

Enhancing Docking Accuracy Through Flexible Pocket-Based Validation: A Case Study On 1eag

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Abstract– Accurate definition of the binding pocket is a crucial step in ensuring the reliability of molecular docking simulations. Precise pocket definition is fundamental to achieving reliable docking predictions, yet few studies have established a reproducible protocol for pocket-based validation on fungal proteases. This study focuses on the structural characterization of the 1EAG protease active site as a preliminary stage for flexible docking validation. The three-dimensional structure of 1EAG was analyzed to identify key active-site residues within 3–5 Å of the co-crystallized ligand (A70). Twelve residues were identified, comprising polar/ionic (58%), hydrophobic (25%), and aromatic (17%) types. These residues were further classified according to their functional interactions, including hydrogen bonding, π - π stacking, and hydrophobic contacts. A simplified pharmacophore model highlighting donor, acceptor, aromatic, and hydrophobic features was constructed to represent the spatial organization of the pocket. The results demonstrate that ASP32 serves as the catalytic hotspot, TYR84 and TYR225 stabilize the ligand through π - π interactions, and hydrophobic residues (ILE and LEU) form the outer pocket contour. Although the present analysis is limited to static pocket characterization, it provides a reproducible framework for the rational development of flexible docking validation protocols.

Keywords: molecular docking, pharmacophore modeling, receptor flexibility, 1EAG, *Candida albicans*.

I. INTRODUCTION

Molecular docking is a cornerstone of structure-based drug discovery (SBDD), enabling the rapid assessment of ligand binding modes and affinities against target proteins. The accuracy of docking predictions critically depends on the precise characterization of the binding pocket, including its geometry and chemical interaction patterns [1–4]. Traditional docking pipelines often rely on rigid receptor models, which neglect the intrinsic flexibility of the binding site. This simplification can lead to pose inaccuracies and erroneous affinity rankings. Consequently, pocket-based validation strategies that explicitly consider interaction features such as hydrogen bond donors/acceptors, aromatic contacts, and hydrophobic surfaces have emerged as powerful approaches to improve docking robustness [5–8].

The present study focuses on 1EAG, a secreted aspartic proteinase 2 (SAP2) from *Candida albicans*, a well-established virulence factor and a frequently studied antifungal drug target [9,10]. The availability of high-resolution crystallographic data through RCSB PDB enables accurate binding pocket mapping and interaction feature extraction [11]. This structural information supports the identification of key residues within 3–5 Å of the reference ligand, forming the basis for the generation of a pharmacophore model that reflects the chemical nature of the active site.

Importantly, 1EAG has been widely used as a benchmark structure in flexible docking and molecular dynamics studies, where the co-crystallized ligand A70 serves as a reliable anchor for interaction mapping [12,13]. Previous reports have demonstrated that interaction-focused validation can highlight conserved binding determinants—such as catalytic aspartates and π - π stacking residues—that are often overlooked by purely RMSD-based approaches [14–16]. This makes 1EAG an ideal case study for

developing and evaluating a flexible pocket-based validation protocol. Although redocking with root-mean-square deviation (RMSD) thresholds below 2 Å is commonly used to assess docking accuracy, this metric alone may fail to capture chemically relevant interactions. Correctly aligned poses can lack key contacts, including catalytic hydrogen bonds or aromatic stacking interactions, resulting in unreliable hit prioritization [17,18].

Furthermore, ignoring local side-chain flexibility can produce ranking biases that are magnified during virtual screening [19]. Pocket-based validation, by incorporating chemical interaction features, provides a more reliable foundation for subsequent docking and scoring [20,21]. Recent studies have shown that integrating receptor flexibility into docking pipelines yields significantly more consistent predictions, particularly in systems exhibiting local conformational changes at the active site [22,23]. Machine learning-assisted approaches further enhance this by predicting pocket deformation and guiding docking search spaces adaptively [24,25]. In parallel, structure-based pharmacophore models have proven to improve hit enrichment by enforcing interaction feature matching during virtual screening [26–28].

