Larry DeLucas, O.D., Ph.D. - Director

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Mission

The X-Ray Crystallography Shared Facility provides CCC members, UAB investigators, and regional scientists access to a premiere facility for protein crystal structure determinations for both aqueous and membrane proteins. The core provides expertise in structural biology support for researchers interested in macromolecular structure-function relationships and drug discovery support via intelligent or structure based drug design. The facility allows the Cancer Center researchers access to the latest crystallography equipment integrated with new methodologies that are currently under development by the X-Ray Director, Dr DeLucas.

Examples include the neural network, a predictive algorithm designed to improve the chances of producing high quality crystals and a new method for HTP quantitative measurements of second viral coefficients, a new high throughput lipid crystallization system and Biacore/calorimetry capabilities as discussed in the research highlights section. Many of these novel techniques are being utilized on campus in hope of discovering new information on the crystallization and structure determination of novel proteins, especially membrane and cytotoxic proteins. As the Facility moves into membrane protein crystallography, a significant amount of resources in the home diffraction lab and SERCAT beamlines will be devoted to this challenging endeavor. The facility maintains four operational bays for in-house diffraction data collection as well as access to two dedicated beamlines at the Argonne National Laboratory. The utilization of these systems positions the facility to rapidly determine protein structures of interest.

Facility Descriptions

Protein Solublization & Stability. The CBSE developed a rapid method for determination of optimum protein solubility/stability solution conditions for aqueous and membrane proteins. The system uses micro columns (ID= 0.4mm X 70mm length) to perform self-interaction chromatography (SIC) to measure second viral coefficients (B_{22}), a thermodynamic term that is a direct measure of protein-protein interaction forces.
The CBSE high-throughput system simultaneously performs multiple B_{22} measurements for the candidate protein using an incomplete factorial solubility screen of all of the possible solution conditions. This information is subsequently input to an artificial neural network that is able to make *in silico* predictions of the solution conditions that provide maximum protein solubility and physical stability (i.e. minimal protein aggregation). In addition, this same technology can provide rapid screening of potential crystallization conditions for those most likely to yield crystals and optimize initial crystallization conditions to produce diffraction-quality crystals.

Protein Crystallization. Facility includes multiple high-throughput nano-crystallization screening systems supporting aqueous and membrane protein crystallization (membrane proteins can be screened in detergent and lipid-based crystallization conditions). The facility is capable of performing up to 15,000 experiments/ day in nanoliter to microliter volumes. The 1,260 sq ft facility contains crystallization equipment, incubators, HTP imaging stations and integrated robotics. Equipment includes a Cartesian HoneyBee, a Rigaku Phoenix System, and a custom nano-crystallization system, the Topaz microfluidic system, a Genomic Solutions capillary counter diffusion system, and a high-throughput membrane protein lipidic crystallization system. These novel & proprietary techniques utilized for optimal crystal growth are described below:

--Through funds received via an internal Health Services Foundation grant, the core is completing the development of a high-throughput *in meso* nanoliter robotic crystallization system for particular use with membrane proteins (this represents the 2nd such system ever developed in the world). The *in meso* method is different from other crystallization techniques in that it employs a highly viscous (grease-like) lipid-based support fluid. To facilitate rapid, economic screening, a high-throughput robotic system was developed using funding provided via an internal UAB HSF award (one of only two such systems known worldwide). The system automatically prepares 1000 membrane protein crystallization experiments using various lipids (in place of detergents) to solubilize/stabilize membrane proteins for crystallization. This approach was recently successful in producing crystals of the beta adrenergic receptor (the second G-protein coupled receptor ever crystallized).

--High-throughput nano-crystallization system in microfluidic chips: the Fluidigm Topaz System enables screening of 400 conditions with less than 20 nl of protein solution.

