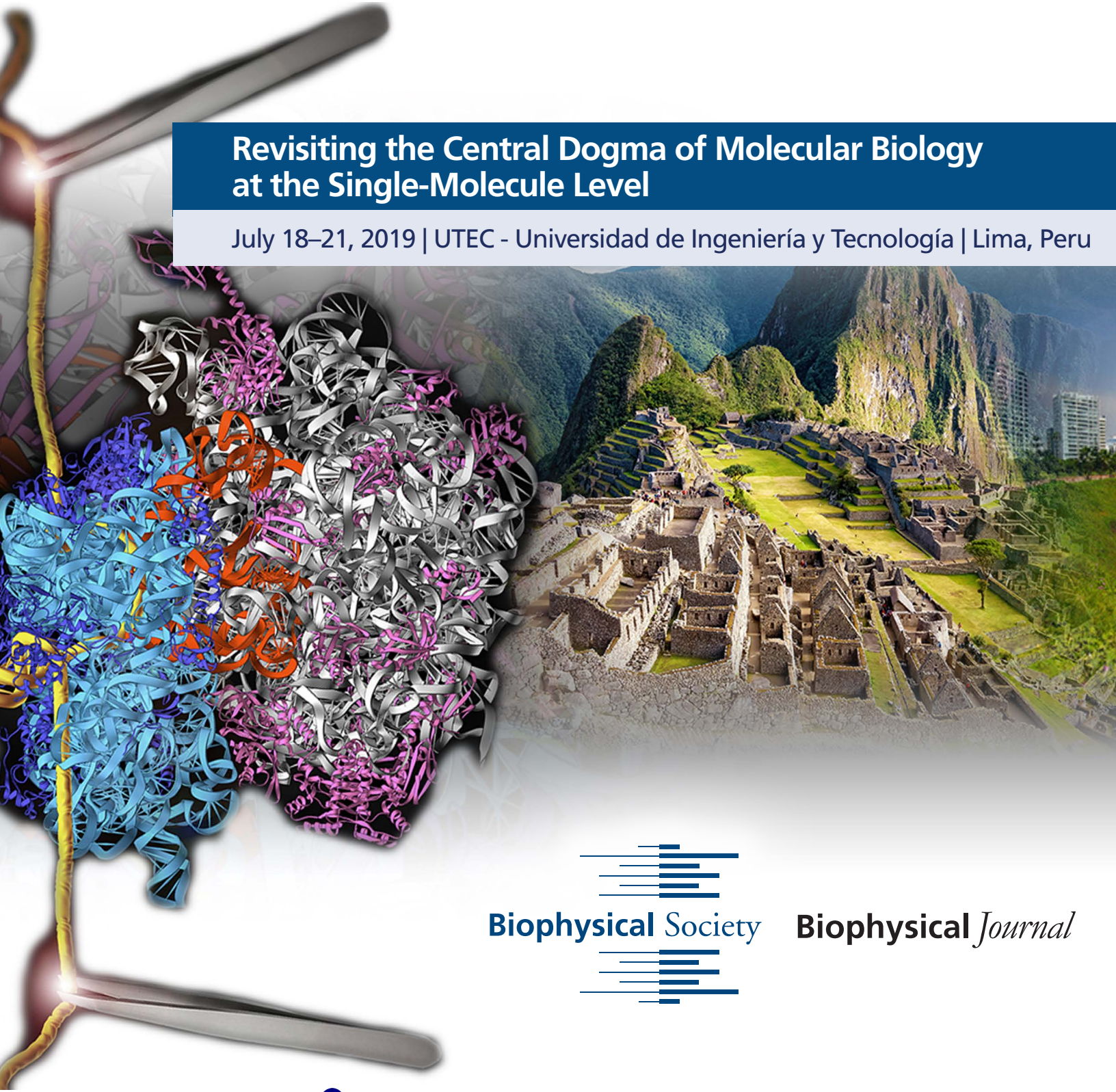
Revisiting the Central Dogma of Molecular Biology at the Single-Molecule Level

July 18–21, 2019 | UTEC - Universidad de Ingeniería y Tecnología | Lima, Peru



Biophysical Society

Biophysical *Journal*

UTEC Universidad de Ingeniería y Tecnología

Instituto Nacional de Salud Ministerio de Salud

CONCYTEC 50 años Consejo Nacional de Ciencia, Tecnología e Innovación Tecnológica

IUBMB International Union of Biochemistry and Molecular Biology

ONi

ZEISS

Beckman Coulter Life Sciences

LUMICKS Capture Molecular Interactions

CHROMA

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Biochemistry

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Thank You to Our Sponsors

Thank you to IUPAB and NSF for Travel Award Support

July 2019

Dear Colleagues,

We would like to welcome you to the Biophysical Society Thematic Meeting titled, *Revisiting the Central Dogma of Molecular Biology at the Single-Molecule Level*. We have assembled a stimulating program, with lectures focused on how recent discoveries by single-molecule manipulation and nanoscale imaging enable molecular level understanding of key molecular processes involved in the central dogma of molecular biology.

The research done at the single-molecule level is inherently interdisciplinary, taking place at the interface of cell biology, physics, biochemistry, and computational biology. This meeting will bring together researchers with a wide range of expertise and interests who use single-molecule tools to address problems in each of these fields. Particular aspects that will be covered by the meeting will include replication, transcription, DNA repair, protein synthesis, chaperone-mediated protein folding/degradation, and molecular motors. In addition, the goal is to feature the latest cutting-edge developments in single-molecule instrumentation and nanoscale visualization, and steered molecular dynamics simulations. We hope that the meeting will promote discussions and foster future interdisciplinary collaborations.

The approximately 150 attendees to this conference will enjoy a full program with 34 lectures, 59 posters, and 20 flash talks, bringing together well recognized scientists from different fields and 15 countries, promising a truly international and multidisciplinary inspiring environment. In addition, we will have two social events where the participants can expand their social networks and meet researchers from different parts of the globe.

We encourage you to enjoy the different social/cultural activities that Lima has to offer as the gastronomic and cultural capital of South America. Particularly, we invite you to experience the diversity and richness of the world-renowned Peruvian cuisine, and to visit the world-heritage sites from pre-Columbian, Inka empire, and Spanish colonial eras located in multiple parts of the city.

We would like to thank our sponsors University of Engineering and Technology (UTEC), National Science Foundation (NSF), Instituto Nacional de Salud (INS), IUPAB, CONCYTEC, Oxford Nanoimaging (ONI), Zeiss, IUBMB, Beckman Coulter, ACS Publications, Chroma, Lumicks, and the Chilean Society of Biochemistry for supporting this Thematic Meeting.

Thank you all for joining our meeting, and we look forward to having a great four-day event together!

Sincerely yours,
Carlos Bustamante — University of California at Berkeley, USA
Daniel Guerra — Cayetano Heredia University, Peru
Victoria Guixé — University of Chile
Rodrigo Maillard — Georgetown University, USA
Edward Málaga-Trillo — Cayetano Heredia University, Peru
Lía Pietrasanta — University of Buenos Aires, Argentina
Piere Rodriguez Aliaga — Stanford University, USA
Julio Valdivia — Universidad de Ingeniería y Tecnología (UTEC), Peru
Christian A.M. Wilson — University of Chile

Biophysical Society Code of Conduct Anti-Harassment Policy

Adopted by BPS Council November 2015

The Biophysical Society (BPS) is committed to providing an environment that encourages the free expression and exchange of scientific ideas. As a global, professional Society, the BPS is committed to the philosophy of equal opportunity and respectful treatment for all regardless of national or ethnic origin, religion or religious belief, gender, gender identity or expression, race, color, age, marital status, sexual orientation, disabilities, veteran status, or any other reason not related to scientific merit. All BPS meetings and BPS-sponsored activities promote a working environment that is free of inappropriate behavior and harassment by or toward all attendees of Society meetings and Society-sponsored activities, including scientists, students, guests, exhibitors, staff, vendors, and other suppliers.

This global policy applies to all locations and situations where BPS business is conducted and to all BPS-sponsored activities and events. This policy does not replace the specific staff policies for situations in which only staff are involved.

Reported or suspected occurrences of harassment will be promptly and thoroughly investigated. Following an investigation, BPS will immediately take any necessary and appropriate action. BPS will not permit or condone any acts of retaliation against anyone who files harassment complaints or cooperates in the investigation of same.

Definition of Harassment
The term "harassment" includes but is not limited to epithets, unwelcome slurs, jokes, or verbal, graphic or physical conduct relating to an individual's race, color, religious creed, sex, national origin, ancestry, citizenship status, age, gender or sexual orientation that denigrate or show hostility or aversion toward an individual or group.

Sexual harassment refers to unwelcome sexual advances, requests for sexual favors, and other verbal or physical conduct of a sexual nature. Behavior and language that are welcome/ acceptable to one person may be unwelcome/offensive to another. Consequently, individuals must use discretion to ensure that their words and actions communicate respect for others. This is especially important for those in positions of authority since individuals with lower rank or status may be reluctant to express their objections or discomfort regarding unwelcome behavior. It does not refer to occasional compliments of a socially acceptable nature. It refers to behavior that is not welcome, is personally offensive, debilitates morale, and therefore, interferes with work effectiveness. The following are examples of behavior that, when unwelcome, may constitute sexual harassment: sexual flirtations, advances, or propositions; verbal comments or physical actions of a sexual nature; sexually degrading words used to describe an individual; a display of sexually suggestive objects or pictures; sexually explicit jokes; unnecessary touching.

Investigative Process
Anyone who feels harassed is encouraged to immediately inform the alleged harasser that the behavior is unwelcome. In many instances, the person is unaware that their conduct is offensive and when so advised can easily and willingly correct the conduct so that it does not reoccur. Anyone who feels harassed IS NOT required to address the person believed guilty of inappropriate treatment. If the informal discussion with the alleged harasser is unsuccessful in remedying the problem or if complainant does not feel comfortable with such an approach, he/she should contact BPS's Executive Director or the Society President, or any BPS Officer. All complaints will be promptly and thoroughly investigated.

All reports of harassment or sexual harassment will be treated seriously. However, absolute confidentiality cannot be promised nor can it be assured. BPS will conduct an investigation of any complaint of harassment or sexual harassment, which may require limited disclosure of pertinent information to certain parties, including the alleged harasser.
No retaliation will be taken against any employee, member, volunteer, exhibitor, or supplier because he or she reports a problem concerning possible acts of harassment. Employees, members, volunteers, exhibitors, or suppliers can raise concerns and make reports without fear of reprisal.

Investigative Procedure
Once a complaint of harassment or sexual harassment is received, BPS will begin a prompt and thorough investigation.

- An impartial investigative committee, consisting of the Past-President, current President, and President-Elect will be established.
- The committee will interview the complainant and review the written complaint. If no written complaint exists, one will be requested.
- The committee will speak to the alleged offender and present the complaint.
- The alleged offender will be given the opportunity to address the complaint, with sufficient time to respond to the evidence and bring his/her own evidence.
- If the facts are in dispute, the investigative team may need to interview anyone named as witnesses.
- The investigative committee may seek BPS Counsel's advice.
- Once the investigation is complete, the committee will report their findings and make recommendations to the Society Officers.

Disciplinary Actions
Individuals engaging in behavior prohibited by this policy as well as those making allegations of harassment in bad faith will be subject to disciplinary action. Such actions range from a verbal warning to ejection from the meeting or activity in question without refund of registration fees and the reporting of their behavior to their employer. Repeat offenders may be subject to further disciplinary action, such as being banned from participating in future Society meetings or Society-sponsored activities. In the event that the individual is dissatisfied with the results of the investigation, he or she may appeal to the President of the Society. Any questions regarding this policy should be directed to the BPS Executive Officer or other Society Officer.

BPS Management Responsibility
Every officer, director, supervisor, and manager is responsible for ensuring that BPS provides an environment free of harassment and inappropriate behavior and that complaints are handled promptly and effectively. The BPS Society Office and Officers must inform the Society membership and all vendors and suppliers about this policy, promptly investigate allegations of harassment, take appropriate disciplinary action, and take steps to assure retaliation is prohibited

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GENERAL INFORMATION

Registration/Information Location and Hours

On Thursday, Friday, Saturday and Sunday, registration will be located on the first floor at the entrance of the Auditorium at UTEC - Universidad de Ingeniería y Tecnología, Jr. Medrano Silva 165, Barranco 15063, Peru. Registration hours are as follows:

Thursday, July 18	8:00 – 18:00
Friday, July 19	8:00 – 18:00
Saturday, July 20	8:00 – 18:00
Sunday, July 21	8:00 – 12:30

Instructions for Presentations

(1) Presentation Facilities:

A data projector will be available in Auditorium. Speakers are required to bring their own laptops and adaptors. It is recommended to have a backup of the presentation on a USB drive in case of any unforeseen circumstances. Speakers are advised to preview their final presentations before the start of each session.

(2) Poster Session:

1) All poster sessions will be held in the cafeteria of UTEC.

2) A display board measuring 100 cm wide x 250 cm high (3.28 feet wide x 8.2 feet high) will be provided for each poster. Poster boards are numbered according to the same numbering scheme as listed in the e-book.

3) Posters should be set up the morning of, Thursday, July 18 and removed by noon Sunday, July 21. All Posters are available for viewing during all poster sessions; however, there will be formal poster presentations at the following times:

Thursday, July 18	14:30 – 15:07	Odd-numbered poster boards
Thursday, July 18	15:07 – 15:45	Even-numbered poster boards
Friday, July 19	14:45 – 15:22	Odd-numbered poster boards
Friday, July 19	15:22 – 16:00	Even-numbered poster boards

4) During the assigned poster presentation sessions, presenters are requested to remain in front of their poster boards to meet with attendees.

5) All posters left uncollected at the end of the meeting will be disposed.

Meals and Coffee Breaks

There will be a two hour Welcome Reception on Thursday evening from 18:00 – 20:00. This reception will be held at the 11th floor of UTEC.

Coffee Breaks (Thursday, Friday, Saturday and Sunday) will be served on the first floor in the Foyer.

A banquet, which includes a cultural show, will be held on Saturday, from 19:30 – 22:00 at the Damajuana Restaurant. Directions to the restaurant will be provided at on-site registration.

Advanced sign-up was required for the Welcome Reception and Banquet. Tickets are required for admittance to both functions and will be provided at onsite registration.

Smoking

Please be advised that smoking is not permitted at UTEC - Universidad de Ingeniería y Tecnología.

Name Badges

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

Internet

Wifi will be provided at the venue. Attendees will receive account number and password at registration.

Contact

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from July 18-21 during registration hours.

In case of emergency, you may contact the following:

Carla Chavez
Cell: +51 980 968 157
Email: cchavez@utec.edu.pe

Julio Valdivia
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Revisiting the Central Dogma of Molecular Biology at the Single-Molecule Level

Lima, Peru
July 18-21, 2019

PROGRAM

Thursday, July 18, 2019

8:00 - 18:00	**Registration/Information**	**Auditorium Entrance, 1st Floor**
9:00 - 9:30	Piere Rodriguez-Aliaga, Stanford University, USA Carlos Heeren, UTEC, Peru Carlos Bustamante, University of California, Berkeley, USA ***Opening Remarks***	
Session I	**Molecular Motors** Shixin Liu, The Rockefeller University, USA, Chair	
9:30 - 10:15	Carlos Bustamante, University of California, Berkeley, USA ***Co-temporal Force and Fluorescence Measurements Reveal a Ribosomal Gear-shift Mechanism of Translation Regulation by mRNA Secondary Structures***	
10:15 - 10:30	Shixin Liu, The Rockefeller University, USA * ***Functional Plasticity in Ring-shaped Molecular Motors***	
10:30 - 11:00	**Coffee Break**	
11:00 - 11:15	Stefan Niekamp, University of California, San Francisco, USA* ***Tracking Dynein Stepping Along Microtubules with Nanometer Accuracy Using Three-color Imaging***	
11:15 - 11:30	Juan P. Castillo, University of California, Berkeley, USA * ***Challenging a Viral DNA Packaging Motor with Modified Substrates***	
11:30 - 11:45	Arne Gennerich, Albert Einstein College of Medicine, USA * ***Molecular Mechanism of Cytoplasmic Dynein Tension Sensing***	
11:45 - 12:15	**Flash Talks**	
12:15 - 14:30	**Lunch (**on own)	
14:30 - 15:45	**Poster Session I**	**Cafeteria, 1st Floor**
15:45 - 16:15	**Coffee Break**	**Foyer, 1st Floor**
Session II	**Nanoscale Imaging of Cellular Processes I** Lía Pietrasanta, University of Buenos Aires, Argentina, Chair	
16:15 - 16:45	Jie Xiao, Johns Hopkins University, USA ***Gene Regulation and Chromosome Conformation***	
16:45 - 17:15	Fernando Stefani, University of Buenos Aires, Argentina ***Far-field Fluorescence Nanoscopy with Sub-10 nm Resolution***	

17:15 - 18:00	Simon Scheuring, Weill Cornell Medicine, USA ***High-speed Atomic Force Microscopy: Structural Dynamics of Single Unlabeled Proteins***	
18:00 - 20:00	**Welcome Reception**	**11th Floor**

Friday, July 19, 2019

8:30 – 18:00	**Registration/Information**	**Auditorium Entrance, 1st Floor**
Session III	**Nanoscale Imaging of Cellular Processes II** Edward Málaga-Trillo, Cayetano Heredia University, Peru, Chair	
9:00 - 9:45	Tomas Kirchhausen, Harvard University, USA ***Imaging Subcellular Dynamics from Molecules to Multicellular Organisms***	
9:45 - 10:15	Melike Lakadamyali, University of Pennsylvania, USA ***Superresolution Imaging of Chromatin Organization***	
10:15 - 10:30	María Benítez-Jones, New York University, USA * ***Single-Molecule Tracking of DNA Repair Factory Dynamics in Live Cells***	
10:30 - 10:45	Elena Kudryashova, The Ohio State University, USA * ***Toxicity Amplification Mechanism of Actin Crosslinking Toxin Revealed by Single-Molecule Imaging***	
10:45 - 11:15	**Coffee Break**	**Foyer, 1st Floor**
Session IV	**Biopolymers** Christian A.M. Wilson, University of Chile, Chile, Chair	
11:15 - 11:45	Márcio Santos Rocha, Universidade Federal de Viçosa, Brazil ***DNA Interactions with Drugs and Small Molecules Investigated by Single Molecule Force Spectroscopy***	
11:45 - 12:00	J. Andres Rivas Pardo, Universidad Mayor, Chile * ***The Power of the Force: Mechano-physiology of the Giant Titin***	
12:00 - 12:30	**Flash Talks**	
12:30 - 14:45	**Lunch (**on own)	
14:45 - 16:00	**Poster Session II**	**Cafeteria, 1st Floor**
16:00 - 16:30	**Coffee Break**	**Location**
Session V	**Protein Synthesis** Christian Kaiser, Johns Hopkins University, USA, Chair	
16:30 - 16:45	Tatsuya Morisaki, Colorado State University, USA * ***Visualization and Quantification of Translation Dynamics in Living Cells at Single Molecule Resolution***	

16:45 - 17:00	Christian Kaiser, Johns Hopkins University, USA * ***The Ribosome Cooperates with Chaperones to Guide Multidomain Protein Folding***
17:00 - 17:15	Scott Blanchard, Weill Cornell Medicine, USA * ***Endogenous Ribosomal RNA Sequence Variation Can Modulate Stress Response Gene Expression and Phenotype***
17:15 - 18:00	Ruben Gonzalez, Columbia University, USA ***Ribosomes in Action: The Role of Dynamics in the Mechanism and Regulation of Translation***

Saturday, July 20, 2019

8:30 – 18:00	**Registration/Information**	**Auditorium Entrance, 1st Floor**
Session VI	**Protein Folding and Allostery** Piere Rodriguez-Aliaga, Stanford University, USA, Chair	
9:00 - 9:45	Matthias Rief, Technische Universität München, Germany ***Single Molecule Mechanics of Protein Folding and Binding***	
9:45 - 10:15	Rodrigo Maillard, Georgetown University, USA ***Activation of a Protein Kinase via Asymmetric Allosteric Coupling of Structurally Conserved Signaling Modules***	
10:15 - 10:45	Mauricio Baez, University of Chile, Chile ***Folding Free Energy Barriers of Topologically Knotted Proteins***	
10:45 - 11:15	**Coffee Break**	**Foyer, 1st Floor**
Session VII	**Protein Processing Machines** Rodrigo Maillard, Georgetown University, USA, Chair	
11:15 - 11:45	Christian A.M. Wilson, University of Chile, Chile ***Studying the Mechanical Properties of Protein Translocation by Optical Tweezers and Nanorheology***	
11:45 - 12:15	Piere Rodriguez-Aliaga, Stanford University, USA ***A Finely Tuned Molecular Motor: Mechanochemistry and Power Efficiency in the AAA+ Protease Machine ClpXP***	
12:15 - 12:30	Erik Jonsson, University of California, Berkeley, USA * ***Direct Observation of Substrate Translocation and Conformational Dynamics in the 26s Proteasome***	
12:30 - 12:45	Gabriel Lander, The Scripps Research Institute, USA * ***Mechanisms of Mitochondrial Machines of Mass Destruction***	
12:45 - 14:45	**Lunch (**on own)	
14:45 - 16:00	**Free Time**	
16:00 - 16:30	**Coffee Break**	**Foyer, 1st Floor**

