University of Central Florida

UCF Biophysics Group

Biophysics Mini-Conference

to Mark the 1st Biophysics Week

(Visit http://www.biophysics.org to learn more)

Monday, March 7, 2016

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Poster Session: 1:00-2:00 pm
UCF Physical Sciences Bldg. Atrium

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Plenary Session: 2:00-5:00 pm
UCF Physical Sciences Bldg., room 445

Plenary presentations will be given by a high school student, an undergraduate student, two graduate students, a postdoctoral researcher, and a professor. The schedule and the abstracts are provided below. Coffee and refreshments will be provided.

Monday, March 7, 2016
Biophysics Mini-Conference

1:00-2:00
Poster Presentations

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Molecular Mechanism and Physiological Function of DbpA

Jared J. Childs, Riley C. Gentry, Jirair Gevorkyan, Anthony F. T. Moore, Aliana López de Victoria, Yulia V. Gerasimova and Eda Koculi

Department of Chemistry, University of Central Florida

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A Novel Coarse-Grain Model that Captures Protein Backbone Structure and its Application to Simulate the Self-Assembly of HIV Capsid Proteins

Jeff Weber and Bo Chen

Department of Physics, University of Central Florida

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Conformations of Double-Stranded DNA: The Effect of Breathing Bubbles

Aiqun Huang and Aniket Bhattacharya

Department of Physics, University of Central Florida

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Translocation of Vesicles through Nanopores

Y. Lin, Y. Rudzevich, A. Wearne, K. Nemec, J. Morales, O. Lupan, S.A. Tatulian and L. Chow

Department of Physics, University of Central Florida

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Morphological Changes in Healthy and Malaria Infected Erythrocytes Probed by High Pressure Microscopy

Silki Arora, Jennifer Mauser, Debopam Chakrabarti and Alfons Schulte

Department of Physics and Burnett School of Biomedical Sciences, University of Central Florida

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The Molecular Basis of the Rous Sarcoma Virus Capsid Tubular Assembly by Solid State NMR

Jaekyun Jeon, Xin Qiao, Daniel Huang, Ivan Hung, Peter L. Gor'kov, Zhehong Gan, Fangqiang Zhu and Bo Chen

Department of Physics, University of Central Florida
National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL
Department of Physics, Indiana-Purdue University, Indianapolis, IN

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Isotope-Edited FTIR Reveals Distinct Aggregation and Structural Behaviors of Unmodified and Pyroglutamylated Amyloid β Peptides

Greg Goldblatt, Jason O. Matos, Jeremy Gornto and Suren A. Tatulian

Department of Physics, University of Central Florida

2:00-5:00pm:

Plenary Presentations

2:00-2:25pm:
Transmembrane Pore Formation by Amyloid Beta Oligomers

Claire Weaver

Paul J. Hagerty High School senior, Oviedo, FL

Amyloid beta (Aβ) peptide is associated with initiation and progression of Alzheimer's disease (AD). It is derived from the amyloid precursor protein (APP) *via* proteolytic cleavage by β- and γ-secretases. The cytotoxic "full-length" Aβ peptide has 42 amino acid residues: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA. Various fragments of Aβ, such as $A\beta_{25\text{-}35}$, have been found in AD brain. $A\beta_{25\text{-}35}$ (underlined in above sequence) causes neuronal damage and synaptic dysfunction. These peptides can misfold to create seeds, which trigger further misfolding and aggregation of other Aβ peptides and the tau protein. Several hypotheses exist that attempt to explain Aβ cytotoxicity. Popular ideas include:

- Aβ's erratic binding to several receptors causes deregulation, and ultimately results in neuronal necrosis or apoptosis.
- Aβ embeds into neuronal membranes and forms pores, causing membrane leakage and imbalance in ionic homeostasis, eventually leading to neuronal necrosis.