--High-throughput Capillary Counter Diffusion System for Liquid-Liquid diffusion for crystallization and Co-Crystallization growth. The Genomic Solution's HoneyComb (this system, originally designed, fabricated and patented by the CBSE was licensed by the university's Research Foundation to Genomic Solutions in January 2008) provides high-throughput preparative Liquid-Liquid diffusion for crystallization and Co-Crystallization studies.

--An in-house incomplete factorial crystallization screening kit that provides a balanced screen tool to cover entire “crystallization space” with a higher success rate than commercial screens.

--A Cartesian HoneyBee Crystallization system and the New Rigaku Phoenix System capable of performing up to 15,000 experiments/ day in nanoliter to microliter volumes.

--A predictive algorithm program to perform *in-silico* virtual screens that optimize protein solubility/stability or crystallization screening conditions.
[1]

Biomolecular Analysis:

BIAcore 2000. The BIAcore 2000 is located in CBSE 221, a 324 sq. ft. wet laboratory. BIACORE 2000 is an instrumental technique to detect and quantify the binding kinetics of interacting partners, from which binding constants can be obtained. This is an automated instrument that allows assays to be conducted in small volumes from a 96 well microtiter plate. It differs from calorimetry in that the principal information obtained is rate constants and binding constants of interacting pairs of native, folded biomolecules, including large multi-molecular complexes under a variety of conditions or post-translational modifications.

Applications and Key Features are: (1) Measures intermolecular binding kinetics in real time under a variety of solution conditions; (2) Allows determination of equilibrium binding constants;

(3) Only very small amounts of sample required (mg per experiment); (3) Can be used to perform epitope mapping studies; Because the signal given is directly correlated to the size of the binding complex formed, it becomes possible to detect the presence or absence of multiple binding sites on a molecule.

Robotic Capillary Differential Scanning Calorimetry (Cap-DSC). The Cap-DSC is located in CBSE 220, a 608 sq. ft. wet laboratory. DSC measures the heat absorbed during macromolecular unfolding and thereby provides the thermodynamic stability of the molecule. Practically, it can facilitate determination of whether or not the protein is properly or completely folded, identify the presence of domains and the extent of interaction between them, lead to improved buffer conditions for maintaining the folded state, and provide insights into the affects of ligands and binding partners on the stability and structure of the molecule, including identification of binding in a high-throughput mode (a yes/no answer). In conjunction with self interaction chromatography (SIC), DSC can confirm that the conditions predicted by SIC, do, in fact, reduce aggregation. Up to 50 samples in 24 hours can be analyzed with as little as 200 mg per sample. Practical applications of the instrument are:

Other laboratory expertise and equipment include: Dynamic Light Scattering, Circular Dichroism and Isothermal Titration Calorimetry.

X-Ray Diffraction. The X-Ray facility provides the state-of-the-art resources required to obtain diffraction data from macromolecule crystals. Computational & graphic capabilities allow for rapid determination of 3-D molecular structures from the diffraction data. The 2,116 sq. ft X-Ray diffraction facility includes multiple operational bays and a 646 sq. ft computer / modeling facility. The computing / modeling space has a central network system for data processing, transfer and structure determination graphics in a HTP pipeline approach with a Linux computer cluster.

X-ray equipment includes: 2 5kW rotating anode 9Cu) X-ray generators fitted with advanced multi-layer x-ray focusing optics, 3 R-axis IV 300mm image plate systems, and 3 X-stream cryrogenic crystal cooling systems. D*TREK & HKL2000 are available for data processing and full crystallographic program suites like CNS and CCP3i are in place for 3-D structure determination as well as molecular graphic capabilities. The x-ray core is a member of the Southeast Regional Collaborative Access Team (SERCAT) allowing the UAB Researchers access to two of the most powerful beamlines at the Advanced Photon Source, Argonne National Laboratory in Chicago, IL.

