REVIEW

Biological cryo-electron microscopy in China

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Abstract: Cryo-electron microscopy (cryo-EM) plays an increasingly more important role in structural biology. With the construction of an arm of the Chinese National Protein Science Facility at Tsinghua University, biological cryo-EM has entered a phase of rapid development in China. This article briefly reviews the history of biological cryo-EM in China, describes its current status, comments on its impact on the various biological research fields, and presents future outlook.

Keywords: electron microscopy; cryo-EM; transmission electron microscope; history; China; Tsinghua University; EM methodology

A Brief History

In 1950, Professor Ling-Chao Tsien (钱临照) was ushered into a warehouse of the former Kuomintang (Chinese Nationalist Party) government Broadcasting Company in Nanjing, where he unpacked a cargo container full of unassembled parts of a transmission electron microscope (TEM). These TEM parts, manufactured by the British company Metropolitan-Vickers, were purchased and hastily left behind by the fleeing Kuomintang government on its way to Taiwan. Professor Tsien was so excited about the serendipitous finding that he immediately decided to assemble the microscope despite lack of an installation handbook. In no more than 4 years, he and his co-workers published the first electron microscopy (EM) research article in China,[1] in which they described the fine structural defects of aluminum foil. This article marked the beginning of an era in China that relied on EM to examine the world at nanoscale. Application of EM soon became popular in China, especially due to its powerful utility in the characterization of inorganic materials for the steel and engineering industry.

A number of pioneering scientists in materials science and physics have made notable contributions to the development of China's EM community. Beginning in the 1980s, a few leading electron microscopists in inorganic material research, including Ling-Chao Tsien, Ke-Hsin Kuo (郭可信), Fanghua Li (李方华), Duan Feng (冯端), and Renhui Wang (王仁卉), realized the huge potential of high-resolution EM in life sciences (Fig. 1). They invited foreign scientists in the field of cryo-EM to visit China and give lectures and workshops to young students and encouraged the younger generation to devote themselves into cryo-EM of biological application. Nowadays, more than two-thirds of the established Chinese scientists in the field of

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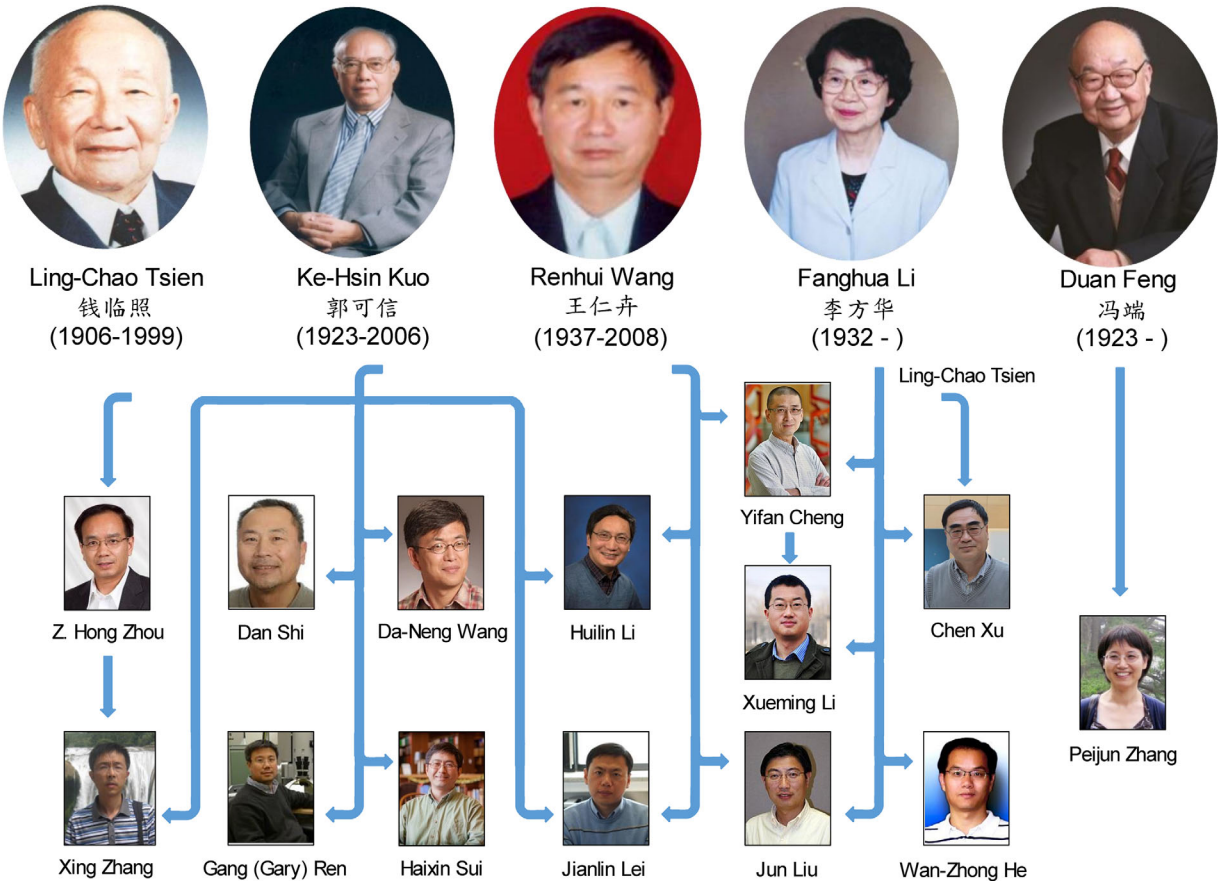

Figure 1. A family tree of the Chinese electron microscopists. Five individuals pioneered EM-based research in China and encouraged a number of their students to go into biological cryo-EM.

cryo-EM were directly trained or largely influenced by these pioneers (Fig. 1).

At about the same period, a few young Chinese scientists from biology or biophysics launched their own expedition into cryo-EM as the technology was developed in its premature phase. In the late 1970s, Jin-Ju Chang (张锦珠) joined Jacques Dubochet's group at European Molecular Biology Laboratory (EMBL) and became one of the very first few scientists studying frozen-hydrated biological specimens using TEM.[2,3] Yun Li (李云)—working with Kevin Leonard at EMBL in 1980—studied mitochondria cytochrome c1 reductase complex in its single particle state using negative-staining EM.[4] Naiqian Cheng (程乃乾)—working with James Deatherage at the University of Arizona—was among the first group of scientists studying the ordered insect muscle fibers using TEM.[5] Da-Neng Wang (王大能) joined Werner Kuhlbrandt's lab at EMBL and solved the first atomic structure of light harvesting complex II in its 2D crystalline forms by electron crystallography.[6] Wei Xu (徐伟) studied the bacteriophage prohead using cryo-EM at Purdue University with Timothy Baker.[7]

In the 1990s, a few research groups in China began to explore the application of EM in structure determination of biological macromolecules. Sen-Fang Sui (隋森芳) at Tsinghua University led a small group of students to grow two-dimensional crystals of proteins on lipid monolayers[8] and studied their structures and interactions with membranes using TEM.[9,10] Wei Xu at the Institute of Biophysics (IBP), Chinese Academy of Sciences (CAS) investigated two-dimensional crystals of membrane proteins. Jing-Qiang Zhang (张景强) at Sun Yat-sen University and Qi-Bin Yang (杨奇斌) at Xiangtan University examined virus structures using cryo-EM technology. Ke-Hsin Kuo and Fanghua Li also dedicated a portion of their limited research funds to the development and application of cryo-EM in addition to their major focus on materials science TEM. Facing meager research funding and the lack of high-end instruments, these groups managed to build their own hardware and software tools for their pioneering research, published several cryo-EM papers to report research carried out solely in China, and trained the first batch of graduate students. Many of these students, exemplified by Yifan Cheng (程亦凡), later became successful scientists in the cryo-EM field (Fig. 1).

