BIOGRAPHICAL SKETCH

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NAME: Andy Nguyen

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

POSITION TITLE: Ph.D. Candidate

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
San Bernardino Valley College, San Bernardino, CA	A.A.	08/2014	05/2017	Liberal Arts: Biological and Physical Sciences
University of California, Los Angeles, Los Angeles, CA	B.S.	09/2017	06/2021	Biochemistry
University of Pennsylvania, Philadelphia, PA	Ph.D.	08/2021	05/2028 (Expected)	Biochemistry, Biophysics, and Chemical Biology

A. Personal Statement

I am pursuing the molecular understanding of eukaryotic transcription regulation through cryo-electron microscopy (cryo-EM) single particle and biochemical approaches. My research experience ranges across different disciplines such as molecular biology, computational genetics, biochemistry, and structural biology. The culmination of my research experiences has laid the foundation for my scientific training and equipped me with the resources necessary to answer fundamental biological questions that I am pursuing for my thesis work as a Ph.D. candidate at the University of Pennsylvania.

My scientific endeavors started as a junior in high school, where I joined the lab of Dr. Kerby Oberg at Loma Linda University. It was during this initial research experience where my scientific curiosity jump-started, and seeing directly how my research could benefit a population highly motivated me. My eagerness to continue making scientific discoveries carried on throughout my undergraduate career, leading me to conduct research at various institutions such as Loma Linda University, the University of California, Los Angeles (UCLA), the University of California, Irvine (UCI), and the University of California, San Francisco (UCSF). Notably, from each experience, I've had the opportunity to communicate my findings at numerous scientific symposiums and conferences, winning multiple poster presentation awards. My extensive research experiences during my undergraduate career left me with a desire to pursue questions of my own and engage in independent research as a Ph.D. student at the University of Pennsylvania, where I joined Dr. Kenji Murakami's lab for my thesis work. My current thesis work revolves around elucidating the molecular mechanism of the *S. cerevisiae* Hir complex's H3 deposition onto chromatin. In the Murakami lab, I've learned multiple biochemistry and structural biology skills, such as protein purification, sample grid preparation, and cryo-EM data processing. As someone who is the first in my family to pursue higher education and graduate from university, I will apply the same courage and resourcefulness to make scientific discoveries.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments
2021-Present: Graduate Research Assistant, University of Pennsylvania
2021: Undergraduate Research Assistant, Williams Lab, University of California, San Francisco
2020: Undergraduate Research Assistant, Lee Lab, University of California, Irvine
2019-2021: Undergraduate Research Assistant, Quinlan Lab, University of California, Los Angeles
2017-2021: Student Members of the American Chemical Society Member, University of California, Los Angeles
2016-2017: Research Assistant, Oberg Lab, Loma Linda University

Honors
2023-2025: Diversity Supplement, National Institute of Health
2023: National Science Foundation Graduate Research Fellowship Program, Honorable Mention
2021: University of California, San Francisco Summer Research Training Program Symposium, San Francisco, CA, Poster Presentation
2021: Koret UC LEADS Research & Leadership Symposium, Irvine, CA, Poster Honorable Mention
2021: University of California, Los Angeles Undergraduate Research Week, Los Angeles, CA, Oral Presentation
2020: The Annual Biomedical Research Conference for Minority Students, Anaheim, CA, Poster Presentation
2020: Koret UC LEADS Research & Leadership Symposium, Berkeley, CA, Poster Honorable Mention
2020: American Chemical Society National Meeting & Expo, San Francisco, CA, Poster Presentation
2020: University of California, Los Angeles Undergraduate Research Week, Los Angeles, CA, Poster Presentation
2020: American Chemical Society National Meeting & Expo, Philadelphia, PA, Poster Presentation
2019: Southern California Conferences for Undergraduate Researchers, San Diego, CA, Poster Presentation
2019–2021: University of California's Leadership Excellence through Advanced Degrees Fellowship
2017–2021: University of California, Los Angeles Achievement Scholarship
2017–2021: Dell Scholarship
2017: Making Hope Happen Scholarship
2017: Asian & Pacific Islander American Scholarship
2017: RIMS AVID Scholarship
2017: Loma Linda University School of Medicine Health Disparities Research Symposium, Loma Linda, CA, Poster Presentation
2016: Loma Linda University School of Medicine Health Disparities Research Symposium, Loma Linda, CA, Poster Presentation

