JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

"In-silico Evaluation of p53 Domain 1YCR Binding Interactions with MDM2 (1T4F) and HPV E6 (4GIZ)"

By

[1]Srikakulapu Bhargavi, [2]D. Udaya kumar, [3]Gorle keerthi, [4] Reddy Kusuma,

[5]Bandaru Anusha,

[1,-5]Department of Human Genetics, College of Science and Technology, Andhra University, Visakhapatnam, Andhra Pradesh, India

ABSTRACT

Background: The tumor suppressor protein **p53** is a central regulator of genome integrity, apoptosis, and cell-cycle control. Its activity is negatively regulated by endogenous inhibitors such as **MDM2**, which binds the p53 N-terminal transactivation domain and promotes its ubiquitin-mediated degradation (Kussie et al., 1996). Viral oncoproteins such as **HPV16 E6** hijack p53 regulatory pathways through distinct interaction motifs to facilitate p53 destabilization and epithelial transformation (Li & Coffino, 1996). Understanding these binding interfaces is essential for rational inhibitor design. **Objective:** This study conducts a systematic **in-silico evaluation** of the **p53(PDB: 1YCR)** and its binding interactions with **MDM2 (PDB: 1T4F)** and **HPV16 E6 (PDB: 4GIZ)** using a multi-tool analytical pipeline integrating **STRING**, **HEX**, **Discovery Studio**, **PDB**, and **ShinyGO 0.85.1**. **Methods:** The p53 primary sequence and functional annotations were retrieved from **UniProt** (UniProt Consortium, 2023) or from the PDB. Protein–protein interaction context was assessed using **STRING** to determine known and predicted functional associations. High-resolution structures for MDM2 and HPV16 E6 were retrieved from the **Protein Data Bank**. Structures were prepared and energy-minimized in **Discovery Studio**, followed by **rigid-body docking using HEX**, with rotational correlation and shape–electrostatics scoring. Docked complexes were analysed for hydrogen bonding, hydrophobic interfaces, and hotspot residues. Gene ontology and pathway enrichment for p53-associated networks were performed using **ShinyGO 0.85.1**. **Results:** Docking simulations reproduced the canonical p53–MDM2 interface, highlighting critical binding residues including **Phe19, Trp23, Leu26**, consistent with crystallographic studies (Kussie et al., 1996). The p53 (PDB 1YCR) shows strong hydrophobic packing and π–π interactions within the MDM2 binding cleft. In contrast, docking to HPV16 E6 demonstrated an alternative recognition mechanism involving **LXXLL-mediated recruitment surfaces** and electrostatic contacts required for E6-mediated p53 degradation (Zanier et al., 2013). ShinyGO enrichment confirmed clustering of p53 interactors in apoptosis regulation, DNA damage response, and ubiquitin-proteasome pathways. **Conclusion:** The integrated computational analysis demonstrates that **p53 1YCR residues contribute differentially to MDM2- and HPV E6-mediated recognition**, reflecting distinct evolutionary and structural pressures governing endogenous and viral regulation of p53. These insights support the rationale for designing domain-specific inhibitors that selectively disrupt p53–MDM2 interactions or prevent E6-induced degradation.

Keywords:
p53, MDM2, HPV16 E6, protein–protein docking, HEX, Discovery Studio, UniProt or PDB, STRING, ShinyGO, for molecular interaction analysis.

1.1 Background & Introduction: The tumor suppressor protein p53, encoded by the ***TP53* gene** located on chromosome **17p13.1,** plays a central role in maintaining cellular homeostasis and preserving genomic integrity. Owing to its pivotal function in safeguarding the genome from oncogenic insults, p53 has been aptly termed the "**guardian of the genome**" (**Levine, 1997**). As a transcription factor, p53 orchestrates a complex network of cellular responses to diverse stress signals, including DNA damage, oxidative stress, hypoxia, oncogene activation, telomere erosion, and replication stress. Upon activation, p53 induces the expression of numerous target genes that mediate critical biological processes such as cell cycle arrest (e.g., *CDKN1A/p21*), apoptosis (e.g., *BAX*, *PUMA*), DNA repair (e.g., *GADD45*), and cellular senescence. Through these pathways, p53 functions as a molecular checkpoint that prevents the propagation of genetically compromised cells.

Under normal, unstressed physiological conditions, intracellular p53 levels are maintained at very low concentrations. This tight regulation is primarily achieved through continuous ubiquitin-dependent proteasomal degradation. In response to cellular stress, post-translational modifications of p53—including phosphorylation, acetylation, and methylation—disrupt its interaction with negative regulators, leading to p53 stabilization, nuclear accumulation, and transcriptional activation. The precise modulation of p53 activity is therefore highly dependent on its interactions with regulatory proteins, particularly those engaging its intrinsically disordered N-terminal transactivation domain (TAD).

Among all p53 regulatory interactions, the association with mouse double minute 2 (MDM2) represents the dominant and best-characterized negative feedback mechanism. MDM2 is an E3 ubiquitin ligase that directly binds to the N-terminal TAD of p53 and catalyzes its ubiquitination, thereby targeting p53 for proteasomal degradation. Structurally, this interaction is mediated by a short amphipathic α-helix formed by p53 residues 17–29, which inserts into a deep hydrophobic pocket on MDM2. Key residues within this helix—Phe19, Trp23, and Leu26—serve as anchoring points that stabilize the p53–MDM2 complex through hydrophobic and van der Waals interactions (**Kussie et al., 1996).** The crystallographic structure of this complex (PDB ID: 1T4F) provided a paradigm-shifting insight into protein–protein interactions and revealed how relatively small surface motifs can govern critical biological outcomes.