Session VIII	**Nucleic Acids Machines** Eli Rothenberg, New York University, USA, Chair
16:30 - 16:45	Wei Ting (Chelsea) Lee, New York University, USA * ***Superresolution Imaging of Replication-associated G4 DNA in Human Cells***
16:45 - 17:00	Nikos Hatzakis, University of Copenhagen, Denmark * ***Direct Observation of CRISPR-Cas12 as Conformational Sampling Reveals How Conformational Activation Promotes Catalysis and Resetting of the Endonuclease Activity***
17:00 - 17:15	Ian Nova, University of Washington, USA * ***Detecting Single Steps During Transcription and a Half-translocated Pause Complex of E. coli RNA Polymerase Using Nanopore Tweezers***
17:15 - 18:00	Michelle Wang, Cornell University, USA ***Molecular Highways – Torsional Consequences of DNA Motor Proteins***
19:30	Banquet Damajuana Restaurant

Sunday, July 21, 2019

8:30 – 12:30	**Registration/Information** **Auditorium Entrance, 1st Floor**
Session IX	**Extracting Kinetics from Molecular Processes** Mauricio Baez, University of Chile, Chile, Chair
9:00 – 9:45	Gijs Wuite, Vrije Universiteit Amsterdam, the Netherlands – Jubilee Lecturer ***Single Molecule Manipulation and Imaging of Complex DNA-Protein Transactions***
9:45 – 10:15	Olga Dudko, University of California, San Diego, USA ***Mechanical Fingerprints of Biomolecules, Decoded***
10:15 - 10:30	Sara Tafoya, LUMICKS, USA * ***Using a System's Equilibrium Behavior to Reduce Its Energy Dissipation in Non-Equilibrium Processes***
10:30 - 11:00	**Coffee Break** **Foyer, 1st Floor**
Session X	**Nanoscale Imaging of Cellular Processes III** Tomas Kirchhausen, Harvard University, USA, Chair
11:00 - 11:30	Eli Rothenberg, New York University, USA ***A Single-Molecule View of Mammalian DNA Double-strand Break Repair***
11:30 - 12:15	Xiaowei Zhuang, Harvard University, USA – Jubilee Lecturer ***Imaging at the Genomic-scale: From 3D Organization of the Genomic DNA to Cell Atlas of Complex Tissues***
12:15 - 12:30	Carlos Bustamante, University of California, Berkeley, USA **Closing Remarks and *Biophysical Journal* Poster Awards**

**Short talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

CO-TEMPORAL FORCE AND FLUORESCENCE MEASUREMENTS REVEAL A RIBOSOMAL GEAR-SHIFT MECHANISM OF TRANSLATION REGULATION BY MRNA SECONDARY STRUCTURES

Carlos Bustamante[1]; Varsha Desai[1]; Harry Noller[2]; Laura Lancaster[2];
[1]University of California, Berkeley, Berkeley, CA, USA
[2]University of California, Santa Cruz, Santa Cruz, CA, USA

Ribosome translocation on mRNAs is often interrupted by secondary structures that represent mechanical barriers and that play a central role in translation regulation. Here, we investigate how ribosomes couple their internal conformational changes with the activity of translocation factor EF-G to unwind mRNA secondary structures using high-resolution optical tweezers with single-molecule fluorescence capability. We find that hairpin opening occurs during EF-G catalyzed translocation and is driven by the forward rotation of the small subunit head. Moreover, we modulate the magnitude of the hairpin barrier by force and surprisingly find that ribosomes respond to strong barriers by shifting their operation to an alternative 7-fold slower kinetic pathway prior to translocation. This shift into a slow gear results from an allosteric switch in the ribosome that may allow it to exploit thermal fluctuations to overcome mechanical barriers. Finally, we observe that ribosomes occasionally open the hairpin in two successive sub-codon steps, revealing a previously unobserved translocation intermediate.

FUNCTIONAL PLASTICITY IN RING-SHAPED MOLECULAR MOTORS

Shixin Liu[1];
[1]Rockefeller University, Laboratory of Nanoscale Biophysics and Biochemistry, New York, NY, USA

Ring ATPases represent a large and diverse group of molecular machines that couple their nucleotide hydrolysis activity to a mechanical task. I will discuss our single-molecule work on the eukaryotic replicative helicase CMG. Using correlative single-molecule fluorescence and force microscopy, we found that when uncoupled from a DNA polymerase, CMG opens a single-stranded (ss) DNA gate to traverse a forked junction and reside on double-stranded (ds) DNA. Surprisingly, CMG undergoes rapid diffusion on dsDNA and can transition back onto ssDNA for continued fork progression. These results reveal unexpected plasticity in the CMG operation, enabling the ring motor to adapt to changing conditions and flexibly transition between distinct functional modes.

TRACKING DYNEIN STEPPING ALONG MICROTUBULES WITH NANOMETER ACCURACY USING THREE-COLOR IMAGING

Stefan Niekamp[1]; Nico Stuurman[1,2]; Ronald D Vale[1,2];
[1]University of California, San Francisco, Department of Cellular and Molecular Pharmacology, San Francisco, CA, USA
[2]Howard Hughes Medical Institute, San Francisco, CA, USA

Cytoplasmic dynein is a minus-end directed microtubule-based motor that belongs to the AAA family of proteins and is responsible for the transportation of many cargos in cells and plays a key role in mitosis. Unlike the well-known kinesin or myosin motor domains, which are globular and compact, the dynein motor domain contains a small microtubule-binding domain (MTBD) that is spatially separated by a ~135 A long coiled-coil from its large catalytic AAA ring. From previous work, in which the AAA rings of a dimeric dynein were labeled with fluorescent probes, it is known that dynein moves through uncoordinated stepping of the AAA ring domains. However, recent structural studies have shown that the relative orientation of the AAA ring and MTBD is quite flexible. Thus, to fully understand how dynein is walking, it is inevitable to follow the MTBD. Moreover, how different domains of dynein move relative to each other during every step cycle is unknown. Addressing these questions requires high-resolution, multicolor imaging. We therefore developed new methods for three-color image registration and distance measurements that enable us to determine distances between three colors with sub-nanometer accuracy. Moreover, we designed small fluorescent probes that allow us to track dynein about fifteen times longer than with common fluorescent dyes. With these new tools at hand we are beginning to see new patterns in dynein motility emerging. Together, we have developed new methods for three-color imaging with nanometer accuracy that provide insights into the mechanism of dynein stepping.

CHALLENGING A VIRAL DNA PACKAGING MOTOR WITH MODIFIED SUBSTRATES

Juan P. Castillo[1]; Alexander Tong[1]; Sara Tafoya[1]; Paul Jardine[2]; Carlos Bustamante[1];
[1]University of California Berkeley, Berkeley, CA, USA
[2]University of Minnesota, Minneapolis, MN, USA

The DNA packaging motor of the bacteriophage phi29 is a powerful molecular machine that couples the free energy of ATP hydrolysis to DNA translocation. This motor is composed by a pentameric ring ATPase that follows a dwell-burst scheme. In each turn of the mechanochemical cycle, ADP is exchanged for ATP during the dwell time, followed by a translocation burst that is 10 base pairs (bp) in size, which is composed of four consecutive sub-steps of 2.5 bp. Several models can explain what determines the burst size of the motor: the B-form DNA has 10.5 bp per turn of the double helix, suggesting that the structure of the substrate is the determining factor; however, the non-integer nature of the sub-steps during the burst suggests that is the fixed conformational change of the ATPase what sets the burst size. Yet another possibility is that the DNA packaging motor switches the local conformation of the DNA substrate from B-form to A-form during packaging. To test the above hypotheses we challenged the phi29 DNA packaging motor with different substrates bearing the A-form of nucleic acids, using high resolution optical tweezers assay. Our results show indeed that the motor is able to adapt its operation to translocate these different substrates by reducing the size of the burst such that it follows the new helical pitch. We propose a mechanistic model where the motor establishes a critical contact with the substrate at every turn of the double helix during subsequent dwells. Such event is a strong interaction that interrupts the last power stroke during the burst when the motor packages A-form substrates, and it serves as a resetting point for the ring ATPase to complete the turnover cycle.

MOLECULAR MECHANISM OF CYTOPLASMIC DYNEIN TENSION SENSING

Arne Gennerich; Lu Rao[1]; Florian Berger[2]; Matthew P Nicholas[1];
[1]Albert Einstein College of Medicine, Anatomy and Structural Biology, Bronx, NY, USA
[2]Rockefeller University, New York, NY, USA

Cytoplasmic dynein is the most complex cytoskeletal motor protein and is responsible for a vast array of biological functions. Essential to dynein's function is its capacity to respond anisotropically to tension, so that its microtubule-binding domains bind microtubules more strongly when under backward load than forward load. The structural mechanisms by which dynein senses directional tension, however, are unknown. Using a combination of optical tweezers, mutagenesis, and chemical cross-linking, we show that three structural elements protruding from the motor domain—the linker, buttress, and stalk—together regulate directional tension-sensing. We demonstrate that dynein's anisotropic response to directional tension is mediated by sliding of the coiled-coils of the stalk, and that coordinated conformational changes of dynein's linker and buttress control this process. We also demonstrate that the stalk coiled-coils assume a novel registry during dynein's stepping cycle. We propose a revised model of dynein's mechanochemical cycle which accounts for our findings.

GENE REGULATION AND CHROMOSOME CONFORMATION

Jie Xiao
Johns Hopkins University, Baltimore, Maryland, USA

No Abstract

FAR-FIELD FLUORESCENCE NANOSCOPY WITH SUB-10 NM RESOLUTION

Fernando D. Stefani[1,2];
[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Centro de Investigaciones en Bionanociencias (CIBION), Buenos Aires, Argentina
[2]Universidad de Buenos Aires, Departamento de Física, Facultad de Ciencias Exactas y Naturales, Buenos Aires , Argentina

Far-field fluorescence nanoscopy is a family of methods that has revolutionized biological imaging by providing sub-diffraction spatial resolution while keeping the low invasiveness of visible light interrogation. Making use of on-off switching of molecular emission, these methods break any fundamental limitation to the achievable spatial resolution. In practice, however, the resolution is limited by the total number of excitation-emission or on-off cycles that a molecule can perform or withstand. Under biological conditions, the lateral resolution is typically limited to about 20 – 50 nm. Axial resolution is typically worse, in the range of 60 – 120 nm. Imaging with this level of detail has constituted a significant advance in the field, enabling the discovery and characterization of sub-cellular structures and pathways in their natural environment. Still, resolving supramolecular protein structures, as well as protein-protein interactions in full detail requires another push to the resolution to get into sub-10 nm regime, which is the typical size of structural proteins and complexes. Here, three recent advances from our lab that aim to achieve biological imaging with sub-10 nm resolution will be presented. First, a new and simpler implementation of MINFLUX will be described. Second, a successful combination of STED-FRET will be shown, which is able to super-resolve biomolecular direct interactions. Finally, a TIRF nanoscopy based on DNA-PAINT that can deliver sub-10 nm in three dimensions, and that can be implemented on any wide-field single molecule fluorescence microscope, will be presented.

HIGH-SPEED ATOMIC FORCE MICROSCOPY: STRUCTURAL DYNAMICS OF SINGLE UNLABELED PROTEINS

Simon Scheuring[1,2];
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The advent of high-speed atomic force microscopy (HS-AFM(1)) has opened a novel research field for the dynamic analysis of single bio-molecules: Molecular motor dynamics (2,3), membrane protein diffusion (4), assembly (5) and conformational changes (6) could be directly visualized. Further developments for buffer exchange (7) and temperature control (8) during HS-AFM operation provide breakthroughs towards the performance of dynamic structural biochemistry using HS-AFM. I will exemplify the power of HS-AFM for the quantitative analysis of function-related structural dynamics on the membrane deformation complex ESCRT-III (5), a glutamate transporter homologue (6), and ligand-gated ion channels (9,10). Finally, I will introduce high-speed AFM line scanning (HS-AFM-LS) and high-speed AFM height spectroscopy (HS-AFM-HS) that reache millisecond and microsecond temporal resolution, respectively, of single molecule dynamics (11). References:1) Ando, Chem Rev 2014, 114(6):3120-882) Kodera, Nature 2010, 468(7320):72-63) Uchihashi, Science 2011, 333(6043):755-84) Casuso, Nat Nanotechnol 2012, 7(8):525-95) Chiaruttini, Cell 2015, 163(4):866-79.6) Ruan, PNAS 2017, 114(7):1584-15887) Miyagi, Nat Nanotechnol 2016, 11(9):783-908) Takahashi, Small 2016, 12(44):6106-61139) Ruan, 2018 115(41):10333-1033810) Marchesi, Nature Communications, 2018, 9(1):397811) Heath and Scheuring, Nature Communications, 2018, 9(1):4983

IMAGING SUBCELLULAR DYNAMICS FROM MOLECULES TO MULTICELLULAR ORGANISMS

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Frontier optical-imaging modalities exemplified by the lattice light-sheet microscope invented by Eric Betzig sets new visualization standards for analyzing and understanding sub-cellular processes in the complex and dynamic three-dimensional environment of living-cells in isolation and within tissues of an organism. By using ultra-thin sheets of light to rapidly illuminate biological samples with extremely low photon doses, 3D experiments previously limited to seconds or minutes by photo-bleaching or by photo- toxicity, can now be done at diffraction limited resolution and high-temporal precision with unprecedented duration of minutes or hours. We believe this ability to image with minimal perturbations is ideally suited to support hypothesis-generating research geared towards new discoveries. The talk will illustrate our use of lattice light-sheet microscopy to 'see' in three dimensions processes that mediate and regulate the biogenesis of organelles in living cells maintained in tissue culture conditions and will also describe our most recent efforts using lattice light sheet microscopy with adaptive optics to investigate with subcellular precision process in cells within tissues of a living zebrafish embryo.

SUPER-RESOLUTION IMAGING OF CHROMATIN ORGANIZATION

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Nucleosomes help structure chromosomes by compacting DNA into fibers. Chromatin organization plays an important role for regulating gene expression; however, due to the nanometer length scales involved, it has been very difficult to visualize chromatin fibers in vivo. Using super-resolution microscopy, quantitative analysis and simulations, we have been gaining new insights into chromatin organization at nanometer length scales in intact nuclei. For example, we found that nucleosomes assemble into heterogeneous groups of varying sizes, which we named "clutches," in analogy with "egg clutches". Clutch organization is highly cell specific and i will give various examples of this specificity. Overall, our results reveal how the chromatin fiber is formed at nanoscale level and link chromatin fiber architecture to cell state.

SINGLE-MOLECULE TRACKING OF DNA REPAIR FACTORY DYNAMICS IN LIVE CELLS

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DNA double-stranded breaks (DSBs) are regarded as the most cytotoxic DNA lesions and failure to repair DSBs can lead to genetic disorders, aging, and cancer. In mammalian cells, DSBs are repaired via two vital pathways: non-homologous end joining (NHEJ) and homologous recombination (HR). Formation of DSBs initiates an elaborate DNA Damage Response (DDR) signaling cascade, which changes the chromatin environment around DSBs and propagates global cellular signaling events. Central to DDR signaling is the recruitment of DSB-related chromatin modulators and repair factors, such as p-53 binding protein-1 (53BP1). Together, these form a distinct macromolecular assembly known as the repair foci, within which the repair process occurs. Although the proper progression and regulation of the DSB repair process is central to cellular viability, little is known about the recruitment and exclusion of DNA repair factors to the repair foci. How do these dynamics correlate with the DDR and the choice between repair pathways? To address this knowledge gap, we have developed single-molecule imaging assays that enable us to track the dynamics of individual repair proteins at DSB repair foci in living cells. Using this approach, we have monitored the recruitment and retention of key repair factors to the liquid condensate structure of 53BP1 foci for perturbed and unperturbed states inside the nucleus of a living cell. Our study revealed that 53BP1 forms biomolecular liquid condensates at DSB foci via distinctive phase separation dynamics and observed that repair factors exhibit novel modes of diffusion at the surface and within the 53BP1 condensates for unperturbed and perturbed states. We conclude that the liquid-like dynamic properties of these 53BP1 condensates are essential for the effective repair of DSBs.

TOXICITY AMPLIFICATION MECHANISM OF ACTIN CROSSLINKING TOXIN REVEALED BY SINGLE-MOLECULE IMAGING

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Efficiency of actin-targeting toxins is hampered by an overwhelming abundance of the target: cytoskeletal actin is among the most abundant proteins in eukaryotic cells. Consequently, toxins employ sophisticated mechanisms of toxicity amplification. One such mechanism is demonstrated by the actin crosslinking domain (ACD)-containing toxins of Vibrio cholerae, Vibrio vulnificus, and Aeromonas hydrophila, which catalyze the formation of covalently crosslinked actin oligomers with actin subunits connected by side-chain amide bonds. Since ACD-produced oligomers are non-polymerizable, crosslinking bulk amounts of actin eventually leads to failure of its functions and cell rounding; however, this mechanism requires high doses of toxin to be effective. Conversely, our data imply that ACD-conferred cytotoxic effects are evident when only 2-6% actin is crosslinked, suggesting that low doses of actin oligomers are highly toxic. We discovered that ACD toxicity is amplified via a “gain-of-function” mechanism whereby ACD-crosslinked actin oligomers act as potent secondary toxins that directly inhibit proteins involved in nucleation, elongation, severing, and branching of actin filaments. Affected actin-regulatory proteins possess multiple G-actin-binding domains either organized in tandem in a single polypeptide or through oligomerization of several polypeptides and, therefore, serve as a multivalent platform for high-affinity interaction with actin oligomers. Single-molecule TIRFM and bulk actin polymerization assays revealed that actin oligomers bind with abnormally high affinity and potently inhibit formins, Ena/VASP, Spire, and NPFs of the Arp2/3 complex. In live cells, single-molecule speckle (SiMS) microscopy corroborated these findings and revealed potent inhibition and halted dynamics of these proteins in lamellipodia leading to massive disarray of the cytoskeleton upon low-dose ACD treatment. This study redefines ACD as an indirect, universal inhibitor of tandem-organized G-actin-binding proteins that overcomes the abundancy of actin by redirecting the toxicity cascade towards less abundant targets whose inhibition by actin oligomers leads to disorganization of actin cytoskeleton disabling normal cellular functions (published in Science-2015 and Current Biology-2018).

DNA INTERACTIONS WITH DRUGS AND SMALL MOLECULES INVESTIGATED BY SINGLE MOLECULE FORCE SPECTROSCOPY

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In this talk we will present some recent studies from our group concerning the DNA interactions with drugs and other small relevant molecules. We will discuss how single molecule force spectroscopy measurements can be useful to determine the possible binding modes, the changes induced on the DNA structure and the physical chemistry of the DNA-ligand interactions. In particular, we present a recently developed quenched-disorder statistical model which allows one to extract the relevant physicochemical (binding) parameters of the interactions from pure mechanical (force-extension) measurements performed with the DNA-ligands complexes. Such a model in principle works well for any type of interaction that occurs between small ligand molecules and DNA, from intercalation to covalent binding, allowing a robust characterization of the interactions with a single experimental technique.

THE POWER OF THE FORCE: MECHANO-PHYSIOLOGY OF THE GIANT TITIN

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Single-molecule methods using recombinant proteins have generated transformative hypotheses on how mechanical forces are generated and sensed in biological tissues. However, testing these mechanical hypotheses on native molecules in their natural environment remains inaccessible to conventional genetics, biophysics and molecular biology tools. To overcome these limitations, here we demonstrate a genetically engineered knock-in mouse model carrying a HaloTag-TEV insertion in the protein titin, the main determinant of myocyte stiffness. Using our system, we have specifically severed the titin filament by digestion with TEV protease, and found that the response of muscle fibers to length changes requires mechanical transduction through titin's intact polypeptide chain. HaloTag-based covalent tethering has enabled directed examination of the dynamics of native titin under physiological forces using recently developed magnetic tweezers. At physiological pulling forces lower than 10 pN, titin domains are readily recruited to the unfolded state, and produce 41.5 zJ mechanical work during refolding. Our results support an active role of titin in muscle contraction in coordination with actomyosin motors. Insertion of the HaloTag-TEV cassette in proteins with mechanical roles opens new grounds to explore the molecular basis of cellular force generation, mechanosensing and mechanotransduction.

