BIOGRAPHICAL SKETCH

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NAME: Uhn-Soo Cho

eRA COMMONS USER NAME (credential, e.g., agency login): uhnsoocho

POSITION TITLE: Associate Professor of Biological Chemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Harvard Medical School, Boston, MA	Postdoc	2012	Structural Biology
University of Washington, Seattle, WA	Ph.D.	2007	Biological Structure
Korea University, Seoul, South Korea	M.S.	2000	Biochemistry
Korea University, Seoul, South Korea	B.S.	1998	Agricultural Biology

A. Personal Statement

My research interests focus on elucidating the biological function of physiologically important and structurally challenging proteins or protein complexes based on biochemical and structural observations. I was first introduced to structural biology during college and have since used structural biology as a main tool to answer my scientific questions. As a graduate student in Dr. Wenqing Xu's laboratory at the University of Washington, I pursued the structural and biochemical understanding of protein phosphatase 2A (PP2A), a major Ser/Thr protein phosphatase in eukaryotes. I determined the first crystal structure of a PP2A holoenzyme and provided the structural basis for understanding substrate recognition and complex formation regulated by methylation (Cho & Xu, 2007, **Nature**; Cho et. al., 2007, **PLoS Biology**). As a postdoctoral fellow in Dr. Stephen C. Harrison's laboratory at Harvard Medical School, I have focused on elucidating the mechanism of initial kinetochore assembly at centromeres, using structural and biochemical approaches. I have determined crystal structures of two major players in the first stage of kinetochore assembly in budding yeast: the Cse4:H4/Scm3 complex (Cho & Harrison, 2011, **PNAS**) and Ndc10 (Cho & Harrison, 2011, **Nat.Struct.Mol.Biol**). As a result of my functional studies, I have outlined a potential sequence of events that occur during initial kinetochore assembly. Currently, I am an associate professor in the Biological Chemistry department at the University of Michigan. As an independent scientist, I have been interested in and focused on three major projects; (1) elucidating the mechanism of initial kinetochore assembly and epigenetic regulation in eukaryotes, (2) the structural study of methane monooxygenase, and (3) understanding the structure-function relationship of human Sestrin2 and its signaling mediators in mTORC1 regulation. My contributions to each project led me to publications as a corresponding author in high-impact journals [(1) An et. al., 2015**, J.Mol.Biol.;** An et al., 2017**, eLife.;** An et al., 2018**, Structure**; Park et al., 2019, **Nat.Comm**.; (2) Lee et. al., 2013, **Nature**; Kim et. al., 2019, **Sci. Adv.**; (3) Kim et. al., 2015, **Nat. Comm.**].

Over the last couple of years, our laboratory has incorporated cutting-edge, state-of-the-art single particle cryo-electron microscopy (cryo-EM), in my laboratory, to determine the structures of protein complexes in more challenging, high-risk/high-reward areas of research. Our efforts recently bring forth a couple of publications by determining the cryo-EM structure of human Mixed-Lineage Leukemia (MLL) 1 bound to the nucleosome (Park et. al., 2019, **Nat.Comm.**; Lee et. al., 2021, **Nat. Comm.**). This

demonstrates our independent capability to perform cryo-EM structural studies. We now have an access to the UM cryo-EM facility (200KeV Talos Arctica, 200 KeV Glacios, and two 300 KeV Titan Krios) as well as equip two GPU-accelerated workstations (each contains 4x2080Ti). Recently, I further gained my personal experience in and was in charge of maintaining and aligning the cryo-electron microscope during my sabbatical leave at Pohang University of Science and Technology (POSTECH; South Korea) from July 2020 to February 2021.

B. Positions and Honors

Professional Experience

2020 – 2021 Visiting Professor in the Life Science department at Pohang University of Science and Technology (POSTECH, South Korea)
2019 – Associate Professor in Biological Chemistry Department, Univ. of Michigan Medical School
2012 – 2019 Assistant Professor in Biological Chemistry Department, Univ. of Michigan Medical School
2010 – 2013 Special Fellow in the Leukemia & Lymphoma Society (Harvard Medical School)
2007 – 2012 Postdoctoral Fellow (PI: Stephen C. Harrison, Harvard Medical School)
2002 – 2007 Ph.D. Graduate Student (PI: Wenqing Xu, University of Washington)
2001 – 2002 Postmaster research scientist (PI: Thomas C. Terwilliger, Los Alamos National Laboratory
2000 – 2001 Research scientist (PI: Kyung-Hyun Kim, Korea University)
1998 – 2000 Master Graduate Student (PI: Kyung-Hyun Kim, Korea University)

Honors and Awards

2016 - 2017 Junior faculty development award, American Diabetes Association
2015 Basil O'Connor Start Scholar Research Award, March of Dimes Birth Defect Foundation
2012 BSSP (Biological Sciences Scholars Program) Scholar Award (Univ. of Michigan)
2010 - 2013 Special Fellow in the Leukemia & Lymphoma Society

Unpaid scientific appointment

None

C. Contributions to Science

1. Understanding CENP-A recruiting mechanism at centromeres

Assembling the proper kinetochore complex is essential for the faithful chromosome segregation during mitosis. CENP-A, a centromere-specific histone H3 variant, plays a key role in marking centromeres and recruiting the rest of the kinetochore components to the centromeric region. Therefore, how CENP-A is specifically recruited into centromeres is a key question that must be addressed in order to understand kinetochore assembly. Using budding yeast (point centromere) as well as fission yeast and human (regional centromere), we address these fundamental questions using structural and biochemical approaches. In order to understand how CENP-A specifically localizes to centromeres, we determined the crystal structures of key components in the process: protein phosphatase 2A and shugoshin (1), a CENP-A^{Cse4} specific chaperone—the CENP-A^{Cse4}:H4/Scm3 complex, and a CENP-A^{Cse4} recruiting factor—Ndc10. Crystal structures of key components in CENP-A^{Cse4} recruitment and subsequent functional assays allow us to propose the recruitment mechanism of CENP-A^{Cse4} at the point centromere. CENP-A localization in regional centromeres, our main interest in this proposed research, employs a different recruiting factor—the Mis18 holo-complex—for selective recruitment of CENP-A to centromeres. We recently determined the crystal structure of Mis16 in complex with histone H4 (2) and Eic1 (4), one of the components in the Mis18 complex, and found that Mis16 plays a centromere-specific role in the selective recognition of CENP-A. We also

elucidate the nuclear translocation mechanism of histone proteins via karyopherin protein called Kap123 in budding yeast (3). We will continue to investigate the physiological role of the Mis18 holo-complex in CENP-A recruitment using biochemical, structural, and genetic approaches.

1. **Cho, U.S.** and Xu, W (2007) Crystal structure of a protein phosphatase 2A heterotrimeric holoenzyme. ***Nature (Article)*** 445, 53-57
2. An, S., Kim, H.S., and **Cho, U.S.** (2015) Mis16 recognizes both histone H4 and Scm3sp to specifically recruit CENP-A^{Cnp1} into centromeres, ***J. Mol. Biol.*** 427(20):3230-3240
3. An, S., Yoon J., Kim, H.S., Song, J.J., and **Cho, U.S.** Structure-based nuclear import mechanism of histone H3 and H4 mediated by Kap123***, eLife***, DOI: 10.7554/eLife.30244
4. An, S., Koldewey, P., Chik, J., Subramanian, L., and **Cho, U.S.**: Eic1 binding to Mis16 redirects the biological function of Mis16 from a histone H4 chaperone to a CENP-A assembly factor ***Structure*** 26: DOI:https://doi.org/10.1016/j.str.2018.04.012, 2018.

2. Allosteric regulation mechanism of methane monooxygenase (MMO)

The chemical conversion of methane to methanol has the great potential to utilize natural gas as an environmentally friendly energy source. This reaction, however, demands high temperatures and high pressure, which limits the availability of natural gas as an alternative energy. When making methanol, methane monooxygenases uses methane as their sole carbon source. The detailed chemical mechanism of this reaction, however, is still obscure due to the lack of understanding in how MMO activity is regulated by its regulatory subunits. We previously determined the first crystal structure of the MMOH-MMOB complex and elucidated the molecular mechanism by which MMOB enhances the catalytic activity of MMOH, which helps substrates fit into the active site by inducing a conformational change in MMOH and its active site (5). We recently determined the crystal structure of the MMOH-MMOD complex, where MMOD functions as an inhibitor (6). The structure indicated that MMOD binds the same binding pocket of MMOH where MMOB associates. However, MMOD induces different conformational changes in MMOH, thereby disrupting the active site geometry in contrast to the activation of the di-iron center geometry as in the case of MMOB. Competition with MMOB and disruption of the di-iron center geometry causes MMOD to function as an inhibitor.

5. Lee, S.J., McCormick, M.S., Lippard, S.J., and **Cho, U.S.** (2013) Control of substrate access to the active site in methane monooxygenase. ***Nature,*** 494, 380-384
6. Kim, H.S., An, S., Park, Y.R., Jang, H, Park, S, Lee, S.J.*, and **Cho, U.S.*** (2019) MMOD-induced conformational changes of hydroxylase in soluble methane monooxygenase. ***Sci. Adv.***;5(10): eaax0059

3. Structural and functional understanding of human Sestrin2

Sestrins are stress-inducible proteins upregulated by reactive oxygen species and other environmental stresses. Two important physiological functions of Sestrins have been identified: antioxidant activity and mTORC1 inhibitory function. However, the molecular mechanism behind it is barely understood. I led the research to determine the crystal structure of human Sestrin2 (8). Recently, we determined the crystal structure of human Sestrin2. The determined structure provides the molecular basis of how this enzyme performs two seemingly independent functions. Our current interest is the structural elucidation of the GATOR2 complex, a five-component protein complex that transfers the inhibitory signal of Sestrin2 to mTORC1 (7). Since both of Sestrin's antioxidant and mTORC1 regulation functions are heavily implicated in aging and diabetes, our study will have a strong impact on elucidating Sestrin's role in pathogenesis of human diseases.

