Program Booklet

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Table of Contents

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HKUST JOCKEY CLUB INSTITUTE FOR ADVANCED STUDY

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IAS Symposium on Biological Cryo-EM

June 19-21, 2023

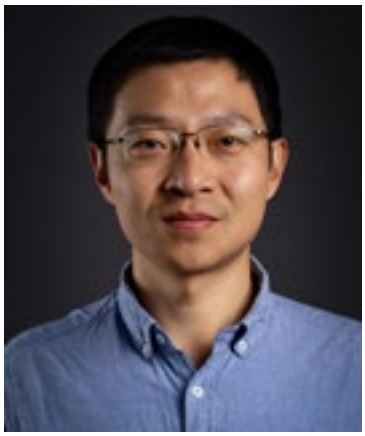
Venue: Kaisa Group Lecture Theater (IAS LT), Lo Ka Chung Building, Lee Shau Kee Campus, The Hong Kong University of Science & Technology, Clear Water Bay, Kowloon, Hong Kong, China

Format of Talk: 25-minute presentation *plus 5-minute Q&A session (*Talks indicated with "(On-site only)" means these talks will be held on-site only, not to be broadcast on Zoom.)*

		June 19, 2023 (Monday)			June 20, 2023 (Tuesday)			June 21, 2023 (Wednesday)	
		Title	Speaker		Title	Speaker		Title	Speaker
Morning	08:45 - 09:15	Registration			Session Chair: Yanxiang ZHAO (The Hong Kong Polytechnic University)			Session Chair: Wilson Chun Yu LAU (The Hong Kong Polytechnic University)	
	09:15 - 09:30	**Opening Remarks** Joy Lo CHEUNG (Chairlady, Lo Kwee Seong Foundation) Yung Hou WONG (Dean of Science, HKUST)		09:00 - 09:30	**D2-#1: Msp1/ATAD1: The Molecular Vacuum Cleaner**	Lan WANG (HKUST)	09:00 - 09:30	**D3-#1 (Online): Beyond Cas Nucleases: The Mechanism of an RNA-Guided Protease**	Ailong KE (Cornell University)
		Session Chair: Lan WANG (HKUST)		09:30 - 10:00	**D2-#2: Cryo Electron Tomography – A Powerful Tool Exploring Ultra-Structure in Biology**	Yingyi ZHANG (HKUST)	09:30 - 10:00	**D3-#2 (Online): Structural Basis of DNA Double-Strand Break Repair by NHEJ**	Yuan HE (Northwestern University)
	09:30 - 10:00	**D1-#1: Molecular Secrets of DNA Replication, Repair and Recombination**	Wei YANG (US National Institutes of Health)	10:00 - 10:30	**D2-#3: Towards the Visual Proteomics of C. *reinhardtii* Using High-throughput Collaborative *in situ* Cryo-ET**	Xianjun ZHANG (Thermo Fisher Scientific)	10:00 - 10:30	**D3-#3 (Online): Mechanisms of Programmable DNA Transposition**	Elizabeth KELLOGG (Cornell University)
	10:00 - 10:30	**D1-#2: Activation of an Origin of Replication Visualised by Cryo-EM**	Alessandro COSTA (The Francis Crick Institute)	10:30 - 11:00	Group Photo Taking & Coffee Break (Venue: Lobby, G/F)		10:30 - 10:50	Coffee Break (Venue: Lobby, G/F)	
	10:30 - 10:50	Coffee Break (Venue: Lobby, G/F)		11:00 - 11:30	**D2-#4 (On-site only): Single Particle cryo-EM of INO80**	Yifan CHENG (University of California, San Francisco (IAS Senior Visiting Fellow))	10:50 - 11:20	**D3-#4 (Online): Structural Insights into Transcription Preinitiation Complex Assembly on Chromatin**	Yanhui XU (Fudan University)
	10:50 - 11:20	**D1-#3: DNA Replication – From Yeast to Human**	Bik-Kwoon TYE (HKUST (IAS Senior Member) & Cornell University)	11:30 - 12:00	**D2-#5: Cryo-EM Driven Paradigm Shift of ABC Transporter Mechanism**	Maofu LIAO (Southern University of Science and Technology)	11:20 - 11:50	**D3-#5: CryoEM Reveals Unpredictable Structural Details of Protein and RNA**	Wah CHIU (Stanford University)
	11:20 - 11:50	**D1-#4: Cryo-EM Structure of the Human Pre-replication Complex**	Yuanliang ZHAI (The University of Hong Kong)	12:00 - 12:30	**D2-#6: Cryo-EM Illuminates the Mechanism of SGLT Inhibitors**	Lei CHEN (Peking University)	11:50 - 12:20	**D3-#6: Structural Insights into the Organization of Membrane Skeleton in Red Blood Cells**	Ning GAO (Peking University)
	11:50 - 12:20	**D1-#5: Molecular Basis of Transcription Elongation, Blockage, and Damage Repair**	Dong WANG (University of California, San Diego)	12:30 - 14:00	Lunch (By Invitation Only; Venue: UniQue, Conference Lodge)		12:30 - 14:00	Lunch (By Invitation Only; Venue: UniBistro)	
	12:30 - 14:00	Lunch (By Invitation Only; Venue: China Garden Restaurant)							
Afternoon		Session Chair: Maofu LIAO (Southern University of Science and Technology)						Session Chair: Tao NI (The University of Hong Kong)	
		Title	Speaker					Title	Speaker
	14:00 - 14:30	**D1-#6 (On-site only): *In Situ* Structures of Macromolecular Assemblies by CryoFIB and CryoET**	Peijun ZHANG (University of Oxford)	14:30 - 18:30	Boat Trip to GeoPark in Sai Kung (By Invitation Only) *Coach departs from Lo Ka Chung Building at 14:30		14:00 - 14:30	**D3-#7 (On-site only): Cryo-EM Analysis of the Nuclear Pore Complex from *Xenopus laevis***	Yigong SHI (Tsinghua University & Westlake University)
	14:30 - 15:00	**D1-#7 (On-site only): *In Situ* Assembly and Membrane Fusion of Enveloped Viruses**	Sai LI (Tsinghua University)				14:30 - 15:00	**D3-#8: FACT Caught in the Act of Manipulating the Nucleosome**	Keda ZHOU (The University of Hong Kong)
	15:00 - 15:30	**D1-#8 (On-site only): Mechanism of α-carboxysomes Formation**	Tao NI (The University of Hong Kong)				15:00 - 15:30	**D3-#9: Structural Basis of Substrate Recognition and Thermal Protection by a Small Heat Shock Protein**	Wilson Chun Yu LAU (The Hong Kong Polytechnic University)
	15:30 - 15:50	Coffee Break (Venue: Lobby, G/F)					15:30 - 15:50	Coffee Break (Venue: Lobby, G/F)	
	15:50 - 16:20	**D1-#9: Technology Development for *In Situ* Structural Study of Macromolecular Machinery**	Fei SUN (Institute of Biophysics, Chinese Academy of Sciences)				15:50 - 16:20	**D3-#10 (On-site only): The Nucleocytoplasmic Shuttling of Tonicity-Responsive Transcription Factors**	Yanxiang ZHAO (The Hong Kong Polytechnic University)
	16:20 - 16:50	**D1-#10: Single Molecule Localization by Interferometric & Cryogenic Imaging**	Wei JI (Institute of Biophysics, Chinese Academy of Sciences)				16:20 - 16:50	**D3-#11: Chemical Approaches to Decode Histone Epigenetics**	David Xiang LI (The University of Hong Kong)
	16:50 - 17:20	**D1-#11: Metallo-Supramolecular Branched Polymer Protects Particles from Air-water Interface in Single-Particle Cryo-Electron Microscopy**	Shangyu DANG (HKUST)				16:50 - 17:20	**D3-#12: Structural and Molecular Basis of Age-Related Memory Impairment**	Ruben HERVAS MILLAN (The University of Hong Kong)
	17:20 - 17:50	**D1-#12 (On-site only): Incorporating Electrospray Ionization in Cryo-EM Specimen Preparation**	Hongwei WANG (Tsinghua University)	18:30 - 20:30	Banquet (By Invitation Only; Venue: Sai Kung)		17:20 - 17:50	**D3-#13: The Cryo-EM Structure of a Viral Nucleosome**	Yang LIU (The University of Hong Kong)
	18:15 - 20:30	Banquet (By Invitation Only; Venue: Tseung Kwan O) *Coach departs from Lo Ka Chung Building at 18:15 and returns at 20:30					17:50 - 18:00	**Closing Remarks** Bik-Kwoon TYE (HKUST (IAS Senior Member) & Cornell University)	

Speakers

*(*Listed in alphabetical order of speakers' last name)*



Speaker: Lei CHEN

Affiliation: College of Future Technology, Peking University

Email: chenlei2016@pku.edu.cn

Title: Cryo-EM Illuminates the Mechanism of SGLT Inhibitors

Biosketch:

Prof. Lei Chen received his PhD from Tsinghua University. He was working on the mechanism of AMPK. After that, he moved to Oregon Health and Science University as a postdoctoral researcher in the lab of Eric Gouaux, studying the mechanism of AMPA receptors. He started his own lab at Peking University in 2016. His lab focuses on the molecular mechanism of proteins involved in human diseases, especially metabolic diseases and cardiovascular diseases.

Speaker: Yifan CHENG

Affiliation: Department of Biochemistry & Biophysics, Howard Hughes Medical Institute/ University of California, San Francisco

Email: yifan.cheng@ucsf.edu

Title: Single Particle Cryo-EM of INO80

Biosketch:

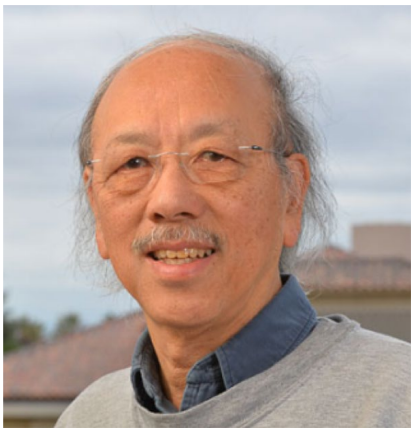
Prof. Yifan Cheng is currently an Investigator of Howard Hughes Medical Institute and a Professor at Department of Biochemistry and Biophysics, University of California San Francisco (UCSF). He received his Ph.D. degree in 1991 from Institute of Physics, Chinese Academy of Sciences (CAS). From 1991 to 1996, he continued his research in solid state physics and electron microscopy as a postdoctoral fellow at University of Oslo (NTNF Fellow) and Max-Planck-Institute of Metal Research (Alexander von Humboldt Fellow). In 1996, he changed his research direction to structural biology, and received further training in cryo-electron microscopy (cryo-EM) from Professors Kenneth Taylor at Florida State University and Yoshinori Fujiyoshi at Kyoto University. In 1999, he joined the laboratory of Thomas Walz to set up a cryo-EM operation at Harvard Medical School. He joined the faculty of University of California San Francisco in 2006 and has stayed there ever since. He has been an HHMI Investigator since 2015. He is the recipient of the Christian B. Anfinsen Award from The Protein Society (2018), elected members of the American Academy of Arts and Science (2019) and National Academy of Sciences (2020)

Prof. Cheng's laboratory uses cryo-EM to study structures of biological macromolecules, particularly integral membrane proteins and dynamic complexes. In addition, development of cryo-EM methodology for structural biology is also a long-lasting interest of his laboratory. Previous works of his laboratory include developments of algorithms to correct electron beam-induced image motion and determination of the first atomic structure of TRPV1 by single particle cryo-EM, etc.

Speaker: Wah CHIU

Affiliation: Department of Bioengineering, Stanford University

Email: wahc@stanford.edu



Title: CryoEM Reveals Unpredictable Structural Details of Protein and RNA

Biosketch:

Prof. Wah Chiu is a pioneer in methodology development for cryogenic electron microscopy (cryo-EM). His work has made multiple transformational contributions in developing single particle cryo-EM as a tool for the structural determination of molecular machines at atomic resolution in the last three decades. His lab has solved many cryo-EM structures including viruses, chaperonins, membrane proteins, ion channels, antigen-antibody complexes, protein-RNA complexes and RNA in collaboration with many scientists around the world. He continues to establish high standard testing and characterization protocols for cryo-EM instrumentation and to develop new image processing and modeling algorithms for cryo-EM structure determination. His current research focuses on developing cryogenic electron tomography (cryo-ET) to determine near atomic resolution structures of molecular complexes in situ.

