NATIONAL HEART, LUNG, AND BLOOD INSTITUTE PROTEOMICS CENTERS: Translating Proteomics Knowledge and Tools to Advance Biology and Medicine

On behalf of NewsSpots from NHLBI Proteomics Centers Program

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http://www.nhlbi-proteomics.org/
Dr Catherine Costello is Principal Investigator at the Boston University School of Medicine Cardiovascular Proteomics Center, where researchers are analysing and identifying proteins that may be modified or created by cardiovascular disease caused by metabolic disorders such as obesity and diabetes.

To begin, could you explain the overarching mission of the Cardiovascular Proteomics Center (CPC)? What is your position within the Center and what excites you most about your role?

It is our hypothesis that the specificity of detecting cardiovascular disease of metabolic causes will be greatly increased by a targeted proteomic approach to detect the effects of abnormal metabolism on proteins. Our present goal is to refine the proteomics pipeline and bioinformatics tools that we have built over the last 10 years by examining which proteins within the diseased heart and vasculature are modified and what modifications occur in response to metabolic disease – and then to identify a subset of these modified proteins in the plasma that show potential for use as tissue-specific biomarkers of the disease.

My roles are Professor of Biochemistry, Biophysics and Chemistry; Founding Director of the Boston University School of Medicine Center for Biomedical Mass Spectrometry; and Principal Investigator of the NHLBI Contract. I have overall responsibility to frame and supervise the research programme, coordinate the efforts of the laboratories and work with the investigators to interpret and communicate the results to NHLBI and the wider community. I am excited by the potential for direct translation of fundamental research into approaches that will be useful for diagnosis and treatment of patients and by the opportunities for training the next generation of researchers.

How does the Center’s work build on current understandings of oxidative chemical reactions in CVD?

Our project takes advantage of our discoveries that multiple covalent oxidative and reactive lipid and glycation modifications occur on plasma and urinary proteins and on proteins present in amyloid deposits in patients with systemic amyloid disease or pulmonary hypertension. We have identified far more than the usual range of examined modifications and have used our strong backgrounds in mass spectrometry, biology, physiology, biochemistry and organic chemistry to model the reactions and define the products.

How closely does the CPC work with the remaining six Centers of the NHLBI Proteomics Program?

We are physically closest to the Harvard-Broad Center. We are currently discussing the possibility for some metabolomic studies to be conducted with them. Last summer our two laboratories jointly hosted the semi-annual meeting of the investigators from all the NHLBI Proteomics Centers. We planned a three-day programme of lectures by invited speakers and investigators from the NHLBI Centers, as well as poster sessions. Well over 100 scientists attended the meeting. We have shared our data interpretation software with some of the Centers. We also shared the results of our evaluation of current commercial systems for quantitative proteomics and often provide advice on sample preparation, high performance mass spectrometry and bioinformatics.

How is the Center responding to challenges facing biomedical research today?

A major challenge is the uncertainty of sustained or new funding to support ongoing or planned research and to guarantee the survival of databases and tools that have been developed to promote biomedical research. We are working with our colleagues and professional societies to bring attention to this situation and to arrange for means to protect the future of research. We are encouraging cooperation among institutions to share resources and results. We are training promising investigators and are trying to support programmes that encourage young students, especially minorities, to consider and prepare for careers in research and medicine. Finally, we are organising courses and workshops to educate researchers and more general audiences.

What are your hopes for the future of the CPC?

We will first quantify changes in the abundances and modifications of heart and vascular tissue proteins in mouse models of human metabolic disease and then assess the occurrence of similar changes in members of the human population who are at risk of, or subject to, heart failure as a consequence of metabolic disease. The expected results of the proposed work will be a set of markers of metabolic dysfunction that should serve as candidate early biomarkers for the development of cardiac dysfunction as a result of metabolic syndromes as well as proven antibody and tandem mass spectrometric methods for the detection and quantification of the key candidates. These will provide new and powerful approaches to the detection and monitoring of metabolic cardiovascular disease. As it becomes appropriate and timely, we will extend these to other types of cardiovascular disease and to other classes of diseases.
Cardiovascular quest

Within the NHLBI Cardiovascular Proteomics Center, Boston University School of Medicine researchers have constructed a state-of-the-art laboratory for investigating oxidative post-translational modifications in metabolic cardiovascular disease.

CARDIOVASCULAR DISEASES (CVD) are the biggest cause of deaths worldwide. Metabolic conditions and diseases such as obesity, diabetes and hyperlipidemia are major causes for CVD in the Western world. Inflammation, oxidative stress, enhanced accumulation of lipids and lipid peroxidation in the heart and vasculature are at the root of diastolic heart failure, hypertension and cardiac and vascular hypertrophy, stiffening and dysfunction. However, the early detection and monitoring of the adverse effects of metabolic disease on the heart and vasculature remain elusive.

TRADITION IN CVD RESEARCH

Now, researchers at the NHLBI Cardiovascular Proteomics Center (CPC) at Boston University School of Medicine (BUSM) are hoping to find new and powerful approaches to the detection and monitoring of metabolic CVD by analysing and identifying proteins that may be modified or created by CVD. Investigators at the CPC propose that the ability to detect CVD caused by metabolic conditions will be vastly improved by using proteomics to examine the effects of abnormal metabolism on proteins. Led by Dr Catherine Costello, the team aims to define a set of markers of metabolic dysfunction that may provide early evidence for the development of CVD as a result of metabolic syndromes, and to develop antibody and tandem mass spectrometric methods for the identification and quantification of the key candidates.

BUSM has a long tradition in cardiovascular research and is the host institution for the Framingham Heart Study, another NHLBI project that has followed CVD development over six decades in three generations of participants. Costello affirms that the NHLBI proteomics programme has provided the opportunity for BUSM to broaden the talents, resources and instrumental capabilities within its NIH-GM Research Resource for Mass Spectrometry in Biology and Medicine, allowing the CPC to undertake more extensive proteomics studies than would otherwise have been feasible.

KEY ACHIEVEMENTS

Since its establishment in 2002, the CPC has made a number of breakthroughs, not least the successful construction of a cryogenic mass spectrometer that exceeded performance expectations and a state-of-the-art proteomics laboratory. They have developed approaches for identification and quantification of post-translational protein modifications that have been widely adopted in the field and identified the sites, types and extent of oxidative post-translational modifications on proteins of interest. Moreover, they have described fluorinated alternatives to expensive ICAT reagents that offer the advantage of clean separations based on polarity. The mechanisms of fragmentation of peptides, proteins, glycoproteins and glycans have been explored, and the team’s results have facilitated the use of these new approaches for the structural determination of unknowns. New software has been created to exploit the strengths of the Center’s laboratory instruments, many of which make use of public resources and are compatible with shared use as much as possible.

Furthermore, Costello points out that the group is now applying the proteomics methods developed in their cardiovascular studies to other diseases of interest to the NHLBI, such as pulmonary hypertension, sickle cell disease and systemic amyloidosis, as well as diseases that involve autoimmune responses to circulating antigens, such as rheumatoid arthritis and antibiotic-resistant Lyme disease.

COLLABORATING ON PROTEOMICS

Proteomics is still a new and rapidly developing field which has evolved greatly since the first NHLBI Centers were established in 2002. Costello believes that it is essential that there is national and international cooperation in the field in order to make the most efficient use of resources and to share knowledge that can advance the science. The CPC collaborates with scientists locally and around the world and hosts visits from investigators: “We are active in publishing our cardiovascular research and in presenting lectures and tutorials and organising sessions at professional meetings such as the American Heart Association, the Society for Free Radical Biology, the American Heart Failure Society and the International Foundation for Heart Research,” Costello adds. She is confident that the NHLBI programme has tied basic and clinical investigators together in a common purpose so that the possibilities for translational research are greatly enhanced: “This gives rise to a much more comprehensive research programme than any of us could have undertaken alone and stimulates new ways of thinking among both experienced and young investigators”.

CATHARINE E COSTELLO is Professor of Biochemistry, Biophysics and Chemistry at Boston University; Founding Director of the BU Center for Biomedical Mass Spectrometry; President of the Human Proteome Organization, and VP of the International MS Foundation. Her research develops and applies MS-based methods for investigating post-translational modifications of proteins, cardiovascular disease, protein folding disorders, glycobiology and bioactive lipids.

