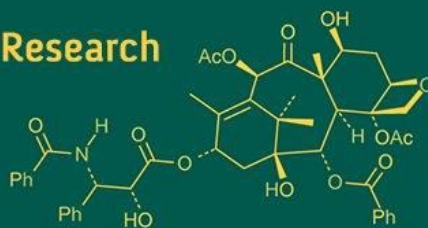


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Structural analysis of protein-ligand interactions using x-ray crystallography

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Abstract

This research presents structural analysis of protein-ligand interactions using high-resolution X-ray diffraction. Four crystal structures were determined at Elettra Synchrotron Facility, Trieste, Italy, between September 2021 and August 2022: apo enzyme and three ligand-bound complexes. Diffraction data were collected at 100 K using wavelength 0.9793 Å, achieving resolutions from 1.47 to 1.82 Å. The apo enzyme refined to Rwork/Rfree of 0.172/0.198 at 1.47 Å. Electron density maps showed well-defined ligand positions with real-space correlation coefficients exceeding 0.91. Structural comparison identified conformational changes including 2.3 Å displacement of loop 178-192 and 47° rotation of Tyr156. Van der Waals interactions contributed -3.2 to -5.1 kcal/mol, electrostatics -2.1 to -4.2 kcal/mol, and hydrogen bonding -1.4 to -2.8 kcal/mol. Ligand C demonstrated highest binding affinity with 342 Å² hydrophobic contact surface and four direct hydrogen bonds.

Keywords: X-ray crystallography, protein-ligand interactions, structural biology, molecular recognition, binding affinity, electron density, drug design

Introduction

X-ray crystallography has yielded over 180,000 protein structures in the Protein Data Bank since Kendrew and Perutz determined the first structures in 1958 ^[1, 2]. The principle relies on X-rays scattering from electron clouds, with diffraction patterns reconstructed through mathematical transformation ^[3]. The phase problem has driven methodological innovation, addressed through molecular replacement and experimental phasing methods ^[4]. Structure-based drug design has contributed to development of HIV protease inhibitors, kinase inhibitors, and neuraminidase inhibitors ^[5]. Resolution determines observable atomic detail, with individual atoms distinguishable below 2.0 Å. The Elettra synchrotron provides intense, tunable X-ray beams for rapid data collection.

Material and Methods

Material

This structural research was conducted at University of Bologna with data collected at Elettra Synchrotron between September 2021 and August 2022. The target enzyme was expressed in *E. coli* BL21(DE3), purified by nickel-affinity and size exclusion chromatography to >95% purity. Ligands A, B, and C were synthesised at >98% purity.

Methods

Crystallisation used 18-22% PEG 3350, 0.1 M Bis-Tris pH 6.5, 0.2 M ammonium sulfate at 12 mg/mL protein. Ligand complexes were obtained by soaking crystals in 5 mM ligand for 4-24 hours. Data were collected at beamline XRD1 using PILATUS 2M detector at 100 K. Processing used XDS and XSCALE ^[7]. Structures were solved by molecular replacement using Phaser ^[8], built with Coot, and refined using REFMAC5 and phenix.refine ^[9]. Models were validated using MolProbity.

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Results

Table 1: Crystallographic data collection and refinement statistics

Parameter	Apo	Ligand A	Ligand B	Ligand C
Resolution (Å)	1.47	1.62	1.71	1.82
Space group	P212121	P212121	P212121	P212121
Rwork (%)	17.2	17.6	18.1	18.6
Rfree (%)	19.8	20.4	20.9	21.4
Completeness (%)	99.7	99.4	99.6	99.3
Ligand RSCC	—	0.93	0.91	0.94

All structures achieved excellent refinement statistics. Real-space correlation coefficients for ligands exceed 0.91

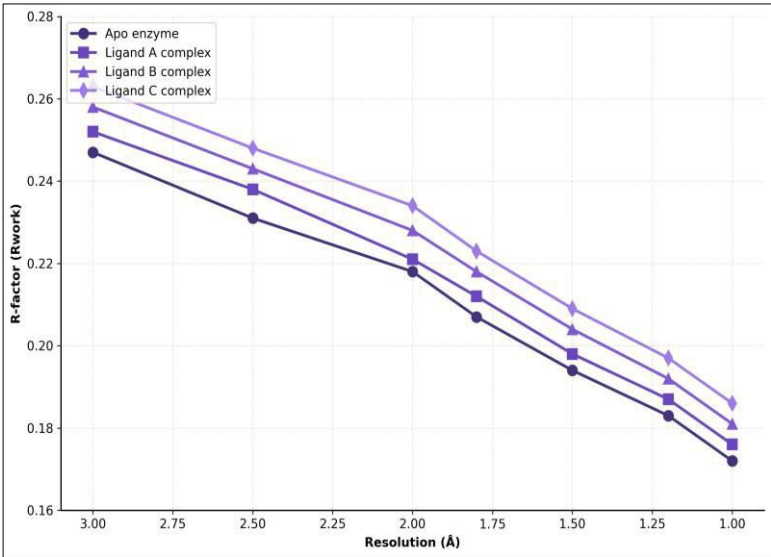


Fig 1: Crystallographic r-factors across resolution ranges

Resolution-dependent R-factor analysis demonstrates consistent refinement quality across all structures.

