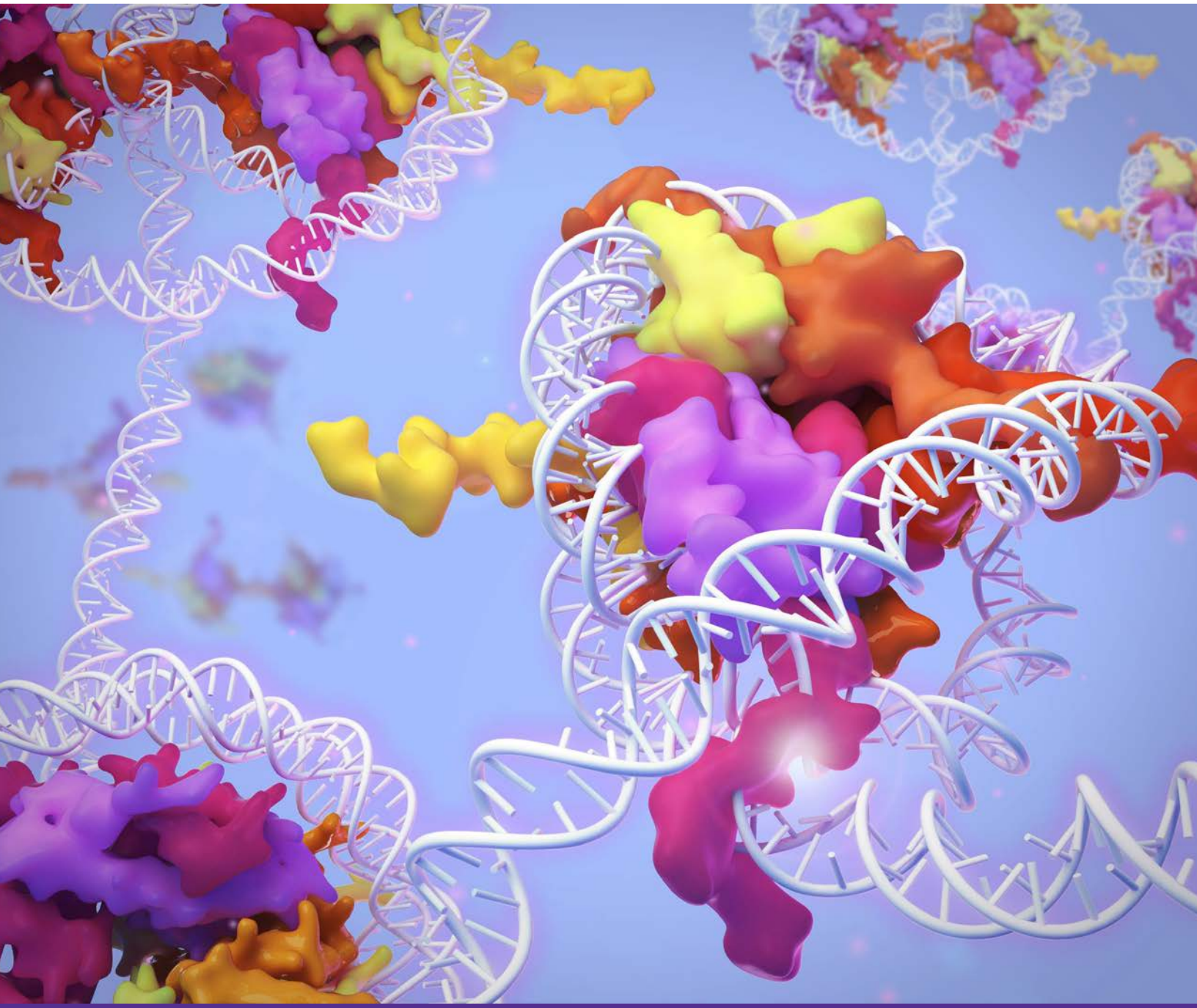
NORTHWESTERN UNIVERSITY FEINBERG SCHOOL OF MEDICINE

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR GENETICS



EIGHTH ANNUAL RETREAT
SEPTEMBER 13, 2024

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ITINERARY

SIMPSON QUERREY BIOMEDICAL RESEARCH CENTER POTOCSNAK ATRIUM

8:30 - 9:00am	Poster Set-Up
9:00 - 11:00am	Poster Session
11:00 - 11:55am	Lunch

SIMPSON QUERREY BIOMEDICAL RESEARCH CENTER AUDITORIUM

12:00 - 12:20 PM	Ali Shilatifard, PhD, Chairman, Biochemistry and Molecular Genetics, Simpson Querrey Institute for Epigenetics *Department Overview and Welcoming Remarks*
12:25 - 12:45 PM	Iris Titos Vivancos, PhD, Assistant Professor, Biochemistry and Molecular Genetics *"A gut feeling for behavior"*
12:45 - 12:50 PM	Q&A
12:50 - 1:10 PM	Shannon Lauberth, PhD, Associate Professor, Biochemistry and Molecular Genetics *"The role of noncoding RNAs and RBPs in cancer: Shedding light on the transcriptomic dark matter"*
1:10 - 1:15 PM	Q&A
1:15 - 1:35 PM	Feng Yue, PhD, Director, Institute for Artificial Intelligence in Medicine-Center for Advanced Molecular Analysis; Duane and Susan Burnham Professor of Molecular Medicine; Professor, Biochemistry and Molecular Genetics, Pathology *"3D genome alteration in human cancer"*
1:35 - 1:40 PM	Q&A
1:40 - 1:45 PM	*Introduction of Keynote*
1:45 - 2:35 PM	Keynote Speaker: Karen Cichowski, PhD, Professor, Medicine, Harvard Medical School; Professor, Medicine/Genetics, Brigham And Women's Hospital; Co-Director of the Landry Cancer Biology Consortium *"Strategies for developing combinatorial cancer therapies"*
2:35 - 2:50 PM	Q&A
2:50 - 3:00 PM	Lillian Eichner, PhD, Assistant Professor, Department of Biochemistry and Molecular Genetics and Clara Peek, PhD, Assistant Professor, Biochemistry and Molecular Genetics, Medicine (Endocrinology) *BMG Retreat Committee Presentation of Awards and Closing Remarks*
3:00 - 3:15 PM	Shuttlebus Boarding in rear of Simpson Querrey Biomedical Research Center, 340 E. Huron
3:15 PM	Shuttlebus departs to DuSable Harbor. The Anita Dee II is docked on the South side of the Chicago River at Lake Shore Drive, 200 N Breakwater Access, Chicago, IL 60601
4:00 - 6:00 PM	Reception aboard the Anita Dee II

KEYNOTE SPEAKER

Karen Cichowski, PhD

Professor, Medicine, Harvard Medical School;
Professor, Medicine/Genetics, Brigham And Women's Hospital;
Co-Director of the Landry Cancer Biology Consortium

Karen received her Ph.D. in genetics at the University of Pennsylvania and completed her post-doctoral training in cancer biology at MIT.

