How to Boost Your Structure-Based Drug Design

Structural analysis of drug-target interactions is a valuable tool to optimize new drugs for efficiency and safety. For decades, the required level of detail could only be obtained through X-ray crystallography or NMR – unfortunately, methods not applicable to many relevant targets. Nobel-prize winning cryo-electron microscopy (cryo-EM) has emerged as an exciting alternative.

John Barker, SVP and Global Head of Protein Sciences, Structural Biology at Evotec

To obtain sufficient resolution in X-ray crystallography, proteins must be grown into suitable, well-ordered crystals – a challenging task, especially for membrane-bound proteins like G-protein coupled receptors (GPCRs), transporters or ion channels.

"More than 50% of our targets are membrane proteins, so the limitations of X-ray crystallography used to be a serious issue for us," explains John Barker, SVP and Global Head of Protein Sciences, Structural Biology at Evotec. *"Sometimes, we analyzed a protein because it crystallized better than others, even if it was not at the top of our list."*

Cryo-EM: the crystal-free technology

Cryo-EM could be a solution for difficult targets, because the technology does not need any protein crystals. Instead, homogeneous protein samples are flash-frozen on a grid, where resulting single particles are analyzed by electron microscopy at ultra-low temperatures under near-vacuum conditions.

In 2017, the pioneers of cryo-EM protein analysis, Jacques Dubochet, Joachim Frank and Richard Henderson, were

honored with the Nobel prize. And the success continues – only recently, structural information for hERG, an important neuron and cardiac potassium channel, was obtained, challenging existing models and providing new insight to observed toxicity issues.

From cryo-EM image to 3D structure

Statistically, proteins take on different orientations upon freezing, allowing binning of single particles into orientation categories, averaging them for analysis, and calculating a three-dimensional image of the protein structure.

With sufficient refinement, even different conformations of the same protein can be distinguished, such as open and closed states of ion channels. *"The insight we get on protein dynamics is exciting, as every piece of information opens the door to so many new questions,"* Barker says.

From "blobs" to atomic resolution

Interestingly, cryo-EM is not a new approach. It has been around since the 1990s, but was ill-reputed in the community because cryo-EM data were typically of low resolution. *"We used to call it 'blob-ology' because that's what proteins looked like in cryo-EM images,"* Barker recalls.

Only about ten years ago, radical improvements in detector hardware were made and combined with new software tools to process large amounts of data. And it didn't take long until a set of high-resolution structures, visualizing detailed protein features, were published.

After this tipping point, the technology quickly picked up speed. Dedicated tools for cryo-EM sample preparation emerged, including nanodisc scaffolds to stabilize membrane protein activity by maintaining surrounding lipids.

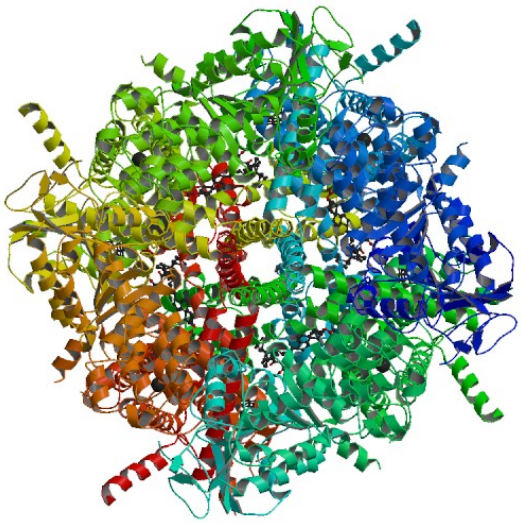
New cryo-EM centers

Electron microscopes at eBIC (source: Diamond Light Source)

Scientific institutes have geared up to meet the needs of the emerging cryo-EM community. Close to Evotec's UK site at Abingdon, a dedicated electron bio-imaging center (eBIC) has been set up at Diamond, the UK's national synchrotron science facility. It includes five of the latest-technology cryo-EM instruments and has one set aside for industry use only.

Right next door to Diamond, the Rosalind Franklin Institute was opened in 2017 to foster interdisciplinary research with impact on industry applications. A nice fit for Evotec: *"We are thrilled that groundbreaking new developments are made just down the road,"* Barker says.

Cryo-EM at Evotec



3.1A resolution structure of the Human TRPM4 ion channel in lipid nanodiscs in a calcium-bound state (Source: RCSB PDB Protein Bank)

Without the need for protein crystals, cryo-EM sample preparation is a lot faster and easier. *"We can spend months crystallizing a protein,"* Barker states. *"For cryo-EM, you still need to prepare a homogeneous sample, but we have the potential to cut the time needed for delivering structural information. Combining this structural information with powerful software tools such as AI will certainly accelerate drug discovery – helping get new drugs to the clinic faster."*

Still, Evotec expands their protein purification team, as the number of projects and collaborations is burgeoning. As Barker points out: "*There are so many new targets that we could not work on before. And the knowledge on protein crystallization sample prep that our scientists have accumulated over the years, nicely translates to cryo-EM applications*."

The holy grail?

So is cryo-EM the holy grail for protein structure analysis? As with every method, there are certain limitations. Because images of individual particles are noisy, tens or hundreds of thousands of them must be averaged to improve signals. This works better for larger particles, because they are easier to visualize.

Barker explains: *"We currently observe a detection limit of about 100 kDa, which is fine for ion channels and larger protein complexes – while unfortunately, GPCRs are only about 30-40 kDa in size. But a lot of them form functional dimers, and we know many groups are addressing this topic."*

Of course, one technology will not cover all applications. While sample preparation is faster with cryo-EM, the actual measurements take hours compared to just minutes for X-ray crystallography, making X-ray the ideal tool for high-throughput ligand screening.

New developments

Meanwhile, progress in the X-ray field also keeps moving – new high-energy X-ray free-electron lasers (XFEL) have been developed, enabling exciting applications such as dynamic snapshots of enzyme turnover in microcrystals. With the European XFEL facility in Hamburg – close to Evotec's headquarters – and the XFEL sample preparation hub at Diamond, the company is perfectly located to interact with the leading experts, and reap the benefits of this emerging technology.

"Eventually, I believe both methods will continue to co-exist and complement each other. And our teams are not at all biased – we always consult our clients on the best integrated and cross-disciplinary approach for their particular question," Barker concludes.

Discover how cryo-EM can speed up your drug discovery process by enabling structure-based drug design of breakthrough targets and novel applications.

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