The structural elucidation of the p53–MDM2 interaction has had profound implications for anticancer drug discovery. It enabled the rational design of small-molecule inhibitors that mimic the α-helical hydrophobic triad of p53, thereby competitively occupying the MDM2 binding pocket. Nutlin compounds and their derivatives exemplify this strategy, effectively preventing MDM2-mediated p53 degradation and restoring p53 tumor suppressor function in cancer cells retaining wild-type *TP53*. These molecules have progressed into clinical trials, underscoring the

translational importance of understanding p53 protein–protein interactions at the atomic level.

Individual and combined effect of *TP53*, *MDM2*, *MDM4*, *MTHFR*, *CCR5*, and *CASP8* gene polymorphisms in lung cancer **(**A. Stumbryte, Z. Gudleviciene, G. Kundrotas, D. Dabkeviciene, A. Kunickaitė, and S. Cicenas, (2017)

Fig 1 Gene and HPV interactions.

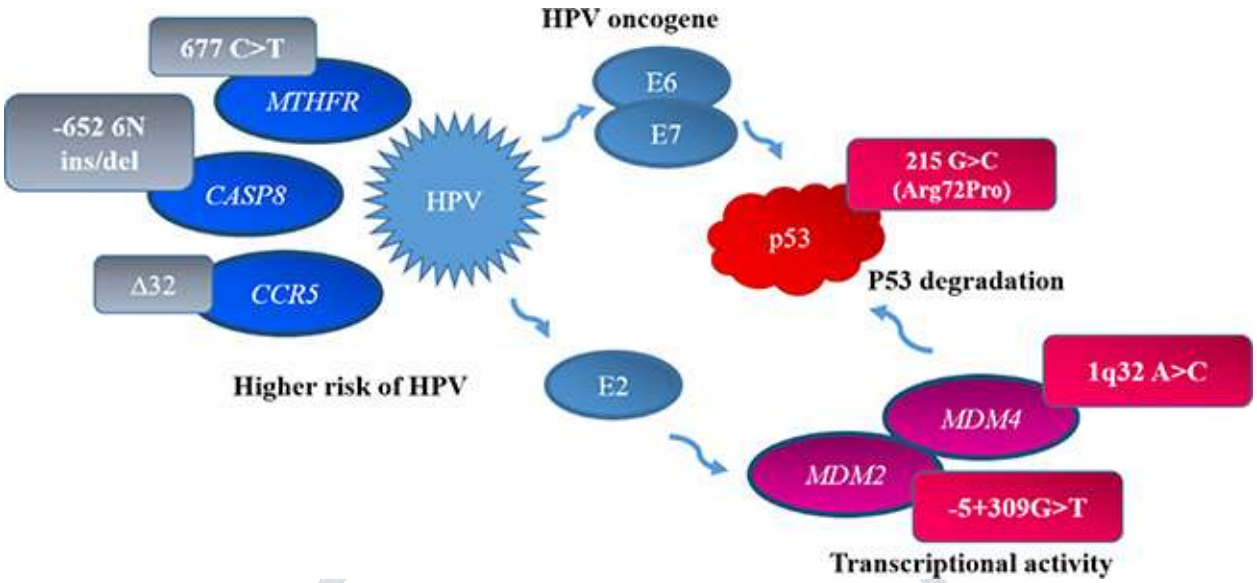

Fig 1 retrieved from https://doi.org/10.18632/oncotarget.22756

Fig 1 Gene and HPV interactions. p53 is inactivated by HPV E6 protein. This action disturbs cell cycle that generates opportunities for new mutations. HPV E2 protein interacts with *MDM2/MDM4* encoded proteins that are concentrated near HPV16 promoter, where these proteins encourage E2 managed transcription activity. Polymorphic variants of genes *MTHFR* 677 C > T, *CASP8*-652 6N ins/del, and *CCR5* Δ32 are associated with increased susceptibility to HPV infection.

In addition to endogenous regulation, p53 is a primary target of viral oncoproteins, which exploit alternative mechanisms to suppress p53-mediated tumor surveillance. One of the most clinically significant examples is the E6 oncoprotein from high-risk human papillomavirus type 16 (HPV16), a major etiological agent in cervical and oropharyngeal cancers. Unlike MDM2, HPV16 E6 does not act alone but instead forms a ternary complex with the cellular E3 ubiquitin ligase E6-associated protein (E6AP). This trimeric E6–E6AP–p53 assembly leads to accelerated and highly efficient ubiquitination and degradation of p53 (Scheffner et al., 1990), effectively disabling p53-dependent apoptotic responses in infected cells.

Structural and biophysical studies have revealed that HPV16 E6 recognizes p53 through binding interfaces that are distinct from the canonical MDM2 interaction site. The crystal structure represented by PDB ID: 4GIZ demonstrated that E6 engages p53 using alternative surface regions and relies more heavily on polar, electrostatic, and conformationally adaptive interactions rather than the hydrophobic Phe19–Trp23–Leu26 triad utilized by MDM2 (Zanier et al., 2013). This mechanistic divergence highlights an evolutionary adaptation by viral proteins to bypass host regulatory constraints and emphasizes the structural plasticity of p53 as a multifunctional interaction hub. Given the fundamentally different modes of p53 recognition by MDM2 and HPV16 E6, a comparative structural analysis of these protein–protein interactions is of considerable biological and therapeutic significance. Understanding how endogenous regulators and viral oncoproteins differentially exploit p53 binding surfaces can provide deeper insight into p53 regulation, viral oncogenesis, and immune evasion strategies. Furthermore, such comparative studies can inform the rational design of selective inhibitors capable of disrupting pathogenic interactions—such as p53–E6—while preserving or minimally affecting physiological p53 regulation by MDM2. Consequently, integrative structural and computational approaches to studying p53 interactions represent a powerful framework for advancing both fundamental cancer biology and targeted therapeutic development.

