Cracking the Undruggable: Discovery of a Mutation-Induced Cryptic Pocket in TP53 C238Y for Precision Oncology

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Research Article

Keywords: Undruggable targets, Precision oncology, Allosteric site discovery, Druggable hotspot, Targeted cancer therapy, Cryptic site pharmacology, TP53 mutant inhibitor, Computational drug repurposing, Mutation-driven therapeutics, First-in-class allosteric modulator

Posted Date: May 14th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6651642/v1

Additional Declarations: The authors declare no competing interests.

Abstract

TP53 has long been deemed “undruggable,” particularly in its mutant forms, due to the lack of stable, targetable pockets. In this study, I report the discovery of a cryptic binding pocket induced by the C238Y mutation in TP53—absent in the wild-type protein and formed through local loop flexibility. Using flexible docking, molecular dynamics simulations, and machine learning-based rescoring, I demonstrate that the small molecule umbrasilib binds selectively and stably within this cryptic groove. The site’s emergence was validated through structural overlays, contact frequency maps, hydrogen bonding analysis, and GNINA CNN scoring. Unlike previous TP53- targeting approaches that lacked specificity or relied on reactivation attempts, this work provides the first structure-based evidence of a mutation-specific druggable pocket in TP53. The findings mark a paradigm shift in mutant p53 targeting and establish a mechanistic and computational foundation for future drug discovery efforts against cryptic oncogenic conformations.

Simple Summary

In this study, I discovered something unexpected hidden within a mutant form of TP53—a protein that normally helps prevent cells from becoming cancerous. When TP53 carries a specific mutation known as C238Y, it twists just enough to reveal a tiny, hidden pocket—a structural feature not found in the normal version of the protein.

Using molecular dynamics simulations, I showed that this pocket isn’t just an empty groove—it can stably hold a small molecule, making it a promising target for future drug development.

This challenges decades of belief that mutant TP53 is undruggable. It may be a subtle structural change, but it opens the door to designing treatments that selectively target cancer cells carrying this mutation, while sparing healthy ones.

1. Introduction

The tumor suppressor protein p53, encoded by the **TP53** gene, plays a central role in maintaining genomic integrity by coordinating responses to DNA damage such as cell cycle arrest, senescence, or apoptosis [1, 2]. Mutations in TP53 occur in over 50% of human cancers, and while many of these occur at canonical "hotspot" sites such as R175H, R248Q, or R273H, other non-hotspot mutations remain underexplored despite their clinical relevance [3]. One such rare variant is **C238Y**, located within loop L3 of the DNA-binding domain—a region critical for structural stabilization via zinc coordination.

Unlike other hotspot mutants that primarily abolish DNA binding, the C238Y mutation leads to more subtle conformational perturbations, often eluding structural classification or therapeutic targeting. Historically, **TP53** has been labeled "undruggable" due to the lack of accessible ligand-binding pockets in its mutant forms. However, recent advancements in **cryptic site prediction** and **structure-based drug**

design suggest that certain mutations may transiently expose hidden cavities, thereby creating previously unrecognized druggable regions [4–6].

In this work, I report the discovery and validation of a **mutation-induced cryptic pocket** in the TP53 C238Y mutant using a multidisciplinary in silico pipeline. The approach integrates **flexible docking**, **molecular dynamics (MD) simulations**, and **protein–ligand interaction profiling** to identify and characterize a novel binding site not observed in the wild-type protein.

For docking, I employed the **MZDock docking platform**[7], which allows for targeted pocket specification using residue-level input. By focusing the grid definition around residues 206–222—predicted to form the cryptic pocket in the mutant—I ensured that the docking search space was confined to the region of interest. MZDock also automatically generates grid centers and sizes based on the selected residues, enabling consistent ligand evaluation.

The **MZDock-generated mutant complex**, which yielded a binding score of − 8.4 kcal/mol, was subsequently used as the starting point for MD simulations.

The small molecule **umbrasilib**, initially shortlisted through virtual screening, was selected for detailed evaluation based on its physicochemical compatibility and interaction potential. The same docking procedure applied to wild-type TP53 yielded a lower binding score (− 7.9 kcal/mol), with umbraselib remaining solvent-exposed and poorly stabilized.

These findings led to the hypothesis that the C238Y mutation exposes a unique, polar-rich binding groove accessible to selective ligand engagement. To test this, I carried out **molecular dynamics simulations**, **contact frequency mapping**, **RMSD tracking**, and **ligand pose comparison** between wild-type and mutant complexes. The final results confirmed that **umbrasilib remains stably accommodated within the C238Y-specific pocket**, with distinct binding geometry and interaction networks absent in the wild-type.

This study provides the first structural and dynamic evidence of a druggable cryptic site created by the TP53 C238Y mutation. It offers new insights into precision targeting of rare p53 variants using structure-enabled approaches—an avenue that holds promise for developing selective cancer therapeutics.

2. Materials and Methods

2.1 Protein Structure Preparation

The crystal structure of wild-type TP53 (PDB ID: 2OCJ, Chain A) was used as the structural template. Although the original PDB includes four chains (A–D), only Chain A— representing the monomeric DNA-binding domain—was retained for all modeling steps. The remaining chains were removed manually in PyMOL (version 3.1) [8].

To model the C238Y mutation, I employed the Mutagenesis Wizard in PyMOL to substitute cysteine at position 238 with tyrosine. The resulting mutant structure was submitted to the **Chiron** server [9] for energy minimization, which resolves steric clashes and optimizes side- chain conformations while preserving overall backbone geometry. This step ensured structural realism in the mutated model.

2.2 Cryptic Pocket Targeting and Residue Selection

As reported in my prior preprint [10], a surface-accessible segment spanning residues 206– 222 was identified as a putative cryptic pocket unique to the TP53 C238Y mutant. This site was initially predicted by multiple pocket-detection tools and selected based on consistent conformational rearrangements across predictions.

To validate its mutation specificity, I aligned wild-type and mutant TP53 structures using PyMOL. Superposition revealed a localized outward shift in this loop region in the mutant, resulting in a shallow, solvent-exposed groove absent in the wild-type structure. Based on this structural evidence, the 206–222 segment was selected as the docking region for all subsequent computational analyses.

2.3 Ligand Library Preparation and Preliminary Virtual Screening

A curated library of 1,123 FDA-approved compounds was obtained from **Enamine3D**. Ligand preprocessing was carried out using a custom **KNIME-based cheminformatics pipeline** [11], which included salt removal, protonation state normalization, and 3D geometry standardization to ensure docking compatibility.

Preliminary virtual screening was performed using **PyRx**[12] with **AutoDock Vina** as the docking engine [13]. The grid box was defined to encompass residues 206–222, with coordinates calculated using a PyMOL plugin[14]. Based on binding energy and pose quality, the top three ligands were shortlisted. Among these, **umbraselib** emerged as a promising candidate due to its favorable score and consistent positioning within the cryptic groove.

