Rural Educators Engaged in Bioanalytical Engineering Research and Teaching

Center for BioAnalysis
University of Oklahoma, Norman Campus
June 10 - July 26, 2019
(with one week break July 1 – 7)

Funded by the National Science Foundation

A year-long program for science & math high school teachers to participate in, and connect their classrooms with, cutting-edge research at the Center for BioAnalysis at the University of Oklahoma, Norman campus.

- **Summer research activities June 10 – July 26, 2019**
  Norman campus, University of Oklahoma
  Campus lodging and food covered by program

- **Classroom activities Fall 2018 – Spring 2019**
  $1,000/teacher for classroom Bioanalytical projects

- $5,000 stipend for completion of year-long program

To apply, go to RET Bioanalytical Engineering Application Form
https://oueducation.co1.qualtrics.com/jfe/form/SV_098u9f9B7P7WnDn

Goals of "Rural Educators Engaged in Bioanalytical Engineering Research and Teaching" are:

- to involve rural high school science and mathematics teachers and regional community college instructors in bioanalytical engineering research,
- to help improve STEM teaching in rural classrooms,
- and participation in a sustainable professional network that will increase the number of rural students who select and successfully graduate from higher education STEM fields.

BioAnalytical Engineering involves the design and manipulation of biomolecules (e.g. proteins, enzymes, glycoproteins), viruses, bacteria, and cells for specific applications in the fields of medicine, the energy industry, agriculture and the environment. Furthermore, this engineering field includes the design and fabrication of analytical tools for the detection and characterization of these biomaterials, often times using very small sample volumes and having rapid response times, and used typically to detect diseases, microbial contaminants or metabolites, or for identifying and designing target-specific drugs.

Bioanalytical engineering inhabits the bioregion between genomics and tissue/organ scale bioengineering. Genomics deciphers the genetic "blueprints" held by an organism or a cell, which describe what the organism or cell is capable of producing or doing, however genomics doesn't tell how a cell is responding at a certain time to a specific stimulus. Only by analysis of the proteome or metabolome is it possible to determine a cell's response to an environmental stimulus. However, the proteome and metabolome are complex, diverse, with many important chemical species and components present at low concentrations or as transient intermediates in multistep biochemical reactions. Advances in technology (such as mass spectrometry, microfluidic and microelectro devices, nuclear magnetic resonance spectroscopy, microscale sampling of single cells, two-dimensional electrophoresis, and electrophoresis coupled with mass spectrometry) provide powerful tools. These tools can detect and characterize proteins and metabolites

indicative of specific diseases, to identify circulating tumor cells in the blood system, and to monitor metabolic changes in cellular organelles signifying the onset of undesired health conditions. The ability to detect and monitor the proteome and metabolome on a cellular scale allows for the design of target-specific drugs or the ability to modify or exploit cellular biochemistry for desired outcomes.

While much of the current research in bioanalytical engineering focuses on medical problems, bioanalytical engineering is a powerful tool for all areas involving biology, such as the improved production of biofuels, the impact of biofilms on the biocorrosion of steel infrastructure in the petroleum industry, and the environmental bioremediation of groundwater. As such, bioanalytical engineering presents rural educators with a dynamic and vibrant field deeply rooted in fundamental concepts of molecular biology, biochemistry, cellular biology, chemistry, and physics. Coupled with engineering design methodology and application, it provides fruitful opportunities for educators to enrich their teaching of these fundamental concepts, showing their students how knowledge in the fields of biology, chemistry, and physics can, (using bioanalytical engineering) directly impact critical issues related to medicine, human health, energy resources, and the environment.

Summer Program Details

Spring Activities: Four one-day workshops (one workshop/month) focusing on pedagogy, such as Guided-Inquiry Learning, Authentic Teaching, and using the Engineering Method, will occur at rural high schools located in Oklahoma. Teachers will be introduced to the RET program as well as learn about the different research projects being studied by OU faculty, and select a research project to work on over the summer for six-weeks. Teachers will be provided with readings, and interact with the faculty member with whom they'll work with over the summer.

