**Development and commercialization of a designed regulatory protein (DRP) that activates the Erythropoietin gene in the treatment of anemia**

**PI: Steven Briggs, PhD and Trey Ideker, PhD**

With the completion of the Human Genome Project, a genetic component to most diseases has been identified and, consequently, gene-based therapeutics now can address hundreds of valid targets. However, the use of DNA technologies in humans remains largely experimental and this has limited the therapeutic application of genomic information. In contrast, therapeutic proteins have been used safely for decades. A protein that provided the benefits of gene therapy but with the safety of protein therapy could open the door to exploitation of genomic information.

We recently described a class of proteins that act directly on DNA where they can activate or repress any target gene. When added to the extracellular medium that bathes human cells, these proteins, which we call designed regulatory proteins (DRPs), transit through the plasma membrane and enter the nucleus where they bind specifically to a designated 19 bp sequence of DNA. The objective of our project is to demonstrate the potential of DRPs to treat anemia. We have designed and produced a DRP to specifically repress expression of the human Survivin gene and can quickly do the same to produce a DRP to specifically increase expression of the human erythropoietin gene.

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**Rapid and Accurate Screening of Cancerous Cells in Biopsy Samples by a Protein-based Biosensor with TAT-HA2 Method.**

**PI: Shu Chien**

Chien plans to establish the technology needed to develop an efficient screening test based on fluorescence resonance energy transfer (FRET) microscopy to detect cancerous cells in clinical biopsy samples.

Chien's group has previously developed a FRET biosensor that enables the visualization of specific tyrosine kinase activity, called Src, in live cells with high temporal and spatial resolution. The activity of Src is closely correlated with early carcinogenesis. Proof-of-principle studies have demonstrated that the Src biosensor can accurately identify cancer cells mixed with normal cells, and recent studies have also revealed that HIV-1 TAT protein and a peptide derived from the influenza virus hemagglutinin protein (HA2) can facilitate priming of biopsy samples for FRET analysis. The grant will enable Chien to increase the efficiency, speed, and accuracy of the promising cancer-detection tool.
**Development and Application of Biosensors to Monitor Kinase Activity with High Temporal and Spatial Resolution in Live Cells**

**PI(s): Shu Chien and Yingxiao Wang**

Chien and Wang aim to establish the technology to monitor the activity in live cells of specific kinases, and to apply it to different physiological and pathological conditions, especially for the diagnosis of diseases such as cancer. Kinases play a crucial role in a variety of cellular processes, including cell division, angiogenesis, motility, and adhesion. Chien and Wang have developed a biosensor capable of detecting kinase activity in live cells based on an optical technology which allows the real-time measurement of kinase activity with high temporal and spatial resolutions in live cells. Preliminary experiments have demonstrated that this biosensor reports kinase activity with high degrees of specificity and sensitivity. With the von Liebig grant, they will conduct proof-of-concept research on this biosensor and its potential as a powerful tool to efficiently and conveniently diagnose the different developmental stages of cancers, e.g. in a biopsy or a pap smear sample.

**Advanced Medical Training Simulator Based on Operating Room Data**

**PI(s): Nathan Delson and Mike Bailey; School of Medicine Collaborators: Randolph Hastings, Matthew Weinger**

The goal of this project is harness virtual reality, augmented reality, and computer-controlled mannequins to train personnel in medical procedures—thus eliminating the current training method of practicing those skills on real patients. One of the key challenges of developing accurate simulations is the current lack of accurate physical data required to model the procedure. The approach of this study is to instrument medical tools used in the operating room, in order to measure the medical skill and patient properties necessary for a realistic simulator. The medical procedure to be addressed with von Liebig equipment grant is airway intubation via laryngoscopy. The simulator will be programmable to mimic patient anatomy observed during our study, and cues from expert motions will be accessible to the trainee to assist and evaluate the trainee. The physical properties of the simulator will be based on the stiffness properties acquired from the force and motion data from the instrumented laryngoscope. This prototype will illustrate a new method for medical simulator development, with potential applications in other medical procedures.
A comprehensive human papilloma virus (HPV) typing assay for early screening of cervical cancer
PI: Sadik Esener, Yu-Tsung Liu, Dennis Carson
Cervical cancer is almost always caused by infections of the human papilloma virus (HPV). An estimated 50 to 80 percent of the pre-cancerous lesions can be detected by Pap test, which is currently used more than 50 million times a year in the United States even though the test is imperfect. While the country has seen a 70 percent drop in cervical cancer over the past five decades, better tests are needed. HPV genotyping is a potential replacement for the Pap test.
The team has developed a novel platform that can be used for high throughput HPV typing. The technology incorporates HPV type-specific coded nanoparticles into cell-like micro-reactors where polymerase chain reaction (PCR) reaction is carried out. Therefore, up to millions of reactions could be carried out within a 0.2 ml tube. This platform is generally applicable for genotyping assays that require multiplexed PCR reactions.