In this work, we provide experimental evidence that $A\beta_{25\text{-}35}$ forms ion-conducting pores in lipid vesicle membranes. The anionic charge of the membrane aids the formation in pores. Moreover, increasing fractions of cholesterol augment peptide-induced calcium release from the vesicles, implying stabilization of the pores by cholesterol. Further studies will include solid-state NMR to identify the atomic-resolution structure of $Ab_{25\text{-}35}$ pores.

2:25-2:50pm

Structural Pathways of Aggregation of Unmodified and Pyroglutamylated Amyloid Beta Peptides

Jacqueline Williams

UCF senior (Biomed track) and Research and Mentoring Program, University of Central Florida, Orlando, FL

The amyloid beta (Aβ) peptide is causatively linked to Alzheimer's disease (AD). Recent studies have pointed to soluble oligomers of Aβ and not fibrillar Aβ as the neurotoxic entities in AD. Aβ is derived from the amyloid precursor protein, a bitopic glycoprotein in neuronal membranes, by cleavage in the transmembrane and extracellular juxtamembrane regions by γ- and β-secretases, respectively. The peptide occurs in varying forms in terms of the number of amino acid residues and posttranslational modifications. Poor sequence specificity of γ-secretase leads to variations in the C-terminus, resulting in 40, 42, and 49 amino acids long Aβ molecules, with the 42- and 40-residue species ($A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$) being the most prevalent forms. N-terminally truncated and pyroglutamylated (at Glu_3 or Glu_{11}) Aβ peptides (Aβ-pE) have been identified in AD brain at significant quantities and have been shown to possess augmented cytotoxicity. The molecular bases for distinct toxicities of $A\beta_{1\text{-}42}$ *vs*. $A\beta_{1\text{-}40}$ and unmodified Aβ *vs*. pyroglutamylated Aβ remain to be elucidated. In this project the secondary structures of $A\beta_{1\text{-}40}$, $A\beta_{pE\ 3\text{-}40}$ and, their equimolar combination were investigated by Fourier transform infrared spectroscopy. Using ^{13}C-labeled $A\beta_{1\text{-}40}$ allowed dissection of individual structures in combined samples. We have found that $A\beta_{pE3\text{-}40}$ inhibits formation of the cross β-sheet structure typical to fibrils. This inhibition may enhance formation of off-pathway lower molecular weight aggregates of enhanced toxicity.

2:50-3:15pm

DNA inside a Nano-Channel: Equilibrium and Non-Equilibrium Conformations

Aiqun Huang[1], Walter Reisner[2], and Aniket Bhattacharya[1]

[1]Department of Physics, University of Central Florida, Orlando, FL
[2]Department of Physics, McGill University, Canada

We study the conformations of a single DNA molecule confined in a nano-channel. First, under equilibrium state, depending on the relative ratio of the persistence length to the channel width, we show that only two distinct regimes for the extension of the molecule exist. In the first regime the DNA is pictured as a series of blobs, and in the second regime the DNA chain deflects back and forth off the wall. Secondly, we study the conformations of the DNA when dynamically compressed by a sliding piston. We are interested in the monomer density profile when the piston is pushing from one end of the molecule and being retracted away later. We also show interesting behaviors of the DNA when pushed inside a very narrow nano-channel.

3:15-3:40pm

Site-Specific Structure and Dynamics of the Tubular Assembly of Rous Sarcoma Virus Capsid Protein by ssNMR and TEM

Jaekyun Jeon[1], Xin Qiao[1], Daniel Huang[1], Ivan Hung[2], Peter L. Gor'kov[2], Zhehong Gan[2] Fangqiang Zhu[3], and Bo Chen[1]

[1] *Department of Physics, University of Central Florida, Orlando, FL*
[2] *National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL*
[3] *Department of Physics, Indiana-Purdue University, Indianapolis, IN*