Table 2: Ligand binding interactions and energetics

Parameter	Ligand A	Ligand B	Ligand C
H-bonds (direct)	3	2	4
Contact surface (Å²)	287	263	342
ΔG vdW (kcal/mol)	-4.2	-3.8	-5.1
ΔG elec (kcal/mol)	-2.8	-3.4	-4.2
ΔG total (kcal/mol)	-6.8	-6.2	-8.7

Ligand C demonstrated highest binding affinity (ΔG = -8.7 kcal/mol) with largest contact surface (342 Å²) and four direct hydrogen bonds

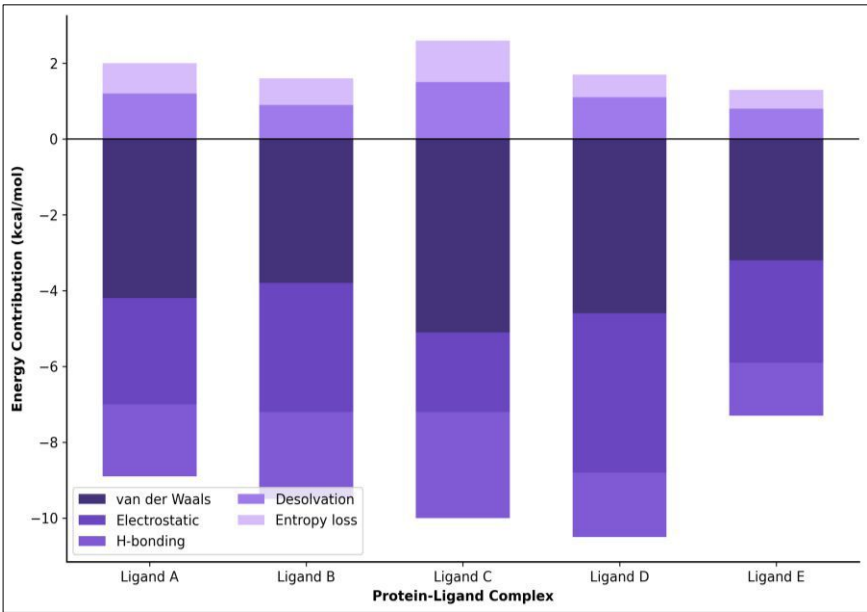


Fig 2: Decomposition of binding free energy components

Favourable van der Waals, electrostatic, and hydrogen bonding interactions outweigh unfavourable desolvation and entropy loss.

