University of Central Florida

UCF Biophysics Group

Biophysics Mini-Conference

to Mark the 2nd Biophysics Week

(https://www.biophysics.org/BiophysicsWeek/GetInvolved/tabid/6655/Default.aspx)

Wed., March 8, 2017, 11:30 am – 5:00 pm

Physical Sciences, room 160

POSTERS

(11:30am-12:30pm)

Protein Disulfide Isomerase is Essential for the Disassembly and Activity of Heat-Labile Toxin but not Ricin.

Jessica Guyette[1], Michael Taylor[1], David Curtis[1], Suren A. Tatulian[2], and Ken Teter[1]

[1]Burnett School of Biomedical Sciences, College of Medicine, [2]Department of Physics, College of Sciences, University of Central Florida, Orlando, Florida

ABSTRACT: *Escherichia coli* heat-labile (LT) toxin and ricin toxin are both AB toxins that contain a catalytically active A subunit and a cell-binding B subunit. The catalytic subunit is connected to the rest of the toxin by a disulfide bond. LT produces watery diarrhea through elevated levels of intracellular cAMP, while ricin is a lethal bioterrorism agent that inhibits protein synthesis. Both toxins enter the cell through receptor-mediated endocytosis and undergo retrograde transport to the endoplasmic reticulum (ER), where reduction and disassembly must occur before the catalytic subunits can exit the ER to engage their cytosolic targets. Toxin reduction is facilitated by oxidoreductases such as protein disulfide isomerase (PDI) that are found in the ER, but it is currently unknown whether toxin reduction is sufficient for holotoxin disassembly. Here, we report that the reduction of LT does not lead to toxin disassembly, whereas the reduction of ricin allows its A chain to separate from the B chain. PDI was specifically required to disassemble the reduced form of LT. Cells lacking PDI were therefore resistant to LT but not ricin. It is known that PDI partially unfolds when it binds to the AB-type cholera toxin (CT), and this unfolding allows PDI to act as a wedge for disassembly of the reduced but intact CT holotoxin. We accordingly predicted that PDI would unfold upon contact with LT but not ricin. Surprisingly, however, binding to either ricin A chain or ricin B chain led to the unfolding of PDI. We are currently determining the functional role of PDI unfolding in LT and ricin disassembly. These structural studies will provide important insight into the different interactions of AB toxins with host proteins.

Micro-Spectroscopy of Bio-Assemblies at the Single Cell Level

Jeslin Kera[1,2], Debopam Chakrabarti[2], and Alfons Schulte[1]

[1] Department of Physics and College of Optics & Photonics,
[2]Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, Florida

ABSTRACT: Confocal absorption microscopy has the benefits of requiring no labels and low light intensity for excitation while providing a signal from the contrast generated by the attenuation of propagating light due to absorption. This enables spatially resolved measurements of single live cells and bio-molecules in nanoliter solutions. We present experiments on model systems over the spectral range from the near-infrared to the ultraviolet. The spectral identification of biomolecules with characteristic absorption bands in the ultraviolet at spatial resolution in the micron range will be discussed.

Mechanics and Dynamics of Cation-Induced Actin Bundles

Nicholas Castaneda[1], Tianyu Zheng[1], Hector Rivera-Jacquez[1], Hyun Joo Lee[2], Jaekyung Hyun[2], Alexander Balaeff[1], Qun Huo[1], and Hyeran Kang[1]*

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[2]Nano-Bio Electron Microscopy Research Group, Korea Basic Science Institute (KBSI) Cheongju-si, Chungcheongbuk-do, South Korea

ABSTRACT: The assembly of actin filaments into bundles plays an essential role in mechanical strength and dynamic reorganization of cytoskeleton. Divalent counterions at high concentrations promote bundle formation through electrostatic attraction between charged filaments. Although it has been hypothesized that specific cation interactions may contribute to salt-induced bundling, molecular mechanisms of how salt modulates bundle assembly and mechanics are not well established. Here we determine the mechanical and dynamic properties of actin bundles at varying divalent cation concentrations. Using total internal reflection fluorescence (TIRF) microscopy, we measure the bending stiffness of actin bundles determined by persistence length analysis. We characterize real-time formation of bundles by dynamic light scattering intensity and direct visualization using TIRF microscopy. Our results show that divalent cations modulate bundle stiffness as well as time-dependent average bundle size. Furthermore, molecular dynamic simulations propose specificity for cation binding on actin filaments. The work suggests that cation interactions serve a regulatory function in actin bundle mechanics and dynamics.