Since the year 2000, China has invested more resources in upgrading EM facilities and funding research projects in universities and research

institutes. Well-trained young scientists returned to China from a few leading cryo-EM laboratories in the world and began their independent research careers at Tsinghua University, Peking University, Sun Yat-sen University, University of Science and Technology of China, IBP of CAS, National Institute of Biological Sciences (NIBS), Shanghai Institute of Biochemistry and Cell Biology, and other academic institutions. The cryo-EM research community gradually matured, bringing worldwide attention to rapid cryo-EM development in China. In 2008, the First Kuo KH International Symposium of Cryo-EM, co-chaired by Da-Neng Wang and Sen-Fang Sui, was held successfully at Tsinghua University with more than 150 participants from all over the world. This event formally marked the beginning of a rapid rise of the cryo-EM community in China. Since then, the Kuo KH International Symposium has become one of the most popular cryo-EM meetings in the world.

The EM Arm of the National Protein Science Facility

The origin of National Protein Science Facility

Between November 2002 and July 2003, severe acute respiratory syndrome (SARS) epidemic swept throughout the greater China region, with more than 8000 confirmed cases worldwide and a death toll of 672 in Hong Kong and mainland China. China was apparently unprepared for such a traumatic experience as the general public deserted shops and avoided gathering. During the process of identifying the disease causing agent and clinical treatment, the Chinese government keenly realized the importance of basic research in biology and medicine. In 2005, Zihe Rao (饶子和), then Director of the IBP, and Fuchu He (贺福初), then Vice-President of the Chinese Academy of Military Medical Sciences (CAMMS) successfully lobbied the government to invest on basic research through construction of a centralized biological research facility. The Chinese Commission of Development and Reform, a *de facto* funding agency, decided to allocate 1.1 billion Chinese Yuan (about 130 million US Dollars then) on a National Protein Science Facility (the Facility). The original idea was to build a state-of-the-art facility at IBP of CAS in Beijing, which scientists from China and all over the world would use for their cutting-edge research. Due to the construction of the Shanghai Synchrotron Radiation Facility and other reasons, the leadership of CAS changed their mind in 2006 and proposed to build the Facility in Shanghai instead of Beijing. This change caused an uproar among scientists in Beijing, particularly those at CAMMS and Tsinghua.

To retain the Facility in Beijing, Fuchu He approached Yigong Shi (施一公), who had just began the relocation of his laboratory from Princeton to Beijing, and Zhixin Wang (王志新), who joined the Tsinghua faculty after serving as the Director of IBP from 1999 to 2003. Together, Wang and Shi convinced the administration of Tsinghua University about the importance of retaining the Facility in Beijing, perhaps to a location conveniently accessible by scientists from Zhongguancun (中关村) and neighboring institutions. With strong support from the Ministry of Education and Beijing municipal government, Tsinghua University formed an alliance with CAMMS to compete with CAS for the right to build the Facility in Beijing instead of Shanghai. After several rounds of discussion, the final outcome was a compromised solution: the Chinese Commission of Development and Reform decided to split the funds evenly between Tsinghua/CAMMS and CAS in 2008. CAS would spend its share of 550 million Yuan on a facility in Shanghai (Shanghai Facility), located next to the Shanghai Synchrotron Radiation Facility in the Pudong District. The Shanghai Facility received an additional allocation of approximately 200 million Yuan from the local government.

The decision to split the Facility between two distant locations proved to be correct, because scientists in each location faced the competitive pressure to achieve higher scientific productivity and originality. In addition, the local governments competed to provide more financial support. The Beijing municipal government was more generous, providing 1:1 matching funds to the allotment from the central government and making the total investment 1.1 billion Chinese Yuan. Once the solution was reached between Tsinghua/CAMMS and CAS, Peking University and IBP asked to have a share of the Beijing Facility. It took some time for all parties to agree to a negotiated consensus. In the end, CAMMS had half of the total funds, with Tsinghua and Peking Universities splitting the remaining half. Despite its affiliation with CAS, IBP also received a small share of the funds that were earmarked for research instrument development. In the end, the Beijing Facility, also known as the Pilot Hub of Encyclopedic ProteomIX (PHOENIX), consists of three sites: a main location at CAMMS that focuses on proteomics, an arm on biological EM at Tsinghua, and a second arm on biological nuclear magnetic resonance (NMR) at Peking University. Binglin Gu (顾秉林), a former President of Tsinghua University, vividly described the Beijing Facility as "one body and two wings," or "一体两翼," where the body refers to CAMMS and two wings Tsinghua and Peking Universities.

A focus on EM at Tsinghua

Assignment of the biological EM arm to Tsinghua University was anything but a random choice. At the beginning of the millennium, hard X-ray free-electron laser (XFEL) technology began to show promise as a powerful tool for structural elucidation of

