The 7th Chesapeake Bay Area Single Molecule Biology Meeting

SATURDAY, MAY 13, 2023

Homewood Campus, Mudd Hall

Organizers: Christian Kasier; Tatjana Trcek; Bin Wu; Jie Xiao, JHU

JOHNS HOPKINS
UNIVERSITY

CbaSmB Committee

Songon An, UMBC

Inhee Chung, GWU

Hoi Sung Chung, NIDDK

Adrian Ferré-D'Amaré, NIH

Christian Kaiser, JHU

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Daniel Larson, NIH

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Rodrigo Maillard, Georgetown

Keir Neumann, NIH

Antonina Roll-Mecak, NIH

Tatjana Trcek, JHU

Bin Wu, JHU

Jie Xiao, JHU

SCHEDULE

Saturday, May 13, 2023 / Mudd Hall, Room 26

	Meeting Registration
9:00-9:25am	Check in, Poster set up
9:25-9:30	Opening Remarks **Jie Xiao, Ph.D.**, Johns Hopkins University
9:30-10:00am	Keynote speaker **Sua Myong, Ph.D.,** Johns Hopkins University
10:10 - 10:25 AM	**Inhee Chung, Ph.D.,** George Washington University, MAxSIM: Multi-Angle-Crossing Structured Illumination Microscopy with Height-Controlled Mirror for 3D Topological Mapping of Live Cells
10:30 - 10:45 AM	**Do-Hyeon Kim, Ph.D.,** Johns Hopkins University A Super-photostable Organic Dye for Long-term Live-cell Single-molecule Imaging
10:50 - 11:15 AM	Coffee break
11:15 - 11:30 AM	**Shuaixin He,** Johns Hopkins University Cas9 cleavage-induced transcription repression requires VCP-proteasome-dependent RNAP2 removal
11:35 - 11:50 AM	**Bok-Eum Choi, Ph.D.,** NIH/NIDDK Tether-free single-molecule FRET reveals conformational heterogeneity and dynamics of gRNA during Cas9 ribonucleoprotein assembly
11:55 - 12:10 PM	**Maggie Rodgers, Ph.D.,** NIH/NIDDK Small RNAs and Hfq capture unfolded RNA target sites during transcription
12:10 - 1:10 PM	Lunch, Poster Review, and CbaSMB committee meeting
1:10 - 1:25 PM	**Ian Morgan, Ph.D.,** NIH Unraveling Type II Topoisomerase Inhibition by Antibacterials at the Single-Molecule Level

1:30 - 1:45 PM	**Brett O'Brien,** Johns Hopkins University Single molecule force spectroscopy reveals structural heterogeneity in single repeat expanded HTT mRNAs
1:50 - 2:05 PM	**Myung Hyun Jo, Ph.D.,** Johns Hopkins University Overstretch tension sensors reveal integrin force loading rates
2:10 - 2: 25PM	**Qing Tang, Ph.D.,** U Penn Detyrosination enrichment on microtubule subsets is established by the interplay between a stochastically-acting enzyme and microtubule stability
2:30 – 2:45 PM	**Jae-Yeol Kim, Ph.D.,** NIH Dynamics of ternary complex formation of an idp and two binding partners and their allosteric effects studied by three-color single-molecule fret
2:45 - 3:15 PM	Coffee break
3:15 - 3:30 PM	**Danfeng Cai, Ph.D.,** Johns Hopkins University Single molecule imaging of YAP transcription condensates
3:35 - 3:50 PM	**Benjamin Donovan, Ph.D.**, NIH Real-Time Visualization of Spliceosome Assembly in Live Cells
3:55 - 4:10 PM	**Jason Lyu, Ph.D.,** Johns Hopkins University FtsN mediates the spatiotemporal coordination of septal cell wall synthesis and degradation in E. coli
4:15 - 4:45 PM	Keynote Speaker **Taekjip Ha, Ph.D.,** Johns Hopkins University
5:00 – 6:30 pm	Poster session and happy hour
7:30 to 7:45 pm	Poster awards
7:45 – 9:00 pm	Karaoke party

OPENING REMARKS | Mudd Hall Atrium

9:25-9:30 am

Jie Xiao, Ph. D., Johns Hopkins University School of Medicine

KEYNOTE SPEAKERS | Mudd 26

9:30-10:00 am

Sua Myong

Taekjip Ha

SUA MYONG, PH.D., Johns Hopkins University

Sua Myong obtained both B.S and Ph.D. at University of California, Berkeley. Her thesis work involved studying one-carbon metabolism measured by mass spectrometry gas chromatography. She received her postdoctorate training in the laboratory of Taekjip Ha at University of Illinois at Urbana Champaign. She used single molecule fluorescence detection to study DNA and RNA motor protein mechanisms. She started her independent position in the bioengineering department at University of Illinois in 2009, then moved to Biophysics Department at Johns Hopkins University in 2015. Her lab develops and applies single molecule and single cell techniques to study biological pathways involved in human diseases including cancer and neurodegeneration. Her current research includes four themes. First, her lab studies the role of G-quadruplex (G4) in gene expression, both at the level of transcription and translation in e.coli and mammalian cells. G4 is a non-canonical nucleic acid structure that arises from guanine rich sequences. Her recent study revealed an unexpected role of G4 in enhancing transcription when formed in the context of transcription mediated R-loop, suggesting a switch-like function of G4 and R-loop. Second, they are investigating a mechanism of telomere maintenance in collaboration with Patricia Opresko lab at University of Pittsburgh. Telomere is a nucleoprotein complex that caps the ends of chromosome for genomic integrity. They have elucidated stepwise and directional binding mechanism and dynamic motions imparted by the shelterin proteins when bound to telomeric DNA. Third, they are seeking to understand the molecular underpinning of liquid-liquid phase separation which appears to be a universal mechanism by which cellular ribonucleoprotein granules (P-bodies, stress granules) form. Loss of liquid-like property of such granules is implicated in incurable neurodegenerative diseases such as Amyotrophic Lateral Sclerosis and Frontotemporal Dementia. Fourth, in collaboration with Anthony Leung lab, they are studying the role of Poly ADP-ribose (PAR) in protein condensation. Their recent work demonstrated a catalyst-like mechanism of PAR in inducing FUS phase separation by transient physical interaction. Her work is supported by NIGMS, NINDS, NIA and NCI.

Many faces of SM

Abstract:

Eight years of my life at Hopkins have been incredibly fun, meaningful, special, and blessing in many ways. I will discuss several lines of research that started at Hopkins including some that would not have happened if I were not at Hopkins. My talk will be in a format of a story telling that features many superheroes, best actors and actresses and supporting roles, all of whom were essential in producing beautiful cinema of biophysics research. I look forward to expressing my gratitude to my lab members, collaborators and the Hopkins network and the Chesapeake Bay Single Molecule community.

Mudd 26 4:15-4:45 pm

TAEKJIP (TJ) HA, PH.D., Johns Hopkins University

Dr. Taekjip Ha is a Bloomberg Distinguished Professor of Biophysics and Biomedical Engineering at Johns Hopkins University and an investigator with the Howard Hughes Medical Institute. He develops and uses single molecule and single cell measurement tools to study life at high resolution.

Dr. Ha received a bachelor in Physics from Seoul National University in 1990 and Physics Ph.D. from University of California at Berkeley in 1996. After postdoctoral training at Stanford, he was a Physics professor at University of Illinois at Urbana-Champaign until 2015.

Dr. Ha serves on Editorial Boards for Science. He is a member of the National Academy of Science and the National Academy of Medicine, and a fellow of the American Academy of Arts and Sciences. He received the 2011 HoAm Prize in Science and was elected as President of the Biophysical Society in 2021.

Fantastic Single Molecule Technologies and Where to Find Them

Abstract:
Owing to their unique abilities to manipulate, label, and image individual molecules in vitro and in cellulo, single-molecule techniques provide previously unattainable access to elementary biological processes. In this talk, I will describe how our mechanistic studies of DNA helicases led to development of new research tools, including very fast CRISPR, GOLD FISH, isothermal DNA amplification, co-transcriptional RNA folding and optohelicase engineering.

TALKS | Mudd 26

Session I

9:30-10:50 am

Chair: James Liu,
Janelia Research Farm, HHMI

INHEE CHUNG, PH.D., George Washington University

MAxSIM: Multi-Angle-Crossing Structured Illumination Microscopy with Height-Controlled Mirror for 3D Topological Mapping of Live Cells

Authors:
Pedro Felipe Gardeazabal Rodriguez, Yigal Lilach, Abhijit Ambegaonkar, Teresa Vitali, Haani Jafri, Hae Won Sohn, Matthew Dalva, Susan Pierce, Inhee Chung

Abstract:
Mapping 3D plasma membrane topology in live cells can bring unprecedented insights into cell biology. Wide-field-based super-resolution methods such as 3D-structured illumination microscopy (3D-SIM) can achieve twice the axial (~400 nm) and lateral (~100 nm) resolution of widefield microscopy in real time in live cells. However, twice-resolution enhancement cannot sufficiently visualize nanoscale fine structures of the plasma membrane. Axial interferometry methods including fluorescence light interference contrast microscopy and its derivatives (e.g., scanning angle interference microscopy) can determine nanoscale axial locations of proteins on and near the plasma membrane. Thus, by combining super-resolution lateral imaging of 2D-SIM with axial interferometry, we developed multi-angle-crossing structured illumination microscopy (MAxSIM) to generate multiple incident angles by fast, optoelectronic creation of diffraction patterns. Precision of MAxSIM axial localization is significantly enhanced by placing cells on a bottom glass substrate, locating a custom height-controlled mirror (HCM) at a fixed axial position above the glass substrate, and optimizing the height reconstruction algorithm for noisy experimental data. The HCM also enables imaging of both the apical and basal surfaces of a cell. MAxSIM with HCM offers high-fidelity nanoscale 3D topological mapping of cell plasma membranes with near-real-time (~0.5 Hz) imaging of live cells and >20 Hz 3D single-molecule tracking.

DO-HYEON KIM, PH.D., Johns Hopkins University

A Super-photostable Organic Dye for Long-term Live-cell Single-molecule Imaging

Authors:
Do-Hyeon Kim, Soon Heok Lee, Hong Minh Triet, Yeji Park, Myung Hyun Jo, Sina Jazani, Taekjip Ha, Yong Tae Chang, Sung Ho Ryu

Abstract:
Organic dyes have emerged as popular fluorophores for live-cell imaging, owing to their advantageous properties such as broad excitation and emission spectra, high brightness, photostability, photo activatability, small size, and ease of chemical modification and genetic conjugation. The innovations of organic dyes have enabled to localize single molecules and visualize nanostructures in live cells beyond the diffraction limit.

Nevertheless, the visualization of single molecules in live cells is still limited to short-term timescales due to photobleaching and the requirement of live-cell compatible imaging conditions. This limitation hinders the exploration of previously unseen molecular events occurring over long-term timescales. While exceptional photostability has been achieved using ST647 or metafluorphore, the necessity of depriving oxygen and manipulating redox reactions prohibits their use in live-cell imaging as these conditions are critical to the biological processes of cells.

We introduce a new super-photostable organic dye called ZF550, which exhibits a photobleaching lifetime of ~331 seconds and a single-molecule localization precision of 25 nanometers in a growth medium without the need for any additives. The photobleaching lifetime of ZF550 is ~207-fold and ~55.2-fold higher than that of AF647 and TMR, respectively. Furthermore, the superior photostability and high brightness of ZF550 were invariant to oxygen levels and a wide range of pH.

ZF550 provides a powerful tool for long-term live-cell single-molecule imaging, as we demonstrated by observing the dynamic single-molecule interactions of epidermal growth factor receptor with clathrin-coated structures and integrin receptor with focal adhesions on the plasma membrane of a live cell under physiological conditions.

Session II

11:15-12:10 pm

Chair: Tatjana Trcek,
Johns Hopkins University

SHUAIXIN HE, Johns Hopkins University School of Medicine

Cas9 cleavage-induced transcription repression requires VCP-proteasome- dependent RNAP2 removal

Authors:
Shuaixin He, Bin Wu

Abstract:
DNA double-strand break (DSB) jeopardizes genome integrity and endangers cell viability. Actively transcribed genes are especially important for cellular function and need to be reliably repaired if broken. DSB triggers transcription repression of the broken gene to prevent accumulation of truncated transcripts and clear the DNA for repairing. However, it remains elusive how fast the repression is initiated and how far it influences the neighboring genes. We used a recently developed very-fast CRISPR (vfCRISPR) to generate DSB at specific genomic locus in seconds, we employed MS2 technology to monitor transcription in live cells, and we used chromatin immunoprecipitation (ChIP) to measure the occupancy of RNA polymerase on the cut- and neighboring genes. Together, we characterized the DSB induced transcription repression and investigated its molecular mechanism. We observed that a single Cas9 cleavage rapidly represses transcription of the damaged gene in minutes, which occurs simultaneously with recruitment of damage repair protein 53BP1. Besides, transcription repression spreads bi-directionally over genome from DSB for hundreds of kilo-bases in an attenuated manner. Different from what literature suggested, the single DSB induced transcription repression did not significantly depend on PRC1-mediated H2AK119 mono-ubiquitination. Instead, the poly- ubiquitination/proteasome was evoked to remove elongating polymerases. Furthermore, the Cohesin complex, which plays important roles in genomic structure maintenance, contributes to repression propagation over genome, even though did not influence the cut-gene. Taken together, these findings showed that DSB induced by vfCRISPR triggers rapid transcription repression that is regulated by proteasome dependent RNAP2 turnover and long-range chromosome organization.