VISUALIZATION AND QUANTIFICATION OF TRANSLATION DYNAMICS IN LIVING CELLS AT SINGLE MOLECULE RESOLUTION

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While transcription processes have been imaged with single gene resolution in living cells for the past decade, it has been a challenge to directly visualize translation processes from single mRNAs in living cells. We have developed a technique to image real-time translation at single mRNA resolution in living cells utilizing multi-epitope tags and antibody-based fluorescent probes. First, by utilizing this novel technique, we have quantified the mobility of translation sites, translation initiation rates, elongation rates, and polysome occupancies at single mRNA resolution for the first time. Second, by extending this technique to a multiplex format, we have shown that the vast majority of polysomes act independently of one another, but a small fraction of polysomes formed complexes in which two distinct mRNAs can be translated simultaneously. Third, by employing this technique, we have investigated another long-standing question in gene expression regulation - translation shutoff during stress response. While it is known that global mRNAs shut off translation and get incorporated into the granules such as stress granules (SGs) and P-bodies during stress, it has not been clear when and where mRNAs shut off translation, and how mRNAs interact with these granules due to a lack of experimental techniques with sufficient spatiotemporal resolution. To address this, we have quantified the dynamic interactions between individual mRNAs, SGs, and P-bodies, along with the translation activity from each mRNA. Interestingly, we found that translating mRNAs only interact with these granules dynamically while non-translating mRNAs can form stable associations with these granules. Also, contrary to the notion of a fluid liquid phase within SGs, we discovered a subset of mRNAs inside granules that were apparently rigidly bound such that there was little to no intragranular mobility.

THE RIBOSOME COOPERATES WITH CHAPERONES TO GUIDE MULTIDOMAIN PROTEIN FOLDING

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Co-translational folding likely simplifies the conformational search problem for large proteins, but the events leading to correctly folded, functional structures remain poorly characterized. Domain-wise folding and help from chaperones are particularly important for multi-domain proteins, which constitute a large fraction of all proteomes. Using optical tweezers, we have dissected the complete folding pathway of elongation factor G, a multi-domain protein that requires chaperones for folding. Early during synthesis, interactions with the ribosome reduce inter-domain misfolding and, depending on nascent chain length, can either reduce or increase folding rates of the N-terminal G-domain. Successful completion of G-domain folding is crucial because it is a prerequisite for folding of the next domain. Unexpectedly, co-translational folding does not proceed unidirectionally: unfolded polypeptide emerging from the ribosome can denature an already folded domain. The chaperone trigger factor protects against denaturation, thus helping multi-domain proteins overcome inherent challenges during co-translational folding. In contrast, neither the ribosome nor the second major nascent chain-binding chaperone, the Hsp70 protein DnaK, prevent denaturation. Interestingly, we find that the energetic coupling among the three C-terminal domains prevents domain-wise folding from continuing. Instead, folding of domains III, IV and V can occur only synthesis is complete and the protein is released from the ribosome. DnaK binds to longer nascent chains and keeps them in a state competent for efficient post-translational folding. Our single-molecule experiments define the folding pathway of a complex multi-domain protein and demonstrate how the ribosome and two differentially acting chaperones together modulate nascent chain folding.

ENDOGENOUS RIBOSOMAL RNA SEQUENCE VARIATION CAN MODULATE STRESS RESPONSE GENE EXPRESSION AND PHENOTYPE

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Prevailing dogma holds that ribosomes are uniform in composition and function. Here, we show that nutrient limitation-induced stress in E. coli changes the relative expression of ribosomal DNA operons to alter the ribosomal RNA (rRNA) composition within the actively translating ribosome pool. The most upregulated operon encodes the unique 16S rRNA gene, rrsH, distinguished by conserved sequence variation within the small ribosomal subunit. rrsH-bearing ribosomes affect the transcription and translation of functionally coherent gene sets and alter the levels of the RpoS sigma factor, the master regulator of the general stress response. These impacts are associated with phenotypic changes in antibiotic sensitivity, biofilm formation, and cell motility, and are regulated by stress response proteins, RelA and RelE, as well as the metabolic enzyme and virulence-associated protein, AdhE. These findings establish that endogenously encoded, naturally occurring rRNA sequence variation in the ribosome can modulate ribosome function, central aspects of gene expression regulation, and cellular physiology.

RIBOSOMES IN ACTION: THE ROLE OF DYNAMICS IN THE MECHANISM AND REGULATION OF TRANSLATION

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Over the past two decades, stunning breakthroughs in the field of structural biology have continued to produce groundbreaking high-resolution structures of the ribosome and the rest of the cellular translation machinery (TM) that translates messenger RNAs (mRNAs) into proteins, an essential step in the central dogma of molecular biology. Comparative analyses of these static structures reveal the remarkable conformational flexibility of the TM and hint at the significant structural rearrangements that evidently accompany its functional cycle. Unfortunately, the experimental observation and characterization of these conformational dynamics is significantly impeded by the size and complexity of the TM, severely limiting our understanding of the contributions that dynamics make to its function. In my talk, I will present recent and ongoing work form my research group in which we have combined single-molecule fluorescence imaging with complementary structural and biochemical approaches to overcome these challenges and elucidate the precise roles that the conformational dynamics of the TM play in driving and controlling protein synthesis. In particular, I will focus on how thermally driven fluctuations of the ribosome and other essential components of the TM contribute to the mechanism and regulation of translation. Because the ribosome is the target of over half of all currently prescribed antibiotics and because of the growing list of human diseases to which dysregulation of translation has been causally linked, our findings hold great promise for informing the development of next-generation antibiotics and small-molecule therapeutic agents that function by modulating the conformational dynamics of the TM. To this end, I will close my talk by discussing what we envision lies ahead as single-molecule fluorescence imaging continues to evolve and expand such that it can address increasingly complex mechanistic and regulatory aspects of this fundamental biological process.

SINGLE MOLECULE MECHANICS OF PROTEIN FOLDING AND BINDING

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Proteins are amazing molecular machines that can fold into a complex three dimensional structure. Even though powerful structural methods have allowed us taking still photographs of protein structures in atomic detail, the knowledge about the folding pathways and dynamics as well as material properties of those structures is rather limited. Over the past years, our group has developed single mechanical methods to study the dynamics and mechanics of protein structures. In my talk I will discuss how these methods can be used to investigate and control the conformational mechanics of individual proteins. Examples include protein folding as well as protein-protein interactions and enzyme mechanics.

ACTIVATION OF A PROTEIN KINASE VIA ASYMMETRIC ALLOSTERIC COUPLING OF STRUCTURALLY CONSERVED SIGNALING MODULES

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Cyclic nucleotide binding (CNB) domains are universally conserved signaling modules that regulate the activities of diverse protein functions. Yet, the structural and dynamic features that enable the cyclic nucleotide binding signal to allosterically regulate other functional domains remain unknown. We use optical tweezers and molecular dynamics to monitor in real time the pathways of signals transduced by cAMP binding in protein kinase A (PKA). Despite being structurally conserved, we find that the response of the folding energy landscape to cAMP is domain-specific, resulting in unique but mutually coordinated regulatory tasks: one CNB domain initiates cAMP binding and cooperativity, while the other triggers inter-domain interactions that lock the active conformation. Moreover, we identify a new cAMP-responsive switch, whose stability and conformation depends on cAMP occupancy. We show that this dynamic switch is a signaling hub, a previously unidentified role that amplifies the cAMP binding signal during the allosteric activation of PKA.

FOLDING FREE ENERGY BARRIERS OF TOPOLOGICALLY KNOTTED PROTEINS

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Knots in proteins are present in about 1% of all structures known, begging the question as to the importance of the knot in their particular folding mechanism and its possible functional significance. Yet, there is a lack of elementary knowledge about spontaneous knot formation in a polypeptide chain—an event that can potentially impair its folding—and about the effect of a knot on the stability of the folded state and on the kinetic barrier that separates it from the unfolded state. Moreover, at molecular level, the folding mechanism of knotted proteins remain controversial as the formation of knotted proteins has been explored by in silico simulations using coarse-grained force fields that do not consider specific contacts proposed to be key for knotting. To answer these questions, we have used optical tweezers to mechanically manipulate and untie the knotted protein from different pulling directions. This approach allowed us to quantitative characterize the folding mechanism of natural and artificial knotted proteins, and extract the free energy to form a knot by chance in the denatured state and the free energy barrier associated with the formation of a knot during the folding of the protein. We found that threading a polypeptide chain increase the folding barrier in several kcal/mol. This increment is comparable in magnitude with the energy cost to form a knot in the denatured state supporting that most of the entropic cost to form a knot is not compensated during the folding reaction. These results support that a knot is an unavoidable topological constrain, whose energy cost is transferred to the folding barrier.

STUDYING THE MECHANICAL PROPERTIES OF PROTEIN TRANSLOCATION BY OPTICAL TWEEZERS AND NANORHEOLOGY

Christian A.M. Wilson[1]; Maira Rivera[1]; Francesca Burgos-Bravo[1]; Diego Quiroga-Roger[1]; Nathalie Casanova-Morales[1]; Mauricio Baez[1]; María Paz Ramírez[1]; Hilda M. Alfaro-Valdés[1]; [1]University of Chile, Biochemistry and Molecular Biology department, Santiago, Chile

Approximately one third of the proteins produced in mammalian cells fold and assemble in the Endoplasmic Reticulum (ER). In ER, proteins are translocated to the lumen where they acquire their tertiary/quaternary structure. The folding and transport of many proteins is facilitated by the action of chaperones. Immunoglobulin heavy-chain-binding protein (BiP) is a 75-kDa Hsp70 monomeric ATPase motor-chaperone that plays broad and crucial roles maintaining proteostasis inside the cell as protein translocation. Its malfunction has been related with the appearance of many and important health problems. It is unknown what kind of molecular motor BiP works like, since the mechanochemical mechanism that BiP utilizes to perform its work during posttranslational translocation across the ER is not fully understood. One novel approach to study both structural and catalytic properties of BiP considers that the viscoelastic regime behavior of the enzymes and their mechanical properties are correlated with catalysis and ligand binding. Structurally, BiP is formed by two domains, and to establish a correlation between BiP structure and catalysis and how its conformational and viscoelastic changes are coupled to ligand binding, catalysis, and allosterism, optical tweezers and nano-rheology techniques are used. We recently developed a method to measure how BiP binds to its substrate using optical tweezers and we found that BiP bind to the unfolded state with higher affinity in the ADP state. Without single-molecule approaches, it is very difficult to learn about how BiP binds to its substrate, since the substrate of BiP is an unfolded peptide, and if we unfold the substrate, we may also unfold BiP. By nanorheology we observed that the folded state of the protein behaves like a viscoelastic material, getting softer when it binds nucleotides but stiffer when it binds peptide substrate. The explanation for this mechanical behavior is related to the ATPase cycle of BiP.

A FINELY TUNED MOLECULAR MOTOR: MECHANOCHEMISTRY AND POWER EFFICIENCY IN THE AAA+ PROTEASE MACHINE CLPXP

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ATP-dependent proteases of the AAA+ family, such as ClpX from Escherichia coli and the eukaryotic 26S proteasome, play a central role in protein degradation and protein homeostasis. Given its extensive biochemical and structural characterization, ClpX is a paradigm for the study of the operating principles of eukaryotic and prokaryotic protease machines of the AAA+ family. ClpX is a hexameric ring-shaped motor that transforms the energy from ATP into mechanical force to unfold proteins and translocate the unfolded polypeptide through its narrow axial pore into an associated protease (ClpP). Previously we showed that ClpX translocates its substrate in cycles composed of a dwell phase, during which the substrate does not move, and a burst phase, where ClpXP translocates the polypeptide by increments of 1–4 nm. However, the molecular mechanism by which ClpXP couples the energy from ATP hydrolysis into mechanical work is still incomplete. Here we used biochemical and single-molecule assays with optical tweezers to dissect i) the complete mechanochemical cycle of ClpXP, and ii) its mechanism of power generation. We show that ADP release and ATP binding happen non-sequentially during the dwell, while ATP hydrolysis and phosphate release occur during the burst. ADP release is the rate-limiting transition of the ATP cycle, and therefore it determines the duration of the dwell phase. Next, using ClpX mutants, we show that the size of the amino acids that form the highly-conserved translocating loops—which contact the protein substrate and propel its translocation along the narrow ClpX pore—has been evolutionarily optimized to maximize ClpX power generation, the coupling between chemical and mechanical cycles, and the efficiency of protein unfolding and translocation. Finally, we show that the conformational resetting of these loops between consecutive bursts determine ADP release from individual ATPase subunits, and therefore the overall duration of the motor's cycle.

DIRECT OBSERVATION OF SUBSTRATE TRANSLOCATION AND CONFORMATIONAL DYNAMICS IN THE 26S PROTEASOME

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The 26S proteasome is a molecular machine that selectively destroys proteins targeted for degradation. It does so by recognizing ubiquitin modifications attached to substrate proteins, followed by engagement of the polypeptide and its mechanical unfolding and tanslocation into a proteolytic core. The precise nature of how the proteasome reads the ubiquitin code, generates mechanical force, and processes its substrates, as well as the conformational landscape it explores during degradation, has remained elusive. Here we employ single-molecule FRET to observe the interactions between fluorescently labeled proteasomes and substrates, and also study the intra-molecular conformational dynamics of dual labeled proteasomes. In order to circumvent previous limitations of working with endogenous holoenzymes, we reconstitute proteasomes from subcomplexes that were recombinantly produced with non-standard amino acid reprograming for fluorescent dye labeling. Fluorescently labeled substrates are enzymatically modified with ubiquitin chains, and we can observe their molecular trajectories as they pass through the various stages of proteasomal engagement, translocation, unfolding, and deubiquitination. By monitoring the conformational state of the proteasome using a FRET pair designed to capture the transition between substrate-free and -engaged states, we observe a highly dynamic holoenzyme at various stages of substrate processing. These observations offer important new insights into the operation of the proteasome and its underlying mechanisms for ATP-dependent degradation.

MECHANISMS OF MITOCHONDRIAL MACHINES OF MASS DESTRUCTION

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Mitochondrial AAA+ quality control proteases regulate diverse aspects of mitochondrial biology through specialized protein degradation, but the underlying molecular mechanisms that define the diverse activities of these enzymes remain poorly defined. The mitochondrial AAA+ proteases YME1 and AFG3L2 reside in the inner mitochondrial membrane but expose their enzymatic domains to the intermembrane space and matrix, respectively. Using cryo-EM, we show that these hexameric complexes use a hand-over-hand mechanism of substrate translocation through a sequential ATP hydrolysis cycle. The basic translocation mechanism we describe is likely to be evolutionarily conserved from bacteria to humans. AFG3L2 is of particular interest, as genetic mutations localized throughout AFG3L2 are linked to diverse neurodegenerative disorders. We used cryo-EM to determine a substrate-bound structure of the catalytic core of human AFG3L2. This structure identifies multiple specialized structural features within AFG3L2 that integrate with conserved structural motifs required for hand-over-hand ATP-dependent substrate translocation to engage, unfold, and degrade targeted proteins. Our results provide a molecular basis for neurological phenotypes associated with different AFG3L2 mutations and establish a structural framework to understand how different members of the AAA+ superfamily achieve specialized, diverse biological functions.While a hand-over-hand translocation is emerging as the conserved mechanism by which ATP hydrolysis drives substrate translocation within the classical clade of AAA+ proteins, the operating principles of the distantly related HCLR clade remains poorly defined. We determined a cryo-electron microscopy structure of Y. pestis, revealing that although sequential ATP hydrolysis and hand-over-hand substrate translocation are conserved in this AAA+ protease, Lon processes substrates through a distinct molecular mechanism involving structural features unique to the HCLR clade. We define a previously unobserved translocation mechanism that is likely conserved across HCLR proteins and reveal how distinct structural configurations of distantly-related AAA+ enzymes can power hand-over-hand substrate translocation.

SUPER-RESOLUTION IMAGING OF REPLICATION-ASSOCIATED G4 DNA IN HUMAN CELLS

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DNA G-quadruplex (G4) structures are noncanonical secondary structures formed within guanine-rich DNA that play important roles in various genomic metabolic processes such as DNA replication. Despite their importance, the specific impacts of DNA G4 formation and regulation during replication remain elusive in part due to the limitations of conventional in vitro and ensemble experimental techniques. To address this knowledge gap, we developed a platform to quantitatively visualize the spatial associations between DNA G4 structures and individual replisome complexes in human cells, by coupling multi-color single-molecule localization fluorescence microscopy (SMLM) with robust triple-correlation (TC) analysis. We showed that during normal replication, DNA G4 predominantly form at newly unwound single-stranded DNA (ssDNA) in between MCM helicase and nascent DNA. By measuring nascent DNA signals at individual replication fork level, we observed a reduced nascent DNA incorporation at G4-associated forks (RF-G4) compared to regular replication forks, indicating that RF-G4s can block replication progression locally. Interestingly, the loading of RPA onto ssDNA is also restricted specifically at RF-G4s but not in regular forks, suggesting that G4 accumulation at forks may result in the suppression of RPA-mediated replication stress response. Importantly, we found that the FANCJ helicase is required to maintain normal replication fork progression by counteracting the abundance of RF-G4s, and the deficiency of FANCJ results in an increased amount of gH2AX signal at RF-G4s, indicating local replication fork collapse and formation of DNA double strand breaks. These findings suggest an important regulatory role of FANCJ in the processing of DNA G4 structures during replication in order to maintain normal replication and preserve genomic integrity.

DIRECT OBSERVATION OF CRISPR-CAS12 AS CONFORMATIONAL SAMPLING REVEALS HOW CONFORMATIONAL ACTIVATION PROMOTES CATALYSIS AND RESETTING OF THE ENDONUCLEASE ACTIVITY

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Cas12a, also known as Cpf1, is a type V-A CRISPR-Cas RNA-guided endonuclease that is used for genome editing based on its ability to generate specific dsDNA breaks. Here, we combined cryoEM structures and single molecule FRET to provide a complete mechanistic understanding of endonuclease structural dynamics role in function and resetting (1). Cryo-EM readout provided the structures of intermediates of the cleavage reaction, and identified protein regions that sense the crRNA-DNA hybrid assembly triggering catalytic activation. Combined with single molecule readout of my group(2-4) and specifically FRET allowed us to directly observe the protein conformational dynamics along the entire reaction pathway. Parallel single molecule imaging provided the directionality of conformational transitions as well as the complete thermodynamic and kinetic characterisation of the conformational activation leading to function. These findings illustrate why Cas12a cuts its target DNA and unleashes unspecific cleavage activity degrading ssDNA molecules after activation and how other crRNAs displace the R-loop inside the protein after target DNA cleavage terminating indiscriminate ssDNA degradation. We proposed a model whereby the conformational activation of the enzyme results in indiscriminate ssDNA cleavage. The displacement of the R-loop by a new crRNA molecule will recycle Cas12a specifically targeting new DNAs. 1. S. Stellaet al., Conformational Activation Promotes CRISPR-Cas12a Catalysis and Resetting of the Endonuclease Activity, Cell175, (2018) 1856.2. M. Liet al., Single Enzyme Experiments Reveal a Long-Lifetime Proton Leak State in a Heme-Copper Oxidase, J. Am. Chem. Soc.137, (2015) 16055.3. S. Veshaguriet al., Direct observation of proton pumping by a eukaryotic P-type ATPase, Science351, (2016) 1469.4. K. Bavishiet al., Direct observation of multiple conformational states in Cytochrome P450 oxidoreductase and their modulation by membrane environment and ionic strength, Sci Rep-Uk8, (2018).

DETECTING SINGLE STEPS DURING TRANSCRIPTION AND A HALF-TRANSLOCATED PAUSE COMPLEX OF E. COLI RNA POLYMERASE USING NANOPORE TWEEZERS

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[2]University of Washington, Molecular Engineering and Sciences, Seattle, WA, USA
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Single-molecule Picometer Resolution Nanopore Tweezers (SPRNT) is a technique that enables observation of single enzyme movement along nucleic acids under an applied force. SPRNT provides measurements of enzyme position along a nucleic acid substrate with sub-Angstrom spatial and millisecond temporal resolution, while simultaneously providing the DNA sequence within the enzyme. We use SPRNT to monitor many E. coli RNA Polymerase (RNAP) core complexes during transcription elongation and pausing with an assisting force. We determine that during elongation at low [NTP], RNAP primarily stalls in a post-translocated state, with brief deviations forward to a hyper-translocated state and backwards to a pre-translocated state. The rates and frequencies of these transitions vary significantly with DNA sequence and the magnitude of assisting force. During transcription pausing at an elemental pause sequence, we observe transitions between five distinct enzyme states (backtracked, pre-, half-, post-, and hyper-translocated), including a half-translocated state between pre and post. We develop a model for RNAP pausing and elongation by varying the applied force and monitoring RNAP mutants with SPRNT.