Table I. *Distribution of Cryo-TEMs in China*

Institution	Cryo-EM model	Status	Max acceleration voltage (kV)	Type of electron gun	Cameras and auxiliary parts	Date of commission
Tsinghua University	Titan Krios	Operational	300	S-FEG	K2 Summit, Eagle 4k	Jun, 2009
	Titan Krios	Operational	300	S-FEG	Falcon II, GIF Quantum 964 (UltraScan 895), STEM	Sep, 2015
	Titan Krios	Operational	300	X-FEG	Falcon II, Image Cs-corrector, Phase plate	Dec, 2015
	Tecnai Arctica	Operational	200	S-FEG	Falcon II, Phase plate	May, 2015
	Tecnai F20	Operational	200	S-FEG	DE-20, UltraScan 895, Orius 830, STEM	Oct, 2009
	Tecnai Spirit	Operational	120	LaB_6/W	UltraScan 895	Mar, 2013
	Tecnai Spirit	Operational	120	LaB_6/W	Eagle 4k, Orius 832	Jul, 2014
	Tecnai Spirit	Operational	120	LaB_6/W	Eagle 4k, iCorr	Jun, 2015
Institute of Biophysics	Titan Krios	Operational	300	S-FEG	K2 Summit, Falcon III	Dec, 2009
	Talos F200C	Operational	200	S-FEG	Ceta 4k	Oct, 2014
	Tecai 20	Operational	200	LaB_6/W	UltraScan 894	May, 2002
	Tecnai Spirit	Operational	120	LaB_6/W	Eagle 2k, iCorr	Dec, 2010
National Center for Protein Science Shanghai	Titan Krios	Operational	300	S-FEG	K2 Summit, GIF Quantum 963, Image Cs-corrector	Nov, 2013
	Tecnai F20	Operational	200	S-FEG	Falcon II, Eagle 4K	Oct, 2013
	Tecnai Spirit	Operational	120	LaB_6/W	Eagle 4K	Oct, 2013
Peking University	Tecnai F30	Operational	300	S-FEG	UltraScan 895, GIF Tridiem 863 (UltraScan 894)	2009
Xiamen University	Tecnai F30	Operational	300	S-FEG	Falcon II, Eagle 4k, STEM	Mar, 2014
	Tecnai Spirit	Operational	120	LaB_6/W	Orius 832	Sep, 2013
Harbin Veterinarian Research Institute	Talos F200C	Operational	200	S-FEG	Ceta 4k	2016
Institut Pasteur of Shanghai	Tecnai F20	Operational	200	S-FEG	Eagle 4k	2010
University of Science and Technology of China	Tecnai F20	Operational	200	S-FEG	Eagle 4k	Nov, 2010
	Tecnai Spirit	Operational	120	LaB_6/W	Eagle 2k	Jun, 2014
Huazhong Agricultural University	Talos F200C	Operational	200	X-FEG	Falcon II	May, 2016
Institute of Zoology	Tecnai F20	Operational	200	S-FEG	Eagle 4k	2014

Table I. *Continued*

Institution	Cryo-EM model	Status	Max acceleration voltage (kV)	Type of electron gun	Cameras and auxiliary parts	Date of commission
Wuhan Institute of Virology	Tecnai 20	Operational	200	LaB_6/W	SIS Cantega G2 2K	2007
National Institute of Biological Sciences	Tecnai Spirit	Operational	120	LaB_6/W	UltraScan 895	Jul, 2011
Sun Yat-sen University	JEM-2010	Operational	200	LaB_6/W	UltraScan 895	Jan, 2005
National Institute for Viral Disease Control and Prevention, China CDC	Tecnai F20	Operational	200	S-FEG	UltraScan 994, Orius 832	Apr, 2016
Institute of Pathogen Biology	Tecnai Spirit	Operational	120	LaB_6/W	Orius 832	2009
	Tecnai F30	To be installed	300	S-FEG	Eagle 4k	
Zhejiang University	Titan Krios	To be installed	300	S-FEG	K2 Summit, UltraScan 994	
	Talos F200C	To be installed	200	S-FEG	Ceta 4k	
	Tecnai Spirit	To be installed	120	LaB_6/W	Eagle 4k	
Nankai University	Talos F200C	To be installed	200	X-FEG	Falcon II, Ceta 4K	
Peking University First Hospital	Tecnai 20	To be installed	200	LaB_6/W	Eagle 4k	
Peking University	Titan Krios	To be installed	300	X-FEG	GIF Quantum LS 967, Falcon III, Phase plate	
	Talos Arctica	To be installed	200	X-FEG	K2 summit, Ceta 4k, STEM, Phase plate	
	Tecnai Spirit	To be installed	120	LaB_6/W	Orius 832	
Institute of Biophysics	Titan Krios	Ordered	300	X-FEG	GIF Quantum LS 967, Falcon III, Phase plate	
	Tecnai Spirit	Ordered	120	LaB_6/W	SIS Morada	
Lanzhou University	Talos F200C	Ordered	200	S-FEG	Ceta 4k, STEM	
Guizhou University	Talos F200C	Ordered	200	X-FEG	Ceta 4k	
Xi'an Jiaotong University		Purchase planned	200			
Southern University of Science and Technology		Purchase planned	300, etc.			
Fudan University		Purchase planned	300, etc.			
ShanghaiTech University		Purchase planned	300, etc.			
Sichuan University		Purchase planned	300, etc.			
Wuhan University		Purchase planned	300, etc.			

macromolecules. A large number of scientists from several disciplines had been lobbying the Chinese government for the construction of a national XFEL facility. Unfortunately, even under optimal circumstances, such an XFEL facility would be completed for operation no earlier than the year 2025 and thus offered little help to the ever-increasing demands of structural biologists in China. Nonetheless, the Shanghai Synchrotron Radiation Facility—a third-generation light source—was under construction in 2006, and Peking University had already built a national NMR facility with support from several branches of the central government. Thus, despite booming structural biology in China, complete absence of any high-end TEM with field-emission gun (FEG) for biological EM in 2007 formed a sharp contrast to the other two major methods of structure determination. Importantly, EM-based methodologies—including single-particle cryo-EM, cryo-electron tomography (cryo-ET), and electron crystallography—had begun to show remarkable potential. In early 2007, a consensus was quickly reached among key scientists at Tsinghua—particularly Shi, Sui, and Wang—to build a centralized EM facility at Tsinghua. The University administration fully supported the decision. On behalf of Tsinghua, Sui delivered a proposal for such a facility to members of a review panel in May 2007.

The Tsinghua Facility now has three Titan Krios, one Tecnai Arctica, one Tecnai F20, and three Tecnai Spirit TEMs (Table I). All microscopes are fully operational. One of the three Titan Krios (Titan1) is equipped with a K2 Summit direct electron detector, and the other two Titan Krios (Titan2 and Titan3) will have their K2 detectors installed by Fall 2016. In addition, Titan2 has an energy filter, and Titan3 will have the most complete auxiliary components including an energy filter, an image Cs-corrector, and a phase plate. The facility also has an FIB/SEM dual-beam system equipped with a cryo-SEM preparation workstation and a correlative light electron microscopy workflow. In addition to the microscopes, the facility has a complete set of cryo-EM sample preparation equipment including high-pressure freezers, freeze substitution systems, ultramicrotomes, freeze plungers, plasma cleaners, and high-vacuum evaporators. An accompanying computing facility of 10,000 CPU cores and 10 PB storage has been installed and will be further expanded.

The EM Team and the First Titan at Tsinghua

The most important prerequisite for building a world-class EM facility is to recruit top-notch scientists. Coincidentally, three young scientists Haixiao Gao (高海啸), Jianlin Lei (雷建林), and Ning Gao (高宁), all from the laboratory of Joachim Frank then at Wadsworth, wrote to Sui and Shi in May 2007, expressing a strong desire to build a unified laboratory at Tsinghua University. Jianlin Lei, trained in physics at both undergraduate and PhD levels, has strengths in EM instrumentation and methodology. Ning Gao has rich experience with protein biochemistry and EM sample preparation. Haixiao Gao had received comprehensive training in cryo-EM and was the leader of the trio. Shi interviewed the team in Room 418 of the Schultz Laboratory at Princeton University, where Haixiao Gao gave an impressive presentation on behalf of all three. As the only interviewer, Shi was convinced of the ambitious EM-based research plan presented by Gao and formally invited the three to apply for tenure-track faculty positions at Tsinghua University. After a prearranged interview, the three were simultaneously offered independent positions, with ample start-up funds.