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The heart of innovation

Dr Jennifer Van Eyk heads up one of the seven Proteomics Centers of the National Heart, Lung, and Blood Institute, focusing on technological innovation in proteomics in the arena of heart failure

The breadth of expertise in our team in proteomics, mass spectrometry, protein and subproteome separation, protein and PTM assessment, peptide and lectin array, bioinformatics, database development and cell modelling is unparalleled. This is magnified by the breadth of expertise of our exceptional Clinical Researchers. Principal Investigators work together in each technology programme and then move to collaborate extensively with the Clinical Researchers. This approach drives the integrative and collaborative nature of our Center.

How does the Center provide rapid paths to translational medicine?

The extensive collaboration between clinical, basic and technological laboratories allows our Center to address unique questions. It enables the rapid integration of cutting-edge proteomic technologies into top-notch scientific programmes addressing issues that will impact the diagnosis and care of heart failure patients and ultimately drive novel therapies.

Could you account for the current gaps in understanding of heart failure? To what extent does the Center’s work on protein modifications close the knowledge gap?

Heart failure is a clinical syndrome in which cardiovascular function is insufficient to support the body’s metabolic needs. Our Principal Investigators apply state-of-the-art proteomic methods and develop new approaches and techniques to investigate the biological and clinical aspects of heart failure. The burden of heart failure is substantial and increasing, with 5-6 million people affected in the US. Several factors contribute to its aetiology and evolution, including ischaemia, hypertension, diabetes and genetic predisposition. Regardless of therapeutic efforts, mortality and morbidity remains high.

Many gaps in understanding heart failure remain and it is the goal of this Center to identify how heart failure impacts signalling cascades and mitochondrial, contractile, cell surface and cell secretory subproteomes, emphasising PTMs to discover novel ways by which protein modifications either contribute to disease or could be targeted to improve disease outcome. We develop new methods and approaches for quantitative analysis of a number of PTMs, which are integrated with bioinformatics and cellular modelling to allow development and testing of new therapies.

Could you elaborate on the Center’s past successes in developing innovative technologies and the significance of these achievements?

Our recent successes include development of proteomic methodology, software, myocyte modelling and publicly-available proteomic databases which have been applied to a number of biological questions in heart, lung, and blood. One such database is the human protein reference database (www.hprd.org), which is annotating every human protein and is widely used in all of the medical sciences fields.

The group has filed 11 patent applications in the areas of technology applications and biomarker discoveries concerning ischaemic heart disease and has produced hundreds of peer-reviewed papers and a book on clinical proteomics.

What is the Center’s long-term mission?

Our exceptional group of scientists will continue to develop and disseminate new tools and proteomic technologies focused on central questions relevant to heart failure. Our strengths lie not only in our excellence in proteomics in terms of analytical approaches, bioinformatics, systems modelling and range of functional assays but also in the breadth of animal models and unique human samples brought to us by world-class cardiologists, epidemiologists and physiologists. Looking ahead, our Center will continue to push the frontiers forward, providing accelerated intellectual payoffs and innovation in technological and data analysis resources for the broader scientific community.
Advancing knowledge of the heart

The Johns Hopkins University Proteomic Innovation Center in Heart Failure breaks new ground to uncover the underlying molecular causes of cardiovascular disease.

CARDIOVASCULAR DISEASE (CVD) claims millions of deaths worldwide each year. One of the forms it can take is heart failure, in which the heart stops pumping blood properly. Lifestyle is a major risk factor for heart failure, but there are a number of other factors, such as diabetes and genetic predisposition. The incidence of heart failure is on the rise worldwide, and although numbers in the West are proportionately nearly double those in less industrialised parts of the world, the incidence even in those regions is increasing significantly.

When it is not fatal, the condition of heart failure is severely debilitating, reducing quality of life and necessitating frequent medical interventions at a major cost to health services.

As changes to genes and proteins in heart cells play a role in the development of the condition of heart failure, advances in proteomic knowledge are key to unravelling the mechanisms responsible. The Johns Hopkins University (JHU) Proteomic Innovation Center in Heart Failure is a center of excellence in state-of-the-art research into innovative proteomic technologies, methods and targets. Its mission is to obtain new insights into the mechanisms of heart failure; to pinpoint biomarkers for new diagnostic aids; and to enable the development of targeted and timely treatments.

Dr Jennifer Van Eyk, Professor of Medicine, Biological Chemistry and Biomedical Engineering at JHU, is the Proteomics Center's Director. She oversees the research of Principal Investigators who work collaboratively on a number of challenging projects in search of biological, clinical and technological advances before moving on to work with Clinical Researchers at the JHU Medical School to bring about changes for heart failure patient treatments: “We believe that the focused application of existing and emerging proteomics approaches will revolutionise our understanding of heart failure, resulting in identification of new paradigms, potential therapeutics and new biomarkers for the prognosis and risk assessment of patients,” she explains. “The highly collaborative and integrated nature of this group of investigators allows us to keep focused on clinically-relevant problems and to provide rapid paths to translational medicine.”

TECHNICAL AND CLINICAL CHALLENGES

The JHU Proteomics Center is currently developing methods for identifying and quantifying the changes in amino acids in certain important proteins after post-translational modification has occurred, exploring the crosstalk between signalling cascades and the end effector molecules. Developing workflows for improved separation and enrichment of proteins and peptides and mass spectrometry methods to extend detection and quantification in tissues and body fluids is another workstream; further work at the Center is dealing with integration of sophisticated cell modelling with quantitative proteomic data and functional endpoints to obtain insights into the interplay between subproteomes of the human cardiac myocyte.

A bioinformatics project is focused on improving protein identification, maximising protein characterisation, and involving the creation of databases of human cardiac proteins, including their post-translational modifications and a separate cardiac proteome peptide database. The objectives of this research are to improve integration between Center projects and to help identify targets for further investigation.

The Center is undertaking a number of studies, using experimental animal models and myocardial and blood samples from patients with heart failure in order to understand the operation of proteins involved in heart muscle contraction in heart muscle disease arising from diabetes, the action of acetylation on mitochondria and its effects on metabolism that lead to heart failure, G protein signalling in heart failure, and the development of a patient risk stratification method based on plasma biomarker identification.

Combining state-of-the-art and pioneering methods with innovative biology in search of improved heart failure treatment is the cornerstone of the JHU Proteomics Center philosophy, as Van Eyk affirms: “Our investigators believe, as I do, that technological innovation can leap science forward, especially if focused on key clinical questions with the end goal of clinical application”.

DR JENNIFER VAN EYK earned her PhD in Biochemistry at the University of Alberta, Edmonton, Alberta, Canada and is the current Director of the Johns Hopkins NHLBI Proteomics Innovation Center in Heart Failure at the Johns Hopkins School of Medicine in Baltimore, Maryland. Director, Bayview Proteomics Center, and Professor of Medicine in the Division of Cardiology and the departments of Biological Chemistry and Biomedical Engineering. Her laboratory is a leader in the field of clinical proteomics, which integrates cutting-edge proteomics and drives innovation in heart and vascular disease.

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Could you begin by summarising the Harvard-Broad Proteomics Center’s strategic aims and objectives and how the three core components of the Center’s approach feed into these goals?

Our Center is focused on pathways and biomarkers in ischaemic heart disease. Our approach integrates three core components: Proteomic Technology Development led by Dr Steven Carr, Molecular Mechanistic and Functional Studies led by Dr Anthony Rosenzweig and myself and Application of Proteomics Approaches to Clinical Questions led by Dr Marc Sabatine and myself. The overall goal is to identify new metabolites and proteins that mark disease activity, shed insight into disease progression and ultimately provide targets for therapeutic intervention. We believe that we have a cohesive multidisciplinary approach to this problem. While there are some divisions in the tasks, all of the scientists contribute to each of the core components – we are pleased with the level of cross-fertilisation.

What is your background in myocardial ischaemia? What led you to head up the Harvard-Broad Proteomics Center?

