*EDITORIAL, FIRST SCIENCE AND POLICY ISSUE, JSCAS*

The Journal of the South Carolina Academy of Sciences (JSCAS), in order to better serve SCAS members, is undergoing a significant change in both the publication frequency and the focus of articles published in the Journal. SCAS has begun expanding its efforts relating to science and technology research developments occurring in South Carolina. We are particularly interested in SC based research that has national and international implications. This complements the traditional role of SCAS in science education at all levels - middle school through to Ph.D. The Journal will maintain an on-line distribution format but is moving to four issues per year. The goal is to target the Journal at matters directly impacting SCAS members and science in South Carolina. We anticipate publishing a Science and Policy issue, a Science Education issue, a Research in South Carolina issue, and a Governors (SCAS) Award Special Issue each year. The Journal will now include peer-reviewed articles as well as provocative invited editorials and reports relating to the state of science in South Carolina.

One of the first steps in these developments in this first science and policy issue of the JSCAS. This includes 6 articles representing state-of-the art developments in SC and the efforts of state private and public organizations to help support these efforts and encourage them to flourish. Our goal is to provide a forum not only for well-established research entities but others that are more developmental but showing promise for the future. As well as agencies concerned with generating support, growth and maturation for these efforts. It is anticipated that JSCAS will act as a facilitator and serve as a catalyst in stimulating these interactions.

AUTHORS AND TOPICS:

John R Rose. A call for a Statewide Bioinformatics Institute

Alvin Fox, Karen Fox USC and Marvin Vestal, Virgin Instruments LLC: Proteomics – universal biomarkers for inherited and infectious diseases

Bill Mahoney, SCRA: With the help of South Carolina research universities, SCRA demonstrates Leadership in applied research and commercialization within SC

Lucia A. Pirisi-Creek, and Scott Little.SC INBRE: A catalyst for biomedical research development in South Carolina.

Prakash Nagarkatti and Don Dipette, USC: Inflammation: the key to health and disease

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The Editor-in-Chief, David Ferris [USC Upstate], Section Editors for the SCAS Journal: Alvin Fox, [USC] - Science and Policy, Val Dunham [Coastal Carolina] - Research in South Carolina. Hans-Conrad zur Loye [USC] - Governors (SCAS) Award Special Issue

# A CALL FOR A STATEWIDE BIOINFORMATICS INSTITUTE

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At present bioinformatics research and education capacity in South Carolina is distributed amongst a large number of colleges and universities as well as government laboratories, such as the Hollings Marine Laboratory and the Department of Energy Savannah River National Laboratory. We argue that to attain national-level recognition, we need to provide support for a statewide virtual community. Significant synergy is possible through collaboration in on-going research and joint research proposals for external funding. Furthermore, education resources could be used more effectively for training students in this important area. A statewide bioinformatics institute that connects researchers and educators in the various colleges, universities, and government laboratories would advance the capacity and competiveness of bioinformatics in South Carolina.

During the last 10-15 years we have witnessed a tremendous increase world-wide in the number of organizations and educational institutes engaged in bioinformatics research and development. Bioinformatics is important because it helps us to understand the molecular basis for health and disease. In general, bioinformatics addresses the representation and interpretation of information in biological systems. The main areas targeted by current bioinformatics research include genomics (genome sequencing, gene prediction, gene splicing, effects of single nucleotide polymorphisms, and analysis of gene expression), proteomics (protein structure, characterization of protein function, and modeling of protein-protein interactions) as well as a number of other “omics”.

Advances in molecular biology, genomic and proteomic technologies, and powerful computational capabilities have led to the development of new methods and tools for analyzing and viewing genomic and proteomic data. For example, DNA microarray technology has made it possible to analyze the expression of tens of thousands of genes simultaneously. More recently, protein microarrays are making it possible to analyze protein-protein interactions. These tools can be used to understand the interactions between genes and proteins in order to elucidate the regulatory networks that form the basis for health and disease. These are high throughput technologies, generating high volumes of data. The advent of these technologies has required bioinformaticians to develop new approaches for analyzing and efficiently storing large amounts of data.

Bioinformatics is an area that is heavily dependent on cyberinfrastructure. In addition to hardware and software needs, there is a critical need to train future educators and researchers in the use and development of bioinformatics-specific cyberinfrastructure tools. It is also an area that fundamentally requires a multidisciplinary approach. The required expertise crosses the boundaries of traditional research domains. The types of problems that bioinformaticians address frequently require expertise in biology, statistics, computer science and mathematics. The cyberinfrastructure that will be required to support a statewide network will have to accommodate disciplines with varying degrees of cyber-sophistication.

The fact that bioinformatics research and education capacity in South Carolina is distributed amongst a large number of colleges and universities as well as government laboratories, reflects in part the bottom-up development of this field as well as the historical lack of top-down coordination. These islands of bioinformatics researchers and educators have come to understand that significant synergy is possible through collaboration in on-going research and joint research proposals for external funding. As a group in South Carolina we understand that collectively we are much more competitive with larger institutions in neighboring states than we are if we operate as individual research groups. This vision can be realized only through the creation of a sustainable multi-institutional statewide bioinformatics network supported by a solid cyberinfrastructure of hardware, software, and people. It will take ten or more years and significant funds, and a concerted effort on the part of the South Carolina bioinformatics community and their home institutions to achieve this goal. Nonetheless, features of a nascent statewide network are emerging. Our goal is to nurture and encourage activities that will lead to a statewide bioinformatics institute comprised of networks of researchers and educators in South Carolina supported by a solid cyberinfrastructure.

There are two immediate goals of this call for a statewide institute. The first goal is to begin the process of creating a sustainable multi-institutional statewide bioinformatics network starting with small groups of South Carolina institutions and leveraging existing cyberinfrastructure. The second goal is to integrate cyberinfrastructure research, training, and education in multidisciplinary collaborations involving teams from South Carolina institutions.

Progress along these lines is already evident. There is currently a loose network of bioinformatics researchers and educators in South Carolina. Early statewide coordination was fostered by the Bioinformatics Core of the South Carolina INBRE program, which involves USC, MUSC, and Clemson as mentor institutions and Claflin, College of Charleston, Furman University, and Winthrop University as mentored institutions. Development of bioinformatics capacity was a principal goal of the INBRE Bioinformatics Core and to this end the Core held training workshops and statewide research meetings. A statewide meeting, held in Columbia on August 18, 2006 (45 attendees from 9 institutions), provided an opportunity for bioinformatics researchers from universities and government labs to meet and plan the creation of a statewide bioinformatics institute and the resources of which would improve the competitiveness of researchers for larger external grants. A second research meeting was held at Clemson University on January 18-19, 2007 (90 attendees, 12 institutions). Discussions at this meeting also focused on the need for greater collaboration and sharing of resources as well as the infrastructure that would be required. Another example of statewide coordination is the South Carolina Marine Genomics Consortium. Member institutions include the College of Charleston, Clemson, the Hollings Marine Lab, and MUSC. The focus of this statewide group is the prediction and evaluation of climate change and environmental stressors on marine ecosystems through the use of cyber-enabled genomic and bioinformatics approaches.

Most research projects have learning and training aspects. However, these components are often subservient to research. In the case of the proposed institute, a consequence of the fact that it will be comprised primarily of educational institutions is that the education and learning components will be as important as the research. The research effort should be directed so that there is a synergy with training and education. A statewide bioinformatics institute should have as one of its primary goals the integration of research, training, and education at the undergraduate and graduate levels. In addition to being the lifeblood of any research enterprise in academia, these students are also the future scientific workforce.

One can imagine training and education activities in such an institute organized according to whether the focus is internal, i.e., training of institute team members, or external, i.e., training/education of non-institute students, faculty, and staff. Within the envisioned institute, collaboration should be managed so that there is a fine-scale integration of research, training, and education with an inward focus. A statewide bioinformatics institute would involve researchers from many traditional disciplines including biology, statistics, computer science, math, and biostatistics. The greatest research and training success could be attained by organizing research activities to encourage tightly coupled interactions between groups from different traditional disciplines. The desire would be for the teams to cross-pollinate and essentially bootstrap their collective training and education in the research areas pertinent to their research projects.

A statewide institute could also serve as a training and educational clearing house. Research performed by members of the institute should be explicitly leveraged to support training and education with an outward focus. This could be accomplished through the development of training workshops and, in some cases, formal undergraduate or graduate courses based on the research. Such courses could be team taught and made available to students in many institutions by real-time simulcast via videoconferencing using the distance learning facilities at the member institutions. The target audience of the workshops would be undergraduate and graduate students as well as faculty and staff in bioinformatics-related disciplines (biology, computer science, statistics, etc.) The target audience of the formal courses would be undergraduate and graduate students in bioinformatics-related disciplines.

The bioinformatics community in South Carolina now exists as a connected group for which a plan to create a statewide virtual community is relatively straightforward. The INBRE program facilitated the development of this statewide coordination, but it cannot be said to have caused it. The demand was strong and remains strong for exactly the kind of statewide cyber-based community that we propose. There is a critical need for a new push for the development of a bioinformatics cyber community to advance the capacity and competitiveness of bioinformatics in South Carolina.

# PROTEOMICS: UNIVERSAL BIOMARKERS FOR INHERITED AND INFECTIOUS DISEASES

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## INTRODUCTION

We shall discuss here the potential of proteomics (which defines the amino acid sequence of all proteins expressed by a specific cell type under specific growth conditions) and biomarker discovery, for diagnosis of inherited diseases (or cancer) and non-culture based biodetection of infectious diseases (in clinical samples) or environmental monitoring. Many of the instrumental developments in proteomics have come from the field of analytical chemistry. Thus the work is highly relevant to all with an interest in biomedical science, biology or chemistry. However since our research is particularly focused on bacteriology, emphasis will be placed here in the microbiology arena. Also recognizing that the audience of JSCAS is multi-disciplinary (and that the journal is read by administrators, researchers and teachers (including professors and their undergraduate or graduate students) I shall make no apologies that wherever possible I shall provide brief explanations of the principles behind technical terms.

The US Market for molecular infectious disease diagnostic technology is predicted to be approaching $4 billion in 2010 (http://www.clpmag.com/issues/articles/2007-11_08.asp). The market for protein biomarker discovery was $290 million in 2005 which is predicted to rise to $745 million in 2011 (Biobusiness, Biomarket Trends (genengnews.com, March 1 2007). While proteomics has great potential for the US and worldwide, as noted below, there are particular opportunities for SC. The bases of these developments are the independent revolutions that have occurred in the fields of molecular biology and analytical chemistry leading to the current inter-relatedness of genomics, proteomics and bioinformatics.

The molecular biology revolution included the development of cloning, the polymerase chain reaction (PCR) and use of restriction enzymes for recognition of sequence differences among organisms employing genetic markers. Indeed in 1993, Kary B. Mullis, received the Nobel Prize in Chemistry for the discovery of PCR. Dr. Mullis went to high school in Columbia; so SC has a history in this area. The process of marker discovery has been greatly aided in recent years by whole genome sequencing (i.e. determining the entire genetic or DNA code for an organism) also allowing a more systematic approach to biomarker discovery. While it is well known that the human genome has been sequenced and annotated, it should also be emphasized that the genomes of many common mammalian and other multi-cellular (eukaryotic) species and single celled organisms (including most common human pathogens) have also been sequenced. What used to take large groups of investigators months or years (and millions of dollars) can now be accomplished in days or weeks (depending on the size of the genome) at a fraction of the cost; although it is still expensive.

## DNA AND PROTEIN MARKERS

It is anticipated the automated instrumental identification of peptide markers for human pathogens will be considerably less labor-intensive than current DNA-based approaches. Discovery of DNA markers e.g. using PCR involves: a. first defining putative sequences from the genome; b. next primers (DNA sequences that are complementary and recognize these sequences) are designed; c. PCR is tested with relevant clinical samples; d. off-line sequencing of PCR product is often performed to confirm identity in initial set-up experiments. In the case of protein markers a. (PCR) and b. (primer design) are not required and d. (sequencing) is performed on-line as part of the proteomic analysis. In other words there is only one instrumental marker discovery/analysis step in a proteomics-based approach. Genetic or protein markers for inherited diseases or cancer are determined in a similar fashion (markers discerning diseased versus normal cells).

At USC we are focusing our efforts on basic research in marker discovery (with support from the Sloan Foundation). However we also are extrapolating these concepts to helping assess the utility of automated instrumentation developed by Virgin instruments LLC (Boston, MA). Prototypes will be evaluated and modifications suggested for specific applications by Automated Methods LLC, Columbia, SC. Recent advances in proteomics technology provide accurate molecular weight (M.W.) and sequence information on peptides from protein digests with high speed and sensitivity. These advances include new mass spectrometers developed at Virgin together with more efficient methods for interfacing separations with mass spectrometry with microbial biomarker methodology developed at USC These systems provide practical solutions to the problems that have severely limited the applications of proteomics for clinical analyses. The focus of current R & D is on reproducibly detecting, identifying, and quantifying human and microbial biomarkers in: 1) plasma, serum, urine and other body fluids in the important 1 pg/mL to 1 ng/mL range and 2) environmental samples (e.g. with biodefense and homeland security applications). This new family of instruments employs 5 khz lasers providing data acquisition 25 times faster than any existing commercial mass spectrometer.