Haixiao Gao included a Titan Krios microscope as a must-have item on her list of requirements. The price tag of approximately 4 to 5 million US dollars, or approximately 35 million Chinese Yuan, was an astronomical number to Tsinghua University. With strong support from Zhixin Wang and Sen-Fang Sui, Shi turned to the Tsinghua University administration for help in early May 2008. The possibility of installing Asia's first operational Titan Krios on Tsinghua campus and the huge research potential convinced the leadership, which decided to make an investment of 30 million Yuan after listening to a brief presentation by Shi. These funds were supposed to cover both the purchase, room preparation, and the operation of the Titan microscope. Facing a gap of funds, Shi *et al.* had to negotiate with FEI to lower the purchase price. Gratifyingly, within 2 weeks of the very first meeting with the FEI team, Shi signed a formal Titan Krios purchasing contract on behalf of Tsinghua University in June 2008. This Titan Krios microscope (Titan1) arrived in early March 2009 and became fully operational in June, the first in Asia.

To fully utilize the first Titan Krios microscope in China and Asia, Tsinghua decided to recruit additional faculty members in cryo-EM. Hong-Wei Wang (王宏伟) performed his PhD thesis research on cryo-EM in the laboratory of Sen-Fang Sui and received his postdoctoral training with Eva Nogales at the Lawrence Berkeley National Laboratory. Wang received several excellent job offers including one from Tsinghua in 2008 but chose to begin his independent career at Yale University instead. Convinced by Shi of the once-in-a-lifetime opportunity to build a world-class cryo-EM facility, Wang returned to Tsinghua in 2011 and quickly became a major driving force behind Tsinghua's continued effort of hiring EM faculty members and expanding the EM facility. Ye Xiang (向烨), a postdoctoral scientist with Michael Rossmann at Purdue University, was recruited to Tsinghua in 2013. Xueming Li (李雪明), a postdoctoral fellow with Yifan Cheng at UCSF and an inventor of the semiautomated low-dose acquisition program UCSF-Image4 and the motion

Table II. *Representative Publications of Biological Cryo-EM From Mainland China*

No.	Year	Structure	Resolution (Å)	Corresponding author(s)	Reference(s)
1	2008	DegP chaperone-protease	8.5	Zengyi Chang, Sen-Fang Sui	Jiang J et al, *PNAS* 105, 11939–11944
2	2009	DegP chaperone-protease	18	Sen-Fang Sui	Shen Q et al, *PNAS* 106, 4858–4863
3	2010	Optimized locally adaptive nonlocal means denoising filter	methodological development	Chang-Cheng Yin	Wei D et al, *J Struct Biol* 172, 211–218
4	2011	Cytoplasmic Polyhedrosis Virus	3.9	Jingqiang Zhang, Ping Zhu	Cheng L et al, *PNAS* 108, 1373–1378
5	2011	RsgA-30S ribosome-GMPPNP complex	9.8	Jianlin Lei, Ning Gao	Guo Q et al, *PNAS* 108, 13100–13105
6	2012	Transcribing cypovirus	4.1	Ping Zhu, Lingpeng Cheng	Yang C et al, *PNAS* 109, 6118–6123
7	2012	*N*-ethylmaleimide-sensitive factor (NSF)	9.2	Sen-Fang Sui	Chang L et al, *Nat Struct Mol Biol* 19, 268–275
8	2013	Immature ribosomal small subunit from *rimm* gene deleted *E. coli* strain	12.9	Jianlin Lei, Ning Gao	Guo Q et al, *Nucleic Acids Res* 41, 2609–2620
9	2013	Wild Rabbit Hemorrhagic Disease Virus	6.5	Dong Zheng, Hai Pang, Fei Sun	Wang X et al, *PloS Pathog* 9, e1003132
10	2013	Assembly intermediate of 50S ribosome subunit	10.7	Jianlin Lei, Ning Gao	Li N et al, *Nucleic Acids Res* 41, 7073–7083
11	2013	Human Dicer	26	Jennifer A Doudna, Hong-Wei Wang	Taylor D et al, *Nat Struct Mol Biol* 20, 662–670
12	2014	50S ribosome ObgE GMPPNP Complex	5.5	Suparna Sanyal, Jianlin Lei, Ning Gao	Feng B et al, *PLoS Biol* 12, E1001866
13	2014	Yeast exosome	23	Ailong Ke, Hong-Wei Wang	Liu J et al, *Nat Struct Mol Biol* 21, 95–102
14	2014	Human γ-secretase	4.5	Sjors H.W. Scheres, Yigong Shi	Lu P et al, *Nature* 512, 166–170
15	2014	30-nanometer chromatin fiber	11	Ping Zhu, Guohong Li	Song F et al, *Science* 344, 376–380
16	2014	50S ribosome EngA Complex	5	Jianlin Lei, Ning Gao	Zhang X et al, *Nucleic Acids Res* 42, 13430–13439
17	2014	Eukaryotic transcription core Mediator complex	31	Gang Cai	Wang X et al, *Cell Res* 24, 796–808
18	2014	Cotranslational chaperone with ribosome	4.9	Jianlin Lei, Ning Gao	Zhang Y et al, *Nat Struct Mol Biol* 21, 1042–1046
19	2015	Phycobilisome	21	Jindong Zhao, Sen-Fang Sui	Chang L et al, *Cell Res* 25, 726–737
20	2015	Human γ-secretase	3.4	Sjors H.W. Scheres, Yigong Shi	Bai X et al, *Nature* 525, 212–217
21	2015	Influenza virus RNA polymerase complex	4.3	Hong-Wei Wang, Yingfang Liu	Chang S et al, *Mol Cell* 57, 925–935
22	2015	Mammalian mechanosensitive Piezo1 channel	4.8	Ning Gao, Bailong Xiao, Maojun Yang	Ge J et al, *Nature* 527, 64–69