This research is to elucidate the assembly mechanism of retroviral capsid (CA) protein. The retroviral CA protein is derived from the cleavage of Gag protein in the maturation process, and self-assembles into a polymorphic fullerene-like form enclosing the viral genome materials. Retroviral CA proteins, for instance, HIV and RSV CA, share little sequence similarity and a common 3D structure, but form distinct CA assemblies *in vivo* and a range of similar assemblies *in vitro*. Due to the strong polymorphism, such assemblies are difficult to be studied by conventional structural biology techniques such as X-ray diffraction and cryo-electron microscopy. Solid-state NMR spectroscopy is the ideal platform to study these CA protein assemblies to obtain site-specific structural and dynamic information. However, it is challenging to make sequential assignments based on multidimensional spectra acquired with these polymorphic samples and the protein sizes about 25.6 kDa (> 230 residues), showing highly overlapping peaks and broadened line widths in the spectra.

We were able to sequentially assign 233 residues of the 237 residue RSV CA protein in its tubular assembly by combining spectra acquired at high fields and strategic sparsely and selectively labeled samples. We demonstrated that highly uniform tubular assembly can be prepared in suitable conditions. Screened by TEM, our tubular assembly exhibits sharp 6-fold symmetry under diffraction, indicating quality similar to quasi-crystalline samples. With such samples, we obtained spectra with good resolution where linewidth of ^{13}C and ^{15}N are 0.4 ppm and 0.6 ppm, respectively. A series of ssNMR spectra were acquired with uniformly ^{13}C and ^{15}N labeled samples, including 2D and 3D NCACX, NCOCX and CANCX. In spite of the sharp linewidth, signals with ^{15}N resonances between 116-120 ppm still exhibit considerable congestion. To further resolve signals unambiguously, sparsely labeled samples (1, 3-^{13}C and 2-^{13}C labeled glycerol) were prepared. In addition, two samples with selective residue uniformly ^{13}C labeled (leucine and arginine) were prepared to further consolidate the assignments, since they are the most populated residues in the sequence and show up in the most congested spectral region. Combined, we were able to sequentially assign 234 out of the 237-residue CA protein in its tubular assembly, with assistance of the Monte Carlo Simulated Annealing (MCSA) algorithm. Based on the sequential assignments, secondary structure and dynamics were derived by Talos N.

3:40-4:15pm

Molecular Insights into the Mechanism Employed by a DEAD-Box Protein to Achieve Temporal and Spatial Specificity

Aliana López de Victoria and Eda Koculi

Department of Chemistry, University of Central Florida, Orlando, FL

DbpA is an *Escherichia coli* DEAD-box RNA helicase involved in RNA isomerization in the peptidyl transferase center. Ribosome assembly is a very coordinated process. The time DbpA reaches the ribosomal RNA and the time it stays bound there influences the order of binding of other proteins to the peptidyl transferase center and the formation of alternative RNA structures. We used surface plasmon resonance to determine microscopic rate constants of DbpA association and dissociation to a fragment of the ribosomal RNA. This is the first time that the rates of association and of dissociation a DEAD-box protein to its cognate RNA have been measured. The binding of DbpA to the RNA is a one-step binding process. Moreover, both the microscopic rate constants of association and dissociation are very large. Furthermore, we measured the equilibrium constant (K_D) of DbpA binding to the RNA in the presence of various monovalent salt concentration. The plot of log (K_D) versus log [K^+] is linear and its slope suggests that 2 to 3 K^+ are released upon protein binding to RNA.