The in-house CCC x-ray facility is increasingly being used to screen crystals prior to shipment to the synchrotron facility where higher quality data can be collected. A robotic crystal handling and remote data collection capability was added in June, 07. This feature has dramatically increased the core facility's access to synchrotron data collection. Instead of receiving a limited allotment of synchrotron time (21 days/yr) this new capability provides access to the synchrotron at any time with wait times averaging less than 7 days for data collection. As the Facility moves into membrane protein crystallography, significant resources in the home diffraction lab and SERCAT beamlines will be devoted to this challenging endeavor.

An exciting new development at SERCAT is the recent announcement that the SERCAT organization was recently awarded an NIH instrumentation grant for ~$500K to fund the purchase and implementation of a state-of-the-art microdiffractometer. This will provide important capability to collect very high quality diffraction data from very small macromolecular crystals or obtain multiple data sets from a single large crystal by shooting the crystals at several spots with a micro sized beam. Both of these capabilities will extend the ability of users to overcome difficulties incurred when crystals simply do not grow large or only one or two crystals are available for study, neither of which are rare circumstances. Micro-diffractometry is a new technique that is proving to be substantially rewarding to the field of macromolecular crystallography. SERCAT should have this capability near the beginning of 2010.

Research Information (examples of current projects):

1) Dr Craig Smith (CCC-member, Department of Vision Sciences)

RXRα and UAB Rexinoids for Breast Cancer Prevention. RXR (Retinoid X Receptor) is a family of nuclear receptor proteins that modulate gene transcription in response to binding of small molecule ligands or hormones. The biological effect of the ligand binding occurs by way of a large conformational change in the RXR that is sensed by coactivators of gene transcription. Drs. Donald Muccio and Wayne Brouillette (UAB Chemistry Department) synthesized a derivative of vitamin A as a potential chemo-preventive drug. This molecule 9-cis UAB30 (9cUAB30), is currently in phase I clinical trials for breast cancer. Experiments by Dr. Clinton Grubbs have shown that 9cUAB30 reduces the incidence of carcinogen induced mammary tumors in mice. Unlike other compounds having similar effects, it does not significantly raise triglyceride levels. The UAB team has demonstrated that 9cUAB30 binds strongly to the nuclear receptor protein RXRα. The structure-based analysis of the RXRα/9cUAB30 complex (figure 1) is being used to characterize protein-drug interactions for the purpose of designing next generation chemo-preventive drugs with enhanced selective biological effects. Thus far, structures of eleven RXRα/ligand complexes have been determined, where the ligands are 9cUAB30 or structurally related analogs. In the past year, two publications [2,3] resulted form this work.

Human Anaplastic Lymphoma Kinase (ALK). ALK is a receptor Tyrosine Kinase (TK) normally expressed in neural tissues during embryogensis. Chromosomal translocations generate a fusion protein, typically with nucleophosmin (NPM) homodimerization domain to create a constitutively activated kinase domain that stimulates anti-apoptotic and mitogenic signaling pathways (PI-3K/AKT, JAK/STAT, and PLCγ). The transforming activity of NPM /ALK is dependent on its kinase activity which is targeted for inhibition as an avenue of chemotherapy

for cancers such as anaplastic large cell lymphoma (ALCL), inflammatory myofibroblastic tumors, and diffuse large B cell lymphoma. M olecular modeling of the protein was performed and the model that resulted appeared to be superior to the one previously available and should be useful to inhibitor design efforts.

This work led to an improved molecular model and hopefully this will aid them in their efforts to design and identify lead inhibitor compounds.

Future plans include publication (with collaborators) in an appropriate peer-reviewed journal.