MOLECULAR HIGHWAYS – TORSIONAL CONSEQUENCES OF DNA MOTOR PROTEINS

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Fundamental biological processes require the concurrent occupation of DNA by numerous motor proteins and complexes. Thus, collisions, congestion, and roadblocks are inescapable on these busy 'molecular highways'. The consequences of these traffic problems are diverse, requiring complex cellular mechanisms to resolve threats to genome stability and ensure cellular viability. Additionally, the molecular highways are continuously and dynamically restructured during these processes, altering highway topology and 'traffic' flow. These alterations of DNA topology generate torsion, which can be present genome-wide and significantly facilitate or inhibit gene expression. Historically, quantitative studies of torsion have been technically challenging, and our lab has developed real-time, single molecule techniques to decipher the actions of multiple players while also simultaneously allowing the ability to mechanically control, alter, and measure DNA topology. These single molecule precision measurements have enabled novel insights into the complex coordination of cellular machineries and the fundamental role of DNA mechanics and topology.

SINGLE MOLECULE MANIPULATION AND IMAGING OF COMPLEX DNA-PROTEIN TRANSACTIONS

Gijs Wuite[1];
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The genetic information of an organism is encoded in the base pair sequence of its DNA. Many specialized proteins are involved in organizing, preserving and processing the vast amounts of information on the DNA. In order to do this swiftly and correctly these proteins have to move quickly and accurately along and/or around the DNA constantly rearranging it. In order to elucidate these kind of processes we perform single-molecule experiments on model systems such as restriction enzymes, DNA polymerases and repair proteins. In this presentation I will show (Super-resolution) Quadruple Trap Correlative Tweezers-Fluorescence Microscopy (CTFM), a single-molecule approach capable of visualizing individual DNA-binding proteins on densely covered DNA and in presence of high protein concentrations. Moreover, proteins on DNA can be visualized on multiple DNA strand. Using this instrument we have investigated human non-homologous end joining (NHEJ). NHEJ is the primary pathway for repairing DNA double-strand breaks (DSBs) in mammalian cells. Such breaks are formed, for example, during gene-segment rearrangements in the adaptive immune system or by cancer therapeutic agents. Although the core components of the NHEJ machinery are known, it has remained difficult to assess the specific roles of these components and the dynamics of bringing and holding the fragments of broken DNA together. I will present data using dual and quadruple-trap optical tweezers combined with fluorescence microscopy, on how human XRCC4, XLF and XRCC4–XLF complexes interact with DNA in real time. We find that XRCC4–XLF complexes robustly bridge two independent DNA molecules and that these bridges are able to slide along the DNA. These observations suggest that XRCC4–XLF complexes form mobile sleeve-like structures around DNA that can reconnect the broken ends very rapidly and hold them together. (Brouwer et al., Nature, doi:10.1038/nature18643 , 2016)

MECHANICAL FINGERPRINTS OF BIOMOLECULES, DECODED

Olga K. Dudko[1];
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The capacity of biological macromolecules to function as exceedingly sophisticated and efficient cellular machines – switches, assembly factors, pumps, or motors – is realized through their conformational transitions. Conformational transitions can be induced, monitored, and manipulated in single-molecule force experiments. The relationship between the applied force and molecular extension, which is revealed in these experiments, identifies a given biomolecule and thus serves as the molecule's mechanical fingerprints. I will present a set of analytically tractable approaches to interpreting single-molecule force spectroscopy measurements in terms of kinetic rates, activation barriers, and pathways. On the fundamental side, being rooted in non-equilibrium statistical mechanics, these analytical approaches help reveal the unifying principles underneath the bewildering diversity of biomolecular behaviors. On the practical side, the analytical solutions that result from these approaches are well-suited for a direct fit to experimental data, yielding the key parameters that govern biological processes at the molecular level.

USING A SYSTEM'S EQUILIBRIUM BEHAVIOR TO REDUCE ITS ENERGY DISSIPATION IN NON-EQUILIBRIUM PROCESSES

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[3]Rockefeller University, Biochemistry, Biophysics, Chemical Biology, and Structural Biology Genetics and Genomics , New York, NY, USA
[4]Simon Fraser University, Department of Physics, Vancouver, BC, Canada
[5]Howard Hughes Medical Institute, Berkeley, CA, USA

Cells must operate far from equilibrium, utilizing and dissipating energy continuously to maintain their organization and to avoid stasis and death. However, they must also avoid unnecessary waste of energy. Recent studies have revealed that molecular machines are extremely efficient thermodynamically when compared to their macroscopic counterparts. However, the principles governing the efficient out-of-equilibrium operation of molecular machines remain a mystery. A theoretical framework has been recently formulated in which a generalized friction coefficient quantifies the energetic efficiency in non-equilibrium processes. Moreover, it posits that to minimize energy dissipation, external control should drive the system along the reaction coordinate with a speed inversely proportional to the square root of that friction coefficient. Here, we demonstrate the utility of this theory for designing and understanding energetically efficient non-equilibrium processes through the unfolding and folding of single DNA hairpins.

A SINGLE-MOLECULE VIEW OF MAMMALIAN DNA DOUBLE-STRAND BREAK REPAIR

Eli Rothenberg
New York University, New York, NY, USA

Chromosomal breaks (DSBs) are the most genotoxic type of DNA damage, and are caused by Ionization Radiation (IR), chemotherapeutic agents as well as from normal cellular processes. The induction of DSBs result in the recruitment of various DNA repair and signaling factors to form microscopic repair foci. Within these foci different repair factors function as a multi component repair factory, supporting the regulation and progression of the DSB repair process. The organization and dynamics of the different factors within repair foci and the precise mechanisms by which they promote repair are still subject to much uncertainty, posing critical constraints to our understanding of the DSB repair processes.
In our recent and ongoing efforts, we have developed and utilized various single-molecule methods, including biochemical assays, super-resolution microscopy and live cell imaging to address fundamental gaps in our knowledge of mammalian DNA repair pathways. Our studies revealed distinctive features of the molecular kinetics along with unique interaction landscapes that encompasses interactions at the DNA-protein level and chromatin level, which together mediate and regulated the DSB repair process.

IMAGING AT THE GENOMIC-SCALE: FROM 3D ORGANIZATION OF THE GENOMIC DNA TO CELL ATLAS OF COMPLEX TISSUES

Xiaowei Zhuang
Harvard University, Cambridge, MA, USA

No Abstract

POSTER ABSTRACTS

THURSDAY, JULY 18
POSTER SESSION I
14:30 – 15:45
Cafeteria, 1st Floor

All posters are available for viewing during all poster sessions; however, below are the formal presentations for Thursday. Presenting authors with odd-numbered poster boards should present from 14:30 – 15:07 and those with even-numbered poster boards should present from 15:07 – 15:45. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Alarcón Rodríguez Paiva, Rodrigo	**1-POS**	**Board 1**
Albano, Juan M.R.	**2-POS**	**Board 2**
Bai, Lihui	**3-POS**	**Board 3**
Bousova, Kristyna	**4-POS**	**Board 4**
Cané, Lucia	**5-POS**	**Board 5**
Castillo, Juan P.	**6-POS**	**Board 6**
Chou, Chia-Fu	**7-POS**	**Board 7**
Christensen, Sune	**8-POS**	**Board 8**
Cooper, Justin	**9-POS**	**Board 9**
de Camargo Rodrigues, Luiz Fernando	**10-POS**	**Board 10**
de Silva, Chamaree	**11-POS**	**Board 11**
Diaz Celis, Cesar	**12-POS**	**Board 12**
Dos Santos Junior, Claudio Victor	**13-POS**	**Board 13**
Ells, Zachary	**14-POS**	**Board 14**
Erban, Radek	**15-POS**	**Board 15**
Espinoza Huertas, Keren	**16-POS**	**Board 16**
Fernandez Sampedro, Miguel	**17-POS**	**Board 17**
García, María José	**18-POS**	**Board 18**
González Lizarraga, María Florencia	**19-POS**	**Board 19**
Halma, Matthew	**20-POS**	**Board 20**
Hanke, Andreas	**21-POS**	**Board 21**
Herrera-Asmat, Omar	**22-POS**	**Board 22**
Jabak, Adam	**23-POS**	**Board 23**
Kapka-Skrzypczak, Lucyna	**24-POS**	**Board 24**
Karpova, Tatiana	**25-POS**	**Board 25**
Kruszewski, Marcin	**26-POS**	**Board 26**
Ladokhin, Alexey	**27-POS**	**Board 27**
Leal, Mateus	**28-POS**	**Board 28**
Leicher, Rachel	**29-POS**	**Board 29**

Posters should be set up the morning of July 18 and removed by noon July 21.

1-POS Board 1

DEVELOPMENT OF A STABLE HETEROLOGOUS EXPRESSION SYSTEM OF THE RNA POLYMERASE OF MYCOBACTERIUM TUBERCULOSIS BY GENOMIC INTEGRATION IN E. COLI

Rodrigo Alarcón Rodríguez Paiva[1]; Daniel Guerra Giraldez[1];
[1]Universidad Peruana Cayetano Heredia, Faculty of Science and Philosophy, Lima, Peru

The RNA polymerase of Mycobacterium tuberculosis is an enzyme of high biomedical interest due to its importance as drug target and the occurrence of polymorphisms linked to drug resistance. In order to facilitate its study, an inducible expression system was developed previously in E. coli. This system consists of the 5 genes coding for the holoenzyme subunits distributed among two low-copy plasmids. Unfortunately, this system presents several difficulties, and frequently a low yield of active M. tuberculosis protein. When different colonies of the same expression clone were tested simultaneously, they showed high heterogeneity in their level of protein production and specific activity. Possibly, this heterogeneity is caused by non-constant copy number of the plasmids and consequent variability in the stoichiometry of the subunits in the expressing cell. Moreover, the expression of these heterologous proteins exerts great stress over the bacteria, evidenced by a strong inhibition of its growth. We hypothesize that this stress contributes to the low expression and heterogeneity in protein production. In this project, we integrated the genes for α, β, β' and ω subunits, which constitute the apoenzyme of M. tuberculosis, into the E. coli genome using a viral recombination site. We hypothesized that with a single copy of the genes and with the superior stability of genomic DNA, the problems presented would be solved. The σA subunit was introduced with a low copy plasmid to allow eventual production of holoenzymes with different σ factors. A second plasmid was introduced, bearing a GFP gene under the control of a promoter exclusively recognized by the ARN polymerase of M. tuberculosis. We observed a strong signal corresponding to the activity of the heterologous enzyme, exclusively when its expression was induced. This new expression system can have applications in detection of inhibitors, transcription and protein regulation studies, among others.

2-POS Board 2

MD SIMULATIONS TO EXPLORE THE CX26 CONNEXON EMBEDDED INTO A POPC BILAYER

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[2]Instituto de Fisica de Buenos Aires-UBA-CONICET, Fisica, Buenos Aires, Argentina
[3]Univeristy of Campinas, Chemestry, Campinas, Brazil
[4]Instituto de Fisica del Plasma-UBA-CONICET, Fisica, Buenos Aires, Argentina
[5]University of Utah, Biomedical Informatics, Salt Lake City, UT, USA

GAP junctions provide a communication pathway between adjacent cells. They are formed by paired connexons that reside in the plasma membrane of their respective cell and their activity could be modulated by the bilayer composition. In this work, we studied the dynamic behavior of a Cx26 embedded in a POPC lipid bilayer, focusing on two factors: membrane protein interaction and ion flux though the connexon pore. We analyzed extensive atomistic Molecular Dynamics simulations for two different conditions: with and without calcium ions. We found lipid pockets surrounding the Cx26 but we did not find specific interactions with the protein, suggesting entropic effects. Furthermore, the shape and electrostatic properties of the Cx26 were also characterized taking special attention to the pore region. With these calculations, we were able to identify and explain differential chlorine flux through the pore for each system.

3-POS Board 3

A TUG-OF-WAR MECHANISM DRIVES THE ALLOSTERIC ACTIVATION OF PROTEIN KINASE A

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[2]University of California, San Diego, Pharmacology, San Diego, La Jolla, CA, USA

Protein Kinase A (PKA) is an important regulatory enzyme in many signal transduction pathways. This study focuses on the mechanisms of allosteric communication between the PKA regulatory and catalytic subunits. The regulatory subunit establishes two sets of interactions with the catalytic subunit to form an inactive holoenzyme: 1) via the inhibitory sequence that docks into the active site of the catalytic subunit and is stabilized by ATP and two Mg^{2+} ions, and 2) via surface contacts between two cyclic nucleotide binding (CNB-A and CNB-B) domains. We use optical tweezers to investigate the crosstalk between the inhibitory sequence, ATP and Mg^{2+} with the surface contacts established between the CNB domains and the catalytic subunit. When ATP and Mg^{2+} are in saturation, we find that the CNB domains bound to the catalytic subunit are in equilibrium between two conformational states, a predominant conformation in which the CNB domains unfold independently of each other and at high force, and a minor conformation wherein the CNB domains unfold near-simultaneously or cooperatively and at low force. In the absence of ATP or Mg^{2+}, a redistribution of the PKA ensemble occurs where the CNB domains only unfold cooperatively and at low force. Moreover, we investigate how the CNB-B domain controls the interaction of the CNB-A domain with the catalytic subunit. We show that the truncated CNB-A domain has a stronger interaction with the catalytic subunit compared to the CNB-A domain as part of the regulatory subunit. This result indicates that the CNB-B domain weakens the interaction between the CNB-A domain and the catalytic subunit. Altogether, this study portrays a PKA allosteric activation mechanism in which the CNB-A domain experiences a tug-of-war due to stabilizing effects conferred by the IS and ATP-Mg^{2+}, and destabilizing effects due to the presence of the CNB-B domain.

4-POS Board 4

ALLOSTERIC MODULATIONS IN SYNTHETIC FUSION PROTEINS

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The design of chimeric synthetic proteins utilizing individual protein domains or artificial mini-proteins as building blocks is an important advance for the formation of new functional biomolecules for medical applications and biotechnology. It is increasingly anticipated that synthetically fused domains from different protein sources, whether derived from nature or from in silico predictions, could lead to improved properties of specifically engineered proteins through allosteric modulation of fused domains. We decided to prepare two fusion molecules in their domain order: the "forward" and the "reverse" one, to study how the domains influence each other in their different orders, and whether the order in the new protein molecules is crucial. To study the domains allosteric modulations we have proposed novel two-domain molecules composed of a well structurally characterized domain of the Postsynaptic Density Protein 3 (PDZ3; part of ZO-1) and the artificial protein Tryptophan-Cage. To decipher changes in new biomolecules we used biochemical, biophysical and structural (nuclear magnetic resonance - NMR) analyses. To determine changes in binding specificities of fusions, we used a peptide derived from PDZ3 natural binding partner molecule Junctional Adhesion Molecule A (JAM-A). Based on biophysical characterizations and NMR results, we have found out that domains order in the context of the protein can be a fundamental factor for allosteric modulation and it can determine the protein-specific function.

5-POS Board 5

GLYCOPHORIN IS NOT A RECEPTOR FOR HLYA IN HUMAN ERYTHROCYTES

Lucia Cané[1]; Sandrine Genetet[2]; Isabelle Moure-Chanteloup[2]; Mariano Ostuni[2]; Pablo Schwarzbaum[3]; Vanesa Herlax[1];
[1]Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), Facultad de Ciencias Médicas. Universidad Nacional de La Plata., La Plata, Argentina
[2]Inserm, INTS, Unité Biologie Intégrée du Globule Rouge., Université Paris Diderot., Paris, France
[3]Instituto de Química y Fisico-Química Biológicas (IQUIFIB) "Prof. Alejandro C. Paladini"., Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Capital Federal, Argentina

Alpha hemolysin (HlyA) is a hemolytic and cytotoxic protein secreted by uropathogenic strains of E. coli. HlyA acts on a variety of cell types from several species, including human red blood cells (RBCs); and also binds to and disrupts protein-free liposomes. Studies that have explored the binding of HlyA to membranes and the characterization of a putative toxin-specific receptor have produced contradictory results. Regarding RBCs, glycophorins were characterized as putative receptors in horse RBCs, though other studies indicated that the binding of HlyA to rabbit RBCs occurred in a nonsaturable manner and that the toxin did not interact with a specific protein receptor. On the other hand, we have previously demonstrated that HlyA induces a diminish in human RBCs deformability, and the release of lytic and nonlytic ATP, effects that have been associated to the interaction of ligands with glycophorin.In this context, the aim of our study was to characterize if the interaction of HlyA with human glycophorins mediate the hemolytic and sublytic effects of the toxin. For this purpose we measure the hemolytic activity of HlyA on RBCs pretreated with different antibodies (iH4 (nanoantibody against $Y_{52}PPE_{55}$), anti-GPA/GPB, anti-GPA) and on GPA/GPB null RBCs. For our surprise, the hemolytic activity of the toxin was similar to control RBCs in all the conditions tested, indicating that glycophorin is not necessary for HlyA lytic activity. Moreover, we performed FACS assays using antibodies anti-GPA (R18) and anti-band3 complexed with glycophorin (Wr), on treated and untreated RBCs. Results showed a slight increment in fluorescence intensity when toxin interacts with RBCs, indicating conformational changes of both GPA and Band 3 when the toxin interact with RBCs. In conclusion, results indicate that HlyA-GPA or GPB interaction is not necessary for HlyA lytic activity.

6-POS Board 6

CHALLENGING A VIRAL DNA PACKAGING MOTOR WITH MODIFIED SUBSTRATES

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[2]University of Minnesota, Minneapolis, MN, USA

The DNA packaging motor of the bacteriophage phi29 is a powerful molecular machine that couples the free energy of ATP hydrolysis to DNA translocation. This motor is composed by a pentameric ring ATPase that follows a dwell-burst scheme. In each turn of the mechanochemical cycle, ADP is exchanged for ATP during the dwell time, followed by a translocation burst that is 10 base pairs (bp) in size, which is composed of four consecutive sub-steps of 2.5 bp. Several models can explain what determines the burst size of the motor: the B-form DNA has 10.5 bp per turn of the double helix, suggesting that the structure of the substrate is the determining factor; however, the non-integer nature of the sub-steps during the burst suggests that is the fixed conformational change of the ATPase what sets the burst size. Yet another possibility is that the DNA packaging motor switches the local conformation of the DNA substrate from B-form to A-form during packaging. To test the above hypotheses we challenged the phi29 DNA packaging motor with different substrates bearing the A-form of nucleic acids, using high resolution optical tweezers assay. Our results show indeed that the motor is able to adapt its operation to translocate these different substrates by reducing the size of the burst such that it follows the new helical pitch. We propose a mechanistic model where the motor establishes a critical contact with the substrate at every turn of the double helix during subsequent dwells. Such event is a strong interaction that interrupts the last power stroke during the burst when the motor packages A-form substrates, and it serves as a resetting point for the ring ATPase to complete the turnover cycle.

7-POS Board 7

GEOMETRIC CUES OF BACTERIA CELL-DIVISION REGULATING PROTEINS REVEALED THROUGH MICROFLUIDIC CONFINEMENT

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Dynamic pattern formations are often encountered in biological systems, constrained by boundary conditions imposed by cell geometry, such as eukaryotic polar differentiation in embryonic development. In many Proteobacteria, septum positioning at mid-cell is ascribed to the sensing capability of the cell-division regulating Min proteins, which undergo dynamic pole-to-pole oscillations. It is currently uncertain whether these oscillations are driven by curvature-mediated localization of MinD around membranes with high negative curvature (diffusion-and-capture hypothesis, or DCH) or whether geometric boundaries imposed by membrane patches determine the reaction-diffusion propagation axis of the Min proteins. To explore the geometric cues behind the distribution of Min proteins during its oscillation, we use microfluidic confinement to gently reshape round mutants of the rod-shaped bacterium Escherichia coli, which reveals a dominant distribution of MinD around positively curved regions of the cell periphery, in sharp contrast to DCH. We then construct a phenomenological formalism based on the principle of wave spreading and the permissive modes of chemical waves indicating that the global membrane geometry, rather than the local curvature, is the primary cue for the localization of membrane-bound MinD, a finding well supported by our experimental observations. Our study suggests a framework for quantitative analysis extendable to other wave-like biopatterning systems without solving the reaction-diffusion equations.

8-POS Board 8

INDUSTRIAL ENZYME ENGINEERING: A JOURNEY FROM SINGLE MOLECULES TO TONS

Philip M Lund[1,2]; Søren S Nielsen[2]; Amalie S Kallenbach[2]; Nikos Hatzakis[2]; **Sune M. Christensen**[1];
[1]Novozymes A/S, Protein Engineering, Lyngby, Denmark
[2]University of Copenhagen, Chemistry, Copenhagen, Denmark

Enzymes are ubiquitous in biology and underpin all forms of life as we know it. Protein engineering can be applied to repurpose naturally occurring enzymes to act as catalysts in industrial processes and as functional constituents in consumer products. Enzyme performance in application is a macroscopic phenomenon emerging from a complex cascade of events transcending length scales from individual molecules to the ensemble. For heterogenous reactions, comprising the bulk of commercially interesting processes, enzyme performance includes, e.g., surface binding, orientation, conformational changes, diffusion, stability, catalytic activity and colloidal effects. The ideal enzyme product should excel at all these levels. Molecular scale in-depth understanding of structure-function relationships is a critical element in engineering such an enzyme. However, in most cases unambiguous information of this kind is extremely difficult to obtain by conventional approaches. Single molecule techniques can provide answers to many of these problems. I will discuss our efforts in this area with emphasis on a recent single particle tracking study of engineered lipase mobility on a triglyceride substrate.