Microbiological applications will be used to give an example of the applicability of the technology. However, as noted above markers can be derived from any form of life (e.g. human, bacterium, parasite or virus). Bacterial species share specific genes (and encoded proteins) of characteristic sequence distinguishing them from other bacterial species. Differences in DNA sequence are generally detected by real-time PCR. Since the differences are small for closely related species; direct (automated Sanger sequencing) or indirect approaches (e.g. restriction digestion) are often used to detect these sequence variants.

Many forms of mass spectrometry have been successfully employed for identification of cultured microorganisms, but none of these approaches provide the sensitivity, specificity, simplicity and speed required for automated clinical identification or detection of infectious agents in human body fluids without culture which is a work in progress.

## MARKER DISCOVERY

Discovery of useful biomarkers by the proposed methods requires two steps. First, fractionation, separation, and analysis protocols must be optimized for potential biomarkers for particular strains and species to be detected at clinically relevant level in body fluids without culture. Second, peptides produced by digestion of proteins from cultured organisms must be identified and their MS-MS (tandem mass spectrometry) spectra recorded, interpreted, and stored in a searchable database together with all available information including the source, strain and species of the organism. This protocol must be sufficiently rapid, robust, and simple to allow its use in a clinical setting. Thus, while limited separation and fractionation may be sufficient for the initial discovery phase, it is important to establish a protocol using proteins from the cultured samples that can be extended to reliable detection of these potential biomarkers at low levels in body fluids. The ultimate goal is detection of specific biomarkers for previously characterized pathogens, at clinically relevant concentrations, within one hour after receipt of a body fluid and to characterize fluids containing previously unknown or emerging pathogens within 48 hours.

Successful completion of this work may revolutionize clinical microbiology allowing laboratory diagnosis in real-time (with equivalent sensitivity to PCR) but also real-time identification of protein sequence variants. This could totally change the way that treatment of infectious diseases is performed in the US. The instruments would also revolutionize battlefield biodetection and counter-terrorism efforts for biological warfare agents (e.g. anthrax). Instrumentation might be purchased by every hospital and/or first responder (urban/battlefield) in the US. Each instrument, depending on sales, would be in the $200,000- one million range. Ancillary products will include disposable reagents, operator training, and up-datable data-bases of markers.

There has been a revolution in mass spectrometry leading to sequencing of the expressed protein products of genomes (proteomics). Indeed the 2002 Nobel Prize in Chemistry was awarded to Koichi Tanaka and John B. Fenn for their development respectively of matrix assisted time of flight ionization/desorption (MALDI) and electrospray ionization (ESI) mass spectrometry (MS). In both cases large molecules (including proteins and DNA) are analyzed in native form from aqueous solutions in a mass spectrometer. Scientists, whose research does not focus on mass spectrometers, are often thinking of an older technology (gas chromatography-mass spectrometry [GC-MS]). GC-MS (and more advanced GC-MS-MS) requires extensive chemical work-up to convert a marker (usually a small molecule such a fatty acid) into a suitable form for analysis in the gas phase. Indeed in the clinical microbiology field GC is now routinely used in reference laboratories for whole cell fatty acid profiling after prior growth in culture media (after conversion to FAMES, fatty acid methyl esters]). GC-MS provides additional structure information on these profiles.

Microbiologists are often not well versed in performing organic chemical reaction schemes and thus fatty acid profiling is limited to laboratories with an emphasis on microbial biochemistry. However fatty acid profiling is still considered a gold standard in taxonomy and classification and widely used in reference laboratories. There are also several companies that will provide a fatty acid profile for a fee (e.g. MIDI Inc., Newark, DE). Sample preparation for fatty acid analysis takes several hours. By comparison a recent proteomics method for identification of Bacillus anthracis developed at US takes a few minutes. The difference in time taken for the two analyses (hours versus minutes) provides a perspective on how things have changed and potential for the future.

Additionally the presence or absence of a fatty acid monomer provides considerably less specificity than a peptide sequence.

Alternatively, the genomic revolution has given us a vast array of molecular biology tools for discrimination of well-known pathogens as well as emerging infections by the presence or absence of genes or for closely related organisms, small changes in DNA sequence. It is anticipated that protein-sequence based discrimination will be as important for the next generation of clinical microbiologists and biomedical researchers.

In the newer so-called soft ionization MS technology, introduced in the 1990s and 2000s, biomolecules are analyzed without any separation of components or after separation employing high performance liquid chromatography (LC) or electrophoresis. This is performed in the liquid phase which is often aqueous in nature. Small molecules can be analyzed but the real power of the technique is in being amenable to analysis of larger molecules (e.g. peptides/proteins) without chemical pre-treatment.

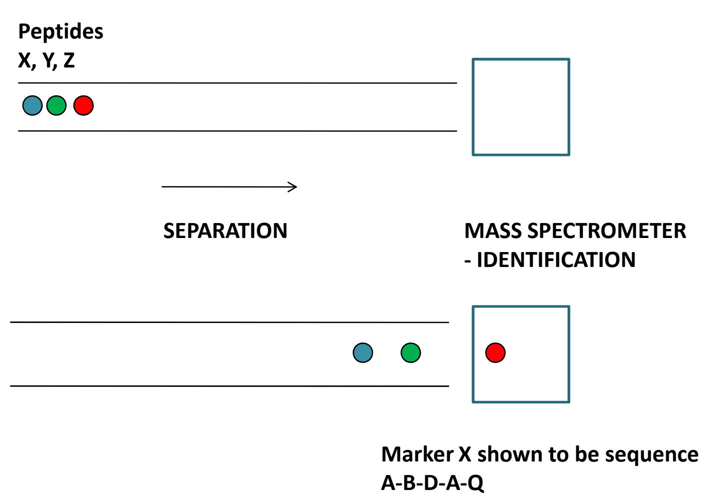
For the non-mass spectrometrist, it should be pointed out that nowadays the analysis of these large molecules is primarily based on MALDI MS or ESI MS. In the former case, the sample is spotted, with a matrix, on a metal plate and allowed to air dry. When struck with a laser beam, after the plate is inserted into the MS, the matrix absorbs the light, transferring it to the molecule of interest (e.g. proteins or peptides). Generally, only a singly ionized species is produced having a single charge. In contrast, ESI MS is performed in solution and the sample is sprayed into the MS using a syringe pump. As the droplets evaporate, charges are transferred to molecules present within the droplet. Ions are produced that can have multiple charge states. Since mass analyzers generally separate by the mass-to-charge ratio, simple spectra are generated for MALDI (molecules having only one charge) but ESI spectra (reflecting mixtures of molecules each having one, few or multiple charges) are more complex. Thus MALDI MS has been more popular with biologists and biomedical researchers because of the simplicity of the spectra. However ESI MS often allows the analysis of larger molecules. An extensive knowledge of chemistry, in performing MALDI or ESI MS, is not required since the molecule is analyzed in its native form without chemical treatment. Indeed as mentioned above, in certain applications it is not necessary to employ a separation stage (i.e. LC or electrophoresis) and the sample can be analyzed directly in the MS with minimal sample pre-treatment.

Another independent but equally important instrumental advance has been the commercial introduction of the tandem mass spectrometer (MS-MS, also in the 1990s-2000s) allowing routine sequencing of peptides. Peptides are identified in two distinct stages. First the molecular weight of the peptide is determined; they are volatilized in the MS as intact molecules. Then, for MS-MS analysis, the peptides are broken into a series of constituent mixture of peptides by breaking them at each peptide bond in the chain. For example, in the following illustration purposely simplified for clarity: a tri-peptide A-C-D (alanine-cysteine-aspartate) in sequence might generate alanine and cysteine-aspartate on MS-MS analysis. The observation of a mass equivalent to A suggests that alanine is the terminal amino acid. This is confirmed by the difference in mass between ACD and CD suggesting that CD make up amino acids 2, 3 of the peptide. The finding of a dipeptide of mass of AC suggests C is linked to A., i.e. is at position 2. The sequence is thus A-C-D. Generally the analysis is more complex (and the spectra more difficult to interpret) since the molecules are larger (usually 10-30 mers) and fragmentation is more complex.

PROTEIN PROFILING

Direct extraction of bacterial vegetative cells or spores followed by MALDI MS analysis has become popular for bacterial identification, since it is simple to perform and mass spectra are readily interpreted. However, only high abundance peptides that are of low mass and ionize readily are observed (e.g. 2-10,000 mass range). Generally the spectra are plotted as the amount of each protein present (as defined by its molecular weight, MW); Unfortunately MW alone is not sufficient to identify a characteristic biomarker and one must rely on the entire spectrum, this often referred to as mass profiling or fingerprinting. These spectral comparisons can be made by eye-balling but generally pattern recognition-based computer programs are employed; unfortunately there is often considerable variability in the spectra from run-to-run or between samples complicating data interpretation.

Alternatively the sequences of individual proteins can be determined using MS-MS. The presence of an individual marker can be determined with great confidence and one does not have to depend on the consistency of the mass profile which can sometimes be problematic. For example, in our recent work, the MWs of small acid soluble proteins (SASPs) were measured using MALDI MS and confirmed by ESI MS. ESI-MS-MS analysis was employed for the generation of sequence-specific information. The analysis consists of simply extracting the samples and analyzing the extract directly into the MS-MS instrument. ESI-MS revealed a prominent doublet of SASPs for all strains in these studies. The first SASP varied in mass and sequence between B. anthracis versus B. cereus/B. thuringiensis. The second SASP had the same MW for all strains correlating with species (or clade; there are two for B. cereus) and served as an internal standard allowing comparison between mass spectra in this study and previous ones. The entire sample extraction and analysis takes under 10 min.



## PROTEOMICS

It should be emphasized protein profiling is distinct from classical proteomics based approaches which involve more time-consuming sample processing. Proteomics often employs 2D gel electrophoresis to isolate individual protein spots which are then digested in situ, usually with trypsin, to generate peptides of characteristic masses that are subsequently analyzed using MALDI MS analysis. The sequences of each peptide in the tryptic digest can then be identified by MALDI MS-MS analysis. Alternatively, after tryptic digestion of whole cells, the mixture of peptides is subjected to on-line liquid LC-ESI-MS-MS analysis (either one or two dimensional). In either case, separation (electrophoresis or chromatography respectively) is important in reducing the complexity of mixtures for analysis by the mass spectrometer but increases the learning curve in implementing the MS technology for routine applications.

Proteomics is quite time consuming and technically demanding and is best used for comparing the relatedness of two strains or species (or cancer versus normal cells). Bioinformatics can be used to relate identified peptides to those predicted to be present in proteins coded by whole genomes. In theory, a novel strain could be categorized in this fashion. This requires bioinformatics analysis of multiple strains of each pair or group of organisms to be discriminated which is complex and labor intensive. Alternatively LC-MS-MS, or 2D-gel electrophoresis/MS-MS, could be used for the process of marker discovery. Once the markers have been discovered, simple MS or MS-MS assays (performed in aqueous solution) could be employed for routine analysis. The analogy is the discovery of DNA markers by whole genomic comparison followed by real-time PCR for diagnostic applications.

Sensitivity and specificity are both of particular importance; in trace detection of microbial markers in complex biological matrices such as infected body fluids or tissues. Indeed there is usually a separation (e.g. LC for proteins) or PCR amplification of the target (DNA) marker in clinical diagnosis. In both instances this serves to increase the concentration of the marker relative to background derived from other components of the matrix, this simplifies the analysis.

Real-time PCR is the current leading non-culture-based technology for determination of infection. More discriminating PCR-MS (mass spectrometry) for bacterial DNA markers was developed in the US through collaboration between the University of South Carolina and Pacific Northwest National Laboratory. In this case the mass accuracy is sufficient to discriminate two PCR products differing by a single nucleotide substitution (e.g. adenine to thymine [9 mass units] or guanine to cytosine [40 mass units]). An automated commercial PCR-MS instrument was subsequently introduced by Ibis Biosciences Inc., Carlsbad, CA based on these principles. PCR-MS has several additional stages, versus PCR, including post-PCR sample clean-up and robotic transfer from PCR to MS module. Thus PCR-MS is currently performed as a reference laboratory technique. For example, it has been successfully used for determining nucleotide composition, for strain typing in epidemiological studies of outbreaks of respiratory infections with Streptococcus, Hemophilus or Neisseria.

## CONCLUDING REMARKS

Once simple automated instruments are widely available, diagnosis of disease variants or bacterial infection using protein markers will involve minimal sample preparation and would be complementary (but simpler to perform) than widely used molecular biology approaches that often involve multiple sample processing steps (e.g. PCR/off-line sequencing). However, the use of mass spectrometers is still daunting to many in the microbiological, biological and biomedical communities. Hopefully this review will contribute to removing some of the mystery behind what is ultimately a simple tool that is highly amenable to unattended sample preparation and computer-based decision making. Genomics is reaching maturity but high-through-put proteomics still has great potential for growth. SC has an opportunity to not only benefit from these developments but to be ahead of the curve and indeed lead them in the US to fruition.