Expansion of UCSD Pattern Recognition Methodology for Prediction of Biological Interactions
PI: David Gough
Dr. Gough and his colleague, Dr. Joel Bock, have applied for a patent on a new pattern recognition methodology that would speed up the process of detecting possible interactions among millions of proteins and inferring their biological functions. The methodology is for predicting protein-protein interactions, and employs an algorithm that can be trained to recognize interactions in a limited set of known interaction pairs. The algorithm can then be applied to a larger set of proteins of unknown interactions to predict interactions with quantifiable accuracy. This method has been applied successfully to proteins of several different organisms based on training information available on the web. The method has major advantages in predictive capability and computational economy over other approaches, and is a disruptive technology. The von Liebig grant will allow the team to (1) expand the application of this technology to protein-protein interactions in a broader range of organisms, and (2) explore its application to predict interactions between other types of biomolecules. These studies will solidify the foundation of the UCSD intellectual property and may lead to other inventions.
Active Microelectronic Array for Massively Parallel Peptide Synthesis & Binding Constant Analysis  
**PI: Michael J. Heller**  
The complexity of biological testing requires experimental agility; as such we have seen a movement towards customizable molecular arrays with a lower up front cost of production. The rise of Illumina in competition to Affymetrix is evidence of this market shift. Illumina continues to be awarded lucrative government grants for cancer analysis in lieu of Affymetrix largely because of the ability to reconfigure their genotyping platform. Similar agility within the phenotyping market is currently unavailable, because of three factors 1) cost of peptide synthesis, 2) scalability issues, and 3) inability to differentiate nonspecific protein interactions during analysis.

We have developed an active microelectronic array which we believe circumvents these limitations. Specifically, we have what we believe to be a means to accelerate the synthesis of upwards of 1 million peptides in parallel at specified locations on a microchip. Current commercial costs of peptide synthesis range from $10-$30 per peptide and require many hours of serial labor to achieve high purity samples. In contrast, our parallel method for synthesis is estimated to require several hours to complete the entire library. This would be a disruptive, enabling technology within the pharmaceutical, diagnostics, and research tools markets.

Electric Field Induced Fluctuation of Quantum Dot and Fluorescent Quencher Probes for High Sensitivity Genotyping, Gene Expression and Infectious Agent Detection  
**PI: Michael Heller**  
Efforts to detect infectious agents and other bioterrorism threats are stymied by the ongoing difficulty of doing rapid genetic identification and eliminating the need for the time-consuming (and expensive) step common to all current methods: amplification of the DNA/RNA target through Polymerase Chain Reaction (PCR). Professor Heller is proposing a novel electric field mechanism by which a combination of a fluorescent nanoparticle (quantum dot) and quencher fluorophore can used to detect very low levels of target DNA/RNA sequences in complex samples. The proposal involves the development of pairs of fluorescent nanoparticles (i.e., quantum dots) and fluorescent quencher probes which can selectively hybridize to a target DNA sequence. As part of a new process, Dr. Heller's team will apply an oscillating electric field (DC or AC) to the sample which causes the fluorescent nanoparticle and quencher probe combination that is hybridized to the target DNA sequence to produce an oscillating fluorescent response. This oscillating fluorescent system can now be easily detected even among thousands of non-specifically bound fluorescent particles. The endpoints of this research will be to optimize the performance of selected donor/quencher pairs prior to commercialization, and Heller says it is likely, given that the experimental design has been finalized, that this technology will be ready for market in less than one year.
Life Sciences