7. Kim, J.S., Ro, S.H., Kim, M., Park, H.W., Semple, I.A., Park, H., **Cho, U.S.**, Wang, W., Guan, K.L., Karin, M. and Lee, J.H. (2015) Sestrin2 inhibits mTORC1 through modulation of GATOR complexes. ***Sci. Rep.*** 5, 9502; DOI:10.1038/srep09502

8. Kim, H.S., An, S.J., Ro, S.H., Teixeira, F., Park, G., Kim, C., Cho, C.S., Kim, J.S., Jakob, U., Lee, J.H. and **Cho, U.S.** (2015) Janus-faced Sestrin2 controls ROS and mTOR signaling through two separate functional domains, ***Nat. Comm.*** doi: 10.1038/ncomms10025
9. Lee, J.H., **Cho, U.S.**, and Karen, M. (2016) Sestrin regulation of TORC1: Is Sestrin a leucine sensor? ***Sci. Signaling***, 0(431), re5.
10. Cho, C.S., Kowalsky, A.H., Namkoong, S., Park, S.R., Wu, S., Kim, B., James, A., Gu, B., Semple, I.A., Tohamy, M.A., Solanki, S., **Cho. U.S.**, Greenson, J.K., Shah, Y.M., Kim, M., Lee, J.H. (2019) Concurrent activation of growth factor and nutrient arms of mTORC1 induces oxidative liver injury. ***Cell Discov.;***5:60.
11. Cho, C.S., Kim, H.S., Semple, I., Ho, A., Namkoong, S., Segales, J., Munoz-Canves, P., Karin, M., Nam, K*., **Cho, U.S.***, and Lee, J.H.* Sestrins are not critical for leucine-induced mTORC1 activation, Submitted

4. Cryo-EM structures of epigenetic regulators in nucleosome recognition

Chromatin modifications contribute to the spatial and temporal regulation of gene expression. Many epigenetic regulators utilize the nucleosome as a substrate to epigenetically activate or suppress gene transcription. Our goal is to elucidate the regulatory mechanisms of these epigenetic regulators by determining cryo-EM structures of these regulators bound to the nucleosome. Recently, we have determined the cryo-EM structure of human Mixed-Lineage Leukemia (MLL) 1 bound to nucleosome (12-13). This structure describes the first portrait of human MLL1 bound to nucleosome and provides the molecular mechanism by which the methyl-transferase activity of MLL1 can be modulated by the way they interact with the nucleosome.

12. Park, S.H. Ayoub, A., Lee, Y.T., Kim, H.S.,[1] Zhang, W., Zhang, B., An, S.J., Zhang, Y, Cianfrocco, M., Su, M., Dou, Y,* and **Cho, U.S.*** Cryo-EM structure of the human MLL1-nucleosome complex, ***Nat. Comm.*** 2019 Dec 5;10(1):5540.
13. Lee, Y.T., Ayoub, A., Park, S.H., Sha, L., Xu, J., Mao, F., Zheng, W., Zhang, Y., **Cho, U.S.**, Dou, Y. Mechanism for DPY30 and ASH2L intrinsically disordered regions to modulate the MLL/SET1 activity on chromatin. ***Nat Comm.*** 2021 May 19;12(1):2953.

Complete List of Published Work in My Bibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/uhn-soo.cho.1/bibliography/40465915/public/?sort=date&direction=ascending

D. Research Support

a. Current Research Support

NIH R01 (PI: Cho, Uhn-Soo; Co-I: Junhee Lee) 09/19/2016-08/31/2022
NIH, NIDDK
Title: The molecular mechanisms of nutrient- and stress-dependent mTORC1 regulation mediated by human Sestrin2

The major goal of this support is to define the biochemical and structural properties of each signaling component within the Sesn-dependent signaling cascade and reveal druggable structural motifs that are critical for the functionality of this signaling pathway

NIH R01 (Multi-PIs: Yali Dou, Uhn-Soo Cho, and Min Su) 04/01/2020-03/31/2025
NIH, NCI
Title: Structural insights into the MLL core complexes

The major goal of this support is to determine the epigenetic role of each MLL1 core complex in histone H3 Lys4 methylation, which plays an important role in gene activation and human diseases. My role within this proposal is to determine the cryoEM structure of MLL1 core complex bound to nucleosome in the presence and/or absence of each key MLL1 core complex.

NIH R01 (PI: Shigeki Iwase, Co-I: Uhn-Soo Cho) 06/01/2020-05/31/2025
NIH, NINDS
Title: A Neuron-specific Methyl-histone Regulatory Complex
The proposed study is designed to identify methyl-histone regulations that are unique to neuronal cells. The obtained knowledge may be used to form the basis upon which abnormal methyl-histone regulations can be selectively targeted in the brain without affecting other organs.

CB and CG shared Resource Small project (PI: Uhn-Soo Cho) 06/01/2021-05/31/2022
The Rogel cancer center, UM
Title: Cryo-EM structural studies of the histone H3 Lys9 methyltransferase Clr4/SUV39H complex
The major goal of this support is to collect cryo-EM data for R01 submission. The funds will be used to pay for the cryo-EM beamtime to screen and collect the microscope data.

b. Recently Completed Research Support

C1 Gas Refinery (PI: Lee, Seung-Jae; Co-I: Cho, Uhn-Soo) 01/01/2018-12/31/2020
National Research Foundation of Korea
Title: Catalytic and allosteric influence of auxiliary components on methane monocxygenase
The major goal of this support is to determine the crystal and cryo electron microscopic structures of soluble methane monooxygenase hydroxylase in complex with it auxiliary subunits, such as an inhibitory subunit (MMOD) and reductase (MMOR).

UM Rogel Cancer Center: Research Grant (PI: Cho, Uhn-Soo; Ragunathan, Kaushik) 02/01/2018-01/31/2019
University of Michigan
Title: Structure & dynamics of oncogenic histone chaperone FACT & nucleosome complex
The major goal of this support is to support the preliminary data preparation for the cryo electron microscopy structural study of the FACT-nucleosome complex.

Biomedical Research Council (BMRC) Bridging Fund (PI: Cho, Uhn-Soo) 09/01/2017-08/31/2018
University of Michigan
Title: Molecular mechanisms of Mis18 complex-mediated CENP-A recruitment and early-stage kinetochore assembly at centromeres
The major goal of this support is to prepare and support the R01 application in the kinetochore-related project.

The Biomedical Research Council (BMRC) bridging fund (PI: Cho, Uhn-Soo) 09/01/2016-08/31/2018
University of Michigan
Title: Molecular mechanisms of Mis18 complex-mediated CENP-A recruitment and early-stage kinetochore assembly at centromeres.
The major goal of this support is to bridge the funding gap for the structural studies of early kinetochore assembly at centromeres.

BIOGRAPHICAL SKETCH

NAME: Sojin An

eRA COMMONS USER NAME: Sojin An

POSITION TITLE: Research Lab Specialist Intermediate, Department of Biological Chemistry, University of Michigan

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Department of Biochemistry, Yonsei University, Seoul, South Korea	Doctor of Philosophy (Ph.D.)	02/2007	Biochemistry
Department of Nutritional science, Ewha Woman's university, Seoul, South Korea	Master of Science (M.S.)	02/2001	Nutritional science
Department of Nutritional science, Ewha Woman's university, Seoul, South Korea	Bachelor of Science (B.S.)	02/1999	Nutritional science

A. Personal Statement

I am a highly motivated and experienced Protein Structural Biologist / Biochemist with over ten years of research experience. My research interests have revolved around the development of innovative drugs against cancers and metabolic diseases and exploring their underlying mechanism through a biochemical and structural biology approach. I currently have a full time position in the Department of Biological Chemistry as a Research Lab Specialist Intermediate at the University of Michigan. During my Ph.D training, my research focused on characterizing two types of human Acyl-CoA:cholesterol acyltransferase (ACAT) isoforms, ACAT-1 and ACAT-2 as a target of atherosclerosis and metabolic disease. I developed and screens to find ACAT isoform-specific inhibitors, and validated compounds in cell systems and the animal models to find drug candidates as a part of a medicinal chemistry research team. After I earned my Ph.D, I moved to the field of x-ray crystallography hoping to gain a structural biology skill set to aid my skills in drug development. I was able to solve x-ray crystal structures of 10 proteins related to epigenetic inheritance, centromere biology and metabolic disease. This work led to 1 international research award, and 8 publications in international peer reviewed journals. Specifically, I investigated the catalytic mechanism of histone methyltransferase Ash1L proposing auto-inhibitory regulation, the mechanism of CENP-A recruitment to the centromere by the fission yeast Mis18 complex, and the nuclear translocation mechanism of histone H3 and H4 in both human and yeast models. I also uncovered how human sestrin2, an important target of metabolic disease, has two distinct functions: reactive oxygen species reduction and inhibition of the mechanistic target of mTORC1. This work was appreciated by being published in *Nature Communications 2015*. I have been trained in cryo-electron microscopy (Cryo-EM) for last 2 years and have been working on the structural study of several nucleosome bound complexes using Cryo-EM techniques such as the FACT (facilitates chromatin transcription) complex and the LSD1-histone-demethylation complex. For the first project, I was able to directly purify the endogenous FACT complex, an oncogenic histone chaperone, bound to nucleosome from S. pombe using TAP-tag purification. We expect the structure of the FACT complex in association with nucleosomes will pave the way for the development of small molecules which can be used as a broad spectrum cancer therapeutic agent to supplement existing treatment approaches. For the second project, I successfully reconstituted both of canonical and neuronal form of LSD1 complex bound to nucleosome. Through this study we will determine how the neuron-specific LSD1 complex recognizes nucleosomes in a distinct manner compared to canonical form and regulate neuronal specific gene transcription. In parallel, I crystallized the LSD1-CoREST complex in the presence of 'TargetD, a candidate drug compound to treat sickle cell anemia and beta-thalassaemia. I plan to determine a series of the LSD1-CoREST complex structures bound with 'TargetD' derived compounds designed based on the determined crystal structures. Recently I started to tackle the Cryo-EM structural study of Nucleosome complex with other proteins including Sirt6, nuclear NAD^+-dependent deacetylase of histone H3 and Nhp6, DNA-binding protein which is required for Nucleosomal DNA binding of FACT complex.

B. Positions and Honors

Postdoctoral Fellow / Research Lab Specialist Intermediate 2013 – current
University of Michigan Medical School, Ann Arbor, MI:
Advisor: Dr. Uhn-Soo Cho (uhnsoo@med.umich.edu)
"Protein Structural Study on Epigenetics, Centromere Biology and Metabolic Disease"

- Investigated the mechanism of CENP-A recruitment to centromere by fission yeast Mis18 complex (related PDB ID : 4XYH, 4XYI, 5WJC)
- Uncovered how human sestrin2 has a two distinct functions, Reactive oxygen species (ROS) reduction and inhibition of the mechanistic target of rapamycin complex-1 (mTORC1) (related PDB ID : 5CUF)
- Researched nuclear translocation mechanism of histone H3 and H4 by Kap123, yeast homologue of human importin4 (related PDB ID : 5VCH, 5VE8, 5W0V)
- Studied the molecular insight of the mechanism of hemoglobin catalyzed H2S oxidation. (related PDB ID : 5UCU, 5XBK)
- Recently working on the structural study of the FACT (facilitates chromatin transcription) complex and the LSD1-histone-demethylation complex bound to Nucleosome using Cryo-EM techniques
- Maintained and managed laboratory and instructed laboratory techniques to undergraduate and graduate students.