C. Contributions to Science

High School Research: I spent two summers in the laboratory of Dr. Kerby Oberg at Loma Linda University. During my junior year of high school, I worked on Podoconiosis, the condition resulting in lymphedema of the feet and legs. It was previously suggested that mineral particles in soil entered the lymphatic system, causing immune-related obstructions that resulted in Podoconiosis. However, the specific mineral properties in the soil, as well as why certain geographic locations had higher numbers of individuals with Podoconiosis remained unknown. Thus, I measured the cellular response of macrophages to Podoconiosis-associated soils using flow cytometry. My preliminary data showed that Podoconiosis-associated soils were indeed toxic, similarly to a known macrophage toxin. Working with Dr. Oberg for a second time as a senior in high school, I was tasked with studying LMX1B, a transcription factor that is expressed in low levels in humans with Nail-Patella Syndrome (NPS), a genetic disorder that results in small and poorly developed nails and kneecaps. The mechanism of LMX1B in NPS was not characterized. Thus, I isolated 2 LMX1B-bound cis-regulatory modules (LARM) and performed site-directed mutagenesis to match the LARM regions of NPS patients. I measured the activity of mutated LARM using a chick limb bioassay and fluorescence microscopy. I found that these mutations that NPS patients had diminished the activity of these LARMs, which resulted in lower levels of LMX1B and caused NPS. My work resulted in a poster presentation at LLU and a co-authorship in a publication that was published in Nature Communications.

A) Haro E, Petit F, Pira C, Spady C, Yorozuya L, Gray A, Escande F, Jourdain AS, **Nguyen AT**, Fellman F, Good JM, Francannet C, Manouvrier S, Ros M, Oberg K. The Identification of Limb-Specific Lmx1b Auto-Regulatory Modules with Nail-Patella Syndrome Pathogenicity. *Nature Communications*. 2021

Undergraduate Research: A significant portion of my undergraduate studies was spent working in Dr. Margot Quinlan's lab at the University of California, Los Angeles. My work centered around Spire, a protein that builds actin filaments and establishes cell polarity in Drosophila melanogaster, and Myosin-V, a motor protein that travels along actin filaments like those built by Spire. Previous research proposed that Spire and Myosin-V bind to one another, however, the significance of this interaction is unknown. I validated the binding between Spire and Myosin-V using a pulldown assay. Notably, mutating either Spire or Myosin-V at the predicted interface greatly reduced the building affinity of the two proteins. Furthermore, I measured actin assembly kinetics using a pyrene assay with Spire and different concentrations of Myosin-V. My results revealed that increasing concentration of Myosin-V had no effect on Spire's actin assembly, suggesting that Myosin-V does not affect the activity of Spire. During the summer of 2020, I worked in the lab of Dr. Grace Lee at the University of California, Irvine. Previous work in the Lee lab observed that heterochromatic DNA recombination and repair proteins evolved in concert with heterochromatin content size in Drosophila melanogaster. However, mammalian species have large differences in levels of heterochromatin, and it remains unknown whether the dependence seen in Drosophila melanogaster holds true in primates. I identified 23 genes involved in heterochromatin repair based on the literature, obtained the reference sequences for 16 primate species using the National Center for Biotechnology Information (NCBI) database, and analyzed primate homologs of these genes using the Phylogenetic Analysis by Maximum Likelihood (PAML) program. My results indicated that there was no prevalent adaptive evolution of mammalian heterochromatic DNA recombination and repair genes for primates, suggesting these genes did not evolve in concert with the size of heterochromatic DNA. During the summer of 2021, I worked with Dr. Allison Williams at the University of California, San Francisco. Peptidoglycan is a major constituent of the bacterial exoskeleton, which provides the cell with structural rigidity and more. Only a small handful of protein peptidoglycan-binding domains have been identified. As such, discovering such novel protein domains and determining how they maintain their peptidoglycan substrate would provide a new tool for combating antibiotic resistance. I trained deep neural networks to classify the mode of recognition of peptidoglycan macromolecules, and I worked to develop a deep learning algorithm to identify peptidoglycan-binding domains and predict where these proteins bind on peptidoglycan.