Dr. Cichowski is the Founder and Director of the Center for Developing Targeted Cancer Therapies at Dana-Farber/Brigham and Women's Cancer Center and is a Professor of Medicine and Ludwig Investigator at Harvard Medical School. For more than 20 years her laboratory has been deconstructing mechanisms that underlie Ras-driven cancers and using this insight to devise new therapeutic strategies for these recalcitrant malignancies. Through her efforts as the Scientific Director of the NF Therapeutic Consortium and beyond, her discoveries have served as the foundation for multiple clinical trials in nervous system, lung, and breast cancers. In addition to her scientific endeavors, she is the Associate Director of Planning at the Dana Farber/Harvard Cancer Center, where she plays a leadership role in developing new strategic initiatives.

STRATEGIES FOR DEVELOPING COMBINATORIAL CANCER THERAPIES

The RAS pathway is one of the most commonly deregulated pathways in human cancer. Approximately 30% of all tumors harbor activating mutations in either KRAS, NRAS or HRAS, and even more possess alterations in other genes that enhance RAS signaling. While RAS was thought to be undruggable for decades, agents that directly inhibit KRAS and/or specific KRAS oncoproteins have now been developed. Nevertheless, not all patients respond to current clinical agents and responses are often temporary. As such there is an urgent need to develop more effective therapies for these malignancies, which will likely require combinatorial approaches. Vertical strategies designed to target different nodes within the RAS pathway to achieve deeper suppression represents one promising approach. However, additional options are emerging, which may circumvent challenges associated with potent pathway suppression. In this presentation, various promising combinatorial strategies for RAS pathway driven cancers will be discussed, including new approaches for treating breast, colon and lung cancers. Importantly, by dissecting the mechanism by which these agents function we have uncovered nodal points of convergence between distinct oncogenic pathways, which represent key therapeutic vulnerabilities, and have identified biomarkers that can be used for patient selection.

Iris Titos Vivancos, PhD

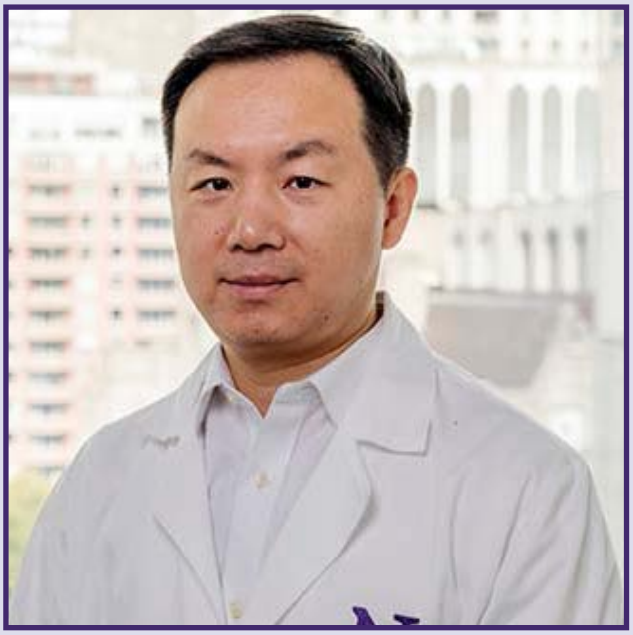
Assistant Professor, Biochemistry and Molecular Genetics, Northwestern University

"A gut feeling for behavior"

Shannon Lauberth, PhD

Associate Professor, Biochemistry and Molecular Genetics, Northwestern University

"The role of noncoding RNAs and RBPs in cancer: Shedding light on the transcriptomic dark matter"



Feng Yue, PhD

Director, Institute for Artificial Intelligence in Medicine- Center for Advanced Molecular Analysis; Duane and Susan Burnham Professor of Molecular Medicine; Professor, Biochemistry and Molecular Genetics, Pathology Northwestern University

"3D genome alteration in human cancer"

POSTER SUBMISSIONS

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26	Olivia Vidal-Cruchez, PhD	*WNK1 kinase controls the Krebs cycle and cell proliferation*
27	Qixuan Wang	*Spatial 3D genome organization reveals intratumor heterogeneity in primary glioblastoma samples*
28	Josiah Wong	*The role of SUV39H1/2-mediated H3K9me3 in maintaining 3D genome organization in acute myeloid leukemia*

Koke Abe, PhD

Postdoctoral Fellow, Lauberth Lab, Biochemistry and Molecular Genetics, Northwestern University

Kouki Abe, Brian Maunze, Pedro-Avila Lopez, Jessica Xu, Nefertiti Muhammad, Guang-Yu Yang, David Katz, Yaping Liu, Shannon M. Lauberth

Downstream-of-gene (DoG) transcripts contribute to an imbalance in the cancer cell transcriptome

Downstream-of-gene (DoG) transcripts are an emerging class of noncoding RNAs. However, it remains largely unknown how DoG RNA production is regulated and whether alterations in DoG RNA signatures exist in major cancers. Here, through transcriptomic analyses of matched tumors and nonneoplastic tissues and cancer cell lines, we reveal a comprehensive catalog of DoG RNA signatures. Through separate lines of evidence, we support the biological importance of DoG RNAs in carcinogenesis. First, we show tissue-specific and stage-specific differential expression of DoG RNAs in tumors versus paired normal tissues with their respective host genes involved in tumor-promoting versus tumor-suppressor pathways. Second, we identify that differential DoG RNA expression is associated with poor patient survival. Third, we identify that DoG RNA induction is a consequence of treating colon cancer cells with the topoisomerase I (TOP1) poison camptothecin and following TOP1 depletion. Our results underlie the significance of DoG RNAs and TOP1-dependent regulation of DoG RNAs in diversifying and modulating the cancer transcriptome.