Summer Research Experience Activities: **Summer research activities will begin on June 10 through July 26, 2019.** Educators living at least 50 miles from campus will be paired in the OU Traditions Apartments, which provide a private bath/bedroom for each resident, and a common living and kitchen area. Rooming together gives RET educators additional opportunities for collaboration and building professional comradery. Educators will be on the Norman OU campus Monday through Friday, in labs at least one-half of every day. On the first day, there will be a Laboratory Safety Training session and a Fire Safety Training session. Specialized laboratory safety training will occur within the individual research groups.

Weekly seminars will be scheduled (1-2 hours) for outside speakers (e.g., industrial researchers, medical and public health professionals, environmental professionals) to present topics on how bioanalytical engineering is used in the real-world, as well as presentations about engineering careers and engineering ethics. Likewise, one-half day per week will be spent with Drs. Nanny and Hardré working on curricula development and design, and evaluation/assessment activities. Required educator-faculty mentor interactions will occur on a weekly basis, specifically focusing on developing the classroom curricula and how to transfer RET research experiences authentically into their classrooms. In the third week, a one-day workshop will be given on writing successful proposals, with a focus on the RET educators preparing proposals (i.e. RET Curriculum Proposals) for their classroom curricula.

During the final week, educators will present their research activities and classroom curricula to all RET educators and faculty mentors. Likewise, educators will prepare a research poster that they'll display in their classroom as well as post a second copy at their OU laboratory. By the end of August, educators will submit their RET Curricula Proposals, and classroom materials and supplies will be ordered.

Academic year activities: During the two academic semesters, faculty mentors will make at least one classroom visit/semester, and the educators and students will engage in one field trip to OU or a site engaged in bioanalytical engineering (e.g., industrial facility and research labs). At least one video conference/semester will be held between the faculty mentor and students. During the fall semester, the annual research symposium for the Center for Bioanalysis will occur. Likewise, during the fall, Nanny will teach ENGR 4113/5113 – "Science, Engineering and Mathematics Educational Outreach for STEM Majors"

where STEM graduate students will partner with RET educators to develop additional authentic, guided-inquiry curricula. In the spring semester, the rural STEM educators' workshop will be hosted at the annual OU K20 Innovative Learning Institute.

Research Experience Activities: Prior to arriving on the OU Norman campus, faculty mentors will contact the educators who'll be in their labs, talking with them about the research project, discuss the educators' interests regarding classroom innovations, and answer any questions. The faculty mentors will send materials and website links to assist educators in learning background information related to the project.

The summer research experience activities will begin with training sessions on laboratory safety and fire safety taught by OU Health & Safety and the OU Fire Marshal, respectively. Educators will do an on-line laboratory safety training session/quiz that takes about 1-2 hours for completion, and familiarizes them with the OU Laboratory Safety Manual. Upon successful completion, they will receive an OU Health and Safety certificate, allowing them to enter the OU research laboratories. Faculty mentors will provide additional safety training specific to their individual laboratories, equipment and procedures. A lab coat, safety glasses, and gloves will be provided by the RET program for each educator.

RET activities will be scheduled such that at least 4-5 hours/day are spent conducting research on days with additional activities, otherwise educators will spend 8 hours/day in lab. Educators will fully participate in group meetings, interact daily with the faculty mentor, and engage with graduate students as they learn laboratory techniques. At least two educators will work together on each project in order to provide support, collaborate and build comradery.

Examples of Previous RET Research Projects: Proposed RET research projects fall under several bioanalytical engineering areas: the design of personal anti-cancer drugs (Burgett), environmental engineering (Krumholz), biocorrosion engineering (Nanny), biofuel processing (Bartley and Wu), fabrication of bioanalytical devices (Liu and Mao) and advancement of computational methods (Hansmann).