Table II. *Continued*

No.	Year	Structure	Resolution (Å)	Corresponding author(s)	Reference(s)
23	2015	PrgJ-NAIP2-NLRC4 inflammasome complex	6.6	Sen-Fang Sui, Jijie Chai	Hu Z et al, *Science* 350, 399–404
24	2015	Eukaryotic MCM complex	3.8	Yuanliang Zhai, Bik-Kwoon Tye, Ning Gao	Li N et al, *Nature* 524, 186–191
25	2015	Toll-like receptor 13	4.87	Jiawei Wang, Hong-Wei Wang, Jijie Chai	Song W et al, *Nat Struct Mol Biol* 22, 782–787
26	2015	Human γ-secretase	3.4	Yigong Shi	Sun L et al, *PNAS* 112, 6003–6008
27	2015	Mammalian apoptosome	3.8	Sjors H.W. Scheres, Yigong Shi	Zhou M et al, *Genes Dev* 29, 2349–2361
28	2015	Voltage-gated calcium channel Cav1.1 complex	4.2	Nieng Yan	Wu J et al, *Science* 350, aad2395
29	2015	Rabbit ryanodine receptor RyR1	3.8	Sjors H.W. Scheres, Yigong Shi, Nieng Yan	Yan Z et al, *Nature* 517, 50–55
30	2015	Ribosome PoTC•EF-G (S588P)•GDPNP complex	4.3	Qiang Xie, Ning Gao, Yan Qin	Zhang D et al, *Nucleic Acids Res* 43, 10525–10533
31	2015	SecM-stalled ribosome	3.3	Ning Gao, Sen-Fang Sui	Zhang J et al, *Elife* 4, 09684
32	2015	50S ribosome bound with HflX	4.5	Jianlin Lei, Suparna Sanyal, Ning Gao	Zhang Y et al, *Nat Struct Mol Biol* 22, 906–913
33	2015	Yeast spliceosome complex	3.6	Yigong Shi	Yan C et al, *Science* 349, 1182-91 Hang J et al, *Science* 349, 1191–1198
34	2015	Cypovirus	12	Hongrong Liu, Lingpeng Cheng	Liu H et al, *Science* 349, 1347–1350
35	2015	Human dendritic cell receptor DEC205	14.6	Yongning He	Cao L et al, *PNAS* 112, 7237–7242
36	2015	Alpha-SNAP-SNARE assembly in 20S particle	7.35	Hong-Wei Wang, Sen-Fang Sui	Zhou Q et al, *Cell Res* 25, 551–560
37	2016	FIRT: Filtered iterative reconstruction technique with information restoration	methodological development	Kai Zhang, Fa Zhang, Fei Sun	Chen Y et al, *J Struct Biol* 195, 49–61
38	2016	Niemann-Pick C1 (NPC1)	4.43	Qiang Zhou, Nieng Yan	Gong X et al, *Cell* 165, 1567–1578
39	2016	Yeast cytoplasmic exosome complex	4.2	Hong-Wei Wang	Liu J et al, *Cell* Res 26, 822–837
40	2016	Yeast 26S proteasome	4.6	Yigong Shi, Feng Wang	Luan B et al, *PNAS* 113, 2642–2647
41	2016	Ebola GP binds to Fab Fragments	6.7	Nancy J. Sullivan, Ye Xiang	Misasi J et al, *Science* 351, 1343–1346
42	2016	Group II intron in complex with its reverse transcriptase	3.8	Rajendra Kumar Agrawal, Marlene Belfort, Hong-Wei Wang	Qu G et al, *Nat Struct Mol Biol* 23, 549–557

Table II. *Continued*

No.	Year	Structure	Resolution (Å)	Corresponding author(s)	Reference(s)
43	2016	Yeast U4/U6.U5 tri-snRNP	3.8	Yigong Shi	Wan R et al, *Science* 351, 466–475
44	2016	Spinach photosystem II-LHCII supercomplex	3.2	Mei Li, Xinzheng Zhang, Zhenfeng Liu	Wei X et al, *Nature* 534, 69–74
45	2016	Late nuclear pre-60S ribosome	3.08	John L. Woolford, Ning Gao	Wu S et al, *Nature* 534, 133–137
46	2016	Bacteriophage Φ29 emptied particles	10.1	Ye Xiang	Xu J et al, *Nature* 534, 544–547
47	2016	EF4 bound to PRE 70S ribosome	3.2	Ning Gao, Yan Qin	Zhang D et al, *Nat Struct Mol Biol* 23, 125–131
48	2016	EV71 mature viron in complex with the Fab	4.8	Zhong Huang, Yao Cong	Ye X et al, *PloS Pathog* 12, e1005454
49	2016	Intact ATM/Tel1 kinase	8.7	Weiwu Wang, Gang Cai	Wang X et al, *Nat Commun* 7, 11655
50	2016	Human 26S proteasome	3.5	Yigong Shi	Huang X et al, *Nat Struct Mol Biol*, publish online
51	2016	Yeast spliceosome B^{act} complex	3.5	Yigong Shi	Yan C et al, *Science*, publish online
52	2016	Yeast spliceosome C complex	3.4	Yigong Shi	Wan R et al, *Science*, publish online
53	2016	DeepPicker: A deep learning approach for fully automated particle picking in cryo-EM	methodological development	Tian Xia, Xueming Li, Jianyang Zeng	Wang F et al, *J Struct Biol*, publish online

correction program MotionCorr,[11] joined Tsinghua in 2014. Jiawei Wang (王佳伟), a classically trained X-ray crystallographer, shifted his research focus to EM data analysis. A critical mass on EM methodology and application began to take shape at Tsinghua. Such a build-up would have been flawless if it were not for the unexpected and sudden departure of Haixiao Gao at the beginning of 2010.

Other Cryo-EM Facilities in China

The emergence of direct electron detectors has fundamentally changed the impact of EM on the various biological research fields. Soon after the operation of Titan1 at Tsinghua, IBP and the Shanghai Facility installed their own 300 kV Titan Krios microscopes, each recently upgraded with a K2 direct electron detector (Table I). The bright prospects of EM-based research spread rapidly in China, with a number of institutions quickly committed to the purchase of 300 kV cryo-TEMs. These institutions include Fudan University and Shanghai Tech University in Shanghai, Peking University, Southern University of Science and Technology in Shenzhen, Sichuan University in Chengdu, Wuhan University, Xiamen University, Zhejiang University in Hangzhou, and the Institute of Pathogen Biology of CAS (Table I). IBP has recently ordered a second Titan Krios microscope. In most places, accompanying microscopes operating at 200 kV and/or 120 kV have also been planned. A number of other institutions have already purchased or plan to purchase a 200 kV cryo-TEM and are keen to the possibility of purchasing a 300 kV cryo-TEM. Currently, there are seven TEMs operating at 300 kV in mainland China, of which five are Titan Krios (Table I). The number of Titan Krios and equivalent 300 kV microscopes in operation is likely to reach double digits in China by the end of 2017, when the total number of operational cryo-TEMs will exceed 50.

Making an Impact in Biological Research

Sen-Fang Sui was admitted into the freshman class of Tsinghua University in 1964; but his undergraduate education was interrupted by the tumultuous 10 years of the Cultural Revolution that began in 1966. Recovering from the wound, China opened to the outside world in 1979 and allowed students to pursue education in the West. In the 1980s, studying abroad was both rare and prestigious for a handful of lucky Chinese students; the vast majority of those who had such opportunities chose to continue their career development in the West, in part due to the inadequate research support in China at that time. After obtaining a PhD degree in biophysics at the

Technical University of Munich in Germany in 1988, Sui returned to Tsinghua University and began his independent, pioneering research endeavor on biological cryo-EM in 1989. The concept of research was primitive in China during that era; Sui became a principal investigator in his own laboratory, which is among the first batch of independent laboratories at Tsinghua and in China.

