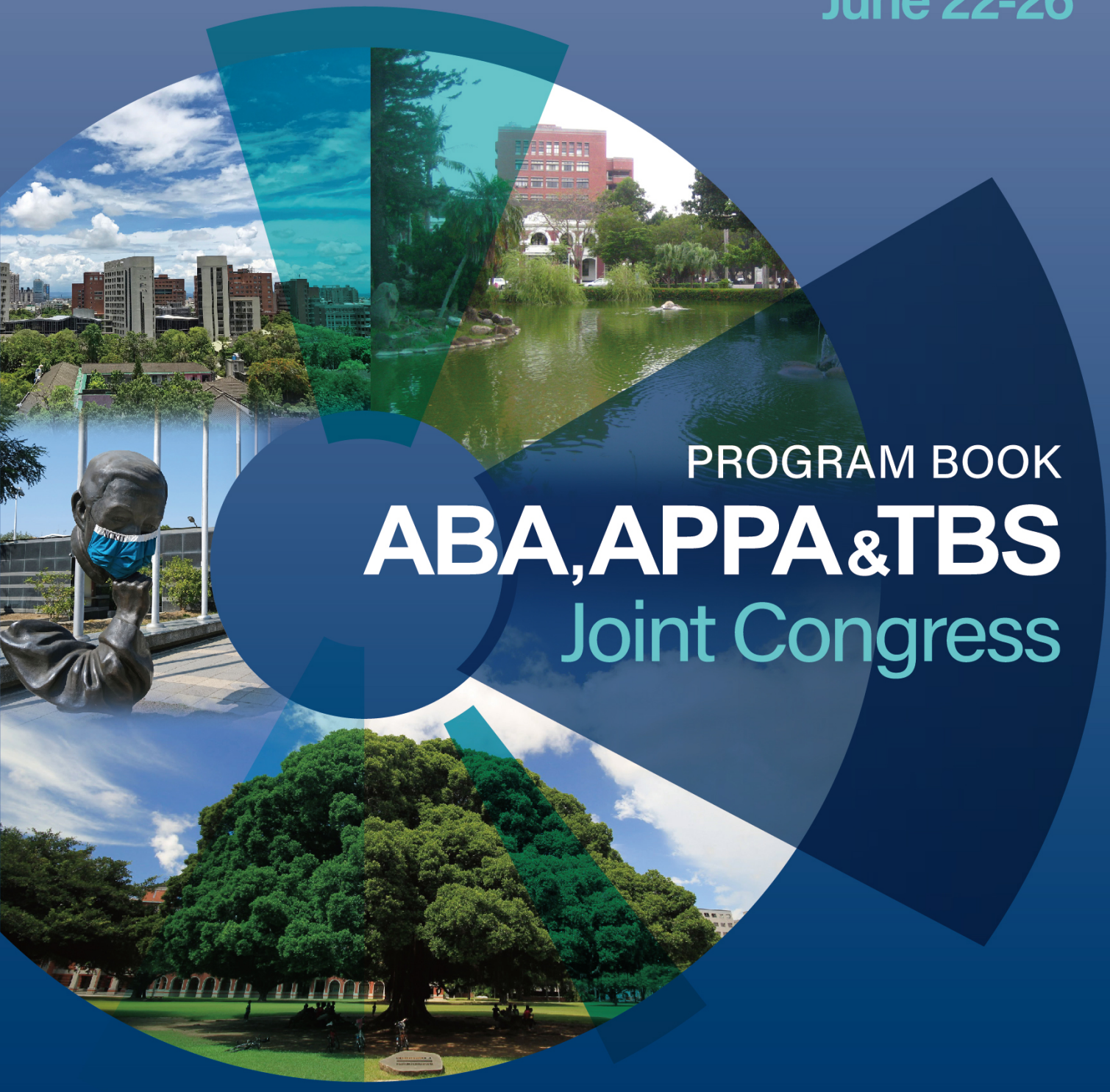
2022
June 22-26



PROGRAM BOOK

ABA,APPA&TBS
Joint Congress

aba-appa-tbs.org

Conference Venue

International Conference Center, National Cheng Kung University,
Tainan, Taiwan (On-Site) and Online Meeting (Cisco Webex)

全自動流式細胞分選儀

CYTOFLEX SRT

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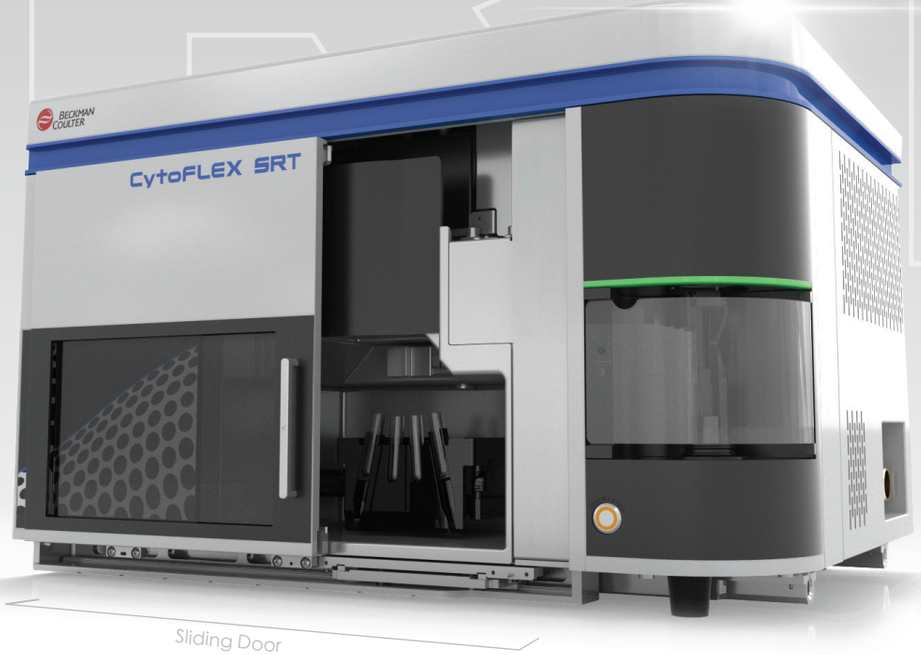

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ABA, APPA and TBS Joint Congress

JUNE 22-26, 2022

NCKU, TAINAN, TAIWAN

Organizers

Asian Biophysics Association

Asia Pacific Protein Association

Biophysical Society of R.O.C.

Local-Organizers

Biophysical Society of R.O.C.

Department of Biochemistry and Molecular Biology, National Cheng Kung University, Taiwan

Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Taiwan

Local Co-organizers

Ministry of Science and Technology Research Promotion Center of Life Science

University Center for Bioscience and Biotechnology, National Cheng Kung University

National Synchrotron Radiation Research Center (NSRRC)

College of Marine Sciences, National Sun Yat-sen University

Biochemical Technology Education Foundation (BTEF)

Foundation of Health Science

Content

Welcome Message

From the President of the Asian Biophysics Association

Dear delegates,

In my role as President of the Asian Biophysics Association (ABA), I am delighted to welcome you to the joint meeting between Asian Biophysics Association, Asia-Pacific Protein Association and the Taiwan Biophysical Society. The objectives of the Asian Biophysics Association are to promote biophysical research and education and to disseminate the knowledge and techniques in biophysics within and among Asian nations and regions. By bringing together the three organizations, the meeting offers delegates a rich experience for networking and hearing about some of the best protein and biophysics research coming out of our region. The hybrid format promises to give delegates the best opportunity for accessing the meeting, and for those attending in person, the wonderful location offers a great cultural experience in addition to the science. I express my gratitude and thanks to the local organizers, as I know first-hand how much work goes on behind the scenes to organize these meetings.

Finally, I wish the meeting the best of success and for delegates to enjoy an outstanding few days of quality science.

Sincerely,

Danny Hatters,
Department of Biochemistry and Pharmacology,
The University of Melbourne
President of the Asian Biophysics Association

Welcome Message

From the President of the Asia Pacific Protein Association

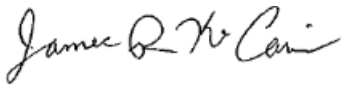
On behalf of the Council of the Asia Pacific Protein Association, I would like to welcome you to the Asia Biophysics Association, Asia Pacific Protein Association, and Taiwan Biophysics Society Joint Congress for 2022. This is the sixth conference of the Asia Pacific Protein Association and the first time to have a joint conference with the Asia Biophysics Association and the Taiwan Biophysics Society, although it is traditional to co-organize the APPA conference with that of the local host society. We are very excited about the outstanding program that our hosts in the Taiwan Biophysics Society have produced.

The Asia Pacific Protein Association started from the Pacific Rim International Conference on Protein Science in Yokahama, Japan in 2004, which was followed by the 2nd PRICPS conference together with the Asia Oceania Human Proteome Organization conference in Cairns, Australia in 2008. At that second conference, the Asia Pacific Protein Association was formally organized to promote conferences and collaboration in protein research in the region. Subsequent conferences were held in Shanghai, China in 2011, Jeju, Korea in 2014 and Bangsaen, Thailand in 2017. In 2020, we were scheduled to hold the World Conference on Protein Science with the Protein Society and the Protein Science Society of Japan in Hokkaido, Japan, which was unfortunately canceled due to the Covid-19 pandemic. We are extremely grateful to the Taiwan Biophysics Society and the Asia Biophysics Association for helping to restart the APPA conferences after this set-back.

In addition to the conferences, the APPA has joined in workshops at national protein organization conferences, such as one held nearly every year at the Protein Science Society of Japan annual symposia. In addition, we have organized the Asia Pacific Protein Association Young Scientist Award to recognize outstanding young scientists in the Asia Pacific region.

This year's conference with the Asia Biophysics Association and the Taiwan Biophysics Society promises to be an outstanding event, especially for those of us itching to get back to learning about outstanding research in protein science and biophysics after the pandemic. We are fortunate that the conference is organized both online and onsite, allowing those who cannot make the quarantine requirements to attend in person to still participate, while allowing those who can attend in person to enjoy the beauty of Tainan, in addition to the joy of getting together to discuss research and build collaborations. I would like to thank the hosts at National Cheng Kung University and the Organizing Committee for helping to organize this wonderful event.

I wish for you all to enjoy the lectures at the ABA, APPA & TPS Joint Congress, and hope we can all learn from each other and build new friendships and collaborations.



James R. Ketudat Cairns

President, Asia Pacific Protein Association

Welcome Message

From the President of the Biophysical Society of R.O.C.

Dear Attendees,

It is of immense pleasure to inform you all that for the first time, the **ABA, APPA & TBS Joint Congress 2022** will be a joint event of the Asian Biophysics Association (ABA), Asia-Pacific Protein Association (APPA), and the Taiwan Biophysical Society (TBS). On behalf of the organizing committee, it is my great pleasure to welcome you all to the ABA, APPA & TBS Joint Congress 2022, scheduled for **June 22 to 26** in Tainan, Taiwan. The main theme of this joint congress will be "Biophysics and Protein Science."

This joint conference will facilitate an insight into the recent trends in research focusing on the amalgamation of cutting-edge technologies from distinct researchers and collaborators to tackle current and future objectives. We will have the opportunity to discover various tactics employed by the young and brilliant researchers to carry on their research during this COVID disease phase. We believe that such scientific interactions especially after 2 years of gap in a post-pandemic scenario may motivate the young minds and facilitate the research community to interact closely to discuss their research plans and future collaborations.

The Taiwan Biophysical Society in collaboration with the Department of Biochemistry and Molecular Biology, National Cheng Kung University will host this grand event. A total of seven sessions will be arranged, covering a broad range of research topics in Biophysics: Biophysics and Medicine, Membrane Biophysiscs, Structural Biology, Bioinformatics, Biophysics in Neuroscience, Imaging and Super-resolution Microscopy, and Biophysical studies on Enzymes or Viral Infection Proteins.

Apart from the main objective of the conference, there will be an opportunity to explore the scenic area around Pingtung for all attendees in a post-conference tour. We will visit the National Museum of Marine Biology and Aquarium which is the most notable museum and research institution for marine biology in Taiwan. The museum also has an 81 metre underwater moving track, the largest underwater tunnel in Asia. The museum also has several major divisions including the experiment center for aquatic life, public facilities, research facilities, maintenance facilities, an international conference center, and an academic research center.

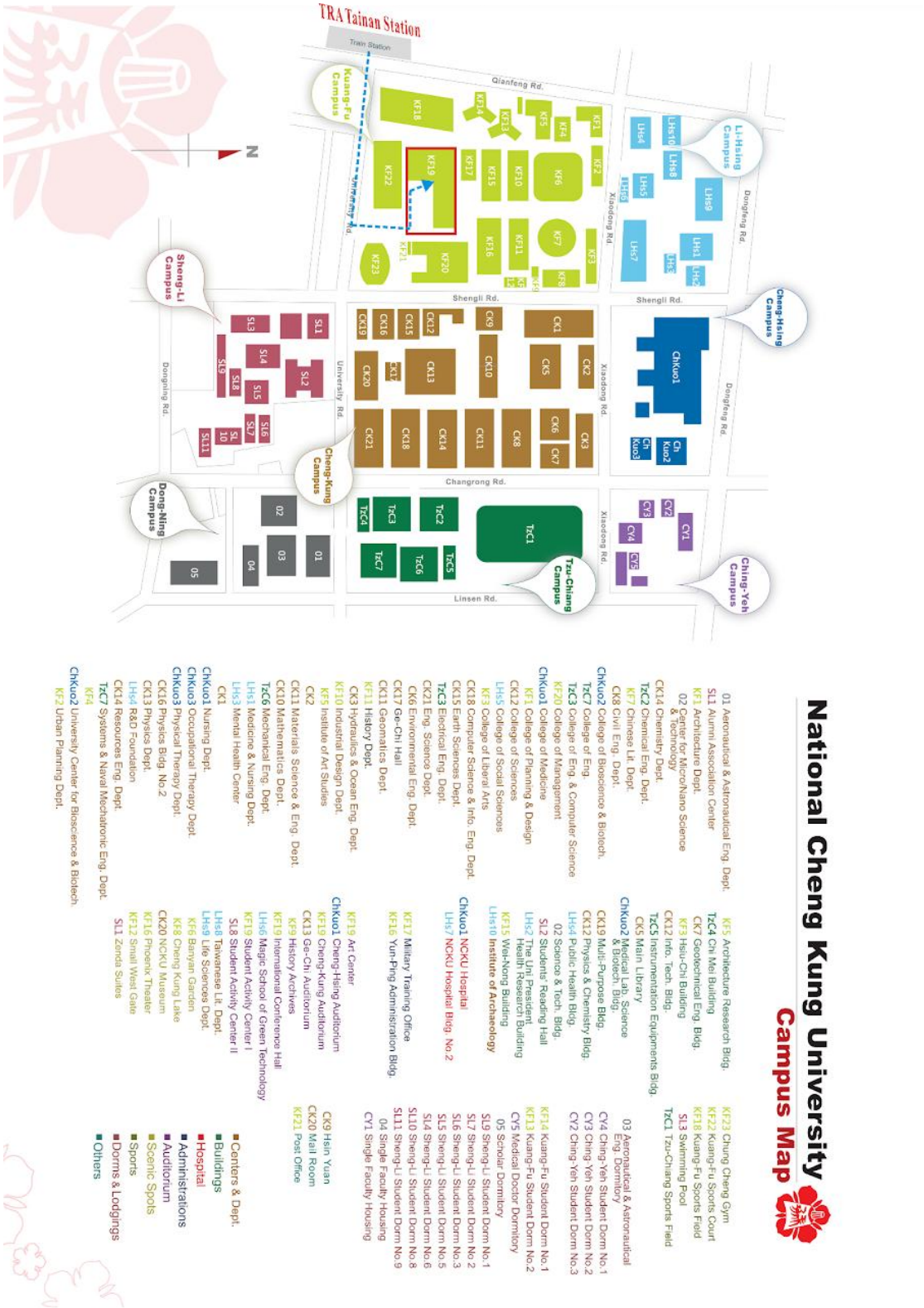
I cordially invite you all to participate in this event and make it a grand success. It will be a great platform to explore the impactful innovations in the Biophysics field and a destination to enrich your knowledge base. Please join us in Tainan, Taiwan. We look forward to seeing you there!

Yours Sincerely,

Dr. Woei-Jer Chuang
President, Biophysical Society of R.O.C.
Associate vice president, National Cheng Kung University (NCKU)
Director, NCKU's Transfer and Business Incubation Center
Professor & Chairman, Department of Biochemistry and Molecular Biology, NCKU

Conference Venue

International Conference Center, Kuang-Fu Campus,
National Cheng Kung University
No.1, University Road, Tainan City 701, Taiwan



The meeting will be held in the B1 floor of International Conference Center, 2nd Lecture Room.

Accommodation

Zenda Suites

國一高速公路 National Highway No.1

- 南下/大灣交流道車程約10分鐘
  Coming from north, exit at Dawan Interchange then follow directions to the Tainan railway station, about 10 minutes driving.
- 北上/仁德交流道車程約15分鐘
  Coming from south, exit at Rende Interchange, follow directions to the city center, about 15 minutes driving.

台鐵 Taiwan Railway

台南火車站後站步行約10分鐘
Exit from Tainan Railway Station rear exit and walk along Dasyue Road, about 10 minutes walk.

高鐵 Taiwan High Speed Rail

台鐵沙崙支線到達台南火車站，出台南火車站後站步行約10分鐘
Take the shuttle at THSR station Tainan to the stop of NCKU Tzu-Chiang Sports Field. Turn right down Dasyue Road, about 10 minutes walk.

市公車 City Bus

- 成大自強校區站: 請搭2號、5號、0左，0右號
  NCKU Tzu-Chiang Campus: Tainan 0L, 0R, 2, 5
- 成大會館站:請搭6路
  Zenda Suites: Tainan 6

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SCIENTIFIC PROGRAM COMMITTEE

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Jie-Rong Huang 黃介嶸
Ming-Daw Tsai 蔡明道
Rita Pei-Yeh Chen 陳佩燁
Shang-Te Danny Hsu 徐尚德
Yun-Ru Ruby Chen 陳韻如

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Shang-Lin Yang 楊尚潾
Cheng Chen 張禎
Ting-Yu Wen 溫婷伃
Cheng-Fu Yen 顏丞甫

SCHEDULE OVERVIEW

Program at a Glance

June 22 (Wed) (GMT +8)	June 23 (Thu) (GMT +8)	June 24 (Fri) (GMT +8)	June 25 (Sat) (GMT +8)	June 26 (Sun) (GMT +8)
	08:00-09:00 APPA Lecture Yuji Goto Chair: Yun-Ru Ruby Chen	08:00-09:00 TBS Lecture Weontae Lee Chair: Ping-Chiang Lyu	08:00-09:00 ABA Lecture Ming-Daw Tsai Chair: Woei-Jer Chuang	08:00-17:30
	09:00-10:30 Biophysics and Medicine I Chair: Rita P.-Y. Chen	09:00-10:30 Structural Biology II Chair: Jie-rong Huang	09:00-9:30 High Resolution Cryo-EM Shangyu Dang Chair: Shang Rung Wu	ePoster Session
	09:00-09:30 Shabih Shakeel	09:00-09:30 Xianyang Fang	09:30-10:00 Coffee Break	
	09:30-10:00 Jack Chun-Chieh Hsu	09:30-10:00 Kuen-Phon Wu	10:00-12:00 Biophysical Studies on Infectious Microbes Chair: Po-Huang Liang	Congress Excursion
	10:00-10:30 Zengyi Chang	10:00-10:30 Chun-Hua Hsu	10:00-10:30 Danaya Pakotiprapha	
	10:30-10:50 Coffee Break	10:30-11:00 Coffee Break	10:30-11:00 Kuakarun Krusong	
	10:50-12:20 Structural Determination of Therapeutic Targets Chair: Shang-Rung Wu	11:00-11:30 APPA Young Scientist Award Rui Zhou Chair: Erinna Lee	11:00-11:30 Ming-Hon Hou	
	10:50-11:20 Shang-Te Danny Hsu			
	11:20-11:50 Susumu Uchiyama	11:30-12:00 Special Talk (Cytiva) Katherine Shen Chair: Shang-Rung Wu	11:30-12:00 Lily Hui-Ching Wang	
11:00-13:20 Registration	11:50-12:20 Saeko Yanaka			
	12:20-13:20 Lunch Time	12:00-13:10 Lunch Time	12:00-13:10 Lunch Time	
13:20-13:30 Opening Remark	13:20-14:50 Membrane Biophysics Chair: Nien-Jen Hu	13:10-15:10 Biophysics in Protein Misfolding and Neuroscience Chair: Yun-Ru Ruby Chen	13:10-13:30 TBS General Meeting	
13:30-14:30 IUPAB Lecture Takayuki Uchihashi Chair: Shang-Te Danny Hsu				
14:30-16:00 Taiwan-Japan Bilateral Biophysics Workshop Chair: Shang-Te Danny Hsu	13:20-13:50 Ichia Chen	13:10-13:40 Danny Hatters	13:30-15:00 Biophysics and Medicine II Chair: Rita P.-Y. Chen	
14:30-15:00 Seiji Kojima	13:50-14:20 Kaavya Krishna Kumar	13:40-14:10 Samir Maji	13:30-14:00 Yu-heng Lau	
15:00-15:30 Hung-Wen Li	14:20-14:50 Wipa Suginta	14:10-14:40 Jianxing Song	14:00-14:30 Che Yang	
15:30-16:00 Tomoya Tsukazaki	14:50-15:10 Coffee Break	14:40-15:10 Pingbo Huang	14:30-15:00 Wei-Chun Kao	
16:00-16:30 Coffee Break	15:10-17:10 Structural Biology I Chair: Shu-Ying Wang	15:10-15:40 Coffee Break	15:00-15:30 Coffee Break	
16:30-18:00 Chair: Shang-Te Danny Hsu	15:10-15:40 Bostjan Kobe	15:40-17:10 Bioinformatics Chair: Lee-Wei Yang	15:30-16:30 Felicia Chen-Wu Memorial Lecture Zihe Rao Chair: David Tai-Wei WU	
16:30-17:00 Yu-Yuan Hsiao	15:40-16:10 Hee-Jung Choi	15:40-16:10 Sun Choi		
17:00-17:30 Takeshi Haraguchi	16:10-16:40 John (Zenghui) Zhang	16:10-16:40 Carmay Lim		
17:30-18:00 Manuel Maestre Reyna	16:40-17:10 Guang Zhu	16:40-17:10 Akio Kitao	16:30-17:00 Poster Awards Closing Remarks	
18:10-19:10 Poster Presentation (On-site Poster)	17:20-18:20 Poster Presentation (On-site Poster)	17:20-18:40 Oral Presentation for Selected Poster (On-site Poster)		
19:10 ~ Welcome Reception	18:40 ~ TBS Council Meeting	19:00 ~ Congress Banquet		

SCIENTIFIC PROGRAM

DAY 1 **June 22 (Wed) (GMT +8)**

11:00-13:20		Registration
13:20-13:30		Opening Remark
13:30-14:30		**IUPAB Lecture** *Chair: Shang-Te Danny Hsu (徐尚德), Research Fellow, Institute of Biological Chemistry, Academia Sinica, Taiwan* **Dynamic Structural Biology Driven By High-Speed Atomic Force Microscopy** Takayuki Uchihashi (内橋貴之) Professor, Department of Physics, Nagoya University, Japan
14:30-18:00		**Taiwan-Japan Bilateral Biophysics Workshop** *Chair: Shang-Te Danny Hsu (徐尚德), Research Fellow, Institute of Biological Chemistry, Academia Sinica, Taiwan*
14:30-15:00	**IL-1**	**Structure-based functional studies of stator, the energy-converting membrane protein complex in the bacterial flagellar motor** Seiji Kojima (小嶋誠司), Professor, Division of Biological Science, Graduate School of Science, Nagoya University, Japan
15:00-15:30	**IL-2**	**SWI5-SFR1 Accessory Proteins Stimulate RAD51 Recombinase Filament Assembly by Reducing its Extension Units to DNA** Hung-Wen Li (李弘文), Professor, Department of Chemistry, National Taiwan University, Taiwan
15:30-16:00	**IL-3**	**Crystal structure of YeeE, a membrane protein, engaged in thiosulfate uptake** Tomoya Tsukazaki (塚崎智也), Professor, Division of Biological Science, Nara Institute of Science and Technology, Japan
16:00-16:30		Coffee Break
16:30-17:00	**IL-4**	**APE1 exonuclease distinguishes various DNA substrates by an induced space-filling mechanism** Yu-Yuan Hsiao (蕭育源), Professor, Department of Biological Science & Technology, National Yang Ming Chiao Tung University, Taiwan

17:00-17:30	**IL-5**	**Functional analysis of plant-specific myosin XIs** Takeshi Haraguchi (原口武士), Assistant Professor, Department of Biology, Graduate School of Science, Chiba University, Japan
17:30-18:00	**IL-6**	**Decrypting cryptochrome signal transduction via time-resolved structural analysis** Manuel Maestre Reyna (馬左仲), Visiting Scholar, Institute of Biological Chemistry, Academia Sinica, Taiwan
18:10-19:10		Poster Presentation (For On-Site Poster)
19:10 -		Welcome reception

SCIENTIFIC PROGRAM

DAY 2 **June 23 (Thu) (GMT +8)**

08:00-09:00		**APPA Lecture** *Chair: Yun-Ru Ruby Chen (陳韻如), Research Fellow, Genomics Research Center, Academia Sinica, Taiwan* **Linking protein folding and misfolding under macromolecular crowding** Yuji Goto (後藤祐児), Professor, Global Center for Medical Engineering and Informatics, Osaka University, Japan
09:00-10:00		**Biophysics and Medicine I** *Chair: Dr. Rita P.-Y. Chen (陳佩燁), Research Fellow and Deputy Director, Institute of Biological Chemistry, Academia Sinica, Taiwan*
09:00-09:30	**IL-7**	**Structures of protein complexes involved in Fanconi Anemia DNA Repair** Shabih Shakeel, Lab Head & Principal Research Fellow, School of Biomedical Sciences, The University of Melbourne, Australia
09:30-10:00	**IL-8**	**Translational shutdown and immune evasion by SARS-CoV-2 NSP14 protein** Jack Chun-Chieh Hsu (許淳傑), Postdoctoral Associate, Department of Immunobiology, Yale School of Medicine, USA
10:00-10:30	**IL-9**	**Exploring proteins in living cells: Discovery of the reversible regrowth-delay body in non-dividing and non-growing bacterial cells** Zengyi Chang (昌增益), Professor, School of Life Science, Peking University, China
10:30-10:50		Coffee Break
10:50-12:20		**Structural Determination of Therapeutic Targets** *Chair: Shang-Rung Wu (吳尚蓉), Associate Professor, School of Dentistry and Institute of Oral Medicine, National Cheng Kung University, Taiwan*

10:50-11:20 **IL-10** **To bind or not to bind: the tale of CoV spikes**
Shang-Te Danny Hsu (徐尚德), Research Fellow, Institute of Biological Chemistry, Academia Sinica, Taiwan

11:20-11:50 **IL-11** **Thermodynamic, kinetic and structural investigations on antigen-IgG-FcγRIIIa interactions**
Susumu Uchiyama (内山進), Professor, Department of Biotechnology, Osaka University, Japan

11:50-12:20 **IL-12** **Multifaceted observation of conformational dynamics and interactions of antibodies**
Saeko Yanaka (谷中冴子), Assistant Professor, Institute for Molecular Science, National Institute of Natural Science, Japan

12:20-13:20 Lunch Time

13:20-14:50 **Membrane Biophysics**
Chair: Nien-Jen Hu (胡念仁), Associate Professor, Institute of Biochemistry, National Chung Hsing University, Taiwan

13:20-13:50 **IL-13** **Glutamate transporters have a chloride channel with two hydrophobic gates**
Ichia Chen (陳乙嘉), Postdoctoral Fellow, Genomics Research Center, Academia Sinica, Taiwan

13:50-14:20 **IL-14** **Structural insights into G-protein coupled receptor activation**
Kaavya Krishna Kumar, Postdoctoral Fellow, School of Medicine, Stanford University, USA

14:20-14:50 **IL-15** **Structural Insights into Chitin Utilization by Marine Vibrio species**
Wipa Suginta, Professor, School of Biomolecular Science & Engineering, Vidyasirimedhi Institute of Science and Technology, Thailand

14:50-15:10 Coffee Break

15:10-17:10 **Structural Biology I**
Chair: Shu-Ying Wang (王淑鶯), Associate Professor, Department of Microbiology and Immunology, National Cheng Kung University, Taiwan

15:10-15:40	**IL-16**	**Signalling by cooperative assembly formation (SCAF) by TIR domains in innate immunity and cell death pathways** Bostjan Kobe, Professor, School of Chemistry & Molecular Biosciences, University of Queensland, Australia
15:40-16:10	**IL-17**	**Structural basis of Neuropeptide Y1 receptor activation** Hee-Jung Choi (최희정) (崔熙貞), Professor, Biological Science, Seoul National University, Korea
16:10-16:40	**IL-18**	**Hot spots and mutational effect in protein-protein interaction** John (Zenghui) Zhang (張增輝), Professor, Shenzhen Institute of Synthetic Biology, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China NYU-ECNU Center for Computational Chemistry at NYU Shanghai, Shanghai, China
16:40-17:10	**IL-19**	**G-quadruplex structures formed by human telomeric DNA and C9orf72 hexanucleotide repeats** Guang Zhu (朱廣), Professor, Department of Chemical and Biological Engineering, The Hong Kong University of Science and Technology, Hong Kong
17:20-18:20		Poster Presentation (For On-Site Poster)
18:40 -		TBS Council Meeting

SCIENTIFIC PROGRAM

DAY 3 **June 24 (Fri) (GMT +8)**

08:00-09:00		**TBS Lecture** *Chair: Ping-Chiang Lyu (呂平江), Professor and Senior Vice President, Department of Life Science and Institute of Bioinformatics and Structural Biology, National Tsing Hua University, Taiwan* **Uncovering membrane protein structure and dynamics by cryo-EM and time-resolved serial femtosecond crystallography** Weontae Lee (이원태) (李元泰), Professor, Department of Biochemistry, College of Life Science and Biotechnology, Yonsei University, Korea
09:00-10:30		**Structural Biology II** *Chair: Jie-rong Huang (黃介嶸), Associate Professor, Institute of Biochemistry and Molecular Biology, National Yang Ming Chiao Tung University, Taiwan*
09:00-09:30	**IL-20**	**Structural mechanism for modulation of functional amyloid and biofilm formation by Staphylococcal Bap protein switch** Xianyang Fang (方顯楊), Assistant Professor, School of Life Sciences, Tsinghua University, China
09:30-10:00	**IL-21**	**Structural polymorphism of α-synuclein amyloid fibrils implicated in Parkinson's disease** Kuen-Phon Wu (吳昆峯), Assistant Research Fellow, Institute of Biological Chemistry, Academia Sinica, Taiwan
10:00-10:30	**IL-22**	**Proline Isomerization and Molten Globular Property of TgPDCD5 Confer its Regulation of Heparan Sulfate Binding** Chun-Hua Hsu (徐駿森), Professor, Department of Agricultural Chemistry, National Taiwan University, Taiwan
10:30-11:00		Coffee Break

11:00-11:30		**APPA Young Scientist Award** *Chair: Erinna Lee, Associate Professor, Laboratory Head, La Trobe Institute for Molecular Science & Visiting Scientist, Olivia Newton-John Cancer Research Institute, Australia*
		Structural and functional insight into human γ-secretase regulation Rui Zhou (周瑞), R.A., School of Life Sciences, Tsinghua University, China
11:30-12:00		**Special Talk** *Chair: Shang-Rung Wu (吳尚蓉), Associate Professor, School of Dentistry and Institute of Oral Medicine, National Cheng Kung University, Taiwan*
		Purifying samples and functional study for Cryo-EM Katherine Shen (沈家珮), Senior Product Specialist, Cytiva
12:00-13:10		Lunch Time
13:10-14:40		**Biophysics in Protein Misfolding and Neuroscience** *Chair: Yun-Ru Ruby Chen (陳韻如), Research Fellow, Genomics Research Center, Academia Sinica, Taiwan*
13:10-13:40	**IL-23**	**Problematic proteins in neurodegenerative disease: Sticky protein misassembly mechanisms** Danny Hatters, Professor, School of Biomedical Sciences, The University of Melbourne, Australia
13:40-14:10	**IL-24**	**Liquid-Liquid phase separation of proteins and polypeptides** Samir Maji, Professor, Department of Biosciences & Bioengineering, Indian Institute of Technology Bombay, India
14:10-14:40	**IL-25**	**ATP modulates phase separation of SARS-CoV-2 N protein druggable by HCQ** Jianxing Song, Associate Professor, Department of Biological Sciences, National University of Singapore, Singapore
14:40-15:10	**IL-26**	**TMC1, LHFPL5, and the mechanotransduction complex in hair cells** Pingbo Huang (黃平波), Professor, Department of Chemical and Biological Engineering, The Hong Kong University of Science and Technology, Hong Kong

15:10-15:40		Coffee Break
15:40-17:10		**Bioinformatics** *Chair: Lee-Wei Yang (楊立威), Director and Professor, Institution of Bioinformatics and Structural Biology, National Tsing Hua University, Taiwan*
15:40-16:10	**IL-27**	**Comprehensive computational studies of membrane proteins for the structural and functional elucidation and drug discovery** Sun Choi (최선) (崔善), Professor, College of Pharmacy & Graduate School of Pharmaceutical Sciences, Ewha Womans University, Korea
16:10-16:40	**IL-28**	**From Quantum-derived Principles Governing Cysteine Reactivity to Combating the COVID-19 Pandemic** Carmay Lim (林小喬), Distinguished Research Fellow, Institute of Biomedical Sciences, Academia Sinica, Taiwan
16:40-17:10	**IL-29**	**Conformational Change and Dissociation/Association of Biological Macromolecules Investigated by Parallel Cascade Selection Molecular Dynamics and Markov State Model** Akio Kitao (北尾彰朗), Professor, School of Life Science & Technology, Tokyo Institute of Technology, Japan
17:20-18:40		Oral Presentation for Selected Poster (For On-Site Poster)
19:00 -		Congress Banquet

SCIENTIFIC PROGRAM

DAY 4 **June 25 (Sat) (GMT +8)**

08:00-09:00		**ABA Lecture** *Chair: Woei-Jer Chuang (莊偉哲), Chair Professor and Vice President, Department of Biochemistry and Molecular Biology, National Cheng Kung University, Taiwan* **Dynamics in Enzyme Catalysis by Cryo-EM and XFEL** Ming-Daw Tsai (蔡明道), Academician & Distinguished Visiting Chair, Institute of Biological Chemistry, Academia Sinica, Taiwan
09:00-09:30		**High Resolution Cryo-EM** *Chair: Shang-Rung Wu (吳尚蓉), Associate Professor, School of Dentistry and Institute of Oral Medicine, National Cheng Kung University, Taiwan*
09:00-09:30	**IL-30**	**Application of MSBP improves particle distribution and orientation in single-particle cryo-EM** Shangyu Dang (黨尚宇), Assistant Professor, Division of Life Science, The Hong Kong University of Science and Technology, Hong Kong
09:30-10:00		Coffee Break
10:00-12:00		**Biophysical Studies on Infectious Microbes** *Chair: Po-Huang Liang (梁博煌), Research Fellow, Institute of Biological Chemistry, Academia Sinica, Taiwan*
10:00-10:30	**IL-31**	**Regulation of *Acinetobacter baumannii* p-hydroxyphenylacetate degradative gene cluster by HpaR transcription factor** Danaya Pakotiprapha, Assistant Professor, Department of Biochemistry, Mahidol University, Thailand
10:30-11:00	**IL-32**	**Design of HIV-1 protease inhibitors** Kuakarun Krusong, Associate Professor, Department of Biochemistry, Chulalongkorn University, Thailand

11:00-11:30	**IL-33**	**Nucleocapsid capsid protein is a potential target for drug discovery against coronavirus** Ming-Hon Hou (侯明宏), Distinguished Professor & Principal Investigator, Institute of Genomics & Bioformatics, National Chung Hsing University, Taiwan
11:30-12:00	**IL-34**	**Evolution of SARS-CoV-2 spike protein push forward to cross-species infection** Lily Hui-Ching Wang (王慧菁), Associate Professor, Institute of Molecular & Cellular Biology, National Tsing Hua University, Taiwan
12:00-13:10		Lunch Time
13:10-13:30		TBS General Meeting
13:30-15:00		**Biophysics and Medicine II** *Chair: Rita P.-Y. Chen (陳佩燁), Research Fellow and Deputy Director, Institute of Biological Chemistry, Academia Sinica, Taiwan*
13:30-14:00	**IL-35**	**Synthetic control of molecular flux into protein cages** Yu-Heng Lau, Senior Lecture & Research Fellow, School of Chemistry, The University of Sydney, Australia
14:00-14:30	**IL-36**	**Targeting molecular interface to de novo design of precision immunogens for respiratory syncytial virus** Che Yang (楊哲), Scientist, Molecular Modelling & Design, Novo Nordisk A/S, Denmark
14:30-15:00	**IL-37**	**Cryo-EM structure of the actinobacterial respiratory supercomplex: an efficient generator and versatile drug target.** Wei-Chun Kao (高偉鈞), Scientist, Institute of Biochemistry & Molecular Biology, University of Freiburg, Germany
15:00-15:30		Coffee Break
15:30-16:30		**Felicia Chen-Wu Memorial Lecture** *Chair: Dr. David Tai-Wei WU (吳台偉), Research Fellow and Director, Institute of Chemistry, Academia Sinica, Taiwan* **Living of SARS-CoV-2 inside the cell: Understand SARS-CoV-2 replication and transcription from structures** Zihe Rao (饒子和), Professor, School of Medicine, Tsinghua University & Academician, Chinese Academy of Sciences, China
16:30-17:00		Poster Awards & Closing Remarks

LIST OF ABSTRACTS

IL-9 Zengyi Chang (昌增益)

Exploring proteins in living cells: Discovery of the reversible regrowth-delay body in non-dividing and non-growing bacterial cells

IL-10 Shang-Te Danny Hsu (徐尚德)

To bind or not to bind: the tale of CoV spikes

IL-11 Susumu Uchiyama (内山進)

Thermodynamic, kinetic and structural investigations on antigen-IgG-FcγRIIIa interactions

IL-12 Saeko Yanaka (谷中冴子)

Multifaceted observation of conformational dynamics and interactions of antibodies

IL-13 Ichia Chen (陳乙嘉)

Glutamate transporters have a chloride channel with two hydrophobic gates

IL-14 Kaavya Krishna Kumar

Structural insights into G-protein coupled receptor activation

IL-15 Wipa Suginta

Structural Insights into Chitin Utilization by Marine Vibrio species

IL-16 Bostjan Kobe

Signalling by cooperative assembly formation (SCAF) by TIR domains in innate immunity and cell death pathways

IL-17 Hee-Jung Choi (최희정) (崔熙貞)

Structural basis of Neuropeptide Y1 receptor activation

IL-18 John (Zenghui) Zhang (張增輝)

Hot spots and mutational effect in protein-protein interaction

IL-19 Guang Zhu (朱廣)

G-quadruplex structures formed by human telomeric DNA and C9orf72 hexanucleotide repeats

TBS Lecture

Weontae Lee (이원태) (李元泰),
Uncovering membrane protein structure and dynamics by cryo-EM and time-resolved serial femtosecond crystallography

IL-20 Xianyang Fang (方顯楊)
Structural mechanism for modulation of functional amyloid and biofilm formation by Staphylococcal Bap protein switch

IL-21 Kuen-Phon Wu (吳昆峯)
Structural polymorphism of α-synuclein amyloid fibrils implicated in Parkinson's disease

IL-22 Chun-Hua Hsu (徐駿森)
Proline Isomerization and Molten Globular Property of TgPDCD5 Confer its Regulation of Heparan Sulfate Binding

APPA Young Scientist Award: Rui Zhou (周瑞)
Structural and functional insight into human γ-secretase regulation

IL-23 Danny Hatters
Problematic proteins in neurodegenerative disease: Sticky protein misassembly mechanisms

IL-24 Samir Maji
Liquid-Liquid phase separation of proteins and polypeptides

IL-25 Jianxing Song
ATP modulates phase separation of SARS-CoV-2 N protein druggable by HCQ

IL-26 Pingbo Huang (黃平波)
TMC1, LHFPL5, and the mechanotransduction complex in hair cells

IL-27 Sun Choi (최선) (崔善)
Comprehensive computational studies of membrane proteins for the structural and functional elucidation and drug discovery

IL-28 Carmay Lim (林小喬)

From Quantum-derived Principles Governing Cysteine Reactivity to Combating the COVID-19 Pandemic

IL-29 Akio Kitao (北尾彰朗)

Conformational Change and Dissociation/Association of Biological Macromolecules Investigated by Parallel Cascade Selection Molecular Dynamics and Markov State Model

ABA Lecture Ming-Daw Tsai (蔡明道)

Dynamics in Enzyme Catalysis by Cryo-EM and XFEL

IL-30 Shangyu Dang (黨尚宇),

Application of MSBP improves particle distribution and orientation in single-particle cryo-EM

IL-31 Danaya Pakotiprapha

Regulation of *Acinetobacter baumannii* p-hydroxyphenylacetate degradative gene cluster by HpaR transcription factor

IL-32 Kuakarun Krusong

Design of HIV-1 protease inhibitors

IL-33 Ming-Hon Hou (侯明宏)

Nucleocapsid capsid protein is a potential target for drug discovery against coronavirus

IL-34 Lily Hui-Ching Wang (王慧菁)

Evolution of SARS-CoV-2 spike protein push forward to cross-species infection

IL-35 Yu-Heng Lau

Synthetic control of molecular flux into protein cages

IL-36 Che Yang (楊哲)

Targeting molecular interface to de novo design of precision immunogens for respiratory syncytial virus

IL-37 Wei-Chun Kao (高偉鈞),

Cryo-EM structure of the actinobacterial respiratory supercomplex: an efficient generator and versatile drug target.