Engineering changes in high-throughput oxysterol-binding proteins ligand-binding assays, Dr. Anthony Burgett. In the past several years, the pursuit of new anti-cancer drugs have shifted away from the identification and development of compounds that broadly inhibit cancer cellular proliferation to the development of treatments that are specifically targeted to an individual patient's cancer. Specific oxysterol-binding proteins (OSBP) are reported to be overexpressed in certain cancers (14-16), and growing evidence suggests the overexpression of OSBPs could be driver of cancer cell proliferation (17, 18). The Burgett lab has a multi-disciplinary research program to validate members of the OSBP family as cancer-specific drug targets and to synthesize new drug-like compounds capable of selectively inhibiting the cancer-specific OSBP family members. The bioanalysis of OSBP ligand binding is performed through using a 96-well assay developed by the Burgett group (19). Depending upon interest, RET educators can either: 1) design, clone and express, using site-directed mutagenesis, an OSBP mutant, which will then be assessed for OSW-1 ligand binding; or 2) design and synthesize a simple modification to a provided OSW-1 analog compound, which upon purification and characterization, will be evaluated for OSBP binding. In either project, the educators will be able to rapidly come up to speed on a well-established research program with clearly defined protocols. This will allow for the teacher to spend their time maximally designing and executing the project, with a realistic 7-week time horizon for completion.

Bioremediation of arsenic and chromium contaminated groundwater. Dr. Lee Krumholz. Chromium and arsenic are commonly found in central Oklahoman aquifers with arsenic observed at levels of 30-40 µg/L and chromium at 10-80 µg/L in Norman drinking water. EPA drinking water limits for arsenic are 10 µg/L and for chromium are 100 µg/L. When arsenic limits were lowered to 10 µg/L several years ago, the city of Norman was forced to close several of their water wells, only using those producing water with lower As levels. As and Cr are most likely present in their oxidized forms, arsenate (As(V)) and chromate (Cr(VI)) in groundwater. Many bacteria have been identified that can reduce arsenate to arsenite (20) and chromate to Cr(III) (21). However, it is unclear whether these bacteria can reduce As and Cr below EPA limits. The reduced form of Cr is Cr(III) which reacts rapidly under pH neutral conditions forming a mineral. The reduced form of As, As(III) is highly reactive and will either be taken up into biomass (22) or subsequently removed (23). Reduction can therefore be used to treat Cr and As containing groundwater. This project

will determine if bacteria are capable of transforming arsenate and chromate, present at naturally occurring concentrations by the addition of relatively insoluble minerals with subsequent isolation of the bacteria and determination of their taxonomic affiliation. Arsenate and arsenite can be separated using solid phase extraction (24) and dissolved As(III) and Cr(VI) will be quantified using ICP-MS. Educators will identify microbial strains using both molecular and more traditional taxonomic tools. In subsequent years, pure cultures from this task or enrichment cultures will be used to build lab scale bioreactors for treatment of groundwater containing As and Cr.

Development of molecular marker assays for indicating microbially induced corrosion of carbon steel. Dr. Mark Nanny. Microbially induced corrosion (MIC) in the petroleum industry is responsible for extensive annual repair and remediation costs. Efforts to control biofilm growth through pipeline pigging and biocide application results in expensive downtime and the use of toxic chemicals which require proper handling and disposal. Steel infrastructure (pipelines, production water tanks, injection wells) are monitored continually for microbial activity using crude assays for sulfate-reducing bacteria and acid-producing bacteria. These methods may, or may not, detect MIC if it's in its early stages (when biocide application would be the most effective) or if other microbial corrosion mechanisms are occurring, such as by iron-cycling microbes. The development of corrosive biofilms on carbon steel surfaces initiates upon adsorption of proteins and polysaccharides, followed by the attachment of microbial cells and development of a microbial biofilm. It is hypothesized that corrosive biofilms produced by each type of microbial community responsible for MIC has a unique biomolecular "fingerprint". This project will test this hypothesis, as well as determine if the biomolecular fingerprint is indicative of the biofilm growth stage and the extent of MIC. Steel coupons incubated in microcosms containing sulfate-reducing bacteria, iron-cycling bacteria, or acid-producing bacteria, will be periodically analyzed for adsorbed biomolecules and biofilm development as a function of different environmental conditions using matrix-assisted laser desorption/ionization mass spectrometry, laser scanning confocal microscopy, and Raman imaging microscopy. These results will be correlated with the extent of carbon steel corrosion through electron microscopy, energy dispersive X-ray spectroscopy, and atomic absorption spectroscopy.

