

# Research and Innovation Conference

## Bridging Bioinformatics, Structural Biology and Molecular Genomics

### PROGRAM

Introduction by  
Track Chairs  
1:30

**Simon Sherman**, *Director, Nebraska Informatics Center for the Life Sciences and Professor, Eppley Cancer Institute, UNMC*  
**Gloria Borgstahl**, *Professor, Eppley Institute for Cancer Research and Allied Diseases*

1:40-2:20

**Yury O. Chernoff**  
"Infectious Proteins, Protein Assembly Disorders and Structural Inheritance"

2:20-2:50

**Paul Sorgen**  
"Expression, Reconstitution, and Characterization of the Connexin43 Carboxyl Terminus Attached to the 4th Transmembrane Domain"

Break

2:50-3:10

3:10-3:50

**Lars C. Pedersen**  
"The X-family Polymerases: Filling the gap between structure and function"

3:50-4:30

**Ashok Deniz**  
"Single-Molecule Fluorescence: A Novel Tool for Structural Studies of Intrinsically Disordered Proteins"

junction protein connexin43 attached to the 4th transmembrane domain (TM4-Cx43CT) was used as a model system (residues G178-I382). The purification was optimized for structural analysis by nuclear magnetic resonance (NMR) because this method is well suited for small membrane proteins and proteins that lack a well-structured three-dimensional fold. The TM4-Cx43CT was purified to homogeneity with a yield of ~6 mg per liter from C41(DE3) bacterial cells, reconstituted in the anionic detergent 1-palmitoyl-2-hydroxy-sn-glycero-3-[phospho-RAC-(1-glycerol)], and analyzed by circular dichroism and NMR to demonstrate that the TM4-Cx43CT was properly folded into a functional conformation by its ability to form  $\alpha$ -helical structure and associate with a known binding partner, the c-Src SH3 domain, respectively.

#### "The X-family Polymerases: Filling the gap between structure and function"

The mammalian X-family polymerases can fill short gaps in DNA within the base excision repair pathway or rejoining of double strand breaks in DNA through non-homologous end-joining. Structural information now exists for polymerases b, l, m and TdT that provides insight into the range of substrates accommodated by these enzymes and the gradient of template dependence from Pol b to TdT. In addition, the use of non-hydrolyzable analogs has allowed us to obtain all-atom pre-catalytic complexes leading to a greater understanding of the catalytic mechanism utilized by DNA polymerases.

#### "Single-Molecule Fluorescence: A Novel Tool for Structural Studies of Intrinsically Disordered Proteins"

Intrinsically disordered proteins (IDPs) are increasingly found to play major roles in cell biology and disease. IDPs are relatively unstructured by themselves, but can gain stable structure by interaction with binding partners. We are utilizing single-molecule fluorescence methods to probe these complex and highly dynamic molecules. These state-of-the-art technologies allow direct measurements of structural distributions and dynamics, while avoiding loss of information due to ensemble averaging. In one example, we investigated the structural dynamics of the yeast prion protein Sup35, whose regulatable amyloid formation is believed to have functional significance. We have utilized single-molecule FRET as a molecular ruler, coincidence to interrogate intermolecular interactions, and correlation analysis to probe the conformational dynamics of this protein. Together, the data show that it populates an ensemble of compact and rapidly fluctuating conformations. Our results favor an amyloid formation mechanism for this protein involving conformational changes to an amyloid structure within the context of oligomeric species. I will also briefly discuss an example of IDP induced folding, and emerging microfluidic and multicolor single-molecule FRET methods that extend current single-molecule measurement capabilities. The combined single-molecule fluorescence approach provides a powerful platform for detailed studies of structure, interactions and biology of this important class of proteins.

### ABSTRACTS

#### "Infectious Proteins, Protein Assembly Disorders and Structural Inheritance"

Amyloids are fiber-like ordered cross- $\beta$  aggregates, causing certain diseases in humans and provide a basis for infectious or heritable proteins (prions). Yeast prions, that are extensively used as a model for studying amyloids, propagate via the chaperone machinery which normally protects cells from environmental stresses. This suggests that prion-like aggregation originated from the processes involved in feedback regulation of protein activity and/or protection of some proteins from destruction during unfavorable conditions. Specificity of amyloid conversion is apparently controlled by very short amino acid stretches. Identification of such stretches may enable us to search databases for potentially amyloidogenic and/or infectious proteins. As an additional mechanism of heritable change, prion formation may contribute to heritable variability at the population level.