The focus of investigation in my group has centred on the signals that drive plaque generation in coronary arteries, which ultimately leads to myocardial ischaemia. Our group has gravitated towards an interest in understanding the downstream sequelae of atherosclerosis (myocardial dysfunction) in addition to the initiating vascular events.

Can you explain how simultaneous assessment of multiple biomarkers examining different pathophysical axes will provide complementary information to improve diagnosis and clarify prognosis of myocardial ischaemia?

If a number of biomarkers are all reporting on the same pathway, they are unlikely to add diagnostic or prognostic information when combined (I sometimes say that this is a case where ‘one plus one plus one only gives you one’). By contrast, biomarkers that report on different physiological aspects of a disease process (‘orthogonal’ markers) are far more likely to provide improved diagnostic/prognostic accuracy. Furthermore, they provide additional hints as to relevant pathways and disease biology.

What do you hope to achieve by investigating the synergy between discovery efforts for clinical biomarkers and mechanistic models?

Those biomarkers that are also in causal pathways are of particular importance since they might ultimately serve as surrogates to assess therapeutic interventions for ischaemic heart disease.

Why are your research efforts focused on carefully phenotyped human models, and how were these clinical cohorts of patients selected?

Even state-of-the-art proteomics platforms are simply not able to deal with large patient sample sizes at this point. We have chosen carefully phenotyped cohorts – human ‘perturbational studies’ (exercise challenge, ‘planned heart attacks’) in which each person serves as their own biological control. Each individual is very closely characterised, something that is impossible in a population-based study. We try to ‘win’ with phenotyping over numbers.

The Center works with several hospitals and medical centres. Could you describe the extent of this relationship and the benefits afforded from such an association?

Our programme combines three hospital-based research programmes with one of the most important genomic institutes in the world. The hospital-based research benefits from the ‘genomic platforms’ at the Broad Institute. By contrast, the Broad ‘high-throughput, big science’ expertise is complemented by disease-based research with insights into the clinical setting. We are hopeful that this will catalyse the translation of our findings to the clinic.

To what extent has the Center proved the hypothesis that an unbiased proteomics discovery platform will yield novel biomarkers and insights into pathways activated upon myocardial injury?

In a recently published manuscript in Nature Biotechnology, we published a number of potential novel markers of myocardial injury. We have already validated several of the markers in additional heterogeneous patient cohorts. A manuscript describing these further validation efforts is currently being finalised for submission.

The next goal of course is understanding these markers better: from a clinical perspective, do they add useful information on top of existing clinical parameters? From a biological perspective, do they contribute in a causal manner to disease pathophysiology? Those are the next key questions.

What would you underline as the Center’s key success to date?

Our Nature Biotechnology manuscript integrates a cohesive plan to go from proteomics discovery to initiate the validation process.
Pathways to treating myocardial ischaemia

The Harvard-Broad NHLBI Proteomics Center is comprised of a consortium of institutions working together to better understand ischaemic heart disease by identifying new proteins and pathways that mark disease activity in order to provide novel targets for treatment.

**ISCHAEMIC HEART DISEASE** is characterised by reduced blood supply in the heart muscle, usually due to atherosclerosis of the coronary arteries. It is the most common cause of death in the Western world and a major cause of hospital admissions. In patients with ischaemic heart disease, small biochemicals and proteins are the end result of a chain of regulatory changes that occur in response to physiological stressors, disease processes or drug therapy; these circulating metabolites and proteins serve as both biomarkers and regulatory signals, such as in the control of blood pressure.

**CONSORTIUM OF INSTITUTIONS**

Now, researchers at the Harvard-Broad Proteomics Center are using proteomics technologies to investigate these biochemicals and proteins, with an aim to establish an infrastructure for the discovery and validation of novel pathways and biomarkers triggered by myocardial ischaemia. Led by Dr Robert E Gerszten, the multidisciplinary group of investigators at the Center bring expertise in basic science, diagnosis and treatment of acute coronary syndromes, epidemiology and bioinformatics, basic and clinical chemistry and proteomics. The Center is comprised of a consortium of cooperating institutions, including the Massachusetts General Hospital, Brigham and Women's Hospital, the Thrombolysis in Myocardial Infarction Study Group, Harvard Medical School, the Beth Israel Deaconess Medical Center, and the Broad Institute.

The current work of the Center builds on some of Gerszten's initial observations and three of his team's working hypotheses: first, that a proteomics approach will assist in the discovery of novel biomarkers and pathways originating from myocardial injury; second, that combining discovery efforts for clinical biomarkers and mechanistic models will help identify the best pathways for further evaluation in both; and third, that by assessing multiple biomarkers which report on different pathophysiological pathways, the team will obtain complementary information to improve diagnosis and prognosis, guide therapy, and provide targets for the treatment of myocardial ischaemia.

**PHENOTYPED PATIENT COHORTS**

The Center’s cutting-edge technologies are matched with unique patient cohorts from the clinical cardiovascular population at the Massachusetts General Hospital. For Gerszten, their goal is not to capture all cardiovascular patients presented to the Hospital but rather to focus their efforts on extremely well-phenotyped patients. Of particular interest to the researchers are “perturbational” studies in humans, for example, cardiac exercise testing; such investigations elicit robust phenotypes in affected individuals to serve as the springboard for analyses that range from genomics to proteomics and biochemical profiling. They then take their findings and explore the usefulness of the markers in clinical trials with the TIMI Group.

**PLASMA ANALYSIS**

The Harvard-Broad group is now building on ongoing work in which they have established liquid chromatography-tandem mass spectrometry (LC-MS)-based discovery protocols to identify differentially expressed low abundance constituents of plasma and tissue, as well as targeted LC-MS-based assays, to move quickly from discovery to initial target validation. Their studies have helped pioneer the application of novel mass spectrometry and liquid chromatography techniques to plasma analysis.

In parallel with the team’s profiling efforts, the Center has developed statistical software for functional pathway trend analysis and used it to demonstrate significant coordinate changes in specific pathways. Such analysis has allowed them to gain insight into the functionally relevant cellular mechanisms contributing to disease pathways and increases the likelihood that prospective biomarkers will be validated in other patient cohorts, leading ultimately to the development of novel therapeutic interventions for ischaemic heart disease.

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Clinically actionable research

Dr Garry Nolan describes the Stanford University Proteomics Center’s overarching mission, with particular attention to its focus on improving treatment for autoimmune diseases

Could you begin by explaining the mission of the Stanford University Proteomics Center?

Stanford University’s Proteomics Center focuses on autoimmune and inflammatory diseases that affect the immune system such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), allergy, cancer, infectious diseases and, more recently, diseases that attack the heart and lungs. Our overarching mission is to develop, validate and disseminate cutting-edge technologies for studying such diseases.

In what way does the Center’s cell- and blood-based analysis advance existing understanding of RA, SLE, and related disorders?

RA and SLE have been studied extensively and are known to be driven by inflammatory cytokines such as TNF, IL1, and IL6 (in RA) and interferon alpha and BAFF (in SLE). The genetics of both diseases are also actively being studied. Surprisingly, these new discoveries have yet to lead to patient-specific or even patient-tailored therapies. The pathogenesis of PAH is even more poorly understood. One of our major goals is to build on our many successes in SLE and RA to better understand the mechanistic underpinnings of PAH.

How does your work on RA and SLE link to research on PAH?

SLE and RA are classic autoimmune diseases. Self molecules are targeted by T and B lymphocytes which then attack organs and blood elements. Both diseases, as well as the related disease systemic sclerosis – also called scleroderma – are associated with interstitial lung disease, accelerated atherosclerosis and PAH. Moreover, a subset of PAH patients develop serum autoantibodies, suggesting that a large subgroup of PAH patients may have autoimmunity as the driving factor. A corollary to this is that patients with PAH whose disease is driven by autoimmune mechanisms may benefit from immunosuppressive therapy or even bone marrow transplantation.

Could you elaborate on the cutting-edge technologies developed at the Center?