Graduate Research: My ongoing predoctoral research is focused on determining the molecular basis of HIRA/Hir function in health aging through its canonical histone chaperone function in H3.3/H3 deposition during nucleosome gap-filling. A key rationale for leveraging yeast lies is the high degree of evolutionary conservation between the human HIRA and yeast Hir complexes. Yeast offers significant experimental advantages, including rapid growth and facile genetic manipulation. These features would allow me to design precise functional assays that are sensitive to single-residue mutations in key functional domains of the Hir complex. Proper HIRA/Hir function is essential for proper transcriptional regulation and for preserving chromatin homeostasis, which is thought to sustain epigenomic plasticity in non-dividing cells. Beyond this role, HIRA also influences cellular senescence and the establishment of the senescent-associated secretory phenotype, which is implicated in age-related diseases such as cancer.

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	SAN BERNARDINO VALLEY COLLEGE	
2014	Strategies for College Success	A
2014	Beginning Walking for Fitness	A
2014	General Psychology	A
2014	College Spanish I	A
2015	Interpersonal Communication	A
2015	United States History: 1865 to Present	A
2015	Intermediate Algebra	A

YEAR	COURSE TITLE	GRADE
2015	American Politics	A
2015	Biological Anthropology	A
2015	Introductory Chemistry	A
2015	Beginning Physical Fitness	A
2015	College Algebra	A
2016	College Composition	A
2016	General Chemistry I	A
2016	Intermediate Composition and Critical Thinking	B
2016	Plane Trigonometry	A
2016	Introduction to the Theatre	A
2017	Introduction to Philosophy	A
2017	Child Growth & Development	A
	UNIVERSITY OF CALIFORNIA, LOS ANGELES	
2017	History of Neoliberalism	A
2017	Differential & Integral Calculus	C+
2017	Issues in Human Physiology: Diet & Exercise	A-
2018	Chemical Energetics & Change	C
2018	Integration & Infinite Series	B
2018	Introduction to Statistical Reasoning	B+
2018	Organic Chemistry I: Structure & Reactivity	B-
2018	Introduction to Asian Civilizations: History of Japan	A
2018	Calculus of Several Variables	B-
2018	General Chemistry Laboratory II	A-
2018	Organic Chemistry II: Reactivity, Synthesis, and Spectroscopy	A-
2018	Cell & Molecular Biology	A
2019	Organic Chemistry Laboratory I	A-
2019	Genetics, Evolution, and Ecology	A-
2019	Mechanics and Energy	A-
2019	Introduction to Structure, Enzyme, and Metabolism	A-
2019	Organic Chemistry III: Reactivity, Synthesis, and Spectroscopy	B+
2019	Organic Chemistry III: Reactivity, Synthesis, and Spectroscopy Honors Content	A
2019	Physiology and Human Biology	B+
2019	Biochemical Methods I	B
2019	Journal Club Seminar	A
2019	Lab & Scientific Method	A
2019	Thermodynamics, Fluids, Wave, Light, & Optics	B+
2020	Metabolism & Regulation	B
2020	Journal Club Seminar	A
2020	Research Apprenticeship	Pass
2020	Genetics	A-
2020	Biochemistry: DNA, RNA, & Protein Synthesis	A+
2020	Journal Club Seminar	A
2020	Electricity, Magnetism, & Modern Physics	A+
2020	Contemporary Health Issues	A
2020	Physical Chemistry: Chemical Thermodynamics	A-
2020	Journal Club Seminar	A
2020	Research Integrity	A
2020	Research Group Seminar	A

YEAR	COURSE TITLE	GRADE
2021	Careers in Chemistry & Biochemistry	Pass
2021	Physical Biochemistry	A
2021	Journal Club Seminar	A
2021	Principles of Epidemiology	B+
2021	Biochemical Methods II	A-
2021	Journal Club Seminar	A
2021	Research Apprenticeship	Pass
2021	Career Exploration in the Life Sciences	Pass
	UNIVERSITY OF PENNSYLVANIA	
2021	Cell Biology	A
2021	Macromolecular Biophysics: Principles & Method	A
2021	Macromolecular Crystallography: Methods & Application	A-
2021	Lab Rotation	A-
2022	Structural & Mechanistic Biochemistry	A
2022	Data Analysis & Scientific Inference	A+
2022	Cryo-EM	A+
2022	Lab Rotation	A
2022	Pre-Dissertation Research	A
2022	Vaccines & Immunology	A-
2022	Introduction to Bioinformatics	A
2023	Candidacy Exam Preparation	A
2023	Pre-Dissertation Research	A

