Advances in cryo-electron microscopy (cryoEM) and X-Ray crystallography for structure-based drug discovery

P. Rubach[1,2], A. Wlodawer[3], W. Minor[2]

[1]Institute of Information Systems and Digital Economy, Warsaw School of Economics, Warsaw, Poland, [2]Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, Virginia, USA,[3]Center for Structural Biology, National Cancer Institute, Frederick, MD 21702, USA

pawel.rubach@sgh.waw.pl

Macromolecular X-ray crystallography (XRC), nuclear magnetic resonance (NMR), and cryo-electron microscopy (cryoEM) are the primary techniques for determining atomic-level, three-dimensional structures of macromolecules essential for drug discovery. With advancements in artificial intelligence (AI) and cryoEM, the Protein Data Bank (PDB) [1] is solidifying its role as a key resource for 3D macromolecular structures. This position underscores the growing need for enhanced quality metrics and robust validation standards for all experimental structures. This presentation examines recent advancements in cryoEM [2] and X-ray crystallography [3], analyzing structure quality metrics (i.a. see Fig.1: Q-score and CC_{MASK} vs. Resolution for cryoEM structures), resolution improvements, ligand and water molecule identification, refinement software, and the identification of duplicate submissions that are not necessarily duplicates [4]. Nearly 800 protein crystal structures determined at a resolution of 2.5Å or better are present in the current PDB release without any water molecules, while some other depositions exhibit unusually low or high solvent occupancies [5]. This study also includes an in-depth analysis of crystal structures' geometrical and quality parameters, such as: R, R-free, clashscore, rotamer and Ramachandran outliers as well as the PQ1 measure performed on a dataset of medicinally important cAMP-dependent protein kinases. In particular, we will show how the different metrics unevenly change when these structures are re-refined by PDB-REDO [6]. Finally, we will discuss the role of both X-ray crystallography and cryoEM techniques in drug discovery in light of two significant findings: the demonstration that cryoEM can resolve hydrogen atom positions and water networks [7], and the report of an atomic-resolution (1.09Å) protein structure (8RQB) that reveals double conformations [8].

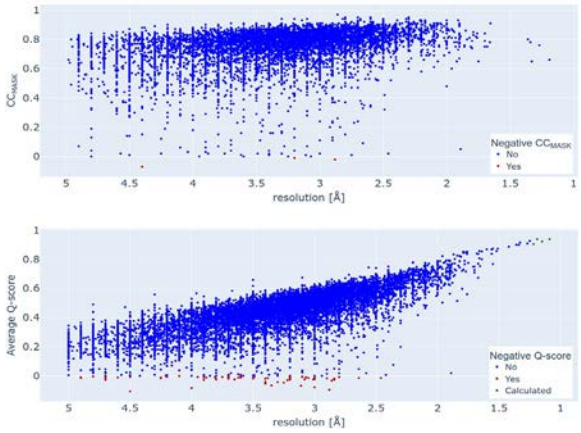

Figure 1. Q-score and CC_{MASK} vs. Resolution for cryoEM structures.

[1] S. K. Burley et al., Protein Sci., vol. 31, no. 1, pp. 187–208, 2022, doi: 10.1002/pro.4213.

[2] P. Rubach, K. A. Majorek, M. Gucwa, K. Murzyn, A. Wlodawer, and W. Minor, Expert Opin. Drug Discov., vol. 0, no. 0, pp. 1–14, doi: 10.1080/17460441.2025.2450636.

[3] V. Bijak et al., Expert Opin. Drug Discov., vol. 18, no. 11, pp. 1221–1230, Nov. 2023, doi: 10.1080/17460441.2023.2246881.

[4] A. Wlodawer et al., Acta Crystallogr. Sect. Struct. Biol., vol. 81, no. 4, Art. no. 4, Apr. 2025, doi: 10.1107/S2059798325001883.

[5] A. Wlodawer et al., IUCrJ, vol. 11, no. 6, Art. no. 6, Nov. 2024, doi: 10.1107/S2052252524009928.

[6] R. P. Joosten, F. Long, G. N. Murshudov, and A. Perrakis, IUCrJ, vol. 1, no. Pt 4, pp. 213–220, Jul. 2014, doi: 10.1107/S2052252514009324.

[7] R. Hussein et al., Science, vol. 384, no. 6702, pp. 1349–1355, Jun. 2024, doi: 10.1126/science.adn6541.

[8] B. Küçükoğlu et al Nat. Commun., vol. 15, no. 1, p. 8062, Sep. 2024, doi: 10.1038/s41467-024-52403-5.[1] S. K. Burley *et al.*, *Protein Sci.*, vol. 31, no. 1, pp. 187–208, 2022, doi: 10.1002/pro.4213.