

Organizing Committee

Vince Conticello, Emory University, USA

Edward Egelman, University of Virginia, USA

Louise Serpell, University of Sussex, United Kingdom

Jerson Silva, Federal University of Rio de Janeiro, Brazil

Ting Xu, University of California, Berkeley, USA

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October 2015

Dear Colleagues,

We would like to welcome you to the Biophysical Society Thematic Meeting, *Polymers and Self Assembly: From Biology to Nanomaterials*, co-sponsored by the Brazilian agencies FAPERJ and CNPq. These Thematic Meetings are an opportunity for scientists who might not normally meet together to gather and exchange ideas in different locations around the world. Previous Thematic Meetings have been held in China, Singapore, the United States, India, South Korea, Ireland, Poland, Turkey, Taiwan and Spain. Future meetings are already scheduled for South Africa, Canada and Switzerland.

Our meeting is aimed at bringing together biophysicists who study protein polymers, both those occurring normally, such as bacterial flagellar filaments, F-actin and microtubules, and those occurring pathologically, such as amyloid, with materials scientists, chemists and physicists who work on synthetic peptides, polymers and designed structures. Great advances that have recently been made in cryo-EM, which now allow many polymers to be readily solved at a near-atomic resolution, helped stimulate the organization of this meeting, but we look forward to presentations using many different techniques that are helping to elucidate the structure of these self-assembled polymers. We hope that you will all actively take part in the discussions following each talk, in the poster sessions, and in the informal exchanges that will be possible during the coffee breaks, the banquet and the excursion. We also hope that you will enjoy the beautiful surroundings of Rio de Janeiro!

The Organizing Committee

Vince Conticello

Edward Egelman

Louise Serpell

Jerson Silva

Ting Xu

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Registration

The registration and information desk will be located on Sunday in the Miramar (upper level) and Monday through Thursday in the Lobby outside of Plaza/Excelsior and Mar da Barra meeting Rooms. Registration hours are as follows:

Sunday, October 25	16:00 – 18:00
Monday, October 26	8:00 – 17:00
Tuesday, October 27	8:00 – 17:00
Wednesday, October 28	8:00 – 17:00
Thursday, October 29	8:00 – 12:00

Instructions for Presentations**Presentation Facilities**

A data projector will be made available in the Auditorium. Speakers are required to bring their laptops. Speakers are advised to preview their final presentations before the start of each session.

Poster Sessions

- 1) All poster sessions will be held in Mar da Barra. Posters in each poster session will be on display from 8:00 – 22:00 on the day of the assigned poster session.

All posters should be set up in the morning of October 26 and **MUST** be removed by 22:00 on October 27.

- 2) During the poster presentation sessions, presenters are requested to remain in front of their posters to meet with attendees.
- 3) A display board measuring 3 feet 2.5 inches (height) by 3 feet 1.8 inches (wide) will be provided for each poster. Poster boards are numbered according to the same numbering scheme as in the program book.
- 4) All posters left uncollected at the end of the meeting will be discarded.

Coffee Break

Coffee breaks will be held outside of the meeting rooms where tea and coffee will be provided free of charge to all participants.

Smoking

Smoking is not permitted inside the buildings of the Windsor Excelsior Hotel.

Meals

The welcome reception, coffee breaks, and banquet are included in the registration fee.

Social Events

Welcome Reception with light hors d'oeuvres will be held in the Miramar at the Windsor Excelsior on Sunday, October 25, 2015 from 18:00 – 20:00.

Name Badges

Name badges are required to enter all scientific sessions and poster sessions. Please wear your badge throughout the conference.

Contact

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from October 25 – October 29 during registration hours. You may also contact Dorothy Chaconas at DChaconas@biophysics.org.

Polymers and Self Assembly: From Biology to Nanomaterials
Windsor Excelsior
Rio de Janeiro, Brazil
October 25-30, 2015

PROGRAM

The meeting will take place at the Windsor Excelsior Hotel. Scientific sessions will be held in the Plaza/Excelsior room and the poster sessions in the Mar da Barra room.

Sunday, October 25, 2015

16:00 – 18:00	Registration/Information	Lobby Miramar
18:00 – 20:00	Opening Reception	Miramar

Monday, October 26, 2015

8:00 AM – 17:00	Registration/Information	Foyer of Mar da Barra
Session I	Louise Serpell, University of Sussex, United Kingdom, Chair	
9:00 – 9:15	Welcome/Opening Remarks	
9:15 – 9:45	Gillian Fraser, University of Cambridge, United Kingdom <i>Building a Flagellum on the Bacterial Cell Surface</i>	
9:45 – 10:15	Marie-France Carlier, Centre National de La Recherche Scientifique (CNRS), France <i>Self-Assembly of Actin in Cell Motility: From Molecules to Movement</i>	
10:15 – 11:00	Coffee Break	
11:00 – 11:30	Enrique De La Cruz, Yale University, USA <i>Cation Release Modulates Actin Filament Mechanics and Drives Severing by Vertebrate Cofilin</i>	
11:30 – 11:45	Frederico Gueiros Filho, Instituto de Quimica-USP, Brazil <i>Filament Capping Regulates the Bacterial Tubulin-like Cytoskeleton*</i>	
11:45 – 13:30	Lunch on own	

*Short talks selected from among submitted abstracts

Session II	Louise Serpell, University of Sussex, United Kingdom, Chair	
13:30 – 14:00	Edward Egelman, University of Virginia, USA <i>Cryo-EM of Protein Polymers at Near- Atomic Resolution Yields New Insights</i>	
14:00 – 14:30	Richard Garratt, Univesrity of Sao Paulo, Brazil <i>How to Build a Septin Filament</i>	
14:30 – 15:00	Robert Robinson, Institute for Molecular & Cell Biology, Singapore <i>The Varied Geometries of ParM Cytomotive Filaments in Bacterial Plasmid Segregation</i>	
15:00 – 15:15	Kildare Miranda, Federal University of Rio de Janeiro, Brazil <i>Asymmetry of Polyphosphates Polymers in Ion-rich Organelles*</i>	
15:15 – 15:45	Coffee Break	
15:45 – 17:45	Poster Sessions I	Mar da Barra
18:00 – 20:00	Dinner on own	

Tuesday, October 27, 2015

8:00 – 17:00	Registration/Information	Foyer Mar da Barra
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Session III	Vince Conticello, Emory University, USA, Chair	
9:00 – 9:30	Anna Rising, Karolinska Institutet, Sweden <i>Spider Silk Assembly is Mediated by a Lock and Trigger Mechanism</i>	
9:30 – 9:45	Jan Johansson, Karolinska Institutet, Sweden <i>Development of Recombinant Spider Silk Proteins with Tunable Assembly Properties for Biomimetic Spinning*</i>	
9:45 – 10:15	Thomas Scheibel, University of Bayreuth, Germany <i>Structural Proteins: Self- Assembling Biopolymers for Various Applications</i>	
10:15 – 10:30	Guillaume Lamour, University of British Columbia, Canada <i>Nanomechanics of Amyloid-like Polymers Made of Self-assembled Mouse Prion Proteins*</i>	
10:30 – 11:00	Coffee Break	

**Short talks selected from among submitted abstracts*

11:00 – 11:30	Louise Serpell, University of Sussex, United Kingdom <i>Exploiting Amyloid Fibrils as Functional Bionanomaterials</i>	
11:30 – 12:00	Jerson Silva, Federal University of Rio de Janeiro, Brazil <i>Hydration and Cavities in Amyloid Fibrils and Oligomers Modulated by Hydrostatic Pressure</i>	
12:00 – 12:15	Cong Liu, Chinese Academy of Sciences, China <i>Structure-based Design of Amyloids with Novel Functions for Nanomaterials*</i>	
12:15 – 12:30	Francesco Ruggeri, École Polytechnique Fédérale de Lausanne (EPFL), Switzerland <i>Amyloids Structural and Nanomechanical Characterization at the Individual Aggregate Scale*</i>	
12:30 – 14:00	Lunch on own	
Session IV	Vince Conticello, Emory University, USA, Chair	
14:00 – 14:30	Claudio Fernandez, National University of Rosario, Argentina <i>Biophysics and Structural Biology of Neurodegeneration: The Case of Alpha-Synuclein</i>	
14:30 – 15:00	Debora Foguel, Medical Biochemistry Institute, Brazil <i>Transthyretin-Related Diseases: From the Patient to the Protein</i>	
15:00 – 15:30	Monica S. Freitas, Federal University of Rio de Janeiro, Brazil <i>Protein Misfolding Pathway Probed by Solution and Solid- State NMR</i>	
15:30 – 15:45	Jean-Marie Ruysschaert, Universite Libre de Bruxelles, Belgium <i>Lipid Nanoparticles and Amyloids Activate Receptors of the Innate System*</i>	
15:45 – 16:30	Coffee Break	
16:30 – 18:30	Poster Session II	Mar da Barra
20:30	Banquet	Porcao Rio Restaurant

**Short talks selected from among submitted abstracts*

Wednesday, October 28, 2015

9:00 – 17:00	Registration/Information	Foyer Mar da Barra
Session V	Ting Xu, University of California, Berkeley, USA, Chair	
9:00 – 9:30	Dek Woolfson, University of Bristol, United Kingdom	<i>New Peptide- Based Assemblies and Materials by Design</i>
9:30 – 10:00	Vince Conticello, Emory University, USA	<i>Protein Assemblies by Design</i>
10:00 – 10:30	Aline Miller, University of Manchester, United Kingdom	<i>Self- Assembling Peptide Based Materials for Regenerative Medicine</i>
10:30 – 11:00	Coffee Break	
11:00 – 11:30	Akif Tezcan, University of California San Diego, USA	<i>Protein Self- Assembly by Rational Chemical Design</i>
11:30 AM – 12:00	Joel Schneider, National Institutes of Health, USA	<i>Racemic Hydrogels from Enantiomeric Peptides: Predictions from Linus Pauling</i>
12:00 – 12:15	Maité Paternostre, Institute of Integrative Biology of the Cell, France	<i>pH Dependent Peptide Self-Assemblies: A Mechanism as Old as Viruses*</i>
12:15 – 12:30	Ivan Korendovych, Syracuse University, USA	<i>Short Peptides Self-assemble in the Presence of Metals to Produce Catalytic Nanomaterials*</i>
12:30 – 12:45	Gina-Murela Mustata, Simmons College, USA	<i>Designer Peptides Self-assemble on Graphene to Create Remarkably Stable, Precisely Organized Substrates*</i>
13:00 – 15:00	Lunch on own/Excursion to Corcovado	

*Short talks selected from among submitted abstracts

Thursday, October 29, 2015

9:00 AM – 17:00	Registration/Information	Foyer Mar da Barra
Session VI	Jerson Silva, Federal University of Rio de Janeiro, Brazil, Chair	
9:00 – 9:30	Cait MacPhee, University of Cambridge, United Kingdom <i>Bacterial Strategies for Protein Self- Assembly at Interfaces</i>	
9:30 – 9:45	Sarah Perrett, Chinese Academy of Sciences, China <i>Self-assembly of Protein Nanofibrils that Display Active Enzymes*</i>	
9:45 – 10:00	Markus Weingarth, Utrecht University, Netherlands <i>The Supramolecular Organization of a Peptide-based Nanocarrier at High Resolution*</i>	
10:00 – 10:30	Ting Xu, University of California Berkeley, USA <i>Hybrid Biomaterials Based on Natural and Synthetic Polymers: From Basics to Applications</i>	
10:30 – 11:00	Coffee Break	
11:00 – 11:30	Ronald Zuckermann, University of California Berkeley, USA <i>Synthesis, Folding and Assembly of Sequence- Defined Peptoid Polymers</i>	
11:30 – 11:45	Jon Parquette, The Ohio State University, USA <i>Immobilization of RubisCO by Self-assembled Nanotubes*</i>	
11:45 – 13:30	Lunch on own	
Session VII	Jerson Silva, Federal University of Rio de Janeiro, Brazil, Chair	
13:30 – 14:00	Mibel Aguilar, Monash University, Australia <i>Supramolecular Self-Assembly of β-Peptides: New Materials with Tunable Morphology and Chemical Function</i>	
14:00 – 14:30	C.J. Brinker, Sandia National Laboratories, USA <i>Inorganic Polymerization at Cellular Interfaces</i>	
14:30 – 15:15	Coffee Break	
15:15 – 15:45	Tom Russell, University of Massachusetts Amherst, USA <i>Interfacial Assembly of Synthetic and Natural Nanoparticles</i>	

**Short talks selected from among submitted abstracts*

15:45 – 16:15

Dave Adams, University of Liverpool, United Kingdom
Multicomponent Supramolecular Hydrogels

16:15

Closing Remarks and Biophysical Journal Poster Awards

SPEAKER ABSTRACTS

Building a Flagellum on the Bacterial Cell Surface.

Gillian M. Fraser¹, Paul M. Bergen¹, Lewis D. Evans^{1,3}, Simon Poulter¹, Eugene M. Terentjev², Colin Hughes¹, Daniel Nietlispach³.

¹University of Cambridge, Cambridge, United Kingdom, ²University of Cambridge, Cambridge, United Kingdom, ³University of Cambridge, Cambridge, United Kingdom.

Bacteria build helical propellers, called flagella, on their surface. Biologists and physicists have long found flagella fascinating as they illustrate beautifully how complex structures self-assemble to operate as nanomachines on the cell surface. During flagellum assembly, thousands of subunits destined for the growing structure are made inside the cell, then unfolded and exported across the cell membrane. Like other biological functions, this initial phase of export consumes energy produced by the cell. But then the subunits pass into a channel at the centre of the growing flagellum on the outside of the cell, and must transit a substantial distance to the flagellum tip where they crystallise into the structure. In this way the flagellum grows at a constant rate to several times the length of the cell.

The mystery has been how are flagellar subunits passed down the long channel far outside the cell where there is no discernable energy source to propel them?

I will describe a simple and elegant mechanism that allows constant rate growth of the flagellum outside the cell by harnessing the entropic force generated by the unfolded subunits themselves as they link in a chain that is pulled to the flagellum tip. I will go on to present new NMR data that reveal structural changes in the membrane export machinery as flagellar subunits bind prior to capture into the export chain.

Reference

Evans LDB, Poulter S, Terentjev EM, Hughes C and Fraser GM (2013) A chain mechanism for flagellum growth. *Nature* 504: 287-290

Self-Assembly of Actin in Cell Motility: From Molecules to Movement

Marie-France Carlier

Emory University, France

No abstract

Cation Release Modulates Actin Filament Mechanics and Drives Severing by Vertebrate Cofilin**Enrique De La Cruz**

Yale University, USA

The polymerization of the protein actin into helical filaments powers many eukaryotic cell movements and provides cells with mechanical strength and integrity. The actin regulatory protein, cofilin, promotes actin assembly dynamics by severing filaments and increasing the number of ends from which subunits add and dissociate. I will present results from biochemical and biophysical studies focused on defining in chemical and physical terms how cofilin binds and fragments actin filaments. The experimental data are well described by a model in which the cofilin-linked dissociation of filament-associated cations introduces discontinuities in filament topology and mechanical properties that promote fracture preferentially at junctions of bare and decorated segments along filaments.

Filament Capping Regulates the Bacterial Tubulin-Like Cytoskeleton**Frederico Gueiros Filho¹**¹Instituto de Química – USP, Brazil*See abstract: Pos-18 Board-18*

Cryo-EM of Helical Polymers

Edward Egelman

University of Virginia, USA

Cryo-EM has undergone a revolution, driven by direct electron detectors, and a near-atomic level of resolution can now be reached for many biological samples. While complexes such as the ribosome can be solved at higher resolution and more readily by cryo-EM than they can be by crystallography, they can still be crystallized. However, a vast number of complexes of biological interest are helical polymers, and most of these can never be crystallized. I will describe the application of cryo-EM to helical assemblies in four different areas: 1) *Vibrio cholera*, the organism responsible for cholera, uses a Type Six Secretion System in pathogenesis. We now understand in detail how parts of this system assemble and work. 2) Type IV pili are essential for the infectivity of bugs such as *Neisseria meningitidis*. We have shown for *Campylobacter jejuni* (responsible for most food-borne illnesses in the world) that the conserved flagellin protein can be assembled into different quaternary structures by small amino acid changes. We show the same thing for Type IV pilins. 3) Flexible filamentous plant viruses are responsible for half of the viral agricultural crop damage, but have resisted all attempts at structure determination since the studies of J.D. Bernal >75 years ago. We have solved the structure of two members of this family, bamboo mosaic virus (BaMV) and wheat streak mosaic virus (WSMV) and show how, because they are completely non-toxic, they can be used in biotechnology, in everything from medical imaging to serving as platforms for vaccines. 4) Viruses that infect hyperthermophilic archaea can survive in nearly boiling acid or organic solvents. We now understand how the stability of DNA in SIRV2 and AFV1 is achieved. AFV1, like Ebola, is a filamentous membrane-enveloped virus, and we present the first atomic structure of such a virus.

How to Build a Septin Filament

Richard Garratt.