For 1EAG, the well-defined active site and co-crystal structure provide an excellent context for deriving interaction anchors. Flexible pocket-based validation has been proposed as a lightweight yet effective strategy for improving docking reliability without requiring long molecular dynamics simulations [29,30]. While most existing workflows rely solely on RMSD evaluation, few have implemented a streamlined and reproducible pocket-based protocol suitable for fungal protease targets [31,32]. This gap underscores the need for a systematic yet practical approach.

The present study aims to (i) establish a reproducible flexible pocket-based validation protocol for 1EAG, (ii) map key residues within a 3–5 Å radius of the reference ligand to build a minimal pharmacophore model, and (iii) generate residue tables and interaction maps to support subsequent docking experiments. The novelty of this work lies in prioritizing interaction patterns rather than ligand RMSD as the primary metric of docking validity. This focused protocol is expected to provide a practical and broadly applicable foundation for virtual screening against fungal protease targets. This work therefore aims to enhance docking accuracy through a flexible pocket-based validation approach, using 1EAG as a model system for fungal proteases.

II. MATERIALS AND METHOD

A. Research Design

This study employed a descriptive computational approach focusing on structural characterization of the 1EAG protease active pocket. All procedures were performed *in silico* between March and May 2025 using open-source molecular visualization and docking preparation tools.

B. Materials and Tools

The 3D structure of 1EAG (PDB ID: 1EAG) was obtained from the Protein Data Bank. Structural analysis and pocket visualization were conducted using PyMOL (v3.1.6.1), while preliminary grid preparation was assisted by AutoDockFR (ADFR). ADFR was used only to visualize grid coverage around the identified pocket, without performing docking simulations.

C. Procedure

The analysis workflow was designed to identify residues contributing to the binding-site architecture and to classify them according to interaction type. The workflow was designed to identify residues contributing to the active site topology and to categorize them according to interaction types relevant for pharmacophore generation. The co-crystallized ligand (A70) was identified and used as the geometric reference for pocket mapping. Active-site residues within 3–5 Å of A70 were selected and classified based on chemical properties: polar/ionic, aromatic, and hydrophobic. Hydrogen bonds were detected using PyMOL's distance measurement function with a 3.5 Å cutoff, while aromatic and hydrophobic contacts were validated through spatial proximity analysis. A simplified pharmacophore model representing donor, acceptor, aromatic, and hydrophobic features was constructed.

D. Data Analysis

All residues were tabulated by residue name, distance, and interaction type. The spatial distribution of residues was analyzed qualitatively through PyMOL visualization.

E. Visualization and Figure Preparation

All structural representations were generated in PyMOL using white background for clarity. Hydrogen bonds were colored cyan, π - π stacking magenta, and hydrophobic contacts green. L'analyse du phénomène *Chronifuati* s'inscrit à la croisée de plusieurs champs théoriques : l'appropriation populaire des technologies, le journalisme citoyen et alternatif, ainsi que les recherches sur la communication en contexte de sous-développement. Ces trois axes permettent de situer le phénomène congolais dans une perspective plus large et de mettre en évidence ses spécificités.

III. RESULTS AND DISCUSSION

A. Results

1. Identification of Key Active Site Residues

Active site mapping of 1EAG revealed twelve key residues located within a 3–5 Å radius from the co-crystallized ligand A70. These residues comprise seven polar/ionic residues, three hydrophobic residues, and two aromatic residues. Polar/ionic residues were concentrated at the catalytic center, aromatic residues were positioned internally, and hydrophobic residues formed the outer contour of the pocket, supporting ligand stabilization.