Ravi Bandaru

Data Analyst, Liu Lab, Biochemistry and Molecular Genetics, Northwestern University

Ravi Bandaru, Haizi Zheng, Hailu Fu, Li Wang, Rashmi S. Hegde, Yaping Liu

Predicting High-Order Chromatin Organization From Cell-Free DNA Co-Fragmentation Patterns Using Generative Adversarial Networks

Epigenetics, including the three-dimensional (3D) genome structure, plays a crucial role in gene regulation. Aberrations in 3D genome structure may contribute to disease development, including cancer. Hi-C, the current gold standard for characterizing chromosome conformation, has limitations—it requires fixed cells, which only permits the study of the 3D genome in a static state, complicating longitudinal monitoring of 3D genome aberrations.

Cell-free DNA (cfDNA) fragments present a promising solution to this issue. CfDNA, composed of short, circulating DNA fragments found in plasma, is released into the extracellular environment through apoptosis and necrosis. CfDNA is already being utilized to dynamically monitor genetic and epigenetic variations, such as DNA methylation and nucleosome positioning. However, methods for studying the 3D genome within cells using plasma cfDNA have not yet been developed.

CfDNA fragmentation is non-random and has associations with epigenetic factors. Recent research by Snyder et al. demonstrated that deeply sequenced cfDNA fragments could successfully reveal nucleosome occupancy across the genome. These findings suggest the potential for using cfDNA fragments to characterize other aspects of the epigenome, including the 3D genome.

Our objective is to develop a computational approach to infer the 3D genome using cfDNA fragments, enabling non-invasive and longitudinal monitoring of 3D genome aberrations.

Mannan Bhola, MS

Data Analyst, Lauberth Lab, Biochemistry and Molecular Genetics, Northwestern University

Mannan Bhola, Kouki Abe, Paola Orozco, Homa Rahnamoun, Pedro Avila-Lopez, Elijah Taylor, Nefertiti Muhammad, Bei Liu, Prachi Patel, John Marko, Anne C. Starner, Chuan He, Eric L. Van Nostrand, Alfonso Mondragón, and Shannon M. Lauberth

RNA Interacts with Topoisomerase I to Adjust DNA Topology

Topoisomerase I (TOP1) is an essential enzyme that relaxes DNA to prevent and dissipate torsional stress during transcription. However, the mechanisms underlying the regulation of TOP1 activity remain elusive. Using eCLIP and UV-RIP-seq in human colon cancer cells along with RNA EMSAs, BLI, and in vitro RNA binding assays, we identify TOP1 as an RNA binding protein (RBP). We show that TOP1 directly binds RNA in vitro and in cells and that most RNAs bound by TOP1 are mRNAs. Using a TOP1 RNA binding mutant and TOP1cc-seq to map TOP1 catalytic activity, we reveal that RNA opposes TOP1 activity as RNAPII commences transcription of active genes. We further demonstrate the inhibitory role of RNA in regulating TOP1 activity by employing DNA supercoiling assays and magnetic tweezers. These findings provide insight into the coordinated actions of RNA and TOP1 in regulating DNA topological stress intrinsic to RNAPII-dependent transcription.

Marcelo Bonini, PhD

Professor of Medicine (Hematology and Oncology) and Biochemistry and Molecular Genetics, Northwestern University

Flavio R. Palma, Diego R. Coelho, Kirthi Pulakanti, Marcelo J. Sakiyama, Yunping Huang, Jeanne M. Danes, Alison Meyer, Cristina M. Furdui, Douglas R. Spitz, Ana P. Gomes, Benjamin N. Gantner, Sridhar Rao, Vadim Backman, **Marcelo G. Bonini**

Histone H3.1 is a chromatin embedded redox sensor triggered by tumor cells developing adaptive phenotypic plasticity and multi-

Chromatin structure is regulated through histone post-translational modifications as well as histone variants. Although highly homologous, histone variants display unique amino acid sequences associated with specialized functions in gene transcription regulation. Abnormal incorporation of histone variants into chromatin contributes to cancer initiation, progression, metastasis, as well as therapy resistance. The current study reports a novel epigenetic function of histone H3.1 as a redox sensor embedded in heterochromatin. We found that because of its unique Cys96 residue, H3.1 is sensitive to and depleted by mitochondrial H2O2 in phenotypically plastic breast cancer cells undergoing epithelial to mesenchymal transition. We also found that increased levels of H2O2 in the nucleus promotes the exchange of H3.1 by H3.3 at specific promoter regions. This process facilitates opening of silenced chromatin domains to activate the transcription of genes associated with phenotypic plasticity and drug resistance. Scavenging nuclear H2O2 or amino acid substitution of H3.1(C96S), suppressed phenotypic plasticity, restored sensitivity to chemotherapeutic drugs and induced remission of metastatic breast cancer lesions in vivo. These results support the hypothesis that increased levels of H2O2 produced by mitochondria of breast cancer cells directly promote redox-regulated H3.1-dependent epigenetic remodeling leading to drug resistance and metastasis.

Bercin Cenik, PhD

Postdoctoral Fellow, Shilatifard Lab, Biochemistry and Molecular Genetics, Northwestern University

Bercin K Cenik, Yuki Aoi, Marta Iwanaszko, Benjamin C Howard, Grant D Andersen, Ali Shilatifard

TurboCas: A method for locus-specific dynamic labeling of genomic regions and their protein interactome

The regulation of gene expression is a complex process that requires the concerted action of transcription factors and chromatin binding proteins. Because this process is both unique to a given locus and varied in response to changing cellular conditions, dynamically mapping the chromatin binding activity at individual promoters and other regulatory loci is crucial to understanding how cis-regulatory elements control gene expression. Earlier methods could only characterize the static binding activity of a single given protein. However, the recent emergence of proximity labeling, a technique for interrogating protein-protein interactions, and the advances brought about by CRISPR technology have enabled the development of new methods that can dynamically map the chromatin binding and secondary association of multiple proteins at given loci. In this study we describe TurboCas, a method that leverages the latest generation of proximity labeling enzymes, miniTurbo, in combination with catalytically dead Cas9 to label chromatin-binding proteins efficiently, dynamically and in a site-specific manner. We demonstrate the use of TurboCas to identify proteins binding the promoter of the heat shock responsive FOS gene. We go on to cross-validate our hits through an independent platform using RNA polymerase II and Cyclin T1 immunoprecipitation. Using these two methodologies, we identify both canonical regulators of heat shock response and members of previously uncharacterized novel pathways as general heat shock regulators. TurboCas represents a significant improvement over previous locus-targeted proximity labelling methods, with the potential not only to deepen our understanding of regulatory pathways in cellular stress response, but more broadly to advance the transcriptional regulation and chromatin biology fields.