4:15-5:00pm

Remote Catalysis: Coupling Long Range Electron Transfer to Radical-Mediated Chemistry

Victor Davidson

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

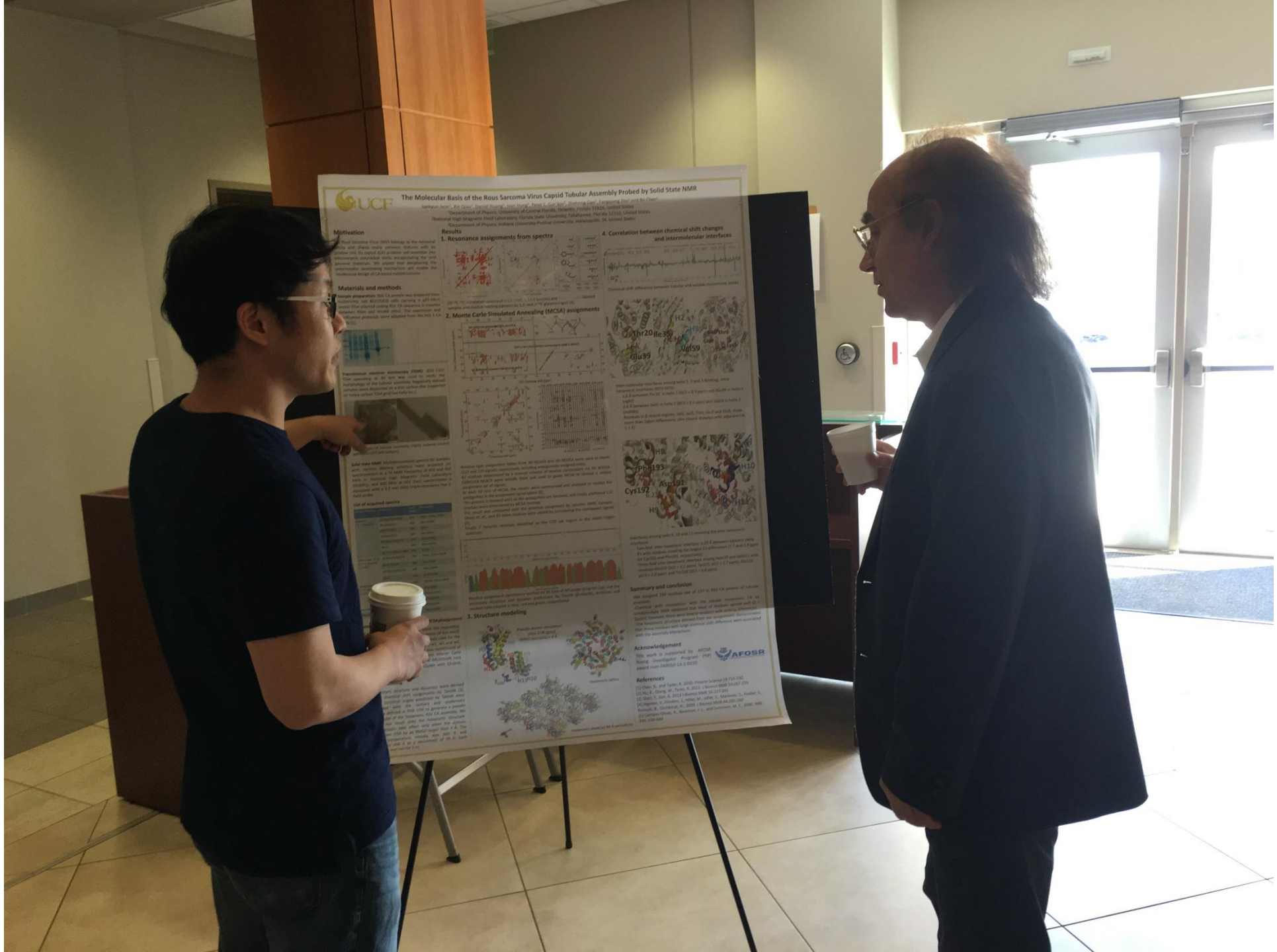
The diheme enzyme, MauG catalyzes the completion of tryptophan tryptophylquinone (TTQ) biosynthesis within a precursor protein of methylamine dehydrogenase (MADH), thus generating a catalytic and redox center in the protein from the otherwise inert tryptophan residues. This posttranslational modification is a six-electron oxidation that requires crosslinking of two tryptophan residues, oxygenation of a tryptophan residue and oxidation of the resulting quinol to TTQ. During these reactions MauG cycles through a unique *bis*-Fe(IV) intermediate with one heme as Fe(IV)=O and the other as Fe(IV) with the two axial ligands provided by histidine and tyrosine residues. The crystal of the MauG-preMADH complex reveals that the hemes of MauG

