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MUTATION-INDUCED ALTERATIONS IN LIGAND BINDING OF CARBOXYPEPTIDASE A1: A COMPARATIVE MOLECULAR DOCKING STUDY IN PANCREATITIS

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ABSTRACT

Pancreatitis is a complex inflammatory disorder in which genetic variations in digestive enzymes can influence protein stability and interaction behavior.[1–4] Carboxypeptidase A1 (CPA1), a zinc-dependent metalloprotease involved in protein digestion, has been associated with mutation-driven structural alterations in pancreatic disease.[5–8] The present study aimed to evaluate the structural impact of a Ser34→Ala substitution in CPA1 through comparative molecular docking analysis. The wild-type and mutant protein structures were prepared and docked with phenylalanine, paracetamol, and ibuprofen using Auto Dock Vina.[12] Binding affinities and zinc–ligand distances were analyzed to assess mutation-induced changes in interaction patterns. Docking results revealed modest, ligand-dependent variations in binding affinity between the two protein forms. Phenylalanine exhibited slightly stronger predicted binding to the mutant structure, whereas paracetamol showed nearly identical affinities in both forms. Ibuprofen demonstrated the highest overall binding affinity but displayed a marginally stronger interaction with the wild-type protein. In all cases, Zn–O distances in the best-ranked docking poses exceeded 5 Å, indicating the absence of direct zinc coordination and suggesting peripheral binding within the catalytic cavity.[5,21]

These findings suggest that the Ser34→Ala substitution induces subtle modifications in the ligand-binding environment without significantly disrupting the zinc-centered catalytic architecture of CPA1.[5,7] The study highlights the importance of mutation-aware structural evaluation in understanding enzyme–ligand interactions and provides a computational foundation for further experimental investigation in pancreatitis-related protein variants.

KEYWORDS: Pancreatitis, Carboxypeptidase A1, Molecular docking, AutoDock Vina, Mutation analysis, Drug–protein interaction.

1. INTRODUCTION

1.1 Pancreatitis

Pancreatitis is an inflammatory disease of the pancreas characterized by autodigestion of pancreatic tissue due to premature activation of digestive enzymes within pancreatic acinar cells.[1–3] The condition may present as acute or chronic pancreatitis and is associated with significant morbidity and mortality.[1,2] Common etiological factors include gallstones, alcohol abuse, metabolic abnormalities and genetic predisposition.[2–4] At the molecular level, pancreatitis is strongly linked to dysregulation of pancreatic digestive enzymes and their activation pathways.[4,13–17]

Under normal physiological conditions, pancreatic enzymes are synthesized as inactive zymogens and are activated only after reaching the intestinal lumen.[13,16] Disruption of this tightly regulated process, particularly due to genetic mutations, can result in intracellular enzyme activation, leading to inflammation, cellular stress and tissue damage.[4,10,18–20]

1.2 Role of Pancreatic Enzymes and CPA1 in Pancreatitis

The pancreas plays a vital role in digestion through the regulated secretion of a diverse set of digestive enzymes, including proteases, lipases and amylases.[30,32,33] Under normal physiological conditions, these enzymes are synthesized as inactive precursors (zymogens) and are activated only after reaching the intestinal lumen, thereby protecting pancreatic tissue from autodigestion.[13,30] Pancreatitis arises when this tightly controlled process is disrupted, leading to premature enzyme activation, cellular injury, inflammation and progressive pancreatic damage.[1–4,13]

Several pancreatic enzymes have been implicated in the pathogenesis of pancreatitis. Trypsinogen, encoded by the *PRSS1* gene, has historically been considered a central player,

as its premature conversion to trypsin can initiate a cascade of digestive enzyme activation within the pancreas.[4,13] Mutations in *PRSS1* often result in increased trypsin activity or resistance to degradation, directly contributing to pancreatic injury.[4,13] Similarly, chymotrypsinogen (CTRB), elastase (CEL) and pancreatic lipase have been associated with pancreatitis through mechanisms involving enzymatic imbalance, altered secretion or impaired degradation.[14–16] However, these enzymes primarily contribute to disease through excessive or dysregulated catalytic activity.[14–16]