BIOGRAPHICAL SKETCH

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NAME: Kenji Murakami

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

POSITION TITLE: Associate Professor of Biochemistry and Biophysics, the University of Pennsylvania

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
The University of Tokyo, Japan	B.Sc.	03/1999	Physics
The University of Tokyo, Japan	Ph.D.	03/2004	Biophysics
Teikyo University, postdoctoral fellow, Japan		01/2008	Biophysics
Stanford University, postdoctoral fellow, Stanford, CA		12/2014	Biophysics

A. Personal Statement

Research: I have focused my entire career on structural biology (cryo-electron microscopy), and protein biochemistry. I studied macromolecular complexes in cytoskeleton during my PhD (Murakami et al., Cell, 2010; Murakami et al., PNAS, 2008), and eukaryote transcription initiation during my postdoc study with Dr. Roger Kornberg (Murakami et al., Science, 2013; Murakami et al., PNAS, 2015; Murakami et al., Mol. Cell, 2015). Since 2015, our laboratory group has been working for research focused on transcription, chromatin, and DNA repair using S. cerevisiae as a model system (Damodaren et al., PNAS, 2017; Fujiwara et al., PNAS, 2019; van Eeuwen et al., Sci Adv, 2021, van Eeuwen et al., Nat Commun, 2021; Yang et al., Mol Cell, 2022; Gorbea et al., Mol Cell, 2023). My research group has broad expertise including cryo-electron microscopy, cross-linking mass spectrometry, and computational (integrative model building) techniques, as well as biochemical, genetic, and genomic experiments. In addition, we employ these techniques to other macromolecular complexes of a bacterial respiratory supercomplex (Steimle et al., Nat Commun, 2021), a centromere nucleosome (Allu et al., Curr Biol, 2019), an NPF-bound human Arp2/3 complex (Zimmet et al., Sci Adv, 2020), a complex for Terpene biosynthesis (Faylo et al., Nat Commun, 2021; Faylo et al., Biochemistry 2022), and Epstein-Barr virus (EBV) and latent infection (Mei et al., Journal of Virol., 2022) through collaborations. Most recently, we have been studying the connection between transcription and histone deposition by the Hir histone chaperone complex (Kim et al., Mol Cell, 2024). I have not published or created research products under another name.

Teaching and Mentoring: I am involved in a significant amount of teaching and mentoring. Since 2015, I have been involved in teaching electron microscopy in BMB508 and eukaryote transcription in BMB509. Since 2018, I co-direct a cryo-EM practical course BMB634, and develop a curriculum to train graduate students, undergraduate students, and postdocs to foster a cryo-EM community on campus. Since 2015, in my own lab, (1) I trained seven graduate students, and five undergraduate students; (2) I mentored four research specialists; (3) I trained six postdocs for our own or collaborative projects. Currently, there are four graduate students, and two postdocs in my lab. During 2015–present, I supervised students and postdocs leading to publications (19 research articles and 2 reviews) and grant writing for external funding agency (NSF and F31 fellowships).

Ongoing Research Support

NIH R01 GM123233 09/01/17-03/31/27
Title: The Mechanism of Transition from Transcription Initiation to Elongation
Role: PI
Major Goals: This R01 grant aims to determine the mechanism of transcription initiation to elongation using biochemical and structural approaches.

NSF MCB-2131806 02/01/22-01/31/27
Title: Investigations into the dynamic DNA recognition and processing during eukaryotic nucleotide excision repair
Role: Co-PI
Major Goals: This proposal aims to obtain comprehensive structural understanding of DNA unwinding process for nucleotide excision repair by combining a set of complementary structural and biophysical tools including fluorescence lifetime-based conformational analysis.

BSF 2023286 10/01/24-09/31/26
Title: Integrated structural determination of the transcription-coupled histone deposition complex
Role: Co-PI
Major Goals: The proposal aims to address how the histone chaperone Hir complex is recruited to the sites of active transcription by a combination of in vivo cross-linking mass spectrometry, yeast genetics, and biochemistry.

The American Cancer Society RSG-22-116-01-DMC 01/01/23-12/31/26
Title: Molecular dissection of nucleotide excision repair
Role: PI
Major Goals: This proposal aims to develop a new paradigm for anticancer therapy and development of drugs that selectively target nucleotide excision repair (NER) pathway. We will identify synthetic interactions between XPB drug and cancer mutations in other NER genes.