Felicia Chen-Wu Memorial Lecture: Zihe Rao (饒子和)

Living of SARS-CoV-2 inside the cell: Understand SARS-CoV-2 replication and transcription from structures

LIST OF POSTER PRESENTATION

Poster Abstract List:

I. Biophysics and Medicine (BM)		
No.	Name/Topic	Presentation Type
BM-01	**Ting-Yi Yu** Functional analysis between AtSAP5 and AtTrx3 in the reduced salicylic acid immunity pathway	Online
BM-02	**Ya-Ting Wang** The Inhibition and Activation Effects of Integrin-targeting Drugs	On-site
BM-03	**Yun-Chu Chou** The Molecular Mechanism of Fluorescence Thermometer: Local Dynamic Motions of Tryptophan governs the Dependence of Fluorescence Intensity on Temperature	On-site
BM-04	**Mei-Ling Shih** Engineering of Interleukin-2 Family Cytokines to Improve its Half-life and Stability for T Cell Therapy	On-site
BM-05	**Shang-Lin Yang** Study and optimize the functional characterization of recombinant mutant tissue-type plasminogen activator fused with platelet-targeting disintegrin	On-site+ Online
BM-06	**Manoj Kumar Sriramoju** Biophysical characterization of cancer-associated BAP1 mutants reveals an inherent tendency to form fibrillar aggregates	On-site
BM-07	**Pin-Shin Kuo** Design and Application of Antimicrobial/Anticancer Peptides	On-site+ Online
BM-08	**Atsushi Yabushita** Primary reaction of red fluorescence protein observed by fastscan femtosecond spectroscopy system	On-site

II. Biophysical Studies on Enzymes or Viral Infection Proteins (BS)		
No.	Name/Topic	Presentation Type
BS-01	**Cheng-Yu Li** Structural and functional elucidation of adenylation domain of nonribosomal peptide synthetase-like protein in Aspulvinone E biosynthesis	Online
BS-02	**Yong-Sheng Wang** The novel conformational landscape of PEDV S trimeric glycoprotein	On-site+ Online
BS-03	**Zi-Wen Weng** Towards atomic insights into the interplay between SARS-CoV-2 E and M proteins.	On-site
BS-04	**Dan-Ni Wu** Vaccinia virus EFC protein A28 interact with virus envelope protein A26 to deactivate fusion activity and conduct endocytosis	Online
BS-05	**Yan-Liang Chen** Switching natural cofactor NAD^+ into NMN^+ in 3α-hydroxysteroid dehydrogenase/carbonyl reductase from *Comamonas testosteroni*	On-site
BS-06	**Yun-Hao Chou** The study of thermodynamic stability of 3α-Hydroxysteroid Dehydrogenase/Carbonyl Reductase from *Comamonas testosteroni* by differential scanning fluorimetry	On-site
BS-07	**Yu-Shian Wang** Characterizing the interaction between ALG-2-interacting protein X and galectin-3 in promoting HIV-1 budding	Online
BS-08	**Wei-Chun Huang** Functional investigation of the polysaccharide repeating units flippase Wzx of *salmonella* SL1344	On-site+ Online
III. Imaging and Super-resolution Microscopy (IM)		
No.	Name/Topic	Presentation Type
IM-01	**Yi-Teng Hsiao** Direct visualization of chromatin dynamics in live cells by label-free interference microscopy	Online

IV. Membrane Biophysics (MB)		
No.	Name/Topic	Presentation Type
MB-01	**Samuel Herianto** Bottom-up creation of liposome-based artificial cells: from cell-free gene expression to the reconstitution of membrane synthesis in liposomes	On-site
MB-02	**David Tzu-Wei Wang** Studying the Effect of Cholesterol on the Structure of Membrane-Spanning Region of Amyloid-Precursor Proteins by Electron Spin Resonance Spectroscopy	Online
V. Structure Biology (SB)		
No.	Name/Topic	Presentation Type
SB-01	**Yin-Chen Mao** Enzymatic activity and structural study of human mitochondrial genome maintenance exonuclease 1	On-site
SB-02	**Tsong-Wei Chen** The role of DNA polymerase γ accessory subunit in mitochondrial DNA degradation	On-site
SB-03	**Chun-Ru, Wu** Structural study of human RNA:m^5C methyltransferase NSUN2	On-site
SB-04	**Deepu Dowarha** Expression in *Pichia pastoris* and characterization of chlorotoxin, an anti-glioma migration agent	On-site+ Online
SB-05	**Wei-Jiun Tsai** Structure of the unique cytolysin-mediated translocation apparatus of Group A *Streptococcus*	On-site+ Online
SB-06	**Tzu-Lu Lin** Characterization of the structure–function relationship of antimicrobial peptides, RR14	On-site+ Online
SB-07	**Tzu-Lu Lin** Characterization of the structure–function relationship of antimicrobial peptides to develop new antibacterial agents	On-site+ Online
SB-08	**Revansiddha Hanamant Katte** A Novel Disulfide Bond Engineering of Fibronectin Type III Domain Enhances Thermostability and Solubility of VEGFR2-Specific Antagonist	On-site

SB-09	**Kai-Cheng Chang** Toward structural and Functional Study of Spo11: the Initiator of Meiotic Recombination	On-site
SB-10	**Yuen Ting Chan** Vitroprocines, tyrosine-polyketide antibiotics, biosynthesized via newly discovered α-oxoamine Synthase and oxidoreductase	Online
SB-11	**Roshan Satange** Targeting DNA mismatches by two distinct intercalators exhibit synergistic effect against mismatch repair deficient cancers	On-site
SB-12	**Cheng-Wei Huang** Receptor recognition of A-type trisaccharide by the receptor-binding domain of the spike protein of SARS-CoV-2	On-site
SB-13	**Ning-En Chang** Structure-function relationships of human coronavirus spike proteins by cryo-EM, mass spectrometry and molecular modeling	On-site
SB-14	**Yan-Ju Lu** A Seven-Bladed β-Propeller Protein can Catalyze a Benzilic Acid Rearrangement in Newly Ascorbate Catabolic Pathway	Online
SB-15	**Yao-Tsung Chang** Structural and functional insights into the Rhodostomin ^{48}ARGDXP motif for the recognition specificity of integrins	On-site
SB-16	**Yun-Yu Chiu** Structural analysis of RNA/DNA hybrid recognition by DEAD-box helicase DDX41	On-site
SB-17	**Chia-Yun Wu** The exoribonuclease activity of TREX1 in RNA and DNA/RNA hybrid metabolism	Online
SB-18	**Tsai-Hsuan Han** The mechanism study of the clinical DNA intercalator-Doxorubicin targeting DNA duplexes for developing potential cancer therapy	On-site
SB-19	**Kuan-Wei Huang** Allosteric Inhibition mechanism of PCMPS and PCMB on Lassa Fever Virus NP exonuclease	Online

SB-20	**Monika Jain** Structural insights into human mitochondrial RNA decay promoted by Suv3 helicase	On-site
SB-21	**Piotr Draczkowski** Structural basis of proteasomal recognition of a branched ubiquitin chain.	On-site
SB-22	**Yu-Xi Tsai** GlycoSHIELD of the spike proteins of human coronaviruses NL63 and 229E	On-site
SB-23	**Chih-Hsuan Lai** Structural Insight into ZFAND1 and p97 Interaction	On-site
SB-24	**Wei‑Tung Wang** Expression, Purification and Characterization of *At*WAT1	On-site
SB-25	**Sin-Hong Lew** Assembly of phospholipid nanodiscs for structural studies of *At*GTR1	On-site
SB-26	**Yi-Shiang Wang** A Molecular Basis for Malfunction in Disease Mutant A97S of Transthyretin	On-site+ Online
SB-27	**Yu-Chen Chen** Roles of The Conserved Traits in Proteins' Intrinsically Disordered Regions: Using Galectin-3 As an Example	Online
SB-28	**Nuzhat Parveen** Pentamidine and its derivatives inhibiting cell proliferation by blocking the interaction between S100A1 and RAGE V domain	Online
SB-29	**Shen Wang** Dissecting Role of Unstructured N-terminal Region of Peptidoglycan Hydrolase MepS in Adaptor-mediated Degradation	On-site+ Online
SB-30	**Hsuan-Yu Huang** Structural basis of transcription activation by the OmpR/PhoB family response regulator PmrA	On-site
SB-31	**Chao-Cheng Cho** Structural Insights into Inhibition of Human DNA Methyltransferases	On-site

SB-32	**Yu-Chuan Chiu** Biochemical characterization of the Hemolytic Mechanism of α-hemolysin from *Vibrio campbellii*	On-site
SB-33	**Hao-Ting Chang** Refinement of BAP1 Structural Ensembles by SAXS-restrained Molecular Dynamics Simulations	On-site
SB-34	**Tsung-lun Hsieh** Charged residues on the intrinsically disordered region of galectin-3 contribute to the regulation of liquid-liquid phase separation	Online
SB-35	**Jai Prakash Singh** The catalytic activity of TCPTP is autoregulated by its intrinsically disordered C-terminal tail and activated by integrin alpha-1	On-site
SB-36	**Hao-Yu Cheng** Structural and functional characterization of *Arabidopsis thaliana* NPF4.6	On-site
SB-37	**Hsing-Mien Hsu** Development of potent human monoacylglycerol lipase (MAGL) inhibitors for the treatment of neuroinflammation	On-site+ Online
SB-38	**Hsing-Mien Hsu** Developing new inhibitors of human glutaminyl cyclase (hQC) as possible agents for the treatment of Alzheimer's disease	On-site+ Online
SB-39	**Min-Hao Wu** Structural Insights into the Stereospecific Cyclization Reaction Catalyzed by Deoxypodophyllotoxin Synthase	On-site
SB-40	**Sashank Agrawal** Structural insights into the pathogenesis of transthyretin amyloidosis	On-site+ Online
SB-41	**Jun-Yu Liang** Structural Prediction and Mechanistic Analysis of Sugar *O*-methyltransferase by Molecular Dynamics Simulation Methods	On-site
SB-42	**Bor-Shiuan Hu** An Insight into Mutation Effects and Protein Structure of Enzymes Based on X-ray Crystal Structures of Drug Methyltransferase	On-site

LECTURE

TAKAYUKI UCHIHASHI 内橋貴之

Professor
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EDUCATION

1997-1998	Doctoral Program, Department of Electronic Engineering, Graduate School of Engineering, Osaka University
1995-1997	Doctoral Program, Department of Physics, Graduate School of Science, Hiroshima Unuversity
1993-1995	Master's Program in Physics, Graduate School of Science, Hiroshima University

WORK EXPERIENCE

2017-present	Professor, Graduate School of Science, Nagoya University
	Visiting professor of Exploratory Research Center
2006-2008	Associate Professor, Graduate School of Natural Sciences, Kanazawa University
2004-2006	Assistant Professor, Graduate School of Natural Sciences, Kanazawa University
2002-2004	Senior Scientist, SFI Nanoscience Institute, Trinity College Dublin
1998-2000	Atom Technology Research Institute Postdoctoral Fellow (NEDO Fellow, Ishikawa Group)

HONORS and AWARDS

2019	IEEE 3M Nano 2019, Best Conference Paper Award, "RealTime Nanoscale Visualization of Biomolecules at Work with Hig-Speed Atomic Force Microscopy", Takayuki Uchihashi, August 7, 2019
2013	2013 Science and Technology Minister's Commendation Science and Technology Award (Development Category) "Development of High-Speed Atomic Force Microscope" Toshio Ando, Takayuki Uchihashi, Tetsuyuki Kodera, April 16, 2013

SELECTED PUBLICATIONS

1. High-speed atomic force microscopy for nano-visualization of dynamic biomolecular processes. T Ando, T Uchihashi, T Fukuma. Progress in Surface Science 83 (7-9), 337-437
2. Protein Needles Designed to Self-Assemble through Needle Tip Engineering. K Kikuchi, T Fukuyama, T Uchihashi, T Furuta, YT Maeda, T Ueno Small, 2106401
3. Molecular Origin of the Anomalous pH Effect in Blue Proteorhodopsin M Sumikawa, R Abe-Yoshizumi, T Uchihashi, H Kandori. The Journal of Physical Chemistry Letters 12, 12225-12229

Dynamic Structural Biology Driven By High-Speed Atomic Force Microscopy

Takayuki Uchihashi

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Most biological phenomena in the cell are elicited by a cascade of extensive dynamic molecular processes, including protein conformational changes, binding and dissociation, assembly and degradation. A fundamental understanding of complex biological processes is inherently reducible to understanding the dynamics of a small number of molecules at each step in such a cascade. Since protein motions are usually asynchronous and often have a multimodal distribution that cannot be directly assessed by ensemble averaging methods, it is necessary to monitor and analyze the dynamic behavior of individual molecules using a dynamic structural biology approach based on single molecule observation.

Among various microscopic techniques for characterizing protein structures and functions, high-speed atomic force microscopy（HS-AFM）is a unique technique in that allows direct visualization of structural changes and molecular interactions of proteins without any labeling in a liquid environment. Since its emergence in 2001[1)], it has been applied to the dynamics analysis of various types of proteins, including motor proteins, membrane proteins, DNA-binding proteins, amyloid proteins, and artificial proteins[2), 3)] and now has now become a versatile tool indispensable to drive research based on dynamic structural biology.

In this talk, I will review several recent bioimaging applications realized by HS-AFM and show what kind of dynamics phenomena can be observed by HS-AFM. This review provides overviews of several recent bioimaging applications achieved by HS-AFM, classified into imaging studies of conformational dynamics and protein-protein interactions. Recent instrumental developments to extend the capabilities of HS-AFM, especially molecular manipulation by localized force application, one of the key features of AFM, will also be discussed.

(1) T. Ando et al,*PNAS* 98, 12468-12472 (2001).
(2) T. Ando, T. Uchihashi, S. Scheuring, *Chem. Rev.* 114, 3120-3188 (2014).
(3) T. Uchihashi and C. Ganser, *Biophys. Rev*.12, 363-369 (2020).

IL1

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EDUCATION

1994-1999	Graduate Student, Division of Biological Science, Graduate School of Science, Nagoya University, Japan
1990-1994	Undergraduate, major in Molecular Biology, Department of Molecular Biology, Faculty of Science, Nagoya University, Japan.

WORK EXPERIENCE

2020-present	Professor, Division of Biological Science, Graduate School of Science, Nagoya University, Japan
2013-2020	Associate Professor, Division of Biological Science, Graduate School of Science, Nagoya University, Japan
2011-2013	Lecturer, Division of Biological Science, Graduate School of Science, Nagoya University, Japan
2005-2011	Assistant Professor, Division of Biological Science, Graduate School of Science, Nagoya University, Japan
2004-2005	Research Scientist, Dynamic NanoMachine Project, International Cooperative Research Project (ICORP), Japan Science and Technology Agency (JST), Osaka, Japan

HONORS and AWARDS

2016	Kobayashi Rokuzo memorial award, Japanese Society for Microbiology.
2000-2002	Postdoctoral Fellowship for Research Abroad, Japan Society for the Promotion of Science.
1997-1999	Research Fellowship for Young Scientists (DC2), Japan Society for the Promotion of Science.

SELECTED PUBLICATIONS

1. Nishikino T, Sagara Y, Terashima H, Homma M* and **Kojima S***. ***J. Biochem.*** (2022) 171:443-450.
2. Homma M*, Terashima H, Koiwa H, **Kojima S**. ***J. Bacteriol.*** (2021) 203: e00159-21.
3. Terashima H*, **Kojima S**, Homma M*. ***J. Bacteriol.*** (2021) 203:e00016-21.
4. **Kojima S***, Imura Y, Hirata H, Homma M. ***Genes Cells*** (2020) 25:279-287.
5. Carroll BL, Nishikino T, Guo W, Zhu S, **Kojima S**, Homma M*, Liu J*. ***eLife*** (2020) 9:e61446.

Structure-based functional studies of stator, the energy-converting membrane protein complex in the bacterial flagellar motor

Seiji Kojima[1]

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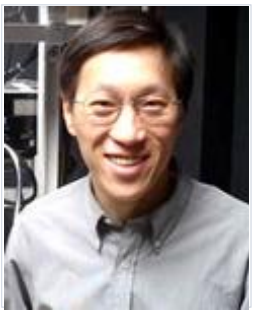
Many motile bacteria can swim in liquid or swarm on the hard surface by rotating the specific motility organ named flagellum. Rotation of the flagellum is driven by a rotary motor at its base embedded in the cell surface, and energy source of the motor is the ion-motive force across the membrane. The flagellar stator is responsible for energy conversion in the motor and transmits the rotational force to the rotor. It is a unique ion-conducting membrane protein complex composed of two kinds of proteins, A subunit and B subunit, whose cryo-EM structures were reported recently. The stator becomes active only when incorporated into the flagellar motor and the periplasmic domain of B subunit is anchored to the cell wall via the peptidoglycan binding (PGB) motif. From the functional studies based on the crystal structures of the periplasmic region of stator ($MotB_C$ or $PomB_C$), it has been revealed that the conformational change in the characteristic N-terminal α-helix of $MotB_C$ or $PomB_C$ allows the stator to efficiently conduct ions and bind to the PG layer (1). The plug and the following linker region between the PGB and transmembrane (TM) region of B subunit function in regulating the ion conductance. Recently we found that the plug segment physically contacts A subunit ring and prevents function of the stator (2). Additional new insights (3), obtained from the structure-based functional studies of the stator, will be discussed, to unravel the enigmatic energy-converter complex in the flagellar motor.

(1) Kojima S, et al., Structure (2018) 26, 590-598.

(2) Homma M, et al., J. Bacteriol. (2021) 203: e00159-21.

(3) Nishikino T, et al., J. Biochem. (2022) 171:443-450.

IL2



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Professor
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EDUCATION

2000	Ph.D., Chemistry, University of California, Berkeley, U.S.A
1993	B.S., Chemistry, National Taiwan University, Taiwan

WORK EXPERIENCE

2014-present	Professor Department of Chemistry, National Taiwan University, Taiwan
2018	Visiting Professor Department of Biology, MIT, USA
2007-2014	Associate Professor Department of Chemistry, National Taiwan University, Taiwan
2004- 2007	Assistant Professor Department of Chemistry, McGill University, Canada
2000-2004	Postdoctoral Fellow Department of Biochemistry, Brandeis University, USA

SELECTED PUBLICATIONS

1. Lei, K.-H., Yang, H.-L., Chang, H.-Y., Yeh, H.-Y., Nguyen, D. D., Lee, T.-Y., Lyu, X., Chastain, M., Chai, W., Li, H.-W.* & Chi, P.*, "Crosstalk between CST and RPA regulates RAD51 activity during replication stress" *Nature Comm.*, 12, 6412 (2021).
2. Lin, Y.-Y., Li, M.-H., Chang, Y.-C., Fu, P.-Y., Ohniwa, R. L., Li, H.-W.* & Lin, J.-J.*, "Dynamic DNA Shortening by Telomere-Binding Protein Cdc13" *J. Am. Chem. Soc.*, 143, 5815-5825 (2021).
3. Li, W.-C., Lee, C.-Y., Lan, W.-H., Woo, T.-T., Chen, C.-Y., Yeh, H.-Y., Chang, H.-Y., Liu, H.-C., Chen, C.-L., Chuang, C.-N., Hsueh, Y.-P., Li, H.-W.*, Chi, P.* & Wang, T.-F.*, "Trichoderma reesei Rad51 tolerates mismatches in hybrid meiosis with diverse genome sequences" *Proc. Natl. Acad. Sci. U.S.A.*, 118, e2007192118 (2021).
4. Lan, W.-H., Lin, S.-Y., Kao, C.-Y., Chang, W.-H., Yeh, H.-Y., Chang, H.-Y., Chi, P.* & Li, H.-W.* "Rad51 Facilitates Filament Assembly of Meiosis-Specific Dmc1 Recombinase" *Proc. Natl. Acad. Sci. U.S.A.*, 117, 11257-11264 (2020).
5. Lin, Y.-H., Chu, C.-C., Wang, P.-Y., Fan, H.-F., Cox, M. M.*, & Li, H.-W.*, "A 5'-to-3' Strand Exchange Polarity is Intrinsic to RecA Nucleoprotein Filaments in the Absence of ATP Hydrolysis", *Nucleic Acids Res.*, 47, 5126-40 (2019).

SWI5-SFR1 Accessory Proteins Stimulate RAD51 Recombinase Filament Assembly by Reducing its Extension Units to DNA

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[2]*Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan*
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Recombinases play an essential role in homologous recombination (HR), a major pathway to repair DNA double-strand breaks. Recombinases are highly conserved throughout the evolution, as seen as RecA in bacteria, RadA in Archaea, and Rad51 and Dmc1 in eukaryotes and humans. In HR reactions, the formation of recombinase-DNA filament is an essential step, subject to tight regulation. However, the assembly mechanism of the recombinase nucleoprotein filament remains elusive. Earlier electron microscopy studies and kinetic investigation suggest that recombinases may assemble to DNA in multimers, but no direct evidence is available. Here, we used single-molecule passive force-clamp optical tweezers to directly measure the assembly of recombinases on DNA. We determined the assembly recombinase unit and its associated dwell time during filament extension. The result shows that E. coli RecA assembles to DNA mainly in a hexamer unit. In contrast, mouse RAD51 assembles in octamers. Surprisingly, in the presence of auxiliary proteins, SWI5-SFR1, the assembly unit of mouse RAD51 is reduced to tetramers. SWI5-SFR1 also reduces RAD51 disassembly events. The combination of the reduction of RAD51 assembly units and RAD51 disassembly events by SWI5-SFR1 stimulates RAD51 recombination activity. These results form the molecular basis responsible for the recombinase-DNA filament assembly and offer insights into regulation strategies.

IL3

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EDUCATION

2004	Ph.D. in science; Graduate School of Science, Kyoto University, Japan (advisor Koreaki Ito)

WORK EXPERIENCE

2018-present	Professor Nara Institute of Science and Technology, Japan
2013-2018	Associate Professor (PI) Nara Institute of Science and Technology, Japan
2010-2013	Assistant Professor Graduate School of Science, The university of Tokyo (advisor Osamu Nureki), Japan
2008-2010	Assistant Professor The institute of Medical Science, The university of Tokyo (advisor Osamu Nureki), Japan
2006-2008	Postdoctoral Fellow Department of Biological Information, Tokyo Institute of Technology (advisor Osamu Nureki), Japan

HONORS and AWARDS

1. The Young Scientists' Prize (MEXT, 2012)
2. Early Research in Biophysics Award (Biophysical Society of Japan, 2010)
3. Young Scientist Award (PSSJ, 2009)

SELECTED PUBLICATIONS

1. Tanaka Y and **Tsukazaki T**. [News and Views] A snapshot of membrane protein insertion. ***EMBO Rep.*** 20, e49034 (2019)

2. ***Tsukazaki T**. [Review] Structural Basis of the Sec Translocon and YidC Revealed Through X-ray Crystallography. ***The Protein Journal*** 38, 249-261 (2019)

3. Inoue M, Sakuta N, Watanabe A, Zhang Y, Yoshikaie K, Tanaka Y, Ushioda R, Kato Y, Takagi J, **Tsukazaki T**, Nagata K, and *Inaba K. Structural Basis of Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase 2b Regulation via Transmembrane Helix Interplay. ***Cell Rep.*** 27, 1221–1230

Crystal structure of YeeE, a membrane protein, engaged in thiosulfate uptake

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In the global biological sulfur cycle, thiosulfate can be used for cysteine synthesis in bacteria as an inorganic sulfur source. We found that YeeE, a membrane protein, is involved in thiosulfate uptake. We determined the crystal structure of YeeE at 2.5-Å resolution, which possesses an unprecedented hourglass-like architecture with thiosulfate in the positively charged outer concave side. YeeE is composed of loops and 13 helices, including 9 transmembrane α helices. Four characteristic loops are buried toward the center of YeeE and form its central region surrounded by the nine helices. Additional electron density maps and successive molecular dynamics simulations imply that thiosulfate can remain temporally at several positions in the proposed pathway. We propose a plausible mechanism of thiosulfate uptake via three important conserved cysteine residues of the loops along the pathway. In addition, I would like to introduce our recent study.

(1) Tanaka et al. (2020) *Sci. Adv.* DOI: 10.1126/sciadv.aba7637

IL4

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EDUCATION

2005-2010	Ph.D., Bioinformatics and Structural Biology, National Tsing Hua University, Taiwan
2003-2005	M.S., Biochemistry National Chung Hsing University, Taiwan
1999-2003	B.S., Chemistry., Kaohsiung Medical University, Taiwan

WORK EXPERIENCE

2021-present	Professor, Institute of molecular medicine and bioengineering, National Yang Ming Chiao Tung University, Hsinchu, Taiwan
2018-2021	Associate Professor, Institute of molecular medicine and bioengineering, National Chiao Tung University, Hsinchu, Taiwan
2010-2013	Assistant Professor, Institute of Molecular Medicine and Bioengineering, National Chiao Tung University, Hsinchu, Taiwan

SELECTED PUBLICATIONS

1. Huang, K.W.; Chu, Y.Y.; Hua, T.U.; Chiu, T.Y.; Liu, J.U.; Tu, C.I.; Hsu, K.C.; Chu, J.W.*; **Hsiao, Y.Y.***, Targeted Covalent Inhibitors Allosterically Deactivate the DEDDh Lassa Fever Virus NP Exonuclease from Alternative Distal Sites. *J. Am. Chem. Soc. Au* (2021). 1, 12, 2315–2327 ; doi.org/10.1021/jacsau.1c00420 (通訊作者)
2. Liu, T.C.; Guo, K.W.; Chu, J.W.*; **Hsiao, Y.Y.***, Understanding APE1 Cellular Functions by the Structural Preference of Exonuclease Activities. *Comput. Struct. Biotechnol. J.* (2021). Jun 24;19:3682-3691. doi: 10.1016/j.csbj.2021.06.036., (通訊作者)
3. Liu, T.C.; Lin, C.T.; Chang, K.C.; Guo, K.W.; Wang, S.Y.; Chu, J.W.; **Hsiao, Y.Y.***, APE1 distinguishes DNA substrates in exonucleolytic cleavage by induced space-filling. *Nat Commun.* 12, 601 (2021). https://doi.org/10.1038/s41467-020-20853-2. (通訊作者)
4. Cheng, H. L.; Lin, C. T. ; Huang, K. W.; Wang, S.; Lin, Y. T.; Toh, S. I.; **Hsiao, Y. Y.*** Structural insights into the duplex DNA processing of TREX2. *Nucleic Acids Res* 2018, doi: 10.1093/nar/gky970. (通訊作者)
5. Huang, K. W.; Liu, T. C.; Liang, R. Y.; Chu, L. Y.; Cheng, H. L.; Chu, J. W.; **Hsiao, Y. Y.*** Structural basis for overhang excision and terminal unwinding of DNA duplexes by TREX1. *PLoS Biol* 2018, 16, e2005653. (通訊作者)

APE1 Exonuclease Distinguishes Various DNA Substrates by an Induced Space-Filling Mechanism.

Tung-Chang Liu[1,2], Chun-Ting Lin[1], Kai-Cheng Chang[1], Kai-Wei Guo[1,2], Shuying Wang[3], Jhih-Wei Chu[1,4] and Yu-Yuan Hsiao[1,2,4]*

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The 3'-to-5' exonuclease activity of Apurinic/apyrimidinic endonuclease 1 (APE1) is responsible for processing matched/mismatched terminus of duplex DNA in various DNA repair pathways, as well as for nucleoside analogs removal associated with drug resistance. However, how APE1 exonucleolytically recognizes and processes the terminus of duplex DNA without base preference remain unclear. We determined the first two APE1-dsDNA complex structures, which displayed a dsDNA end-binding mode. Integration of our structures, biochemical assays, and molecular dynamics simulation reveals the general rules of APE1 in handling various dsDNA substrates. The DNA binding-induced RM (Arg176 and Met269) bridge formation in active site and DNA-binding modes transition between matched and mismatched termini of dsDNA compose the exquisite machinery for substrate selection, binding, and digestion. Our studies pave the way for understanding the dsDNA terminal-processing-related cellular functions and drug resistance mechanisms of APE1.

(1) Liu, T.C.; Lin, C.T.; Chang, K.C.; Guo, K.W.; Wang, S.Y.; Chu, J.W.; Hsiao, Y.Y.*, APE1 distinguishes DNA substrates in exonucleolytic cleavage by induced space-filling. Nat Commun. 12, 601 (2021). https://doi.org/10.1038/s41467-020-20853-2.

(2) Liu, T.C.; Guo, K.W.; Chu, J.W.*; Hsiao, Y.Y.*, Understanding APE1 Cellular Functions by the Structural Preference of Exonuclease Activities. Comput. Struct. Biotechnol. J. (2021). Jun 24;19:3682-3691. doi: 10.1016/j.csbj.2021.06.036.

IL5

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EDUCATION

2011-2014	Ph.D. course Dept. of Nanobiology, Graduate School of Advanced Integration Science, Chiba University, Japan
2009-2011	Master course Dept. of Nanobiology, Graduate School of Advanced Integration Science, Chiba University, Japan
2004-2009	Bachelor course Dept. of Natural Sciences, The Faculty of Liberal Arts, The Open University of Japan. Japan

WORK EXPERIENCE

2022-present	Assistant Professor, Dept. of Biology, Chiba University, Japan
2015-2022	Postdoctoral Researcher, Dept. of Biology, Chiba University, Japan.
2014-2015	Postdoctoral Researcher, Dept. of School of Computer Science and System Engineer, Kyushu Institute of Technology, Japan.

HONORS and AWARDS

11 October, 2018, MCRC Symposium Best Poster, Chiba University.

SELECTED PUBLICATIONS

1. Haraguchi T, Ito K, Morikawa T, Yoshimura K, Shoji N, Kimura A, Iwaki M, and Tominaga M. Autoregulation and dual stepping mode of MYA2, an Arabidopsis myosin XI responsible for cytoplasmic streaming. ***Scientific reports.*** 2022 Feb 24;12(1):3150.
2. Haraguchi T, Tamanaha M, Suzuki K, Yoshimura K, Imi T, Tominaga M, Sakayama H, Nishiyama T, Murata T, Ito K. Discovery of the fastest myosin, its amino acid sequence, and structural features. ***Proc. Natl. Acad. Sci. USA.*** 2022 Feb 22;119(8)
3. Duan Z, Tanaka M, Kanazawa T, Haraguchi T, Takyu A, Era A, Ueda T, Ito K, Tominaga M. Characterization of ancestral myosin XI from Marchantia polymorpha by heterologous expression in Arabidopsis thaliana. ***Plant J.*** 2020 Oct;104(2):460-473.
4. Haraguchi T, Ito K, Duan Z, Rula A, Takahashi K, Shibuya Y, Hagino N, Miyatake Y, Nakano A, Tominaga M. Functional Diversity of Class XI Myosins in Arabidopsis thaliana. ***Plant Cell Physiol.*** 2018 Nov 1;59(11):2268-2277.

Functional analysis of plant-specific myosin XIs

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Myosins are motor proteins that convert chemical energy, ATP, to physical force to move actin filaments. *Arabidopsis thaliana* (*At*) has 13 genes belonging to the myosin XI. It has been suggested that *At* myosin XIs are involved in organelle transport and cytoplasmic streaming. However, velocities and enzymatic activities of each *At* myosin XIs have been poorly understood. Therefore, we measured velocities and enzymatic activities and tissue expression of 13 *At* myosin XIs. We suggested that *At* myosin XIs have different enzymatic activities and specific roles.

Chara cells with very fast velocity (70 μm s^{-1}), it has been suggested that a myosin XI with a velocity of 70 μm s^{-1} exists in *Chara* cells. However, the velocity of the previously cloned *Chara corallina* myosin XI (*Cc*XI) was about 20 μm s^{-1}. Recently, the genome sequence of *Chara braunii* has been published. We cloned four myosin XI (*Cb*XI-1, 2, 3, and 4) and measured their velocities. We showed that the velocity of *Cb*XI-1 and *Cb*XI-2 are 3 times faster than that of *Cc*XI. We proposed that *Cb*XI-1 and *Cb*XI-2 would be the main contributors to cytoplasmic streaming in *Chara* cells.

(1) Haraguchi T, Ito K, Duan Z, Rula S, Takahashi K, Shibuya Y, Hagino N, Miyatake Y, Nakano A, Tominaga M. Functional Diversity of Class XI Myosins in Arabidopsis thaliana. Plant Cell Physiol. 2018 Nov 1;59(11):2268-2277. doi: 10.1093/pcp/pcy147.

(2) Haraguchi T, Tamanaha M, Suzuki K, Yoshimura K, Imi T, Tominaga M, Sakayama H, Nishiyama T, Murata T, Ito K. Discovery of ultrafast myosin, its amino acid sequence, and structural features. Proc Natl Acad Sci U S A. 2022 Feb 22;119(8):e2120962119. doi: 10.1073/pnas.2120962119.

IL6

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EDUCATION

2006-2011	Ph.D. Biochemistry and Structural Biology, Philipps University Marburg, Marburg, Germany
2005-2006	M.S. Thesis in Biochemistry and Photobiology, Philipps University Marburg, Marburg, Germany
2001-2005	M.S. in Biochemistry, Valencia Literary University, Valencia, Spain
1999-2001	B.S. in Pharmacy, Valencia Literary University, Valencia, Spain

WORK EXPERIENCE

2020	Visiting Scientist, Taiwan Protein Project (TPP), Institute of biological Chemistry (IBC), Academia Sinica (AS), Taiwan
2016-2019	Visiting Assistant Professor, TPP, IBC, AS, Taiwan
2012-2016	Postdoctoral Fellow, IBC, AS, Taiwan (with Andrew H.-J. Wang)
2011-2012	Lab Leader, Phillips University Marburg, Germany (with Lars-Oliver Essen).
2006	Research Assistant, Phillips University Marburg, Germany (with Lars-Oliver Essen).

HONORS and AWARDS

1. Best presentation award at the CNDY 2013 workshop. Dec. 2013.
2. 3rd prize in the poster contest during the IITC Symposium. Sept. 2011.
3. Excellence Prize in the workshop attached to the E-MeP-Lab Master class in “System approaches to membrane proteins”. Mar. 2007.

SELECTED PUBLICATIONS

1. **Maestre-Reyna M**, Yang C-H, Nango E, Hang W-C, Putu EPGN, Wu W-J, Wang P-H, Franz-Badur S, Saft M, Emmerich H-J, Wu H-Y, Lee C-C, Huang K-F, Chang Y-K, Liao J-H, Weng J-H, Gad W, Chang C-W, Pang AH, Sugahara M, Owada S, Hosokawa Y, Joti Y, Yamashita A, Tanaka R, Tanaka T, Luo FJ, Tono K, Kiontke S, Schapiro I, Spadacini R, Royant A, Yamamoto J, Iwata S, Essen L-O, Bessho Y, Tsai M-D. Serial crystallography captures dynamic control of sequential electron and proton transfer events in a flavoenzyme. *Nature Chemistry.* (2022), https://doi.org/10.1038/s41557-022-00922-3.
2. **Maestre-Reyna M**, Huang W-C, Wu W-J, Singh PK, Hartmann R, Wang P-H, Lee C-C, Hikima T, Yamamoto M, Bessho Y, Drescher K, Tsai M-D, Wang AH-J, Vibrio cholerae biofilm scaffolding protein RbmA shows an intrinsic, phosphate-dependent autoproteolysis activity. *IUBMB Life.* 2021 Jan, 73, 418–431 (2021).

Decrypting cryptochrome signal transduction via time-resolved structural analysis

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Cryptochrome photoreceptors (CRY) are members of the photolyase/cryptochrome superfamily (PCSf), a group of ubiquitous light driven flavoenzymes. Most PCSf activity depends on a flavin adenine dinucleotide chromophore (FAD, with DNA photolyase involved in light-driven DNA repair. Conversely, CRYs modulate plant growth, regulate circadian rhythms, and have been linked to magnetoreception via a poorly understood mechanism of signal transduction involving radical pair formation.

Here, we solved the cryptic CRY signal transduction mechanism by obtaining a 3D structural movie of the post-ET events in *Cra*CRY via time-resolved serial femtosecond crystallography at the SACLA X-ray free electron laser, and possibly elucidating the molecular basis of magnetoreception, the worst understood biological sense.

APPA Lecture

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EDUCATION

1982	D. Sci., Osaka Univ., Graduate School of Science, Biochemistry
1979	M. Sci., Osaka Univ., Graduate School of Science, Biochemistry
1977	B. Sci., Osaka Univ., Faculty of Science, Biology

WORK EXPERIENCE

2022-present	Specially Appointed Researcher, Graduate School of Engineering, Osaka University
2020-2022	Specially Appointed Professor, Global Center for Medical Engineering and Informatics Osaka University
2020--present	Professor Emeritus, Osaka University
1998-2020	Professor, Institute for Protein Research, Osaka University
1989-1998	Associate Professor, Faculty of Science, Department of Biology Osaka University

SELECTED PUBLICATIONS

1. Breakdown of supersaturation barrier links protein folding to amyloid formation. Masahiro Noji, M. Samejima, T., Yamaguchi, K., So, M., Yuzu, K. Chatani, E., Akazawa-Ogawa, Y., Hagihara, Y., Kawata, Y., Ikenaka, K., Mochizuki, H., Kardos, K., Otzen, D., Bellotti, V., Buchner, J., and Goto, Y. Commun Biol 4, 120 (2021).

2. Heating during agitation of β2-microglobulin reveals that supersaturation breakdown is required for amyloid fibril formation at neutral pH. Noji, M., Sasahara, K., Yamaguchi, K., So, M., Sakurai, K., Kardos, J., Naiki, H. and Goto, Y. (2019) J. Biol. Chem. 294: 15826-15835.

3. Heat of supersaturation-limited amyloid burst directly monitored by isothermal titration calorimetry. Ikenoue, T.*, Lee, Y.-H.*, Kardos, J., Yagi, H., Ikegami, T., Naiki, H. and Goto, Y. (2014) Proc. Natl. Acad. Sci. USA, 111(18), 6654-6659. *equal contribution.

4. Direct observation of amyloid fibril growth monitored by thioflavin T fluorescence. Ban, T., Hamada, D., Hasegawa, K., Naiki, H. and Goto, Y. (2003) J. Biol. Chem. 278(19), 16462-16465.

5. Mapping of the core of the β_2-microglobulin amyloid fibril by H/D exchange. Hoshino, M., Katou, H., Hagihara, Y., Hasegawa, K., Naiki, H. and Goto, Y. (2002) Nature Struct. Biol. 9(5), 332-336.

LINKING PROTEIN FOLDING AND MISFOLDING UNDER MACROMOLECULAR CROWDING

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The thermodynamic hypothesis of protein folding, known as the “Anfinsen’s dogma” states that the native structure of a protein represents a free energy minimum determined by the amino acid sequence. However, inconsistent with the Anfinsen’s dogma, proteins misfold to form amyloid fibrils, which are ordered aggregates associated with diseases such as Alzheimer’s and Parkinson’s diseases. Here, we present a general concept for the link between folding and misfolding (1). We show that folding and amyloid formation are separated by the supersaturation barrier of a protein. Thus, the breakdown of supersaturation links the Anfinsen’s intramolecular folding universe and the intermolecular misfolding universe.