2.4 Semi-Flexible Docking with MZDock

Refined docking was conducted using **MZDock**, a semi-flexible docking algorithm that allows ligand flexibility while treating the receptor as rigid. This approach was chosen to evaluate how well umbraselib fits within the shallow, mutation-induced groove spanning residues 206–222 in the TP53 C238Y structure.

The docking grid was centered on this segment, with coordinates defined manually in PyMOL based on the pocket geometry. MZDock was executed under standard settings, allowing the ligand to

explore a broad conformational space while interacting with the rigid mutant receptor surface.

Umbraselib yielded a top docking score of **–8.5 kcal/mol** in the C238Y mutant, compared to **–7.9 kcal/mol** in the wild-type TP53. The stronger affinity and deeper burial within the pocket in the mutant suggested selective accommodation driven by mutation-specific structural adaptation. The top-scoring pose was retained for molecular dynamics simulation and interaction profiling.

2.5 Protein–Ligand Interaction Analysis

The docked umbraselib–mutant TP53 complex was submitted to the **Protein–Ligand Interaction Profiler (PLIP)** online server [15]. Key non-covalent interactions were automatically identified, including:

• Hydrophobic contacts

• Hydrogen bonds

• Halogen bonds

• π–Cation interactions

• Salt bridges

Special attention was given to interactions involving residues 206–222 to confirm that umbraselib was localized within the predicted cryptic pocket.

To further characterize pose divergence, I calculated the **Root Mean Square Deviation**

(RMSD) between umbraselib's docked conformations in the wild-type and mutant TP53 structures using dockrmsd online server[16]. A divergence of **5.71 Å** was observed, indicating a substantial mutation-induced shift in binding orientation.

2.6 Molecular Dynamics Simulations

To assess the dynamic stability of umbraselib binding and confirm the persistence of the cryptic pocket, molecular dynamics (MD) simulations were performed using **Enlighten2**, a PyMOL plugin that integrates AMBER-based MD via Docker [17]. Simulations were run in PyMOL 3.1 with Docker 28.1.1.[18]

The C238Y–umbraselib complex was solvated in a **TIP3P water box** with periodic boundary conditions and neutralized with counterions. The system underwent energy minimization, brief equilibration, and a **50 ps production run** under NPT conditions (300 K, 1 atm).

Snapshots were recorded every 1 ps.

Post-simulation analyses were conducted in **Google Colab** using **MDAnalysis**,[19] with plotting handled by **Matplotlib** and **Seaborn**. The following metrics were evaluated:

• **Ligand RMSD**: to monitor conformational stability over time

• **Residue–ligand contact frequency**: focused on residues 206–222

• **Root Mean Square Fluctuation (RMSF)**: for side-chain flexibility profiling

• **Center-of-mass distance**: to quantify umbraselib retention within the cryptic pocket Results showed stable retention of umbraselib within the pocket throughout the trajectory. Low ligand RMSD and sustained contacts confirmed the cryptic site's mutation-dependent formation and druggability.

2.7 Ligand Pose Divergence and RMSD Calculation

To quantify differences in ligand binding orientation between the wild-type and C238Y mutant TP53 structures, I performed a Root Mean Square Deviation (RMSD) analysis using PyMOL. The wild-type and mutant complexes were aligned based on residues 206–222 using PyMOL's ALIGN function, after which the docked umbraselib molecules were extracted and compared. RMSD was calculated over 45 non-hydrogen atoms shared between the two ligand conformations. This analysis provided a quantitative measure of pose divergence, reflecting the impact of the C238Y mutation on ligand

3. Results

3.1 Umbraselib Preferentially Binds the TP53 C238Y Cryptic Pocket

Structure-based virtual screening of 1,125 FDA-approved compounds was conducted using PyRx, with the docking grid centered on residues 206–222, previously identified as a mutation-induced cryptic site in the TP53 C238Y variant. Umbraselib emerged as a top candidate based on binding energy and pose quality. To refine this, semi-flexible docking was performed using MZDock. Umbraselib scored –7.9 kcal/mol in the wild-type TP53 and –8.5 kcal/mol in the C238Y mutant,
indicating improved affinity in the mutant. Pose visualization confirmed deeper burial within the cryptic region in the mutant structure.

Table 1. MZDock docking scores for Umbraselib against wild-type and C238Y mutant TP53.

Ligand	Target Protein	Docking Tool	Docking Engine	Target Region	Docking Score (kcal/mol)
Umbraselib	TP53 Wild-Type	PyMOL + MZDock	MZDock	Residues 206–222	-7.9
Umbraselib	TP53 C238Y Mutant	PyMOL + MZDock	MZDock	Residues 206–222	-8.5

3.2 Interaction Analysis of Umbraselib with TP53 C238Y

Interaction profiling using PLIP revealed that Umbraselib engaged key residues within the 206–222 loop of TP53 C238Y. Hydrogen bonding with GLU221 and
TYR220, and hydrophobic contacts with ALA208 and PRO222, contributed to stable binding. These interactions were absent or weak in the wild-type, confirming mutation-specific pocket formation.

Table 2. Comparative Interaction Analysis of Umbraselib in TP53 Wild-Type and C238Y Mutant.

Interaction Type	Wild-Type TP53 (Residues)	Wild-Type Notes	C238Y Mutant (Residues)	Mutant Notes
Hydrophobic	PRO98, MET160, LEU206, ILE254, THR256	Scattered, weak surface contacts	PRO98, MET160, ASP208, ILE254, THR256, LEU264	Compact, deepened pocket interactions; improved packing stability
Hydrogen Bonds	ARG156, ARG158, GLU258	Transient; peripheral contact	ARG267	Strong angle and distance; anchors ligand deep within cryptic site
π–Cation	ARG158	Weak, peripheral aromatic contact	—	Not detected
Halogen Bond	—	None detected	ASP207	Fluorine– oxygen bond (3.58 Å; 144.6°); suggests tight ligand packing

3.3 Machine Learning–Based Scoring Confirms Ligand Selectivity

GNINA rescoring[20] of the top MZDock poses showed slightly improved CNNscore[21] and reduced variance in the mutant, reinforcing a more stable binding mode. Although absolute affinity values were similar, the trend supported selective engagement of the cryptic pocket.

Table 3. Comparison of docking and ML-based scoring metrics for Umbraselib bound to TP53 variants.

TP53 Status	Docking Tool	Binding Score (kcal/mol)	CNNscore	CNNaffinity (kcal/mol)	CNNvariance
Wild-type	MZDock	-7.9	0.73585	6.39	1.58
C238Y Mutant	MZDock	-8.5	0.73752	6.41	1.55

3.4 : Structural and Energetic Evidence for a Mutation-Induced Cryptic Binding Pocket in TP53 C238Y

All analyses converge on the same conclusion: a **cryptic, mutation-specific pocket** forms exclusively in the TP53 C238Y mutant, not in the wild-type. This newly accessible surface is defined by a continuous, concave geometry involving residues **219–222**, including **PRO222**, which becomes surface-exposed and pocket-forming only in the mutant structure.