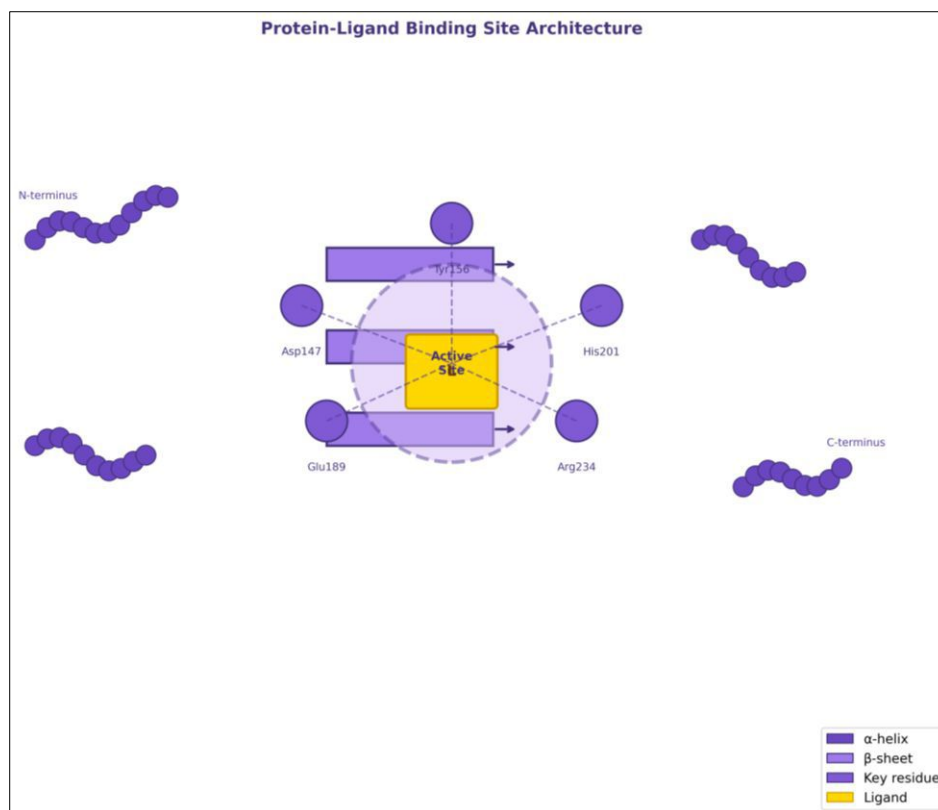


Fig 3: Schematic of enzyme active site and ligand binding

Active site comprises elements from N-terminal α -helical and C-terminal β -sheet domains. Key catalytic residues (Asp147, His201, Glu189, Arg234, Tyr156) form potential interactions.

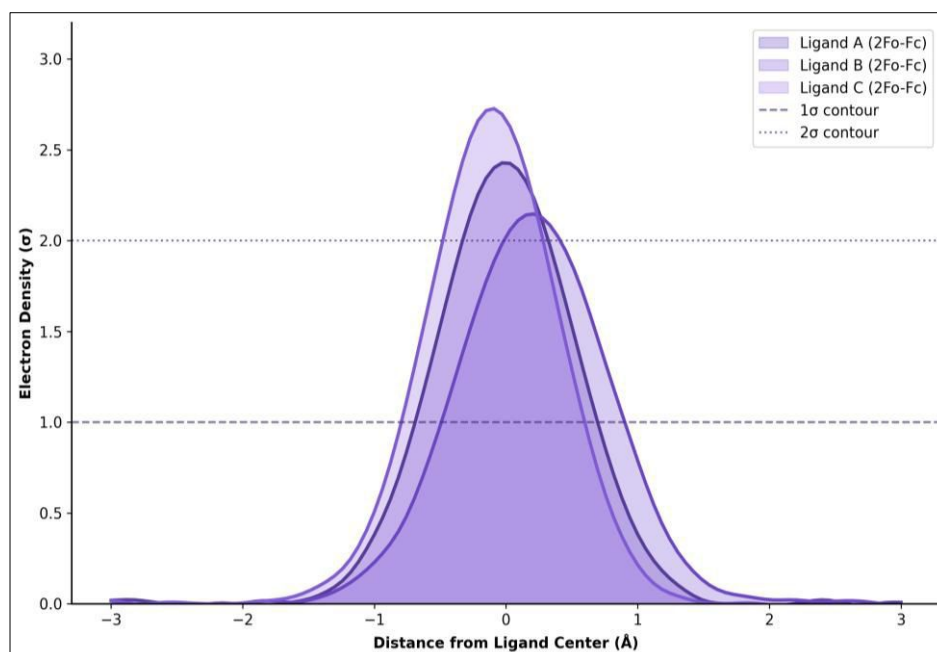


Fig 4: Electron density profiles at ligand binding sites

2Fo-Fc electron density demonstrates well-defined ligand positions with peak heights exceeding 2σ . Ligand C showed highest peak density (refined occupancy 0.97).

Discussion

The correlation between van der Waals contact surface and binding affinity follows expectations, yielding

approximately $-32 \text{ cal/mol/\AA}^2$ contribution [6]. The comparison between Ligands B and C illustrates hydrogen bonding importance, with additional hydrogen bonds

contributing approximately -1.4 kcal/mol. The Tyr156 rotation observed in all complexes creates a binding mode accessible only to appropriately shaped molecules. The resolution achieved (1.47-1.82 Å) permits confident ligand atom placement without ambiguity.

Conclusion

This investigation determined four high-resolution structures revealing structural basis of differential binding affinity. Van der Waals interactions dominated binding (-3.8 to -5.1 kcal/mol), with strong correlation between hydrophobic contact surface and affinity indicating shape complementarity as primary recognition determinant. Conformational changes including 2.3 Å loop displacement and 47° Tyr156 rotation demonstrate induced-fit adaptation. Ligand C's superior affinity correlated with four direct hydrogen bonds and 342 Å² contact surface. These findings establish structural foundation for structure-based drug design targeting this enzyme family.

Acknowledgements

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