BOK-EUM CHOI, PH.D., NIH/NIDDK

Tether-free single-molecule FRET reveals conformational heterogeneity and dynamics of gRNA during Cas9 ribonucleoprotein assembly

Authors:
Bok-Eum Choi, Hugh Wilson, Quan Wang

Abstract:
CRISPR-Cas9, which uses a programmable guide RNA (gRNA) as a template to cleave DNA at specific sites, has become an indispensable gene editing tool. The dynamic nature and conformational heterogeneity of RNA may be critical to Cas9 assembly and catalytic functions, but are inaccessible via conventional structural methods. Here, we use the recently developed ABEL-FRET spectroscopy to determine the in-solution conformational ensemble and dynamics of gRNA along the maturation pathway of Cas9. ABEL-FRET provides a tether-free platform for single-molecule FRET measurements with ultrahigh resolution and can differentiate the assembly state of individual gRNA molecules based on their hydrodynamic profiles. Our measurements reveal previously unknown conformational heterogeneity and fluctuations of gRNA both before and after Cas9 binding. Specifically, gRNA by itself exists in several distinct and interconverting conformations with kinetics dependent on Mg2+ concentrations. Cas9 seems capable of binding to the heterogenous ensemble of gRNA conformations, and the resulting holoenzyme adopts a relatively homogenous gRNA conformation. Furthermore, the 5'-end of gRNA, in the Cas9-bound state, exhibits conformational dynamics at the millisecond timescale which is completely suppressed after binding of target DNA. This observed disorder-to-order transition is consistent with existing crystal structures and suggests a possible structural basis of a previously proposed 'checkpoint' along the Cas9 reaction pathway. These results deepen our understanding of Cas9 mechanism and may pave the way for the optimization of gRNA design for efficiency and specificity.

MAGGIE RODGERS, PH.D., NIH/NIDDK

Small RNAs and Hfq capture unfolded RNA target sites during transcription

Authors:
Dr. Margaret Rodgers, Brett O'Brien, and Sarah Woodson

Abstract:
Many ribonucleoproteins (RNPs) function by targeting an RNA via complementary base pairing to specifically guide RNA folding, perform nucleotide modification, or carry out splicing. Targeting often occurs during transcription of the target RNA and can be challenged by co-transcriptional folding and binding of proteins that mask the target site sequence. Nevertheless, RNPs successfully find their target sites during a short time window in transcription before the nascent RNA is assembled into a mature RNP. To characterize the mechanism of RNP targeting during transcription, we examined association of a bacterial small RNP (sRNP), containing a small RNA and the RNA chaperone, Hfq, with a target mRNA transcript using single-molecule Colocalization Co-transcriptional Assembly (smCoCoA). By incorporating site-specific fluorophores in the DNA template, we measured the timing of target site synthesis using Protein Induced Fluorescence Enhancement (PIFE) to pinpoint the location of RNA polymerase on the DNA. Using colocalization, we measured stable and dynamic recruitment of the sRNP and correlated sRNP binding to transcription of the target site. We uncovered that as soon as the target site is transcribed, the sRNP captures the target RNA in proximity to the RNA polymerase exit channel before the transcript even has a chance to fold. This mechanism for sRNP recruitment explains why we observe that co-transcriptional targeting is more efficient than post-transcriptional targeting on a folded, full-length mRNA. Many other RNPs, including those involved in ribosome and spliceosome assembly, may utilize similar mechanisms to facilitate rapid and efficient targeting during transcription.

Session III
1:10-2:45 pm

Chair: Jan Rios-Grant
Johns Hopkins University School of Medicine

IAN MORGAN, PH.D., NIH

Unraveling Type II Topoisomerase Inhibition by Antibacterials at the Single-Molecule Level

Authors:
Ian L Morgan, Neil Osheroff, Keir C. Neuman

Abstract:
Type II topoisomerases are an essential class of enzymes that are the targets of some of the most widely prescribed and effective antibacterials in clinical use. In cells, these enzymes help to resolve the topological problems that arise on DNA during transcription, replication, and recombination. Antibacterial drugs that target these enzymes inhibit their activity and lead to the accumulation of DNA breaks. While many biochemical and structural studies have addressed these antibacterials' mechanism of inhibition, resolving drug binding/unbinding kinetics in situ has been technically challenging. Therefore, we designed a high-throughput single molecule magnetic tweezers assay that directly measures drug binding/unbinding during topoisomerase activity. At physiological drug concentrations, we show that E. coli topo IV activity inhibition is directly related to the drug off-rate. Our results suggest that drug off-rate is an important factor to consider when optimizing new lead antibacterial compounds, and our single molecule magnetic tweezers assay provides a convenient way to measure it.

BRETT O'BRIEN, Johns Hopkins University

Single molecule force spectroscopy reveals structural heterogeneity in single repeat expanded HTT mRNAs

Authors:
Brett O'Brien, Roumita Moulick, Gabriel Jimenez, Nandakumar Rajasekaran, Christian M. Kaiser and Sarah A. Woodson

Abstract:
Huntington's disease is caused by expansion of the CAG repeat tract located in the first exon of the huntingtin gene, leading to toxic aggregation of the translated protein in neurons. Recently, cellular models have demonstrated the ability of expanded CAG repeat mRNAs to self-assemble into aggregates that sequester essential nuclear proteins supporting a toxic gain of function of the mRNA product [1, 2]. Information regarding the structure of these repeat expanded mRNAs is limited to ensemble methods, which lack the resolution needed to uncover the structural basis of their aggregation potential. Here, we use optical tweezers to monitor the unfolding/refolding pathways of individual CAG repeat mRNA fragments from exon one of the huntingtin gene (fHTTN). Our results show that both the unfolding forces and cooperativity of unfolding decrease significantly as the number of CAG repeats were increased, causing heterogeneity in force-extension curves (FECs). Both force ramp and passive force clamp assays revealed occupancy of several intermediates separated by one to two 'slipped' CAG repeats. Furthermore, we show that the presence of the native flanking CCG repeat sequence significantly enhances the available conformational space of the mRNA, increasing its aggregation potential. Finally, we show that the force-extension behavior of tethered fHTTN mRNAs is significantly perturbed when pulled in the presence of freely diffusing fHTTN mRNAs. Together, these results contribute new insights regarding the structural basis for the aggregation of expanded CAG repeat mRNAs, providing a platform that is well-suited for testing the effect of proteins and small molecules on force-extension behavior.

MYUNG HYUN JO, PH.D., Johns Hopkins University

Overstretch tension sensors reveal integrin force loading rates

Authors:
Myung Hyun Jo, Olivia Yang, Paul Meneses, Sushil Pangeni, and Taekjip Ha

Abstract:
Understanding the mechanisms by which cells sense and respond to mechanical forces is critical for deciphering cellular processes. One key aspect is the detection of single-molecule forces acting on membrane receptors, such as integrins. Here, we introduce overstretch tension sensor (OTS), that can detect and record a wide range of force levels (20 – 70 pN) with minimal structural changes (~3 nm in length). The OTS is based on stretching-induced denaturation of double-stranded nucleic acid oligos and allows for rare single-molecular force transmission events in dense ligand conditions. Importantly, the OTS enables the detection of multiple force levels. We measured that integrin force increases from 20 to 36 pN over a duration of ~5-20 s in adhering cells.

QING TANG, PH.D., University of Pennsylvania

Detyrosination enrichment on microtubule subsets is established by the interplay between a stochastically-acting enzyme and microtubule stability

Authors:
Qing Tang, Sebastian Sensale, Charles Bond, Andy Qiao, Siewert Hugelier, Arian Arab, Gaurav Arya, Melike Lakadamyali

Abstract:
Microtubules in cells consist of functionally diverse subpopulations carrying distinct post-translational modifications (PTMs). Akin to the histone code, the tubulin code regulates a myriad of microtubule functions ranging from intracellular transport to chromosome segregation. Yet, how individual PTMs only occur on subsets of microtubules to contribute to microtubule specialization is not well understood. In particular, microtubule detyrosination, which is the removal of the C-terminal tyrosine on α-tubulin subunits, marks the stable population of microtubules and modifies how microtubules interact with other microtubule-associated proteins to regulate a wide range of cellular processes. Previously, we found that, in certain cell types, only a small subpopulation of microtubules is highly enriched with the detyrosination mark (~30%) and that detyrosination spans most of the length of a microtubule, often adjacent to a completely tyrosinated microtubule. How the activity of a cytosolic detyrosinase, Vasohibin (VASH) leads to only a small subpopulation of highly detyrosinated microtubules is unclear. Here, using quantitative super-resolution microscopy, we visualized nascent microtubule detyrosination events in cells consisting of 1-3 detyrosinated α-tubulin subunits after Nocodazole washout. Microtubule detyrosination accumulates slowly and in a disperse pattern across the microtubule length. By visualizing single molecules of VASH in live cells, we found that VASH engages with microtubules stochastically on a short time scale suggesting limited removal of tyrosine per interaction, consistent with the super-resolution results. Combining these quantitative imaging results with simulations incorporating parameters from our experiments, we propose a stochastic model for cells to establish a subset of detyrosinated microtubules via a detyrosination-stabilization feedback mechanism.

JAE-YEOL KIM, PH.D., NIH

Dynamics of ternary complex formation of an idp and two binding partners and their allosteric effects studied by three-color single-molecule fret

Authors:
Jae-Yeol Kim, Hoi Sung Chung

Abstract:
Intrinsically disordered proteins (IDPs) often have more than one binding domain, suggesting they can interact with multiple binding partners simultaneously. Ternary complexes formed by an IDP and two binding partners have been observed by NMR, but it is difficult to obtain more detailed kinetic information from ensemble measurements. In this work, we used three-color single-molecule FRET to directly visualize the formation and dissociation of a ternary complex. We studied binding of an IDP (p53 transactivation domain (TAD)) and two competitive binding partners (TAZ2 of CBP and MDM2). By labeling TAD with Alexa 488(donor) and Alexa 594(acceptor 1) and TAZ2 with CF680R(acceptor2), it is possible to detect the formation of not only two binary complexes (TAD-TAZ2 and TAD-MDM2) but also the ternary complex (TAD-MDM2-TAZ2). We found that exchange of the two binding partners can happen via both a competitive pathway (i.e., complete dissociation of one partner and binding of the other) and pathway involving the ternary complex. The latter is called an “allosteric pathway”, in which allosteric interactions of the two binding partners modulate binding and dissociation kinetics. The presence of this pathway allows for more efficient transcription initiation by rapid binding of TAZ2 in the presence of the high concentration of MDM2.

Session IV

2:45-4:45 pm

Chair: Fran Harris
Johns Hopkins School of Medicine

DANFENG CAI, PH.D., NIH

Single molecule imaging of YAP transcription condensates

Authors:
Danfeng Cai, Peng Dong, Jennifer Lippincott-Schwartz, Zhe Liu

Abstract:
YAP/TEAD signaling is essential for organismal development, cell proliferation, and cancer progression. As a transcriptional coactivator, how YAP activates its downstream target genes is incompletely understood. YAP forms biomolecular condensates in response to hyperosmotic stress, concentrating transcription-related factors to activate downstream target genes. Super-resolution imaging using assay for transposase-accessible chromatin with photoactivated localization microscopy revealed that the YAP nuclear condensates were areas enriched in accessible chromatin domains organized as super-enhancers. Using single molecule tracking, we found that YAP condensates slowed YAP diffusion within condensate boundaries, a possible mechanism for promoting YAP target search. These results reveal that YAP condensate formation is a highly regulated process that is critical for YAP/TEAD target gene expression.

BENJAMIN DONOVAN, PH.D., NIH

Real-Time Visualization of Spliceosome Assembly in Live Cells

Authors:
Benjamin Donovan, Gloria Garcia, Daniel Larson

Abstract:
How the spliceosome selects the correct splice site among a vast pool of highly similar non-functional sites is not clear. U2AF is a splicing factor primarily involved in initial 3' splice site (3'SS) recognition. The prevailing view is that U2AF remains bound until after A-complex formation at bona fide splice sites although the context in which a bound U2AF commits to splicing is not clearly defined. We initially approached this problem by measuring U2AF binding in vitro to every 3'SS. We find that 3'SSs contain low-affinity U2AF binding sites, indicating that sequence alone does not impart the specificity required for accurate splice site selection. Despite this narrow distribution of RNA binding affinities, we observe a broad distribution of U2AF dwell times in live-cell single-molecule tracking assays which we show, through a combination of in vitro and in vivo single-molecule assays, reflects a wide range of processes, from initial binding site sampling to involvement in spliceosome assembly. We are currently identifying the spliceosome components that regulate U2AF occupancy during assembly. In particular, U2AF pull-down mass-spectrometry experiments identify the RNA helicase DDX42 as interacting with U2AF. DDX42 and DDX46 compete for the same region on the SF3B1 component of the U2 snRNP. Interestingly in orbital tracking assays, where U2AF binding and pre-mRNA splicing are observed simultaneously, DDX42 knockdown stabilizes U2AF binding while DDX46 knockdown destabilizes binding. Together, these results provide new insight into how U2AF binding is interrogated during spliceosome assembly to ensure highly-specific 3'SS recognition.