In contrast, carboxypeptidase A1 (CPA1) represents a distinct and increasingly important mechanistic pathway in pancreatitis.[8–11,17] CPA1 is a zinc-dependent exopeptidase responsible for cleaving C-terminal aromatic and aliphatic amino acids from peptide substrates during the final stages of protein digestion.[5,6,21,30] Like other pancreatic proteases, CPA1 is synthesized as an inactive proenzyme and is physiologically activated outside the pancreas.[30,32] Notably, recent genetic and molecular studies have identified CPA1 mutations as strong risk factors in hereditary, idiopathic and early-onset pancreatitis.[8–11]

What distinguishes CPA1 from many other pancreatic enzymes is that disease association is not primarily driven by increased enzymatic activity. Instead, pathogenic CPA1 variants are frequently linked to protein misfolding, reduced structural stability, impaired intracellular trafficking and abnormal molecular interactions.[8,11,18] These defects can induce endoplasmic reticulum stress, trigger unfolded protein responses and ultimately lead to pancreatic acinar cell injury, even in the absence of excessive proteolytic activity.[18–20] Importantly, even single-point mutations in CPA1 have been shown to produce significant conformational alterations that affect ligand binding and local active-site architecture.[8,11]

This unique mechanism makes CPA1 an especially suitable target for structure-based computational investigations.[12,24,25] Studying ligand interactions with both wild-type and mutant CPA1 provides valuable insight into how subtle structural perturbations influence binding behavior without necessarily abolishing enzymatic function.[12,28] Consequently, CPA1 serves as an ideal molecular model for understanding mutation-induced changes at the protein–ligand interface in pancreatitis, justifying its selection for the present docking-based comparative analysis.[8,10,12]

1.3 Rationale for Selecting CPA1

The selection of Carboxypeptidase A1 (CPA1) for the present study was guided by its suitability as a structural and computational model rather than by general disease background alone. One of the primary reasons for choosing CPA1 is its consistent identification as a high-risk genetic factor in multiple forms of pancreatitis, making it a clinically relevant target for structure-based investigations.[8–11,17]

CPA1 contains a well-defined, zinc-coordinated catalytic pocket with clearly resolved active-site residues.[5–7,21] This structural clarity is particularly advantageous for molecular docking studies, as it allows precise grid placement, reproducible docking poses and reliable interpretation of ligand–protein interactions.[12,24,25,35] The presence of a metal-dependent active site further enables exploration of coordination-based interactions that are often critical in enzyme–ligand binding.[5,7,21]

Another key factor is the pronounced structural responsiveness of CPA1 to single amino acid substitutions. Even minor point mutations can induce localized conformational changes without completely disrupting the overall fold of the protein.[8,11,29] This property makes CPA1 especially suitable for comparative docking studies, where subtle differences between wild-type and mutant structures can be directly correlated with changes in binding affinity or interaction patterns.[12,24]

The availability of high-resolution crystal structures of CPA1 further strengthens its selection.[6,22] High-quality structural data reduce modeling errors, enhance docking accuracy and ensure that observed differences between wild-type and mutant forms arise from genuine structural variation rather than computational artifacts.[22,24,25]

Importantly, CPA1 occupies a functional position at the interface of endogenous substrate processing and potential drug interactions.[5,30,34] Alterations in its peripheral structural environment may influence binding not only of physiological ligands but also of small-molecule drugs. This dual relevance provides a strong rationale for evaluating both natural and therapeutic ligands in a comparative docking framework.[12,34]

Together, these attributes establish CPA1 as an optimal and technically robust target for investigating mutation-induced changes in ligand binding within the context of pancreatitis.[8,10,12]