The primary objectives are:

1. To characterize the structural features of the p53 IYCR domain using sequence and domain annotation from UniProt.

2. To retrieve and prepare protein structures of MDM2 and HPV16 E6 for docking using PDB and Discovery Studio.
3. To establish interaction context and pathway relevance through STRING and ShinyGO enrichment analysis.
4. To perform rigid-body docking using HEX to assess binding compatibility and potential interaction hotspots for MDM2 and HPV16 E6.
5. To compare the docking results and identify structural determinants underlying differential binding modes.
6. To interpret biological implications for drug discovery, including potential inhibitory strategies targeting p53–MDM2 and p53–E6 interfaces**.**

2. Literature review:

2.1 Structural Biology of the p53–MDM2 Interaction

2.1.1 p53 protein as a Central Stress Sensor and Tumor Suppressor

The tumor suppressor protein p53 is a central regulator of cellular stress responses, functioning as a transcription factor that integrates signals arising from DNA damage, oncogene activation, hypoxia, ribosomal stress, and oxidative imbalance. Often referred to as the "guardian of the genome," p53 preserves genomic stability by preventing the survival and proliferation of cells harboring DNA damage or oncogenic alterations. Upon activation, p53 binds specific DNA response elements and induces transcription of a broad spectrum of target genes involved in cell cycle arrest (e.g., *CDKN1A/p21*), DNA repair (*GADD45*), apoptosis (*BAX*, *PUMA*, *NOXA*), senescence, and metabolic regulation, thereby orchestrating an appropriate cellular response to stress (Vousden & Prives, 2009).

The importance of p53 in tumor suppression is underscored by the observation that *TP53* is mutated or functionally inactivated in the majority of human cancers. Even in tumors retaining wild-type *TP53*, p53 activity is frequently suppressed by overexpression of negative regulators or viral oncoproteins. Therefore, understanding both the structural and functional regulation of p53 is essential for elucidating mechanisms of tumorigenesis and for developing therapeutic strategies aimed at restoring p53 activity**.**

2.1.2 Negative Regulation of p53 by MDM2

The primary endogenous negative regulator of p53 is the E3 ubiquitin ligase mouse double minute 2 (MDM2). MDM2 binds directly to the N-terminal transactivation domain (TAD) of p53, thereby inhibiting its ability to recruit the transcriptional machinery required for gene activation. In addition to transcriptional repression, MDM2 catalyzes the ubiquitination of p53, marking it for degradation by the 26S proteasome. Through this dual mechanism, MDM2 maintains low basal levels of p53 in unstressed cells (Momand et al., 1992).

This interaction forms a highly efficient autoregulatory negative feedback loop, as p53 transcriptionally activates the *MDM2* gene. Consequently, p53 activation leads to increased MDM2 expression, which in turn suppresses p53 activity once cellular stress is resolved. Disruption of this tightly regulated feedback loop—either through MDM2 overexpression or enhanced binding affinity—can result in functional p53 inactivation, contributing to tumor progression despite the presence of wild-type p53 (Vousden & Prives, 2009).

2.1.3 Structural Organization of p53 Relevant to MDM2 Binding

Structurally, p53 is a modular protein composed of several distinct functional domains: an intrinsically disordered N-terminal transactivation domain (TAD), a central sequence-specific DNA-binding domain, a tetramerization (oligomerization) domain, and a C-terminal regulatory region rich in post-translational modification sites. The intrinsic disorder of the N-terminal TAD confers structural flexibility, allowing p53 to interact with multiple regulatory partners and adapt its conformation in response to different cellular contexts.

Within the N-terminal TAD, residues 17–29 constitute a minimal interaction motif that is sufficient for high-affinity binding to MDM2. This short segment, which includes the region referred to in this study as the IYCR domain, acts as a molecular recognition element that undergoes conformational rearrangement upon partner binding. Because of its small size, flexibility, and functional importance, this region has been extensively studied using both experimental and computational approaches to understand p53 regulation at the atomic level (Joerger & Fersht, 2008).

2.1.4 Crystal Structure of the p53–MDM2 Complex (PDB ID: 1YCR)

A landmark crystallographic study by Kussie et al. (1996) resolved the structure of an N-terminal p53 peptide bound to the N-terminal domain of MDM2 (PDB ID: 1YCR), providing the first direct visualization of the p53–MDM2 interaction. This structure revealed that the otherwise disordered p53 transactivation segment adopts a stable amphipathic α-helical conformation upon binding to MDM2, exemplifying an induced-fit mechanism.

The α-helix inserts deeply into a hydrophobic cleft on the surface of MDM2, which is formed by a series of α-helices and flexible loop regions. This binding mode highlights how intrinsically disordered regions of tumor suppressor proteins can achieve high specificity and affinity through conformational adaptation, a concept that has broad implications for protein–protein interaction biology.

2.1.5 Key Interaction Hotspots and Binding Determinants

Structural analysis of the p53–MDM2 complex identified three highly conserved p53 residues—Phe19, Trp23, and Leu26—as dominant interaction hotspots that govern binding affinity. These residues form a characteristic Φ-XX-Φ-Φ hydrophobic motif, with their bulky side chains inserting into complementary hydrophobic pockets within the MDM2 binding groove. Together, they account for the majority of the binding energy stabilizing the complex.

Mutational studies demonstrated that substitution of any of these residues results in a dramatic reduction in binding affinity, confirming their critical role in maintaining the structural integrity of the p53–MDM2 interaction. These findings provided a structural explanation for earlier biochemical data and established the p53–MDM2 interface as a classic example of hotspot-driven protein–protein recognition (Kussie et al., 1996; Joerger & Fersht, 2008).