JASON LYU, PH.D., NIH

FtsN mediates the spatiotemporal coordination of septal cell wall synthesis and degradation in E. coli

Authors:
Zhixin Lyu, Atsushi Yahashiri, Gabriela M. Kaus, Xinxing Yang, Joshua W. McCausland, David S. Weiss, Jie Xiao

Abstract:
Bacterial cell division requires coordinated cell wall synthesis and hydrolysis while maintaining the integrity of the cell envelope. However, the simultaneous regulation of these opposing activities in time and space is not well understood. Recently, my work has demonstrated that FtsN forms a complex with the essential septal peptidoglycan (sPG) polymerase FtsW and transpeptidase FtsI to maintain their synthesis activities. Overexpression of FtsN's SPOR domain resulted in a chaining phenotype that indicates the SPOR domain protects the glycan strand from cleavage by lytic transglycosylases (LTs). Using single-molecule tracking (SMT), we observed that truncation of FtsN's SPOR domain or deletion of three Amidases led to a large portion of FtsN and FtsW moving away from the sPG synthesis track and towards the Z-track. Additionally, processive movement of FtsN stopped when six LTs were deleted. These findings suggest that FtsN's SPOR domain binds to the transient denuded glycan strand, protecting it from cleavage by LTs and marking the site for new synthesis. When sufficient lipid II substrates are supplied, the FtsWI synthase complex binds with FtsN and begins to synthesize new sPG processively, pulling FtsN away from the denuded glycan strands. This allows LTs to degrade them in the vicinity of the active complex, coupling sPG synthesis and hydrolysis spatially and enzymatically. Through this coordinated mechanism, new sPG strands' synthesis and old sPG strands' hydrolysis could be synchronized spatiotemporally, ensuring the cell wall's integrity during cell division.

Poster Session & Happy Hour
5:00-6:30 pm

Poster 1

GIULO AGNETTI, JHU Cardiology & Cell Biology

New Mechanisms and Therapeutic Strategies to Preserve Desmin Homeostasis in the Heart

Authors:
Zixiao Li1, Joseph Oldam1, Jessica L. Semel1, Christopher Pantelis1, Seungho Jun1, Krishna K Singh1, Patrick Calhoun1, Gizem Kiceli1, Hikmet Kadioglu1, Kenneth Bedi2, Kenneth B. Margulies2, Nazareno Paolocci1,3, Giulio Agnetti1,4

Abstract:
Background: Cardiac ischemia/reperfusion (I/R) injury is a leading cause of acute and long-term mortality. We reported that desmin is increasingly cleaved and misfolded in patients with HF of ischemic origin. Both desmin loss and its aggregation are detrimental for the heart and we now examined their contribution to cardiac I/R injury, as well as testing new therapeutic strategies aimed at reducing desmin adverse remodeling in the heart.

Methods and Results: Oxidative stress induces desmin cleavage and aggregation, acutely, in a cell-specific and dose-dependent fashion while elevation of O-linked N-acetyl-glucosamine (O-GlcNAc) prevents these toxic effects. Desmin's single cysteine play a protective role from I/R injury which is independent from its loss or gain of function. While we show that the new, cardiac, Transmembrane Protease Serine 13 (TMPRSS13) interacts with desmin with oxidative stress, we develop a new platform to test drugs targeting desmin phase separation and its aggregation.

Conclusions: We provide data on two new therapeutic strategies to target desmin loss of, and gain of toxic function, and on a new platform to screen drugs targeting desmin pathobiology.

Poster 2

MICHAEL BANCO, NIH

Cryo-EM Structures of the DEAH-Box Helicase DHX36 Reveal the Initiation of Unwinding

Authors:
Michael T. Banco, Tapas Paul, Jiansen Jiang, Sua Myong and Adrian R. Ferré-D'Amaré

Abstract:
G-quadruplexes (G4s) are nucleic acid structures formed by guanine-rich sequences adopting a helical arrangement of stacked tetrads. DHX36 is a G4-specific helicase that associates with guanine-rich sequences and exhibits high affinity to G4 structures. Previously, our structure of the Bos taurus DHX36 bound to a DNA G4 derived from the cMyc oncogene highlighted, without ATP, the G4 was pulled by a single nucleotide. Further support for this observation was demonstrated by smFRET studies describing conformational fluctuations of DHX36 bound to a DNA G4, which corresponded to the distance of a single nucleotide. Contrary to the behavior of DNA G4s, similar measurements of RNA G4s illustrated a single conformational state, implying that ATP-independent remodeling by DHX36 progresses differently for DNA and RNA G4s. Here, we determined multiple single-particle cryo-EM reconstructions of DHX36 bound to a DNA or RNA variant of the cMyc G4 that details the initiation of unwinding. Intriguingly, our reconstruction highlights that the RNA G4 was not pulled by a single nucleotide, suggesting DHX36 predominantly exist in a non-pulled state with RNAs. These reconstructions along with smFRET measurements reveal the interplay between helicase domains responsible for the differences in ATP-independent remodeling. Altogether, we provide mechanistic insights of DHX36 unwinding G4s that can be extended to other DEAH-box helicases, which play critical roles in all facets of nucleic acid metabolism in eukaryotes. This work was funded, in part, by the intramural program of the National Heart, Lung and Blood Institute, NIH. M.T.B. is a recipient of the Lenfant Fellowship.

Poster 3

ARITRA BASU, Johns Hopkins University

Exploring the spatial distribution of mRNAs that engage in heterotypic RNA-RNA interactions using single-molecule FISH

Authors:
Aritra Basu, Ziqing Ye, Grzegorz Kudla, Tatjana Trcek

Abstract:
mRNA localization is a universal process that controls the spatial organization of gene expression. Importantly, mis-regulation of mRNA localization can cause human neuromuscular disorders, and death in the Drosophila and Xenopus germlines. Certain RNA-RNA interactions have been shown to play important roles in mRNA localization, such as the role of oskar mRNA dimerization in localizing oskar to the Drosophila posterior. However, the abundance of such interactions in cells has been poorly characterized. In recent years, advancements in next-generation RNA sequencing have allowed scientists to capture RNA-RNA interactions transcriptome-wide. However, during the sequencing process, spatiotemporal information about those RNAs' cellular expression is lost. We explored datasets generated from two sequencing methods to capture RNA base-pairing interactions (PARIS-seq) and protein-mediated RNA interactions (RIC-seq) and showed that a large fraction of genes is enriched in both datasets, indicating a potentially conserved population of heterodimer-forming RNA species across cell types. Here, using a combination of smFISH, super-resolution microscopy, and single molecule counting techniques, we aim to characterize the spatial localization patterns of these sequencing-predicted heterodimer-forming RNA sequences in vivo. Our analysis of this dataset has revealed a longer average 3'UTR length, and a fraction of the transcripts contain extensive GC-rich repeats predicted to enable G-quadruplex formation. In addition, preliminary results suggest that RPS29, an mRNA coding for a ribosomal protein on the heterodimer list, localizes around the nuclei in HeLa as well as in HEK-293T cells and aggregates into clusters, which may imply a novel mechanism for subcellular mRNA spatial organization.

Poster 4

CHRISTOPHER BOHRER, NIH/NCI

Synthetic analysis of chromatin tracing and live-cell imaging indicates pervasive spatial coupling between genes

Authors:
Christopher H. Bohrer and Daniel R Larson

Abstract:
The role of the spatial organization of chromosomes in directing transcription remains an outstanding question in gene regulation. Here, we analyze two recent single-cell imaging methodologies applied across hundreds of genes to systematically analyze the contribution of chromosome conformation to transcriptional regulation. Those methodologies are (1) single-cell chromatin tracing with super-resolution imaging in fixed cells; and (2) high-throughput labeling and imaging of nascent RNA in living cells. Specifically, we determine the contribution of physical distance to the coordination of transcriptional bursts. Leveraging the variability in distance inherent in single-cell imaging, we show that physical distance–but not genomic distance–between genes on individual chromosomes is the major factor driving co-bursting. By combining this analysis with live-cell imaging, we arrive at a corrected transcriptional correlation of $\varphi \approx 0.3$ for genes separated by < 400 nm. We propose that this surprisingly large correlation represents a physical property of human chromosomes and establishes a benchmark for future experimental studies.

Poster 5

BROOKE BRITTON, Johns Hopkins School of Medicine

Conformational Changes in the Essential E.coli Septal Cell Wall Synthesis Complex Suggest and Activation Mechanism

Authors:
Brooke M. Britton, Remy A. Yovanno, Sara F. Costa, Joshua McCausland, Albert Y. Lau, Jie Xiao, Zach Hensel

Abstract:
The bacterial divisome, a macromolecular machine composed of more than thirty proteins in E. coli, orchestrates the essential process of cell wall constriction during cell division. Novel antimicrobial strategies can target protein-protein interactions within the divisome and will benefit from insights into divisome structure and dynamics. We combined structure prediction, molecular dynamics simulation, single-molecule imaging, and mutagenesis to construct a model of the core complex of the E. coli divisome composed of the essential septal cell wall synthase complex formed by FtsW and FtsI, and its regulators FtsQ, FtsL, FtsB, and FtsN. We observed extensive interactions in four key regions in the periplasmic domains of the complex. FtsQLB scaffold FtsI in an extended conformation with the FtsI transpeptidase domain lifted away from the membrane through interactions among the C-terminal domains. FtsN binds between FtsI and FtsL in a region rich in residues with superfission and dominant negative mutations. Mutagenesis experiments in cellulo and in silico revealed that the essential domain of FtsN functions as a tether to tie FtsI and FtsL together, impacting interactions between the anchor-loop of FtsI and the putative catalytic region of FtsW, suggesting a mechanism of how FtsN activates the cell wall synthesis activities of FtsWI.

Poster 6

XINLEI (LILY) CHEN, Johns Hopkins University

Exploring the Structure and Activity of Bacterial Cell Division Proteins

Authors:
Xinlei Chen, Amilcar Perez, Jie Xiao

Abstract:
The bacteria cell maintains its shape through a rigid structure surrounding the inner membrane, the peptidoglycan (PG) cell wall. Our goal is to study the structure and the activity of PG synthases such as PBP1b and its regulators such as FtsN during bacterial cell division. FtsN is an activator in bacterial cell division. Previous in-vivo imaging studies suggest FtsN may form a multimer via binding of its SPOR domain. Here, I studied the oligomeric properties of the FtsN-SPOR domain in-vitro using biochemical assays including crosslinking, native gel electrophoresis, blue native gel electrophoresis, and western blotting, all of which revealed no obvious indications of physiologically relevant multimerization. In addition to FtsN, I also helped establish an in-vitro single molecule PG synthesis assay using PBP1b as tool for our studies. This assay would fill in gaps in knowledge such providing measurements of changes in enzyme conformation, processivity, and on-off rates during PG synthesis. Using previously constructed strains containing plasmids containing IPTG-inducible PBP1b-mutant proteins containing Cysteine to Serine mutations for future Cy3-maleimide labelling, I tested for IPTG induction. 12 out of 13 induction strains were shown to have retained IPTG induced expression, which allows for us to continue purification of these proteins and preparation of in-vitro single molecule cell wall synthesis assay. This project will aid in guiding new ways of targeting antibiotics resistant bacteria by providing new mechanisms to target during cell wall synthesis.

Poster 7

YI-LAN CHEN, Johns Hopkins University

Coordinated methylation and ribosomal RNA processing by U14 snornp

Authors:
Yi-Lan Chen and Sarah A. Woodson

Abstract:
Small nucleolar RNA-protein complexes (snoRNPs) guide pseudouridylation or 2'-O-methylation of the pre-rRNA, and in some cases, assist pre-rRNA processing. U14 snoRNP is recruited to the pre-18S rRNA during early-stage 40S assembly and is thought to aid rRNA folding and assembly. U14 snoRNA base pairs with two conserved sites in the 18S rRNA that are separated by ~380 nt, including a variable expansion segment (ES3). Studying how U14 snoRNP recognizes and coordinates these distant binding sites will show how the snoRNP facilitates pre-rRNA folding. Here, we observed in real-time how U14 snoRNP binds and methylates different variants of the pre-rRNA target sites. We found that U14 methylates a pre-rRNA fragment containing both binding sites faster than a pre-rRNA fragment containing only the methylation site, which indicates that U14 snoRNP binding to both sites promotes methylation. Longer sequences between the two binding sites delayed the methylation, further supported the theory above. Furthermore, using single-molecule fluorescence microscopy, we observed that U14 snoRNP stably binds to the pre-rRNA and causes the target to sample 3-4 conformational states. The transitions between these states become more dynamic upon adding the methyl donor S-adenosylmethionine (SAM). The target with both sites has more overall dynamic events implied interaction between two binding sites. We aim to decipher the functional consequences of the different pre-rRNA states using engineered pre-rRNA fragments and snoRNP mutants. The results will help us understand how U14 snoRNP facilitates the folding of ES3, thus safeguarding its structure despite changes in the sequence of ES3 during evolution.

Poster 8

GABOR HARAMI, NIH/NHLBI

The human RMI1-2 complex facilitates closing of the Topoisomerase IIIa DNA gate and alleviates the inhibitory effect of DNA tension

Authors:
Gábor M. Harami, Hajnalka Harami-Papp, Ian L. Morgan, Xi Yang, János Pálinkás, Mihály Kovács, Keir C. Neuman

Abstract:
Human TOP3α topoisomerase (TOP3A) forms the so called TRR complex with the RecQ-mediated genome instability proteins 1 and 2 (RMI1-2) proteins and alters the topology of various DNA structures during replication, meiosis, and homologous recombination-based DNA repair processes. Mutations in any of these proteins lead to severe genomic and developmental defects, underscoring their essential functions. In vitro, RMI1 enhances the DNA decatenation activity of TOP3A and was proposed to stabilize the open state of the TOP3A DNA gate to facilitate DNA strand passage. Strikingly, our single-molecule experiments of gate opening/closing kinetics indicate that RMI1-2 facilitates the closing of the TOP3A gate and increases both the DNA cleavage and ligation rates, resulting in an overall faster enzymatic cycle. Furthermore, TRR complex activity is less sensitive to tension applied on the DNA than TOP3A alone, suggesting that in the cell RMI1-2 may aid TOP3A-mediated processing of physiologically occurring entwined and highly strained DNA structures, such as anaphase bridges.