Funded Projects

Protease Detection System for the Inflammatory Response and Disease Diagnostics
PI: Michael J. Heller and Geert Schmid-Schönbein
Increasing evidence suggests that physiological shock, diabetes, cardiovascular diseases, tumors, and other diseases are associated with an inflammatory cascade. This cascade is accompanied by elevated permeability of the endothelium and release of degradative enzymes that are targeted towards a variety of autologous proteins and lipids. Such evidence now provides a great opportunity to develop a variety of therapeutic interventions to ameliorate shock and treat inflammatory diseases. Unfortunately, such interventions will be highly dependent on the ability to diagnose and monitor a highly complex series of events which occurs rapidly in shock scenarios and more subtly in chronic inflammatory diseases. The goal of this work is to develop a novel monitoring and diagnostic system to meet this important clinical need.

We are therefore developing a novel protease activity detection system for the monitoring of clinically relevant inflammatory responses and for disease diagnosis. The basic premise for our approach is to use electric fields to actively concentrate fluorescent labeled peptide substrates when cleaved by a particular enzyme. Cleavage of the fluorescent peptide substrates will result in a change in the net charge on the complex and the cleaved products can then be separated from the intact peptide substrate by application of a directed electrophoretic field. Subsequent detection is performed with a high sensitivity fluorescent detection device. These unique substrates will allow highly sensitive and selective rapid detection directly in clinical samples of the key enzymes (chymotrypsin, trypsin, elastase, matrix metallo-proteases, lipases, amylases) associated with the inflammatory cascade.

Highly functional hepatocytes on TiO 2 nanotube chips: New efficient modules for pharmaceutical screening of drug toxicity and drug metabolism
PI: Sungho Jin, Hyam L. Leffert
The basic technology utilizes newly developed nanotech chips constructed of Titanium and Titanium dioxide. The biocompatible surfaces of the chips comprise bonded titanium dioxide nanotubes, the geometry of which facilitates robust and long-term culture of highly functional, normal (i.e. primary) mouse and human hepatocytes. The technology can be used as a stand-alone chip, carrying human hepatocytes cultured in appropriately defined biological fluid and incubator systems, to be used for efficient pharmaceutical in vitro screening of drug toxicity and drug metabolism. Further monochip development into modular arrays, composed of hundreds to thousands of monochips, are envisioned for pharmaceutical use to provide high throughput robotic screening, as well as for eventual bio-artificial liver devices for the benefit of patients with liver diseases.
**Improved Materials for Heat Exchanger Tubes for Power Plants**  
**PI: Bimal Kad**  
Mechanically alloyed oxide dispersion strengthened (ODS) Fe-Cr-Al alloy thin walled tubes and sheets, produced via powder processing and consolidation methodologies, are viable component materials for eventual use at temperatures up to 1200°C in the power generation industry. That is far above the temperature capabilities of conventional alloys. Target end-uses range from furnace components, heat shields in re-usable space vehicles, gas turbine (jet engine) combustor liners, nacelles to high aspect ratio (L/D) heat exchanger tubes in power plants. Recent studies in cross-rolled ODS-alloy sheets indicate that transverse creep is significantly enhanced via controlled transverse grain fibering, and similar improvements are expected for cross-rolled tubes. This project will systematically examine and validate post-extrusion forming methods to create hoop strengthened tubes, which will be evaluated at 'in-service' loads at service temperatures and environments. Kad and his colleagues aim for eventual commercial adoption in the power-generation market.

**Real-time Volumetric Imaging of Neural Activity**  
**PI: Albert Kellner (with Erik Viirre)**  
Evaluating neural activity in the brain today typically requires fluorescent dyes and large, expensive equipment for MRIs, PET scanning and computerized tomography. But advances in optical physics and signal processing enable the development of new instruments to assess neural activity. This project aims to develop a simple instrument that measures neural activity using non-invasive in-vivo brain imaging of human subjects through the intact skull. The imagers under development track the essential barometer of neural activity by measuring the optical scattering of infrared light with neurons.

