BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Kang, Jin Young

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

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Korea Advanced Institute of Science and Technology (KAIST)	BS	03/2000	02/2005	Chemistry
Korea Advanced Institute of Science and Technology (KAIST)	PHD	03/2005	08/2011	Biochemistry

A. Personal statement

During my Ph.D. study, I determined crystal structures of Toll-like receptor (TLR) 2 complexes bound to various ligands and elucidated how TLR2 recognizes different ligands and forms a heterodimer complex with different partner TLRs. As a postdoc in the Darst lab at Rockefeller University, I changed my gear to cryo-EM and studied high-resolution structures of *E.coli* RNA polymerase elongation complexes (ECs). Mainly my theme was on transcriptional pausing and its inhibition (anti-pausing) mechanisms. As an independent PI, I will continue investigating the molecular mechanism of transcription by using cryo-EM and understanding the fundamental dynamics of an RNA polymerase interacting with various surrounding factors such as DNA, RNA, and diverse transcription factors.

B. Positions and Honors

Positions and employment

2011-2012 Postdoctoral associate (PI: Jie-Oh Lee), Dept. of Chemistry, KAIST, South Korea
2012-2014 Postdoctoral associate (PI: Roderick MacKinnon), The Rockefeller University, NY, USA
2014-2017 Postdoctoral associate (PI: Seth Darst), The Rockefeller University, NY, USA
2017-2019 Research associate (PI: Seth Darst), The Rockefeller University, NY, USA
2019-current Assistant professor, KAIST, South Korea

Honors

2019 POSCO Science Fellowship
2018 NYKB (New York Korean Biologists) Award (NYBK 10th Annual Conference)
2017 Travel award (FASEB conference on 'Mechanism and Regulation of Prokaryotic Transcription')
2012 Best Publication Award (Department of Chemistry, KAIST)
2010 New Star Women Scientist Award (Women's Bioscience Forum, South Korea)

C. Contributions to Science

1. Activation mechanism of Toll-like receptor 2 (TLR2)

My Ph.D. thesis focused on the structural determination of TLR2 complexes. TLRs are the representative innate immune receptors recognizing various molecules from pathogens such as bacterial membrane components and viral genomic double-stranded RNA. Despite their biological importance, structural studies of TLRs were elusive due to their low expression level and poor crystallizabitiy. The research group, including myself, devised a 'hybrid LRR technique' and engineered the TLR proteins for the structural study by X-ray protein crystallography. As a result, the first activated TLR complex structure, the TLR2-TLR1-triacyl lipopeptide complex, was determined and revealed TLR2 and the partner TLR1 form 'M'-shape dimer in response to the ligand. Furthermore, my colleagues and I crystallized TLR2-TLR6-diacyl peptide, TLR2-lipoteichoic acid, and TLR2-PE-DTPA and showed that TLR1 and TLR6 aid TLR2 in recognizing different ligands (triacyl and diacyl peptide), and the core diacyl-cysteine chemical group of the ligands play critical roles in dimer assembly, which can initiate an immune response from the cell. The hybrid LRR technique was sooner utilized to solve other TLR structures in my and other laboratories, and the results confirmed our model that activated TLRs form 'M'-shape dimers to initiate signaling. The structures of TLR family proteins and their working mechanism are summarized in the review paper my Ph.D. supervisor, and I wrote.

a. HM Kim, SC Oh, KJ Lim, J Kasamatsu, **JY Heo**, BS Park, H Lee, OJ Yoo, M Kasahara, and J,-O Lee*. Structural Diversity of the Hagfish Variable Lymphocyte Receptors. J. Biol. Chem. 282(9), 6726-6732, (2007).
b. MS Jin, SE Kim†, **JY Heo**†, ME Lee, HM Kim, S,-G Paik, H Lee, and J,-O Lee*. Crystal Structure of TLR1-TLR2 Heterodimer Induced by Binding of a Tri-Acylated Lipopeptide. Cell 130, 1071-1082 (2007). [F1000 Prime Recommended: F1000Prime.com/1091080]
c. **JY Kang** and J,-O Lee*. Structural Biology of the Toll-Like Receptor Family. Annu. Rev. Biochem. 80, 917-941 (2011).
d. **JY Kang**†, X Nan†, MS Jin, S,-J Youn, YH Ryu, S Mah, SH Han, H Lee, S,-G Paik*, and J,-O Lee*. Recognition of Lipopeptide Patterns by Toll-like Receptor 2-Toll-like Receptor 6 Heterodimer. Immunity 31, 873-884 (2009).

2. Mechanism of transcriptional pausing in prokaryotic RNA polymerase (RNAP).

I studied the fundamental mechanism of transcriptional pausing by prokaryotic RNAPs in Seth Darst laboratory at the Rockefeller University during my postdoc. Prokaryotic RNA polymerases are known to pause due to the DNA/RNA sequences (elemental pause), and the paused enzymes can stay longer at the position when additional factors are present such as RNA secondary structure formation or RNA polymerase backtracking (RNA hairpin pause or backtrack pause). First, I determined the cryo-electron microscopy (cryo-EM) structure of E.coli RNAP elongation complex (EC) in the presence and absence of HK022 Nun protein. This structure was the first reported cryo-EM structure of E.coli RNAP without crystal packing force and revealed the whole transcription bubble structure. Moreover, it showed that the bacteriophage HK022 Nun protein stalls the transcription of λ DNA by wedging its c-terminal peptide into the EC at pausing sites. Then, I determined the cryo-EM structure of RNA hairpin-stabilized paused RNAP elongation complex (EC) and showed the RNA hairpin located in the RNA exit channel of RNAP rearranges the domains of the enzyme globally and facilitates the transcriptional pausing. Furthermore, I determined the structures of the ECs bound with NusG and RfaH, which belong to the universally conserved transcription factor family. The structures showed that both NusG and RfaH bind to the clamp, protrusion, and lobe domains RNAP elongation complex, maintaining the efficient elongation complex conformation. This study suggested how NusG and RfaH have different reactivity toward RNA hairpin pause and backtrack pause from structural and biochemical studies. Besides, I determined the loading structure of Mfd onto the EC. Mfd recognizes a stalled EC on the damaged DNA, removes the RNAP from the lesion using ATP hydrolysis energy, and recruits DNA repair factors such as UvrABC. Using cryo-EM, I observed seven different conformations of the Mfd:EC complex and arranged them in the Mfd loading and ATP hydrolysis pathway. Besides the biological knowledge on transcription-coupled DNA repair, this work displayed how cryo-EM resolves the target protein complexes' heterogeneity and provides dynamic structural information.