2. Rationale for Ligand Selection

2.1 Phenylalanine as an Endogenous Reference Ligand

Phenylalanine was selected as the reference ligand due to its direct functional relevance to Carboxypeptidase A1 activity. CPA1 exhibits a strong preference for cleaving C-terminal aromatic amino acids during protein digestion, with phenylalanine being one of its primary physiological targets.[5,6,30,32] Consequently, phenylalanine represents a biologically meaningful ligand for examining native substrate recognition within the CPA1 active site.[5,6]

Incorporating phenylalanine as a reference ligand enables assessment of whether the introduced mutation influences interactions with a natural substrate-related molecule. This approach provides a reliable baseline for comparing binding behavior between the wild-type and mutant proteins, allowing mutation-induced effects on ligand recognition and binding stability to be interpreted in a biologically relevant context.[8,11,12]

2.2 Paracetamol and Ibuprofen as Analgesic Drug Ligands

Paracetamol and ibuprofen were chosen as representative analgesic agents due to their widespread clinical use in the management of pain associated with pancreatitis.[3,33] These two compounds exhibit distinctly different physicochemical characteristics, allowing evaluation of how chemical diversity influences ligand binding to CPA1.[12,24] Paracetamol is comparatively small and polar, with binding behavior largely governed by hydrogen-bonding and polar interactions.[33,34] In contrast, ibuprofen possesses a bulkier and more hydrophobic structure, with binding dominated by hydrophobic contacts and van der Waals interactions.[33,34]

The inclusion of both ligands enables a systematic comparison of mutation-dependent effects across chemically distinct drug classes.[12,25] Although neither paracetamol nor ibuprofen serves as a natural substrate for CPA1, mutation-induced alterations in the enzyme's active-site environment may modulate interactions with therapeutic compounds.[8,11] Exploring these interactions provides insight into how structural changes associated with pancreatitis could potentially influence drug binding behavior and therapeutic outcomes.[4,34]

3. MATERIALS AND METHODS

3.1 Protein Preparation

The three-dimensional structure of wild-type Carboxypeptidase A1 (CPA1) was retrieved from the Protein Data Bank in PDB format and used as the native reference structure for the

present study.[22] This experimentally resolved crystal structure represents the physiologically functional form of the enzyme and is referred to as the wild-type protein throughout the analysis.[6,22]

Protein preparation was performed to ensure structural accuracy and compatibility with molecular docking protocols.[12,24] Initially, crystallographic water molecules (HOH) and other non-essential heteroatoms were removed from the structure, as these components originate from crystallization conditions and do not represent stable interactions in solution.[22,24] Their presence during docking may introduce artificial steric constraints or non-physiological hydrogen-bond interactions.[24,25] In contrast, the catalytically essential Zn^{2+} ion located within the CPA1 active site was deliberately retained. CPA1 is a zinc-dependent metalloprotease, and the Zn^{2+} ion plays a critical role in maintaining active-site geometry and facilitating ligand coordination.[5–7,21] Removal of this metal ion would distort the catalytic pocket and compromise the biological relevance of docking results.[5,21]

Following structural cleaning, hydrogen atoms were added to the protein to establish appropriate protonation states of amino acid residues.[12,23] As X-ray crystallographic structures typically lack explicit hydrogen atoms, this step is essential for accurately modeling electrostatic interactions, hydrogen bonding, and ligand recognition.[22,23] Local energy minimization was subsequently performed to relieve steric strain introduced during hydrogen addition and to stabilize the protein structure without altering its overall fold.[24,28]

To investigate mutation-induced effects on ligand binding, a disease-relevant point mutation was introduced using the PyMOL Mutagenesis Wizard. Specifically, the serine residue at position 34 was replaced with alanine (Ser34 → Ala). The lowest-energy rotamer was selected to minimize steric clashes and ensure local structural stability.[29] The mutant structure was saved as a separate file to preserve the integrity of the wild-type model and enable direct comparative analysis.