In the first half of 1990s, the Sui laboratory focused on two-dimensional (2D) electron crystallography and developed strategies for protein 2D crystallization on lipid layers. Using these strategies, his laboratory investigated the structures of avidin on biotinylated lipid monolayers[8] and C-reactive protein on ligand-modified lipid monolayers.[12] In the second half of 1990s, Sui turned his attention to the single-particle reconstruction technique.[13] Since then, his laboratory has solved several cryo-EM structures including DegP oligomers[14,15] and the NSF and 20S particle.[16] In particular, the cryo-EM structures of different oligomeric states of DegP represent the first of its kind in China (Table II).

EM methodology and technology

Xueming Li had worked on EM for materials science in his early career. In 2009, he changed his research field to structural biology with the specific aims of improving the efficiency and resolution of biological cryo-EM. Obtaining a large number of particles to boost the overall signal-to-noise ratio and enhancing signal recording efficiency at high frequency are two key strategies for achieving atomic resolutions in cryo-EM. As a postdoctoral fellow in the laboratory of Yifan Cheng at UCSF, Li introduced the general purpose graphic processing unit (GPGPU) into 3D reconstruction[17] and developed the data collection system named UCSFImage.[11] These efforts resulted in the acceleration of single-particle cryo-EM data processing by more than 10-fold. His studies on a new generation of detector (i.e., electron counting camera), and a new motion correction procedure, greatly facilitate cryo-EM reconstruction of small biomolecules at near atomic resolution.[18,19] Since joining Tsinghua University in the spring of 2014, Li has focused on the development of new methods and technologies, aiming to make atomic-resolution cryo-EM analysis more accessible and convenient for a wide range of biological samples. Relying on the deep learning algorithm, Li and his collaborators, Jianyang Zeng (曾坚阳) and Tian Xia (夏天), have developed a fully automated particle-picking program (Table II).[20]

Fei Sun (孙飞) of IBP has been collaborating with Fa Zhang (张法) of the Institute of Computation Technology, CAS, to build an automatic pipeline for high-resolution electron tomography (ET). They developed a feature-based tilt series alignment algorithm ATOM_align[21] for plastic section ET and a fiducial marker-based tilt series algorithm Marker-Auto[22] for cryo-ET. To improve the contrast and quality of ET reconstruction, they developed three different reconstruction algorithms: ASART,[23] FIRT,[24] and ICON.[25] Notably, ICON combines both nonuniform fast Fourier transformation and compressed sensing techniques and significantly recovers the missing information in both the angular intervals and the missing wedge.

Chromatin organization

The organization and dynamics of the 30 nm chromatin fiber play a critical role in regulating DNA accessibility for gene transcription. Ping Zhu (朱平) of IBP started his cryo-EM training as a postdoctoral fellow at Florida State University in 1999. He had been working on the structures of envelope glycoproteins (ENV) on the surface of AIDS viruses[26,27] before returning to IBP. Primarily using cryo-EM, Zhu seeks to understand the molecular mechanism of viral assembly, infection, and replication, and the higher order structure of 30 nm chromatin fiber. Zhu et al. determined the cryo-EM structure of 30 nm chromatin fiber reconstituted from 12 nucleosome arrays at a resolution of 11 Å (Table II)[28]. The *in vitro* reconstituted 30 nm chromatin fibers are organized as a left-handed double helix twisted by repeating tetranucleosome units in an H1-dependent manner. These results provide critical insights into fundamental structural aspects of the chromatin fiber and constitute an important step toward understanding of how chromatin fiber is organized and regulated.

Immune response by the inflammasome

Jijie Chai (柴继杰) is interested in mechanistic understanding of the nucleotide-binding domain (NBD)- and leucine-rich repeat (LRR)-containing proteins (NLRs), which are crucial for the cytosolic immunosurveillance system of mammals.[29,30] NLRs function as pattern recognition receptors to perceive pathogen-associated molecular patterns (PAMPs) or host-derived danger components. This results in the formation of high-molecular-weight complexes termed inflammasomes that activate the proinflammatory caspases-1. Recognition of bacterial pathogens by NLR apoptosis inhibitory proteins (NAIPs) induces activation of NLR family CARD domain-containing protein 4 (NLRC4) followed by formation of the NAIP-NLRC4 inflammasomes.[31,32] Recent cryo-EM studies[33,34] of the mouse Naip2-Nlrc4 inflammasome provide the first view of how inflammasomes are assembled and activated (Table II). In the cryo-EM structures, the complex formed a wheel-like architecture containing 10 or 11 Nlrc4 protomers; however, only one Naip2 molecule was incorporated into the complex. Structural comparison between an inactive Nlrc4[35] and the activated

Nlrc4 suggests a model for the activation of the Naip2-Nlrc4 inflammasome, in which Nlrc4, once activated by Naip2, exposes a previously hidden surface for interaction with a solvent-exposed receptor surface of an inactive Nlrc4, leading to Nlrc4 self-activation.

Intramembrane proteases and γ-secretase

Yigong Shi has been working on intramembrane proteases since 2005. X-ray crystallography was employed to solve the structures of the serine protease rhomboid and the zinc metalloprotease S2P.[36,37] The ultimate target is human γ-secretase, which comprises the catalytic component presenilin and three additional membrane proteins. Human γ-secretase is intricately linked to Alzheimer's disease (AD), and most AD-derived missense mutations map to presenilin.[38] Although the archeal homolog of presenilin was bagged by X-ray crystallography,[39] the intact γ-secretase defied many years of crystallization attempt. The calculated molecular weight of γ-secretase is only about 160 kDa, a challenging size for cryo-EM analysis. Through a close collaboration between the Shi laboratory at Tsinghua and Xiaochen Bai and Sjors Scheres at the Laboratory of Molecular Biology of MRC, the structure of γ-secretase was determined at an overall resolution of 3.4 Å (Table II).[40] The vast majority of the side chains within the membrane exhibit unambiguous EM density for specific assignment. Atomic modeling of human γ-secretase allows mechanistic understanding of functions and disease-derived mutations.

Ion channels

Nieng Yan (颜宁) seeks to elucidate the structural mechanism of the Na^+ and Ca^{2+} channels in the excitation–contraction (E–C) coupling of skeletal and cardiac muscles, including the voltage-gated Na^+ and Ca^{2+} (Na_v and Ca_v) channels and the ryanodine receptor RyRs.[41] The Na_v channels are responsible for the initiation and propagation of action potential in excitable membranes. The action potential-induced conformational changes of $Ca_v1.1$ activate RyR1, releasing Ca^{2+} from sarcoplasm into cytoplasm. In contrast, RyR2 is activated by the $Ca_v1.2$-permeated Ca^{2+} influx, a mechanism known as calcium-induced calcium release (CICR). Yan *et al*. determined the crystal structure of a bacterial Na_v channel Na_vRh in a potentially inactivated state (Table II).[42] The homotetrameric Na_vRh exhibits asymmetry in the selectivity filter and the voltage-sensing domains (VSDs) in the structure. Using cryo-EM, Yan's group elucidated structures of the rabbit $Ca_v1.1$ channel complex at resolutions ranging from 4.3 to 3.6 Å.[43,44] The structures exhibit conformational shifts and reveal mechanistic insights into voltage-dependent structural changes of the Ca_v channels. In collaboration with Yigong Shi and Sjors Scheres, Yan's group also determined the cryo-EM structures of the rabbit RyR1 at near-atomic resolutions in closed states and 5.7 Å resolution in the open state (Table II).[45,46] The dilation of the cytoplasmic gate is induced by the conformational changes of the adjoining Central domain, which represents the major transducer in the long-range allosteric gating of RyRs.[46] These structures lay a solid foundation for mechanistic understanding of the E–C coupling and the function and disease mechanism of Na_v and Ca_v channels.