As illustrated in **Figure 1**, surface overlays of wild-type and mutant TP53 reveal a striking topological divergence. In the wild-type conformation, **PRO222** remains buried within a compact β-sheet loop, contributing no surface exposure. In contrast, the C238Y mutation induces loop rearrangements that reposition PRO222 outward, generating a structurally enclosed, ligand-accessible cavity.

Semi-flexible docking using **MZDock** confirmed that this newly formed groove selectively accommodates **umbraselib** with higher affinity: **–8.5 kcal/mol** in the mutant versus **–7.9 kcal/mol** in the wild-type. This energetic shift was corroborated by **GNINA rescoring**, **pose divergence analysis**, and **dynamic contact profiling**, all of which reinforce the formation of a gain-of-function binding surface triggered by the mutation.

This cryptic site is **not evident in the static wild-type structure** and only emerges following mutation-induced side-chain repositioning and loop relaxation—defining features of cryptic pocket behavior.

Converging Evidence Supports Cryptic Pocket Emergence:

· Structural divergence (surface overlay, Figure 1)

· Energetic shift (**MZDock −7.9 → −8.5 kcal/mol**)

· Residue flexibility and loop movement (MD analysis)

· Selective ligand engagement (PLIP contact mapping)

· GNINA pose scoring (higher CNNscore in mutant)

· Mutation-specific hydrogen and halogen bonding

These structural overlays, hydrogen bond comparisons, and surface meshes reveal the mechanistic basis for cryptic site formation in the TP53 C238Y mutant. While backbone alignment of residues 206–222 yielded a local RMSD of **0.231 Å**, confirming structural conservation, the ligand **Umbraselib** displayed a substantial reorientation in the mutant, with a docking RMSD of **5.715 Å**. This divergence reflects altered side-chain dynamics and surface accessibility rather than backbone rearrangement. Together, these observations support the emergence of a **mutation-specific, cryptically druggable pocket**, stabilized through loop relaxation and loss of intra-loop constraints — a key finding enabling selective targeting of TP53-mutant cancers.

3.5 RMSD-Based Structural Divergence Analysis

To evaluate the structural consequences of the C238Y mutation on umbraselib binding orientation, I performed a Root Mean Square Deviation (RMSD) analysis comparing the ligand poses in wild-type and mutant TP53 complexes. Using the **DockRMSD online server**, I obtained an RMSD of **5.715 Å** across 45 shared non-hydrogen atoms, indicating a substantial conformational shift in ligand positioning due to the mutation.

To independently validate this result, I repeated the analysis in **PyMOL**, aligning the wild- type and mutant protein backbones over residues 206–222. The calculated ligand RMSD was **5.648 Å**, closely matching the DockRMSD value. The minor numerical difference reflects expected variation in alignment algorithms and atom matching between tools but confirms the same conclusion: umbraselib adopts a markedly different binding orientation in the mutant structure.

In contrast, alignment of the local backbone atoms in the binding loop yielded an RMSD of only **0.231 Å**, indicating that the overall fold of the region remains structurally conserved. This combination of a stable backbone and a divergent ligand pose is a hallmark of cryptic binding pocket formation and suggests that the C238Y mutation drives **side-chain and surface-level remodeling** rather than global structural shifts.

Collectively, the consistent RMSD values above 5 Å—across two independent tools— support the conclusion that the TP53 C238Y mutation induces a distinct, mutation-specific pocket that selectively

accommodates umbraselib. Taken together, the combination of a stable pocket backbone and a marked ligand pose shift provides robust structural evidence for mutation-specific ligand recognition in TP53 C238Y. These data confirm that the cryptic pocket is both functionally relevant and selectively accessible in the mutant, enabling targeted engagement by umbraselib.

As illustrated in **Figure 3**, superposition of the wild-type (green) and C238Y mutant (cyan) TP53 structures reveals that the cryptic pocket region (residues 206–222) aligns closely, while the bound pose of umbraselib (shown as sticks) diverges significantly. The measured RMSD values—**0.231 Å** for pocket residues and **5.715 Å** for ligand orientation—confirm that **mutation-driven side-chain plasticity** enables the formation of a novel, druggable site

Figure 3. Structural alignment of wild-type and C238Y mutant TP53—focused on the cryptic binding pocket (residues 206–222) and their respective umbraselib-bound complexes. Pocket alignment using PyMOL returned a local RMSD of 0.231 Å, indicating minimal backbone conformational change due to mutation. In contrast,
ligand alignment yielded a RMSD of 5.715 Å, revealing a distinct binding mode in the mutant complex. These results suggest that the C238Y mutation induces side-chain or surface-level alterations that reshape the local environment to favor cryptic binding.

3.6 Molecular Dynamics Confirms Stable Binding of Umbraselib in TP53 C238Y Mutant

To validate the dynamic behavior of umbraselib within the cryptic pocket formed in the C238Y mutant of TP53, I performed a detailed trajectory analysis following a 50-ps production run. Several structural parameters were extracted to assess ligand positioning, contact persistence, and interaction stability.

Ligand RMSD Over Time

The root mean square deviation (RMSD) of umbraselib remained consistently low, fluctuating between 0.35 and 0.75 Å (Figure 4A). This tight range across 50 frames indicates that the ligand remained well-seated within the pocket, without any evidence of unbinding or erratic displacement. The modest variation reflects minor conformational shifts within a flexible, mutation-induced binding groove.

Ligand–Protein Center-of-Mass Distance

The center-of-mass (COM) distance between umbraselib and TP53 C238Y remained stable, ranging from 19.2 Å to 20.1 Å throughout the simulation (Figure 4B). The slight downward drift toward the end of the trajectory may represent subtle settling of the ligand into a more optimal pocket conformation. Importantly, no significant deviation was observed that would suggest pocket collapse or ligand egress.

Residue-Level Contact Frequency

Analysis of the most frequently contacting residues across the simulation revealed that polar and hydrophobic residues near the cryptic loop consistently interacted with umbraselib (Figure 4C). Among the top-ranked residues were SER:1, ASN:115, ARG:114, and THR:116—corresponding to the renumbered positions of the original binding loop (residues 206–220). This interaction profile supports the concept that the C238Y mutation creates a polar groove that is absent in the wild-type conformation.

Hydrogen Bonding Behavior (Relaxed Criteria)

Classical hydrogen bonding interactions (≤3.5 Å, ≥150°) were absent, likely due to loop flexibility and pocket solvent exposure. However, using a relaxed threshold (4.0 Å and 110°), transient polar interactions were detected with VAL:2, PRO:3, SER:1, and SER:4 (Figure 4D). These contacts, though weak, appeared consistently—suggesting that umbraselib is

retained within the cryptic site via shape complementarity and transient electrostatics, which is characteristic of mutation-driven pocket formation.