NIH R15GM147899 07/01/22-6/30/25
Title: Mechanisms of DNA damage processing the initiation of Nucleotide Excision Repair
Role: Co-PI
Major Goals: This proposal aims to understand synthetic lethal interactions on the nucleotide excision repair pathway using yeast genetics and human cell lines.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2024-present	Epigenetics Institute, University of Pennsylvania, Philadelphia, PA
2024-present	Associate Professor of Biochemistry and Biophysics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA
2023-present	Institute of Structural Biology, University of Pennsylvania, Philadelphia, PA
2020-present	Penn Center for Genome Integrity, University of Pennsylvania, Philadelphia, PA
2015-2023	Assistant Professor of Biochemistry and Biophysics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA
2008-2014	Postdoctoral fellow, Stanford University, CA (advisor: Dr. Roger Kornberg)
2004-2007	Postdoctoral fellow, Teikyo University, Japan (advisor: Dr. Takeyuki Wakabayashi)
1999-2003	Graduate Research, The University of Tokyo, Japan (advisor: Dr. Takeyuki Wakabayashi)
1995-1999	Undergraduate Research, The University of Tokyo, Japan (advisor: Dr. Takeyuki Wakabayashi)

Other Experience and Professional Memberships

2024-	Ad hoc member, NIH/NHLBI Program Project Review Study Section
2023-	Ad hoc member, The Macromolecular Structure and Function C (MSFC) Study Section
2020-	Ad hoc member, American Heart Association, Study Section

Honors

2015	McCABE Fellow Award, USA
2010	Kazato (JEOL) Research Award, Japan
2009	Kanae Foundation Postdoctoral Fellowship, Japan
2009	JSPS Postdoctoral Fellowship, Japan
2008	Uehara Memorial Foundation Postdoctoral Fellowship, Japan

C. Contributions to Science

1. Development of *in vitro* reconstituted transcription initiation from *S. cerevisiae*. During my postdoctoral work, I worked on the molecular mechanism of transcription initiation in the laboratory of Roger Kornberg at Stanford University. At the time, the pre-initiation complex (PIC), comprising RNA polymerase II (Pol II) and a full set of general transcription factors (GTFs), had only been previously obtained with nuclear extract or with partially purified GTFs assembled on immobilized promoter DNA. I developed a highly efficient in vitro reconstituted system of a 31-protein PIC from highly purified yeast PIC components, that enabled definitive biochemical, biophysical, and structural studies of the entire PIC machinery (Murakami et al., JBC, 2013). Based on this development, we have succeeded in tracking a pol II molecule during transcription initiation using optical tweezers, and thereby directly observing all of the major steps in initiation including promoter opening, start site scanning, and promoter escape at single base-pair resolution (a collaboration with the Steven Block lab at Stanford University, Fazal et al., Nature, 2015). Also our biochemical development enabled structural studies of the entire transcription initiation machinery. In the first of such studies, we have determined the structure of the PIC in the closed state by cryo-electron microscopy(cryo-EM) at 15 Å resolution (Murakami et al., Science, 2013) and sub-nanometer resolution (~6 Å) using an independent analysis pipeline coupled to a more advanced direct electron detector (Murakami et al., 2015, PNAS, 2015).

a. Fazal, F.*, Meng, C.*, **Murakami, K.***, Kornberg, R.D., Block, S.M. Real-Time Observation of the Initiation of RNA Polymerase II Transcription. Nature, 525: 274-277 (2015). (* equally contributed)

b. **Murakami, K.**, Calero, G., Brown, C.R., Liu, X., Davis, R.E., Boeger, H., and Kornberg, R.D. Formation and Fate of a Complete, 31-Protein, RNA polymerase II Transcription Initiation Complex. J. Biol. Chem. 288, 6325-6332 (2013).

c. **Murakami, K.*,** Tsai, K-L.*, Kalisman, N., Bushnell, D.A., Asturias, F.J., Kornberg, R.D. *Structure of an RNA polymerase II preinitiation complex*. Proc Natl Acad Sci U S A, 112, 13543–13548 (2015). (* equally contributed)

d. **Murakami, K.***, Elmlund, H.*, Kalisman, N.*, Bushnell, D.A., Adams, C.M. Azubel, M., Elmlund, D., Levi-Kalisman, Y., Liu, X., Levitt, M., and Kornberg, R.D. Architecture of an RNA Polymerase II Transcription Pre-Initiation Complex. Science, 1238724 (2013). (* equally contributed)