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Nanoscale Investigation of Biophysicochemical Responses to Induced Stress in *Rhodococcus opacus*

Briana Lee[1] and Laurene Tetard[1,2]

[1]NanoScience Technology Center, University of Central Florida, 12424 Research Parkway, Orlando, FL, 32826, [2]Department of Physics, University of Central Florida, 4000 Central Florida Blvd., Orlando, FL, 32816

ABSTRACT: Creating a more in-depth knowledge base of bacterial existence and evolution in a chemical, physical, and biological sense has become increasingly relevant, especially with the growing number of treatment-resistant bacteria. Some of the most important factors regarding how bacteria become infectious relate to the biomechanical properties: stiffness, adhesion, and binding interactions. Our study aims to investigate the nano-molecular measurements of these factors, establish chemical compositions of cell walls, and understand the biophysicochemical responses associated to stress on bacterial systems.

Here we focus on the plant-based bacteria Rhodococcus opacus and their response to varying growth adaptations. First, we study the physical attributes of the bacterial system with atomic force microscopy (AFM). Additionally, we use mechanical measurements to understand the biomechanical properties associated with the bacterial cell membranes. This allows for the measurement of stiffness and adhesion, Young's modulus, and relative hydrophobicity/hydrophilicity. We use Raman spectroscopy to understand the biophysicochemical properties and identify/quantify molecular details, which can be further explored with nanoscale-IR spectroscopy. By exploring all associated properties and bacterial responses to stress at the nanoscale level, we propose a new approach with exciting implications, such as potential clues for the development of more potent treatments for resistant bacteria.

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Biophysical Characterization of Membrane Pores Formed by Amyloid β_{25-35}

Nabin Kandel and Suren A. Tatulian

Department of Physics, University of Central Florida, Orlando, FL 32816

ABSTRACT: Amyloid β (Aβ) peptide contributes to Alzheimer's disease (AD) by a yet unidentified mechanism. In brain tissue, Aβ occurs in various forms, including a undecapeptide Aβ(25-35) (GSNKGAIIGLM), which exerts neurotoxic effect through mitochondrial dysfunction and/or Ca^{2+}-permeable pore formation in cell membranes. This work was aimed at biophysical characterization of pores formed by Aβ(25-35) in membranes of unilamellar vesicles composed of a zwitterionic lipid 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC), an acidic lipid 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG), and varying fractions of cholesterol. Vesicles were made by extrusion, in a buffer containing 6 mM Quin-2, a Ca^{2+}-dependent fluorophore. External Quin-2 was removed using a Sephadex G-50 column, and 6 mM $CaCl_2$ was added externally to the vesicles. Aβ(25-35) was incubated in an aqueous buffer for 3 hours to form oligomers, and was added to the vesicles, resulting in gradual increase in Quin-2 fluorescence, interpreted in terms of membrane pore formation by the peptide, Ca^{2+} influx and binding to intravesicular Quin-2. The positive and negative controls involved addition of a non-fluorescent calcium ionophore Br-A23187 or blank buffer, respectively. The pore forming activity of Aβ(25-35) was dependent on the lipid composition of the vesicles and the ionic strength of the buffer. High ionic strengths (150 mM NaCl and above) significantly suppress pore formation, indicating the importance of electrostatic interactions between the cationic peptide (Lys28) and anionic membranes. CD spectra of Aβ(25-35) in same vesicle samples displayed a minimum around 210 nm and a shoulder at 222 nm, whereas the peptide in a buffer without lipid showed a minimum around 206 nm, indicating membrane-induced α-helix formation. Cholesterol exerts a complex effect on Aβ(25-35) pore and is interpreted in terms of interactions with both membrane lipids and Aβ(25-35). Combined with FTIR analysis, these studies provide the structure and function of membrane pores formed by Aβ(25-35).

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Effect of Metal Ions on Polyelectrolyte Mechanics

Angie M. Diaz, Xiaoyan Lu, James Heidings, Lei Zhai*, and Hyeran Kang*

NanoScienceTechnology Center, University of Central Florida, Orlando, FL 32826

ABSTRACT: Polyelectrolytes based hydrogel fibers can mimic extracellular matrix, and have various biomedical applications such as drug delivery and tissue scaffolding. Metal ions have been shown to stabilize hydrogel fiber networks through the interactions between the carboxylic group of the polyacrylic acid and metal ions. However, how ions modulate the mechanical properties of individual polyelectrolyte polymers is still unknown. In this study, electrospun polyacrylic acid (PAA) with chitosan (CS) is used as a model system to evaluate how ferric ions (Fe^{3+}) affect both fiber length and bending stiffness using dark field microscopy images and persistence length analysis. We demonstrate that increasing ferric ions leads to a reduction in fiber length and an increase of bending stiffness. Our work suggests that metal ions can regulate single polyelectrolyte fiber mechanics, thereby providing designs to fabricate hydrogel with controllable stiffness.