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# WITH THE HELP OF SOUTH CAROLINA RESEARCH UNIVERSITIES, SCRA DEMONSTRATES LEADERSHIP IN APPLIED RESEARCH AND COMMERCIALIZATION WITHIN SC

SCRA
Collaborating To Advance Technology

Bill Mahoney, CEO, SCRA

## SCRA EXPERTISE DELIVERS ASSURED OUTCOMES

Now in our 25th year of operation, SCRA has expertise in building multi-organizational teams from industry, government and academia. We create teams to solve our clients' problems – by carefully choosing partners to fill technology gaps -- so that the delivery of technology-based solutions for our clients' complex challenges is assured.

In addition to creating teams and managing applied research programs, SCRA adds value and provides technology as a contributor based on deep domain expertise. We develop and demonstrate new technologies and apply off-the-shelf technologies in innovative ways to deliver client solutions. Our experienced management techniques keep researchers focused on relevant outcomes. SCRA utilizes the reach of the team to achieve broad implementation of technology breakthroughs and best-practice methodologies. Our processes and people combine to deliver assured outcomes across entire industries.

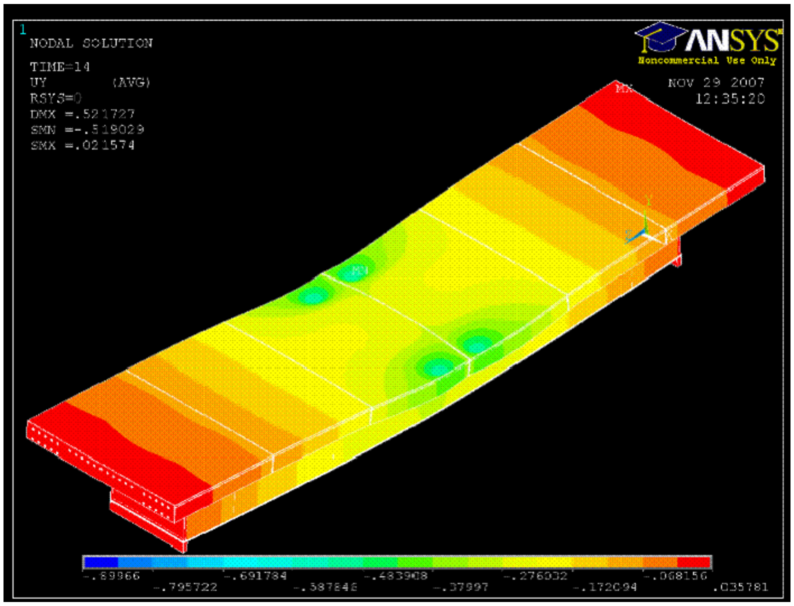
## PROFILES OF SCIENTIFIC RESEARCH AND COMMERCIALIZATION COLLABORATIONS WITHIN SOUTH CAROLINA

To illustrate the breadth, depth and scope of collaboration among South Carolina's research universities and SCRA, here are a few recent profiles of activity, from both a research and a commercialization perspective.

## VANADIUM: A STUDY FOR LIGHTER WEIGHT AND HIGHER PERFORMANCE BRIDGES FOR THE US ARMY

Dimitris C. Rizos, PhD., Assistant Professor at the University of South Carolina (USC) Department of Engineering and Information Technology has partnered with the US Army Corp of Engineers to design a prototype non-standard, fixed bridge for extensive military testing and possible civilian use. The project objective is to research high strength vanadium steel for the replacement construction of non-standard fixed bridges on military supply routes that become damaged or destroyed during combat. This application has a clear commercial analog in the highway bridge construction industry. The technical characterization focuses on weight savings and increased span lengths realized through the use of the Vanadium. A cost comparison, risk analysis, and structural design assessment and analysis have been included in the study.

Dr. Rizos' team at University of South Carolina has developed a 200-foot bridge model that accommodates 60-ton military vehicles. Computer simulations and Finite Element Modeling are being utilized to evaluate the 200-foot segment of the bridge for various load cases, which checks the design against anticipated design loads and load combinations.




*Above: Example of Simulation Testing Model. Simulations may include stress tests, deflection rates, bridge connection strength*

The overall outcome of this work with vanadium will benefit warfighter protection and also provide civilian spin-offs. For example, improved energy absorption in vehicles travelling over the bridges will provide better soldier protection. Other benefits include increased awareness of vanadium's structural benefits among designers and producers of vehicles and buildings. Increased demand for these steels benefits efficiency of US and South Carolina steel-makers.

The Advanced Technology Institute (ATI), an SCRA affiliate, is the Program Manager for the Vanadium Technology Partnership. ATI has strength in achieving program goals set forth jointly by the U.S. Army and industry. The approach also focuses on maximizing return while minimizing risk.

## COPPER STUDIES FOR IMPROVED MEDICAL PATIENT CARE; EXPANDED MARKETS FOR COPPER PRODUCTS

Another collaborative program, in progress for the US Department of Defense (DoD) and the Copper Development Association, utilizes the inherent antimicrobial properties of copper. Two studies underway include extensive work being performed in South Carolina. One study is focused on the ability of copper alloy surfaces to kill deadly pathogens and impede cross-contamination. Clinical trials are underway in both South Carolina and in New York City to complete a pilot conversion of touch surfaces in healthcare facilities. The second study is designed to demonstrate the effectiveness of copper components in heating, ventilating and air-conditioning (HVAC) systems to reduce the incidence of harmful microbes that spread throughout buildings and other indoor air environments. University leads on this program are Charles E. Feigley, Ph.D., C.I.H, Professor in the Department of Environmental Health Sciences at the University of South Carolina and Michael G. Schmidt, Ph.D. Dr. Schmidt is the Director of the Office of Special Programs and Professor and Vice Chair Dept of Microbiology and Immunology at the Medical University of South Carolina.

This outstanding program team for DoD, in support of Army Medical Research, is also being implemented by SCRA affiliate ATI. The team will assure outcomes of both improved patient care and expanded applications and markets for copper products. The program brings together a world-class team of medical researchers and practicing clinicians including hospital sites committed to conducting a series of multiple clinical trials at the Sloan-Kettering Cancer Center in New York, and at affiliated hospitals in Charleston: the Medical University of South Carolina and the Ralph H. Johnson Veterans Affairs Medical Center. Project team members from the University of South Carolina's Arnold School of Public Health and the School of Engineering is coordinating activities with fabricators and manufacturers identified by the Copper Development Association to implement copper touch surfaces in selected locations in the three hospitals.

An Independent Advisory Board is monitoring the study progress. The program consists of four program phases: a theoretical study and the establishment of baseline data sets in a lab environment; operation and monitoring of standard and "copperized" HVAC systems in real-world settings; trials conducted in hospital and/or military settings; and integration of copper microbial control with the concepts of green building design.

NUTRITION RESEARCH CONSORTIUM LINKS SOUTH CAROLINA SCIENTISTS AT CLEMSON, MUSC AND USC

The Nutrition Research Consortium (NRC) is administered through SCRA's Public Interest Sector. Based in South Carolina, this consortium helps link nutrition researchers throughout the State. The group addresses nutrition concerns that affect not only South Carolinians, but people throughout the country. With more than 50 of the State's nutrition scientists participating in NRC research and outreach activities, NRC focus areas are wide-ranging, from early detection and prevention of childhood obesity to molecular nutrition studies on energy balance. Additional focus areas include obesity and hospital-based nutrition to prevent patient complications and reduce re-admissions.

Marilyn Laken, PhD., RN, MUSC Professor, cites that the NRC has provided pilot funding for faculty at all three South Carolina research universities: Clemson University, the Medical University of South Carolina and the University of South Carolina – which were absolutely critical to gathering data to help the NRC obtain funding for large grants. For example, funding for nutriceuticals led to a grant to test a naturally- occurring substance for cancer prevention. Another grant will produce a videotape to help instruct caregivers how to feed elderly family members with dementia. The grant is specifically tailored to the African American community, since previous studies only included care to Caucasians. Some grants lead to even larger National Institute of Health grant opportunities to help people within South Carolina. NRC enables important studies that specifically address the needs of South Carolina citizens.

Some of the most important work conducted by NRC simply involves connecting the dots within the universities -- by maximizing their ability to be interdisciplinary. The research universities combine their complementary strengths through the NRC to collaborate, which further increases the potential for extramural funding. For example, University of South Carolina's Sarah Wilcox, an associate professor in USC's Department of Exercise Science and an expert in physical fitness, and MUSC's Dr. Laken combined expertise on a recent program. Their efforts secured a Centers for

Disease Control grant for AME churches to implement a physically fit program throughout the State. USC's Russ Pate, PhD, Professor of exercise science at the Arnold School of Public Health introduced MUSC's Dr. Laken to Dr. Wilcox. The CDC grant led to an NIH grant to improve physical fitness. Another DoD grant was obtained to better enable recruits to complete military training -- after the NRC discovered that 43% of women and 18% of men would fail the US Army's weight requirements.


A strong suit of NRC is its ability to foster trust, which allows the group to identify key collaborators among our universities. Dr. Laken emphasizes how the research universities are working together to foster and commercialize innovative research in nutrition and disease prevention, attracting large-scale research grants to help people in South Carolina.

SCRA AFFILIATE, SC LAUNCH!, FACILITATES APPLIED RESEARCH, PRODUCT DEVELOPMENT AND COMMERCIALIZATION PROGRAMS TO STRENGTHEN SOUTH CAROLINA'S KNOWLEDGE ECONOMY

SC Launch! began less than two years ago as a collaboration among SCRA and South Carolina's research university foundations: Clemson University, The University of South Carolina and the Medical University of South Carolina. SC Launch! was created to facilitate applied research, product development and commercialization programs to strengthen South Carolina's Knowledge Economy. After one year of formal operation, this collaboration extended even further with business, academic and economic development entities as Resource Partners in the SC Launch! Resource Network. These partners are integral to the SC Launch! program because they provide an eco-system of support to start-up companies and entrepreneurs with business counseling, mentoring and training.

The SC Launch! mission is to:

- Fulfill the Innovation Centers Act passed by the South Carolina legislature in 2005;
- Help create start-ups that leverage intellectual property from and partnerships among the research university foundations and the public and private sectors;
- Generate professional-grade research and development and Knowledge Economy jobs in South Carolina;
- Establish a continuing forum to foster greater dialogue between the state's research university foundations and industry;
- Focus SC Launch! client efforts on the development, testing and implementation of new advances in knowledge-based industries;
- Promote the development of knowledge economy industries and applied research facilities in South Carolina.

What is the Knowledge Economy?

A knowledge-driven economy is one in which the generation and exploitation of knowledge play the predominant part in the creation of wealth. In South Carolina, the Knowledge Economy is growing around high-technology industries such as life science, advanced materials and alternative energy. More than 60% of US workers are knowledge workers.

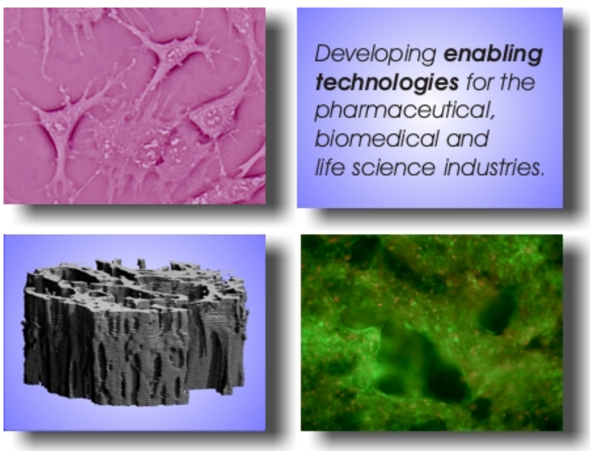
PROGRAMS THAT HELP POSITION SOUTH CAROLINA START-UPS TO PROPEL THE SC TECHNOLOGY BASE

SC Launch! is involved in each step of the innovation pipeline, from discovery assessment through development and deployment, continuing with support during later commercialization activities. Programs include a pre-company and university initiative; an SBIR/STTR Matching grant program, and innovation prizes awarded through The New Ideas for a New Carolina contest, through which SC Launch!, New Carolina, Think Tec and other Knowledge Economy sponsors support innovators and inventors in commercializing their ideas.