Mechanosensitive channel piezo

Trained as an X-ray crystallographer, Maojun Yang (杨茂君) began to use cryo-EM to study membrane proteins since 2010. Mechanosensitive channels are unique membrane transporters that are activated by mechanical forces and transport specific molecules down a concentration gradient across a membrane. Mouse Piezo1 is the first mechanosensitive channel found in mammals[47] and has broad functions in many physiological processes relating to mechanical forces. In close collaboration with Ning Gao and Bailong Xiao at Tsinghua, Yang determined the cryo-EM structure of Piezo1 at 4.8 Å resolution (Table II).[48] In contrast to the prediction of a homotetramer, Piezo1 forms a trimeric propeller-like structure, with three distal blades forming the extracellular domains and a central cap constituting the transmembrane domain which includes 42 transmembrane helices. This structure shed exciting new light on the functional mechanism of mechanosensitive channels.

Photosynthesis

In plant photosynthesis, a series of supramolecular machineries embedded in the thylakoid membrane of the chloroplast mediate the initial biochemical reactions driven by light energy. Among them, photosystem II (PSII) is the most upstream machinery capable of converting light energy and splitting water molecules under ambient temperature. PSII is powered by a series of peripheral light-harvesting complexes termed LHCII. As a graduate student, Zhenfeng Liu (柳振峰) in the laboratory of Wenrui Chang (常文瑞) at the IBP solved the first X-ray structure of plant major LHCII.[49] However, little was known about how the LHCII complexes assemble with the PSII core complexes at specific locations, so as to deliver excitation energy efficiently. Through close collaborations with Xinzheng Zhang and Mei Li at the IBP, Liu determined the structure of spinach PSII–LHCII supercomplex at 3.2 Å resolution using single-particle cryo-EM (Table II).[50] This unveiled intricate innerworkings of a 1.1 MDa homodimeric supramolecular system containing 50 protein subunits, 210 chlorophylls, 56 carotenoids, and many other cofactors. The energy transfer pathways between three different LHCII complexes and the PSII core are suggested.

Ribosome biogenesis

Ning Gao is interested in ribosome biogenesis and since 2008 has been employing cryo-EM to study the dynamic ribosome assembly pathways[51,52] and the roles of various assembly factors.[53–55] In particular, eukaryotic ribosome assembly is orchestrated by hundreds of auxiliary factors, making it an ideal system to fully explore the powerful potential of cryo-EM. With the recent hardware and software breakthroughs in the field, Gao *et al*. have obtained a series of structures of the endogenous premature ribosomal particles isolated from yeast nuclei, and one specific state was solved at an overall resolution of 3.0 Å (Table II).[56] This structure enabled atomic modeling of over 20 assembly factors, which provide a structural framework for understanding the diverse roles of pre-60S assembly factors. Gao also uses cryo-EM as a primary tool to study the function of less-characterized ribosome-binding proteins in translation control, such as the ribosome-associated cotranslational chaperones[57] and heat-shock-induced ribosome-splitting factor HflX.[58]

RNA splicing and spliceosome

The genetic material DNA is first transcribed into precursor messenger RNA (pre-mRNA) by eukaryotic RNA polymerase. Pre-mRNA undergoes splicing by the spliceosome to generate mature mRNA, which is translated into protein by the ribosome. The spliceosome is a multi-mega-Dalton ribonucleoprotein (RNP) complex, with its components undergoing rapid flux during each cycle of pre-mRNA splicing. Compared to the relatively stable structures of RNA polymerase and the ribosome (both determined by X-ray crystallography), the spliceosome exhibits exceptional conformational flexibility and compositional dynamics. Relying on about 200,000 particles, the Shi laboratory determined the structure of an intact spliceosome from the yeast *S. pombe* at an overall resolution of 3.6 Å (Table II).[59,60] This structure was followed up with near-atomic cryo-EM structures of the *S. cerevisiae* spliceosome at two distinct states of the pre-mRNA splicing cycle.[61,62] Together with the structures of the U4/U6.U5 tri-snRNP,[63,64] the molecular mechanisms of the yeast spliceosome are documented at unprecedented clarity.

RNA metabolism

One of the major focuses by Hong-Wei Wang has been the structure and mechanism of macromolecules involved in RNA metabolism. He revealed the architecture of yeast RNA degradation exosome complex using single particle EM[65] and later discovered the presence of two distinct routes within the complex for RNA substrate recruitment.[66] In a recent study, Wang *et al*. pushed the resolution of the exosome complex to near-atomic and discovered an interaction mode of Ski7 protein with the entire complex as well as structural elements involved in RNA substrate-induced conformational switch of the complex.[67] Wang *et al*. determined the architecture of human Dicer and RISC-loading complex in the RNA interference pathway.[68] Using the phase-plate cryo-EM technology, his group revealed the various conformational states of human Dicer induced by specific RNA substrates and cofactors (Table II).[69] He also solved the long-sought structure of the group II intron RNA in complex with its intron-encoded reverse transcriptase at near-atomic resolution, which reveals the mechanism of intron self-splicing and has implications for both the spliceosome and telomerase.[70]

Virus structures

Ye Xiang of Tsinghua University has been working on virus structures using a combination of X-ray crystallography and cryo-EM. The bacterial virus phi29 is an ~15 MDa asymmetric complex system consisting of ~9 components in different copies varying from several to several hundreds. Xiang used X-ray crystallography to solve the structure of each individual component[71–74] and cryo-EM to determine the structures of complex viral particles at different states (Table II).[74,75] The ultimate goal is to obtain atomic details of the whole complex at different states, including the prohead, the mature virion, and the postinfection virion. Such structural information may help decipher the mechanisms of the viral capsid assembly, genome packaging, host cell recognition, host cell wall and membrane penetration, and genome release. Ping Zhu of IBP determined the cryo-EM structures of a cypovirus virus (CPV) in its nontranscribing state at 3.9 Å resolution[76] and in the transcribing state at 4.1 Å,[77] which provide a detailed glimpse into the transcription process (Table II). Fei Sun studied the structure of rabbit hemorrhagic disease virus (RHDV) that belongs to Lagovirus, a branch of calicivirus. The cryo-EM structure of RHDV was solved to 6.5 Å resolution with the core region at 5.5 Å, which enabled modeling of a semi atomic model and identification of the N-terminal arm of the capsid protein that was unavailable in the crystal structure.[78]