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Assignments of 3D ssNMR Spectra Strategy from the Studies of Rous Sarcoma Virus Capsid Protein

Xin Qiao, Jaekyun Jeon, and Bo Chen

Department of Physics, University of Central Florida, Orlando, FL 32816, USA

ABSTRACT: Assignments of 3D spectra are the prerequisite for structural characterization of proteins by solid state NMR (ssNMR). Due to the presence of anisotropy, it is very challenging to make accurate assignments of the spectra of proteins larger than 150 amino acid residues. In this work, we summarize strategies to enhance the assignments of the 3D ssNMR spectra, based on the sequence assignment of the tubular assembly of Rous sarcoma virus (RSV) capsid protein. Specifically, we find that the N frequency of Cb is the best match between different 3D spectra including NCACX, NCOCX and CANCX, while those of other sites in the same residue can display larger discrepancy. Moreover, the secondary shift of Ca is the largest among all carbons in the residues. We show that the congested resonance of Gly and Ala residues can be effectively assigned by the degeneracy setting in the auto-assignment program Mcassign. We also carefully analyzed Lys, Leu, Gln, and Arg, which exhibit similar chemical shift pattern. In our work, we selectively labeled Leu and Arg to distinguish these residues. However, we find that intraresidue cross-peaks sometimes exhibit considerable mismatched N resonance frequencies, confirmed by inspecting the NCACX spectra of Leu and Arg selectively labeled samples; the underlying reason is not clear.

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PLENARY PRESENTATIONS

12:30-1:20 pm

A Tale to TeLL: The C-terminus of Bax Proves to Be a Potent Anti-Cancer Agent for Metastatic Disease

O. Flores[1], A. Carr[1], D. Nierenberg[1], A. Showlater[1], R. Bassiouni[1], A. Limaye[1], S. Santra[2], P. Vishnubhotla[3], S. Litherland[4], L Barr[4], A. S. Khaled[3], J. M. Perez[5], and A. R. Khaled[1]

[1]Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL, 32827, [2]Department of Chemistry, Pittsburg State University, Pittsburg, KS 66762, [3]Orlando VA Medical Center, Orlando, FL 32827, [4]Translational Research, Florida Hospital Cancer Institute, Orlando, FL 32804, [5]Cedar Sinai Medical Center, Los Angeles, CA 90035

ABSTRACT: Current FDA-approved medications for metastatic breast cancer focus on estrogen synthesis disruption, mitotic spindle disassembly, epidermal growth factor receptor blockage, or various kinase inhibition, in an attempt to hinder the cancer's growth. Unfortunately, over time drug resistance can result and patients often suffer from detrimental side effects such as neuropathy, cardiomyopathy, and depression. It is evident that new avenues of treatment must be investigated in order to attain a lasting and safe solution that prevents cancer recurrence and spread.

Our research led to the discovery of the CT20p, a peptide derived from last twenty amino acids of the apoptotic protein, Bax. CT20p displays selective toxicity, killing cancer cells in a manner distinct from the parent protein and independent of the apoptotic machinery. CT20p has a hydrophobic nature and the inherent ability to self-assemble under cell-free conditions into oligomers with pore-forming capacity. However, CT20p's mechanism of action involves interference with a protein-folding macromolecular complex called chaperonin-containing TCP-1 (CCT). In normal cells, CCT folds only about 10% of the intracellular proteins. In contrast, in cancer cells CCT is the essential supplier of critical proteins like actin, tubulin, STAT3, cyclins, Ras and others. We found that CCT levels increased in cancer cells compared to normal cells, especially with advanced cancer stage, and that this correlated with susceptibility to killing by CT20p. Hence. CT20p is a promising candidate for cancer therapy, not only because of its specificity towards tumorigenic cells expressing CCT and its innocuous effects on non-carcinogenic cells, but also because its hydrophobicity and small size permits delivery using targeted nanoparticles (NPs). An HBPE (hyperbranched polyester) nanoparticle containing a water-soluble and biodegradable core capable of encapsulating the CT20p was developed. HBPE-NPs can be designed via assorted surface functionalization to target specific plasma membrane markers associated with cancer cell types. Incubation of prostate and breast cancer cells with CT20p-HBPE-NPs caused cell death accompanied by dramatic changes in cell morphology and loss of adhesion molecules and cytoskeletal proteins as a result of CCT inhibition. In xenograft cancer models, CT20p-HBPE-NPs caused significant tumor regression.

In summary, our research yielded a novel therapeutic agent that targets an intracellular complex that is highly expressed in cancer. Expectations are that as a result of inhibiting the protein folding activity of CCT, CT20p impairs the growth and progression of cancer cells and also elicits a protective immune response by turning the dying cancer cells into potent immune stimulators.