Our Center is at the forefront of inventing and validating new methods for characterising patients and subsetting diseases. These include methods to study blood cells, including phosphoflowcytometry, high-throughput immunophenotyping using transcription (HIT), reverse phase lysate arrays and, more recently, CyTOF mass cytometry, and techniques for measuring thousands of autoantibodies simultaneously, including protein and peptide arrays, Intel Arrays and mass spectrometry. Some of these methods are already being used extensively in phase I to III human clinical trials. They have successfully led to invention of kinase inhibitors for RA with Rigel Therapeutics and development of antigen-specific therapies for juvenile diabetes and multiple sclerosis with Bayhill Therapeutics. One of our goals is to develop our methods for point-of-care diagnostics and for Clinical Laboratory Improvement Amendments-certified assays in hospitals and clinics.

One research project at the Center is characterising serum autoantibodies and cytokines to define the autoimmune targets associated with PAH initiation and progression. Why is it important to develop ‘clinically actionable’ diagnostics for this disease?

Our Center only works on diseases and models that translate to the human condition. Dr Robinson has coined the term ‘clinically actionable,’ which means that a discovery made in rodent models or in studying human samples can directly affect diagnosis, management and therapeutic intervention. Almost all of our investigators are clinically active; doing research just for the sake of research does nothing to help our patients with such devastating illnesses.

What hopes do you have for the future of autoimmune and proteomics research?

There are several holy grails. First is the development of antigen-specific immunotherapies for autoimmune disease. As with allergy desensitisation, we plan to identify target autoantigens, then to develop tolerising therapies to turn off the immune response to the self-antigens while leaving global immunity intact. Second is the development of research-grade assays that allow us to subset patients and to predict response to targeted therapies. Only about a third to a half of patients respond to anti-cytokine therapies like Enbrel or Actemra, at a cost of $20,000 per year. This is not sustainable as a society nor is it in the best interests of our patients. And finally, we hope to convert a subset of our assays from research-grade to clinical-grade, bringing them all the way to the marketplace.

How significant is the NHLBI Program?

The Proteomics Center Program has been instrumental in the development of all of the technologies I’ve just described. As National Institutes of Health funding becomes more and more difficult to obtain, it is critical that programmes like this one remain in place.
The Stanford University Proteomics Center develops technologies beyond the state of the art for exploring and counteracting diseases caused by rogue immune system functions.

A HEALTHY IMMUNE SYSTEM protects the body against infections. When it malfunctions, it produces antibodies that attack healthy tissues instead of invading bacteria and viruses, occasioning autoimmune disease. There are about 100 autoimmune diseases currently known, all generally debilitating and disabling and some deadly. They tend to affect women much more than men. Though more prevalent in Western societies, their incidence is rising worldwide; worryingly, because their underlying mechanisms are largely undiscovered, they are difficult to diagnose, and current treatments cannot reverse them.

Some of the best known autoimmune diseases are Rheumatoid Arthritis (RA), Multiple Sclerosis (MS) and Systemic Lupus Erythematosus (SLE, commonly known as Lupus). There are many millions of sufferers, with an estimated 23 million at least in the US alone. Because of the sheer numbers of victims and the financial and other burdens that autoimmune diseases bring, it is important that new biological knowledge about these diseases is shared with the world; this is a core tenet of Stanford University Proteomics Center research.

The Center concentrates on RA; Pulmonary Arterial Hypertension (PAH), an incurable lung disease; MS; and SLE, developing new technologies and techniques for cellular and proteomic analysis that can be directly translated to clinical solutions. Dr Garry Nolan, head of the Nolan Laboratory at the Stanford Proteomics Center, believes that the innovation afforded by the Center comes largely as a result of the National Institutes of Health Proteomics Center Program: “Breakthroughs follow the invention of transformative methodologies, and they almost always require risk-taking,” he affirms. “Programs such as this allow Stanford Investigators to take risks in inventing new methodologies and to be creative in their interactions with each other and investigators at other Centers.”

COLLABORATING LABORATORIES
Each of the individual projects at the Center is autonomous, but the different laboratories, with their varying philosophies, expertise and disease interests, converge to expedite development of new treatments that can be used to treat autoimmune diseases: “We study blood cells, signalling pathways, autoantibodies and cytokines specifically because defects in all of these molecules or pathways are ‘drug-able,’ or can be used to improve diagnosis and prognosis,” states Nolan.

The Utz laboratory focuses on developing technologies for high-throughput studies of biological samples and purified proteins and is currently exploring microfluidic assays in collaboration with the Santiago Microfluidics Laboratory at Stanford University, leveraging the microfabrication techniques used in microelectronics to obviate the problem of evaporation of solvents during protein analysis and to allow for very rapid creation of assays for proteomics analysis. In this work, the connective tissue autoimmune diseases RA and SLE are the focus. The Steinman Laboratory looks into the pathogenesis of autoimmune diseases and is developing microarrays for detecting autoantibodies, especially for MS and neuromyelitis optica. The Robinson laboratory is developing antigen arrays for simultaneous profiling of autoantibody responses against large panels of candidate autoantigens to identify new autoantigens and develop autogen-specific tolerising therapies for SLE and RA. The Chu Laboratory is focused on how cells respond to DNA damage from ionising and ultraviolet radiation in cancer. The Tibshirani laboratory is mining, standardising and classifying proteomics data from multiple inconsistent sources to create vast standardised datasets that can be integrated with genomics data for ready interrogation.

Nolan reveals how the Center achieves synergies: “For example, Dr Kodadek is a renowned chemist, Dr Robinson is an expert in multiplexed proteomics assays, and my laboratory is constantly pushing the edge of single cell multiparametric techniques. Our projects are highly integrated in that analytes identified in Dr Utz’s lab using lysate arrays and high-throughput immunophenotyping using transcription are then used to generate flow cytometry reagents for my lab. Similarly, Dr Kodadek’s peptoid libraries are used to identify flow reagents and immobilise them in planar arrays with Drs Robinson and Utz. While each of our labs is excellent in its own area, by combining expertise through interdisciplinary research, we can address questions that no single lab could ever answer.”

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DR GARRY NOLAN is the Rachford and Carlota A Harris Professor, and his laboratory is in the Department of Microbiology and Immunology, Baxter Laboratory for Stem Cell Biology. He is the Director of the Proteomics Center at Stanford University. His research interests include development and application of advanced single cell analysis systems using mass spectrometry and other methods. Disease areas of interest are autoimmune and inflammatory processes, normal haematopoietic development, and cancer. Kinases, intracellular and intercellular signalling systems, network-based analysis, and biocomputational approaches are key to the lab’s studies.
Dr Merry L Lindsey directs pioneering cardiovascular proteomics research at an NHLBI Proteomics Center investigating the responses of the extracellular matrix following myocardial infarction. This research could drive the development of better treatment strategies for heart attack patients.

To begin, how would you sum up the defining goals of UTHSCSA Cardiovascular Proteomics Center?

The major goal of our Center is to develop novel proteomic technologies to identify predictive markers of adverse remodelling of the left ventricle following myocardial infarction (MI), with a focus on extracellular matrix (ECM) fragment generation as a key initiating event.

How would you quantify the impact of the NHLBI’s recognition of UTHSCSA in selecting the institution as the home to one of its seven Proteomics Centers?

The impact is tremendous. Selecting our Center sent the message that the ECM environment is an important component to understand. We are the only Center focused on examining ECM responses in the heart following a heart attack, and we are thrilled to represent the ECM community.

Multidimensional approaches are integral to the studies undertaken at the Center. Could you offer an example of such a holistic strategy in practice, both in terms of personnel, disciplines, and tools?

Our Center epitomises the multidimensional approach. We couple physiology, pathology, biochemistry, cell biology, engineering, and computational approaches to our proteomic techniques, which provides a systems biology look at the post-myocardial infarction response. Along the same lines, the investigators in our Center each have complementary non-overlapping roles, which provides a remarkable array of expertise.

In terms of translational medicine, what success have you had in the development of therapeutic strategies to prevent, slow, or reverse the progression to MI?

As a result of funding by the NHLBI, we have identified several target peptides that have translational potential. We are currently recruiting patients into a study that will tell us whether the changes we see in our mouse model also occur in humans who have a heart attack.

What have you revealed about the biological mechanisms at work when the left ventricle responds to injury and how a greater understanding of these characteristics can be applied?

We have shown that the protein matrix metalloproteinase 9 (MMP-9) mediates several components of the remodelling process. In particular, the inflammatory and fibrotic responses are highly MMP-9 dependent.

