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Scaffolds in Medicinal Chemistry

How cryoEM can improve the design of scaffolds in medicinal chemistry

Medicinal chemists can modify and manipulate the central common core of protein scaffolds (also known as 'privileged structures') to achieve new target specificities. A traditional drug discovery process relies on the observation of validated lead compounds on the physiological or pathological state of a test biological system. After initial Iterative synthesis and biological testing on protein targets, the later stage optimization of safety and efficacy is often the last hurdle to bringing therapeutics into the clinic (1, 2).

Modern genetics and high-throughput molecular biology approaches have identified an array of novel cellular targets, necessitating the discovery and design of new drug molecules to treat a myriad of diseases. In the initial stages of a modern drug discovery process, small molecule modulators are selected based on the level of activity in a suitable target assay. This selection may require no prior knowledge of the ligand or the target and is extrapolated from hit identification strategies such as biophysical or biochemical testing.

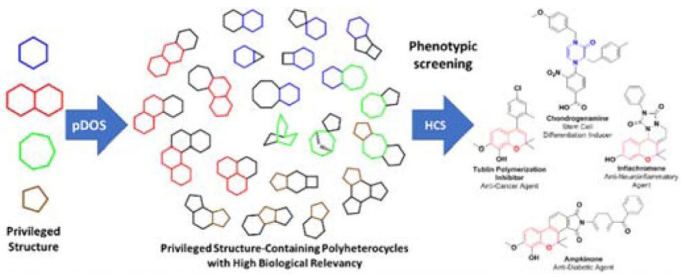

Figure 1. Privileged structure image (Kim, et al. 2014) https://pubs.acs.org/doi/10.1021/ja508343a. Further permissions related to the material excerpted should be directed to the ACS.

In other cases, direct visualization of the target and ligand interactions are critical for program progression. Structural enablement can be accomplished by nuclear magnetic resonance (NMR), X-ray crystallization and more recently cryogenic transmission electron microscopy (cryoTEM/cryoEM). This is often supported by follow-on structures of elaborated compounds or strategic mutagenesis (3, 4).

How cryoEM can improve the design of scaffolds.

CryoEM is a biophysical technique that relies on structure determination of non-crystalline frozen, hydrated biological macromolecules using high energy electrons. Until recently cryoEM was described as "blobology" due to resolution limitations that were not informative for structure-guided drug design. However, developments on several fronts including improved EM instrumentation, direct electron detectors, and advances in image processing software and algorithms have enabled structure determination of biological macromolecules to atomic or near atomic resolution.

CryoEM provides several advantages over other structural methods. Multiple proteins or protein complexes can be captured, with larger species (>500 kDa) often providing superior data for high-resolution determination.

In this whitepaper, we explore how modern approaches, such as cryoEM and related techniques, can be used to improve the design of scaffolds within medicinal chemistry.

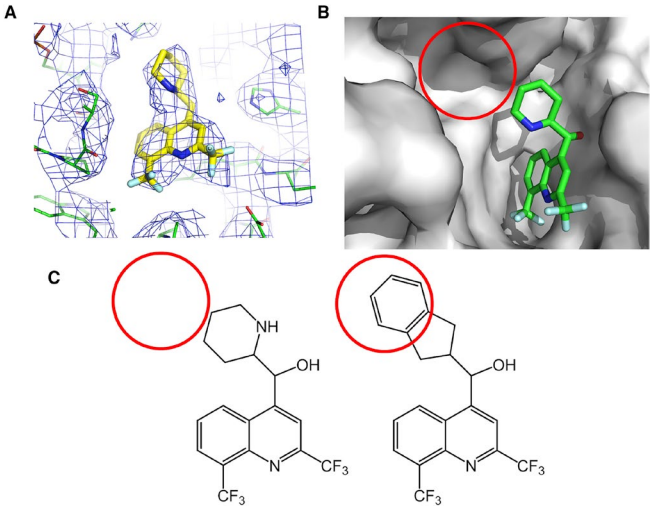

Figure 2. Using Cryo-EM Structures to Guide SBDD (9)

(A) Density for the antimalarial mefloquine as visualized in the 3.2 Å cryo-EM map (A and B) were generated with PyMOL, from map EMD-8,576 and PDB: 5UMD.