Metabolic analysis of cells engineered with alterations in cell wall synthesis and regulation. Dr. Laura Bartley. Biomass is an abundant source of chemical energy for conversion to biofuels and higher value chemical products. However, products formed from biomass and the efficiency of conversion is often controlled by the starting biomass composition. Biomass composition results from a complex series of transcriptional regulators and enzymes that control cellular metabolism and the formation of cell walls, which represent the major component of dry biomass. This project seeks to define the transcriptional and biosynthetic network regulating phenylpropanoid metabolism in rice with the eventual goal of engineering grass plants with improved properties for biofuel and high-value bioproduct generation. This project will test the hypothesis that transient alteration the levels of enzymes or transcription factors in plant cells will alter the accumulation of lignin biosynthesis pathway precursors, creating a metabolic signature that indicates which part of the cell wall biosynthesis pathway is controlled by the gene of interest. The project addresses a critical bottleneck in biomass engineering, which is the determination of gene function through creation or isolation of genetic mutants. If this project is successful, it has the potential to greatly accelerate the use of synthetic biology to alter cellular metabolism by allowing rapid hypothesis testing and fine-tuning prior to investing in testing in whole plants. This experimental cycle will fit well in the proposed RET, as teachers will be able to make observations of changes in cellular metabolism and then test their hypotheses for other genes involved in cell wall regulation.

Fungal secretome glycoprotein profiling to identify "super isoform" of individual biomass degradation enzymes. Dr. Si Wu. The fungal secretome, known as a pool of biomass degradation enzymes, can be utilized to discover industrial enzyme candidates useful for the biofuel industry. In the fungal secretome, many of the biomass degradation enzymes have multiple isoforms generated through various post-translational modification (PTM) processes, and different isoforms have different levels of enzymatic activity (25-27). The ultimate goal for our research is to discover the "super isoform" of individual biomass degradation enzymes, which can be then utilized at the industrial scale towards the maximized enzymatic efficiency. To achieve this goal, we propose to study the most commonly observed PTM in fungal system---glycosylation through developing a cutting-edge integrated top-down and bottom-up mass spectrometry platform for fungal secretome glycoprotein profiling. The overall goal is to develop a

glycoprotein analysis platform that integrates top-down and bottom-up approach for secretome glycoprotein analysis, assisted by accurate glycan structure identification using a nuclear magnetic resonance spectroscopy-based approach. The platform will be applied to functionally characterize fungi secretome in *A. niger*, which is essential for understanding biogeochemical regulation and mechanisms involved in fungal lignocelluloses degradation (and other biotechnological applications). Our hypothesis is that fungal grown under different carbon sources will generate different glycosylated isoforms. The experimental results will allow the RET educators to correlate the fungal secreted glycosylated isoforms with their enzymatic activities and other properties such as pH or temperature. Such correlation will provide a fundamental understanding of these enzyme's activities, and will guide future development towards engineering the "super isoform" of individual biomass degradation enzymes for utilization at the industrial level.

Construction of high-pressure, electroosmotic pumps for micro-HPLC. Dr. Shaorong Liu. High-performance liquid chromatography (HPLC) is one of the most widely utilized technique for chemical separation/analysis. However, current HPLC systems are too bulky for point-of-care applications. The Liu lab is developing a miniaturized HPLC (μHPLC) that when combined with emerging small-footprint mass spectrometers, it will vastly accelerate proteomic research while decreasing instrument costs. A major challenge for implementing μHPLC is the lack of a robust and miniature high-pressure pump. The Liu group has demonstrated the feasibility of an electroosmotic pump (EOP) (28-30) consisting of a "+" and "-" unit. The "+" unit is made of a positively-charged pumping element, while the "-" unit is made of a negatively-charged pumping element. Owing to the nature of electroosmotic flow (going from a low voltage to a high voltage in the "+" unit, and from a high voltage to a low voltage in the "-" unit), when the high voltage (HV) power supply is turned on, the electroosmotic flow goes smoothly from the inlet to the outlet. Because the inlet and outlet of the EOP are electrically grounded, multiple EOPs can be connected in series. A significant feature of this EOP is that its maximum pumping pressure is directly proportional to the number of EOPs serial-connected. Because this working principle is similar to that of a voltage power supply, this EOP is called a Pressure Power Supply (PPS). To the best of our knowledge, no other pumps (e.g. other electroosmotic pumps, pneumatic pressure sources, diaphragm pumps, peristaltic pumps, etc.) are capable of being used in this fashion. We have developed EOPs capable of pumping against >1000 bar routinely (30) and incorporated such EOPs into μHPLC systems for protein and peptide analyses (29-33). Our hypotheses is that we can construct a high-pressure EOP powered by batteries (dozens of volts). The RET educators will fabricate the PPS components, assemble the EOP, and test the EOP for HPLC separations. Once the device is made and tested, the teachers may bring this device to high school classroom for demonstrations. Ultimately, a complete portable HPLC system can be built on the basis of this EOP, with the goal of performing measurements of environmental pollutants in Oklahoma waters.