2. Quantitative Data of Active Site Residues

The minimum interaction distance between the key residues and the ligand ranged from 2.54 Å to 4.90 Å, with ASP32 exhibiting the shortest distance, indicating its role as the central catalytic residue. The key residue interactions and their spatial relationships with the co-crystallized ligand are visualized in Figure 2, where hydrogen bonds, π - π stacking, and hydrophobic contacts are depicted in cyan, magenta, and green, respectively. The quantitative parameters of these interactions are summarized in Table 1. Table 1 summarizes the classification of these residues based on their chemical properties, interaction distances, interaction types, and functional roles.

Table 1. Classification of key active-site residues in 1EAG based on distance, interaction type, and functional role.

No	Residue	Category	Min Distance (Å)	Interaction Type	Functional Role
1	ASP32	Polar/Ionic	2.54	H-bond / ionic	Catalytic residue; donor/acceptor H-bond
2	ASP86	Polar	3.46	H-bond / ionic	Hydrogen bond bridge
3	GLU193	Polar (acidic)	3.30	H-bond / ionic	Hydrogen bond acceptor
4	ARG195	Polar (basic)	4.90	H-bond / ionic	Donor H-bond and charge anchor
5	ASN131	Polar	4.06	H-bond	Ligand orientation stabilizer
6	TYR84	Aromatic	3.42	π - π / H-bond	π - π stacking and donor H-bond
7	TYR225	Aromatic	3.65	π - π / hydrophobic	Aromatic surface stabilizer
8	LEU216	Hydrophobic	3.67	Hydrophobic	Complex stabilization
9	ILE30	Hydrophobic	3.98	Hydrophobic	Pocket cavity filler
10	ILE82	Hydrophobic	3.38	Hydrophobic	Pocket contour
11	THR221	Polar	3.51	H-bond	Secondary hydrogen bonding
12	ASP120	Polar/Ionic	4.64	H-bond / ionic	Peripheral interaction

3. Visual Representation and Residue Composition

Three-dimensional visualization using PyMOL clearly illustrates the clustering of polar and ionic residues at the catalytic center, the positioning of aromatic residues internally, and the hydrophobic residues outlining the pocket. As shown in Figure 1, the overall structure of 1EAG exhibits a clearly defined catalytic cavity, with polar residues highlighted near the central axis of the protein, confirming the pocket localization derived from ligand A70. Chemical composition analysis indicated that 58% of the residues were polar/ionic, 25% hydrophobic, and 17% aromatic. Correlation between distance and functional roles showed that residues with interaction distances < 3.5 Å predominantly participated in catalytic hydrogen bonding, whereas those > 4 Å were structurally supportive. These spatial relationships are further simplified in the pharmacophore model (Figure 3), which highlights donor (blue), acceptor (red), aromatic (violet), and hydrophobic (yellow) regions, providing a concise representation of the pocket's chemical diversity.

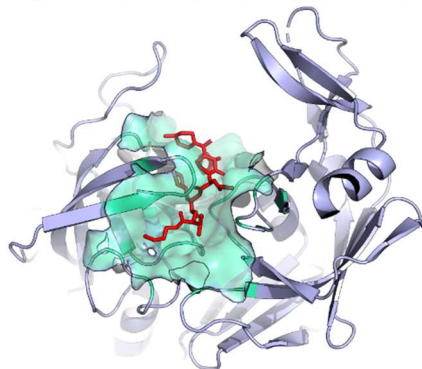


Figure 1. Overall structure of 1EAG and active site location.