Could you define the impact of ECM fragment generation in adverse remodelling of the left ventricle following MI?

The impact of ECM peptide generation is huge – the idea that these peptides can serve as upstream signalling molecules to initiate cascades has not previously been studied in the cardiovascular setting.

Has work at the Center resulted in the identification of biomarkers that may delineate a patient’s vulnerability to heart failure, and if so, at what stage are these investigations?

Right now, we have identified several biomarkers that increase in our mouse model, but whether these markers can serve as predictors and whether they also occur in humans is what we still need to discover.

What technological tools have been pioneered at the Center, and to what extent have they enabled scientific discovery?

The crucial technological tool that we have developed is decellularisation of the left ventricle, which allows us to remove the cellular compartment and focus in on the outside environment. Without this technology, it would be difficult, if not impossible, to target the ECM.

Looking ahead to the next few years, where will your research priorities lie at the Center?

Our research priorities are clear. First, we need to test and validate the peptides we have already identified. Second, we need to continue adding to the list of candidates because we need to increase our odds of having the most informative markers. Third, we need to understand the mechanisms of how the peptides are working, which will provide additional insight for developing novel therapeutic strategies.

Finally, how would you sum up the contribution made by your participating scientists?

The students and fellows who are working in our Center are amazing. They are at the forefront of pushing our studies to completion. Without their efforts, this project would not be moving as quickly as it is. Our trainees are earning numerous awards and accolades, including their own grant funding, and I am extremely proud of them. Second, we have received tremendous support from the other Centers. In particular, Dr Van Eyk (Johns Hopkins), Dr Cathy Costello (Boston University), and Dr Peipei Ping (UCLA) have been wonderful consultants for us.
EACH YEAR, MILLIONS of people worldwide suffer heart attacks. In a third of these individuals, the damage results in heart failure, a progressive condition in which the heart cannot pump enough blood to meet the body’s oxygen needs. Half of these individuals will die within five years. Although progress has been made over the last 40 years to improve short-term survival after heart attacks, more work is needed to identify the three out of 10 patients who will need more intensive treatment.

THERAPIES FOR HEART FAILURE

Now, researchers at the University of Texas Health Science Center at San Antonio (UTHSCSA) Cardiovascular Proteomics Center are developing new proteomic methods using state-of-the-art mass spectrometry equipment to identify predictive markers of unfavourable remodelling of the left ventricle following a heart attack. Using multidimensional approaches, they aim to examine the mechanisms whereby the left ventricle responds to injury and apply the knowledge gained to develop therapeutic strategies to prevent, slow, or reverse the progression to heart failure.

Led by Dr Merry L Lindsey, the Center includes faculty members from the School of Medicine and Graduate School of Biomedical Sciences at UTHSCSA, from the College of Engineering at the University of Texas at San Antonio, and from Torrey Pines Institute for Molecular Studies.

FOCUSING ON THE EXTRACELLULAR MATRIX

Lindsey’s team is focusing on the extracellular matrix (ECM) – the outside environment surrounding cells, whose turnover regulates how the heart responds to injury. ECM fragment generation is a key initiating event of cardiac remodelling. This particular focus distinguishes the Center from other research into the ECM, which is usually targeted towards searching for cancer biomarkers.

The scientists at the Center are investigating how ECM proteins are produced and processed post-myocardial infarction to regulate the left ventricular remodelling process and exploring whether ECM proteomics could drive the development of better treatment strategies for heart attack patients. They are also looking at optimum operating procedures for measuring ECM and screening for altered levels following a heart attack. The aim is to identify peptides that can be used as biomarkers of prognosis in patients of myocardial infarction, leading ultimately to a blood test that will predict who will develop heart failure.

ADDRESSING SHORTCOMINGS IN COMMUNICATION

A main outcome of the Center’s work is the dissemination of their results to the general, scientific, and medical communities. Lindsey recognises that there are shortcomings in the current status of medical research dissemination to the public and is implementing measures at the Center to engage directly with the local community: “We very much believe that, as scientists, we do not do a good job to disseminate our results to the public. Our Center is very aware of the need to improve our communication,” she explains. To address this, she and her colleagues recently completed a commercial that they uploaded on YouTube. "This video did not contain any scientific jargon and was aimed at letting the non-scientific community know that we are actively engaged in efforts to find a cure for heart failure," she recalls with enthusiasm. "This is a first-step measure to reach out more to the community. The key messages that we need to get out are that research is worth supporting and that we are making strides, even if the individual day-to-day steps are small."

The aim is to produce peptides that can be used as biomarkers of prognosis in patients of myocardial infarction, leading ultimately to a blood test that will predict who will develop heart failure.
What does it mean for University of Texas Medical Branch (UTMB) to be awarded one of the seven national Proteomics Centers funded by the NHLBI?

Our research activities utilising proteomics were very much stimulated by the award. An especially notable impact was the subsequent recruitment of two senior professors, Carol Nilsson and Mark Emmett, both of whom have considerable proteomics and mass spectrometry (MS) experience, especially with Fourier Transform – ion cyclotron resonance (FT-ICR) mass spectrometry. Furthermore, this recruitment was supported with the purchase by UTMB of a high performance Bruker 12 Tesla FT-ICR MS. Dr Nilsson subsequently obtained a US $2 million scholar award from the Cancer Prevention and Research Institutes of Texas to conduct proteomic studies. A further result of the NHLBI award is the considerable increase in scientific exchange between the Houston Medical Center and UTMB. Many Houston Medical Center researchers now recognise UTMB’s strong expertise in the area of proteomics-related research and have initiated collaborations.

How much is understood about the correlation between ageing and inflammatory diseases?

There are many immune cell functions that decline with ageing, including thymus involution, diminution of naïve T-cells and reduction in T-cell diversity. In addition, functional activities of macrophages, neutrophils, eosinophils and dendritic cells that play important roles in airway inflammation also decline. Moreover, there is a reduction in anti-inflammatory molecules such as lipoxin A4 levels, which function as important contributors to inflammation resolution.

To what extent do you interact with the other NHLBI Proteomics Centers?

The regional isolation of our Center, and the fact that we are the only lung-focused Center, has not allowed much interaction with other NHLBI Centers as yet. However, our Center is very interactive with the Medical Institutions in Houston as well as local investigators at UTMB. We have multiple proteomics collaborations, both ongoing and completed. For example, major ongoing proteomics projects include a proteomics study of eosinophilic esophagitis with Carla Davis at Baylor College of Medicine; a discovery investigation of the biology of low and high grade glioblastoma multiforme with Dr Kenneth Aldape of the Brain Tumor Center at MD Anderson Cancer Center; investigations on the effect of cysteinyl-S-nitrosylation on the toxicity of C. difficile endotoxins; proteomic studies of COPD with Farrah Kheradmand at Baylor College of Medicine; and investigations on the molecular injury that occurs upon hypoxia/ischaemia, followed by reperfusion in a rat brain model of this condition. This study resulted in the identification and quantification of several critical proteins that implicate calcium signalling as a major factor contributing to the injury induced by reperfusion.

What is the relationship between computational biology and proteomics?

The relationship between computational biology and proteomics is and will remain critically important. Computational biology is used to explore protein-protein and protein-ligand interactions at both the structural and temporal scales to develop a finer understanding of how they operate at the atomic and molecular level as well as to build interaction hypotheses that can be tested experimentally. From a computational perspective, the identification of biomarker panels from proteomics data can be exceedingly challenging, and new algorithms for this that exploit sophisticated new machine learning methods on high performance computing (HPC) are fast emerging. Another HPC application of computational biology that is revolutionising the discovery of novel therapeutics is computer docking, a virtual screening method that scans very large virtual libraries of small molecules against the atomic structures of proteins involved in disease. Highly ranked candidates can then be tested for pharmacological activity in cell culture and animal models using proteomics.

How would you sum up the brightest points of your tenure so far and the discoveries of most promise?