Poster 9

FRANCES HARRIS, Johns Hopkins University School of Medicine

RNAP Promoter Search and Transcription Kinetics in Live E. coli Cells

Authors:
Kelsey Bettridge, Frances Harris, Nicolás Yehya, and Jie Xiao

Abstract:
Bacterial transcription has been studied extensively in vitro, which has provided detailed molecular mechanisms of transcription. The in vivo cellular environment, however, may impose different rules on transcription than the homogenous and well-controlled in vitro environment. How an RNA polymerase (RNAP) molecule searches rapidly through vast nonspecific chromosomal DNA in the three-dimensional nucleoid space and identifies a specific promoter sequence remains elusive. Transcription kinetics in vivo could also be impacted by specific cellular environments including nucleoid organization and nutrient availability. In this work, we investigated the promoter search dynamics and transcription kinetics of RNAP in live E. coli cells. Using single-molecule tracking (SMT) and fluorescence recovery after photobleaching (FRAP) across different genetic, drug inhibition, and growth conditions, we observed that RNAP's promoter search is facilitated by nonspecific DNA interactions and is largely independent of nucleoid organization, growth condition, transcription activity, or promoter class. RNAP's transcription kinetics, however, are sensitive to these conditions and mainly modulated at the levels of actively engaged RNAP and the promoter escape rate. Our work establishes a foundation for further mechanistic studies of bacterial transcription in live cells.

Poster 10

ALEXANDRA HOLLÓ, NIH

Mechanistic Insights into The Regulation of Human Myosin-7a

Authors:
A. Holló, N. Billington, A. M. Kengyel, J. R. Sellers and R. Liu

Abstract:
Human myosin-7a (Myo7a) is an actin-based motor protein essential for vision and hearing. It plays critical roles in the development and functions of actin rich protrusions. Previous studies using Drosophila homolog showed that myosin-7a is a monomeric, high duty ratio motor and can move processively upon dimerization. However, characterization of full-length mammalian myosin-7a has been limited by the difficulty of expressing and purifying stable, intact protein.

Here, we report the production of a complete human myosin-7a holoenzyme in insect cells and study its regulation by intra- and intermolecular mechanisms. We found that human myosin-7a utilizes regulatory light chain (RLC), calmodulin and calmodulin like protein 4 (CALML4) as the light chain subunits. CALML4 is recently discovered highly enriched in stereocilia and identified as a deafness candidate gene. We show that CALML4 plays a key role in regulating the dynamic binding of calmodulin to myosin-7a in response to Ca2+ signaling. The cochlea expresses two myosin-7a splicing isoforms differed by a short N-terminal extension. Using biochemistry and in vitro motility assays, we show that the N-terminal extension greatly influences the enzymatic and mechanical behaviors of mammalian myosin-7a. We propose that the hair cell regulates its mechanosensitivity by adjusting the expression levels of the two myosin-7a isoforms. Finally, using single molecule motility assays, we show that in the presence of MyRIP, a known myosin-7a binding protein, purified full-length myosin-7a move processively on actin in vitro.

Together, our results provide new molecular insight into how myosin-7a functions in hair cell stereocilia and neuroretina.

Poster 11

JIHEE HWANG, Johns Hopkins University

DNA supercoiling-mediated G4/R-loop formation tunes transcription yield by controlling the access of RNA polymerase

Authors:
Jihee Hwang, Chung-ing Lee, Tapas Paul and Sua Myong

Abstract:
Transcription can mechanically modify DNA topology and structure, generating non-canonical DNA structures dynamically. Resulting from ongoing transcription, these modifications in turn may provide instant feedback to the transcription machinery. But, the underlying mechanism has not been clear.

Here, we investigated how the representative non-canonical B DNA structures generated by transcription, such as G-quadruplex (GQ), R-loop, and DNA super helicity, can regulate the transcription itself. Gel-based assay and molecular beacon assay indicate the GQ forming sequence located promoter in supercoiled DNA enhanced the R-loop formation. The GQ-induced R-loop structure results in the relaxation of super helical turns, and hence, down-regulates transcription. In addition, the distance of the GQ forming sequence from the transcription start site determines the degree of relaxation of the coiled DNA. By observing the dynamics of GQ/R-loop formation in real-time at the molecular level, negative super helicity is required to form an R-loop structure accelerated by GQ formation in DNA. Our results clarify GQ-stabilized R-loop can act as a regulator of the DNA topological state, thereby control the transcription process.

Poster 12

SINA JAZANI, Johns Hopkins University, School of Medicine

Fluorescence lifetime analysis of smFRET with contribution of pfife on donor and acceptor

Authors:
Sina Jazani, Taekjip Ha

Abstract:
Single-molecule fluorescence resonance energy transfer (FRET) is a powerful technique based on dipole-dipole interaction between donor and acceptor fluorophores to observe inter- and intra-molecular dynamics in real time with sensitivity to macro-molecular distances (~2.5-10 nm). That said, some fluorophores have an inherent characteristic known as protein induced fluorescence enhancement (PIFE). PIFE is a photo-physical feature of dyes undergoing cis-trans transitions and occurs for protein-dye interactions closer than 3 nm where FRET is less sensitive.

Here, the challenge is uncoupling the PIFE effect in the FRET data. Ignoring the PIFE effect in the analysis of the FRET data may lead to misinterpretation of the system under investigation. As a solution to this problem, we develop a computational framework based on Bayesian statistics to analyze the fluorescence lifetime signals of the donor and acceptor channels which allows us to uncouple the PIFE effects from the FRET. Our framework can extract any changes in the FRET efficiency simultaneously with any changes in the fluorescence lifetimes of the donor and acceptor due to the PIFE effect. In addition, our framework can provide other parameters, such as the donor and acceptor excitation rates, background photon rates, and detectors' cross-talk ratios. Our framework extracts all these parameters by analyzing a single photon arrival time trace with only a few thousand photons.

Poster 13

HAOYUAN JING, Johns Hopkins University

Dynamin condensates mediate the coupling between synaptic vesicle exocytosis and ultrafast endocytosis

Authors:
Haoyuan Jing, Jian Liu

Abstract:
Sustainable neuron transmissions rely on the clathrin-independent ultrafast endocytosis which recycles exocytic synaptic vesicle components and preserves the identity of the active zone in a timely manner. The formation of ultrafast endocytic membrane pits is coupled with the synaptic vesicle exocytosis both spatially next to the active zone and temporally within 50ms and can be explained by our lateral compression model. However, it is still unclear how the endocytic membrane pits evolve and pitch off from the membrane in the absence of the clathrin. In vitro, the ultrafast endocytosis also benefits from the local high concentration of dynamin which forms a condensate inside the active zone. Although the formation of the ultrafast endocytic membrane pit does not rely on the dynamin condensate, the endocytic membrane pit pinching off is significantly slowdown in the absence of the dynamin condensate. Considering the curvature sensing and generating features of the dynamin, our theoretical model unravels that the endocytosis can complete in an ultrafast manner, within 0.2s, or in a slow manner, up to few hundred seconds. The interplay between the dynamin condensates and the endocytic membrane pits is essential for the timeous recycle of synaptic vesicles to maintain neuron activities.

Poster 14

HUIJIN LEE, Johns Hopkins University

A single molecule TIRF-based platform to study DNA supercoiling effect on DNA processing enzymes

Authors:
Huijin Lee, Jihee Hwang, Taekjip Ha

Abstract:
DNA topology plays an important role in regulating DNA metabolic pathways. DNA supercoiling changes the properties of the DNA double helix, and alters the binding specificity of proteins. Thus, proteins may differentially interact with DNA under various supercoiling states. A biophysical investigation of how these proteins recognize DNA defects and distortions and differentiate various topological states could further our understanding of protein-DNA interactions. We have developed a new single molecule total internal reflection fluorescence (smTIRF) -based platform to investigate how DNA topology affects protein-DNA interactions. We are currently studying MutS, which detects a mismatched base pair to initiate mismatch repair, and CRISPR-Cas9 as candidate proteins. We have generated plasmids that are site-specifically labeled with one or two fluorophores and a biotin. We prepared them in three different topological states: “Linear”, “relaxed circular”, and “negatively supercoiled” using restriction digestion (linear) or DNA gyrase (negatively supercoiled). For MutS study, we designed a 2.5 kb plasmid with a single GT mismatch and an adjacent Cy5 fluorophore. We were able to observe FRET between Cy3-labelled MutS and Cy5 suggesting that MutS bound to the mismatch region. In the presence of ADP, MutS bound the negative supercoiled DNA with a longer residence time compared to the linear or relaxed circular DNA, suggesting that negative supercoiling helps mismatch recognition. For CRISPR-Cas9, we created a 2.5 kb plasmid with Cy3 and Cy5 fluorophores, positioned to show a reduced FRET value upon DNA unwinding during R-loop formation induced by dCas9. The R-loop formation rate was about the same for all three DNA topological states when the guide RNA sequence fully matched the DNA sequence. When we introduced one base pair mismatch either in a PAM (protospacer adjacent motif)-proximal position or in a PAM-distal position, we observed the highest R-loop formation rate for the negatively supercoiled DNA and the rate decreased progressively for the open circular DNA and the linear DNA. Our observation confirm that DNA negative supercoiling assists its unwinding by Cas9 and suggests that off-target effects in the cell can be exacerbated by negative supercoiling. Collectively, our smTIRF-based platform allows high throughput investigation of supercoiling effects on DNA protein interactions as we demonstrate using two different biological systems.

Poster 15

TING-WIE LIAO & **NICOLÁS YEHYA**, Johns Hopkins University

Single Molecule Measurements of DNA Topology Effects on Transcription Kinetics

Authors:
Ting-Wei Liao*, Nicolás Yehya*, Jie Xiao, Taekjip Ha (*Equal Contribution)

Abstract:
Bacterial chromosomes are organized spatially and topologically within cells, taking on conformations that affect gene expression and transcription behavior. Nucleoid- associated proteins facilitate this organization by bending, looping, and coating DNA to form chromosomal topologically isolated domains (TIDs) at various different length levels, starting from 10 kbp. This chromosomal organization within the TIDs localizes supercoiling to different portions of the chromosome. Topological constraint of supercoiling dynamically changes the mechanical properties of DNA. The relationship between this and transcription is one of coupling, where supercoiling density determines transcriptional activity, but transcriptional activity also changes supercoiling density. To probe these effects and to test our hypothesis that the formation of TIDs significantly modulates gene expression profiles we use a single-molecule imaging approach on a synthetic transcription system. To obtain a quantitative understanding of how the topological state of a TID impacts RNA polymerase (RNAP) transcription kinetics, we must make isolated measurements of initiation and elongation rates, which necessitates the use of single molecule techniques. We have constructed a topologically-constrained DNA system mimicking a TID using single molecule protein induced fluorescent enhancement (smPIFE) in vitro to track the loading and elongation of individual RNAPs. This platform makes use of robust smPIFE signals to monitor kinetic parameters of transcription, which will be able to give an understanding of the effects of chromosome topology and RNAP cooperatively along DNA.

Poster 16

YUAN LOU, Johns Hopkins University

Co-transcriptional folding facilitates self-cleavage of the glmS ribozyme

Authors:
Yuan Lou and Sarah A. Woodson

Abstract:
The glmS ribozyme, located in the 5' UTR of the B. subtilis glmS mRNA, regulates expression of the downstream open reading frame through ligand-induced self-cleavage and mRNA decay. The ribozyme consists of a highly conserved catalytic core and a downstream peripheral domain. Although the glmS ribozyme has been studied extensively, typically using full-length, refolded RNA, the kinetics of ribozyme cleavage during transcription is not fully understood. Here, by using a TIRF-based single-molecule platform, we monitored transcription and ribozyme self-cleavage simultaneously. By fitting the single-molecule results to a mathematical model, we showed that self-cleavage can occur during transcription even before the entire peripheral domain is synthesized. Our data also revealed that high metabolite concentration slows down transcription, which allows the ribozyme core itself to fold into an active but metastable structure before the downstream domain becomes available. DMS foot printing and RNA structure prediction further revealed how slow sequential folding favored native folding of the core through fraying of misfolded local structures. Taken together, we propose a model for co-transcriptional cleavage of the glmS ribozyme. Excess metabolite facilitates proper folding of the ribozyme core to trigger self-cleavage at an early stage during transcription, creating a longer time window in which a ribonuclease can degrade the glmS mRNA before ribosomes load. Our results emphasize the importance of co-transcriptional folding of RNA tertiary structure for mRNA stability control.