Speaker: Alessandro COSTA

Affiliation: Macromolecular Machines Laboratory,
The Francis Crick Institute

Email: alessandro.costa@crick.ac.uk

Title: Activation of an Origin of Replication Visualised by Cryo-EM

Biosketch:

Dr. Alessandro Costa obtained a “Laurea” degree in Biotechnology at the University of Padua (2004) and a PhD in Structural Biology at Imperial College London (2008). He trained as a postdoc at the University of Oxford and later as an EMBO fellow at the University of California, Berkeley. In 2012, he established his own group at the London Research Institute Clare Hall Laboratories, CRUK. In 2015 he joined the Francis Crick Institute where he is now Principal Group Leader.

Dr. Costa’s research focuses on DNA replication. Recent work involved time-resolved cryo-EM imaging to establish the mechanism for loading the eukaryotic replicative helicase onto DNA. Current work focuses on replication origin activation. His lab also develops methods to optimize protein-DNA complex assembly for cryo-EM imaging.

Speaker: Shangyu DANG

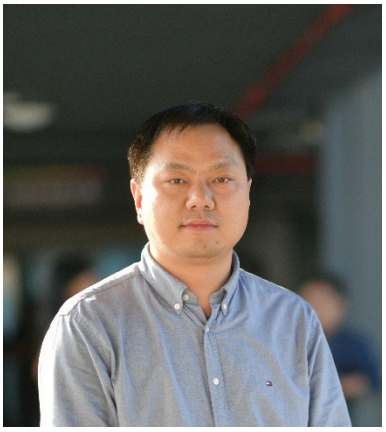
Affiliation: Division of Life Science,
The Hong Kong University of Science and Technology

Email: sdnag@ust.hk

Title: Metallo-Supramolecular Branched Polymer Protects Particles from Air-water Interface in Single-Particle Cryo-Electron Microscopy

Biosketch:

Prof. Shangyu Dang received his PhD from Tsinghua University in 2014. He then moved to University of California San Francisco for postdoc training. He now is an Assistant Professor in the Division of Life Science at the Hong Kong University of Science and Technology. The research in his lab focuses on molecular mechanisms of the biological macromolecules, with particular interests in membrane proteins and protein-DNA complex through multiple approaches including single-particle cryo-EM, cryo-ET, biochemistry, and electrophysiology. In addition, his lab is also interested in new method development in single particle cryo-EM and cryo-ET field to solve the recurring problems and expand their application.



Speaker: Ning GAO

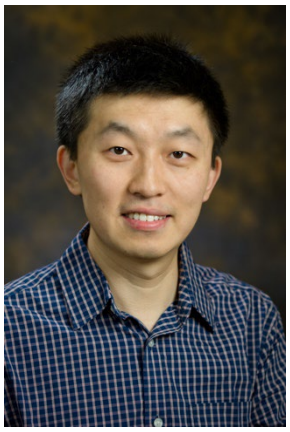
Affiliation: School of Life Sciences, Peking University

Email: gaon@pku.edu.cn

Title: Structural Insights into the Organization of Membrane Skeleton in Red Blood Cells

Biosketch:

Prof. Ning Gao received his PhD in Biomedical Sciences from the State University of New York at Albany in 2006. He worked in Tsinghua University from 2008-2017, and is currently a Professor in the School of Life Sciences, Peking University. The general research interest of his laboratory is to understand mechanistic details of cellular molecular machines, using cryo-EM technique as a major tool. In the past a few years, his lab has primarily focused on ribosome biogenesis in prokaryotic and eukaryotic cells, towards answers to a few fundamental questions, such as “how the ribosome is made in the cell”, and “how ribosome biogenesis, or the translation capacity of a cell is regulated by various well-characterized growth-control pathways”. Meantime, part of his research effort is also devoted to structural studies of non-conventional biological assemblies.



Speaker: Yuan HE

Affiliation: Department of Molecular Biosciences, Northwestern University

Email: yuanhe@northwestern.edu

Title: Structural Basis of DNA Double-Strand Break Repair by NHEJ

Biosketch:

Prof. Yuan He obtained his Bachelor's degree from Beijing Technology and Business University in China and PhD from Northwestern University. After doing a post-doctoral research with Prof. Eva Nogales at the University of California, Berkeley, Prof. He returned to Northwestern University as an Assistant Professor to set up his independent lab. His lab is interested in studying the molecular mechanisms by which large, multi-subunit complexes engage in DNA-centric processes. Current research topics include two main area: (1) how eukaryotic gene transcription is regulated by different stages and (2) how various types of DNA damage are repaired and why deficiencies in these repair pathways lead to pathology of cancer predisposition or accelerated aging.

Speaker: Ruben HERVAS MILLAN

Affiliation: School of Biomedical Sciences,
The University of Hong Kong

Email: ruhm@hku.hk

Title: Structural and Molecular Basis of Age-Related Memory Impairment

Biosketch:

Prof. Ruben Hervas Millan joined the School of Biomedical Sciences as Assistant Professor in March 2021. He graduated from the Autonomous University of Madrid with a BS in Biochemistry in 2006. From 2007 to 2013, he trained in Biophysics at the Cajal Institute in Madrid as a graduate student. After his PhD, he undertook postdoctoral training at the Stowers Institute for Medical Research in the USA. Prof. Hervas' lab integrates structural, biochemical and biophysical approaches, in combination with animal models, to provide structural and mechanistic information about functional amyloid assemblies linked with memory persistence and animal development.

Speaker: Wei JI

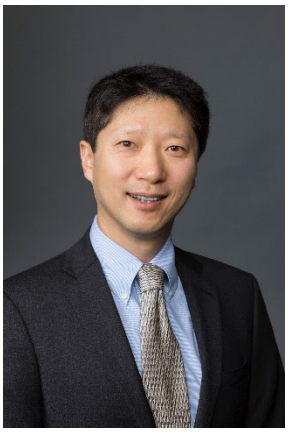
Affiliation: Institute of Biophysics,
Chinese Academy of Sciences

Email: jiwei@ibp.ac.cn

Title: Single Molecule Localization by Interferometric & Cryogenic Imaging

Biosketch:

Dr. Wei Ji received his PhD from Institute of Biophysics (IBP), Chinese Academy of Sciences. He joined IBP as an engineer and tried to improving the resolution of SMLM with a drive to do something original and risky. And he still focuses on this field as a Principal Investigator of IBP now, with the aim to invent more and better imaging tools for life sciences.



Speaker: Ailong KE

Affiliation: Department of Molecular Biology and Genetics, Cornell University

Email: ailong.ke@cornell.edu

Title: Beyond Cas Nucleases: The Mechanism of an RNA-Guided Protease

Biosketch:

Prof. Ailong Ke is Robert J. Appel Professor in Molecular Biology and Genetics at Cornell University. He received his BS degree from the University of Science and Technology of China in 1995, and PhD in Biophysics with Cynthia Wolberger from the Johns Hopkins University School of Medicine in 2002. After a three-year post-doctoral training with Nobel Laureate Prof. Jennifer Doudna at UC Berkeley, he started his independent career as an Assistant Professor at Cornell University in 2005 and rose to Full Professor in 2017. Since independence, Prof. Ke has been working in the areas of RNA 3'-end processing and degradation, metabolite-sensing riboswitches, and more recently, the CRISPR-Cas immunity system. He also strives to apply the mechanistic understanding to genome editing applications in eukaryotic cells. Prof. Ke holds key patents in CRISPR-Cas3 and related fields. He has published over 50 papers in journals such as Nature, Science, Cell, Molecular cell, NSMB, PNAS, and RNA. He received the Cornell Provost Research Innovation award in 2018 and RNA society Mid-Career award in 2019.

Speaker: Elizabeth KELLOGG

Affiliation: Department of Molecular Biology and Genetics, Cornell University

Email: ehk68@cornell.edu

Title: Mechanisms of Programmable DNA Transposition

Biosketch:

Since the Kellogg lab opened in 2019 at Cornell University, they have contributed significantly to mechanistic understanding of DNA transposons using cryo-EM. Their major goal is to understand how transposons reshape genomes and how transposons can be repurposed as genome-editing tools. The Kellogg lab's first significant scientific contribution includes the structural and mechanistic characterization of a unique cut and paste transposase, called the P-element transposase, published in NSMB (2019). The Kellogg lab also formulated the first model of transposition that could explain the behavior of programmable, CRISPR-associated transposons, which was recently published in Science (2021) and PNAS (2022). Using a combination of biochemistry, cryo-EM, and genetic assays, they described a highly regulated and complex biochemical process that would result in programmable DNA insertions while simultaneously avoiding previous insertion sites. In addition, the Kellogg lab is exploring avenues to re-engineer CAST (and related) elements using protein design.

Speaker: Wilson LAU

Affiliation: Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University

Email: wilson-cy.lau@polyu.edu.hk

Title: Structural Basis of Substrate Recognition and Thermal Protection by a Small Heat Shock Protein

Biosketch:

Prof. Wilson CY Lau is specialized in single-particle cryo-EM and has made significant contributions to the studies of membrane proteins and macromolecular assemblies. Prof. Lau has gained invaluable, multidisciplinary research experience in internationally recognized laboratories during his graduate study and postdoctoral research in Canada and the United States. He was trained as a structural biologist with Prof. John Rubinstein (University of Toronto) and later with Prof. Wah Chiu (Baylor College of Medicine). He was also awarded the prestigious AXA Research Fund Fellowship to support his postdoctoral work with Prof. Michael Huen (University of Hong Kong). Prof. Lau's research is internationally recognized and has been recognized by F1000Prime. He is the lead author on high profile publications in *Nature*, *PNAS* and *Nature Communications*. His recent work focuses on studies of molecular chaperones and metabolic enzymes with applications for structure-based discovery of novel antimicrobial drugs.

Speaker: Sai LI

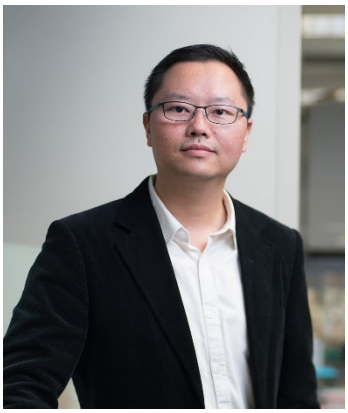
Affiliation: School of Life Sciences, Tsinghua University

Email: sai@tsinghua.edu.cn

Title: *In Situ* Assembly and Membrane Fusion of Enveloped Viruses

Biosketch:

Over the past decade, Prof. Sai Li has focused on developing cryo-ET technics and studying pathogenic enveloped viruses. His main work includes: 1) Developing high-resolution in situ structural analysis methods. He pushed the resolution of nuclear pore complex structures to 9Å and discovered a new luminal ring structure (*Cell Res.* 2020); resolved the S protein from the surface of SARS-CoV-2 to a resolution of 4.3Å (*PNAS*, 2023). He developed phase plate cryo-ET and resolved the glycoprotein structure from the surface of HRPV, discovering a novel membrane fusion protein with a molecular weight of 57kDa (*Nat. Commun.* 2019). 2) He systematically studied the in situ structures and mechanisms of assembly and infection of various enveloped viruses, including the molecular structure of intact SARS-CoV-2 (*Cell* 2020; ESI hot article), revealing the membrane fusion process of Rift Valley fever virus (*Nat. Commun.* 2018), and resolving the in situ structures of Lassa virus (*PLoS Pathog.* 2016) and Hantavirus (*Cell Rep.* 2016). In his recent reviews, he summarized how methodological advances over the past decade have hatched the cryo-ET resolution revolution (*Annu. Rev. Biophys.* 2023) and how this brought new insights into virology (*TiBS* 2022). He was also invited to give keynote talks at international conferences such as 3DEM Gordon Research Conference in 2021 and International Virus Assembly Symposium in 2022.