To clarify the role of supersaturation in vivo, we studied the mechanism of dialysis-related amyloidosis (DRA), a serious complication among long-term hemodialysis patients caused by amyloid fibrils of β2-microglobulin (β2m) (2). Although high serum β2m levels and a long dialysis vintage are the primary and secondary risk factors for the onset of DRA, respectively, patients with these do not always develop DRA, indicating that there are additional risk factors. We found that serum albumin prevented amyloid fibril formation based on macromolecular crowding effects, and that the decreased serum albumin concentration in dialysis patients is a tertiary risk factor for the onset of DRA. The model was constructed assuming accumulative effects of three risk factors and may be useful for predicting the onset of DRA. Furthermore, the model suggested the importance of monitoring temporary and accumulated risks to prevent the development of amyloidoses in general.

(1) Noji, M. et al. Breakdown of supersaturation barrier links protein folding to amyloid formation. Commun. Biol. 4, 120 (2021). https://doi.org/10.1038/s42003-020-01641-6

(2) Nakajima, K. et al. Macromolecular crowding and supersaturation protect hemodialysis patients from the onset of dialysis-related amyloidosis. bioRxiv doi: https://doi.org/10.1101/2022.02.01.478730

IL7

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EDUCATION

2014	PhD in Genetics, University of Helsinki, Helsinki, Finland Grade: eximia cum laude approbator Specialisation: structural biology
2006	MSc Biotechnology, Jamia Millia Islamia (A Central University), New Delhi, India 1st Division with distinction Class of 2006 topper
2006	MSc Bioinformatics, Jamia Millia Islamia (A Central University), New Delhi, India 1st Division with distinction

WORK EXPERIENCE

2021-present	Laboratory Head at The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia (0.8 FTE)
2021-present	Associate Professor/Principal Research Fellow at the Department of Biochemistry and Molecular Biology, University of Melbourne, Melbourne, Australia (0.2 FTE)
2017-2020	MRC Career Development Fellow in Dr. Lori Passmore's group at MRC Laboratory of Molecular Biology, Cambridge, UK

SELECTED PUBLICATIONS

1. Sijacki T, Alcón P, Chen ZA, McLaughlin SH, **Shakeel S**, Rappsilber J, Passmore LA. **Accepted**. The DNA-damage kinase ATR activates the FANCD2-FANCI clamp by priming it for ubiquitination. **Nature Structure & Molecular Biology**.

2. Laurinmäki P*****, **Shakeel S***, Ekström JO*****, Mohammadi P, Hultmark D, Butcher SJ. **2020**. Structure of Nora virus at 2.7 Å resolution and implications for receptor binding, capsid stability and taxonomy. **Scientific Reports** 10(1):19675. *Equal sharing first authors.

3. Farrell DP, Anishchenko I, **Shakeel S**, Lauko A, Passmore LA, Baker D, DiMaio F. **2020**. Deep learning enables the atomic structure determination of the Fanconi Anemia core complex from cryoEM. **International Union of Crystallography Journal (IUCrJ)** 7(5).

4. Alcón P*****, **Shakeel S***, Chen ZA, Rappsilber J, Patel KJ, Passmore LA. **2020**. FANCD2-FANCI is a clamp stabilized on DNA by monoubiquitination of FANCD2 during DNA repair. **Nature Structural and Molecular Biology** 27(3):240-248. *Equal sharing first authors. F1000Prime recommended.

Structures of Protein Complexes Involved in Fanconi Anemia DNA Repair

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The Fanconi Anemia (FA) pathway repairs DNA damage caused by endogenous and chemotherapy-induced DNA crosslinks. Genetic inactivation of this pathway impairs development, prevents blood production, and promotes cancer. The key molecular step in the FA pathway is the monoubiquitination of a heterodimer of the FANCD2-FANCI (D2-I) substrate proteins by the FA core complex - a megadalton multiprotein E3 ubiquitin ligase. Monoubiquitinated FANCD2-FANCI (ubD2-I) then initiates removal of the DNA crosslink. Lack of molecular insight into the FA core complex and ubD2-I limits a detailed explanation of how this DNA repair pathway functions. The FA core complex contains eight different subunits, most with unknown structure and no substantial homology to proteins of known structure. Moreover, the functions of many of the subunits are unclear. We aim to understand the mechanism of the FA core complex by biochemically reconstituting the specific monoubiquitination reaction, and by determining structures of the FA core complex and its product, ubD2-I. We now report purification and structural characterization of an intact FA core complex and ubD2-I. Together our data allow us to propose a model for the architecture of the entire FA core complex, providing new insight into the structure and function of its subunits and how its E3 ligase activity leads to remodelling of D2-I into a DNA clamp.

(1) Shakeel S*, Rajendra E*, Alcón P, O'Reilly F, Chorev D, Maslen S, Degliesposti G, Russo CJ, He S, Hill CH, Skehel M, Scheres SHW, Patel KJ, Rappsilber J, Robinson CV, Passmore LA. 2019. Structure of the Fanconi anaemia monoubiquitin ligase complex. Nature 575:234–237.
(2) Alcón P*, Shakeel S*, Chen ZA, Rappsilber J, Patel KJ, Passmore LA. 2020. FANCD2-FANCI is a clamp stabilized on DNA by monoubiquitination of FANCD2 during DNA repair. Nature Structural and Molecular Biology 27(3):240-248.
(3) Farrell DP, Anishchenko I, Shakeel S, Lauko A, Passmore LA, Baker D, DiMaio F. 2020. Deep learning enables the atomic structure determination of the Fanconi Anemia core complex from cryoEM. International Union of Crystallography Journal (IUCrJ) 7(5).

IL8

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EDUCATION

2016	PhD, Department of Biochemistry, Duke University School of Medicine, USA
2009	M.S., Institute of Biochemical Sciences, National Taiwan University, Taiwan
2007	B.S., Department of Horticulture, National Taiwan University, Taiwan

WORK EXPERIENCE

2013	Teaching Assistant for Physical Biochemistry, Duke University School of Medicine, Durham, NC
2009-2011	Research Assistant, Institute of Biological Chemistry, Academia Sinica, Taiwan
2006	Research Intern, Institute of Biochemical Sciences, National Taiwan University, Taiwan

SELECTED PUBLICATIONS

1. **Hsu, J.C.**, Laurent-Rolle, M., Pawlak, J.B., Xia, H., Kunte, A., Hee, J.S., Lim, J., Harris, L.D., Wood, J.W., Evans, G.B., Grove, T.L., Shi, P.-Y., Almo, S.C., and Cresswell, P., Viperin triggers ribosome collision- dependent translation inhibition to restrict viral replication. Molecular Cell, 2022; 82(9):1631- 1642.e6. DOI: 10.1016/j.molcel.2022.02.031.
2. **Hsu, J.C.**, Pawlak, J.B., Laurent-Rolle, M., and Cresswell, P., Protocol for assessing of translational regulation in mammalian cell lines by OP-Puro labeling. STAR Protocols, (invited submission under review)
3. **Hsu, J.C.**, Lloyd, T., Pawlak, J.B., the Yale IMPACT research team, Almo, S.C., Cresswell, P., Grove, T.L., and Laurent-Rolle, M., Detection of antiviral nucleoside 3'-deoxy-3',4'-didehydro-cytidine in sera from COVID-19 and flu patients. (in preparation)
4. **Hsu, J.C.**, Pawlak, J.B., Cresswell, P., and Laurent-Rolle, M., Ubiquitination of SARS-CoV-2 nonstructural proteins promotes viral pathogenesis. (in preparation)
5. **Hsu, J.C.***, Pawlak, J.B.*, Han, P., Sengupta, D., Laurent-Rolle, M., and Cresswell, P., SARS-CoV-2 viral proteins promote immune evasion by translational inhibition of MHC class I. (in preparation) ***These authors made an equal contribution**
6. Pawlak, J.B., **Hsu, J.C.**, Han, P., Xia, H., Shi, P.-Y, Cresswell, P., and Laurent-Rolle, M., CMPK2 is a mitochondrial antiviral factor restricting flavivirus replication. (in preparation)

Translational shutdown and immune evasion by SARS-CoV-2 NSP14 protein

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The ongoing COVID-19 pandemic has had a devastating worldwide impact on health and economy. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of COVID-19. There is still no effective treatment, and the full extent of COVID-19 pathogenesis remains unclear. In particular, mechanisms for SARS-CoV-2 evasion of host immune surveillance remain poorly understood. In this study, we show that SARS-CoV-2 inhibits host protein synthesis and that several SARS-CoV-2 nonstructural proteins (NSPs) exert this activity, including NSP14. We demonstrate that the translation inhibition activity of NSP14 is conserved in human coronaviruses. The bifunctional enzyme NSP14 has exoribonuclease (ExoN) and N7-methyltransferase (N7-MTase) activities, which are both required for SARS-CoV-2 replication. We find that mutations in the ExoN or N7-MTase active sites abolish its translation inhibition activity. Consequently, the translation inhibition by NSP14 abolishes CD8+ T cell responses and the type I interferon-dependent induction of interferon-stimulated genes (ISGs). Together, we demonstrate that SARS-CoV-2 employs a translation inhibitor to evade both host innate and adaptive immune responses, which provides new insights into understanding the biology of COVID-19 pathogenesis.

(1) **Hsu, J.C.**, Laurent-Rolle, Pawlak, J.B., Wilen, C.B., and Cresswell, P., Translational shutdown and immune evasion by SARS-CoV-2 NSP14 protein. Proceedings of the National Academy of Sciences of the USA, 2021; 118 (24) e2101161118; DOI: 10.1073/pnas.2101161118

IL9

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EDUCATION

1985-1992	Ph.D., Biochemistry, Baylor College of Medicine, USA
1984-1985	M.S., Biochemistry, Shanghai Institute of Biochemistry, Chinese Academy of Sciences, China
1980-1984	B.S., Biology, East China Normal University, Shanghai, China

WORK EXPERIENCE

2005-present	Director, Center of Protein Sciences, Peking University, China
2003-present	Professor and Doctoral Supervisor, School of Life Sciences, Peking University, China
2007-2013	Vice Dean, the School of Life Sciences, Peking University, China
2002	Visiting Professor at Harvard Medical School, USA
1998-2003	Professor and doctoral supervisor (Biochemistry and Molecular Biology), Department of Biology, Tsinghua University, China
1996-1998	Associate Professor, Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing, China
1992-1995	Postdoctoral Fellow, Howard Hughes Medical Institute (HHMI), USA

SELECTED PUBLICATIONS

1. Chang, Z and Fu X (2022) Biogenesis of Secretory Proteins in Eukaryotic and Prokaryotic Cells. Reference Module in Life Sciences (Book), https://doi.org/10.1016/B978-0-12-821618-7.00048- 1.

2. Chang Zai, Probing into living cells of proteins, 2021, Scientific Bulletin (invited review), 66: 4524-4538.

3. Xinmiao Fu, Anastasia N Ezemaduka, Xinping Lu and Zengyi Chang (2021) The nematode worm Caenorhabditis elegans 12-kDa small heat shock proteins with little in vitro chaperone activity play crucial roles for its dauer formation, longevity and reproduction. Protein Science, 30:2170-2182.

4. Yang Liu#, Jiayu Yu#, Mengyuan Wang#, Qingfang Zeng, Xinmiao Fu, Zengyi Chang (2021) A high-throughput genetically directed protein crosslinking analysis reveals the physiological relevance of the ATP synthase “inserted” state. FEBS Journal, 288:2989-3009.

Exploring proteins in living cells: Discovery of the reversible regrowth-delay body in non-dividing and non-growing bacterial cells

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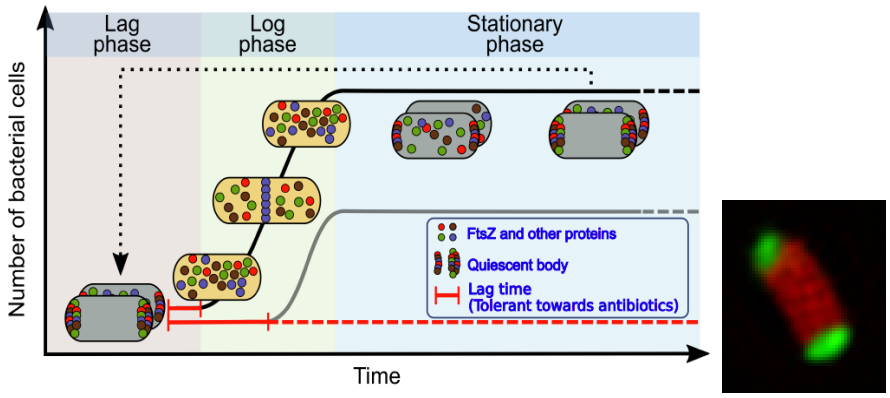
Almost since the 1940s when antibiotics were applied to treating bacterial infections, it was noticed that there is always a small number of bacterial cells that will survive the antibiotic treatment, either in the test tube or in the living body. This phenotype of drug tolerance bacteria was termed persistence. Such persistent bacteria are considered as the major source from which antibiotic resistant bacteria are derived. Such persistent bacterial cells have been of great interest both for the purpose of finding ways to eradicate pathogenic bacteria and for understanding the molecular nature of the dormant phenomenon in life. However, due to the difficulty of identifying this type of bacteria that exist in an extremely low level in the population but lack any identifiable molecular or subcellular marker, progress of understanding the cell biology and molecular biology of this group of bacterial cells are seriously hindered.

We have been trying to understand the dynamic assembly/disassembly process of the FtsZ protein in forming the Z-ring structure that play an essential role for the division of the bacterial cells, by mainly performing unnatural amino acid-mediated protein photocrosslinking analyses in living cells. We accidentally observed that the partner proteins with which the FtsZ protein interact are different in actively dividing log phase cells and the non-dividing late stationary phase cells are rather different. Further studies via live cell imaging analysis revealed that the FtsZ proteins are present in two clearly identifiable subcellular structures located at the cell poles. We subsequently demonstrated that this subcellular structure is composed of multiple important proteins that are involved in cell division, transcription, translation and other key metabolic processes.

Our live cell imaging analysis further demonstrated the following. 1）This subcellular structure start to appear in the stationary phase non-dividing cells, in more cells upon longer incubation. 2）When these cells are placed under optimal growth conditions, these subcellular structure will dissolve, with the released proteins to be able to function again, in reawakening cells, but will retain in cells that fail to regrow. In view of these properties, we designate this subcellular structure as Regrowth-delay Body. 3）Bacterial cells containing the regrowth-delay body will effectively survive the treatment of such antibiotics as ampicillin, while those that do not contain are effectively killed (be lysed). 4）The regrowth-delay body are formed in bacterial cells cultured either in liquid or on solid medium, and not only in *E. coli*, but also in other pathogenic bacteria, indicating that is might be commonly formed in bacteria that enter the dormant state.

It is presumed that either blocking the formation or facilitattingf the dissolution of the regrowth-delay body would help to eradicate the persistant pathogenic bacterial cells that are tolerant to multiple antibiotics.

(1) Yu, J., Liu, Y., Yin, H., and Chang, Z. (2019). Regrowth-delay body as a bacterial subcellular structure marking multidrug-tolerant persisters. *Cell Discovery* 5:8; doi.org/10.1038/s41421-019-0080-3.



IL10

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EDUCATION

2004	Ph.D., Chemistry, Utrecht University, The Netherlands
2000	M.Sc., Life Sciences, National Tsing Hua University, Taiwan
1998	B.Sc., , Physics and Life Sciences (Double Major), National Tsing Hua University, Taiwan

WORK EXPERIENCE

2022-present	Research Fellow, Institute of Biological Chemistry, Academia Sinica, Taiwan
2018-present	Adjunct Associate Professor, Institute of Biochemical Sciences, National Taiwan University (NTU), Taiwan
2017-2022	Associate Research Fellow, Institute of Biological Chemistry, Academia Sinica, Taiwan
2010-2011	Assistant Professor, Institute of Bioinformatics and Structural Biology, National Tsing Hua University, Taiwan

HONORS and AWARDS

2022	Outstanding Research Award, Ministry of Science and Technology, Taiwan
2021–2023	Academia Sinica Presidential Scholars Program
2020–2024	Career Development Award Academia Sinica

SELECTED PUBLICATIONS

1. Yang TJ, Li TN, Huang RS, Pan MY, Lin SY, Lin S, Wu KP, Wang LH, (Hsu STD)* (2022-06) *JOURNAL OF CELL BIOLOGY* 221(6), e202201094 "Tumor suppressor BAP1 nuclear import is governed by transportin-1."
2. Chen T, Yeh HW, Chen PP, Huang WT, Wu CY, Liao TC, Lin SL, Chen YY, Lin KT, (Hsu STD), Cheng HC* (2022-05) *Biochimica et Biophysica Acta - General Subjects* 1866(5), 130099 "BARD1 is an ATPase activating protein for OLA1"
3. Puri S, Chen SN, Chiu YH, Draczkowski P, Ko KT, Yang TJ, Wang YS, Uchiyama S, (Hsu STD)* (2022-05) *Journal of Molecular Biology* 434(9), 167553 "Impacts of cancer-associated mutations on the structure-activity relationship of BAP1"
4. Puri S, (Hsu STD)* (2022-04) *Biochemical and Biophysical Research Communications* 599, 57-62 "Oxidation of catalytic cysteine of human deubiquitinase BAP1 triggers misfolding and aggregation in addition to functional loss"

To bind or not to bind: the tale of CoV spikes

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Spike proteins of coronaviruses are responsible for host recognition and viral entries. Understanding the structure-function relationships of spike proteins requires atomic insights into the protein structure and the domain motions, which can be characterized in great detail by cryo-electron microscopy (cryo-EM) and a better description of the complex glycan shield that constitutes a significant part of the overall structures.[1,2] In this talk, I shall discuss our recent efforts in studying the structures of SARS-CoV-2 spike variants and developing better neutralizing antibodies against these spike variants.[3-5] By integrating EM, mass spectrometry, and biolayer interferometry, we have established a robust workflow to characterize the effect of the mutations of different variants of concern/interest, thereby providing detailed insights into the molecular basis of immunity escapes. We also demonstrated the broad-spectrum neutralizing effects of a panel of monoclonal antibodies that can be used as a cocktail to maximize their efficacies.[3-5] Importantly, we showed that the immunity escape of SARS-CoV-2 VOCs can be achieved by alternating the glycosylation patterns within the hyper-antigenic supersite within the N-terminal domain of the spike protein distant to the receptor-binding domain.

(1) Yang TJ, Chang YC, Ko TP, Draczkowski P, Chien YC, Chang YC, Wu KP, Khoo KH, Chang HW*, Hsu STD* (2020). Cryo-EM analysis of a feline coronavirus spike protein reveals a unique structure and camouflaging glycans. *Proc. Natl. Acad. Sci, U. S. A.*, 117, 1438-1446.

(2) Kuo CW, Yang TJ, Chien YC, Yu PY, Hsu STD*, Khoo KH* (2022). Distinct shifts in site-specific glycosylation pattern of SARS-CoV-2 spike proteins associated with arising mutations in the D614G and Alpha variants. *Glycobiology*, 32(1), 60-72.

(3) Su SC, Yang TJ, … Lin YL*, Tao MH*, Hsu STD*, Wu HC* (2021). Structure-guided antibody cocktail for prevention and treatment of COVID-19. *PLoS Pathogens*, 10(17):e1009704.

(4) Yang TJ, …, Hsu STD* (2021). Effect of SARS-CoV-2 B.1.1.7 mutations on spike protein structure and function. *Nat. Struc. Mol. Biol.*, 28, 731- 739.

(5) Yang TJ, … Wu HC, Hsu STD* “Structure-activity relationships of B.1.617 and other SARS-CoV-2 spike variants” *BioRxiv* **doi:** 10.1101/2021.09.12.459978

IL11

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EDUCATION

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WORK EXPERIENCE

2017-present	Professor, Department of Biotechnology, Graduate School of Engeering, Osaka University, Japan
2012-2017	Associate Professor, Department of Biotechnology, Graduate School of Engeering, Osaka University, Japan
2005	Visiting Scientist, Department of Chemistry, Cambridge University (Carol Robinson Lab.), UK
2001-2012	Assistant Professor, Dept. Biotech, Graduate School of Engeering, Osaka University, Japan
1999-2001	Postdotoral Researcher, RRF Research Institute, Inc.

SELECTED PUBLICATIONS

1. Yamaguchi Y, et al. The Fab portion of immunoglobulin G has sites in the CL domain that interact with Fc gamma receptor IIIa. MAbs. 14(1):2038531 (2022).
2. Abe K, Kabe Y, et al. Pro108Ser mutation of SARS-CoV-2 3CLpro reduces the enzyme activity and ameliorates the clinical severity of COVID-19. Sci Rep. 12(1):1299 (2022).
3. Yoneda S, et al.. Influence of Protein Adsorption on Aggregation in Prefilled Syringes. J Pharm Sci. 110(11):3568-3579 (2021).
4. Yoneda, et al. Development of syringes and vials for delivery of biologics: current challenges and innovative solutions. *Expert Opin Drug Deliv.* 18, 459-470 (2021).
5. Melo GB, et al. Critical analysis of techniques and materials used in devices, syringes, and needles used for intravitreal injections. *Prog Retin Eye Res.* 18:100862. (2020).
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Thermodynamic, kinetic and structural investigations on antigen-IgG-FcγRIIIa interactions

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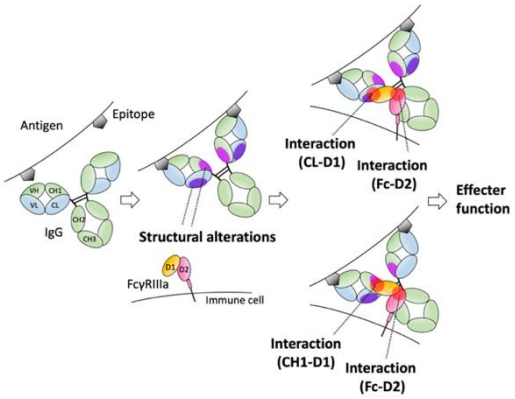
Mass spectrometry (MS) have been widely used for protein characterizations in both science and industry sectors. I'll introduce our recent studies on the protein primary and higher order structure analysis and on protein-protein interactions, mainly on the research about the antigen-immunoglobulin G (IgG)-Fc gamma receptor IIIa (FcγRIIIa) interactions. The thermodynamic and kinetic effects of antigen binding on IgG-FcγRIIIa interactions were investigated using bio-layer interferometry. As a result, binding of protein antigens to IgG significantly decreased a dissociation rate constant (koff) of IgG toward FcγRIII. We then attempted to clarify the reason of the difference by structural investigations using Hydrogen/deuterium exchange mass spectrometry (HDX-MS) of antigen-IgG binary complexes. Binding of antigens to IgG changed the deuterium uptake behavior of some segments in Fab besides the binding sites for antigens. Protein antigens caused the decreases in deuterium uptake of the segments in two domains, constant light (CL) domain and the first constant (CH1) domain. We further conducted HDX-MS and crosslinking mass spectrometry (XL-MS) to identify the binding sites between Fab and FcγRIIIa and also spatial arrangement of IgG and FcγRIIIa in the complex. Finally, we revealed that Fab has two binding sites for FcγRIIIa in CL and CH1. The binding sites in Fab were located in the same segments where structural alterations occurred by antigen-binding. We concluded that antigen-Fab interactions regulate the antibody effector functions thorogh the structural alterations in Fab to stabilize the formation of the ternary complexes for FcγRIIIa activations.



(1) Yogo R, et al., Sci Rep. 9(1):11957 (2019).

(2) Yamaguchi Y, et a., MAbs. 14(1):2038531 (2022).

IL12

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EDUCATION

2011-2013	JSPS Research Fellowship for Young Scientists (DC2)
2013	Ph.D. The University of Tokyo
2008	B.S. The University of Tokyo

WORK EXPERIENCE

2015-present	Assistant Professor, Institute for Molecular Science, National Institutes of Natural Sciences , Japan
2015-2017	Research Assistant Professor, Institute for Molecular Science, National Institutes of Natural Sciences, Japan
2013-2015	Suntory Foundation for Life Sciences, Bioorganic Research Institute
2013-2015	JSPS Research Fellowship for Young Scientists (PD)

HONORS and AWARDS

2021	the Pharmaceutical Society of Japan Award for Young Scientists
2015	Inoue Research Award for Young Scientists
2013	Young Scientist Award, the Protein Science Society of Japan
2013	Poster Award for Young Scientists Ⅰ, the Nuclear Magnetic Resonance Society of Japan
2011	Merck Award for Young Biochemistry Researcher, Merck Millipore Corporation

SELECTED PUBLICATIONS

1. Yanaka, S., Yagi, H., Yogo, R., Onitsuka, M. and Kato, K. Glutamine-free mammalian expression of recombinant glycoproteins with uniform isotope labeling: an application for NMR analysis of pharmaceutically relevant Fc glycoforms of human immunoglobulin G1. J. Biomol. NMR in press (2022)
2. Yanaka, S., Nishiguchi, S., Yogo, R., Watanabe, H., Shen, J., Yagi, H., Uchihashi, T. and Kato, K. Quantitative visualization of the interaction between complement component C1 and immunoglobulin G: The effect of CH1 domain deletion. Int. J. Mol. Sci. 23, 2090 (2022)
3. Yanaka, S., Yamaguchi, Y., Takizawa, T., Miyanoiri, Y., Yogo, R., Shimada, I. and Kato, K. NMR assignments of the N-glycans of the Fc fragment of mouse immunoglobulin G2b glycoprotein. Biomol. NMR Assign. 15, 187–192 (2021)

Multifaceted observation of conformational dynamics and interactions of antibodies

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Antibodies are glycoproteins that trigger effector functions upon antigen recognition and are responsible for the elimination of foreign substances through interactions with complements and Fc receptors. The interactions of antibodies with these molecules are governed by the coordination of their multiple domains, which are linked by flexible segments, enabling dynamic spatial arrangement of the domains. Moreover, the glycans attached to antibodies exhibit various glycoforms and are closely linked to the effector functions. To provide mechanistic insights into the antibody functions, it is crucial to characterize conformational dynamics of antibody molecules constituting form the carbohydrate and polypeptide chains.

To investigate the structural dynamics and interactions of antibodies, we integrated experimental and theoretical approaches including molecular dynamics (MD) simulation, nuclear magnetic resonance spectroscopy, small-angle x-ray scattering and high-speed atomic force microscopy. The experimentally validated MD simulation revealed the dynamic allosteric network within the antibody molecule. Our study shows that subtle changes in the glycan structures affect the intramolecular network, causing structural changes at unexpected distal sites and thereby modulating the effector functions.

(1) Yanaka, S., Nishiguchi, S., Yogo, R., Watanabe, H., Shen, J., Yagi, H., Uchihashi. T., Kato, K., “Quantitative Visualization of the Interaction between Complement Component C1 and Immunoglobulin G: The Effect of C_H1 Domain Deletion” *Int. J. Mol. Sci.* 76, 12-17 (2022).

(2) Yanaka, S., Yogo, R., Inoue, R., Sugiyama, M., Itoh, S.G., Okumura, H., Miyanoiri, Y., Yagi, H., Satoh, T., Yamaguchi, T., Kato, K., “Dynamic Views of the Fc Region of Immunoglobulin G Provided by Experimental and Computational Observations” *Antibodies* 8, 39 (2019).

IL13

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EDUCATION

2018-2021	Doctor of Philosophy (Medicine), Transporter Biology Group, School of Medical Sciences, University of Sydney (USyd)
2017	Bachelor of Science (Advanced) (Honours) in Pharmacology, Transporter Biology Group, School of Medical Sciences, USyd
2014-2016	Bachelor of Science (Advanced), Majors: Pharmacology and Biochemistry. USyd

WORK EXPERIENCE

2021-2022	Postdoctoral Researcher, Genomics Research Center, Academia Sinica, Taiwan.

SCHOLARSHIPS and AWARDS

2020	- Postgraduate Research Support Scheme (PRSS). USyd
2019	- Edith Mary Rose (Travelling) Scholarship. Usyd - Grant to attend the course “Cryo 3D Electron Microscopy” International School of Crystallography, Erice - Postgraduate Research Support Scheme (PRSS). USyd - Travel bursary for attending the Asian Biophysics Association Symposium. Australian Society for Biophysics
2018	- Postgraduate Research Support Scheme (PRSS). USyd - Research Training Program Stipend Scholarship (RTP) (2018-2021) - University of Sydney Academic Merit Prize

SELECTED PUBLICATIONS

1. Chen, I., Wu, Q., Font, J., Ryan, R. The twisting elevator mechanism of glutamate transporters reveals the structural basis for the dual transport-channel functions. **Current Opinion in Structural Biology (2022)**
2. Chen, I*, Pant S*., Wu, Q*., Cater, R., Sobti, M., Vandenberg, R., Stewart, A., Tajkhorshid, E., Font, J. and Ryan R. *co-authors. Glutamate transporters have a conserved chloride channel with two hydrophobic gates. **Nature (2021)**
3. Freidman, N., Chen, I., Wu, Q., Briot, C., Holst, J., Font, J., Vandenberg, R., Ryan, R. Amino acid transporters and exchangers from the SLC1A family: structure, mechanism and roles in physiology and cancer. **Neurochemical Research (2020)**

Glutamate transporters have a chloride channel with two hydrophobic gates

Ichia Chen, Shashank Pant, Qianyi Wu, Rosemary Cater, Meghna Sobti, Robert Vandenberg, Alastair G. Stewart, Emad Tajkhorshid, Josep Font & Renae Ryan

Glutamate is the most abundant excitatory neurotransmitter in the central nervous system, therefore its precise control is vital for maintaining normal brain function and preventing excitotoxicity[1]. Removal of extracellular glutamate is achieved by plasma membrane-bound transporters, which couple glutamate transport to sodium, potassium and pH gradients using an elevator mechanism[2-5]. Glutamate transporters also conduct chloride ions via a channel-like process that is thermodynamically uncoupled from transport[6-8]. However, the molecular mechanisms that allow these dual-function transporters to carry out two seemingly contradictory roles are unknown. Here we report the cryo-electron microscopy structure of a glutamate transporter homologue in an open-channel state, revealing an aqueous cavity that is formed during the transport cycle. By studying functional properties combined with molecular dynamics simulations, we show that this cavity is an aqueous-accessible chloride permeation pathway gated by two hydrophobic regions and is conserved across mammalian and archaeal glutamate transporters. Our findings provide insight into the mechanism by which glutamate transporters support their dual function and add a crucial piece of information to aid mapping of the complete transport cycle shared by the SLC1A transporter family.

1 Vandenberg, R. J. & Ryan, R. M. Mechanisms of glutamate transport. *Physiol Rev* **93**, 1621-1657, doi:10.1152/physrev.00007.2013 (2013).

2 Reyes, N., Ginter, C. & Boudker, O. Transport mechanism of a bacterial homologue of glutamate transporters. *Nature* **462**, 880-885, doi:10.1038/nature08616 (2009).

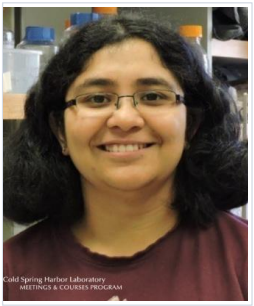
3 Ryan, R. M. & Vandenberg, R. J. Elevating the alternating-access model. *Nat Struct Mol Biol* **23**, 187-189, doi:10.1038/nsmb.3179 (2016).

4 Zerangue, N. & Kavanaugh, M. P. Flux coupling in a neuronal glutamate transporter. *Nature* **383**, 634-637, doi:10.1038/383634a0 (1996).

6 Fairman, W. A., Vandenberg, R. J., Arriza, J. L., Kavanaugh, M. P. & Amara, S. G. An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. *Nature* **375**, 599-603, doi:10.1038/375599a0 (1995).

7 Ryan, R. M. & Mindell, J. A. The uncoupled chloride conductance of a bacterial glutamate transporter homolog. *Nat Struct Mol Biol* **14**, 365-371, doi:10.1038/nsmb1230 (2007).

IL14



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EDUCATION

PhD	Molecular and Structural Biology, University of Sydney, Australia
M.S.	Human Genetics, Sri Ramachandra Medical College & Research Institute (SRMC&RI)
B.S.	Chemistry, Stella Maris College (Autonomous), Chennai (University of Madras).

WORK EXPERIENCE

2015-present	Postdoctoral Fellow, Stanford University, Stanford, USA
2012-2015	Research Officer, Walter and Eliza Hall Institute, Melbourne, Australia
2010-2011	Laboratory demonstrator (Part-time position), School of Molecular Bioscience, University of Sydney, Australia
2012	Research assistant (Part-time), School of Pharmacology, University of Sydney, Australia
2007-2008	Clinical Trial Coordinator, Department of Urology and Renal Transplantation, Sri Ramachandra University, Chennai, India.

SELECTED PUBLICATIONS

1. Wei T, Chandy M, Zhang A, **Krishna Kumar K**, Justesen JM, Chen IY, Wo H, Khanamiri S, Yang JY, Seidl FJ, Burns NZ, Liu C, Sayed N, Shie J, Yeh C, Yang K, Lau E, Lynch KL, Rivas M, Kobilka BK, Wu JC. *Cannabinoid receptor 1 antagonist genistein attenuates marijuana-induced vascular inflammation.* Cell. 2022. Apr; S0092-8674(22)00443-3. doi: 10.1016/j.cell.2022.04.005.

2. Lerch MT, Matt RA, Masureel M, Elgeti M, **Krishna Kumar K**, Hilger D, Foys B, Kobilka BK, Hubbel WL. *Viewing rare conformations of the β2 adrenergic receptor with pressure-resolved DEER spectroscopy.* PNAS. 2020. Dec; 117(50): 31824-31831. doi: 10.1073/pnas.2013904117.

3. Hilger D*, **Krishna Kumar K***, Hu H*, Pedersen MF, Giehm L, Mathiesen JM, Skiniotis G, Kobilka BK. *Structural insights into differences in G protein activation by Family A and Family B GPCRs*. Science. 2020. Jul; 369(6503).doi: 10.1126/science.aba3373. *Equal contribution

4. Kato HE, Zhang Y, Hu, H, Suomivuori C, Kadji FM, Aoki J, **Krishna Kumar K**, Fonseca R, Hilger D, Huang W, Latorraca NR, Inoue A, Dror RO, Kobilka BK, Skiniotis G. *Conformational transitions of a neurotensin receptor1-Gi1complex*. Nature. 2019. Aug; 572(7767): 80-85.doi: 10.1038/s41586-019-1337-6.

Structural insights into G-protein coupled receptor activation

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Glucagon is a hormone that plays important roles in the regulation of glucose homeostasis. It exerts its physiological effects by activating the Family B G-protein-coupled-receptor (GPCR), glucagon receptor (GCGR), which signals predominantly through the adenylyl-cyclase stimulatory family of G-protein, G_s. To understand the activation mechanism of GCGR, we determined the structure of the GCGR-G_s complex. In the GCGR-G_s structure, TM6 outward movement results in the formation of a sharp kink, as opposed to a gradual bend seen in Family A. By comparing Family A and Family B G-protein engagement properties, we propose a distinct activation mechanism for Family B GPCRs.

IL15

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----------EDUCATION----------

1995-1999	Ph.D. (Biochemistry), The University of Edinburgh, UK
1992-1994	M.Sc. (Biochemistry), Mahidol University
1987-1992	B.Sc. (Genetics), Chulalongkorn University

----------WORK EXPERIENCE----------

2015-present	Full Professor of Biochemistry (Awaiting official endorsement by His Majesty King Maha Vajiralongkorn)
2007-present	Associate Professor, Suranaree University of Technology
2004-2007	Assistant Professor, Suranaree University of Technology
1999-2000	Post-Doctoral Research Fellow, Membrane Biology Group, The University of Edinburgh, U.K
1994-2004	Lecturer, Suranaree University of Technology (Paid leave of absence for Ph.D. study and postdoctoral research in the UK from 1995-2000)

----------HONORS and AWARDS----------

2019	Mahidol University Alumni Award for 'Outstanding Research'
2014	Outstanding Research Award in Biochemistry and MolecularB Biology (Professor. M.R. Jisnuson Svasti BMB Award)

----------SELECTED PUBLICATIONS----------

1. Suginta W*, Sanram S, Aunkham A, Winterhalter M, Schulte A. (2021) The C2 entity of chitosugars is crucial in molecular selectivity of the Vibrio campbellii chitoporin.J Biol Chem. In Press. DOI:https://doi.org/10.1016/j.jbc.2021.101350

2. Vibulcharoenkitja P, Suginta W, Schulte A* Electrochemical N-Acetyl-β- D-glucosaminidase Urinalysis: Toward Sensor Chip-Based Diagnostics of Kidney Malfunction. Biomolecules. 2021, 11(10):1433.

3. Kitaoku Y, Fukamizo T, Kumsaoad S, Ubonbal P, Robinson RC, Suginta W* (2021) A structural model for (GlcNAc)2 translocation via a periplasmic chitooligosaccharide binding protein from marine Vibrio bacteria. J Biol Chem. In press.

Structural Insights into Chitin Utilization by Marine Vibrio species

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Chitin is the most abundant biopolymer in marine ecosystems. However, there is no accumulation of chitin in the ocean-floor sediments, since marine bacteria *Vibrios* are mainly responsible for a rapid turnover of chitin polysaccharide. The catabolic pathway of chitin in the *Vibrio* system is a multi-step process that involves chitin attachment and degradation, followed by chitooligosaccharide uptake across the bacterial membranes, and the catabolism of the transport products to carbon and nitrogen sources. In this study will focus on the structural basis of chitin degrading enzymes, including endochitinase and exo-*N*-acetylglucosaminidase, as well as chitin transporter, and solute binding protein. Crystal structures of these enzymes/proteins in complex with chitooligosaccharides, together with detailed functional characterization provide mechanistic understanding how such molecular machinery works in an extremely coordinating manner in order to enable the bacteria to efficiently utilize chitin as their sole source of energy.

IL16

BOSTJAN KOBE

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EDUCATION

1994	PhD., University of Texas Soutwestern Medical Center at Dallas, USA
1989	BSc (Hons)., University of Ljubljana, Slovenia

WORK EXPERIENCE

Professor of Structural Biology and Australian Laureate Fellow,
School of Chemistry and Molecular Biosciences, Institute for Molecular Bioscience,
Australian Infectious Diseases Research Centre, University of Queensland, Australia

HONORS and AWARDS

2020	Slovenian Ambassador of Science
2018	Fellow of the Australian Academy of Science
2018	Beckman Coulter Discovery Science Award (ASBMB)
2017	School of Chemistry and Molecular Biosciences RHD Supervision Award
2017	Australian Research Council Laureate Fellowship

SELECTED PUBLICATIONS

1. Shi et al (2022) Structural basis of SARM1 activation, substrate recognition, and inhibition by small molecules. *Mol Cell* 82: 1643-1659
2. Figley et al (2021) SARM1 is a metabolic sensor activated by an increased NMN/NAD(+) ratio to trigger axon degeneration. *Neuron* 109: 1118-1136
3. Clabbers et al (2021) MyD88 TIR domain higher-order assembly interactions revealed by microcrystal electron diffraction and serial femtosecond crystallography. *Nat Commun* 12, 2578.
4. Horsefield et al (2019) NAD+ cleavage activity by animal and plant TIR domains in cell death pathways, *Science* 365, 793–799.
5. Ve et al (2017) Structural basis of TIR-domain-assembly formation in MAL- and MyD88-dependent TLR4 signaling. Nat Struct Mol Biol 24: 743-751
6. Williams et al (2014) Structural basis for assembly and function of a heterodimeric plant immune receptor. *Science* 344, 299-303.

Signalling by cooperative assembly formation (SCAF) by TIR domains in innate immunity and cell death pathways

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TIR (Toll/interleukin-1 receptor) domains are key components of innate immunity and cell-death pathways in animals, plants and bacteria (1). Signaling depends on association of TIR domains. We reconstituted large assemblies of the TLR (Toll-like receptor) adaptor TIR domains and determined the structures of the filamentous assemblies of the TIR domains of TLR adaptor MAL (2) and the TLR4:MAL complex (unpublished) by cryo-electron microscopy (cryoEM), and MyD88 by micro-electron diffraction and serial femtosecond crystallography (3). We found that TIR domains involved in cell-death pathways, including those from the human TLR adaptor SARM1, involved in axon degeneration, those from plant immune receptors (NLRs), and those from bacteria, possess self-association-dependent NAD-cleavage activity (4-7). Crystal and cryoEM structures of these proteins shed light on the structural basis of their enzymatic activities. Our studies unify the mechanism of function of TIR domains as a nucleated and hierarchical process of "signaling by cooperative assembly formation" (SCAF) with prion-like features that leads to the activation of effector enzymes and show that some TIR domains can themselves function as effector enzymes (1). The structures will be useful for therapeutic development against neurodegenerative and inflammatory diseases and for development of improved resistance in agricultural crops.