(B) The structure revealed an empty pocket within the ligand binding site (red circle).

(C) Left panel: the red circle identifies the position where mefloquine could be modified to extend into the pocket shown in (B). Right panel: the bicyclic piperidine replacement extends into the pocket identified by the red circle. This structure guided modification of mefloquine resulted in derivatives with a 2-fold potency enhancement towards the parasite (8).

The ability to study molecules in native-like lipid environments is particularly advantageous for notoriously difficult targets such as membrane-bound receptors, ion channels and enzymes.

Since proteins are vitrified in a solution state, multiple conformations of a target molecular machine can be captured and visualized from a single dataset. Additionally, unanticipated binding sites for physiological ligands or drug molecules can be captured even if they induce unforeseen conformational changes.

Since the method eliminates the need for 3D crystals of the target proteins, the timeline for cryoEM structure determination for liganded complexes is often shorter and more predictable compared to apo target proteins. More than half of the structures submitted in public repositories such as PDB in 2020 were better than 3.5Å resolution and the number of structures at better than 2.5Å resolution has been rapidly increasing. Importantly, due to the often specific and potent interactions between drug-like leads and their target proteins, the local resolution around the small molecule interaction interface is often higher than that reported for the global resolution of the map, providing the most valuable information to the medicinal chemistry team at the highest level of structural detail.

New possibilities for early-stage drug discovery

CryoEM can provide critical information at various stages of a structure-guided drug discovery project. At early stages, even low resolution (4.0-8.0Å) cryoEM maps can provide critical insights into the target activation and modulation and conformational changes resulting from protein-ligand or protein-protein interactions.

At resolution between 4.0-5.0Å cryoEM maps are sufficiently high quality to be used for in silico screening and docking to identify preferred pharmacophores and essential interactions that can be integrated with other rational drug design strategies. Coupled with appropriate activity assays and receptor pharmacology, low resolution cryoEM structures are vital for discovery of initial hits.

The limits in resolution are often inherent in the samples due to conformational heterogeneity in solution. However, this challenge can often be overcome by collecting larger data sets, thereby increasing the number of particles belonging to each conformational class. CryoEM structures between 3.5-4.0Å resolution can have well defined density for drugs and bulkier groups of a ligand. Aromatic rings can be useful as an anchor for modeling the correct orientation of the small molecule.

New possibilities for targeting molecular complexes

CryoEM structures with near atomic resolution are becoming routine thanks to continuous improvements and developments in image processing software, together with the introduction of new algorithms to mitigate the issues of conformational heterogeneity. The power of cryoEM for structure-guided drug discovery is manifested in several recent studies where unknown structures of important targets were solved, opening new possibilities of targeting some of these molecular complexes in diseases (5).

Seminal work has come from the laboratory of Yifan Cheng (6), delivering the high-resolution structure of the TRPV1 channel with two known drugs (5). The Scheres lab (7) has resolved the high-resolution structure of the Tau filament. In the later work, the cryoEM structure of Tau filament from an Alzheimer's patient's brain revealed the molecular code for the protein aggregation that, in future, may be targeted by small molecule inhibitors.

Similarly, Wong et. al showed that bicyclic piperidine extension of the antimalarial drug mefloquine fills the empty cavity in a drug-binding site on the plasmodium falciparum 80s ribosome. At a moderate resolution of at 3.2Å, this structure-guided modified inhibitor shows improved parasiticidal effects (8) For more seminal work, refer to (9, 10, 11, 12).

Summary

The “resolution revolution” in CryoEM has resulted in an explosion of structures of biological macromolecules of both academic and drug industry interests. Many protein complexes and protein-ligand structures have been solved with atomic resolution details providing new insights into the mechanistic understanding of the biological roles and disease relevance of these molecular machines. CryoEM is taking a new leap and many pharmaceutical companies are investing in cryoEM for drug discovery. With continued developments on various fronts, particularly high-throughput capabilities for sample preparation and use of AI for grid screening and data collection, cryoEM is becoming an integrated part of many structure-guided drug discovery projects in the near future.