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1:20-2:10 pm

Studying Molecular Motors: from Molecules to Mice

Stephen King

Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL, 32827,

ABSTRACT: Our ultimate goal is to understand how intracellular transport occurs and how defects in transport may lead to the onset and progression of neurodegenerative disease. One cytoskeletal motor that plays an important role in intracellular transport is cytoplasmic dynein, which generates force as it moves toward the minus ends of microtubules. We have previously used biochemical and biophysical approaches to identify novel aspects of dynein motor force generation as well as the ability of a different molecule, dynactin, to enhance the ability of cytoplasmic dynein to take multiple steps without dissociating from the microtubule, called processivity. . Processive long-range movements are especially important in the axonal processes of neurons where cytoplasmic dynein moves retrograde cargo over distances ranging from microns to meters. Genetic lesions in components of the cytoplasmic dynein and dynactin motor machinery have been shown to alter axonal transport and in some cases to result in severe neurodegenerative diseases such as Charcot Marie Tooth disease (CMT) Perry syndrome, amyotrophic lateral sclerosis (ALS), and distal spinal and bulbar muscular atrophy (dSBMA). We are now utilizing both in vitro and in vivo assays of dynein and dynactin function to test our models for how dynein-based cargo transport normally occurs and how it may be altered during neurodegenerative disease.

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2:20-3:10 pm

Proteins that Control Intracellular VLDL Trafficking and Secretion

Shadab A. Siddiqi

Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL

ABSTRACT: Aberrant secretion of very low-density lipoproteins (VLDL) by the liver causes dyslipidemia, which is associated with severe metabolic disorders such as type-2 diabetes, atherosclerosis, hepatic steatosis etc. The rate-limiting step in the secretion of VLDLs from the liver is their transport from the endoplasmic reticulum (ER) to the Golgi, which represents a potential therapeutic target in controlling VLDL secretion. We have identified a distinct ER-derived vesicle, VLDL transport vesicle (VTV), which facilitates the targeted delivery of VLDLs from the ER to the Golgi. To find out the factors that regulate the biogenesis of these vesicles, detailed proteomic and biochemical analyses were performed. Our data revealed that two small Mr proteins, cideB and SVIP were present in the VTV but not in other ER-derived vesicles. Morphological data coupled with co-immunoprecipitation analyses revealed that both cideB and SVIP specifically interact with VLDL structural protein, apolipoproteinB100. To examine the roles of these proteins in VTV biogenesis, we carried out an in vitro ER-budding assay. Our results demonstrate that either blocking or knockdown of cideB and SVIP abrogates VTV biogenesis and VLDL secretion from hepatocytes. We conclude that cideB and SVIP control VLDL secretion from the liver through modulation of VTV biogenesis and their identification is critical for the development of novel therapeutics for dyslipidemia.

3:10-4:00 pm

Preliminary Findings for the Structure of a Tail Fiber Protein from the Marine Cyanophage P-SSM2