(1) Nimma et al (2021) Front Immunol 12: 784484
(2) Ve et al (2017) Nat Struct Mol Biol 24, 743
(3) Clabbers et al (2021) Nat Commun 12, 2578
(4) Horsefield et al (2019) Science 365, 793
(5) Figley et al (2021) Neuron 109, 1118
(6) Shi et al (2022) Mol Cell 82, 1643-1659
(7) Manik et al (2022) bioRxiv 2022.05.07.491051

IL17

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EDUCATION

1993-1997	PhD., Biochemistry/Structural Biology, Seoul Nat'l University, South Korea
1991-1993	M.S., Biochemistry, Seoul Nat'l University, South Korea
1987-1991	B.S., Chemistry, Seoul Nat'l University, South Korea

WORK EXPERIENCE

2020-present	Associate Dean for Student Affairs, College of Natural Sciences, Seoul National University, South Korea
2012-present	Assistant Professor / Associate Professor / Professor, School of Biological Sciences, Seoul National University, South Korea
2000-2012	Postdoc & Research Associate, Departments of Structural, Biology/Molecular and Cellular Physiology, Stanford University, USA (PI: William Weis)

SELECTED PUBLICATIONS

1. Choi H.-K. †, Kang H. †, Lee C., Kim H.G., Phillips B.P., Park S., Tumescheit C., Kim S.A., Lee H., Roh S.-H., Hong H., Steinegger M., Im W., Miller E.A.*, **Choi H.-J.***. Yoon T.-Y.* Evolutionary balance between foldability and functionality of a glucose transporter. (2022) *Nat. Chem. Biol.*, DOI 10.1038/s41589-022-01002-w (*corresponding author)
2. Park C. †, Kim J. †, Ko S.-B., Choi Y.K., Jeong H., Woo H., Kang H., Bang I., Kim S.A., Yoon T.-Y., Seok C., Im W., **Choi H.-J.*** Structural basis of neuropeptide Y signaling through Y1 receptor. (2022) *Nat. Commun.* 13, 853. (*corresponding author)
3. Park C., Zheng X., Park C.Y., Kim J., Lee S.K., Won H., Choi J., Kim Y.-G.*, **Choi H.-J.*** Dual conformational recognition by Z-DNA binding protein is important for the B-Z transition process. (2020) *Nucleic Acids Res.*48 (22), 12957-12971. (*corresponding author)
4. Kim Jinuk, Han W., Park T., Kim E.J., Bang I., Lee H.S., Jeong Y., Roh K., Kim Jeesoo, Kim J.-S., Kang C., Seok C., Han J.-K., **Choi H.-J.*** Sclerostin inhibits Wnt signaling through tandem interaction with two LRP6 ectodomains. (2020) *Nat. Commun.* 11, 5357. (*corresponding author)
5. Kang, H., Yang, H.S., Ki, A.Y., Ko, S.B., Kim, K.W., Shim, C.Y., **Choi, H.-J.***, Chung, K.Y.* (2020) Conformational dynamics and functional implications of phosphorylated beta-arrestins. *Structure* **28**(3), 314-323. (*corresponding author)

Structural basis of Neuropeptide Y1 receptor activation

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Neuropeptide Y (NPY) is highly abundant in the brain and involved in various physiological processes related to food intake and anxiety, as well as human diseases such as obesity. However, the molecular details of the interactions between NPY and its receptors are poorly understood. Here, I present a cryo-electron microscopy structure of the NPY-bound neuropeptide Y1 receptor (Y_1R) in complex with G_i protein. The NPY C-terminal segment forming the extended conformation binds deep into the Y_1R transmembrane core, where the amidated C-terminal residue Y36 of NPY is located at the base of the ligand-binding pocket. Furthermore, the helical region and two N-terminal residues of NPY interact with Y_1R extracellular loops, contributing to the high affinity of NPY for Y_1R. In addition, a key residue for Y_1R-specific binding and activation will be discussed in this talk.

(1) Park C & Kim J et al. (2022) Structural basis of neuropeptide Y signaling through Y1 receptor. *Nat. Commun.* 13, 853.

IL18

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----------EDUCATION----------

1982-1987	University of Houston, Ph. D in Chemical Physics
1978-1982	East China Normal University, B.S. in Physics

----------WORK EXPERIENCE----------

2021-present	Chair Professor, Faculty of Synthetic Biology, Shenzhen Institute of Advanced Technology, Chinese Academy of Science
2013-present	NYU global network professor，Director of NYU-ECNU Center for Computational Chemistry
2009-present	Professor, ECNU
2005	Visiting Professor, Institute of Atomic and Molecular Science, Taiwan
2001-2008	Founding Director, Institute of Theoretical & Computational Chemistry, Nanjing University

----------AWARDS and Fellowships----------

2021	Fellow of the Royal Society of Chemistry (United Kingdom)
1999	Overseas Assessor of Chinese Academy of Science
1995	Alfred P. Sloan Research Fellow
1995	Camille Dreyfus Teacher-Scholar

----------SELECTED PUBLICATIONS----------

1. Wei, M., Zhang, X., Pan, X., Wang, B., **Ji, C.**, Qi, Y., and **Zhang, J. Z. H.** HobPre: accurate prediction of human oral bioavailability for small molecules. *J. Cheminform.* 14, 1 (2022)

2. Pan, X., Wang, H., Zhang, Y., Wang. X., Li, C., **Ji, C.**, and **Zhang, J. Z. H.** AA-score: A new scoring function based on amino acid-specific interaction for molecular docking. *J. Chem. Inf. Model.* 62, 2499–2509 (2022)

3. Tian, S., **Ji, C.**, and **Zhang, J. Z. H.** Molecular basis of SMAC-XIAP binding and the effect of electrostatic polarization. *J. Biomol. Struct. Dyn.* 39, 1588-1599 (2021)

4. Pan, X., Wang, H., Li, C., **Zhang, J. Z. H.**, and **Ji, C.** MolGpka: A web server for small molecule pKa prediction using a graph-convolutional neural network. *J. Chem. Inf. Model.* 61, 3159–3165 (2021)

Computational study of protein-ligand and protein-protein interactions

John Z.H. Zhang

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NYU-ECNU Center for Computational Chemistry at NYU Shanghai

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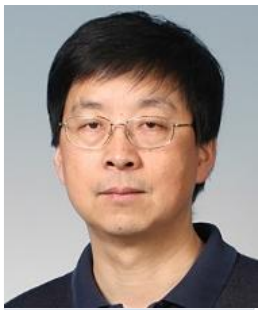
Protein-ligand and protein-protein interactions are fundamental processes in biology and their accurate prediction remains a grand challenge in computational biology. In this talk, we present some recent work in protein-ligand and protein-protein interactions. The reported work involved development of machine learning methods to accurately predict complex structures in protein-ligand systems as well as methods to predict binding energies and mutational effects in protein-protein interaction.

IL19



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EDUCATION

1992	Ph.D, Institute of Physical Science, University of Maryland, College Park, MD. USA
1987	MS, Department of Physics, Old Dominion Univ. Va. USA
1982	BS, Department of Physics, Northwest University, Xian, China

WORK EXPERIENCE

2012-present	Professor, Division of Life Science, The Hong Kong University of Science and Technology
2002 – 2011	Associate Professor, Department of Biochemistry, The Hong Kong University of Science and Technology
1994 – 2001	Assistant Professor, Department of Biochemistry, The Hong Kong University of Science and Technology
1993 – 1994	Post-doc, NIDDK, NIH, USA

SELECTED PUBLICATIONS

1. Chen, Xingxiang, et al (2021) Simultaneous Real-Time Three-Dimensional Localization and FRET Measurement of Two Distinct Particles, ***Nano Lett*** 2021 Sep 22;21(18):7479-7485. doi: 10.1021/acs.nanolett.1c01328. Epub 2021 Sep 7
2. Yanyan Geng,†, Peiyuan Qian*, Bo Zhou* and **G Zhu*** (2021) Crystal structure of parallel G-quadruplex formed by the two-repeat ALS and FTD related GGGGCC sequence, ***Nucleic Acid Research*** (IF 16.5) 49(10), pp. 5881-5890
3. Aftab Amin, et al (2020) An Essential and Cell-Cycle-Dependent ORC Dimerization Cycle Regulates Eukaryotic Chromosomal DNA Replication, ***Cell Reports*** 30, 3323–3338
4. Y Geng, C Liu, B Zhou, Q Cai, H Miao, X Shi & **G Zhu*** (2019) The Crystal Structure of an Antiparallel Chair-type G-quadruplex Formed by Bromo-substituted Human Telomeric DNA. ***Nucleic Acids Res*****. 47**, 10 5395–5404
5. C Liu, B Zhou, Y Geng, DY Tam, R Feng, H Miao, N Xu, X Shi, Y You, Y Hong, BZ Tang, PK Lo, V Kuryavyi* & **G Zhu*** (2019) A Chair-type G-quadruplex Structure Formed by a Human Telomeric Variant DNA in K+ solution. ***Chemical Science*****, 10**, 218 – 226,

G-quadruplex Structures Formed by Human Telomeric DNA and C9orf72 Hexanucleotide Repeats

LIU Changdong[1†], XU Naining[1], FUNG Chun Po[1], SUEN Monica Ching[1], Yan Bin[1], Huang Zeguo[1], Qian Peiyuan[2] and ZHU Guang*[1,2]

[1]Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong SAR, China
[2]Hong Kong Branch of Guangdong Southern Marine Science and Engineering Laboratory (Guangzhou), Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong SAR, China
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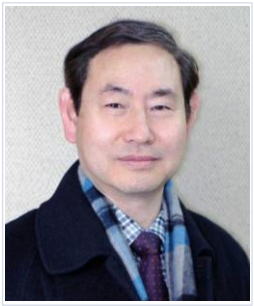
It is known that the G-quadruplex forming sequences are enriched in telomeres, promoters and in the first intron of genes. Many human diseases, such as cancer, acquired immune deficiency syndrome (AIDS) and amyotrophic lateral sclerosis (ALS), are closely related to the formation of G-quadruplexes, which represent promising drug targets of the potential therapeutics for these diseases.

My group has been interested in the structure-functional study of G-quadruplexes in above diseases and human DNA replication, and in search for specific inhibitors targeting these G-quadruplexes. Here, I will briefly present some results from this endeavor, including a novel G-quadruplex fold in K+ solution formed by a human telomeric variant, and its structure determined by NMR and x-ray crystallography. We also showed that the parallel d(G4C2)2 G-quadruplex, which is involved in ALS, folded as a symmetric tetramer, while the antiparallel d(G4C2)2 adopted the topology of an asymmetric dimer. Additionally, we demonstrated human origin recognition complex subunit 6 (hsOrc6) could also interact with the G-quadruplex DNA. We have also screened marine natural products to target these G-quadruplexes. Our results expand the repertoire of known G-quadruplex folding topologies and may provide potential targets for the structure-based anticancer and anti-ALS/FTD drug design. These works were supported by GRF(16103719, 16101120, 16101121), SMSEGL20SC01-H, AoE/M-403-16 and AOE/ M-401/20.

(1) Y Geng, C Liu, Q Cai, Z Luo, N Xu, CP Fung, B Yan, N Li, P Qian*, B Zhou* & **G Zhu*** (2021) Crystal struc ture of parallel G-quadruplex formed by the two-repeat ALS and FTD related GGGGCC sequence, ***Nucleic Acid Res.*** 49(10), pp. 5881-5890
(2) NN Xu, Y You, CD Liu, M Balasov, T Lee, Y Geng, C Fung, H Miao, H Tian, T Choy, X Shi, Z Fan, B Zhou, K Akhmetova, R Ud Din, H Yang, Q Hao, I Chesnokov,* & G Zhu* (2020) Structural Basis of DNA Replication Origin Recognition by Human Orc6 Protein Binding with DNA, *Nucleic Acids Res*. **48**(19):11146-11161.
(3) C Liu, B Zhou, Y Geng, DY Tam, R Feng, H Miao, N Xu, X Shi, Y You, Y Hong, BZ Tang, PK Lo, V Kuryavyi* & **G Zhu*** (2019) A Chair-type G-quadruplex Structure Formed by a Human Telomeric Variant DNA in K+ solution. ***Chemical Science*, 10**, 218 – 226,

TBS Lecture



WEONTAE LEE (이원태) (李元泰)

Professor
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EDUCATION

1994	Postdoc, Medical Physics Department of Medical Physics University of Toronto, Canada
1992	Ph.D. Biophysics/Physics, Department of Physics, Faculty of Science, University of Alabama, Birmingham, AL, USA
1984	MS. Physics, Department of Physics Education Seoul National University, Seoul, Korea

WORK EXPERIENCE

2018-present	Director, Institute of Science Education for Gifted & Talented, Yonsei University
2019-2020	Dean, College of Life Science and Biotechnology, Yonsei University, Seoul, Korea
1997-present	Professor, Department of Biochemistry, Yonsei University, Seoul Korea
2006-2008	Director, Functional Genomics Program Yonsei University, Seoul Korea

PROFESSIONAL ACTIVITIES

1. Organizing Chair, 2024 ICMRBS, 2020-Present
2. Organizing Chair/Advisory Board Member, Pacifichem, 2010-present

SELECTED PUBLICATIONS

1. 208 Peer Reviewed Papers including Nature, PNAS, Mol Cell, NSB
2. A High-Affinity Peptide Ligand Targeting Syntenin Inhibits Glioblastoma.
Haugaard-Kedström, L. M., Clemmensen, L. S., Sereikaite, V., Jin, Z., Fernandes, E. F. A., Wind, B., Abalde-Gil, F., Daberger, J., Vistrup-Parry, M., Aguilar-Morante, D., Leblanc, R., Egea-Jimenez, A. L., Albrigtsen, M., Jensen, K. E., Jensen, T. M. T., Ivarsson, Y., Vincentelli, R., Hamerlik, P., Andersen, J. H., Zimmermann, P., Weontae Lee, Kristian Strømgaard. 2021 Feb 11, In: Journal of Medicinal Chemistry. 64, 3, p. 1423-1434 12 p.
3. NMR spectroscopy uncovers direct interaction between BAF60A and p53
Han, J., Kim, T., Moon, S., Park, J. H., Lee, W., Ko, Y. J., Ryu, K. S., Yun, J. H. & Lee, W., 2021 Jan 1, In: Biochemical and Biophysical Research Communications. 534, p. 815-821 7 p.

TBS Lecture

Uncovering membrane protein structure and dynamics by cryo-EM and time-resolved serial femtosecond crystallography

Jihye Yun[1], Jahyun Park[1], Yunseok Heo[1], Ji-Joon Song[2], Sam-Yong Park[3] and Weontae Lee[1*]

[1]Structural Biochemistry and Molecular Biophysics Laboratory, Department of Biochemistry, Yonsei University, Seoul, Republic of Korea; [2]Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Korea; [3]Drug Design Laboratory, Graduate School of Medical Life Science, Yokohama City University, Tsurumi, Yokohama 230-0045, Japan

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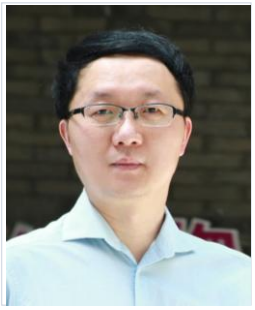
With dramatic advances of both hardware development and experimental techniques, studies on membrane protein structure and dynamic motion have become feasible from microcrystals at room temperature as well as non-crystalline EM condition. Non-cryogenic protein structures with time-resolved frame determined at ambient temperature could disclose significant information about membrane protein structure together with real-time motion in the membrane environments. In addition, Cryo-EM technique with advances of electron detector device and image processing methods enables to determine high-resolution structures of membrane proteins without crystallization. Therefore, SFX and Cryo-EM have become powerful new methods complimentary to conventional X-ray crystallography in studying structure and dynamics of proteins, particularly for membrane receptors and GPCRs. In this presentation, structure and dynamics of membrane proteins which are transport proteins and GPCRs will be discussed.

1.Jahyun Park, *et al*., (2022) *Nature*, In Press

2,Ji-Hye Yun, et al., (2021) *PNAS*, 118(13), e2020486118

3.Ji-Hye Yun, *et al*., (2020) *Science Advances*, 6(6), eaay2042

IL20



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EDUCATION

2002-2008	Ph.D., Biochemistry and Molecular Biology, Institute of Biophysics Chinese Academy of Sciences, Beijing, China
1998-2002	B.S., Chemistry, College of Chemistry and Molecular SciencesWuhan University, Wuhan, China

WORK EXPERIENCE

2015-present	Assistant Professor, School of Life SciencesTsinghua University
2013-2015	Research Fellow, The Small Angle X-ray Scattering Core Facility,National Cancer Institute at Frederick, NIH
2009-2013	Postdoctoral Associate, Protein-Nucleic Acid Interaction Section, National Cancer Institute at Frederick, NIH

SELECTED PUBLICATIONS

1. Burkhard Endeward[#], Yanping Hu[#], Guangcan Bai, Guoquan Liu, Thomas F. Prisner[*], **Xianyang Fang**[*]. Long-range distance determination in fully deuterated RNA with pulsed EPR spectroscopy. ***Biophysical Journal*** **2022，** 121(1):37-43.

2. Xiaolin Niu[#], Ruirui Sun[#], Zhifeng Chen, Yirong Yao, Xiaobing Zuo, Chunlai Chen[*], **Xianyang Fang**[*]. Pseudoknot length modulates the folding, conformational dynamics and robustness of Xrn1 resistance of flaviviral xrRNAs. ***Nature Communications*** **2021,** 12(1): 6417.

3. Junfeng Ma, Xiang Cheng, Zhonghe Xu, Yikan Zhang, Jaione Valle, Shilong Fan, Xiaobing Zuo, Iñigo Lasa, **Xianyang Fang**[*]. Structural mechanism for modulation of functional amyloid andbiofilm formation by Staphylococcal Bap protein switch. ***The EMBO Journal*** **2021**, 40(14): e107500. (**Research Highlight on *Nature Chemical Biology* 2021, 17: 839**)

4. Youqi Tao[#], Jingfei Xie[#], Qinglu Zhong[#], Yongyao Wang[#], Shengnan Zhang, Feng Luo, Fengcai Wen, Jingjing Xie, Jiawei Zhao, Xiaoou Sun, Houfang Long, Junfeng Ma, Qian Zhang, Jiangang Long, **Xianyang Fang**, Ying Lu, Dan Li, Ming Li, Jidong Zhu, Bo Sun[*], Guohui Li[*], Jiajie Diao[*], Cong Liu[*]. A novel partially-open state of SHP2 points to a "multiple gear" regulation mechanism. ***Journal of Biological Chemistry*** **2021,** 296: 100538.

5. Jaione Valle, **Xianyang Fang**, Iñigo Lasa. Revisiting Bap multidomain protein: more than sticking bacteria together. ***Frontiers in Microbiology*** **2020**, 11:613581.

Structural mechanism for modulation of functional amyloid and biofilm formation by Staphylococcal Bap protein switch

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Beijing Advanced Innovation Center for Structural Biology, School of Life Sciences, Tsinghua University, Beijing 100084, China

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The Staphylococcal Bap proteins sense environmental signals (such as pH, [Ca^{2+}]) to build amyloid scaffold biofilm matrices via unknown mechanisms. We here report the crystal structure of the aggregation-prone region of *S. aureus* Bap which adopts a dumbbell-shaped fold. The middle module (MM) connecting the N-terminal and C-terminal lobes consists of a tandem of novel double-Ca^{2+}-binding motifs involved in cooperative interaction networks, which undergoes Ca^{2+}-dependent order-disorder conformational switches. The N-terminal lobe is sufficient to mediate amyloid aggregation through liquid-liquid phase separation and maturation, and subsequent biofilm formation under acidic conditions, such processes are promoted by disordered MM at low [Ca^{2+}] but inhibited by ordered MM stabilized by Ca^{2+}-binding, with inhibition efficiency depending on structural integrity of the interaction networks. These studies illustrate a novel protein switch in pathogenic bacteria and provide insights into the mechanistic understanding of Bap proteins in modulation of functional amyloid and biofilm formation, and development of anti-biofilm therapeutics.

IL21

KUEN-PHON WU 吳昆峯

Assistant Research Fellow
Institute of Biological Chemistry, Academia Sinica, Taiwan
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EDUCATION

2010	Ph.D., Dept. of Chemistry & Chemical Biology, Rutgers University, Piscataway, NJ
2002	M.Sc., Dept. of Life Sciences, National Tsing Hua University, Hsinchu, Taiwan
2000	B.Sc., Dept. of Life Sciences, National Tsing Hua University, Hsinchu, Taiwan

WORK EXPERIENCE

2019-present	Joint Appoint Assistant Professor, Institute of Biochemical Sciences, National Taiwan University, Taiwan
2018-present	Secretary in general, Taiwan Magnetic Resonance Society
2018-present	Assistant Research Fellow, Institute of Biological Chemistry, Academia Sinica, Taiwan
2017-2018	MOST-awarded assistant research scholar, Institute of Biological Chemistry, Academia Sinica, Taiwan
2012-2017	Post-doctoral fellow, Department of Structural Biology, St. Jude Children's Research Hospital, Memphis, TN
2010-2012	Post-doctoral fellow, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ

SELECTED PUBLICATIONS

1. Huang PC, Chen SK, Chiang WH, Ho MR, **Wu KP**. Structural basis for the helical filament formation of Escherichia coli glutamine synthetase. Protein Sci. 2022 May;31(5):e4304.
2. Yang TJ, Li TN, Huang RS, Pan MY, Lin SY, Lin S, **Wu KP**, Wang LH, Hsu SD. Tumor suppressor BAP1 nuclear import is governed by transportin-1. J Cell Biol. 2022 Jun 6;221(6):e202201094.
3. Weston KP, Gao X, Zhao J, Kim KS, Maloney SE, Gotoff J, Parikh S, Leu YC, **Wu KP**, Shinawi M, Steimel JP, Harrison JS, Yi JJ. Identification of disease-linked hyperactivating mutations in UBE3A through large-scale functional variant analysis. Nature communications. 2021 Nov 23;12(1):6809.
4. Chiu YH , Ko KT, Yang T-J, **Wu KP**, Ho M-R, Draczkowski P, and Hsu SD. Direct Visualization of a 26 kDa Protein by Cryo-Electron Microscopy Aided by a Small Scaffold Protein. Biochemistry. 2021 Apr 13;60(14):1075-1079

Structural polymorphism of alpha-synuclein amyloid fibrils implicated in Parkinson's disease

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Human α-synuclein (aSyn) is an intrinsically disordered protein, and aggregations of its amyloid fibrils are associated with Parkinson's disease (PD). Apart from familial aSyn mutations, accumulated environmental divalent ions exacerbate aSyn aggregation and accelerates symptoms in aging PD patients. Here, we explored the effects of divalent ions on an aSyn, aggregation-prone mutant variant. From the disordered states to fibrillar structure, paramagnetic nuclear magnetic resonance (NMR) revealed that binding of ions to the aSyn C-terminal (residues 110-140) relaxed the aSyn conformation, resulting in more aggressive fibrillogenesis. Cryo-electron microscopy structures of aSyn with or without these divalent ions revealed substantial differences in amyloid folds and fibril assemblies. We characterized N1 (residues 61-66), N2 (residues 69-79), and N3 (residues 89-95) segments in the central non-amyloid β component (NAC) crucial for forming localized structural contacts during early-step aggregation. Our work establishes the contacts governing aSyn misfolding from disordered monomer to aggregated fibril and provides insights into the structural changes elicited by divalent ions.

IL22

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----------EDUCATION----------

1998-2002	Ph.D., Biochemical Sciences, National Taiwan University, Taiwan
1996-1998	M.S., Biochemical Sciences, National Taiwan University
1992-1996	B.S., Chemistry, National Chung-Hsing University

----------WORK EXPERIENCE----------

2016-present	Professor
2012-2016	Associate Professor
2007-2012	Assistant Professor
2005-2006	Academia Sinica distinguished postdoctoral scholarship
2003-2004	NHRI distinguished postdoctoral scholarship

----------SELECTED PUBLICATIONS----------

1. JV Kumar, TS Tseng, YC Lou, SY Wei, TH Wu, HC Tang, YC Chiu, CH Hsu*, and C Chen* (2022). Structural Insights into DNA Binding Domain of Vancomycin-resistance-associated Response Regulator in Complex with Its Promoter DNA from *Staphylococcus aureus*. **Protein Science**, 31(5), e4286.
2. MH Lin, CC Cho, YC Chiu, CY Chien, YP Huang, CF Chang, and CH Hsu* (2021). Elucidating the Tunability of Binding Behavior for the MERS-CoV Macro Domain with NAD Metabolites. **Communications Biology**, 4, 123.
3. YC Chiu, TS Hsu, CY Huang, and CH Hsu* (2021). Molecular Elucidation of a Urate Oxidase from *Deinococcus radiodurans* for Hyperuricemia and Gout Therapy. **International Journal of Molecular Sciences**, 22(11):5611.
4. SC Chen, LC Ye, TM Yen, RX Zhu, CY Li, SC Chang, SH Liaw*, and CH Hsu* (2021). Crystal Structures of *Aspergillus oryzae* Rib2 Deaminase: the Functional Mechanism Involved in Riboflavin Biosynthesis. **IUCrJ**, 8(Pt 4):549-558.
5. YC Chiu, TS Hsu, CY Huang, and CH Hsu* (2021). Structural and Biochemical Insights into a Hyperthermostable Urate Oxidase from *Thermobispora bispora* for Hyperuricemia and Gout Therapy. **International Journal of Biological Macromolecules**, 188, 914-923.
6. YC Chiu, MC Tseng, and CH Hsu* (2021). Expanding the Substrate Specificity of Macro Domains toward 3"-isomer of O-Acetyl-ADP- ribose. **ACS Catalysis**, 11(17), 11075-11090. (Featured on cover)

Proline Isomerization and Molten Globular Property of TgPDCD5 Confer its Regulation of Heparan Sulfate Binding

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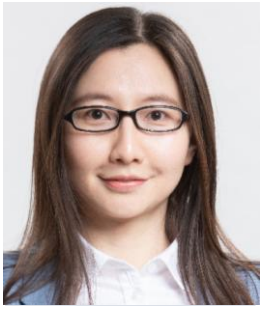
Toxoplasmosis is a zoonotic infection caused by the parasite *Toxoplasma gondii* with a wide range of clinical syndromes in humans. The infection can be dangerous for pregnant women and people with weak immune systems. The secreted protein TgPDCD5 (Programmed cell death protein 5 from *T. gondii*) was demonstrated to lead the increasing apoptosis of host macrophages via heparan sulfate-mediated endocytosis. However, the detailed mechanism of the entry of TgPDCD5 into the host cell remained mysterious.

Taking advantage of various biophysical tools and NMR spectroscopy, we found that TgPDCD5 adopts a helix-bundle structure connected to an extended N-terminal helix and with the properties of a molten globule, a loosely packed and highly dynamic conformational ensemble. Furthermore, NMR perturbation showed that the heparin/heparan sulfate-binding ability of TgPDCD5 is not only standing on the ground of an HSPG (heparan sulfate proteoglycan)-binding motif but contributed from the core region of TgPDCD5. Since a Pro107 residue is adjacent to the HSPG-binding motif, we speculate that proline isomerization may toggle TgPDCD5 between two conformations: an open conformation of the core region and HSPG-binding motif in the cis form, and a closed conformation promoted by the trans form. In addition to acting as a structural switch, the heterogeneous proline recruits another secretory cyclophilin homolog TgCyp18, which accelerates the interconversion rate between the two conformers, thereby regulating the heparan/heparin-binding of TgPDCD5. The data provide atomic insight into the mechanisms that underpin the functionality of this binary switch and elucidate its remarkable efficiency. The results also reveal new heparan sulfate-binding surfaces, expanding the versatility of molten globular properties and highlighting the noncanonical cellular entry of pathogenic TgPDCD5.

APPA Young Scientist Award



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----------EDUCATION----------

2013-2018	School of Life Sciences, Tsinghua University Program of Tsinghua-Peking Joint Center for Life Sciences Project: Structural and Functional Study of Human γ-secretase Supervisor: Prof. Yigong Shi
2009-2013	B.S., School of life Sciences, Northwest Agriculture & Forestry University

----------WORK EXPERIENCE----------

2020-present	R.A., School of Life Sciences, Tsinghua University Project: Molecular Basis of Modulation of γ-secretase by Regulatory Proteins or Small Molecules Drugs
2018-2020	Postdoc., School of Life Sciences, Tsinghua University Project: Structural Basis of Substrate Recognition by γ-secretase Advisor: Prof. Yigong Shi

----------SELECTED PUBLICATIONS----------

1. Jin Chen[#], Jiaoni Wang, Yumeng Wang, Bojun Jia, Xuefei Guo, Guanghui Yang, Peng Xu, Paul Greengard, **Rui Zhou***, Yigong Shi*. Modulation of amyloid precursor protein cleavageby gamma-secretase activating protein through phase separation[J]. **Proceedings of the National Academy of Sciences**, 2022. In press. ([#] co-first author, * corresponding author)
2. Guanghui Yang[#], **Rui Zhou**[#], Xuefei Guo, Chuangye Yan, Jianlin Lei, Yigong Shi*. Structural basis of γ-secretase inhibition and modulation by small molecule drugs[J]. **Cell**.2021;184(2):521-533.e14. (https://pubmed.ncbi.nlm.nih.gov/33373587/)
3. **Rui Zhou**[#], Guanghui Yang[#], Xuefei Guo, Qiang Zhou, Jianlin Lei, Yigong Shi*. Recognition of the amyloid precursor protein by human γ-secretase[J]. Science, 2019; 363(6428): eaaw0930. (https://pubmed.ncbi.nlm.nih.gov/30630874/)
4. Guanghui Yang[#], **Rui Zhou**[#], Qiang Zhou, Xuefei Guo, Chuangye Yan, Meng Ke, Jianlin Lei, Yigong Shi *. Structural basis of Notch recognition by human γ-secretase[J]. **Nature**, 2018; 565(7738):192-197. (https://pubmed.ncbi.nlm.nih.gov/30598546/)

APPA Young Scientist Award

Structural and functional insight into human γ-secretase regulation

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γ-secretase represents a potential therapeutic target for Alzheimer's Disease (AD). My talk will focus on a central biological question: how γ-secretase is affected by different factors thus contributing to AD pathogenesis?

To investigate the effect of AD-associated mutations, systematic characterization of the cleavage products from 138 PS1 mutations was performed. Our work provides a valuable resource for the research field, and indicates there is no single cause for AD. By covalent crosslinking method, we determined the structures of γ-secretase in complex with APP/Notch fragment, which uncovered the molecular basis of substrate recognition by human γ-secretase. New structural elements were found to form to facilitate the substrate cleavage. Additionally, we determined structures of γ-secretase bound to three representative inhibitors and a classic modulator. This work revealed underpinnings for the design and improvement of substrate-selective small molecules that still hold the promise for AD treatment. Recently, we found GSAP, γ-secretase activating protein, undergoes phase separation, thus modulating γ-secretase activity. All these works structurally and functionally help researchers gain a better understanding towards AD and facilitate related therapeutic intervention.

IL23

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EDUCATION

1998-2002	**Ph.D.,** Department of Biochemistry and Molecular Biology, University of Melbourne, Australia
1993-1996	**B.Sc. (Hons),** Botany and Genetics, University of Melbourne, Australia

WORK EXPERIENCE

2020-present	Promotion to Professor
2019-2023	NHMRC Senior Research Fellowship
2017	MDHS Faculty Research Fellowship
2017-2019	Deputy Head of Department
2012-2016	ARC Future Fellowship
2015	Promotion to Associate Professor

HONORS and AWARDS

2015	**McAulay-Hope Prize for Original Biophysics** –award from the Australian Society to recognise true originality and innovation in the field of biophysics, rather than the use of existing techniques or applications
2009	**Applied Biosystems Edman Award** –award from the Australian Society for Biochemistry and Molecular Biology (ASBMB) to a biochemist or molecular biologist with no more than 7 years postdoctoral experience, in recognition of their outstanding research work

SELECTED PUBLICATIONS

1. Cox, D., Ormsby, A. R., Reid, G. E., and Hatters, D. M. (2022) Protein painting reveals pervasive remodeling of conserved proteostasis machinery in response to pharmacological stimuli, bioRxiv, 2022.2005.2014.491969.
2. Raeburn, C. B., Ormsby, A., Moily, N. S., Cox, D., Ebbinghaus, S., Dickson, A., McColl, G., and Hatters, D. M. (2021) A biosensor to gauge protein homeostasis resilience differences in the nucleus compared to cytosol of mammalian cells, bioRxiv, 2021.2004.2019.440383.
3. Kriachkov, V., McWilliam, H. E. G., Mintern, J. D., Amarasinghe, S. L., Ritchie, M., Furic, L., and Hatters, D. M. (2022) Arginine-rich C9ORF72 ALS Proteins Stall Ribosomes in a Manner Distinct From a Canonical Ribosome-Associated Quality Control Substrate, bioRxiv, 2022.2002.2009.479805.

Problematic proteins in neurodegenerative disease: Sticky protein misassembly mechanisms

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Protein misfolding and aggregation is a hallmark of neurodegenerative diseases including Huntington's and Motor Neuron Disease. Our research has focused on how inappropriate protein aggregation leads to dysfunction in cell biology. Here, I will present two unpublished mini-stories based on our recent research[1,2]. The first will be on how abnormal dipeptide repeat (DPR) products formed in C9ORF72-associated amyotrophic lateral sclerosis leads to toxicity. We show that the arg-rich DPRs, which are most toxic to cell culture and animal models of disease, are promiscuous binders to other cellular proteins compared to the other less toxic or inert DPRs, especially those involved in translation. We show that the arg-rich DPRs lead to ribosome stalling. Through genome-wide CRISPR screens, we show that the canonical mechanisms for sensing and clearing stalled ribosomes are distinct to those involved in stalled arg-rich DPR-mediated stalls.

The second ministory focuses on what features of unfolded states of globular proteins mediates their inappropriate aggregation. We develop a new conceptual framework to defining how patterns of "stickers" within the unfolded state direct co-aggregation with self, and with other cellular proteins. Our work also finds evidence that the engagement of protein quality control systems to proteins prone to unfolding are governed by specific sticker patterns.

(1) Kriachkov, V., McWilliam, H. E. G., Mintern, J. D., Amarasinghe, S. L., Ritchie, M., Furic, L., and Hatters, D. M. (2022) Arginine-rich C9ORF72 ALS Proteins Stall Ribosomes in a Manner Distinct From a Canonical Ribosome-Associated Quality Control Substrate, bioRxiv, 2022.2002.2009.479805.
(2) Ruff, K. M., Choi, Y. H., Cox, D., Ormsby, A. R., Myung, Y., Ascher, D. B., Radford, S. E., Pappu, R. V., and Hatters, D. M. (2021) Sequence grammar underlying unfolding and phase separation of globular proteins, bioRxiv, 2021.2008.2020.457073.

IL24

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EDUCATION

2003	Ph.D. in Biological Chemistry from the Department of Biological Chemistry, Indian Association for the Cultivation of Science (IACS), Jadavpur, Kolkata, India.
1998	M.Sc. in Chemistry (Major in Organic Chemistry) from the Department of Chemistry, University of Calcutta, Kolkata, India.
1996	B.Sc. In Chemistry (Organic, Inorganic and Physical Chemistry), University of Calcutta, Kolkata, India

WORK EXPERIENCE

2018-present	**Professor**, Department of Biosciences and Bioengineering, IIT Bombay, Powai, Mumbai 400076, India
2014-2018	**Associate Professor**, Department of Biosciences and Bioengineering, IIT Bombay, Powai, Mumbai 400076, India
2013	**Visiting Fellow,** Lab Fur PhysikalischeChemie, ETH Zurich, Switzerland
2009-2014	**Assistant Professor,** Department of Bioscience and Bioengineering, IIT Bombay, Powai, Mumbai 400076, India

HONORS and AWARDS

1. Elected Fellow of Indian Academy of Sciences, Bangalore, 2022
2. P.B Rama Rao Memorial Award, 2021

SELECTED PUBLICATIONS

1.Krishnan, R., Ranganathan, S., **Maji, S. K**., Padinhateeri, R., (2022), Role of non-specific interactions in the phase-separation and maturation of macromolecules. **PLOS Computational Biology** doi.org/10.1371/journal.pcbi.1010067 **(IF: 4.4)**

2.Chatterjee, D., Jacob, R.S., Ray, S., Navalkar, A., Singh, N., Sengupta, S., Gadhe, L., Kadu, P., Datta, D., Paul, A., Mehra, S., Pindi, C., Kumar, S., Singru, P.S., Senapati, S.K. and **Maji, S. K**., (2022), Co-aggregation and secondary nucleation in the life cycle of human prolactin/galanin functional amyloids. **eLife** 11: e73835 **(IF: 8.1)**

3.Deshmukh, P.P., Malankar, G.S., Sakunthala, A., Navalkar, A., **Maji, S. K.,** Murale, D.P., Saravanan R. and Manjare S.T. (2022), Efficient Chemodosimeter for Hg(II) Via Diselenide Oxidation, **Dalton Trans** 51, 2269-2277 **(IF: 4.3)**

Liquid-Liquid phase separation of proteins and polypeptides

Manisha Poudyal[1]†, Komal Patel[1]†, Ajay Singh Sawner[1], Laxmikant Gadhe[1], Pradeep Kadu[1], Debalina Datta[1], Semanti Mukherjee[1], Soumik Ray[1], Ambuja Navalkar[1], Siddhartha Maiti[1,2], Debdeep Chatterjee[1], Riya Bera[1], Nitisha Gahlot[1], Ranjith Padinhateeri[1] and Samir K. Maji[1]*

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Liquid-liquid phase separation (LLPS) has emerged as a critical biological mechanism for the formation of membrane-less organelles in cells. The cell sequesters various macromolecules (such as proteins and nucleic acids) to form liquid condensates for performing various biological functions of the host organism. LLPS has also emerged as a crucial nucleation mechanism for protein aggregation associated with various neurodegenerative disorders such as Parkinson's and Alzheimer's disease. These condensates are dynamic entities, maintained by multivalent interactions facilitated by the presence of unstructured and intrinsically disordered domains of proteins. We hypothesized that LLPS might be a generic property of proteins and polypeptides under high crowding and/or with conditions favoring high intermolecular interactions. To demonstrate this, we studied more than 20 proteins/polypeptides and showed that depending upon the sequence and polypeptide length, proteins/polypeptides undergo LLPS at a unique critical concentration and phase space. We further established that both exposed hydrophobic surface and charge-based interaction could be the driving force for protein/polypeptide LLPS. Using both single components and combinations of protein multicomponent (co-LLPS) systems, we establish that extent of intermolecular interactions determines the propensity of protein/polypeptide LLPS.

KEYWORDS: Liquid-liquid phase separation; protein condensates; protein droplets.

IL25

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EDUCATION

1994	Ph.D., Biophysics, the Chinese Academy of Sciences, China.

WORK EXPERIENCE

2002-2008	Assistant Professor. Jointly appointed at Department of Biochemistry, School of Medicine and Department of Biological Sciences, Faculty of Science, National University of Singapore, Singapore.
1997-2002	Senior Research Scientist at Biotechnology Research Institute, National Research Council, Canada.
1996-1997	Postdoctoral Research Associate at Department of Biochemistry, University of Connecticut Health Center, School of Medicine, USA.
1994-1996	Postdoctoral Research Associate at Protein Engineering Institute, CEA (Commission of the Atomic Energy) Saclay, France.

SELECTED PUBLICATIONS

1. Dang, M., & **Song, J*** (2022) CTD of SARS-CoV-2 N protein is a cryptic domain for binding ATP and nucleic acid that interplay in modulating phase separation. ***Protein Science***. 31, 345–356. **(Citations: 2)**.
2. Dang, M., & **Song, J*** (2021) Structural basis of anti-SARS-CoV-2 activity of HCQ: specific binding to N protein to disrupt its interaction with nucleic acids and LLPS. ***QRB Discovery***, 2: e13, 1–9 **(Citations: 1).**
3. Dang, M., Lim, L., Kang, J., & **Song, J*** (2021) ATP biphasically modulates LLPS of TDP-43 PLD by specifically binding arginine residues. ***Communications Biology***, *4*(1) **(Citations: 5).**
4. Kang, J., Lim, L., & **Song, J*** (2019) ATP binds and inhibits the neurodegeneration-associated fibrillization of the FUS RRM domain. ***Communications Biology***, *2*(1) **(Citations: 39)**.
5. Kang, J., Lim, L., Lu, Y., & **Song, J*** (2019) A unified mechanism for LLPS of ALS/FTLD-causing FUS as well as its modulation by ATP and oligonucleic acids. ***PLOS BIOLOGY***, *17*(6), 33 pages **(Citations: 48)**.