SC Launch! also focuses to identify, nurture and help grow high-impact knowledge economy companies. SC Launch! offers funding through grants, loans and equity investments up to $200,000 per entity. SC Launch! zone managers and Resource Partners throughout the State work with prospective companies to prepare them for the rigors involved in acquiring follow-on financing from angel and venture capital investors. Additional, important programs include support of landing parties – companies that relocate to South Carolina and make a commitment to grow the knowledge economy here; and demonstration projects, which have included important work in exploration and application of alternative energy throughout our State. One such company, Selah Technologies, began with research from Clemson University. Selah is a nanomaterials innovator and manufacturer with two distinct and compelling platform nanotechnologies. Its mission is to consistently deliver high quality nanomaterials and nanotechnology-enabled products to the global marketplace in an ethically sound manner. The company produces Selah Dots ® - carbon-based quantum dots that have a broad range of potential applications that include tumor-guided surgery and use in biosensors.

## PRE-COMPANY AND UNIVERSITY INITIATIVES ARE AN IMPORTANT PRIORITY

SC Launch! makes pre-company and university initiatives a priority to assist in turning promising research into commercial realities. Here are a few recent examples:




Clemson University and KIYATEC take drug discovery out of the lab and into the marketplace. The KIYATEC - Clemson University interaction is one that demonstrates the benefits of business and South Carolina university research working hand-in-hand to apply an important technology. The technology was originally invented in Clemson University's Department of Bioengineering as a means to create 3-D tissue constructs (i.e. "tissue engineering") for implantation to repair or replace diseased tissue. While a worthy goal and one that will be possible some day, KIYATEC management, led by CEO Matthew Gevaert, Ph.D., recognized it to be not commercially feasible in the near future because of today's financial and regulatory environment. They did, however, recognize the value of the technology for an application which will be quicker to market but still quite valuable - drug discovery - and are focusing their efforts on this much more commercially viable path forward. Through an interaction in which each group contributed ideas aligned with their unique "raison d'être" - discovery in one case and product in the other - they are creating a solution that neither alone would have generated.

Another example of promising South Carolina technology and its link back into potential SC university research projects is illustrated with SensorTech. SensorTech offers a new, patent-pending contact sensing technology that can accurately measure force, pressure, torque, or impact, and has the ability to be formed into any shape and size. Here's how this venture began: Andrew Clark, Ph.D., completed the Technology Entrepreneurship Certificate program at Clemson University's Spiro Institute for Entrepreneurial Leadership – which was designed for folks like Andrew who are earning a graduate or doctorate-level degree in engineering and science and want to complement that study with an understanding of entrepreneurship.

Dr. Clark had also invented a new bioengineering technology as part of his doctoral program in Bioengineering. He disclosed it to Clemson, the University patented it and the

Spiro Institute arranged for MBA students to work with the Clemson Office of Technology Transfer to explore its market opportunity.

The next year, when he was ready to graduate and form his company, SensorTech, with Chuck Pringle, the Spiro Institute arranged for another team of MBA students to assist the young company in developing information needed for the launch.

Andrew Clark is a good example of a top bioengineering PhD graduate from a South Carolina university who has chosen to stay in SC and work for an entrepreneurial venture here because of these connections – a prime example of their value. Similarly, Joanna Isbill, an undergrad in materials science who is pursuing a dual MBA and MS in Bioengineering at Clemson, served as the team leader on the SensorTech project. With her experience with SensorTech, she is considering an entrepreneurial career path for herself as well. These strategies, programs and collaborations provide an opportunity for these highly educated students to find career opportunities in SC rather than leaving the State.

## THE UNIVERSITY OF SOUTH CAROLINA AND CARBONIX TACKLE THE DIFFICULT TASK OF TAMING A COMMON ALLERGY

Another important connection between scientific research and commercialization in South Carolina is happening between CarboNix and the University of South Carolina. CarboNix was formed specifically out of research technology performed in the Chemical Engineering department at USC by Dr. Mike Matthews. In an effort to commercialize this technology, Dr. Tony Bocanfuso, working with the USC Technology Transfer Office, contacted Al Quick, a USC College of Engineering grad and local entrepreneur, to take a look. Al was already a seasoned, retired senior executive from a major corporation and a veteran of a high-tech start-up that was sold after nine successful years in business. Dr. Matthews, currently the Chemical Engineering chair at USC and Al, agreed that there was a commercial opportunity in the technology and partnered to form CarboNix. While both men have technical backgrounds and management experience, in CarboNix Mike provides the technical expertise and Al provides the business background and start-up company experience. The USC Research Foundation owns stake in the venture through both equity and royalty from the technology licensing and CarboNix holds exclusive rights to the technology.

It's a perfect formula - Mike provides the technology and Al provides the business expertise. The technology was originally based on a sterilization technique that does not require high temperatures, harsh chemicals or radiation. Dr. Matthews found that he could achieve their desired results of killing bacteria and bacterial spores using liquid carbon dioxide.

While it appears that there is a significant medical opportunity commercially for this technology, it will take time to gain required agency approvals. Since it is often difficult to interest investors in a venture that is most likely five years out, the two will continue with that path for the long term. Meanwhile, the team began looking at more near-term revenue streams using essentially the same technology. They believe that they have identified an application that may provide a near-term revenue stream and won't require the lengthy FDA approval cycle. Their work focuses on the tenet that many people suffer from allergies and asthma, frequently triggered by certain proteins in dust mite excretion. These attacks result in many lost work and school days. Dust mites are present in the bedding of almost every home and are difficult to control. CarboNix has identified a process using liquid carbon dioxide to not only kill the mites but more importantly denature the protein in the dust mite waste that causes these attacks. Work is currently underway to commercialize the process.

The team hopes to generate jobs and significant revenue to further strengthen the knowledge economy in South Carolina. If work goes as planned, it's a unique application of basic science to a serious problem – dust mite allergies – that will come from South Carolina university research. The Company has engaged several experts from other Schools at USC, including its Public Health, Medicine and Business departments, to participate in the activity. This is a great example of how a new focus on commercializing the technologies developed in our South Carolina research universities can play a significant role in economic development for the state. With a greater focus on developing technologies and commercializing them, Quick is confident that companies like CarboNix will continue to spring up all over the state with help of partners like SC Launch! and others.

## SBIR/STTR MATCHING GRANT PROGRAM AWARDS INNOVATION IN SOUTH CAROLINA

The SC Launch! SBIR/STTR Phase I Matching Grant Program is designed to award matching funds to South Carolina-based companies that have been granted a Federal Small Business Innovation Research Phase 1 award. Since this program was initiated in June, 2006, seven South Carolina companies have received awards:

- Cell & Tissue Systems
- First String Research
- Innegrity
- Microbial Fuel Cell Technologies
- Tetramer Technologies
- Selah Technologies
- Sensor Electronic Technology

## SC LAUNCH! CIRCLE OF INNOVATION

SC Launch! provides an opportunity for individuals and organizations to have a direct impact on South Carolina's competitiveness agenda. The Industry Partners Act allows individual or corporate donors to take a South Carolina tax credit against income taxes, insurance premium taxes and certain license fees for 100% of the contribution amount. This enables Circle of Innovation members to be in the vanguard of South Carolina's shift to a Knowledge Economy.

## CONCLUSION: DELIVERING ASSURED OUTCOMES AND POSITIONED FOR FURTHER GROWTH

Since its incorporation in April, 2006, SC Launch! has supported (as of January, 2008):

- 150 applications
- 37 companies who have received funding
- 81 companies who have received support services

Program results include:

- Loans and equity investments to 14 companies
- 23 Pre-company/university grants
- 4 landing parties who have relocated to South Carolina
- 7 demonstration projects
- 7 SBIR matching grants
- 25 innovation prizes with New Carolina

Four SC Launch! companies have secured $30M in follow-on venture capital

# SC INBRE: A CATALYST FOR BIOMEDICAL RESEARCH DEVELOPMENT IN SOUTH CAROLINA

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## INTRODUCTION

At the very core of a university's mission is the process in which research - the generation of new knowledge - in a given discipline is coupled with the teaching of that discipline to students. This is true for the sciences, as well as for the arts, where technical and artistic limits are expanded by the scholarly work conducted by professors/mentors and their students.

The integration of research with teaching is traditionally at the center of graduate education, while undergraduate learning is still perceived as being primarily classroom-based, particularly by the public at large. However, there is ample evidence that such integration can also occur at the undergraduate level, with considerable benefits to the student, the institution, and ultimately the community. In the scientific and technical arena, the benefits of integration of research with undergraduate education are tangible and well documented (1-5). These benefits are not limited to sharpening a student's skills in the chosen discipline: research training has far-reaching effects on the overall maturity, work, and life skills of the trainees, as well as on their satisfaction with their own undergraduate experience. Graduates who have been involved in structured research in their undergraduate studies are more likely to continue on to graduate school and become researchers, and also more likely to work in an area closely related to their major. These individuals perceive themselves as being more inquisitive, better equipped to tackle and resolve problems, and more confident in their own abilities and leadership (1-5). In addition, there is compelling evidence that undergraduate research training is an effective means to recruit and retain students, particularly minority students, in science careers (6-9). In short, research training is highly beneficial to the training of a workforce prepared to adapt and respond to the demands of a knowledge-based economy. Therefore, predominately undergraduate institutions are seeking to increase their offerings in terms of research training not only to compete for the best students, but also to provide a high quality education that is responsive to the demands of the marketplace.

Research universities act as catalysts for economic development by providing a trained technical workforce in their geographic regions. A thriving academic research enterprise also stimulates ties to other research-intensive institutions and industry. Federally funded research support, needed to build and sustain a research enterprise, goes to relatively few institutions and is confined to a limited number of states. The Experimental Program to Stimulate Competitive Research (EPSCoR) at the National

Science Foundation, and other federal agencies, is committed to a more equitable distribution of research funds and opportunities across the national landscape. Based on estimates by the 2003 Bureau of Census, the Carnegie Foundation, and the National Science Foundation, the 27 EPSCoR jurisdictions (including South Carolina) have 20% of the U.S. population and 25% of all research universities, which employ 18% of academic scientists and engineers in the country; yet only receive 10% of the total federal research funding. These schools also educate a large number of MS and Ph.D. level scientists and engineers who go on to postdoctoral positions at non-EPSCoR states. Therefore, EPSCoR support benefits not only the states and institutions that are targeted directly, but the entire research enterprise in the country, by increasing the size, quality, and diversity of the postdoctoral fellow applicant pool.

In the biomedical research arena, the NIH response to the congressional mandate for an EPSCoR-like initiative is the Institutional Development Awards (IDeA) program at the National Center for Research Resources. This program funds a single IDeA Network for Biomedical Research Excellence (INBRE) grant in each of the 23 IDeA states and Puerto Rico, and provides support for Centers of Biomedical Research Excellence (COBRE) at research-intensive institutions.

## SC INBRE: GOALS AND STRUCTURE

SC INBRE is a network of academic institutions working together to develop the biomedical research infrastructure in South Carolina. INBRE provides support for target faculty having the capacity to build sustainable programs that can increase student participation in research, with particular attention to the recruitment and retention of underrepresented minorities in biomedical research. SC INBRE, a five-year, $17.3 million program, began in 2005 building upon BRIN, a five-year program devoted to building research infrastructure at predominately undergraduate institutions (PUIs). The network has evolved over the nine years since its inception. Dr. John Baynes, the original Principal Investigator of BRIN, deserves much credit for this program which has far-reaching beneficial effects on the biomedical research infrastructure in South Carolina.

South Carolina’s three Comprehensive Research Universities (CRUs) – the University of South Carolina (USC), the Medical University of South Carolina (MUSC), and Clemson University, serve as mentor institutions to build the biomedical research enterprise at four Predominately Undergraduate Institutions (PUIs) - Claflin University, the College of Charleston, Furman University and Winthrop University, within the network. Twenty-four designated “outreach” institutions are linked to the network by the SC INBRE Outreach Core (Figure 1). The Administrative Core of SC INBRE is housed under the umbrella of the SC EPSCoR/IDeA State Office. The SC INBRE Program Coordinator, Dr. Scott Little, is the Director of the State Office, in which both INBRE Administrative Core staff and SC EPSCoR administrative staff are housed. This arrangement keeps overhead costs to a minimum and enables cooperation and integration across the various EPSCoR and IDeA programs in South Carolina.

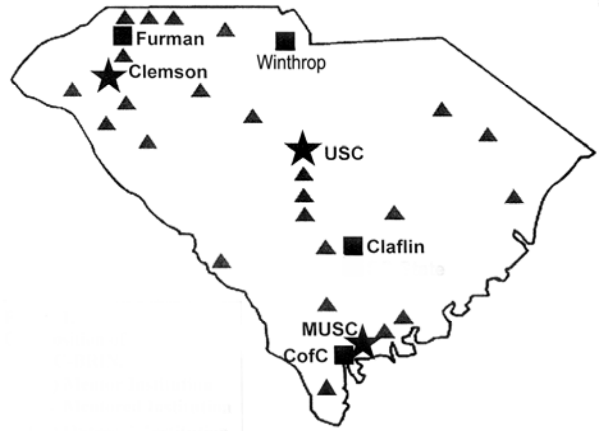


Figure 1: Seven institutions comprise the INBRE Network (stars: CRUs; squares, PUIs). The approximate location of outreach institutions is represented by triangles.