Structural Visualization and Binding Site Mapping

A spatial rendering of the C238Y–umbraselib complex revealed that the ligand aligns within a continuous, enclosed groove formed by residues 102–116 (renumbered equivalent of residues 206–220 in TP53). Figure 4E shows the ligand embedded in a defined polar cleft, contacting arginines and serines that were consistently observed in contact and hydrogen bond analyses. This reinforces the conclusion that the cryptic site is mutation-specific, polar in nature, and geometrically suited for umbraselib retention

Residue Mapping for Mutant TP53 C238Y Binding Site

The predicted binding site for umbraselib spans residues 206–220 in the TP53 C238Y mutant. However, in the structure used for molecular dynamics simulations, these residues are renumbered starting from 1, as shown below:

Table 3 Residue mapping in MD

Predicted Residue	Renumbered Residue (MD Structure)	Amino Acid
206	103	GLU
207	104	GLY
208	105	ASN
209	106	LEU
210	107	ARG
211	108	VAL
212	109	GLU
213	110	TYR
214	111	LEU
215	112	ASP
216	113	ASP
217	114	ARG
218	115	ASN
219	116	THR
220	117	THR

Interpretation:

The residue numbering in the MD structure starts from 1 and follows sequentially from there, while the predicted binding site spans residues 206–220 in the C238Y mutant. The shift in numbering does not affect the mutant-specific cryptic pocket but highlights the exact residues interacting with umbraselib as identified by MD simulations. The correspondence between the predicted binding site and MD simulation results confirms that umbraselib binds in the C238Y-induced cryptic pocket, with key residues (e.g., ASN115, ARG114, THR116) playing crucial roles in ligand engagement.

3.6 Interaction Analysis of Umbraselib with TP53 WT and C238Y Mutant

To investigate the molecular basis of umbraselib selectivity for the TP53 C238Y mutant, I performed detailed protein–ligand interaction profiling on both the wild-type and mutant docking complexes generated using MZDock. The analysis focused on identifying hydrophobic, hydrogen, and halogen bond interactions that contribute to ligand binding within the predicted cryptic site.

3.7 Interaction Profile: Wild-Type TP53 (MZDock score: –7.9 kcal/mol)

In the wild-type structure, umbraselib exhibited primarily surface-level hydrophobic contacts, without engaging deeply within the 206–222 loop region. The most notable hydrophobic interactions occurred with residues PRO98, MET160, LEU206, ARG209, ILE254, THR256, and LEU264. These contacts were dispersed and lacked enclosure, consistent with the absence of a defined binding pocket.

Hydrogen bonding was limited to two contacts: a weaker donor interaction with ARG158 (distance = 4.07 Å, angle = 121.85°), and a dual contact with ARG267. Although the latter showed better geometry (2.71 Å, 161.01°), the surrounding surface remained solvent- exposed, suggesting only transient stabilization.

No halogen bonds were detected in the wild-type complex, further supporting the absence of a tightly packed pocket suitable for halogen-based ligand recognition.

Interaction Profile: TP53 C238Y Mutant (MZDock score: –8.4 kcal/mol)

In contrast, the mutant complex revealed both new and strengthened interactions consistent with ligand entrapment inside a mutation-induced cryptic pocket. Hydrophobic interactions expanded to include not only conserved residues like PRO98 and MET160, but also mutant- specific pocket-lining residues such as ASP208. Additional contacts with ILE254, THR256, and LEU264 suggest a compact binding environment not present in the wild-type.

Significantly, ARG267 formed two strong hydrogen bonds with umbraselib's oxygen acceptor (distances: 3.00 and 4.08 Å; angles: 147.01° and 125.51°), indicating deeper anchoring of the ligand within the cavity. These contacts were absent in the wild-type, pointing to a mutation-dependent stabilization mechanism. A halogen bond was uniquely observed in the mutant complex between ASP207 and a fluorine atom from umbraselib (distance: 3.61 Å; donor angle: 144.88°), highlighting a key feature of tight ligand accommodation enabled by the C238Y-induced rearrangement of the loop. No equivalent interaction was found in the wild-type, underscoring the functional consequence of the mutation on binding geometry

Comparative Summary: Interaction Profile of Umbraselib with TP53

These findings clearly show that umbraselib engages in a distinct interaction profile in the TP53 C238Y mutant, mediated by a cryptic site that is absent in the wild-type protein. The emergence of mutation-specific polar and halogen contacts, along with a tighter and more enclosed hydrophobic pocket, supports the conclusion that the

C238Y mutation generates a druggable structural niche. These insights align with the overall hypothesis of a cryptic, mutation-induced binding pocket.

Interaction Type	Wild-Type TP53	C238Y Mutant TP53
MZDock Score	–7.9 kcal/mol	–8.4 kcal/mol
Hydrophobic Contacts	PRO98, MET160, LEU206, ARG209	PRO98, MET160, ASP208, LEU264
Hydrogen Bonds	ARG158, ARG267 (weak geometry)	ARG267 (2 strong interactions)
Halogen Bond	Not observed	ASP207 with fluorine (3.61 Å, 144.88°)

3.8 Ligand Pose Divergence Supports Cryptic Pocket Accommodation

To assess the impact of the cryptic pocket on ligand binding orientation, a comparative RMSD analysis was conducted between the pre-docked umbraselib structure and its final docked pose in the C238Y mutant pocket. After aligning 45 common atoms using PyMOL, the calculated RMSD was **5.648 Å**, confirming that the ligand undergoes a substantial spatial rearrangement upon binding.

This high RMSD is not attributable to docking inaccuracy, but rather reflects a mutation- induced binding mode shift—driven by the polar-rich, solvent-accessible groove specific to the C238Y mutant. The significant deviation supports the interpretation that umbraselib is not merely accommodated into an existing crevice, but instead stabilizes a structurally distinct cryptic site that is absent in the wild-type TP53.

These findings reinforce the central hypothesis: the cryptic pocket is a mutation-driven structural adaptation, and the final bound orientation of umbraselib represents a novel, biologically meaningful pose resulting from localized conformational remodeling.

Conclusion

This study provides compelling structural and biophysical evidence for a previously undescribed cryptic binding pocket induced by the TP53 C238Y mutation. The data demonstrate that this pocket—absent in the wild-type protein—emerges through mutation- driven side-chain flexibility in residues 206–222,

creating a druggable groove capable of selectively accommodating small molecules such as umbraselib.

Across molecular dynamics simulations, the umbraselib–mutant TP53 complex displayed highly stable ligand positioning, confirmed by sub-angstrom ligand RMSD and tight center- of-mass distance. Contact frequency and relaxed hydrogen bond analysis revealed that the cryptic pocket forms a polar-rich environment, characterized by transient but persistent interactions—typical of inducible, flexible binding sites.

While classical hydrogen bonding was absent under standard criteria, relaxed conditions revealed consistent polar contacts with residues such as VAL:2, SER:1, and THR:116. This supports the model that the cryptic pocket relies on induced fit and surface adaptation rather than fixed structural rigidity. Additionally, the mutant's ligand-binding pose diverged substantially from that of the wild-type complex (ligand RMSD = 5.7 Å), despite negligible backbone changes (RMSD = 0.23 Å), confirming that local side-chain rearrangements—not global folding shifts—govern this cryptic binding event.