University of Sao Paulo, Brazil

Monomeric septins polymerize into membrane associating hetero-filaments which are involved in membrane remodelling events and barrier formation. The human genome includes 13 genes coding for septins that can be divided into four different groups leading to hundreds of different possible combinations for hetero-filament formation. The filaments themselves are stabilized via two different types of inter-subunit interface (G and NC) which alternate along the main axis. In an attempt to understand the rules which underpin spontaneous filament assembly we have used crystallographic approaches allied to a series of complementary biophysical techniques. In essence, the problem of self assembly can be reduced to understanding the structural basis for specificity at each of the five different interfaces which appear between individual septins along a filament composed of four different monomers. We demonstrate that a C-terminal coiled-coil domain is important for the recognition of partner septins at one of the NC interfaces as well as contributing to the formation of higher-order assemblies. Studies of septins bound to both GTP and GDP show that the two types of interface are interconnected as a result of an unexpected shift in the register of a central β -sheet strand on GTP hydrolysis. This is predicted to affect membrane binding. In summary, our data suggest mechanisms for self-assembly, filament bundling and the importance of GTP binding and hydrolysis for membrane association.

phosphorylated by S-phase cyclin-Cdk1-Cks1. The processivity is modulated by phosphorylation/dephosphorylation of a priming site and a diversional site by two kinases and a phosphatase of stress pathways. Both the priming site and the diversional site compete for binding to Cks1. This mechanism demonstrates how external signals can be integrated into the Cdk1 control system via multi-branched signal-processing modules based on multisite phosphorylation networks. Such transistor-like modules are possibly ubiquitous and could regulate many cellular events.

The Varied Geometries of ParM Cytomotive Filaments in Bacterial Plasmid Segregation

Robert Robinson

Institute for Molecular & Cell Biology, Singapore

No abstract

Asymmetry of Polyphosphates Polymers in Ion-rich Organelles

Kildare Miranda, Wendell Girard-Dias, Wanderley De Souza
Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Understanding mechanisms involved in osmoregulation control in protozoan parasites has been a challenge for many research groups. Over the past years, a number of key players in cell signaling in trypanosomatid parasites have been identified. Among these, inorganic polyphosphate (PolyP) polymers have proven to play important roles in cell physiology, both as an energy source, stored in its constituent phosphoanhydride bonds, and as a polyanion that might activate a number of physiological processes. A number of methods for PolyP localization and quantification are available, including DAPI-staining followed by microscopic visualization and quantification, P-NMR analysis, enzymatic assay using recombinant exopolyphosphatases and analytical electron microscopy (AEM). From the AEM point of view, X-ray microanalysis combined with elemental mapping as well as energy filtered TEM have been the most employed techniques carried out to explore the two-dimensional composition and distribution of (poly)ions (including polyphosphate stores) within cells. In this work, we combined different three-dimensional electron microscopy techniques with X-ray microanalysis using more sensitive detectors to generate three-dimensional nanoscale elemental maps of polyphosphate-rich organelles present in the protozoan parasite *Trypanosoma cruzi*. We showed a heterogeneous three-dimensional distribution of ions within the shell of polyphosphate polymers forming segregated nanochemical domains with an auto exclusion pattern for the cations. This is the first direct evidence for the asymmetric distribution of cations bound to a polyphosphate polymer, raising questions about polyphosphate assembly mechanisms and its influence on the functional role of polyphosphate in cell physiology. In addition, these strategies were used here to explore the three-dimensional elemental distribution are novel for biological materials and may be applied to future studies in a wide variety of biological samples.

Spider Silk Assembly is Mediated by a Lock and Trigger Mechanism**Anna Rising**

Karolinska Institutet, Sweden

*No abstract***Development of Recombinant Spider Silk Proteins with Tunable Assembly Properties for Biomimetic Spinning****Jan Johansson**^{1,2}, Anna Rising^{1,2}.¹Karolinska Institutet, Huddinge, Sweden, ²SLU, Ultuna, Sweden.

Spiders use specialized glands to make different types of protein-based silks with remarkable biochemical and mechanical properties, and artificial spider silk could be an ideal source for generation of novel high performance biomaterials. Spider silk fibres contain crystalline β -sheet regions, which mediate mechanical stability and that are formed within fractions of a second in the end of the spinning duct, but the soluble silk proteins (spidroins) can be stored at huge concentrations in the silk gland for long times, without aggregating prematurely. These properties have so far not been mimicked by recombinant spidroins. Spidroins contain unique repetitive segments, which determine the mechanical properties of the silk, as well as non-repetitive N- and C-terminal domains (NT and CT), which regulate conversion of the dope into fibres. We have studied the physiological regulation of spider silk formation and the molecular actions of NT and CT in detail. NT employs an evolutionarily conserved pH dependent three-step mechanism to decouple dimerization from locking of the dimer structure – a mechanism that ensures both rapid β -sheet aggregation and prevention of premature silk assembly. CT, in contrast, gets destabilised and converts into amyloid-like fibrils in a pH and CO₂ dependent manner, a hitherto unique mechanism that we suggest is important for nucleating the formation of β -sheets in the silk fibres.

We now use this knowledge to develop novel miniature spidroins with optimal properties in terms of solubility, yields upon recombinant production, and stability. A first generation of novel, designed minispidroins that show very high expression yields and solubility, and that can convert into fibres using a biomimetic spinning procedure have been generated. These minispidroins also allow high-resolution studies of spidroin structures in soluble and fibrillar states.

Structural Proteins: Self-Assembling Biopolymers for Various Applications**Thomas Scheibel.**

University of Bayreuth, Bayreuth, Germany.

Proteins reflect one fascinating class of natural polymers with huge potential for technical as well as biomedical applications. One well-known example is spider silk, a protein fiber with excellent mechanical properties such as strength and toughness. During 400 million years of evolution spiders became outstanding silk producers. Most spider silks are used for building the web, which reflects an optimized trap for flying prey. Another example of an outstanding protein fiber is mussel byssus. Some marine species like the blue mussel (*Mytilus galloprovincialis*) are able to settle among seabed stones, piers and harbor walls. These mussels have successfully adapted to changes in tides, wind and sun. Their success is based on a unique anchorage, the mussel byssus. Byssus threads show unusual mechanical properties, since they resemble soft rubber at one end and rigid nylon at the other, and these properties are found with a seamless and gradual transition. We have developed biotechnological methods using bacteria as production hosts which produce structural proteins mimicking the natural ones. Besides the recombinant protein fabrication, we analyzed the natural assembly processes and we have developed spinning techniques to produce protein threads closely resembling natural silk or mussel fibers. Importantly, we can employ the bio-inspired proteins also in other application forms such as hydrogels, particles, non-woven mats, foams or films. Our bio-inspired approach serves as a basis for new materials in a variety of medical, biological, or chemical applications.

Nanomechanics of Amyloid-Like Polymers Made of Self-Assembled Mouse Prion Proteins

Guillaume Lamour, Calvin Yip, Hongbin Li, Joerg Gsponer.
University of British Columbia, Vancouver, BC, Canada.

Amyloids are made of several polypeptides of the same protein that self-assemble into highly-ordered fibrillar nanostructures characterized by a cross-beta sheet conformation. Their outstanding mechanical properties combined with great thermodynamic stability make them excellent candidates for the development of future biomaterials with nanotechnological applications. Amyloids were first discovered in the context of brain pathologies. They are involved in infectious prion diseases (e.g., mad cow disease, Creutzfeldt-Jakob), but they also play a role in noninfectious nonprion diseases (e.g. Parkinson's, Alzheimer's). What distinguishes amyloid fibrils formed by prions from those formed by other proteins is not clear. On the basis of previous studies on yeast prions that correlated high intrinsic fragmentation rates of fibrils with prion propagation efficiency, it has been hypothesized that the nanomechanical properties of prion amyloid such as elastic modulus and strength may be the distinguishing feature. Here, we demonstrate that fibrils formed by mammalian prions are relatively soft (0.1-1GPa) and clearly in a different class of rigidities when compared to nanofibrils formed by nonprions (over 2GPa). Using a new bimodal nanoindenting technique of atomic force microscopy called AM-FM mode, we estimated the radial modulus of PrP fibrils at lower than 0.6GPa, consistent with the axial moduli derived by using an ensemble method (built upon polymer physics equations that calculate the persistence length by measuring fibril shape fluctuations). We also show, by using sonication-induced fibril scission, that the mechanical strength of prions fibrils (10-150MPa) is significantly lower than that of nonprions (250-800MPa). Our results have far-reaching implications for the understanding of protein-based infectivity and the design of future amyloid biomaterials.

Reference: Lamour et al. ACS Nano. 2014. pubs.acs.org/doi/abs/10.1021/nn5007013

Exploiting Amyloid Fibrils as Functional Biomaterials**Louise Serpell**

University of Sussex, Brighton, United Kingdom

Amyloid fibrils are known to be composed of a cross- β structural core that is hydrogen bonded along the length of the fibre to form a highly ordered and repetitive structure. It is clear however, that side chains play an important role in driving self-assembly and strengthening the overall structure via internal interactions between the β -sheets as well as stacking within sheets. Our work utilizes electron microscopy, X-ray fibre diffraction and biophysical and spectroscopic techniques to examine the structure of amyloid fibrils.

Research into the self-assembly of short amyloidogenic peptides has provided a novel architecture in the form of a cross- β nanotube formed by an amphipathic peptide. Our work has highlighted the important central role for the aromatic side chains phenylalanine and tyrosine in the internal interactions within the amyloid protofilament. Most recently, we have shown oxidation leads to covalent linking of the tyrosine side chains may play a very significant role in the structure and stability of amyloid fibrils in diseases including Alzheimer's disease. We have also shown that charge interactions play an important role and recently investigated the functionalization of amyloid fibrils using the lysine residues to promote silicification. This presentation will focus on recent insights into the contribution of primary sequence to the architecture of the amyloid fibrils and how these extremely stable structures may be further exploited as templates for further functionalization.

Hydration and Cavities in Amyloid Fibrils and Oligomers Modulated by Hydrostatic Pressure**Jerson Silva**

Federal University of Rio de Janeiro, Brazil

*No Abstract***Structure-Based Design of Amyloids with Novel Functions for Nanomaterials****Cong Liu**¹, Dan Li¹, Bin Dai¹, Xiang Zhang¹, Michael Sawaya², David Eisenberg².¹Chinese Academy of Sciences, Shanghai, China, ²UCLA, Los Angeles, CA, USA.

Protein can self-assemble into amyloid aggregates with highly ordered hierarchical structure. Amyloid was firstly identified as pathological entities in a variety of devastating human diseases including Parkinson's, Alzheimer's, and Huntington's diseases¹. Recently, more and more proteins are found to self-assemble into amyloid with diverse physiological functions, including signal transduction, hormone storage, RNA granules formation, and cell surface adhesion². Given the favorable properties including high thermal stability, stiffness and biocompatibility, amyloid is acquiring utility as a new class of bionanomaterials. In this work, we developed a general method for the design of functional amyloids with distinct functions, based on the atomic structures of amyloids. We further illustrate the method with two applications³⁻⁵. In the first one, we designed amyloid fibrils with lysine condensed and exposed on the fibril surface. We show that designed fibril is capable of capturing carbon dioxide from flue gas. In the second one, we used a newly identified amyloid architecture -- amyloid-like nanosheet as a platform to design a series of effective enhancers for retrovirus transduction. The work demonstrates the potency of the structure-based design method for development of amyloid-based nanomaterials with novel functions. 1. Eisenberg D, Jucker M (2012) The amyloid state of proteins in human diseases. *Cell* 148(6):1188–1203. 2. Maji SK, et al. (2009) Functional amyloids as natural storage of peptide hormones in pituitary secretory granules. *Science* 325(5938):328–332. 3. Li D, et al. Structure-based design of functional amyloid materials. *J. Am. Chem. Soc.*, 2014 Dec 31;136(52):18044-51. 4. Li D, et al. Designed amyloid fibers as materials for selective carbon dioxide capture. *PNAS*, 2014, 111, 191-1965. Dai B, et al. Tunable assembly of amyloid-forming peptides into nanosheets as a retrovirus carrier. *PNAS*, 2015 doi:10.1073/pnas.1416690112.

Amyloids Structural and Nanomechanical Characterization at the Individual Aggregate Scale

Francesco Simone Ruggeri¹, Sophie Vieweg², Giovanni Longo¹, Annalisa Pastore³, Hilal Lashuel², Giovanni Dietler¹.

¹École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, ³King's College, London, United Kingdom. ²École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland,

Aging of the population has increased the visibility of several neurodegenerative disorders such as Parkinson's and Ataxia diseases. Their onset is connected with insoluble fibrillar protein aggregates, called amyloids. However, these structures were also discovered in many physiologically beneficial roles (functional amyloids) including bacterial coatings and adhesives. During their aggregation, monomeric proteins undergo internal structural rearrangements leading to the formation of fibrils with a universal cross beta-sheet quaternary structure. This conformation is independent of the monomeric initial structure and is the fingerprint of amyloids. Strong evidence indicates that neurodegeneration is produced by the intermediate species of fibrillization. This poses the problem of investigating the early stages of the inter-conversion of monomers into amyloid fibrils. In our work, we investigated amyloids structural and mechanical properties by single molecule Atomic Force Microscopy (AFM) based methods. Infrared nanospectroscopy (nanoIR), simultaneously exploiting AFM and Infrared Spectroscopy, can characterize at the individual aggregate scale the conformational rearrangements of proteins during their aggregation. Whereas, AFM Quantitative Imaging can map the nanomechanical properties of amyloid aggregates at the nanoscale. In this way, we correlate the secondary structure of amyloid intermediates and final aggregates to their nanomechanical properties. Our results directly demonstrate, for the first time at the individual amyloid species scale, that the increase of beta-sheet content is a fundamental parameter determining the growth of amyloids intrinsic stiffness.[1] Nanoscale chemical characterization of amyloidogenic structures is central to understand how proteins misfold and aggregate, to unravel the structural rearrangement of monomers inside amyloid fibrils and to target pharmacological approach to neurodegenerative disorders. Finally, it is central to measure and quantify the ultra-structural properties of amyloid fibrils in order to appreciate their full potential as biomaterials. 1 Ruggeri, Nat. Commun., 2015

Biophysics and Structural Biology of Neurodegeneration: The Case of Alpha-Synuclein**Claudio Fernandez**

National University of Rosario, Argentina

*No abstract***Transthyretin-Related Diseases: From the Patient to the Protein****Debora Foguel**

Medical Biochemistry Institute, Brazil

*No abstract***Protein Misfolding Pathway Probed by Solution and Solid-State NMR****Mônica Santos de Freitas.**

Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Amyloidosis is a clinical dysfunction caused by extracellular accumulation of proteins that are normally soluble in their original structure, but suffered structural modifications generating insoluble and abnormal fibrils that impair the proper functioning of tissues. Although many challenges have been overcoming in the field of amyloidosis many questions are still waiting for answers. The limitation of many techniques approaches applied to study fibrillar formation has slowed the advances in the understanding of how soluble proteins suffer conformational changes that result in aggregation. In this way, solid-state NMR has been pointed out as a good tool to improve the knowledge about formation of fibers and aggregates. Solid-state NMR spectroscopy of proteins has undergone a great improvement due to the recent development of methods for resonance assignments, distance measurements and determination of torsion angles. The resolution has increased due the higher static magnetic fields, improved decoupling techniques and higher Magic Angle Spectroscopy (MAS) frequencies. These advances were obtained due to the development of several strategies for ^{15}N and ^{13}C proteins assignments in the last years. In this work we are interested to evaluate the pathway involved in fibril formation, following structural features that could indicates how soluble proteins undergo conformational changes that result in aggregation. The combination of Solution and Solid-State NMR has been a valuable tool to get informations concerning the first steps regarding the misfolding pathway.

Solid-State NMR Structural Characterization of Peptide Assemblies: Oligomeric A β (1-42) and Designer Peptide Nanofibers**Anant K. Paravastu.**

Georgia Institute of Technology, Atlanta, USA.

Our group has focused on probing structures of self-assembled peptides using solid-state NMR spectroscopy. For the 42-residue Alzheimer's amyloid- β peptide (A β (1-42)), emerging understanding of environment-dependent assembly pathways has made it possible to produce oligomeric samples with a stable homogeneous molecular structure. We seek to understand how oligomeric structures are distinct from fibrillar structures in order to provide a structural basis for differing neuronal toxicity profiles. We are also interested in designer peptides with amino acid sequences that were rationally designed to promote specific self-assembled molecular structures. We will show how the combination of solid-state NMR and constrained molecular dynamics computer simulations could provide the specific structural information necessary to test proposed structural models from the literature. For 150 kDa A β (1-42) oligomers, we will show data in support of an antiparallel β -sheet structure that is distinct from the in-register parallel motif commonly observed for amyloid fibrils. We will present a structural model for RADA16-I designer nanofibers that is composed of parallel β -sheets, unlike the antiparallel β -sheet structure proposed in the literature. For the MAX8 designer peptide, we will present direct evidence of proposed β -hairpin formation. For the SAF-p1/p2 system, we will show inter-molecular side chain contacts that are consistent with an α -helical coiled-coil nanofiber structure. We will use these results to evaluate our overall ability to predict and control self-assembled peptide molecular structures.