ATP modulates phase separation of SARS-CoV-2 N protein druggable by HCQ

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ATP, the universal energy currency for all living cells but absent in viruses, mysteriously has concentrations >mM, much higher than required for its previously-known functions (1). SARS-CoV-2 is the coronavirus causing the ongoing pandemic with >521 millions of infections and >6.26 millions of deaths. Its nucleocapsid (N) is the only structural protein which plays essential roles in key steps of the viral life cycle by binding various viral and host-cell nucleic acids. In this context, any small molecules capable of interfering in its interaction with nucleic acids may manifest the anti-SARS-CoV-2 activity. N protein with highly conserved sequences in all variants including Delta and Omicron is composed of both folded N-/C-terminal domains (NTD/CTD) as well as three long intrinsically-disordered regions (IDRs). Here, I will discuss our results: 1) N protein undergoes liquid-liquid phase separation (LLPS) driven by interacting with nucleic acids. Unexpectedly, ATP biphasically modulates LLPS and appears to be evolutionarily hijacked by SARS-CoV-2 to promote its life cycle (2). 2) CTD and IDRs have been decoded to be cryptic ATP and nucleic-acid-binding domains (3). 3) Hydroxychloroquine (HCQ) specifically binds NTD and CTD to inhibit their interactions with nucleic acids, as well as to disrupt LLPS. Most importantly, HCQ-binding residues are identical in SARS-CoV-2 variants and therefore HCQ is likely effective to different variants. The unique structure of the HCQ-CTD complex offers a promising strategy for further design of anti-SARS-CoV-2 drugs with better affinity and specificity (4,5). The findings define N protein to be a key drug target and indicate that LLPS is indeed druggable by small molecules, thus opening up a promising direction for future drug discovery/design by targeting LLPS in general.

(1) Song J. (2021) Adenosine triphosphate energy-independently controls protein homeostasis with unique structure and diverse mechanisms. *Protein Sci.* 31: 345-356
(2) Dang M, Li Y, Song J. (2021) ATP biphasically modulates LLPS of SARS-CoV-2 nucleocapsid protein and specifically binds its RNA-binding domain. *Biochem Biophys Res Commun*. 19: 50-55.
(3) Dang M, Song J. (2022) CTD of SARS-CoV-2 N protein is a cryptic domain for binding ATP and nucleic acid that interplay in modulating phase separation. *Protein Science*. 31:345–56.
(4) Dang M, Song J. (2021) Structural basis of anti-SARS-CoV-2 activity of HCQ: specific binding to N protein to disrupt its interaction with nucleic acids and LLPS. *QRB Discovery*, 2: 1–9.
(5) Dang M, Song J. (2022) A review of the effects of ATP and hydroxychloroquine on the phase separation of the SARS-CoV-2 nucleocapsid protein. *Biophysical Reviews*. In press.

IL26

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EDUCATION

1993	PhD, Department of Physiology, Univ. of Cincinnati.
1986	MS, Biology Dept., Peking University.
1983	BS, Biology Dept., Peking University.

WORK EXPERIENCE

2003-present	Assistant Professor, Associate Professor, ad full Professor, Division of Life Science, HKUST
1994-2002	Postdoc/Research Associate, UNC-Chapel Hill

SELECTED PUBLICATIONS

1. Cao X*, Zhou Z*, Tian Ye, Liu Z, Cheng KO, Chen X, Hu W, Wong YK, Li X, Zhang H, Hu, R, and **Huang P**. Opposing roles of E3 ligases TRIM23 and TRIM21 in regulation of ion channel ANO1 protein levels, **Journal of Biological Chemistry**, 296, 100738, 2021 (Featured as "**Editors' Picks**" due to "providing an exceptional contribution to the field").

2. Zhou Z, Yu X, Jiang B, Feng W, Tian Y, Liu Z, Wang J, and **Huang P**. Alternative splicing of 3 genes encoding mechanotransduction-complex proteins in auditory hair cells. **eNeuro,** 8(1**)**, 0381-20.2020, 2021.

3. Yu X, Zhao Q, Li X, Chen Y, Tian Y, Liu S, Xiong W, and **Huang P**. Deafness mutation D572N of TMC1 destabilizes TMC1 expression by disrupting LHFPL5 binding. **Proc Natl Acad Sci U S A,** 117(47), 29894, 2020.

4. Li X, Yu X, Chen X, Liu Z, Wang G, Li C, Wong EYM, Sham, MH, Tang J, He J, Xiong W, Liu Z, and **Huang P**. Localization of TMC1 and TMHS in auditory hair cells in neonatal and adult mice. **The FASEB Journal,** 33 (6), 6838, 2019.

5. Hu W, Yu X, Liu Z, Sun Y, Chen X, Yang X, Li X, Lam WK, Duan Y, Cao X, Steller H, Liu K, and **Huang P**. The complex of TRIP-Br1 and XIAP ubiquitinates and degrades multiple adenylyl cyclase isoforms. **eLife**, 6: e28021, 2017

6. Xie C, Cao X, Chen X, Dong Wang, Zhang WK, Sun Y, Hu W, Zhou Z, Wang Y, **Huang P**. Mechanosensitivity of wild-type and G551D cystic fibrosis transmembrane conductance regulator (CFTR) controls regulatory volume decrease in simple epithelia. **The FASEB Journal,** 30(4): 1579**,** 2016 (recommended by Faculty1000 Prime).

TMC1, LHFPL5, and the mechanotransduction complex in auditory hair cells

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The channel that governs mechanotransduction (MT) by auditory hair cells in the inner ear has been studied intensively for 4 decades, but its molecular identity remains enigmatic. Recently, transmembrane channel-like protein 1 (TMC1) and lipoma HMGIC fusion partner-like 5 (LHFPL5) are recognized as two critical components of the mechanotransduction complex in inner-ear hair cells. Our recent work examined the localization, physical and functional interactions, and alternative splicing of TMC1 and LHFPL5 by using multiple approaches, including our newly developed ultrasensitive microbead-based single-molecule pulldown (SiMPull) assay. We demonstrated that LHFPL5 physically interacted with and stabilized TMC1 in both heterologous expression systems and in the soma and hair bundle of hair cells. Moreover, the semi-dominant deafness mutation D572N in human TMC1 (D569N in mouse TMC1) severely disrupted LHFPL5 binding and destabilized TMC1 expression. Thus, our findings reveal previously unrecognized physical and functional interactions of TMC1 and LHFPL5 and provide insights into the molecular mechanism by which the D572N mutation causes deafness. Notably, these findings identify a missing link in the currently known physical organization of the mechanotransduction macromolecular complex. In addition, our findings on the alternative splicing of TMC1 and LHFPL5 genes reveal the potential complexity of the MT-complex composition and provide critical insights for guiding future research on the function, regulation, and trafficking of TMC1, and LHFPL5.

This work was supported by Hong Kong RGC GRF16102417, GRF16100218, and GRF16102720, GRF16101321, NSFC-RGC Joint-Research Scheme N_HKUST614/18, Shenzhen Basic Research Scheme (JCYJ20170818114328332), SMSEGL20SC01-K, and in part by the Innovation and Technology Commission (ITCPD/17-9).

IL27

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----------EDUCATION----------

1999	Ph.D., Medicinal Chemistry, State University of New York at Buffalo, USA
1987	B.S. College of Pharmacy, Ewha Womans University, Korea

----------WORK EXPERIENCE----------

2005 - present	Professor, College of Pharmacy, Ewha Womans University, Korea
2020 - presen	Director, "Global AI Drug Discovery Center
2021 – present	Ewha Fellow, Ewha Womans University
2015-2017	Department Chair, Department of Pharmacy, College of Pharmacy, Ewha Womans University, Korea

----------HONORS and AWARDS----------

- ***Faculty Best Research Award*** from Ewha Womans University (2020)
- ***Presidential Citation for the Order of Science and Technology Merit*** designated by Ministry of Science and ICT of Korea (2019)
- ***NokAm Research Award*** from the Pharmaceutical Society of Korea (2019)
- ***Chun Moon Woo Medicinal Chemistry Research Award*** from the Division of Medicinal Chemistry of the Pharmaceutical Society of Korea (2019, 2020)

----------SELECTED PUBLICATIONS----------

1. "Transmembrane 4 L Six Family Member 5 Senses Arginine for mTORC1 Signaling" J. W. Jung, S. J. Y. Macalino, M. Cui, J. E. Kim, H. J. Kim, D. G. Song, S. H. Nam, S. Kim, S. Choi*, J. W. Lee*, ***Cell Metabolism*** **2019**, *29(6)*, 1306-1319.

2. "In Vivo Albumin Traps Photosensitizer Monomers from Self-Assembled Phthalocyanine Nanovesicles: A Facile and Switchable Theranostic Approach" X. Li, S. Yu, Y. Lee, T. Guo, N. Kwon, D. Lee, S. C. Yeom, Y. Cho, G. Kim, J.-D. Huang*, S. Choi*, K. T. Nam*, and J. Yoon*, ***J. Am. Chem. Soc.*** **2019**, *141(3)*, 1366-1372 (Cover).

3. "N-terminus-independent activation of c-Src via binding to a tetraspan(in) TM4SF5 in hepatocellular carcinoma is abolished by the TM4SF5 C-terminal peptide application," H. E. Song, Y. Lee, E. Kim, C. Y. Cho, O. Jung, D. Lee, E. G. Lee, S. H. Nam, M. Kang, S. J. Y. Macalino, J. Kim, J. W. Jung, S. W. Kwon, S. Choi*, J. W. Lee*, ***Theranostics*** **2021**, *11(16),* 8092-8111.

Comprehensive computational studies of membrane proteins for the structural and functional elucidation and drug discovery

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Transmembrane 4 L six family member 5 (TM4SF5) is a transmembrane protein known to interact with other TM4SFs, growth factor receptors, signaling proteins, and integrins, leading to uncontrollable cell growth and proliferation which eventually results in fibrosis and cancer. TM4SF5 contains 4 transmembrane (TM) helices, two extracellular loops (short (SEL) and long extracellular loop (LEL)), an intracellular loop, and N- and C-terminal cytosolic tails. The LEL region of TM4SF5 has previously been noted as integral to its function and interactions with molecular partners. Recently, we have identified TM4SF5 as an arginine sensor for mTORC1 activation and subsequent phosphorylation of its downstream effectors. Mutations of conserved residues in the LEL region were found to significantly affect mTORC1 function supporting the involvement of TM4SF5 in this pathway. To investigate important structural features of TM4SF5, we first generated a homology model using CD81 as template. Based on sequence conservation and mutational studies in the LEL region, we applied molecular dynamics (MD) simulations for the apo wild-type and mutant (W124A and Y126S) structures. Protein-protein docking with mTORC1 and MD simulation of ensuing complexes were also done. Trajectory and network analysis allowed us to distinguish the importance of each conserved residue to TM4SF5 structural integrity and function in the mTORC1 pathway. We also performed docking studies to predict the binding mode of L-arginine and TSAHC in the TM4SF5 LEL region. Information from the resulting protein-ligand complexes can be used for the design of potent TM4SF5 inhibitors.

(1) J. W. Jung, S. J. Y. Macalino, M. Cui, J. E. Kim, H. J. Kim, D. G. Song, S. H. Nam, S. Kim, S. Choi*, J. W. Lee*, ***Cell Metabolism*** **2019**, *29(6)*, 1306-1319.
(2) H. E. Song, Y. Lee, E. Kim, C. Y. Cho, O. Jung, D. Lee, E. G. Lee, S. H. Nam, M. Kang, S. J. Y. Macalino, J. Kim, J. W. Jung, S. W. Kwon, S. Choi*, J. W. Lee***,*** ***Theranostics*** **2021**, *11(16),* 8092-8111.

IL28

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EDUCATION

1979-1984	Ph.D. (Chemical Physics), University of Minnesota, Minneapolis
1976-1979	B.Sc. (Chemistry) First Class Honors, Royal Holloway College, London University

WORK EXPERIENCE

2008-present	Distinguished Research Fellow, IBMS, Academia Sinica, Taipei
1999-present	Professor (joint appointment), Chemistry Department, NTHU, Hsinchu
1998-2008	Research Fellow, Institute of Biomedical Sciences, Academia Sinica
1995-1999	Assoc. Professor (joint appointment), Chemistry Department, National Tsing Hua University (NTHU), Hsinchu

HONORS and AWARDS

2018-2022	Academia Sinica Investigator Award
2016-2019	National Science Council Outstanding Scientist Award

SELECTED PUBLICATIONS

1. Factors Allowing Small Monovalent Li^{+} to Displace Ca^{2+} in Proteins. Cédric Grauffel*, Wei-Hsiang Weng, and Carmay Lim*, (**2022**) *submitted*.
2. A CA19-9-targeting antibody with site-specifically linked ^{177}Lu-bound chelator bundles for radioimmunotherapy. Li-An Chen, Yueh-Hsiang Yu, Wei-Ting Tian, Wei-Chen Lin, Cédric Grauffel, Chun-Yi Wu, Chuan-Lin Chen, Carmay Lim, Hsing-Mao Chu, Tse Wen Chang, and Chi-Jiun Peng* (**2022**) *submitted*
3. A γ-tubulin complex-dependent pathway suppresses unscheduled ciliogenensis by promoting cilia disassembly. Sahana Shankar, Zi-Ting Hsu, Artur Ezquerra, Chien-Chien Li, Tzu-Lun Huang, Etienne Coyaud, Cédric Grauffel, Brian Raught, Carmay Lim, Jens Lüders*, Su-Yi Tsai*, and Kuo-Chiang Hsia* (**2022**) *submitted*.
4. Allosteric coupling between TM4 and selectivity filter regulates extracellular pH gating of TALK1 potassium channels. Wen-Hao Tsai (蔡文豪), Cédric Grauffel, Ming-Yueh Huang (黃名鉞), Sandra Postić, Marjan Slak Rupnik, Carmay Lim (林小喬), Shi-Bing Yang (楊世斌)*, *J. Biol.Chem.* (**2022**).

From Quantum-derived Principles Governing Cysteine Reactivity to Combating the COVID-19 Pandemic

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Our research interests are to

1. unravel the principles governing biological processes and use them to identify novel drug targets, guide drug design, and elucidate drug mechanisms, and
2. develop new methods for studying macromolecular interactions.

I will first give an overview of our research work.[1-2] Then I will describe the key factors governing cysteine reactivity in proteins from quantum mechanical/continuum calculations, & how they led to a novel multi-targeting strategy against SARS-CoV-2.[3-4]

1. Competition Among Metal ions for Protein Binding Sites: Determinants of Metal Ion Selectivity in Proteins. Todor Dudev & Carmay Lim, *Chem*. *Rev*. 2014 114: 538. Share link: https://pubs.acs.org/doi/abs/10.1021/cr4004665
2. Calcium in signaling: Its specificity and vulnerabilities towards biogenic and abiogenic metal ions. Todor Dudev, Cedric Grauffel, and Carmay Lim, *J. Phys. Chem. B* (2021) *125*, 10419-10431 (invited contribution to the *Special Issue 125 Years of The Journal of Physical Chemistry*). Share link: https://doi.org/10.1021/acs.jpcb.1c05154
3. Multi-targeting of functional cysteines in multiple conserved SARS-CoV-2 domains by clinically safe Zn-ejectors. Karen Sargsyan, Chien-Chu Lin, Ting Chen, Cédric Grauffel, Yi-Ping Chen, Wei-Zen Yang, Jian-Jong Liang, Chun-Che Liao, Yi-Ling Lin, Hanna S. Yuan, and Carmay Lim, *Chem. Sci.* (2020), *11,* 9904-9. Share link: https://doi.org/10.1039/D0SC02646HS
4. Synergistic inhibition of SARS-CoV-2 replication using disulfiram/ebselen and remdesivir. Ting Chen, Cheng-Yin Fei, Yi-Ping Chen, Karen Sargsyan, Chun-Ping Chang, Jian-Jong Liang, Chun-Che Liao, Yi-Ling Lin, Hanna S. Yuan, and Carmay Lim, *ACS Pharmcol. Transl. Sci.* (2021) 4, 898–907 Share link: https://doi.org/10.1039/D0SC02646H

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EDUCATION

1994	D.Sc, Chemistry, Kyoto University, Japan
1991	M.Sc, Chemistry, Kyoto University, Japan
1989	B.Sc, Physics and Chemistry, Kyoto University, Japan

WORK EXPERIENCE

2017-present	Professor, School of Life Science and Technology, Tokyo Institute of Technology
2003-2017	Associate Professor, Institute of Molecular and Cellular Biosciences, The University of Tokyo
2002-2003	Research Scientist, Quantum Bioinformatics Group, Japan Atomic Energy Research Institute

SELECTED PUBLICATIONS

1. Satoshi Yamashita, Misao Mizuno, Kazuhiro Takemura, Akio Kitao, and Yasuhisa Mizutani*. Dependence of Vibrational Energy Transfer on Distance in a Four-Helix Bundle Protein: Equidistant Increments with the Periodicity of α Helices. *The Journal of Physical Chemistry B* 2022 *126* (17), 3283-3290

2. Duy Phuoc Tran, Yuta Taira, Takumi Ogawa, Ryoga Misu, Yoshiki Miyazawa, Akio Kitao. Inhibition of the hexamerization of SARS-CoV-2 endoribonuclease and modeling of RNA structures bound to the hexamer. *Scientific Reports* 2022 12(1), 3860

3. Mohamed Marzouk Sobeh and Akio Kitao*. Dissociation Pathways of the p53 DNA Binding Domain from DNA and Critical Roles of Key Residues Elucidated by dPaCS-MD/MSM. . *Chem. Inf. Model.* 2022 62(5), 1294–1307

4. Hiroaki Hata, Duy Phuoc Tran, Mohamed Marzouk Sobeh, Akio Kitao. Binding free energy of protein/ligand complexes calculated using dissociation Parallel Cascade Selection Molecular Dynamics and Markov state model. *Biophysics and Physicobiology* 2021 18, 305-316

Conformational Change and Dissociation/Association of Biological Macromolecules Investigated by Parallel Cascade Selection Molecular Dynamics and Markov State Model

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Conformational change, and dissociation and association of biological macromolecules such as proteins and DNAs are closely associated with their functions. Molecular dynamics (MD) simulation is considered to be a powerful tool to observe such processes in atomic detail but their time scales tend to be longer than typical length of MD simulation. Parallel cascade selection molecular dynamics (PaCS-MD) is an efficient conformational sampling method that can observe relatively slow processes within tens of ns (1-3). By analyzing trajectories obtained by PaCS-MD with the Markov state model (MSM), we can conduct analysis of pathways of conformational changes, and dissociation and association of two molecules (4-7). Also, this combination, which we call PaCS-MD/MSM, enables us to calculate free energy profile of these processes, rates of the processes. In this talk, we will report latest progress of PaCS-MD/MSM and applications, which include the investigation of protein conformational changes, and dissociation and association process of small compounds, proteins, and DNA (8-11).

(1) R. Harada, A. Kitao, J. Chem. Phys. 139, 035103 (2013).
(2) R. Harada, A. Kitao, J. Chem. Theory Comput. 11, 5493 (2015).
(3) K, Takaba, D.P. Tran, A. Kitao J. Chem. Phys. 152, 225101 (2020).
(4) D.P. Tran, K. Takemura, A. Kitao, J. Chem. Theory Comput. 14, 404 (2018).
(5) D.P. Tran, A. Kitao, J. Phys. Chem. B, 123 2469 (2019).
(6) Y. Inoue et al., Structure 27, 965 (2019).
(7) D.P. Tran, A. Kitao, J. Chem. Theory Comput. 16, 2835 (2020).
(8) H. Hata et al., Sci. Rep. 10, 2351 (2020).
(9) H. Hata et al., Biophys. Physicobiol. 18, 305 (2021).
(10) M.M. Sobeh, A. Kitao, J. Chem. Inf. Model. 62, 1294 (2022).
(11) D.P. Tran et al., Sci. Rep. 12, 3860 (2022).

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----------EDUCATION and WORK EXPERIENCE ----------

Professor Ming-Daw Tsai received B.S. degree from National Taiwan University (1972) and Ph.D. from Purdue University (1978), and served in the faculty of the Department of Chemistry and Biochemistry, The Ohio State University in 1981-2006. Subsequently he moved to the Institute of Biological Chemistry of Academia Sinica, Taiwan. His research interests include mechanistic enzymology of phosphoryl transfer enzymes including DNA polymerases, kinases and phospholipases, and structure-function relationship of proteins in DNA damage response and cancer signaling, including ankyrin repeat proteins and FHA domain proteins. He probes mechanistic problems by applying emerging methodologies in structural biology, including NMR, X-ray crystallography, MS, and recently cryo-EM and XFEL, leading to 300 publications. He was elected to Fellow, American Association for the Advancement of Science (AAAS, 1992), Academician, Academia Sinica (2012), and Fellow, The World Academy of Science (TWAS, 2014).

----------SELECTED PUBLICATIONS----------

1. Maestre-Reyna M, Yang CH, Nango E, Huang WC, Ngurah Putu EPG, Wu WJ, Wang PH, Franz-Badur S, Saft M, Emmerich HJ, Wu HY, Lee CC, Huang KF, Chang YK, Liao JH, Weng JH, Gad W, Chang CW, Pang AH, Sugahara M, Owada S, Hosokawa Y, Joti Y, Yamashita A, Tanaka R, Tanaka T, Luo F, Tono K, Hsu KC, Kiontke S, Schapiro I, Spadaccini R, Royant A, Yamamoto J, Iwata S, Essen LO*, Bessho Y*, (Tsai MD)* (2022-06) *Nature chemistry* 14(6), 677-685 "Serial crystallography captures dynamic control of sequential electron and proton transfer events in a flavoenzyme."
2. (Tsai MD)*, Wu WJ, Ho MC (2022-05) *Annual review of biophysics* 51, 19-38 "Enzymology and Dynamics by Cryogenic Electron Microscopy."
3. Choi SH, Jeon B, Kim N, Wu HH, Ko TP, Ruszczycky MW, Isiorho EA, Liu YN, Keatinge-Clay AT, (Tsai MD), Liu HW (2021-12) *Journal of the American Chemical Society* 143(48), 20291-20295 "Evidence for an Enzyme-Catalyzed Rauhut-Currier Reaction during the Biosynthesis of Spinosyn A."
4. Chang YC, Chen CY, (Tsai MD)* (2021-07) *Jove-Journal of Visualized Experiments* 173, e62772 "Preparation of High-Temperature Sample Grids for Cryo-EM"

ABA Lecture

Dynamics in Enzyme Catalysis by Cryo-EM and XFEL

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Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan

In the past 5-10 years, breakthrough has occurred not only in cryo-EM, but also in X-ray free electron laser (XFEL). Both techniques have broken new grounds in the solution of high-resolution structures of macromolecular complexes. What is less known is their applications in the dynamic properties of enzyme catalysis. The goal of this lecture is to illustrate these applications of cryo-EM in several enzymes, and XFEL in the DNA repair by a DNA photolyase. The latter is based on time-resolved serial femtosecond X-ray crystallography (TR-SFX) at time scales from picoseconds to microseconds.

IL30

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EDUCATION

2009-2014	Ph.D. in Structural Biology and Biochemistry, School of Life Sciences, Tsinghua University, Beijing, China
2005-2009	B.S. in Biological Sciences, Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing, China

WORK EXPERIENCE

2019-present	Assistant Professor, Division of Life Science, The Hong Kong University of Science and Technology, Hong Kong SAR, China
2014-2018	Postdoctoral Scholar, Department of Biochemistry and Biophysics, University of California San Francisco, USA

SELECTED PUBLICATIONS

1. Cheng, J., Li, N., Huo Y., **Dang, S.**, Tye, B.[#], Gao, N.[#], Zhai, Y.[#]. Structural insight into the MCM double hexamer activation by Dbf4-Cdc7 kinase. *Nature Communications* 13:1396 (2022)
2. Fine, M.[#], Li, X.[#], **Dang, S.[#]**. Structural insights into group II TRP channels. *Cell Calcium* 86, (2020).
3. Feng, S.***, Dang, S.***,** Han, T.N., Ye, W., Jin, P., Cheng, T., Li, J., Jan, Y.N., Jan, L.Y.[#], Cheng, Y.[#]. Cryo-EM studies of TMEM16F calcium-activated ion channel suggest features important for lipid scrambling. *Cell Reports* 28, 567-579 (2019).
4. **Dang, S.**, van Goor, M.K., Wang, Y., Julius, D.[#], Cheng, Y.[#], van der Wijst, J.[#]. Structural insight into TRPV5 channel function and modulation. *PNAS* 116 (18) 8869-8878 (2019).
5. **Dang, S.***, Feng, S.*, Tien, J.*, Peters, C.J., Bulkley, D., Lolicato, M., Zhao, J., Zuberbühler, K., Ye, W., Qi, L., Chen, T., Craik, C.S., Jan, Y.N., Minor, D.L., Jr., Cheng, Y.[#], Jan, L.Y.[#]. Cryo-EM structures of the TMEM16A calcium-activated chloride channel. *Nature* 552, 426-429 (2017).
6. **Dang, S.***, Wu, S.*, Wang, J., Li, H., Huang, M., He, W., Li, Y.-M., Wong, C.C.L., Shi, Y.. Cleavage of amyloid precursor protein by an archaeal presenilin homologue PSH. *PNAS* 112 (11) 3344-3349 (2015).
7. Li, X.*, **Dang, S.***, Yan, C., Gong, X., Wang, J., Shi, Y.. Structure of a presenilin family intramembrane aspartate protease. *Nature* 493, 56-61 (2013).

Application of MSBP improves particle distribution and orientation in single-particle cryo-EM

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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 to prepare good quality frozen cryo-EM grids, which often is a trial-and-error process. Among many issues, preferred particle orientation and sample damaging by air-water interface (AWI) are common problem in practice. 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 applying MSBP keeps majority of particles away from air-water interface and mitigates preferred orientation, as demonstrated by analysis of both apoferritin and hemagglutinin (HA) trimer. Together, the use of MSBP is a simple method and could be applied to improve particle distribution for high-resolution structure determination using single-particle cryo-EM.

IL31

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EDUCATION

2003-2008	Ph.D. (Biochemistry), June 2008, A.M. (Biology), June 2005, Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA
1998-2002	B.Sc. (Biochemistry), First Class Honours, Department of Biochemistry, Chulalongkorn University, Bangkok, Thailand

WORK EXPERIENCE

2020-present	Associate Professor of Biochemistry, Mahidol University
2015-2020	Assistant Professor of Biochemistry, Mahidol University
2012-2015	Lecturer, Mahidol University
2009-2012	Post-doctoral research, Harvard University and The City College of New York

HONORS and AWARDS

2020	M.R. Jisnuson Svasti Young Protein Scientist of Thailand Award
2013	Dissertation Award from the National Research Council of Thailand (Excellent category, ระดับดีเด่น)

SELECTED PUBLICATIONS

1. Permsirivisarn P, Yuenyao A, Pramanpol N, Charoenwattanasatien R, Suginta W, Chaiyen P, **Pakotiprapha D***. Mechanism of transcription regulation by *Acinetobacter baumannii* HpaR in the catabolism of *p*-hydroxyphenylacetate. *FEBS J*. 2021; Dec 30.

2. Pakparnich P, Sudsumrit S, Imwong M, Suteewong T, Chamchoy K, **Pakotiprapha D**, Leartsakulpanich U, Boonyuen U. Combined effects of double mutations on catalytic activity and structural stability contribute to clinical manifestations of glucose-6-phosphate dehydrogenase deficiency. *Sci Rep.* 2021; 11(1):24307.

3. Tanramluk D, **Pakotiprapha D**, Phoochaijaroen S, Chantravisut P, Thampradid S, Vanichtanankul J, Narupiyakul L, Akavipat R, Yuvaniyama J. MANORAA: A machine learning platform to guide protein-ligand design by anchors and influential distances. *Structure*. 2022; 30: 181–189.

4. Kraithong T, Sucharitakul J, Buranachai C, Jeruzalmi D, Chaiyen P, **Pakotiprapha D***. Real-time investigation of the roles of ATP hydrolysis by UvrA and UvrB during DNA damage recognition in nucleotide excision repair. *DNA Repair (Amst)*. 2021; 97: 103024.

Regulation of *Acinetobacter baumannii* p-hydroxyphenylacetate degradative gene cluster by HpaR transcription factor.

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HpaR is a transcription regulator in the MarR family that controls the expression of the gene cluster responsible for conversion of *p*-hydroxyphenylacetate to pyruvate and succinate for cellular metabolism. To obtain insights into the mechanism of regulation by HpaR, we determined the structures of *Acinetobacter baumannii* HpaR (*Ab*HpaR) and its complex with cognate DNA. Our structural and biochemical characterizations revealed that *Ab*HpaR binds upstream of the divergently transcribed *hpaA* gene and the *meta*-cleavage operon, as well as the *hpaR* gene, thereby repressing their transcription by blocking access of RNA polymerase. DNA binding specificity of HpaR is achieved via a combination of both direct and indirect DNA sequence readouts. The presence of 3,4-dihydroxyphenylacetate (DHPA), which is the substrate of the *meta*-cleavage reactions weakens DNA binding by HpaR and likely leads to expression of the target genes. Based on our findings, we propose a model for how *A. baumannii* controls transcription of HPA-metabolizing genes, which, unlike in *E. coli*, is independent of global catabolite repression and could be beneficial for metabolic engineering towards bioremediation applications.

(1) Permsirivisarn et al. *FEBS J* . 2021. doi: 10.1111/febs.16340. (online ahead of print).

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EDUCATION

2000-2005	Doctorate of Philosophy, Biological Sciences, Imperial College London, South Kensington, UK
1998-2000	Master of Science, Biochemistry (GPA: 3.92/4.00), Chulalongkorn University, Bangkok, Thailand
1994-1998	Bachelor of Science, Biochemistry (high 2nd class honours; GPA: 3.45/4.00), Chulalongkorn University, Bangkok, Thailand

WORK EXPERIENCE

2019-present	**Head of Structural and Computational Biology Research Unit**, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand
2018-present	**Associate Professor,** Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand
2013-2017	**Assistant Professor,** Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand
2007-2012	**Lecturer,** Department of Biochemistry, Chulalongkorn University, Bangkok, Thailand

SELECTED PUBLICATIONS

1. **Krusong, K.**, Ismail, A., Wangpaiboon, K., and Pongsawasdi, P. “Production of large-ring cyclodextrins by amylomaltases” **Molecules**, 2022, Vol. 27(4), 1446
2. Rungnirundorn, T., **Krusong, K.**, Kalayasiri, R. and Maes, M. “Leukocyte telomere length is not shortened in methamphetamine dependence or methamphetamine-induced psychosis but is increased following traumatic events” **World J Biol Psychiatry, 2022**
3. Trithavisup, K., Shi, Y.C., **Krusong, K.** and Tananuwong, K. “Molecular structure and properties of cassava-based resistant maltodextrins” **Food Chem, 2022,** Vol. 369, 130876
4. Sangwongchai, W., **Krusong, K.**, Thitisaksakul, M. “Salt tolerance at vegetative stage is partially associated with changes in grain quality and starch physiochemical properties of rice exposed to salinity stress at reproductive stage” **J Sci Food Agric, 2022,** Vol 102(1), pp. 370-382.
5. Sangwongchai, W., Tananuwong, K., **Krusong, K.** and Thitisaksakul, M. “Yield, grain quality and starch physiochemical properties of 2 elite Thai rice cultivrs grown under varying production systems and soil characteristics” **Foods, 2021,** Vol. 10 (11), pp. 2601

DESIGN OF HIV-1 PROTEASE INHIBITORS

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Human immunodeficiency virus type-1 (HIV-1) protease is the main target in the suppression of Acquired immunodeficiency syndrome (AIDS) progression by cleaving viral polyproteins contributing to mature structural and functional proteins. Darunavir (DRV), approved by the Food and Drug Administration (FDA), is one of the potent HIV-1 protease inhibitors (PIs). DRV interacts with HIV-1 protease via both hydrogen-bonding and hydrophobic interactions within the active site. The mutations in HIV-1 protease were found to decrease DRV susceptibility. In this study, we design and screen 20 darunavir analogs in silico. The docking results showed that the top 5 analogs could interact with the HIV-1 protease at the active site significantly better than the darunavir. Daraunvir analogs have been synthesized and tested for HIV-1 protease inhibition by fluorogenic assay.

IL33

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EDUCATION

1999-2003	PhD., Biological Chemistry, National Taiwan University, Taiwan

WORK EXPERIENCE

2015-present	Distinguished Professor, Institute of Genomics and Bioinformatics, National Chung Hsing University, Taiwan
2013-present	Professor, Institute of Genomics and Bioinformatics, National Chung Hsing University, Taiwan
2008-present	Joint Professor, TIGP-MBAS, Institute of Biological Chemistry, Academia Sinica, Taiwan
2014-2020	Director, Institute of Genomics and Bioinformatics, National Chung Hsing University, Taiwan

HONORS and AWARDS

2020	17th National Innovation Award for development of broad spectrum antiviral drug against coronaviruses
2020	Ministry of Science and Technology (MOST) Outstanding Research Award
2015	The Young Scholars' Creativity Award from the foundation for the advancement of outstanding scholarship

SELECTED PUBLICATIONS

1. Jia-Ning Hsu, Jyun-Siao Chen, Shan-Meng Lin, Yi-Jheng Chen, Jhen-Yi Hong, U-Ser Jeng, Shun-Yuan Luo, **Ming Hon Hou* (April 2022)** Targeting the N-terminus domain of the coronavirus nucleocapsid protein induces abnormal oligomerization via allosteric modulation. ***Frontiers in Molecular Biosciences,*** Volume 9, Article 871499, 2022
2. Chun-Wei Lin, Kuo-Yang Huang, Ching-Hsiung Lin, Bing-Yen Wang, Chew-Teng Kor, **Ming-Hon Hou**, and Sheng-Hao Lin **(February 2022)** Video-Assisted Thoracoscopic Surgery in Community-Acquired Thoracic Empyema: Analysis of Risk Factors for Mortality. ***Surgical Infections*** Vol. 23, No. 2. 191-198
3. Tai-Lin Chen, Anilkumar S. Patel, Vicky Jain, Ramajayam Kuppusamy, Yi-Wen Lin, **Ming-Hon Hou**, Tsann-Long Su*, and Te-Chang Lee* **(August 2021)** Discovery of Oral Anticancer 1,2-Bis(hydroxymethyl)benzo[g]pyrrolo[2,1-a]phthalazine Hybrids That Inhibit Angiogenesis and Induce DNA Cross-Links. ***Journal of Medicinal Chemistry***, volume 64, 17, pages 12469–12486.

Nucleocapsid capsid protein is a potential target for drug discovery against coronavirus

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The COVID-19 pandemic has claimed over 1 million human lives, infected another 50 million individuals, and wreaked havoc on the global economy. The crisis has spurred the ongoing development of drugs targeting its etiological agent, the SARS-CoV-2. The nucleocapsid protein (N protein) is located inside the virus particle and is one of the most abundant structural proteins in coronaviruses (CoVs). It binds to the viral RNA genome to form a virion core comprising a ribonucleoprotein (RNP) complex that assumes a long helical structure. The RNP is essential for maintaining the RNA in an ordered conformation for replication and transcription. The CoV N protein also plays a critical part in viral RNA synthesis. In addition to its role in viral processes, the CoV N protein is also involved in regulating cellular processes, such as gene transcription, interferon inhibition, actin reorganization, host cell cycle progression, and apoptosis. Moreover, the N protein is an important diagnostic marker and immunodominant antigen in host immune responses. Recent studies suggest that N proteins of coronaviruses and other viruses could be useful antiviral drug targets against infections caused by these viruses because they serve many crucial functions during the viral lifecycle. Here, we will present new strategies for structure-based drug development against coronaviruses by interfering with RNA-binding and self-association properties of N protein.

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EDUCATION

2017-2019	Bachelor of Laws, National Tsing Hua University
1998-2004	Ph.D., Department of Basic Medical Sciences, National Cheng Kung University, Taiwan
1993-1997	Bachelor, Department of Biology, National Cheng Kung University, Taiwan

WORK EXPERIENCE

2021-present	Professor, Institute of Molecular and Cellular Biology, National Tsing Hua University, Taiwan
2015-2021	Associate Professor, Institute of Molecular and Cellular Biology, National Tsing Hua University, Taiwan
2010-2015	Assistant Professor, Institute of Molecular and Cellular Biology, National Tsing Hua University, Taiwan
2009-2010	Project Leader, Biozentrum, University of Basel, Basel, Switzerland

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1. Yang TJ, Li TN, Huang RS, Pan YC, Lin SY, Lin S, Wu KP, **Wang LH**, and Hsu SD. Tumor suppressor BAP1 nuclear import is governed by transportin-1. ***Journal of Cell Biology***, 2022, In press
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Evolution of SARS-CoV-2 spike protein push forward to cross-species infection

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Angiotensin-converting enzyme 2 (ACE2) is an angiotensin-converting enzyme that controls blood pressure and is the receptor for SARS-CoV and SARS-CoV-2. We consider it is interesting to identify ACE2 variants in humans and primates and explore viral susceptibilities of corresponding variants. With extensive data mining, we identified 396 amino acid variants of ACE2 in humans, among which 9 variants were located on the interphase between ACE2 and viral spike protein. To characterize the interaction between ACE2 and viral spike proteins, we developed a novel viral receptor-binding domain (RBD)-ACE2 attachment assay. RBD attachment was significantly reduced in human ACE2 variants E37K and M82I and completely abolished in variant D355N. For non-human primates, no new variant among the 6 apes and 20 Old World monkeys (OWMs) was identified. In contrast, New World monkeys (NWMs) and prosimian species shared several variants that cause a substantial reduction in binding affinity. Based on these findings, we suggested that the common ancestor of primates was strongly resistant to SARS-CoV-2, and the same was the Philippine tarsier, whereas apes and OWMs, like most humans, are susceptible for wild type SARS-CoV-2(1). With the emergence of new viral variants of concern, we developed a pseudovirus infection assay based on delta and omicron variants (2). Strikingly, ACE2 dependency of viral delta and omicron variants reduced significantly. In addition, both delta and omicron variants increased their infectivity to wild-type resistant ACE2 variants, including human D355N and major resistant primate variants. In short, our data raised a caveat for cross-species infection and transmission of SARS-CoV-2 variants of concern, which should be closely monitored in this pandemic of COVID-19.

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IL35

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----------**EDUCATION and WORK EXPERIENCE** ----------

Yu Heng Lau is a Senior Lecturer and Westpac Research Fellow in the School of Chemistry at the University of Sydney. Yu Heng completed a PhD in organic chemistry at the University of Cambridge, then moved to Harvard Medical School as a Sir Henry Wellcome Postdoctoral Fellow in bioengineering. In mid-2017, he was recruited back to Sydney where he has established a research program that spans the fields of medicinal chemistry and synthetic biology.

Yu Heng is recognised for developing new chemical methods for peptide cyclisation to target oncogenic protein-protein interactions. He has also established genome-scale engineering methods for the reprogramming of synthetic microorganisms, and protein engineering methods for manipulating *in vivo* self-assembly. Currently, his research program on controlling biomolecular architectures covers two main research themes: 1) Shape-controlled peptides as cancer therapeutic leads that target genome stability at telomeres, and 2) Protein nanocage architectures as synthetic organelles for controlling biocatalysis.

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Synthetic control of molecular flux into protein cages

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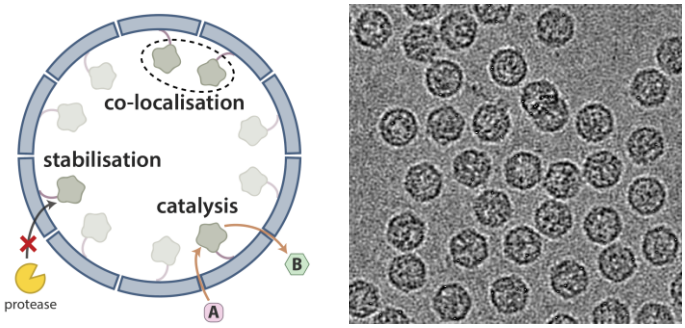
Living cells use compartments to organise the vast and seemingly incompatible plethora of biochemical reactions required for metabolism. In my lab, we aim to emulate Nature's organisation principles by using biological cage-like compartments as macromolecular nanoreactors for controlling catalysis.