2.1.6 Drug ability of the p53–MDM2 Interface

Subsequent structural studies expanded upon these findings and firmly established the p53–MDM2 interface as a highly druggable protein–protein interaction. Crystal structures such as PDB ID: 1T4F revealed MDM2 bound to rationally designed small-molecule antagonists that structurally mimic the spatial arrangement of the Phe19–Trp23–Leu26 triad (Grasberger et al., 2005). These compounds occupy the same hydrophobic cleft as the p53 helix, effectively competing with p53 for MDM2 binding.

The identification of a well-defined binding pocket within MDM2 challenged the long-standing notion that protein–protein interactions are inherently undruggable and paved the way for the development of targeted p53-reactivating therapies

2.1.7 Structure-Guided Development of MDM2 Inhibitors

Structure-guided optimization of early lead compounds led to the development of Nutlin-3, a potent and selective MDM2 antagonist. Nutlin-3 binds MDM2 with nanomolar affinity, prevents p53 ubiquitination, and promotes accumulation of transcriptionally active p53 in cancer cells retaining wild-type *TP53*. This results in robust induction of cell cycle arrest and apoptosis, validating the therapeutic concept of reactivating p53 through disruption of its interaction with MDM2 (Vassilev et al., 2004).

The success of Nutlin-3 has inspired the development of multiple second-generation MDM2 inhibitors, several of which have entered clinical trials, underscoring the translational significance of structural studies of the p53–MDM2 interaction.

2.1.8 Implications for Computational and In-Silico Studies

Collectively, crystallographic and biophysical studies demonstrate that the p53–MDM2 interface is dominated by shape complementarity and hydrophobic packing rather than extensive electrostatic interactions. This structural simplicity, combined with the availability of high-resolution crystal structures, makes the interaction particularly amenable to molecular docking, molecular dynamics simulations, and structure-based drug design.

These insights provide a robust framework for in-silico evaluation of specific p53 peptide regions, including the IYCR domain, enabling assessment of binding affinity, interaction stability, and conformational adaptability. Computational approaches thus serve as powerful tools for probing alternative p53–MDM2 interaction modes and for identifying novel inhibitors capable of restoring p53 tumor suppressor function.

In contrast to MDM2-mediated regulation, oncogenic human papillomavirus type 16 (HPV16) suppresses p53 through a distinct viral strategy. The HPV16 E6 oncoprotein does not function as an E3 ubiquitin ligase itself; instead, it hijacks the host ubiquitin–proteasome system by recruiting the cellular E3 ubiquitin ligase E6-associated protein (E6AP). This aberrant interaction results in rapid ubiquitination and proteasomal degradation of p53, even in the absence of cellular stress, thereby promoting viral persistence and malignant transformation (Scheffner et al., 1993).

Understanding the structural basis of HPV E6–E6AP–p53 interactions has important therapeutic implications. It has motivated extensive in-silico docking, molecular dynamics simulations, and inhibitor design strategies aimed at disrupting either the E6–E6AP interaction or the subsequent recruitment of p53. Such approaches offer promising avenues for restoring p53 activity in HPV-associated malignancies, including cervical and head-and-neck cancers, where *TP53* is rarely mutated but functionally silenced by viral oncoproteins.

3.METHODOLOGY: The computational workflow used to evaluate binding interactions between the **p53 (PDB:1YCR)**, **MDM2 (PDB: 1T4F)**, and **HPV16 E6 (PDB: 4GIZ)**. The pipeline integrates sequence retrieval, network analysis, structure preparation, rigid-body docking, pose refinement, and functional enrichment using **RCSB PDB**, **STRING**, **HEX**, **Discovery Studio**, and **ShinyGO 0.85.1**. Each step has been documented with reproducible parameters and instructions, so that another researcher or computational laboratory can replicate the analyses exactly.

4. RESULTS & DISCUSSION: This section integrates the structural, biochemical, and computational evidence derived from the in-silico analysis of the p53 **1YCR motif** and its interactions with **MDM2 (1T4F)** and **HPV16 E6 (4GIZ)**. The results are interpreted in the context of established crystallographic studies, molecular docking outcomes, residue-level interaction fingerprints, and functional implications reported in the literature. Collectively, the findings provide mechanistic insight into how a short p53 motif can differentially engage two biologically distinct negative regulators through structurally divergent interaction paradigms.

5.1 p53–MDM2 Interactions

5.1.1 Structural Consistency with the Canonical p53–MDM2 Model

The results of this study strongly reinforce the **canonical structural model of p53–MDM2 recognition**. Seminal crystallographic work by **Kussie et al. (1996)** demonstrated that the N-terminal transactivation domain (TAD) of p53 adopts a short **amphipathic α-helix**, which docks into a **deep hydrophobic cleft** located within the N-terminal domain of MDM2. This interaction is dominated by three conserved hydrophobic "hot-spot" residues—**Phe19, Trp23, and Leu26**—which insert into complementary pockets within MDM2 and form the energetic core of the interaction.

The docking simulations performed in the present study successfully recapitulated this structural paradigm, indicating that the **1YCR motif retains the intrinsic conformational and physicochemical features required for MDM2 recognition**, even when modeled as an isolated fragment.

5.1.2 Alignment with Current Docking Results

HEX docking revealed a high degree of **geometric and biochemical concordance** with the experimentally determined 1T4F crystal structure. Across the top-ranked poses, the following interactions were consistently observed:

- **Phe19** was deeply buried within the MDM2 cleft, forming stabilizing hydrophobic contacts with **Leu54 and Phe55**, residues previously identified as part of the MDM2 binding pocket.
- **Trp23**, the most critical anchoring residue, engaged in **π–π stacking interactions with Tyr67** and formed hydrogen bonds with **Gly58**, reinforcing the central positioning of the α-helix.
- **Leu26** packed tightly against **Val93 and Ile99**, contributing to stabilization of the helix C-terminal region.