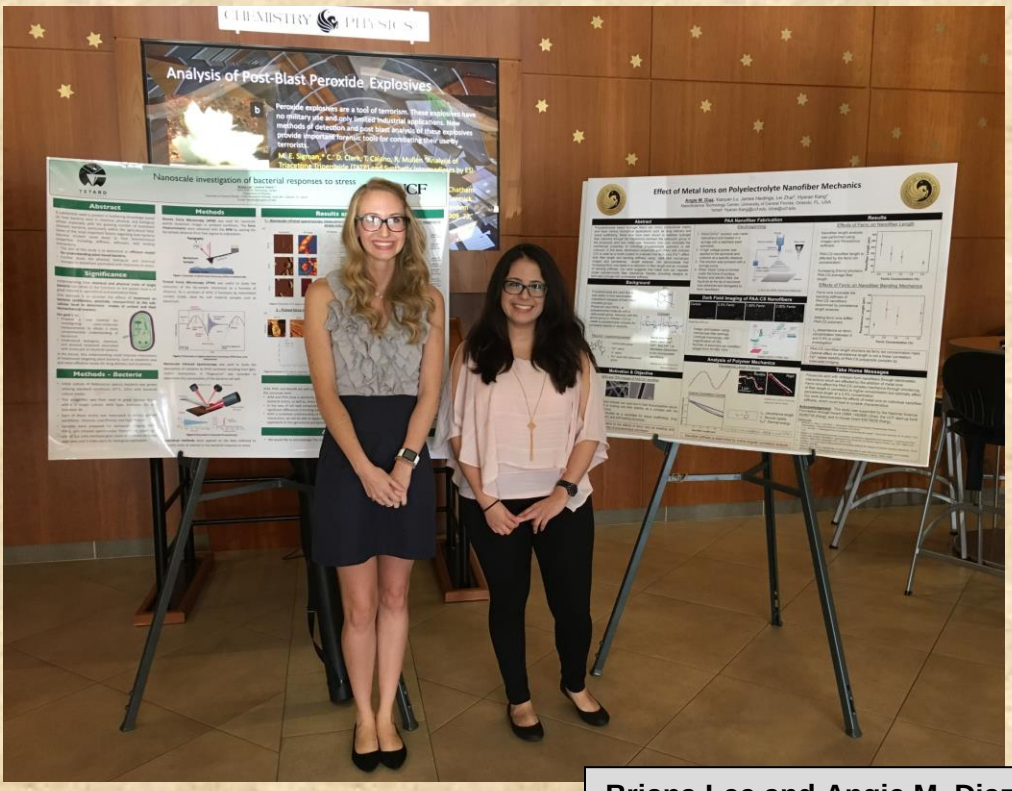
Leon Hardy

Structural and Computational Biology Group, Department of Biological Sciences, University of South Florida, St. Petersburg, Florida

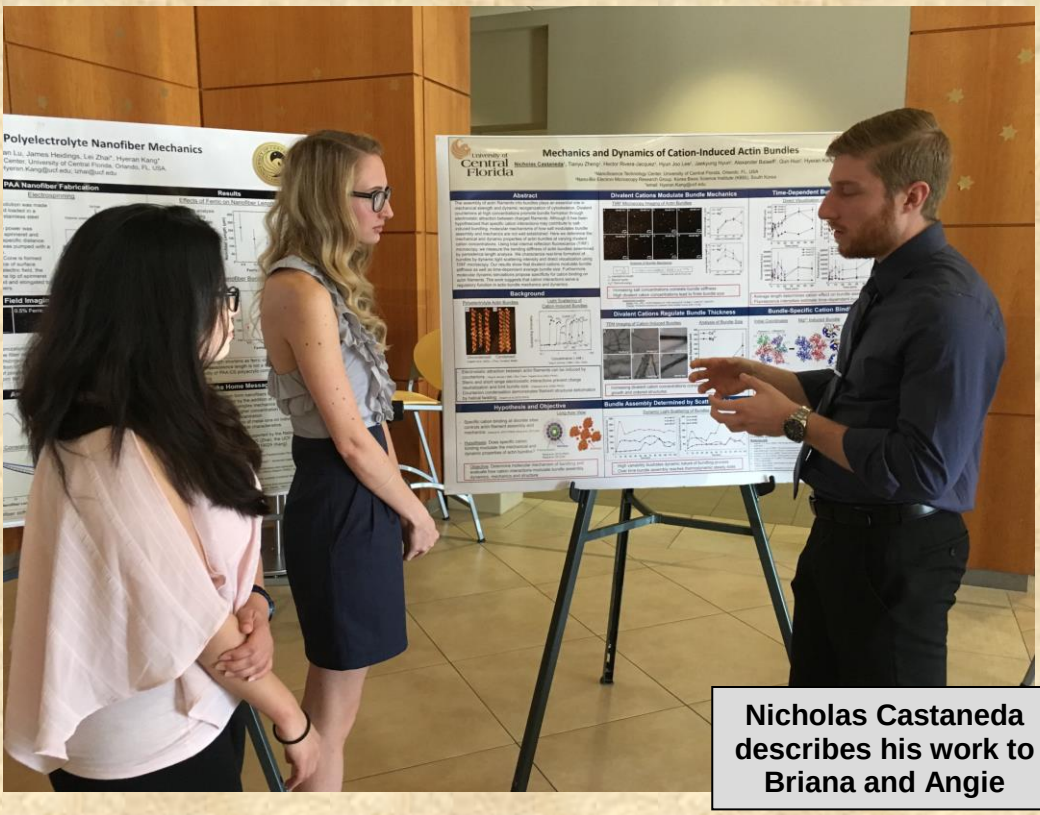
ABSTRACT: Recent X-ray crystallography studies have demonstrated the presence of iron ions within the receptor-binding tips of the tail fiber protein of bacteriophage T4, which infects E. coli. The amino acid sequence of the phage T4 tail fiber protein (gp37) contains seven paired histidine residues (His-X-His motifs), which coordinate the iron ions within the structure of the tail fiber. We have recently identified the presence of six His-X-His motifs in the putative tail fiber protein of a related phage, P-SSM2, which infects Prochlorococcus, a dominant genus of marine bacteria. We speculate that these residues serve to coordinate iron ions in the same manner for P-SSM2, and that only six iron ions would be present instead of seven. However, the structure of the P-SSM2 tail fiber has not yet been determined, therefore, we sought to simulate the structure of this protein. To date, a model of a single monomer has been developed in vacuum and in water, and subsequent work will simulate the structure of this protein as a trimer and its binding strength to iron.