are distant from the site at which preMADH binds. Thus, catalysis does not involve direct contact between the protein substrate and either heme of MauG. Instead, this enzyme performs remote catalysis using a hole-hopping mechanism of electron transfer in which specific intervening amino acid residues of MauG are reversibly oxidized. In this manner, long range electron transfer is efficiently coupled to the radical-mediated chemical reactions that are required for TTQ biosynthesis. If the *bis*-Fe(IV) intermediate is generated in the absence of the substrate, it is unusually stable but eventual oxidizes specific methionine residues on MauG which inactivates the enzyme. The unusual mechanisms by which the catalytic and self-inactivating reactions occur, and the implications of these results will be discussed.

☺☺


Translocation of vesicles through nanopores
Morphological Changes in Healthy and Malaria Infected Erythrocytes
Probed by High Pressure Microscopy


PHYSICS
Tri-Fold Presentation Board
Tríptico para presentaciones
Panneau d'affichage à trois battants

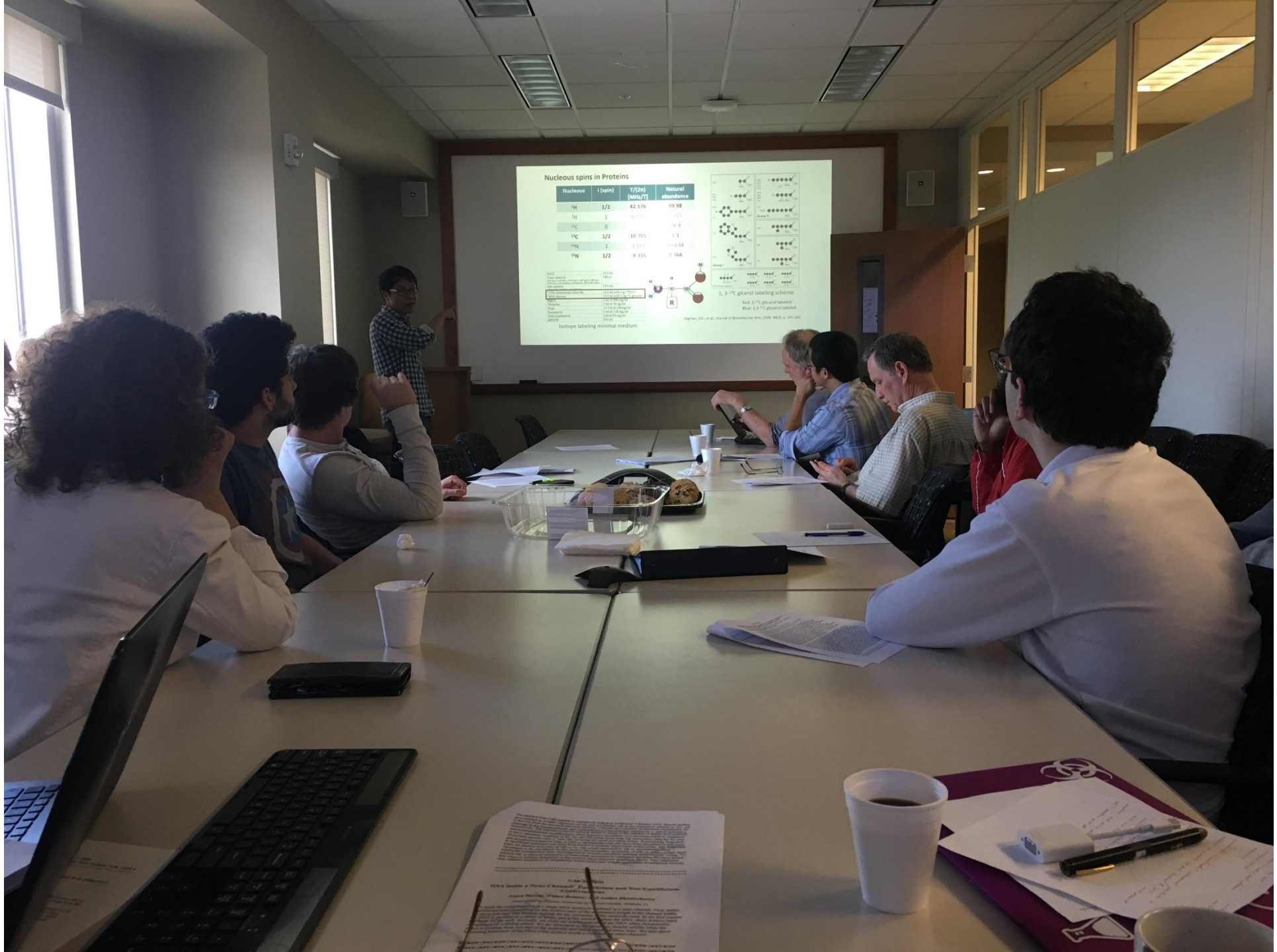

The Molecular Basis of the Rous Sarcoma Virus Capsid Tubular Assembly Probed by Solid State NMR
Motivation
Materials and methods
Results
1. Resonance assignments from spectra
2. Monte Carlo Simulated Annealing (MCSA) assignments
3. Structure modeling
4. Correlation between chemical shift changes and intermolecular interfaces
Summary and conclusion
Acknowledgement
References


Morphological Changes in Healthy and Malaria Infected Erythrocytes
Probed by High Pressure Microscopy
Volume changes in healthy RBCs
Morphological changes in infected RBCs
Conclusions
The Molecular Basis of the Rous Sarcoma Virus Capsid Tubular Assembly Probed by Solid State NMR
Summary and conclusions
Acknowledgment
AFOSR