Poster 17

MIRA MALMOSI, Georgetown University

Signaling in Multimeric Protein Complexes: A Single-Molecule Study of PKA

Authors:
Mira Malmosi, Andrew Hoy, Rodrigo Maillard

Abstract:
Protein kinases are ubiquitous enzyme complexes, consisting of a highly conserved catalytic core and diverse regulatory domains. The diversity of the regulatory domains makes them major targets for drug development, so it is important to understand how they interact with various binding partners and signaling molecules and how these communication channels are disrupted by mutations. During the activation cycle of the Protein Kinase A (PKA) holoenzyme tetramer the regulatory subunits are in largely different conformations depending on whether they are bound to catalytic subunits or to cAMP. To build a comprehensive model of the behavior of macromolecular assemblies such as PKA, we start by studying the smallest possible unit and increase the complexity in a step-wise fashion. Single molecule optical tweezers allows us to selectively probe and manipulate one element in a complex and follow how that single element responds to binding interactions and mutations through conformational and energetic changes. Our findings indicate a stabilization effect for the cAMP-bound regulatory subunits compared to the apo state, where each cyclic nucleotide binding (CNB) domain unfolds separately. The full-length regulatory subunit dimer of PKA unfolds in five steps where the first four correspond to the unfolding of the four CNB domains (two per subunit). The fifth unfolding event occurs at a much higher force and its size is consistent with the partial unfolding of the highly stable dimerization domain of the regulatory subunits via the breakage of one of the disulfide bonds.

Poster 18

DANIËL MELTERS, National Cancer Institute

Single Molecule Analysis of CENP-A Chromatin by High-Speed Atomic Force Microscopy

Authors:
Daniël P. Melters, Keir C. Neuman, Tatini Rakshit, Yamini Dalal

Abstract:
Chromatin accessibility is modulated in a variety of ways to create open and closed chromatin states, both of which are critical for eukaryotic gene regulation. At the single molecule level, how accessibility is regulated in the chromatin fiber composed of canonical or variant nucleosomes is a fundamental question in the field. Here, we developed a single-molecule tracking method where we could analyze thousands of canonical H3 and centromeric variant nucleosomes imaged by high-speed atomic force microscopy. This approach allowed us to investigate how changes in nucleosome dynamics in vitro inform us about chromatin accessibility in vivo. By high-speed atomic force microscopy, we tracked chromatin dynamics in real time and determined the MSD and diffusion constant for the variant centromeric CENP-A nucleosome. Furthermore, an essential kinetochore protein CENP-C reduces the diffusion constant and mobility of centromeric nucleosomes along the chromatin fiber. We subsequently interrogated how CENP-C modulates CENP-A chromatin dynamics in vivo. Overexpressing CENP-C resulted in reduced centromeric transcription and impaired loading of new CENP-A molecules. Thus, changes which alter chromatin accessibility in vitro, also correspondingly alter transcription in vivo. These data suggest a model in which variant nucleosomes encode their own diffusion kinetics and mobility, and where binding partners can suppress or enhance mobility.

Poster 19

EMILY NAKAYAMA, Johns Hopkins University

Enhancing Protein Visualization in Single-Molecule Imaging with the AID2 System

Authors:
Ana De La Cruz, Emily Nakayama, Bin Wu

Abstract:
Single-molecule Imaging of Nascent PeptideS (SINAPS) is a powerful tool that has the ability to visualize translation in live and fixed cells. One of the key components of the SINAPS system is the use of the auxin-inducible degron (AID) and OsTIR1 to induce the degradation of mature proteins. These mature proteins can increase the background signal in cells, making it difficult to detect single proteins and nascent peptides. This increase in background can be a result of high translating mRNA concentrations in cells, which limits the SINAPS AID system to cells with low translating mRNA. Recently, Yesbolatova et al. developed the AID2 system in which an OsTIR1(F74G) mutant has the ability to degrade proteins more rapidly than the original AID system at lower auxin concentrations. In bulk degradation experiments with mCherry tagged with AID, we show that using the OsTIR1(F74G) mutant leads to a decrease in mCherry expression compared to the unmutated OsTIR1. We then combined our SINAPS system with the AID2 system in hopes to remove the cap on high translating cells. In high-RNA expressing cells, the single cell average single protein intensity decreases slightly using the SINAPS + AID2 system, tentatively pointing towards improved protein visualization in single molecule experiments.

Poster 20

TANYA NESTEROVA, Johns Hopkins University

Spatiotemporal Control of sPG Synthesis

Authors:
Amilcar Perez, Longhua Hu, Tanya Nesterova, Jian Liu, Jie Xiao

Abstract:
Bacterial cell division entails the remodeling of the septal cell wall (i.e., septal peptidoglycan (sPG)), in which the synthesis and the degradation of sPG are spatiotemporally coordinated to avoid osmotic lysis. However, the physical mechanisms of such spatiotemporal coordination are not well understood and are believed to hold the key for a better antibacterial treatment targeting to bacterial septal cell wall. It is reported that sPG remodeling is orchestrated by the so-called divisome, which consists of 12 essential proteins, including the sPG synthases, FtsWI and its regulator, FtsQLB. In particular, FtsZ, a bacterial homolog of tubulin, is the central organizer of the divisome: While treadmilling, FtsZ polymers form a discontinuous ring in the midcell and recruit other divisome proteins. Combining theory and experimental testing, we demonstrated that 1) FtsZ treadmilling drives the directed and processive movements of sPG synthases through Brownian ratcheting, 2) FtsZ-mediated Brownian ratcheting facilitates the activation of FtsWI through corralling sPG synthase FtsWI into close and persistent contacts with its regulator FtsQLB, and 3) FtsZ treadmilling speeds modulate the spatial distribution of sPG synthesis along the septum, instead of its total amount. Importantly, compared to those in the FtsZ mutants with different treadmilling speeds, the sPG synthesis is the most uniformly distributed with the septum being most robustly constricted at the wildtype FtsZ treadmilling speeds of 20-30 nm/s. Our finding therefore suggests that FtsZ treadmilling speed may be optimized over evolution for a robust bacterial cell division.

Poster 21

LUIZ PASSALACQUA, NHLBI/NIH

Structural basis for fluorophore activation by a DNA mimic of green fluorescent protein

Authors:
Luiz F. M. Passalacqua, Michael T. Banco, Jared D. Moon, Xing Li, Samie R. Jaffrey, Adrian R. Ferré-D'Amaré

Abstract:
DNA exists primarily as a double helix, in which two complementary strands hybridize, making the overall B-form helical geometry largely independent from nucleotide sequence. Functional DNA molecules, whose sophisticated biochemical or biophysical activities arise from their specific nucleotide sequences, have been isolated through in vitro selection. Analogous to their RNA counterparts (including aptamers, ribozymes, and riboswitches), it is thought that the non-helical elements of functional DNAs adopt non-canonical three-dimensional (3D) folds that endow them with unique biochemical properties. However, only limited information is available on the atomistic basis of functional DNA activity. Lettuce is an in vitro-evolved DNA that binds and activates conditional fluorophores derived from green fluorescent protein (GFP). To extend to DNA previous structural studies of fluorogenic RNAs as well as GFP and other fluorescent proteins, we have characterized Lettuce-fluorophore complexes by X-ray crystallography and cryo-electron microscopy. These reveal that the 53-nucleotide DNA adopts a four-way junction fold in which four DNA stems associate coaxially to form its fluorophore binding site at a G-quadruplex. This fold is stabilized by stacking, extensive nucleobase hydrogen bonding –including by unusual diagonally stacked bases that bridge successive tiers of the main coaxial stack of the DNA– and coordination of monovalent and divalent cations. The coaxial fold of this DNA is distinctly different from those of RNA four-way junctions, that typically adopt L-shaped (e.g., tRNA) or T-shaped folds. Lettuce demonstrates how DNA can form elaborate three-dimensional structures without employing RNA-like tertiary interactions, and suggests that new principles of nucleic acid organization will be forthcoming from the analysis of complex DNAs. We have also shown that Lettuce can be used as a fluorescent reporter to detect in-vitro R-loop formation. This work was partially supported by the intramural program of the National Heart, Lung and Blood Institute, NIH.

Poster 22

TAPAS PAUL, Johns Hopkins University

Vectorial folding of telomere overhang promotes higher accessibility

Authors:
Tapas Paul, Patricia L Opresko, Taekjip Ha, Sua Myong

Abstract:
Human telomere overhang composed of tandem repeats of TTAGGG folds into G-quadruplex (G4). Unlike in an experimental setting in the test tube in which the entire length is allowed to fold at once, inside the cell, the overhang is expected to fold as it is synthesized directionally (5' to 3') and released segmentally by a specialized enzyme, the telomerase. To mimic such vectorial G4 folding process, we employed a super helicase, Rep-X which can unwind DNA to release the TTAGGG repeats in 5' to 3' direction. We demonstrate that the folded conformation achieved by the refolding of full sequence is significantly different from that of the vectorial folding for two to eight TTAGGG repeats. Strikingly, the vectorially folded state leads to a remarkably higher accessibility to complementary C-rich strand and the telomere binding protein POT1, reflecting a less stably folded state resulting from the vectorial folding. Importantly, our study points to an inherent difference between the co-polymerizing and post-polymerized folding of telomere overhang that can impact telomere architecture and downstream processes.

Poster 23

AMILCAR PEREZ, Johns Hopkins University

Modulation between active and in-active states of single molecule cell wall synthases

Authors:
Amilcar Perez, Joshua McCausland, Tanya Nesterova, Longhua Hu, Jian Liu, Jie Xiao

Abstract:
FtsW and FtsI form a protein complex (FtsWI) that is essential for making septal peptidoglycan (sPG) required to divide bacteria. Recently, single molecules studies in cells demonstrated processive motions of FtsWI in the septal ring. In E. coli, FtsWI processivity can be powered by autonomous sPG synthesis activity and occur within the "sPG track". Alternatively, FtsWI processivity can be driven by FtsZ-treadmilling and occurs on the "Z-track". How FtsWI transitions between these two tracks, and whether this is coordinated with different division stages of E. coli cell cycle, remain elusive. To address these questions, we performed single molecule tracking of FtsI or FtsW molecules in vertically oriented E. coli cells. We showed that the distribution of FtsWI on the Z-track predominates the early division stages whereas FtsWI switch onto the sPG track at the end stages. Analyzing transitions between the sPG track and the Z-track revealed an intermediate "immobile" state suggesting the requirement of additional sPG synthesis initiation inputs. Mathematical modelling supports a kinetic pathway for the activation of FtsWI involving lipid II and FtsBLQ. Altogether this data shows that E. coli temporally and spatially separates active populations of FtsWI to integrate this as a function of cell division progression.

Poster 24

MIHIRKUMAR N. PRAJAPATI, NIH/NHLBI

The Conformational Preferences of Type II Topoisomerase During the Strand Passage Activity

Authors:
Mihirkumar N Prajapati, Parth R Desai, Siddhartha Das, Keir C Neuman

Abstract:
Type II topoisomerases are essential enzymes that regulate DNA topology by cleaving and re-ligating both strands of the DNA double helix. This strand passage activity can link/unlink, knot/unknot, or change the supercoiling of DNA assembly, and is critical for DNA replication, transcription, and chromosome segregation. Despite having the potential to both increase and decrease the topological complexity, experiments demonstrate that the enzyme preferentially does later. This preference of action suggests that the strand passage performed by the enzyme is not random, and it somehow gauges the topological situation of the system before acting upon it. The enzyme being very small compared to the typical DNA assembly, it is surprising how it can effectively gauge the global DNA topology and act accordingly. The different theories suggest that the enzyme ascertain the topological condition either by multiple interactions with DNA or they glean it from the local conformational features. The Hooked-Juxtaposition (HJP) model postulates that the higher the hookedness of the DNAs, the higher the chances of Topo-II binding to them and performing the strand passage. With the help of single-molecule magnetic tweezers experiments and Molecular Dynamics (MD) simulations, we test the HJP model. Looking at the preferred crossing geometry based on the high unlinking activity by the enzyme, we show that HJP does not give the complete picture of what the enzyme wants during the strand passage. We also show what features of the DNA crossing play an important role in determining the unlinking activity by the enzyme.

Poster 25

NANDAKUMAR RAJASEKARAN, Johns Hopkins University

Resolving chaperone action on ribosome-bound nascent chains with single-molecule spectroscopy

Authors:
Nandakumar Rajasekaran, Ting-Wei Liao, Taekjip Ha, Christian Kaiser

Abstract:
Proteins begin to fold during their synthesis by the ribosome. Stretches of unfolded polypeptides that accumulate co-translationally are prone to misfolding and aggregation before the domain can fold into its native structure. Molecular chaperones stabilize unfolded proteins, preventing premature folding into non-native states. They also rescue proteins from misfolded structures and protect folded domains against destabilizing interactions. However, the molecular mechanisms of how trigger factor interacts with nascent chains on the ribosome are not well understood. Trigger factor is the first molecular chaperone encountered by nascent proteins emerging from the ribosome. We have developed a single-molecule approach for directly visualizing the interaction of trigger factor with nascent chains. With nascent chains of the E. coli elongation factor G protein, we find that trigger factor dynamically engages with unfolded polypeptides on the timescale of seconds. Binding changes with nascent chain length and is dependent on trigger factor recruitment to the ribosome exit tunnel. Our results suggest how interactions of the trigger factor chaperone with its nascent chain clients is tuned to achieve productive folding. Our experimental system sets the stage for exploring molecular mechanisms of chaperone action on nascent proteins.

Poster 26

SERGEI RUDNIZKY, Johns Hopkins University

Deciphering the nature of genomic conflict using locus-specific chromatin perturbation and capture

Authors:
Sergei Rudnizky, Alberto Marin Gonzalez, Carl Wu, Taekjip Ha

Abstract:
Chromatin modulates DNA accessibility and serves as a fundamental layer in the complex regulation of gene expression and DNA integrity. At functionally important regions, chromatin is remodeled to harbor nucleosome-depleted regions (NDRs) flanked by well-positioned nucleosomes, making it more susceptible to DNA lesions. However, the role of this structure in coordinating genomic conflict between chromatin remodeling, transcription, and DNA damage response (DDR)- is unclear. This stems from the dynamic nature of chromatin that is transcribed and repaired in an asynchronous and position-dependent manner and the inability of current approaches to control DDR in space, time, and high throughput.