Claire Chaikin

PhD Candidate, Peek Lab,
Biochemistry and Molecular Genetics,
Northwestern University

Chaikin, C., Thakkar, A., Steffeck, A., Pfrender, E., Hamlish, N., Hung, K., Zhu, P., Song, W., Meza, G., Dayanidhi, S., Ben-Sahra, I., Bass, J., and Peek, C.

Skeletal Muscle Circadian Clock Regulates HIF1α-dependent Transcription and Glucose Homeostasis during Diet-induced Obesity

Circadian rhythms govern animal physiology, metabolism, and behavior allowing organisms to synchronize internal processes with their environment. Disrupted circadian rhythms lead to exacerbated symptoms of metabolic syndrome. Studies indicate that core clock factor, BMAL1, is required in skeletal muscle for proper insulin-dependent glucose uptake in lean mice; however, BMAL1's role during metabolic stress, such as diet-induced obesity (DIO), remains unclear. Recent work has uncovered a link between the molecular clock and the hypoxia inducible factor (HIF) response pathway, which controls the induction of glycolytic metabolism in skeletal muscle during nutrient stress. Thus, we hypothesized that the molecular clock mediates glucose utilization during DIO via control of HIF activity. Induction of DIO by high-fat diet (HFD) feeding increased muscle HIF1α target gene expression compared to regular chow (RC) fed controls. Furthermore, muscle-specific BMAL1-deficient mice showed impaired glucose tolerance ($p=0.0514$) and reduced HIF1α target gene expression during DIO. HIF1α target genes were not reduced in RC fed mice, suggesting that BMAL1 regulates HIF1α activity during HFD but not RC. Finally, we found that muscle-specific loss of VHL, a negative regulator of HIF activity, restores glucose disposal in muscle-specific BMAL1-deficient mice in DIO. RNA-sequencing of muscle-specific BMAL1-deficient mice and muscle-specific VHL/BMAL1-deficient mice showed HIF1α stabilization, via loss of VHL, rescues ~1/3 of downregulated genes. These data suggest an interaction between the clock and HIF response pathways during DIO that impacts whole body glucose metabolism and skeletal muscle specific gene expression. Ongoing studies are focused on understanding the molecular mechanisms linking BMAL1 and HIF1α.

Evan Couser

PhD Candidate, Eichner Lab,
Biochemistry and Molecular Genetics,
Northwestern University

Evan Couser, Austin T. Klein, Ambryn S. Meehan, Caroline K. McGuire, Marc L. Mendillo, Lillian J. Eichner

CRISPR screen reveals LKB1-specific mediators of therapeutic resistance in NSCLC

LKB1/Stk11 mutant non-small cell lung cancer (NSCLC) is a distinct subtype of NSCLC characterized by poor patient outcome and therapeutic resistance to standard-of-care therapies. Previous work from the Eichner lab uncovered an LKB1-specific mechanism of therapeutic resistance to the MEK inhibitor trametinib that involves upregulation of the kinase Fibroblast Growth Factor Receptor 1 (FGFR1). To determine if other kinases are involved in mediating therapeutic resistance in an LKB1-dependent manner, we carried out a CRISPR screen targeting the mouse kinome in isogenic LKB1-null lung cancer cells or without reconstitution with LKB1. Our screen revealed LKB1-specific essential genes in the context of therapeutic resistance to KRAS pathway inhibition. These hits included both known downstream effectors of LKB1 and proteins whose functions in LKB1 mutant NSCLC are unknown. Here, we validated a top hit from our screen using inducible degradation tag (dTAG) targeting the endogenous protein. We also show that degradation of this protein in combination with KRAS pathway inhibition slows proliferation of LKB1 mutant NSCLC cells in culture.

Jonathan Gurkan

MSTP Student, Singer Lab,
Pulmonary and Critical Care Medicine,
Northwestern University

Jonathan K. Gurkan, Manuel A. Torres Acosta, Anthony M. Joudi, Katheryn A. Helmin, Qianli Liu, Samuel M. Weinberg, Benjamin D. Singer

Metabolic regulation of regulatory T cells through fatty acid metabolism during viral pneumonia

Regulatory T cells (Tregs) are a subset of CD4+ T cells that maintain immune self-tolerance and mediate the resolution of lung injury during viral pneumonia by suppressing overexuberant inflammatory responses and facilitating tissue protection and repair. During viral infection, the lung microenvironment imposes substantial metabolic stress on Tregs. Tregs depend on mitochondrial oxidative phosphorylation, a process largely driven by fatty acid metabolism, to optimally perform their function. While there is preliminary evidence that Tregs are clinically efficacious as a cellular therapy for patients with severe viral pneumonia, there is a need to ascertain the context-specific metabolic mechanisms that promote Treg function to enhance their therapeutic activity. Here, we examined whether carnitine palmitoyltransferase 1 A (CPT1A), the rate-limiting enzyme in mitochondrial long chain fatty acid (LCFA) oxidation, is necessary for Treg cell function during viral pneumonia. While established in the microenvironment of tumors, it is unknown whether Tregs depend on LCFAs for their function during viral pneumonia and whether short chain fatty acids (SCFAs), which fuel oxidative phosphorylation independently of CPT1A, are able to sustain Treg function in LCFA-limited contexts. In preliminary experiments, we bred mice with Treg specific deletion of CPT1A (TregCPT1AKO) and challenged them intratracheally with influenza A and subcutaneously with B16F10 melanoma tumors. While TregCPT1AKO mice exhibit no signs of autoimmunity at homeostasis, they are more susceptible to mortality from severe influenza pneumonia and exhibit slower tumor progression compared with controls. These results suggest that CPT1A is dispensable to maintain homeostatic self-tolerance but is necessary for Treg function during the pathologically-induced microenvironmental mitochondrial stress present in the virally-infected lung and tumor. Therefore, these data support the hypothesis that Tregs require CPT1A-mediatedmitochondrial LCFA metabolism to provide lung tissue protection following severe viral pneumonia.

Irena Gushterova, MA

PhD Candidate, Eichner Lab,
Biochemistry and Molecular Genetics,
Northwestern University

Irena Gushterova, Lillian J. Eichner

Defining the Class IIa HDACs as transcriptional regulators in lung cancer

Recent advances have uncovered important mechanistic insights regarding how LKB1/STK11 functions as a tumor suppressor in the lung, which revealed that transcription appears to play an important role in mediating LKB1 tumor suppressor function. However, a gap remains in our mechanistic understanding of how LKB1 loss elicits its unique transcriptional signature observed in lung tumors. We have identified that the Class IIa HDACs are potent repressors of gene expression in lung cancer cells, and that LKB1 status is a key determinant thereof. We found that Class IIa HDACs dictate chromatin access at regulated genes in an LKB1-dependent manner. By integrating next-generation sequencing datasets, we have defined the core set of genes regulated by the LKB1-Class IIa HDAC axis in both LKB1 mutant lung cancer cells and primary lung tumors from the genetically engineered mouse model (GEMM). Importantly, we found that Class IIa HDACs are dominant regulators of LKB1 mutant lung tumor growth in vivo, and ongoing work is defining Class IIa HDAC-dependent tumor biology.