Structure of Nicotinic Acid Mononucleotide Adenylyltransferase (NaMNAT) and NAD^+ Synthetase (NADS) from
Bacillus anthracis.
Bacterial NaMNAT catalyzes the transfer of the adenylyl moiety of ATP to NaMN to form NaAD. NaAD is catalytically transformed to NAD^+ by the enzyme NAD^+ synthetase. Since NaMNAT and NADS are conserved enzymes and essential to the survival of every bacterium studied to date, they are regarded as a potential targets for the development of antibacterial drugs. We recently determined the apo structure of NaMNAT from *Bacillus anthracis* to 2.3Å resolution. The structure was deposited in the PDB (3DV2) and published in Acta Crystallographica[4]. Several crystal structures of NADS have been determined over the years by several UAB CBSE investigators as either apo structures or complexed with various substrates or substrate analogs. However crystals of complexes with novel synthesized inhibitors have remained elusive. Recently we have begun to analyze possible reasons for this phenomenon and are taking steps to overcome it. At the time of this report there is good evidence we may have been successful.

2) Dr. Larry DeLucas (CCC-member, Director, X-ray Core facility, Department of Optometry)

Ubiquitinin Specific Protease 2a (USP2a). The covalent attachment of ubiquitin to a variety of cellular proteins is thought to target these proteins for intracellular degradation. Although the broader consequences of protein modification of ubiquitin are just beginning to become apparent, the best understood role involves the ATP-dependent degradation of ubiquitinated protein. This degradative pathway is the major route for proteolytic removal of damaged, missfolded, and short-lived proteins and is necessary for the generation of MHC class I peptides for extracellular antigen presentation. USP2a is a deubiquitinating protein implicated in prostate cancer. It is one of more than 60 different ubiquitinin processing proteins (Ubps) that are believed to be involved in maintaining cellular levels of regulatory proteins. Studies have demonstrated that Mdm2 is also a target of USP2a potentially affecting p53-mediated apoptosis (USP2a was identified as a regulator of the Mdm2/p53 pathway that makes a significant contribution to repression of p53 activity *in vivo*).
The CBSE and SRI have initiated a collaboration to develop unique inhibitors of this relatively novel cancer target. The assay results shown in figure 2 demonstrate that purified USP2a is biologically active and amenable to HTP screening at 0.3 ug/ml enzyme concentration. Figure 3 shows crystals obtained for a complex of USP2a-ubiquitinin.

Crystallization/Structure Determination of Human OPG-RANKL Complex. Osteoporosis, the major metabolic bone disease, affects more than 10 million people in the United States and accounts for more than 1.5 million fractures per year. Bone remodeling involves the sequence of osteoclast activation followed by bone resorption and subsequent bone formation. Osteoporosis is one of many pathological conditions which involve a disruption in bone homeostasis, namely, an increase in bone resorption compared to bone formation (others include periodontal disease, rheumatoid arthritis, and most bone metastasis). Osteoprotegerin (OPG) is a soluble decoy receptor that binds to the receptor activator of nuclear factor-kB ligand (RANKL) preventing resorption of bone by inhibiting osteoclast (OC) differentiation. The development of OPG mimetics has been suggested as a possible strategy for treatment of diseases such as osteoporosis. OPG, receptor activator of nuclear factor-kB (RANK), and RANK ligand (RANKL) are proteins that regulate osteoclast formation and differentiation. Secreted from osteoblasts, OPG acts as a decoy receptor, and once bound to RANKL, prevents binding of RANKL to RANK. Blocking RANKL-RANK complex formation then prevents the differentiation of osteoclasts. Since, osteoclasts are responsible for bone resorption, proper balance of the RANK-RANKL-OPG system is crucial to healthy bone metabolism. The development of small molecule, OPG mimetics which target RANKL have been suggested for the development of therapeutic agents to treat diseases resulting in bone loss such as osteoporosis. The crystal structure of OPG will enable new avenues for structure-based rational drug design. Purified protein (figure 4) was demonstrated to be functionally active *in vitro*
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Progress includes; 1) Successful expression and purification of hOPG, 2) Demonstrated use of excipients to improve solubility and decrease aggregation of hOPG, 3) Successful formation of OPG-RANKL complex, 4) Design and production of nine solubility/crystallization variants of hOPG.