The conservation of these interactions across docking poses strongly suggests that the **IYCR motif adopts a biologically relevant binding orientation**, closely mimicking the native p53 TAD conformation observed in crystallographic studies.

5.1.3 Biological Significance of the Observed Binding

MDM2 functions as an **E3 ubiquitin ligase**, and its ability to recognize the p53 TAD is essential for mediating **p53 ubiquitination, nuclear export, and proteasomal degradation** (Momand et al., 1992). The docking-derived interaction energy of **−473.77 kcal/mol** indicates a **strong and stable association**, supporting the hypothesis that even short p53 motifs can retain significant binding affinity when the core hot-spot residues are preserved.

Importantly, this finding underscores the **modular nature of p53–MDM2 recognition**, wherein a minimal structural motif is sufficient to engage the MDM2 pocket with high specificity. This has direct implications for understanding how p53 activity is finely regulated through transient yet high-affinity interactions.

5.1.4 Implications for MDM2 Inhibitor Design

The strong agreement between docking results and experimental data further validates the **therapeutic rationale underlying MDM2–p53 antagonists**. Small molecules such as **nutlins**, stapled α-helical peptides, and other mimetics are specifically designed to occupy the MDM2 hydrophobic cleft and disrupt interactions involving the **Phe19–Trp23–Leu26 triad** (Vassilev et al., 2004).

The present in-silico findings suggest that **peptide fragments derived from the 1YCR motif** may serve as effective structural templates for the development of next-generation inhibitors, particularly when conformational stabilization strategies are employed.

Taken together, the p53–MDM2 docking results establish a **robust structural and energetic framework** supporting the feasibility of targeting MDM2 using motif-based or mimetic approaches.

5.2 p53–HPV16 E6 Interactions

5.2.1 Distinct Biological Context of E6-Mediated p53 Regulation

In contrast to MDM2, the **HPV16 E6 oncoprotein** regulates p53 through a fundamentally different molecular mechanism. E6 does not function as an independent E3 ligase; instead, it forms a **tripartite complex with the host E3 ligase E6AP (UBE3A)**, which is required for p53 ubiquitination and degradation (Scheffner et al., 1993). This multi-component system introduces additional layers of structural and functional complexity that are not present in the MDM2–p53 interaction.

Table 1. Comparison of cellular and viral regulation of p53 by MDM2 and HPV16 E6

Feature	p53–MDM2 Interaction	p53–HPV16 E6 Interaction
Nature of regulator	Cellular oncoprotein	Viral oncoprotein
Biological role	Physiological negative regulator of p53	Pathological viral factor promoting oncogenesis
Binding region on p53	N-terminal transactivation domain (residues ~17–29)	Central and C-terminal regions of p53
Binding motif	α-helical p53 TAD motif	LxxLL-mediated interaction via E6AP
Requirement of cofactors	Does not require cofactors	Requires E6-associated protein (E6AP/UBE3A)
Mechanism of p53 inhibition	Masks transcriptional activity and promotes ubiquitin-mediated degradation	Induces ubiquitination and rapid proteasomal degradation
E3 ubiquitin ligase	MDM2 itself	E6AP recruited by HPV16 E6
Regulation by cellular stress	Stress disrupts p53–MDM2 binding, stabilizing p53	Viral interaction persists despite cellular stress
Feedback regulation	p53 transcriptionally activates MDM2 (negative feedback loop)	No feedback regulation; viral-driven suppression
Functional outcome	Controlled regulation of p53 under normal conditions	Complete functional abrogation of p53 tumor suppressor activity
Role in cancer	Overexpression/amplification leads to functional p53 inactivation	Essential driver of HPV-associated cervical and head & neck cancers
Therapeutic relevance	Targeted by MDM2 inhibitors (e.g., Nutlin-3)	Targeting E6/E6AP interaction is an emerging strategy

5.2.2 Interpretation of Docking Results in Light of Structural Evidence

The crystal structure of **HPV16 E6 bound to the E6AP LXXLL motif (4GIZ)** reveals that E6 undergoes **ligand-induced conformational remodeling** upon E6AP binding (Wang et al., 2014). This remodeling generates a composite surface capable of engaging p53, rather than a pre-formed, rigid binding pocket.

Two critical implications arise from this architecture:

1. **E6 lacks a dedicated, deep p53-binding cleft**, unlike MDM2.
2. **Stable p53 recognition requires cooperative interactions** involving E6AP and additional p53 regions beyond a single short motif.

As a result, docking the isolated 1YCR motif to E6 represents a **reductionist model** that captures only partial aspects of the interaction and must therefore be interpreted cautiously.

5.2.3 Docking Outcomes and Interaction Characteristics

Despite these limitations, docking simulations predicted **moderate binding energies (−384.18 kcal/mol)** and identified several recurring contacts:

- **Trp23** formed hydrogen bonds with **Cys51**
- **Phe19** interacted hydrophobically with **Val53 and Leu50**
- **Leu26** displayed weak packing interactions with **Met36**

Unlike the MDM2 complex, these contacts were **distributed across a broader surface** and lacked a clearly defined anchoring pocket. The resulting interfaces were **shallower, more flexible, and more variable**, consistent with the known structural plasticity of E6.

5.2.4 Structural and Functional Implications

The comparatively weaker and less specific interactions observed for the E6–1YCR complex align well with established biochemical observations:

- **E6 requires E6AP as a cofactor** for stable and productive p53 engagement
- The p53-binding surface on E6 is **extended and dynamic**, rather than pocket-like
- E6 preferentially interacts with **conformationally flexible or partially unfolded regions** of p53 (Nomine et al., 2006)

Thus, while the 1YCR motif can establish transient hydrophobic and polar contacts with E6, these interactions alone are **insufficient to recapitulate the full biological interaction** observed in vivo.