Both wild-type and mutant CPA1 structures were subjected to identical preparation procedures, including hydrogen addition and local energy minimization, to maintain methodological consistency.[12,24] This ensured that any observed differences in docking behavior could be attributed specifically to the introduced mutation rather than to variations in protein preparation.

For molecular docking, a grid box was defined to encompass the zinc-containing catalytic region of CPA1. A grid box dimension of 22 Å was selected to fully cover the active site along with surrounding residues involved in ligand recognition and stabilization.[12] This grid size provides sufficient space to accommodate ligand flexibility and explore alternative binding orientations while avoiding unnecessary inclusion of irrelevant protein regions that could reduce docking accuracy.[12,25] Identical grid parameters were applied to both wild-type and mutant proteins to ensure reliable comparison of docking outcomes.[12]

The Ser34 → Ala substitution represents a non-catalytic, peripheral mutation. Serine-34 is located outside the zinc-coordinated catalytic core of CPA1 and does not directly participate in substrate cleavage or metal coordination, which are primarily governed by residues such as His69, Glu72, His196, and the bound Zn^{2+} ion.[5,6,21] Nevertheless, mutations at non-active-site positions are known to induce subtle conformational changes, altered folding dynamics or long-range structural perturbations.[8,11,29] Replacement of serine with alanine at position 34 removes a polar hydroxyl group and introduces a smaller, nonpolar side chain, enabling assessment of how changes in local side-chain polarity influence overall protein stability and ligand-binding behavior.[29] This approach provides a technically sound and biologically relevant model for studying mutation-induced modulation of ligand interactions in CPA1 without completely abolishing enzymatic function.[8,12]

3.2 Ligand Preparation

Phenylalanine, paracetamol and ibuprofen were obtained in Structure Data File (SDF) format from publicly available chemical databases and subjected to systematic ligand preparation prior to docking.[12,35] Each ligand structure was imported into AutoDockTools, where initial geometry inspection was performed to ensure structural integrity.[12,35] Polar hydrogen atoms were added to accurately represent hydrogen-bond donors and acceptors, which are essential for realistic modeling of ligand–protein interactions.[12,24] Gasteiger partial charges were assigned to all ligand atoms to enable proper electrostatic interaction calculations during docking simulations.[23,35] Aromatic carbon atoms were identified to preserve correct ring geometry and aromaticity, particularly for phenylalanine and ibuprofen.[23,35] Rotatable bonds were defined to allow conformational flexibility of the ligands during docking, while maintaining rigid ring systems where appropriate.[12,35] Following preparation, each ligand was saved in Protein Data Bank, QT (PDBQT) format, which is required for compatibility

with AutoDock Vina and ensures consistent treatment of atomic types, charges and torsional degrees of freedom during docking calculation.[12,35]

3.3 Molecular Docking Protocol

Docking simulations were carried out using AutoDock Vina to evaluate ligand binding to both wild-type and mutant CPA1 structures.[12] A cubic grid box with dimensions of 22 × 22 × 22 Å³ was centered on the zinc-containing active site of CPA1 and applied uniformly to both protein forms to ensure methodological consistency.[12,24] This grid size was selected to fully encompass the catalytic pocket and adjacent residues involved in ligand recognition while allowing sufficient conformational freedom for ligand exploration.[12,25] The docking exhaustiveness parameter was set to 8 to achieve a balance between computational efficiency and sampling accuracy.[12] For each docking run, multiple binding poses were generated, and the pose exhibiting the lowest predicted binding free energy was selected as the most favorable conformation for subsequent interaction analysis.[12,25,35]

4. RESULTS

Table-1

Ligand	WT Binding Energy (kcal/mol)	WT Zn–O Distance (Å)	MT Binding Energy (kcal/mol)	MT Zn–O Distance (Å)
Phenylalanine	–5.4	5.7, 6.4	–5.6	7.5, 9.6
Paracetamol	–5.6	6.9, 9.1	–5.5	6.8, 9.1
Ibuprofen	–6.5	6.5, 8.4	–6.3	6.2, 8.1

Molecular docking was performed to evaluate and compare ligand-binding affinities between wild-type CPA1 and the Ser34→Ala mutant structure.[12] The predicted binding energies and corresponding Zn–O distances for the best-ranked pose of each ligand are summarized in Table 1.

