Changing times in structural biology A personal view from a long-term fellow at KEK

Vincent Olieric
KEK Long-Term Invited Fellow

In November 2020, I was quarantined in my hotel in Narita as part of the prevention measures against COVID-19 when I read the news in the journal Nature: "It will change everything: (Google) DeepMind's artificial intelligence (AI) makes a gigantic leap in solving protein structures"[1]. Eight months later, on July 22, 2021, DeepMind and the European Molecular Biology Laboratory announced the AlphaFold[2] database—likely the most important contribution of AI to the advancement of science to date—giving researchers free and open access to ~365,000 protein structure predictions and making their algorithm available. Not only for individual proteins but machine learning systems also provide accurate predictions for both protein-protein complexes and nucleic acid structures.

What is certainly going to be transformative in structural biology arrives only a couple of years after the cryo-electron microscopy (cryo-EM) "resolution revolution"[3]. Structural biology is changing fast.

I entered the field of structural biology in 2001 at the onset of the dominance of macromolecular X-ray crystallography (MX) at synchrotron radiation as a tool for obtaining structural information of macromolecules. Since 2009, I have been in charge of the protein crystallography X06DA-PXIII beamline at the Swiss Light Source (SLS) at Paul Scherrer Institut (PSI), Switzerland, which just passed 2,000 entries in the Protein Data Bank (PDB). In 2021, I joined KEK as a long-term invited fellow in the Structural Biology Research Center (SBRC) group headed by Pr. Senda, aiming at preparing the future of X06DA-PXIII associated with the SLS2.0 upgrade, as well as training myself in cryo-EM.

Changing practice in structural biology

Knowledge of the structure of large biological molecules, crucial as their function depends on their 3D shape, is addressed by three main techniques. While nuclear magnetic resonance (NMR) is restricted to small macromolecules (< 40 kDa) and cryo-EM thus far limited to low-resolution structures, MX has been the leading method for the structural investigation of large molecules over the past decades. Instrumental in the success of MX were the constant technological advances at synchrotron beamlines to the point that many structures are nowadays generated within minutes in a fully automated manner. However, cryo-EM has made revolutionary progress in recent years owing to tremendous developments in electron detectors and image processing. Without the need for crystals, cryo-EM single-particle analysis now provides high-resolution detail rather than molecular blobs and is growing as the method of choice for structure determination.

During my stay at KEK, I had the chance to practice and experience the power of cryo-EM by screening my samples at the TARA facility at Tsukuba University with help of Dr. Aramaki, at the SBRC cryo-EM facility with Dr. Adachi and Dr. Kawasaki, as well as by following Dr. Shinoda at the new cryo-EM facility in Hokkaido University. Seeing the donut shape structure of the small bacterial sliding clamp—my 81 kDa benchmark protein but also my very first crystallographic structure—on the electron micrographs was remarkable.

The end of the phase problem in crystallography?

The computation of electron density requires phases for the diffracted waves, but diffraction measurements only provide amplitudes—this is known as the "phase problem" in crystallography. With the growth of the PDB, molecular replacement (MR) was developed and became the predominant route to macromolecular structure determination. However, MR requires an accurate search model, so structures without homologs in the PDB were traditionally derived experimentally by finding the positions of heavy atoms that have been added or are intrinsically present in the macromolecules—the so-called experimental phasing method. At least, this was the situation before AlphaFold and the era of highly accurate structure prediction.

My colleagues at SLS and myself have developed experimental phasing at the beamline X06DA-PXIII over the past 10 years, with a particular interest in low energy. The method comes with its own challenges, especially for ways of dealing with absorption. How these have been addressed at X06DA-PXIII and BL-1A at the Photon Factory at KEK, headed by Dr. Matsugaki (Fig. 1) are very different, but triggered a long-term collaboration between the two teams.

Since 2012, we visited KEK on several occasions to evaluate the unique capabilities and incremental developments for low-energy experimental phasing at BL-1A, namely, 3.75 keV with helium atmosphere to reduce absorption, the mini-kappa goniometer for high-completeness measurements with true multiplicity, and the V-shape detector configuration to catch high-angle reflections. In addition, with the help of Dr. Hikita, we shaped crystals as spheres and cylinders using a deep-UV laser to reduce sample absorption. We later identified that the new PSI JUNGFRAU detector, initially developed for X-ray

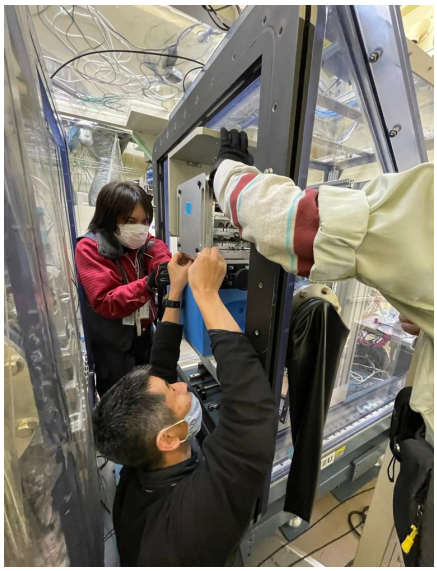

Figure 1 Dr. Matsugaki and his team adjusting the position of the PSI JUNGFRAU detector (BL-1A, Photon Factory, March 28, 2021)

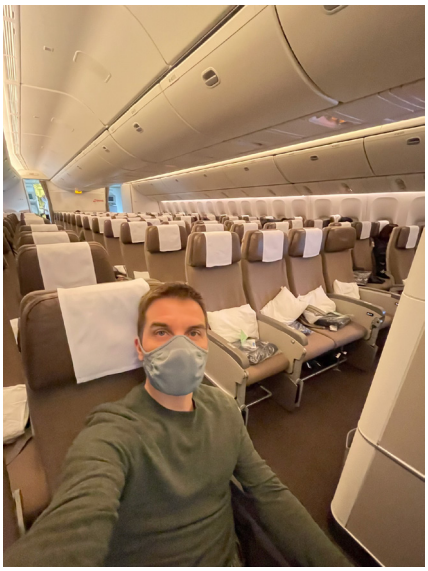

Figure 2 Traveling in times of COVID-19 (ZRH-NRT, November 21, 2020)

free-electron laser (XFEL) applications, performs well at low energy and we were excited to validate our finding at BL-1A in Spring 2020.