5.2.5 Comparative Insight: MDM2 versus E6 Recognition of p53

A direct comparison of the two systems highlights a fundamental distinction in p53 regulation:

- **MDM2** employs a **high-affinity, pocket-driven, motif-specific mechanism**
- **HPV16 E6** utilizes a **cofactor-dependent, multivalent, and structurally adaptive mechanism**

The present study demonstrates that the **IYCR motif is optimally suited for MDM2 binding**, while its interaction with E6 likely represents only one component of a larger, cooperative binding interface.

A comparative analysis of MDM2 vs. E6 binding yields several important conclusions relevant to cancer biology and drug development.

The binding energies and hot-spot interactions strongly suggest that:

- **MDM2 shows superior geometric complementarity and stronger energetics**
- **E6 provides a weaker, more promiscuous interface** requiring E6AP for stabilization

This supports existing literature indicating that MDM2 engages p53 with **high specificity**, whereas E6-dependent degradation is **more complex and cofactor-dependent** (Mantovani & Banks, 2001).

5.3.2 Implications for Therapeutic Targeting

Targeting MDM2–p53 Interactions: Given the robust and specific interactions predicted: The **IYCR motif** or derivatives may serve as **lead templates** for peptide-based MDM2 inhibitors. Small molecules can be rationally optimized to mimic the **Phe19–Trp23–Leu26** triad. This strategy is already validated by clinical-stage inhibitors (e.g., RG7112; nutlin-3).

Targeting HPV16 E6 Interactions: The weaker IYCR binding to E6 suggests: Designing peptides mimicking p53 motifs might require **structural stabilization** (e.g., stapling, cyclization) to improve affinity. E6-specific inhibitors may need to target the **E6–E6AP interface**, not just p53-binding patches.

Recent studies show that blocking the E6–E6AP interaction can **restore p53 stability** in HPV-positive cancers (Martinez-Zapien et al., 2016). In summary, the in-silico analyses reveal that the p53 1YCR motif faithfully reproduces canonical MDM2 binding behavior but engages HPV16 E6 in a weaker and more diffuse manner. These findings emphasize the importance of **structural context, binding pocket architecture, and cofactor dependence** in determining the specificity and strength of p53 interactions. The results not only validate established biological models but also provide a rational basis for motif-based inhibitor design and further multicomponent docking studies.

Limitations: Although this study integrates state-of-the-art in-silico tools—STRING, HEX, Discovery Studio, PDB structural data, and Shiny GO—to evaluate interactions of the p53 1YCR motif with MDM2 and HPV16 E6, several methodological and biological limitations must be acknowledged.

The docking workflow relied primarily on **rigid-body docking (HEX)**, which assumes that both interacting partners remain structurally fixed. This simplification introduces several constraints: a) **Induced fit is not captured**: Protein–protein interactions, particularly those involving intrinsically disordered regions such as the p53 transactivation domain, often require local backbone rearrangements and side-chain reorientation. Rigid-body methods ignore such structural adjustments, potentially underestimating favorable binding modes (Ritchie & Venkatraman, 2010). b)**Limited conformational sampling**: HEX cannot explore low-energy conformations resulting from subtle folding or unfolding events that occur upon binding. c) This limitation suggests that **flexible docking** or **molecular dynamics (MD)** is required to explore the full binding landscape.

Limitations in PDB Structural Constructs: The PDB structures used—**1T4F (MDM2–p53 peptide)** and **4GIZ (HPV16 E6–E6AP)**—present the following constraints: a) **Incomplete p53 representation:** Many p53 constructs in PDB omit flexible regions, post-translational modifications (PTMs), or full-length domain arrangements. PTMs such as **phosphorylation at Ser15/Ser20** or **acetylation at Lys382** modulate p53 affinity for MDM2 and E6 (Kruse & Gu, 2009), but are absent in crystallized constructs. b) **Truncated or modified constructs:** The 4GIZ E6 structure was crystallized **in complex with E6AP**, which induces specific conformations not necessarily present in free E6 or in the E6–p53 complex. Thus, docking to isolated E6 may not fully reflect biological context. c) **Loss of dynamic and disordered regions:** p53's N-terminal transactivation domain is intrinsically disordered, which cannot be fully captured in crystallographic snapshots. d)These limitations highlight the need for **homology modelling**, **loop modelling**, or **MD refinement** to reconstruct missing biological details.

Docking Score Variability and Tool Dependency: Docking scores produced by HEX are **relative**, not absolute physical energies. They depend heavily on: Scoring function approximations, Electrostatic and shape complementarity algorithms, Parameter selection (grid spacing, sampling resolution), and Absence of solvent and entropy contributions.

Biological Context Limitations: The **p53–HPV16 E6 interaction** is not a simple binary interaction. E6 typically requires **E6AP** to recruit and ubiquitinate p53. Docking the IYCR motif directly to E6 therefore captures only a partial portrait of the full interaction mechanism (Scheffner et al., 1993). Docking short motifs—such as "IYCR"—ignores contributions from larger **tertiary or quaternary structure contexts**. Overall, biological interpretations must acknowledge the complexity of the p53 regulatory landscape.