a. **JY Kang**, PDB Olinares, J Chen, EA Campbell, A Mustaev, BT Chait, ME Gottesman, and SA Darst*. Structural basis of transcription arrest by coliphage HK022 Nun in an Escherichia coli RNA polymerase elongation complex. eLife 6, e25478 (2017). [F1000 Prime Recommended: F1000Prime.com/727423159]
b. **JY Kang**, TV Mishanina, MJ Bellecourt, RA Mooney, SA Darst* and R Landick*. RNA polymerase accommodates a pause RNA hairpin by global conformational rearrangements that prolong pausing. Mol. Cell 69, 802-815 (2018).
c. **JY Kang**, RA Mooney, Y Nediakov, J Saba, TV Mishanina, I Artsimovitch, R Landick and SA Darst*. Structural basis for transcript elongation control by NusG/RfaH universal regulators. Cell 173, 1650–1662 (2018).
d. **JY Kang***, TV Mishanina*, R Landick and SA Darst*. “Mechanisms of transcriptional pausing in bacteria.” Journal of Molecular Biology, 431:4007-4029 (2019).
e. **JY Kang**†, E Llewellyn†, J Chen, PDB Olinares, J Brewer, BT Chait, EA Campbell, and SA Darst*. “Structural basis for transcription complex disruption by the Mfd translocase.” eLife, 10:e62117 (2021).
e. PDB Olinares, **JY Kang**, E Llewellyn, C Chiu, J Chen, B Malone, RM Saecker, EA Campbell, SA Darst, and BT Chait*. “Native mass spectrometry-based screening for optimal sample preparation in Single-Particle Cryo-EM.” Structure, https://doi.org/10.1016/j.str.2020.11.001 (2021).

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing

Jin Young Kang (PI) 01/01/2020-12/31/2021
Posco Science Fellowship, TJ Park Foundation
Goal: To understand th molecular mechanism of the colibactin biosynthesis
Role: PI

Jin Young Kang (PI) 06/01/2019-06/18/2021
Basic Science Research Program, National Research Foundation of Korea (NRF)
2021R1C1C100656011
Goal: To understand the molecular mechanism of transcription regulation by secondary structure of nascent RNA
Role: PI

Completed Research Support

Darst (PI) 05/01/2016-04/30/2021
National Institute of General Medical Sciences
R35GM118130
Goal: To understand the mechanism of transcription and its regulation
Role: Investigator

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NAME: Minjae Kim

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

POSITION TITLE: Graduate Student

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Korea Advanced Institute of Science and Technology (KAIST)	BS	03/2019	Chemistry

A. Personal Statement

I am currently studying the dynamics of colibactin biosynthesis by determining atomic structures of enzymes in colibactin biosynthetic gene cluster. In this research, I aim to elucidate the structure of colibactin biosynthetic enzymes involved in colibactin's genotoxicity using cryo-electron microscopy (cryo-EM). In addition, I try to characterize the structure of the enzyme-substrate complex and confirm the interaction between the enzyme and the substrate at the atomic level. The determined structures would lay the foundations for solving the structure of flexible multi-domain mega-enzymes and will provide the structural basis for understanding the fundamental mechanism of colorectal cancer formation.

B. Positions, Scientific Appointments, and Honors

None

C. Contributions to Science

None

D. Additional Information: Research Support and/or Scholastic Performance Ongoing

Jin Young Kang (PI)
Posco Science Fellowship, TJ Park Foundation
01/01/2020-12/31/2021
Goal: To understand th molecular mechanism of the colibactin biosynthesis
Role: Investigator

BIOGRAPHICAL SKETCH

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NAME: Jinwoo Kim

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

POSITION TITLE: Graduate Student

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Korea Advanced Institute of Science and Technology (KAIST)	BS	03/2021	Chemistry

A. Personal Statement

I am currently interested in the biosynthetic pathway of natural products. In particular, I am interested in colibactin, which is a genotoxin produced by certain *E. Coli* strains found in the human gut. The colibactin is synthesized by a hybrid polyketide synthase–nonribosomal peptide synthetase (PKS–NRPS) assembly line. Although each protein was biochemically characterized, the molecular level insight into how the assembly line proteins are organized is elusive. In this study, I am going to reconstruct the biosynthetic pathway of the colibactin *in vitro*, so that the pre-colibactins can be attached to a specific protein of interest in the PKS, ClbC, and solve the cryo-EM structure(s) of the ligand bound complex. The structure will reveal how the ClbC's every domain orchestrates the biosynthesis to form the desired product.

B. Positions, Scientific Appointments, and Honors

None

C. Contributions to Science

None

D. Additional Information: Research Support and/or Scholastic Performance Ongoing

Jin Young Kang (PI)
Posco Science Fellowship, TJ Park Foundation
03/01/2021-12/31/2021
Goal: To understand th molecular mechanism of the colibactin biosynthesis
Role: Investigator