9-POS Board 9

APPLICATION OF SUPER RESOLUTION RADIAL FLUCTUATION (SRRF) IMAGING TO MEASUREMENT OF SINGLE-MOLECULE DNA HYBRIDIZATION KINETICS.

Justin Cooper[1,2];
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Super-resolution radial fluctuations (SRRF) is a novel algorithm based super-resolution imaging technique that incorporates radial intensity gradients and temporal intensity fluctuations to calculate and project SRRF images with resolution up to 2x below the diffraction limit in real time. SRRF, has been used for imaging sub-diffraction intracellular structures and for performing time-resolved super resolution measurements of live cell dynamics. However, no application to single-molecule localization and tracking techniques has been shown. Here, we characterize the capabilities of the SRRF algorithm on measuring DNA hybridization kinetics from a DNA capture surface via single-molecule localization and tracking. We find that SRRF data has some obvious advantages such as a substantial increase in the signal-to-background ratio of single-molecule data. This is a side effect of the image radially transform which yields low radially values for regions which contain randomized intensity values on the distances scales of the radiality calculation. This is particularly usefully when measuring hybridization kinetics with slow on rates which necessitate higher solution phase concentrations and resulting in higher background levels. We also find that with sparse data such as these, molecule localization can be performed by calculation of the 1st moment centroids of the SRRF point spread functions (PSF) which can deliver spatial resolution similar to non-linear fitting of the optical PSF but with much faster computational times. Finally, we explore the ability for SRRF to resolve individual molecules in high DNA surface density conditions which result in many overlapping optical PSFs. We find that measuring single-molecule data following the SRRF radiality transform allows for resolving much higher densities of molecules than diffraction limited images thus allowing for faster collection of statistically significant population data.

10-POS Board 10

STUDYING FOUR HUMAN HSP70 CHAPERONE MEMBERS USING SMALL ANGLE X-RAY SCATTERING (SAXS)

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[2]Universidade de São Paulo, Institute of Chemistry of São Carlos, São Carlos, Brazil

Molecular chaperones are responsible for proteostasis and cellular protein quality control, preventing misfolding and aggregation of client proteins, especially under stress conditions, also aiding in refolding processes, protein traffic, signalling and aggregates' solubilization. They are known as Hsps (Heat Shock Proteins) and are intimately related to protein misfolding pathologies such as Parkinson and Alzheimer diseases and also some forms of cancer, being currently studied as potential drug targets. The Hsp70 family collaborate with cochaperones and nucleotide exchange factors to form a central component of the cellular chaperone machinery, having hundreds of client proteins, and are composed of a Nucleotide Binding Domain (NBD) and a Peptide Binding Domain (PBD) connected by a flexible linker. They present an ATP-dependent non-equilibrium mode of substrate binding that requires large domain rearrangements, which makes them suitable for Small Angle X-Ray Scattering (SAXS) experiments, as high resolution information is limited due to their dynamic nature and size. SAXS is a low resolution technique that provides general structural parameters, such as protein radius of gyration, maximum dimension and information on the particle shape. With such information, a low resolution three-dimensional model may be generated by means of computational methods. The objective of this study is to compare the structural modifications of the human Binding immunoglobulin protein (hBip), which is an Hsp70 homologue found in the endoplasmatic reticulum, by nucleotide binding and also to compare different human Hsp70 chaperones from different cell compartments and with varying sequence similarities. From the scattering profiles and analysis slight structural differences can be observed, which may be related to different client specificities, organelle environments and equilibrium properties with nucleotides.This work was supported by Conselho Nacional de Aperfeiçoamento de Pessoal de Nível Superior (PROEX - 0487), by FAPESP (17/26131-5) and by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

11-POS Board 11

OBSERVING DNA FLEXIBILITY USING TETHERED PARTICLE MICROSCOPY

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Tethered Particle Microscopy is a single-molecule biophysical technique that is used to study DNA flexibility by fixing one end of the strand to a microscope slide and attaching a one micrometer synthetic bead to the other end. Using an Olympus CX31 microscope and a digital microscope imager, the precise movement of the bead is recorded. This movement is then monitored using a particle tracking software to determine the characteristics of the DNA molecule. This in turn provides insight into the DNA's flexibility and looping. In this experiment, 2D displacement of the bead over a few minutes of each particle was recorded to see the range of motion of tethered lambda-DNA in real time.

12-POS Board 12

NUCLEOSOMAL STATES REGULATES DISASSEMBLY AND ASSEMBLY OF NUCLEOSOMES

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The nucleosome is the structural unit of chromatin and consists of an octamer of histone proteins, wrapped by DNA, which forms ~1.7 turns. Two copies of four histones compose the octamer core: H2A, H2B, H3, and H4. H2A/H2B form a dimer and two dimers of H3/H4 forms a tetramer. Nucleosome dynamics (assembly and disassembly) are fundamental for genomic organization and regulation, and those properties rely on octamer plasticity, DNA sequence, post-translational modifications, regulatory factors, and molecular motors (RNA polymerase, remodelers). We examined the force dependent disassembly of single nucleosomes assembled onto the 601 positioning sequence using single molecule optical trapping. We observed the nucleosome disassemble to form bare DNA in a stochastic manner that generates hexasomes and tetrasomes. Force distribution of the unwrapping of the inner DNA turn indicates that in vitro assembled nucleosomes exists in at least three states characterized by different unwrapping and rewrapping trajectories. The three nucleosomal states were also observed in nucleosomes assembled on the natural 5S positioning sequence, which showed similar unwrapping/rewrapping trajectories and force distributions compared to the 601 sequence. This indicates that nucleosomal states are independent of the DNA sequence and they result from the intrinsic plasticity of the octamer core. These distinct nucleosome states suggest a mechanism that can regulate DNA accessibility to the transcription machinery.

13-POS Board 13

AFM TIPS FUNCTIONALIZED WITHETHYLENEDIAMINEBYPECVD AND THEINTERACTION OF GLUTARALDEHYDECROSSLINKED SURFACES IN AQUEOUS SOLUTIONS

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Atomic force microscopy(afm) force distance curves were used to evaluate the efficiencyof using amino precursor coatings produced byplasma-enhanced chemical vapor deposition (pecvd) to immobilize glutaraldehyde (ga) on silicon nitrate substrates. Deposition was performed in a capacitive reactor using ethylenediamine (eda) as precursor as it contain both carbon as well as amine groups, c h (nh) . Coatings were characterized byfast fourier infrared spectroscopy(ftir) and showed characteristic amine peaks. Glutaraldehyde was immobilized on coated substrates at different concentrations and the crosslinking interaction forces between the surface and the tip were evaluated bymeasuring the adhesion force using afm force distance curves and characteristic covalent force occurrence probability. Increased as the ga concentration increases. Topographic images of eda functionalized silicon wafers were acquired before and after deposition of glutaraldehyde, in air and in pbs solution. Images showed a drastic decrease of the roughness in air. All results indicate that amine surfaces functionalization performed bypecvd with eda is a promisor path for biorecognition surfaces in biomedical and biosensors applications among others

14-POS Board 14

SINGLE MOLECULE STUDIES OF DOXORUBICIN-DNA INTERACTIONS UNDER HIGH TENSION PROVIDES NEW INSIGHTS TO ITS BINDING MECHANISM

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Understanding the binding mechanisms of cancer drugs to DNA at the single molecule level plays a key role to design new and improved cancer drugs for the future. We use counter-propagating laser beams in a dual-beam optical tweezers setup to quantify the binding properties of an anti-cancer drug, Doxorubicin, to DNA. Prior studies that used optical tweezers to study Doxorubicin interactions with DNA at low forces observed intercalation in the micromolar range. The dual-beam setup provides us the capabilities to extend these studies to higher forces. When the DNA was stretched past the over stretching transition in the presence of Doxorubicin, it was observed that melting the DNA felicitated more intercalation in the nanomolar concentration range. We use the lengthening of DNA upon binding to Doxorubicin in conjunction with the McGhee - von Hippel one dimensional lattice binding model to quantify the binding affinity. Our study suggests that during the transcription process when the DNA is under high tensions forming transcription bubbles Doxorubicin can effectively bind in lower concentrations than previously believed.

15-POS Board 15

MULTI-RESOLUTION SIMULATIONS OF INTRACELLULAR PROCESSES WITH SINGLE-MOLECULE DETAIL

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Molecular dynamics (MD) approaches, based on the rules of classical mechanics, are commonly used to study the behaviour of complex biomolecules in biological applications. They are given as systems of differential equations for the time evolution of positions and velocities of particles, representing either individual atoms or groups of atoms, describing parts of a biomolecule. One of the main limitations of all-atom MD simulations is that their direct application to the modelling of intracellular behaviour is restricted to modelling processes in relatively small domains over relatively short time intervals. In particular, intracellular processes which include transport of molecules between different parts of a cell, are usually only modelled by a much coarser modelling approach, including Brownian dynamics (BD) and other stochastic reaction-diffusion models. In my presentation, I will discuss connections between MD and BD, focusing on the development, analysis and applications of multi-resolution methods for spatio-temporal modelling of intracellular processes. These methods use detailed MD simulations in localized regions of particular interest (in which accuracy and microscopic details are important) and a coarser (less-detailed) model in other regions where accuracy may be traded for simulation efficiency. Three types of multi-resolution methodologies will be considered in detail: describing the whole biomolecule (biological structure) of interest by the detailed modelling approach which is coupled with a coarse model for the solvent molecules which are far away from the biomolecule; describing different parts of a biomolecule by using models with different level of resolution;considering the region with the most detailed model as a fixed part of the physical space and allowing the biomolecule of interest to pass between this region and its surroundings, where a coarse-grained modelling approach is used.These multiscale methodologies are applied to investigate models of three intracellular processes, describing dynamics of (a) calcium, (b) actin and (c) DNA.

16-POS Board 16

TRANSCRIPTIONAL DYNAMIC OF MYCOBACTERIUM TUBERCULOSIS RNA POLYMERASE

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Mycobacterium tuberculosis (Mtb) is an infectious agent that causes more than one million of annual deaths worldwide. RNA polymerase (RNAP) is one of the most important therapeutic targets to fight Mtb, therefore a detailed characterization of this protein and its activity is necessary to understand the mechanisms of antibiotic resistance and contribute to the development of new treatments against this bacterium. Given the slow growth of Mtb, its regulatory mechanisms are probably very different from what is observed in the currently most well-known transcription systems (mainly E. coli and Archaea). Using optical tweezers, we have manipulated individual molecules of Mtb-RNAP in order to evaluate its activity during transcription elongation. Elongation activity of Mtb-RNAP was evaluated with saturant concentrations of NTPs at 20°C. We obtained 13 traces at 5pN and 5 traces at 15pN. A total velocity of 5.78±2.17 nt/s at 5pN and a velocity of 2.53±0.78 nt/s at 15pN were calculated. The free pause velocity (instant rate of transcription) at 5pN was 6.48 ± 0.40 nt/s, and, it was 7.13 ± 0.39 nt/s at 15 pN. There is no significant difference between velocities at different forces; however, the free pause velocity of Mtb is significantly lower than the E. coli velocity (±14 nt/s). We report, for the first time, a description of the Mtb RNAP's dynamics. The average speed as number of nucleotides incorporated per second, the distribution of pause times, the pause-free velocity, and the force dependence of these variables are reported too. These results bring the first clues about the differences in transcriptional dynamic between Mtb and E. coli. Moreover, this novel biochemical approach will allow subsequent studies in the regulation of gene expression, interaction with inhibitors and the functional consequences of polymorphisms associated with antibiotic resistance.

17-POS Board 17

RHODOPSIN FLUORESCENCE SPECTROSCOPY ANALYSIS PROVIDES NEW INSIGHTS INTO MAMMAL EVOLUTION TO DIURNAL VISION.

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Visual rhodopsin are G protein-coupled receptors located in the rods of the retina that have a key role in the dim light vision. This membrane protein consists of an 11-cis-retinal chromophore bound to a seven transmembrane protein by means of a protonated Schiff base linkage. After illumination the retinal release from the receptor (and at the same time the decay of the active form, Meta II) result in a Trp fluorescence increase when measured by the fluorescence spectroscopy that differs in terms of half-life. Traditionally different in vivo and in vitro models, like mouse and bovine rhodopsin respectively, were thoroughly used to characterize it physiologically and biochemically. Keeping in mind that a large body of works about human genetic diseases related with opsins where studied on this models, the comparison with the human rhodopsin should be seriously taken into account. In this thesis bovine, murine and human rhodopsins were immunopurified and biochemically characterized and important biochemical differences in the retinal release rates were found, mainly between the diurnal (human and bovine) vs nocturnal (mouse) species. In addition, we also found a novel relevant amino acid position that appears to be significantly associated with rhodopsin molecular adaptation to the nocturnal (L290) and the diurnal (I290) niches throughout different terrestrial therian mammal species. We also demonstrate that L290I substitution increase the rate of retinal release, thus based on this results and previous studies we hypothesize that the important difference among diurnal vs nocturnal retinal release could be related to the variation into light intensity perception through species and an equilibrium between retina protection under intense light and dark adaption under dim-light.

18-POS Board 18

PULMONARY SURFACTANT DYNAMIC AND BIOPHYSICS BEHAVIOR FROM LUNG INJURED BY MECHANICAL VENTILATION.

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The alveolar epithelial lining fluid is fundamental for proper respiratory system functionality, in there is located the pulmonary surfactant. Pulmonary surfactant is frequently associated to different degrees of pulmonary injury such lung infection, trauma or induced by mechanical ventilation. Specifically, the respiratory support by mechanical ventilation is strongly associated to a particular kind of lung injury called Ventilation Induced Lung Injury (VILI). During VILI the mechanical stress is transmitted to the pulmonary tissue and the alveolar space affecting the composition and function of pulmonary surfactant.In the present work we study the dynamic performance of the pulmonary surfactant in isolated rat lungs that develops acute lung injury (ALI) by VILI, evaluating the correlations with composition, structural and ventilator parameters of the lung. Our study involves a rat model with different degrees of ALI by changes in ventilator parameters (from protective to harmful mechanical ventilation). The use of isolated lung enables us to control the gas exchange as well as to collect the pulmonary surfactant and lung histology after the protocol. To evaluate the effect of VILI, we measured lung mechanics gas exchange and pulmonary histology. Then the pulmonary surfactant composition and dynamic properties were evaluated using HPLC and Langmuir-Blodgett trough, respectively. The histological analysis shows increased deterioration by alveolar collapse and septum thickness enlargement during the severe VILI. This septum thickness enlargement can be related to the occurrence of pulmonary edema. The phospholipid profile was impaired in the pulmonary surfactant under VILI, mainly related to the phosphatidylcholine. So far, we were able to produce an isolated model of lung ALI by VILI where investigate the effect of mechanical ventilation on pulmonary surfactant at the alveolar interphase. In the future we plan to address the tissue effects by autofluorescence tissue imaging and supramolecular organization of pulmonary surfactant using giant unilamellar vesicles.

19-POS Board 19

ANTI-AGGREGATION AND DISAGGREGATION PROPERTIES OF THE NON-ANTIBIOTIC TETRACYCLINE INCYCLINIDE AGAINST THE PARKINSON DISEASE PROTEIN ALPHA-SYNUCLEIN

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Objectives: Parkinson's disease (PD) is a progressive neurodegenerative condition with age being the main risk factor for its development. This disease has a multifactorial basis and a multi-target treatment is required. The escalating cost of developing new compounds has reinvigorated interest in drug repositioning to accelerate bench to bedside transition. With this in mind, we previously demonstrated that the tetracycline doxycycline (DOX) reshapes oligomeric species of the Parkinson disease protein alpha-synuclein (aS) reducing their toxicity, seeding capacity and propensity to form toxic fibrillar species. However, the antibiotic activity of DOX represents a possible hurdle for its repositioning in long-term treatments. Thus, we sought out to find a non-antibiotic DOX analogue with potent anti-amyloidogenic properties, making this drug an ideal candidate for repurposing to treat PD and conceivably other amyloid-associated disorders. Methods: In order to detect putative anti-amyloidogenic ready to use molecules, we used chemioinformatic techniques to extract a novel structural motif (Cb-IM) capable of interacting with cross-b structures and screened a number of pre-existing compounds using this strategy. Incyclinide was selected among tetracyclines because: i) it contains this motif in a planar structure, ii) passes the BBB, and iii) is ready for repurposing. Using a combination of biophysical techniques (fluorescence and infrared spectroscopy, scattering second order, electron microscopy, atomic force microscopy) together with cell biology approaches, we characterized its impact against aS toxic aggregates. Results: Incyclinide had an exceptional ability to reshape aS oligomers towards less toxic and non-seeding species. Moreover, Incyclinide was able to disrupt mature fibrils and was more efficient than DOX at inhibiting neuroinflammatory processes. Conclusion: The anti-amyloidogenic and anti-neuroinflammatory properties of Incyclinide, together with its ability to cross the BBB, position Incyclinide as an ideal drug to be repurposed in PD and possibly in other amyloid-associated diseases.

20-POS Board 20

COMPLEX DYNAMICS UNDER TENSION IN A HIGH-EFFICIENCY FRAMESHIFT STIMULATORY STRUCTURE

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Specific structures in mRNA can stimulate programmed ribosomal frameshifting (PRF). PRF efficiency can vary enormously between different stimulatory structures, but the features that lead to efficient PRF stimulation remain uncertain. To address this question, we studied the structural dynamics of the frameshift signal from West Nile virus (WNV), which stimulates −1 PRF at very high levels and has been proposed to form several different structures, including mutually incompatible pseudoknots and a double hairpin. Using optical tweezers to apply tension to single mRNA molecules, mimicking the tension applied by the ribosome during PRF, we found that the WNV frameshift signal formed an unusually large number of different metastable structures, including all of those previously proposed. From force-extension curve measurements, we mapped two mutually exclusive pathways for the folding, each encompassing multiple intermediates. We identified the intermediates in each pathway from length changes and the effects of anti-sense oligomers blocking formation of specific contacts. Intriguingly, the number of different structures that could be occupied by the WNV frameshift signal was maximal in the range of forces applied by the ribosome during −1 PRF. Furthermore, the occupancy of the pseudoknotted conformations was far too low for static pseudoknots to account for the high levels of −1 PRF. These results support the hypothesis that conformational heterogeneity plays a key role in frameshifting and suggest that fluctuations between different conformers under tension are linked to efficient PRF stimulation.

21-POS Board 21

KINETIC PATHWAYS IN KNOTTED SUPERCOILED DNA INDUCED BY TYPE-II TOPOISOMERASES AND SITE-SPECIFIC RECOMBINASES

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The topological state of covalently closed, double-stranded DNA is defined by the knot type K and the linking-number difference DLk relative to unknotted relaxed DNA. DNA topoisomerases are essential enzymes that control the topology of DNA in all cells. In particular, type-II topoisomerases change both K and DLk by a duplex-strand-passage mechanism and have been shown to simplify the topology of DNA to levels below thermal equilibrium at the expense of ATP hydrolysis. It remains a key question how small enzymes are able to preferentially select strand passages that result in topology simplification in much larger DNA molecules. Using numerical simulations, we consider the non-equilibrium dynamics of transitions between topological states (K, DLk) in DNA induced by type-II topoisomerases modeled as a hairpin-like gate segment. For a biological process that delivers DNA molecules in a given topological state (K, DLk) at a constant rate we fully characterize the pathways of topology simplification by type-II topoisomerases in terms of stationary probability distributions and probability currents on the network of topological states (K, DLk). Surprisingly, only a small number of intermediate topological states contribute to the pathways, namely those that dominate the conditional equilibrium distribution P(K|DLk), the distribution of K for given DLk. Our results show that topology simplification by type-II topoisomerases in DNA results from a combination of two effects: Enhanced juxtaposition probability between gate and transfer segments, and enhanced probability for an unknot to stay unknotted after strand passage. Another important class of enzymes capable of changing DNA topology are recombinase proteins, which change the degree of DNA supercoiling, and knot or catenane type, by a segment breakage and rejoining mechanism of specific DNA sites. Using numerical simulations, we fully characterize the distribution of topological states (K, DLk) of recombination products induced by tyrosine recombinases acting on inversely repeated DNA sites.