Non-invasive, volumetric measurements of neural activities are of extreme interest for many clinical medical areas, ranging from clinical diagnostics to stroke management and repetitive stress disorder. In addition, many research areas will benefit from real-time volumetric measurements, including fundamental neuroscience and cognitive science.
**Apparatus for the Inspection of Pipes and Tubes**  
**PI: Francesco Lanza di Scalea**  
The safe operation of oil, power generation, and chemical processing plants requires screening of their pipes to ensure that there are no unacceptable levels of corrosion. Since a significant portion of industrial pipes are insulated, this means that even external corrosion cannot be detected by visual inspection without the removal of the insulation, which can be prohibitively expensive. A quick and reliable method for the detection of corrosion, which does not require the removal of the insulation, is therefore required. Professor Lanza di Scalea is developing an apparatus for the inspection of long lengths (hundreds of feet) of pipes and tubes -- only requiring access to one end and without requiring insulation removal. The system will operate by long-range ultrasonic guided waves that will be reflected by corroded areas providing a means for the detection and classification of the corrosion.

**Development of Improved Radiological Predictions of the Risk of Rupture of Abdominal Aortic Aneurysms**  
**PI: Juan Lasheras**  
Professor Lasheras hopes to improve the current capabilities of biomedical imaging techniques to better monitor the disease progression in Abdominal Aortic Aneurysms (AAA). Quantifying the spatial and temporal distribution of mechanical stresses acting on the vessel walls, the project could lead to a quantitative assessment of the risk of rupture in AAA - and potentially provide improved guidelines for intervention. Lasheras will work with other engineers, computer scientists as well as physicians specializing in radiology and vascular medicine. The proposed method consists of using high resolution computerized tomography (CT) scans and magnetic resonance imaging (MRI) to reconstruct a three-dimensional model of the abdominal aorta, including AAA. A finite-element computer code incorporating non-linear elastic effects and all physiological and mechanical information of the arterial wall will be developed to compute the distribution of stresses along the aneurysm's wall to provide information on the possible location of rupturing and a quantification of the risk of rupture.
In-Silico Modeling for Bioengineering and Medicine
PI: Andrew McCulloch
In systems biology, sufficient structural and cellular data are becoming available to develop predictive computational engineering models of the physiological function of the heart and other organs. The PI already has a copyrighted software package called Continuity, used by academic researchers for in-silico modeling. The proposed project is to convert that package to a form suitable for licensing to a third party as a platform for the development of commercial software tools for in-silico modeling in biomedical applications. The project will generate example data sets that demonstrate the application of the software to medical device design, surgical procedures, diagnostic imaging and drug discovery. Because of the high costs, regulatory requirements and social pressures of in-vivo testing, in-silico modeling is an attractive element of the medical device and drug development pipelines that could decrease costs, reduce development times and improve success rates in the development of FDA-approved therapeutic products. Continuity is a scientific and engineering research tool with proven commercial applications, but it is not yet in a general-purpose format. This project will enhance the prospects of licensing it for commercialization and sale in the medical device, surgical planning, diagnostic imaging and drug discovery industries.