Recently, our laboratory has studied a family of self-assembling bacterial protein cages known as encapsulins.[1] These nanosized protein cages can non-covalently encapsulate any given cargo protein of interest when that cargo is fused to a short peptide that acts as a tag for encapsulation. Despite significant bioengineering efforts, our fundamental understanding of such nanoreactor systems is still remarkably limited, especially in terms of the biophysical parameters that govern their stability and molecular flux through their pores.

I will outline our systematic analysis of 24 designed variants based on the T. maritima encapsulin protein organelle, each featuring pores of different size and charge.[2] We combine cryo-EM, molecular dynamics, and stopped flow kinetics, to uncover the complex interplay of factors that determines the kinetics of such nanoreactor systems.



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EDUCATION

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WORK EXPERIENCE

2020-present	**Novo Nordisk**, Molecular modelling and design department, Scientist
2020	**EPFL**[1], Post-Doctoral Fellow (Principal Investigator: Bruno E Correia, Ph.D.)
2015-2020	**EPFL**[1], Doctoral Researcher (Ph.D. Research Supervisor: Bruno E Correia, Ph.D.)
2014-2015	**DCB**[4], Associate Research Fellow (Employer: Chuan-Long Hsu, Ph.D.)
2011-2013	**Academia Sinica, Taiwan**, Master Assistant (Research Supervisor: Rita R.-Y. Chen, Ph.D.)

SELECTED PUBLICATIONS

1. Assessing and enhancing foldability in designed proteins. Listov D, Lipsh-Sokolik R, **Yang C**, Correia BE, Fleishman SJ. Protein science (under review), biorxiv: *doi.org/10.1101/2021.11.09.467863*

2. Bottom-up de novo design of functional proteins with complex structural features. **Yang C***, Sesterhenn F*, Bonet J, Aalen EV, Scheller L, Abriata LA, Cramer JT, Wen X, Rosset S, Georgeon S, Jardetzky T, Krey T,Fussenegger M, Merkx M & Correia BE (2021). *equal contribution. Nature Chemical Biology, *doi.org/10.1038/s41589-020-00699-x*

3. De novo protein design enables the precise induction of RSV-neutralizing antibodies. Sesterhenn F*, **Yang C***, Bonet J, Cramer JT, Wen X, Abriata LA, Kucharska I, Chiang CI, Wang Y, Castoro G, Vollers SS, Galloux M, Rosset S, Corthésy P, Georgeon S, Villard M, Descamps D, Delgado T, Rameix-Welti MA, Más V, Ervin S, Eléouët JF, Riffault S, Bates JT, Julien JP, Li Y, Jardetzky T, Krey T & Correia BE (2020). *equal contribution. Science, *doi: 10.1126/science.aay5051*

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Targeting molecular interface to de novo design of precision immunogens for respiratory syncytial virus.

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Traditional vaccine development efforts are facing a roadblock, failing to induce protective immunity against several pathogens. This failure is generally attributed to the fact that such vaccine approaches do not elicit antibody responses focused towards sites where the pathogen is vulnerable, allowing it to escape the host immune response. The main effort in this field throughout the last few decades has been the design of immunogens that spotlight neutralization epitopes for efficient recognition through the immune system.

We describes a “bottom-up” functional protein design strategy, aiming at centering the design process around the functional module. This generalized methodology allows the modularly-assembly of protein secondary structural elements to accommodate the functional binding motifs extracted from natural proteins. By this means, we designed the *de novo* protein to stabilize RSV epitopes outside of their native environment (RSVF) and applied those designed proteins as synthetic immunogens. Using a cocktail of three epitope-focused immunogens, we show the consistent elicitation of neutralizing antibodies in mice and non-human primates targeting the presented antigenic sites. Besides, a cocktail formulation of multiple epitope-focused immunogens is able to reshape serum antibody specificities under conditions of preexisting immunity in non-human primates.

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(2) Yang C*, Sesterhenn F*, *et al*. Bottom-up de novo design of functional proteins with complex structural features. Nat. Chem. Bio., 2021.

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EDUCATION

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WORK EXPERIENCE

2013-present	Postdoctoral Positions, Albert-Ludwigs-Universität Freiburg, Germany
2010-2013	Doctoral Researcher, Albert-Ludwigs-Universität Freiburg, Germany
2009-2010	Visiting Doctoral Researcher, University of Leeds, United Kingdom
2007-2008	Doctoral Researcher, IMPRS, Max-Planck-Institut für Biophysik, Frankfurt am Main, Germany

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[1] W.-C. Kao, C. Ortmann de Percin Northumberland, T. C. Cheng, J. Ortiz, A. Durand, O. von Loeffelholz, O. Schilling, M. L. Biniossek, B. P. Klaholz, and C. Hunte Structural basis for safe and efficient energy conversion in a respiratory supercomplex. *Nat. Commun.* 13, 545 (2022).

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Cryo-EM structure of the actinobacterial respiratory supercomplex: an efficient generator and versatile drug target.

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Respiratory chain cytochrome (cyt) *bc* complexes (complex III) and cyt *c* oxidases (complex IV) are major generators of the proton motive force that fuels ATP synthesis via transporting electrons obtained from metabolism. They are the enzymes which catalyse the last two steps of the conversion of molecular oxygen to water in cells, therefore complex III and complex IV, or their homologous enzymes are indispensable in aerobic lives. In a series of redox reactions catalysed by these complexes, free radical as well as reactive oxygen species may be produced as harmful byproducts. Respiratory chain complexes are proposed to assemble into supercomplexes to optimise the energy conversion efficiency, but less is known at the level of atomic detail. Here, we report the 2.8-Å resolution cryo-EM structure of the obligate complex III_2-IV_2 (cyt *bcc*-*aa*$_3$) supercomplex of the actinobacterium *Corynebacterium glutamicum* [1]. The resolved catalytic position of menaquinol as well as proton channels provide insights in the basis for concerted release of electrons and protons limiting wasteful and deleterious bypass reactions in the cyt *bcc* complex. A previously unknown menaquinone binding Q_c site and a tightly bound lycopene indicate the presence of a built-in free radical handling system. The conformational states of four conserved key protonable groups provide the basis for controlled proton uptake, loading and release and thus for effective proton pumping in cyt *c* oxidases. Our results show how safe and efficient energy conversion is achieved in a respiratory supercomplex. The well-resolved conformations of inhibitor as well as native substrates in the cryo-EM structure of the supercomplex may aid the rational design of drugs against actinobacteria that cause diphtheria and tuberculosis [2].

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[2] W.-C. Kao and C. Hunte, Quinone binding sites of cyt *bc* complexes analysed by x-ray crystallography and cryogenic electron microscopy. *Biochem. Soc. Trans*. **50**, 877-893 (2022)

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----------EDUCATION----------

1979-1982	Graduate Student, University of Science and Technology, Chinese Academy of Science
1973-1977	Undergraduate Student, University of Science and Technology, Chinese Academy of Science

----------WORK EXPERIENCE----------

Professor of Tsinghua University, Member of the Chinese Academy of Sciences, Member of the American Academy of Arts and Sciences，Member of the Standing Committee of the CPPCC National Committee, Member of the Presidium of Academic Divisions of CAS, Honorary President of Biophysics Society of China, Founding President of China Union of Life Science Societies. He was president of Nankai University, Director-General of the Institute of Biophysics of CAS, and President of International Biophysical Union (IUPAB).

He has revealed fundamental structure-function and mechanistic insights to the replication/transcription, assembly and host invasion of coronavirus, retrovirus, influenza virus, picornavirus, herpesvirus, Africa-Swine-Fever-virus and other disease-causing viruses, and uncovered how Mycobacterium tuberculosis achieves metabolite/energy transport and drug resistance. This has led to new therapeutic targets and innovative drug designs. To date, Zihe Rao has published more than 400 peer reviewed research papers, including 23 papers in Science, Nature and Cell, with over 25，000 citations (retrieved from Google Scholar). He also has 38 innovation patents.

----------SELECTED PUBLICATIONS----------

1. Yan, L.; Ge, J.; Zheng, L.; Zhang, Y.; Gao, Y.; Wang, T.; Huang, Y.; Yang, Y.; Gao, S.; Li, M.; Liu, Z.; Wang, H.; Li, Y.; Chen, Y.; Guddat, L. W.; Wang, Q.; Rao, Z.*; Lou, Z.*, Cryo-EM Structure of an Extended SARS-CoV-2 Replication and Transcription Complex Reveals an Intermediate State in Cap Synthesis. Cell 2021, 184 (1), 184-193 e10. PMID:33232691
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Living of SARS-CoV-2 inside the cell: Understand SARS-CoV-2 replication and transcription from structures

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Till June 2022, the pandemic of Coronavirus Disease 2019 (COVID-19) has caused over 532 million infections and over 6.3 million deaths worldwide. It has become the most devastating challenge to global health for a century. As the causative agent of COVID-19, SARS-CoV-2 encodes 16 non-structural proteins (nsp1-nsp16) that assemble a set of protein machineries, the Replication-Transcription Complexes (RTCs), that play central roles in virus replication and transcription cycle inside the host cells.

In the early of COVID-19 outbreak, we rapidly initiated the structural study of SARS-CoV-2 RTCs, aiming to dissect the key mechanisms for SARS-CoV-2 lives in human cells and provide structural information to discover potent antivirals. With great efforts from joint collaborations, we successfully determined the structure of the central RTC (C-RTC) composed by nsp12 (RNA-dependent RNA polymerase, RdRp) with cofactors nsp7 and nsp8[1], providing the first picture for the world to visualize this key antiviral target. We also elucidated how C-RTC catalyzes and how Remdesivir (RDV) inhibits the synthesis of RNA, through determining the structure of C-RTC in complex with template-product duplex RNA and the active form of RDV[2]. Subsequently, we presented the structure of the elongation RTC (E-RTC), showing how nsp13 (helicase) unwinds the high-ordered structure in genome to yield the functional template for RNA synthesis in C-RTC[3]. After that, we discovered a key intermediate state of RTC towards mRNA capping [Cap(-1)'-RTC], demonstrating the nsp12 NiRAN is indeed the key enzyme to catalyze the second capping action and presenting nsp9 is an "adaptor" for the further recruitments of capping enzymes into RTC[4]. Very recently, we successfully assembled Cap(0)-RTC by Cap(-1)'-RTC and

nsp10/nsp14 complex and determined its structures in a monomeric and a dimeric form. The monomeric Cap(0)-RTC structure shows the assembly of a co-transcriptional capping complex (CCC) to RTC for mRNA capping, while most interestingly, the dimeric form reasons an *in trans* backtracking mechanism for proofreading[5]. Other RTCs responsible for key steps for SARS-CoV-2 living inside cells have also been determined. These works not only provide a basis to understand SARS-CoV-2 proliferates in the host cells through a structural biology lens, but also shed the light for antiviral development against the rapid emerging of SARS-CoV-2 variants.

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2 Wang, Q. *et al.* Structural Basis for RNA Replication by the SARS-CoV-2 Polymerase. *Cell* **182**, 417-428.e413, doi:10.1016/j.cell.2020.05.034 (2020).

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5 Yan, L. *et al.* Coupling of N7-methyltransferase and 3'-5' exoribonuclease with SARS-CoV-2 polymerase reveals mechanisms for capping and proofreading. *Cell*, doi:10.1016/j.cell.2021.05.033 (2021).

POSTER SESSION

Functional analysis between AtSAP5 and AtTrx3 in the reduced salicylic acid immunity pathway

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Phytohormone salicylic acid (SA) plays an important role in the plant defense responses against biotic stresses. In the SA immunity pathway, AtNPR1 is a master regulator. In normal condition, AtNPR1 forms oligomeric state. While SA-induced redox oscillation, the oligomeric AtNPR1 will be reduced by TRX-h3/5 to form monomeric state and to enter nucleus for regulating defense gene expression. However, the reduced pathway in SA signaling pathway is still uncleared. AtSAP5, an interacting protein with TRX-h3/5, contains several Cys residues. Therefore, AtSAP5 might have the ability to reduce TRX-h3. In our results, AtSAP5 could interact with TRX-h3, and AtSAP5 also could reduce TRX-h3. In order to realize the interacting region between TRX-h3 and AtSAP5, the deletion mutants of AtSAP5 were constructed. The result showed that A20 domain of AtSAP5 has the strongest interaction ability. In reducing ability test, the A20 domain could reduce TRX-h3, but A20 domain lost reducing ability while mutating the conserved residues Cys. In conclusion, AtSAP5 could interact with TRX-h3 via the A20 domain and reduce TRX-h3 to involve in the SA immunity pathway. The results provided a novel regulating mechanism in SA-induce redox oscillation.

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The Inhibition and Activation Effects of Integrin-targeting Drugs

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Integrins are transmembrane proteins that mediate many important cellular responses. The dysfunction of integrins is associated with many diseases, and there are some integrin targeted drugs developed for therapeutic. However, it was also found that the treatment of RGD-mimetic integrin inhibitors caused proangiogenic effect and hampered their efficacy as anticancer agents[1]. We hypothesized that the binding of RGD inhibitors not only inhibits the interactions of integrins with ECM but also induces the activation of integrins. Disintegrins, rhodostomin and trimucrin, are snake venom proteins with an RGD motif that binds to integrin and blocks the interaction of integrin with ECM. A trimucrin KGDRR mutant was reported that did not prime the binding of platelets to fibrinogen and induce the exposure of the ligand-induced binding site (LIBS) of integrin αIIbβ3[2]. But the linker region and the RGD region of RR mutant composed of continuous charged amino acids, it may cause potential non-specific binding effect. Therefore, we make some mutation of disintegrin variants and try to understand the inhibition and activation effects. According to the cell adhesion and platelet aggregation assay, the results showed that the specificity of disintegrin variants for activated integrins is not the main cause of integrin activation, but mainly due to the interaction between specific amino acid sites and integrins. The regions of disintegrin variants that affect the activation of integrins are C-terminal region and the second amino acid after the RGD, while the second amino acid after RGD is positive charged can avoid the activation effect. The linker region and the first amino acid after RGD of disintegrin variants does not affect the activation of integrins, mutating the originally continuous charged amino acids may avoid the potential risk of nonspecific binding. The results of this study will serve as the basis for design of integrins-specific inhibitors without activating integrins.

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The Molecular Mechanism of Fluorescence Thermometer: Local Dynamic Motions of Tryptophan governs the Dependence of Fluorescence Intensity on Temperature

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Bovine serum albumin (BSA) is a protein often used in many biochemical research. Because of the presence of intrinsic tryptophan (Trp) fluorophores, BSA has many potential applications in fluorescence-based biosensors. Recent experimental studies showed that BSA exhibited a dependence of its fluorescence intensity on temperature in the range of 20 to 50 Celsius degrees, suggesting its potential use as molecular thermometer. However, the underlying mechanism is still not clear. To understand the molecular mechanism, we use molecular dynamics (MD) simulation to explore the temperature-dependent structural dynamics of BSA at several elevated temperatures: 303K, 313K, 323K, and 353K. We found that the global structural dynamics of BSA is mostly intact within the temperature range explored. In addition, the secondary structures in the vicinity of Trp134 do not change significantly. Interestingly, we observe a correlation between a dihedral motion of the Trp134 with the temperature, suggesting a thermal effect on the local dynamics of the residue, rather than the global structure of the protein. We, therefore, hypothesize that Trp fluorescence intensities correlate with the population change among different dihedral conformers of the Trp134–a rotamer model. To mimic the local structural dynamics of Trp134 within the global protein context for biologically relevant timescale, we used a “truncated” structure along with a RMSD biasing technique which allow us to propagate simulation timescale to sub-milliseconds (sub-ms). Our simulation results support our rotamer hypothesis. Furthermore, we carry out a simple theoretical analysis based on the rotamer model, which can successfully explain the temperature dependence on the fluorescence emission. This work combine both atomistic simulation and theoretical model to explain experimental phenomenon. We expect to see our approach being generalized to predict and design luminescent properties of other fluorescent proteins.

Engineering of Interleukin-2 Family Cytokines to Improve its Half-life and Stability for T Cell Therapy

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The treatment of cancer has evolved with generations, such as surgery, chemotherapy, and radiotherapy to the current adoptive cell therapy (ACT). ACT is known as cellular immunotherapy, attacking cancer with our immune cells. The interleukin-2 (IL-2) family cytokines play important roles in differentiation and proliferation of immune cells for these therapies. Since the half-lives of the IL-2 family cytokines in the human body and T cell culture media are very short, their treatments of cancers are required for high dose and multiple injections. It is known that human serum albumin (HSA) offers a safe solution for optimized cell performance and can increase the half-life and solubility of its fusion protein. To improve the half-life, performance, and solubility of IL-2 family cytokines in T cell culture media, we proposed to fuse the IL-2 family cytokines, including IL-2, IL-4, IL-7, IL-15, and IL-21, with HSA to prolong their half-lives and enhance their stability. Cell proliferation analysis of the mouse cytotoxic T lymphocyte cell (CTLL-2) induced by HSA-IL-2 and HSA-IL-15 showed that specific activities of HSA-IL-2 expressed in *Pichia pastoris* and ExpiCHO were similar to that of the commercial IL-2 from Prospec. In contrast, the activity of HSA-IL-15 expressed in ExpiCHO exhibited 10-fold higher than that of HSA-IL-15 expressed in *Pichia pastoris*. Interestingly, cell proliferation analysis of TF-1 cell showed that specific activity of the unglycosylated HSA-IL-4 N38A mutant has 3-fold higher than that of glycosylated HSA-IL-4. Stability test showed that the formulation of IL-2 family cytokines were important for their half-lives. The results of this study will be used to improve cell media for T cell therapy.

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Study and optimize the functional characterization of recombinant mutant tissue-type plasminogen activator fused with platelet-targeting disintegrin

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Thrombosis is the underlying cause for most cardiovascular events. Use of antithrombotic drugs are recommended as first-line treatment or prevention of a dangerous blood clot. However, antithrombotic therapy is often associated with significant medical complications, particularly excessive bleeding. In our previous study, integrin αIIbβ3-specific disintegrin mutant, trimucrin(RR), has been successfully designed to inhibit platelet aggregation with a low risk of bleeding. Based on the above reason, bifunctional fusion proteins are proposed by incorporating trimucrin(RR) into tenecteplase(TNK), a genetically engineered mutant tissue-type plasminogen activator(tPA), with different types of linkers. In this study, TNK、TNK-RR and RR-TNK were expressed in the ExpiCHO system and purified by affinity chromatography. Platelet aggregation and whole blood thrombolysis assay were subsequently conducted to evaluate the effects of TNK、TNK-RR and RR-TNK. The results from the platelet aggregation inhibitory activity showed that TNK-$(G_4S)_3$-RR and TNK-$(PA)_7$-RR had the better IC50 value, but they still exhibited 4.71 and 5.28-fold decreases in comparison with RR. Furthermore, the assessment of thrombolytic activity indicated that TNK-$(PA)_7$-RR had the equivalent efficacy to TNK. Taken together, these findings suggested the important role of the linker region and provide new insights into the design of antithrombotic drugs.

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Biophysical characterization of cancer-associated BAP1 mutants reveals an inherent tendency to form fibrillar aggregates

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Spontaneous accumulation of genetic mutations over time in genes that control the cell cycle can eventually deregulate cell signaling, causing normal cells to transform into cancer cells. Deubiquitinating enzymes (DUB), such as ubiquitin carboxyl-terminal hydrolases (UCHs) that are involved in removing ubiquitin from the target protein are known to play a crucial role in cell cycle regulation. Mutations in these UCHs are often associated with various ailments like cancer and neurodegeneration. BRCA-associated protein-1 (BAP1) is a family member of UCHs involved in cell cycle regulation, kinase activation, protein degradation, and DNA repair, and is a critical tumor suppressor gene that is mutated in various human cancers including uveal melanoma, pleural mesothelioma, renal cell carcinoma. About 60% of somatic mutations are reported to be present in one of the three domains, UCH domain, of BAP1 making it a significant cause of cancers. Herein, we have selected five of the naturally occurring mutations in the UCH domain of BAP1 and carried out biophysical characterization along with the wild-type protein. Our results show significantly perturbed structures in these mutants. They exhibit partial unfolding at body temperature and also have an inherent tendency to undergo aggregation. The rate of formation of aggregation propensity is enhanced in most of these cancer-associated variants and they also tend to form oligomers. Further, the negative stain transmission electron microscope images of these BAP1 variants reveal the formation of long protofibrils suggesting the loss of their function and their role in the development of cancers.

Design and Application of Antimicrobial/Anticancer Peptides

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With the abuse of antibiotics in medicine, the anti-microbial resistance of microorganisms is becoming more and more serious. So there is an urgent need for countermeasures against these bacterial infections. Nonetheless, the difficulty in developing new antibiotics results in low profit from the perspective of pharmaceutical companies. Thus, replacing antibiotics has become a consideration in all fields. Antimicrobial peptides, AMPs for short, are an alternative to a novel class of antibiotics.

In our previous investigation, we have designed five types of cationic AMPs. Among them, pepD with a unique sequence pattern “BBHBBHHBBH” (B: basic, H: hydrophobic) showed excellent antibacterial efficacy against a wide range of pathogens. Therefore, we set pepD as a template for further design to improve their efficacy (MIC/MBC of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*), safety (hemolysis and CC50 of HEK293), and stability (plasma stability), hoping to obtain a low-cost, highly safe, and stable AMP. Our strategy is to shorten AMPs, simplify sequences, and replace the amino acids to receive more potent and selective AMPs while reducing manufacturing costs and toxicity. Besides, we also want to figure out the most active structure that could perform the best. Our result showed that the minimum peptide length is 11 amino acids, and the stereochemistry of AMP is essential, which should have the same chiral properties. Substituting Leucine with Isoleucine would not affect the antibacterial activity and could reduce cytotoxicity, increasing the AMP safety in the in-vivo application. Surprisingly, the non-canonical amino acid replacement prolonged their half-life in plasma without affecting their safety and antimicrobial ability. At last, we also found that our antimicrobial peptides have anticancer activity.

Primary reaction of red fluorescence protein observed by fastscan femtosecond spectroscopy system

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We have developed a 10fs visible pulse laser whose bandwidth is broad enough to cover whole absorption band of the red fluorescence protein (RFP) to be studied in the present work. The ultrashort pulse enables us to observe molecular vibration in time domain which visualizes vibrational dynamics and structural dynamics during the photoreaction of RFP.

The transient absorption spectroscopy using ultrashort pulse has advantage of its ability to measure electronic dynamics and vibrational dynamics simultaneously in a single shot, which helps to visualize its primary reaction from two viewpoints of electronic state transition and molecular structure dynamics. However, this measurement takes about 30 minutes for single scan of measurement to observe the whole femtosecond region with accuracy resolving molecular vibration in time domain. Because of this long measurement time which results in serious damage accumulation, this method is thought to be not applicable for ultrafast spectroscopy of protein samples.

We have developed a fastscan femtosecond spectroscopy system which enables us to perform this measurement in five seconds for each scan, and it was applied for ultrafast spectroscopy of the RFP sample in the present work. The fluorescence lifetime of RFP was reported to be 2.42 ns.[1] In the present study with femtosecond resolution, we have observed the primary reaction of RFP with lifetime of <100 fs and vibrational dynamics proceeding in femtosecond region.

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Structural and functional elucidation of adenylation domain of nonribosomal peptide synthetase-like protein in Aspulvinone E biosynthesis

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Nonribosomal peptide synthetase (NRPS) is a large multimodular protein category that orchestrates the production of the secondary metabolite containing peptide bonds. Interestingly, the quinone structure-containing natural product could be synthesized by one kind of NRPS-like protein, a monomodular protein composed of A-T-TE tri-domain, and had a lack of participation of the C domain in the condensation stage. In *Aspergillus terreus*, an NRPS-like protein named ApvA is confirmed to synthesize melanin, Aspulvinone E. The A domain in ApvA (ApvA-A) is responsible for adenylating 4-hydroxyphenylpyruvic acid (4-HPPA), an aromatic α-keto acid converted from L-tyrosine by aminotransferase. However, the substrate-binding information of the aromatic α-keto acid adenylated domain is still poorly understood.

Hence, we aimed to elucidate the substrate specificity and catalytic mechanism of ApvA-A. The gene of ApvA-A was successfully cloned from the fungal genome, and recombinant protein could be produced in high purity. Analytical gel filtration data revealed that ApvA-A exists as a dimer form in solution. In addition, various 4-HPPA analogs, including L-Tyr, L-Phe, and L-DOPA, were applied to an activity assay of ApvA-A, which is measured by the hydroxylamine trapping continuous MesG-pyrophosphate method. The data showed the activities of L-Tyr and L-DOPA were both lower than that of 4-HPPA, while L-Phe as substrate was not detectable. Structural information of ApvA-A is also attempted to approach via both protein crystallography and molecular simulation. Crystal structure determination of ApvA-A is in progress. A complex model of ApvA-A and 4-HPPA was further constructed by using molecular docking, demonstrating that A216, F260, and A333 of ApvA-A could create a hydrophobic surrounding in the substrate-binding site. The aromatic ring of 4-HPPA could interact with F260 via the π-π stacking. Moreover, E220 could form a hydrogen bond with the hydroxyl group in the para position of 4-HPPA. The result indicated that the para-hydroxyl group in the aromatic ring is crucial to substrate recognition, and the α-keto group would have an influence on substrate identification.

The novel conformational landscape of PEDV S trimeric glycoprotein

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Porcine epidemic diarrhea virus (PEDV), which belongs to alphacoronavirus, causes enteric disorders in piglets with an exceptionally high fatality, bringing substantial economic losses to the pork industry. The spike (S) protein of PEDV mediates virus attachment to the target cell and subsequent membrane fusion. The structures of the S proteins of two different PEDV strains have been reported with opposing orientations of the N-terminal domain, known as the D0 domain, which is unique to alphacoronaviruses[1,2]. Here, we integrated cryo-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET) to determine in situ on intact viruses and recombinant forms the molecular structures of the S protein of a domestic PEDV strain. The recombinant PEDV S proteins were produced in a human cell line and a porcine cell line, which represents the native host environment for the post-translational modifications, i.e., glycosylation. Aided by mass spectrometry-based glycopeptide analysis, we identified a N-glycan that likely contributes to the regulation of the up/down switching of the D0 domain of the PEDV S protein. Subsequent mutagenesis and cryo-EM structure analyses confirmed our postulation. Taken together, our findings suggested a functional role of the D0 up conformation in increasing the virulence of PEDV, and the structural contributions of N-glycosylation to promote the D0 up conformation.

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Towards atomic insights into the interplay between SARS-CoV-2 E and M proteins.

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SARS CoV-2 is composed of four proteins, namely the spike protein (S), membrane protein (M), envelope protein (E), and nucleoprotein (N). The first three are integral membrane glycoproteins. M and E proteins are crucial for viral assembly, budding, and envelope formation, but the underlying mechanism remains elusive not least because of the lack of detailed structural information of the E and M proteins. Our goal is to resolve the structure of full-length E and M proteins in their native membrane environment by incorporating it into a nanodisc. The E protein was chemically synthesized and purified by size exclusion chromatography (SEC) in detergent micelle to homogeneity for structural investigations. The mammalian cell expressed recombinant M protein readily aggregated during purification. We eventually succeeded in optimizing the purification of the primarily dimeric M protein. The E and M protein were individually incorporated into a phospholipid nanodisc for biophysical and structural analyses. In particular, the E protein was found to form a well-defined pentameter in a nanodisc based on negative staining electron microscopy (NSEM) and cryo-electron microscopy (Cryo-EM) thereby paving the way towards detailed structural characterizations of the atomic details of the interplay between the E and M proteins.

Vaccinia virus EFC protein A28 interact with virus envelope protein A26 to deactivate fusion activity and conduct endocytosis

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Vaccinia virus is a member of poxviridae family that produces two types of virions known as single-membraned intracellular mature virus (MV) and double-membraned extracellular enveloped virus (EV). (1) MVs are the most abundant and essential vaccinia virus in the infected cells, enters the cells either through endocytosis or plasma membrane fusion. The virus envelope protein A26 acts as an acid-sensitive fusion suppressor for MV, also downrugulate EV titers with its expression. The viral A26 gene is conserved in all vertebrate poxivurus and appears to be evolutionally adaption, revealing the importance of A26-controlled mature virions. Previous founding showed that viral A26 protein will bind with A16/G9 of viral entry-fusion complex (EFC), which contributes to suppression of virus-triggered membrane fusion. (2) The EFC is a complex to conduct membrane fusion function. It comprises at least eleven transmembrane proteins that are conserved in all poxviruses. Among these, A28 protein is the core component of EFC, the complex is not able to assemble well on the host cell surface without A28. Although it has already known A26 acts as an acid-suppressor to switch the entry pathway through endocytosis of MV. However, the full framework of virus entry mechanism still needs to clarify, because lack of direct evidence to prove A26 interacts with EFC core member A28.

To understand how A28-A26 interaction during the viral fusion pathway, we apply NMR spectroscopy under different pH treatments. With our published A28 NMR structure, (3) we are able to observe the difference between ssociation/disassociation, also identify the interacting interfaces on both A28 and A26 proteins. The NMR results showed the N-terminal of A28 and the C-terminal of A26 will associate to form oligomers at neutral/basic pH to deactivate EFC fusion activity. This is consistent with the results of oligomer forms observe on MS spectra and blue-native-page. This study provides a comprehensive understanding of the virus/host fusion mechanism in association with switchable virus entry to endocytosis at low pH.

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Switching natural cofactor NAD^+ into NMN^+ in 3α-hydroxysteroid dehydrogenase/carbonyl reductase from *Comamonas testosteroni*

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Cofactor engineering is significant in the regulation of metabolic pathways by orthologous pathway. Lowering costs and greater stability make nicotinamide mononucleotide (NMN^+) a wonderful candidate for cofactor engineering. *Ct*.3α-HSD/CR catalyzes the stereospecific oxidoreductive reaction of NAD^+ with androsterone to form NADH and androstanedione. However, 3α-HSD/CR acting on NMN^+ shows a significant decrease in activity. To switch the cofactor specificity of NAD^+ into NMN^+ for *Ct*.3α-HSD/CR, we use a rational design to replace the residues for enhancing the interactions with NMN^+ and reducing the interactions of AMP moiety of NAD^+ based on the crystal structure and catalytic mechanisms. Therefore, single mutation of T11A/K/R, I13A/K/R, D41A/I/Q, A70I/Q/K, and I112A/K variants of 3α-HSD/CR are prepared and purified in homogeneity.

The catalytic efficiency of WT with NAD^+ is similar to T11A/K/R variants but decrease in other variants from 3.6-fold for I112A mutant to 29000-fold for I13R mutant. Among these mutants, mutation on A70 shows a promising result in switching cofactor specificity of NAD^+ into NMN^+. The catalytic efficiency for NAD^+ significantly decreases with a concomitant increase for NMN^+. Kinetic study on A70K variant toward NMN^+ show a lower K_m and a 17-fold increase in catalytic efficiency compared with WT. The cofactor specificity ratio of NMN^+ and NAD^+ for A70K and WT 3α-HSD/CRs is $5.5x10^{-2}$ and $2.3x10^{-7}$, respectively. A70K variant demonstrated a $2.4x10^5$-fold increase in the relative cofactor specificity. Substitute lysine for A70 may enhance the electrostatic interaction with the phosphate group of NMN^+ but block the binding site for NAD^+, resulting in a significant decrease for NAD^+ but an increase for NMN^+ in catalytic efficiency.

In structural analysis of 3α-HSD/CR variants, variants of T11K, T11R, I13K and A70I show a red shift of the maximum wavelength by intrinsic protein fluorescence, indicating the changes of protein structure. Meanwhile, the thermal unfolding of T11K, T11R, I13A, I13K, and A70I mutants appear lower Tm values measured by differential scanning fluorimetry, suggesting the mutation causes the lower thermodynamic stability.

The study of thermodynamic stability of 3α-Hydroxysteroid Dehydrogenase/Carbonyl Reductase from *Comamonas testosteroni* by differential scanning fluorimetry

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Protein stability is an important issue for studying enzyme activity, optimizing the storage or purification conditions, and the drug development (1,2). The structural studies of a protein can help better characterizing its structure-function relationship. Hydroxysteroid dehydrogenase (HSD) takes part in the biosynthesis of steroid hormones and related diseases like breast cancer and prostate cancer. However, the energetic of the protein stability of HSD is mostly unknown in present. Here we characterize the protein stability of 3α-hydroxysteroid dehydrogenase/carbonyl reductase (3α-HSD/CR) from *Comamonas testosteroni* as functions of temperature, pH and denaturants by intrinsic fluorescence measurement and differential scanning fluorimetry (DSF). Thermal unfolding of *C.t.*3α-HSD/CR to obtain thermal parameters of ΔH_D and T_m from pH 4.5 to 9.7 is performed by DSF. It shows that the protein is most stable in neutral environments but unstable in both acidic and alkaline environments. The change in heat capacity on denaturation (ΔCp) is determined from the dependence of ΔH_D on T_m by varying pH or the concentration of urea. We then determined the protein stability curves as functions of temperature and pH for *C.t.*3α-HSD/CR based on the obtained values of ΔH_D, T_m and ΔCp by Gibbs Helmholtz equation. The stability curves ($\Delta G_u(T)$) show that the protein has the highest stability of 7.37 kcal mol^{-1} at 26.5°C, pH 7.6 in the range of pH 4.5 to 7.6. Meanwhile, the pH dependence of $\Delta G_u(T)$ indicates that there are two proton uptake by the protein when unfolded. The urea- and GdmCl-induced protein denaturation also gives the similar $\Delta G_u(22°C)$ values. In summary, *C.t.*3α-HSD/CR is most stable in neutral environments and its stability is linked to the pH values. Consistent ΔCp values can be obtained by DSF from varying pH or the concentration of urea. The robustness of the DSF results is supported by its consistency to the results of denaturant-induced denaturation.

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Characterizing the interaction between ALG-2-interacting protein X and galectin-3 in promoting HIV-1 budding

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The human immunodeficiency virus (HIV) is a pandemic virus these decades and will lead to acquired immune deficiency syndrome (AIDS) that attacks the body's immune system. The virus infection, replication, assembling, then budding out by endosomal sorting complex required for transport (ESCRT) to spread to other cells. The previous study reported that Galectin-3 will associate with ALG-2-interacting protein X (Alix) one of the ESCRT to promote HIV-1 viral budding, however, the mechanism of their interaction is still unclear. More research suggest that liquid-liquid phase separation (LLPS) is vital to keeping a cell's physiological function. LLPS is driven by intrinsically disorder region (IDR) and multivalency characteristics, which can form the liquid-like droplet to improve reaction efficiency. We know the N-terminal of Galectin-3 and the C-terminal of Alix are intrinsically disordered. Thus, we hypothesize that their interaction is through LLPS to promote HIV-1 viral budding. To confirm our hypothesis, we're going to purify the C-terminal of Alix and N-terminal of Galectin-3 to analyze the interaction with different biophysical tools.

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Functional investigation of the polysaccharide repeating units flippase Wzx of *salmonella* SL1344

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Non-typhoidal salmonella is one of the common foodborne pathogens. Although most of the infection only causes slightly gastrointestinal disorder, more systemic severe invasions such as bacteremia also frequently occur in immunocompromised or elderly patients. Nowadays, antibiotic resistance occurs rapidly; several *Salmonella* strains also developed resistance against third-generation antibiotics such as ciprofloxacin. To catch up with the pace of this important issue, new methods and targets are being developed to conquer bacterial infections.

The outer membrane of gram-negative bacteria contains several kinds of polysaccharides, and they are roughly classified into two groups. One is a membrane-bounded form like lipopolysaccharide (LPS), and the other is a membrane-associated form such as exopolysaccharide (EPS). These polysaccharides are tightly related to stress resistance, outer membrane stability, and virulence of the bacteria. Wzx belongs to the polysaccharide transporter (PST) family and is important to the LPS biosynthesis.[1] Wzx serves as flippases to translocate oligosaccharides of bacterial polysaccharides units from the inner leaflet of the inner membrane toward the periplasmic side. In the present study, we chose two Wzx family flippases annotated as Enterobacterial Common Antigen (ECA) flippase

(WzxE) and O antigen flippase (Wzx_B) for study. Both of them were predicted to have 12 transmembrane helices and are topologically similar to each other. Earlier research indicated that, unlike other membrane transporters, Wzx protein is an ATP-free and ion-based antiporter with high substrate specificity.[2] To clarify the mechanism, the *in vitro* liposome flippase assays are designed. Two Wzx knockout strains of *Salmonella* were also generated to examine phenotype changing of LPS production, motility, and virulence.

Both two knockout strains present different degrees of motility changing. Wzx_B knockout present delated swimming motility which is compared with wildtype. WzxE knockout did not alter the velocity of motility; however, the pattern of bacteria growing on the plate was changed and seemed to stay at the interface of soft agar and air but not penetrate into soft agar. The LPS patterns analyzed by Tricine SDS PAGE gel of the two knockout strains were both altered that the Wzx_B knockout strain was lack of middle and long LPS and the WzxE knockout strain was affected only on middle-sized LPS. Virulence decreasing of Wzx_B knockout strain was also observed via bacterial invasion assay. Furthermore, using Swiss-Port structure simulation, we plan to mutate several charged residues at the inner part of WzxE and Wzx_B, which might be crucial for activity and substrate recognition. Our preliminary results indicated that the K146A substitution of Wzx_B lost the flippase activity so that the O-antigen of LPS was unmatured. Other charged amino acids predicted to be crucial will also be mutated to comprehend the importance of the WzxE and Wzx_B flipping mechanism.

Through these functional studies of the Wzx protein family, more insights into the mechanisms for different types of bacterial polysaccharide synthesis could be obtained. Since bacterial LPS and virulence are tightly related, the Wzx protein family might also be a potential new target for antibiotics development in the future.

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Direct visualization of chromatin dynamics in live cells by label-free interference microscopy

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The organization of chromatin and its dynamics play crucial roles in harmonizing cell functions. However, measuring the chromatin compaction quantitatively in live cells remains challenging. To probe the chromatin compaction, a few fluorescence-based approaches have been demonstrated, including FLIM-FRET microscopy [1] and fluorescence anisotropy microscopy [2]. Unfortunately, fluorescence-based measurements are complicated by the photobleaching effect. There are also concerns about potential artifacts due to fluorophore labeling. In this work, we present a novel optical microscope technique to resolve the chromatin organization in the unlabeled live cell nuclei. A highly sensitive optical interference microscopy, coherent brightfield microscopy (COBRI), is used to directly record the dynamic scattering signal of chromatin at a high speed. By analyzing the temporal fluctuations of the scattering signal, the chromatin density and the level of chromatin compaction are accurately estimated with the sub-micrometer spatial resolutions. We refer to this new imaging strategy as “DYNAMICS imaging” [3]. The reconstructed chromatin density map is highly correlated to the fluorescence image of chromatin. In addition, the chromatin compaction changes induced by chemical drug treatments are successfully detected. We employ the DYNAMICS imaging to investigate the chromatin conformation changes of local DNA damage induced by laser microirradiation. Our data show that the chromatin is decondensed at the damage site, which is consistent with the DNA damage responses reported previously. We monitor the chromatin remodeling dynamics at the damaged site over a broad timescale (from seconds to hours) by continuous DYNAMICS imaging. Exploiting the ultrastable scattering signal, our DYNAMICS imaging supports noninvasive long-term observation of chromatin dynamics in the live cells.

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Bottom-up creation of liposome-based artificial cells: from cell-free gene expression to the reconstitution of membrane synthesis in liposomes

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The bottom-up approach in creating liposome-based artificial cells that can mimic sophisticated properties of natural cells has attracted much attention(1). In particular, liposomes have been reported to be capable of functionally reconstituting foundational features of biological cells such as the ability to grow, divide, communicate, fuse, replicate the DNA/RNA, and undergo shape deformation(1-3). Nevertheless, reconstitutions of these features were mostly demonstrated through encapsulating purified proteins or biomolecules. Here, we have successfully demonstrated the synthesis of a kinase, phosphatidylinositol-5-phosphate 4-kinase type-2 alpha (hPIP5K2A), from its genes using the cell-free protein synthesis (CFPS) system in both bulk solution and liposomes. The synthesized hPIP5K2A was confirmed by SDS-PAGE and LR-MALDI-TOF analysis. Compared to cell-based protein synthesis (using Flag-PIP5K2A-His6) in HEK293T cells, the CFPS system offers unique advantages such as less laborious and time savings. Furthermore, the *in vitro* kinase assay and thin-layer chromatography (TLC) analysis confirmed the enzymatic activity of hPIP5K2A kinase, indicating that cell-free expressed hPIP5K2A was functional. In the future, we attempt to perform the PI(4,5)P2 biosynthesis from the PI5P pathway, confirming the functional activity of expressed hPIP5K2A kinase within liposomes. Finally, clathrin-mediated endocytosis will be reconstituted in presence of the newly synthesized PI(4,5)P2 within liposomes. Overall, this study demonstrates the functional activity of the protein synthesized in the cell-free system, paving a way for creating an artificial cell with a custom function for reconstituting cell-like phenotypes.