Postdoctoral Research fellow 2009 – 2013
Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, South Korea:
Advisor: Dr. Ji-Joon Song (songj@kaist.ac.kr)
"Protein Structural Study on Epigenetics"

- Investigated the catalytic mechanism of histone methyltransferase Ash1L proposing auto-inhibitory regulation. (related PDB ID : 3OPE)
- Researched the mechanism of non-coding RNA, Xist recognition by EZH2 histone methyltransferase through NMR
- Researched nuclear translocation mechanism of Asf1/histone H3:H4 by human importin 4 (related PDB ID : 5XAH, 5XBK)
- Instructed laboratory techniques to graduate students, technicians and other personnel.

Research assistant fellow 2002 - 2009
National Research Lab. of Lipid Metabolism & Atherosclerosis, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, South Korea:
Advisor: Dr. Tae-Sook Jeong (tsjeong@kribb.re.kr)
"Medicinal chemistry to develop anti-atherosclerotic lead compounds from natural resources"

- Developed Acyl-CoA:cholesterol acyltransferase (ACAT) isotype specific inhibitors as anti-atherosclerotic agent through enzyme characterization, activity guided natural resources fractionation and lead oriented chemical synthesis.
- Investigated molecular mechanism(s) and developed pharmacological therapy in metabolic disease using various in vivo and in vitro systems.

C. Awards and recognition

- Scholarship Award, Keystone Symposia Scholarship from Keystone Symposia, USA 2011
- Research Award, Korean Biophysical Society, South Korea 2011
- Brain Korea 21 Postdoctoral Fellowship, Korea Ministry of Education, Science and Technology, South Korea, 2009-2012
- Basic Research Award, Korean Society of Lipidology and Atherosclerosis, South Korea 2008

D. Selected publication

Peer-Reviewed and Research Articles (*Authors equally contributed)

1. Lee SH, Hyeon DY, Yoon SH, Jeong JH, Han SM, Jang JW, Nguyen MP, Chi XZ, **An S**, Hyun KG, Jung HJ, Song JJ, Bae SC, Kim WH, Hwang D, Lee YM: RUNX3 methylation drives hypoxia-induced cell proliferation and antiapoptosis in early tumorigenesis. Cell Death Differ. 28(4):1251-1269. (2021)

2. Raiymbek G, **An S**, Khurana N, Gopinath S, Larkin A, Biswas S, Trievel RC, Cho US, Ragunathan K: An H3K9 methylation-dependent protein interaction regulates the non-enzymatic functions of a putative histone demethylase. Elife. 9:e53155 (2020)
3. Park SH, Ayoub A, Lee YT, Xu J, Kim H, Zheng W, Zhang B, Sha L, **An S**, Zhang Y, Cianfrocco MA, Su M, Dou Y, Cho US**:** Cryo-EM structure of the human MLL1 core complex bound to the nucleosome. Nat Commun. 10(1):5540. (2019)
4. Kim H, **An S**, Park YR, Jang H, Yoo H, Park SH, Lee SJ, Cho US: MMOD-induced structural changes of hydroxylase in soluble methane monooxygenase. Sci Adv. 5(10):eaax0059 (2019)
5. Chik JK, Moiseeva V, Goel PK, Meinen BA, Koldewey P**, An S**, Mellone BG, Subramanian L, Cho US: Structures of CENP-C cupin domains at regional centromeres reveal unique patterns of dimerization and recruitment functions for the inner pocket. J Biol Chem. 294(38):14119-14134 (2019)
6. **An S**, Koldewey P, Chik J, Bardwell J, Subramanian L, Cho US: Eic1, a component of the oligomeric Mis18 holo-complex, converts Mis16 from a histone H4 chaperone to a CENP-ACnp1 assembly factor. Structure. 26(7):960-971. (2018)
7. Yoon J, Kim S, **An S**, Leitner A, Jung T, Aebersold R, Herbert H, Cho US, Song JJ: Integrative structural investigation on the architecture of human Importin4_histone H3/H4_Asf1a complex and its histone H3 tail binding. J Mol Biol. 430, 822-841. (2018)
8. **An S***, Yoon J*, Kim H, Song JJ, Cho US: Structure-based nuclear import mechanism of histones H3 and H4 mediated by Kap123. ELife. 6, e30244. (2017)
9. Kim H*, **An S***, Ro SH*, Teixeira F, Jin Park G, Kim C, Cho CS, Kim JS, Jakob U, Hee Lee J, Cho US: Janus-faced Sestrin2 controls ROS and mTOR signaling through two seperate functional domains. Nat Communications. 6, 10025-10036. (2015)
10. **An S**, Kim H, Cho US: Mis16 Independently Recognizes Histone H4 and the CENP-A(Cnp1) Specific Chaperone Scm3sp. J Mol Biol. 427, 3230-3240. (2015)
11. **An S**, Yeo KJ, Jeon YH, Song JJ: Crystal structure of human histone methyltransferase Ash1L catalytic domain and its implications on the regulatory mechanism. J Bio Chem. 286, 8369-8374. (2011)
12. **An S**, Han JI, Kim MJ, Park JS, Han JM, Baek NI, Chung HG, Choi MS, Lee KT, Jeong TS: Ethanolic extracts of Brassica campestris spp. rapa roots prevent high-fat diet-induced obesity via beta(3)-adrenergic regulation of white adipocyte lipolytic activity. J Med Food. 13, 406-414. (2010)
13. Han JM, Kim MJ, Baek SH, **An S**, Jin YY, Chung HG, Baek NI, Choi MS, Lee KT, Jeong TS: Antiatherosclerotic effects of Artemisia princeps Pampanini cv. Sajabal in LDL receptor deficient mice. J Agric Food Chem. 57, 1267-1274. (2009)
14. **An S**, Jang YS, Park JS, Kwon BM, Paik YK, Jeong TS: Inhibition of acyl-coenzyme A:cholesterol acyltransferase stimulates cholesterol efflux from macrophages and stimulates farnesoid X receptor in hepatocytes. Exp Mol Med. 40, 407-417. (2008)
15. **An S**, Park YD, Paik YK, Jeong TS, Lee WS: Human ACAT inhibitory effects of shikonin derivatives from Lithospermum erythrorhizon. Bioorg Med Chem Lett. 17, 1112-1116. (2007)
16. **An S***, Cho KH*, Lee WS, Lee JO, Paik YK, Jeong TS: A critical role for the histidine residues in the catalytic function of acyl-CoA: cholesterol acyltransferase catalysis: evidence for catalytic difference between ACAT1 and ACAT2. FEBS Lett. 580, 2741-2749. (2006)
17. Cho KH*, **An S***, Lee WS, Paik YK, Kim YK, Jeong TS: Mass-production of human ACAT-1 and ACAT-2 to screen isoform-specific inhibitor: A different substrate specificity and inhibitory regulation. Biochem Biophys Res Commun. 309, 864-872. (2003)

Review Articles

1. **An S** and Song JJ: The coded functions of noncoding RNAs for gene regulation. Mol. Cells. 31, 491-496. (2011)

E. PATENTS

1. Tae-Sook Jeong, LEE Woo-Song, KIM Hyoung-Chin, Yang-Kyu Choi, Ju-Ryoung Kim, **So-Jin An**, Kyoung-Ran Im, Ki-Chang Jang, Og-Sung Moon, Jun-Seock Son. Administering terpenoids selected from ferruginol derivatives, dehydroabietinol, kayadiol and delta-cadinol, that inhibit acyl Coenzyme A: cholesterol acyltransferases and oxidation of low-density lipoproteins, for the treatment of hyperlipemia or atherosclerosis. US Patent No. 7825162 Application No. 12/265,

088 (2010/11/2)

2. Tae-Sook Jeong, LEE Woo-Song, KIM Hyoung-Chin, Yang-Kyu Choi, Ju-Ryoung Kim, **So-Jin An**, Kyoung-Ran Im, Ki-Chang Jang, Og-Sung Moon, Jun-Seock Son. Phenanthrene derivative; acylcoenzime A-cholesterol acyl transferase inhibitor; anti-oxidative activity to low density lipoproteins (LDL); hyperlipidemia and atherosclerosis caused by the LDL oxidation and the synthesis and accumulation of cholesteryl ester. US Patent No. 7820212 Application No. 12/181, 583 (2010/10/26)
3. Tae-Sook Jeong, LEE Woo-Song, KIM Hyoung-Chin, Yang-Kyu Choi, Ju-Ryoung Kim, **So-Jin An**, Kyoung-Ran Im, Ki-Chang Jang, Og-Sung Moon, Jun-Seock Son. Abietane diterpenoid compound, and composition comprising extract of torreya nucifera, or abietane diterpenoid compounds or terpenoid compounds isolated from them for prevention and treatment of cardiovascular disease. US Patent No. 7517542 Application No. 10/591,282 (2009/4/14)

BIOGRAPHICAL SKETCH
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NAME**: Dou, Yali**

eRA COMMONS USER NAME (credential, e.g., agency login): yalidou

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Peking University – Beijing, China	B.S.	7/1996	Medicine
University of Rochester – Rochester, NY, USA	Ph.D.	5/2001	Molecular Biology, Genetics (Mentor: Martin A. Gorovsky)
The Rockefeller University – New York, NY, USA	Postdoc	10/2006	Biochemistry (Mentor: Robert G. Roeder)

A. PERSONAL STATEMENT

We have a strong research interest on chromatin biology, particularly, on the function of histone-modifying enzymes in fundamental cellular processes. Our research mainly focuses on how histone modifying enzymes regulate the epigenomic landscape and cell fate commitment and how their dysregulation leads to human diseases. We have discovered the molecular mechanisms by which the MLL family histone methyltransferases and histone acetyltransferase MOF regulate cell specific transcription programs. Based on the basic biochemical and structural studies, we have developed the first-in-class MLL1 inhibitors and demonstrated the causal role of histone modification in important cellular processes. We have published over 100 papers, with many in high impact journals. In addition to research, I served and is serving as a regular reviewer for the NIH MGA (2015-2019), CG (2021-2025) and the American Cancer Society DMC study sections. I am a regular ad hoc reviewer for NCI P01 and Special Emphasis grants as well as foundation grants from NSF and LLS. I am currently on the editorial boards of Journal of Biological Chemistry and Molecular Cancer Research.