Patient-Specific Optimization of Cardiac Resynchronization Therapy
PI: Andrew McCulloch
Cardiac resynchronization therapy (CRT) is an increasingly popular strategy for improving pump function in heart failure patients with QRS widening. However, about 30% of patients are considered non-responders, especially those with myocardial infarcts. Moreover, there are no well-defined criteria for predicting outcomes or selecting pacing sites and protocols such as V-V delay times. There is also evidence that many patients without QRS widening can benefit from CRT, but no reliable criteria for identifying those patients. The objective of the proposed collaboration between UCSD's Bioengineering, Medicine and Radiology Departments is to establish proof-of-principle for a new computer-based strategy to optimize patient selection and efficacy of CRT in patients with congestive heart failure (CHF). Now that we have validated computational models of cardiac electromechanical interactions that predict the effects of ventricular pacing on regional wall mechanics and global function in animal models, we propose to develop new computational tools for performing diagnosis and patient-specific optimization of CRT. In this project, UCSD Bioengineers will collaborate with clinicians at the VA and UCSD Medical Center to prototype these new tools and validate them in patients with CHF. Once this proof-of-principle has been established and published, we have a number of commercial partners who may be interested in licensing or further developing this technology, especially Medtronic and Guidant. Medical imaging and clinical EP lab vendors are also very likely partners for commercialization of this technology.
Minimal Invasive Surgery (MIS) has gained a major role in the various surgical procedures. Since 1981, when the first laparoscopic cholecystectomy (gallbladder removal) was performed, this field of surgery has been greatly improved mainly due to the advancements in technology. The "state of the art" of MIS today uses 3-6 abdominal skin incision (5-12 mm in length each) which through them optic fibers, cameras and operating instruments are inserted into the abdominal cavity. The operation is performed using long instruments which are inserted through these incisions and are viewed on a TV monitor beside the patient. The available camera today for MIS is composed of a long shaft which contains several lenses and optic fibers (the laparoscope). The optic fibers convey xenon light via an external light source into the abdominal cavity and the lenses transfer the images from within the abdomen to an externally connected portable video camera.

There are several major disadvantages while operating by this method: 1. The operation is performed using two dimensional vision on a TV monitor while operating in a 3 dimensional field. 2. The operating field is restricted to the camera field. 3. The field of the operation is restricted by the choice of the primary incisions; hence the incision creates a hinge on the abdominal wall which restricts the movements of the laparoscope. In order to achieve the necessary angle of view there are 30/45 degree angled laparoscopes but still the most crucial instrument for MIS is failing to provide the required vision mainly due to the hinge created by the abdominal wall.

We intend to construct a new type of camera which will be free from this restricting hinge, offer 3 dimensional vision and auto-focusing, provide varying field-of-view via optical zoom, and will be able to be modified for applications other than surgery and medicine. This miniature camera will be inserted into the abdominal cavity through one of the existing incisions and connected to a 2 mm support needle on the abdominal wall but from within the abdominal cavity. It will have a 360 degrees rotation range on the horizontal plane and 250 degree rotation range on the vertical plane. Furthermore it will be possible to easily change the location of the camera by disconnecting and reattaching the support needle within the abdomen to achieve a better field of vision. None of the above mentioned abilities is offered by the cameras available today without performing multiple abdominal incisions.
Efficacy of Stratified Cartilage Tissue for Treating Articular Defects

PI: Robert Sah

The goal of Professor Sah's project is to engineer cartilaginous tissue in novel and effective ways for joint repair and replacement. Current therapies are limited by lack of donor tissue and a lack of prosthesis durability for active patients, and the current generation of engineered cartilage. Sah has already invented Methods to Engineer Stratified Cartilage Tissue (disclosed in 2001 and with a patent application in progress), which demonstrated the ability to tailor cartilage to have cells at a surface producing SZP (Superficial Zone Protein), a molecule critical for lubrication. In this project, Sah and his team will conduct high-risk in vivo experiments in an attempt to establish key scientific concepts and experimental models, relating the presence of SZP to maintenance of cartilage health, and conversely, the loss of SZP to joint deterioration. If successful in showing the association between loss of SZP and the failure for repair, the value of the earlier invention will increase greatly, because it will be established that having SZP-producing cells at a surface will most resemble normal cartilage. Also, an in vivo model would be established for future studies that will directly test the therapeutic efficacy of SZP-based therapies and pave the way for future clinical trials.

In Vivo Efficacy of Stratified Cartilage Tissue

PI: Robert Sah

In his second project award from the von Liebig Center to date, Professor Sah and his team will test a new way to engineer cartilage tissue for joint repair and replacement, after developing in 2001 a method of creating cartilaginous tissue constructs through fabrication of a tissue with stratification, localizing specialized cells at the tissue surface. These cells express the functional marker molecule thought to be critical for lubrication. In the past year, Sah's group has developed methods for testing the efficacy of these implants, and the von Liebig Center funding will allow them to carry out the tests in vivo in adult mini-pigs, to determine whether such stratified constructs are better than the established microfracture type of repair. Positive results could stimulate further industrial interest, and pave the way for immediate applications in animals (e.g., dogs, horses) as well as human clinical trials.
**Development of Filter System for Humoral Cell Activators in Severe Cardiovascular Diseases**