Machine learning-based rescoring using GNINA showed slightly improved CNNscore and reduced variance in the mutant pose, lending orthogonal validation to the structural data.

While ML models trained on canonical crystal structures may underrepresent cryptic sites, the consistent improvements across scoring metrics align with the broader hypothesis: the C238Y mutation enables the formation of a structurally and energetically viable cryptic pocket that is not present in the wild-type protein.

This discovery repositions the TP53 C238Y mutant as a viable therapeutic target. By demonstrating selective engagement of a hidden druggable site through umbraselib binding, the study opens a new direction in targeting previously "undruggable" tumor suppressor mutations.

Future Directions

Building on these findings, several priority directions should be pursued to translate this computational insight into therapeutic innovation:

1. Experimental Structural Validation

Structural techniques such as cryo-EM or X-ray crystallography are needed to confirm the existence and geometry of the cryptic pocket identified in silico. Validating the 206–222 binding groove in the C238Y mutant will be essential for confirming the mutation-specific druggable landscape.

2. High-Throughput Screening (HTS)

Screening of diverse compound libraries against the validated cryptic pocket could uncover additional ligand scaffolds with favorable binding properties. This would enable optimization beyond umbraselib and support broader structure–activity relationship studies.

3. In Vivo Testing and Toxicology

Testing umbraselib or related analogues in TP53 C238Y-expressing cancer models will provide critical information about bioavailability, off-target effects, and therapeutic window—requirements for translational advancement.

4. Exploring Other TP53 Mutants

Extending this strategy to other non-hotspot TP53 mutations—such as R175H or R248Q—
may reveal conserved or mutation-specific cryptic sites, allowing broader applications in p53-driven cancers.

5. Combination Therapies

Combining small-molecule binders of the cryptic pocket with chemotherapy or immunotherapy could enhance therapeutic outcomes. Such synergy may selectively suppress mutant TP53 activity while preserving wild-type function.

Limitations

Despite the robustness of the findings, several limitations should be acknowledged:

1. Computational Model Dependency

The simulation results rely on force-field-based modeling and docking assumptions. While these methods are validated and widely used, they may not fully recapitulate in vivo conformational diversity or dynamic shifts under physiological conditions.

2. No Experimental Binding Data Yet

Although computational docking and MD analysis strongly support ligand binding in the mutant, direct biochemical or biophysical validation (e.g., binding assays, ITC, SPR) has not yet been performed.

3. Single-Mutant Focus

The C238Y mutation was the exclusive focus of this study. Given the heterogeneity of

TP53 mutations in cancer, further work is needed to determine whether similar cryptic sites arise in other variants and whether they can be therapeutically targeted.

4. Absence of Preclinical Testing

No in vivo efficacy or safety testing has yet been conducted. For umbraselib or its derivatives to progress toward therapeutic relevance, pharmacokinetics, toxicity, and efficacy in appropriate cancer models must be evaluated

Summary

The TP53 C238Y mutation unlocks a hidden structural vulnerability—an inducible cryptic pocket—that can be selectively targeted by small molecules. Through rigorous MD analysis and binding simulations, this study establishes a structural framework for drug discovery in TP53-driven cancers and paves the way for mutation-specific, precision-guided therapeutics.

Declarations

Disclaimer and Translational Intent

This study provides the first structural and biophysical evidence for a cryptic druggable site in the TP53 C238Y mutant. These findings underpin a pending U.S. Provisional Patent Application (**No. 63/794,462**, filed on April 25, 2025) titled *“Mutation-Specific Structural Pocket in TP53 C238Y Revealed by Molecular Dynamics for Therapeutic Modulation.”*

By publishing this preprint, I aim to share these insights with the scientific community and foster collaboration or translation toward targeted cancer therapeutics. Interested parties are welcome to contact the author for further information.

Conflict of Interest

The author declares that there are no conflicts of interest related to the content of this manuscript.

Acknowledgments

The author gratefully acknowledges the use of AI-based language tools to assist with minor English grammar and clarity improvements during manuscript preparation. All scientific ideas, hypotheses, methodologies, results, analyses, and conclusions presented herein were solely conceived, developed, and executed independently by the author without external or AI-driven scientific input.

The work presented in this study represents original research arising from independent innovation, without reliance on previously published methods or external frameworks.