SC INBRE is built upon three specific aims: 1) development of research infrastructure for regenerative medicine at South Carolina's three CRUs, 2) development of biomedical research capacity at four PUIs; and 3) establishment of a statewide biomedical research network through bioinformatics and outreach cores. The network has established a "pipeline" for undergraduate students trained in the biomedical sciences (with particular emphasis on minority students) increasing the likelihood of matriculation into graduate programs in the biomedical sciences and the pursuit of scientific careers.

Salary and research support by SC INBRE provides target faculty with the means to build their research programs, create an environment in which to train students in research, and remain competitive beyond direct INBRE support. Target faculty receive course release time, and are asked to identify and work with research and career development mentors. Progress of target faculty toward their stated goals is monitored and encouraged by both institutional and statewide external advisory committees.

Support for CRU target faculty and core facilities fosters an increase in the number of investigators in the specific thematic area of regenerative medicine, building upon existing strengths and in accord with stated institutional goals for research enhancement. The goal, and condition for "graduation" from SC INBRE support, for a CRU target faculty is the establishment of an independent research program funded at the R01 level, or equivalent. Other important measures of success are the number and quality of publications produced, presentations given, and students trained, as well as evidence that the faculty members have established productive collaborations, and contribute to the overall development of a cohesive regenerative medicine research program in South Carolina.

SC INBRE support to PUI target faculty fosters the development of research educators equipped to provide their undergraduate students with well-structured and effective research training. The areas of research supported at the PUIs are not necessarily aligned with the regenerative medicine theme of SC INBRE, and in fact encompass a variety of themes and disciplines. Target faculty at the PUIs are also expected to achieve research independence, in order to sustain programs initiated with INBRE resources. Research independence dictates a need for enhanced instrumentation, requiring sustained support of maintenance contracts and technical personnel. Faculty development at PUIs aims at enabling these faculty members to compete for extramural funding opportunities such as NIH/AREA, NSF/RUI, NSF/MRI and NSF/REU grants. Thanks in part to INBRE support, Furman University and the College of Charleston were able to submit strong applications to the Howard Hughes Medical Institute for major grants in support of undergraduate research programs (pending). An important measure of success for PUI faculty, in addition to publications and presentations, is clear evidence of increased undergraduate student participation in research, with student co-authorship in papers and presentations. Hence, SC EPSCoR/IDeA respects the character and traditions of PUIs, and does not seek to transform these institutions into comprehensive research universities.

An NIH required component of SC INBRE is a Bioinformatics Core to provide access to scientific literature and specific data analysis tools to all investigators and students throughout the network. The SC INBRE Bioinformatics core has evolved during the years, in response to the growing needs of network institutions. The BRIN Bioinformatics core had primarily provided access to the scientific literature and some data analysis tools to investigators at all network institutions. In addition to continuing those services, in the first two years of SC INBRE the Bioinformatics core has provided a host of workshops, training sessions, and scientific symposia (see also Dr. John Rose's article, in this issue). During this past year, the Core has evolved again, to add to its information support and training portfolio an active service component: the SC INBRE Biotechnology Core provides service and support for the conduction of experiments involving the application of genomics and proteomics methodologies, including gene expression analysis by any of the available microarray platforms and related data analysis services. The core taps into and augments the resources of existing genomics/proteomics facilities within the network, to bring the application of these state-of-the-art techniques within easy reach of researchers across the state (10).

## SC INBRE: PROGRESS TO DATE

During the past three years, 30 faculty members have been targeted for INBRE support in South Carolina. Ten of these were new hires made possible by leveraging efforts and resources of EPSCoR and IDeA. These new hires are aligned with South Carolina EPSCoR/IDeA's strategy to develop the state's intellectual and scientific resources, by providing support for new junior faculty who bring with them expertise in specific areas not yet represented within our targeted areas of science and technology excellence. These investigators have produced 23 applications for extramural support, 11 of which originated at PUIs; and for the first time since the beginning of SC INBRE, faculty filed four patent applications, two licenses and two inventions across the network. Approximately 150 students, mostly undergraduate, have received research training in the past three years, and minority student participation in research has increased across the board, with several institutions (such as Winthrop University) exceeding their initial goals for minority student participation in SC INBRE supported research. SC INBRE administrative staff maintains a student tracking database used for program review and reporting purposes. These data will be used to prepare a competitive renewal of SC INBRE in 2009.

The Outreach Core provided summer research experiences for undergraduate students, collaborative faculty research programs, and postdoctoral academic career development (PACD). The PACD initiative, supported by INBRE and EPSCoR provides funding for one semester in the laboratory and one semester in the undergraduate classroom for selected postdoctoral fellows. This allows for increased exposure to the classroom for aspiring academic scientists, while also enhancing the research experience of undergraduate students. PACD scholars submit formal research, educational training, and career development plans for approval by the Outreach and Administrative Cores. The PACD initiative provided preliminary data used in two applications for postdoctoral training grants submitted to the NIH K-12 program: one from MUSC and Claflin University (funded) and the other from USC and Benedict College (pending). The Outreach Core has also provided support for research symposia and workshops, including the Annual Meeting of the South Carolina Academy of Science and the South Carolina Bioengineering Summit. Much of this support is in the form of travel for undergraduate students and invited speakers.

The success of SC INBRE, however, goes well beyond the numbers of faculty developed, students trained, and grants secured. SC INBRE has established a climate of cooperation, sharing of resources, and communication among network institutions that had traditionally operated in a somewhat insular fashion. The benefits of this shift are far reaching and their full impact on the biomedical research landscape in South Carolina will become much more evident with every year of implementation. In fact, one of the challenges for the SC INBRE administrative core is to track or measure the impact of the program, and communicate these results to the institutions, the NCRR and the public. Continuous evaluation and assessment of the program's impact is vital to our competitiveness for renewal of SC INBRE, and to the continuation of IDeA as a whole within NIH.

## INBRE AS A CATALYST FOR CHANGE

The requirement for institutional commitment is key to the success of the program, because EPSCoR and INBRE are intended to stimulate sustainable change beyond the funding period. The $17.3 million of federal INBRE support has been matched with over $33 million in commitments by the participating institutions and the State. INBRE network institutions must fully embrace new research paradigms into their culture. Changes in the daily activities of faculty members and students involved in the program, the curriculum, and hours of research training are key metrics used for evaluation of the impact of the program. A shift in tenure and promotion criteria to encourage research and reward it properly is a metric for success. Tenure-track vs. non-tenure track lines, and working relationships within a department and with the higher administration all are subject to evaluation.

Compliance issues can be complicated and again require a high level of commitment by the institution. An essential step toward building research competitiveness is the establishment of necessary sponsored programs administration, including grants accounting personnel to ensure compliance with federal and state assistance regulations. The establishment and management of an Internal Review Board for research on human subjects, a Biosafety Committee, and an Institutional Animal Care and Use Committee are required for a participating INBRE institution.

In all cases, SC INBRE has served as a springboard for tremendous growth in biomedical research programs at network PUIs, with the addition of new faculty, the renovation of existing research facilities, and the implementation of new research training programs, all of which was made possible with INBRE funding and institutional resources.

It is evident that South Carolina INBRE has stimulated a profound change in research culture. The challenge, as we move forward, is to consolidate and sustain the newly developed culture and secure its future beyond the current funding period. Participating institutions are well aware of this challenge and, we believe, well equipped to take it on. We hope that other PUIs will participate in the competitive process necessary for renewal of funding for the South Carolina INBRE network.

Information about the SC INBRE and EPSCoR programs, including details on core facilities, programs, and resources available to faculty and students throughout the state can be found on the South Carolina EPSCoR/IDeA website at www.scepscoridea.org.

ACKNOWLEDGEMENTS

A sincere thank-you to the South Carolina EPSCoR/IDeA State Committee and their staff (Alysia Bridgman, Isabel Sanchez, Gray Ladd, and Lee Snelgrove); and the Office of Sponsored Awards Management at USC and staff member Andrea Ceselski. They do all the work needed to manage and evaluate SC INBRE, and provided us with the information needed to assemble this article. This paper was supported by the SC INBRE grant, 5P20RR016461 and NSF EPSCoR grant, EPS-0447660. The contents of this article are the sole responsibility of the Authors and do not represent the official position of the NIH or NSF.

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Visit: http://www.scepscoridea.org/INBRE/BiotechnologyCore.html for specific information on how to gain access to SC INBRE Biotechnology Core services. Join the Core's mailing list online (also from this page) to receive updates on Core's offerings, symposia, funding opportunities, and other activities.

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# INFLAMMATION: THE KEY TO HEALTH AND DISEASE

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## INTRODUCTION

Recently, National Institutes of Health (NIH) awarded a 5 year $ 6 million grant to the University of South Carolina (USC) to establish a Center of Excellence for Complementary and Alternative Medicine Research on Autoimmune and Inflammatory Disease. The University of California Los Angeles and the Mount Sinai School of Medicine were the only other institutions awarded centers in 2007. Previously centers have been awarded at Harvard Medical School, Massachusetts General Hospital, Oregon State and Temple universities, and the universities of Maryland, North Carolina and California-San Francisco. The underlying focus of the NIH Center at USC is to study the mechanisms by which plant products suppress inflammation so that they can be used as preventive or therapeutic modalities against autoimmune diseases (http://camcenter.med.sc.edu/). The goal of this review is to provide an understanding of inflammation, discuss its role in health and disease, and provide an overview of how the NIH Center award and research in inflammation at the USC School of Medicine (SOM) provides the niche to bring together many research focus areas in basic and clinical sciences by providing a platform for multidisciplinary collaborations and research advancement.

Inflammation which is defined clinically as heat, pain, redness, and edema, actually results from a physiological response to tissue injury and infection (Oke and Tracey, 2007). Inflammation is a double-edged sword—while it is critical in restoring tissue homeostasis following damage secondary to invading pathogens, foreign bodies, and trauma, inflammation can also trigger acute and chronic diseases. This list includes major pathological disorders such as autoimmune diseases, allergies, cardiovascular diseases, neurodegenerative diseases and cancer. Thus, inflammation plays a critical role in the pathogenesis of a wide range of diseases.

Inflammation can be classified as either acute or chronic. During acute inflammation, the body responds to harmful stimuli through movement of plasma and the white blood cells of the immune system, called leukocytes, from the blood into the injured tissues. Acute inflammation plays a critical role in clearing infections and in tissue healing. If the inflammation persists for prolonged periods, it is known as chronic inflammation. This can lead to a progressive shift in the nature of immune cells that are present at the site of inflammation and can trigger tissue destruction, injury and organ failure. During inflammation, the cells of the immune system release a large number of chemical mediators known as chemokines and cytokines. Some of the important cytokine mediators include interleukin (IL)-1, IL-6, tumor necrosis factor (TNF), and interferon gamma (IFN). In the early stages of inflammation, the predominant cell type infiltrating the tissues is the neutrophil. In contrast, accumulation and activation of macrophages is the hall mark of chronic inflammation. In addition, lymphocytes also contribute towards the development of inflammation by producing cytokines and chemokines.

Autoimmune diseases are disorders in which the immune system, for reasons that are not clear, starts destroying an individuals own cells, tissues or organs by triggering inflammation. These diseases include more than 80 serious, chronic illnesses that involve almost every human organ system. Collectively, they affect 15-20 million people in the USA. They are more common in women and are considered to be among the 10 leading causes of death in women in the US under the age of 65 years. Currently, there is no known cure for autoimmune diseases. Prominent examples of these diseases include Coeliac disease, diabetes mellitus type 1 (IDDM), systemic lupus erythematosus (SLE), Sjögren's syndrome, multiple sclerosis (MS), Hashimoto's thyroiditis, Graves' disease, idiopathic thrombocytopenic purpura, myesthenia gravis and rheumatoid arthritis (RA).

The main objective of newly funded NIH Center at USC is to conduct research that would determine whether various plant-derived compounds possess immunosuppressive activity and to determine their efficacy against autoimmune diseases. Initially, the NIH Center will pursue three projects. Project one, led by Dr. Prakash Nagarkatti from the USC SOM, will investigate the effect of resveratrol (trans-3,5,4'-trihydroxystilbene) on experimental allergic encephalomyelitis (EAE), a model for human MS. Resveratrol, a polyphenolic compound found in plant products including red grapes, exhibits anticancer, antioxidant, and anti-inflammatory properties. Recently, we demonstrated that resveratrol treatment decreased the clinical symptoms and inflammatory responses in an experimental MS model (Singh *et al.*, 2007). Resveratrol was shown to act through the Aryl Hydrocarbon receptor (AhR) and estrogen receptors (ER) found on immune cells which, in turn, triggered apoptosis (programmed cell death) in these cells. Resveratrol administration also led to significant down-regulation of certain cytokines and chemokines including TNF-alpha, interferon-gamma, and the interleukins (IL)-2, IL-9, IL-12, IL-17, for instance (Singh *et al.*, 2007). These studies suggest that resveratrol and other plant-derived products may be benefiscial in the treatment of not only autoimmune diseases but also other inflammatory disorders as well. Project two, led by Dr. Mitzi Nagarkatti, will investigate the effect of compounds isolated from hemp oil on the suppression of the immune response which may be beneficial in the treatment of autoimmune hepatitis. Project three, led by Dr. Lorne Hofseth from the USC College of Pharmacy will test the efficacy of American ginseng on colitis and colon cancer. Preliminary studies suggest that ginseng is very effective in suppressing colitis and development of colon cancer in an experimental model. The Center will also provide core resource facilities, which will enable, the screening of the potential toxic effects of plant-derived compounds on the immune system. These facilities will be led by Drs Narendra Singh and Robert Price from the USC SOM. The Center will also create training opportunities for new investigators to pursue research on CAM and establish the basis upon which to initiate clinical trials on compounds that exhibit efficacy against specific autoimmune diseases.