Yue He

PhD Candidate, Shilatifard Lab, Biochemistry and Molecular Genetics, Northwestern University

Yue He, Saeid Mohammad Parast, Jacob Martin Zeidner Sarah Gold, Ali Shilatifard

Characterization of a novel function for ELOA as an elongation factor regulating cellular senescence

Cellular senescence is a typically irreversible process characterized by cell cycle arrest, morphological changes, and secretion of inflammatory factors. Physiologically, cellular senescence contributes to tissue dysfunction, neoplastic transformation, and age-related diseases. Many transcription factors related to RNA Polymerase II (RNAPII) have been reported to be involved in the induction of cellular senescence. However, the underlying mechanisms (and potential targets for therapeutic interventions) remain largely elusive. Here, by combining next-generation sequencing and biochemical assays, we demonstrate that depletion of the transcription factor SPT6 promotes cellular senescence-associated gene expression phenotypes, including suspended proliferation and robustly detectable β -galactosidase activity. Through a genome-wide CRISPR screen, we identified the transcriptional elongation factor ELOA as a regulator of cellular senescence. Interestingly, loss of ELOA rescued the proliferation defect caused by SPT6 depletion. ELOA knockout also led to downregulation of the senescence entry marker p21, suggesting a direct role for ELOA in the transcriptional regulation of cellular senescence. Overall, our results shed light on ELOA as a potential therapeutic target in future approaches to limit or reverse cellular senescence.

Leila Iravani, MS

Research Technician, Shilatifard Lab, Biochemistry and Molecular Genetics, Northwestern University

Yuki Aoi, **Leila Iravani,** Isabella C. Mroczek, Benjamin C. Howard, and Ali Shilatifard

SPT5 loss eliminates transcription elongation via Cullin 3 recognition

Transcription elongation by RNA polymerase II (Pol II) is an intricate process that involves various transcription elongation factors. Among the essential and evolutionary conserved transcription elongation factors is SPT5, a component of the DRB-sensitivity-inducing factor (DSIF) complex, which plays important roles in the promoter-proximal pause-release and rate of elongation. Furthermore, SPT5 functions in Pol II stabilization: We have previously shown that rapid depletion of SPT5 leads to the degradation of RPB1, the biggest subunit of Pol II, mediated through the Cullin 3 (CUL3) E3 ubiquitin ligase activity. The active CUL3 complex comprises a scaffold cullin protein, a RING finger protein interacting with an E2 ubiquitin-conjugating enzyme, and an adaptor protein that recognizes its substrates. However, the detailed mechanism of Pol II degradation upon SPT5 loss via CUL3 is unclear. Here, using a proteomic screening for Pol II-interacting proteins, we identify the factors that are involved in Pol II degradation in the absence of SPT5. We will discuss the important role of these proteins in Pol II transcription and turnover pathways.

Marta Iwanaszko, PhD

Research Assistant Professor, Shilatifard Lab, Biochemistry and Molecular Genetics, Northwestern University

Parast S, **Iwanaszko M,** Das M, Wang S, Ramani V, and Shilatifard A.

Transcriptomic and Splicing Alterations in Aging Mouse and Human Tissues: Insights from Short- and Long-Read RNA Sequencing

Aging involves a gradual decline in molecular, cellular, and physiological functions, leading to diminished vitality and the onset of age-related diseases. Despite advances in omics technologies, the functional status of basal transcription processes in aging remains insufficiently understood. To address this, we conducted a comprehensive transcriptomic analysis of the brains and other tissues of young (3 months) and old (24 months) mice using bulk short-read and long-read RNA sequencing.

We developed a novel algorithm capable of distinguishing old tissues from young based on intronic coverage in large and extra-large exons. Contrary to recently reported changes in the slope of the intronic coverage, indicating difference in the speed of elongation, we did not observe that phenomenon but rather a stable change in the amplitude of coverage over large and extra-large introns, which globally decreased with aging especially in tissues with low regenerative ability, like brain.

Our analysis revealed that the aged brain exhibited an enriched representation of genes connected to phagosome and microglia phagocytosis, synapse pruning, immune response, and neutrophil activation. In contrast, downregulated genes in the aged brain were associated with processes such as nervous system development, neuron projection, synapse organization, and gliogenesis.
We validated these findings using published human RNA-seq data and observed similar characteristics in the aging human brain, corroborating our mouse model results. Furthermore, significant changes were noted in the spliceosome during aging, correlating with short-read RNA-seq data and showing significant downregulation of genes and isoforms in the aged brain. Additionally, the aged brain showed significant overrepresentation of mono-exonic isoforms and novel intron retention isoforms.
Our biochemical studies showed a significant decrease in the association of Med23, a mediator complex subunit, with RNAPII in aged mice, suggesting an altered transcriptional machinery.

Our findings underscore the importance of studying mRNA splicing events and transcriptional dynamics in the context of aging and provide a robust framework for identifying age-related transcriptomic changes. The observed reduction in mRNA dynamics and the altered regulatory role of RNA-binding proteins in the aged brain suggest a reshaping of the transcriptome with aging, driven by changes in splicing and mRNA processing.

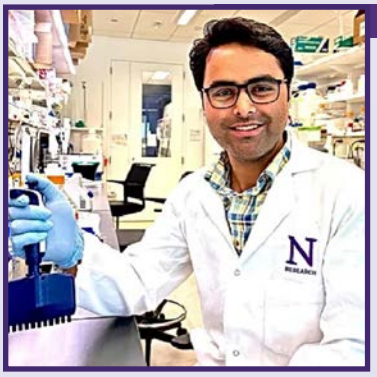
Austin Klein

PhD Candidate, Mendillo Lab,
Biochemistry and Molecular Genetics,
Northwestern University

Austin Klein, Roger Smith, Josiah Wong, Hannah Mubarak, Ibrahim Ahmad, David Amici, Marc Mendillo

Identification of TMED2 as a Critical Factor for ER Stress in Triple-Negative Breast Cancer