Phenylalanine exhibited a binding energy of –5.4 kcal/mol toward the wild-type enzyme and –5.6 kcal/mol toward the mutant structure. Although the mutant demonstrated a slightly stronger predicted affinity, the difference (0.2 kcal/mol) was modest and falls within the typical scoring uncertainty range of docking algorithms.[12,25] Notably, Zn–O distances in both structures exceeded 5 Å, indicating that the ligand does not directly coordinate the catalytic zinc ion in the highest-ranked docking orientation.[5,21]

Paracetamol showed nearly identical binding energies for wild-type (–5.6 kcal/mol) and mutant CPA1 (–5.5 kcal/mol), suggesting minimal influence of the Ser34→Ala substitution

on its interaction profile. Similar to phenylalanine, Zn–O distances remained greater than 6 Å, confirming the absence of direct zinc coordination in the best-scoring pose.[5,7,21]

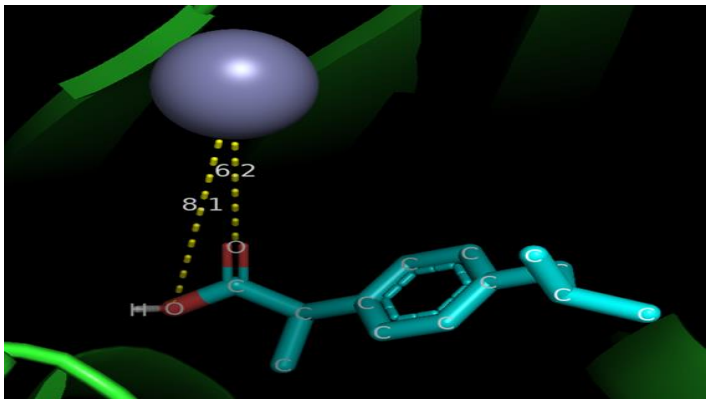

Fig.1: Zn-O distance in mutant type CPA1 and ligand Ibuprofen.

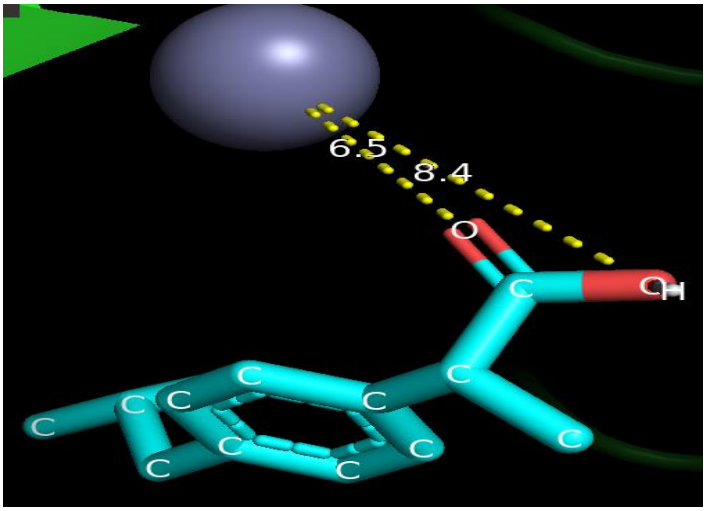

Fig. 2: Zn-O distance in Wild type CPA1 and ligand Ibuprofen.