Lipid Nanoparticles and Amyloids Activate Receptors of the Innate System

Jean-Marie Ruyschaert, Malvina Pizzuto, Caroline Lonez

Universite Libre de Bruxelles, Bruxelles, Belgium.

Toll-like receptors are major members of the Pattern Recognition Receptors (PRRs) from the innate immune system, which recognize bacterial or viral components. It was recently demonstrated that those receptors, that usually recognized molecular patterns characteristic of pathogens, are activated by non bacterial lipid and protein aggregates (amyloids) structurally different from the natural ligands. We will illustrate this aspect with two examples related to nanoparticles and neurodegenerative diseases. It is tempting to speculate that amyloid fibrils represent a new class of danger signals detected by the innate immune system, through sensing of their common cross- β structure, a motif common to all amyloids irrespective of their origin and sequence. The immune system responds more specifically to structural features of fibrils rather than to an aggregated state or to a specific sequence motif.

It is hard to believe that nanoparticles which are so different from natural ligands do activate receptors the same way natural ligands do. How lipid and protein nanoparticles made of a large number of molecules activate pattern recognition receptors is still unknown but it is very likely that it proceeds via a new mechanism quite different from what has been described so far for monomeric natural ligands. Implications in nanotechnologies and nanomedicine will be briefly discussed.

1-Lonez C, Vandenbranden M, Ruyschaert JM.-Adv Drug Deliv Rev. 2012,64,1749-58

2- Lonez C, Bessodes M, Scherman D, Vandenbranden M, Escriou V, Ruyschaert JM.

Nanomedicine. 2014 -10(4):775-82-

New Peptide-Based Assemblies and Materials by Design**Dek Woolfson**

University of Bristol, United Kingdom

*No abstract***Protein Assemblies by Design****Vincent Conticello.**

Emory University, Atlanta, USA.

Structurally defined materials on the nanometer length-scale have been historically the most challenging to rationally construct and the most difficult to structurally analyze. Sequence-specific biomolecules, i.e., proteins and nucleic acids, have advantages as design elements for construction of these types of nano-scale materials in that correlations can be drawn between sequence and higher order structure, potentially affording ordered assemblies in which functional properties can be controlled through the progression of structural hierarchy encoded at the molecular level. However, the predictable design of self-assembled structures requires precise structural control of the interfaces between peptide subunits (protomers). In contrast to the robustness of protein tertiary structure, quaternary structure has been postulated to be labile with respect to mutagenesis of residues located at the protein-protein interface. We have employed simple self-assembling peptide systems to interrogate the concept of designability of interfaces within the structural context of nanotubes and nanosheets. These peptide systems provide a framework for understanding how minor sequence changes in evolution can translate into very large changes in supramolecular structure, which provides significant evidence that the designability of protein interfaces is a critical consideration for control of supramolecular structure in self-assembling systems.

Self-Assembling Peptide Based Materials for Regenerative Medicine

Aline Miller.

University of Manchester, Manchester, United Kingdom.

The development of highly functional, tailored soft materials is arguably one of the most important challenges of material science for the next decade. Self-assembling peptides have been highlighted as one of the most promising building blocks for future material design where individual molecules are held together via strong, yet irreversible bonds, imparting strength to the material. The translation of these soft materials into commercial applications is starting to become a reality with the advent of routine procedures for peptide synthesis and purification in both the lab and industrial scale, thus making them easily accessible at a reasonable cost. Consequently design rules for the self-assembly route of the different peptide systems and final material structure and properties are emerging, but these typically provide bare materials that lack the ability to adapt to their environment. Here several different strategies developed in our group will be outlined for the fabrication of functional, responsive and active materials based on ionic-complementary self-assembling octa-peptides. Several examples of the different types of functionalities that can be incorporated will be outlined, thus covering a wide range of application areas including controlling cell culture, targeted and temporal release of therapeutics, biosensors and biocatalysis for fine chemical manufacturing.

Protein Self-Assembly by Rational Chemical Design

F. Akif Tezcan.

University of California, San Diego, La Jolla, USA.

Proteins represent the most versatile building blocks available to living organisms for constructing functional materials and molecular devices. Underlying this versatility is an immense structural and chemical heterogeneity that renders the programmable self-assembly of protein an extremely challenging design task. To circumvent the challenge of designing extensive non-covalent interfaces for controlling protein self-assembly, we have endeavored to use rational, chemical bonding strategies based on metal coordination and disulfide bonding. These approaches have resulted in discrete or infinite, 1-, 2- and 3D protein architectures that display structural order over large lengths scales, yet are dynamic and stimuli-responsive, and possess emergent physical and functional properties.

Racemic Hydrogels from Enantiomeric Peptides: Predictions from Linus Pauling

Joel Schneider.

National Cancer Institute-NIH, Frederick, USA.

We have reported that hydrogel materials can be prepared from self-assembling beta-hairpin peptides. For example, the 20-residue peptide MAX1 rapidly self-assembles into a hydrogel network of monomorphic fibrils whose molecular structure was recently determined by solid state NMR. The enantiomer of MAX1, namely DMAX1 assembles affording a hydrogel of identical crosslink density, mesh size, and mechanical rigidity to the MAX1 gel. Surprisingly, the gelation of a 1 wt % equimolar solution of peptide enantiomers occurs more rapidly resulting in a racemic hydrogel network whose mechanical rigidity is over four-fold greater than gels prepared from either pure enantiomer. Keeping in mind that the total amount of peptide in the racemic gel is equal to that of either pure enantiomeric gel, this observation is truly unexpected and suggests that biomolecular chirality, at the level of the monomer, is directly influencing the mechanical properties of the self-assembled hydrogel. We interrogated the self-assembly process and resulting fibrillar and network morphologies of the racemic gel employing CD spectroscopy, isotope-edited FTIR, transmission electron microscopy labeling experiments, small angle neutron scattering, diffusing wave spectroscopy, solid state NMR and molecular modeling to uncover the molecular basis for this behavior. We show that the enhancement in hydrogel rigidity does not result from an increase in network crosslink density, as one might predict. Instead, the racemic gel is more mechanically rigid because each fibril in its network is, itself, more rigid. In light of the NMR structure of pure MAX1 fibrils, the mechanism of enantiomeric assembly and their molecular arrangement in the solid state will be presented. The mode of molecular assembly uncovered in our studies was predicted by Linus Pauling in 1953 in the course of deriving models of the pleated beta-sheet, a fold ubiquitous in protein structure.

Dynamic Mechanisms Underlying Ubiquitin Ligation

Brenda Schulman^{1,2}.

²St. Jude Children's Research Hospital, Memphis, USA. ¹Howard Hughes Medical Institute, Memphis, TN, USA,

Post-translational modification by ubiquitin-like proteins (UBLs) is a predominant eukaryotic regulatory mechanism. The vast reach of this form of regulation extends to virtually all eukaryotic processes that involve proteins. UBL modifications play critical roles in controlling the cell cycle, transcription, DNA repair, stress responses, signaling, immunity, plant growth, embryogenesis, circadian rhythms, and a plethora of other pathways. UBLs dynamically modulate target protein properties including enzymatic activity, conformation, half-life, subcellular localization, and intermolecular interactions. Moreover, the enzymatic process of UBL ligation to proteins is itself dynamic, with the UBL moving between E1/E2/E3 enzyme active sites and ultimately to a target. With roughly 300 members, the largest E3 family consists of Cullin-RING ligases (CRLs), which regulate a staggering number of biochemical pathways and biological processes. CRL activity is under fascinating conformational control, with different orientations of the catalytic RING domain mediating different activities. In my presentation, I will discuss recent results from the lab addressing how the dynamic conformations underlie regulation of and by this large family of dynamic ubiquitin E3 ligases.

pH Dependent Peptide Self-Assemblies: A Mechanism as Old as Viruses

Maité Paternostre

Institute of Integrative Biology of the Celle, France

See abstract: Pos- 34 Board 34

Short Peptides Self-Assemble in the Presence of Metals to Produce Catalytic Nanomaterials

Caroline Rufo¹, Yurii Moroz¹, Olga Makhlynets¹, Jan Stoehr², Jenny Hu², William DeGrado², **Ivan Korendovych¹**.

¹Syracuse University, Syracuse, USA, ²UCSF, San Francisco, CA, USA.

Enzymes fold into unique three-dimensional structures, which underlie their remarkable catalytic properties. The requirement that they be stably folded is a likely factor that contributes to their relatively large size (> 10,000 Dalton). However, much shorter peptides can achieve well-defined conformations through the formation of amyloid fibrils. To test whether short amyloid-forming peptides might in fact be capable of enzyme-like catalysis, we designed a series of 7-residue peptides that act as Zn²⁺-dependent esterases. Zn²⁺ helps stabilize the fibril formation, while also acting as a cofactor to catalyze acyl ester hydrolysis. The fibril activity is on par with the most active to date zinc-protein complex. Such remarkable efficiency is due to the small size of the active unit (likely a dimer of 7-residue peptides), while the protein is at least 15-fold larger in molecular weight. The observed catalytic activity is not limited to ester hydrolysis. We have designed copper binding peptides that are capable oxygen activation.

These results indicate that prion-like fibrils are able to not only catalyze their own formation – they also can catalyze chemical reactions. Thus, they might have served as intermediates in the evolution of modern-day metalloenzymes. These results also have implications for the design of self-assembling nanostructured catalysts including ones containing a variety of biological and nonbiological metal ions.

Designer Peptides Self-Assemble on Graphene to Create Remarkably Stable, Precisely Organized Substrates

Gina-Mirela Mustata^{1,6}, Meni Wanunu^{1,2}, Gevorg Gregoryan^{3,4}, Jian Zhang³, William DeGrado⁵.

⁶Simmons College, Boston, MA, USA, ¹Northeastern University, Boston, MA, USA, ³Dartmouth College, Hanover, NH, USA, ²Northeastern University, Boston, MA, USA, ⁴Dartmouth College, Hanover, NH, USA, ⁵University of California, San Francisco, San Francisco, CA, USA,

We present a study of designed self-assembly of 2D peptide monolayer crystals on the surface of graphene and graphitic interfaces and their properties in various biologically significant conditions. Atomic force microscopy imaging of dried peptides adsorbed on graphitic surfaces reveals an amorphous monolayer structure that contains voids due to drying. After rehydration, the peptide monolayer reorganizes into highly ordered domains comprised by parallel arranged peptides that are oriented on the graphitic structure with C3 symmetry, in close agreement with computational predictions. The monolayers are remarkably stable in a wide range of pH, ionic strengths, urea concentrations, and temperatures. Importantly, we find that alternating peptides that do not contain aromatic residues organize similarly, and conclude that aromatic residues are not essential for this organization. The monolayers are highly stable to proteolytic digestion when full coverage is acquired, while voids in the layer become seeds to slow degradation from the void inwards. A striking quality of these substrates is the preference to bind double stranded DNA imposing a preferred alignment to match their own molecular arrangement on the graphene surface.

This system of designed peptide-coated graphene surfaces, with its stability over a wide range of situations, presents new opportunities for the design of structures and systems that are significant in the study of various biological entities and processes, such as specific binding or designed catalysis.

Bacterial Strategies for Protein Self-Assembly at Interfaces**Cait MacPhee**

University of Cambridge, United Kingdom

*No abstract***Self-Assembly of Protein Nanofibrils that Display Active Enzymes****Sarah Perrett.**

Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

The ability of proteins to self-assemble into beta-sheet-rich aggregates called amyloid fibrils is considered to be universal, although certain polypeptide sequences have a particularly high propensity to adopt these conformations. In many cases the formation of amyloid fibrils is deleterious and associated with the progression of disease, but there are also examples of proteins for which the cross-beta structure represents the functional conformation. Ure2 is the protein determinant of the yeast prion [URE3]. Ure2 consists of an N-terminal prion-inducing domain that is disordered in the native state, whereas the C-terminal functional domain has a globular fold with structural similarity to glutathione transferase enzymes. The C-terminal domain shows enzymatic activity in both the soluble and fibrillar forms of Ure2. We have used a variety of biophysical approaches to investigate the structure of Ure2 fibrils and their mechanism of assembly. We have also created chimeric constructs where the prion domain is genetically fused to other enzymes of different sizes and architectures. These chimeric polypeptide constructs spontaneously self-assemble into nanofibrils with fused active enzyme subunits displayed on the amyloid fibril surface. We can measure steady-state kinetic parameters for the appended enzymes in situ within fibrils, and compare these for the identical protein constructs in solution. We have also applied microfluidic techniques to form enzymatically-active microgel particles from the chimeric self-assembling protein nanofibrils. The use of scaffolds formed from biomaterials that self-assemble under mild conditions enables the formation of catalytic microgels whilst maintaining the integrity of the encapsulated enzyme. In combination with microfluidic trapping techniques, these approaches illustrate the potential of self-assembling materials for enzyme immobilization and recycling, and for biological flow-chemistry. The design principles can be adopted to create countless other bioactive amyloid-based materials with diverse functions.

The Supramolecular Organization of a Peptide-Based Nanocarrier at High-Resolution

Markus Weingarth¹.

¹Utrecht University, Utrecht, Netherlands,

See abstract: Pos-48 Board 48

Hybrid Biomaterials Based on Natural and Synthetic Polymers: From Basics to Applications

Ting Xu

University of California Berkeley, USA

No abstract

Synthesis, Folding and Assembly of Sequence-Defined Peptoid Polymers

Ronald Zuckermann

University of California Berkeley, USA

No abstract

Immobilization of RubisCO by Self-Assembled Nanotubes

Jon Parquette

The Ohio State University, Columbus, USA

See abstract: Pos-33 Board 33

Supramolecular Self-Assembly Of β -Peptides: New Materials With Tunable Morphology And Chemical Function

Mibel Aguilar.

Monash University, Clayton, Vic, Australia.

Supramolecular self-assembly is rapidly emerging as a powerful strategy for the design of new materials in biomedicine and engineering applications. Peptide self-assembled systems offer significant advantages including biological compatibility, ease of synthesis, low toxicity and functionalisability. However, the control over essential features such as chemical, structural and metabolic stability, the scale and relatively slow rate of self-assembly remain significant challenges. We have designed helical N-acetyl- β 3-peptides that spontaneously undergo supramolecular self-assembly to form fibers ranging in size from nano- to macro-scale [1,2] via a unique head-to-tail fashion which is driven by a 3-point H-bond motif associated with the 14-helical structure of N-acetyl- β 3-peptides. In addition, the unique helical structure of the peptide monomer allow the introduction of a wide variety of functions via modification of the side chains of the component amino acids.

We have exploited this symmetry to design a lateral supramolecular self-assembly motif to link the fibres in a controlled manner. We have shown that these materials can be chemically modified in two ways, either by decorating the fibres with functional payloads or by insertion of functional payloads into the peptide sequence, and that the resulting materials exhibit biological activity. We also demonstrate that the superstructure morphology of a self-assembled β 3-peptide, can be tuned to yield a range of morphologies by different solvents.

1. Del Borgo MP, Mechler AI, Traore D, Forsyth C, Wilce JA, Wilce MCJ, Aguilar MI & Perlmutter P, 'Supramolecular Self-Assembly of N-Acetyl capped β -Peptides Leads to Nano-to Macroscale Fibre Formation'. *Angewandte Chemie Int Ed.* 2013, 52 8266-.
2. Seoudi RS, Del Borgo MP, et al, Hierarchical morphology of a self-assembling tri- β 3 peptide controlled by solvent". *New J Chem.* 2015, 39, 3280.

Inorganic Silica and Rare Earth Phosphate Polymerization at Cellular Interfaces**C Jeffrey Brinker**^{1,2}.¹Sandia National Labs, Albuquerque, NM, USA, ²Univeristy of New Mexico, Albuquerque, NM, USA.

Our work explores the cellular processing of nanoscale materials to form new bio/nano interfaces and organisms. We have shown that yeast, bacterial, and mammalian cells introduced into self-assembling solutions of phospholipids and soluble silica, direct the formation of unique silica@cell interfaces and architectures through cellular response pathways. The association of silica with cellular interfaces has been further explored in recent work, where we have discovered a process, Silica-Cell-Replication, wherein mammalian cells direct their exact replication in silica. The silica cell replicas preserve nm-to-macro-scale cellular features on both the cell surface and interior after drying at room temperature - and largely after calcination to 600 °C. The process is self-limiting and self-healing, and remarkably generalizable to any cells of interest—from red blood cells to neurons. Our current hypothesis is that, due to comparable hydrogen bonding strengths, silicic acid molecules replace bound water at cellular interfaces and are amphotericly catalyzed by proximal proteins and other membrane bound components to form a self-limiting, defect-free, nm-thick silica encasement that resists drying stress and preserves key features of biofunctionality. We recently reported the exceptional ability of rare earth oxide nanoparticles to desphosphorylate mammalian endosomal compartments following non-specific internalization by macropinocytosis or phagocytosis. The dephosphorylation pathway, which is shared by all trivalent rare earth oxides, involves the irreversible formation of highly insoluble rare earth phosphates from any bio-available phosphorous source. Questioning whether rare earth oxides (REO) would desphosphorylate bacterial cell membranes, we tested a library of REO nanoparticles against Gram negative *Escherichia coli* and *Salmonella enterica* and Gram positive *Staphylococcus aureus* and found in all cases the formation of rare earth phosphates with needle or urchin-like structures and retained viability for exposure levels up to 100 µg/ml. These rare earth phosphate modified bacteria represent a new living biotic/abiotic material/phenotype.