While the USC School of Medicine has many areas of research strengths, three specific research areas have been identified for further development. These areas include cancer, cardiovascular diseases and neuroscience. It is interesting to note that inflammation is a common thread that weaves throughout the pathogenesis of diseases represented in these areas.

## INFLAMMATION AND CANCER

Although inflammation is a necessary response to clear infections and to repair tissue injury, chronic inflammation has been shown to correlate an increased risk of developing cancer. Recent studies have revealed that inflammation is a critical component of tumor progression (Coussens and Werb, 2002). Inflammation functions at all three stages of tumor development: initiation, progression and metastasis. Inflammation contributes to the initiation of cancer by triggering the release of a variety of cytokines and chemokines which in turn cause oxidative damage, DNA mutations, and other changes in the microenvironment. Such changes make it more conducive for cell transformation and the increased survival and proliferation of tumor cells. Such novel insights are leading to the use of anti-inflammatory agents as therapeutic approaches to prevent cancer development and progression. The recognition of the importance of inflammation to oncogenesis has led to clinical trials investigating the use of anti-inflammatory drugs, such as COX-2 specific inhibitors for cancer prophylaxis and treatment. A NIH think tank on cancer biology has recently dealt with this topic at length (http://dcb.nci.nih.gov/thinktank/Executive_Summary_of_Inflammation_and_Cancer_Think_Tank.cfm).

## CARDIOVASCULAR DISEASES AND INFLAMMATION

It is becoming increasingly clear that inflammation of blood vessels is one of the major factors that increase the incidence of cardiovascular diseases, including atherosclerosis, hypertension, stroke and myocardial infarction or heart attack. Initiation and progression of vascular inflammation is a complex process involving macrophages of the immune system. The proinflammatory mediators produced by macrophages increase tissue oxidative stress and lipid retention, which participate directly in vascular remodeling (Yan and Hansson, 2007). Normally, endothelial cells (ECs), which line the blood vessel, resist adhesion by leukocytes. However, triggers of atherosclerosis, such as consuming a high-saturated-fat diet, smoking, hypertension, hyperglycemia, obesity, or insulin resistance, can initiate the expression of adhesion molecules by ECs, thus allowing the attachment of leukocytes to the arterial wall (Libby, 2006). After adhering to the endothelium, blood monocytes penetrate the endothelial lining and mature into macrophages, and engulf modified lipoproteins. Cholesterol esters accumulate in the cytoplasm, and the macrophages become foam cells through lipid uptake which characterizes the early stages of atherosclerosis. Also, the macrophages multiply and release several growth factors and cytokines, thereby amplifying and sustaining proinflammatory mediators (Libby, 2006). Thus, inflammation is central to the progression from fatty streak to complex plaque. Recent studies suggest that drugs commonly prescribed to lower cholesterol such as statins also reduce inflammation, suggesting an additional beneficial effect of such drugs.

Similar inflammatory tissue processes are also involved in the pathogenesis of hypertension. It is becoming increasingly clear that known mediators that increase blood pressure, such as angiotensin-II, also increase oxidative stress and inflammation both of which contributes to the target organ damage to the heart, brain, kidney, and blood vessels secondary to the hypertensive process. Our laboratory has recently demonstrated that certain endogenous neuropeptides, such as calcitonin gene-related peptide, improve hypertension by vasodilation and inhibiting oxidative stress and inflammation (Bowers et al., 2005). These studies provide an opportunity for the development of new pharmacologic targets to treat hypertension and its deadly consequences.

## INFLAMMATION AND NEURODEGENERATIVE DISEASES:

There is growing evidence that links immune system and the CNS. For example, various immune cells can traverse the blood-brain barrier. During the development of the CNS, blood monocytes populate the brain to differentiate into microglia. Invading lymphocytes can attack target antigens in the CNS such as during MS or produce growth factors that might protect neurons against degeneration. Immune molecules, such as interleukins and chemokines, are also expressed at high levels in neurons and may be involved in the communication of neurons with glial cells. Moreover, the inflammatory reflex is a neurophysiological mechanism that regulates the immune system (Oke and Tracey, 2007). The efferent branch of the reflex include the cholinergic anti-inflammatory pathway involving the vagus nerve, which inhibits inflammation by suppressing cytokine synthesis through the release of acetylcholine in immune organs, the liver, and the gastrointestinal tract. Thus, such a neurological control mechanism regulates inflammation via acetylcholine and suppresses production of proinflammatory cytokines (Oke and Tracey, 2007).

Inflammation during neurodegenerative disorders can be triggered by a number of causes including protein aggregates, molecules released from or associated with injured neurons or synapses, and dysregulation in the mechanisms that control inflammation. The resulting inflammatory responses may modulate neurodegenerative pathways with a potential beneficial or detrimental effect. Emerging evidence suggests that inflammation may account for chronic neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and Creutzfeldt–Jakob disease (Minghetti, 2005). In these diseases, inflammation is atypical and occurs in the absence of robust leucocyte infiltration. In these diseases, resident microglia which are the macrophages of brain parenchyma appear to play a major role. In healthy normal brain, microglia are present in an inactive phase as compared with other tissue macrophages, but subtle microenvironmental changes can induce microglia to react rapidly, change morphology and acquire an array of functions, including phagocytosis and secretion of inflammatory mediators. In addition to microglia, reactive astrocytes contribute to the process by restricting the area of lesion and releasing local mediators. This localized process, is distinct from inflammation seen in other tissues and is often referred to as ‘neuroinflammation’ (Minghetti, 2005). This unique neuroinflammation is a double-edged sword which is both neuroprotective as well as can trigger neurodegenerative disorders (Minghetti, 2005). Thus, understanding the dynamic relationship between beneficial and detrimental effects of neuroinflammation is central to the prevention and treatment of neurodegenerative diseases.

## TREATMENT OF INFLAMMATION

Historically, anti-inflammatory drugs were discovered when certain plants and their extracts were found to relieve pain, fever and inflammation (Rainsford, 2007). Salicylates were discovered in the mid-19th century from Willow and this enabled the synthesis of acetyl-salicylic acid leading to development of Aspirin. Subsequent research in 19th-

20th centuries led to the development of the non-steroidal anti-inflammatory drugs (NSAIDs), most of which were initially organic acids, but later non-acidic compounds were developed. The major adverse effects associated with NSAIDS were the associated gastro-intestinal (GI) toxicity. In the 1990's two cyclo-oxygenase (COX) enzyme systems controlling the production of prostaglandins (PGs) and thromboxane (TxA2) were discovered. COX-1 produces PGs and TxA2 which play a role in gastrointestinal, renal, and vascular functions, and COX-2 produces PGs which are involved in inflammation, pain and fever. This led to the discovery of inhibitors of the COX enzymes. While COX-2 inhibitors were enthusiastically received due to their low GI side effects, there are recent concerns regarding an increase in cardiovascular toxicity of these agents. Because inflammation plays a crucial role in the pathogenesis of a wide range of diseases including autoimmune diseases, allergies, cancer, cardiovascular diseases and neurodegenerative diseases as discussed above, one can imagine how crucial it is to discover new anti-inflammatory drugs, and the potential impact such discoveries will have on human health and disease.

## CONCLUDING REMARKS :

Inflammation is a process that enables the host to fight and overcome infections, cancer and help repair damaged tissues. Interestingly, however, inflammation has become one of the hottest areas of medical research because it also plays a significant role in the pathogenesis of a large number of human diseases, including autoimmune diseases, allergies, cardiovascular diseases, neurodegenerative diseases and cancer. It is indeed distressing that despite extensive research, highly effective treatment modalities do not exist to treat inflammation. The concept that inflammation contributes to the underlying cause of such varied diseases is so intriguing because it suggests the possibility to treat major human ailments through a single inflammation-reducing agent. Thus, it is not surprising that the NIH in its new roadmap initiatives for 2008 has identified inflammation as one of the key topics. The NIH website (http://nihroadmap.nih.gov/2008initiatives.asp) states, "While significant breakthroughs have occurred in our understanding of inflammation, research is needed to further understand inflammatory processes. Because inflammation is broadly implicated in many diseases and conditions, this initiative would be valuable in uncovering as-yet-unknown immune mechanisms and mediators of inflammation as well as genetic factors, environmental triggers, and the relationship of inflammation to disease".

The USC SOM is excited about the recognition of its research efforts afforded by the NIH to initiate and develop the Center for Autoimmune and Inflammatory diseases with the research focus on inflammation, and we believe that this initiative provides us with the niche not only to advance research on inflammation but also extend it to other areas of research including cancer, cardiovascular diseases and neurodegenerative disorder.

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# POLYMER-LAYERED OXIDE NANOCOMPOSITES

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## POLYMER NANOCOMPOSITES

Polymer nanocomposites are composite materials that consist of nanoscale additives dispersed in a polymer matrix where, typically, a layered material capable of exfoliation into nano-sized platelets is incorporated into the polymer. Since the composites are mixed on the nanometer length scale, they often exhibit enhanced properties compared to their macroscale counterparts, such as improved strength, stiffness, thermal stability, biodegradability, flame resistance, and gas barrier (Schmidt *et al*. 2002). Types of polymer matrices studied include, but are not limited to, vinyl polymers (polymethacrylate, polystyrene), condensation polymers (Nylon, polyethylene terephthalate), polyolefins (polypropylene, polyethylene), epoxides, rubber, and other specialty polymers such as polypyrrole and polyaniline (Ray and Okamoto 2003). Additives that are potential candidates for polymer nanocomposites include natural, commercial, and synthetic clays, layered silicic acids, layered hydroxides, layered double hydroxides, layered alumino-phosphonates and other metal oxides (Utracki *et al.* 2007). However, the vast majority of nanocomposite research is directed towards polymer matrices containing layered silicate platelets of nanometer thickness and high aspect ratio and these types of nanocomposites will be highlighted in later sections.

Nanocomposite materials are useful in a wide variety of applications in medical, automotive, fiber, textile, coatings, electronics, and packaging industries, among others. Research in this area has increased substantially after a Toyota research team developed one of the first successful montmorillonite–Nylon-6 nanocomposites in an effort to use Nylon-6 based parts in engine compartments and for automotive applications (Kojima *et al.* 1993, Usuki *et al.* 1993). Since then, layered-oxides, specifically layered silicates, have attracted a great interest as additives (Schmidt *et al*. 2002, Ray and Okamoto 2003, Kojima *et al.* 1993, Usuki *et al.* 1993, Alexandre *et al.* 2002, Alexandre and Dubois 2000, Bharadwaj *et al.* 2002, Carrado 2003, Chang *et al.* 2003, Chang and Yeong 2002, Dai and Huang 1999, Fornes *et al.* 2002, Garces *et al.* 2000, Gilman *et al.* 2000, Gopakumar *et al.* 2002, Grunlan *et al.* 2004, Hoffman *et al.* 2000, Imai *et al.* 2003, Jan and Lee 2004, Jordan *et al.* 2005, Matayabas *et al.* 2000, Pinnavaia and Beall 2000, Sekelik *et al.* 1999, Strawhecker and Manias 2000, Kim and Kim 2007, LeBaron *et al.* 1999, Tsai 2000).

Researchers at Eastman Chemical Company expanded on the work of Toyota, by incorporating montmorillonite, ion-exchanged with alkylammonium compounds, into polyethylene terephthalate (PET) via melt-blending and in-situ polymerization (Matayabas *et al.* 2000a, Matayabas *et al.* 2000b). They found that at even a low weight loading of montmorillonite clay, the oxygen permeability decreased dramatically. This was an important finding for Eastman, a leading producer of PET, as their motivation was to increase the gas barrier of PET for use in food and beverage packaging. Although research in polyester-clay nanocomposites is in a relatively early stage, over 28 patents for PET-clay nanocomposites were issued between the years of 1991 and 1998, suggesting that this a thriving area of research (Tsai 2000). Earlier methods of incorporating montmorillonite clay into PET were usually based on melt-blending, or melt-intercalation by extrusion. Typically these method do not facilitate full exfoliation of the clay layers but, nonetheless, there were some successful well-exfoliated nanocomposites produced where exfoliation and enhancement in properties were achieved (Vidotti *et al.* 2004, Yan *et al.* 2004). More recently, analogous to the research developed by Eastman, layered clays like montmorillonite, talc, and mica were ion-exchanged with compatibilizers or surfactants and incorporated into PET by in-situ polymerization to improve gas barrier (Sekelik *et al.* 1999, Ke and Yongping 2005, Ke *et al.* 1999, Chang *et al.* 2004, Tsai *et al.* 2005). Most recently, layered double hydroxides (LDHs) were ion-exchanged with anionic surfactants dodecylsulfate, dodecylbenzene sulfate, and octylsulfate and incorporated into PET by melt-extrusion (Lee *et al.* 2006). Only the dodecylsulfate LDH was well exfoliated in the PET matrix, and consequently the thermal and mechanical properties were enhanced. This research is encouraging as only a select few PET nanocomposites are prepared with a layered oxide other than a layered silicate. However, gas barrier properties were not studied for this nanocomposite system.