To overcome these limitations, we developed a strategy based on light-activated very fast CRISPR (vfCRISPR) to simultaneously induce double-strand breaks in hundreds of human NDRs to perturb their chromatin structure with high spatiotemporal control. Using time-resolved MNase-seq and Cut&Run-seq, we monitored the fate of damaged chromatin and chromatin-binding proteins over time and the effects of DDR on the transcription of the heat-shock-induced Hsp70 model gene. Finally, we are developing a chromatin pulldown approach with the potential to perturb, capture and dissect any chromatin locus of interest. The developed approaches can be broadly applied to genomic regions of high relevance to health and disease, providing an exciting opportunity to understand the fundamentals of genomic transactions in vivo at unprecedented resolution.

Poster 27

RIQUEZ-SANDOVAL, Johns Hopkins University

Single Molecule Time Domain Sequencing of Full-length Proteins & Proteoforms

Authors:
Edgar Manriquez-Sandoval, Charles E. F. Millard, Yilin Shen, & Stephen D. Fried

Abstract:
Proteomics has largely benefited from high-throughput mass spectrometry where single molecule techniques have struggled to introduce multiplexity, flexibility, and feasibility. Recent developments in single molecule protein sequencing (SMPS), based on N-terminal amino acid recognizers, have now emerged as a viable alternative to mass spectrometry for single protein studies; with proteome-wide capabilities on the horizon. Protein sequences are decoded by identifying the lifetime and intensity of fluorescence as recognizers bind and unbind N-terminal amino acids of peptides bound to a semiconductor surface with millions of wells, coined Time Domain Sequencing (TDS). However, TDS relies on peptides for sequencing and is incapable of performing top-down-like protein identification. Here we present a labeling technique that will enable full-length protein sequencing by TDS which utilized positive selection of C-termini.

Poster 28

ASHESH SHARMA, UMBC

Modulating size distribution of glucosomes to understand their functional contributions to cellular redox homeostasis

Authors:
Ashesh Sharma, Minjoung Kyoung, Songon An

Abstract:
Glucosomes are liquid-liquid phase separated condensates formed by phosphofructokinase liver type (PFKL) and other rate-determining enzymes in glycolysis and gluconeogenesis in human cells. Spatially, glucosomes are assembled into three different sizes at subcellular levels (i.e., small-, medium-, and large-sized glucosomes). Functionally, they are active in regulating glucose flux between energy metabolism and building block biosynthesis in a size-specific manner. Specifically, medium-sized glucosomes have been associated with diverting glucose flux into the pentose phosphate pathway in response to redox perturbation or enzymatic inhibition. However, the heterogeneous distribution of different glucosome sizes in both single-cell and population levels complicates our efforts to mechanistically investigate their size specific functional contribution in live cells. In this work, we have investigated PFKL fusion protein strategies and oxidant treatments to achieve homogeneous distribution of medium-sized glucosomes in Hs578T cells. By the fusion of a light sensitive oligomerizing protein, Arabidopsis thaliana cryptochrome 2 (Cry2), to PFKL-mCherry, we were able to reversibly induce the formation of medium-sized glucosome using light. Meanwhile, we have observed population-wide upregulation of medium-sized glucosomes with the treatment of hydrogen peroxide and oxidized glutathione, respectively. Taking them together, we have identified how to induce medium-sized glucosomes using light and oxidant treatment, and how to modulate glucosome size-distribution in human cells in both single-cell and population levels. We will utilize these systems to gain mechanistic insights into the functional role of medium-sized glucosomes in the context of redox homeostasis.

Poster 29

SARA SOHAIL, Laboratory of Chemical Physics, NIDDK/NIH

Single molecule FRET imaging and deep learning reveal concentration dependence of aggregation pathways during Aβ42 aggregation

Authors:
Sara H Sohail, Janghyun Yoo, Hoi Sung Chung

Abstract:
Protein aggregation into amyloid fibrils is the hallmark of several devastating neurodegenerative diseases. Gaining an understanding of disease etiology hinges on our ability to understand the molecular mechanics of how soluble monomers assemble to form insoluble fibrils consisting of thousands of constituent monomers. Although amyloid fibril formation is a highly specific self-assembly process, growth patterns and resultant fibril morphologies are highly dependent on solution conditions. Using fluorescence lifetime imaging and deep learning, we have recently shown that amyloid assembly occurs via heterogeneous aggregation pathways resulting in a mixture of co-present mature fibrils with unique morphologies and physicochemical properties (Meng et al., PNAS_2022_e2116736119). Bulk biophysical methods are unable to fully characterize these mixtures of fibril polymorphs. Here, we further develop and use Förster Resonance Energy Transfer (FRET) imaging to monitor the entire aggregation pathway of the Alzheimer's Disease related peptide amyloid β 42 (Aβ42) at the single fibril level in real-time. We incubated a mixture of donor-labeled, acceptor-labeled, and unlabeled Aβ42 monomers, which resulted in the formation of fibrils with diverse FRET efficiency values, indicating structural heterogeneity. Single-fibril images reveal that increasing monomer concentration promotes the formation of more homogeneous fibrils. Fibrils formed at lower concentrations show assemble via highly heterogeneous pathways. Deep learning methods (https://github.com/hoisunglab/FNet) enable segmentation of single fibrils within images of highly overlapping fibrils, allowing for quantitative analysis of the aggregation process in terms of fibril growth rate and photon density for each fibril over the course of fibril assembly.

Poster 30

YONGLEI SUN, NIH

Elucidating the origin of extra noise in single-molecule Förster resonance energy transfer measurements

Authors:
Yonglei Sun, Quan Wang

Abstract:
Single-molecule Förster resonance energy transfer (smFRET) is a powerful technique that probes the nanometer distance between the donor-acceptor fluorophore pair on a single biomolecule. To unleash the potential of smFRET as a structural biology tool, it is important to conduct smFRET efficiency measurements with the highest possible precision. Typically, the measured widths of smFRET efficiency histograms are several-fold broader than the fundamental limit imposed by Poisson noise (shot-noise) in the photon counting process, and the origin of the extra broadening is unknown. In this work, we aim to elucidate the cause of extra noise that limits smFRET experiments. Recently, by using a tether-free smFRET modality known as ABEL-FRET, we achieved an unprecedented narrow distribution that approaches the fundamental shot-noise limit, suggesting that the act of immobilization in conventional smFRET modalities may contribute to the extra broadening. Current work focuses on comparing the same sample measured with ABEL-FRET and other smFRET modalities to fully quantify the amount of broadening induced by immobilization and other technical sources. This work is expected to provide important guidelines for high-resolution probing of biomolecular structures and dynamics using smFRET.

Poster 31

WILSON SUN, Johns Hopkins University

DEAD-box Protein CsdA Refolds Nascent rRNA

Authors:
Wilson Sun, Sarah Woodson

Abstract:
Ribosome assembly in bacteria cells is coupled with rRNA transcription and is completed within about 2 minutes. During the assembly process, the rRNA folds, and ribosomal proteins bind to the rRNA in a hierarchical order. For the small subunit ribosome in E. coli, ribosomal protein S4 is one of the initial proteins to stably bind the rRNA. Unexpectedly, S4 binding during transcription is mainly transient and non-productive (Rodgers and Woodson, 2019). A 30-minute refolding is required for the rRNA to stably incorporate S4, which is significantly slower than assembly in vivo. In the cell, the nascent rRNA may be refolded by RNA chaperones, such as ATP-dependent DEAD-box proteins that locally unwind RNA duplexes (Bhaskaran and Russell, 2007). According to the generalized iterative annealing mechanism, the DEAD-box protein repeatedly unfolds the RNA, leading to the accumulation of rRNA in the native conformation (Chakrabarti et al., 2017). We developed a smFRET method to monitor the specific interactions between S4 and newly transcribed rRNA. After the addition of 50 nM DEAD-box protein CsdA, S4 stably binds to the nascent rRNA at a level comparable to that of pre-folded rRNA. This effect cannot be achieved by a helicase inactive mutant of CsdA. Excess CsdA disrupts the structure of the pre-folded rRNA and hampers S4 binding, as predicted by the generalized iterative annealing mechanism. Conversely, the native S4 – rRNA complex impedes unfolding by CsdA. Therefore, formation of a stable RNP may be a natural checkpoint for terminating repetitive unfolding. Our study illuminates the role of DEAD-box proteins in ribosome biogenesis and provides insight into how the rRNA structural integrity is maintained during the early stages of assembly.

Poster 32

GORDON SUN, Johns Hopkins University

Dynamic Self-Organization of Mitochondria Facilitates Asymmetric Damage Inheritance during Cell Division

Authors:
Gordon Sun, Christine Hwang, Tony Jung, Jian Liu, Rong Li

Abstract:
Mitochondria are essential organelles in eukaryotic cells and their inheritance is crucial during cell division. In cells undergoing asymmetric cell division, selective inheritance of fittest mitochondria has been thought to be a means of prolonging the proliferative capacity of stem-like cells for the population. However, the mechanism by which mitochondria are remodeled for asymmetric inheritance is not yet fully understood. To investigate this, we characterized various aspects of mitochondrial behavior in response to aggregation of misfolded proteins in mitochondrial matrix, including fission/fusion, diffusive motion, and fission/fusion site placement, using high-resolution live-cell imaging of dividing budding yeast cells. Using these parameters, we developed an in-silico agent-based model to evaluate the impact of mitochondrial dynamics on the inheritance of damage-bearing mitochondria. The findings reveal biased mitochondrial fission as a mechanism for reducing the inheritance of damaged mitochondria in the bud by facilitating the clustering of proteins aggregates. This mechanism is particularly important when diffusive mobility in mitochondrial matrix is limited with a discontinuous inner matrix.

Poster 33

EWA SZCZESNA, NINDS/NIH

Combinatorial readout of the tubulin code by katanin

Authors:
Ewa Szczesna, Elena A. Zehr, Steven W. Cummings, Agnieszka Szyk, Kishore K. Mahalingan, Yan Li, Antonina Roll-Mecak

Abstract:
Cells functionalize microtubules with spatiotemporally complex patterns of posttranslational modifications. How effectors interpret this tubulin modification code is largely unknown. Here, we show that katanin, a microtubule severing AAA ATPase mutated in microcephaly and critical for cell division, axonal elongation and cilia biogenesis, responds precisely, differentially and combinatorially to three chemically distinct modifications: glycylation, glutamylation, and tyrosination, but is insensitive to tubulin acetylation. Glutamylation and glycylation act as antagonistic rheostats with glycylation being protective of microtubules. Katanin exhibits graded and divergent responses to glutamylation on the α- and β-tubulin tails, and these act combinatorially. The structure of the katanin hexamer central pore constrains the polyglutamate chain patterns on β-tails that can productively be recognized. In contrast, elements distal to the katanin AAA core sense α-tubulin tyrosination, and detyrosination downregulates severing. The multivalent microtubule recognition that enables katanin to read multiple tubulin modification inputs explains in vivo observations and illustrates how effectors can integrate tubulin code signals to produce diverse functional outcomes. A.R.M is supported by the intramural programs of the National Institute of Neurological Disorders and Stroke (NINDS) and the National Heart, Lung and Blood Institute (NHLBI).

Poster 34

QING TANG, University of Pennsylvania

Detyrosination enrichment on microtubule subsets is established by the interplay between a stochastically-acting enzyme and microtubule stability

Authors:
Qing Tang, Sebastian Sensale, Charles Bond, Andy Qiao, Siewert Hugelier, Arian Arab, Gaurav Arya, Melike Lakadamyali

Abstract:
Microtubules in cells consist of functionally diverse subpopulations carrying distinct post-translational modifications (PTMs). Akin to the histone code, the tubulin code regulates a myriad of microtubule functions ranging from intracellular transport to chromosome segregation. Yet, how individual PTMs only occur on subsets of microtubules to contribute to microtubule specialization is not well understood. In particular, microtubule detyrosination, which is the removal of the C-terminal tyrosine on α-tubulin subunits, marks the stable population of microtubules and modifies how microtubules interact with other microtubule-associated proteins to regulate a wide range of cellular processes. Previously, we found that, in certain cell types, only a small subpopulation of microtubules is highly enriched with the detyrosination mark (~30%) and that detyrosination spans most of the length of a microtubule, often adjacent to a completely tyrosinated microtubule. How the activity of a cytosolic detyrosinase, Vasohibin (VASH) leads to only a small subpopulation of highly detyrosinated microtubules is unclear. Here, using quantitative super-resolution microscopy, we visualized nascent microtubule detyrosination events in cells consisting of 1-3 detyrosinated α-tubulin subunits after Nocodazole washout. Microtubule detyrosination accumulates slowly and in a disperse pattern across the microtubule length. By visualizing single molecules of VASH in live cells, we found that VASH engages with microtubules stochastically on a short time scale suggesting limited removal of tyrosine per interaction, consistent with the super-resolution results. Combining these quantitative imaging results with simulations incorporating parameters from our experiments, we propose a stochastic model for cells to establish a subset of detyrosinated microtubules via a detyrosination-stabilization feedback mechanism.