Cystic Fibrosis Trans-membrane Regulator Protein. The cystic fibrosis trans-membrane conductance regulator (CFTR) protein plays a key role in chloride conductance in normal airway cells. Single point mutations of CFTR can cause improper processing of the protein within epithelial cells or unresponsiveness to other interacting molecules. We previously demonstrated the ability to express biologically active, full-length CFTR in human cells. In the past year we have utilized a proprietary protein expression protocol that allows preparative production (in 10-liter bioreactors) of native CFTR to be used in crystallization studies. A second objective is to purify the protein without loss of biological activity. Purified protein is currently being used to support biological/biophysical investigations by other CFF-funded investigators and for the production of three-dimensional crystals of CFTR to support future x-ray structural studies. The crystallization and x-ray structure determination of CFTR will provide valuable information, at the atomic level, regarding normal and abnormal functional states of this protein. This information is key to developing new strategies for therapeutic intervention.

Chemokine Receptor-1. CCR-1 is a prime therapeutic target for treating autoimmune diseases. Chemokines constitute a large family of small proteins characterized by a well-conserved three-dimensional structure involving two highly conserved cysteine bridges. These small proteins are responsible for the orchestration of leukocyte recruitment to sites of inflammation or lymphoid tissue. One such chemokine, myeloid progenitor inhibitor factor 1alpha (MIP1α), mediates its effects through binding to CCR-1 and CCR-5 receptors. CCR-1 receptors are expressed on lymphocytes such as monocytes and peripheral blood monocytes. These receptors belong to the GPCR superfamily and have been viewed as attractive therapeutic targets by the pharmaceutical industry, mainly because of their central role in regulating leukocyte trafficking. The premise is that drugs can inhibit the directed migration and activation of immune cells via specific and highly potent chemokine receptor antagonists. Autoimmune diseases like multiple sclerosis and rheumatoid arthritis are characterized by interactions between invading T lymphocytes and tissue macrophages that result in extensive inflammation, tissue damage, and chronic disease pathologies. Numerous studies have

demonstrated CCR-1 expression in these cell types, and a variety of evidence provide strong in vivo concept validation for the role of this receptor in animal models of these diseases.

During the past year numerous unsuccessful attempts were made to produce three-dimensional crystals of purified CCR-1. The lack of success is not surprising since this GPCR, like the majority of GPCRs, does not have significant intra- and extra-cellular domains extruding from the membrane that are available for necessary lattice interactions required for the production of three-dimensional crystals. In an effort to address this deficiency, recent efforts are focused on production of a complex of CCR-1 with bound agonist, MIP1-α on the extracellular side and G-protein bound to the intracellular side of CCR-1. Initial data have confirmed successful complex formation. Future studies will involve scale-up of the expression for all three proteins in an effort to produce milligram quantities of the complex to support crystallization trials. This project involves collaboration between Drs. DeLucas and Kappes (UAB) and Dr. John Sondek at the University of North Carolina, Chapel Hill.

KISS1 and Metastasis Suppression. A new and exciting area of cancer research involves the discovery of metastasis suppressors such as KISS1 and BRMS1. Dan Welch and others have shown that these molecules suppress the spread of tumor cells to discontinuous sites but do not inhibit primary tumor growth. KISS1 Metastin is a proteolytic product of KISS1 and appears to mediate metastasis suppression by binding to a GPCR. BRMS1 may act partly through molecular association with histone deacteylases. We have had several milligrams of the 54-mer peptide KISS1 Metastin synthesized and attempts have been made to crystallize it with no success thus far. We are currently interested in cloning and expressing in significant quantities the BRMS1 256 residue protein for crystallization attempts. We hope these efforts will provide crystal structures so that structure/function information will help explain how metastasis suppressor proteins function.