B. POSITIONS AND HONORS:

Positions and Employment:

10/2006-8/2012 Assistant Professor of Pathology and Biological Chemistry, University of Michigan
9/2012-8/2017 Associate Professor of Pathology and Biological Chemistry, University of Michigan
9/2017-5/2020 Professor of Pathology and Biological Chemistry, University of Michigan
6/2020- Professor of Medicine, Biochemistry and Molecular Medicine, University of Southern California
6/2020- Co-Leader, Genomic and Epigenomic Regulation Program, USC Norris Comprehensive Cancer Center

Professional Membership and services (selected):

BIOGRAPHICAL SKETCH

NAME: Sojin An

eRA COMMONS USER NAME: Sojin An

POSITION TITLE: Research Lab Specialist Intermediate, Department of Biological Chemistry, University of Michigan

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Department of Biochemistry, Yonsei University, Seoul, South Korea	Doctor of Philosophy (Ph.D.)	02/2007	Biochemistry
Department of Nutritional science, Ewha Woman's university, Seoul, South Korea	Master of Science (M.S.)	02/2001	Nutritional science
Department of Nutritional science, Ewha Woman's university, Seoul, South Korea	Bachelor of Science (B.S.)	02/1999	Nutritional science

A. Personal Statement

I am a highly motivated and experienced Protein Structural Biologist / Biochemist with over ten years of research experience. My research interests have revolved around the development of innovative drugs against cancers and metabolic diseases and exploring their underlying mechanism through a biochemical and structural biology approach. I currently have a full time position in the Department of Biological Chemistry as a Research Lab Specialist Intermediate at the University of Michigan. During my Ph.D training, my research focused on characterizing two types of human Acyl-CoA:cholesterol acyltransferase (ACAT) isoforms, ACAT-1 and ACAT-2 as a target of atherosclerosis and metabolic disease. I developed and screens to find ACAT isoform-specific inhibitors, and validated compounds in cell systems and the animal models to find drug candidates as a part of a medicinal chemistry research team. After I earned my Ph.D, I moved to the field of x-ray crystallography hoping to gain a structural biology skill set to aid my skills in drug development. I was able to solve x-ray crystal structures of 10 proteins related to epigenetic inheritance, centromere biology and metabolic disease. This work led to 1 international research award, and 8 publications in international peer reviewed journals. Specifically, I investigated the catalytic mechanism of histone methyltransferase Ash1L proposing auto-inhibitory regulation, the mechanism of CENP-A recruitment to the centromere by the fission yeast Mis18 complex, and the nuclear translocation mechanism of histone H3 and H4 in both human and yeast models. I also uncovered how human sestrin2, an important target of metabolic disease, has two distinct functions: reactive oxygen species reduction and inhibition of the mechanistic target of mTORC1. This work was appreciated by being published in *Nature Communications 2015*. I have been trained in cryo-electron microscopy (Cryo-EM) for last 2 years and have been working on the structural study of several nucleosome bound complexes using Cryo-EM techniques such as the FACT (facilitates chromatin transcription) complex and the LSD1-histone-demethylation complex. For the first project, I was able to directly purify the endogenous FACT complex, an oncogenic histone chaperone, bound to nucleosome from S. pombe using TAP-tag purification. We expect the structure of the FACT complex in association with nucleosomes will pave the way for the development of small molecules which can be used as a broad spectrum cancer therapeutic agent to supplement existing treatment approaches. For the second project, I successfully reconstituted both of canonical and neuronal form of LSD1 complex bound to nucleosome. Through this study we will determine how the neuron-specific LSD1 complex recognizes nucleosomes in a distinct manner compared to canonical form and regulate neuronal specific gene transcription. In parallel, I crystallized the LSD1-CoREST complex in the presence of 'TargetD, a candidate drug compound to treat sickle cell anemia and beta-thalassaemia. I plan to determine a series of the LSD1-CoREST complex structures bound with 'TargetD' derived compounds designed based on the determined crystal structures. Recently I started to tackle the Cryo-EM structural study of Nucleosome complex with other proteins including Sirt6, nuclear NAD^+-dependent deacetylase of histone H3 and Nhp6, DNA-binding protein which is required for Nucleosomal DNA binding of FACT complex.

B. Positions and Honors

Postdoctoral Fellow / Research Lab Specialist Intermediate 2013 – current
University of Michigan Medical School, Ann Arbor, MI:
Advisor: Dr. Uhn-Soo Cho (uhnsoo@med.umich.edu)
"Protein Structural Study on Epigenetics, Centromere Biology and Metabolic Disease"

- Investigated the mechanism of CENP-A recruitment to centromere by fission yeast Mis18 complex (related PDB ID : 4XYH, 4XYI, 5WJC)
- Uncovered how human sestrin2 has a two distinct functions, Reactive oxygen species (ROS) reduction and inhibition of the mechanistic target of rapamycin complex-1 (mTORC1) (related PDB ID : 5CUF)
- Researched nuclear translocation mechanism of histone H3 and H4 by Kap123, yeast homologue of human importin4 (related PDB ID : 5VCH, 5VE8, 5W0V)
- Studied the molecular insight of the mechanism of hemoglobin catalyzed H2S oxidation. (related PDB ID : 5UCU, 5XBK)
- Recently working on the structural study of the FACT (facilitates chromatin transcription) complex and the LSD1-histone-demethylation complex bound to Nucleosome using Cryo-EM techniques
- Maintained and managed laboratory and instructed laboratory techniques to undergraduate and graduate students.

Postdoctoral Research fellow 2009 – 2013
Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, South Korea:
Advisor: Dr. Ji-Joon Song (songj@kaist.ac.kr)
"Protein Structural Study on Epigenetics"

- Investigated the catalytic mechanism of histone methyltransferase Ash1L proposing auto-inhibitory regulation. (related PDB ID : 3OPE)
- Researched the mechanism of non-coding RNA, Xist recognition by EZH2 histone methyltransferase through NMR
- Researched nuclear translocation mechanism of Asf1/histone H3:H4 by human importin 4 (related PDB ID : 5XAH, 5XBK)
- Instructed laboratory techniques to graduate students, technicians and other personnel.

Research assistant fellow 2002 - 2009
National Research Lab. of Lipid Metabolism & Atherosclerosis, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, South Korea:
Advisor: Dr. Tae-Sook Jeong (tsjeong@kribb.re.kr)
"Medicinal chemistry to develop anti-atherosclerotic lead compounds from natural resources"

- Developed Acyl-CoA:cholesterol acyltransferase (ACAT) isotype specific inhibitors as anti-atherosclerotic agent through enzyme characterization, activity guided natural resources fractionation and lead oriented chemical synthesis.
- Investigated molecular mechanism(s) and developed pharmacological therapy in metabolic disease using various in vivo and in vitro systems.

C. Awards and recognition

- Scholarship Award, Keystone Symposia Scholarship from Keystone Symposia, USA 2011
- Research Award, Korean Biophysical Society, South Korea 2011
- Brain Korea 21 Postdoctoral Fellowship, Korea Ministry of Education, Science and Technology, South Korea, 2009-2012
- Basic Research Award, Korean Society of Lipidology and Atherosclerosis, South Korea 2008

D. Selected publication

Peer-Reviewed and Research Articles (*Authors equally contributed)

1. Lee SH, Hyeon DY, Yoon SH, Jeong JH, Han SM, Jang JW, Nguyen MP, Chi XZ, **An S**, Hyun KG, Jung HJ, Song JJ, Bae SC, Kim WH, Hwang D, Lee YM: RUNX3 methylation drives hypoxia-induced cell proliferation and antiapoptosis in early tumorigenesis. Cell Death Differ. 28(4):1251-1269. (2021)

2. Raiymbek G, **An S**, Khurana N, Gopinath S, Larkin A, Biswas S, Trievel RC, Cho US, Ragunathan K: An H3K9 methylation-dependent protein interaction regulates the non-enzymatic functions of a putative histone demethylase. Elife. 9:e53155 (2020)
3. Park SH, Ayoub A, Lee YT, Xu J, Kim H, Zheng W, Zhang B, Sha L, **An S**, Zhang Y, Cianfrocco MA, Su M, Dou Y, Cho US**:** Cryo-EM structure of the human MLL1 core complex bound to the nucleosome. Nat Commun. 10(1):5540. (2019)
4. Kim H, **An S**, Park YR, Jang H, Yoo H, Park SH, Lee SJ, Cho US: MMOD-induced structural changes of hydroxylase in soluble methane monooxygenase. Sci Adv. 5(10):eaax0059 (2019)
5. Chik JK, Moiseeva V, Goel PK, Meinen BA, Koldewey P**, An S**, Mellone BG, Subramanian L, Cho US: Structures of CENP-C cupin domains at regional centromeres reveal unique patterns of dimerization and recruitment functions for the inner pocket. J Biol Chem. 294(38):14119-14134 (2019)
6. **An S**, Koldewey P, Chik J, Bardwell J, Subramanian L, Cho US: Eic1, a component of the oligomeric Mis18 holo-complex, converts Mis16 from a histone H4 chaperone to a CENP-ACnp1 assembly factor. Structure. 26(7):960-971. (2018)
7. Yoon J, Kim S, **An S**, Leitner A, Jung T, Aebersold R, Herbert H, Cho US, Song JJ: Integrative structural investigation on the architecture of human Importin4_histone H3/H4_Asf1a complex and its histone H3 tail binding. J Mol Biol. 430, 822-841. (2018)
8. **An S***, Yoon J*, Kim H, Song JJ, Cho US: Structure-based nuclear import mechanism of histones H3 and H4 mediated by Kap123. ELife. 6, e30244. (2017)
9. Kim H*, **An S***, Ro SH*, Teixeira F, Jin Park G, Kim C, Cho CS, Kim JS, Jakob U, Hee Lee J, Cho US: Janus-faced Sestrin2 controls ROS and mTOR signaling through two seperate functional domains. Nat Communications. 6, 10025-10036. (2015)
10. **An S**, Kim H, Cho US: Mis16 Independently Recognizes Histone H4 and the CENP-A(Cnp1) Specific Chaperone Scm3sp. J Mol Biol. 427, 3230-3240. (2015)
11. **An S**, Yeo KJ, Jeon YH, Song JJ: Crystal structure of human histone methyltransferase Ash1L catalytic domain and its implications on the regulatory mechanism. J Bio Chem. 286, 8369-8374. (2011)
12. **An S**, Han JI, Kim MJ, Park JS, Han JM, Baek NI, Chung HG, Choi MS, Lee KT, Jeong TS: Ethanolic extracts of Brassica campestris spp. rapa roots prevent high-fat diet-induced obesity via beta(3)-adrenergic regulation of white adipocyte lipolytic activity. J Med Food. 13, 406-414. (2010)
13. Han JM, Kim MJ, Baek SH, **An S**, Jin YY, Chung HG, Baek NI, Choi MS, Lee KT, Jeong TS: Antiatherosclerotic effects of Artemisia princeps Pampanini cv. Sajabal in LDL receptor deficient mice. J Agric Food Chem. 57, 1267-1274. (2009)
14. **An S**, Jang YS, Park JS, Kwon BM, Paik YK, Jeong TS: Inhibition of acyl-coenzyme A:cholesterol acyltransferase stimulates cholesterol efflux from macrophages and stimulates farnesoid X receptor in hepatocytes. Exp Mol Med. 40, 407-417. (2008)
15. **An S**, Park YD, Paik YK, Jeong TS, Lee WS: Human ACAT inhibitory effects of shikonin derivatives from Lithospermum erythrorhizon. Bioorg Med Chem Lett. 17, 1112-1116. (2007)
16. **An S***, Cho KH*, Lee WS, Lee JO, Paik YK, Jeong TS: A critical role for the histidine residues in the catalytic function of acyl-CoA: cholesterol acyltransferase catalysis: evidence for catalytic difference between ACAT1 and ACAT2. FEBS Lett. 580, 2741-2749. (2006)
17. Cho KH*, **An S***, Lee WS, Paik YK, Kim YK, Jeong TS: Mass-production of human ACAT-1 and ACAT-2 to screen isoform-specific inhibitor: A different substrate specificity and inhibitory regulation. Biochem Biophys Res Commun. 309, 864-872. (2003)