The COVID-19 pandemic forced us to revise our plan on shipping the JUNGFRAU to KEK. The conditions to enter Japan became more stringent and I also had to give up on my attempt to come to KEK in the summer of 2020 for cryo-EM experiments. Japan finally reopened its borders on October 1, 2020, but for long-term VISA holders only. In the meantime, Dr. Wang, head of the MX team at PSI, had fully supported my plan for a 1-year stay at KEK, and together with my Japanese colleagues, we started the VISA application process—a daunting experience in the time of COVID. Here I want to thank the dedicated administrative support from Sukegawa-san and Zeniya-san from SBRC, Arimoto-san, and Katsuki-san from the KEK international office, as well as Sugaya-san from the user office.

In November 2020, my colleague Dr. Leonarski shipped the JUNGFRAU detector, a 323 kg package worth 677,920 CHF of equipment. With negative COVID PCR tests, my colleague Dr. Tomizaki and I traveled to Japan on an empty airplane (Fig. 2). After 2 weeks' quarantine, we started our beamtime at BL-1A with remote support from Switzerland—online communication tools imposed by the pandemic are here to stay. Thanks to the generous commissioning beamtime, we kept the JUNGFRAU at BL-1A for an additional 6 months! Overall, the experiments were very successful as we could solve many crystal structures experimentally. In August 2021 however, we found out that all those structures could also be solved by MR with AlphaFold generated models—for us, the impact of structure predictions with AlphaFold was immediate.

Structural biology in the era of structure prediction

Computational methods are expected to play a major role now that structure predictions have reached the accuracy of experimentally determined models. They will ease solving structures, by facilitating at the front-end crystallization with the design of stable protein constructs, and at the back-end by helping MR and model building. But how long will researchers still attempt to determine structures experimentally, independent of the method used? These advances are going to transform structural biology and broader life science research similarly to the release of the human genome sequence 20 years ago. We are entering an era where every biologist is a structural biologist.

The future of MX is certainly worth thinking about at the time where most synchrotrons around the world upgrade to fourth-generation designs. Crystallography remains highly suited to yield precise atomic coordinates of macromolecules under a few hundred kDa in size and facilitate the development of new therapeutics. At both new synchrotron sources and XFELs, time-resolved methods will be used to capture high-resolution dynamic information along reaction pathways as a function of time, temperature, pressure, and other perturbations. Visualizing molecular movies of drugs in action will be one of the next challenges.

From the increased throughput of crystallographic structure determination with enhanced remote capabilities to the resolution revolution in cryo-EM, the advent of cryo-electron diffraction or the prospect of using cryo-electron tomography to study the structure of macromolecules in situ—realizing the Cell Atlas at the structural level—and lately, the development of AI tools that can predict structure with high accuracy, structural biology is undergoing radical changes at a fast pace!

My time in Japan has been both scientifically and personally very rewarding. Knowing that the Japanese borders only opened two months from March 2020, I feel extremely fortunate and privileged to have had the opportunity to spend 2021 in Tsukuba. I very much enjoyed living at the Ninomiya house from where I commuted to KEK by bike. While missing gatherings and other events with colleagues, the COVID-19 pandemic made me discover Japan with a unique perspective. I often felt as if I were the only foreigner in the country! I want to warmly thank all the people, both in Switzerland and in Japan, who helped me with this unforgettable experience!

References

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2. Jumper, J. *et al.* Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583–589 (2021).
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PF トピックス一覧（8 月～ 10 月）

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9. 22 【プレスリリース】放射光でついに見えた磁気オクタポール ～熱を電気に変える新たな担い手～
9. 27 【物構研トピックス】物構研ロゴ商標登録のお知らせ
9. 27 【トピックス】日本鉄鋼協会 2021 年澤村論文賞を総研大の原野貴幸氏、木村正雄教授らが受賞しました
9. 30 【プレスリリース】極めて安定な天然赤色色素を分解できるバクテリアを発見
10. 5 【プレスリリース】昆虫のさやばね内部に十字型の影をもつ球晶構造を発見
10. 6 【トピックス】KEK 一般公開特設ページに Q & A を追記しました
10. 7 【物構研トピックス】2021 年夏の研究系技術職員仕事体験をオンライン開催しました
10. 14 【物構研トピックス】第 9 回対称性・群論トレーニングコースをハイブリッド開催しました
10. 14 【物構研トピックス】KEK 公開講座「生命の謎を探る“ハイテクな顕微鏡たち”」を開催しました
10. 18 【プレスリリース】岡山県産鉱物「逸見石」が示す新奇な磁性 特徴的な結晶構造が量子力学的なゆらぎを生み出す
10. 27 【プレスリリース】金属が破壊する瞬間に出現する不思議な原子配列を発見
10. 28 【プレスリリース】岡新しい半導体物質「硫化ホウ素シート」の生成に成功