Speaker: Xiang David LI

Affiliation: Department of Chemistry,
The University of Hong Kong

Email: xiangli@hku.hk

Title: Chemical Approaches to Decode Histone Epigenetics

Biosketch:

Prof. Xiang David Li received his BS in Chemistry at Fudan University in 2003, and PhD in 2008 from the University of Hong Kong under the guidance of Professor Dan Yang. He has then spent three years as a postdoctoral fellow with Professor Tarun Kapoor at Rockefeller University. In 2011, he moved back to the University of Hong Kong to start his independent career as an Assistant Professor and was promoted to Associate Professor in 2017 and Professor in 2020. Research in the Li laboratory focuses on the development and application of chemical tools and methods to study histone modifications.

Speaker: Maofu LIAO

Affiliation: School of Life Sciences,
Southern University of Science and Technology

Email: liaomf@sustech.edu.cn

Title: Cryo-EM Driven Paradigm Shift of ABC Transporter Mechanism

Biosketch:

Prof. Maofu Liao received his PhD from Albert Einstein College of Medicine in 2006, and performed his postdoctoral research at University of California, San Francisco. In 2013, he determined the cryo-EM structure of TRPV1 which is the first near-atomic resolution membrane protein structure produced by non-crystallographic method. In 2014, he joined the faculty in the department of Cell Biology at Harvard Medical School. His research focuses on using cryo-EM and biochemical approaches to understand the structural dynamics and functional mechanisms of macromolecular machines. He has made a series of important contributions particularly in the field of membrane protein biology. In 2022, he moved back to China and joined the Southern University of Science and Technology as a Chair Professor in the School of Life Sciences.

Speaker: Yang LIU

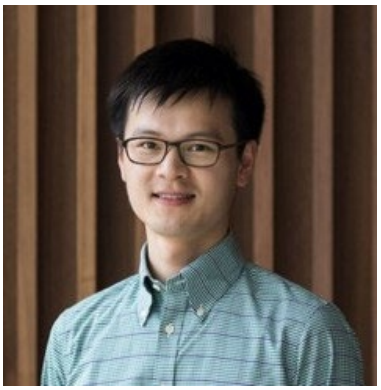
Affiliation: School of Biomedical Sciences,
The University of Hong Kong

Email: yangliu9@hku.hk

Title: The Cryo-EM Structure of a Viral Nucleosome

Biosketch:

Prof. Yang Liu is currently an Assistant Professor in the School of Biomedical Sciences at the University of Hong Kong. She received her BS and MS degrees from Sun-Yat Sen University and PhD from the University of Guelph. She then worked as an HHMI postdoc research fellow in chromatin biology at the University of Colorado at Boulder. Prof. Liu joined the University of Hong Kong as an Assistant Professor in 2022.



Speaker: Tao NI

Affiliation: School of Biomedical Sciences,
The University of Hong Kong

Email: taoni@hku.hk

Title: Mechanism of α-carboxysomes Formation

Biosketch:

Prof. Tao Ni is an Assistant Professor in the School of Biomedical Sciences at the University of Hong Kong from July 2022. Prior to joining to HKU, he was a postdoctoral research fellow at the University of Oxford and visitor scientist at eBIC working on HIV capsid and carboxysomes using cryoEM and cryoET subtomogram averaging. He completed his Bachelor in Biological Sciences degree in Nankai University (2008-2012) and PhD in structural biology in the University of Oxford (2012-2016). His group in HKU focuses on the interface between host and pathogens (virus and parasites) using cryo-electron microscopy and tomography methods.

Speaker: Yigong SHI

Affiliation: School of Life Sciences,
Westlake University

Email: syg@westlake.edu.cn

Title: Cryo-EM Analysis of the Nuclear Pore Complex from *Xenopus Laevis*

Biosketch:

Prof. Yigong Shi mainly uses biochemical and biophysical methods to study the molecular mechanism of cell apoptosis, the structure and function of important membrane proteins, and intracellular biological macromolecules. He analyzes the key complex structure of eukaryotic messenger RNA splicing bodies revealing molecule layer mechanisms. Prof. Shi is a recipient of the Searle Scholar Award, the Rita Allen Scholar Award, the Irving Sigal Young Investigator Award (2003), the Raymond and Beverly Sackler International Prize in Biophysics (2010), the Qiu Shi Outstanding Scientist Award of the Qiushi Foundation (2010), the CC Tan Life Science Achievement Award (2010), the Gregori Aminoff Prize of the Royal Swedish Academy of Sciences (2014), the Wu Jieping-Paul Janssen Medicine and Pharmaceutical Science Award (2014), the 'You Bring Charm to the World Award'(2015-2016), the Science and Technology Achievement Prize of the Ho-Leung-Ho-Lee Foundation (2016), the Future Science Prize of the Life Sciences Prize (2017), the achievement award of the VCANBIO Award for Biosciences and Medicine (2017), and Tan Kah Kee Science Awards (2020). He was also nominated as a Foreign Associate of the European Molecular Biology Organization in 2013.

Speaker: Fei SUN

Affiliation: Institute of Biophysics,
Chinese Academy of Sciences

Email: feisun@ibp.ac.cn

Title: Technology Development for *In Situ* Structural Study of Macromolecular Machinery

Biosketch:

Prof. Fei Sun received his PhD from Tsinghua University. He joined the Institute of Biophysics, Chinese Academy of Sciences in 2006 and is currently a Principal Investigator of the Laboratory of Biological Electron Microscopy and Structural Biology. He is also the Chief scientist and director of the Center for Biological Imaging, Core Facilities for Protein Sciences at the Chinese Academy of Sciences.

The research interests of his team are mainly related with the structures and functions of biological macromolecules including membrane proteins and supra macromolecular assemblies. The aim of his group is to utilize and develop advanced biological imaging approaches, especially cryo-electron microscopy, to study the architecture of the biological system, in vitro and in vivo, from nano-scale to meso-scale. Currently they are focusing on molecular mechanism of bio-membrane dynamics, structure and function of supra macromolecular assembly and bio-imaging methodology development. In recent years, together with his colleagues and collaborators, he has got great achievements in both scientific research and methodology developments. He has authored 150 peer-review papers with ~100 in his supervision with the H-index of 37.

Speaker: Bik-Kwoon TYE

Affiliation: Cornell University /
The Hong Kong University of Science & Technology

Email: biktye@ust.hk

Title: DNA Replication – From Yeast to Human

Biosketch:

Prof. Bik Tye received her PhD from the Massachusetts Institute of Technology. She is professor *emeritus* at Cornell University and senior member of the Institute for Advanced Study at the Hong Kong University of Science and Technology. She is a pioneer in eukaryotic DNA replication by using forward yeast genetics in the 1980's to identify DNA replication initiation genes that are required for origin-specific mini-chromosome maintenance. These include the MCM2-7 genes that encode the catalytic core of the replisome and MCM10, which is the last critical factor essential for the conversion of the pre-initiation complex to the active replisome. She is a late comer to the use of structural biology to solve functional biological problems by riding on the waves of the resolution revolution powered by cryo-electron microscopy.

Speaker: Dong WANG

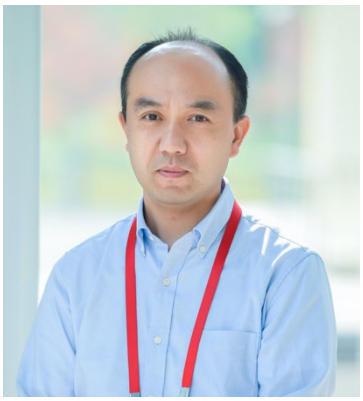
Affiliation: Division of Pharmaceutical Sciences, University of California, San Diego

Email: dongwang@ucsd.edu

Title: Molecular Basis of Transcription Elongation, Blockage, and Damage Repair

Biosketch:

Prof. Dong Wang obtained his BS at Peking University and PhD with Dr. Stephen Lippard at MIT. His role in Lippard group was to investigate the effect of nucleosome structure and epigenetic status change on DNA repair of platinum DNA damage. He then joined Dr. Roger Kornberg's group at Stanford University as a postdoc fellow. His postdoc research focused on understanding the molecular mechanism of Pol II transcription elongation and fidelity. Prof. Wang joined University of California, San Diego in 2010 and promoted to full professor in 2019.



Speaker: Hongwei WANG

Affiliation: School of Life Sciences, Tsinghua University

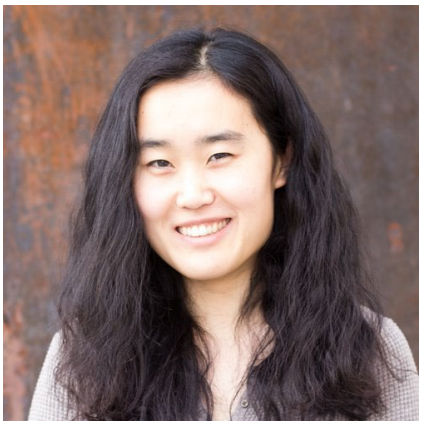
Email: hongweiwang@tsinghua.edu.cn

Title: Incorporating Electrospray Ionization in Cryo-EM Specimen Preparation

Biosketch:

Prof. Hongwei Wang received his BS and PhD from Tsinghua University in 1996 and 2001, respectively. Subsequently, he worked as a Postdoctoral Fellow, advancing to Research Scientist in 2006 at Lawrence Berkeley National Laboratory. He joined Yale University as a Tenure-Track Assistant Professor of Molecular Biophysics and Biochemistry in 2009 and returned to his alma mater as a Professor of Life Sciences in 2011.

Prof. Wang has been working on the development and application of cryo-EM. His current research focuses on methodology development for more efficient and high-resolution cryo-EM, the coordination mechanism of cytoskeleton and membrane systems, and the mechanism and regulation of nucleic acid quality control.



Speaker: Lan WANG

Affiliation: Division of Life Science,
The Hong Kong University of Science and Technology

Email: lanwang@ust.hk

Title: Msp1/ATAD1: The Molecular Vacuum Cleaner

Biosketch:

Prof. Lan Wang studied Chemistry as an undergraduate student at Tsinghua University. Fascinated by molecular mechanisms, she went on to study the mechanism of DNA repair at Harvard with Prof. Gregory Verdine. Upon completion of her PhD at Harvard, Prof. Wang went on to join Prof. Peter Walter's lab at UCSF, where she made a series of discoveries on different aspects of cellular protein homeostasis. Prof. Wang's postdoctoral work earned her a few recognitions and awards including the Damon Runyon Cancer Research Foundation Fellowship Award and the UCSF PBBR Postdoc Independent Research Award. In 2022, she joined the Division of Life Science at HKUST to continue her study on protein homeostasis.

Speaker: Yanhui XU

Affiliation: Institutes of Biomedical Sciences, Fudan University

Email: xuyh@fudan.edu.cn

Title: Structural Insights into Transcription Preinitiation Complex Assembly on Chromatin

Biosketch:

Prof. Yanhui Xu received his PhD from Tsinghua University in Prof. Zihe Rao lab, where he studied the mechanism of virus fusion. He went to Princeton University as a postdoctoral scholar in Prof. Yigong Shi lab, where he studied the structural basis for assembly of protein phosphatase 2A holoenzyme. He moved to Fudan University in 2008 as a principal investigator. His lab was investigating the structure and function of transcription regulation by using biochemistry and cryo-electron microscopy.

Speaker: Wei YANG

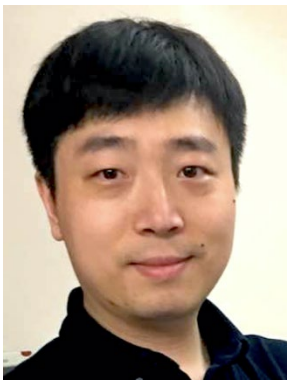
Affiliation: Intramural Research Program,
US National Institutes of Health

Email: weiy@niddk.nih.gov

Title: Molecular Secrets of DNA Replication, Repair and Recombination

Biosketch:

Dr. Wei Yang is a Distinguished Investigator at the US National Institutes of Health since 1995. She uses a combination of structural and biochemical methods to elucidate molecular mechanisms underpinning DNA replication, repair and recombination. She was born in Shanghai, China and began her undergraduate studies in biochemistry at Fudan University. She received a BA degree from SUNY at Stony Brook and her PhD from Columbia University mentored by Wayne Hendrickson. She was a postdoctoral fellow in the lab of Tom Steitz at Yale University. She is a recipient of the 2011 Dorothy Crowfoot Hodgkin award and the 2017 Mildred Cohn Award, and is an elected member of the National Academy of Sciences and American Academy of Arts and Sciences.