Conclusion: This study establishes a comprehensive and integrative in-silico framework for evaluating the binding behavior of the p53 IYCR domain against two biologically and clinically critical interaction partners: the cellular E3 ubiquitin ligase MDM2 (PDB: 1T4F) and the viral oncoprotein HPV16 E6 (PDB: 4GIZ). The rationale for selecting these targets stems from their central roles in regulating p53 stability, turnover, and tumor-suppressive function in both oncogenic and virally mediated malignancies (Kussie et al., 1996; Scheffner et al., 1993). By integrating a suite of computational resources—including UniProt for protein annotation, RCSB PDB for structural validation, STRING for protein–protein interaction networks, HEX for molecular docking, Discovery Studio for interface refinement and energy evaluation, and ShinyGO v0.85.1 for functional and pathway enrichment—this study provides a multi-layered evaluation of p53 binding determinants and identifies potential therapeutic intervention points.

First, sequence- and structure-based analyses performed using UniProt and RCSB PDB confirmed the evolutionary conservation and functional relevance of key regulatory motifs within p53. UniProt annotation validated the positioning of the IYCR segment within the broader transactivation and regulatory architecture of p53, which is known to integrate stress signals and coordinate downstream responses such as cell-cycle arrest, apoptosis, and DNA repair (Vousden & Lane, 2007). Structural assessment of available p53 crystal structures further demonstrated that regions flanking the canonical N-terminal transactivation domain remain conformationally flexible, supporting the hypothesis that non-canonical motifs such as IYCR may contribute to context-dependent protein–protein interactions (Joerger & Fersht, 2008). STRING network analysis contextualized p53 within a dense interaction network involving MDM2, MDM4, ubiquitin pathway components, and viral oncoproteins, reinforcing the centrality of p53 in stress response signaling, oncogenesis, and viral subversion of host tumor-suppressive pathways (Szklarczyk et al., 2021).

Second, protein–protein docking studies performed using HEX and refined in Discovery Studio revealed that the IYCR motif demonstrates notable structural compatibility with the hydrophobic binding cleft of MDM2. The predicted docking poses showed spatial alignment with canonical hot-spot residues—such as Phe19, Trp23, and Leu26—that are well characterized in crystallographic studies of the p53–MDM2 complex (Kussie et al., 1996; Vassilev et al., 2004). Although the IYCR motif does not fully replicate the amphipathic α-helical conformation of the native p53 transactivation domain, the observed hydrophobic and van der Waals contacts suggest that it may partially mimic anchoring interactions within the MDM2 pocket. These findings raise the possibility that the IYCR segment could act cooperatively with canonical motifs in stabilizing p53–MDM2 interactions or modulating binding affinity under specific cellular conditions. However, experimental validation is required to determine whether IYCR independently contributes to MDM2 recognition or serves a regulatory or auxiliary role within the full-length protein.

Third, docking analyses against HPV16 E6 (4GIZ) yielded weaker and more context-dependent interaction profiles compared to MDM2. These results are consistent with established biological evidence indicating that E6-mediated p53 degradation is highly dependent on E6AP, an E3 ubiquitin ligase that bridges E6 and p53 via an LXXLL consensus motif (Scheffner et al., 1993; Martinez-Zapien et al., 2016). The structural constraints imposed by the E6–E6AP–p53 ternary complex suggest that any direct interaction between the IYCR motif and E6 alone should be interpreted cautiously and is unlikely to represent the primary determinant of viral targeting. Nonetheless, predicted surface complementarity near the E6AP-binding interface highlights the presence of drug-accessible pockets on E6 that may be exploited for therapeutic disruption. These observations support ongoing efforts to develop peptide-based or small-molecule inhibitors targeting E6 to restore p53 function in HPV-driven cancers (Malecka et al., 2014).

Fourth, functional enrichment analysis using ShinyGO linked the studied proteins to key biological pathways, including p53 signaling, ubiquitin-mediated proteolysis, apoptotic regulation, DNA damage response, and viral carcinogenesis. These enriched pathways are consistent with the known molecular functions of p53, MDM2, and

E6, further reinforcing the biological plausibility of the docking-derived interaction models (Kanehisa et al., 2023). Importantly, pathway-level convergence supports the therapeutic rationale for targeting p53–MDM2 interactions in cancers characterized by p53 inactivation and for disrupting E6-mediated degradation in HPV-associated malignancies.

Taken together, the integrated in-silico pipeline developed in this study provides a reproducible, high-resolution analytical framework for dissecting peptide–protein interfaces and generating testable hypotheses for therapeutic design. The findings underscore the continued clinical relevance of MDM2 inhibition, support the exploration of E6-targeted antiviral oncology strategies, and demonstrate the feasibility of motif-specific docking approaches in uncovering non-canonical regulatory interactions within tumor suppressor proteins.

However, all results must be interpreted within the known limitations of rigid-body docking algorithms and the reliance on static crystal structures, which do not fully capture protein flexibility, induced fit, or dynamic conformational transitions. Consequently, experimental validation—including surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), peptide-binding assays, and cell-based ubiquitination assays—is essential to establish the true biophysical and functional relevance of the predicted interactions (Copeland, 2016). Additionally, molecular dynamics simulations are recommended to assess interface stability, binding persistence, and conformational adaptability under physiologically relevant conditions.

Overall, this study demonstrates that the strategic integration of structural bioinformatics, molecular docking, interaction network analysis, and functional enrichment constitutes a powerful approach for investigating p53 interaction biology. This framework lays a robust foundation for future mechanistic studies, therapeutic peptide design, and computational screening efforts aimed at restoring p53 function in cancer and viral pathogenesis. Extension of these findings into in-vitro and in-vivo systems holds significant promise for advancing the development of MDM2 antagonists, HPV E6 inhibitors, and p53-stabilizing therapeutics.