Interfacial Assembly of Synthetic and Natural Nanoparticle

Thomas P. Russell.

Polymer Science and Engineering Department, University of Massachusetts, Amherst, USA. Nanoparticles will segregate to the interface between two immiscible fluids to reduce the interfacial energy. Due to the size of the nanoparticles, the assemblies can range from disordered energy holding the particle at the interface is small and is, in fact, comparable to thermal energies. This gives rise to a disordered, liquid-like assembly, as opposed to colloidal particles, where the assemblies can order and crystallize. This provides a unique platform to probe assemblies of both synthetic nanoparticles, like cadmium selenide or silica, and natural nanoparticle, like cow pea mosaic virus and tobacco mosaic virus. In addition, since these assemblies are at a liquid/liquid interface, the nanoparticles are easily accessed by chemicals dispersed in one or both of the fluids and chemistries can be performed on the nanoparticles, as for example, crosslinking chemistries or attachment of ligands that reduce the interfacial tension further by the formation of nanoparticle surfactants. This reduction in the interfacial energy per nanoparticle increases the force holding the nanoparticles at the interface and allows the assemblies to be jammed at the interface when the interfacial area is reduced. This opens the possibility of shaping the liquid phase by use of an external field, like shear or an electric field, where, upon removal of the field, the interfacial area decreases, jamming the particles at the interface and locking-in non-equilibrium shapes of the fluids. Integration of functionality into the ligands allows the assemblies to be responsive to a range of external stimuli.

Multicomponent Supramolecular Hydrogels

Dave Adams, Emily R. Draper, Edward Eden, Tom O. McDonald.
University of Liverpool, Liverpool, United Kingdom.

Low molecular weight gelators (LMWG) are molecules that self-assemble into one-dimensional fibrous structures. Entanglement of these fibres leads to the immobilisation of the solvent and the formation of a gel. These supramolecular gels are attracting significant interest as they have unusual properties, for example forming gels at low LMWG concentrations and being able to reversibly go from a gel to a solution. In most cases, gels are formed from a single LMWG. Mixing different LMWG (which each form gels independently) is interesting. Conceptually, depending on how these LMWG assemble, mixing LMWG could be used to adjust the properties of the final gels, or to prepare systems with higher information content, for example by the selective positioning of specific functional groups in space. However, for this approach to be useful, it is not only necessary to simply mix two LMWG, but to be able to finely control the assembly of both such that, ideally, their location in space is finely controlled. This is extremely difficult to do. Here, we will describe a range of mixed dipeptide-based LMWG systems. We will show how fibrous structures form in these systems and show how we can control how different types of fibrous networks are built up in multicomponent systems. We will show how to control the type of networks formed, and how this can be used to control the properties of the gel. We will also show the selective removal of one of the networks.

POSTER ABSTRACTS

POSTER SESSION I
Monday, October 26
15:45 – 17:45
Mar da Barra

All posters being presented in Poster Session I should be set up the morning of October 26.

Ebtisam Aldaais	2-POS	Board 2
Wendel Alves	4-POS	Board 4
Andres Barco	6-POS	Board 6
Fillipe Bronze	8-POS	Board 8
Éverton D'Andréa	10-POS	Board 10
Viviane De Paula	12-POS	Board 12 <i>withdrawn</i>
Wojciech Dzwolak	14-POS	Board 14
Deborah Fygenson	16-POS	Board 16
Frederico Gueiros Filho	18-POS	Board 18
Miklós Kellermayer	20-POS	Board 20
Eda Koculi	22-POS	Board 22
Andriy Kovalenko	24-POS	Board 24
Mayra Marques	26-POS	Board 26
Tracy Melvin	28-POS	Board 28
Miklos Nyitrai	30-POS	Board 30
Garegin Papoian	32-POS	Board 32
Maité Paternostre	34-POS	Board 34
Régis Pomès	36-POS	Board 36
Warren Ruder	38-POS	Board 38
Juliana Santana	40-POS	Board 40
Katarina Siposova	42-POS	Board 42
Christophe Tarabout	44-POS	Board 44
Gabriela Valle	46-POS	Board 46
Markus Weingarth	48-POS	Board 48

2-POS Board 2

A Theoretical Study of Micellar Ligand-Receptor Binding Regulations: The Effect of Receptors Density on The Binding to A Distinctive Ligand**Ebtisam A. Aldaais**^{1,2}, Mark J. Uline¹.¹University of South Carolina, Columbia, SC, USA, ²University of Dammam, Dammam, Eastern Province, Saudi Arabia.

A variety of interactions between drug delivery devices, and local cells and tissues, impact clinical outcomes in terms of both therapeutic action and biological response. The further development of design objective micelles for drug delivery applications is associated with understanding the competitions of interactions in the system. The mean-field approximation is used in this study to generalize a molecular theory that determine the competition between electrostatic, van der Waals and steric interaction, and consequently, determining the ligand-receptor binding protocols. The micelles are designed to target cancer cells primarily through electrostatic binding as several cancers are known to flip negatively charged lipids to the outer-leaflet [1, 2]. Cancer cells and healthy cells have the same kinds of receptors, however some of these receptors are overexpressed in cancer cells, such as epidermal growth factor receptors (EGFR). Thus, the developed theory considers the influence of different receptor densities on the ligand-receptor binding. The molecular reorganization on the surface of the micelle is a design variable that needs to be considered for enhanced targeting. We show that size (curvature) is strongly coupled to the way polymers express ligands to the surface, and our molecular theory platform is uniquely suited to address these issues. According to the theory, charge regulation stabilizes polybases on micelles at the cost of chemical free energy and gaining in the van der Waals attractive interactions. As a result, the bonds formation between ligands and receptors with different densities are affected by the proper choice of temperature, ligand-polybases density and spacer. The developed theory should enable the prediction of a design guideline for the creation of therapeutic micelles.

4-POS Board 4**Self-Assembly Pathway of Peptide Nanotubes Formed by a Glutamic Acid-Based Bolaamphiphile**

Wendel Alves¹, Emerson R. Silva¹, Valeria Castelletto², Mehedi Reza³, Janne Ruokolainen³, Rohanah Hussain⁴, Ian W. Hamley².

¹Universidade Federal do ABC, Santo André, São Paulo, Brazil, ²University of Reading, Reading, Berkshire, United Kingdom, ³Aalto University School of Science, FI-00076, Finland, ⁴Diamond Light Source Ltd, Didcot, United Kingdom.

The self-assembly of peptide-nanotubes formed by an L-glutamic acid-based bolaamphiphile is shown to proceed via a remarkable mechanism where the peptide conformation changes from b-sheet to unordered. The kinetics of this process are elucidated via X-ray scattering and UV circular dichroism methods. The reverse transition from “unordered” to b-sheet structures is triggered by UV radiation [1].

Here, we use small-angle X-ray scattering (SAXS) and synchrotron radiation circular dichroism (SRCD) to examine the structure and the self-assembly pathway of an amino acid-based bolaamphiphile, the linear octamer EFL4FE (E = L-glutamic acid, F = L-phenylalanine and L = L-leucine). The hydrophobic region of the sequence is composed of four leucine repeats, an aliphatic amino acid often found in molecular zippers in protein aggregates. This aliphatic core is complemented by two phenylalanine moieties which are designed to assist lateral association due to p-p stacking interactions between aromatic rings. The polar heads are made from L-glutamic acid moieties endowed with carboxyl groups. We found a remarkable and unexpected pathway of nanotube formation in which initial b-sheet structures are disrupted.

Reference:

[1] Silva, E.R.S.; Alves, W.A.; Castelletto, V.; Reza, M.; Ruokolainen, J.; Hussain, R.; Hamley, I.W. Chem. Commun. DOI: 10.1039/c5cc03640b

6-POS Board 6**Rheological Properties of Peptide-Based Hydrogel-Glycosaminoglycan Mixtures**

Andres Barco^{1,2}, Eileen Ingham^{1,2}, John Fisher^{2,1}, Robert P. Davies³, Hazel Fermor^{1,2}.

¹Institute of Medical and Biological Engineering, Leeds, United Kingdom, ²Institute of Medical and Biological Engineering, Leeds, United Kingdom, ³Department of Oral Biology, Leeds, United Kingdom.

Peptide-based hydrogels are of high interest as a class of biomaterials for their potential use in regenerative medicine. Mixing these hydrogels with materials that may enhance their properties, such as glycosaminoglycans (GAGs), has the potential to extend their range of applications.

The aims of this study were to investigate the physical properties of self-assembling peptide hydrogels based on three peptides of the P-11 series in combination with chondroitin sulphate using rheology.

The hydrogel mixtures of the three peptides were investigated in two different salt solutions at a temperature of 37°C, in order to determine their suitability for a range of applications. Peptide alone, peptide in combination with chondroitin sulphate at two different molar ratios and chondroitin sulphate alone were investigated. A Malvern Kinexus pro rheometer was used to carry out the measurements. An amplitude sweep at two frequencies (1Hz & 20Hz) was run to determine a suitable strain value within the linear viscoelastic region (LVER). This strain value was used to run a frequency sweep across a range of frequencies (1-20Hz) to determine the elastic and viscous modulus of the material.

The results indicated that all of the variables (peptide, salt concentration, and chondroitin sulphate molar ratio) had a significant effect on the mechanical properties of the hydrogels. However, one of the peptide-hydrogel mixtures, P11-8, showed greater mechanical strength in both salt solutions and molar ratios when compared to other peptide hydrogel mixtures. This peptide-hydrogel mixture will be investigated further in glycosaminoglycan depleted model tissues.

8-POS Board 8**Efficient Bio Incorporation of Bulky-Tryptophan Analogs in Recombinant Proteins Expressed in *E. coli*: Obtaining α -Synuclein Labeled with 5-Hydroxy-Tryptophan**

Fellipe Bronze¹, Wellington P. Souza¹, Marcelo Marcondes^{1,2}, Jaap Broos², Vitor Oliveira¹.
¹UNIFESP, Sao Paulo, Brazil, ²University of Groningen, Groningen, Netherlands.

A-Synuclein oligomerization/aggregation has been implicated in many progressive neurodegenerative diseases. Labeling the α -synuclein with spectroscopic probes has been a useful tool to investigate the oligomerization/aggregation of this protein in vitro and even in vivo. Among the available labeling methods, biosynthetic incorporation of non-canonic amino acids is an attractive strategy to introduce new properties in recombinant proteins. In the present work we describe an efficient method to bio incorporate bulky tryptophan (Trp) analogs in proteins expressed in *E. coli*. Furthermore, this method is compatible with bacterial vectors based on the T7-RNA polymerase/promoter that are widely used for protein expression.

The first target protein for this study was the recombinant α -synuclein mutant (V26W) cloned in one T7-based vector, the pET26b. The probe to be incorporated in this target was 5-hydroxy-Tryptophan (5HW) in the place of the regular W at position 26 introduced by mutagenesis (mutation V26W). In fact, there are few descriptions that 5-hydroxy-tryptophan (5HW) could be bio incorporated in recombinant proteins in *E. coli*, and with a large range of efficiencies.

Nevertheless, we verified that such inefficient 5HW bio incorporation in T7-based systems is because that the central T7-RNA polymerase in this system has the same control as the target protein, resulting in formation of inactive T7-RNA polymerase. To overcome this problem we developed a two-step expression protocol that resulted in incorporation efficiencies of 5HW higher than 90% in α -synuclein mutant (V26W).

The results also show other possibilities for the bio incorporation of bulkier tryptophan analogs (Ex: nitro-Trp, 5Br-Trp, F-Trp). Bulkier Trp analogs may not be recognized by regular *E. coli* Tryptophanyl-tRNA synthetase. But, the co-expression of a more permissive synthetase makes possible the incorporation of such bulkier Trp analogs.

10-POS Board 10**Structural Determination of Hypothetical Proteins Conserved in Kinetoplastids**

Éverton D'Andréa¹, Anne Diehl², Peter Schmieder², Yvette Roske³, Hartmut Oschkinat², José Ricardo M. Pires¹.

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Chagas Disease, Sleeping Sickness, and Leishmaniasis are among the so called neglected diseases. Genome sequencing of the kinetoplastids *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major* open up new perspectives for drug research against these diseases. In each diploid genome ca. 10,000 gene pairs were identified and around 50 % code for proteins of unknown function. Proteomic studies for *T. cruzi* confirmed the expression of several of these proteins in a life-cycle dependent manner. In the present work using bioinformatic tools available we mined the Trityp Databank to end up with a list of 197 proteins up to 30 kDa, conserved in kinetoplastids, without orthologues in mammals, plant or fungi, without transmembrane regions and without homologous sequences in the PDB suitable for structural studies by solution NMR/X-Ray Crystallography. From this search, 17 were obtained commercially cloned into the pUC57 plasmid. Q4DY78 gene was subcloned into pGEX-4T2 and expressed as a soluble protein. ¹⁵N HSQC spectrum showed it was folded. Samples unlabelled, isotopically ¹⁵N labelled, and ¹³C and ¹⁵N double labelled were prepared. A set of NMR experiments was performed on a Bruker Avance III 800 spectrometer. Spectra were acquired and processed using TopSpin® Software (v 3.1). Resonance assignment was performed using Sparky (v 3.114). Secondary structure was determined using CSI (v 2.0). ARIA (v 2.3) is going to be used to calculate the structure. Q4D6Q6 gene was expressed into a pET-28a vector as a soluble protein. ¹⁵N HSQC spectrum showed the protein was folded. Crystals were obtained and X-Ray crystallographic structure was calculated with a resolution of 1,46 Å. DALI search showed similar homology (RMSD = 3,1 Å, Z score = 7.6, 11% identity) with the GTP Cyclohydrolase I Feedback Regulatory Protein (GFRP) (rat) which is the rate-limiting enzyme in the biosynthesis of tetrahydrobiopterin.

12-POS Board 12

Withdrawn

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14-POS Board 14

Competition or Forced Collaboration? On the Relationship Between Two Types of Insulin Amyloid Seed.**Wojciech Dzwolak**^{1,2}, Weronika Surmacz-Chwedoruk^{2,3}, Viktoria Babenko¹.¹University of Warsaw, Warsaw, Poland, ²Polish Academy of Sciences, Warsaw, Poland,³Institute of Biotechnology and Antibiotics, Warsaw, Poland.

Cross-seeding of fibrils of bovine insulin (BI) and LysB31-ArgB32 human insulin analog (KR) induces self-propagating amyloid variants with infrared features inherited from mother seeds. We have shown recently that when native insulin (BI or KR) is simultaneously seeded with mixture of equal amounts of both templates (i.e., of separately grown fibrils of BI and KR), the phenotype of resulting daughter fibrils is as in the case of the purely homologous seeding: heterologous cotemplates accelerate the fibrillation but do not determine infrared traits of the daughter amyloid [1]. This implies that fibrillation-promoting and structure-imprinting properties of heterologous seeds become uncoupled in the presence of homologous seeds. We argue that explanation of such behavior requires that insulin molecules partly transformed through interactions with heterologous fibrils are subsequently recruited by homologous seeds. The selection bias toward homologous daughter amyloid is exceptional: more than 200-fold excess of heterologous seed is required to imprint its structural phenotype upon mixed seeding. Our study captures a snapshot of elusive docking interactions in statu nascendi of elongation of amyloid fibril and suggests that different types of seeds may collaborate in sequential processing of soluble protein into fibrils. Furthermore, it demonstrates that the actual nature of events taking place upon integration of soluble protein with amyloid tips may be much more complex than is currently assumed.

[1] W. Surmacz-Chwedoruk, V. Babenko, W. Dzwolak, *J. Phys Chem B*. 118 (2014) 13582-13589

16-POS Board 16

Spontaneous Nucleation of DNA Nanotubes of Defined CircumferenceJohn E. Devany¹, **Deborah K. Fygenson**^{1,2}.¹Physics Dept., University of California, Santa Barbara, CA, USA, ²Biomolecular Science & Engineering Program, University of California, Santa Barbara, CA, USA.

Nanotubes are an important structural primitive in both cell biology and DNA nanotechnology. Like cellular “microtubules”, DNA nanotubes can grow to lengths that are thousands of times their diameter (~10 nm) without exceeding their persistence length (~10 μ m), and thus truly bridge the molecular and material scales. DNA nanotubes are accordingly being investigated for a variety of applications: as templates for nanowires, pathways for molecular locomotion and rigidifying elements in active gels. To optimize quality, simplify placement, guide sequence design and rationalize solution conditions for such applications, fundamental understanding of and control over DNA nanotube self-assembly kinetics is needed.