Cancers must adapt to a diverse set of stressors in order to proliferate and survive. These include but are not limited to endoplasmic reticulum (ER) stress. The proteostasis network (PN) comprises molecular chaperones, co-chaperones, and a variety of additional support proteins which enable cells to cope with these myriad stresses. Recently, I have used an unbiased screen of proteostasis factors to identify genes most critical for adapting to chronic, growth-associated stress. Notably, this screen used tool compounds to induce ER stress at doses ~1000x lower than most acute stress studies which highlighted factors most important for surviving chronic stress. In my preliminary data, I found: (1) TMED2, a transmembrane protein involved in vesicular transport between the Golgi and ER, was among the most critical factors for surviving growth-associated ER stress in all conditions; and (2) TMED2 is essential in a subset of particularly aggressive triple-negative breast cancers (TNBCs), even in the absence of exogenous stressors; (3) TMED2 and TMED10 are strongly coessential across cancer cell lines; and (4) TMED2 is upregulated in many cancer types, including TNBCs. From these data, I predict that a subset of TNBCs have high levels of cell-autonomous ER stress and are thus dependent on TMED2, and that loss of TMED2 will suppress TNBC proliferation, and render TNBC tumors more susceptible to existing proteotoxic stress-inducing therapeutics.



Mushtaq Ahmad Nengroo, PhD

Postdoctoral Fellow, Ben-Sahra & Mendillo Labs, Biochemistry and Molecular Genetics, Northwestern University

Mushtaq A. Nengroo, Austin T. Klein, Heather S. Carr, Olivia Vidal-Cruchez, Eunus S. Ali, Umakant Sahu, Peng Gao, John M. Asara, Marc L. Mendillo, Issam Ben-Sahra

Succinate controls de novo purine synthesis and cancer cell growth

The de novo purine synthesis pathway is fundamental for nucleic acid biosynthesis and cellular energetics. While various signaling pathways have been implicated in regulating enzymes supporting purine synthesis, the specific impact of metabolites derived from cellular metabolism on this process and cell growth remains unclear. Here, we identify succinate, a key substrate of the succinate dehydrogenase (SDH) complex, as a regulator of de novo purine synthesis and cell proliferation. A co-essentiality network analysis using the DepMap database shows that the SDH complex, which oxidizes succinate into fumarate in the tricarboxylic acid (TCA) cycle, shares essentiality with genes involved in de novo nucleotide synthesis. Loss of SDH activity leads to succinate accumulation, which reduces the rate of de novo purine synthesis and compromises cell growth. Mechanistically, succinate interacts with and inhibits the synthetase activity of methylenetetrahydrofolate dehydrogenase, cyclohydrolase and formyltetrahydrofolate synthetase 1 (MTHFD1), an important enzyme in the one-carbon metabolism pathway that provides 10N-formyl-tetrahydrofolate, essential for purine synthesis. Thus, our findings define a previously unrecognized metabolic function of mitochondrial succinate in suppressing purine synthesis and cellular proliferation under metabolic stress conditions.

Timothy Pan

PhD Candidate, Gao Lab,
Biochemistry and Molecular Genetics,
Northwestern University

Timothy Pan, Minhua Wang, Lina Lu, Cheng-Kai Shiau, Yueying He, Hsiao-Yun Lin, Arvind Bhimaraj, Keith Youker, Ruli Gao

Long-read single-cell RNA sequencing atlas of the human heart

Heart disease remains the leading cause of global mortalities. Single-cell RNA-sequencing has emerged as a promising approach to dissect the progression of heart disease by analyzing gene expression signatures. However, due to technological boundaries, these approaches fail to distinguish consequential transcript isoforms of the cardiac transcriptome that are essential for the development of effective therapies. To address this, we applied our long-read single-cell Nanopore RNA-sequencing technology onto fresh frozen tissues from the left ventricles of nonfailing and failing hearts to construct a comprehensive catalogue of cardiac cell type and cell state specific transcript isoforms. Our data showed numerous genes that present as more than one dominant isoform, including those involved in cell type specific phenotypes and housekeeping functions. Comparative analysis between normal and diseased-associated cell states identified several genes with contrasting transcript usage across conditions. Furthermore, to facilitate visualization and navigation of our findings, we created an online static database with Shiny. Our long-read atlas provides an extensive catalogue of cell type and cell state specific transcript isoforms in human hearts and serves as a unique resource for future studies.

Saeid Parast, PhD

Postdoctoral Fellow, Shilatifard Lab, Biochemistry and Molecular Genetics, Northwestern University

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A role for NELF and its suppressor in reversible cellular senescence

Negative elongation factor (NELF) is a transcriptional regulator that is primarily known for its role in the promoter-proximal pausing of RNA polymerase II (RNAPII). We have previously demonstrated that acute depletion of the NELF subunit NELF-C does not result in the genome-wide release of RNAPII. Here, we show that acute depletion of NELF-C results in a reversible cellular senescence phenotype, with robustly detectable β -galactosidase activity and upregulated expression of senescence-associated genes. We also identify a small subset of genes for which NELF-C depletion induces RNAPII release. Our genetic suppressor screen identifies suppressor of NELF (SOF) as a factor mediating NELF depletion-induced cellular senescence. Mechanistically, SOF alters the kinetic coupling between elongation and splicing of nascent transcripts in the absence of NELF, which results in intron retention and other splicing defects. Despite reports implicating splicing defects in aging-related phenomena, mechanisms have remained elusive. The findings presented here indicate that the crosstalk between splicing factors and the elongation factors SOF and NELF may be critical to understanding the transcriptional regulation of gene expression in cellular senescence and aging.

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A novel function for a master regulator of molecular circadian rhythm in control of RNAPII pause-release

RNA polymerase II (RNAPII) transcribes eukaryotic genes in a tightly regulated, multistep process. Following transcriptional initiation and promoter escape, transcriptionally engaged RNAPII pauses downstream of promoters. RNAPII must then be released from this promoter-proximal paused state to begin productive elongation within gene bodies. A variety of factors contribute to the regulation of RNAPII pause release (and therefore gene expression) in response to various environmental stresses such as heat shock and serum starvation. Here we demonstrate global RNAPII pausing (indicating impaired RNAPII pause release) upon serum starvation in the human colorectal cancer cell line DLD-1. However, we also identify a small subset of genes for which serum starvation conversely induces RNAPII pause release. This subset includes circadian clock-associated genes. We then characterize the protein interactome of RNAPII purified from DLD-1 cells with and without serum starvation, observing an enriched association of RNAPII with CLOCK in response to serum starvation. CLOCK is a master regulator of molecular circadian rhythm. Furthermore, we demonstrate that acute CLOCK depletion via a degron system results in global RNAPII pausing with robust accumulation of RNAPII proximal to promoters and loss of the Ser2-phosphorylated, elongating form of RNAPII from gene bodies. Thus, the results of this study indicate a novel function for CLOCK in control of RNAPII pause-release that may have broader implications for regulation of molecular circadian rhythm.