Speaker: Yuanliang ZHAI

Affiliation: School of Biological Sciences,
The University of Hong Kong

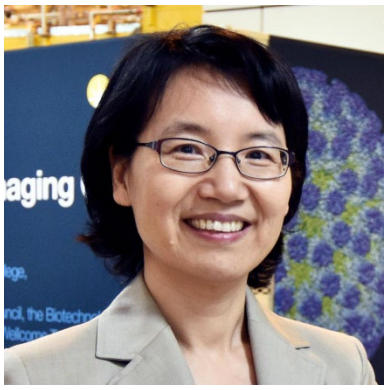
Email: zhai@hku.hk

Title: Cryo-EM Structure of the Human Pre-replication Complex

Biosketch:

Prof. Yuanliang Zhai graduated with a PhD in Biochemistry from the Hong Kong University of Science and Technology (HKUST) in 2010 and then held his postdoctoral fellow of the Division of Life Science before working as a Research Assistant Professor in 2013. From 2015 to 2018, he was a Junior Fellow of the Institute for Advanced Study at HKUST. Since 2018, he has been an Assistant Professor of the School of Biological Sciences at the University of Hong Kong.

Prof. Zhai's research focuses on the regulation of eukaryotic DNA replication and chromosome dynamics in both yeast and human cells. His lab uses a variety of approaches such as cryo-electron microscopy (cryo-EM), chemical biology, and biochemistry to understand the molecular mechanisms of large multiunit complexes involved in origin licensing, helicase activation, chromatin replication, and related DNA-centric processes.



Speaker: Peijun ZHANG

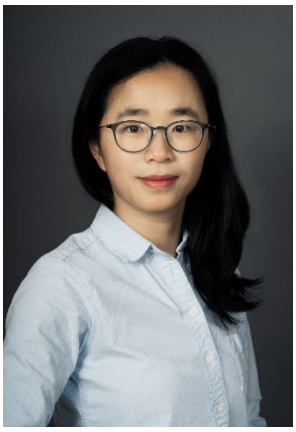
Affiliation: Nuffield Department of Medicine, University of Oxford

Email: peijun.zhang@strubi.ox.ac.uk

Title: *In Situ* Structures of Macromolecular Assemblies by CryoFIB and CryoET

Biosketch:

Prof. Peijun Zhang is a Professor of Structural Biology at the University of Oxford and the founding director of eBIC (the UK National Electron Bio-imaging Centre) at the Diamond Light Source. She is a leading expert in the fields cryoEM and cryo-electron tomography of molecular complexes and assemblies, especially investigating these *in situ* in the native cellular context. Her research is aimed at an integrated, atomistic understanding of molecular mechanisms of virus and bacterial infections by developing and combining novel technologies for high-resolution cryoEM and cryo-electron tomography with complementary computational and biophysical/biochemical methods. Her current research efforts focus on HIV-1 and SARS-CoV-2 infections and bacterial chemotaxis signaling pathways.



Speaker: Xianjun ZHANG

Affiliation: Thermo Fisher Scientific

Email: xianjun.zhang@thermofisher.com

Title: Towards the Visual Proteomics of C. reinhardtii Using High-throughput Collaborative *In Situ* Cryo-ET

Biosketch:

Dr. Xianjun Zhang received her PhD from ShanghaiTech University. She was investigating the structure of GPCRs by using X-Ray crystallography and single particle electron microscopy. After that, she moved to the University of Southern California as a postdoctoral scholar in Raymond Stevens lab. She was collaborating with Prof. Grant Jensen at Caltech to study the insulin vesicle secretory pathway in situ by using Cryo-electron Tomography and MicroED. She joined Thermo Fisher Scientific in 2021 as the Application Development Scientist.

Speaker: Yingyi ZHANG

Affiliation: Biological Cryo-EM Center, Laboratory Animal Facility (CWB), Hong Kong University of Science and Technology

Email: yizhang@ust.hk

Title: Cryo Electron Tomography – A Powerful Tool Exploring Ultra-Structure in Biology

Biosketch:

Dr. Yingyi Zhang received her PhD from Max-Planck Institute of Biophysics in Germany. She was investigating the structure of serotonin receptor by using single particle cryo-electron microcopy and cryo electron tomography and sub-tomogram averaging. After that, she moved to the Hong Kong University of Science and Technology as the facility manager at the Biological Cryo-EM Center, LAF(CWB).

Speaker: Yanxiang ZHAO

Affiliation: Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University

Email: yanxiang.zhao@polyu.edu.hk

Title: The Nucleocytoplasmic Shuttling of Tonicity-Responsive Transcription Factors

Biosketch:

Prof. Yanxiang Zhao earned her PhD from the Rockefeller University and currently serves as Professor in the Department of Applied Biology and Chemical Technology. Her lab conducts structure-based mechanistic studies of molecular machineries involved in various cellular processes including autophagy, telomere maintenance and nuclear import. Her lab employs a combination of NMR, x-ray crystallography and cryo-EM methods to dissect the assembly of protein machineries with the ultimate goal to understand their functional roles in human physiology and diseases.

Speaker: Keda ZHOU

Affiliation: School of Biomedical Sciences,
The University of Hong Kong

Email: kedazhou@hku.hk

Title: FACT Caught in the Act of Manipulating the Nucleosome

Biosketch:

Prof. Keda Zhou received his PhD from Colorado State University in the US. He was studying chromatin through biochemical and biophysical approaches under the supervision of Prof. Karolin Luger. Later on, he moved to the University of Colorado Boulder with Luger's lab. Since then, he leveraged the power of single particle cryo-EM to study several important chromatin related structures including centromeric chromatin, histone chaperone FACT and chromatin remodeler. Prof. Zhou joined the University of Hong Kong as a tenure-track Assistant Professor in 2022.

Talk Abstracts

*(*Listed in chronological order according to the symposium schedule)*

D1-#1

Molecular Secrets of DNA Replication, Repair and Recombination

Wei Yang*, Lan Liu, Jinseok Kim, Eric Li, Yang Gao and Marty Gellert

Laboratory of Molecular Biology, NIDDK, National Institutes of Health, US

Email: wei.yang@nih.gov

The processes and chief players in DNA replication, repair and recombination (the DNA 3Rs) have been identified for many years. Because of tight regulation of replication and sparse occurrence of repair and recombination events, macromolecular machineries involved in the DNA 3Rs are often assembled based on need and dynamic. To structurally elucidate how these dynamic and sometime rare events occur, tricks to enrich low population and stabilizing transient states are essential in addition to the cutting-edge techniques in cryogenic electron microscopy. In this talk, I will present examples of how we overcome roadblocks and share salient lessons we have learnt.

D1-#2

Activation of an Origin of Replication Visualised by Cryo-EM

Alessandro Costa

Macromolecular Machines Lab, The Francis Crick Institute, UK

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In eukaryotes, DNA loading and activation of the MCM replicative helicase are temporally separated to ensure that chromosomes are copied only once per cell cycle[1]. Before replication initiation, MCM is loaded onto duplex DNA at replication origins forming an inactive head-to-head double hexamer[2]. Upon S phase transition, helicase activation occurs in two steps, involving limited opening of the double helix first, and then topological separation of the two DNA strands[3,4]. I will describe how imaging *in vitro* reconstituted reactions informs the mechanism of origin activation.

References:

[1] Costa, A. & Diffley, J. F. X. The Initiation of Eukaryotic DNA Replication. Annual review of biochemistry, doi:10.1146/annurev-biochem-072321-110228 (2022).
[2] Li, N. et al. Structure of the eukaryotic MCM complex at 3.8 A. Nature 524, 186-191, doi:10.1038/nature14685 (2015).
[3] Miller, T. C. R., Locke, J., Greiwe, J. F., Diffley, J. F. X. & Costa, A. Mechanism of head-to-head MCM double-hexamer formation revealed by cryo-EM. Nature 575, 704-710, doi:10.1038/s41586-019-1768-0 (2019).
[4] Douglas, M. E., Ali, F. A., Costa, A. & Diffley, J. F. X. The mechanism of eukaryotic CMG helicase activation. Nature 555, 265-268, doi:10.1038/nature25787 (2018).
[5] Lewis, J. S. et al. Mechanism of replication origin melting nucleated by CMG helicase assembly. Nature 606, 1007-1014, doi:10.1038/s41586-022-04829-4 (2022).

D1-#3

DNA Replication – From Yeast to Human

Bik Tye

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Email: biktye@ust.hk

The DNA replication mechanism is highly conserved in eukaryotes yet must adapt to the diverse life styles of the myriads of species within the kingdom. I will discuss two specific DNA replication complexes, the origin recognition complex, ORC [1, 2] and the MCM double hexamer bound by its activator, the Dbf4-Cdc7 kinase [3], as examples of how the DNA replication machinery evolves to adapt to the very different life cycles adopted by yeast and human.

References:

[1] Li, N, Lam WH, Zhai Y, Cheng J, Zhao Y, Gao, N and Tye, BK. *Nature* 559:217-222 (2018) Structure of the Origin Recognition Complex Bound to DNA Replication Origin

[2] Lee CSK, Cheung MF, Li J, Zhao Y, Lam WH, Ho V, Rohs R, Zhai Y, Leung D, Tye BK. *Nat Commun* 12:33 (2021) Humanizing the Yeast Origin Recognition Complex.

[3] Cheng J, Li N, Huo Y, Dang S, Tye BK, Gao N, Zhai Y. *Nat Commun* 13:1396 (2022) Structural Insight into the MCM Double Hexamer Activation by Dbf4-Cdc7 Kinase.

D1-#4

Cryo-EM Structure of the Human Pre-replication Complex

Yuanliang Zhang

School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, P. R. China

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The minichromosome maintenance (MCM) 2-7 double hexamer (DH) contains two heterohexameric rings joined at the N-terminal ends. It is loaded onto duplex DNA by the origin recognition complex and serves as the scaffold for the assembly of the bidirectional replisomes as well as the catalytic core of the CMG (Cdc45-MCM2-7-GINS) helicase. Replisome assembly is accompanied by MCM-DH remodeling and uncoupling that respectively melts and separates the origin DNA strands. To better understand the role of MCM2-7 in this melting process, we recently determined a 2.59-Å cryo-electron microscopy structure of the human MCM-DH (hMCM-DH), also known as the pre-replication complex (pre-RC). The overall structure of the hMCM-DH is similar to that of the yeast MCM-DH but shows a more constricted central channel with a diameter of only 13 Å at the hexamer interface. This unusual conformation untwists and stretches the DNA strands such that almost a half turn of the bound duplex DNA is distorted with 1 base pair completely separated, generating an initial open structure (IOS) at the hexamer junction. Disturbing the IOS inhibits DH formation and replication initiation. Mapping of hMCM-DH footprints indicates that IOSs are distributed across the genome in large clusters aligning well with initiation zones designed for stochastic origin firing. This work unravels an intrinsic mechanism that couples DH formation with initial DNA melting to license replication initiation in human cells. Our findings also highlight that although general mechanisms obtained from yeast could be applied to human cells, the detailed regulation of pre-RC assembly is drastically different between yeast and human.

D1-#5

Molecular Basis of Transcription Elongation, Blockage, and Damage Repair

Dong Wang[1, 2, 3]

[1]Division of Pharmaceutical Sciences, Skaggs School of Pharmacy & Pharmaceutical Sciences; The University of California, San Diego, US

[2]Department of Cellular and Molecular Medicine, The University of California, San Diego, US

[3]Department of Chemistry and Biochemistry, The University of California, San Diego, US

Email: dongwang@ucsd.edu

During transcription elongation, RNA polymerase moves along DNA template, recognizes the template base, and synthesizes RNA with a high fidelity. Transcription elongation process is subject to pausing and arrest by various obstacles such as pause-inducing DNA sequences or secondary structures, DNA modifications, DNA lesions, DNA-binding proteins and small molecules. Here we will present our recent progress in understanding the structural basis of lesion recognition and repair. In particular, we will focus on recent results related to transcription blockage, recognition of DNA lesions and unnatural base pairs, as well as transcription-coupled repair.