22-POS Board 22

IN SINGULO STUDY OF THE MODULATION OF TRANSCRIPTION ELONGATION OF MTBRNAP BY MTBGREA

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RNA polymerase of Mycobacterium tuberculosis (MtbRNAP) is a target of antibiotics to treat Tuberculosis. While recently structural approaches have provided new insights into the transcription mechanism in Mtb, wherein transcriptional factors play a key role during elongation, functional biophysical studies of this central enzyme have lagged behind. MtbGreA, a transcription factor known to reduce backtracking events, is thought to play a role in both initiation and elongation, while MtbCarD, another essential factor, has been proposed to be involved only in initiation and in early elongation stages. We present the first single molecule trajectories of Mtb RNAP using high resolution optical tweezers, and investigate the effect of MtbGreA on MtbCarD during transcription elongation. We found that MtbGreA reduces the pause-free velocity (17.2 ±0.3 bp/sec versus 21.5 ± 0.3 bp/sec) and decreases both the frequency and duration of pauses of the enzyme. In contrast, Mtb CarD increases the duration and density of transcriptional pauses, but this effect is abolished by MtbGreA. A model to rationalize these observations is presented.

23-POS Board 23

EXPLORING THE EFFECTS OF CHIRALITY OF DNA-THREADING INTERCALATORS AT SINGLE MOLECULE LEVEL USING OPTICAL TWEEZERS

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Using optical tweezers we have been able to study the DNA binding interactions of small molecules and prospective anti-cancer drugs at the single molecule level. A specific type of these small molecules, known as threading intercalators, has a flat planar moiety in between the molecule's bulky side chains. In order for them to bind with DNA, they have to thread their bulky side chains in between the base pairs. Due to this threading requirement, these molecules tend to have high binding affinities and slow kinetics. We have been investigating the binding properties of the ruthenium-based threading intercalator LL-[μ-bidppz(phen)$_4$Ru$_2$]$^{4+}$, or LL-P for short. This complex has the exact chemical components but an opposite chirality to the previously studied DD-P complex. Our data shows that the change in chirality affects the DNA binding kinetics, the association and dissociation rates, to at least a threefold in favor of the left handed LL-P complex. The data also confirms that both the left-handed and the right-handed molecules have a similar binding affinity as a result of their common intercalating moiety. This comparison leads us to a better understanding of how chirality can affect the binding of similar small molecules to DNA. It also will contribute an insight towards improved designs of potential anti-cancer drugs.

24-POS Board 24

SILVER BUT NOT GOLD NANOPARTICLES INDUCE CHANGES IN GLOBAL DNA METHYLATION AND DNA DAMAGE IN TWO TUMOR CELL LINES

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Nanoparticles (NPs) are defined as particles having all dimensions of 100 nm or less. Metallic NPs are used in an increasing number of applications in industry, science and medicine. Among metallic NPs foreseen to be widely used in medicine are gold nanoparticles (AuNPs) due to their low toxicity and silver NPs (AgNPs), due to their strong antimicrobial activity. The aim of this study was to compare effect of AgNPs and gold NPs (AuNPs) on global DNA methylation and formation of DNA damage in two cell lines, A2780 and 4T1, a widely used model of human ovarian carcinoma and murine mammary carcinoma, respectively. The cells were exposed to AgNPs coated with citrate (AgNPs(cit) or PEG (AgNPs(PEG), or AuNPs and global DNA methylation and formation of DNA damage was investigated. AgNPs but AuNPs decreased global DNA methylation and increased formation of DNA lesions in both cell lines. The effect was dependent on type of NPs used, its coating and cell line used. In conclusion, the effects of NPs strongly depends on NPs nature and cellular context. Epigenetic changes observed upon action of AgNPs may play a crucial role in NPs-induced changes in protein expression. This work was supported by National Science Centre grant No. 2014/15/B/NZ7/01036 (MK, LKS), statutory funding for INTC (BS, SMW, MW) and IRH (MK, LKS).

25-POS Board 25

SINGLE MOLECULE TRACKING REVEALS FAST INTERDEPENDENT CYCLING OF TRANSCRIPTION FACTOR ACE1P AND CHROMATIN REMODELER RSC AT CUP1 PROMOTER IN YEAST

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We investigate the molecular links between the dynamic binding of Transcription Factors (TF) and chromatin remodeling and transcription. For several TFs their residence time at the specific sites is critical for the regulation of transcription and increase in residence time improves the transcriptional output. RSC chromatin remodeler affects the transcription of the yeast metallothionein-encoding CUP1. Using Single Molecule Tracking, we show that the chromatin remodeler RSC decreases the residence time of the TF, and speeds up the search process of the TF Ace1p for its Response Elements (RE) at the CUP1 promoter. We quantify smFISH CUP1 mRNA data using a gene bursting model, and demonstrate that RSC regulates transcription bursts of CUP1 by modulating TF occupancy. We show by SMT that RSC binds to activated promoters transiently. Therefore, transient binding of Ace1p and rapid bursts of transcription at CUP1 may be dependent on short repetitive cycles of nucleosome mobilization. This type of regulation reduces the transcriptional noise and ensures a homogeneous response of the cell population to heavy metal stress

26-POS Board 26

POSTTRANSLATIONAL MODIFICATIONS OF HISTONE H3 IN TUMOR CELLS TREATED WITH SILVER AND GOLD NANOPARTICLES

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Nanoparticles (NPs) are defined as particles having all dimensions less than 100 nm. The small size of NPs results in unusual chemical and physical properties different from a bulk material. One of the most generally used NPs are silver nanoparticles (AgNPs). First of all, they have strong antimicrobial activity and are widely used in various medical and general applications, among others, in cosmetics, odour resistant textiles etc. The aim of this study was to compare effect of AgNPs and gold NPs (AuNPs) on histone H3 posttranslational modifications. Histone molecule posttranscriptional modifications are responsible for chromatin compaction and repackaging. Two cell lines: A2780 and 4T1. Both cell lines are widely used as a model cells of human ovarian carcinoma and murine mammary carcinoma, respectively. The cells were exposed to AgNPs coated with citrate (AgNPs(cit) or PEG (AgNPs(PEG), or AuNPs and thereafter the histone H3 acetylation on Lys9 and H3 methylation on Lys4, Lys9, Lys29 was investigated. All NPs tested decreased H3 methylation, while no effect was observed for H3 acetylation. Modification of histone H3 methylation dependent on type of NPs used, its coating, site of methylation and cell line used. In conclusion, whereas a simple comparison of different NPs in one particular cell line gave similar results, the comparison between cell lines revealed additional factors that might affect cellular response to nanoparticles. This work was supported by National Science Centre grant No. 2014/15/B/NZ7/01036 (MK, LKS), statutory funding for INTC (BS) and IRH (MK, LKS).

27-POS Board 27

EXTENDING CENTRAL DOGMA ONE STEP FURTHER: INSERTION OF MEMBRANE PROTEINS

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The conversion of a protein structure from a water-soluble to membrane-inserted form is one of the least understood cellular processes. Examples include the cellular action of various bacterial toxins and colicins, tail-anchor proteins and multiple proteins of the Bcl-2 family, bearing pro-apoptotic and anti-apoptotic functions. In our lab we study anti-apoptotic regulator Bcl-xL and diphtheria toxin translocation (T) domain. The latter undergoes conformational change in response to endosomal acidification, inserts into the lipid bilayer and translocates its own N-terminus and the attached catalytic domain of the toxin across the membrane. Our goal is to describe at the molecular level the mechanisms of protonation-dependent conformational switching of the T domain, which serves as a model for membrane insertion/translocation transitions of structurally related proteins (e.g., Bcl-xL, Bax and other apoptotic regulators). Here we present our progress toward this objective, including structural, kinetic and thermodynamic characterization of the insertion pathway of the T domain using both experimental (e.g., Fluorescence Correlation Spectroscopy, FRET, Depth-Dependent Fluorescence Lifetime Quenching, NMR, X-ray crystallography, HD exchange Mass Spectrometry) and computational approaches (e.g., All-Atom Molecular Dynamics Simulation).
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28-POS Board 28

THREE-DIMENSIONAL HELICOIDAL MESOSCOPIC MODEL FOR DNA WITHIN THE FRAMEWORK OF THE TRANSFER INTEGRAL TECHNIQUE

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The Peyrard-Bishop model is a two-dimensional physical statistics method proposed in 1989 for calculating thermodynamic properties of DNA and RNA molecules. This model can be used with several theoretical techniques such as molecular dynamics and path integrals, but its main application still lies within the original method which is the transfer-integral technique. It also used to predict hydrogen bonds and stacking interaction in oligonucleotides from melting temperature experiments, complementing experimental techniques such as NRM and X-ray diffraction. One of the key simplifications of the model is to ignore the helical structure of DNA, nevertheless it has been highly successful in predicting melting temperatures and other important properties of DNA. Here, we add the missing helical torsion to the original 2D Hamiltonian while maintaining the existing framework of numerical techniques for solving the classical partition function. We introduce some approximations to avoid an excessive increase in degrees of freedom which would make the problem intractable. This allows us to use the same transfer integral technique for both the 2D and 3D models. The 3D model shows similar anti-crossings in the eigenvalue spectra as the 2D models leading to very sharp increases in the average strand displacement. The approximations introduced in the 3D model were validated by comparing them with the numerical integration of the exact 3D Hamiltonian. With this new 3D approach we are now able to use mesoscopic models to investigate structural properties of DNA such as the helical rise distance and torsion angle from melting temperatures, in the same way as we obtained hydrogen bonds from the 2D model. Funding: Capes, CNPq and Fapemig.

29-POS Board 29

SINGLE-MOLECULE INVESTIGATION OF NUCLEOSOME ENGAGEMENT BY HISTONE METHYLTRANSFERASE PRC2

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Epigenetic marks play a key role in the regulation of gene expression. These post-translational modifications contribute to heterochromatin and euchromatin states, which control gene expression profiles that can determine cellular differentiation and development. Failure to maintain the proper epigenetic landscape during cell differentiation and division can result in disease states such as cancer. The players that mediate the maintenance of these reversible chromatin marks are often essential proteins in the cell. Uncovering the mechanisms of these molecular complexes and their dynamic interplay with chromatin is imperative for understanding how epigenetics contribute to cellular homeostasis. To this end, I have developed an optical-tweezers-based experimental platform for analyzing chromatin dynamics and multiplexed chromatin modifications. Specifically, I am investigating the interaction between the histone H3K27 methyltransferase, Polycomb Repressive Complex 2 (PRC2) and polynucleosomal substrates harboring either wildtype histones or oncogenic mutants, containing either naïve or methylated histone tails, and with different linker DNA lengths. I found that PRC2 has multiple binding modes on nucleosomal DNA. Importantly, the single-molecule data suggest that PRC2 has the ability to bridge non-adjacent nucleosomes. This finding has fundamental implications for the mechanism of spreading of heterochromatic marks on chromatin substrates.

FRIDAY, JULY 19
POSTER SESSION II
14:45 – 16:00
Cafeteria, 1st Floor

All posters are available for viewing during all poster sessions; however, below are the formal presentations for Friday. Presenting authors with odd-numbered poster boards should present from 14:45 – 15:22 and those with even-numbered poster boards should present from 15:22 – 16:00. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Lotierzo, Mayra	**30-POS**	**Board 30**
Machado, Fredderico	**31-POS**	**Board 31**
Machin, María Belén	**32-POS**	**Board 32**
Maturana, Patricia del Valle	**33-POS**	**Board 33**
Matute, Ricardo	**34-POS**	**Board 34**
Medina, Luciana	**35-POS**	**Board 35**
Mikkelsen, Evelyn	**36-POS**	**Board 36**
Molina, Gissela	**37-POS**	**Board 37**
Morten, Michael	**38-POS**	**Board 38**
Mustafa, Golam	**39-POS**	**Board 39**
Nimigean, Crina	**40-POS**	**Board 40**
Ontiveros, Mallku	**41-POS**	**Board 41**
Ovando-Vázquez, Cesaré	**42-POS**	**Board 42**
Palomino Cardenas, Andrea Carmeli	**43-POS**	**Board 43**
Perez Moron, Nestor Alonso	**44-POS**	**Board 44**
Pillco Valencia, Yeny	**45-POS**	**Board 45**
Rios Colombo, Natalia	**46-POS**	**Board 46**
Robeson, Luka	**47-POS**	**Board 47**
Rouzina, Ioulia	**48-POS**	**Board 48**
Salazar, Paula	**49-POS**	**Board 49**
Sanchez-Castro, Ana	**50-POS**	**Board 50**
Scanavachi, Gustavo	**51-POS**	**Board 51**
Silva, Marcus Vinícius	**52-POS**	**Board 52**
Solis-Sarmiento, Julio	**53-POS**	**Board 53**
Sosa, Robert	**54-POS**	**Board 54**
Vondrasek, Jiri	**55-POS**	**Board 55**
Weng, Xiaoli	**56-POS**	**Board 56**
Zegarra, Victor	**57-POS**	**Board 57**
Zevallos Aliaga, Dahlin	**58-POS**	**Board 58**
Ziraldo, Riccardo	**59-POS**	**Board 59**
Meireles, Fernando Augusto	**60-POS**	**Board 60**

Posters should be set up the morning of July 18 and removed by noon July 21.

30-POS Board 30

THE INTERFERENCE OF ORGANIC SOLVENTS IN THE CRYSTALLINE STRUCTURE OF NONIONIC CUBOSOMES

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[1]USP, São Paulo, Brazil

Cubosomes are nanoparticles composed of a specific combination of some types of amphiphilic lipids, such as phytantriol, and a nonionic polymer used as a stabilizer for the cubic phase. They have a high hydrophobic area and potential for the drug delivery system. Due of their unique structure, these nanoparticles possess the ability to incorporate highly hydrophobic drugs. A challenge for the encapsulation of hydrophobic molecules is the use of organic solvents in the sample preparation process. In this study we performed the Bottom-Up protocol for the production of cubosomes. To investigate the structural influence of the organic solvents, Small Angle X-ray Scattering (SAXS), Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) were used. The phytantriol cubosomes have a crystallographic cubic phase Pn3m with lattice parameter at ~ 6.83 nm and size of ~ 220 nm. Acetone presented interference in the structure of the nanoparticles in concentrations 1: 1 and 1: 2. Chloroform interacted with the nanoparticles, destroying them at even lower concentrations, from 1: 500. Ethanol destroyed the cubosome structure from the 1: 5 concentration. The octane presents a transition from cubic to hexagonal phase in concentration 1:35. DMSO interfered in the structure only at concentrations 1: 2 and 1: 1. All proportions presented were calculated by volume. The organic solvents tested are suitable for the encapsulation of hydrophobic drugs in the cubosome, but at high concentrations they destroyed the cubic structure of the nanoparticles.

31-POS Board 31

EFFECT OF CHARGE REGULATION ON THE INTERACTION POTENTIALS OF PROTEINS

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[2]Universita Politecnica delle Marche, Department of Life and Environmental Sciences, Ancona, Italy

Protein Charge regulation is a subject that has been studied by various researchers groups around the world. The main idea of this methodology developed in the 50's is to consider a possible fluctuation in the value of the charges on the protein surface, generating a charge distribution not constant in this ensemble. This distribution may be affected by the presence of other macromolecules, such as proteins and polymers, or even due to changes in the medium, such as the addition of salts in the solution or pH variation. The aim of this study is to observe the effects of charge regulation from protein-protein interaction potentials using SAXS technique.We chose Random Phase Approximation (RPA) the closure relation to solve the Ornstein-Zernike equation. In this closing relation, the total potential of interaction between the proteins is divided in two contributions: Potential of reference, given by the potential of hard sphere; Potential of perturbation, given by Coulomb potential, Kirkwood potential (coming from the charge regulation theory) and attractive potential Yukawa type. Simulations were performed for Bovine Serum Albumin and Lysozyme and compared with experimental measurements. For the former, the term of charge regulation did not significantly influence the attractive potential (Yukawa type). For pHs 4 and 5, for example, despite the inclusion of the variance term of the charge, the value of potential at the contact, J, was not changed. As for lysozyme, in the pH's far from the isoelectric point (pI), the contact potential had its value diminished, thus showing that the regulation of charges may be an important factor for this protein. Due to these results, the hypothesis of charge regulation will be tested more clearly for proteins of smaller size, such as cytochrome C.

32-POS Board 32

DOXYCYCLINE DERIVATIVES BINDS EFFICIENTLY TO ALPHA-SYNUCLEIN FIBRILS: TOWARDS NOVEL POSITRON EMISSION TOMOGRAPHY PROBES

María Belén Machin[1]; Julian Sandobal[2]; Gustavo Messina[2]; Guillermo Casale[2]; Juan E Ferrario[3]; Maria Bastianello[4]; Oscar Varela[5]; Cesar Avila[1]; Rosana Chehin[1];
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[4]Cemic, Hospital Universitario- CONICET, Ciudad Autónoma de Buenos Aires, Argentina
[5]Centro de Investigación de Hidratos de Carbono (CIHIDECAR), UBA- CONICET, Ciudad Autónoma de Buenos Aires, Argentina

The results of several studies have shown that the evolution and prognosis of Parkinson's disease (PD) correlates with α-synuclein (AS) aggregation in brain. This information indicates that to monitor progression of the patient with PD by positron emission tomography (PET), a specific in vivo neuroimaging marker would be needed to label the α-synuclein fibers. However, nowadays, no PET probes are available for selective detection of pathological α-synuclein in this disorder. Objective: The aim of this study was to evaluate novel chemical entities that could have high affinity and selectivity for AS aggregates and which could serve as compounds for PET radiotracers development. We have previously reported that doxycycline can bind α-synuclein aggregates but not monomeric α-synuclein. For this reason, we propose the doxycycline and novel synthetic analogues as candidates for α-synuclein imaging PET radiotracers. Methods: Doxycycline analogues were synthesized chemically from doxycycline, called DA-3, DA-4 and DA-5. The binding affinity of these compounds to α-synuclein fibrils was determined by competition binding assays using thioflavin T dye. K_i values were calculated from EC_{50} values using the equation $K_i = EC_{50}/ (1 + [\text{radioligand}]/ K_d)$. Equations were fitted to the experimental data by nonlinear regression. Results: In vitro binding assays demonstrated that these compounds have affinity for insoluble AS filaments. DA-4 and DA-5 show more affinity than doxycycline, in contrast with DA-3. Conclusions: These results suggest that DA-4 and DA-5 would be promising candidates as PET α-synuclein imaging radiotracers. The data described here could provide valuable information for the design of new doxycycline probes which bind preferentially to α-synuclein fibrils.

33-POS Board 33

UNRAVEL THE WAY OF ACTION DE NOVO DESIGNED PEPTIDES IN GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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Antimicrobial peptides are small molecules that display antimicrobial activity against a wide range of pathogens. In previous work, by using model membranes we studied Peptide 6 that show no antimicrobial activity and Peptide 6.2 which exhibited antibacterial activity. In the present work, we aimed to unravel the way of action of theses peptide by studying its interaction with bacteria using Escherichia coli and Staphylococcus aureus.Zeta Potential experiments showed that both peptides were able to interact with a bacterial envelope. However, the effects on P6.2 were much more noticeable in both bacteria. Interesting, besides the CIM obtained for P6.2 were similar on both bacteria, the affinity seems to be higher in the case of E.coli.The ability of both peptides to disrupt the bacterial membrane was also studied. In the case of E.coli, besides both peptides were able to damage the outer membrane (OM), 5 times concentration of P6 was needed in order to obtain comparable results than those obtained with P6.2. Also, P6.2 exhibited faster kinetics of damage. The same behavior was obtained when internal membrane disruption was evaluated. When permeabilization of the cytoplasmic membrane of S.aureus was evaluated, again P6.2 exhibit higher and faster damage. When results were compared in both bacteria, E.coli showed faster kinetics and a lower amount of peptide to obtain the full permeabilization. Those results could be explained due to the OM in E.coli its higher exposure to the media whereas in S.aureus peptides requires pass through peptidoglycan to reach the cytoplasmic membrane. Also, these difference could explain the differences observed in Zeta potential. All data put together allows postulating, in a physiologic model, that the lower affinity of P6 for bacterial envelope results in a minor final concentration of the peptide in the bacterial membrane unable to trigger the antimicrobial activity.

34-POS Board 34

EXPLORING THE ROTATION OF THE GAMMA SHAFT OF F1-ATPASE IN SINGLE-MOLECULE EXPERIMENTS

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Single-molecule imaging experiments provide information that is not available from ensemble experiments. We are interested in the interpretation of dynamical studies imaging and manipulation in F1-ATPase single-molecules. One key question that has arisen in single molecule stalling experiments is the erratic behavior a rotor angle of 55° between the binding and hydrolysis dwell angles of 0 and 80°, respectively. In our previous theoretical work, we used the elastic property of the rotor-stator structure to treat the experiments on controlled rotation. Our modelling suggests that there has to be a change in the bonding network, for example, of hydrogen bonds, as the system transitions between the two dwell points, perhaps at 55°, as indicated by an unusual stalling behavior around that angle. In the present work, we performed full-atomistic molecular dynamics (MD) simulations on the F1-ATPase to explore the rotation of the gamma shaft of F1-ATPase, thus capturing the main conformational changes from an “open” to a “closed” conformation of the catalytic beta subunits, which are associated to the rotation of the gamma shaft of F1-ATPase in single-molecule experiments. Acknowledgements R.A.M. acknowledges the FONDECYT Grant No. 1181260. This work used the Extreme Science and Engineering Discovery Environment (XSEDE), which is supported by National Science Foundation grant number ACI-1548562.