2. Visualizing transition from transcription initiation to elongation *de novo*. At the University of Pennsylvania, we have been pursuing *in vitro* reconstitution of the complete process of initiation from the PIC to elongation using highly-purified yeast proteins (Fujiwara and Murakami, Methods, 2019). This biochemical development allowed for visualizing key steps of the initiation-to-elongation transition *de novo* by cryo-EM single particle analysis for the first time (Yang et al., Mol. Cell, 2022). Another remarkable finding from this work is that the initially-transcribing PIC (ITC) persists longer than previously thought, at least in the yeast system (Fujiwara et

al., PNAS, 2019). The long-persisting nature of the ITC is consistent with our earlier single-molecule optical trapping studies (see section 1) (Fazal et al., Nature, 2015). Addition of elongation factors (i.e., the capping enzyme and/or Spt4/5) increased the frequency of promoter escape and the assembly of a follow-on pre-initiation complex (PIC) for re-initiation (Fujiwara et al., PNAS, 2019). Currently, we are extending this in vitro transcription system to study the connection between transcription and chromatin, specifically focusing on the +1 nucleosome. As the first study of this kind, we have recently determined the structure of the Hir/HIRA complex, the conserved histone chaperone responsible for the assembly of the +1 nucleosome in all eukaryotes (Kim et al., Mol Cell, 2024).

a. Fujiwara, R., Damodaren, N., Wilusz E.J., and **Murakami, K**. The capping enzyme facilitates promoter escape and assembly of a follow-on pre-initiation complex for re-initiation. Proc Natl Acad Sci U S A, 116, 22573-22582 (2019).

b. Yang, C., Fujiwara, R., Kim, H.J., Basnet, P., Zhu, Y., Gorbea, J., Steimle, S., Garcia, B.A., Kaplan, C.D., and **Murakami, K.** Structural visualization of de novo transcription initiation by Saccharomyces cerevisiae RNA polymerase II. Molecular Cell 82, 660-676 (2022).

c. Yang.C., Basnet, P., Sharmin, S., Shen, H., Kaplan, C.D., **Murakami, K**. Transcription start site scanning requires the fungi-specific hydrophobic loop of Tfb3. Nucleic Acids Research: gkae805, (2024).

d. Kim, H.J., M.R., van Eeuwen, T., M. Ricketts, D., Basnet, P., Zhang, A.L., Vogt, A., Sharmin, S., Kaplan, C.D., Garcia, B.A., Marmorstein, R.†, **Murakami, K.**† Structure of the Hir histone chaperone complex. Molecular Cell, 2024. 84, 601-2617. (2024).

3. Mechanism and structure of Mediator. Mediator, an assembly of more than 20 proteins, is required for the regulation of RNA polymerase II (pol II) transcription, relaying signals from gene-specific activators to the PIC. Through a collaboration with Asturias lab, we determined the first entire structure of Mediator (Tsai et al., *Nature*, 2017). Also I have significant contributions to structure determination of the Mediator kinase module (Li et al., Science Advances, 2021) and in a form complexed with the core Mediator (Chao et al., Mol. Cell., 2024). Through these studies, we have revealed the MED13 IDR obstructs the recruitment of RNA Pol II/MED26 onto the core MED by direct occlusion of their respective binding sites. Also at a UAS (enhancer in humans), we revealed the Mediator-PIC form a dimer through the Mediator tail module, induced by a homodimeric activator protein localized near the dimerization interface, consistent with the emerging picture of PIC assembly *in vivo* (Gorbea et al., Mol. Cell. 2023).

a. Tsai, K-L., Yu, X., Gopalan, S., **Murakami, K.**, Conaway, R.C., Conaway, J.W., and Asturias, F.J. Atomic models of Mediator and holoenzyme: implications for the Mediator transcription regulation mechanism. Nature, 544(7649):196-201 (2017).

b. Gorbea, J. Palao III L., Chen, S.F. , Kim, H.J., Snyder, L., Chang, Y.W. †, Tsai, K-L. †, and **Murakami, K**†. Structural basis of a transcription pre-initiation complex on a divergent promoter. Molecular Cell 83, 574-588 (2023).