Although PET is the polymer traditionally used for packaging materials, research into biodegradable packaging materials is being developed for environmental reasons. Montmorillonite was mixed into a biodegradable potato starch by melt blending (Avella *et al.* 2005), where complete intercalation of the starch into the interlayer galleries was achieved, as well as and increase in tensile strength and modulus. The nanocomposite films formed complied with European regulations for biodegradable materials and therefore could be used as alternative packaging materials. Gas barrier was not measured for these nanocomposites.

Epoxy-clay nanocomposites have also been well established for over a decade. Typically the clay layers are expanded with onium ions and then the epoxide, curing agent, or a mixture of both, are intercalated into the interlayer gallery regions of the clay (Messersmith and Giannelis 1994, Wang and Pinnavaia 1998). This has been a proven method to exfoliate the clay layers into the epoxy matrix which improves mechanical properties, thermal stability, and solvent resistance.

Similarly, the most successful polystyrene-clay nanocomposites were prepared by melt-intercalating the polymer above its melting point into the interlayer galleries that had been previously expanded by quaternary alkylammonium ions (Via *et al.* 1996). Another polystyrene (PS) nanocomposite was prepared by covalently modifying layered mesostructured aluminosilicates (LMAS) with hexadecyl groups and mixing the organo-alumniosilicate with polystyrene by microcompounding (Chastek *et al.* 2005). The PS-LMAS had increased elastic modulus and strength. Although the LMAS were well dispersed, they appeared as stacks according to transmission electron microscopy images.

Poly(styrene-butadiene), a synthetic rubber copolymer, was intercalated into dioctadecyldimethyl ammonium exchanged montmorillonite by mixing the copolymer and the organoclay at 120 ºC (Laus *et al.* 1997, Laus *et al.* 1998). The nanocomposite formed showed an increased storage modulus. Natural rubber reinforced with organo-montmorillonite resulted in a 350% increase in strength without sacrificing the elasticity (Arroyo *et al.* 2003). Other research in rubber nanocomposites has indicated that silane modified kaolinite increases the compatibility between the hydrophilic clay and the rubber matrix, thus reinforcing the rubber (Dai and Huang 1999).

A polypropylene-bentonite nanocomposite is a yet another example of a clay that has been organically modified with a quaternary organic salt and that was added to a polymer matrix (Dai and Huang 1999). Bentonite, a layered aluminosilicate similar in structure to montmorillonite, was mixed with polypropylene using a batch mixer at 150-210 ºC at a 1-5 wt. % loading. The main result obtained was that the nanocomposites containing organo-bentonite had a higher thermal stability than nanocomposites formed with natural clays. The authors explain this phenomenon by the formation of a nano structure that reduced diffusion of oxygen into the material.

While the above nanocomposite materials mostly utilized layered silicates to reinforce the polymer matrix, other layered oxides are being incorporated into polymer matrices for use as electrolyte materials and conducting nanocomposites, among others. An example of a PET-LDH nanocomposites was briefly presented earlier, but other LDH polymer nanocomposite systems are being studied. In contrast to silicates, LDHs have a positive charge on brucite-like Mg(OH)2 layers, which can be compensated for by anions or polymeric anions (Wilson *et al.* 1999). This results in an interesting layer chemistry of the materials and makes them attractive for applications such as ion-exchange, catalysis, and even as antacids (Constantino and Pinnavaia 1995, Ookubo *et al.* 1993, Playle *et al.* 1974). Early research in polymer-LDH research was based on intercalation of anionic polymers poly(styrenesulfonate) (PSS) and poly(vinylsulfonate) (PVS) into the galleries of a carbonate-containing LDH, confirmed by an increase in the interlayer spacing by X-ray diffraction (Wilson *et al.* 1999). Similarly, another LDH was modified by ion-exchange with sodium dodecylsulfate or polyoxyethylene sulfate to form biocompatible nanocomposites (Yang *et al.* 2005). Making use of the anion exchange capacity of LDHs, a poly(ethylene oxide) (PEO)-LDH was prepared for potential use as a polymer electrolyte (Liao and Ye 2004). An oligo(ethylene oxide) (OEO) modified LDH was made by a template method and then subsequently mixed with PEO. The LDH layers remained well exfoliated due to the compatibility between the OEO and the PEO. The PEO-LDH nanocomposite exhibited a substantial enhancement in conductivity compared to the pristine PEO.

Recently, nanosheets of layered metal oxides have attracted interest as additives for nanocomposite electrolytes because of their physicochemical properties and ability to be intercalated with various species (Pang *et al.* 2005). Specifically, polyaniline-vanadium oxide nanocomposites are being explored because of their mixed electronic charge-transport properties (Wu *et al.* 1996).

A polyaniline-V2O5 nanocomposite was formed by in-situ polymerization of aniline intercalated into the layered V2O5 under hydrothermal conditions (Pang *et al.* 2005). Polyaniline- V2O5 nanocomposite sheets formed with a thickness between 10-20 nm and lateral dimensions on the range of hundreds of nanometers to several microns. Poly(ethylene oxide) (PEO) was also intercalated into a lithium trivanadate, LiV3O8, to prepare a solid polymer electrolyte with potential applications in lithium batteries.53 PEO is widely doped with lithium salts but efforts are underway to improve the conductivity of the doped-PEO. LiV3O8 is a promising material for lithium batteries because the structure allows for reversible ion-exchange of lithium cations. When the PEO is mixed with the LiV3O8 under semi-hydrothermal conditions, the PEO partially exfoliates the LiV3O8 layers. The PEO- LiV3O8 nanocomposite showed a higher ionic conductivity than LiV3O8 and other lithium salt polymer electrolytes (Yang *et al.* 2005). This research is a step towards the incorporation of other layered lithium containing metal oxides into polymers to form conducting nanocomposites. PEO was also introduced into the interlayer galleries of $HNbWO_6 \cdot 1.5\ H_2O$ using melt-intercalation for another potential solid polymer electrolyte (Sairam and Viswanathan 2002). $HNbWO_6 \cdot 1.5\ H_2O$ has a tetragonal structure with layers of $NbWO_6$ slabs made up of Nb/W oxygen octahedra, separated by interlayer water molecules. It was found that as the intercalation time (i.e. heating of a $HNbWO_6 \cdot 1.5\ H_2O$ -PEO pressed pellet to 75 ºC) was increased, the conductivity also increased.

Finally, and most recently, layered zirconium phosphates were synthesized and exfoliated into platelets with a high aspect ratio (>1000) for incorporation into various polymer matrices (Alberti *et al.* 2007, Sun *et al.* 2007, Zhang *et al.* 2007). The zirconium phosphates (ZrP) have an advantage over layered clays because of synthetic control over the dimensions and surface functionalities (Sun *et al.* 2007). A layered ZrP, $Zr(HPO_4)_2 \cdot H_2O$ was exfoliated using tetrabutylammonium hydroxide ($TBA^+OH^-$), and then the exfoliated platelets were isolated after centrifugation. These $TBA^+$ exchanged platelets were then re-dispersed into acetone and mixed with an epoxy monomer. The acetone was evaporated and a curing agent was added to form an epoxy nanocomposite containing well exfoliated ZrP platelets with high aspect ratio (Sun *et al.* 2007). Similarly, ZrP was exfoliated using alkylamines and intercalated with an acrylamide monomer, which was subsequently polymerized to form a polyacylamide-ZrP nanocomposite (Zhang *et al.* 2007). This nanocomposite had improved thermal stability, most likely from the retardant effect of the exfoliated ZrP layers. A Nafion membrane was also prepared with the addition of the same ZrP to enhance the stability of proton conductivity at higher temperatures, by increasing the stiffness of the composite membrane (Alberti *et al.* 2007).

These examples of successful nanocomposites reiterate the fact that research in this area is promising and a plethora of nanocomposite materials are being explored for a wide variety of applications. However, there are still obstacles present, such as incomplete exfoliation of the oxide, incompatibility of the oxide and the polymer, and the sacrifice of some properties for the enhancement of others. Ongoing research aims at finding ways to overcome these obstacles and to produce quality nanocomposites with improved gas barrier property and ideally, enhancements in mechanical properties as well. As an in-depth example of one type of polymer nanocomposite, a summary of the structure, properties, and preparation of PET based polymer nanocomposites is given below.

## 1.2. POLYETHYLENE TEREPHTHALATE

Polyethylene terephthalate (PET) is a polyester that is used in a variety of industrial applications, especially in the food and beverage industry as packaging material. Therefore it is imperative that the PET packaging retains a barrier to gases such as carbon dioxide and oxygen, as well as water vapor. PET has many desirable properties for a packaging material including clarity, color, processability, chemical resistance, recyclability and, most importantly for food and beverage containers, tastelessness (Matayabas and Turner 2000). However, PET alone has minimal gas barrier and, therefore, using it as packaging for foods and beverages that are sensitive to oxygen or loss of carbon dioxide is problematic. Thus, there has been a recent thrust in nanocomposite research to improve the gas barrier of PET, so that the shelf-life of products like beer, wine, and tomato-based products can be extended. Matayabas *et al.* 2000, Sekelik *et al.* 1999, Kim and Kim 2007, Matayabas and Turner 2000).

PET has been produced commercially for over 50 years, and is manufactured from ethylene glycol (EG) and terephthalic acid (TPA) or dimethylterephthalate (DMT) (Kim and Kim 2007). The polymerization of PET requires two main steps, transesterification or direct esterification, followed by polycondensation. Both esterification processes produce BHET, an oligomeric precursor to PET. In the final step excess EG is removed upon heating BHET to about 280ºC during polycondensation to form PET. This reaction scheme is shown in Figure 1.3.

Once PET is formed, there are two main methods of dispersing the additives to prepare nanocomposites: melt-blending or in-situ polymerization (Kim and Kim 2007, LeBaron *et al.* 1999, Tsai 2000, Ray and Okamoto 2003). Melt-blending involves mixing the polymer and the additive above the melting point of the polymer under high shear force. Melt-blending is typically used industrially to produce large scale quantities of PET, so it is advantageous to produce nanocomposites by the same method. However, the main difficulty lies in successfully exfoliating the layered additives by shear force alone. Few have reported the successful formation of PET nanocomposites by this method (Davis *et al.* 2001, Lyatskaya and Balazs 1998). For in-situ polymerizations, a PET monomer is intercalated into the layered structure, and subsequently polymerized. The most important advantage of this method is the fact that exfoliation of the layers is promoted and maintained by the presence of the intercalated polymer. As discussed earlier, exfoliation is a key to producing quality nanocomposites. The main disadvantage is that large-scale production of nanocomposites by this method is a difficult and time-consuming task.

## 1.3. LAYERED OXIDES

As previously mentioned, layered oxides are good candidates for nanocomposite additives because their layered structure allows for exfoliation and subsequent incorporation into polymer matrices. Simply stated, layered oxides are any oxygen-containing layered material such as, cuprates, titanates, phosphonates, niobates, or silicates. However, layered silicates are the most utilized layered material in the vast majority of ongoing research in layered-oxide nanocomposites. Layered silicates, or phyllosilicates, represent a large class of clay minerals that are distinguished by layers of silicate sheets coordinated to other metal-oxygen sheets. Groups in this class include micas, kaolins, vermiculites, chlorites, talc, pyrophyllite, and smectites. Smectites are the largest and one of the most widely studied groups because they are common in temperate soils, and have a high cation exchange capacity and a large aspect ratio (Carrado *et al.* 2001, Carrado 2004, Kloprogge *et al.* 1999). Two of the most researched smectites of this structure type are montmorillonite and hectorite. Montmorillonite is a readily available clay, while hectorite is an easily synthesized clay via mild conditions. Montmorillonite and hectorite are the preferred layered materials for polymer nanocomposite systems (Alexandre and Dubois 2000, Carrado 2003, Chang *et al.* 2003, Gopakumar *et al.* 2002, Loo and Gleason 2004, Maiti *et al.* 2002, Matayabas and Turner 2000, Nielsen 1967, Pinnavaia and Beall 2000, Strawhecker and Manias 2000).