Poster 35

RAQUEL MERINO URTEAGA, Johns Hopkins University

Single-molecule analysis of cytosolic DNA sensor sequestration by chromatin binding

Authors:
Raquel Merino Urteaga, Alexander Strom, Xinyu A. Feng, Jungsan Sohn, Taekjip Ha

Abstract:
Cyclic-G/AMP synthase (cGAS) initiates the innate immune response against cytosolic double-stranded DNA in eukaryotic cells. Recent findings showed that cGAS is predominantly localized in the nucleus. It is believed that nuclear cGAS is tightly tethered to chromatin in an inactive state. Cryo-EM reports suggest that a cGAS monomer binds to the acidic patch of the nucleosome core and nucleosomal DNA. These studies have shown that two nucleosomes can be held together by two cGAS protomers. However, the dynamics involved in the interaction between cGAS and nucleosomes remains poorly understood and most studies have only used the catalytic domain of cGAS (cGASCAT). Here, we utilized single-molecule FRET to study the interaction between cGAS and a nucleosome core particle (NCP). This assay revealed that cGASCAT binds with high affinity to an NCP, with Kd=0.72nM, and remains associated with the nucleosome for 13 seconds. By contrast, the full-length protein (cGASFL) displays a lower affinity (Kd=1.35nM) and exhibits heterogeneous dissociation kinetics. Notably, a subset of cGASFL can remain stably tethered to nucleosomes. We used a dinucleosome construct with an internal FRET pair to monitor its conformational rearrangements upon cGAS binding. This revealed that cGAS draws the nucleosomes closer together stably over time. Additionally, cGASFL displayed positive cooperativity when mediating dinucleosome compaction. Real-time measurements reveal the existence of an intermediate state of transiently compacted dinucleosomes. These studies provide insight into the dynamic picture of cGAS-nucleosome interaction, the generation of oligomeric assemblies between cGAS and chromatin, and highlight the mechanism ruling cGAS nuclear sequestration.

Poster 36

QILAN WEI, Affiliation

The Effect of Nucleotide Modification on 30S Ribosome Assembly

Authors:
Qilan Wei and Sarah A. Woodson

Abstract:
Chemical modifications are widely present in different types of RNA. In ribosomal RNA (rRNA), modified nucleotides are concentrated in functionally important regions. RsmB is a methyltransferase that modifies C967 of the E. coli 16S rRNA. Deletion of RsmB impairs pre-rRNA processing. However, the precise roles of m5C967 and RsmB in ribosome assembly are unknown. RsmB's target site is only accessible during the early steps of 16S 3' domain assembly, which indicates that methylation may occur during nascent rRNA folding and initial ribosomal proteins (r-proteins) recruitment. Here we examined whether the m5C967 can change the binding of r-proteins, and whether RsmB can work as a chaperone to help the folding of rRNA and further assist the binding of r-proteins during transcription. Using electrophoretic mobility shift assays (EMSA) and single-molecule total internal reflection fluorescence (TIRF) microscopy, we found that the primary assembly r-protein uS7 prefers to bind to RNA-RsmB complex than RNA alone, and similarly, RsmB also has a higher affinity to RNA-uS7 complex. m5C967 itself does not affect the binding affinity of uS7. In the future, we will test whether RsmB can modify the rRNA during transcription and fold the nascent rRNA into its native structure. We will also determine whether m5C967 improves the binding of other 3'domain r-proteins that have direct interactions with the methylation site. Our results will illuminate how rRNA modifications and their modification enzymes accelerate the assembly process and stabilize rRNA-protein complexes.

Poster 37

BERSABEL WONDIMAGEGNHU, Johns Hopkins University

The molecular mechanism for TERRA recruitment and annealing to telomere

Authors:
Bersabel Wondimagegnhu, Tapas Paul, Ting-Wei Liao, Chun-Ying (Jim) Lee, Sua Myong

Abstract:
Telomeric repeat containing RNA (TERRA), a noncoding RNA that is transcribed from telomeres. TERRA trans anneals by invading the telomeric duplex to form an R-loop. Despite the evidence of TERRA's role in telomere structure and function, our molecular understanding of TERRA is limited. Here, we elucidate the molecular mechanism underlying TERRA recruitment and invasion into telomeres in the context of shelterin, RAD51 and RNaseH1 using single molecule (sm) assays. Using smFRET we show that TERRA trans anneals to telomeric DNA and exhibits dynamic movement that is stabilized by TRF2. Additionally, TERRA annealing to the telomeric duplex forms a triplex structure. Sm-colocalization assays show that the presence of non-TERRA sequence, which represents the sub-telomeric DNA and the presence of G-quadruplex telomeric overhang enhances TERRA binding to telomeric duplex. We also studied the mechanism underlying TERRA recruitment and invasion by Rad51 and shelterin proteins. Our result shows that RAD51-TERRA complex invades telomere duplex more efficiently than TERRA alone. Additionally, TRF2 increases TERRA affinity to telomeric duplex and protects it from RNaseH1 digestion. In contrast, TRF1 represses TERRA binding to telomeric duplex and doesn't provide protection against RNaseH1. We demonstrate the molecular mechanism underpinning TERRA recruitment and annealing to telomere.

Poster 38

HAOTIAN WU, UMBC

Hyperosmotic stress alters enzyme stoichiometry of glucosomes

Authors:
Haotian Wu, Spencer Jaquet, Erin Kennedy, Songon An, Minjoung Kyoung

Abstract:
Glucose metabolism is one of the nexuses connecting both energy metabolism and building block biosynthesis in living cells. In our previous study, human enzymes in glucose metabolism, including PFKL, PKM2, etc., are found to be spatially organized into membraneless compartments named glucosomes. However, how glucosomes respond to environmental changes remain to be investigated. To study how hyperosmotic pressure affect glucosomes, we applied cell media with various NaCl concentrations to live cells. Our observations indicate that the partitioning of enzymes into glucosomes is highly sensitive to changes in NaCl concentration, resulting in significant alterations to enzyme stoichiometries in glucosomes. Furthermore, we observed that under the 200 mM NaCl condition, glucosomes exhibit greater reversibility in terms of enzyme composition ratios compared to the 300 mM condition. These results suggest that glucosomes' metabolic activities may be affected by hyperosmotic pressure.

Poster 39

ZIQING YE, Johns Hopkins University

KuantifAir: Unsupervised learning-based detection of mRNA clustering behavior in vivo

Authors:
Ziqing Ye, Siran Tian, Jeff Liao, Aritra Basu, Isabel Betances, Tatjana Trcek

Abstract:
mRNA localization is a universal process that controls the spatial organization of gene expression. One mechanism by which a cell can regulate mRNA localization is through mRNA dimerization mediated by sequence-specific Watson-Crick base pairing. For example, a dimerization motif drives bicoid mRNA localization to the Drosophila embryo anterior (Wagner 2001). To better understand the biological roles of mRNA multimerization in mRNA spatial localization requires a reliable way to detect mRNA multimers in cells while preserving their spatial information. This naturally calls for an in vivo imaging approach, where multimers of fluorescently-labeled molecules can be detected through inspecting the fluorescent intensities. However, one major challenge is the inherent fluorescent intensity variations introduced during the imaging process. To address this issue, we introduce KuantifAir, an unsupervised learning-based program that enables quantification of mRNA clustering behavior in vivo in a parameter-independent manner, when combined with single molecule fluorescent in situ hybridization (smFISH). We present the full automated KuantifAir workflow, and illustrate that it yields consistent quantification results independent of arbitrary program parameters. We show that KuantifAir combined with smFISH captures clustering behavior of mRNAs with dimerization motif insertions in both Drosophila embryos and transfected human U2OS cells. In addition, KuantifAir is versatile and flexible, has good compatibility with existing spot-detection programs, and has potentials for many other applications in the single molecule imaging field both in vivo and in vitro where fluorescent intensity scales with the number of fluorescently-labeled molecules in one resolvable spot.

Poster 40

MARTIN YEPES, Johns Hopkins School of Medicine

Investigating the role of E. coli FtsZ membrane tethers in relaying treadmilling dynamics and activating cell wall synthesis

Authors:
Martin Yepes Trujillo, Joshua McCausland, Piet de Boer, Jie Xiao

Abstract:
Bacterial cell wall synthesis during cell division is an important target in antibiotic development. The assembly of the divisome begins with the formation of treadmilling FtsZ filaments in the cytosol and culminates in the synthesis of septal peptidoglycan (sPG) by FtsI in the periplasm. FtsZ relies on its membrane tethers FtsA and ZipA to relay its position through the inner membrane to the periplasm. The dynamics of FtsA and ZipA remain poorly understood despite these two proteins being a key checkpoint in cell division. Neither ΔzipA or ΔftsA cells are viable, but cells expressing a gain-of-function variant FtsAR286W (FtsA*) exhibit a “superfission” phenotype, which compensates for the deletion of ZipA. Previous work has shown that FtsI has fast or slow modes of movement that are coupled to FtsZ treadmilling or active sPG synthesis, respectively, and that the ratio between these two directionally moving populations is sensitive to upstream effects on the divisome. In this study, we monitor the moving dynamics of single FtsI and FtsA molecules in various genetic backgrounds including wt, ftsA*, and ftsA* / ΔzipA through single molecule tracking (SMT). We find that a higher fraction of FtsI is actively synthesizing sPG in cells expressing FtsA*, while FtsA is mostly stationary in all backgrounds. Our study suggests that FtsA’s oligomeric state plays an important role in relaying the dynamic information of the Z-ring to sPG synthases.

Poster 41

JASMIN ZARB, Johns Hopkins University School of Medicine

Probing Mismatch Type Dependence of DNA Mismatch Repair at the Single Molecule Level

Authors:
Jasmin Zarb, Tunc Kayikcioglu, James A. London, Richard Fishel, Taekjip Ha

Abstract:
During DNA replication, DNA polymerases can incorrectly incorporate a non-complementary nucleotide against the parental DNA strand, leading to mutations and ultimately debilitating diseases if left unrepaired. All organisms possess a mismatch repair system that scans, recognizes, and repairs DNA mismatches. MutS is one of the first repair proteins to arrive at a mismatch. Upon recognition, MutS exchanges bound ADP molecules to ATP, and undergoes a conformational change to form a sliding clamp and slide away from the mismatch. MutS will then recruit other repair proteins to the site and resolve the error. Previous in vivo studies from our lab suggest that the mismatch repair efficiency in E.coli is hypervariable not only for different mismatched bases, but also for their local sequence context. Deletion studies showed that MutS is responsible for the observed hypervariability. To understand the molecular mechanism underlying sequence dependence of MutS, we employed single molecule FRET (fluorescence resonance energy transfer) to directly detect MutS binding to a mismatch, sliding clamp formation, and dissociation. The binding characteristics of E. coli MutS showed a striking dependence of the mismatch type and the overall residence time of MutS on a mismatch-containing DNA invitro was strongly correlated with the in vivo mismatch repair efficiencies. Furthermore, the sliding clamp formation yield in the presence of ATP was correlated within vivo mismatch repair efficiencies. These data support in vivo repair trends and help clarify mechanistic basis by which mismatches are differentially repaired. Future experiments will be conducted using additional DNA mismatches with different sequence contexts to understand the generalizability of this observation

Poster 42

JAKUB ZIAK, Johns Hopkins School of Medicine

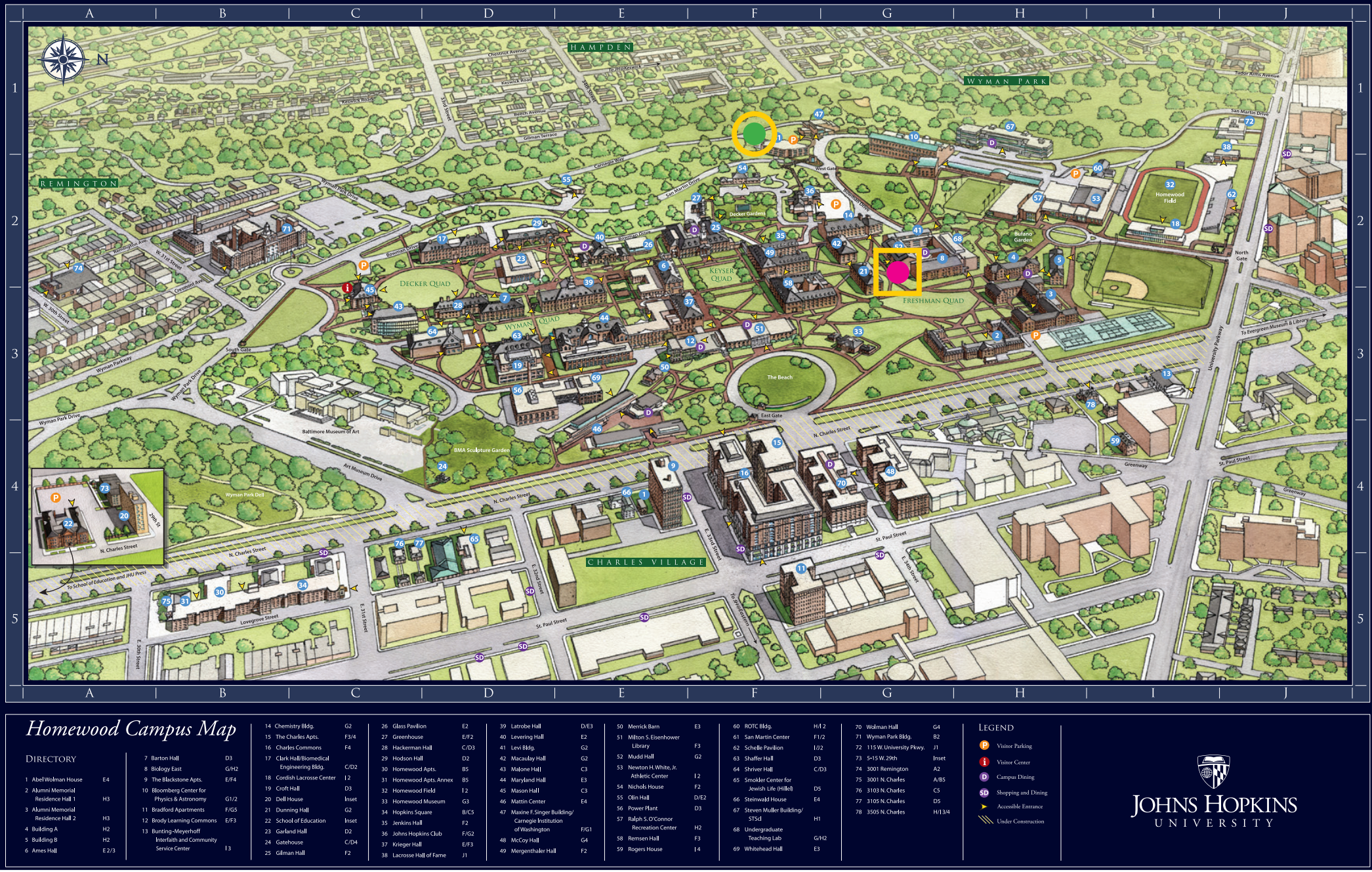
Molecular regulators of cortical layer 2/3 excitatory neuron connectivity at the single neuron resolution

Authors:
Jakub Ziak, Sriram Sudarsanam, Brian Trigg, Joelle Dorskind, Yijun Xu, Soraia Barao, Ulrich Mueller and Alex Kolodkin

Abstract:
The cerebral cortex is a laminated structure that plays a central role in thought, memory, motor skills and sensory processing. The establishment of functional cortical connectivity requires that neurons elaborate complex and unique axon branching patterns. However, the intracellular signaling pathways, receptors, cell adhesion molecules and extracellular ligands that regulate laminar-specific interstitial axon branching in the cortex are poorly understood. Strikingly, experimental approaches that allow for studying mouse neuronal connectivity in a quantitative fashion at the single cell level are limited.