Review Articles

1. **An S** and Song JJ: The coded functions of noncoding RNAs for gene regulation. Mol. Cells. 31, 491-496. (2011)

E. PATENTS

1. Tae-Sook Jeong, LEE Woo-Song, KIM Hyoung-Chin, Yang-Kyu Choi, Ju-Ryoung Kim, **So-Jin An**, Kyoung-Ran Im, Ki-Chang Jang, Og-Sung Moon, Jun-Seock Son. Administering terpenoids selected from ferruginol derivatives, dehydroabietinol, kayadiol and delta-cadinol, that inhibit acyl Coenzyme A: cholesterol acyltransferases and oxidation of low-density lipoproteins, for the treatment of hyperlipemia or atherosclerosis. US Patent No. 7825162 Application No. 12/265,

088 (2010/11/2)

2. Tae-Sook Jeong, LEE Woo-Song, KIM Hyoung-Chin, Yang-Kyu Choi, Ju-Ryoung Kim, **So-Jin An**, Kyoung-Ran Im, Ki-Chang Jang, Og-Sung Moon, Jun-Seock Son. Phenanthrene derivative; acylcoenzime A-cholesterol acyl transferase inhibitor; anti-oxidative activity to low density lipoproteins (LDL); hyperlipidemia and atherosclerosis caused by the LDL oxidation and the synthesis and accumulation of cholesteryl ester. US Patent No. 7820212 Application No. 12/181, 583 (2010/10/26)
3. Tae-Sook Jeong, LEE Woo-Song, KIM Hyoung-Chin, Yang-Kyu Choi, Ju-Ryoung Kim, **So-Jin An**, Kyoung-Ran Im, Ki-Chang Jang, Og-Sung Moon, Jun-Seock Son. Abietane diterpenoid compound, and composition comprising extract of torreya nucifera, or abietane diterpenoid compounds or terpenoid compounds isolated from them for prevention and treatment of cardiovascular disease. US Patent No. 7517542 Application No. 10/591,282 (2009/4/14)

2006-	Member, American Association of Cancer Research
2006-	Member, American Chemical Society
2013	NIH Target I special emphasis panel (ZES1 LWJ-D TG)
2013	NIH special emphasis panel ZRG1 BST-N (50) R
2013	NIH MGA study session, ad-hoc
2015-2019	Member, MGA study section, NIH
2016-2019	Member, DMC study section, ACS
2017	Reviewer, NCI Program Project I (P01) ZCA1 RPRB-F (O1) P
2017	Reviewer, Leukemia and Lymphoma Society TRP grant
2018-2019	NCI U54 Center grant on pediatric fusion onco-proteins
2018-2019	Bloodwise, UK
2019	AAAS international grant review, 2019
2019-2021	AACR-CRUK transatlantic postdoctoral fellowship
2019	NIH Director's New Innovator Award (DP2)
2021-2015	Member, CG study section, NIH
2021	Member, NCI special emphasis panel SEP-3: Research Answers to NCI Provocative Questions

Editorial Boards

2011-2013	Associate Member, Editorial Board, Journal of Clinical & Experimental Pathology
2013-	Member, Editorial Board, Molecular Cancer Research, AACR
2016-2021	Member, Editorial Board, Journal of Biological Chemistry, ASBMB

Honors:

2004-2007	The Irvington Institute for Immunological Research Fellowship
2007	Biomedical Science Scholar, University of Michigan
2010	American Cancer Society RSG Award
2010	AACR Gertrude B. Elion Cancer Research Award
2011	Stand Up to Cancer IRG Award
2012	Leukemia & Lymphoma Society Scholar Award
2014	Dean's Award in Basic Science, University of Michigan
2014	Excellence in Research, University of Michigan

C. Contribution to Science

1. One major focus is biochemical characterization of histone methyltransferase MLL1. We have purified and rigorously defined the mammalian MLL1 complex, reconstituted its H3K4me activity using purified factors and collaboratively solved the crystal structures of the MLL1 and MLL3 core complexes as well as cryo-EM structure of the MLL1-NCP complex. Our studies have provided novel insights on the unique biochemical property of the MLL1 complex on chromatin and how its enzymatic activity is regulated. The work directly leads to the development of the first small molecule inhibitor that specifically blocks MLL1 methyltransferase activity.

a. **Dou Y**, Milne TA, Tackett AJ, Smith ER, Fukuda A, Wysocka J, Allis CD, Chait BT, Hess JL, Roeder RG: Physical association and coordinate function of the H3 K4 methyltransferase MLL1 and the H4 K16 acetyltransferase MOF. ***Cell*,** 121(6): 873-885, 2005. PM15960975

b. Li Y, Han J, Zhang Y, Cao F, Liu C, Li S, Wu J, Hu C, Wang Y, Shuai, J, Chen J, Cao L, Li D, Shi P, Tian C, Zhang J, **Dou Y**, Li Guo, Chen Y and Lei M. Structural basis for activity regulation of MLL family methyltransferases. ***Nature***, 530: 447-452, **2016**. doi:10.1038/nature16952

c. Park SH, Ayoub A, Lee YT, Xu J, Kim H, Zhang W, Zhang B, An S, Zhang Y, Cianfrocco MA, Su M, **Dou Y ***, and Cho US*. Cryo-EM structure of the human Mixed Lineage Leukemia-1 complex bound to the nucleosome. ***Nature Communication***, 10(1): 5540. doi: 10.1038/s41467-019-13550-2, 2019. *Co-correspondence.

d. Lee Y, Ayoub A, Park SH, Sha L, Xu J, Mao F, Zheng W, Zhang Y, Cho US and **Dou Y**. Mechanism for DPY30 and ASH2L intrinsically disordered regions to modulate MLL/SET1 activity on chromatin. ***Nature Communication***, in press.

2. The second major focus is to understand the molecular basis of malignant transformation as the result of HOXA9 overexpression as well as the role of MLL and other H3K4 methyltransferases in this process. The mechanistic studies paved the way for identification of novel therapeutic targets in AML with poor prognosis. We have shown that targeting the MLL1 complex by small molecule inhibitors and biologics is a valid strategy for inhibiting $HOXA9^{high}$ leukemia.

a. Gupta A, Xu J, Lee S, Kurosawa K, Werner M, Koide A, Ruthenburg AJ, **Dou Y*** and Koide S*. Facile target validation in an animal model using monobodies. ***Nature Chemical Biology***, in press. *co-correspondence.

b. Sun Y, Miao H, Mao F, Zou Z, Zhou B, Cai S, Ge K, Dressler G, Levine RL, Armstrong SA, **Dou Y *** and Hess JL*. HOXA9 reprograms the enhancer landscape during leukemic transformation. ***Cancer Cell***, *https://doi.org/10.1016/j.ccell.2018.08.018*. *co-correspondence.

c. Cao F, Townsend EC, Karatas H, Xu J, Li L, Lee S, Liu L, Chen Y, Ouillette P, Zhu J, Hess JL, Atadja P, Lei M, Qin ZS, Malek S, Wang S*, **Dou Y***: Targeting MLL1 H3K4 methyltransferase activity in mixed-lineage leukemia. ***Molecular cell*,** 53 (2), 247-61, 2014. PM24389101. *co-correspondence.

d. Rao, RC and **Dou Y**. Hijacked in cancer: the MLL/KMT2 family of methyltransferases. ***Nature Review in Cancer***, Vol 15, 334-346, 2015. PM25998713.

3. The third major focus is to understand epigenetic regulation in cell fate determination in stem cells. We found that histone acetyltransferase MOF is important in ESC self-renewal via regulating the core transcription network and maintaining active chromatin structures. We found that histone modifications restrict cell fate plasticity and thus, preventing cell fate reversal in development.

a. Li X, Li L, Pandey R, Byun JS, Gardner K, Qin Z, **Dou Y**: The histone acetyltransferase MOF is a key regulator of the embryonic stem cell core transcriptional network. ***Cell Stem Cell*,** 11(2): 163-178, 2012. PM22862943

Selected as 'Issue Highlight' and previewed by *Cell Stem Cell*, 11(6): 139-140.

b. Zhang H, Gayen S, Xiong J, Zhou B, Shanmugam AK, Sun Y, Karatas H, Liu L, Rao RC, Wang S, Nesvizhskii AI, Kalantry S and **Dou Y**: MLL1 inhibition reprograms epiblast stem cells to naïve pluripotency. ***Cell Stem Cell***, 18(4): 481-494, 2016. PM26996599.

c. Le TPK, Tsan YC, MaoF, Kremer D, Sajjakulnukit P, Zhang L, Zhou B, Tong X, Bhanu NV, Choudhary CR, Garcia BA, Yin L, Smith GD, Saunders TL, Bielas S, Lyssiotis CA and **Dou Y**. Histone acetyltransferase MOF regulates quiescence in ground-state pluripotency through fatty acid oxidation. ***Cell Stem Cell***, 27(3):441-458, 2020.

4. The fourth major focus is biochemical characterization of the mammalian histone acetyltransferase MOF (also called MYST1 or HAT8). We have identified two mammalian MOF complexes, a canonical MOF complex related to the Drosophila dosage compensation MSL complex and a non-canonical MOF-MSL1v1 complex. We found that the MOF-MSL1v1 complex has novel and distinct enzymatic activities from the MOF-MSL complex. We found that two MOF complexes play distinct functions in transcription regulation.

a. Li X, Wu L, Corsa CAS, Wu L and **Dou Y**. Two mammalian MOF complexes regulate transcription activation through distinct mechanisms. ***Molecular Cell***, 36: 290-301(2009).

Selected as 'Issue Highlight' and previewed by *Molecular Cell*, 36 (2) 174-175, 2009.

b. Li X, Corsa CAS, Pan PW, Wu L, Ferguson D, Yu X, Min J, **Dou Y**. MOF and H4 K16 acetylation play important roles in DNA damage repair by modulating recruitment of DNA damage repair protein Mdc1. ***Mol Cell Biol.,*** 30(22): 5335-47 (2010).

c. Wu L, Zee BM, Wang Y, Garcia BA, **Dou Y**: The RING Finger Protein MSL2 in the MOF Complex Is an E3 Ubiquitin Ligase for H2B K34 and Is Involved in Crosstalk with H3 K4 and K79 Methylation. ***Molecular Cell,*** 43(1): 132-144, 2011.