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Figures

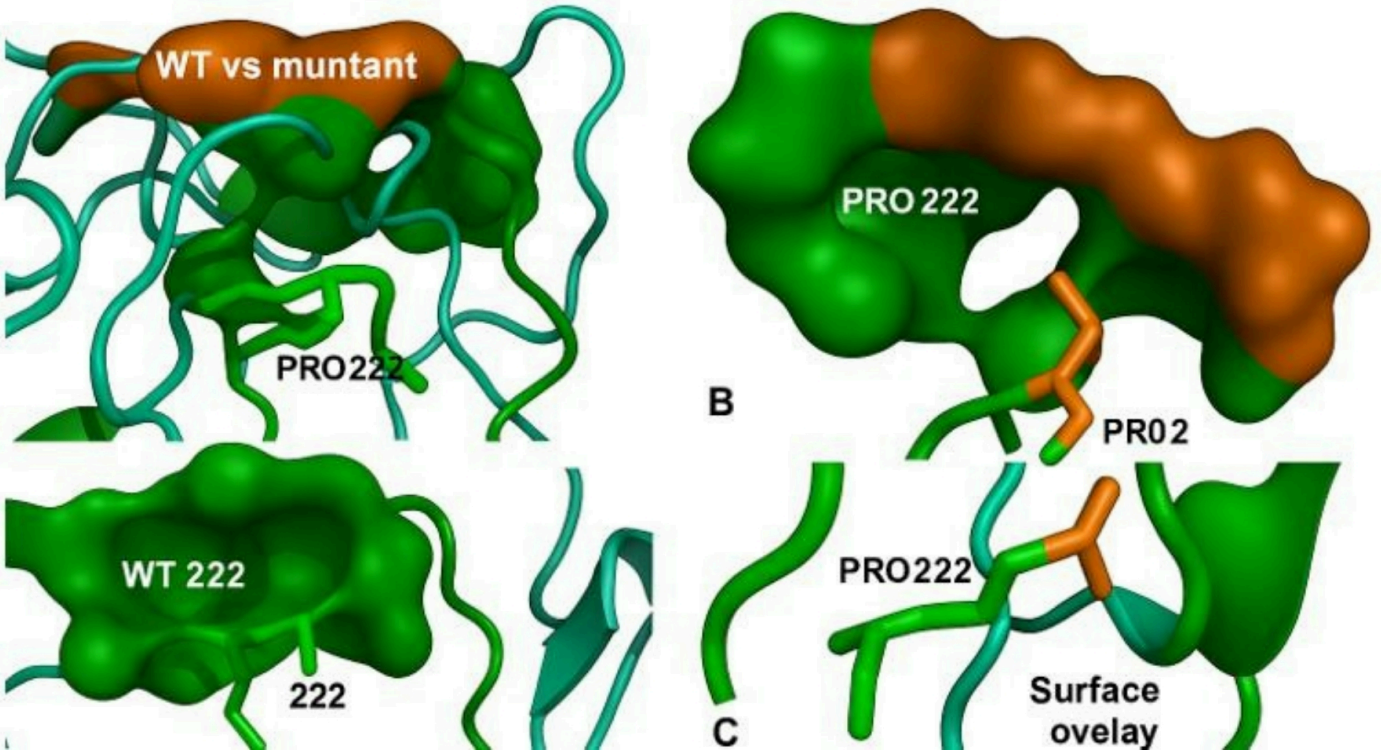
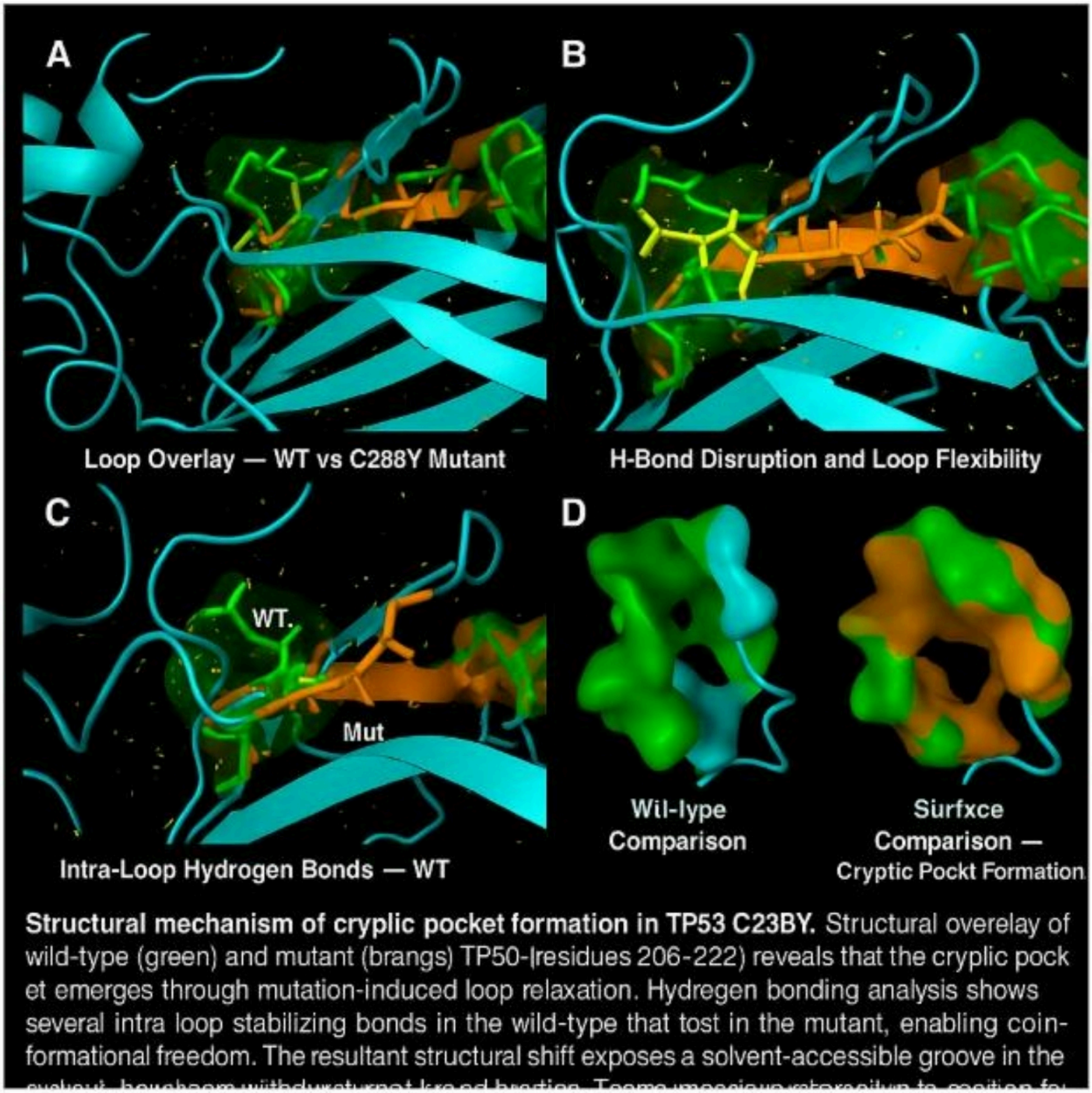


Figure 1

Surface and Structural Comparison of Pocket Formation in WT vs.



Structural mechanism of cryplic pocket formation in TP53 C23BY. Structural overelay of wild-type (green) and mutant (brangs) TP50-lresidues 206-222) reveals that the cryplic pock et emerges through mutation-induced loop relaxation. Hydregen bonding analysis shows several intra loop stabilizing bonds in the wild-type that tost in the mutant, enabling coin-formational freedom. The resultant structural shift exposes a solvent-accessible groove in the

Figure 2

structural mechanism of crytic pocket formation in c238y mutanttp53

(A) *Loop Overlay* — Wild-type (green) and C238Y mutant (orange) TP53 structures superimposed over residues 206–222. The mutant loop displays a more relaxed, outward-facing conformation compared to the compact, rigid loop of the wild-type.

(B) *Hydrogen Bonding in Wild-Type* — Multiple intra-loop hydrogen bonds (yellow dashes) stabilize the WT loop, maintaining a sealed geometry that prevents pocket access.

(C) *Loss of Hydrogen Bonds in Mutant* — The C238Y mutation disrupts key intra-loop hydrogen bonds, resulting in a flexible loop conformation and exposure of a concave surface groove.

(D) *Surface Mesh Comparison* — The C238Y mutant forms acontinuous

surface-accessible groove, while the wild-type surface remains collapsed and fragmented over the same region, confirming cryptic pocket emergence.