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Studying the Effect of Cholesterol on the Structure of Membrane-Spanning Region of Amyloid-Precursor Proteins by Electron Spin Resonance Spectroscopy

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More than 90% of Alzheimer's disease (AD) occurs sporadically without genetic linkage found among its patients. Aging, hypertension, high cholesterol content, and diabetes are the known non-genomic risk factors of AD(1). The aggregation of peptides named amyloid-β (Aβ) peptides is the initial event in the pathogenesis of AD. The Aβ peptides are catabolic products cleaved from a type I membrane protein called amyloid precursor protein (APP) by two membrane proteases named β-secretase and γ-secretase. β-Secretase cuts at the N-terminus of the Aβ peptides. The cleavage by γ-secretase happens at the transmembrane region of APP without a specific cutting site leading to several Aβ peptides that are different in their C-terminal sequence with varying propensities of aggregation and toxicities. The peptide Aβ42, which has 42 amino acid residues, is the most toxic peptide and the primary form in Aβ aggregate in the AD brain. Since cholesterol content is one risk factor of sporadic AD, we aim to explore whether its change can affect the structure of the APP transmembrane region, thereby modulating the γ-secretase cutting behavior. We synthesized a series of cysteine mutated $A\beta_{22\text{-}55}$ peptides containing the transmembrane region of APP and identified the structural change of embedded peptides on liposomes composed of DOPC and different content of cholesterol, using spin-label (R1) and electron spin resonance (ESR) techniques including double electron-electron resonance (DEER), electron spin echo envelope modulation (ESEEM), and continuous-wave (CW) ESR. Our results show that cholesterol decreases the structural flexibility of the APP transmembrane region in the lipid bilayer. The segment of 36-40 (sequence VGGVV), which is a GG kink-containing region, formed an α-helical structure rather than a 3_{10} helix. The relative orientation of V36 and V40 varies in the presence of cholesterol and the distance of these two residues is the closest in the presence of 10% cholesterol. Additionally, the distance distributions extracted from the CW-ESR spectra showed that the distance between G29R1-V36R1 was shorter than that between V40R1-I47R1. Together, this study provides spectroscopic evidence showing how the cholesterol content modulates the secondary structure of APP-based peptides in the lipid bilayer.

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Enzymatic activity and structural study of human mitochondrial genome maintenance exonuclease 1

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By producing key proteins involving in mitochondrial oxidative phosphorylation system, mitochondrial DNA (mtDNA) plays a vital role in cellular energy production. Hence, quality control of mtDNA is important for the fitness of our cells. Mitochondrial genome maintenance exonuclease 1 (MGME1), a single-stranded DNA (ssDNA)-specific exonuclease, is found to participate in mtDNA replication and degradation – both are crucial mtDNA quality-controlling processes. As an exonuclease, MGME1 exhibits a unique bidirectionality *in vitro*, being capable of degrading ssDNA in either direction. In this study, we aim to investigate the structural basis of this bidirectionality. Five putative substrate-interacting residues of the enzyme were therefore substituted to alanine to examine their role in processing either 5'-overhang or 3'-overhang DNA duplexes. The result showed that MGME1 uses distinct sets of residues for recognizing substrate when working in different directions. Intriguingly, we found two of our MGME1 variants exhibit significant higher 5′- but diminished 3′-exonulcease activity comparing to wild-type enzyme. We thus propose a working model of MGME1 focusing on its interplay with substrate and cleaved product when acting in 5′-to-3′ direction. More investigations are required to uncover the structural mechanism underlining the bidirectionality of MGME1.

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The role of DNA polymerase γ accessory subunit in mitochondrial DNA degradation

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DNA polymerase γ (POLG), the sole replicase in mitochondria, is consisting of a catalytic subunit POLGA and two copies of the accessory subunit named POLGB, forming a heterotrimeric holoenzyme. In which POLGB increases DNA synthesis rate by partially suppressing the 3′-exonuclease (*i.e.*, proofreading) activity of POLGA, thus enabling processive DNA synthesis with the ability to proofread retained. Moreover, POLGB plays a key role at replication fork where the holoenzyme needs to coordinate with mitochondrial DNA helicase Twinkle (TWNK) for maintaining the progression of replication fork. How POLGB mediates such a coordination is unclear. Apart from mitochondrial DNA (mtDNA) synthesis, POLG is also responsible for damaged-induced degradation of the genome, in which fragmented mtDNA is unwound by TWNK and then digested by POLG through its 3′-exonuclease activity. Whereas cellular depletion of POLGB does not significantly affect the degradation process, the accessory subunit is currently excluded from the mtDNA degradation model. However, recent studies suggest that assembly of POLG holoenzyme is crucial for preventing POLGA from being turned over. In addition to that POLGB may mediate the coordination with TWNK, it raises the question: if POLGB is an absolute component of POLG, what is its role in mtDNA degradation? To answer this question, we included the accessory subunit in POLGA nuclease assay and found that it largely enhanced the exonuclease activity of POLGA, particularly in digesting 3′-overhang DNA duplex which mimics the substrate in mtDNA degradation. The data thus suggests that POLGB introduces distinct regulatory effects to the POLG holoenzyme when it acts as a replicase or nuclease. More studies are required to understand the underlying structural mechanism.

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Structural study of human RNA:m^{5}C methyltransferase NSUN2

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NSUN2—belonging to NOL1/NOP2/SUN domain (NSUN) family—introduces 5-methylcytosine (m^{5}C) to various RNA, including tRNA, mRNA and non-coding RNA, hence playing a fundamental role in RNA metabolism and function. For example, NSUN2-mediated methylation protects tRNA from being cleaved, accordingly, loss of NSUN2 leads to reduced protein translation which is linked to stress response. Further, NSUN2-dependent mRNA methylation, mostly located in untranslated region, can regulate translation efficiency, stability, and transport of the mRNA. NSUN2 methylates RNA in a sequence- and structure-dependent manner, however, the structural basis of the substrate recognition is unclear. In addition, besides the canonical RNA-recognition motif (RRM) and the catalytic m^{5}C methyltransferase (MTase) domain, NSUN2 possesses a large C-terminal (C-ter) extension spanning around 300 residues with unknown function. Intriguingly, this C-ter extension is NSUN2 exclusive, absent from other RNA:m^{5}C MTase members. We thus speculate that the C-ter extension of NSUN2 may contribute to the sequence- and/or structure-specific recognition of its substrates. To tackle this question, full-length and C-ter truncated NSUN2 proteins are purified for activity and structural analysis. RNA substrates for the *in vitro* study are designed to mimic the structure of variable loop of tRNA, representing the primary targeting site of NSUN2 in tRNA m^{5}C modification. We anticipate our study will uncover the structural basis of substrate recognition by NSUN2 and the role of the unique C-ter extension in its function.

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Expression in *Pichia pastoris* and characterization of chlorotoxin, an anti-glioma migration agent

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Chlorotoxin (CTX), a scorpion-derived disulfide-rich peptide, is an efficacious tumor-selective targeting agent. However, it is time-consuming and laborious to obtain a high yield of correctly folded CTX. Thus, to facilitate studying the molecular determinants of CTX, we established a high-level expression system in *Pichia pastoris* and used a migration assay to determine the bioactivity of CTX. It was reported that the C-terminal fragment (residues 29-36) of CTX exhibited anti-cell migration activity. Sequence alignment of CTX-like peptides revealed three varied regions: The N-terminus, loop-2, and C-terminus regions. Structural analysis of CTX elucidated that the C-terminal region is sandwiched between the N-terminal, loop-2, and the β-strand 2 regions. Hence, we hypothesized that these regions might cooperatively interact with CTX-targeting proteins. Accordingly, based on sequence alignment, we altered the N-, C-terminus, loop-1, loop-2, and β-strand 2 regions of CTX. We successfully expressed wild-type CTX and eight mutants with 0.7-11.2 mg/L yields in *Pichia pastoris*. The functional analysis showed that the M1R, Y29K, and R36APY mutants decreased the activity of inhibiting glioma cell migration. Crystal structure analysis showed that the 3D structures of CTX mutants (M1R and Y29K) are identical to the wild-type, indicating the importance of these residues in regulating CTX function. Our results suggest that the residues, M1 (N-terminal), Y29 (β-strand 2), and R36 (C-terminus) of CTX are located in a structurally close position and are crucial to its bio-activity. These results can be a platform for the advancement of CTX mutants for cancer detection and treatment.

Structure of the unique cytolysin-mediated translocation apparatus of Group A *Streptococcus*

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Effector delivery is crucial to the pathogenesis of many bacteria, including *Streptococcus pyogenes*, which applies Streptolysin O (SLO) to translocate NAD^+-glycohydrolase (NADase) into the host cytosol during infection. The translocation of NADase does not depend on the pore formed by SLO, yet can be delivered into the host cytosol by the monomeric SLO. The translocated NADase acts as an effector to hydrolyze cytosolic NAD^+ to deplete host energy storage, which enhances the ability of GAS to resist phagocytic killing. However, the structure of this unique CMT apparatus is unrevealed, and whether the interaction between NADase and SLO is critical to CMT activity remains unclear. We determined the crystal structure of NADase/SLO complex and pinpointed the key residues that are of central importance to the cooperative activities of the NADase/SLO. Small-angle X-ray scattering (SAXS) was conducted to depict the solution structure of the protein complex. By SAXS-based analysis, we revealed that the dynamic interplay between these toxins is fundamental to the functioning of the complex. The structure-guided complex-disruptive mutant strain was generated, and the essentialness of the NADase/SLO interaction in CMT activity and in resisting to phagocytic killing were demonstrated by *in vitro* infection model. The importance of NADase/SLO complex in the pathogenesis of GAS was further revealed by mouse infection model. Our studies convey the structure-functional relationship of a NADase/SLO complex underpinning the augmented virulence of the bacterial pathogenesis.

Characterization of the structure–function relationship of antimicrobial peptides, RR14

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The rapid emergence of antibiotic-resistant bacteria is now a severe health problem all around the world. Antibiotic-resistant bacteria increase mortality and morbidity, as well as incur high healthcare costs. Therefore, developing new antibiotic agents with less probability to induce evolved resistance is urgently needed. Antimicrobial peptides are feasible candidates in developing potent therapeutics against the infections of antibiotic-resistant bacteria. Our study aims to investigate the antibacterial activity and possible mode of actions of the synthetic peptide, RR14. We utilized biochemical and biophysical techniques coupling with computational methods to characterization the structure–function relationship of the synthetic peptide, RR14. The membrane-permeabilizing ability of RR14 was carried out by calcein leakage assay and propidium iodide uptake assay. The results showed that RR14 was potent in killing *E. coli, A. baumannii and S. aureus.* To analyze the solution structure of RR14, we monitored its secondary structures by circular dichroism (CD) experiments. Moreover, the tertiary structures of RR14 was determined by solution-state nuclear magnetic resonance (NMR). Furthermore, the orientation of RR14 in DPC micelles was investigated by paramagnetic relaxation enhancement (PRE) experiments. As the result, functional important residues of RR14 be explored. Taken together, we disclose the detailed structure and function relationships of RR14 which would benefit to the development of new and novel bactericidal agents.

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Characterization of the structure–function relationship of antimicrobial peptides to develop new antibacterial agents

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The rapid emergence of antibiotic-resistant bacteria is now a severe health problem all around the world. Antibiotic-resistant bacteria increase mortality and morbidity, as well as incur high healthcare costs[1, 2]. Therefore, developing new antibiotic agents with less probability to induce evolved resistance is urgently needed. Antimicrobial peptides are feasible candidates in developing potent therapeutics against the infections of antibiotic-resistant bacteria[3]. Our study aims to investigate the antibacterial activity and possible mode of actions of the synthetic peptides, AL, LK and IL that designed by AI. We utilized biochemical and biophysical techniques coupling with computational methods to characterization the structure–function relationship of the synthetic peptides. The membrane-permeabilizing ability of the peptides was carried out by calcein leakage assay. To analyze the solution structure of the peptides, we monitored their secondary structures by circular dichroism (CD) experiments. So far, we have verified the membrane-permeability of the peptides against different surface-charge liposomes. LK exerted stronger disrupting ability against negative-charged POPG liposome but with weaker activity against neutral charged POPC liposome, revealing the selectivity of LK against bacterial membranes instead of mammalian cells. By analyzing the CD data, we also have confirmed that the peptides adapt the α-helical conformations in membrane mimic environment, indicating the bioactive conformational transition (from unstructured to α-helical conformation) of antimicrobial peptides is required for binding to phospholipid membranes. In the future, we will test antibacterial activity and analyze the solution structure to optimize the peptides.

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(3) M. Zasloff, *Nature* **415**, 389–395 (2002).

A Novel Disulfide Bond Engineering of Fibronectin Type III Domain Enhances Thermostability and Solubility of VEGFR2-Specific Antagonist

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The fibronectin type III domain (Fn3) is a protein scaffold that was successfully used to develop targeting therapeutics with high affinity and specificity. C7 is a potent anti-angiogenic agent targeting vascular endothelial growth factor receptor 2 (VEGFR2); however, it exhibited low thermostability and aggregation problem. We here proposed to incorporate disulfide bonds into C7 for solving these problems. The bioinformatics tool Disulfide by Design 2.0 (DbD2) and the structure-based approach were used to design disulfide bonds for C7. Our findings showed that the criteria of χ3 torsion angle played the most important role in the success of introducing disulfide bonds. The incorporation of disulfide bonds into C7 increased the Tm values up to 52 ^{0}C and the solubility up to 6.7-fold. In particular, we found a C7 mutant with high activity in inhibiting the VGFR binding to VEGFR2 with the IC_{50} of 17.6 nM, as well as with the Tm value of 96.3 ^{0}C and the soluble of 5.9 mg/L. These results demonstrated that the incorporation of disulfide bonds into Fn3 can improve the thermostability and solubility of C7 and maintain its VEGFR binding affinity, making it applicable in the design of drugs using Fn3 scaffold.

Toward structural and Functional Study of Spo11: the Initiator of Meiotic Recombination

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Meiotic recombination is an essential process for sexually reproducing organisms, which not only increases genetic diversity, but also ensures faithful chromosome segregation. This unique DNA recombination event takes place during the prophase I of meiosis, and is triggered by the formation of DNA double-stranded break (DSB). Spo11, a protein conserved from yeast to vertebrates is suggested to be the initiator of meiotic recombination by introducing programed DSB formation across the genome. Spo11 shows considerable sequence similarity to the A subunit of topoisomerase VI (TopVI), an A_2B_2 tetrameric protein responsible for resolving DNA topological problems in archaea. TopVI performs reversible cleavage of a DNA duplex through a catalytic tyrosine residing in the WHD domain of its A subunit, forming a pair of phosphotyrosyl bonds with DNA, leaving a protein-linked DSB with two-base 5' DNA overhangs. Subsequently, a second DNA duplex is captured and transferred through the DSB, resulting in changes in DNA topology. Given that the catalytic tyrosine found in TopVI A subunit is also present in Spo11, scientists speculated that Spo11 may share functional similarity with TopVI either in the DNA cleavage activity or structure. Besides Spo11, another protein that shows high sequence similarity to TopVI B subunit was identified and named TopVI B-like protein (TopVI-BL). With this finding, it was further suggested that Spo11 may interact with TopVI-BL and assemble a structure like TopVI for function.

To elucidate the DNA cleavage mechanism of Spo11, we aim to obtain the structure of Spo11. However, the study of Spo11 has been hampered by the difficulties in producing soluble protein for analysis. Fortunately, we have successfully established a robust protocol for expressing and purifying soluble Spo11 for further crystallization. However, crystals were not observed after screening for thousands of crystallization conditions. To overcome this bottleneck, we attempt to test whether the inclusion of a Spo11-targeting nanobody may facilitate the crystallization process. After screening a nanobody library, we have obtained a nanobody with high affinity toward Spo11. The Spo11-nanobody was then used for crystallization. Although crystals are are not yet observed, images of homogeneous particles were observed under negative staining EM and cryo-EM. 3D reconstruction is currently underway.

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Vitroprocines, tyrosine-polyketide antibiotics, biosynthesized via newly discovered α-oxoamine Synthase and oxidoreductase

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The PLP-dependent α-oxoamine synthases are generally believed to be responsible for elongating polyketides or catalyzing the condensation of amino acids and acyl-CoA thioester substrates. An example is the production of the sphingolipid precursor 3-ketodihydrosphingosine (KDS), generated via condensation of L-serine and palmitoyl-CoA by serine palmitoyltransferase (SPT). Subsequently, KDS reductase (KDSR) catalyzes the reduction of KDS to dihydrosphingosine. Both of enzymes play crucial roles involved in the biosynthesis of ceramide ranging from yeasts and plants to animals. Vitroprocines, L-tyrosine (or L-phenylalanine)-polyketide derivative, a potentially powerful new antibiotic recently discovered from marine *Vibrio* sp. QWI-06. It exhibits significant inhibitory activity against the pathogen *Acinetobacter baumannii*, which causes serious nosocomial infections in Taiwan. Therefore, we have reasonable suspicion that at least one SPT-like enzyme could conduct the biosynthesis of vitroprocines. By using bioinformatics analysis, we identified certain putative α-oxoamine synthases (VsAOS) from marine *Vibrio* sp. QWI-06. Recently, we have proposed that VsAOS-1 acts strictly in the condensation of L-glycine to C12 and C16 fatty acids ***(Chang et al., Colloids Surf. B: Biointerfaces, 2021)***. Furthermore, we also discovered a brand new α-oxoamine synthase (VsAOS-2) as well as a human KDSR-like reductase (VsOR) from marine *Vibrio* sp. QWI-06, which were responsible for the decarboxylative condensation of L-tyrosine to lauroyl-CoA, following the reduction of ketone group, to form the vitroprocine-type compounds **1** ***(Liaw et al., Organic Letters, 2022)***. I am the co-author of these two papers. Together, our findings on the biosynthetic enzymes of vitroprocines shed light on the biosynthetic logic for the potential antibiotics, engineering of bioactive natural product and further biocatalyst development.

Targeting DNA mismatches by two distinct intercalators exhibit synergistic effect against mismatch repair deficient cancers

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Combination chemotherapy is one of the most useful treatment methods to achieve a synergistic effect and reduce the toxicity of a single drug dose. In this study, we used a combination of two DNA intercalators, actinomycin D (ActD) and echinomycin (Echi) to investigate their binding capabilities with DNA duplexes containing different mismatches embedded within canonical Watson-Crick base pairs. We found that ActD and Echi significantly stabilized homopyrimidine T:T mismatches. The increased stability of DNA duplex-drug complexes is mainly due to the cooperative binding of two drugs to the mismatch duplex, which contains intermolecular stacking interactions between the two different drug molecules and DNA. Since pyrimidine mismatch repair is less efficient in mismatch repair (MMR)-deficient cancer cells, we found that the equimolar combination of ActD and Echi exhibited enhanced synergistic effects against MMR-deficient cell lines. We further accessed the clinical potential of the two-drug combination approach in a xenograft mice model for the treatment of MMR-deficient cancers. The current study provides a novel approach for the development of combination chemotherapy for the treatment of cancers related to DNA mismatches.

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Receptor recognition of A-type trisaccharide by the receptor-binding domain of the spike protein of SARS-CoV-2

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Infection of the novel severe acute respiratory syndrome coronavirus (SARS-CoV-2) is responsible for the spread of the devastating COVID-19 pandemic. To initiate viral infection, the receptor-binding domain (RBD) of the spike protein of SARS-CoV-2 binds to the host receptor protein, ACE2, to trigger conformational changes within the spike protein and subsequent viral entry. There is good evidence to indicate that individuals with a blood type A are more likely to get COVID-19 infection than other those with other blood types, implying that the blood type antigens, which are carbohydrates, may also serve as (co)receptors to mediate SARS-CoV-2 infections. To investigate the molecular basis of blood type recognition by the RBD of SARS-CoV-2 spike protein, we generated ^{13}C and ^{15}N enriched RBD by an *E. coli*-based bacterial expression system and an Expi293-based mammalian expression system for solution NMR spectroscopy studies. The latter expression afforded the possibility to introduce appropriate post-translationally modified N-glycans on the RBD that may be important for blood type recognition. We showed by NMR saturation transfer difference (STD) evidence of preference for ATS over BTS (blood B antigen) by the RBD. Methyl NMR spectroscopy showed localized chemical shift perturbations in the RBD upon ATS binding. Furthermore, the effects of glycosylation manifested in significant and localized chemical shift perturbations in the methyl resonances of the RBD. Our findings demonstrated the ability to introduce $^{13}C/^{15}N$ labeling to mammalian cell-expressed recombinant proteins in high efficiencies. The results also paved the way towards detailed structural analysis of how the RBD of SARS-CoV-2 may interact with carbohydrate-based host receptors and the effect of glycosylation.

Structure-function relationships of human coronavirus spike proteins by cryo-EM, mass spectrometry and molecular modeling

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Human coronaviruses (hCoVs) belong to the order *Nidovirales,* which are zoonotic pathogens that evolve to infect humans. CoVs use highly glycosylated spike proteins to bind to host receptor(s), the first step towards host membrane fusion and viral entries. As the post-translationally modified glycans constitute a significant part of the overall structures of the spike proteins, understanding their structures and dynamics in the context of receptor and immunity recognition is essential for developing preventions and antivral treatments. Here, we focused on three hCoV spike proteins in the genus *Betacoronavirus*, including SARS-CoV, MERS-CoV and hCoV-HKU1. We produced the recombinant spike proteins by a mammalian protein expression system. The folding stabilities over a range of experimental conditions were evaluated by differential scanning calorimeter (DSC) and differential scanning fluorimeter (DSF). We also employed single particle analysis by cryo-electron microscopy (cryo-EM) to determine four distinct structures of the MESR-CoV spike protein that differed in the different conformations of the receptor-binding domain (RBD). To characterize the chemical compositions and structures of the surface glycans, we used mass spectrometry to determine site-specifically the glycoforms of individual N-linked glycans. We further implemented a modelling procedure by GlycoSHIELD (1) to quantitatively describe the shielding effect of the N-glycans on the spike proteins. Our results revealed that the local antigenicity of the spike proteins negatively correlated with the extent of protein surface shielding by the N-glycans. Collectively, our study provided a robust workflow to determine the molecular structure of CoV spike proteins in the context of the shielding effect of glycosylation with the ability to predict potential (co)receptor binding sites and epitopes for antibody binding.

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A Seven-Bladed β-Propeller Protein can Catalyze a Benzilic Acid Rearrangement in Newly Ascorbate Catabolic Pathway

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A novel pathway of L-ascorbate catabolism in *Cupriavidus necator* for utilization of L-ascorbic acid as carbon source was discovered (1). 2,3-diketo-L-gulonate mutase (DkgM) is involved in the aforementioned pathway to convert 2,3-diketo-L-gulonate (DKG) into 2-carboxy-L-lyxonolactone (Clyx lactone) via a benzilic acid rearrangement (2). Since the noncanonical catalytic mechanism and unkown structural features, the study on structural-function relationship of DkgM becomes attractive.

Based on the result of genome mining, we cloned a DkgM candidate from *Burkholderia lata* (*Bl*DkgM) for further characterization. Crystal structure of *Bl*DkgM was determined to 1.69-Å resolution and revealed that the enzyme adopts a seven-bladed β-propeller protein fold. Since one asymmetric unit of the crystal contains a six-molecular assemble, several methods including AUC, SEC-MALS, SAXS experiments were carried out to confirm the hexameric form of *Bl*DkgM in solution. Incubation of *Bl*DkgM with DKG leads to the production of 2-carboxy-L-lyxonolactone was evidently supported by ^{1}H NMR spectroscopy. However, the enzyme lost its activity after EDTA treatment. Notably, we further found that adding some of divalent metal ions, especially Ca^{2+}, successfully rescue the enzyme activity of *Bl*DkgM. In the complex structure of *Bl*DkgM, two residues, H36 and H91, are suggested to coordinate with metal ion; E178 plays as a general base to deprotonate C_5 hydroxyl group. Finally, the catalytic mechanism was proposed to illustrate the first enzyme-catalyzed benilic acid rearrangement.

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Structural and functional insights into the Rhodostomin ^{48}ARGDXP motif for the recognition specificity of integrins

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The affinity of integrin for RGD-containing protein is regulated by the residue and conformation of the RGDX motif. To systematically study the structure-function relationships of the RGDX motif on integrins, we used Rhodostomin (Rho) containing ^{48}ARGDXP sequence as the protein scaffold. The effect of the RGDX sequence on their integrins binding activities was $\alpha5\beta1$ > αIIb$\beta3$ > $\alpha V\beta3$. In particular, the ^{48}ARGD**D**P was the most selective inhibitor of integrin αvβ3, the ^{48}ARGD**W**P was the most potent inhibitor of integrin αIIbβ3, and the ^{48}ARGD**P**P was the inactive inhibitor of all integrins. X-ray structural analyses of ^{48}ARGD[M/D/W/P]P showed a similar core structure but different surface property or RGD conformation. The ^{48}ARGDDP and ^{48}ARGDWP showed a negatively charged and highly hydrophobic surface, while the ^{48}ARGDPP disrupted the turn structure of the RGDX motif. The docking of the ^{48}ARGDWP into αIIbβ3 showed that the W52 residue formed a cation-π interaction with β3 R214 and a hydrogen bond with αIIb Y190. The docking of the ^{48}ARGDDP into αvβ3 showed that the D52 residue formed a hydrogen bond with the β3 R214 residue. In contrast, this hydrogen bond was absent in the D52-integrin αIIbβ3, α5β1, and αvβ6 complexes, and was replaced by repulsive hydrophobic interactions. Mutagenesis of integrin αvβ3 showed that the β3-R214G mutant caused a 15.3-fold decrease in binding of ^{48}ARGDDP as compared to ^{48}ARGDMP. These results provide structural and functional evidence that the affinity of the RGDX motif of medium disintegrin depends on the turn structure and sequence, and the interactions on the α/β subunit play important roles in regulating the recognition and specificity of integrin.

Structural analysis of RNA/DNA hybrid recognition by DEAD-box helicase DDX41

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Pattern recognition receptors (PRRs) sense molecular features of invading bacteria and viruses to trigger inflammatory signaling during innate immune response. Acting as an intracellular PRRs, DEAD-box protein 41 (DDX41) triggers type I interferon response via stimulator of interferon genes (STING) upon binding to its substrates/ligands, including double-stranded DNA (dsDNA), cyclic dinucleotides (CDN) and RNA/DNA hybrid. Intriguingly, as a member of RNA helicase family, DDX41 does not response to dsRNA but preferentially reacts to RNA/DNA hybrid. Further, apart from its role in immune response, DDX41 also locates to the transient RNA/DNA hybrid region during transcription, known as R-loop, where it plays a key role in regulating R-loop level, presumably by resolving RNA/DNA hybrid through the helicase activity. However, structural information of how DDX41 specifically recognizes RNA/DNA hybrid is lacking. Here, aiming to reveal the structural basis of DDX41 in recognizing RNA/DNA hybrid, we have purified the protein to homogenous for activity and structural studies. Based on helicase and substrate-binding assays, we are currently designing substrates/ligands for the structural analysis.

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The exoribonuclease activity of TREX1 in RNA and DNA/RNA hybrid metabolism

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TREX1 is a DNase belonging to the DEDDh exonuclease family and has been confirmed as RNase by in vitro study recently. Dysfunctional TREX1 leads to DNA/RNA hybrids accumulation in the cytoplasm and triggers the immune response, and TREX1-inactivated cells infected by RNA virus produced more IFN-β than normal cells. All evidence suggests that TREX1 involves processing cytoplasmic RNA or DNA/RNA hybrids, but the detailed mechanism remains unclear. Therefore, we aim to reveal how TREX1 works on RNA and DNA/RNA hybrids by biochemical and structural approaches.

Our study indicates that TREX1 can work on ssRNA and DNA/RNA hybrids without sequence preference but cannot act on dsRNA. Our TREX1-NMP complex shares a similar overall structure as TREX1-dNMP complexes, only with an additional hydrogen bond between 2'-OH and TREX1, suggesting that TREX1 degrades RNA similar to degrading DNA. However, our activity shows TREX1 has higher activities on DNA than RNA, which may cause by the difference in the substrate-binding ability or product release rates. Our further structural and biochemical study identified that the main reason for the difference is the binding ability. In conclusion, TREX1 can degrade RNA and DNA/RNA hybrids without sequence preference but with structural selectivity. TREX1 has different activities in digesting DNA and RNA caused by the different binding affinity against DNA or RNA substrates. Our study provides a molecular basis for TREX1 metabolizing ssRNA and DNA/RNA hybrids.

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The mechanism study of the clinical DNA intercalator- Doxorubicin targeting DNA duplexes for developing potential cancer therapy

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Cancer is one of the leading causes of death in the world. Current chemotherapy or targeted therapy uses small molecule compounds to inhibit tumor growth and target immune cells for anti-tumor effects and regulation. Doxorubicin (Dox) is an anthracycline antibiotic widely used to treat various cancers. Dox can inhibit topoisomerase II by inserting into double-stranded DNA (e.g. CpG site) [1] and generating free radicals that impair the DNA repair function of cancer cells. In this study, we designed DNA oligonucleotides d(CGATATAC/GTATATCG) containing both TA and CG sites and determined their crystal structures in complex with Dox. We found that Dox intercalated both the CpG and TpA sites. Strong H-bonds exist between G2-N2 and Dox-O10/O12, C7-N1 and Dox-O6, and T6-O2 and Dox-N3' at the CpG site. Intermolecular H-bond interactions are also found between T4-O4' and Dox-N3', and A5-O3' and Dox-O14 at the TpA site. Detailed structural analysis reveals that Dox at the CpG site can cause extensive strong H-bond interaction at CpG site compared to the TpA site. In addition, the chromophore of Dox produces a π-stacking interaction with neighboring bases and can enhance the stability of the molecular structure. We then screened the toxicity of free Dox and Dox-DNA complex against cancer cell lines using the cell cytotoxicity assay. The results showed that the DNA-Dox complex could inhibit the growth of cancer cells more than the drug alone, suggesting that the specific DNA can be used as a drug carrier [2]. In conclusion, our crystal structure analysis and the drug carrier system discussed in this study can provide a completely new strategy for existing cancer treatment.

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Allosteric Inhibition mechanism of PCMPS and PCMB on Lassa Fever Virus NP exonuclease

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Essential for suppressing the host immunity, NP exonuclease (NPexo) in Lassa fever virus is a key drug target. To reduce virulence by obstructing the NPexo function, we establish that targeted covalent inhibitors are a viable strategy by systematically elucidating the impact of organomercurials like p-chloromercuriphenyl sulfate (PCMPS) and p-hydroxymercuribenzoate (PCMB). The unprecedented structural information resolved here for complexed wild-type and mutant NPexo consistently show PCMPS or PCMB making a covalent linkage with alternative cysteine residues distal to the active site. Although the protein crystal structures are only slightly perturbed, all-atom molecular dynamics simulations in explicit solvent uncover that the effects of PCMPS sulfonate interacting with nearby arginine residues can propagate through the structural network and impact the active site conformation. The measured NPexo activities and substrate binding due to PCMPS and PCMB are in line with this allosteric mechanism, which also provides guidelines for using organomercurials to study protein functions.

Keywords : DEDDh exonuclease, NP exonuclease, antiviral inhibitor, Molecular dynamics, Organomercurial

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Structural insights into human mitochondrial RNA decay promoted by Suv3 helicase

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Human Suv3 is a helicase of superfamily 2 that plays a vital role in maintaining mitochondrial homeostasis. Dimeric Suv3 is a significant component of the mitochondrial degradosome, working cooperatively with exoribonuclease PNPase for efficient RNA unwinding and degradation in mitochondria. However, the molecular mechanism of how Suv3 is assembled into a homodimer and interacts with PNPase to unwind and degrade RNAs with secondary structures remains elusive. Here, we observed that Suv3 forms a homodimer via the C-terminal tail (residues 723-786). We showed that dimeric Suv3 preferentially binds to and unwinds DNA/DNA, DNA/RNA, and RNA/RNA duplexes with an extended 3' overhang (≥ 10 nucleotides). The C-terminal tail (CTT)-truncated Suv3 (Suv3ΔC) becomes a monomeric protein that binds to and unwinds duplex substrates with ~6-7-fold lower activities relative to dimeric Suv3. Moreover, dimeric Suv3, but not monomeric Suv3ΔC, binds RNA with high nanomolar affinities with or without the presence of ATP or ADP, and can interact with PNPase, indicating that dimeric Suv3 assembly ensures its continuous association with RNA and PNPase during ATP hydrolysis cycles for efficient RNA degradation. We observed that the S1 domain of PNPase and the N-terminal domain of Suv3 (residues 48-170) are involved in protein-protein interactions and participate in mitochondrial RNA degradosome assembly. We further determined the crystal structure of the apo-form of Suv3ΔC and low-resolution SAXS structures of the Suv3 dimer and Suv3-PNPase complex, revealing that Suv3 caps on the top of PNPase via interactions with S1 domains and forms a dumbbell-shaped degradosome complex with PNPase. Our results suggest that only dimeric Suv3 efficiently unwinds dsRNA and further passes the unwound RNA to PNPase via interactions between Suv3 and PNPase. Collectively, these results indicate that Suv3 forms a hetero-pentamer complex with PNPase so that this mitochondrial degradosome can efficiently unwind and degrade bulk RNAs in a processive manner and maintain mitochondrial genome integrity and homeostasis.

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Structural basis of proteasomal recognition of a branched ubiquitin chain.

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Ubiquitin is involved in virtually every aspect of cell biology due to its ability to form a spectrum of conjugates of different architecture and function. In addition to the well-established functional role of homotypic linear Ub conjugates, the functional importance of heterotypically branched Ub chains is emerging[1]. In particular, the K11/K48-branched Ub chains help accelerate substrate turnover by the proteasome; they play essential roles in cell cycle progression and response to proteotoxic stress[2]. Meanwhile, K11/K48-branched Ub chains are preferred substrates for the proteasome-associated deubiquitnase, UCH-L5, whose action competes against the proteasome[3]. Despite their proven importance little is known about the mechanism by which the K11/K48-linked chains achieve their distinct cellular functions.

Herein we report the cryo-EM structure of the human 26S proteasome in complex with a K11/K48-branched Ub chain and UCH-L5. The structure unveiled the molecular basis of how the proteasome recognizes the K11/K48 branching point, and how the branching provides avidity to enhance substrate binding compared to the canonical linear Ub chains. In binding to the K11/K48-branched Ub chain, the regulatory particle of the proteasome undergoes a series of hitherto uncharacterized conformational changes pertinent to substrate recognition. Our findings therefore provide new insights into the mechanism by which the proteasome decodes the complex Ub chain topologies beyond the poly-K48 Ub chains.

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GlycoSHIELD of the spike proteins of human coronaviruses NL63 and 229E

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The ongoing COVID-19 pandemic spurs global efforts of vaccine developments to combat the culprit, SARS-CoV-2. The pressing global health concern has resulted in an explosion of structural studies on the trimeric SARS-CoV-2 spike (S) glycoprotein, which mediates viral entry into host cells and therefore serves as the main target of neutralizing antibodies to prevent infection. In contrast, human coronavirus (HCoV) NL63 and HCoV-229E, which belong to the *Alphacoronavirus* lineage and cause milder symptoms, lack comprehensive structural understanding. To bridge this gap, we produced stabilized variants of the HCoV-NL63 and HCoV-229E S proteins in a human HEK293 cell line to study their glycosylation patterns by mass spectrometry (MS). A python program was written to automate the MS data analysis downstream of the popular program Byonic™. Our analyses identified 34 and 30 N-glycans for the HCoV-NL63 S protein and HCoV-229E S protein, respectively, which resulted in a much more densely decorated glycoSHIELD compared to that of SARS-CoV-2 S protein. Biolayer Interferometry (BLI) showed that the receptor binding domain (RBD) of HCoV-NL63 S protein binds to the receptor ACE2 significantly more weakly compared to its counterpart of SARS-CoV. Furthermore, the trimeric ectodomain of the SARS-CoV S protein binds to ACE2 much more strongly than does the isolated RBD. The reported cryo-EM structure of the ectodomain of HCoV-NL63 S protein has a closed RBD conformation that is unable to bind to ACE2, a finding that was confirmed by our BLI analysis. We are working on identifying the potential activator(s) of the HCoV-NL63 S protein to become ACE2 binding-competent, to which the surface N-glycans may make important contributions.

Structural Insight into ZFAND1 and p97 Interaction

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Arsenite-induced stress granule (SG) formation can be cleared by the ubiquitin-proteasome system (UPS) aided by the ATP-dependent unfoldase p97. ZFAND1 is recruited by p97 to help SG clearance. ZFAND1 contains two An1-type zing fingers (ZF1 and ZF2), followed by a ubiquitin-like domain (UBL). To understand the structural basis of ZFAND1 recruitment by p97, we integrated nuclear magnetic resonance (NMR) spectroscopy, cryo-electron microscopy (cryo-EM), and X-ray crystallography to determine the atomic structures of the individual domains, and to characterize the interaction between ZFAND1 and p97. ^{15}N spin relaxation dynamics analysis indicated independent domain motions for ZF1, ZF2, and UBL. The solution structures of the three domains were independently determined by triple resonance NMR spectroscopy. ZF1 and ZF2 each contained two C3H1 zinc fingers to coordinate zinc atoms. UBL shares only 12 % sequence identity with ubiquitin, and the loops of UBL differed significantly to that of ubiquitin, which hindered the initial attempt to solve the crystal structure by molecular replacement. We eventually used AlphaFold to help determine the crystal structure of UBL, which showed a distant spatial arrangement for N-terminal α-helix compared to that of the solution structure. ZFAND1 transiently interacts with p97. Aided by chemical cross-linking, cryo-EM showed significant conformational rearrangements within the N-terminal domain of p97 upon binding to ZFAND1, which could not be defined by cryo-EM due to the intrinsic dynamics. We therefore used methyl NMR spectroscopy to demonstrate that UBL is primarily responsible for the transient binding to p97. This work paved the way toward a better understanding of the interplay between the proteasome, p97, and ZFAND1 in the context of SG clearance.

Expression, Purification and Characterization of *At*WAT1

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Auxin plays an important role in the growth of plants, so how auxin is transported in plants has been extensively studied. *Arabidopsis thaliana* Wall Are Thin 1 (*At*WAT1), a transmembrane protein mainly located in plant vesicle membrane, has been found to transport auxin from the vacuole to cytosol driven by proton gradient. However, it is still unclear how *At*WAT1 specifically selects auxin and determines the direction of transport. The objective of this study was to establish a platform to prepare the recombinant *At*WAT1 for subsequent structural and biological activity analysis. We expressed *At*WAT1 in *Saccharomyces cerevisiae* and solubilized it by using the detergent, DDM, from yeast membrane fractions. The solubilized *At*WAT1 was purified for negative stain transmission electron microscopy (TEM) analysis to build the 3D model by single particle reconstitution. In addition, styrene-maleic acid lipid particles (SMALP) technique was also utilized for *At*WAT1 purification and liposome reconstitution. The molecular sizes of the *At*WAT1 in SMALP is similar to the detergent-solubilized *At*WAT1. Overall, this study would contribute to the preparation of soluble recombinant *At*WAT1 for biochemical studies and answer the molecular characteristics of this important auxin transporter in plants.

Assembly of phospholipid nanodiscs for structural studies of *At*GTR1

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The amphipatic membrane scaffold proteins (MSPs) can encircle phospholipid to form nanoscale discoidal bilayer particles, termed nanodiscs. Nanodiscs have been exploited to provide native-like lipid environment for membrane proteins. After integration of membrane protein, the nanodiscs are stable, soluble, possess homogenous size and are compatible to biochemical and structural analysis. The *Arabidopsis thaliana* Glucosinolate Transporter 1 (*At*GTR1), is a glucosinolate transporter but also transports various substrates, including jasmonic acid-isoleucine and gibberellin. The structure of *At*GTR1 has not been determined yet and the molecular mechanism of *At*GTR1 remains unclear. In this study, we reconstitute *At*GTR1 fused with GFP (*At*GTR1-GFP) into MSPE3D1 nanodiscs for further structural characterization. We overexpressed and purified MSPE3D1 from *Escherichia coli* for preparing phospholipid nanodiscs. In addition, the *At*GTR1-GFP is expressed in *Saccharomyces cerevisiae* and extracted from membrane fractions by detergents for chromatographic purification. Solubilized *At*GTR1-GFP is reconstituted into nanodiscs using phosphatidylcholine (PC). The *At*GTR1-GFP nanodiscs showed a molecular size of 300 kDa in size exclusion chromatography. Negative--staining transmission electron microscopy (TEM) analysis also revealed the particle size of AtGTR1-GFP nanodiscs was around 12 nm. The *At*GTR1-GFP nanodiscs will be subjected to cryogenic electron microscopy (cryo-EM) for structure determination.