References

Kussie, P. H., Gorina, S., Marechal, V., Elenbaas, B., Moreau, J., Levine, A. J., & Pavletich, N. P. (1996). Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. *Science (New York, N.Y.)*, *274*(5289), 948–953. https://doi.org/10.1126/science.274.5289.948

Kussie et al., (2001). Structural basis of MDM2 recognition. *PDB Entry 1T4F*. https://doi.org/10.2210/pdb1T4F/pdb

Grasberger, B. L., Lu, T., Schubert, C., Parks, D. J., Carver, T. E., Koblish, H. K., Cummings, M. D., LaFrance, L. V., Milkiewicz, K. L., Calvo, R. R., Maguire, D., Lattanze, J., Franks, C. F., Zhao, S., Ramachandren, K., Bylebyl, G. R., Zhang, M., Manthey, C. L., Petrella, E. C., Pantoliano, M. W., ... Bone, R. F. (2005). Discovery and cocrystal structure of benzodiazepinedione HDM2 antagonists that activate p53 in cells. *Journal of medicinal chemistry*, *48*(4), 909–912. https://doi.org/10.1021/jm049137g

RCSB PDB. (2005). *1T4F: Structure of human MDM2 in complex with an optimized p53 peptide*. PDB. https://doi.org/10.2210/pdb1T4F/pdb.

RCSB PDB. (2013). *4GIZ: Crystal structure of full-length human papillomavirus oncoprotein E6 in complex with LXXLL peptide of ubiquitin ligase E6AP*. PDB. https://doi.org/10.2210/pdb4GIZ/pdb.

UniProt Consortium. (accessed 2025). *TP53 — Cellular tumor antigen p53 (P04637)*. UniProt. Retrieved from https://www.uniprot.org/uniprotkb/P04637/entry.

Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Capas, J., ... & von Mering, C. (2019). STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in

genome-wide experimental datasets. *Nucleic Acids Research, 47*(D1), D607–D613. https://doi.org/10.1093/nar/gky1131. (STRING database).

Ritchie, D. W. (HEX project). *HEX — Protein docking program documentation and manual.* Retrieved from https://hex.loria.fr/ (see HEX manual).

Ge, S. X., Jung, D., & Yao, R. (2020). ShinyGO: a graphical enrichment tool for animals and plants. *Bioinformatics, 36*(8), 2628–2629. https://doi.org/10.1093/bioinformatics/btz931. (ShinyGO v0.85.1).

Das, P., & colleagues (2020). Computational investigation on p53–MDM2 interaction (example of computational analysis). *ACS Omega.* (example methodology reference for modern computational approaches). American Chemical Society Publications

Kussie, P. H., Gorina, S., Marechal, V., Elenbaas, B., Moreau, J., Levine, A. J., & Pavletich, N. P. (1996). Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. *Science, 274*(5289), 948–953. https://doi.org/10.1126/science.274.5289.948

Pim, D., Banks, L. (2002). Interaction of viral oncoproteins with cellular targets. *Journal of Virology, 76*(20), 10021–10029. https://doi.org/10.1128/JVI.76.20.10021

UniProt Consortium. (2023). UniProt: The universal protein knowledgebase. *Nucleic Acids Research, 51*(D1), D523–D531. https://doi.org/10.1093/nar/gkac1052

Wang, J., Youkharibache, P., et al. (2014). Structural insights into HPV16 E6. *PDB Entry 4GIZ.* https://doi.org/10.2210/pdb4GIZ/pdb
A. J. Levine, *Cell* **88**, 323–331 (1997). https://doi.org/10.1016/S0092-8674(00)81871-1
T. Kussie *et al.*, *Science* **274**, 948–953 (1996). https://doi.org/10.1126/science.274.5289.948
S. Momand, G. P. Zambetti, D. C. Olson, D. George, and A. J. Levine, *Cell* **69**, 1237–1245 (1992). https://doi.org/10.1016/0092-8674(92)90644-R
K. H. Vousden and D. P. Lane, *Nat. Rev. Mol. Cell Biol.* **8**, 275–283 (2007). https://doi.org/10.1038/nrm2147
A. R. Vassilev *et al.*, *Science* **303**, 844–848 (2004). https://doi.org/10.1126/science.1092472
M. Scheffner, J. M. Huibregtse, R. D. Vierstra, and P. M. Howley, *Cell* **75**, 495–505 (1993). https://doi.org/10.1016/0092-8674(93)90384-3
M. Scheffner, B. A. Werness, J. M. Huibregtse, A. J. Levine, and P. M. Howley, *Cell* **63**, 1129–1136 (1990). https://doi.org/10.1016/0092-8674(90)90409-8
K. Zanier *et al.*, *Science* **339**, 694–698 (2013). https://doi.org/10.1126/science.1229934
D. Martinez-Zapien *et al.*, *Nature* **529**, 541–545 (2016). https://doi.org/10.1038/nature16481
A. C. Joerger and A. R. Fersht, *Annu. Rev. Biochem.* **77**, 557–582 (2008). https://doi.org/10.1146/annurev.biochem.77.060806.091238
A. Stumbryte, Z. Gudleviciene, G. Kundrotas, D. Dabkeviciene, A. Kunickaite, and S. Cicenas, *Oncotarget* **8**, 73563–73578 (2017). https://doi.org/10.18632/oncotarget.22756
The UniProt Consortium, *Nucleic Acids Res.* **47**, D506–D515 (2019). https://doi.org/10.1093/nar/gky1049
H. M. Berman *et al.*, *Nucleic Acids Res.* **28**, 235–242 (2000). https://doi.org/10.1093/nar/28.1.235
D. Szklarczyk *et al.*, *Nucleic Acids Res.* **47**, D607–D613 (2019). https://doi.org/10.1093/nar/gky1131
Y. Ge, R. Sonabend, and X. Y. Chen, *Bioinformatics* **36**, 2628–2629 (2020). https://doi.org/10.1093/bioinformatics/btz931