35-POS Board 35

DOXYCYCLINE INDUCES A LESS TOXIC TAU AGGREGATED STRAIN: A NOVEL TARGET FOR REPURPOSING THIS ANTIBIOTIC AS NEUROPROTECTOR.

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Objective: Tauopathies are a diverse group of neurodegenerative diseases characterized by a progressive deposition of abnormally phosphorylated tau protein aggregates, in characteristic brain regions. Tau can adopt multiple propagating conformers in vitro, nowadays called "strains".Based on doxycycline's neuroprotective effect reported for a Parkinson disease model, we explored whether doxycycline is able to interact with tau.For this reason, we studied if doxycycline was able to induce changes in tau amyloid aggregation process and phosphorylation patterns. The neuroprotective effect of doxycycline was evaluated on an in vivo model of tauopathy.Methods: For the in vitro assays, we used the heparin-induced tau fibrillation model; to perform Transmission Electron Microscopy (TEM), Thioflavin T fluorescence spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and protease digestion of aggregated tau species; in the presence and in the absence of doxycycline. We performed in vitro phosphorylation of tau using Glycogen synthase kinase-3 beta (GSK3-beta). And to evaluate doxycycline neuroprotective effect we used a C. elegans tauopathy model.Results: Our results show that doxycycline interacts with tau inducing the formation of morphologically differentiated species, observed by TEM. These novel species display different beta-sheet structural arrangement according to FTIR studies and have different protease digestion pattern.Doxycycline does not interfere on the GSK3 beta activity, as tau phosphorylation pattern remains the same.Our data demonstrate that doxycycline could revert the low mobility phenotype induced by the over expression of human tau by the nematode C. elegans.Conclusions: Our results reveal that doxycycline shows a neuroprotective effect over a C. elegans tauopathy model, and the mechanism of toxicity reduction would be the interference over tau amyloid fibrillogenesis. The presence of doxycycline might induce a novel and less toxic tau aggregation strain, making this antibiotic a good candidate to be repurposed as neuroprotector for tauopathies.

36-POS Board 36

NTP-ASE ACTIVITY OF ZIKA VIRUS NS3 HELICASE AND ITS COUPLING WITH RNA UNWINDING ACTIVITY

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[3]Fundación Instituto Leloir-IIBBA-CONICET, Ciudad de Buenos Aires, Argentina

Zika virus (ZIKV) nonstructural protein 3 (NS3) is a motor protein that unwinds double-stranded RNA driven by the free energy derived from the hydrolysis of nucleoside triphosphates (NTPs). The aim of this work is to characterize the NTPase activity of ZIKV NS3 helicase, to determine the specificity order for the substrates: ATP, CTP, GTP and UTP, and to establish the ability of these four substrates to drive the unwinding activity. To initiate the characterization of NS3 NTPase activity we studied the hydrolysis of NTPs under steady-state conditions. Initial rates of reaction were determined measuring the Pi released from NTP hydrolysis. The substrate curves obtained for ATP, GTP, CTP and UTP were, in all cases, well described by hyperbolic functions whose parameter values (k_{cat} and K_M) were obtained by non-linear regression analysis. The order of specificity of NS3 for these nucleotides, which was evaluated according to the value of the ratio k_{cat}/K_M, was ATP > GTP > CTP ≅ UTP. Additionally, different nucleotides were tested as substrates for the RNA unwinding activity of NS3 helicase. RNA unwinding reactions were evaluated by fluorescence spectroscopy using fluorescently labeled RNA oligonucleotides. Results indicate that NS3 helicase acts as an energy transducer using any of the four nucleotides tested as substrates. That is, in all four cases the helicase couples its catalytic properties (NTPase activity) with the ability to perform the mechanical work of translocation along single stranded RNA and unwinding of double stranded RNA.

37-POS Board 37

EFFECT OF NATURAL PLANT EXTRACTS IN THE NEURAL DEVELOMENT OF ZEBRAFISH EMBRYOS

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Natural products used by Peruvian traditional medicine are a potential source of active ingredients for the development of new drugs. The present study focused on the evaluation of extract 7158, obtained from a UPCH collection of native plants used in Peruvian traditional medicine. This plant extract has been shown to produce psychoactive effects in behavioral tests carried out in mice. Due to the numerous advantages of the zebrafish as a powerful experimental model in vivo, in the present study we began the analysis of the biological activity of the plant extract 7158 using zebrafish embryos. The analysis included pharmacological tests, and the use of light field and fluorescent microscopy to analyze the neuroanatomical and cellular phenotypes induced by the extract. These tests revealed lethal doses of the whole extract between the concentrations of 2 to 10 μg/ml depending on the genetic background of the embryos used, and various morphological alterations in structures of the nervous system. Using confocal microscopy, we discovered a neuroanatomical phenotype related to axonal development at 1 and 2 dpf, and an early cellular phenotype that was characterized by alterations of the blastomeres size and the nuclear DNA distribution. These experiments demonstrate the applicability of zebrafish embryos to study the cellular basis of neurophysiological effects induced by natural products of relevance to the treatment of mental disorders.

38-POS Board 38

SINGLE-MOLECULE IMAGING REVEALS NOVEL INTERACTIONS IN THE ASSEMBLY KINETICS OF THE HUMAN NHEJ REPAIR MACHINERY

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Broken chromosomes, known as double strand breaks (DSBs), are arguably the most dangerous form of DNA damage to the cell; and their aberrant repair can cause massive genomic rearrangements, accelerated tumorigenesis and cell death. Nonhomologous end joining (NHEJ) is the main pathway for the repair of DSBs in mammalian cells, a process facilitated by the assembly of a multicomponent repair machinery. It relies on the efficient detection of broken DNA ends by the heterodimer Ku and the formation of a stable multi-protein complex that is composed of several enzymes and structural proteins, which mediates the synapsis, alignment and ligation of the broken ends. Despite the crucial importance of this complex in facilitating faithful DSB repair, its initial assembly and stability remain undefined. We use single molecule co-localization and smFRET microscopy to measure the assembly of the entire NHEJ complex and associated factors on DNA ends, and determine how its stability depend on various interaction among the different repair factors and DNA substrates. In contrast to current models, our studies show that the assembly process is highly dynamic and reversible, with the core NHEJ proteins exhibiting a novel cooperativity when assembling on exposed DNA ends. Importantly, increased molecular crowding reinforces the stability of these proteins assembled on DNA. Overall, our study directly correlates the stability of the pre-synaptic complex and the efficiency of repair. Thus, our approach of monitoring the formation of individual synapses during DNA repair in real time offers novel mechanistic insights into both the successful NHEJ repair and the dysfunctional regulation of DSBs.

39-POS Board 39

SHORT LOOPED DNA AS A FORCE TRANSDUCER: CONVERSION OF SINGLE MOLECULE FRET SIGNAL INTO FORCE INFORMATION

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A multiplexed, high throughput single-molecule force sensor and transducer concept has been developed that converts fluorescence signal into force information via single molecule Förster resonance energy transfer (smFRET). A double-stranded DNA (dsDNA) loop has been formed by bridging the ends of a ~100 base pair (bp) long dsDNA with a nucleic acid secondary structure (NAS), such as a hairpin or a G-quadruplex (GQ). The looped dsDNA generates a tension across the NAS and unfolds it when the tension is high enough. The FRET efficiency between donor and acceptor (D&A) fluorophores placed across the NAS reports on its folding state. As proof-of-principle measurements, 70 bp, 90 bp and 110 bp long dsDNA constructs were bridged by a DNA hairpin and KCl was titrated to change the tension across the DNA hairpin. Later, the interactions of a GQ structure formed by thrombin binding aptamer (TBA) with a destabilizing protein, Replication Protein A (RPA), and a stabilizing small molecule, an oxazole telomestatin derivative, were studied while the TBA-GQ is maintained under tension by a 110-bp long looped dsDNA. The force required to unfold TBA-GQ was independently investigated with high-resolution optical tweezers (OT) measurements that established the relevant force to be a few pN, which is consistent with the force generated by the looped dsDNA. Since hundreds of such molecules could potentially be imaged simultaneously, it is possible to perform high-throughput force measurements with single molecule sensitivity. The proposed method enables studying NAS, protein, and small molecule interactions using a highly-parallel FRET-based assay while the NAS is kept under an approximately constant force.

40-POS Board 40

CYCLIC NUCLEOTIDE-GATED ION CHANNELS REQUIRE SPECIFIC LIPIDS FOR ACTIVITY

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The function of integral membrane proteins, such as ion channels, depends not only on the physical properties of the surrounding lipid bilayer but also the identity of lipids, which may interact specifically with the protein, allowing for different functional characteristics of the protein. Here, we investigate the lipid requirement of the bacterial cyclic nucleotide-gated (CNG) channel SthK and how the different lipids affect channel function.Density maps from single particle cryo-EM structures of SthK reconstituted into lipid nanodiscs revealed lipids located in close proximity to SthK at the protein-membrane interface. In addition, a lipid density was also identified bound along the outside of the inner pore helix, a location less congruent with it being simply part of the lipid bilayer. The same lipid densities were identified in SthK structures solved in detergent micelles, indicating that the lipids visible in the structure were co-purified with the channel from E. coli.Thin layer chromatography confirmed the presence of lipids bound to SthK in detergent and, together with mass spectrometry on the same sample, we were able to identify these lipids as POPG and cardiolipin. We then systematically analyzed the effects of these lipids on channel activity by using a fluorescence-based stopped-flow flux assay. In the absence of negatively charged lipids SthK shows little to no activity indicating that the co-purified lipids are not sufficient to activate the channel. Increasing the amount of POPG or cardiolipin during the reconstitution leads to significantly increased and stable channel activity. The time course of activation with cAMP and the cAMP concentration needed for half activation were not significantly affected by the type of lipid employed. Our structural data combined with the lipid identification by mass spectrometry and the subsequent functional analyses form the groundwork for future studies on the molecular determinants of protein-lipid interactions in CNG channels.

41-POS Board 41

NATURAL FLAVONOIDS AND CALCIUM TRANSPORT THROUGH BIOLOGICAL MEMBRANES: MOLECULAR TARGET AND MECHANISMS OF ACTION

Mallku Ontiveros[1]; Débora Rinaldi[1]; Mariel Marder[1]; Victoria Espelt[1]; Maximiliano Vigil[1]; Irene Mangialavori[1]; Rolando Rossi[1]; Juan Pablo Rossi[1]; Mariela Ferreira-Gomes[1]; [1]Instituto de Química y Fisicoquímica Biológica (IQUIFIB) UBA-CONICET, Química Biologica, Ciudad Autónoma de Buenos Aires, Argentina

Recently, there has been increasing interest in research on flavonoids from plant sources because of their beneficial properties for health. Several studies have attributed them anti-oxidative and anti-carcinogenic properties. Flavonoids exert their effect in different ways, in particular, there are flavonoids related with changes of intracellular calcium concentration suggesting that they could disturb the calcium transport, including SERCA and PMCA. However, the molecular mechanisms underlying this effect are not yet clear. The present study seeks to investigate the effect of several natural flavonoids on hPMCA4 a P-type ATPase essential for the intracellular Ca^{2+} control in eukaryotic cells. Results obtained with purified PMCA show that some flavonoids inhibited the PMCA activity related to the increase in the number of -OH in the B ring. The most potent inhibitors were quercetin and gossypin. The mechanism of inhibition of these flavonoids was dependent on the Mg^{2+} concentration, suggesting that the real inhibitor is a flavonoid-Mg^{2+} complex. When phosphorylated intermediates (EP) were measured, quercetin led to the increase of EP, which was sensitive to the ADP concentration in the medium, whereas gossypin induced a decrease. These results suggest that gossypin could affect the ATP binding site. We evaluated the effect of these flavonoids monitoring the changes in the cytoplasmic Ca^{2+} in HeK293T and Caco-2 cells that overexpress hPMCA4. Fluorescence microscopy images indicate that both flavonoids distributed widely in the nucleus and the cytoplasm of cells. Results reveal that quercetin and gossypin are incorporated into the cells, showing that the cytoplasmic Ca^{2+} dynamics is affected, probably because PMCA activity is inhibited when quercetin or gossypin pass into the cells. These findings suggest a direct interaction between PMCA cytoplasmic domains and these flavonoids. In conclusion, our results show that PMCA could be involved in the molecular mechanism underlying the flavonoids effects on biological systems.

42-POS Board 42

MODELING METHYLATION IN STACKED GPC-CPG DNA SEQUENCE CONSIDERING BACKBONES

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The incorporation of a methyl group to DNA nucleotides (methylation) is a well know epigenetic regulation phenomena. Methylation can result in gene silencing. The methylation (m) of cytosine base (C) at GpC sequence site, was investigated using first-principles density functional theory calculations to elucidate the changes of the electronic structure. We investigated the bare G-C and G-Cm Watson-Crick base pairs electronic properties. Also, we incorporated a sugar-phosphate to these systems to generate the pG-pC and pG-pCm systems. Beyond these, we investigated the stacked GpC-CpG, GpC-mCpG and GpCm-mCpG systems. Methylated stacked base pairs were analyzed and compared with the non-methylated case (GpC-CpG). The structures were relaxed using conjugated gradient method. We present results for the highest occupied molecular orbital (HOMO), the lowest unoccupied molecular orbital (LUMO) wave functions, charge Mulliken population, and dipole moments. We demonstrate that the backbone or the deoxyribose-phosphate chain plays a crucial role in the spatial distribution of HOMO and LUMO wave functions in the stacked systems. Cytosine methylation results in electronic structure changes, suggesting reactivity modulation affecting the protein binding to methylated DNA, such as transcription factors or histones. Also, dipole moment modification in the studied systems could favor specific protein-DNA interactions due to the methyl group non-polarity, producing water fear DNA regions. Cytosine methylation on the GpC-CpG system results in a stabilization effect. These observations could help explaining the quantum nature of methylation gene silencing.

43-POS Board 43

GENETIC DIVERGENCE OF THE SUBUNIT I OF THE CITOCROMO OXIDASE OF CHARINUS LONGITARSUS (ARACHNIDA: AMBLYPYGI) ENDEMIC SPECIES OF THE CUSCO REGION

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The order Amblypygi is a group of very old arachnids with species reported from the Carboniferous era whose study is quite limited in our continent. In Peru, only three species have been described morphologically: Charinus koepckei, Charinus longitarsus and Heterophrynus elaphus. The environmental degradation of the biosphere of the planet has caused the extinction of species, in some cases even before discovered and described by science. The Peruvian tropical Andes are a critical region for conservation since they contain a great wealth of species susceptible to extinction. Therefore, research and preservation of species, such as Amblypygi, is important because they are extremely sensitive to environmental changes. The collection areas were carried out in the provinces of; La Convención, Calca, Cusco Region during the years 2016 to 2017. The objective of the research is to evaluate the evolutionary molecular history and genetic divergence of the cytochrome oxidase subunit I of C. longitarsus by means of phylogenetic analysis and molecular sequence analysis. The methodology for the design of primers was employed by the NCBI server, "Primer-BLAST". Conclusion, the amplification by PCR reactions, of the cytochrome oxidase subunit I gene is ongoing in several samples collected in La Convención, Cusco. Our preliminary data indicate that the COX-1 gene has the potential to have molecular markers.

44-POS Board 44

IMPLEMENTATION OF A NEW METHOD FOR GRID STEERED MOLECULAR DYNAMICS

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Molecular Dynamic(MD) is a computational technique that was crucial for the development of molecular biology. Nevertheless, the drawback of MD is the high computational cost, in other words, the amount of cores increment at the same time as the number of particles(a core for each 2000 atoms). Therefore, to work with big systems, it is necessary the access of unavailable clusters. For that reason, scientist have develop new methods to resolve this problems , one of them is named Grid Steered Molecular Dynamics(Grid-SMD)[1].The actual technique of Grid-SMD determinates the properties of particles in a rapid-evaluation-grid. This method only consider the electrostatic potential, and evades the Van-der-Waals(VDW) interactions. The present research accomplish an implementation of the VDW potential to the Grid-SMD method to upgrade the molecular description of the technique.To endforce the VDW-Grids, it was created a TCL script to compute VDW energy using the Lennard-Jones potential and the information from CHARMM parameters. The software required for this was VMD to visualize the simulation and NAMD to makes the simulation. The Grid-SMD simulation was develop with the VDW-Grids and the Electrostatic Grid in a multiple grid simulation where the long range force and the short range force was cover.For the testing part, we use the MHC-II system. In the structure, we identify the MHC system and the receptor, a peptide. This simulation showed the bound between the system and the peptide. Furthermore, the time from the simulation was enormously reduced, taking over the system by the VDW-Grids, and making a simulation with the Grids and the peptide. In the other hand, further information needs to be analyze to validate the use of this implementation for interaction studies.

45-POS Board 45

COARSE-GRAINED COMPUTATIONAL SIMULATION OF PHOSPHOLIPID AGGREGATES IN THE PRESENCE OF ANTIMICROBIAL PEPTIDE LL-37

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Antimicrobial peptides (AMPs) are part of the innate immune response found in all living organisms, they have a broad spectrum activity against bacteria, viruses and fungi. This biological compounds in contact with the target microbial membrane can cause cell rupture through several hypothetical mechanisms: barrel, aggregate, carpet and toroidal pore model. The first amphiphilic peptide isolated from human cells is LL-37 and belongs to the cathelicidin family. The AMP LL-37 (LLGDF-FRKSK-EKIGK-EFKRI-VQRIK-DFLRN-LVPRT-ES) has a charge of +6 and an amphipathic alpha-helical structure at physiological pH. Despite several studies, the molecular mechanism of action of LL-37 is still not fully understood, therefore our objective is to investigate the possible mechanism of molecular action of this LL-37 in phospholipid membranes. Experimental measures such as Small-angle X-ray scattering (SAXS) and electronic microscopy suggest that LL-37 destabilizes 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) bilayers and induces the disappearance of multilamellar bilayers and the appearance of micelles in a concentration-dependent manner. We have performed atomistic and coarse-grained MD simulations to bring higher resolution structural information into the experimental picture. We have performed simulations of POPG and POPC bilayer at different concentrations of the peptide to investigate the process of LL-37 induced transition of phospholipidic bilayers. Our simulations show that LL-37 binds to POPG bilayer in a horizontal orientation. At low peptide concentrations of 15:1 (ratio on mass lipid: peptide), LL-37 remains at the headgroup area throughout 3 μs, but above a concentration of 9:1 we observe a slow migration of the peptide to the hydrophobic region of the bilayer. Interestingly, the process appears to take place faster at the atomistic than at the coarse-grained levels, but further sampling is required to verify this claim. We argue that the POPG bilayer is destabilized by LL-37 via a carpet-like mechanism consistent with experimental structural data.

46-POS Board 46

TOWARDS A NEW APPROACH FOR CLASS II BACTERIOCINS: USING SUICIDE PROBES TO STUDY THEIR MECHANISM OF ACTION.

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The study of alternative antimicrobials has become relevant in the last years because of the increasing resistance to common antibiotics. Class II bacteriocins are unmodified membrane-active peptides that act over a narrow spectrum of bacterial targets. They are believed to bind a specific receptor on the membrane that would participate in the formation of a pore, leading to membrane permeabilization and cell death. Objectives: 1) Reveal whether or not the pore structure involves the specific receptor. 2) Study the effect three bacteriocins in some membrane properties using fluorescent spectroscopy. Methods: we designed chimeric peptides fusing the bitopic membrane protein EtpM with different class II bacteriocins: enterocin CRL35, pediocin PA-1 and microcin V. These hybrid proteins EtpM-bacteriocin (also called “suicide probes”) were heterologously expressed in E. coli and E. coli sdaC respectively. We chose E. coli as an expression host because this bacterium is naturally insensitive to enterocin and pediocin, since their specific receptor Man-PTS is not present on its inner membrane. In addition, an sdaC mutant E. coli strain was employed as a receptor-free host for MccV, as it does not express SdaC, the specific membrane receptor for this microcin. The effect of these suicide probes in transmembrane potential and membrane fluidity was also assessed. Results and conclusions: Since the suicide probes kill the expressing host cells, we suggest that the specific receptor is more likeable to act as docking molecule and it could be dispensable for the final step of membrane disruption. These set of chimeric peptides also represent an in vivo system that allows to study the interaction of the bacteriocins with real bacterial membranes, instead of model membranes. In this work we demonstrate how these peptides can depolarize or increase membrane lipids order, even in the absence of the specific receptor.