c. Li, C., Chao, T.C., Kim, H.J., Cholko, T., Chen, S.F., Li, G., Snyder, L., Nakanishi, K., Chang, C., **Murakami, K.**, Garcia, B.A., Boyer, T.G. and Tsai, K.L. Structure and noncanonical Cdk8 activation mechanism within an Argonaute-containing Mediator kinase module. Science Advances 7 (3): eabd4484, 2021.

d. Chao, T.C., Chen, S.F., Kim, H.J., Tang, H.C., Tseng, H.C., Xu, A., Palao III L., Khadka, S., Li, T., Huang, M.F., Lee, D.F., **Murakami, K.**†, Boyer T.G.†, and Tsai K.L.† Structural basis of the human transcriptional Mediator regulated by its dissociable kinase module. Molecular Cell 84(20): 3932-3949 (2024).

4. The mechanisms of TFIIH for transcription and DNA repair. We have been studying the dual functions of the general transcription factor TFIIH in RNA polymerase II transcription initiation and nucleotide excision repair

(NER). We have determined cryo-EM structures of the kinase module of TFIIH (TFIIK) (van Eeuwen et al., Science Advances, 2021), and the core TFIIH in a form of NER initiation (van Eeuwen et al., Nature Communications, 2021). We are also pursuing the mechanisms that couple pol II transcription and DNA repair (Damodaren et al., 2017).

a. Damodaren, N., Van Eeuwen, T., Zamel, J., Lin-Shiao, E., Kalisman, N., and **Murakami, K**. Def1 interacts with TFIIH and modulates RNA polymerase II transcription. Proc Natl Acad Sci U S A, 114, 13230–13235 (2017).

b. van Eeuwen, T., Shim, Y., Kim, H.J., Zhao, T., Basu, S., Kaplan, C.D., Garcia, B.A., Min, J.H. †, and **Murakami, K†.** Structure of TFIIH/Rad4-Rad23-Rad33 in damaged DNA opening in Nucleotide Excision Repair. Nature Communications (2021).

c. van Eeuwen, T., Li, T. , Kim, H-J , Gorbea, C. J., Parker, M.I., Dunbrack, R.L., Garcia, B.A., Tsai, K-L. †, and **Murakami K†.** Structure of TFIIK for phosphorylation of CTD of RNA polymerase II. Science Advances, 7 (15), eabd4420 (2021).

5. Development of structural analysis pipeline. At the University of Pennsylvania, my laboratory has been developing structural analysis pipeline, that mainly involves cryo-EM single-particle analysis, a wide variety of cross-linking mass spectrometry (XL-MS) techniques, and computational modeling using integrative model building. We have pursued a human centromere nucleosome with a centromere-specific natural sequence (a-satellite DNA) with the Black lab (Allu et al., 2019), NPF-Bound Human Arp2/3 Complex in actin cytoskeleton with the Dominguez lab (Zimmet et al., 2020), the respiratory supercomplex III-IV with the Daldal lab (Steimle et al., 2021), the Terpene Biosynthesis with the Christianson lab (Faylo et al., 2021), and Epstein-Barr virus (EBV) latent infection with the Lieberman lab (Mei et al., 2022).

a. Allu, P.K., Dawicki-McKenna, J., Van Eeuwen, T., Slavin, M., Braitbard, M., Xu, C., Kalisman, N., **Murakami, K**., and Black, B. E. Structures of interphase and mitotic forms of the human core centromeric nucleosome complex. Current Biology, 29 (16), 2625-2639 (2019).

b. Zimmet, A., Van Eeuwen, T., Boczkowska, M., **Murakami, K.**, and Dominguez, R. Cryo-EM Structure of NPF-Bound Human Arp2/3 Complex and Activation Mechanism. Science Advances, 6(23), eaaz7651 (2020).

c. Steimle, S., van Eeuwen, T., Ozturk, Y., Kim, H.J., Braitbard, M., Garcia, B.A., Schneidman-Duhovny, D., **Murakami, K.**†, and Daldal, F.† Cryo-EM Structure of Respiratory bc1-cbb3 type CIII2CIV Supercomplex and Electronic Communication Between its Complexes. Nature Communications, 12: 929 (2021). († co-corresponding)

d. Faylo, J, van Eeuwen, T., Kim, H.J., Gorbea, J., Garcia, B., **Murakami, K.,** and Christianson, D. Structural Insight on Assembly-Line Catalysis in Terpene Biosynthesis. Nature Communications, 12: 3487 (2021).

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/kenji.murakami.1/bibliography/public/