polyfunctionalized Pt or Pd porphyrin, whose triplet state emission is strong, occurs in the near infra-red and is sensitive to O2; 2) because 2PA cross sections of metalloporphyrins are small, several strong 2P absorbers will be linked to the core, either directly or to the termini of dendritic arms attached to the porphyrin, providing an efficient 2P antenna; 3) the 2P chromophors will be chosen in such a way that their 2P absorption bands will be in the near infra-red window of tissue (e.g. 700-900 nm), while their emission will be maximally overlapped with the absorption band(s) of the core metalloporphyrin, assuring efficient antenna ->remitter energy transfer (ET); 4) the functional groups on the core metalloporphyrin unused in the coupling of the antenna will be used for attaching the protecting dendrons; these will form an encapsulating jacket, isolating the core from interactions with biological components, controlling diffusion of oxygen and making the entire sensor water-soluble. In addition to being the key to 2P in vivo oxygen imaging, the proposed construct will be an excellent model compound to study triplet state emission and oxygen quenching at the single molecule level. If successful, these experiments will open avenues for novel research, which will span from the studies of protein conformations by small molecule diffusion to the construction of a single-molecule oxygen sensor.

Vohra, Yogesh K University Of Alabama At Birmingham Nanotechnology In Osseointegration Of TMJ Implants R01DE013952 4 years NIDCR

Abstract: We propose the use of nanotechnology approaches for controlling interfaces between Temporomandibular Joint (TMJ) implants and the surrounding tissues. It is estimated that more than 10 million people suffer from the TMJ-related disorder symptoms in the Unites States with a clinical need for TMJ implant designs that will show propensity for osseointegration. We propose the use of nanostructured functionally graded metalloceramic coatings for articulating surfaces and nanostructured hydroxyapatitecoated fixation screws for the TMJ implants. The four specific aims are: (1) development of nanotechnology tools and methods for deposition of metalloceramic coatings having a gradual structural transition from the coating/implant interface (metallic bonding) to the outer implant surface (nanometer-level smooth ceramic) (2) development of nanostructured hydroxyapatite coatings on fixation screws to withstand the shear stresses during insertion of the implants and surface functionalization for osseointegration of the TMJ implant (3) In vitro investigations to compare the adhesion and osteoblastic differentiation of mesenchymal cells (MSC's) on the nanostructured coatings. Test the ability of these coatings to adsorb pro-adhesive proteins from blood, and determine whether functionalizing the surface via application of a synthetic pro-adhesive peptide (RGD) enhances MSC adhesion and differentiation, relative to uncoated materials with adsorbed serum proteins and (4) In vivo investigations to characterize the short term (initial organic and cellular responses) and long term (soft tissue and bone integration) responses of control and proposed biomaterials with and without functionalized surfaces specific to overall biointegration and biocompatibility profiles. A multidisciplinary research team consisting of physicists, a biomedical engineer, a cell biologist, and a biomaterials and dental surgeon has been assembled to address TMJ osseointegration. The primary focus is to improve the fixation, wear resistance and osseointegration for long-term success of TMJ implants and lower the need for multiple or revision surgeries. The novel nanostructured functionally graded surface architectures validated by our in vitro and in vivo studies will have a broader impact on a variety of other metal-on-metal and metal-on-polyethylene implants currently in clinical use.

Walker, Gilbert C University Of Pittsburgh At Pittsburgh Near-Field IR Microscope For Nanoscale Chemical Imaging R21EB003101 3 years NIBIB

Abstract: The goal of this project is to develop an attachment for a scanning probe microscope that enables high throughput, apertureless, near field infrared microscopy (ANSIM). The development of this instrument will enable sensing applications that require the characterization of extremely small regions of complex biological materials on surfaces. The proposed near field instrument will be able to perform IR absorption spectroscopy at less than 100nm lateral spatial resolution. The ANSIM attachment will be designed to be small and portable, so that replicas of this attachment may become a practical add-on to other atomic force microscopes (AFMs). The instrument development program has three principal elements. First, infrared (IR) quantum cascade laser arrays will be assembled with the optics for guiding the IR laser light into and out of the microscope. Second, multi-channel detection and signal processing modules will be built and integrated with a common, commercial instrument. Third, improved designs of the sharp metal tip used in an apertureless near-field scanning infrared microscopes will be tested and optimized. IR spectroscopic chemical imaging of manmade polymeric surfaces at 100nm lateral resolution using apertureless near field microscopy has already been demonstrated in preliminary work. The goal of this exploratory proposal is to determine whether useful information can be acquired for imaging biological molecules, and whether technology that would be readily used by other scientists can be developed. The test measurements will concentrate on imaging peptide layers and their secondary structure, primarily using IR active amide bands. This would be the first time IR near field microscopy has been applied to peptide and protein analysis, and would represent a significant step forward in biochemical imaging using scanning probe microscopy. Polyglutamine (PG) and its aggregates have been chosen for demonstration studies because 1) PG has a relatively simple IR spectrum related to its secondary structure and 2) PG structures have been implicated in diseases associated with the deposition of insoluble aggregations of proteins, known as fibrils, in various human organs. To treat or to prevent such diseases, improved ability to observe the formation and the deposition of such insoluble aggregates is required; IR near field microscopy could have considerable impact in this area.

Weissleder, Ralph Massachusetts General Hospital Magnetic Nanoswitches For Sensing Biomolecules R01EB004626 4 years NIBIB

Abstract: Robust, versatile, and high-throughput biosensors are required for genomic and proteomic research applications, for high throughput screening in drug development, for rapid point of care assays and, potentially for in vivo imaging applications. In particular, there is a need for assay methodologies that a) are highly sensitive, b) minimize sample preparation and allow measurements in turbid/obscure media, c) measure widely different types of molecules using the same format and instrument and, d) can be run in single tube point of care or high throughput screening formats. We have developed a novel magnetic nanosensor technology that uses "magnetic relaxation switches" (MRSW), i.e. magnetic nanoparticles that are induced to switch between dispersed and clustered states by analytes, with changes in the spin-spin relaxation time (T2) of adjacent water molecules. The broad goals of this application are to optimize and extend MRSW methodology, and to develop MRSW assays for key areas of biomedical research and needs. Specific aim 1 involves the optimization of nanoparticles for MRSW assays, and improvements and extensions of the MR based instrumentation the method uses. In Specific Aim 2 we will apply the MRSW methodology to key issues of cancer biology (telomerase and telomere length), microbiology (virus detection and characterization), and panomics.