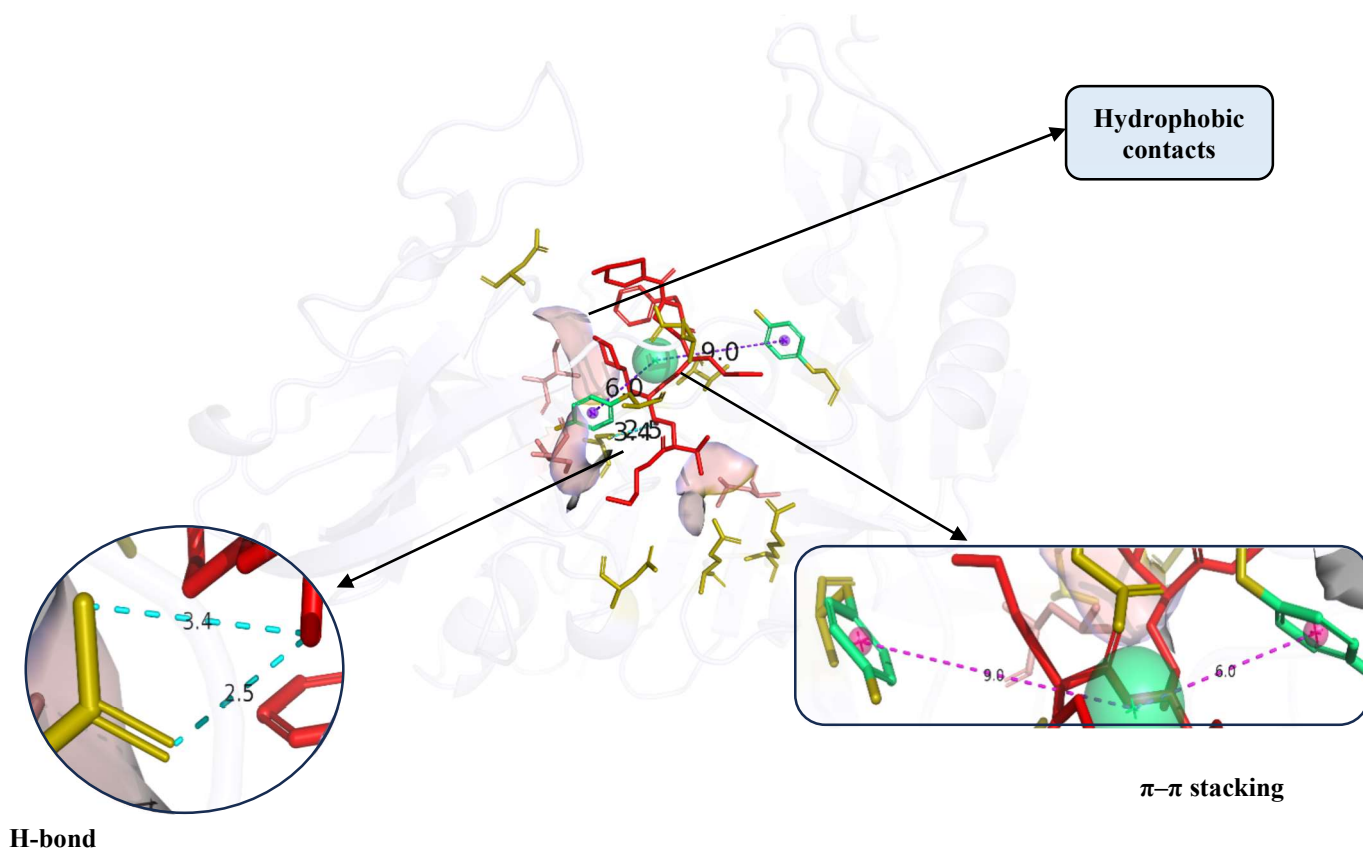


Figure 2. Key residue interactions with ligand A70 (H-bond, π - π stacking, hydrophobic contacts).