Ibuprofen demonstrated the strongest binding among the tested ligands, with binding energies of –6.5 kcal/mol for wild-type and –6.3 kcal/mol for the mutant protein. Despite its relatively higher affinity, Zn–O distances were again greater than 6 Å in both structures, indicating peripheral binding rather than catalytic-site metal coordination.[5,21]

Collectively, these findings indicate that the lowest-energy (highest-affinity) docking poses for all three ligands occur outside direct zinc coordination distance. While minor ligand-specific differences in binding affinity were observed between wild-type and mutant CPA1, the Ser34→Ala mutation does not appear to induce substantial alteration of metal-centered catalytic interactions.[5,8] Instead, the mutation results in subtle modulation of the ligand-binding environment without disrupting overall pocket accessibility.[8,12]

5. DISCUSSION

The purpose of this work was to determine whether replacing serine with alanine at position 34 in CPA1 produces any meaningful change in ligand-binding behavior. Based on the docking results obtained for phenylalanine, paracetamol and ibuprofen, the mutation does not appear to cause a dramatic alteration in the structural integrity of the enzyme. The predicted energy differences between wild-type and mutant forms are present, but they are small in magnitude and remain within the normal variation expected from docking-based scoring systems.[12,25]

With respect to phenylalanine, the mutant protein showed a slightly more favorable binding energy than the wild type. Since Ser34 is positioned away from the zinc-coordinating residues that define the catalytic core—namely His69, Glu72, His196 and the bound Zn^{2+} ion—this difference is unlikely to arise from changes at the metal center itself.[5,6,21] Instead, it may reflect subtle adjustments in the surrounding microenvironment of the binding pocket. Even small alterations in side-chain polarity can influence local packing interactions or hydrogen-bonding patterns, which in turn can shift ligand orientation within the cavity.[8,11,29] The Zn–O distances exceeding 5 Å in both structures confirm that phenylalanine does not directly coordinate the zinc ion in its most stable docking pose.[5,21] This supports the interpretation that binding occurs within the catalytic pocket but without metal chelation.

Paracetamol behaved somewhat differently. The binding energies for the wild-type and mutant structures were almost identical, suggesting that the Ser34→Ala substitution has little effect on its interaction profile. Because paracetamol is relatively small and polar, its stabilization is more likely driven by hydrogen bonding and surface contacts than by deep hydrophobic packing or metal-mediated interactions.[12,24] In both protein forms, the ligand remained well outside coordination distance of the zinc ion, again indicating that the metal center is not directly involved in stabilizing the docked pose.[5,21] This observation is consistent with the pharmacological role of paracetamol, which does not function through inhibition of digestive metalloproteases.[33,34]

Among the ligands examined, ibuprofen showed the strongest predicted affinity overall. The difference between wild-type and mutant structures, however, was still modest. Given the bulkier and more hydrophobic nature of ibuprofen, its binding likely benefits from favorable van der Waals interactions within hydrophobic regions of the pocket rather than from direct interaction with catalytic residues.[24,35] The Zn–O distances greater than 6 Å further indicate

that the ligand occupies a peripheral position relative to the metal center.[5,21] Taken together, these findings suggest that hydrophobic stabilization, rather than catalytic-site coordination, accounts for the slightly stronger binding observed with this compound.

Looking at the data as a whole, the Ser34→Ala substitution does not disrupt the zinc-dependent catalytic architecture of CPA1.[5,6] The metal center remains structurally intact, and none of the tested ligands approach coordination distance in their lowest-energy conformations. The mutation instead appears to introduce subtle changes in the binding environment that may influence ligand positioning or micro-interactions without compromising the overall fold or catalytic geometry of the enzyme.[8,12] This distinction is important, because structural modulation does not necessarily imply functional impairment.