3) Dr. Ming Lou (CCC-member, Department of Microbiology)

Oncolytic Viruses. Vesicular stomatitis virus (VSV) is a prototype of negative strand RNA virus (NSV). Some of NSVs are oncolytic viruses that have been studied as therapies for cancer [5]. Preparation of genetically enhanced oncolytic viruses can be improved when our knowledge of NSV assembly is more in-depth. One of the viral-encoded proteins, nucleocapsid (N), is required for both virus assembly and replication. The viral RNA polymerase only recognizes the viral nucleocapsid protein-RNA complex (RNP) as the template. We developed a co-expression system to produce recombinant N and phoshoprotein (P) in one construct. In our E.coli expression system, N forms a soluble complex of 10 subunits and a 90-base RNA molecule in association with the P protein. After testing several different approaches, we were able to produce single crystals of the N-RNA complex that diffracted X-rays to 3.0 Å resolution at synchrotron sources. The 3D structure of the N protein-RNA complex has been achieved and the position of the RNA molecular has been identified. There are extensive interactions between the neighboring protein subunits, which makes the nucleocapsid protein forming a ribbon like structure. The RNA molecule is snuggled into the cleft formed within the protein subunit and between the boundaries of the subunit. The structure helps to explain how the RNA polymerase recognizes the nucleocapsid protein covered RNA template. This work led to the recent discovery of “hot spots” in the N protein that are responsible for the assembly of the nucleocapsid [6]. This project will support development of new methods for preparation of target specific oncolytic viruses with the potential for treating cancer.

4) Dr. Narayana Sthanam (CCC-member, Department of Optometry)

Structure-Function Relationships of C3-Convertases. The Sthanam lab is involved in studies of structure-function relationships of C3-convertases that are central enzymes for complement activation. The x-ray crystal structures were determined for factor B, and C2, multi-domain serine proteases that contribute the catalytic apparatus for the convertases. Current efforts are concentrated on the structural characterization of co-factors of the convertases C3b and its homologous protein present in cobra venom CVF. The Sthanam lab is presently attempting crystallization of complexes of convertases (C3bBb and CVFBb). A second project involves the bacterial sortase project we have determined the crystal structure of Sortase B, an enzyme essential for the assembly of group B streptococcus pili and attempts are in progress for the crystallization of Sortase A [7]

from the same organism that anchors the pili. We have initiated the investigation of pili components and determined the structure of minor pili component SpaB, which is identified as the essential adhesive component of the pili.

5) Dr. Mark Walter (CCC-member, Director, CCC Structural Biology, Department of Microbiology)

Structure-Function Analysis of IL-22 and Viral Interferon Antagonists. Class 2 cytokines, including IL-22 and the interferons are important in normal function of the immune system. However, cytokine imbalances lead to pathology that can contribute to cell transformation. IL-22 and IFN-γ are major cytokines produced by Th17 and Th1 cells, respectively. IL-22 shares approximately 20% sequence similarity with IL-10, but its mechanisms of receptor engagement are unknown. IL-22 forms complexes with IL-22R1/Il-10R2 and IL-22R1/IL-20R2 complexes. The IL-22R1/IL-10R2 receptor heterodimer is also used by IL-24, which has been implicated as a tumor suppressor protein. This year the Walter laboratory determined the structure (fig. 5) of the IL-22/IL-22R1 complex [8,9]. The structure provides insights into the specificity differences and function of IL-22 compared to IL-24. This work required the Mass Spectroscopy and X-ray Core Facilities. The Walter lab also determined the structure of an interferon-γ binding protein (IFN-γBP), which encoded in the viral genome of poxviruses to disrupt immune function. The structure of the IFN-γBP revealed a novel tetramer structure, which is assembled through a 50 amino acid C-terminal peptide [10]. This worked required the X-ray Core Facility. The structural solution suggests that the C-terminal peptide can tetramerize heterologous proteins, which may be useful in developing proteins that efficiently target tumor antigens or might be useful in other research application where increased binding avidity is required.