We studied the homogeneous (unseeded) nucleation of HX-tiled DNA nanotubes. The HX-tiling scheme, pioneered by Reif and co-workers, provides a versatile means for assembling DNA nanotubes with a defined number of double helices in circumference. Using sequence sets with binding domains of uniform strength that form nanotubes with either six, eight or ten helices in circumference, we looked for a dependence of nucleation rate on helix number.

We detected nanotube nucleation and growth from the increase in fluorescence of a Cy3 molecule that occurs when the DNA to which it is covalently attached becomes double-stranded. We used a real-time PCR machine (Stratagene Mx3005P) to measure fluorescence following an abrupt change in temperature across the melting temperature and verify the rigid, linear nature of the assemblies that result by fluorescence microscopy. We find that nucleation of our DNA nanotubes proceeds in a single step (i.e., without meta-stable intermediates) and at a rate that scales with strand concentration to the 4th power, independent of helix number. The tetramer nature of the critical nucleus suggested by these results is further supported by a similar study on ribbon-forming subsets of the same strands.

18-POS Board 18

Filament Capping Regulates the Bacterial Tubulin-Like Cytoskeleton

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Cell division in bacteria is orchestrated by FtsZ, the tubulin ortholog of prokaryotes. At the time of division, FtsZ self assembles into a contractile protein structure, the Z ring, and this, in turn, remodels the bacterial envelope to achieve cytokinesis. Proteins that bind to FtsZ are key regulators of bacterial division and valuable probes for the understanding of the fundamental rules of FtsZ polymerization. Here we have applied biochemical and structural approaches to determine the mechanism of FtsZ inhibition by MciZ, a 40 amino acid peptide that shuts off cell division during spore development in *Bacillus subtilis*. The crystal structure of the FtsZ-MciZ complex was solved at 3.2 Å resolution and revealed that MciZ binds to the C-terminal polymerization interface of FtsZ, the equivalent of the minus end of tubulin. MciZ prevents the assembly of higher order FtsZ polymers at substoichiometric levels both in vitro and in vivo. This is not due to FtsZ monomer sequestration because FtsZ monomers self-poisoned with MciZ are as effective as MciZ alone at inhibiting FtsZ assembly. Furthermore, EM and fluorescence microscopy showed that MciZ binds to the ends of FtsZ filaments and shortens them. Thus, MciZ is an FtsZ filament capping protein, the first capping protein described for the bacterial cytoskeleton. Capping of the minus end is an unusual way of shortening filaments and indicates that fragmentation and annealing are important reactions for FtsZ filament assembly. The effect of MciZ on FtsZ dynamics also indicates that FtsZ filaments exhibit the same polarity as microtubules and, thus, could undergo treadmilling.

20-POS Board 20

Highly Oriented, Epitaxially Generated Beta-amyloid-based Nanoarray for Nanobiotechnological Applications

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The self-assembly of biomolecular systems are of fundamental appeal for nanobiotechnological applications. However, the lack of easy chemical access and nano- to micro-scale structural order often hinders the nanotechnological use of conventional biomolecules. We have previously shown that the amyloid beta 25-35 peptide (A β 25-35) forms a trigonally oriented network on mica by epitaxial mechanisms. To enhance the chemical reactivity and the structural complexity of this nano-network, here we synthesized and utilized a chemically accessible mutant and various A β -foldamer chimeras. Oriented network of amyloid fibrils was generated by incubating mutant, A β 25-34_N27C peptides or its wild-type mixtures on mica at varying KCl concentrations. To functionalize the fibrils we used maleimido-nanogold (1.4 nm). As evidenced by high-resolution atomic-force microscopy, we were able to tune the average length of the amyloid fibrils by adjusting the peptide and KCl concentrations. In the nanogold-labeled amyloid network spherical particles of 1.4-nm diameter lined up along the oriented fibrils, demonstrating that the fibrils can indeed be chemically addressed and functionalized. The A β -foldamer chimeras were conjugates of an A β 25-29 peptide and a 2-aminocyclopentane-carbonic acid hexamer located at the N- and C-terminals, respectively. We found that the A β -foldamer chimeras formed, on a time-scale of minutes, trigonally oriented network on mica in which the individual fibrils reached lengths up to several microns. Apparently the A β component is responsible for the oriented surface binding and epitaxis, while the foldamer enhances the assembly of extended fibrils. In sum, the oriented amyloid-based network may be used towards the construction of functional biomolecular nanoarrays with complex, tunable geometries and properties.

22-POS Board 22

RNA Structural Isomerization in the Peptide Bond Formation Site: Molecular Mechanism and Physiological Function of DbpA.Jared J. Childs, **Eda Koculi** .

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DbpA is a DEAD-box RNA helicase. Like all the members of this family, DbpA has two RecA-like domains, which use the energy of ATP binding and hydrolysis to unwind short double helix substrates. Different from other members of the DEAD-box family of enzymes, DbpA has a structured C-terminal domain, which imparts the DbpA protein's specificity for helix 92. Helix 92 is located in the peptide bond formation site; therefore, DbpA performs RNA structural isomerization in a region of the ribosome that is crucial for cell survival. Using a number of DbpA constructs, and RNA-DNA and RNA-PEG chimeras we show that the DbpA protein unwinds the double helix substrates by directly loading on them and that the residues outside the double-helix region are mechanistically unimportant for the DbpA catalytic activity. Moreover, our data indicates that the interaction of the DbpA C-terminal domain with the double helix regions near helix 92 determine the double helix substrates that are accessible by the DbpA catalytic core. Hence, during ribosome assembly the role of the DbpA C-terminal domain could be both to tether DbpA to its site of action and to prevent the DbpA catalytic core from unwinding correctly annealed double helices. In vivo pulse-labeling experiments indicate that DbpA acts in two pathways of large subunit assembly. When helicase inactive DbpA, R331A, is expressed in the cell one of the pathways of the large subunit assembly never goes to completion and a dead-end large subunit intermediate accumulates. We are in the process of investigating both protein compositions and RNA structures of various intermediates on large subunit assembly in the presence of R331A DbpA. These experiments will determine the precise RNA structural isomerization that DbpA performs during ribosome assembly.

24-POS Board 24

Molecular Solvation Effects in Formation and Stability of A β - oligomers and Optimization of Misfolding Inhibitors from Multiscale Platform Using Molecular Theory of Solvation**Andriy Kovalenko**^{1,2}, Neil Cashman³, Nikolay Blinov^{2,1}.¹National Institute for Nanotechnology, Edmonton, AB, Canada, ²University of Alberta, Edmonton, AB, Canada, ³University of British Columbia, Vancouver, BC, Canada.

In neurodegenerative diseases associated with accumulation of fibrillar proteins, elucidation of the mechanisms of formation, recognition, and inhibition of neurotoxic aggregates by therapeutic agents is important for development of therapies against these diseases.^{1,2} Molecular modeling provides a valuable insight into the oligomerization mechanisms and structural characteristics of oligomers and amyloid fibrils,³ and can be useful for initial selection of drug candidates for further experimental screening and optimization of drugs and conformational antibodies targeting neurotoxic aggregates for efficient delivery.⁴ Solvation is a major factor in biomolecular processes, including slow exchange and localization of solvent and ions, protein-ligand recognition, and membrane translocation. Statistical-mechanical, 3D-RISM-KH molecular theory of solvation^{5,6} accurately describes solvation effects in protein-ligand recognition protocols.^{7,8} In a single formalism, the 3D-RISM-KH theory efficiently accounts for electrostatic and non-polar forces, including hydrogen bonding, hydrophobicity, structural solvation and desolvation in crowded cellular environment. A new multiscale modeling platform for optimization of molecular recognition and translocation of antiprion therapeutic agents is based on the implementations of the 3D-RISM-Dock protocol in AutoDock suite,⁸ 3D-RISM-KH solvent analysis in the Molecular Operating Environment package,⁹ and multi-time-step molecular dynamics steered with 3D-RISM-KH effective solvation forces in the Amber molecular dynamics package.^{10,11} We apply the new platform to study molecular recognition at the initial stages of oligomerization of A β peptides, structural solvation and desolvation effects on stability of amyloid fibrils, binding modes of antiprion compounds, and optimization of antiprion agents for efficient delivery.

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26-POS Board 26

Structural and Thermodynamics Behavior of Cardiac Troponin C Variants Present in Cardiomyopathic Patients**Mayra Marques**¹, Guilherme A. De Oliveira¹, Adolfo H. Moraes¹, José R. Pinto², Jerson L. Silva¹.¹Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, ²Florida State University, Tallahassee, FL, USA.

Cardiac Troponin C (TnC) is a Ca²⁺-binding protein and plays an important role in regulation of muscle contraction. Mutations in cTnC are implicated in phenotypic characteristics known as hypertrophic and dilated cardiomyopathy (HCM and DCM, respectively). However, the structural mechanisms underlying cardiac dysfunction are unknown. The main goal of this work is to investigate changes in stability and dynamics of seven cTnC variants (A8V, D145E, C84Y and A31S related to HCM; and Y5H, M103I and I148V related to DCM) using an ensemble of thermodynamic and structural approaches. Ca²⁺-titrations monitored by bis-ANS fluorescence revealed that D145E decreased the Ca²⁺-induced hydrophobic exposure, while Y5H, C84Y, I148V and A31S substantially enhance it by the N-domain exposure compared to WT. A8V and M103I did not show any significant differences. Thermostability monitored by circular dichroism revealed similar melting temperatures between apo and holo states for D145E (apo: 66.4 ± 1.4°C, holo: 65.4 ± 1.6 °C) but different values for WT (apo: 65 ± 1.9°C and holo: >90°C) and C84Y (apo: 43.8 ± 1.5°C, holo: 66.6 ± 0.8°C). The scattering pattern obtained from small angle X-ray scattering were used to evaluate conformational changes induced by 5M of urea on WT and D145E at apo and holo states. Our data suggest that Ca²⁺ does not confer stability to D145E showing a similar Kratky profile for apo and holo state. Furthermore, the D145E displayed the most affected shape compared to WT and perturbed residues were located at the C-domain as confirmed by chemical shift perturbation analysis. These observations open up new avenues for the comprehension of the complex behavior of HCM and DCM mutations in cTnC that has heretofore been not evaluated at structural level.

28-POS Board 28

Stretching of Single DNA Molecules under Pressure-Driven Flow in Straight and Curved Microfluidic Channels

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Microfluidic devices are playing an increasingly important role in the manipulation of DNA molecules for bio-medical analysis. In particular, they have the ability to uncoil and stretch single DNA-molecules for subsequent genomic mapping. This unravelling and stretching of DNA is based on the shear forces present in a pressure-driven laminar flow inside a microchannel of dimensions comparable to the length of the DNA. However, diffusion induced by Brownian motion tends to accumulate DNA in the region of maximum flow velocity at the centre of the channel where shear forces vanish and DNA strands start to coil up again. We have found experimentally that this can be mitigated by employing curved microfluidic channels. In particular there is evidence that serpentine-shaped channels deliver more fully extended DNA strands than simple straight channels.

To understand the mechanism behind this improvement we perform numerical simulations combining a computational fluid dynamics model of the microchannel with Brownian dynamics of a coarse-grain model of λ -DNA molecules. Comparing the simulations of a serpentine channel with those of a straight channel supports the experimentally found improvement of DNA stretching in the former. A detailed analysis of the DNA dynamics reveals that the elastic molecular forces opposing the stretching of the molecule are pulling the DNA out of the central flow line towards the inside of a microchannel bend and thus into regions of larger shear forces. This gives rise to larger average DNA extension but it can also be seen in a modified spatial distribution of the molecules over the channel cross section at the output.

30-POS Board 30

Phalloidin Binds to MREB from *Leptospira Interrogans*Szilvia Barko^{1,2}, Eموke Bodis^{1,2}, David Szatmari¹, Robert C. Robinson^{3,4}, **Miklos Nyitrai**^{1,2,5}.¹University of Pecs, Medical School, Pecs, Hungary, ²János Szentágothai Research Center, Pecs, Hungary, ³Institute of Molecular and Cell Biology, A*STAR, Singapore, Singapore, ⁴Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, ⁵MTA-PTE Nuclear-Mitochondrial Interactions Research Group, Pecs, Hungary.

MreB is a bacterial actin-like protein, which is a key player in the maintenance of cell shape and essential coordinator of the cell-wall synthesis. Although its sequence identity is low in comparison to eukaryotic actin, crystallography studies have shown that MreB and actin share a similar three-dimensional structure with conserved nucleotide-binding elements. The exact distribution of MreB in a bacterial cell remains uncertain due to potential mislocalization of the fluorescently tagged protein. Biochemical analyses have been confined mostly to *T. maritima*, *B. subtilis*, and *E. coli* MreBs, although the sequences and functions of MreBs can vary dramatically within a single species. One possible explanation for the limited number of described MreBs is the complicated purification of soluble functional protein. In our work we have purified and characterised MreB from *Leptospira interrogans* using denaturing purification protocol, which solved the previously mentioned problems in preparation. This MreB may carry novel structural and biochemical properties attributed to the special corkscrew shaped cell-type of Spirochetes. Here we show that MreB of *L. interrogans* is able to polymerise in vitro with association rates that depend on ionic strength and buffer conditions rather than the presence of nucleotides. Its cysteines can be labelled with Alexa 488 maleimide and the fluorescence intensity of the fluorophore changes during polymerisation. Surprisingly, it was found that *L. interrogans* MreB can be labelled also with fluorophore conjugated phalloidin. So far phalloidin binding has only been reported for actin. The observed MreB polymers are indistinguishable from that labelled with Alexa maleimide. Binding of phalloidin did not alter the biochemical properties of MreB. We envisage that the phalloidin staining of *Leptospira interrogans* MreB will provide a powerful experimental tool for the in vivo characterisation of the localisation and function of this important actin-like protein.

32-POS Board 32**Molecular Simulations of Acto-Myosin Network Self-Assembly and Remodeling****Garegin Papoian.**

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Acto-myosin networks are an integral part of the cytoskeleton of eukaryotic cells and play an essential role in determining cellular shape and movement. Acto-myosin network growth and remodeling in vivo is based on a large number of chemical and mechanical processes, which are mutually coupled and spatially and temporally resolved. To investigate the fundamental principles behind the self-organization of these networks, we have developed a detailed physico-chemical, stochastic model of actin filament growth dynamics, at a single-molecule resolution, where the nonlinear mechanical rigidity of filaments and their corresponding deformations under internally and externally generated forces are taken into account. Our work sheds light on the interplay between the chemical and mechanical processes governing the cytoskeletal dynamics, and also highlights the importance of diffusional and active transport phenomena. Our simulations reveal how different acto-myosin micro-architectures emerge in response to varying the network composition.

34-POS Board 34

pH Dependent Peptide Self-Assemblies: A Mechanism as Old as Viruses

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External stimuli are powerful tools that naturally control protein assemblies and functions. For example during viral entry and exit changes in pH are known to trigger large protein conformational changes. However, the molecular features stabilising the higher pH structures remain unclear. We report on a decapeptide that self-assembles into either 50nm diameter nanotubes at high pH or 10.7nm diameter nanotubes at low pH. To solve both low and high pH structures we combine complementary technical approaches going from X-ray crystallography, fiber diffraction and vibrational spectroscopy to electron and optical microscopies to have access from the smallest (sub-ångström) to highest (micrometers) level of organization. The peptide conformation switches from a globular one at pH>7.5 to an extended one at pH<6.5. The high pH crystal structure obtained at 0.85Å resolution reveals a histidine-serine H-bond and histidine-aromatic interactions, whereas the low pH molecular structure demonstrates these key interactions have disappeared, likely in favour of cation- π proximities. Interestingly, re-analysing protein structures with pH-dependent functions reveals that these specific interaction networks are present in viral, bacterial and human proteins. The mechanism discovered in this study may thus be generally used by pH-dependent proteins and opens new prospects in the field of nanomaterials.

36-POS Board 36

The Liquid Structure of Elastin AggregatesSarah Rauscher^{1,2}, **Régis Pomès**^{1,3}.¹Hospital for Sick Children, Toronto, ON, Canada, ²Max Planck Institute for Biophysical Chemistry, Goettingen, Germany, ³University of Toronto, Toronto, ON, Canada.

The protein elastin imparts extensibility, elastic recoil, and resilience to diverse tissues including arterial walls, skin, lung alveoli, and the uterus. Elastin and elastin-like peptides are self-aggregating polymers that undergo liquid-liquid phase separation upon increasing temperature and are well-suited for biomaterials applications. Despite the biological importance of elastin and decades of study, the structural and physico-chemical basis for the assembly and mechanical properties of elastin has remained elusive. We provide an atomistic description of the structural ensemble of an elastin-like aggregate using molecular dynamics simulations with a total time exceeding 0.2 ms. The aggregate consists of highly-disordered chains that retain local secondary structure in the form of hydrogen-bonded turns. The polypeptide backbone remains partly hydrated as it is unable to form extensive secondary structure, precluding the formation of a compact, solvent-excluding hydrophobic core. Consistent with the entropic nature of elastic recoil, the aggregated state is stabilized both by the hydrophobic effect and by an increase in conformational entropy upon self-assembly. These findings resolve the long-standing controversy concerning the structure of elastin and reconcile seemingly contradictory features of previous elastin models: it is because aggregated polypeptide chains form extensive intermolecular interactions between non-polar groups that they approach the state in which their chain entropy is maximized. The dramatic increase in conformational disorder upon aggregation is consistent with the Flory theorem, which predicts maximal chain entropy for polymer chains self-aggregating within a polymer melt. The fact that polypeptide chains can aggregate yet retain functionally-essential conformational entropy is of broad relevance to the study of both protein disorder and protein phase separation. The structural ensemble of the elastin-like aggregate obtained here provides the first atomistic view into what may be described as the liquid state of proteins.