4:00-5:00pm

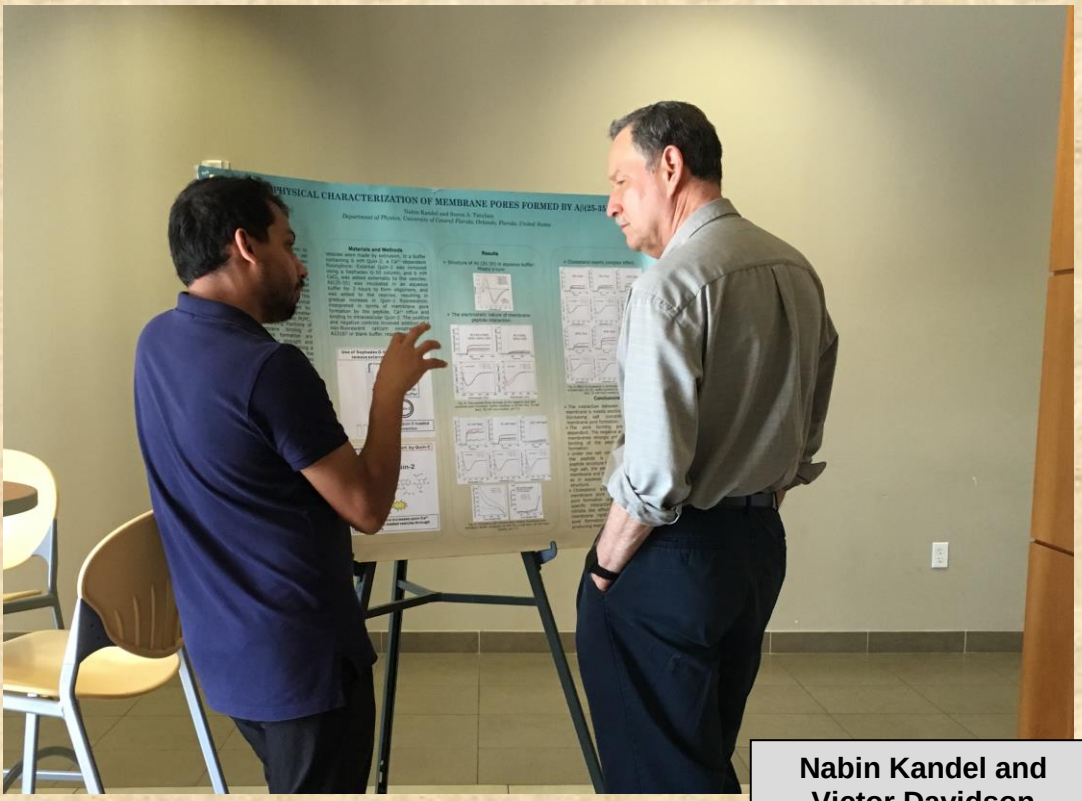
Meeting of the Computational Biology group to discuss inception of a US Southeastern Regional Student Organization with affiliation with the *International Society for Computational Biology* (ISCB).