Qinfen Zhang (张勤奋) of Sun Yat-sen University has used cryo-EM to analyze protein complexes[79] as well as the icosahedral viruses that infect plant, fish, and crab.[80] These studies yield clues for vaccine and medicine design. Although the structures of icosahedral viral capsids have been studied for about 50 years, the precise structures of genome and associated proteins within viral capsid remained unknown. Hongrong Liu (刘红荣) of Hunan Normal University and Lingpeng Cheng (程凌鹏) of Tsinghua University collaborated to resolve this question using single-particle cryo-EM analysis in combination with their

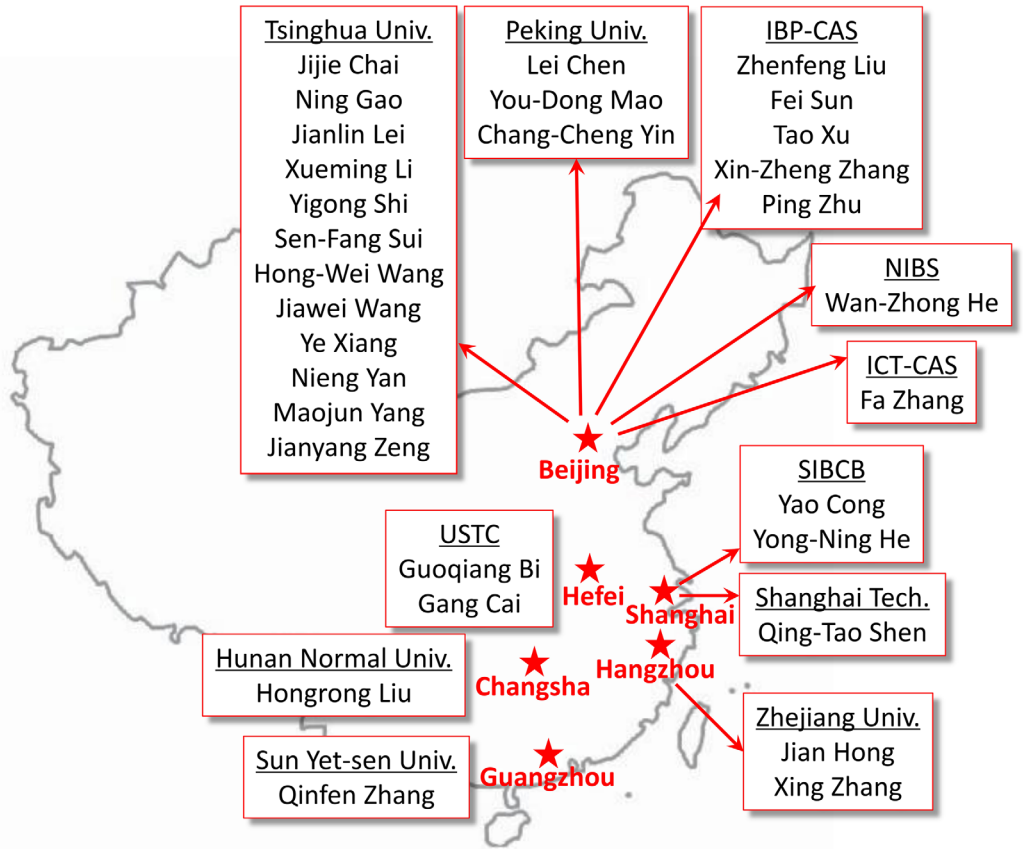

Figure 2. Distribution of the principal investigators in biological cryo-EM in mainland China. More than 24 independent laboratories primarily use cryo-EM to determine 3D structures of proteins and supramolecular complexes. These laboratories are concentrated in Beijing, particularly at Tsinghua University.

newly developed method for the symmetry-mismatch reconstruction of icosahedral virus.[81] The 3D structures of the dsRNA genome and the RNA-dependent RNA polymerases were reconstructed within both transcribing and nontranscribing cypovirus capsid, revealing a dynamic process during virus transcription and challenging the conventional view on the packaging of dsRNA virus genomes inside the rigid capsid. Yao Cong (丛尧) of the Shanghai Institute of Biochemistry and Cell Biology has focused on the protein folding nanomachine TRiC/CCT as well as virus–antibody complexes. In close collaboration with Zhong Huang at the Institute Pasteur of Shanghai, Cong has resolved the near-atomic resolution cryo-EM structure of human enterovirus 71 (EV71) in complex with the Fab of a bivalent broadly neutralizing antibody.[82] The Cong laboratory is also developing the sampling reduced FRM2D method to further accelerate the speed of image alignment.

Future Outlook

The Chinese biological cryo-EM community has expanded tremendously in the last decade, from a few independent laboratories in 2005 to more than 2 dozens in 2015 (Fig. 2). This remarkable expansion is paralleled by the timely procurement of advanced TEMs and fueled by the revolution in camera technology in recent years. There was no 200 kV cryo-TEM with FEG in China in 2008; but 17 cryo-TEMs with FEG were in service operating at 200 or 300 kV by the end of June 2016 (Table I). The research output, as measured by publications in top-notch journals, has increased drastically. Since 2014, 18 original research articles from China, each reporting the cryo-EM structure of a biological macromolecule, were published in the global weekly journals *Nature* and *Science* (Table II). Although cryo-EM has also enjoyed a rapid and healthy growth in the other countries, the growth rate of cryo-EM in China far exceeds that of the world average. This trend is expected to continue for an additional period of 5–10 years.

The development of biological EM in China is strongly tilted toward application, particularly single-particle cryo-EM analysis of supramolecular complexes, which brings an immediate and significant impact on the biological research community worldwide. The flip side of emphasizing application is reflected by apparent weakness in EM methodological development in China, which will likely restrict applications at the forefront of the EM field if left unaddressed. In addition, there is an

ostensible paucity of research activity using cryo-ET in China. These deficiencies would be tolerable for a small nation or a region, but not for a huge country like China that is motivated to play a key role in the evolving research field of biological EM. Thankfully, the Chinese biological cryo-EM community has keenly recognized these shortcomings and is working hard to improve the situation.

Since its inception, X-ray crystallography has played an instrumental role in our understanding of the biological world.[83] Owing to its mature methodology, X-ray crystallography has become a powerful, routine technology for nonspecialists and contributes to the vast majority of the detailed structural information in the protein data bank (PDB). There is no doubt that X-ray crystallography will continue to play an important role in our quest to understand the microscopic world. But the relative roles of X-ray crystallography versus EM have already changed and the trend of such changes is likely to accelerate in the coming years. In 2005, the weighted overall contribution to structural biology by cryo-EM probably represented 1% of that by X-ray crystallography; in 2015, the number was perhaps 80%. Although it is very difficult to predict the scenario in 2025, we would not be surprised if the relative contribution by these two methodologies reciprocates relative to that in 2005. Thankfully, those of us who used to rely exclusively on X-ray crystallography are increasingly inclined to turn to EM for tackling research problems. We are optimistic that the biological EM community will greatly benefit from the joining of the X-ray crystallography experts.

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