Another layered silicate, magadiite, is a member of a class of materials known as layered hydrous sodium (or alkali) polysilicates that also includes kanemite, makatite, kenyaite and octosilicate. Their structure is made up of negatively charged tetrahedral silicate layers balanced by sodium cations. Magadiite has an even higher cation exchange capacity and aspect ratio compared to the smectites, and is easily synthesized under semi-hydrothermal conditions (Feng and Balkus 2003, Kooli *et al.* 2006, Kwon *et al.* 1995, Kwon and Park 2004, Peng *et al.* 2005, Schwieger and Lagaly 2004, Wang *et al.* 2006, Zhang *et al.* 2003). Research has shown that the incorporation of exfoliated magadiite layers into epoxy nanocomposites has increased the tensile strength of the epoxy matrix, while still maintaining transparent optical properties (Wang *et al.* 1996, Wang and Pinnavaia 1998). To date, research of magadiite nanocomposites for improved gas barrier properties is limited.

Theoretically, based on the gas barrier models presented in the following section, these layered silicates alone should improve the gas barrier of nanocomposites. However, unfavorable interactions between the hydrophilic silicates and the hydrophobic polymer may have a negative impact on other physical and mechanical properties. Therefore, the silicates are typically modified by covalently attaching organic functionalities to the surface hydroxyl groups, or by ion-exchanging with alkylammonium cations to expand the layers, and subsequently modify the interlayer surfaces (Peng *et al.* 2005, Zhang *et al.* 2003, Fujita *et al.* 2003, Isoda and Kuroda 2000, Ogawa *et al.* 1998, Okutomo *et al.* 1999, Wang *et al.* 2004).

## 1.4. NANOCOMPOSITE THEORY

There are three scenarios that can occur when a layered oxide is mixed with a polymer (Figure 1.1). First, the oxide can remain ordered and unexfoliated, forming a phase-separated composite. These conventional composites might have improved rigidity, but might sacrifice other properties such as elongation and toughness (LeBaron *et al.* 1999). Insertion of a polymer matrix into the layered silicate structure results in an intercalated nanocomposite, where the silicate layers remain ordered, but interfacial surface area between the silicate and polymer is greatly increased. This improves chemical, structural, and thermal stabilities compared to the polymer alone (Komori and Kuroda 2000). Finally, the best-case scenario would be complete exfoliation of the silicate layers well dispersed into the polymer. In this case, the clay-polymer interactions are maximized, and thus the chemical, physical, and mechanical properties are greatly enhanced. However, complete exfoliation is often a difficult task. Variables such as choice of matrix, process of incorporating the layered additive, choice of additive, treatment of additive (organic modification), and use of dispersing aids must be carefully considered (Matayabas and Turner 2000). Since exfoliation has been determined to have a profound effect on the performance of nanocomposites, a high aspect ratio of the separated layers would further improve the performance. Therefore, nanocomposites containing well-exfoliated silicate layers with the highest aspect ratio, to maximize the clay-polymer interactions, achieve the best results.

Aspect ratio ($\alpha$) is defined as the ratio of the lateral dimensions to the thickness of an exfoliated silicate platelet. Since the platelets are often irregular, the aspect ratio becomes

$$\alpha = \frac{\sqrt{A}}{Z} \qquad (1)$$

where A is the area of the platelet face, and Z is the thickness of the platelet (Auddy 2007, Kloprogge *et al*. 1999, Liu 2005, Ploehn and Liu 2006). It has been found that aspect ratio is crucial for improving certain properties, such as gas barrier (Alexandre and Dubois 2000, Jang and Lee 2004, Tsai 2000, Matayabas and Turner 2000). Nielsen developed the "tortuous path" theory to explain how aspect ratio effects the permeability of gases through a polymer (Alberti *et al.*, 2007). An approximation of the permeability ratio can be represented as

$$\frac{P_n}{P_m} = \frac{\phi_p}{\tau} \tag{2}$$

where Pn is the permeability of the nanocomposite and Pm is the permeability of the unfilled polymer matrix, $\phi$p is the volume fraction of the polymer, and $\tau$ is the tortuosity factor. The tortuosity factor is defined as the distance a gas molecule must travel through a polymer film divided by the thickness of the film. This factor is dependent on the aspect ratio in the following relationship:

$$\tau = 1 + \alpha\phi_f \tag{3}$$

where $\phi$f is the volume fraction of filler additives and therefore equation 2 becomes

$$\frac{P_n}{P_m} = \frac{\phi_p}{1 + \alpha\phi_f} \tag{4}$$

for ideal nanocomposite systems. This model is represented schematically in Figure 1.2, where exfoliated platelets provide a more "tortuous path" for the gas to diffuse through the polymer. A barrier improvement factor (BIF) can be estimated from the permeabilities, where

$$BIF = \frac{P_m}{P_n} \tag{5}$$

and the BIF is used to quantify the barrier improvement of nanocomposites (Liu 2005, Matayabas and Turner 2000, Nielsen 1967). More advanced models were also developed to account for overlapping in "semi-dilute" nanocomposites systems (Matayabas and Turner 2000, Nielsen 1967). Based on calculated aspect ratios of synthesized silicates, the barrier improvement of a nanocomposite can be predicted from this theoretical model and compared to experimental BIF values determined by various methods. More advanced models were also developed to account for overlapping in "semi-dilute" nanocomposites systems (Cussler *et al.*, 1998).

1.5. SUMMARY

Polymer nanocomposites are an exciting and active research area that promises new materials with enhanced properties. These polymer nanocomposites consist of nano-sized additives that are uniformly dispersed in a polymer host matrix, to generate substantial enhancements in physical properties relative to those of the pristine polymers. Research in nanocomposites has shifted towards layered oxides as the preferred additive, as their layered structure allows for exfoliation, or separation of layers, which increases the aspect ratio, and subsequently property enhancements.

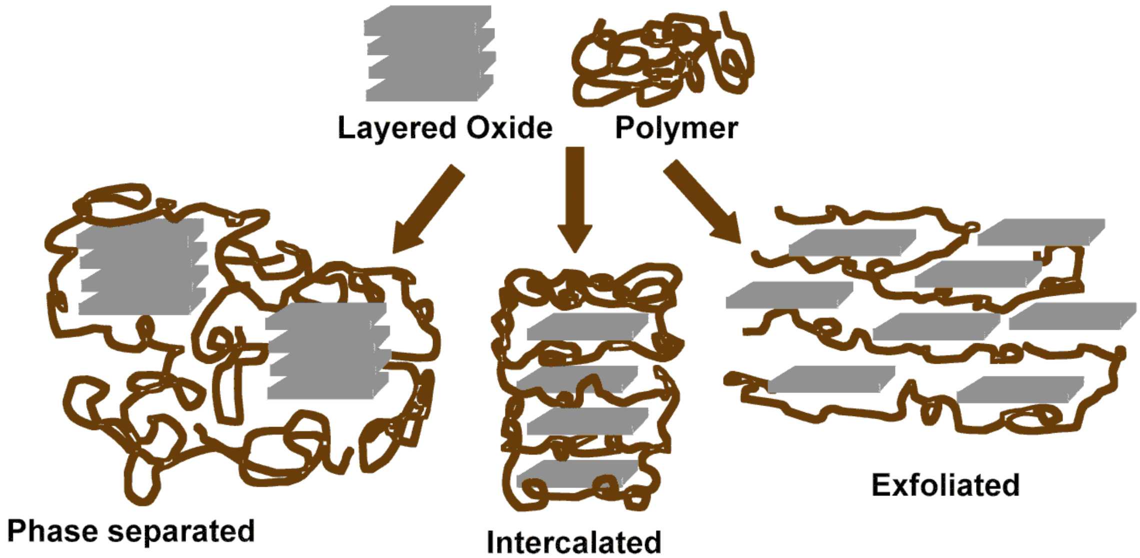


**Figure 1.1.** Schematic illustrations of three possible scenarios for dispersion of layered oxides in a polymer matrix.

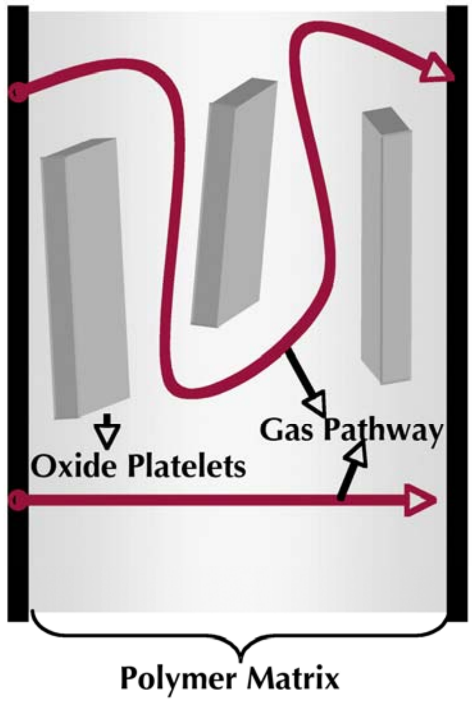


**Figure 1.2.** Illustration of the “tortuous path” model. Oxide platelets increase the path length of the gas, causing a reduction in permeablitity.

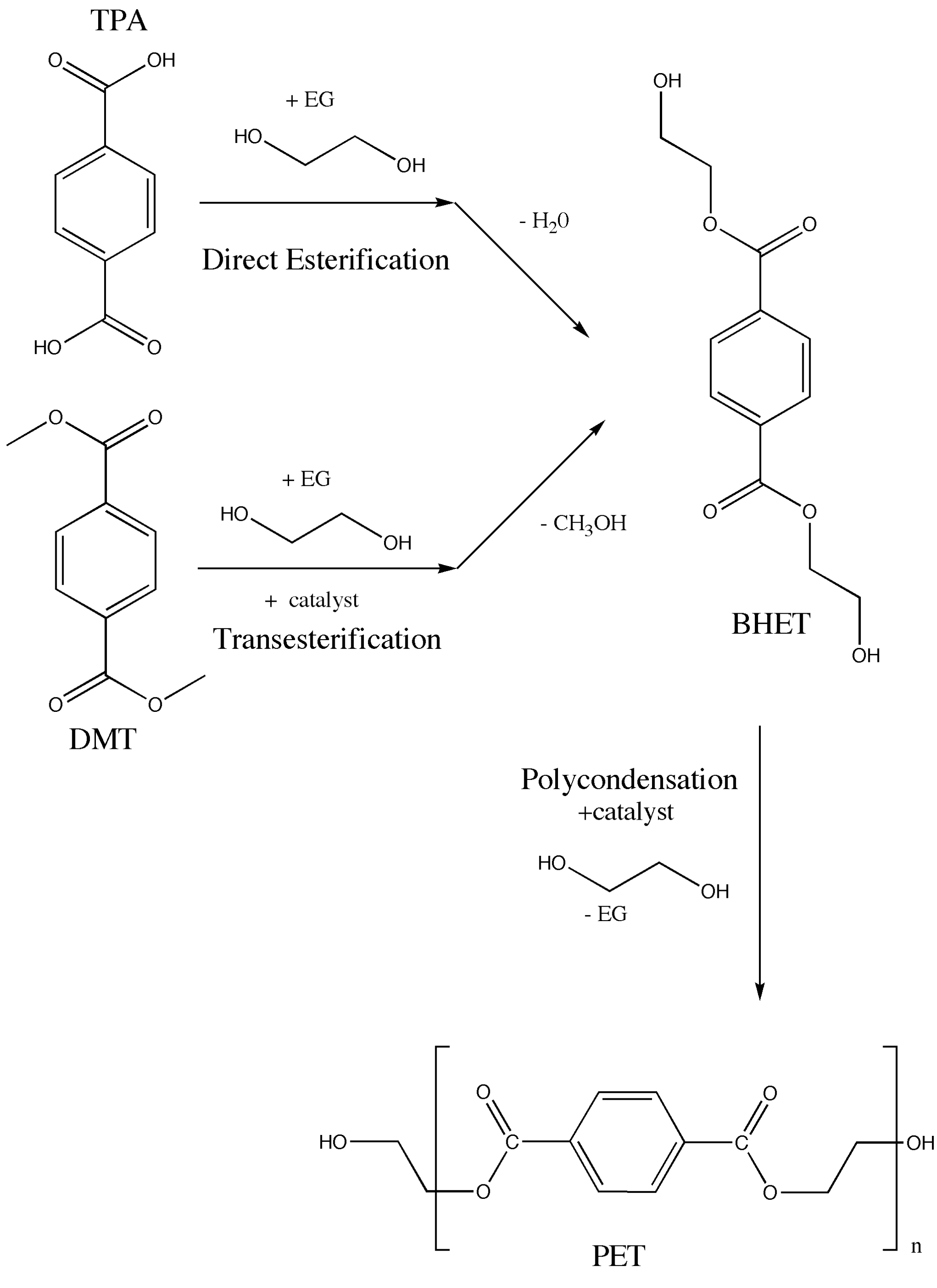


**Figure 1.3.** Reaction scheme for the PET polymerization process.

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