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Mechanisms Restricting CENP-A at Centromeres

Faithful chromosome segregation during mitosis is carried out by the recruitment of the constitutive centromere-associated network (CCAN) and the kinetochore via centromeres. Centromeres are unique chromatin domains epigenetically characterized by a nucleosome containing the histone H3 variant CENP-A. CENP-A nucleosomes are interspersed with H3 nucleosomes within megabases of alpha-satellite DNA. There are ~400 CENP-A nucleosomes in a human centromere comprising ~4% of all centromeric nucleosomes. The core centromeric chromatin is enriched in permissive histone marks like H3K4me2, H3K9ac, and H4K20me1 and is flanked by heterochromatin enriched in repressive marks, H3K9me3 and H3K27me35. Recent complete mapping of the human genome has allowed for the epigenetic characteristics of centromeric DNA to be better understood. Complete mapping of the centromere has shown that β Sat arrays, upon which active centromeres are assembled, are associated with high CpG methylation. However, CENP-A is enriched within the β Sat arrays at distinctly hypomethylated regions known as centromere dip regions (CDRs) consistent with centromeric DNA being associated with more active or open chromatin structure compared to neighboring pericentromeres. The role of DNA methylation in centromere deposition or stability is completely unknown.

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WNK1 kinase controls the Krebs cycle and cell proliferation

The tricarboxylic acid (TCA) cycle, known as the Krebs cycle, is a pivotal metabolon crucial for oxidizing nutrients to fuel cellular bioenergetics. This study focuses on identifying novel kinases that regulate the TCA cycle in proliferating cells, ultimately enabling the development of strategies to inhibit the TCA cycle, reduce ATP production, and suppress cell proliferation. Through a comprehensive yet targeted metabolite profiling approach, we examined the metabolome of human cells treated with 50 kinase inhibitors and discovered that the kinase WNK1 (With-No-Lysine Kinase-1) significantly influences the levels of citrate, a key TCA cycle metabolite. WNK1, typically activated by osmotic stress and chloride, modulates electro-neutral cotransporters by phosphorylating SPAK and OSR1 kinases, facilitating ion transport across cell membranes for homeostasis. However, the connection between WNK1 and cellular metabolism remains largely unexplored. Our investigation reveals that WNK1 inhibition decreases TCA cycle activity partly through increased phosphorylation of pyruvate dehydrogenase (PDH). PDH catalyzes the conversion of pyruvate into acetyl-CoA, a crucial precursor that fuels citrate synthesis, the TCA cycle, and lipid metabolism. Inactivation of PDH via phosphorylation by PDH kinases (PDKs) reduces pyruvate oxidation, thereby limiting acetyl-CoA entry into the TCA cycle, oxygen consumption, and subsequent ATP production. This study uncovers a role for WNK1 in controlling mitochondrial metabolism, underscoring its potential as a therapeutic target in diseases associated with metabolic deregulation, including cancers and metabolic syndrome.

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Spatial 3D genome organization reveals intratumor heterogeneity in primary glioblastoma samples

Glioblastoma (GBM) is the most prevalent malignant primary brain tumor, and is associated with universal poor prognosis. Currently, most genomic studies are conducted at a single tumor site, which does not reflect the complete genetic or epigenetic information across the whole tumor. Chromatin intra-tumoral heterogeneity (ITH) is a characteristic feature of GBM long observed under the microscope, but the ITH of 3D genome organization is poorly understood. To address these gaps, we performed Hi-C experiments in 21 samples obtained from 9 GBM patients, 15 of which were spatially mapped using MRI registered coordinates. We identified extensive inter-tumoral and intra-tumoral heterogeneity in genome compartmentalization and chromatin interactions. Notably, in a patient with 9 spatially mapped samples from both temporal and frontal regions, we accumulated over 6 billion reads and defined ultra-high-resolution, tumor region-specific chromatin interactions, regulatory networks, and key regulators within the same patient. We detected structural variation (SV) and enhancer hijacking across all the samples, and identified recurrent events that affect cancer-related genes such as CDKN2A/B. Finally, we introduce the concept of 'enhancer amputation', defined as the loss of enhancers due to SVs that leads to decreased expression of target genes. To our knowledge, this study represents the first large-scale exploration of the 3D genome in primary GBM patients and the first investigation of 3D genome organization in multiple regions of the same tumor. Our findings provide insights into the ITH of GBM at the 3D genomic level, opening new avenues for understanding and potentially targeting this devastating disease.

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The role of SUV39H1/2-mediated H3K9me3 in maintaining 3D genome organization in acute myeloid leukemia

Three-dimension genome architecture is essential for proper gene regulation and it has been shown that acute myeloid leukemia (AML) has altered 3D genome landscape. However, majority of the previous work has been focused on enhancer-promoter interactions, and the contribution of heterochromatin to higher-order chromosomal structure has been understudied. In this study, we first profiled the genome-wide distribution of H3K9me3, a constitutive heterochromatin mark, in multiple AML cell lines. Through systematic CRISPR/Cas9-mediated genetic knockout of H3K9-specific methyltransferases, we identified SUV39H1/2 as the major depositors in AML cells.
We showed that SUV39H1/H2 DKO reduced global levels of H3K9me3 in multiple AML cell lines, while single KO reduced H3K9me3 to a lesser, but still significant level. Furthermore, removal of SUV39H1/2 hampered cell proliferation and cell clonogenicity and induced changes of gene expression in multiple pathways, including myeloid cell differentiation and immune response. Finally, we performed Hi-C experiments, and showed that SUV39H1/2 DKO lead to global chromatin compartments switches, and in particular enhanced heterophilic interactions. Together, we revealed novel roles of SUV39H1/2 in sustaining leukemic transcriptional programs, heterochromatin identity, and 3D genome organization in AML.

The BMG Retreat Committee would like to thank all of the Department of Biochemistry and Molecular Genetics for participating in this eighth annual retreat. We would like to extend our gratitude to our talented medical illustrator, Brianna Monroe, for the beautiful program cover design and agenda layout. We appreciate the hard work of those who participated in the poster session and talks. Finally, we would like to give special thanks to Linda Jackson for organizing the event and without her, none of this would have been possible.