D1-#6

In situ Structures of Macromolecular Assemblies by CryoFIB and CryoET

Peijun Zhang

Division of Structural Biology, Nuffield Department of Medicine, University of Oxford, UK

Email: peijun.zhang@strubi.ox.ac.uk

Recent development in cryo-electron microscopy (cryoEM) has revolutionized the field of structural biology, allowing protein structures to be determined at the atomic resolution in a close-to-native, frozen-hydrated state, especially using cryoEM SPA method. For studying macromolecular complexes that are intrinsically flexible and dynamic, and often function in higher-order assemblies that are difficult to purify, cryoET and subtomogram averaging (cryoET STA) has emerged as a potent tool to obtain structures of these at near-atomic resolution. The study of such complexes and assemblies in situ using cryoET STA, coupled with cryoFIB/SEM and correlative imaging, opens a new frontier in structural cell biology. I will present our recent studies on the infection of SARS-CoV-2, architecture of native chromatin fibers in intact cells and particulate methane monooxygenase (pMMO) in methanotrophic bacteria, to demonstrate the power of high-resolution in situ structural biology using cell lamellae-based cryoET STA.

D1-#7

In Situ Assembly and Membrane Fusion of Enveloped Viruses

Sai Li

School of Life Sciences, Tsinghua University, P. R. China

Email: sai@tsinghua.edu.cn

We combine cryo-ET, cryo-EM, and mass-spectrometry for in situ structural characterization of emerging enveloped viruses, such as SARS-CoV-2 and influenza. The structure, landscape and site-specific glycan compositions of spike proteins were studied. We also captured snapshots of virus fusion in action, from which intermediate steps of spike-mediated membrane fusion were interpreted. We further explored how enveloped viruses prepare their spike proteins to mediate membrane fusion. The in situ observation and structural analysis have provided novel insights into the enveloped viral assembly and membrane fusion mechanism. Together, these findings have provided structural references of the antigens in situ, which helped with the development of novel vaccines.

References:

[1] Hong, Y., Song, Y., Zhang, Z., and Li, S. (2023). Cryo-Electron Tomography: The Resolution Revolution and a Surge of In Situ Virological Discoveries. ***Annual Review of Biophysics*** *52*, 14.11-14.22. 10.1146/annurev-biophys-092022-100958.

[2] Song, Y., Yao, H., Wu, N., Xu, J., Zhang, Z., Peng, C., Li, S., Kong, W., Chen, Y., Zhu, M., et al. (2022). In situ architecture and membrane fusion of SARS-CoV-2 Delta variant. ***PNAS*** (accepted)

[3] Li, S. (2022). Cryo-electron tomography of enveloped viruses. ***Trends in Biochemical Sciences*** *47*, 173-186. 10.1016/j.tibs.2021.08.005.

[4] Yao, H., Song, Y., Chen, Y., Wu, N., Xu, J., Sun, C., Zhang, J., Weng, T., Zhang, Z., Wu, Z., et al. (2020). Molecular Architecture of the SARS-CoV-2 Virus. ***Cell*** *183*, 730-738 e713. 10.1016/j.cell.2020.09.018.

D1-#8

Mechanism of α-carboxysomes Formation

Tao Ni

School of Biomedical Sciences, The University of Hong Kong, Hong Kong SAR, P. R. China

Email: taoni@hku.hk

Tao Ni[1,3#*], Qiuyao Jiang[2#], Pei Cing Ng[2], Juan Shen[3], Hao Dou[3], Yanan Zhu[3], Julika Radecke[3], Gregory F. Dykes[2], Fang Huang[2], Lu-Ning Liu[2*], Peijun Zhang[3*]

[1]School of Biomedical Sciences, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, P. R. China.
[2]Institute of Systems, Molecular and Integrative Biology, University of Liverpool, UK
[3]Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, UK

Carboxysomes are a family of bacterial microcompartments in cyanobacteria and chemoautotrophs. They encapsulate Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and carbonic anhydrase catalyzing carbon fixation inside a proteinaceous shell. An intrinsically disordered linker protein CsoS2 plays a central role in driving carboxysomes assembly, however, the underlying molecular mechanism is obscure. Here we used cryo-electron microscopy and tomography to study α-carboxysomes from a chemoautotrophic bacterium *Halothiobacillus neapolitanus*. By cryo-ET, we identify that Rubiscos form high-order intertwining spiral assembly inside the carboxysomes and further resolve the structures of native Rubisco at near-atomic resolutions by subtomogram averaging. Surprisingly, CsoS2 interacts only with the Rubiscos close to the shell using its N-terminal region. In addition, high-resolution cryo-EM structures of recombinant shell assemblies revealed that CsoS2 C-terminus interacts with the inner surface of shell, acting as a “molecular thread” stitching through multiple shell protein interfaces with a remarkable highly-conserved repeating motif. Taken together, our findings provide critical knowledge of the assembly principles of α-carboxysomes, which may aid in the rational design and repurposing of carboxysome structures for new functions.

D1-#9

Technology Development for *In Situ* Structural Study of Macromolecular Machinery

Fei Sun

Institute of Biophysics (IBP), Chinese Academy of Sciences, P. R. China

Email: feisun@ibp.ac.cn

With the mature and rapid development of single particle electron cryo-microscopy, structural biology has gone into a new era with more and more supra macromolecular complexes and membrane protein complexes whose structures are well resolved and studied. A new direction has emerged to study the structures of these complexes *in situ* without purifying them from their native environment. The development of cryo-electron tomography has provided such opportunity for high resolution *in situ* structural study. However, there are still various bottlenecks including specimen preparation, data collection and image processing that need to be solved to improve the throughput, efficiency, and resolution. Here I will talk about our past effects in this direction, which include the development of site-specific cryo-focused ion beam (cryo-FIB) technique, the workflow to prepare cryo-lamella of tissue specimen and reconstruction algorithms to deal with missing-wedge problem.

References:

1. ***J Struct Biol.*** 194(2): 218-223, (2016)
2. ***J Struct Biol.*** 195(1): 100-112, (2016)
3. ***J Struct Biol.*** 195(1): 49-61, (2016)
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5. ***J Struct Biol.*** 201(1): 63-75, (2018)
6. ***J Struct Biol.*** 213(3): 107763, (2021)
7. ***Nature Methods*** 20(2): 276-83, (2023)
8. ***Communications Biology*** (in press), 2023

D1-#10

Single Molecule Localization by Interferometric & Cryogenic Imaging

Wei Ji

Institute of Biophysics, Chinese Academy of Sciences, P. R. China

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Remarkable progress in Single molecule localization microscopy (SMLM) has been made in the past decade. Here I would like to present interferometric and cryogenic imaging which exhibit excellent localization precision performances compared to conventional SMLMs. We introduced interferometric SMLMs named ROSE and ROSE-Z. A fluorescence molecule is located by the intensities of multiple excitation patterns of an interference fringe, providing improvement in the localization precision compared to the conventional centroid fitting method at the same photon budget. We demonstrate this technique by resolving a nanostructure down to 5 nm. We also built an ultra-stable super-resolution cryo-FM that exhibits excellent thermal and mechanically stability. We have demonstrated the super-resolution imaging capability of this system. The results suggest that our system is particularly suitable for SMLM and cryogenic super-resolution correlative light and electron microscopy. Based on the cryo-fluorescence imaging technique we developed, we build a cryogenic correlated light, ion and electron microscopy (cryo-CLIEM) that is capable of preparing cryo-lamellae under the guidance of three-dimensional confocal imaging. Moreover, we demonstrate a workflow to preselect and preserve nanoscale target regions inside the finished cryo-lamellae. By successfully preparing cryo-lamellae that contain a single centriole or contact sites between subcellular organelles, we show that this approach is generally applicable, and shall help in innovating more applications of cryo-ET.

References:

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[2] Gu L#, Li Y#, Zhang S#, Zhou M, Xue Y, Li W, Xu T*, Ji W*. Molecular-scale axial localization by repetitive optical selective exposure. Nat. Methods. 18, 369-373(2021).

[3] Gu L#, Li Y#, Zhang S#, Xue Y, Li W, Li D, Xu T*, Ji W*. Molecular resolution imaging by repetitive optical selective exposure. Nat. Methods. 16, 1114-1118(2019).

D1-#11

Metallo-Supramolecular Branched Polymer Protects Particles from Air-water Interface in Single-Particle Cryo-Electron Microscopy

Shangyu Dang

Division of Life Science, The Hong Kong University of Science and Technology, Hong Kong SAR, P. R. China

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Recent technological breakthroughs in single-particle cryo-electron microscopy (cryo-EM) enabled rapid atomic structure determination of biological macromolecules. A major bottleneck in the current single particle cryo-EM pipeline is the preparation of good quality frozen cryo-EM grids, which is mostly a trial-and-error process. Among many issues, preferred particle orientation and sample damage by air-water interface (AWI) are common practical problems. Here we reported a method of applying metallo-supramolecular branched polymer (MSBP) in the cryo-sample preparation for high-resolution single-particle cryo-EM. Our data shows that MSBP keeps a majority of particles away from air-water interface and mitigates preferred orientation as verified by the analyses of apoferritin, hemagglutinin (HA) trimer and various sample proteins. The use of MSBP is a simple method to improve particle distribution for high-resolution structure determination in single-particle cryo-EM.

D1-#12

Incorporating Electrospray Ionization in Cryo-EM Specimen Preparation

Hongwei Wang

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The current method of specimen preparation is not keeping pace with innovations in other major steps of cryo-EM structural biology. We have developed a new specimen preparation method using electrospray ionization equipment, which is an important technique for liquid separations in mass spectrometry. This method provides an alternative solution to the problem of macromolecules absorption to the air-water and solid-water interfaces. By refining the spraying parameters, the method produces an optimal ice thickness for the target macromolecules, due to the effective wetting of charged liquid droplets on the grid surface. We have used this method to prepare cryo-specimens and achieved 3D reconstructions of the 70S ribosome, 20S proteasome, apo-ferritin, and ACE2, at resolutions of 2.8 Å, 2.1 Å, 2.2 Å, and 3.5 Å, respectively. Further analysis of the specimens demonstrated that the macromolecules stay mostly in the middle of the amorphous ice layer, away from the air-water and solid-water interfaces.

D2-#1

Msp1/ATAD1: The Molecular Vacuum Cleaner

Lan Wang

Division of Life Science, The Hong Kong University of Science and Technology, Hong Kong SAR, P. R. China

Email: lanwang@ust.hk

When cells experience stress, the efficiency and faithfulness of protein targeting are compromised, leading to homeostatic imbalances and damage to organelles. Yeast Msp1 (mitochondrial sorting of proteins 1) and mammalian ATAD1 (ATPase family AAA domain–containing 1) are orthologous AAA proteins that, fueled by ATP hydrolysis, recognize and extract mislocalized membrane proteins from the outer mitochondrial membrane. Msp1 and ATAD1 are implicated in multiple diseases in various animal models, including male infertility in fruit flies and neurological disorders in mammals. Through the cryo-EM structures and cell-based assays for protein mislocalization, we show that Msp1 and ATAD1 possess several features that are specifically adapted to removing membrane proteins.

D2-#2

Cryo Electron Tomography – A Powerful Tool Exploring Ultra-Structure in Biology

Yingyi Zhang

Biological Cryo-EM Center, LAF(CWB), The Hong Kong University of Science and Technology,

Hong Kong SAR, P. R. China

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The ultra-structure in biology refers to the fine biological structures, as of a cell, usually containing massive details which are invisible through a conventional microscope, such as all organelles in the cell. Promisingly, with the application of cryo electron microscope, the close details become visible.