#### "Expression, Reconstitution, and Characterization of the Connexin43 Carboxyl Terminus Attached to the 4th Transmembrane Domain"

In recent years, reports have identified that many eukaryotic proteins contain disordered regions spanning greater than 30 consecutive residues in length. In particular, a number of these intrinsically disordered regions occur in the cytoplasmic segments of plasma membrane proteins. These intrinsically disordered regions play important roles in cell signaling events, as they are sites for protein-protein interactions and phosphorylation. Unfortunately, in many crystallographic studies of membrane proteins, these domains are removed because they hinder the crystallization process. Therefore, a purification procedure was developed to enable the biophysical and structural characterization of these intrinsically disordered regions while still associated with the lipid environment. The carboxyl-terminal domain from the gap

### SPEAKER BIOS

**Yury O. Chernoff** is Professor and Associate Chair at School of Biology, Georgia Institute of Technology, Atlanta. He received his Ph.D. in Biology from St. Petersburg State University (Russia), performed postdoctoral research at Okayama University (Japan) and University of Illinois at Chicago (USA), and joined Georgia Tech since 1995. Dr. Chernoff is also an Editor-in-Chief of the journal Prion, and member of editorial boards of The Journal of Biological Chemistry and Gene Expression. He published more

than 60 scientific papers and edited a book. Dr. Chernoff is using yeast models for studying the cellular control of amyloid aggregation and evolution of prion properties. He discovered that prions can be induced by protein overproduction and provided the first evidence for the chaperone role in prion phenomena.

**Paul Sorgen** is an Associate Professor in the Department of Biochemistry and Molecular Biology at UNMC. He obtained his Ph.D. in Biochemistry and Molecular Biology in 1999 at the University of Florida (Dr. Cain). Dr. Sorgen was a postdoctoral fellow at the Albert Einstein College of Medicine with Dr. Girvin from 1999-2003. In 2003 he joined the faculty at the UNMC. His laboratory is focused on the structure and function of membrane proteins and their complexes with other proteins with an emphasis in NMR. A major focus in his lab is on the structure of the gap junction proteins and how specific domains of these proteins regulate the gating of gap junction channels by acidification, phosphorylation, and molecular partner interactions. Dr. Sorgen is Co-Director of the Nebraska Center for Structural Biology and Director of the Analytical Ultracentrifugation shared resource.

**Lars Pedersen** received his BS in chemistry from the University of North Carolina in 1990 and his PhD in biochemistry from the University of Washington in 1994. He then did post-doctoral research at the NIEHS/NIH, studying cytosolic sulfotransferases and enzymes involved in heparan sulfate biosynthesis. Currently, he is the head of the collaborative crystallography Structure Function Group at the NIEHS. In this capacity, he supports ongoing research at the NIEHS, in DNA replication and repair using X-ray crystallography techniques. In addition, his personal research focus is on enzymatic biosynthesis of homogeneous heparan sulfate for improved anti-coagulation and anti-cancer therapeutics.

**Ashok Deniz** is an Associate Professor of Molecular Biology at The Scripps Research Institute in La Jolla, California. His work focuses on the development and application of state-of-the-art single-molecule fluorescence methods to answer key questions in biological folding, misfolding and assembly. Dr. Deniz received his Ph.D. in Chemistry in 1996 from the University of Chicago, using pulsed-laser techniques to investigate short-lived organic species. He then undertook postdoctoral training in the area of Biophysics at the University of California, Berkeley with Peter Schultz (in collaboration with Shimon Weiss, then at LBL). During this time, he was involved in some of the early developments of single-molecule FRET, a "molecular ruler" that has since become very popular for structural studies of biomolecules. In late 2000, he took a position as Assistant Professor in the Department of Molecular Biology at The Scripps Research Institute, and became Associate Professor in early 2007. During recent years, Dr. Deniz has focused on applying single-molecule fluorescence technology to address key issues in biophysics, while continuing to make selected advances in the methodologies. In one area of application, his group has adapted single-molecule methods as novel tools for structural studies of intrinsically disordered proteins, which are involved in several biological and disease processes. Another area of research is into the mechanisms of assembly of biological complexes that function as molecular machinery in the cell. Please visit the Deniz Lab's website at [www.scripps.edu/mb/deniz](http://www.scripps.edu/mb/deniz) for more information about his research and a complete list of publications.