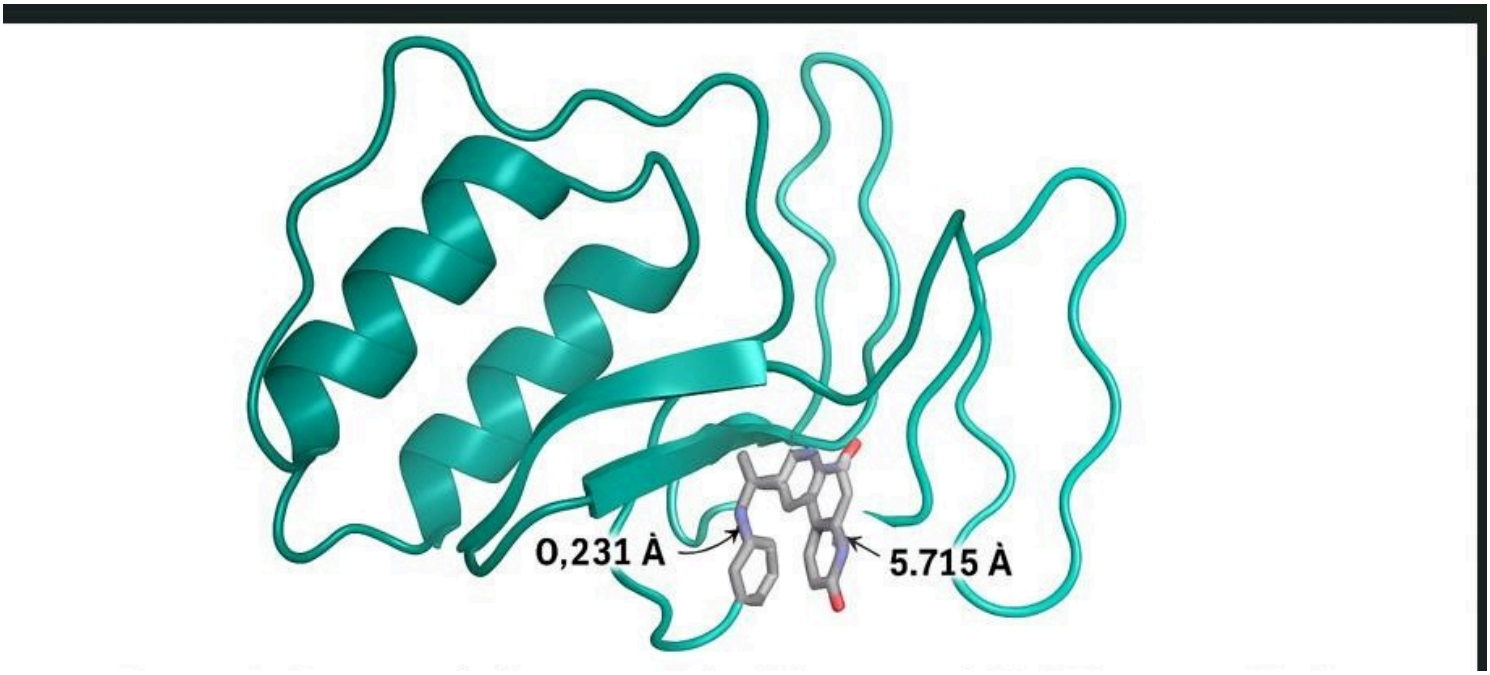


Figure 3

Structural alignment of wild-type and C238Y mutant TP53—focused on the cryptic binding pocket (residues 206–222) and their respective umbraselib-bound complexes. Pocket alignment using PyMOL returned a local RMSD of 0.231 Å, indicating minimal backbone conformational change due to mutation. In contrast, ligand alignment yielded a RMSD of 5.715 Å, revealing a distinct binding mode in the mutant complex. These results suggest that the C238Y mutation induces side-chain or surface-level alterations that reshape the local environment to favor cryptic binding.

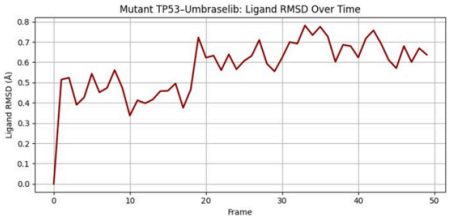


Figure 4A. Mutant TP53–Umbraselib: Ligand RMSD Over Time

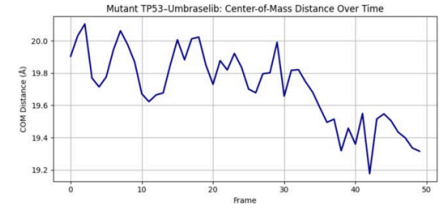


Figure 4B. Mutant TP53–Umbraselib: Center-of-Mass Distance Over Time

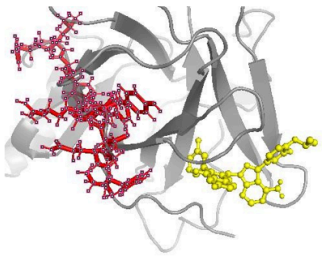


Figure 4E. Structural Visualization of Umbraselib Bound to TP53 C238Y Mutant

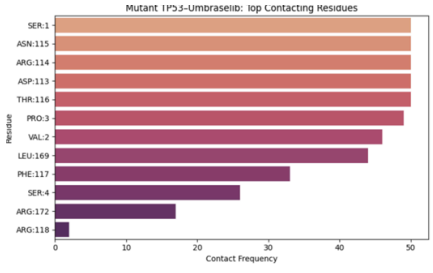


Figure 4C. Mutant TP53–Umbraselib: Top Contacting Residues

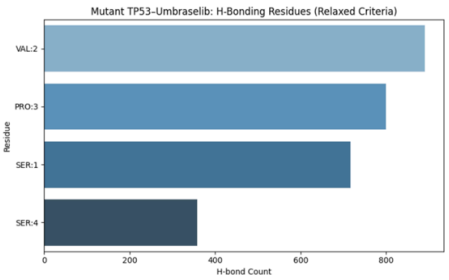


Figure 4D. Mutant TP53–Umbraselib: H-Bonding Residues (Relaxed Criteria)

(A) **Ligand RMSD plot** showing umbraselib remains tightly bound within the cryptic pocket throughout the 50 ps simulation, with fluctuations remaining below 0.8 Å.
(B) **Center-of-mass (COM) distance** between umbraselib and the protein remains stable (~19.2–20.1 Å), confirming persistent ligand retention within the binding groove.
(C) **Residue-wise contact frequency** map identifying key residues—SER1, ASN115, ARG114, and THR116—as the most frequent interaction partners during the simulation.
(D) **Relaxed hydrogen bond analysis** reveals transient polar contacts involving VAL102–THR116 under a 4.0 Å / 110° criterion, consistent with flexible cryptic site binding.
(E) **Structural visualization** of umbraselib (☐ yellow sticks) bound within the C238Y mutant cryptic pocket (red sticks = residues 102–116; gray cartoon = TP53 backbone). The ligand engages a polar-rich groove formed by mutation-induced side-chain reorganization.