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A Molecular Basis for Malfunction in Disease Mutant A97S of Transthyretin

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Native transthyretin (TTR) biologically functions to transport thyroxine in human body. Familial amyloid polyneuropathy (FAP) is a classically hereditary type of a systemic amyloid disease called transthyretin amyloidosis (ATTR). A publicly acceptable model of ATTR hypothesizes that systemic senile and inherited mutations promote dissociation of tetrameric TTR followed by formation of misfolding monomer and polymerization of amyloid fibrils. Ala97Ser (A97S) is the major mutation reported in Taiwanese FAP patients. So far, there is limited structural information about the amyloidogenic mechanism of TTR-A97S located between β strand F and FG loop. Here, we report crystal structure of TTR-A97S determined at 1.35 Å resolution. Structural analysis reveals that TTR-A97S does not influence beta-sheet structure, and this amyloidogenic point mutation shares almost identical structure to TTR-WT (wild-type) except for minor deviations in the vicinity of the mutation site. Indeed, the largest difference is observed in the region around FG loop, and this loop becomes more flexible upon amino acid exchange to Ser. The biochemical analyses demonstrate that significantly decreased stability and enhanced amyloidogenicity for TTR-A97S. Based on calorimetric studies by isothermal titration analysis, TTR-A97S shows stronger binding affinity than wild-type in the first binding event and has similar binding affinity in the second site, explaining TTR-A97S has less monomer than wild-type in the presence of native-state stabilizers. Our findings suggest that TTR-A97S may enhance the local flexibility of FG loop along with distant regions of EF helix, EF loop, and BC loop, thus leading to its destabilization.

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Roles of The Conserved Traits in Proteins' Intrinsically Disordered Regions: Using Galectin-3 As an Example

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Intrinsically disordered proteins (IDPs) do not have tertiary structures, but they have functions. More than half of the proteins have disordered regions, and it is confirmed that many diseases are related to IDPs, such as neurodegenerative disease. Without a constraint from a well-defined structure, the sequence conservation of IDPs is much lower than the folded domain. However, we notice that although their sequences are diverse, many physicochemical properties remain preserved. Therefore, we want to know the significance of IDPs from an evolutionary perspective. The N-terminal domain of Galectin-3 is intrinsically disordered region. After many years of evolution, although N-terminal sequences of Galectin-3 between human and zebrafish are different, they have similar traits. Both have many aromatic and negative charge residues. Hence, we want to take zebrafish Galectin-3 as an example to know whether the conserved traits in IDPs preserve its function.

Pentamidine and its derivatives inhibiting cell proliferation by blocking the interaction between S100A1 and RAGE V domain

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Human S100A1, in S100 protein family, is calcium-binding protein. Upon Ca^{2+} binding to S100A1 EF-hand motifs, the conformation of S100A1 change promoting interaction with target proteins. Receptors for the advanced glycation end products (RAGE) consists of 5 domains: cytoplasmic domain, transmembrane domain, C1, C2 domains and V domain. V domain of RAGE is one of the target proteins for S100A1 binding to its hydrophobic surface and further triggers signalling transduction cascades that induces cell growth, cell proliferation, and tumorigenesis. Here, we utilized nuclear magnetic resonance (NMR) spectroscopy to characterize the interaction between mS100A1 and RAGE V domain. Otherwise, we found Pentamidine, a small molecular as a drug to treat protozoal- depend illness, could interact with mS100A1 via ^{1}H-^{15}N HSQC NMR titrations. According these results, we utilize the HADDOCK program to generate the structures of binary complex, S100A11-RAGE V domain and S100A1- Pentamidine. Overlapping these two structures, we found Pentamidine play a crucial role in blocking the interaction site between S100A1 and the V domain of RAGE [1,2]. Finally, WST-1 cell proliferation assay also supported this result. Further utilizing this report, we successfully synthesize the derivatives of the pentamidine which potentially enhances the therapeutic approach against cancers.

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Dissecting Role of Unstructured N-terminal Region of Peptidoglycan Hydrolase MepS in Adaptor-mediated Degradation

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Bacterial morphogenesis is coupled to the expansion of peptidoglycan (PG), and murein hydrolase MepS cleaves the cross-links for insertion of new PG materials. MepS is modulated by a lipoprotein NlpI and a periplasmic protease Prc. NlpI acts as an adaptor to bring MepS and Prc together, and Prc further degrades MepS into small fragments. To date, the structure of Prc-NlpI complex is solved at 2.3 Å with X-ray crystallography. The solution structure of N-terminal truncated MepS (residues 37-162) is determined by Nuclear Magnetic Resonance spectroscopy (NMR) while the first 36 N-terminal residues of MepS are intrinsically disordered in the NMR spectral screening. Based on calorimetric studies by isothermal titration analysis, interaction between MepS and NlpI is an exothermic reaction but no heat change can be observed in the interaction between MepS and Prc, implying Prc hardly recognizes MepS without NlpI in solution. However, there is no structural evidence to explain how NlpI adaptor protein identifies MepS substrate. Our gel filtration assay clearly demonstrates that incubation of full-length MepS with NlpI results in the formation of tight complex, but N-terminus truncated MepS (MepS-dN36) significantly reduces its ability to interact with NlpI, indicating N-terminal region of MepS is involved in the interaction with NlpI. Compared to full-length MepS, MepS-dN36 shows almost no effect during the titrations of NlpI in ITC experiments, and the degradation efficiency is also decreased *in vitro*. Heteronuclear NMR analyses reveal most of the well-dispersed cross-peaks in NMR spectra of truncate and full-length MepS can be superimposed, indicating that the well-folded domain structure corresponding to residues 37-162 is not perturbed by the N-terminal extension, residues 1-36. Upon addition of well-folded NlpI, a dramatic variation in the 2D spectral features of full-length MepS is detected and the resonances undergo a significant decrease in intensity. In contrast, the truncated MepS spectrum shows marginal chemical shift perturbations (CSPs) in the presence of equimolar NlpI. Recently, we successfully produce several crystals of Prc-NlpI-MepS from crystal

screening, but the X-ray diffraction data shows poor quality. Taken together, our findings suggest the unstructured N-terminal region of MepS is important for efficient NlpI-mediated Prc proteolysis.

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Structural basis of transcription activation by the OmpR/PhoB family response regulator PmrA

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PmrA, an OmpR/PhoB family response regulator, activates the transcription of genes responsible for polymyxin resistance in bacteria by recognizing promoters in which the canonical *-35 element* is replaced by the *pmra box*, a scenario of class-II transcription activation. Here, we report a high resolution cryo-electron microscopy structure of an intact bacterial PmrA-dependent transcription activation complex (TAC) containing a PmrA dimer, an RNA polymerase σ^{70} -holoenzyme, and a class-II *pbgp* promoter DNA. The structure shows a simple recruitment mechanism, in which the RNA polymerase σ^{70} -holoenzyme contacts PmrA mainly via electrostatic interactions. In addition, the PmrA-*pmra box* and σ4-DNA interactions of PmrA-TAC–caused conformational reorientation resulted in a weaker TAC than did the canonical CAP-TAC. The loose PmrA-TAC structure conclusively helps the PmrA and σ^{70} factor to escape from TAC to enter the elongation stage of transcription. Our study provides a structural basis for understanding how PmrA activates transcription via both recruitment and reorientation mechanisms.

Structural Insights into Inhibition of Human DNA Methyltransferases

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Mammalian DNA methylation is a key mechanism of epigenetic regulation established and maintained by DNA methyltransferases (DNMTs), including DNMT1, DNMT3A and DNMT3B. All DNMTs contain a methyltransferase domain for transferring a methyl group to the cytosine C5 position of CpG sites. Dysregulation of DNMTs is related to carcinogenesis in various human cancers. To date, various non-covalent DNMT inhibitors have been identified, but the underlying mechanisms for their inhibitory activity and specificity for each DNMT are unknown. Here, several structurally diverse non-covalent DNMT inhibitors were selected for testing their inhibitory activities against recombinant human DNMT1, DNMT3A and DNMT3B. A natural compound originated from *Peganum harmala*, harmine, efficiently blocks the methyltransferase activity of not only DNMT3B but also DNMT3A and DNMT1 with a similar μM range of inhibitory activity. Binding assay using intrinsic tryptophan fluorescence suggests that harmine directly interacts with DNMT3B. Co-crystal structure of the catalytic domain of DNMT3B in complex with harmine was determined revealing that harmine is bound at the cofactor SAM-binding site, explaining why harmine inhibits all of the three DNMT enzymes. Kinetic assays further confirmed that harmine competes against SAM in inhibiting the activity of DNMT3B. We also found that harmine can induce cytotoxicity effects on castration-resistant prostate cancer (CRPC) model cells with an IC50 of ~ 14 μM, and CPRC cells treated with harmine resulted in un-silencing of the transcription of several hypermethylated tumor-suppressor genes in prostate cancer compared to untreated cells. In summary, our study not only reveals the DNMT inhibition mechanism by harmine but also suggests new directions for developing novel DNMT inhibitors as the anticancer drugs for the treatment of prostate cancer.

Biochemical characterization of the Hemolytic Mechanism of α-hemolysin from *Vibrio campbellii*

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The aquaculture industry plays an important role in the world economy. However, the development of aquaculture was limited due to diseases outbreaks. Among marine pathogens, *Vibrio* bacterium, which infect both aquatic vertebrates and invertebrates, can lead to high host mortality. A *Vibrio*-secreted virulence factor, α-hemolysin (αHL), possess the hemolytic activity on host erythrocytes. αHL can eventually lead to cell lysis, by assembling and forming a pore on the host cell membrane. The key factors inducing protein assembly and pore formation remain unclear. Investigation on the molecular mechanism of αHL would be important for the control of *Vibrio* infections. Here, we overexpressed and purified the recombinant *Vibrio campbellii* αHL (*Vc*αHL) in *Escherichia coli* and studied its biochemical activities. Recombinant *Vc*αHL was slightly assembled on the liposome and revealed weak hemolytic activities at high concentration. Interestingly, presence of several divalent cations significantly enhanced the oligomerization and membrane integration of *Vc*αHL. Moreover, these cations dramatically stimulate the hemolytic activity of *Vc*αHL and the transmembrane permeability of liposome. Negative-staining transmission electron microscopy (TEM) analysis further demonstrated that the divalent cations promote *Vc*αHL oligomerization instead of membrane integration. Truncated *Vc*αHL mutants were established to identify the functional domain responsible for ion-sensing on *Vc*αHL. Overall, these findings would contribute to the development of prevention and treatment methods for hemorrhagic diseases in aquaculture.

Refinement of BAP1 Structural Ensembles by SAXS-restrained Molecular Dynamics Simulations

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The deubiquitinase BRCA1-associated protein 1 (BAP1) is a tumor suppressor implicated in a large number of cancer types. It plays crucial roles in multiple cellular processes, including transcription activation, histone remodeling, and mitochondrial apoptosis. The most frequently mutated ubiquitin carboxyl-terminal hydrolase (UCH) domain of BAP1 is a highly conserved region. In contrast to other human UCHs (UCHL1, UCHL3 and UCHL5) and *Drosophila* UCH (Calypso), there is no experimental structural model available to BAP1 UCH to date. We have recently carried out small angle X-ray scattering (SAXS) analyses of BAP1 UCH variants harboring a number of cancer-associated mutations.[1] A catalytic mutation, C91G, showed a significantly reduced radius of gyration (R_g) compared to that of the wild type (WT). The C91G variant also exhibited a higher melting temperature and a higher degree of unfolding cooperativity. To understand how such a point mutation increase the folding stability and compactness, we generated a homology model of BAP1 UCH for restrained all-atom molecular dynamics (MD) simulations using the SAXS data as restrains.[2,3] For both systems, the experimental R_g values and the overall profiles could be faithfully reproduced by the SAXS-restrained MD simulations. The resulting MD trajectories were used to generate conformational ensembles of both WT and C91G to compare the structural and dynamic differences. Our analyses indicated a few structural motifs that may be responsible for the more loosely packed structure of WT. Such an analytical procedure will be applied to the other BAP1 UCH variants that showed significantly more expanded molecular dimensions according to SAXS. Understanding how disease-associated mutations impact on the folding of BAP1 UCH will help better understand the molecular basis of the structure-activity relationships of this important oncogene regulator.

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Charged residues on the intrinsically disordered region of galectin-3 contribute to the regulation of liquid-liquid phase separation

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The spatio-temporal distribution of biomacromolecules in the cell is possibly explained by the well-regulated liquid-liquid phase separation (LLPS). Here, we study the regulation of LLPS by using galectin-3 (Gal-3) as a model. Gal-3 contains a structured carbohydrate recognization domain (CRD) and a long intrinsically disordered N-terminal domain (NTD). The high dynamic NTD plays a crucial role in LLPS. Although the mechanism of Gal-3 LLPS is roughly understood, its regulation is still unknown. For understanding how Gal-3 NTD regulates LLPS, we searched the clue of evolution by aligning several vertebrates' sequences and found that there are two highly conserved negatively charged residues on the NTD. **We hypothesize that these two charges play a repulsive role intermolecularly, then decrease the ability of LLPS.** To test our hypothesis, we generated negative charge substituted mutants to check their biophysical properties.

The catalytic activity of TCPTP is autoregulated by its intrinsically disordered C-terminal tail and activated by integrin alpha-1

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T-Cell Protein Tyrosine Phosphatase (TCPTP, PTPN2) is a non-receptor type protein tyrosine phosphatase that is ubiquitously expressed in human cells. It is a critical component of various key signaling pathways that are directly associated with the formation of cancer, inflammation, and other diseases. Thus, understanding the molecular mechanism of TCPTP activity regulation is essential for the development of TCPTP-based therapeutics. In spite of that, the structural basis for the regulation of TCPTP's activity has remained elusive. Under basal conditions, TCPTP is inactivated by its own C-terminal tail, but how this inactivation is achieved has been unknown. Furthermore, if it is inactive then how can it be activated inside a cell. To answer these questions, we used nuclear magnetic resonance (NMR) spectroscopy, small-angle X-ray scattering (SAXS), and chemical cross-linking coupled with mass spectrometry (CX-MS) as major tools to show that the C-terminal intrinsically disordered tail of TCPTP functions as an intramolecular autoinhibitory element that controls the TCPTP catalytic activity. However, this is not achieved by completely blocking the active site, but rather the C-terminal tail moves around the active site and dynamically occludes substrates from the TCPTP active site which is akin to a 'windshield wiper' in a car. Activation of TCPTP is achieved by cellular competition, *i.e.*, the intrinsically disordered cytosolic tail of Integrin-α1 displaces the TCPTP autoinhibitory tail, allowing for the full activation of TCPTP. Taken together our work not only defines the completely unique mechanism by which TCPTP is regulated but also reveals that the intrinsically disordered tails of two of the most closely related PTPs (PTP1B and TCPTP) autoregulate the catalytic activity of their cognate PTPs via entirely different mechanisms, which can be exploited to develop TCPTP based specific therapeutics.

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Structural and functional characterization of *Arabidopsis thaliana* NPF4.6

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Abscisic acid (ABA) is a plant hormone that involved in plant development and stress responses, such as drought or pathogen infections. Several members of the NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER FAMILY (NPF) have been identified as ABA transporters in the *Arabidopsis*. Among these transporters, *At*NPF4.6 shows the ABA import activity after phosphorylation at Ser292. However, there is limited understanding about the regulatory mechanism and transport function of *At*NPF4.6. Therefore, we aimed to identify the key residues involved in the ABA transport of *At*NPF4.6. First, we expressed recombinant *At*NPF4.6 in *Saccharomyces cerevisiae* and confirmed the transport activity of *At*NPF4.6 by using a cell-based uptake assay. Kinetic analysis of the ABA transport indicated that *At*NPF4.6 was a high substrate affinity transporter. The pH profile analysis showed that *At*NPF4.6-expressing yeast had a higher ABA transport activity at acidic environment. In addition, *At*NPF4.6 dramatically lost the ABA transport function after the treatment of ionophores, suggesting that *At*NPF4.6 was a proton-coupled transporter. Moreover, we used molecular docking approaches to model the *At*NPF4.6-ABA interaction and found some important residues may participate in the substrate binding of *At*NPF4.6. Uptake assay confirmed that mutations on these residues significantly reduced the ABA transport function of *At*NPF4.6. Together, our results reveal a possible ABA selective and transport mechanism of *At*NPF4.6. More structural studies would be carried on to further understand this interesting transporter in plants.

Development of potent human monoacylglycerol lipase (MAGL) inhibitors for the treatment of neuroinflammation

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Human monoacylglycerol lipase (MAGL) is a serine hydrolase which participates in the metabolic pathway of inflammation by converting the endocannabinoid 2-arachidonylglycerol (2-AG) to arachidonic acid (AA). The central nervous system (CNS) triggers neuroinflammation during infection, trauma, exposure to toxic metabolites, or aging to reduce tissue damage and defend against pathogens. However, severe or persistent neuroinflammation may cause Alzheimer's and other neurogenerative diseases. Reports have shown that MAGL is regarded as an important target for the development of new drugs. In this study, natural compounds from the InterBioScreen (IBS) natural products database were screened by molecular docking software GOLD, Molegro and LibDock. We analyzed the docking results with LigPlot^{+} and PyMOL and found that compound ZINC70706920 is considered a potential inhibitor against MAGL according to its docking score, molecular interaction, and logP value. As for direct drug screening, natural inhibitors from traditional Chinese medicine library were screened via MAGL inhibition assay. The result showed that tst-41 was identified as the strongest inhibitor against MAGL (IC_{50} = 2.4 μM). Moreover, the molecular modeling revealed that S122, I179 and H269 are key residues of MAGL interacting with the Top 10 inhibitors. In conclusion, tst-41 and ZINC70706920 are potential leads against MAGL and our results are helpful in developing new and novel therapeutics to relieve the symptoms of neuroinflammation related diseases.

Developing new inhibitors of human glutaminyl cyclase (hQC) as possible agents for the treatment of Alzheimer's disease

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Alzheimer's disease (AD), a chronic neurodegenerative disease, is commonly observed in people over the age of 65. AD is estimated to affect more than 150 million people worldwide by the year 2050. In addition, 99% of AD drug candidates in clinical trials fail, so the development of drugs against this neurodegenerative disease is regarded as top priority. Some studies have shown that one of the causes of AD is the human glutaminyl cyclase (hQC), which catalyzes the N-terminal cyclization of amyloid beta (Aβ) peptides, and result in a more intense hydrophobic structure. The Aβ is more likely to accumulate and cause neuropathy in the brain after crossing the blood-brain barrier. Therefore, the development of hQC inhibitors could provide a new direction for the treatment of AD. The known hQC inhibitors such as, PQ912, PBD150, and SEN177 are reported with Ki = 0.02, 0.06, 0.023 μM. However, these inhibitors are all chemically synthesized compounds, which have side effects, and only PQ912 has entered the phase II clinical trials. Therefore, there is an urgent need to develop new and effective hQC inhibitors with fewer side effects for the treatment of AD. In this study, computer-aided drug design (CADD) and pharmacophore model were used to screen and design hQC inhibitors from natural product database. Additionally, we employed the established inhibition assay platform to screen inhibitors from traditional Chinese medicine (TCM) library. At present, we successfully expressed and purified hQC protein, confirmed the activity of hQC, and established an inhibition assay platform to screen potential inhibitors. We screened the inhibitor 21359 of hQC through CADD, and identified the best inhibitor was tst-69 from TCM library. As well, we analyzed the detailed molecular interactions of the identified inhibitors with hQC. Conclusively, the identified inhibitors could be potential for development of new and novel agents to treat AD.

Structural Insights into the Stereospecific Cyclization Reaction Catalyzed by Deoxypodophyllotoxin Synthase

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Podophyllotoxin (PPT) is a medically important, naturally occurring aryltetralin lignan that serves as a precursor for the synthesis of clinically active antineoplastic drugs etoposide and teniposide. Owing to the growing demand for PPT, alternative synthetic strategies are being vigorously pursued. Among them, chemoenzymatic approaches using enzymes involved in PPT biosynthetic pathway are considered more efficient than chemistry-based methods in overcoming stereochemical issues arising during PTT synthesis. In particular, the activity of deoxypodophyllotoxin synthase (DPS), which catalyzes the formation of a C–C bond between C6 and C7′ of (−)-yatein to generate the tetracyclic core of (−)-deoxypodophyllotoxin, is crucial for the development of a chemoenzymatic synthesis scheme. DPS belongs to the superfamily of non-heme iron(II)- and 2-oxoglutarate-dependent (Fe/2OG) oxygenases, whose members share a conserved catalytic core featuring a double-stranded β-helix (DSBH) fold and an active site harboring a 2-His-1-carboxylate motif for iron coordination. Since Fe/2OG oxygenases can activate unreactive C–H bond toward various chemical transformations, such as hydroxylation, demethylation, desaturation, and cyclization, these enzymes are known to participate in a wide variety of biochemical reactions. Previous mechanistic studies suggested that DPS-catalyzed cyclization may be achieved through either a benzylic radical or carbocation pathway. However, no structural information on DPS is available. In addition, our recent results indicate that DPS catalyzes the cyclization of not only the native substrate (−)-yatein but also its enantiomer (+)-yatein, raising a question about how DPS carries out the cyclization reaction on both stereoisomers. To elucidate the structural basis of DPS-catalyzed reaction, we have determined two crystal structures which correspond to the substrate- and cofactor-bound states of DPS at 2.05 and 2.09 Å resolution, respectively. Interestingly, the substrate yatein undergoes binding-induced conformational changes upon entering the DPS active site. By adopting the observed U-shaped conformation, the C7′ atom is placed close to the ferrous iron, presumably allowing hydrogen atom transfer to occur. Moreover, steric restriction imposed by the active site, particularly around the C7′ position, appears to define the reaction outcome and product chirality, which ensures stereospecific production of (−)-deoxypodophyllotoxin. Our results provide key structural insights into the DPS-catalyzed ring closure, which may pave a way for utilizing DPS in synthesizing podophyllotoxin-derived drugs.

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Structural insights into the pathogenesis of transthyretin amyloidosis

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Transthyretin-associated amyloidosis (ATTR) is the most common form of familial amyloid polyneuropathy (FAP), a hereditary amyloid disease. The disease is caused by the misfolding of an otherwise native tetrameric protein, Transthyretin (TTR), into the insoluble amyloid fibrils, deposited extracellularly in various body parts. More than 100 pathogenic mutations of TTR have been reported that can cause ATTR with diverse clinical manifestations. The A97S pathogenic mutant, the most common variant in the Taiwanese population, is characterized by a late-onset but rapidly deteriorating amyloidosis with distinct phenotypes involving severe cardiac dysfunctions. The cardiac manifestations of transthyretin amyloidosis with the A97S variant contribute to the fast disease progression, leading to heart failure or death. In this research, we studied the structure and the protein dynamics using X-ray crystallography and solution NMR to gain insights into the disease mechanism of ATTR. We first used biomolecular NMR spectroscopy to show that calcium interacts with and destabilizes native TTR protein. We mapped this residue-specific information at the protein structure to understand the mechanism of TTR aggregation. Furthermore, to gain insights into the disease mechanism of A97S TTR, we solved the protein structure of the tetrameric and monomeric forms of TTR-A97S using X-ray crystallography. We then studied the protein dynamics by solutions NMR to understand the behavior of wild-type and mutant TTR protein in solution. Altogether, our results provide a better understanding of the TTR aggregation pathway leading to amyloidosis and the molecular mechanism of A97S-related TTR pathology.

Structural Prediction and Mechanistic Analysis of Sugar *O*-methyltransferase by Molecular Dynamics Simulation Methods

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Glycosylated natural products as drugs are modified at their sugar moieties by sugar *O*-methyltransferase (SOMT) as a way to improve target specificity and potency. As a member of TylF superfamily, SOMT is a divalent metal-dependent enzyme exhibiting substrate promiscuity while retaining remarkable substrate stereoselectivity and regiospecificity, giving its methylated product as a potent tumor inhibitor. However, the catalytic activity and mechanism in relation to protein structure and dynamics has been unclear. In this study, molecular dynamics (MD) simulation methods was utilized to show six structural conformations of SOMT corresponding to different catalytic stages. Root-mean-square deviation analysis and sequence alignment tool were combined to reveal the structural variations throughout the entire binding and catalytic turnover. The results showed two distinct catalytic pathways characterized by substrate/cofactor binding and release order, consequently revealing the tunnels for substrate and cofactor actions on enzyme. In addition, variations in the octahedral coordination with metal ion were observed in the active site, further inferring the impact of protein conformational changes on enzyme kinetics through possible interactions with key residues. Moreover, MD simulation showed the highly mobile loops that were mostly absent in crystal structures, as well as the substrate/cofactor-binding conformations. The key residues controlling the loop conformations and interactions were also revealed. The study here may illustrate the characteristics of the enzyme superfamily which usually possesses highly conserved sequences, and provide important information about structural and mechanistic relationship for its members, thereby facilitating utilizing SOMT and its related enzymes in drug modification and therapeutic applications.

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(2) H. Kawasaki, N. Soma, R. H. Kretsinger *Sci Rep.* 2019 Jul 23;9(1):10688.

An Insight into Mutation Effects and Protein Structure of Enzymes Based on X-ray Crystal Structures of Drug Methyltransferase

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Methylation is indispensable in cell biology and also drug function. Nature modifies drug functions and specificity through actions of methyltransferases. Our kinetics studies reveal that mutations on a sugar *O*-methyltransferase can lead to interesting positive synergistic correlation between amino acid residues which play lethal or reductive effects on enzyme activity. Hence, X-ray crystallography was utilized in this study to reveal structural determinants of enzyme catalysis. In the present results, we obtained the mutant drug methyltransferases by the protein heterologous expression technology, as well as protein crystals of different shapes by scanning the crystal growing reagents with vapor diffusion protein crystallization methods. Subsequently, we have obtained high-resolution diffraction data for structural analysis, and then obtained the corresponding mutant structures. Based on the protein structures, positional changes of mutated amino acids can be observed in up to five methyltransferase mutants with single or double mutations. Furthermore, the relationship of the structural variations to kinetic data of enzyme activity was analyzed, thus shedding new light on effects of protein conformational changes on enzyme catalysis. Also, the information attend in this study provides an insight into how a methyltransferase binds the substrate and coenzymes by way of loop conformational changes and key residues controlling metal chelation, catalysis, and substrate/coenzyme bindings. The information may be useful for future engineering and subsequent applications of the enzyme in drug modifications.

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(2) Warrier, T.; Kapilashrami, K.; Argyrou, A.; Ioerger, TR.; Little, D.; Murphy, KC.; Nandakumar, M.; Park S.; Gold, B.; Mi, J.; Zhang, T.; Meiler, E.; Rees, M.; Somersan-Karakaya, S.; Porras-De Francisco, E.; Martinez-Hoyos, M.; Burns-Huang, K.; Roberts, J.; Ling, Y.; Rhee, KY.; Mendoza-Losana, A.; Luo, M.; Nathan, CF. *Proc. Natl. Acad. Sci.*, **2016**, 113, E4523-E4530.

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陳映雪博士紀念學術講座

(Felicia Chen-Wu Memorial Lecture)

緣起：

陳映雪博士1939年出生於臺北市，幼年早慧，1951年進入臺北市立第一女子中學（簡稱北一女中）初中部（註一），爾後且直升北一女中高中部，並以第一名優異成績直升臺灣大學化學系。大學期間與吳成文（註二）一路相伴，直至大學畢業結婚後雙雙赴美留學取得博士學位，在美國數所重要大學如耶魯大學、愛因斯坦醫學院、紐約州立大學石溪校區進行學術研究。

1988年陳映雪博士捨棄美國順暢之學術研究，偕同夫婿吳成文院士回臺。吳成文院士啟肇臺灣生物醫學研究之基礎建置，處處有其協助之身影，無論是中研院生醫所、國家衛生研究院，以及臺灣重要的數個學會，陳映雪博士均是最關鍵之助力。尤其是中華民國生物物理學會之籌設與建立，初期的行政繁瑣，在陳映雪博士的規劃與執行中逐一到位完成，成立爾後學會運作得以條貫傳承，陳映雪博士奔走之力，功不可沒。

其實陳映雪博士回臺時已罹患癌症，然她生性活潑熱情，積極堅毅，同時酷愛學術研究，除非親口告知，多數人不知她是嚴重的癌病患者。陳映雪博士與癌症搏鬥13年，經過52次化療，其中尚包括在美國國家衛生研究院執行中的自體骨髓移植之人體試驗。多年後美國國衛院宣佈此人體試驗治療癌症的計畫失敗。而在陳博士經歷此水裡來、火裡去艱苦備嘗之人體試驗回臺，當有同儕或是友人詢問她為何要去嘗試這一個辛苦的人體試驗之際，陳映雪博士的回答是：

我是一位科學的研究者，學術研究乃在希望瞭解過去所不知道的，解鎖釋疑，未來方得以嘉惠人類，如果連我都不敢去嘗試新的治療方法，這怎麼是一個學者的精神？萬一這一次真的成功了，固然欣喜，即令是失敗了，我也是幫助這一個計畫的一份子，與有榮焉。不畏懼，生命才有機會大開大闔，這如同學術研究，哪有實驗室的試驗是一路成功無礙的，不也是藉助無數的失敗所堆積出的成功嗎？

如此足以解讀陳映雪博士十數年抗癌過程中，為何依舊孜孜勤勉於實驗室的學術研究，她即令在醫院中仍指導實驗室的研究如常，同時跟實驗室同仁逐一告知病況，並信心滿滿會早日回去實驗室與大家一起追索學術堂奧。

她每一次住院治療，床榻上總堆滿紙張，每是實驗的數據以及她建議如何修改論文的手寫痕跡，對生命的熱愛、家庭的支持，以及倘佯追求真理新知的學術研究，成為她一路與癌病抗衡的動力，而永不退卻的堅持，正是一位學術人成功的特質，在陳映雪博士身上，展現出對生命與學術研究的價值。

這一位衷情學術研究、熱愛生命的科學家逝世於1997年，得年壽60載。仙去之日，病床上滿是實驗室的工作，她的學術生涯伴隨她到塵世的最後一刻。當時吳成文院士卸任學會理事長，由陳長謙院士繼任，她仍擔任學會監事，為紀念陳映雪博士堅持學術的一生，由時任學會秘書長甘魯生教授提案，學會成立「陳映雪博士紀念學術講座」，除紀念陳映雪博士獨特的生命故事之外，其實最重要的在於彰顯一位學術人堅毅與不畏挑戰的精神。研究過程的挫敗是學術成果得以豐收壯闊的基礎，陳映雪博士秉持研究學術的精神，來面對她的癌症，因而得以不屈不饒。

本講座設立於民國89年（西元2000年），自第六屆生物物理學會年會開始。設立本講座的目的除前述為紀念陳映雪博士獨特之學術人格特質及對學會的貢獻之外，其實更重要之目的為藉助講座之成立，邀請國內外知名科學家參與講座，以引進卓越科學研究現況，在生物物理如此跨領域之新興學門，洞窺學域的發展，幫助臺灣學界引領高峰。

爾故，講座伊始，為以陳映雪博士暨吳成文院士其國際友人、知名科學家創立舞台，而至我國學術成熟、英才濟濟，本講座交由學會完全主導，邀請其認為最適切之國、內外卓著科學家與會，為此日新月異之學術研究開疆拓土。學會如此運作成功，亦表示臺灣學術基礎建置逐次完備，這其實便是上世紀一群自海外歸國科學家如陳映雪博士之心志，仰仗臺灣以科學強健國本之初心。是為記。

歷屆陳映雪博士紀念講座邀約記錄：

★民國89年（2000年）第6屆生物物理學會年會暨新知研討會於清華大學，當年度舉辦第一次「陳映雪博士紀念學術講座」，主講人為美國康乃爾大學講座教授、美國科學院院士Gordon G. Hammes教授。

★民國90年（2001年）第7屆生物物理新知研討會於陽明大學，主講人為美國加州聖地牙哥大學講座教授、美國科學院院士Charles Cantor教授。

★民國92年(2003年) 生物物理學會假圓山飯店擴大舉行「第四屆東亞生物物理研討會」，因之學會建議將陳博士紀念學術演講移至此年度，主講人為美國史丹佛大學講座教授、美國科學院院士暨美國總統科學獎得獎人Lubert Stryer教授。

★民國93年(2004年) 第9屆生物物理新知研討會於中研院，主講人為哈佛大學講座教授、美國科學院院士、中研院院士王惇教授。

★民國94年(2005年) 第10屆生物物理新知研討會於清華大學，主講人為美國加內基麥隆大學講座教授、中研院院士何潛教授。

★民國95年（2006年）年第11屆生物物理新知研討會於臺灣大學，主講人為美國加州大學聖地牙哥校區講座教授、美國科學院院士、中研院院士錢煦教授。

★民國96年（2007年）年第12屆生物物理新知研討會於國家衛生研究院，主講人為國家衛生研究院院長、中研院院士伍焜玉教授。

★民國97年(2008年)年第13屆生物物理聯合研討會於中興大學（惠蓀林場），主講人為美國密西根大學講座教授James K. Coward教授

★民國98年(2009年)第14屆生物物理聯合研討會於成功大學，主講人為曾任生物物理學會理事長、中研院副院長、中研院院士陳長謙教授。

★民國99年（2010年）第15屆生物物理聯合研討會於中研院，主講人為中研院副院長、中研院院士王惠鈞教授。

★民國100年（2011年）第16屆生物物理研討會於東華大學，主講人為中研院化學所所長、中研院院士王寬教授。

★民國101年（2012年）第17屆生物物理聯合研討會於中研院，主講人為斯克里普斯研究所（Scripps Research Institute）Peter Wright教授。

★民國102年（2013年）第18屆生物物理研討會於中研院，主講人為中研院基因體中心特聘研究員張子文教授。

★民國103年(2014年)第19屆生物物理研討會於成功大學，主講人為中研院生物醫學研究所研究員黃太煌教授。

★民國104年（2015年）第20屆生物物理研討會於中研院，主講人為美國麻省理工學院講座教授、美國科學院院士Paul Schimmel教授。

★民國105年（2016年）第21屆生物物理研討會於清華大學，主講人為美國喬治亞大學研究員汪必成教授。

★民國106年（2017年）第22屆生物物理研討會於義守大學，主講人為中研院院士、中研院院長廖俊智教授。

★民國107年（2018年）第23屆生物物理研討會於中興大學，主講人為中研院院士、國衛院創院院長、陽明大學特聘講座吳成文教授。

★民國108年（2019年）第24屆生物物理研討會於宜蘭大學，主講人為中研院院士、中研院特聘研究員蔡明道教授。

★民國110年(2021年)第25屆生物物理研討會於成功大學，主講人為國衛院特聘研究員、生技與藥物研究所所長張俊彥教授(註三)。

註一：當時年代小學進入中學必須參與全市的競試，而北一女中亦尚有初中部，陳博士以優異的成績考入北一女中。

註二：吳成文，中研院院士、國家衛生研究院創院院長，於上一世紀80年代，帶領數十位於國外訓練完備之科學家回臺，創建臺灣生物醫學學術研究之基礎建置，並創立諸多學會，造就我國今日生物醫學學術研究榮景，被譽為我國之「生醫開拓手」，為臺灣生命科學之競爭力貢獻良多。

註三：2020年(民國109年)因COVID-19疫情之故，第25屆生物物理研討會延期至2021年舉行。

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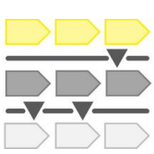

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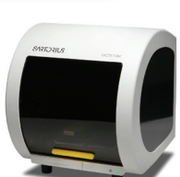
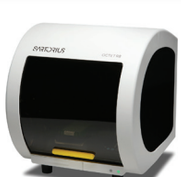

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No. of channels	2	4	8
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Evaporation control	No	No	Yes
Sample plate format	96-well	96-well	96-well
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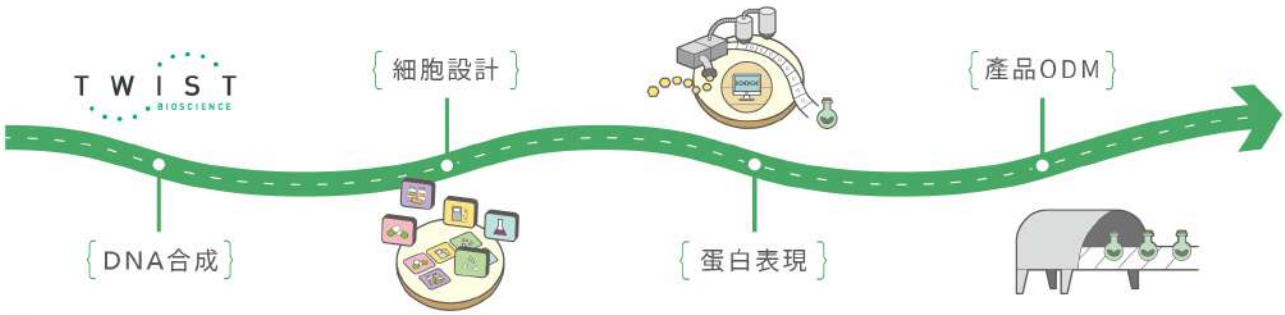

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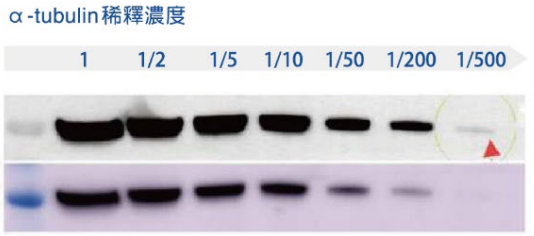
我最快 / 1秒鐘拍完照 下～班～啦～



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小體積 | 2018德國紅點設計大獎，冷光界的iPAD

高靈敏 | 電子式底片，貼覆式感光，訊號不流失

寬動態 | 超大感光畫素，百萬級電子容量，不易過曝

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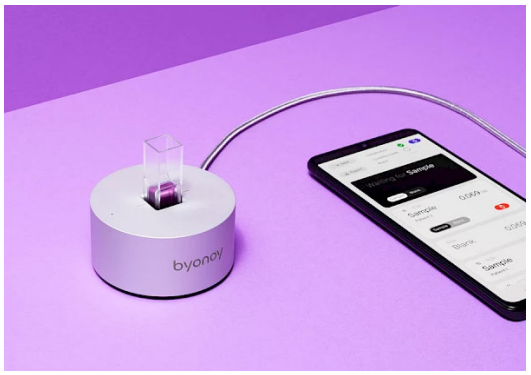

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免疫實驗試劑

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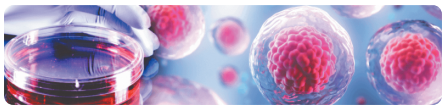
- 胜肽與重組蛋白
- ELISA, ELISPOT, Flow Kit
- 小分子化合物
- 細胞螢光染劑與分析試劑

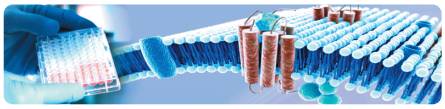


細胞實驗工具

- 初代細胞、細胞株
- 細胞轉染試劑
- 細胞培養基及胎牛血清
- 活細胞影像觀察玻片

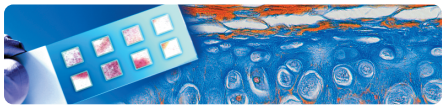
蛋白質體研究工具

- 蛋白質結晶試劑與耗材
- 各式層析膠體與預注管柱
- 蛋白質電泳試劑
- ECL 冷光試劑與轉漬膜



藥物研發試劑

- 藥物功能篩選平台
- 生化與細胞檢測試劑
- 放射性產品
- BioBank 生物樣本庫
- FDA 藥庫、天然物藥庫



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- 活細胞影像暨流體培養系統
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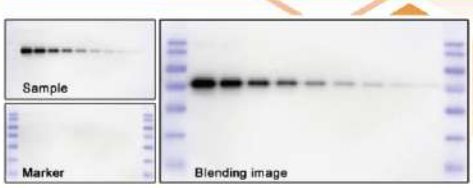

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- 實驗室小型儀器
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