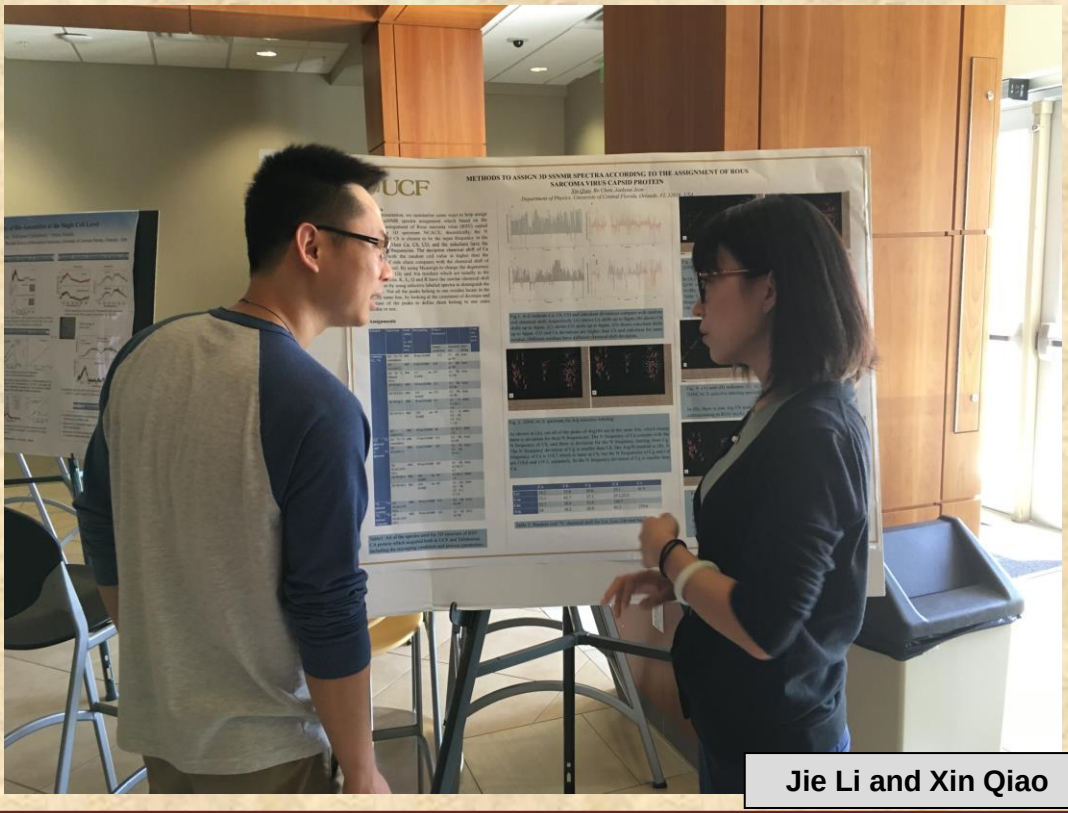
Briana Lee and Angie M. Diaz



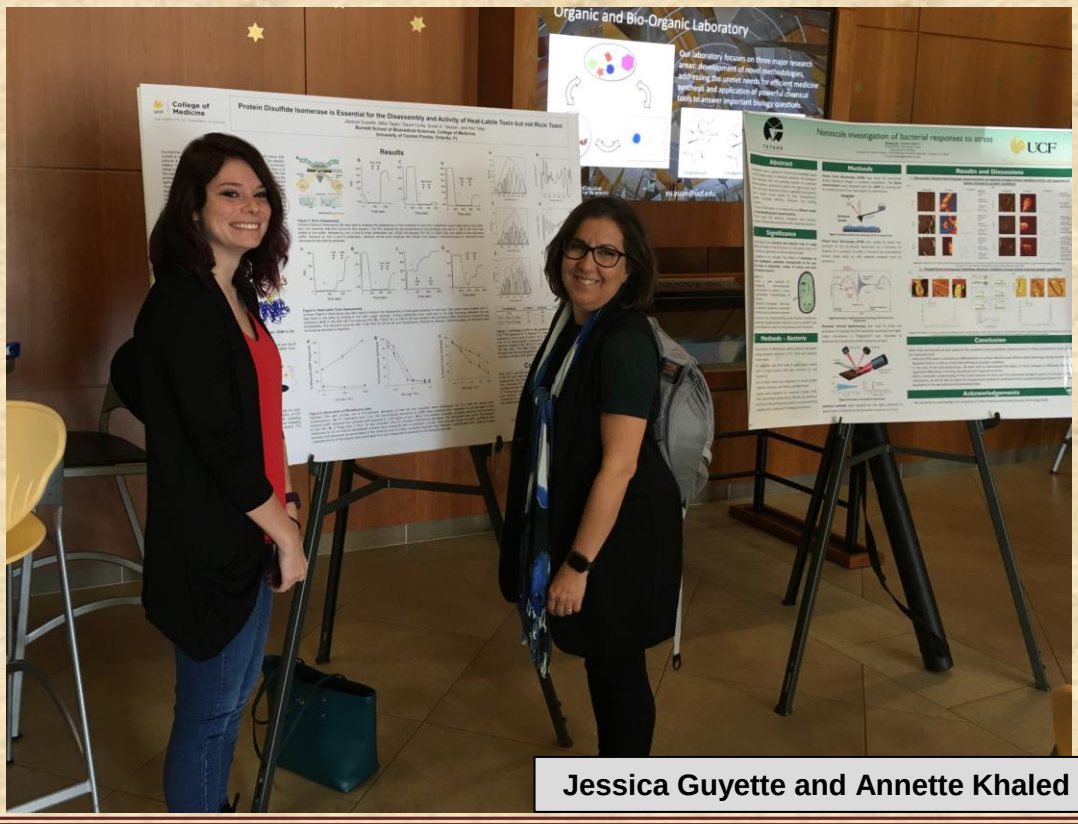
Nicholas Castaneda describes his work to Briana and Angie