6) Christie Brouillette (CCC-member, Department of Chemistry)

Recombinant CFTR: Cooperativity and Structural Domains. The long term objective of this application is to provide structural information on full length CFTR that will facilitate the development of mechanism-based drugs to treat cystic fibrosis. The disease of cystic fibrosis is caused by defects in the function of the cystic fibrosis transmembrane regulator, CFTR, which functions as a chloride ion channel. The Specific Aims of this proposal are designed to help answer mechanistic questions using biophysical tools to study the structural architecture of the protein, such as differential scanning calorimetry (DSC), circular dichroism, and fluorescence. In the past year we have used primarily differential scanning calorimetry (making use of the bioanalysis core) and thermal unfolding assays to detect ligand binding of compounds that effect CFTR function in the cell. We have also used DSC to propose a mechanism for unfolding of the protein that may explain why functionally defective mutations in the protein.

7) Dr Bin Sha (CCC-member, Department of Cell Biology)

Structural and Functional Studies of Molecular Chaperones Hsp40 and Hsp70. Hsp40 can bind non-native polypeptides to function as a molecular chaperone to suppress protein aggregation. Hsp40 can cooperate with Hsp70 to facilitate protein folding and assembly. The N-terminal J-domain of Hsp40 can stimulate the ATPase activity of Hsp70 while the C-terminal peptide-binding fragment of Hsp40 can deliver the non-native polypeptide to Hsp70 for subsequent protein folding. The mechanism of Hsp40 action as a molecular chaperone is unknown. X-ray protein crystallographic and biochemical studies are being carried out on Hsp40 11

to uncover how it interacts with the non-native polypeptides and transport them to Hsp70.

Structural and Mechanistic Studies of Mitochondria Translocons. Protein translocations across mitochondria membranes play critical roles in mitochondria biogenesis. The protein transports from the cell cytosol to the mitochondria matrix are carried out by the translocase of the outer membrane (TOM) complex and the translocase of the inner membrane (TIM) complex. The long-term goal of this project is to carry out structural studies on yeast TOM and TIM complexes to uncover the basic mechanisms by which these translocons facilitate the precursors across the outer and inner mitochondria membranes.

Structural Studies for Unfolded Protein Response (UPR) Related Proteins. Endoplasmic reticulum (ER) stress can lead to protein overloading and protein misfolding within the ER lumen, which could induce the so-called unfolded protein responses (UPR). Several ER-resident stress sensor proteins such as IRE1, PERK and ATF-6 function to transduce the ER stress signals from ER lumen to trigger the UPR. In the normal conditions, the ER luminal domains of these sensor proteins bind the ER molecular chaperone Bip and these interactions inhibit the UPR signaling. In the stressed conditions, the dissociation of these sensor proteins from BiP may initiate the UPR due to the elevated misfolded protein concentration in ER. The UPR can lower the ER stress burden by regulating a number of transcription pathways. One major pathway is to reduce the ER protein influx and the second is to promote protein folding and degradation of the misfolded proteins within ER. Several ER protein chaperones are involved in UPR to facilitate protein dynamics. The Sha lab is currently working on a number of UPR related protein structures
[12]
to reveal the molecular mechanisms how UPR helps the cell to cope with ER stress.

8) Dr. Champion Deivananyagam (CCC-member, Department of Vision Sciences)

Bacteriophage Hyaluronidase HylP. In patients with breast, ovarian and prostate cancers, high levels of hyaluronan (HA) in the tumor-stroma interface have been associated with poor survival rates. Overproduction of HA is also observed on the surface of cancer cells, which enhances tumorigenesis and metastasis via HA-CD44 mediated HA internalization and the subsequent intra-cellular signaling pathways. Administration of bovine testicular hyaluronidase (BTH) caused rapid clearance of HA and reduction in human breast tumor xenografts in SCID mice. In addition, pharmaceutical preparations of BTH (Neopermease®, Hylase® and Wydase®) have been therapeutically used as an adjuvant in chemotherapy since they increase chemoagent permeability by reducing the HA present on the surface of tumor. However, the pharmaceutical preparations of BTH from testicular extracts are not highly purified and the possibility of being contaminated with Bovine Spongiform Encephalopathy (BSE) exists. In this context, the Deivanayagam lab is studying the bacteriophage hyaluronidase HylP, and its potential anti-cancer and adjuvant properties that could be utilized for treatment of breast cancer.

