Cryo-EM Versus X-Ray Crystallography in Resolving Allosteric Conformational States of Multidomain ABC Transporters: A Structural and Mechanistic Benchmarking Study

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Abstract. Multidomain ATP-binding cassette (ABC) transporters adopt distinct allosteric conformational ensembles critical to substrate translocation and drug resistance mechanisms. Despite substantial progress, persistent ambiguities remain regarding which structural methodology—cryo-electron microscopy (cryo-EM) or synchrotron-based X-ray crystallography—more faithfully captures physiologically relevant intermediate states. Here, we systematically benchmarked both approaches against five therapeutically significant ABC transporter homologs, combining molecular dynamics simulations, hydrogen-deuterium exchange mass spectrometry (HDX-MS), and cross-validation with small-angle X-ray scattering (SAXS) datasets. Cryo-EM consistently resolved occluded-intermediate conformations at sub-3-Å resolution, whereas X-ray crystallography introduced lattice-packing artifacts that distorted nucleotide-binding domain (NBD) dimerization interfaces. These findings redefine quality metrics for ABC transporter structural determination and have direct implications for structure-based drug design targeting multidrug resistance efflux pumps.

Keywords: ABC transporters, cryo-electron microscopy, X-ray crystallography, allosteric conformational states, nucleotide-binding domain dimerization, hydrogen-deuterium exchange mass

spectrometry, multidrug resistance efflux pumps, molecular dynamics simulation, small-angle X-ray scattering

Introduction

ATP-binding cassette (ABC) transporters constitute one of the largest and most evolutionarily conserved superfamilies of integral membrane proteins, orchestrating the ATP-driven translocation of chemically diverse substrates across biological membranes in all kingdoms of life. In the context of human pathophysiology, their dysregulation underpins a spectrum of clinically urgent conditions, including cystic fibrosis (CFTR/ABCC7 dysfunction), adrenoleukodystrophy (ABCD1 mutations), and, most consequentially for oncology and infectious disease medicine, the overexpression of efflux pumps such as P-glycoprotein (ABCB1), ABCG2, and MRP1 that confer broad-spectrum multidrug resistance (MDR) to cancer cells and bacterial pathogens alike. As of 2025, MDR phenotypes are implicated in treatment failure in over 50% of relapsed malignancies and represent a leading mechanistic contributor to the global antimicrobial resistance (AMR) crisis—a burden estimated by the WHO to exceed 10 million annual deaths by 2050 if left unaddressed. Structurally, ABC transporters operate through a canonical alternating-access mechanism in which conformational cycling between outward-facing, occluded-intermediate, and inward-facing states is tightly coupled to ATP binding and hydrolysis at the nucleotide-binding domains (NBDs). The precise geometry of these transitions—particularly the transient occluded-intermediate state—constitutes the mechanistic crux of substrate selectivity, and is therefore the primary determinant of rational drug target identification. However, the structural biology community has long grappled with a fundamental methodological dichotomy: X-ray crystallography, the historical gold standard for atomic-resolution structure determination, imposes crystalline lattice constraints that may sterically bias or altogether preclude the capture of dynamic intermediate conformations; whereas cryo-electron microscopy, whose methodological maturation was recognized by the 2017 Nobel Prize in Chemistry and which has since achieved routine sub-2-Å resolutions, operates on vitrified specimens in near-native lipid or detergent environments, theoretically permitting unbiased sampling of conformational heterogeneity through computational particle classification. Despite numerous landmark structures deposited across both modalities in the Protein Data Bank (PDB)—now exceeding 220,000 total entries as of early 2025—no rigorous, experimentally cross-validated benchmarking study has directly adjudicated the relative fidelity of each technique in resolving the full allosteric conformational landscape of a structurally diverse cohort of ABC transporter homologs under matched biochemical conditions. This gap constitutes a significant impediment to reproducibility in structure-based drug discovery pipelines. The present study addresses this deficit through a systematic, multi-technique benchmarking framework. We first describe the selection criteria and biochemical preparation of five representative ABC transporter homologs spanning bacterial, archaeal, and human origins. We then detail the parallel cryo-EM and X-ray crystallographic data

collection and refinement pipelines, followed by cross-validation using orthogonal solution-state methods including HDX-MS and SAXS. Subsequent sections present quantitative comparison of conformational state distributions, NBD dimerization interface geometries, and ligand-binding pocket volumes, culminating in a discussion of methodology-dependent bias and its downstream impact on pharmacophore modeling.

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