We have made significant progress both with regard to technology development and clinical research. These contract activities have been highly translational. For example, Dr John Wiktorowicz has led the development of quantifying S-nitrosylated proteins in physiologic processes due to oxidative stimuli; Dr Brasier’s group has developed a DNA aptamer-selected stable isotope dilution SRM MS procedure for quantifying the cytoplasmic transcription factor RelA; and Dr Garofalo’s group has shown, through proteomic studies, that viral-mediated inhibition of antioxidant enzymes contributes to the pathogenesis of severe respiratory syncytial virus.
A breath of fresh air

The multidisciplinary research team at the University of Texas Medical Branch NHLBI Proteomics Center at Galveston is developing innovative proteomics technologies and isolating biomarkers for the diagnosis and treatment of respiratory diseases like asthma.

**THE GLOBAL INCIDENCE** of asthma, allergy and respiratory viruses is on the rise. Some 20 million people worldwide suffer from asthma, many of whom are children. Causes to explain these increases include the theory that the modern hygienic environment is responsible for a reduction in certain allergens, which leads to reduced immunologic activation mechanisms that prime protective defences early in life. More significantly, scientists have shown that air pollution from cars, factories and power plants is a major cause of asthma attacks. Recent theories have suggested that asthma may be the result of epigenetic events perhaps as a consequence of infectious diseases, for example, respiratory viruses. However, as yet, no definitive answers have been forthcoming.

**POWERFUL TECHNOLOGIES**

The University of Texas Medical Branch (UTMB) NHLBI Proteomics Center at Galveston is now using the power of multiple analytical technologies to carry out proteomics research which is focused on airway inflammation relating to asthma, allergy and respiratory viruses. Headed by Dr Alexander Kurosky, the Center consists of seven multidisciplinary teams of scientists led by 11 investigators to study protein expression associated with signalling pathways important in lung diseases.

One of the main aims of the Center’s work is to develop innovative technologies; consequently, they are in partnership with industrial agencies to help bring innovation to commercial fruition. The researchers are building separation and array-based proteomic technologies to facilitate and enhance protein expression and post-translational modification studies of asthma, chronic obstructive pulmonary disease and respiratory viruses. Excitingly, they have embarked on a collaborative investigation set within a contract with MedImmune, a Pfizer subsidiary, to investigate signalling pathways associated with cytokine stimulation of eosinophils. These investigations are targeted at establishing phenotypes most responsive or non-responsive to MedImmune’s new drug benralizumab. This will be a comprehensive proteomic approach including definitions of post-translational modifications.

**EOSINOPHIL PROTEIN COMPLEXES**

A major ongoing project in Kurosky’s laboratory relates to investigation of the function of protein complexes found in eosinophils and their involvement in inflammatory diseases. Eosinophils are bone marrow-derived granulocytes that are abundant in inflammatory infiltrates of many pathologic processes. Furthermore, other key components of this research relate to cell signalling, especially with regard to eosinophil activation, and to the structure and function of protein complexes found in eosinophils and their subsequent alteration in various disease states, including component dynamic flux. Overall, these studies employ a variety of protein fractionation and characterisation technologies.

Protein complex research represents a challenge for researchers employing proteomic technologies. Many, if not most, cellular proteins form complexes whose properties can be both dynamic and variable depending on the status of the cell, for example, healthy versus diseased. Currently, complex isolation by immunoprecipitation is commonly employed followed by Western blot analysis for confirmation. However, as Kurosky reveals, there is considerable need for additional methodologies to be developed for the characterisation of protein complexes: “At UTMB, with the strong expertise of Dr Massoud Motamedi, we are also investigating methods relating to dynamic cell and tissue imaging of protein complexes”.

**BIOMARKERS FOR TREATMENTS**

Another main goal of the Center’s work is the development and verification of diagnostic and prognostic biomarkers for disease severity and treatment sensitivity. In pursuit of this, Kurosky’s team draws together interdisciplinary and international expertise in proteomics and epidemiology in a bid to isolate biomarkers for the more effective treatment of respiratory diseases. They are building on existing research, which has documented the different phenotypes of asthma in order to better and more effectively apply pharmacologic remedies, in particular, the biochemical understanding of why some individuals are resistant to steroids, a major therapeutic treatment for asthma. “Our Center is actively investigating the biochemical basis for steroid resistance with the aim to identify biomarkers for steroid resistance,” affirms Kurosky, who is encouraged by their work to date: “We have already achieved some progress in this effort through proteomic approaches”.

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**ALEXANDER KUROSKY, PhD, PI** is Director of the UTMB Proteomics Center. He has over 35 years of experience in the areas of protein structure, function, and genetics. His experience includes significant technology development and implementation related to proteins as well as proteomics-related research. Dr Kurosky received his PhD in biochemistry/protein chemistry from the University of Toronto and his postdoctoral training in molecular genetics at UTMB. He has worked with several biotechnology companies in instrument development and has β-tested instrumentation.
Could you outline recent progress in the University of California, Los Angeles (UCLA) Proteomics Center?

The UCLA team, together with The Scripps Research Institute (TSRI) team led by Dr. John Yates, the Royal Institute of Technology (KTH) team led by Dr. Matthias Uhlén, and the European Bioinformatics Institute (EBI) team led by Dr. Rolf Apweiler, has made significant progress.

In the clinical area, we have completed several studies characterising mitochondrial activities in heart failure and left-ventricular assist device (LVAD) patients. In terms of biological mechanisms, we focused on protein degradation and turnover, with the first publications coming out on degradation rates and turnover of proteins in cardiac mitochondria. We also developed a workflow for the absolute quantification of post-translational modifications in mitochondria using multiple-reaction monitoring. In terms of bioinformatics, we have integrated the cardiovascular protein knowledgebase COPaKB with the EBI-based PRIDE, the KTH-based Human Protein Atlas and the TSRI-based Census.

How would you sum up the mission and ethos of the Center?

Our mission is to propel the application of proteomics concepts and technology to advance medicine by addressing the many different biological aspects that underlie cardiovascular diseases. One of our major goals is to develop new technologies and drive innovations that directly address key clinical questions. Much of our effort concerns the creation of knowledgebases that curate and store proteomic data to support the entire scientific community.

Could you highlight how proteomics goes further than genomics in building a more complete picture of the biology of cardiovascular diseases?

Proteomics helps establish disease origin at one of its most miniscule and precise stages and allows us to look at parameters outside the realms of genetic studies, such as protein damage, protein turnover and post-translational modifications.

Our Center is very interested in the study of mitochondria in heart disease and understanding why mitochondria in the heart are different from those elsewhere.

How close is the Human Proteome Atlas to completion? What might its impact be?

The Human Proteome Atlas, headed by Dr. Matthias Uhlén at KTH, is one of the most innovative projects currently in progress. In its first seven years, the project has identified over 8,400 proteins – completion is anticipated around 2015. Proper identification and comprehension of the behaviour of these proteins will allow better understanding of why and how diseases develop, which will aid in both detection and treatment.

International Innovation has featured the work of many of your key investigators, including Drs. John Yates, Rolf Apweiler, Huilong Duan and Mathias Uhlén. How are your investigators selected?

We bring together top researchers from across the globe who value collaboration to achieve a higher purpose. The criterion for selecting investigators is that they are amongst the very best in their respective fields.

Amongst your current projects, are there any areas of discovery about which you are particularly excited?

We have just completed a project with the objective of determining the specificity and efficiency of proteome turnover within cardiac mitochondria by endogenous and exogenous proteolytic mechanisms. Mitochondrial protein homeostasis is an essential component of the functions and oxidative stress responses of the heart. We assessed proteolytic degradation of murine cardiac mitochondria by two-dimensional differential gel electrophoresis
New perspectives on disease

The UCLA Global Proteomic Initiative of Cardiovascular Medicine harnesses efforts worldwide to unravel the complex biological processes involved in diseases caused by circulatory malfunction.

**ABOUT A THIRD** of all deaths are attributable to cardiovascular disease, which also reduces the quality of life for many millions of people. Largely attributed to Western lifestyle factors, the burden of cardiovascular disease, to nation states as well as sufferers, is enormous and now sharply rising in low- and middle-income countries.