Cancer Relevant X-ray Core Pilot Projects funded in 2008:

Pilot Funding Initiatives

In May, 2008 the x-ray core established two different pilot funding initiatives to stimulate expanded use of the core's services. Two budgets, one funded through the Center's CCC core facility is strictly used to fund cancer-related projects. The other budget, funded through the Center for Biophysical Sciences and Engineering, is available to support other biologically-relevant projects. In response to a call for proposals, investigators (faculty at any level) are required to write a brief application for the specific core service(s) they wish to use. New funds and opportunities will be available every 6 months. All applications collected in a given 4-month period are peer-reviewed by a panel of 3 faculty members from within the UAB (with no conflict of interest and each representing a different academic department). Funding is prioritized based on the score obtained in the peer-review process and availability of funds in the given cycle (see pilot projects below for information on projects funded to date). Thus far, several cancer-related projects have been funded via this mechanism.

2008 Pilot Awards

- Heidi Erlandsen (non-CCC member, Institute of Oral Health Research) *Structural and functional studies of pleiotrophin and receptor protein tyrosine phosphatase β/z carbonic anhydrase domain*

- Fang-Tsyr (Fannie) Lin (CCC-member, Department of Cell Biology) *Mechanisms of the LPA2 Receptor Actions Biological importance of the LPA2 Receptor in cancer progression*

2009 Pilot Awards and CBSE Requested Core Services

- Dr. Doug Watson (Biology) *Investigation of a Candidate Crustacean Molt-inhibiting Hormone Receptor*

BiaCore/self-interaction studies; Attending Biacore training PhD student: Hsiang-Yin Chen

- Drs. Jay McDonald and Chen (Pathology) *Cholangiocarcinoma Pathogenesis: Fas-mediated Apoptosis*

BiaCore/self-interaction studies

Nano-crystallization screening; Attending Biacore training Post Doc: Gu Jing

- Dr. Terje Dokland (**Microbiology),** *Self interaction Chromatography and Crystallization of TerS*

Preliminary self-interaction chromatography and crystallization studies; Graduate student: Jenny Chang

- Drs. Paul Sanders and Allen, (**Medicine-Nephrology**) *Crystallization of THP Constructs* (Approved for funding)
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Preliminary nano-crystallization screening

- Dr. Dennis Pillion, (**Pharmacology**/**Toxicology**) *Modulation of Protein-Protein Interactions by non-ionic Surfactants*

Preliminary self-interaction chromatography studies for each of the proposed peptides.

- Dr. Jamil Saad (**Microbiology)** *Crystallization, BiaCore and x-ray Characterization of CaM*

Preliminary BiaCore, crystallization and x-ray diffraction characterization (if crystals are

obtained) studies; Crystallization and x-ray characterization studies will be performed for you by CBSE x-ray core personnel.

Services and Fees *(as of 6-23-09)*

HTP Protein Crystallization Laboratory Fees

Costs per screen	$450
Costs per Image Analysis	
With Hits	$230
Without Hits	$150

HTP Self Interaction Chromatography Fees

Min charge for Sample Run

(includes prep / up to 8 hours) $500

Or w/o Service Center Support $300

Each Additional Run $ 30

Biomolecular Analysis Laboratory Fees

Per sample (includes buffer control)

for DSC measurement $50/sample

Use of the BIAcore per day $100/day

X-ray Diffraction Laboratory Fees

R axis IV image plates $30/day

SERCAT Fee	$50/hr

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