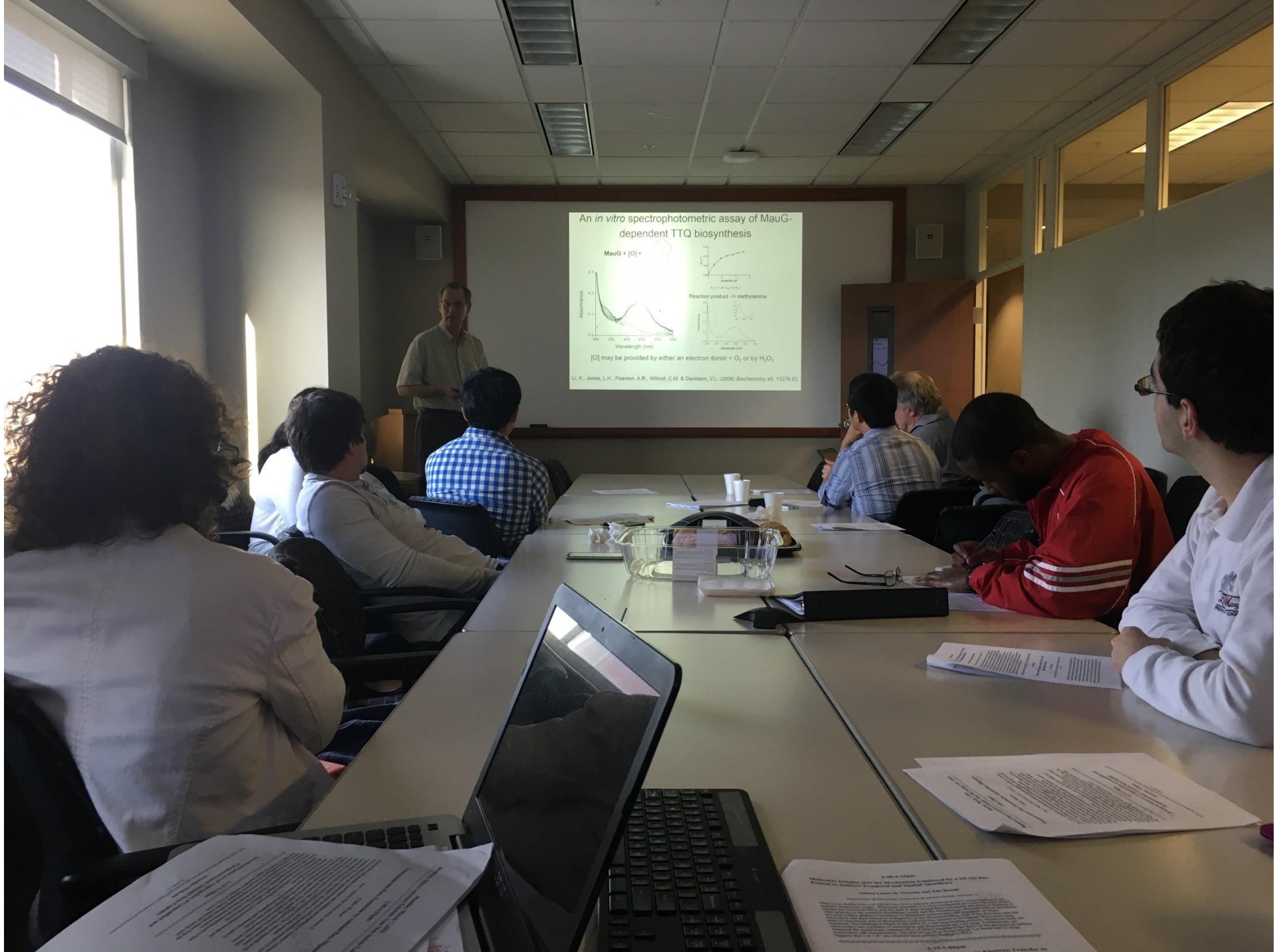

Aβ protein
· Proteolytic cleavage from Amyloid Precursor Protein
· GSNKGAIIGLM
· Full length fragment Aβ42
· Molecular dynamics study:
· •β-barrel motif
· Stabilized by cholesterol


Amyloid Precursor Protein (APP)
Transmembrane Protein
Mutations causing FAD primarily affects the APP, PSEN 1, and PSEN 2 genes
These mutations increase Aβ load in the brain linking Aβ and AD


Equilibrium conformations
Equilibrium conformations


Nucleous spins in Proteins
Nucleous
I (spin)
γ/(2π) [MHz/T]
Natural abundance
1H 1/2 42.576 99.98
2H 1 6.536 0.015
12C 0 0 98.9
13C 1/2 10.705 1.1
14N 1 3.077 99.634
15N 1/2 -4.316 0.366
Isotope labeling minimal medium
1, 3-13C glcerol labeling scheme
Red: 2-13C glycerol labeled
Blue: 1,3-13C glycerol labeled
Higman, V.A., et al., Journal of Biomolecular Nmr, 2009. 44(4): p. 245-260.


E. coli DbpA is Sequence Specific in vitro
UCF
R153
H89
H93
H90
H91
H92
R32
H92
K_d(RNA) = 0.4 nM
K_d(RNA) = 7nM


An *in vitro* spectrophotometric assay of MauG-dependent TTQ biosynthesis
MauG + [O] +
Absorbance
Wavelength (nm)
Reaction product -/+ methylamine
[O] may be provided by either an electron donor + O_2 or by H_2O_2
Li, X., Jones, L.H., Pearson, A.R., Wilmot, C.M. & Davidson, V.L. (2006) *Biochemistry* 45, 13278-83