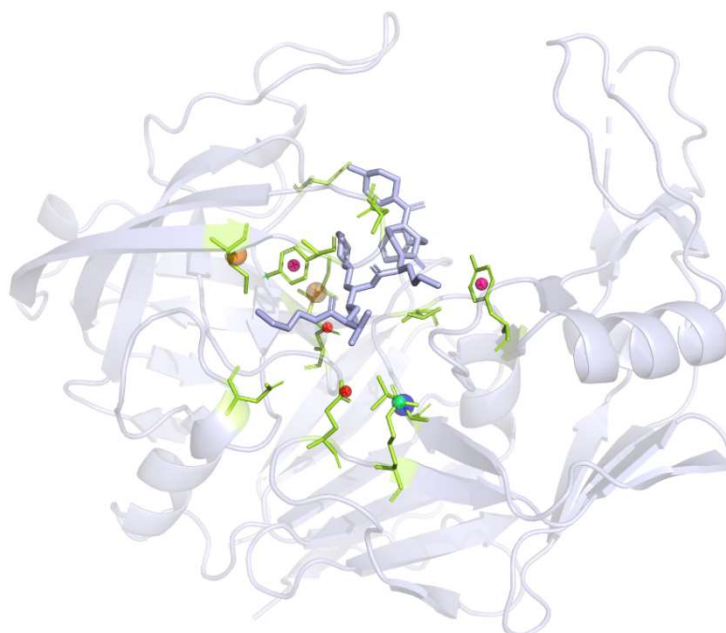


Figure 3. Simplified pharmacophore model (donor, acceptor, aromatic, hydrophobic features).

B. Discussion

The overall spatial arrangement of catalytic and supporting residues suggests a well-organized interaction network typical of aspartic proteases, while aromatic tyrosines provide π - π stacking support. These results are consistent with previously reported structural analyses of similar protease systems, which highlight the critical interplay between polar and hydrophobic residues in ligand stabilization [27, 30-33]. The contour-forming residues (ILE and LEU) align with studies indicating the importance of hydrophobic interactions in maintaining the structural integrity of active sites [28,29]. The observed distribution of key residues mirrors the well-characterized features of aspartic proteases, where aspartate and glutamate residues dominate the catalytic center, while aromatic tyrosines provide π - π stacking support [26,20].

The characterization of key pocket residues provides a robust foundation for defining biologically relevant docking grids and pharmacophore models. Scientifically, these findings emphasize that accurate delineation of the active site is a crucial determinant of flexible docking performance. Practically, this pocket-based strategy enables efficient virtual screening workflows for identifying antifungal compounds targeting SAP2. ASP32, with the shortest interaction distance, represents the catalytic hotspot, while TYR84 and TYR225 enhance ligand stabilization through π - π interactions. The combination of hydrogen bonding, hydrophobic contacts, and aromatic stacking creates a chemically diverse environment that allows the pocket to accommodate structurally distinct ligands—a hallmark of catalytic protease sites [24–26, 31–33, 34].

This study employed a single static crystal structure, which may not fully capture the conformational dynamics of active site residues under physiological conditions. Molecular dynamics simulations and statistical evaluation of binding energy fluctuations were not performed and represent a potential extension to this work. Future studies should incorporate molecular dynamics simulations to assess temporal flexibility and conformational adaptability of the binding pocket. Additionally, pharmacophore-based virtual screening against chemical libraries could identify novel inhibitors targeting SAP2 with improved selectivity and potency. Overall, the residue interaction landscape of 1EAG suggests a conserved catalytic environment similar to other fungal aspartic proteases, validating its use as a benchmark structure for pre-docking pocket analyses [25, 33–38].

IV. CONCLUSION

This study successfully characterized the active pocket of the 1EAG protease by identifying twelve key residues involved in hydrogen bonding, π - π stacking, and hydrophobic interactions. The resulting simplified pharmacophore model highlights the dominant donor, acceptor, aromatic, and hydrophobic features within the catalytic cavity. These findings provide a reliable structural basis for subsequent flexible docking studies targeting SAP2 inhibitors. Although limited to static structural analysis, the methodology establishes a reproducible framework for pocket-based validation prior to docking simulations. This protocol can serve as a reference workflow for structure-based drug design targeting protease families where pocket geometry plays a dominant role in ligand recognition. These insights highlight the potential of flexible pocket-based strategies to improve docking accuracy across structurally diverse protease systems.

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