We are investigating the unique patterns of interstitial axon branching and overall morphology of layer 2/3 callosal projection neurons (CPNs). We have developed in utero electroporation strategies that allow for temporally controlled, sparse and very robust labeling of select excitatory cortical CPNs. Combining these approaches with brain clearing and lightsheet imaging allows for developing single-cell resolution image datasets of complete axonal arbors.

Using these techniques, we show that activation of the serine threonine kinase GSK3β induces excessive axon branching in layer 2/3 CPNs and we identify one of its targets, MAP1B, as a cell-autonomous branch restricting factor. In addition, we find that GSK3β/MAP1B signaling regulates the tyrosination/detyrosination cycle of α-tubulin, which in turn increases or decreases the probability of generating interstitial axon branches in layer 2/3 CPNs. Targeted expression of a fluorescent sensor in layer 2/3 CPNs reveals high levels of tyrosinated α-tubulin in axonal segments enriched for interstitial axon branches. Together, these data describe a cell-autonomous molecular pathway that regulates interstitial axon branching in mammalian cortical neurons.



Homewood Campus Map

Directory

1	Abel Wolman House	E4
2	Alumni Memorial Residence Hall 1	H3
3	Alumni Memorial Residence Hall 2	H3
4	Building A	H2
5	Building B	H2
6	Ames Hall	E 2/3
7	Barton Hall	D3
8	Biology East	G/H2
9	The Blackstone Apts.	E/F4
10	Bloomberg Center for Physics & Astronomy	G1/2
11	Bradford Apartments	F/G5
12	Brody Learning Commons	E/F3
13	Bunting-Meyerhoff Interfaith and Community Service Center	I 3
14	Chemistry Bldg.	G2
15	The Charles Apts.	F3/4
16	Charles Commons	F4
17	Clark Hall/Biomedical Engineering Bldg.	C/D2
18	Cordish Lacrosse Center	I 2
19	Croft Hall	D3
20	Dell House	Inset
21	Dunning Hall	G2
22	School of Education	Inset
23	Garland Hall	D2
24	Gatehouse	C/D4
25	Gilman Hall	F2
26	Glass Pavilion	E2
27	Greenhouse	E/F2
28	Hackerman Hall	C/D3
29	Hodson Hall	D2
30	Homewood Apts.	B5
31	Homewood Apts. Annex	B5
32	Homewood Field	I 2
33	Homewood Museum	G3
34	Hopkins Square	B/C5
35	Jenkins Hall	F2
36	Johns Hopkins Club	F/G2
37	Krieger Hall	E/F3
38	Lacrosse Hall of Fame	J1
39	Latrobe Hall	D/E3
40	Levering Hall	E2
41	Levi Bldg.	G2
42	Macaulay Hall	G2
43	Malone Hall	C3
44	Maryland Hall	E3
45	Mason Hall	C3
46	Mattin Center	E4
47	Maxine F. Singer Building/ Carnegie Institution of Washington	F/G1
48	McCoy Hall	G4
49	Mergenthaler Hall	F2
50	Merrick Barn	E3
51	Milton S. Eisenhower Library	F3
52	Mudd Hall	G2
53	Newton H. White, Jr. Athletic Center	I 2
54	Nichols House	F2
55	Olin Hall	D/E2
56	Power Plant	D3
57	Ralph S. O'Connor Recreation Center	H2
58	Remsen Hall	F3
59	Rogers House	I 4
60	ROTC Bldg.	H/I 2
61	San Martin Center	F1/2
62	Schelle Pavilion	I/J2
63	Shaffer Hall	D3
64	Shriver Hall	C/D3
65	Smokler Center for Jewish Life (Hillel)	D5
66	Steinwald House	E4
67	Steven Muller Building/ STSci	H1
68	Undergraduate Teaching Lab	G/H2
69	Whitehead Hall	E3
70	Wolman Hall	G4
71	Wyman Park Bldg.	B2
72	115 W. University Pkwy.	J1
73	5–15 W. 29th	Inset
74	3001 Remington	A2
75	3001 N. Charles	A/B5
76	3103 N. Charles	C5
77	3105 N. Charles	D5
78	3505 N. Charles	H/13/4

Legend

- Visitor Parking
- Visitor Center
- Campus Dining
- Shopping and Dining
- Accessible Entrance
- Under Construction

JOHNS HOPKINS UNIVERSITY

Map & Directions from San Martin Parking Garage to Mudd Hall

San Martin Parking Garage is marked with a green dot & has a yellow circle around it. Mudd Hall is marked with a pink circle & has a yellow rectangle around it. Starting at San Martin Garage, walk towards the ground level stairs and elevators. Exit the parking lot, taking a left onto the path. Cross the street at the first crosswalk. Walk up the sidewalk in the direction of the dark lacrosse arch & JHU sign. Pass the Hopkins Club on the right. Take the stairs near the benches up to the building. Mudd Hall will be the building to the left.

PARTICIPANTS

Guilio Agnetti, *JHU School of Medicine*
Amelia Alessi, *Johns Hopkins University*
Michael Banco, *NIH*
Aritra Basu, *Johns Hopkins University*
Nils Benning, *Johns Hopkins University*
Christopher Bohrer, *NIH*
Pedro Andrade Bonilla, *NIH*
Gregory Bowman, *Johns Hopkins University*
Brooke Britton, *Johns Hopkins School of Medicine*
Rodrigo Caceres, *Frederick National Laboratory*
Danfeng Cai, *Johns Hopkins Bloomberg School of Public Health*
Nagaraju Chada, *Johns Hopkins University*
Iryna Chelepis, *Johns Hopkins University*
Siqi Chen, *Johns Hopkins School of Medicine*
Jiayi Chen, *NIH/NINDS*
Xin Chen, *Johns Hopkins University*
Yi-Lan Chen, *Johns Hopkins University*
Lily Chen, *Johns Hopkins University*
Austin Chiappetta, *Johns Hopkins University*
Bok-Eum Choi, *NIH/NIDDK*
Hoi Chung Sung, *NIH/NIDDK*
Inhee Chung, *GWU*
Anson Dang, *Johns Hopkins University*
Ana De La Cruz, *Johns Hopkins University*
Ben Donovan, *NIH*
Karen Elmore, *NIH/NINDS*
Chi-Jui Feng, *NIH*
Xinyu Feng, *Johns Hopkins University*
Karen Fleming, *Johns Hopkins University*
Adler Guerrero, *Johns Hopkins School of Medicine*
Stephen Ha, *Johns Hopkins School of Medicine*
Taekjip Ha, *Johns Hopkins University*
Hannah Haller-Hidalgo, *Johns Hopkins University*
Gabor Harami, *NIH*
Fran Harris, *Johns Hopkins School of Medicine*
Shuaixin He, *Johns Hopkins School of Medicine*
Alexandra Hollo, *NIH/NHLBI*
Andrew Hoy, *Georgetown University*
Jihee Hwang, *Johns Hopkins University*
Myung Hyun Jo, *Johns Hopkins University School of Medicine*
Sina Jazani, *Johns Hopkins University School of Medicine*
Gabriel Jimenez, *Johns Hopkins University*
Christian Kaiser, *Johns Hopkins University*
Marina Kalinina, *Johns Hopkins University*
Jee Min Kim, *NIH*
Jae-Yeol Kim, *NIH*
Dohyun Kim, *Johns Hopkins School of Medicine*
Jiwoong Kwon, *Johns Hopkins University*
Minjoung Kyoung, *UMBC*
Daniel Larson, *NIH*
Chun-Ying Lee, *Johns Hopkins University*
Jane Lee, *Johns Hopkins University*
Ye Jin Lee, *Johns Hopkins University*
Huijin Lee, *Johns Hopkins University School of Medicine*
Choon Leng So, *Johns Hopkins Bloomberg School of Public Health*
Jiaxin Li, *Johns Hopkins University*
Ting-Wei Liao, *Johns Hopkins University*
Yuan Lou, *Johns Hopkins University*
Jason Lyu, *Johns Hopkins School of Medicine*
Mira Malmosi, *Georgetown University*
Edgar Manriquez-Sandoval, *Georgetown University*
Maranda McDonald, *Johns Hopkins University*

Daniël Melters, *NCI*
Christian Mendoza, *Johns Hopkins University*
Paul Meneses, *Johns Hopkins University*
Kiyoshi Mizuuchi, *NIH/NIDDK*
Ian Morgan, *NIH NHLBI*
Peter Murray, *Johns Hopkins University*
Sua Myong, *Johns Hopkins University*
Emily Nakayama, *Johns Hopkins School of Medicine*
Blake Nelson, *Johns Hopkins School of Medicine*
Tanya Nesterova, *JHU School of Medicine*
Keir Neuman, *NIH*
Brett O'Brien, *Johns Hopkins University*
Miriam Owoola, *Johns Hopkins University*
Luiz Passalacqua, *NIH*
Tapas Paul, *Johns Hopkins University*
Amilcar Perez, *Johns Hopkins University*
Meeknakshi Pillai, *Johns Hopkins Bloomberg School of Public Health*
Mihirkumar Prajapati, *NIH/NHLBI*
Tatjana Pulisic, *Johns Hopkins University*
Nandakumar Rajasekaran, *Johns Hopkins University*
Rajesh Ranjan, *Johns Hopkins University*
Anand Ranjan, *Johns Hopkins University*
Sergi Regot, *Johns Hopkins University School of Medicine*
Jan Rios, *Johns Hopkins School of Medicine*
Margaret Rodgers, *NIH*
Sergei Rudnizky, *Johns Hopkins School of Medicine*
Adam Rybczynski, *Johns Hopkins University*
Ashesh Sharma, *UMBC*
Prashanta Sharma Roy, *UMBC*
Rebika Shrestha, *Frederick National Lab/NIH*
Sara Sohail, *NIH*
Jungsan Sohn, *Johns Hopkins School of Medicine*
Gordon Sun, *Johns Hopkins University*
Yonglei Sun, *NIH*
Wilson, Sun, *Johns Hopkins University*
Ewa Szczesna, *NIH*
Qing Tang, *University of Pennsylvania*
Xiaona Tang, *Johns Hopkins University*
Derrin Tanrioven, *Johns Hopkins University*
Dmitri Toptygin, *Johns Hopkins University*
Brian Trigg, *Johns Hopkins School of Medicine*
Thomas Turbyville, *Frederick National Lab/NIH*
Raquel Merino Urteaga, *Johns Hopkins University*
Ben Veresko, *Johns Hopkins School of Medicine*
Quan Wang, *NIH*
Yiran Wang, *Johns Hopkins Bloomberg School of Public Health*
Leslie Watkins, *Johns Hopkins School of Medicine*
Qilan Wei, *Johns Hopkins University*
Lukas Whaley-Mayda, *NIH*
Elise White, *Johns Hopkins University*
Clara Wolfe, *Johns Hopkins School of Medicine*
Sarah Woodson, *Johns Hopkins University*
Haotian Wu, *UMBC*
Bin Wu, *Johns Hopkins School of Medicine*
Carl Wu, *Johns Hopkins University*
Jie Xiao, *Johns Hopkins School of Medicine*
Ziqing Ye, *Johns Hopkins University*
Nicolas Yehya, *Johns Hopkins School of Medicine*
Martin Yepes, *Johns Hopkins University School of Medicine*
Jasmin Zarb, *Johns Hopkins School of Medicine*
Elena Zehr, *NIH*
Yaoyuan Zhang, *Johns Hopkins University, Carey Business School*
Maggie Zhu, *Johns Hopkins University*
Jakub Ziak, *Johns Hopkins School of Medicine*

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