As proteins play a role in protective response mechanisms that may trigger cardiovascular problems, advances in knowledge of their biological actions within the heart should advance cardiovascular medicine. So innovations in proteome biology are the target of the National Heart, Lung, and Blood Institute Global Proteomic Initiative of Cardiovascular Medicine, in which scientists work together to discover how these complex diseases start, at the systems and molecular levels: “Proteomics itself is, by definition, large-scale studies of complex systems,” reflects Peipei Ping, Professor of Physiology, Medicine and Cardiology at the David Geffen School of Medicine at the University of California, Los Angeles (UCLA) and co-Director of the Initiative. “The traditional single-pathway, single-target paradigm of cardiac disease, focusing on mitochondria and cardiac proteasomes.

Is pure science your primary concern?

We aim to develop the field of proteomics in a bench-to-bedside manner. We concentrate not only on the noteworthiness of results but also on their significance to healthcare.

Our clinical studies focus on understanding the proteome signatures of late-stage heart failure patients undergoing LVAD and/or cardiac transplantation. The UCLA Heart Transplant Program, led by Dr Mario Deng, Medical Director of Advanced Heart Failure and Heart Transplantation Programs, recently celebrated being only the second center in the world to have completed 2,000 heart transplants.

Our technological advances and new applications will bring about new research in the scientific world and gradually diffuse out to affect the general public.

**COLLECTIVE KNOWLEDGEBASES**

Proteomic mass spectrometry is the domain of the Yates Lab at TSRI. The Lab uses tandem mass spectrometry to identify proteins from complex mixtures and identify sequences by peptide.
Sharing new knowledge with the world

The **NHLBI Proteomics Coordinating and Administration Center** coordinates information from the seven National Heart, Lung, and Blood Institute Proteomics Centers to provide consistent and up-to-date information about achievements and share the innovative proteomics resources they produce.

**What was the impetus for the National Heart, Lung, and Blood Institute (NHLBI) Proteomics Centers Program?**

The ultimate vision for the NHLBI’s Proteomics Centers is to better understand heart, lung, and blood disease biology. Hence, the NHLBI Proteomics Centers Program promotes the application of proteomic technologies to gain a greater understanding of physiological pathways. The Program supports the development of new tools as well as a knowledgebase to facilitate the translation of innovative proteomic approaches to clinical utility. It is intended to complement and enhance the NHLBI’s ongoing research programs, which include substantial investment in clinical research, genomic research, basic biology, technology, training, and education programs.

**Why were the seven Centers at Boston, Johns Hopkins, Harvard-Broad, Stanford, University of Texas Health Science Center, University of Texas Medical Branch at Galveston, and UCLA selected?**

All of these Centers are engaged in developing innovative technologies in proteomics research. In addition, each specialises in a particular biological question relevant to heart, lung, and blood health and diseases.

Each Center consists of a multidisciplinary team of scientists and physician-scientists with a dynamic range of expertise. Moreover, each one focuses on the development of different novel proteomics technologies and answering diverse aspects of healthy and diseased heart, lung, and blood processes.

**Why is it important that the work of the Centers is coordinated?**

The NHLBI Proteomics Coordinating and Administration Center serves an important role as a central synchronising entity and a catalyst among the seven Centers. We aim to support all their scientific goals alongside the mission of the NHLBI on proteomics. We serve them by carrying out administrative duties as instructed by NHLBI. We engage in active communication with the investigator community and ensure that the accomplishments of the Centers elicit a profound impact on the scientific community at large. We also aim to provide the cardiovascular community with proteomic knowledge and tools through effective dissemination of data and technologies generated by each Center, thereby propelling innovations and advancement in cardiovascular medicine.

**What are the challenges posed by coordinating the Centers over such a wide geographic and disciplinary spread? How would you sum up your key successes?**

Creating a vibrant hub to fulfil diversified goals is a challenge. Each Center aims to achieve its own scientific milestones while still supporting a collective mission. Our Administrative Center has actively engaged in showcasing the investigator teams and their accomplishments, as well as advocating for the Centers within the greater scientific community.

We have constructed posters and brochures to highlight the Proteomics Centers; in doing so, we have reached out to a broad scientific community, including at least 10 scientific organisations in the US and more than 15 conferences around the globe.

We have successfully organised three Principal Investigator meetings, nine teleconferences and one -Onci Workshop – the ‘Omics Integration in Biology and Medicine Workshop’, jointly sponsored by NHLBI and National Cancer Institute (NCI) – which drew large public attention.

We developed a website dedicated to the Proteomics Centers which was launched on 15 August, 2011. This website encourages the sharing of expertise, techniques, methods, documentation, information and services with the scientific community. The website has received an overwhelming response – since its launch, over 30,191 hits have been recorded. It has been viewed in 74 different countries and 1,034 cities worldwide.

We have compiled and published a newsletter, NewsSpots, which plays a critical role in communicating and advocating the separate and joint accomplishments and successes of the seven Centers and enhancing their visibility. We have received very positive feedback on it from the scientific community.

**Does more need to be done to disseminate the progress made within proteomics and to explain its relevance especially to cardiovascular and respiratory research?**

Awareness of proteomics is slowly but surely becoming more widespread among the public, but more needs to be done to raise public awareness of Proteomics. In the scientific community, we also have much to do to encourage more researchers to engage proteomics strategies in their studies.

**How would you sum up the contribution of young investigators to the different Centers?**

Young investigators are indispensable; the Centers consider support for creative investigations in proteomics science as critical. Our young investigators are creative and dedicated and will be major forces in moving research forward in the future.
THE PROTEOMICS COORDINATING AND ADMINISTRATION CENTER

Gathering proteomics discovery

The NHLBI Proteomics Centers Program involves multiple Centers spread across the US, with contributing laboratories across the world, drawn together by the Proteomics Coordinating and Administration Center

THE HUMAN GENOME sequencing project provided the precedent of success in a massive task and also a framework that could be used as a reference for acquired knowledge about the Human Proteome. Genomic discovery has led to great strides in biological and medical sciences. Now, expanding information about the proteome is likewise leading to breakthroughs in understanding diseases.

The National Heart, Lung, and Blood Institute (NHLBI) instigates pure scientific research into the prevention and treatment of heart, lung, and blood diseases, seeking to stimulate discoveries about the causes of disease and expedite their translation into clinically-relevant applications to help reduce the burden of heart, lung, and blood diseases and improve health worldwide. To this end, the NHLBI is currently supporting the Proteomics Centers Program, which is coordinated by the Proteomics Coordinating and Administration Center, led by Dr Jun Zhang. The NHLBI has established a consortium of seven highly interactive, multidisciplinary Proteomics Centers to develop and enhance innovative proteomic technologies and apply them to relevant biological questions that will advance knowledge of health and diseases of the heart, lung, and blood. Located at the David Geffen School of Medicine at the University of California, Los Angeles (UCLA), the Administration Center is tasked with harmonising the outputs of the seven Proteomics Centers within the Program and presenting their work to the world.

THE SEVEN CENTERS

The Proteomics Centers are spread across the US, and each addresses different aspects of diseases while ensuring that the common purposes of innovation, relevance to key clinical problems, and wider knowledge sharing are upheld. Each is effectively a network of academic institutions and medical hospitals.

The Boston University Cardiovascular Proteomics Center, led by Dr Catherine Costello, identifies proteins in cardiovascular disease brought on by unfavourable metabolic conditions and diseases, including obesity, diabetes and hyperlipidaemia.

The Harvard-Broad Proteomics Center, directed by Dr Robert Gerstzen, explores myocardial ischaemia, an acute disease caused by insufficient blood flow to the heart.

The Johns Hopkins Proteomics Innovation Center in Heart Failure, led by Dr Jennifer Van Eyk, investigates post-translational protein modifications and other aspects of heart failure.

THE TECHNOLOGIES AND PRINCIPLES generated in the Proteomics Program will help in tackling many diseases

Stanford Proteomics Center, under Dr Garry Nolan, analyses the roles of intracellular and secreted proteins in pulmonary arterial hypertension.

The University of Texas Health Science Center at San Antonio Cardiovascular Proteomics Center, led by Dr Merry Lindsey, investigates the mechanisms whereby the left ventricle of the heart responds to injury and devises strategies for averting progression to heart failure.

The University of Texas Medical Branch NHLBI Proteomics Center at Galveston, led by Dr Alexander Kurosky, focuses on airway inflammation in asthma, allergy and respiratory viruses and protein roles in lung disease.