47-POS Board 47

EXPLORING THE INTERACTION BETWEEN THE SIGNAL PEPTIDE FOR RETICULAR TRANSLOCATION AND THE SEC61 TRANSLOCON USING FORCE SPECTROSCOPY AT THE SINGLE MOLECULE LEVEL

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The Sec61 translocon is a protein transport channel found in the membrane of the endoplasmic reticulum. It allows the translocation of proteins from the cytosol to the reticular lumen, a decisive step in the biosynthesis of most extracellular proteins. These secretory proteins possess a "signal peptide" at their N-terminus, which interacts with the translocon and begins translocation. Mutations in the signal peptide can impede translocation and cause diseases related to the intracellular accumulation of these proteins, indicating an essential role for this signal peptide-translocon interaction in the activation of translocation; however, the binding parameters that characterize these interactions remain to be defined. Single molecule force spectroscopy using optical tweezers was used to measure the force necessary to break the interaction between the signal peptide of Prepro-alpha-factor (PpαF) and Sec61. Dudko-Hummer-Szabo models were applied to the histograms of rupture forces obtained to calculate the mean lifetime of interaction in dependence of the force [$\tau(F)$]. From the latter, the mean lifetime at zero force (τ_0 = 16.7 s) and the parameters of free energy ($\Delta G‡ = 20K_BT$) and distance of the transition state ($\Delta x‡$ = 0.4 nm) of the dissociation process at zero force were obtained. Considering that the translocation rate is 1.1 amino acids/s, it is concluded that the interaction between the signal peptide and the translocon grants sufficient time to allow this process to happen. Next, we will compare these parameters with those from an Ala13Glu mutation in the hydrophobic region of the signal peptide of PpαF, which was reported to decrease the translocation rate up to 50 times, to study whether the loss of translocation efficiency may be related to a change in the dissociation parameters.

48-POS Board 48

SELF-REGULATION OF SINGLE-STRANDED DNA WRAPPING DYNAMICS BY E. COLI SSB MEDIATED THROUGH COMPETITIVE BINDING.

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Single-stranded DNA (ssDNA) binding proteins (SSBs) rapidly bind and protect transiently formed ssDNA intermediates during genomic maintenance and replication processes. E. coli SSB (EcSSB), a homotetramer that wraps the ssDNA in multiple topologies, is a model protein used to study SSB function. Here, we characterize the collective binding dynamics of EcSSB to a long ssDNA template using optical tweezers. We show that EcSSB binds to ssDNA in a biphasic manner where EcSSB initially wraps the ssDNA, and then unwraps from the ssDNA as the substrate becomes saturated with protein. A general two-step kinetic model in which the binding state of EcSSB is regulated through ssDNA occupancy describes the observed competitive binding dynamics. Our results uncover an unwrapped SSB binding mode that is confirmed using AFM imaging. In this mode EcSSB binds ssDNA segments as short as 8 nt and is thus signified as $(SSB)_8$. Furthermore, while EcSSB slowly dissociates from ssDNA through increased ssDNA tension, salt concentration, and displacement by RecA filaments, EcSSB wrapping stimulates rapid dissociation of neighboring proteins on a saturated ssDNA substrate. Taken together, our results suggest a mechanism by which tightly wrapped EcSSB dissociate from ssDNA to self-regulate the protein density ensuring its transient role during genomic maintenance and replication. In addition, the proposed mechanism provides insights on biological implications of the distinct EcSSB binding topologies significantly enhancing the understanding of SSB function.

49-POS Board 49

ENRICHED POLYPHENOL EXTRACTS DERIVED FROM GRAPE POMACE EXERT DIFFERENTIAL INHIBITION ON MEMBRANE-BOUND AND SOLUBLE ACETYLCHOLINESTERASE ACTIVITY

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In this work, the possible inhibition of acetylcholinesterase by phenolic compounds present in methanolic extracts of grape pomace was studied. For that purpose, the human erythrocyte acetylcholinesterase (AChE-E), a membrane-bound variant, was chosen as the model enzyme. The phenolic compounds were significantly less effective in inhibiting AChE-E in its soluble form. To gain a better understanding of the molecular interactions between phenolics with membrane lipids, several biophysical measurements were performed, such as quenching of octadecyl rhodamine B (R18) fluorescence by increasing concentrations of phenolic compounds. The quenching of R18 allowed us to assess the interaction of phenolic compounds with the interfacial region of the membrane. Besides, changes in osmotic resistance of erythrocytes in the presence of polyphenols were also tested. This analysis was complemented with the study of the protective effect of the bilayer to micelle detergent-mediated transition of liposomes. The interaction of each phenolic compound with the lipid moieties of membranes was assessed by infrared spectroscopy, using lipids purified from erythrocyte ghosts and synthetic lipids. To sum up, it was shown that these flavonoids may bind to the red blood cell membrane, which in turn may improve the interaction between polyphenols and AChE. Actually, the differential inhibition of the membrane-bound and the soluble AChE variants by the pomace polyphenols could be explained as either a direct and differential interaction of the phenolic compounds with these isoforms or as the consequence of an indirect effect. In the latter scenario, the enzyme may be inhibited upon alteration of the lipid phase. Even though we cannot rule out a direct interaction of phenolic compounds with AChE, our results allow us to conclude that the polyphenols tested did interact with lipids and this could explain, at least partially, the inhibition of the AChE activity.

50-POS Board 50

APTAMER-MEDIATED INHIBITION OF BACTERIAL INITIATION FACTOR IF3

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Bacterial translation initiation factor IF3 is an essential protein that promotes 30S initiation complex (IC) formation. IF3 binds the 30S ribosomal subunit and modulates the fidelity and speed of the codon-anticodon interaction between the mRNA and initiator tRNA. Although IF3 functions are well understood, the role of the N terminal domain (IF3N) of the factor remains debated. Here, we use Systematic Evolution of Ligands by EXponential enrichment (SELEX) to develop aptamers for IF3N and biophysical methods to study aptamer-mediated inhibition of 30S IC assembly. Purified IF3N from Escherichia coli was used as target for aptamers selection. Five potential aptamers were identified and chemically synthesized. Pull-down assays using target-immobilized magnetic particles suggested four molecules as potential binders. Three aptamers showed dissociation constants in the range of 100-400 nM, as assayed by Microscale Thermophoresis (MST). Intramolecular Förster Resonance Energy Transfer (FRET) showed that all three aptamers are able to bind a double labeled IF3; however, only one molecule was able to drastically reduce the speed of IF3 binding to the 30S subunit. The aptamer affected IF1- and IF2-depended conformational accommodations of IF3 on the 30S subunit. Furthermore, the canonical codon-anticodon association appeared to be affected by the aptamer as assayed by rapid kinetics and MST. Altogether, our results suggest that IF3N positioning contributes with the accommodation of initiator tRNA and therefore, with canonical 30S IC formation. Additionally, the experimental scheme presented here provides a solid alternative for the development of new inhibitors of bacterial translation.

51-POS Board 51

THE IMPACT OF OXIDIZED POPC DERIVATIVES ON LIPID BILAYER HYDRATION AND FLUIDITY: INSIGHTS FROM STEADY-STATE AND TIME-RESOLVED FLUORESCENCE METHODOLOGIES

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The presence of oxidized lipids in natural membranes plays a crucial role in several biological dysfunctions, such as cardiovascular disease, Alzheimer's disease and Type II diabetes. Despite intensive study, the impact of oxidized lipids in the membrane physical properties remains elusive. Here,the influence of oxidized lipids obtained by oxidation of POPC (POPC-OOH, PAzePC and POVPC) on the properties of mimetic membranes of POPC was investigated by steady-state and time-resolved fluorescence techniques,at 23 and 37ºC. Remarkably, our results show a decrease in the fluorescence lifetime of TMA-DPH (a lipid/water interface probe) with increasing of mol% of POPC-OOH,reporting an increase in water penetration at the membrane interface. Moreover, from time-resolved anisotropy data of TMA-DPH,an increase in the average rotational diffusion time,$\langle\varphi\rangle$,of the probe was observed, pointing out to an increase in the microviscosity, η,of the environment around the probe. This is compatible with the location of –OOH group near the carbonyl/polar-head region implying a decrease in free volume for probe rotation and a concomitant increase in the surface area of oxidized membranes. Interestingly, a decrease in the limiting anisotropy, r_{oo}, of TMA-DPH was also observed, meaning that the probe rotational dynamics is confined within a larger wobbling cone in the presence of POPC-OOH. These results are also supported by time-resolved emission spectra (TRES) analysis of Laurdan to evaluate the dipolar relaxation in the same vicinity as TMA-DPH. Furthermore, the presence of the truncated PAzePC and POVPC also increased the hydration degree and the area per lipid at the membrane interface,according to the sequence: POVPC>PAzePC>POPC-OOH. These findings are again supported by Generalized Polarization (GP) and TRES analysis of Laurdan. Finally, PAzePC and POVPC only have a significant impact on the microviscosity values at 37ºC but not at 23ºC, suggesting that their truncated chains can only efficiently approach the membrane interface at higher temperatures.

52-POS Board 52

ALTERATIONS IN AGGREGATION KINETICS OF GLYCATED ERYTHROCYTE BY IN VITRO ANESTHESIA

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The erythrocyte membrane has negative electrical charges. When blood flow is slower or null, the erythrocytes form aggregates similar to stacks of coins called rouleaux. In some vascular diseases, such as diabetes, there are other types of globular aggregates called “clusters”, which could be due in part to the reduction of surface charge by glucose effect. On the other hand, previous studies indicate that propofol could alter the hemorheological properties of the erythrocyte membrane, affecting principally diabetic patients. To evaluate this hemorheological effect of anesthesia, we studied the kinetics of aggregation of in vitro glycated blood samples by incubating them with different commonly used anesthetics (Propofol, Remifentanil and Vecuronium Bromide). For this purpose, red blood cells (RBCs) from healthy donors were preincubated with glucose solutions or phosphate saline solution for 5 hours. Subsequently, incubation was carried out with the different anesthetics and their combinations for 30 minutes (Propofol 4 μg/mL whole blood, Remifentanil 10 ng/mL plasma, Vecuronium bromide 0.15 mg/mL plasma). After washing, the RBCs were suspended in autologous plasma at 40%. To determine the aggregation kinetics of RBCs, the “Agregámetro Eritrocitario de Chip Óptico” was used. This instrument was developed by the research group of Biomedical Physics of the Rosario Institute of Physics (CONICET-UNR) and is based on the laser transmission technique analyzing the intensity of light transmitted as a function of time. These data allow to obtain the sylectograms (intensity vs. time) to obtain the characteristic aggregation parameters for each sample. Results show that Aggregation Amplitude of glycated RBCs were altered in comparison with their respective control. Moreover, greater difference is observed in RBCs incubated with vecuronium bromide and the mixture of remifentanil and vecuronium bromide showing that they are aggregated more rapidly. In conclusion, anesthetics can affect the parameters of aggregation kinetics increase the speed of the aggregation process in glycated red blood cells.

53-POS Board 53

RECENT ADVANCES IN THE DEVELOPMENT OF MOLECULAR TOOLS FOR ANDEAN LUPIN (Lupinus mutabilis Sweet)

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Lupinus mutabilis (Sweet), commonly known as tarwi, chocho or lupin, is an important neglected crop of the Andean region. Development of cultivars with good attributes such as low alkaloid content, high protein content, and disease tolerance requires the utilization of modern breeding tools. The present work represents the first approach to the development of genetic markers from the transcriptome and to the mining of genomic resources of L. mutabilis. By looking at the transcriptome and genome sequences we have identified simple-sequence repeat (SSR) markers and genes of agronomic importance for lupin breeding. The outcomes of the present work will be useful in modern genetic studies of Andean lupin.

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54-POS Board 54

DNA WRAPPING IN OPEN TRANSCRIPTION INITIATION COMPLEXES IS THE MAJOR COMPONENT OF THEIR STABILITY

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The interaction of RNA polymerase (RNAP) and a DNA promoter leads to the formation of the open transcription initiation complex (RPo), the major regulatory checkpoint in transcription. DNA wrapping is a common process in RPo formation and despite its potential importance, wrapping has been problematic to study due to the difficulty to separate the initial binding from the ensuing fast wrapping through techniques such as footprinting, fluorescence, and AFM. We investigate wrapping in RPo using optical tweezers and determine its structure using transmission electron microscopy and single particle analysis. Our results indicate that wrapping is reversible, cooperative, and spontaneous, with a change in free energy similar to the change in free energy of RPo formation (~ -13 kcal/mol), indicating that this process is the principal contributor to the complex's stability. The RPo structure at 17 Å resolution reveals that wrapping encompasses position -76 to +18 along the promoter, with an angle of ~245°, and that RNAP alpha subunit C-terminal domains (aCTDs) interact with the proximal and middle upstream promoter sequences. Also, ~80% of the wrapping energy is contributed by aCTDs in interaction with non-specific upstream DNA sequences and we show that the transcriptional modulator ppGpp reduces the extent and strength of wrapping. Finally, we demonstrate a correlation between wrapping-dependent stability and RNAP promoter escape as well as RPo formation.

55-POS Board 55

WORKING FROM A DISTANCE: ENGINEERING ALLOSTERIC CONTROL ON PDZ3 DOMAIN SELECTIVITY FROM ZO-1 PROTEIN THROUGH DOMAIN FUSION

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A central paradigm within structural biology is the concept of domains. A particular domain can be identified and assigned to specific functional or structural properties. Despite the fact that there is a high but limited number of protein domains, they appear in countless combinations. It is plausible to expect that organization of domains can modulate a function via allostery. We present results of our study on PDZ domain to capture external allostery exerted by adjacent domains by means of combination of theoretical and experimental methods. Our study of protein chimeras consisting of PDZ3 from the ZO-1 protein (homo sapiens) and various fusion domains will help to understand the mechanism of allosteric regulation in PDZ3 specificity by means of the character, position or size of the attached domain. We also provide an overview of molecular design on selected chimeras and methodological background for their theoretical description. These results are compared with biophysical and structural characterizations to elucidate the nature of the allostery in these model systems.

56-POS Board 56

SPATIAL ORGANIZATION OF RNA POLYMERASE AND ITS RELATIONSHIP WITH TRANSCRIPTION IN E. COLI

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Recent studies have shown that RNA polymerase (RNAP) is organized into distinct clusters in E. coli and B. subtilis cells. Spatially organized molecular components in prokaryotic systems imply compartmentalization without the use of membranes, which may offer new insights into pertinent functions and regulations. It has been proposed that the formation of RNAP clusters is driven by active ribosomal RNA (rRNA) transcription and that RNAP clusters function as factories for highly efficient transcription. In this work, we examined these hypotheses by investigating the spatial organization and transcription activity of RNAP in E. coli cells using quantitative superresolution imaging coupled with genetic and biochemical assays. We observed that RNAP formed distinct clusters that were preferentially located in the center of the nucleoid and engaged in active rRNA synthesis under a rich medium growth condition. Surprisingly, a large fraction of RNAP clusters persisted under various conditions in which rRNA synthesis was reduced or abolished, and was only significantly diminished when all RNA transcription was inhibited globally. Moreover, the cellular distribution of RNAP closely followed the morphology of the underlying nucleoid under all conditions tested irrespective of the corresponding transcription activity. These results suggested that RNAP was organized into active transcription centers under the rich medium growth condition; their spatial arrangement at the cellular level, however, was not dependent on rRNA synthesis activity and was likely organized by the underlying nucleoid.

57-POS Board 57

HOW THE INITIATING RIBOSOME COPES WITH PPGPP TO TRANSLATE MRNAS

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Stringent response uses (p)ppGpp to reshape the bacterial proteome by acting pleiotropically on RNA and protein synthesis, allowing bacteria to colonize and persist in host environments. In this work, we study a long-lasting paradox of protein synthesis during bacterial stress. Particularly, ppGpp accumulation during bacterial starvation was shown to inhibit protein synthesis; however, a number of genes are activated, and their mRNAs are translated in vivo at high (p)ppGpp concentrations. A model bridging these sets of observations was lacking. Here, we show that the translation Initiation Factor IF2 senses the cellular ppGpp to GTP ratio and regulates the progression towards protein synthesis. Our results indicate that the affinity of GTP and the inhibitory concentration of ppGpp for 30S-bound IF2 vary depending on the programmed mRNA. Highly translated mRNAs enhanced GTP affinity for 30S complexes, resulting in fast transitions to elongation of protein synthesis. Less demanded mRNAs allowed ppGpp to compete with GTP for IF2, stalling 30S complexes until exchange of the mRNA enhances the affinity for GTP. Altogether, our data propose a novel regulatory mechanism at the onset of protein synthesis that tolerates physiological concentrations of ppGpp, and that bacteria can exploit to modulate its proteome as a function of the nutritional shift happening during infection.

58-POS Board 58

PRODUCTION OF MICROCIN J25 ANTIBACTERIAL PEPTIDE VARIANTS AND ITS ACTIVITY TEST IN THE MYCOBACTERIUM TUBERCULOSIS RNAP.

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The success of rifampin as anti-tuberculosis therapy indicates that the inhibition of RNA polymerase (RNAP) of Mycobacterium tuberculosis (MtbRNAP) is an effective strategy, however, resistance to this antibiotic is increasing. This is why the finding of new inhibitors for MtbRNAP would be a great contribution to the fight against tuberculosis. Microcin J25 (MccJ25) is an antibiotic peptide whose main molecular target is the RNA polymerase of Escherichia coli (EcoRNAP). Biochemical studies have determined that MccJ25 binds and obstructs the secondary channel of EcoRNAP and have identified the regions of MccJ25 that are responsible for this interaction. They also demonstrated that MccJ25 inhibits RNA polymerases from bacteria phylogenetically related to E.coli, but has no inhibitory activity on the RNAPs of bacteria related to the Mycobacterium genus. Computational analysis indicates that the structure of the secondary channel of EcoRNAP and MtbRNAP is generally conserved but with some amino acid sequence differences that might explain their different MccJ25 susceptibility. Here, I propose to produce variants of MccJ25 through randomly modifying its amino acid sequence in two specific locations that are important for its interaction with residues present EcoRNAP but absent in MtbRNAP. The variants will be produced by PCR- mediated overlap method, and the resulting products will be inserted in a plasmid for the intracellular expression within a reporter bacterium. The reporter bacteria express MtbRNAP, and its activity inside the cell is measured thanks to a GFP gene that is expressed under the control of a promoter recognized by the mycobacterial enzyme. This study intends to serve as prove of principle for the use of a reporter bacterium for high-throughput assays of new inhibitors. Although the variants of MccJ25 to be produced are not intended to serve directly as anti-tuberculosis drugs, their discovery and characterization could contribute to the development of new therapies.

59-POS Board 59

SINGLE-MOLECULE TOPOLOGICAL CHARACTERIZATION OF CIRCULAR DNA BY TETHERED-FLUOROPHORE MOTION

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We present a method to determine the topology of single circular DNA molecules as a function of knot type, K, and linking number, Lk. Plasmid DNAs bearing fluorophores conjugated at specific sequence positions are immobilized to glass coverslips and analyzed using using total-internal-reflection fluorescence (TIRF) microscopy. Using topoisomer pools and specific DNA-knot types, respectively generated by topoisomerase-I relaxation and Cre site-specific recombination, we are determining the characteristic fluorophore-emission image distributions belonging to particular DNA topologies. Comparing observed spatial fluorophore distributions with those obtained by Monte Carlo simulation, we seek to show that variations in plasmid topology lead to measurable, single-molecule-level differences in the spatial footprint of conjugated fluorophores.

60-POS Board 60

IN SILICO STUDY OF VIP AND PACAP INTERACTIONS WITH THEIR MEMBRANE RECEPTORS AIMING AT NEUROPEPTIDE OPTIMIZATION FOR HIV-1 INHIBITION IN MACROPHAGES

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Vasoactive Intestinal Peptide (VIP) and Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) are neuropeptides of the glucagon/secretin family of high similarity. They act through interactions with the Vasoactive Intestinal Peptide Receptors 1 and 2 (VPAC1 and VPAC2) and the Pituitary Adenylate Cyclase-Activating Polypeptide Receptor type 1 (PAC1). In the scope of their functions, recent studies have demonstrated the capacity of VIP and PACAP to inhibit replication of HIV-1 in infected macrophages partially. This work aims to build, "in silico", the six possible peptide-receptor interaction systems and to identify hot-spots responsible for their stability. Molecular Modeling, Docking and Molecular Dynamics methods will be applied. Once the dissociation constants of the ligands are estimated, the most promising system will be optimized through Protein Engineering aiming at developing a mutant neuropeptide with higher affinity for one of the receptors, thus increasing its anti-HIV potential. Results from the PACAP/PAC1 system, obtained through comparative modeling using the software MODELLER 9.21, suggest the formation of several H-bonds and salt bridges between the C-terminal of the neuropeptide and the membrane receptor. The analysis package from the software Visual Molecular Dynamics (VMD) shows that PACAP residues Arg-30, Lys-32, Arg-34 and Asn-37 form H-bonds and Lys-29, Lys-32 and Arg-34 form salt bridges. The relevance of some of these interactions was indicated by hot-spots prediction server KFC2, which identified residues Ala-18, Tyr-22, Arg-30, Tyr-31, Gln-33, Arg-34, Val-35, Lys-36, Asn-37 and Lys-38 as hot-spots. Even though the interactions in the static model may hint to the actual "in vivo" interface conformation, molecular dynamics are necessary to determine the stability of these bonds.