



Session II, May 25, Monday

L6

THE STRUCTURAL BASIS OF MOLECULAR FUNCTION: EXPERIMENTAL STUDIES IN DISEASE, REGULATION, AND CATALYSIS

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This study investigates the structural basis of protein function across diverse biological systems, with a focus on how atomic-level architecture governs regulation, catalysis, and disease mechanisms. Combining X-ray crystallography with biochemical and computational approaches, it integrates structural and functional data to elucidate the molecular determinants of protein activity.

The lecture is organized around three main themes. First, it presents a structural-functional analysis of germline missense variants in the Ras-MAPK signaling pathway associated with neurodevelopmental disorders, showing that these variants induce subtle, mechanistically distinct effects compared to oncogenic mutations. Second, it examines enzymes of the α -hydrolase fold, including

haloacid dehalogenase phosphatases and haloalkane dehalogenases, revealing how active-site architecture and access tunnel dynamics shape substrate specificity and catalytic efficiency. Third, it characterizes parasite-derived protease inhibitors from the serpin and cystatin families, uncovering unique structural adaptations.

Overall, it highlights the importance of integrative structural biology in linking molecular structure to biological function.

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REPROGRAMMING HOST UBIQUITINATION: PROTAC-INDUCED DEGRADATION OF ENTEROVIRAL 2C HELICASE

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Enteroviruses (EV), including EV-A71, CV-B3, poliovirus, and EV-D68, are significant human pathogens associated with neurological and respiratory disease, including acute flaccid myelitis and severe respiratory illness [1-3]. Despite their clinical impact, targeted antiviral therapies remain lacking [1].

The nonstructural protein 2C is considered a high-priority antiviral target because it is highly conserved across enteroviruses and is functionally essential [1, 4]. As a superfamily 3 helicase and AAA+ ATPase, 2C contributes to RNA remodeling, viral RNA synthesis, replication organelle formation, membrane rearrangement, genome replication, and encapsidation [5-7]. Mutational analyses show that disrupting 2C function severely impairs viral RNA replication and virion production [5].

Despite its attractiveness as a target, conventional inhibition of 2C is challenging because the protein forms dy-

namic oligomeric assemblies and is often inhibited through allosteric pockets rather than the catalytic site [6, 8]. Existing inhibitors, including fluoxetine and dibucaine, act allosterically by stabilizing an inactive hexameric state [8, 9]. Emerging compounds such as 12b and SJW-2C-227 target conserved 2C pockets and show promising broad-spectrum antiviral activity. Notably, resistance mutations often reduce viral fitness, highlighting the evolutionary constraints on 2C function [10].

To overcome the limitations of classical inhibition, we propose a targeted protein degradation strategy based on proteolysis-targeting chimeras (PROTACs) that recruit the cereblon E3 ubiquitin ligase to induce degradation of 2C. This is a rationale rather than a published 2C-specific result, but the broader antiviral concept is supported by CRBN-recruiting degraders that eliminate viral proteins and can reduce susceptibility to resistance mutations. This

approach could, in principle, enable complete removal of the target protein and may reduce resistance development.

Structure-guided design is supported by X-ray crystallography and cryo-EM studies that define ligandable sites and oligomeric assemblies of 2C [8].

This work establishes a platform for degrader-based antivirals targeting conserved viral proteins, with potential broad-spectrum applicability against enteroviruses.

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DIFFRACTION IMAGES OF OLIVE FLOUNDER INTERFERON GAMMA: MULTI-PURPOSE DATASET FOR TEACHING CRYSTALLOGRAPHY

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Interferon gamma (IFN γ) is an important molecule for the immune system of vertebrates. The Protein Data Bank contains structures of IFN γ from numerous species. We crystallized and collected the diffraction images of IFN γ from the olive flounder - *Paralichthys olivaceus*. The raw diffraction images of native and Selenourea soak of PoIFN γ have been deposited in the Zenodo public repository (native – <https://doi.org/10.5281/zenodo.3369717>, Selenourea soak – <https://doi.org/10.5281/zenodo.19218271>). Initially, we estimated the diffraction limit at 2.3 Å. We solved the phase problem using the single-wavelength anomalous dispersion method and deposited the structure to the PDB under the accession code 6F1E [1].

The manuscript and data is great for teaching multiple stages of structure determination including the practical approaches. First of all, the Materials and Methods contains a

protocol for production of crystals for experimental phasing. In addition, the diffraction images are available for re-production of data reduction and further analysis. The diffraction anisotropy can be treated using modern approaches and result in better data. The structure of PoIFN γ is different from the predicted models or from models of homologous proteins known by the year of its publication (2018). The experimental phases may serve for both phase problem solution and final model validation. The dataset is already used in tutorial for teaching standard paired refinement. Even paired refinement considering diffraction anisotropy can be applied, although this process has not been implemented to any computational crystallographic pipeline, yet.

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