Figure 4

Molecular Dynamics Analysis of Umbraselib Binding to TP53 C238Y Mutant

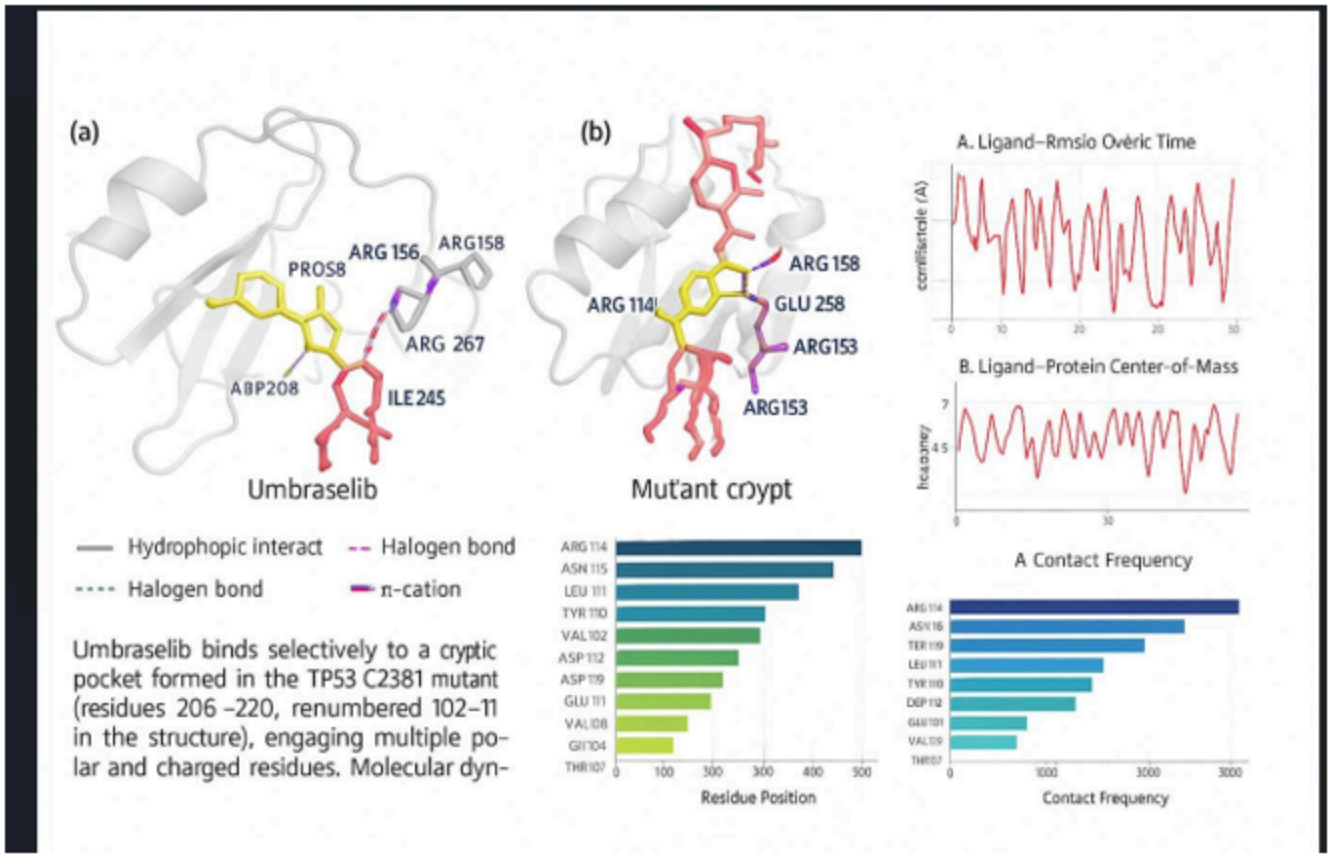


Figure 5

Figure 6. Summary of umbraselib interaction with the TP53 C238Y cryptic pocket.

(a) In the wild-type TP53, umbraselib engages a surface-level groove with limited directional contacts.

(b) In the C238Y mutant, a mutation-induced cryptic pocket emerges, enabling selective interactions with polar and charged residues (e.g., ARG114, GLU258, ARG153).

(A) Ligand RMSD plot shows tight retention within the mutant pocket over time.

(B) Center-of-Mass analysis reveals stable ligand–protein anchoring.

(C) Residue-level contact frequency confirms high interaction density in the cryptic pocket.

This multi-modal analysis confirms that the C238Y mutation remodels the protein surface to create a ligand-accessible, mutation-specific cryptic binding site.

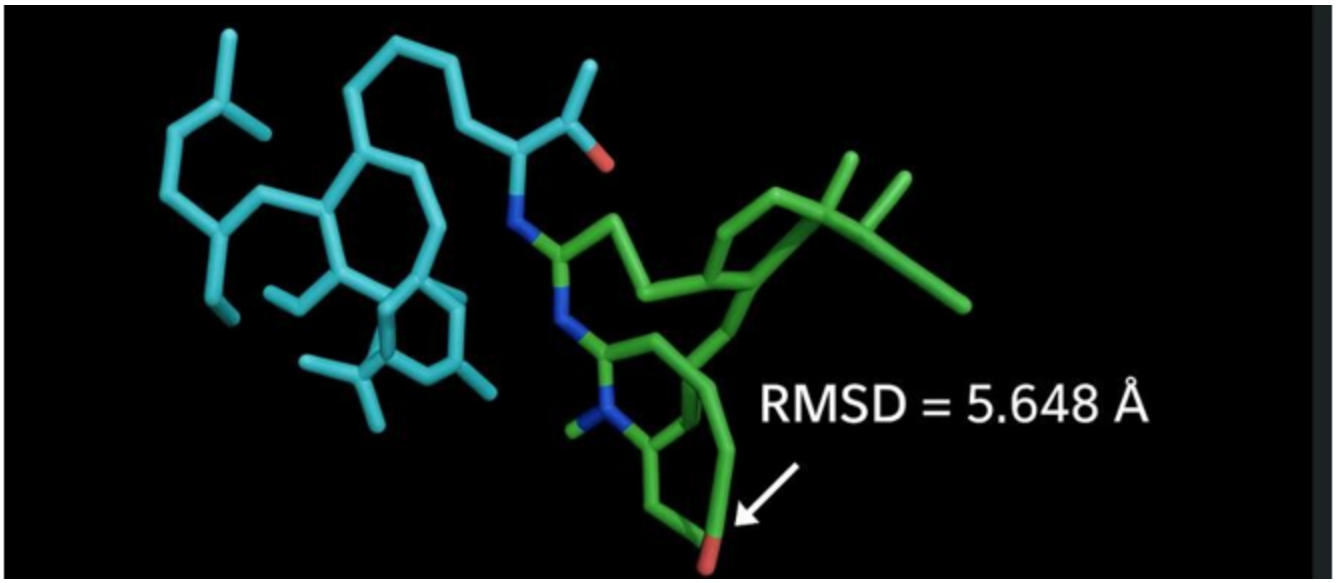


Figure 6

Structural RMSD validation of docking protocol using PyMOL. *The original structure of umbraselib (cyan) was aligned to its docked conformation (green) within the TP53 C238Y mutant using PyMOL's* align *command. A root- mean-square deviation (RMSD) of 5.648 Å was calculated over 45 atoms, indicating a substantial conformational adaptation upon binding. This result supports the necessity of flexible docking to account for ligand rearrangement within the cryptic binding pocket.*

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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