Nabin Kandel and Victor Davidson



Jie Li and Xin Qiao

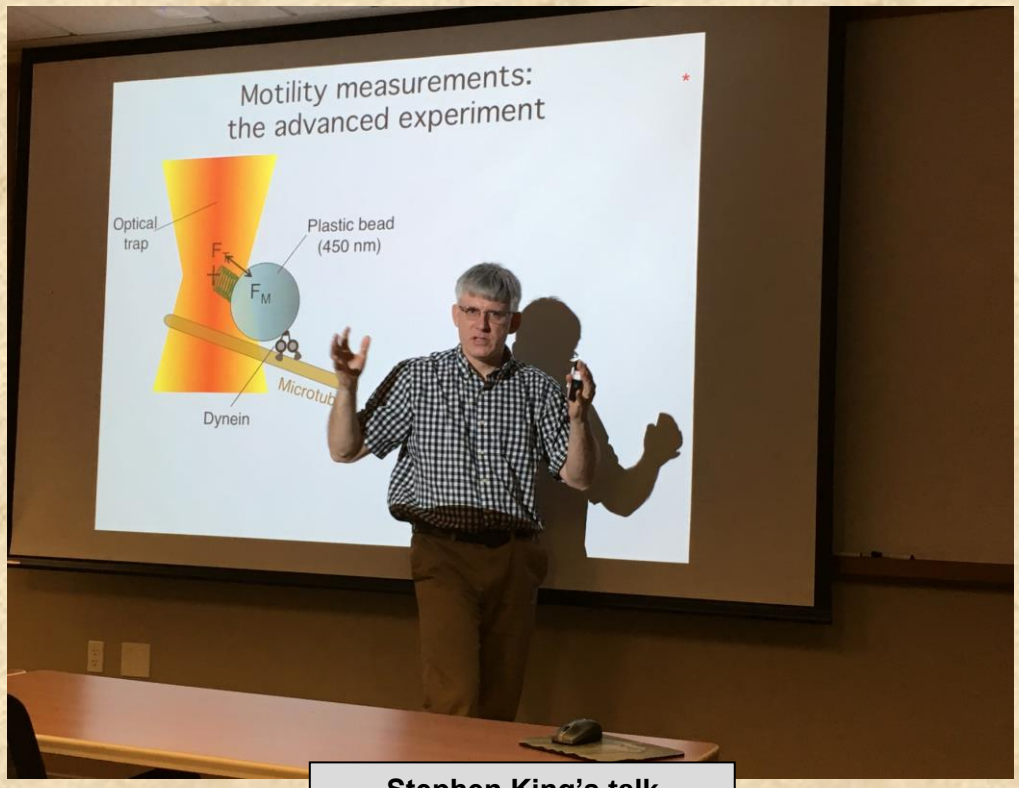


Jessica Guyette and Annette Khaled

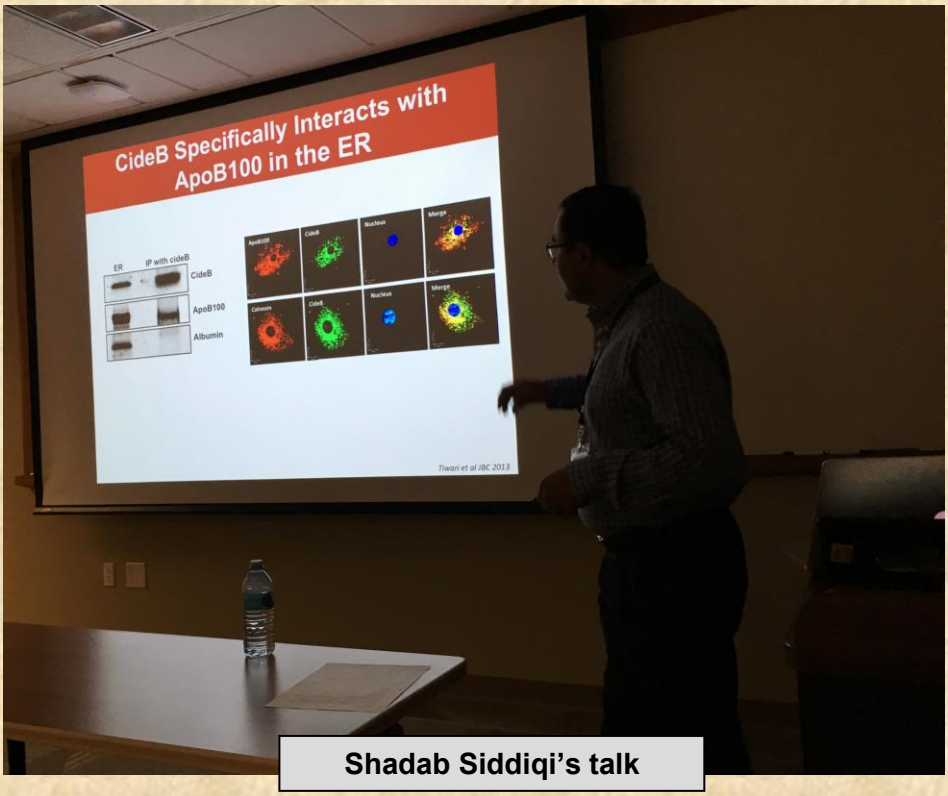
Victor Davidson, Jessica Guyette, and Suren Tatulian

Plenary session just started

Annette Khaled's talk



Stephen King's talk



Shadab Siddiqi's talk

Leon Hardy's talk