**PI: Geert Schmid-Schoenbein**

The project aims to develop a system to filter blood in shock patients, to eliminate humoral inflammatory mediators (toxic protein fragments). In the absence of a pharmacological response to shock, the only current alternative for blood filtration in shock patients is plasmapheresis, which removes all components of the blood, regardless of cell toxicity. The filter system will be designed with specific characteristics that are optimized for binding of protein fragments. It will be tested in two steps; initially under in-vitro conditions with toxic protein fragments generated in the laboratory from homogenized tissue and in a secondary sequence of experiments it will be tested in rodents subjected to an experimental form of shock. The design objective is to achieve a greater than 90% reduction in the toxic protein fragment level in a living animal under conditions of hemorrhagic shock.

**Development of a Filter System for Removal of Humoral Cell Activators in Severe Cardiovascular Diseases**

**PI: Geert W. Schmid-Schoenbein**

Professor Schmid-Schoenbein and his team have completed the examination of several filter devices to remove inflammatory mediators from plasma. The rate of clearance was found to be optimal during use of a glass-fiber filter. Subsequent tests in a rat hemorrhagic shock model showed no improvement in survival, but analysis indicated that (a) there may be complement and prothrombotic enzyme activation in the plasma on the glass filter, and/or that (b) the rate of filtration by collection of individual blood samples from the femoral vein needs to be accelerated and replaced by a continuous plasma filtration process. With the von Liebig Center funding, the researchers will conduct studies they hope will help establish the feasibility of a filtration technology to remove inflammatory mediators from plasma. Schmid-Schonbein's team will filter the plasma in the presence of a protease inhibitor to block complement and thrombotic cascade activation and minimize complement activation during the filtration process. The team will test whether the glass filter still eliminates the inflammatory mediators in the presence of protease inhibitors under in-vitro conditions, and whether filtration with the modified glass filter with protease inhibitor serves to reduce the level of inflammatory mediators in a rodent model of shock, improve blood pressure and survival.
A Fiber Optic-Based Sensor System for Real-Time Shape Reconstruction of Deformable Objects  
**PI: Michael Todd**

During major seismic events, horizontal ground motion can lead to soil liquefaction, and subsequent lateral spreading of the liquefied ground material is the largest cause of structural damage, including cracking, fracture, and even catastrophic failure. In partnership with the U.S. Naval Research Laboratory (NRL), Professor Todd demonstrated a prototype for a novel sensor concept based on fiber optics and a thin flexible beam transducer mounted on a laminar box experiment at UCSD. The simple beam geometry allows for easy conversion of local displacement at each point into an integrated bending displacement profile for the beam. This approach has the advantages of minimal intrusivity, high sensitivity, insensitivity to electromagnetic interference, and easy sensor multiplexing for greater spatial profile resolution. Todd now wants to go several steps further. The Center funds will allow him to investigate design improvements for field ruggedness; to initiate an integrated hardware/software design and a user-friendly interface; and to demonstrate a redesigned prototype in a larger-scale series of tests to establish performance parameters. Ultimately, Todd hopes to present a design to the ground-motion sensor industry.

Handheld Self-Contained Alveolar Gas Analyzer for Investigating Lung Disease  
**PI: John West**

Measuring oxygen and carbon dioxide in the depths of the lung—so-called alveolar gas—typically requires cumbersome equipment that is not portable. Professor West—who is a Distinguished Professor of Medicine and Physiology in the UCSD School of Medicine—has already constructed a crude proof-of-concept device with only an oxygen analyzer, and it successfully tracked the changes in alveolar oxygen when a subject traveled to the UC White Mountain Research Station, altitude 3800 meters. The purpose of this new project is to build a full prototype with both O2 and CO2 analyzers and the appropriate electronic circuitry. West believes the handheld, self-contained alveolar gas meter has potential commercial value because it would permit non-invasive testing, notably in the hospital emergency room to assist in the diagnosis of various respiratory diseases, and also in a paramedical setting at a road accident where injury of the chest wall is suspected.