38-POS Board 38

Engineered Living Cells that Program Self-Assembly to Spawn Artificial Cells

Ruihua Zhang, Sung-Ho Paek, Keith C. Heyde, **Warren C. Ruder**.

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Synthetic biology has made significant strides in creating tools for programming intracellular material assembly. We leveraged this control and harnessed the capacity of *E. coli* to produce biotin to guide polymer self-assembly. Biotin plays an essential role in cell growth although requirements are low (e.g., 1 ng/ml in *E. coli*). Biotin is also widely used in molecular assembly because of its strong attraction to streptavidin, with a K_d around 10^{-15} M. First, we engineered *E. coli* with a synthetic gene circuit that increased biotin production 17-fold. Next, we leveraged biotin's attraction to streptavidin in a competitive binding scheme to create a biotin sensor that is both specific and sensitive in comparison to common biotin assay methods. We used the sensor's underlying biophysical processes to control the assembly of DNA polymers on streptavidin-functionalized microbeads, using biotin to tune this self-assembly. We then encapsulated these beads - along with a cell-free expression system consisting of ribosomes, ATP, and RNA polymerase - within hydrofluorocarbon microdroplets. The entire construct functioned as an artificial cell, whereby the addition of biotin to the system repressed synthetic gene expression. As a result, we used biotin to control cell-free synthetic gene circuits within artificial cells. This work shows the utility of linking self-assembling polymer systems with synthetic biology. We believe this system provides insight into the origins of life as this work shows that living cells can express extracellular molecular signals - in this case, biotin - to control the assembly and function of minimal cells. In the future, this work could impact fields ranging from biophysics to synthetic biology.

40-POS Board 40

Structural Characterization of the Interactions Between B-amyloid₁₋₄₀ Peptide and Alpha-Synuclein Protein Involved in Lewy's Body Diseases**Juliana Santana**

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Protein misfolding and aggregation are features shared by some proteins involved in neurodegenerative diseases. The beta amyloid (AB) peptide, alpha synuclein protein (α -syn) and Tau are related to Alzheimer's Disease (AD), Parkinson's (PD) and tauopathies. Those diseases are implicated in amyloid fibril formation. The symptoms displayed by those diseases are similar and results in cognitive and motor deficit. It has been proposed that the AB₁₋₄₀ and AB₁₋₄₂ peptide may be found in the extracellular environment in which it could form fibers or in the intracellular environment maintaining its oligomeric form depending on the point at which the amyloid precursor protein (APP) has been cleaved. The a-syn protein contains 140 amino acids and initially observed to be localized at presynaptic terminals. In the case of dementia associated with Lewys Bodies both the AB peptide and a-syn protein are found co-aggregated. Thus, leading to the hypothesis that there is a synergism between the peptide and the protein to promote the aggregation into fibrillar structures. This work aims to improve the knowledge regarding the molecular interactions involved between the proteins (a-syn) and peptide AB₁₋₄₀ in the formation of protein aggregates. For that, it has been applied fluorescence spectroscopy, circular dichroism, Nuclear Magnetic Resonance State liquid and Solid State. The AB₁₋₄₀ peptide was purified and the purity analyzed by SDS-PAGE gel. The oligomeric state of association between AB₁₋₄₀ and alpha synuclein has been evaluated by means of fluorescence polarization measurements, while the molecular interactions has been followed by Nuclear Magnetic Resonance.

42-POS Board 42

Curcumin Analogues in Treatment of Alzheimer's Disease

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Current treatment of Alzheimer's disease leads to moderate symptomatic benefits but not to stop disease progression. Curcumin and its derivatives have attracted great interest in the prevention and treatment of Alzheimer's disease (AD), due to the ability inhibit the formation of amyloid-beta ($A\beta$) aggregates and the ability to bind Cu (II) ion. However, the use of curcumin in vivo is limited by its poor bioavailability, instability, and reduced water solubility. We have examined the anti-amyloid activity of newly synthesized curcumin derivatives (curcuminoids) with greater chemical stability comparing to curcumin, while maintaining almost unchanged biological properties of the lead compound. The K2T derivatives were synthesized by introducing the t-butyl ester group on central atom of the diketo moiety and different substituent on the phenyl rings of curcumin frame. The IC₅₀ values determined from the dose-response curves obtained by fitting the average Thioflavin fluorescence assay values by non-linear least-square method were in micromolar range. The character of the aggregates was examined also by atomic force microscopy. The imbalances of metal ions homeostasis, especially Cu²⁺, may favor $A\beta$ aggregation and induce oxidative damage. Thus, our second goal was to examine the ability of curcuminoids to bind Cu²⁺ ions and therefore, to evaluate their antioxidant activity. It was shown that K2T compounds effectively binds Cu²⁺. It was also demonstrated that K2T21 and in some extent K2T31 hold the capacity to scavenge DPPH radicals suggesting the importance of a phenolic group combined with methoxyl moiety in orto position. We concluded that the curcumin derivative K2T21 due to its ability to inhibit $A\beta$ aggregation and bind to Cu²⁺ together with good radical scavenging properties is the best candidate for designing new curcumin-based analogs that can effectively treat AD.

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44-POS Board 44

Material Characterization of Ultra-Short Peptides at Low and High Concentrations in Water**Christophe Tarabout**¹, Carmen Valverde Tercedor¹, Stephan Jokisch², Luca Bertinetti¹, Damien Faivre¹.¹Max planck institut for colloids and interfaces, Potsdam, Germany, ²Universität Bayreuth, Bayreuth, Germany.

Ultra-short peptides can be used for systematic mutation studies because of their simplicity and low cost. Understanding their self-assembly is important for controlling the growth of the structures and creating new biomaterials. Recently, a few teams have used bioinformatic methods to predict their potential for aggregation.

We have taken the opposite approach and characterized a family of related short peptides from a material science point of view. For peptides at low concentrations in water, we observe in rheometry experiments that small changes in the sequence can modify the behavior from shear thinning to shear thickening. For peptides at high concentration, amorphous solids and fibers were studied using DSC, mechanical testing, X-ray diffraction and SEM. For some sequences, with the right amount of water, the materials could exhibit self-healing capacity. We will also report how replacement of counterions, modification of charge, size, aromaticity/aliphaticity, hydrophilicity/hydrophobicity and order of the aminoacid sequence affect the macroscopic material properties. The most noticeable effects are the material resistance to heating and propensity to absorb humidity. This study is a necessary step to understand and tune the properties of small peptides as new materials.

46-POS Board 46

Structural Characterization of the Chemokine CCL20 and its Interaction with the Receptor CCR6**Gabriela Valle**¹, Ana Paula Valente², Viviane De Paula¹.¹Núcleo Multidisciplinar de Pesquisa em Biologia – UFRJ, Polo Xerém, Duque de Caxias, Rio de Janeiro, Brazil, ²Instituto de Bioquímica Médica, CNRMN - UFRJ, Rio de Janeiro, Rio de Janeiro, Brazil.

Chemokines constitute a family of small proteins that regulate the immune response by signaling leukocytes through interaction with their transmembrane G-protein coupled receptors. However, the inappropriate regulation of these proteins is associated with an extraordinary number of pathophysiological disorders. Thus, there is a significant interest in understanding how these receptors work to developing drugs to block its activity. The chemokine CCL20 is a natural ligand of CCR6. CCR6-CCL20 interactions have been shown to be involved in several autoimmune and inflammatory processes. CCR6 is expressed in colorectal cancer and has been shown that the binding of the chemokine CCL20 promotes proliferation and migration of tumor cells in vitro. The aim of this work is to investigate the interaction of CCL20 with a peptide derived from the extracellular domain of the receptor CCR6 by NMR spectroscopy. Here we report the expression, purification and NMR characterization of the recombinant CCL20 and the peptide comprising the first 35 amino acid residues of the CCR6 N-terminal domain (CCR6₁₋₃₅). Following several expression and solubility tests we select the BL21(DE3)plysS strain for the expression of the fusion protein SUMO-CCL20, which was expressed in soluble form in minimal medium isotopically labeled with ¹⁵NH₄Cl for NMR studies. CCL20 was purified by nickel-affinity and reversed-phase chromatography. The CCL20 backbone and side chains resonance assignments will be achieved through analysis of triple resonance experiments. At the same time, the recombinant CCR6₁₋₃₅ peptide was overexpressed in Rosetta(DE3) strain and it is being purified. Chemical shift mapping and backbone dynamics of the interaction with the CCR6 peptide will reveal the binding surface on CCL20. These data could offer new insights into the structure-function relation of the CCL20-CCR6 interaction and may be helpful for the design of novel anti-tumor drugs.

48-POS Board 48

The Supramolecular Organization of a Peptide-Based Nanocarrier at High-Resolution

Mazda Rad-Malekshahi², Koen M. Visscher¹, Joao Rodrigues¹, Renko De Vries³, Wim E. Hennink², Marc Baldus¹, Alexandre Bonvin¹, Enrico Mastrobattista², **Markus Weingarth**¹.
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Nanovesicles self-assembled from amphiphilic peptides are promising candidates for applications in drug delivery. However, complete high-resolution data on the local and supramolecular organization of such materials has been elusive thus far, which is a substantial obstacle to their rational design. In the absence of precise information, nanovesicles built of amphiphilic "lipid-like" peptides are generally assumed to resemble liposomes that are organized from bilayers of peptides with a tail-to-tail ordering.

Using the nanocarrier formed by the amphiphilic *self-assembling peptide 2* (SA2 peptide) as an example, we derive the local and global organization of a multimega-Dalton peptide-based nanocarrier at high-resolution and at close-to physiological conditions.¹ By integrating a multitude of experimental techniques (solid-state NMR, AFM, SLS, DLS, FT-IR, CD) with large- and multiscale MD simulations, we show that SA2 nanocarriers are built of interdigitated antiparallel β -sheets and bear little resemblance to phospholipid liposomes.

Our atomic level study allows analyzing the vesicle surface structure and dynamics as well as the intermolecular forces between peptides, providing a number of potential leads to improve and tune the biophysical properties of the nanocarrier. The herein presented approach is of general utility to investigate peptide-based nanomaterials at high-resolution and at physiological conditions, an important advance towards the tailoring of such materials for medical applications.

I. M. Rad-Malekshahi, K.M. Visscher, J.P.G.L.M. Rodrigues, R. de Vries, W.E. Hennink, M. Baldus, A.M.J.J. Bonvin, E. Mastrobattista, M. Weingarth, J. Am. Chem. Soc., in press, DOI: 10.1021/jacs.5b02919, The Supramolecular Organization of a Peptide-Based Nanocarrier at High Molecular Detail

POSTER SESSION II
Tuesday, October 27
16:30 – 18:30
Mar da Barra

All posters being presented in Poster Session II should be set up the morning of October 26.

Juliana Aguiar	1-POS	Board 1
Andrei Alexandrescu	3-POS	Board 3
Saumya Bajaj	5-POS	Board 5
Zuzana Bednarikova	7-POS	Board 7
Ana Carolina Cintra	9-POS	Board 9
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Yohan Kevorkian	21-POS	Board 21
Sergio Kogikoski, Jr.	23-POS	Board 23
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Carlo Montemagno	29-POS	Board 29
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Christopher Taylor	45-POS	Board 45
Tuane Vieira	47-POS	Board 47

1-POS

Board 1

Single Protein Nanopore as Mass Spectrometer to Polyvinylpyrrolidone

Juliana P. Aguiar, Sheila M. Barros, Dijanah C. Machado, Janilson J. Silva Junior, Maria C. Araujo Melo, Claudio Rodrigues
Universidade Federal de Pernambuco, Recife, Brazil.

Synthetic high-molecular polymers are widely applied in biochemistry, biology and medicine. Many of these applications require a detailed understanding of the structure, morphology and chemical interactions of these compounds under confinement and in aqueous solution. Poly-N-vinylamides such as poly(vinylpyrrolidone) (PVP) are highly water soluble synthetic polymers with a broad mass distribution. PVP is a versatile polymer with innate surface activity. It is very difficult to accurately assay due to its wide molecular weight range and amphiphilic nature. There are a number of analytical methods available for the analysis of synthetic polymers. But analysis of PVP has some difficulty. The classical methods don't differentiate between unique oligomers present in a sample, and don't distinguish impurities and/or additives. Nanometer scale pores, including nanopore formed by *Staphylococcus aureus* α -toxin (α -HL), can be used to detect and quantify nucleic acids, proteins, and synthetic polymers. Binding of analytes to nanopore is random and reversible, and it causes characteristic fluctuations in the ionic current. For this reason, single nanopores inserted in thin membranes have recently been referred to as stochastic biosensors. Stochastic biosensing is an approach that relies on the observation of individual binding events between analyte molecules and a single receptor. Recently we had demonstrated that size of individual molecules in polydisperse polyethyleneglycol (PEG1500) sample is achievable with a single protein nanopore. The results are similar to MALDI-TOF spectrograms. This study aims to apply the nanopore based method to PVP analyzer. PVP was chosen because it wide applicability in pharmaceutical, cosmetic and food industries. We demonstrated that α -HL nanopore is able to detect PVP and could be used as mass spectrometer to this polymer and proposed nanopore based biosensor for molecular mass measurement and/or monitoring different polymers in aqueous solutions.

3-POS

Board 3

Subverting Amyloid Laws of Attraction: Inhibition of Fibril Assembly through Like-Charge Repulsion**Andrei Alexandrescu**

University of Connecticut, Storrs, USA

In amyloid structures like-charges are replicated along the one-dimensional lattice that constitutes the fibril axis. Repulsion between like-charges should oppose fibril assembly. To probe the role of charges in fibrillogenesis we have studied the pH-dependence of amylin, a peptide hormone that forms amyloids in type 2 diabetes. Amylin has two ionizable sites: the alpha-amino group at the N-terminus and His18. Our approach has been to look at the pH-dependence of fibrillization kinetics, in variants that have only one of the two groups. The α -amino group at the unstructured N-terminus of amylin has a pKa near 8.0, similar to random coil models, and makes only small contributions. By contrast, His18, which participates in the intermolecular β -sheet structure, has a pKa lowered to ~ 5.0 from the random coil value of 6.5. His18 acts as an electrostatic switch, inhibiting aggregation in its charged state at acidic pH and favoring fibrillization in its uncharged state at neutral pH. The presence of a charged side-chain at position 18 affects fibril morphology and lowers amylin cytotoxicity towards a mouse model of pancreatic β -cells. We exploited the principle that electrostatic repulsion interferes with fibril formation to design peptide variants Arg1 and Arg2, which incorporate strings of four arginines in the amylin amino acid sequence. The charge-loaded peptides fibrillize poorly on their own, and inhibit fibril elongation of WT-amylin at physiological salt concentrations. The most effective inhibitor Arg-1, inhibits WT-amylin fibril elongation rates with an IC₅₀ of ~ 1 μ M and cytotoxicity with an IC₅₀ of ~ 50 μ M, comparable to other inhibitors reported in the literature. These studies suggest that electrostatic interactions can be exploited to develop new types of inhibitors of amyloid fibrillization and toxicity.

5-POS

Board 5

Nano-encapsulated Assemblies Derived from a Non-enveloped Virus Capsid Protein for Targeted Delivery**Saumya Bajaj**, Manidipa Banerjee.

Indian Institute of Technology Delhi, New Delhi, India.

Viruses exemplify uniform self-assembly - multiple copies of single (or few) coat proteins assemble spontaneously in a precisely regulated manner into highly regular and symmetrical capsid shells. The viral genome is protected within these nano-sized containers. Viruses are stable enough to protect the genomic content, and yet pliable enough to release this payload into host cells. This makes viruses fine candidates for development of biomaterials for drug delivery; the engineering of viral capsid proteins and encapsulation of functional cargo in them is one of the subjects of current research in nanobiotechnology. The aim of this work is to explore the capsid protein of a non-enveloped insect nodavirus, Flock House Virus (FHV), as a building block for nano-encapsulated structures for targeted delivery to cancer cells. FHV is a structurally simple virus, being made up of 180 copies of a single capsid protein alpha that presumably contains all the information needed for higher-order assembly. We have expressed alpha in a heterologous (bacterial) expression system and shown its calcium-templated assembly into morphologically diverse particles, the structures of which we are analyzing through electron microscopy and single-particle reconstruction. To the best of our knowledge, this is the first report utilizing *E. coli* to produce FHV particles. Considering that insect cell-produced FHV VLPs have been shown to be carriers of transposable elements, an expression system where *in vitro* self-assembly is encouraged will be of considerable benefit for its biomedical applications. We have genetically engineered tumor-homing peptide Lyp-1 into a surface exposed loop of alpha, thus generating *in vitro* assembled, nano-encapsulated particles with tumor-binding capability. We are using these viral nanoparticles for targeting cancer cells. Thus, we have developed a candidate functional biomaterial for targeted drug delivery.