Selected as 'Issue Highlight' and previewed by *Molecular Cell*, 43 (1), 5-7, 2009.

d. Wu L, Li L, Qin Z and **Dou Y**: MSL2 mediated H2B K34 ubiquitylation promotes RNA Pol II processivity through regulating PAF1 and pTEFb pathways. ***Molecular Cell***, 54 (6), 920-931, 2014.

5. We have extensive ongoing collaborations in broad areas of immunology, cancer biology and hematology. Here are some representative collaborative publications in the past a few years.

a. Wang W, Kryczek I, Dosta Ll, Lin H, Tan L, Zhao L, Lu F, Wei S, Maj T, Peng D, He G, Vatan L, Szeliga W, Kuick R, Kotarski J, Tarkowski R, **Dou Y**, Rattan R, Munkarah A, Liu JR, and Zou W. Effector T cells abrogate stroma-mediated chemoresistance in ovarian cancer. ***Cell*** 165(5), 1092-1105, 2016.

b. Luo H, Shenoy AK, Li X, Jin Y, Jin, Cai Q, Tang M, Liu Y, Chen H, Reisman D, Wu L, Seto E, Qiu Y, **Dou Y**, Casero Jr. RA, Lu J. MOF acetylates the histone demethylase LSD1 to suppress epithelial-to-mesenchymal transition. ***Cell Report***, 15(12), 2665-78, 2016.

c. Peng D, Kryczek I, Nagarsheth N, Zhao L, Wei S, Wang W, Sun Y, Zhao E, Vatan L, Szeliga W, Kotarski J, Tarkowski R, **Dou Y**, Cho K, Hensley-Alford S, Munkarah A, Liu R, Zou W. Epigenetic silencing of TH1-type chemokines shapes tumor immunity and immunotherapy. ***Nature,*** 527(7577): 249-53. doi: 10.1038/nature15520, 2015.

d. Jones M, Chase J, Brinkmeier M, Xu J, Weinberg DN, Schira J, Friedman A, Malek S, Grembecka J, Cierpicki T, **Dou Y**, Camper SA and Maillard I. Ash1L controls quiescence and self-renewal potential in hematopoietic stem cells, ***J. Clinical. Investigation***, 125 (5), 2007-2020, 2015.

List of Publication Work in Pubmed:
http://www.ncbi.nlm.nih.gov/pubmed/?term=Yali+Dou

D. MAJOR RESEARCH SUPPORTS

R01 NS100156 09/01/2016-08/31/2021
PRDM16 function in neural development
Role: PI
Goal: Examine the novel function of the histone methylation activity of PRDM16 in neurogenesis

R01 CA 232263 02/01/2019 - 01/31/2024
Enhancer Dysregulation in AML
Role: PI
Goal: To characterize how HOXA9 overexpression alters the enhancer landscapes in normal hematopoietic cells that lead to malignant transformation

R01 GM082856 4/2009-09/2024
Epigenetic regulation of transcription by MLL1
Role: PI
Goal: To characterize the function of MLL1 in transcription regulation and stem cell fate

R01 CA250329 4/1/2020-03/31/2025
Structural insights into the MLL core complexes
Role: Contacting PI (PIs: Dou, Cho, Su)

Goal: To determine the role of ASH2L in regulation of histone methylation

P30CA014089-46 Lerman (PI) 12/01/96-11/30/21
USC Norris Comprehensive Cancer Center (Core) Support
Role: Co-Leader of the Genomics and Epigenomics Research Program
Goal: Support the USC Cancer Center.

Foreign Support/Appointment
Dr. Dou has no full-time, part-time, or voluntary position or scientific appointment in any foreign institution.

BIOGRAPHICAL SKETCH

NAME: Shigeki Iwase

eRA COMMONS USER NAME: SIWASE

POSITION TITLE: Associate Professor, Human Genetics Department, The University of Michigan, Medical School

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date	FIELD OF STUDY
University of Tsukuba, Tsukuba, Japan	B.S.	03/2001	Environmental Science
University of Tsukuba, Tsukuba, Japan	Ph.D.	03/2006	Life and Environmental Science
Harvard Medical School, Boston Children's Hospital,	Postdoctoral	08/2011	Chromatin Biology

A. Personal Statement

I have a long-standing passion for identifying new gene-regulatory mechanisms of the brain, focusing on histone methylations. My group has been closely working with Dr. Cho' group (Co-PI) for multiple projects over the past twi years, and the collaborations have been highly productive and exciting.

As a postdoctoral fellow in Yang Shi's group, I uncovered the molecular functions of two orphan histone methyl regulators, SMCX/KDM5C and ATRX, mutated in neurodevelopmental disorders X-linked intellectual disability (ID) and autism spectrum disorder (ASD). I reported that KDM5C encodes the first eraser enzyme for di- and tri-methylated histone H3 lysine 4 (Iwase *et al*. ***Cell***. 2007). I discovered that ATRX is a reader protein for tri-methylated histone H3 lysine 9 (Iwase *et al*. ***Nature Structural & Molecular Biology***, 2011). In both cases, I demonstrated that the missense mutations associated with ID/ASD compromised the eraser or reader function of KDM5C or ATRX. The work made one of the earliest causal links between histone methylation dynamics and human cognitive development.

Having launched my laboratory in 2012, I have expanded experimental approaches to interrogate molecular and cellular roles of histone methylation dynamics in brain development and function. These include new mouse models and genomics tools. For example, we reported a new RNA-seq approach that enabled precise detection of transcription start sites (Agarwal *et al*. ***Nature Communications***, 2015). This new method can be a powerful tool to investigate gene regulatory mechanisms by chromatin regulators. Combining these new approaches with interdisciplinary collaborations, we have found **1)** the mice lacking KDM5C serve as a novel model of ID/ASD syndrome associated with impaired removal of histone methylation (Iwase *et al*. ***Cell Reports***, 2016), **2)** KMT2A mediates impaired brain development in KDM5C-mice (Vallianatos *et al*. ***Communications Biology***, 2020). **3)** KDM5C is a key initiation factor for X-chromosome inactivation, the female-specific epigenetic process (Gayen S *et al*., ***BioRxiv***, 2017, under review in *Nature Genetics*), **4)** roles of LSD1 histone demethylase in genome-wide homeostasis of transcriptional enhancers both in embryonic stem cells and cortical neurons (Agarwal *et al*. ***Genome Research***, 2021), and **5)** impaired cAMP response as a potential mechanism of Potocki-Shaffer Syndrome, which is associated with PHF21A histone binding protein (Porter *et al*. ***Neuroscience***, 2017). **6)** consequences of a new KDM5C mutation associated with ID/ASD (Vallianatos *et al*. ***Frontiers in Molecular Neuroscience,*** 2018). **7)** roles of RAI1, the Smith-Magenis syndrome gene, in synaptic scaling and neuronal activity-driven transcription (Garay *et al*. ***Cell Reports***, 2020). The work has been supported through the following NIH fundings.

Ongoing Research Support

1. R R01NS116008 Iwase (PI) 5/01/2020 – 4/30/2025
   National Institute of Neurological Disorders and Stroke (NINDS)
   Title: A Neuron-specific Methyl-histone Regulatory Complex

Explore neuron-specific regulation of histone H3K4 methylation that underlies normal synapse Development

Completed Research Support

1. R01NS089896 (No cost extension) Iwase (PI) 6/15/2015 – 4/30/2021
   NINDS
   Title: Neutralizing epigenomes in neurodevelopmental disorders
   This project aims to determine histone H3K4me writer enzymes that mediate molecular, cellular, and behavioral abnormalities of a mouse model of X-linked intellectual disability.
2. R21NS104774 Iwase (PI) 5/15/2018 – 4/30/2020
   NIH/NINDS
   Exploring Neuron - Specific Histone Methylation Dynamics
   This project's primary goal is to explore the molecular and cellular roles of a neuron-specific isoform of PHF21A, a putative histone binding protein.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2006–2010 Postdoctoral research fellow, Department of Pathology, Harvard Medical School, Boston, MA Advisor: Yang Shi, Ph.D.
2010–2012 Postdoctoral research fellow, Division of Newborn Medicine, Boston Children's Hospital, Boston, MA. Advisor: Yang Shi, Ph.D.
2012–2019 Assistant professor, Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI
2015– Visiting Scholar, Thesis Examiner, University of Tsukuba, Japan.
2019– Associate professor, Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI

Honors

2004 Japan Society for the Promotion of Science, Scholarship
2008 Jane Coffin Childs Memorial Fund, Postdoctoral Fellowship Award
2010 Japan Society for the Promotion of Science, Postdoctoral Fellowship for Research Abroad
2012 University of Michigan, Biological Sciences Scholars
2013 Cooley's Anemia Foundation, Research Fellowship Award
2014 Basil O'Connor Starter Scholar Research Award
2014 Competitive renewal: Cooley's Anemia Foundation, Research Fellowship Award

Other Experience and Professional Memberships

2011– Member, Society for Neuroscience
2013– Member, The American Society of Human Genetics
2013– Member, American Neurological Association
2013– Ad hoc journal reviewer, *Neuropharmacology*, *Nature Structural & Molecular Biology*, *The New England Journal of Medicine*, *PNAS*, *PLoS Genetics, Frontiers in Neuroscience, Molecular & Cellular Neuroscience, Epigenetics in Human Disease, Cell Reports, Journal of Visualized Experiments (JoVE), Molecular Neurobiology, PLoS Biology, Scientific Reports, Genes, Trends in Neuroscience, Elife, Development, Nature Communications, Molecular Cell, and Nature Neuroscience.*
2017 Guest editor: *Molecular and Cellular Neuroscience*
2017– Chairing symposia "Epigenetic etiology of intellectual disability" at the Society for Neuroscience annual conference at Washington DC (2017), "Chromatin Dysregulation in Neurodevelopmental Disorders Symposium" in American Society for Human Genetics Annual Meeting, at San Diego (2018).
2019– Ad hoc NIH grant reviewer, **1)** NIH Neurodevelopment, Synaptic Plasticity, and Neurodegeneration study section (F03A), **2)** NIGMS, COBRE Phase I, Centers of Biomedical Research Excellence (P20), and **3)** Special Emphasis Panel/Scientific Review Group ZHD1 DSR-H for NICHD IDDRC.