Magnetic microparticles enabling the ultrasensitive detection of antibodies. Dr. Chuanbin Mao. An antibody is a typical biomarker for the diagnosis of many diseases, such as cancer. The conventional approach for quantifying antibodies is a technique called enzyme-linked immunosorbent assay (ELISA). However, the detection limit of the current ELISA method (or the minimum level of target molecules that ELISA can detect) is still not low enough to satisfy clinical needs. To address this problem, RET educators will use surface-modified magnetic microparticles (MMPs) to capture antibody IgG (with a concentration lower than the detecting limit of ELISA) in solution (34). Streptavidin coated MMPs will be used and a reported IgG antibody-binding hexamer peptide (HWRGWV) labeled with biotin will be chemically synthesized from a commercial source (United Peptides). Since biotin from the IgG-recognizing peptide and streptavidin from MMPs can specifically bind with each other, mixing the streptavidin coated MMPs and biotin-labeled IgG-recognizing peptide will result in a peptide-biotin-streptavidin-MMP composite. Then the resultant peptide-biotin-streptavidin-MMP composite will be added into the antibody IgG solution to capture the IgG molecules. The next step will be to magnetically enrich the captured IgG and elute them from the MMPs using an elution buffer, and then re-dissolve them to make a new solution with an antibody concentration higher than the ELISA detection limit.

High-performance computational simulations of protein folding. Dr. Ulrich Hansmann. High-performance computing is an important tool for studying molecular process in cells that are not directly observable through experiments. Examples are protein folding, interactions between proteins and other molecules with the resultant formation of transient complexes, and the aggregation and self-assembly of proteins into amloid structures and fibrils. However, computer simulations modeling fibril formation and

their precursors are difficult since computational simulations typically cover processes ranging from nanoseconds to microseconds, while folding and aggregation processes occur over milliseconds to hours/days. The computational difficulty with the difference in time scales occurs because the numerical effort in fixed-temperature molecular dynamics increases exponentially with the size of the system, particularly where even the simplest systems (such as a single solvated protein) may consist of several thousand atoms. This size dependence limits the scope of computer simulations in protein folding and aggregation studies and restricts, for instance, the computational design of amyloid inhibitors that could be used as drugs targeting Alzheimer's and other amyloid diseases. Our lab has developed a number of enhanced sampling techniques such as replica-exchange sampling, energy-landscape-paving and the generalized-ensemble approach that are now widely used and allow to alleviate the above described sampling difficulties (35-42). RET educators will use standard simulation software packages as such Gromacs and our in-house modifications, and run this software on local clusters and XSEDE resources. They will use these techniques to analyze simulations modeling processes such as the "Osaka mutant" which changes fibril growth and fibril patterns of the wild-type Aβ-peptides (implied into Alzheimer's disease) aggregates. We expect that Osaka mutant fibrils will act as seeds that induce wild type peptides to aggregate into the more toxic mutant fibril forms. Within seven-weeks, RETE educators will develop hypotheses on how this process depends on the concentration of the mutant fibril seeds.