It is also necessary to interpret docking results with appropriate caution. Docking provides a static estimate of binding affinity and does not capture protein flexibility, solvent dynamics, or long-range conformational effects that may occur in vivo.[12,24,25] Small differences in predicted energy values—such as those observed here—should therefore be viewed as suggestive rather than definitive. More detailed computational analyses, including molecular dynamics simulations or free energy calculations, would provide a clearer picture of whether the observed trends persist under dynamic conditions.[26–28] Ultimately, experimental validation through enzymatic assays or structural characterization will be required to determine whether the Ser34→Ala mutation produces measurable functional consequences.

6. CONCLUSION

This study examined whether replacing serine with alanine at position 34 in CPA1 alters the way ligands interact with the enzyme's binding pocket. Based on the docking results obtained with phenylalanine, paracetamol and ibuprofen, the substitution does not appear to compromise the structural integrity of the zinc-dependent catalytic center. The predicted differences in binding energy between the wild-type and mutant proteins were present but relatively small, suggesting that the overall architecture of the enzyme remains stable despite the amino acid change.

Among the tested ligands, ibuprofen consistently showed the strongest predicted affinity, whereas phenylalanine and paracetamol displayed moderate and comparable binding strengths. However, in all cases the ligands remained outside direct coordination distance of the catalytic zinc ion. This indicates that their stabilization is driven by interactions within the

surrounding cavity rather than by metal chelation at the active site. The Ser34→Ala mutation therefore seems to influence the local interaction environment subtly, without fundamentally disturbing catalytic geometry.

These observations highlight an important distinction: not every amino acid substitution leads to major catalytic disruption. In the case of CPA1, the mutation appears to introduce localized structural adjustments rather than global destabilization. Such subtle effects may still be biologically relevant, particularly under dynamic physiological conditions, but they are not indicative of a collapsed or dysfunctional active site.

Because molecular docking provides a static representation of binding behavior, the findings should be interpreted as indicative trends rather than definitive functional outcomes. Further investigation through molecular dynamics simulations, free energy calculations, and experimental enzymatic studies would help clarify whether the small energetic differences observed here translate into measurable biological consequences.

Overall, this work contributes to a more nuanced understanding of how peripheral mutations can shape the ligand-binding landscape of CPA1. By examining both endogenous and therapeutic ligands in parallel, the study provides a structural framework for exploring mutation-specific effects in pancreatitis-associated protein variants and supports the broader value of mutation-aware computational analysis in enzyme research.

7. Future Scope

Although the present work provides useful structural insight into how ligands interact with both wild-type and mutant CPA1, it represents only an initial step toward understanding the broader functional consequences of the Ser34→Ala substitution. The docking results offer a static view of binding behavior, but proteins operate in a dynamic biological environment. For this reason, molecular dynamics simulations would be a logical next direction. Such simulations could help determine how stable the docked complexes remain over time and whether the mutation influences flexibility, pocket rearrangement, or long-range structural communication within the protein.

To obtain more quantitative estimates of binding strength, free energy calculations using approaches such as Molecular Mechanics–Poisson–Boltzmann Surface Area (MM-PBSA) or Molecular Mechanics–Generalized Born Surface Area (MM-GBSA) could be employed.

These methods may clarify whether the small energetic differences observed in docking persist under more rigorous computational treatment.

Computational predictions, however, should ultimately be complemented by experimental work. Expression of the mutant CPA1 in vitro followed by enzymatic activity measurements would provide direct evidence of whether the structural changes observed here translate into altered catalytic performance. Likewise, structural techniques such as circular dichroism spectroscopy or X-ray crystallography could help determine whether the mutation induces measurable conformational changes at the secondary or tertiary structural level.

Another worthwhile direction would be to broaden the range of tested ligands. Including established metalloprotease inhibitors or compounds specifically associated with pancreatitis could reveal mutation-dependent interaction trends that are not apparent from the limited ligand set used in this study. Taken together, these additional computational and experimental efforts would provide a more complete picture of how peripheral mutations in CPA1 influence structure, stability, and ligand recognition, ultimately guiding mutation-aware therapeutic exploration.

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