C. Contribution to Science

C1. Discovery of histone methylation-based mechanisms of neurodevelopmental disorders. Methyl-histone dysregulation emerged as a significant contributor to neurodevelopmental disorders. The mechanisms by which these mutations lead to neurological disorders remain mostly unknown. My previous work identified

the roles of two genes that were mutated in X-linked ID/ASD syndromes; KDM5C and ATRX. In 2007, I reported that KDM5C encodes the first eraser enzyme for di- and tri-methylated histone H3 lysine 4 **(a)**. In 2011, I reported that ATRX is a reader protein for tri-methylated histone H3 lysine 9 **(b)**. In both cases, I headed the projects as the leading author and demonstrated that the missense mutations associated with intellectual disability compromise the enzymatic eraser or reader function of KDM5C or ATRX. The work demonstrated the link between dynamic regulation of histone methylation and human cognitive development for the first time.

More recently, my group generated a novel mouse model of ID/ASD syndrome associated with impaired histone methylation removal by deleting the *Kdm5c* gene **(c)**. We then demonstrated KMT2A writer enzyme mediates abnormal brain development in the *Kdm5c*-eraser mutant mice **(d)**.

a. **Iwase S.***, Lan F.*, Bayliss P., Torre-Ubieta L., Huarte M., Qi H., Whetstine J. W., Bonni A., Roberts M. T., and Shi Y. The X-linked mental retardation gene *SMCX/JARID1C* defines a family of histone H3 lysine 4 demethylases. ***Cell***. 128: 1077-1088 (2007) *equal contribution
b. **Iwase S.**, Xiang B., Ghosh S., Ren T., Lewis P.W., Cochrane J.C., Allis C.D., Picketts D. J., Patel D. J., Li H. and Shi Y. ATRX links atypical histone methylation recognition mechanisms to human brain function. ***Nature Structural & Molecular Biology***. 18: 769-776 (2011). PMC3130887
c. **Iwase S.***†, Brookes E.*, Agarwal S.*, Badeaux A. I., Ito H., Vallianatos C. N., Tomassy G. S., Kasza T., Lin G., Thompson A., Gu L., Kwan K. Y., Chen C., Sartor M. A., Egan B., Xu J.†, Shi Y.† A mouse model of X-linked intellectual disability associated with impaired removal of histone methylation. ***Cell Reports.*** 15, 01547-8 (2016). *equal contribution, †corresponding authors. PMC4749408
d. Vallianatos CN, Raines B, Porter RS, Bonefas KM, Wu MC, Garay PM, Collette KM, Seo YA, Dou Y, Keegan CE, Tronson NC†, **Iwase S†**. Mutually suppressive roles of KMT2A and KDM5C in behaviour, neuronal structure, and histone H3K4 methylation. ***Communications Biol***. 2020 Jun 1;3(1):278. doi: 10.1038/s42003-020-1001-6. PMC7264178. †corresponding authors.

C2. Uncovering biological roles and new regulatory mechanisms of histone methylations.
In Michigan, we identified and characterized the first KDM5C mutation that does not affect enzymatic activity but is associated with ID/ASD **(a)**. Another work was able to describe unexpected roles of LSD1/KDM1A in genome-wide homeostasis of transcriptional enhancers in embryonic stem cells and post-mitotic neurons **(b)**. In collaboration with Kalantry lab, we revealed the key role of SMCX/KDM5C in X-inactivation, the female-specific epigenetic silencing of the X chromosome **(c)**. My laboratory also identified impaired cAMP response as a candidate molecular mechanism of cognitive deficits in Potocki-Shaffer Syndrome **(d)**.

a. Vallianatos C.N., Farrehi C., Friez M.J., Burmeister M., Keegan C.E., **Iwase S.** Altered Gene-Regulatory Function of KDM5C by a Novel Mutation Associated with Autism and Intellectual Disability ***Front Mol Neurosci.*** (2018) PMC5893713
b. Agarwal S†, Bonefas K, Garay PM, Porter RS, Brookes E, Murata-Nakamura Y, Macfarlan TS, Ren B†, **Iwase S†**. LSD1/KDM1A Maintains Genome-wide Homeostasis of Transcriptional Enhancers. ***Genome Research***. (2021). †corresponding authors (PMCID requested)
c. Gayen S, Maclary E, Murata-Nakamura Y, Vallianatos CN, Porter RS, Garay PM, **Iwase S†**, and Kalantry S†. Induction of X-chromosome Inactivation by the Histone Demethylase SMCX/KDM5C ***BioRxiv***. (2017). †corresponding authors (Under revision in *Nature Genetics*)
d. Porter RS, Murata-Nakamura Y, Nagasu H, Kim HG, and **Iwase S**. Transcriptome analysis revealed impaired cAMP responsiveness in *PHF21A*-deficient human cells. ***Neuroscience***: 17, 30365-2 (2017). PMC5708152

C3. Development of new genomics tools for investigating gene regulatory mechanisms. The most RNA-seq methods suffer from underrepresentation of both the 5' and 3' ends of RNAs, which harbor crucial information for gene regulation — transcription start sites (TSS) and polyadenylation sites (PAS). My group reported a novel ssRNA-seq method, which enabled the profiling of both TSS and PAS at near-base resolution **(a)**. We also developed nascent RNA-sequencing to monitor transcriptional response to network activity shifts. This method offers the first genomics tool to obtain the "full-length" transcriptome with a single library and accurately monitor dynamic transcriptional activity in neurons **(b)**.

a. Agarwal S., Macfarlan T.S., Sartor M.A., and **Iwase S**. Sequencing of first-strand cDNA library reveals full-length transcriptomes. ***Nature Communications.*** 6: 6002 (2015). PMC5054741

b. Garay P.M., Chen A., Tsukahara T., Kohen R., Althaus J.C., Wallner M.A., Giger R.J., **Sutton M.A.** †, **Iwase S**†. RAI1 Regulates Activity-Dependent Nascent Transcription and Synaptic Scaling. ***Cell Reports***. 32(6):108002. PMC7418709 (2020). †corresponding authors

C4. Summarizing current knowledge regarding chromatin dysregulation in neurodevelopmental disorders: I was the lead and corresponding author of each of four invited review articles, which comprehensively discussed identified mutations in histone methyl regulators and possible cellular and molecular consequences of such mutations in brain development and function. The work illuminated the emerging field of research investigating the histone methylation dynamics in the brain.

a. Vallianatos C.N. and **Iwase S**. Disrupted intricacy of histone H3K4 methylation in neurodevelopmental disorders. ***Epigenomics*.** 7, 503-519 (2015). (review article) PMC4501478
b. Garay P.M., Wallner M.A., and **Iwase S**. Yin-Yang Actions of Histone Methylation Regulatory Complexes in the Brain. ***Epigenomics*.** 8: 1689-1708. (2016) (review article) PMC5289040
c. Porter R.S., Jaamour F., and **Iwase S**. Neuron-Specific Alternative Splicing of Transcriptional Machineries: Implications for Neurodevelopmental Disorders. ***Molecular and Cellular Neuroscience.*** 87:35-45. (2018) (review article) PMC5828955
d. **Iwase S**, Battaglioli E, Nadif Kasri N, Zhou Z., Bérubé N, and Barco A. Epigenetic etiology of intellectual disability. ***Journal of Neuroscience***. 37(45):10773-10782. (2017) (review article) PMC5678009

Link to full list of publications in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/49720759/?sort=date&direction=descending

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **KAUSHIK RAGUNATHAN**

eRA COMMONS USER NAME (credential, e.g., agency login): **KRAGUNATHAN**

POSITION TITLE: **ASSISTANT PROFESSOR**

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
PSG College of Technology, India	B.Tech	04/2005	Biotechnology
University of Illinois, Urbana-Champaign, IL	Ph.D	05/2012	Biophysics & Computational Biology
Harvard Medical School, Boston, MA	Postdoc	12/2016	Cell Biology
University of Michigan, Ann Arbor, MI	Asst. Professor	01/2017-present	Biological Chemistry

A. PERSONAL STATEMENT

Our bodies consist of billions of genetically identical cells which have the capacity to exhibit distinct phenotypic or epigenetic states. Epigenetic states are established through the collective action of diffusible *trans*-acting factors that can read, write and erase histone modifications. The interactions between histone modifiers and modified histones are weak and transient. Yet, gene expression patterns that emerge from these collective, low-affinity interactions persist for many cell divisions. **My overall program of research tackles this enigma using a unique permutation of genetic, biochemical and biophysical approaches to elucidate the molecular mechanisms of epigenetic inheritance.**

As a new junior faculty member at the University of Michigan in 2017, I was selected to join the Biological Sciences Scholars Program (BSSP), which recruits a cohort of outstanding scientists each year in key areas of life sciences investigation. This award enabled me to forge connections with research areas that were entirely new to me such as: protein biochemistry, *in vitro* reconstitution, single particle tracking in living cells, development of microfluidics based technologies and image processing algorithms. This initial impetus to venture into uncharted scientific territory was instrumental in securing a major award from the National Science Foundation as part of its flagship **Understanding Rules of Life Program (URoL) focused on Epigenetics**. As the PI of this major NSF award, I stitched together a diverse team of researchers (Biteen, Bailey, Khalil and Freddolino) to study how cells make adaptive epigenetic choices that enhance fitness and cell survival. A second focus of my lab is to reconstitute H3K9 methylation dependent protein interactions and to determine how the H3K9me dependent recruitment of chromatin associated factors enforces transcription silencing and epigenetic inheritance. We use an interdisciplinary approach that includes *in vitro* biochemical reconstitution, single particle tracking and single cell lineage tracing methods to determine how epigenetic states are stable and heritable across multiple generations. This line of research is funded by an **R35 Maximizing Investigator's Research Award from NIGMS**.

As part of **my professional service**, I am a co-organizer of the Epigenetics and Bioengineering Conference (EpiBio 2018 and 2021). I am also a co-founder of **the Chromatin Club, a collaborative research collective at the University of Michigan**. Only in its second year, the Chromatin Club has emerged as the pre-eminent forum for exchange of research ideas relating to epigenetics at the University of Michigan. In addition to our monthly efforts in community building through research seminars, we organize an annual symposium where we invite leaders in the field of chromatin biology. The success of the Chromatin Club is evidenced also through financial support that we secured from the University of Michigan Biosciences Initiative to develop and disseminate new tools to advance research in epigenetics.

In addition to my research pursuits, I am passionate about **training the next generation of research scholars**. I have taught a senior level undergraduate biochemistry course and a graduate level course on single molecule approaches in biology. Over the past two and a half years, I have mentored eight undergraduate students in research and currently supervise four graduate students and two post-doctoral researchers. Thus, across my research, teaching/mentoring, and professional service, I am committed to interdisciplinary and collaborative science that advances the field of epigenetics.