7-POS

Board 7

Amyloid Self-Assembly of Insulin in Presence of Glyco-Acridines: In Vitro and In Silico Study

Zuzana Bednarikova^{1,2}, Quan Van Vuong³, Andrea Antosova¹, Pham Dinh Quoc Huy^{3,4}, Katarina Siposova¹, Nguyen Anh Tuan⁵, Suan Li Mai^{3,4}, Zuzana Gazova¹.

¹Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia, ²Faculty of Science, Safarik University, Kosice, Slovakia, ³Institute for Computational Science and Technology, Tan Chanh Hiep Ward, Ho Chi Minh City, District 12, Viet Nam, ⁴Institute of Physics Polish Academy of Sciences, Warsaw, Poland, ⁵University of Science - VNUHCM, Ho Chi Minh City, Viet Nam.

Self-assembly of insulin into amyloid aggregates causes problems in the insulin production, storage and application of insulin pumps. Frequent application of insulin into patients with diabetes may result in formation of deposits consisting of insulin amyloid aggregates. We have investigated the effect of 9 aromatic glyco-acridine derivatives with side saccharide chain connected by different linkers on insulin amyloid self-assembly using ThT assay, FTIR spectroscopy and AFM technique. Our results indicate that glyco-acridines are able to interfere with amyloid self-assembling of insulin depending on their structure. The most effective inhibitory effect was obtained for isothiosemicarbazide derivate I1. The obtained IC₅₀ value was in μM range. The least effective were thiosemicarbazide and cyclic 1,3-thiazolidinone derivatives. In silico calculation point out that the non-polar interactions together with the core of acridine derivatives are the key factors determining ligand's free binding energy to insulin. The contribution of the linker and side chains to the inhibitory activity depends not only on their position relative to the core but also on their flexibility. Our findings can be useful for the design of new small molecule drugs for therapy of amyloid-related diseases.

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9-POS

Board 9

Studying the Antifouling Properties of Bacterial S-layers

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²University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

The mimicking of (the physico-chemical) properties bacterial surface layers (S-layers), the outermost cell envelope component of prokaryotic organisms, enable infinite possibilities for technological processes and scientific studies. S-layers are composed of single (glyco)protein units acting as building blocks, which re-assemble into crystalline arrays when exposed to different types of supports (i.e. lipid films, polymers, silica). This assembly in a regular arrangement in the case of *Lysinibacillus sphaericus* (SbpA) is driven by the presence of divalent cations (Ca²⁺) in the crystallization buffer, which also contributes to its high stability. Among other features, such biomimetic films are characterized by their antifouling activity while forming the crystalline structure. Our study focused on the recrystallization of S-layer from *Lysinibacillus sphaericus* (SbpA) on hydrophobic silicon surfaces. Subsequently, the formed crystalline films were exposed to different chemical treatments (metal chelator-EDTA and pH variations) in order to disrupt the SbpA crystalline structure, without causing full protein removal. In a second step, the ability of the protein layer to bind different molecules (BSA, polyelectrolytes) was investigated. Quartz crystal microbalance with dissipation (QCM-D) technique was used to monitor the real time variations of mass deposited per unit area along the crystallization, as well as the kinetics of the process. Complementary atomic force microscopy (AFM) measurements allowed for a detailed following of the topographical changes and mechanical properties of the structures formed.

Although the stability of the protein crystal was not completely altered by the chemical treatment, the change in its antifouling properties suggests possible a charge rearrangement. This question is currently investigated in our laboratory.

11-POS

Board 11

A Hypothesis to Reconcile the Physical and Chemical Unfolding of Proteins**Guilherme A. De Oliveira**, Jerson L. Silva.

Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

A comprehensive view of protein folding is crucial for understanding how misfolding can cause neurodegenerative diseases and cancer. When using physical or chemical perturbations, NMR spectroscopy is a powerful tool to reveal a shift in the native conformation toward local intermediates that act as seeds for misfolding. Pressure favors the reversible unfolding of proteins by causing changes in the volumetric properties of the protein–solvent system. However, no mechanistic model has fully elucidated the effects of urea on structure unfolding, even though protein–urea interactions are considered to be crucial. Here, we provide NMR spectroscopy and 3D reconstructions from X-ray scattering to develop the “push-and-pull” hypothesis, which helps to explain the initial mechanism of chemical unfolding in light of the physical events triggered by HP. In studying MpNep2 from *Moniliophthora perniciosa*, we tracked two cooperative units using HP-NMR as MpNep2 moved uphill in the energy landscape; this process contrasts with the overall structural unfolding that occurs upon reaching a threshold concentration of urea. At subdenaturing concentrations of urea, we were able to trap a state in which urea is preferentially bound to the protein (as determined by NMR intensities and chemical shifts); this state is still folded and not additionally exposed to solvent [fluorescence and small-angle X-ray scattering (SAXS)]. This state has a higher susceptibility to pressure denaturation (lower $p_{1/2}$ and larger ΔV_u); thus, urea and HP share concomitant effects of urea binding and pulling and water-inducing pushing, respectively. These observations explain the differences between the molecular mechanisms that control the physical and chemical unfolding of proteins, thus opening up new possibilities for the study of protein folding and providing an interpretation of the nature of cooperativity in the folding and unfolding processes.

13-POS

Board 13

Synthesis of CCR6 Sulfopeptides to Probe Specific Contacts with the Chemokine CCL20**Marlon Dias**¹, Fabio C. Almeida², Ana Paula Valente², Viviane S. De Paula¹.²Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. ¹Universidade Federal do Rio de Janeiro-Polo Xerém, Rio de Janeiro, Brazil,

Chemokines are small soluble proteins that stimulate chemotactic cell migration through interactions with chemokine receptors, a family of G protein-coupled receptors expressed in leukocyte cell membranes. In addition to their inherent pro-inflammatory activity, chemokines also contribute to many pathologies including auto-immune diseases and cancer. The N-terminus domains of the chemokine receptors contain tyrosine amino acid residues. Tyrosine sulfation is a post translational modification that enhances protein-protein interactions and the sulfation in the N-terminus regions of the chemokine receptors enhance the binding affinity of chemokine ligands and can modify the selectivity of a receptor. The aim of this work is to characterize the interactions of the chemokine CCL20 with several CCR6-derived extracellular peptides by NMR spectroscopy. In this work we showed a strategy for synthesis of peptides containing sulfotyrosine at one or more specific positions to explore the role of tyrosine sulfation in recognition of the CCL20 by its receptor CCR6. We report the synthesis, purification and analyses of peptides corresponding to residues 16-30 of CCR6 in sulfated and non-sulfated form at the Y18, Y26 and Y27 positions. The non-sulfated peptide and sulfopeptide (sY27) were obtained with success and with high yield. The samples exhibited a major peak on preparative HPLC chromatograms and the correct masses of both peptides were verified by electrospray ionization mass spectrometry. The peptide resonances were assigned by analysis of TOCSY spectra. Next, we will demonstrate that sulfation of this peptide at one or both positions would substantially enhance the affinity of chemokine binding. Our objective is to understand which sulfotyrosine present in the CCR6 sulfopeptide is critical for CCL20 recognition and highlights the potential influence of initial binding interactions on receptor activation.

15-POS

Board 15

Thermal Stability and Self-Assembly of Lysozyme in Water-Miscible Ionic Liquids

Diana Fedunova, Andrea Antosova, Jozef Marek, Erna Demjen, Zuzana Gazova.
Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia.

Ionic liquids (ILs) are widely used as novel solvents in many areas. The water miscible ILs can serve as effective participants in various biological processes. The major advantage of ILs is that their physicochemical properties can be tuned by appropriate combination of cations and anions in order to obtain solvent with desired properties. We have studied effect of imidazolium-based ionic liquids with acetate and tetrafluoroborate as anions on thermal stability of lysozyme and morphology of amyloid fibrils using calorimetry, circular dichroism spectroscopy, AFM and computer image analysis. The reduction of thermal stability of lysozyme is observed at the presence of either ILs in concentration range 1-8% w/w in 2 mM glycine buffer, pH 2.7. The transition temperature and ΔH_c decreases at all studied ILs concentrations comparing to neat lysozyme, but denaturation process is still highly reversible. The decrease of ΔH_c indicates the depletion of native structure at the presence of either ILs. We have shown that ionic liquids with imidazolium as cation and chaotropic anion tetrafluoroborate or kosmotropic anion acetate decrease stability of lysozyme at acidic pH conditions with only slightly stronger effect of chaotropic ion suggesting more complicated mechanism than simplistic Hofmeister phenomena. We exploited obtained results for study of effect of both ILs on lysozyme fibrillization at the same conditions. Lysozyme at low protein concentration doesn't form fibrils at acidified water. Addition of either ILs leads to formation of lysozyme fibrils, with slightly slower kinetics of fibrillization at the presence of acetate ions comparing to tetrafluoroborate and different morphology. Even if tetrafluoroborate-promoted fibrils are matured in shorter time, wider spectrum of fibril types is observed.

Acknowledgements. This work was supported by research grants from the Slovak Grant Agency VEGA, 0176, 0181, 0175, ESF 26110230097 and APVV 0526-11.

17-POS

Board 17

Hofmeister Anions Determine the Stability and Amyloid Self-Assembly of Lysozyme

Zuzana Gazova¹, Slavomira Ponikova¹, Andrea Antosova¹, Erna Demjen¹, Dagmar Sedlakova¹, Jozef Marek¹, Rastislav Varhac², Erik Sedlak^{2,3}.

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Hofmeister series of ions ranks the relative influence of ions on physico-chemical behaviour of biomacromolecules. We have explored an effect of various Hofmeister anions on stability and amyloid self-assembly of hen egg white lysozyme in acidic pH. The kinetics of amyloid fibrillization in the presence of anions suggest that neutralization of positive surface charge of lysozyme due to interaction with anions accelerates lysozyme amyloid self-assembly. The analysis of the conformational properties of formed fibrils has shown that lysozyme forms typical elongated fibrils with high content of β -sheet in presence of sodium chloride. On the other hand, in the presence of both chaotropic perchlorate and kosmotropic sulfate anions the fibrils form clusters with secondary structure of β -turn. Moreover, the acceleration of fibril formation is accompanied by decreased amount of the formed fibrils. Our study shows Hofmeister effect of monovalent anions on: (i) lysozyme stability, (ii) ability to accelerate nucleation phase of lysozyme fibrillization, (iii) amount, and (iv) conformational properties of the formed fibrils.

This work was supported by research grants VEGA 1/0521/12, 2/0181/13, 2/0175/14, APVV 0526-11 and from CELIM (316310) funded by 7FP EU (REGPOT).

19-POS

Board 19

Self-Assembly of Silk Proteins Under a Uniform Flow**Ana Herrera**^{1,2}, Frauke Gräter^{1,2}, Eduardo Cruz-chu¹.¹Heidelberg Institute for theoretical Studies, Heidelberg, Germany, ²Universität Heidelberg, Heidelberg, Baden-württemberg, Germany.

Natural spider's silk fibers have unique properties such as high strength, extensibility and biocompatibility. The arrangement of the proteins subunits into crystalline and non-crystalline phases determines the performance of these fibers. The tendency of silk proteins to aggregate increases under flow. It is believed that shear forces guide fiber assembly on a molecular level, by stretching and aligning the protein chains such that the formation of crystalline regions is enhanced. However, the molecular mechanism of shear-induced assembly of silk is unknown. We performed atomistic molecular dynamics (MD) simulations of fragments of the repetitive unit of silk peptides in explicit water under elongational flow. For single peptides, we examined the dependence of peptide extension on the flow velocity. We recovered a worm-like chain behavior, which reflects the intrinsic stiffness and coiling propensity of silk in water. To analyze the assembly pathway, MD simulations of a set of aligned silk peptides (3x3 and 5x5 arrays) with starting configurations obtained from the single peptides stretching stage were performed. On this basis, we were able to quantify the role of the crystal-forming poly-alanine repeats and their elongation by flow for silk beta-stack formation. Our results help to guide experimental attempts to assemble silk and other polymers into tough fibers.

21-POS

Board 21

Prion-DNA Interaction Probed by Solution and Solid-State NMR

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Amyloidosis is a clinical disorder caused by extracellular deposition of proteins that suffered conformational modifications resulting in insoluble, abnormal fibrils that impair organ function. Prion is an uncommon amyloid disease, since pathogenic form of prion protein can transmit the disease between animals, coming to epizootic events that often spread to different species through various routes, such as inoculation or ingestion of contaminated meat. In the 1980's, prion disease was implicated to a major epizootic event, in which more than 2 million UK cattle were infected. In humans, the number of cases to date has been relative modest, around 200. However, the genetic factors allied with widespread population exposure and the lower incubation time for prion diseases, suggest the need for caution. The main goal of this work is to apply solid-state NMR in order to obtain more information about prion fibrils formation and stabilization. Amyloid proteins are usually organized in ordered fibers, which are characterized by a cross beta-sheet quaternary structure. However the prion protein forms amorphous aggregates that are founded in the brain of infected animal, instead of fibrillar ones. The lack in the prion structural studies in the solid-state NMR field has opened opportunity to begin this work in one of the best laboratories that develop this technology and apply it to study formation of prion protein aggregates and the mechanisms underlying prion conversion. This study has good perspectives since it can help us to obtain atomic information about protein-protein and protein-nucleic acid interactions and the dynamics of monomer-fibers incorporation. Altogether, these information may contribute to the development of new strategies to control prion diseases.

23-POS

Board 23

Self-Assembled Diphenylalanine Modified with Microperoxidase-11 and Glucose Oxidase: Electrochemical Studies Aiming Sensing Applications

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Ever since the discovery of L,L-diphenylalanine micro/nanostructures(FF-MNSs), researchers have been exploring their potential for biosensing purposes. A novel platform for the detection of glucose was obtained by self-assembly of PAH, FF-MNSs, MP11, and GOx onto glassy carbon(GC) electrode surface. The peptide nanostructures were prepared by crystallization of the L,L-diphenylalanine in water, with posterior thermal treatment to obtain different crystal structure (*P6₁* and *P22₁2₁*). The peptides were then modified in solution and used to modify electrodes. The interaction between the FF-MNSs and MP11 was studied by SEM, XRD, Raman, FTIR and EPR spectroscopy. The electroactive area was studied, and the results showed an increase in electroactive area due to the use of FF-MNSs. The charge transfer resistance was studied by electrochemical impedance spectroscopy. The electrodes modified only with the peptides, GC/(Hex+PAH) and GC/(Ort+PAH), had a resistance of 280 and 102Ω, respectively. With MP11 modification, the values changed to 506 and 142Ω, respectively. The band structure of the peptides were calculated and showed that the band-gap energies of the hexagonal is around 3.6eV, and for the orthorhombic this value is larger around 4.0eV. However, the structure of the HOMO and LUMO levels of the orthorhombic structure allows it to be doped, similar to the semi-conductors, and we believe that PAH is acting as dopant for the orthorhombic FF-MNSs, leading to a smaller charge transfer resistance. The electron transfer rate(k_s) were studied using Laviron's equations, and the values for the electrodes modified with the FF-MNSs were higher than the one containing only MP11. Our results show that the energy band-gap of the orthorhombic FF-MNS nanostructures plays a fundamental role in the conductivity and electron transfer rates when modified with MP11. The efficacy as H₂O₂ and glucose sensors was also evaluated.

25-POS

Board 25

New Evidences to Support the Prion-like Behavior of Mutant p53

Adriani Lima, Jerson L. Silva, Guilherme A. De Oliveira.
Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

The human p53 is a nuclear phosphoprotein of 53 kDa activated in response of cellular stress. Upon activation it mediates specific responses, including cell-cycle arrest, apoptosis, and senescence. The majority of p53 mutations associated with cancer was screened in the DNA binding domain of the protein (DBD). The DBD is a domain of 25 kDa and mutations in this region favor non-native conformations, affecting their solubility and thermal stability. The changes in protein conformation can cause inactivation and formation of aggregated p53. To verify the role of aggregation, and to expand the p53 aggregation hypothesis based on the prion like specific mutations were used to stabilize and destabilize p53. We evaluate whether wild type p53 (WT) and mutants; Y220C with a large loss of thermodynamic stability, double mutant (DM) (Y236F / T253I), and quadruple mutant (QM) (M133L / V203A / N239Y / N268D), which stabilizes p53 by 1.6 kcal / mol and 2, 65 kcal / mol, respectively could aggregate as amyloids under near physiological conditions. We also investigated if studied mutants are able to seed aggregation of the wild-type form p53. The aggregation kinetics of p53 was monitored by measuring the binding of the protein to Thioflavin T (ThT) and light scattering. Our results show that the cancer – related mutant Y220C has a faster aggregation kinetics when compared to the wild-type, DM and QM-p53C at 37 ° C. It was also observed that seeds of this mutants (Y220C) accelerate the aggregation of wild p53C compared to seeds obtained from the stabilized mutants. These data support the prion-like behavior of a cancer-related p53 mutants and open up new discussions for the coaggregation vs. prion-like mechanisms of p53 aggregation in cancer.