Learning these ultra-structures and be able to “see” as many details towards their surroundings as possible, is helpful to understand what they are, where they are located, what roles they are playing and their mechanism related to physiological dysfunctions. Endless of scientific questions would be raised and many of them could be addressed.

Cryo electron tomography is such an approach, still developing, aiming of studying the ultra-structures and revealing their related physiology functions. As the development of cryo electron tomography, there are still technical limitations under study.

It is valuable to review the development of cryo electron tomography and its widely application in structural biology, to introduce this technique to prompt application and improvement.

D2-#3

Towards the Visual Proteomics of *C. reinhardtii* Using High-throughput Collaborative *in situ* Cryo-ET

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Cryo-electron tomography (cryo-ET) has become a vital tool in structural biology for determining the 3D structure of macromolecular complexes in their native context inside cells or tissues. Cryo-ET and subsequent subtomogram averaging have produced structures at resolutions sufficient to visualize sidechains (3-5Å), with the specimens being cryo-fixed in vitrified ice 200nm or thinner. While thicker vitrified single cells and tissues from multicellular organisms have been milled using Gallium cryo-focused ion beam (Ga+-cryo-FIB), subtomogram averages of macromolecular complexes within these cryo-FIB milled lamellae have often been limited to resolutions lower than 10 Å due to poor lamella quality, redeposition, and beam-induced motion during tilt-series data acquisition. A next-generation cryo-FIB that uses plasma instead of Gallium (cryo-plasmaFIB) from Thermo Fisher Scientific reduces redeposition and ion beam damage while improving the throughput with automated sample transfers, making it possible to collect large in situ cryo-ET datasets on lamellae of cells and tissues to determine high-resolution molecular atlases or visual proteomics.

In this study, we benchmarked the performance of cryo-plasmaFIB using *S. cerevisiae* as an established model system, which resulted in a 6.5 Å subtomogram average of 80S ribosomes from 7 tomograms acquired on a single lamella, cryo-FIB milled using Xenon plasma. The analysis of Rosenthal-Henderson B-factors of 80S ribosomes from lamellae with varying thicknesses demonstrates an inverse relationship between specimen thickness and signal-to-noise ratio (SNR). As a proof of principle of visual proteomics approaches, we generated a large in situ cryo-ET dataset containing thousands of tomograms from *C. reinhardtii*. We show that we were able to obtain sub-nanometre subtomogram averages of biomolecular complexes ranging in size from megadaltons to several hundred kilodaltons, and hence feasibility to determine the Visual Proteome of *C. reinhardtii*.

However, the increasing number of known and unknown macromolecular complexes in such large datasets poses a challenge for individual efforts to identify, average, classify, and curate macromolecular complexes, calling for a collaborative effort. The availability of large curated molecular atlases will prove instrumental in the development of new computational tools for in situ cryo-ET. Overall, the study highlights the potential of cryo-plasmaFIB for high-throughput and high-resolution cellular cryo-ET and visual proteomics approaches to gain insights into the molecular functioning of fundamental cellular processes.

D2-#4

Single Particle Cryo-EM of INO80

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My laboratory is interested in applying single particle cryo-EM to address some challenging biological questions. I will present a brief introduction of the current technological status of single particle cryo-EM and our recent work on applying single particle cryo-EM to study ATP dependent chromatin remodeling complex INO80.

D2-#5

Cryo-EM Driven Paradigm Shift of ABC Transporter Mechanism

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Present in all kingdoms of life, ATP-binding cassette (ABC) transporters harness the energy of ATP binding and hydrolysis to translocate a multitude of chemically diverse substrates across cellular membranes. Despite decades of studies and many available structures, the molecular mechanisms of most ABC transporters are still poorly defined. The ongoing revolution of cryo-EM has enabled novel approaches for obtaining deep insights into these highly dynamic membrane protein machines. Through our cryo-EM studies of several ABC transporters that perform different functions, we have uncovered how distinct tasks of substrate translocation are accomplished by the unique actions of these transporters. Importantly, our own research experience in the past decade is an excellent demonstration of how single-particle cryo-EM methodology and the mechanistic study of ABC transporters stimulate each other's development, thus emphasizing the extremely versatile nature and yet-to-be-realized potential of cryo-EM in biological research. (https://liao.bio.sustech.edu.cn/)

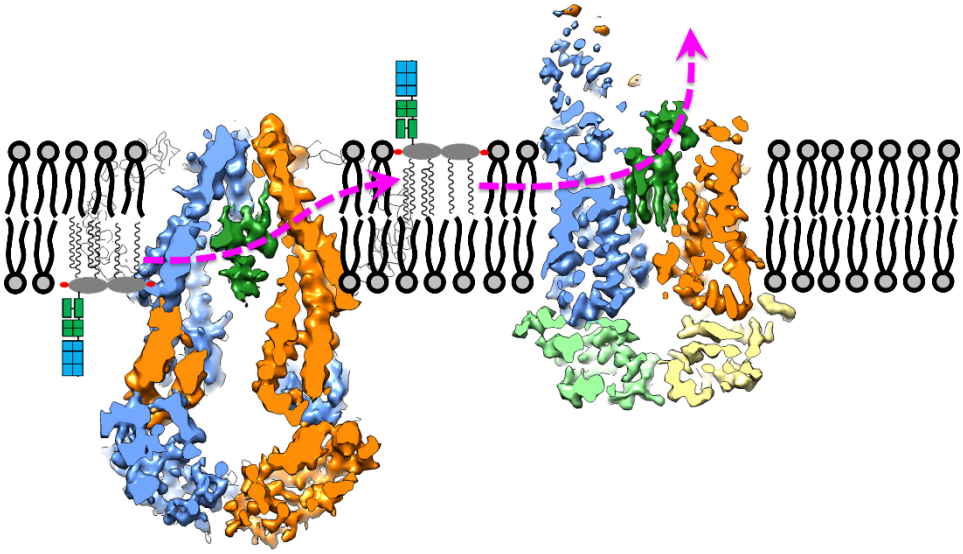

Figure 1. Cryo-EM reveals how lipopolysaccharide is flipped by MsbA(1, 2) (left) and extracted by $LptB_2FGC$(3, 4) (right), two ABC transporters in *Escherichia coli*.

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D2-#6

Cryo-EM Illuminates the Mechanism of SGLT Inhibitors

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Sodium-glucose co-transporters (SGLT) harness the electrochemical gradient of sodium to transport glucose against their chemical gradients. In humans, SGLT1 and SGLT2 are crucial for glucose uptake and homeostasis and are important for human health. Their loss-of-function mutations lead to genetic disease in humans. These proteins are important drug targets. SGLT inhibitors are used for the treatment of diabetes and cardiovascular diseases. SGLTs are challenging targets for cryo-EM due to their small sizes (60-70kDa). We developed a "three-joint-tethering strategy" to increase the molecular weight of SGLTs and create a fiducial marker for image alignment [1]. Using this strategy, we determined the structures of human SGLT1 and SGLT2 with inhibitors bound [2, 3]. We found that SGLT inhibitors lock the transporter in the outward-open conformation to inhibit sugar transport.

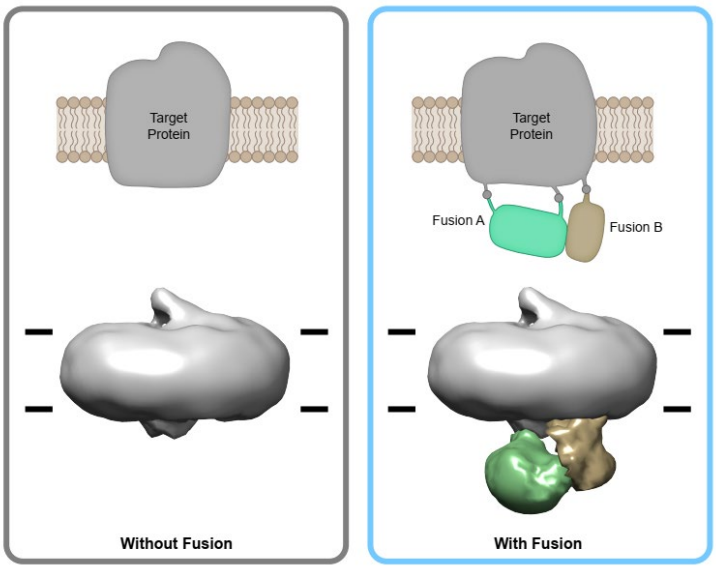

Figure 1. Three-joint-tethering strategy facilitates structure determination of SGLTs

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D3-#1

Beyond Cas Nucleases: The Mechanism of an RNA-Guided Protease

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CRISPR RNA-guided nucleases have been the driving force behind the current revolution of genomic medicine. A new wave of discovery defined an atypical Type III CRISPR-Cas system, in which the RNA-guided RNase established cooption with a Caspase-like protease. Together they function as a CRISPR RNA-guided protease – Craspase. In a Science publication last year, my group and Stan Brouns' group applied cryo-EM and molecular genetics approaches to define the conditions leading to the RNA-guided activation and inactivation of Craspase, in vitro and in vivo, and provide a thorough set of high-resolution mechanistic explanations. Since Craspases do not cleave DNA, they may serve as safe alternatives to Cas nucleases in therapeutics.

D3-#2

Structural Basis of DNA Double-Strand Break Repair by NHEJ

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DNA double-strand breaks, one of the most cytotoxic forms of DNA damage, can be detected and repaired by the fast-responding and tightly regulated non-homologous end-joining (NHEJ) machinery [1, 2]. NHEJ factors are targets for the development of cancer therapeutics and are essential for the generation of antibody and antigen receptor diversity in immune cells [3, 4]. Core NHEJ factors (Ku70/80 heterodimer (Ku), catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs), DNA ligase IV (LigIV), XRCC4 and XLF) form an initial long-range (LR) synaptic complex that transitions into a DNA-PKcs free, short-range (SR) state to align and repair the DSB ends [5]. Using single-particle Cryo-Election Microscopy (Cryo-EM), we have visualized three additional key NHEJ complexes representing different transition states, with DNA-PKcs adopting distinct dimeric conformations within each of them. Integrated modeling with both experimental reconstruction and in silico structural prediction reveals how an accessory NHEJ scaffolding factor, PAXX, stabilizes the LR complex during ATP-dependent DNA-PKcs signaling. Upon DNA-PKcs autophosphorylation, the LR complex undergoes a substantial conformational change, with both Ku and DNA-PKcs rotating outward to promote DNA break exposure and DNA-PKcs dissociation. In addition, we captured a dimeric state of catalytically inactive DNA-PKcs, which resembles structures of other PIKK family kinases, revealing a model of the full regulatory cycle of DNA-PKcs during NHEJ.

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D3-#3

Mechanisms of Programmable DNA Transposition

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Transposons are powerful drivers of evolutionary change, creating large genomic rearrangements using protein-encoded machinery. An important class of Tn7-like transposons uses a guide-RNA sequence to target insertions using a CRISPR-like effector, endowing these CRISPR-associated transposons (CAST) with genome-editing capabilities that have powerful implications for bioengineering and medicine. Transposon-encoded machinery is sufficient to carry out site-specific integrations occurring within a narrow window, with orientation specificity, and target-site immunity. In this talk I will share our recent mechanistic work aimed at explaining the remarkable properties of this self-regulated transposon.

D3-#4

Structural Insights into Transcription Preinitiation Complex Assembly on Chromatin

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We would like to present our recent finding on the +1 nucleosome–bound PIC-Mediator structures [1]. Our previous studies reveal assembly of transcription preinitiation complex (PIC) on TATA box and TATA-less promoters [2] and Mediator facilitates PIC assembly and phosphorylation of Pol II C-terminal domain for transcription activation [3]. These structures were obtained on naked promoters. We therefore investigated how PIC-Mediator complex is assembled in the context of chromatin.

The +1 nucleosome is well positioned about 40 base pairs downstream of the transcription start site (TSS) and is commonly known as a barrier of transcription. Our structural and biochemical studies show that PIC-Mediator prefers binding to T40N nucleosome located 40 base pairs downstream of TSS and contacts T50N but not the T70N nucleosome. The nucleosome facilitates the organization of PIC-Mediator on the promoter by binding TFIIH and Mediator subunits and may contribute to transcription initiation. PIC-Mediator exhibits multiple nucleosome-binding patterns, supporting a structural role of the +1 nucleosome in the coordination of PIC-Mediator assembly. Our study reveals the molecular mechanism of PIC-Mediator organization on chromatin and underscores the significance of the +1 nucleosome in regulating transcription initiation.