The UCLA Proteomics Center, Global Proteomics Initiative of Cardiovascular Medicine, directed by Dr Peipei Ping, is a multinational initiative that is investigating protein mechanisms in cardiovascular diseases.

The Administration Center carries out administrative duties for all these research Centers on behalf of the NHLBI Proteomics Centers Program and is charged with raising the profile of their work.

BRINGING IT ALL TOGETHER

Zhang, supported by Kimberly Bunje, approaches coordination in multiple ways, including the production of regular newsletters for disseminating progress and achievements, and the organisation of workshops and meetings. There is a dedicated website (NHLBI-Proteomics.org) that provides news and information about the Program, progress and events and also, importantly, offers access to the tools, resources and papers produced from the Program and links to external proteomics resources.

“We are committed to assisting the NHLBI Proteomics Centers Program to achieve high impact in advancing heart, lung, and blood medicine,” state Bunje and Zhang.
Leading lights

We profile selected key investigators working within the NHLBI Proteomics Centers, offering a fascinating insight into the aims, findings and likely impact of some of the specific projects underway.

HARVARD-BROAD PROTEOMICS CENTER

Gregory D Lewis, MD, Assistant Professor of Medicine, Harvard Medical School; Director of Cardiopulmonary Exercise Testing, Massachusetts General Hospital

Dr Lewis’ research focuses on combining careful physiologic measurements at rest and during exercise with emerging metabolomics and proteomics approaches to improve phenotyping of cardiovascular diseases. He is interested in defining novel circulating markers of myocardial ischaemia and myocardial performance through ongoing work in the Harvard-Broad Proteomics Center. Another field of interest is in improving diagnostic approaches and treatment of patients with right ventricular and pulmonary vascular dysfunction in the setting of heart failure and pulmonary vascular diseases. His state-of-the-art exercise laboratory focuses on defining haemodynamic response patterns to exercise, coupling this information with non-invasive gas exchange patterns and circulating markers.

BOSTON UNIVERSITY CARDIOVASCULAR PROTEOMICS CENTER

Richard A Cohen, MD, J&L Coffman Professor of Medicine and Professor of Physiology, and Pharmacology and Experimental Therapeutics; Director of the Vascular Biology Unit

Dr Cohen and his group have determined that, in diabetes, hypertension and atherosclerosis, the vasodilator, nitric oxide, is inactivated by superoxide, an oxidant produced in diseased blood vessels, forming peroxynitrite, a potent oxidant. Sustained high levels of peroxynitrite cause irreversible chemical modifications, inactivating important proteins such as manganese superoxide dismutase or sarcoplasmic reticulum calcium ATPase (SERCA). Low levels of reactive oxygen or nitrogen species induce reversible protein modifications that regulate the function of many proteins including SERCA p21ras and sirtuin-1, thereby modulating cell signalling. Within the BU Cardiovascular Proteomics Center, Dr Cohen and his group are identifying oxidative chemical modifications of proteins that may serve as biomarkers for abnormal cell signalling and/or metabolic disease in diseased human arteries, platelets, and blood. Dr Cohen was President of the American Federation for Medical Research; he is an elected Fellow of the American Physiological Society and serves on several editorial boards.

UNIVERSITY OF TEXAS MEDICAL BRANCH NHLBI PROTEOMICS CENTER AT GALVESTON

Allan Brasier, MD; working on the project: The Innate Immune Response in Respiratory Mucosa

One aim of the UTMB NHLBI Proteomics Center is to understand how the respiratory mucosa responds to allergens or respiratory viruses. These agents trigger the innate immune response (IIR), an intracellular signalling network designed to protect the airways but whose dysregulation results in chronic respiratory disease. Dr Brasier’s work seeks to understand the connectivity of the IIR at the systems level. A major challenge for understanding the IIR is quantifying its myriad activation states. This is difficult because the signalling proteins are in low abundance, protein activation involves...
labile post-translational protein modifications, and also there are few available high affinity detection reagents. To address this problem, the Brasier group has developed a multiplex proteomics technique, termed selected reaction monitoring (SRM), to monitor the dynamic IIR. They are subsequently able to quantify changes in subcellular concentrations and detect activating post-translational modifications. This information is combined with dynamic imaging and genome-wide chip-seq approaches to refine predictive mathematical models to study how the pathway is modified in response to respiratory viruses and develop ways to treat airway inflammation.

UT HEALTH SCIENCE CENTER AT SAN ANTONIO CARDIOVASCULAR PROTEOMICS CENTER

Dr Yufang Jin, Associate Professor; working on the project: Computational modelling of left ventricular remodelling

This project aims to develop computational approaches to simulate LV remodelling outcomes when ECM fragment concentrations are adjusted, to determine key parameters that most inform the remodelling process, and to develop software to translate simulations for basic science and clinical applications. Dr Jin’s results are identifying the most informative ECM proteins/fragments by computational analysis of the temporal experimental data, interpolating the spatial and temporal progression of LV remodelling by identifying and quantitatively linking the clustered protein groups, and translating the computational results for basic science and clinical applications by developing computational modules for simulating the LV remodelling process. Her studies will reveal new strategies to globally and individually evaluate ECM, identify candidate peptide biomarkers and establish the equations that describe remodelling. Dr Jin has had a wonderful experience working within the UTHSCSA Proteomics Center. This has provided her with the opportunity to interact with computational experts in the other centers, in particular Dr Ping’s center at UCLA.

Dr Hai-Chao Han, Associate Professor; working on the project: Mechanical testing of the infarcted myocardium

This project aims to develop improved techniques to determine the mechanical strain in post-MI LV to evaluate the structural and functional evolution and better determine ECM effects on LV structure and function. Dr Han has developed and improved techniques to determine mechanical strain using serial imaging for non-invasive same animal measurements and is currently evaluating the time course of changes that occur following a heart attack. His studies will build understanding of how ECM processing regulates remodelling, which will be important for validating particular peptides as potential biomarkers. Dr Han has enjoyed working in the UTHSCSA Proteomics Center. While his primary research focus is on the mechanical properties of blood vessels, his involvement has provided a means to expand his research to identify mechanical components that are in common to the heart and vasculature.

STANFORD UNIVERSITY PROTEOMICS CENTER

Paul J Utz, MD, Associate Professor, Medicine – Immunology & Rheumatology

The Utz lab is developing several methods that will be broadly useful in the proteomics arena. During the first cycle of funding, his lab invented reverse phase lysate microarrays (Chan et al, *Nature Medicine*) for characterising signalling pathways in T lymphocytes. This has led to important discoveries regarding defects in Notch and mTOR pathways in T cell ALL. The Utz and Robinson labs have also developed autoantigen microarrays for profiling serum autoantibodies in over a dozen diseases. In the current funding cycle, the Utz lab is developing three new methods. First, high-throughput immunophenotyping using transcription (HIT) is being expanded to study PAH and to identify analytes for the Nolan lab and CyTOF. Second, the lab has made the surprising and unexpected discovery while measuring disease-associated cytokines that naturally-occurring autoantibodies to these secreted molecules exist and may play an important role in pathogenesis. Finally, his lab has invented Intel peptide arrays (in press in *Nature Medicine*). All of these techniques are expected to impact our studies of human PAH patients, serving as new tools for generating large amounts of data.

JOHNS HOPKINS UNIVERSITY PROTEOMICS CENTER

Anne M Murphy, MD, Center Associate Director, Professor of Pediatrics, Training Program Director, Division of Pediatric Cardiology, School of Medicine

Dr Murphy is a Pediatric Cardiologist with a long-term interest in the structure and function of the cardiac myofilament. Her lab work has focused on how disease related alterations of post-translational modifications of the myofilament result in changes in systolic and diastolic function. Within the Hopkins Proteomics Center, her lab has focused on modifications of the myofilament in diabetic cardiomyopathy and the use of quantitative mass spectrometry approaches to measure the changes in the myofilament phosphoproteome in human heart failure. This work has also led to the discovery of several novel modifications of the cardiac myofilament which are being functionally investigated. Dr Murphy has served on editorial boards of scientific and clinically oriented journals and has been an active professional volunteer for the American Heart Association.