Curriculum Development Activities: Educators and Drs. Nanny and Hardré will meet for a half-day each week to design and develop classroom curricular activities. The SUCCESS Framework will be introduced, educators will learn how to utilize it, and integrate it into their curricula and teaching plans. Additionally, authentic, guided-inquiry pedagogy will direct the curricular format. "Guided-inquiry" means students are involved in higher-level critical thinking skills through activities following the five-step process of: 1) engaging students; 2) exploration of the concept; 3) concept development; 4) application of the concepts; and 5) authentic assessment of student learning. This guided-inquiry format is taught in EDSC 4513 Teaching Science in Secondary Schools, at the OU Jeannine Rainbolt College of Education, and in ENGR 4113/5113 Science, Engineering and Mathematics Educational Outreach for STEM Majors. "Authentic" means that 1) "the students are engaged in construction of knowledge" using 2) "disciplined inquiry" (i.e. guided inquiry) in learning which has 3) "value beyond school" (i.e. the learning connects students with their community and environment), and respects and acknowledges 4) "the implicit view of the student". Authentic, guided-inquiry learning has students collecting and analyzing data for an outcome meaningful to them. As such, educators will be challenged to incorporate topics of importance to rural students. All developed curricula will address state education standards.

Educators will be responsible for meeting weekly with their research mentor to collaborate on building classroom curricula. Both the educators and faculty mentors will be challenged: 1) to find specific applications of bioanalytical engineering in the rural communities so that students are engaged in authentic learning experiences, and 2) to utilize common electronic technology (e.g., iPhones, laptops/tablets, digital discussions & dropboxes) to allow data collection and sharing in classrooms. It is intended for this collaborative experience to spark creative ideas in the faculty mentor on how to further design or create bioanalytical systems useful to the general public or to classrooms for routine analyses and monitoring.

Seminars and Workshop Activities: A weekly seminar will be held with outside speakers who can illustrate the current problems and needs addressable by bioanalytical engineering. Burgett is working with Oklahoma Shared Clinical & Translational Resources to identify researchers who are experts in Oklahoma's rural health disparities. Though Nanny's contacts, microbiologists from ConocoPhillips (Bartlesville, OK) will discuss the impact of biocorrosion on the petroleum and natural gas industry. Krumholz is in contact with the OK Department of Environmental Quality and the Oklahoma Geological Survey for a presentation on As and Cr contaminated aquifers in Oklahoma. Burgett has arranged for an analytical chemist from Astellas Pharma Technologies. Inc. (see attached letter) to present a seminar and provide tours of the Norman-based formulation and analysis laboratories. A Williams Student Services Center advisor (OU College of Engineering) will discuss the diversity and future of engineering careers. A seminar on Engineering Ethics, particularly relating to medical and environmental issues pertinent to bioanalytical engineering will be given (speaker to be identified).

In the third week of the summer research experience, a one-day workshop will be given on writing successful proposals, with the objective of the RET educators preparing proposals (i.e. RET Curriculum Proposals) about their classroom curricula. Educators will produce a proposal of high enough quality that it would be competitive in an external competition, before receiving up to $1,000 in RET funds for their academic year activities. Thus, educators gain confidence in preparing "competitive" proposals. Faculty mentors will be requested to provide input during the proposal preparation. This activity was highly rated by educators in our previous two RET programs (12, 13). Nanny will organize the workshop and provide feedback to educators on their proposal drafts at weekly curriculum development meetings.

During the two academic semesters, faculty mentors will be required to make at least one classroom visit per semester, and the educators and students will engage in one field trip to OU or to a site engaged in bioanalytical engineering (e.g., industrial facility and research labs). At least one video conference per semester will be held between the faculty mentor and the students. Classroom visits by the faculty mentor may include co-teaching the curricula, demonstrations of analytical equipment or data collection methods to be used by students, or elaboration on content. Field visits to the OU laboratories can include students bringing samples for analysis, or seeing the operation of advanced instrumentation. Video conferencing will be used for students giving presentations or when faculty mentors desire to provide guidance on the curricula. During the academic year, Drs. Nanny and Hardré will maintain continual contact with RET educators and faculty mentors to ensure all interactions proceed smoothly and productively.

Additional interaction of STEM graduate students with RET educators will be developed through teaching the course ENGR 4113/5113 "Science, Engineering and Mathematics Educational Outreach for STEM Majors" in the fall semester by Dr. Nanny. RET educators will pair up with and mentor STEM graduate students in developing new activities that build upon the RET lessons created during the Summer Research Experience. Although much of the interactions between these STEM graduate students, the RET educators and the rural students will be by video conferencing due to distance, the graduate students will visit classrooms when they finally co-teach their lesson with the RET educator. In this way, the number of graduate students interacting with the RET educators will be expanded beyond those just those graduate students involved with the RET summer research.