27-POS

Board 27

Viscoelastic Properties of DNA in Vivo Measured by Fluorescence Correlation SpectroscopyRudra Kafle, Liebeskind Molly, **Jens-Christian Meiners**.

University of Michigan, Ann Arbor, USA.

Mechanical quantities like the elasticity of cells are conventionally measured by directly probing them mechanically. This, however, is often impractical, and even impossible when subcellular structures inside living cells are concerned. We use a purely optical method instead; an adaptation of fluorescence correlation spectroscopy (FCS) to measure such mechanical quantities in chromosomal DNA in live E. Coli cells. FCS is a fluorescence technique conventionally used to study the kinetics of fluorescent molecules in a dilute solution. Being a non-invasive technique, it is now drawing increasing interest for the study of more complex systems like the dynamics of DNA or proteins in living cells and cell membranes. Unlike an ordinary dye solution, the dynamics of macromolecules like proteins or entangled DNA in crowded environments is often slow and subdiffusive in nature. This in turn leads to longer residence times of the attached fluorophores in the excitation volume of the microscope and artifacts from photobleaching abound that can easily obscure the signature of the molecular dynamics of interest. We present a method of calculating the intensity autocorrelation function from the arrival times of the photons on the detector that maximizes the information content while correcting for the effect of photobleaching to yield an autocorrelation function that reflects only the underlying dynamics of the sample. This autocorrelation function in turn is used to calculate the mean square displacement of the fluorophores attached to DNA. By using a suitable integral transform of the mean square displacement, we determine the viscoelastic moduli for lambda-DNA solution and the DNA in E. coli. We note that these mechanical quantities evaluated by an optical technique are in good agreement with similar quantities measured by completely different techniques.

29-POS

Board 29

Precision Assembly of Biologically Functional Abiotic/Biotic Materials**Carlo Montemagno.**

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Mankind has crafted its world through the creative manipulation of a small number of fundamental machines. In the agricultural and mechanical ages all the trappings of civilization were crafted from the six fundamental machines of physics: the screw; the wheel and axle; the incline plane; the lever; the pulley; and the wedge. The modern electronic age was established through the addition of five fundamental machines to humankind's toolbox: the diode; the transistor; the inductor; the resistor; and the capacitor. Our civilization is founded on the creative exploitation of the properties of only 11 different building blocks. The ability of living systems to transform matter and actively interact with the environment sets them apart from current systems made by man. This difference in complexity can be attributed to the fact that nature has tens of thousands of building blocks to work with instead of the 11 used by man.

Presented is a new technology that transitions additive manufacturing from 3D space to a four-dimensional, functional space. Through developments in stabilizing a very large set of integral membrane proteins, the suite of tools available to engineer complex systems has been greatly expanded. A new class of printable inks is being produced that integrates peptides and functional proteins with polymer constructs enabling the incorporation of biological metabolism as an intrinsic property into materials. Presented is a new class of materials and devices that possess a functional architecture that transform energy, and collect, process and act on information in response to changes in their local environment.

31-POS

Board 31

Prediction of Cleavage Specificity in Proteases by Biased Sequence Search ThreadingGonca Ozdemir Isik, **Nevra Ozer**.

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The assessment of substrate specificity in proteases is crucial in drug development studies, where interpreting the adaptability of residue positions can be useful in understanding how inhibitors might best fit within the substrate binding sites. In this work, the substrate variability and substrate specificity of the Hepatitis C virus (HCV) NS3/4A serine protease and the Adenovirus 2 (AdV2) cysteine protease was investigated by the computational biased sequence search threading (BSST) methodology. Using available crystal structures, the template structures for the substrate-bound proteases were created in silico by performing various peptide building and docking procedures followed by energy minimization and molecular dynamics simulations. BSST was performed starting with known binding, nonbinding and random peptide sequences that were threaded onto the template complex structures, and low energy sequences were searched using low-resolution knowledge-based potentials. Then, target sequences of yet unidentified potential substrates were predicted by statistical probability approaches applied on the low energy sequences. The results show that the majority of the predicted substrate positions correspond to the natural substrate sequences with conserved amino acid preferences. For NS3/4A serine protease cleavage, significant selection for Pro at P2 and Cys at P1 positions is observed, where these positions are correlated with increased cleavage efficiency hence are probably less tolerant to change. For AdV2 cysteine protease, BSST produces similar significant results for both type 1 (XGX-G) and type 2 (XGG-X) consensus cleavage sites, where P2 and P1' positions have Gly with highest percentage in type 1 (XGX-G) while P2 and P1 positions have Gly in type 2 (XGG-X). Overall, supported by the successful outcomes with the case studies of HCV NS3/4A serine protease and AdV2 cysteine protease here, BSST seems to be a powerful methodology for prediction of substrate specificity in protease systems.

33-POS

Board 33

Immobilization of RubisCO by Self-Assembled Nanotubes

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Biology-inspired catalysts, such as ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO), efficiently reduce CO₂ resulting in products that are subsequently metabolized to energy-rich compounds like glucose by the sequential action of multiple enzymes. However, the use of free or cell-based enzymes as biocatalysts for large-scale industrial processes pose significant limitations due to their incompatibility with reaction conditions that often depart from their physiological states. Although biological catalysts, such as RubisCO, are among the most efficient and ubiquitous catalysts for CO₂ fixation, the use of free or cell-based enzymes as biocatalysts for large-scale industrial processes pose significant drawbacks due to their incompatibility with reaction conditions that often depart from their physiological states. However, cells often compartmentalize various biological reactions to address challenges such as the toxicity of accumulating intermediates, competing reaction pathways and slow turnover rates. **Objective:** In this work, we attempt to mimic biological compartmentalization, where structures such as carboxysomes naturally encapsulate RubisCO and carbonic anhydrase and provide a protected environment to maximize CO₂ assimilation. **Methods:** To initiate such studies, we have attempted to encapsulate RubisCO within self-assembled nanotubes. Conditions were established such that RubisCO was successfully sequestered into a variety of self-assembling nanotubes. **Results:** The encapsulated protein was enzymatically active and was clearly associated with the nanotubes and removed from solution based on a number of criteria. **Conclusion:** These nanostructures were also found to enhance the stability of RubisCO toward proteases and other environmental factors. We expect this research will permit encapsulated biocatalysts to be useful in scalable CO₂ conversions and other processes.

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Board 35

Mutant p53 Aggregates in Glioblastoma Cell Lines

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Glioblastoma Multiforme (GBM) is the most common and aggressive brain tumor in humans. According to World Health Organization (WHO), it is classified as grade IV glioma. This feature is correlated with the high tumor invasiveness to the normal adjacent nervous tissue. p53 is a tumor suppressor protein which plays an essential role in preventing the development of cancer through cell cycle arrest or apoptosis in response to DNA damage. Mutations in TP53 gene is frequently associated to a high probability of cancer development. Therefore it is a great target against cancer. Studies from our group have shown that p53 aggregation into a mixture of oligomers and fibrils sequesters the native protein into an inactive conformation, typical of a prionoid¹. Furthermore, these aggregates are present in tissue biopsies of breast cancer and in several cancer cell lines such as basal cell carcinoma, breast and ovarian carcinoma. Objective: To evaluate p53 aggregation and the gain-of-function phenotype of mutated p53 in glioblastoma multiforme cell lines. Material and Methods: Primary GBM cells U87MG, U138MG, T98G and cells derived from patient biopsies (GBM11) were used to evaluate p53 transcripts and protein levels, protein aggregation assays by confocal microscopy and the response of Temozolomide treatment to the mRNA levels of p53 target genes. Results and Discussion: Mutated p53 accumulates in glioblastoma cell lines, aggregates in the perinuclear space and shows a gain-of-function by increasing O⁶-alkylguanine DNA alkyltransferase expression. Furthermore, glioma cells with mutated p53 (T98G and U138MG) migrates more upon scratch when compared top53 wild type. The gain-of-function of aggregated p53 in glioblastoma cell lines may explain the GBM phenotype, migration and open up new opportunities to target these cells against tumor.

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Board 37

Mechanical Tightening and Untying of a Trefoil-Knotted Protein by Optical Tweezers

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According to the funnel hypothesis, the roughness of the energetic landscape increases along with the topological complexity of proteins and thus, the chance of produce misfolded structures. Knotted proteins are examples of topological complexity, but it is not clear how these proteins deal with the folding because experimentally is difficult to determine how the folding mechanism is coupled with the threading of the polypeptide chain.

To solve this problem, we have studied the mechanical unfolding of a trefoil knotted protein (MJ0366 of *Methanocaldococcus jannaschii*) using the experimental setup of optical tweezers. Two mutants were created, one to pull MJ0366 from the ends of the polypeptide chain to tight the knot, and other mutant in order to untie the protein by pulling from key residues of the structure. When the knot was tightened, MJ0366 showed a two state folding mechanism characterized by the presence of a single unfolding and refolding transitions with average forces of 19.8 pN and 13.6 pN respectively. The contour length was 7.8 nm shorter than expected. This could be associated in part with the presence of the knot in the structure, although the formation of trefoil knot decreases the contour length in about 5 nm. Free energy of unfolding reaction, calculated using the Crooks fluctuation theorem, is 13.6 kcal/mol around 4.4 kcal/mol higher than the obtained in experiments performed with chaotropic agents. In the case of the MJ0366 mutant whose knot is untied upon pulling, preliminary experiments show a complex folding mechanism with multiple transitions of unfolding and refolding. These results suggest that threading of the polypeptide chain creates a rougher landscape for folding, but once the knot is formed, the folding landscape becomes smooth. Funded by Fondecyt: 1151274, Anillo ACT-1107, CONICYT fellowship N°21130254.

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Board 39

k-Casein from Bovine Milk: From Natural to Pathological Assemblies

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Casein is the best-characterised milk protein and, in bovine milk, it constitutes over 70–80% of total protein content. In milk, casein exists as large micelle-like complex that comprise four unrelated proteins (α s1-, α s2-, β - and κ -casein) and calcium phosphate. Crucial for the integrity of the protein complex is the k-casein which is responsible for the steric stability of the casein micelles through coating of the structure. All the caseins, α s1-, α s2-, β - and κ adopt so extremely open and flexible conformations to be considered full members of the class of intrinsically disordered proteins (IDPs). However, k casein presents special structural features that may reflect in different self-association pathways, each one related to a specific function and biological role. In particular, the presence of the two Cys residues, Cys11 and Cys88, creates a complex disulphide bonding pattern between κ -casein molecules causing heterogeneous polymerization. In native environment, the heterogeneous polymers (from monomers to octamers) further associate through several kinds of interactions in multimeric colloidal systems. On the other hand, it has been demonstrated that κ -casein is able to form amyloid fibrils both in vitro and in vivo, and this is probably the principal cause of corpora amylacea (CA) occurring in calcified stones in mammalian glands.

Here, by using several biophysical methods and bioinformatic tools, we investigated how the varying of environmental conditions can determine the different k-casein fate: from natural assembly to pathological amyloid fibrils. Moreover, we demonstrated that the two different self-association regimes are reached in conditions in which specific interactions, hydrogen bond or hydrophobic, became predominant on the others.

41-POS

Board 41

Nanomaterials and Their Applications in Viral and Bacterial Inhibition**Shree Singh**¹, Vida A. Dennis¹.¹Alabama State University, Montgomery, USA, ²Alabama State University, Montgomery, AL, USA.

Use of nanomaterials for a variety of applications has opened new avenues for treatment of pathogenic organisms. This emergence of nanotechnology is also being explored to treat viral and bacterial diseases. It has been noted in recent research articles that metals like silver and gold have anti-microbial and anti-viral activities, but cytotoxic effects of these reactive metals make them unsafe for human applications. The reactivity and behavior of nanomaterials can be modulated by reducing their size or conjugating them with biologically compatible materials. We have used gold nanoparticles of various sizes and shown that they can effectively inhibit RSV growth while exhibit low toxicity. Similarly, Polyvinylpyrrolidone (PVP) conjugated silver nanoparticles showed low toxicity to HEp-2 cells at low concentrations and exhibited 44% RSV inhibition. Carbon nanotubes (CNTs) are emerging nanomaterials for biomedical applications. We have shown that silver-CNTs being the effective nanomaterials in reducing RSV infection in cell culture. Similarly, various nanomaterials such as silver, gold and CNTs are very effective in inhibiting gram positive and gram negative bacteria.

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Board 43

Synthesis, Characterization and Applications of Size Restricted Polycationic Hen Lysozyme Nanoparticles

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We report a novel strategy for synthesis of Hen Egg-White Lysozyme (HEWL) nanoparticles by alkaline pH-induced aggregation at room temperature with subsequent stabilization by intermolecular disulfide bonds [1]. Size heterogeneity of the nanoparticles renders their characterization a formidable challenge. To address this we have used size exclusion and ion exchange chromatography to separate differently sized nanoparticles. Further biophysical methods like steady state fluorescence anisotropy, DLS, TEM, AFM and SAXS were employed to characterize the size-fractionated nanoparticles in detail. Accessible hydrophobic regions in the HEWL nanoparticles were assessed by ANS fluorescence. Pyrene fluorescence sensitivity to solvent polarity has been used to estimate changes in probe microenvironment as a result of conformational change during aggregation of HEWL. Significant drop in steady state anisotropy of fluorescein conjugated fractions clearly shows that aggregates labeled with multiple fluorophores exhibit homo-FRET. Self-quenching of fluorescein limits the brightness available per labeled protein. But in our case the phenomenon of self-quenching is turned into an advantage to study aggregation. The fluorescein conjugated nanoparticles can be used for targeted molecular imaging by attaching appropriate ligands to them. Polycationic HEWL nanoparticles have numerous positive charges on their surface which can facilitate association of polyplexes to various negatively charged cargoes. These nanoparticles were able to condense DNA efficiently as revealed from gel retardation assay. The promising HEWL nanoparticles can be utilized as imaging nanovectors, DNA delivery vehicle and F19 contrast agents. These nanoparticles also offer the advantage of being engineered for use as drug delivery vehicle in future.

[1] Ravi VK, Swain T, et. al., (2014) PLoS ONE 9(1): e87256.
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45-POS

Board 45

Combining Single-Molecule Techniques with Microfluidics to Study Protein Aggregation Linked to Neurodegenerative Disease

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Alzheimer's and Parkinson's diseases, along with numerous other neurodegenerative diseases, are understood to be caused by the aggregation of amyloidogenic proteins in the brain. This protein self-assembly produces cytotoxic species that give rise to brain deterioration. Recent evidence indicates that it is the rare, low-molecular-weight species (oligomers) rather than the more abundant high-molecular-weight fibrils of certain amyloidogenic proteins that are the most cytotoxic in a number of neurodegenerative diseases. Due to their low relative abundance, transient nature and high heterogeneity, oligomers have proven a challenging target for biophysical studies. We have approached this problem by uniting the advantages of single-molecule fluorescence, which can resolve sub-populations of species by probing each individually, and microfluidics. By using a microfluidic device to rapidly dilute protein samples for single-molecule detection, we are able to observe unstable species which would otherwise be invisible. The combination of the two methods enables the determination of the size distribution of the oligomers, their stability and information on their structure as the aggregation proceeds. This fundamental information on the aggregation pathway and the rate constants measured will shed insight into the pathogenesis of Alzheimer's or Parkinson's disease.

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Board 47

Dissection of Prion protein and Lipid InteractionRaiane Santos², Quezia Tedeschi¹, Jerson Silva², **Tuane Vieira**¹.¹IFRJ, Rio de Janeiro, Brazil, ²UFRJ, Rio de Janeiro, Brazil.

Introduction: Transmissible spongiform encephalopathies are a group of fatal diseases, which affect mammals, caused by an abnormal isoform of the prion protein (PrP). Conversion of cellular PrP (PrPC) into the pathological conformer, PrPSc, involves contact between both isoforms and probably requires a cellular factor. Recombinant PrP can be converted to an abnormal form via seeded polymerization in vitro techniques in the presence of lipids.

Objectives: The importance of lipid molecules for conversion has been revealed, but little is known about the structural features implicit in this interaction. A detailed understanding of this interaction may provide new insights into toxic mechanisms associated with this disease.

Material and Methods: In the present work, we used light scattering, FTIR, electron microscopy and fluorescence measurements in order to provide information on the chemical and physical properties of the murine recombinant PrP (rPrP 23-231) interaction with

Phosphatidylethanolamine (PE) and Phosphatidic Acid (PA) vesicles. Results and Discussion: We found that phospholipid vesicles raised rPrP light scattering. PE induced changes on PrPC secondary structure, forming soluble oligomers/aggregates with increased β -sheet. PA induced changes on PrPC secondary structure, forming insoluble aggregates. These aggregates were shown to be fibers with increased β -sheet content. Conclusions: Our results suggest that phospholipids, such as PE and PA, play a role in prion aggregation, acting as a cofactor for its pathogenic conformation. On the other hand, these two lipids seem to trigger PrPC aggregation through different pathways, generating different PrPC aggregated forms. These differences may be also important for disease development.