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D3-#5

CryoEM Reveals Unpredictable Structural Details of Protein and RNA

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Cryogenic electron microscopy (cryoEM) has been advanced to resolve atomic structures of biochemically purified macromolecules with details equivalent to X-ray crystal structures. A unique aspect of cryoEM is to use image processing methods to sort out images of particles with heterogeneous compositions and conformations. This allows us to visualize structures of macromolecules that exist in an ensemble of biochemical states, which are interpretable in their biochemical processes. I will illustrate this approach to show how different folding intermediates of tubulin is seen within the chamber of human chaperonin TRiC and how misfolded and folded states of ribozyme are organized.

D3-#6

Structural Insights into the Organization of Membrane Skeleton in Red Blood Cells

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Spectrin-based membrane skeleton is a ubiquitous structure of metazoan cells. In erythrocytes, this skeleton, as a membrane-associated polygonal network, confers exceptional strength and elasticity to the lipid membrane to tolerate the shear stress in circulation. Defects in membrane skeleton cause a variety of red blood cell disorders. Here, we report the cryo-EM structures of the native spectrin-actin junctional complex, which is a highly decorated short filamentous actin (F-actin) acting as a central organizational unit of the erythrocytic membrane skeleton. The structures reveal general principles underlying the organization and assembly of the skeleton and elucidate specific molecular roles for the components of the junctional complex. While a hetero-tetramer of α- and β-adducin binds to the barbed end of the junctional complex as a flexible cap, tropomodulin and a newly identified factor SH3BGRL2 together create an absolute cap at the pointed end. Three copies of dematin, present in the central actin layers, form parallel ring-like structures encircling the junctional complex. The structures also provide atomic details for the docking of tropomyosin on the junctional complex, including its interactions with the capping proteins and successive attachments with the trunk of the junctional complex. Overall, this work serves as a structural framework for understanding the assembly and dynamics of the membrane skeleton in erythrocytes, and also offers insights into mechanisms of related ubiquitous F-actin binding factors in other types of F-actin systems.

D3-#7

Cryo-EM Analysis of the Nuclear Pore Complex from *Xenopus Laevis*

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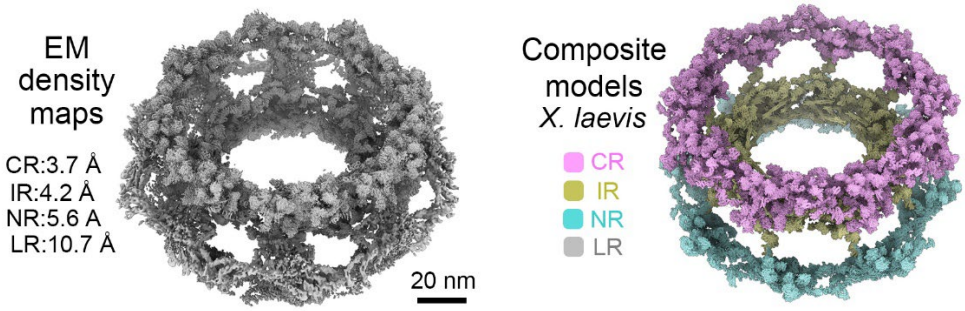
The nuclear pore complex (NPC) resides on the nuclear envelope (NE) and mediates nucleocytoplasmic cargo transport. As one of the largest cellular machineries, a vertebrate NPC has a molecular mass of over 100 MDa and consists of multiple cytoplasmic filaments (CF), a cytoplasmic ring (CR), an inner ring (IR), a nuclear ring (NR), a nuclear basket (NB), and a luminal ring (LR). Each ring scaffold consists of eight repeating subunits. Relying on single particle cryo-EM analysis of the NPC from *Xenopus laevis* (*X. laevis*) [1], we previously obtained a reconstruction of the LR subunit at an average resolution of 10.7 Å [2], which for the first time reveals detailed structural features of the LR. We then obtained reconstructions of the CR subunit, IR subunit, and NR subunit of the *X. laevis* NPC at average resolutions of 3.7-4.7 Å [3], 4.2 Å [4], and 5.6 Å [5], respectively. These reconstructions allow identification of previously unrecognized nucleoporin components and revelation of key interface features among neighboring nucleoporins. These experimental advances, together with AI-based structure prediction, have given rise to a composite atomic model of the NPC with marked improvement over previous models of vertebrate NPC [Figure 1].

Figure 1. The EM reconstructions (left) and composite models (right) of the *X. laevis* NPC.



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D3-#8

FACT Caught in the Act of Manipulating the Nucleosome

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In almost all eukaryotes, the nucleosome profoundly affects all DNA-related processes such as RNA transcription and DNA replication. To pass through as well as maintain the nucleosome during these processes, a histone chaperone called FACT (FAcilitate Chromatin Transcription) is required [1]. For decades, the mechanism on how FACT promotes chromatin transaction while maintaining the nucleosome integrity was elusive. The talk will focus on the structure determination of FACT on a partially disassembled nucleosome [2]. By conquering the "water-air" interface problem, we obtained two cryo-EM structures of human FACT on its substrates. Hydrogen-deuterium exchange (HDX) assay validated the structures in solution. The structures revealed how FACT engages with the nucleosomal DNA and all histones simultaneously. The findings reconciled discrepancies regarding the many roles of FACT and underscored the dynamic interactions between histone chaperones and the nucleosomes.

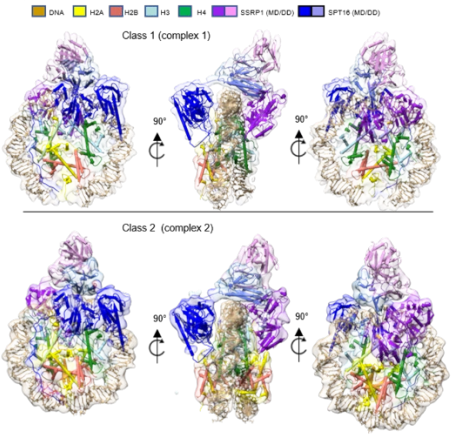

Figure 1. Two cryoEM structures of FACT in complex with "sub-nucleosomes".

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D3-#9

Structural Basis of Substrate Recognition and Thermal Protection by a Small Heat Shock Protein

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Small heat shock proteins (sHsps) bind unfolding proteins, thereby playing a pivotal role in the maintenance of proteostasis in virtually all living organisms. Structural elucidation of sHsp-substrate complexes has been hampered by the transient and heterogeneous nature of their interactions, and the precise mechanisms underlying substrate recognition, promiscuity, and chaperone activity of sHsps remain unclear. Here we show the formation of a stable complex between Arabidopsis thaliana plastid sHsp, Hsp21, and its natural substrate 1-deoxy-D-xylulose 5-phosphate synthase (DXPS) under heat stress, and report cryo-electron microscopy structures of Hsp21, DXPS and Hsp21-DXPS complex at near-atomic resolution. Monomeric Hsp21 binds across the dimer interface of DXPS and engages in multivalent interactions by recognizing highly dynamic structural elements in DXPS. Hsp21 partly unfolds its central α-crystallin domain to facilitate binding of DXPS, which preserves a native-like structure. This mode of interaction suggests a mechanism of sHsps anti-aggregation activity towards a broad range of substrates.

D3-#10

The Nucleocytoplasmic Shuttling of Tonicity-Responsive Transcription Factors

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Maintenance of tonicity balance is essential for cell survival. To counter the deleterious effects of hyper- or hypotonic conditions, cells have developed sophisticated mechanisms to mount an effective adaptive response. Part of this adaptation process involves rapid nucleocytoplasmic shuttling of transcription factors to regulate the expression of tonicity-responsive genes.

Nuclear Factor of Activated T-cells 5 (NFAT5) is one such example in mammalian cells. Hypertonicity triggers rapid nuclear import of NFAT5 to activate the transcription of multiple organic osmolyte transporters. In contrast, hypotonicity renders NFAT5 largely cytosolic, thus turning off the NFAT5-mediated transcription program. The molecular mechanism of this tonicity-regulated nucleocytoplasmic shuttling is not well understood.

Here we report molecular and structural studies to dissect the tonicity-regulated nucleocytoplasmic shuttling process of NFAT5. We first used siRNA screening to confirm that the nuclear import of NFAT5 under hypertonicity requires only karyopherin β1 (KPNB1), but not karyopherin α. We also used proteomics analysis to reveal that the nuclear export of NFAT5 under hypotonicity is driven by exportin-T (XPOT). We mapped an unconventional nuclear localization signal (NLS) in NFAT5 and investigated its interaction with KPNB1 by cryo-EM studies.

Overall, our studies reveal that NFAT5 undergoes tonicity-drive nucleocytoplasmic shuttling through unconventional routes involving KPNB1 and XPOT. Our findings may apply to other tonicity-responsive transcription factors.

D3-#11

Chemical Approaches to Decode Histone Epigenetics

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Histone posttranslational modifications (PTMs), such as phosphorylation, methylation and acetylation, play crucial roles in regulating many fundamental cellular processes, such as gene transcription, DNA replication, DNA damage repair, chromosome segregation and cell differentiation. Increasing evidences have indicated that PTMs of histones can serve as a heritable 'code' (so-called 'histone code'), which provides epigenetic information that a mother cell can pass to its daughters. Histone code is 'written' or 'erased' by enzymes that generate or remove the modifications of histones. Meanwhile, 'readers' of histone code recognize specific histone modifications and 'translate' the code by executing distinct cellular programs necessary to establish the diverse cell phenotypes.

While a large number of PTMs have been identified on histones, the biological significance of vast majority of them remains poorly understood. This is particularly the case for those newly discovered histone modifications such as lysine crotonylation, succinylation, fatty-acid acylation, and the modifications present at histone cores such as methylation at H3 lysine 79. Studies of these new PTMs are hindered by the lack of knowledge about their regulating enzymes (i.e., 'writers' and 'erasers') and functional binding proteins (i.e., 'readers'). To fill this knowledge gap, here I present the development of novel chemical tools and approaches, in combination with the state-of-the-art biochemistry, proteomics and cell biology methods, to comprehensively identify 'writers', 'erasers' and 'readers' of histone PTMs and examine their regulatory mechanisms and cellular functions.

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D3-#12

Structural and Molecular Basis of Age-Related Memory Impairment

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How transient events create persistent memories is a yet unsolved question. Although synapses have been established as the basic units of memory, the molecular processes behind long term memory formation and persistence are poorly understood. Among such processes, the experience-dependent self-assembly of the cytoplasmic polyadenylation element-binding (CPEB) protein is a plausible physical storage unit of long-term memories. While CPEB monomer functions as a translation repressor, its aggregate version activates the translation of synaptic mRNAs involved in memory. Through electron cryo-microscopy as well as functional and biochemical tests, we have recently revealed the atomic structure and biochemical activity of aggregated CPEB from 3-7-day old *Drosophila* brains. CPEB in *Drosophila*, known as Orb2, adopts a hydrophilic amyloid state which boosts the translation of mRNA targets associated with memory. Using this knowledge, we may now delve further into the molecular sources of memory decline. Humans and *Drosophila* alike suffer from age-related memory impairment (AMI). In this talk, I will introduce our preliminary experiments showing that hydrophilic Orb2 amyloid is susceptible to changes with AMI. We identified three major and distinct Orb2 amyloid polymorphs - from young flies with effective memories (“young polymorph”), adult flies with slight AMI (“adult polymorph”), and old flies with extreme AMI (“aged polymorph”). Differences in the prevalence of the three major Orb2 amyloid polymorphs found in each group of flies show that as age increases, so does the presence of the aged polymorph - which has a decreased capability to enhance the translation of mRNA related to memory. We believe these changes between a biochemically active (young polymorph) and inactive (aged polymorph) state of Orb2 amyloid could be one factor leading to AMI. Thus, we hope to further understand the correlation between the conformational state of Orb2 amyloid and AMI by examining the functional and atomic-level structure of Orb2 isolated from *Drosophila* brains during ageing. In the long term, the understanding of the molecules behind memory decline and how we can create structure-based strategies to ameliorate AMI could benefit from this research.