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References:

(1) Barreiro, Eliezer J. 2015. “Chapter 1 Privileged Scaffolds in Medicinal Chemistry: An Introduction,” 1–15.

(2) Duarte, Carolina D., Eliezer J. Barreiro, and Carlos A. M. Fraga. 2007. “Privileged Structures: A Useful Concept for the Rational Design of New Lead Drug Candidates.” Mini Reviews in Medicinal Chemistry 7 (11): 1108–19.

(3) Renaud, Jean-Paul, Ashwin Chari, Claudio Ciferri, Wen-Ti Liu, Hervé-William Rémigy, Holger Stark, and Christian Wiesmann. 2018. “Cryo-EM in Drug Discovery: Achievements, Limitations and Prospects.” Nature Reviews. Drug Discovery 17 (7): 471–92.

(4) Johnson, Rachel M., Anna J. Higgins, and Stephen P. Muench. 2019. “Emerging Role of Electron Microscopy in Drug Discovery.” Trends in Biochemical Sciences 44 (10): 897–98.

(5) Liao, Maofu, Erhu Cao, David Julius, and Yifan Cheng. 2013. “Structure of the TRPV1 Ion Channel Determined by Electron Cryo-Microscopy.” Nature 504 (7478): 107–12.

(6) Cheng, Yifan. 2018. “Membrane Protein Structural Biology in the Era of Single Particle Cryo-EM.” Current Opinion in Structural Biology 52 (October): 58–63.

(7) Fitzpatrick, Anthony W. P., Benjamin Falcon, Shaoda He, Alexey G. Murzin, Garib Murshudov, Holly J. Garringer, R. Anthony Crowther, Bernardino Ghetti, Michel Goedert, and Sjors H. W. Scheres. 2017. “Cryo-EM Structures of Tau Filaments from Alzheimer’s Disease.” Nature 547 (7662): 185–90.

(8) Wong, Wilson, Xiao-Chen Bai, Brad E. Sleebs, Tony Triglia, Alan Brown, Jennifer K. Thompson, Katherine E. Jackson, et al. 2017. “Mefloquine Targets the Plasmodium Falciparum 80S Ribosome to Inhibit Protein Synthesis.” Nature Microbiology 2 (March): 17031.

(9) Reprinted from Cell Chem Biol. 2018 Nov 15;25(11):1318-1325. Scapin G, Potter CS, Carragher B. Cryo-EM for Small Molecules Discovery, Design, Understanding, and Application, with permission from Elsevier.

(10) Scapin G, Dandey VP, Zhang Z, Prosise W, Hruza A, Kelly T, Mayhood T, Strickland C, Potter CS, Carragher B. Structure of the insulin receptor-insulin complex by single-particle cryo-EM analysis. Nature. 2018 Apr 5;556(7699):122-125. doi: 10.1038/nature26153. Epub 2018 Feb 28. PMID: 29512653; PMCID: PMC5886813.

(11) Tan YZ, Carragher B. Seeing Atoms: Single-Particle Cryo-EM Breaks the Atomic Barrier. Mol Cell. 2020 Dec 17;80(6):938-939. doi: 10.1016/j.molcel.2020.11.043. PMID: 33338409.

(12) Kim J, Tan YZ, Wicht KJ, Erramilli SK, Dhingra SK, Okombo J, Vendome J, Hagenah LM, Giacometti SI, Warren AL, Nosol K, Roepe PD, Potter CS, Carragher B, Kossiakoff AA, Quick M, Fidock DA, Mancia F. Structure and drug resistance of the Plasmodium falciparum transporter PfCRT. Nature. 2019 Dec;576(7786):315-320. doi: 10.1038/s41586-019-1795-x. Epub 2019 Nov 27. PMID: 31776516; PMCID: PMC6911266.

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