BMG Retreat Committee: Lillian Eichner, PhD, Assistant Professor, Department of Biochemistry and Molecular Genetics and Clara Peek, PhD, Assistant Professor, Biochemistry and Molecular Genetics, Medicine (Endocrinology)

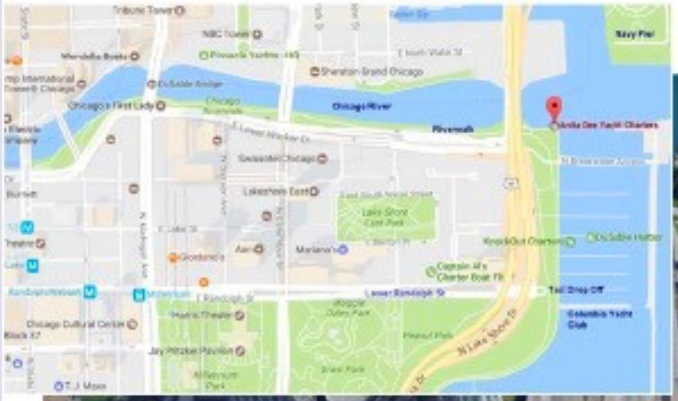
DIRECTIONS TO DOCK

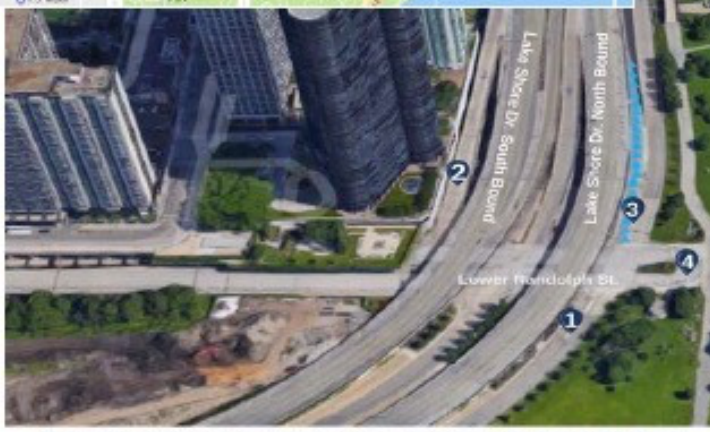
Docking Location: ** *Since the Harbor has no real physical address, GPS and online directions are unreliable.Use the maps along with the directions, below, to get to our location!*

DuSable Harbor- Docked on the South side of the Chicago River at Lake Shore Drive.

200 N Breakwater Access

Chicago, IL 60601





1) Randolph/Wacker Exit Ramp Via Lake Shore Dr. From the South
2) Lower Randolph Exit Ramp Via Lake Shore Dr. From the North
3) DuSable Harbor Lot Access Road
4) Cal-de-Sac at the end of Lower Randolph St.

▸▸▸▸ *Route To Parking Lot*

Directions

Driving (Parking in DuSable Harbor Parking Lot) *Parking ranges from $16-$24

From the West

- Head east, toward the lake, on Lower Randolph Street from Michigan Ave. or Columbus Drive. (If coming from Michigan Ave., stay to the left to access lower Randolph St., do not go to the upper level!)
- Take Randolph Street all of the way to the last stop light before the lake and turn left. (Do not enter cal-du-sac)
- Immediately after you turn, **Merge left** to ramp leading down to parking lot.
- At bottom of ramp, take ticket at the gate (pay upon exit) then drive straight to the end of the lot.
- Park, walk out of opening on the right/east side of the lot. Both yachts will be to your left on the River.

From the North Via Lake Shore Drive

- Take Randolph St. exit off of Lake Shore Drive.
- Turn left at bottom of Randolph St. exit ramp.

- Turn left at second light (Do not enter cal-du-sac).
- Immediately after you turn, **Merge left** to ramp leading down to parking lot.
- At bottom of ramp, take ticket at the gate (pay upon exit) then drive straight to the end of the lot.
- Park, walk out of opening on the right/east side of the lot. Both yachts will be to your left on the River.

From the South Via Lake Shore Drive

- Exit to Randolph/Wacker Dr. off of Lake Shore Drive. Stay in middle lane of ramp.
- At bottom of ramp, proceed straight beyond traffic light.
- **Merge left** to ramp leading down to parking lot.
- At bottom of ramp, take ticket at the gate (pay upon exit) then drive straight to the end of the lot.
- Park, walk out of opening on the right/east side of the lot. Both yachts will be to your left on the River

Alternate Parking Locations

In the event that DuSable Harbor Parking Lot is at capacity, we've listed additional parking options. **Please be sure to inform your guests to allow ample time for travel and parking prior to the event!**

Millennium Park Garage (312)616-0600 5 S. Columbus Dr.	ABM Parking Services (855)234-0260 400 N McClurg	Standard (312)494-9770 505 E. Illinois
ABM Parking Services (312)268-8116 323 E. Wacker	LAZ Parking (312)616-0600 350 E. Monroe	Navy Pier Lots (312)595-5072 600 E Grand Ave.

Dropped Off

- If getting dropped off by taxi, **instead of telling the driver an address, tell them to drop off where lower Randolph Street meets the lakefront, DuSable Harbor, or Columbia Yacht Club.**
- Lower Randolph Street, east to the lakefront. Drop off at DuSable Harbor cul-du-sac
- Walk down to the lake front path and turn left/walk north along the lakefront.
- Our yachts will be straight ahead, at the end of the lakefront path where it ends at the Chicago River.
- Taxis can also go inside to the end of the DuSable Harbor Parking Lot which is closer (directions above in 'Driving'). If they're there for less than 15 mins, there is no charge.

Walking

From the Loop

- If walking from the loop, take any stairway down to the Riverwalk on the **south side** of the Chicago River.
- Walk east toward the lake (From Michigan Ave. Bridge- 10 min walk)
- The south side of the Riverwalk ends at our boats just east of the Lake Shore Drive Bridge

From the Navy Pier

- Walk west on Illinois to Lower Lake Shore Drive, the first street on your left.
- Cross Lower Lake Shore Drive to the west side of the street and turn left.
- Once you cross over the Chicago River, look for the staircase on your right to walk down to Riverwalk
- Turn left to go through tunnel on Riverwalk
- Once you exit tunnel, you'll see both yachts to your left on the River.

From Millennium Park

- Head east on Monroe and cross over to the east side of Lake Shore Drive.
- Turn left or walk north on the lakefront path.
- Once you pass Columbia Yacht Club, keep walking north for 4-5 mins- Path ends at Chicago River and our Yachts.