D3-#13

The Cryo-EM Structure of a Viral Nucleosome

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The organization of genomic DNA into defined nucleosomes is considered as a universal and ancestral feature of all eukaryotes. This concept has been challenged by the identification of histone homologues in the giant virus family *Marseilleviridae*, which are classified as double-stranded DNA viruses (genomes size of 300 kb-2.8 Mb) that encode histones as fused doublets. We demonstrated that viral histone doublets are localized to cytoplasmic viral factories post-infection, essential for viral infectivity, and ultimately found in mature virions. Despite the limited sequence similarity, the cryo-EM structures of viral nucleosome-like particles showed strong similarities to eukaryotic nucleosomes. However, the unique connectors that link the histone doublets contribute to the observed instability of viral nucleosomes. This further expands the range of 'organisms' that require nucleosomes and suggests a specialized function of histones in the biology of these unusual viruses.

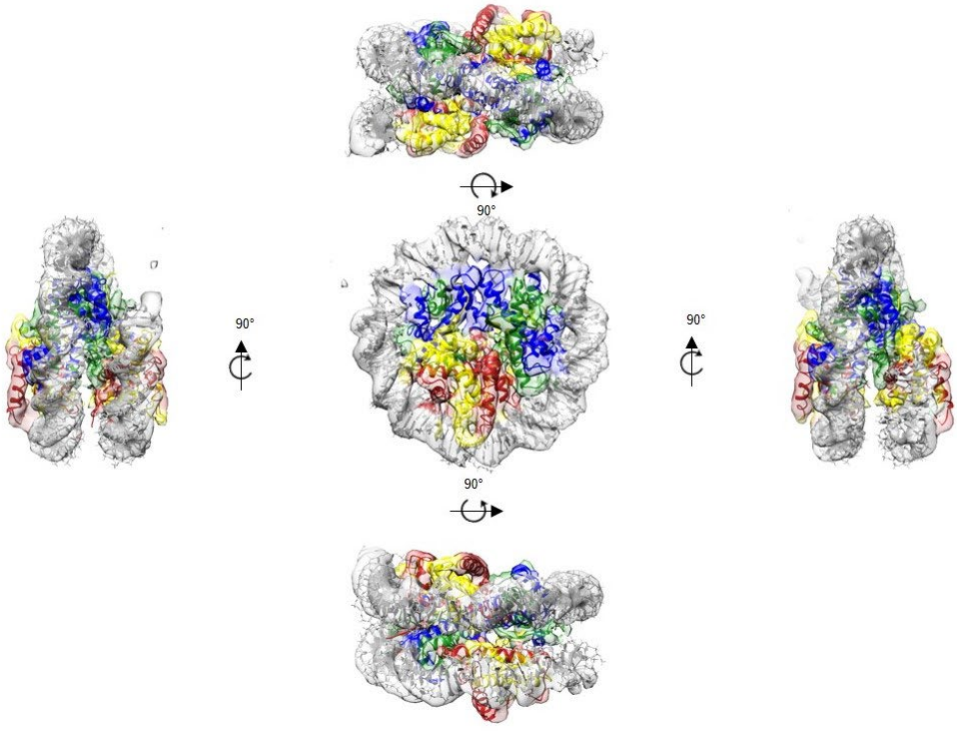

Figure 1. Overview of viral nucleosome-like structures formed by virus-encoded histone doublets.

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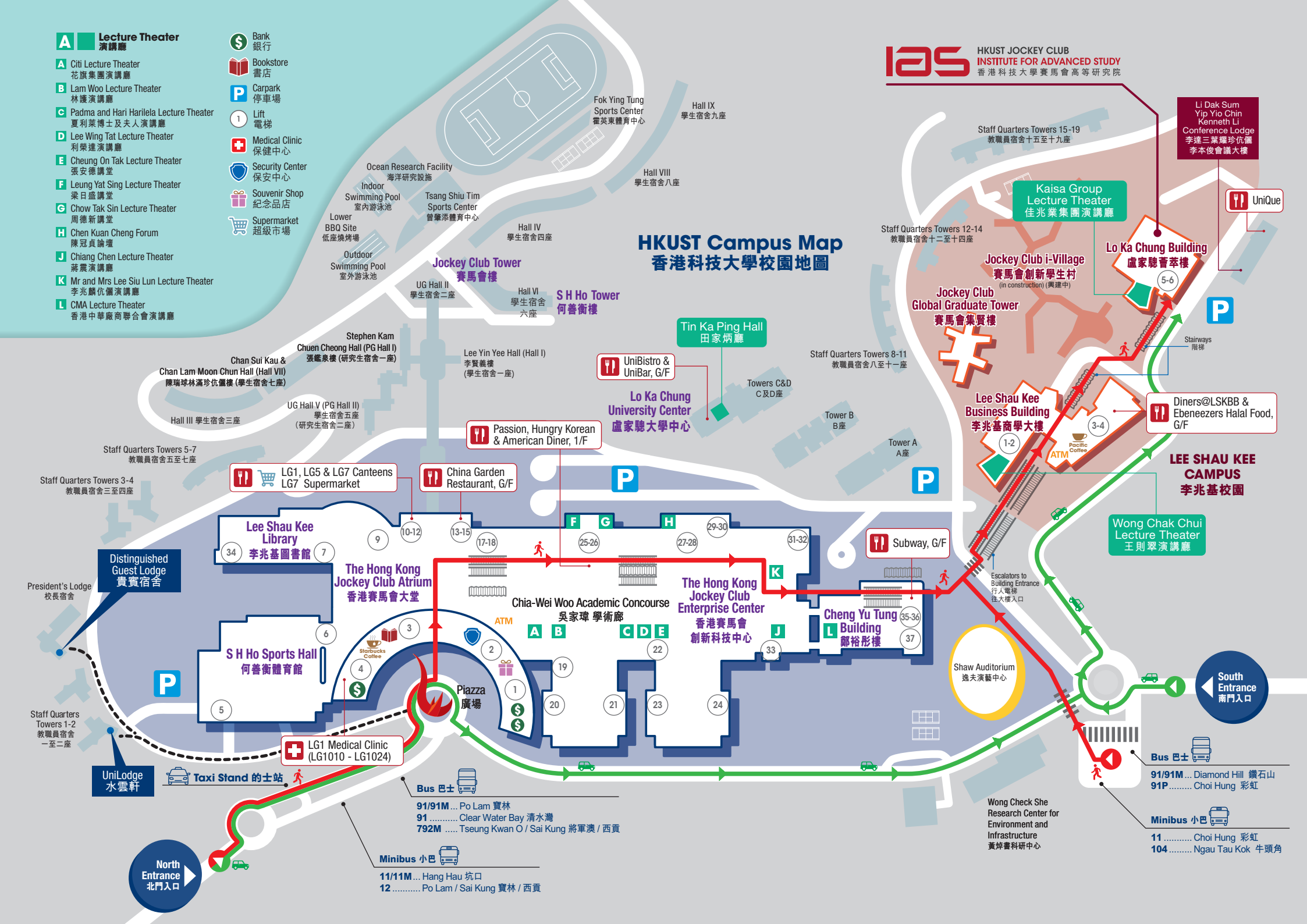
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B Lam Woo Lecture Theater 林護演講廳
C Padma and Hari Harilela Lecture Theater 夏利萊博士及夫人演講廳
D Lee Wing Tat Lecture Theater 利榮達演講廳
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H Chen Kuan Cheng Forum 陳冠貞論壇
J Chiang Chen Lecture Theater 蔣震演講廳
K Mr and Mrs Lee Siu Lun Lecture Theater 李兆麟伉儷演講廳
L CMA Lecture Theater 香港中華廠商聯合會演講廳
Bank 銀行
Bookstore 書店
Carpark 停車場
Lift 電梯
Medical Clinic 保健中心
Security Center 保安中心
Souvenir Shop 紀念品店
Supermarket 超級市場
Fok Ying Tung Sports Center 霍英東體育中心
Hall IX 學生宿舍九座
Hall VIII 學生宿舍八座
Ocean Research Facility 海洋研究設施
Indoor Swimming Pool 室內游泳池
Tsang Shiu Tim Sports Center 曾肇添體育中心
Lower BBQ Site 低座燒烤場
Hall IV 學生宿舍四座
Outdoor Swimming Pool 室外游泳池
Jockey Club Tower 賽馬會樓
UG Hall II 學生宿舍二座
Hall VI 學生宿舍六座
S H Ho Tower 何善衡樓
Stephen Kam Chuen Cheong Hall (PG Hall I) 張鑑泉樓 (研究生宿舍一座)
Chan Sui Kau & Chan Lam Moon Chun Hall (Hall VII) 陳瑞球林滿珍伉儷樓 (學生宿舍七座)
Lee Yin Yee Hall (Hall I) 李賢義樓 (學生宿舍一座)
Tin Ka Ping Hall 田家炳廳
UniBistro & UniBar, G/F
Lo Ka Chung University Center 盧家聰大學中心
Towers C&D C及D座
Tower B B座
Tower A A座
Staff Quarters Towers 8-11 教職員宿舍八至十一座
Staff Quarters Towers 12-14 教職員宿舍十二至十四座
Staff Quarters Towers 15-19 教職員宿舍十五至十九座
Li Dak Sum Yip Yio Chin Kenneth Li Conference Lodge 李達三葉耀珍伉儷李本俊會議大樓
Kaisa Group Lecture Theater 佳兆業集團演講廳
UniQue
Jockey Club i-Village 賽馬會創新學生村 (in construction) (興建中)
Lo Ka Chung Building 盧家聰書萃樓
5-6
Jockey Club Global Graduate Tower 賽馬會集賢樓
Stairways 階梯
Lee Shau Kee Business Building 李兆基商學大樓
1-2
3-4
ATM
Pacific Coffee
Diners@LSKBB & Ebeneezers Halal Food, G/F
LEE SHAU KEE CAMPUS 李兆基校園
Wong Chak Chui Lecture Theater 王則翠演講廳
Escalators to Building Entrance 行人電梯 往大樓入口
Passion, Hungry Korean & American Diner, 1/F
LG1, LG5 & LG7 Canteens
LG7 Supermarket
China Garden Restaurant, G/F
Subway, G/F
Staff Quarters Towers 5-7 教職員宿舍五至七座
Staff Quarters Towers 3-4 教職員宿舍三至四座
Hall III 學生宿舍三座
UG Hall V (PG Hall II) 學生宿舍五座 (研究生宿舍二座)
Lee Shau Kee Library 李兆基圖書館
The Hong Kong Jockey Club Atrium 香港賽馬會大堂
Chia-Wei Woo Academic Concourse 吳家瑋 學術廊
The Hong Kong Jockey Club Enterprise Center 香港賽馬會創新科技中心
Cheng Yu Tung Building 鄭裕彤樓
Distinguished Guest Lodge 貴賓宿舍
President's Lodge 校長宿舍
S H Ho Sports Hall 何善衡體育館
Starbucks Coffee
Piazza 廣場
Shaw Auditorium 逸夫演藝中心
Staff Quarters Towers 1-2 教職員宿舍一至二座
UniLodge 水雲軒
Taxi Stand 的士站
LG1 Medical Clinic (LG1010 - LG1024)
North Entrance 北門入口
South Entrance 南門入口
Bus 巴士
91/91M... Po Lam 寶林
91 ..... Clear Water Bay 清水灣
792M ..... Tseung Kwan O / Sai Kung 將軍澳 / 西貢
Minibus 小巴
11/11M... Hang Hau 坑口
12 ..... Po Lam / Sai Kung 寶林 / 西貢
Bus 巴士
91/91M... Diamond Hill 鑽石山
91P..... Choi Hung 彩虹
Minibus 小巴
11 ..... Choi Hung 彩虹
104 ..... Ngau Tau Kok 牛頭角
Wong Check She Research Center for Environment and Infrastructure 黃焯書科研中心