Listed below are four key publications which highlight my scientific contributions:

a. **Ragunathan, K**., Jih, G., and Moazed, D., "Epigenetic inheritance uncoupled from sequence specific recruitment" *Science,* 348(6230), p. 1258699 (2015)

b. Raiymbek, G., An, S., Khurana, N., Gopinath, S., Biswas, S., Larkin, A., Trievel, R., Cho, US., **Ragunathan, K***. "An H3K9 methylation dependent protein interaction regulates the non-enzymatic functions of a putative histone demethylase" *eLife 9:e53155* (2020) (*corresponding author)

c. Biswas, S., Karslake, J., Chen, Z., Farhat, A., Freddolino, PL., Biteen, JS., **Ragunathan, K***. "HP1 oligomerization compensates for low-affinity H3K9me recognition and provides a tunable mechanism for heterochromatin-specific localization" doi.org/10.1101/2021.01.26.428151 *bioRxiv* (2021) (*senior corresponding author) – *under review in Nature Chemical Biology*

d. **Ragunathan, K**., Liu, C., and Ha, T., "RecA filament sliding on DNA facilitates homology search" *eLife*, 1: p. e00067 (2012)

B. POSITIONS AND HONORS

Positions and Employment

2012-2016 Leukemia and Lymphoma Society Postdoctoral Fellow,
Department of Cell Biology, Harvard Medical School, Boston, MA

2017-present Assistant Professor, Department of Biological Chemistry,
University of Michigan, Ann Arbor, MI

Other Experience and Professional Memberships

2017- present Member, American Institute of Chemical Engineers (AIChE)

2017- present Member, American Society for Biochemistry and Molecular Biology (ASBMB)

Honors

2016 Biological Sciences Scholars Program (BSSP), University of Michigan

2015 HMS Epigenetics Initiative Travel Award

2013 Leukemia and Lymphoma Society Postdoctoral Fellowship

2009 University of Illinois Graduate College Travel Award

2005 Best Graduating Student Award, PSG College of Technology

2004 Indian Academy of Sciences Fellowship in Biological Sciences

2003 Raman Research Institute Fellowship in Physics

C. CONTRIBUTIONS TO SCIENCE

1. Histone modifications can act as carriers of epigenetic memory

The methylation of histone H3 lysine 9 (H3K9me) is associated with the establishment of silent epigenetic states or heterochromatin. Heterochromatin establishment, in organisms ranging from yeast to humans, involves conserved interactions between sequence specific DNA or RNA binding proteins, histone modifiers and modified histones. An important question in this context is whether modified histones can perpetuate epigenetic states in the absence of any inputs from underlying genetic elements. Can the establishment of epigenetic states be fully uncoupled from its subsequent inheritance? I discovered that in the fission yeast, *Schizosaccharomyces pombe*, H3K9 methylated histones can act as carriers of epigenetic memory. This process involves the inactivation of a putative H3K9 demethylase, Epe1 which antagonizes sequence-independent epigenetic inheritance. This provides a unique model system to interrogate what factors

might be specifically involved in epigenetic maintenance uncoupled from its initial establishment. Using this approach, we have discovered factors that couple DNA replication to parental histone transfer and the propagation of epigenetic states.

a. **Ragunathan, K**., G. Jih, and D. Moazed, Epigenetic inheritance uncoupled from sequence specific recruitment" *Science*, 348(6230), p. 1258699 (2015)

Cited as part of *"A timeline of major discoveries and advances in epigenetic research between 1996 and 2016" (Allis,D.C. and Jenuwein,T. Nature Reviews Genetics, 2016); Highlighted in Nat. Rev. Genetics.*

2. Non-enzymatic functions associated with histone modifying enzymes regulate epigenetic inheritance

The deletion of Epe1 bypasses a continuous requirement for DNA sequences and enables histone modifications to serve as carriers of epigenetic memory (Ragunathan et al., Science, 2015). The dominant model is that that Epe1 reverses epigenetic states via enzymatic demethylation of H3K9 methylated histones. However, there is no biochemical evidence to support the notion that Epe1 harbors any *in vitro* enzymatic activity (Tsukada et al, Nature, 2005). Using genetics and *in vitro* reconstitution measurements, we discovered that an auto-inhibited state of Epe1 regulates its capacity to directly interfere with heterochromatin assembly. H3K9 methylation relieves Epe1 from an auto-inhibited state enabling the formation of heterochromatin restricted complex with its binding partner, Swi6. We propose that the formation of a regulatory complex comprised of Epe1 and Swi6 displaces and outcompete other effector molecules. Our results underscore how histone modifying proteins that resemble enzymes have non-catalytic functions which regulate the assembly of epigenetic complexes in cells.

a. Raiymbek, G., An, S., Khurana, N., Gopinath, S., Biswas,S., Larkin, A., Trievel, R., Cho, US., **Ragunathan,K***. "An H3K9 methylation dependent protein interaction regulates the non-enzymatic functions of a putative histone demethylase" *eLife 9:e53155* (2020) (*corresponding author)

3. Mapping biochemical intermediates states associated with chromatin complexes in living cells.

HP1 proteins bind with low affinity but high specificity to histone H3 lysine 9 methylation (H3K9me), forming transcriptionally inactive genomic compartments referred to as heterochromatin. How HP1 proteins traverse a complex and crowded chromatin landscape on the millisecond timescale to bind H3K9me chromatin remains paradoxical. We used a single-molecule approach to visualize an HP1 homolog, the fission yeast Swi6, in its native chromatin environment. By analyzing Swi6 motions, we identify individual mobility states that map to discrete biochemical intermediates. Using mutants that perturb Swi6 H3K9me recognition, oligomerization, or nucleic acid binding, we mechanistically parse how each biochemical property affects protein dynamics. While nucleic acid binding titrates Swi6 away from heterochromatin, as few as four tandem chromodomains are sufficient to restore H3K9me-dependent localization. Our studies propose a new paradigm where HP1 oligomerization stabilizes higher-order complexes to outcompete inhibitory nucleic acid and non-specific chromatin interactions, enabling high specificity H3K9me recognition in cells. Our high-resolution biophysical studies provide a comprehensive framework for *in vivo* biochemistry and reveal how the competing biochemical properties of Swi6 affect H3K9me recognition in living cells.

a. Biswas, S., Karslake, J., Chen, Z., Farhat, A., Freddolino, PL., Biteen, JS., **Ragunathan, K***. "HP1 oligomerization compensates for low-affinity H3K9me recognition and provides a tunable mechanism for heterochromatin-specific localization" *bioRxiv (2021)* doi.org/10.1101/2021.01.26.428151 (*senior corresponding author)

4. Mechanism of RecA mediated homology search and double strand break repair

E.coli RecA, a homolog of the eukaryotic protein Rad51, plays a central role in double strand break repair via homologous recombination. RecA catalyzes a reaction which is referred to as 'strand exchange'. This process involves two stages 1) a search for homology 2) exchange of complementary basepairs between two homologous DNA molecules. The search for homology can be conceptualized in terms of a classical 'needle in a haystack' problem where RecA must locate a homologous sequence amidst a large excess of non-homologous DNA sequences within the genome. My work captured the ability of RecA to slide along DNA templates in search of complementarity. RecA mediated homology recognition requires a seed region containing as few as six complementary basepairs. My data represented one of the first examples of a DNA bound multi-protein complex which can slide along another DNA molecule to facilitate target search. I also developed a single-molecule fluorescence assay with few base-pair and millisecond time resolution to unravel

the mechanistic details of the strand exchange reaction. I determined that the exchange of complementary basepairs mediated by RecA occurs in 3bp intervals. My findings integrated high resolution structural models of RecA with its catalytic function.

a. **Ragunathan, K**., C. Liu, and T. Ha, RecA filament sliding on DNA facilitates homology search. *eLife*, 1: p. e00067 (2012)

Commentary: B.Gibb and E.Greene, "Sliding to the rescue of damaged DNA" eLife 1:e00347 (2012); Highlighted by Cell Leading Edge article on Genome Instability

b. **Ragunathan, K**., C. Joo, and T. Ha, Real-time observation of strand exchange reaction with high spatiotemporal resolution. *Structure*, 19(8): p. 1064-73 (2011)

Commentary by C.Wyman, "Mechanistic insight from Chaos: How RecA Mediates DNA Strand Exchange" Structure 19(8) p. 1031-1032 (2011);

Highlighted by Faculty of 1000

5. Protein-RNA dynamics during 30S ribosomal subunit assembly

The assembly of the 30S ribosomal subunit involves the sequential association of about 20 different RNA binding proteins with the 16S ribosomal RNA. In this work, we identified how protein binding influences the folding landscape of the 16S ribosomal RNA. My contribution involved the use of a three color FRET approach, where I could simultaneously visualize rRNA conformational dynamics in conjunction with ribosomal protein binding. The ability to determine distance changes across multiple coordinates allowed me to measure how the binding of ribosomal proteins-S4, S12, S16 and S20 affect rRNA folding pathways. This model for protein-guided changes in RNA structure reveals how the association of RNA binding proteins can constrain RNA folding pathways allowing access to a constrained subset of intermediate conformations. These models also offer an alternative explanation to induced fit models of RNA-protein binding.

a. Kim, H., S.C. Abeysirigunawarden, K. Chen, M. Mayerle, **K. Ragunathan**, Z. Luthey-Schulten, T. Ha, S.A. Woodson, Protein-guided RNA dynamics during early ribosome assembly. *Nature*, 506(7488): p. 334-8 (2014)

News and Views: K.B.Hall "Molecular Biology: Protein binding cannot subdue a lively RNA" Nature 506, 303-304 (2014)

b. Abeysirigunawardena, S, Kim, H, J. Lai, **K. Ragunathan**, M. Rappe, Z. Luthey-Schulten, T. Ha, S.A. Woodson, "Evolution of protein-coupled RNA dynamics during hierarchical assembly of ribosomal complexes", *Nature Communications,* 8, 492 (2017)

Complete List of Published Work in MyBibliography

https://www.ncbi.nlm.nih.gov/myncbi/kaushik.ragunathan.1/bibliography/public/

D. Research support

Ongoing Research Support

NSF Award Number: 1921677 Ragunathan (PI) 09/01/19-8/31/24

Title: Robustness and Adaptability of the Dynamic Epigenome: A Multiscale Approach

By combining fission yeast genetics, high resolution imaging, synthetic biology and mathematical modeling, our goal is to determine how cells make adaptive epigenetic choices that enable survival under conditions of acute stress. This project is a collaborative effort with funds that are distributed across five investigators situated at the University of Michigan and Boston University.

Role: PI

NIH 1R35GM137832-01 Ragunathan (PI) 09/01/20-8/31/25

Title: Capturing the dynamic epigenome using single molecule and single cell approaches

The major goal of this award is to determine how transient and dynamic interactions between heterochromatin associated proteins and their nucleosome substrates produces stable and heritable gene expression states. Using single molecule and single cell approaches enables us to capture these interactions and visualize complex assembly in real-time with high spatial and temporal resolution.

Role: PI

Completed Research Support

09/01/2017-08/31/2018 Ragunathan (co-PI)

Endowment for the Basic Sciences Innovation Initiative

Title: Single cell approaches to understand the molecular basis of epigenetic clonal variability

Role: co-PI