Theoretical investigation of interactions between HIV-1 Tat and p53 proteins

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Abstract: HIV-1 Tat (transactivator of transcription) protein is the main arsenal of HIV, playing numerous roles during viral infection. This protein is intrinsically disordered, lacking well-defined secondary structures. Such structural plasticity allows HIV-1 Tat to interact with a wide range of proteins and biological molecules, ultimately leading to immune system collapse or severe tissue damage. Proteomic studies have previously revealed that p53, often referred to as the "guardian of the genome," interacts with Tat through its tetramerization domain. Since p53 plays a pivotal role in determining cell fate, its interaction with Tat is of broad interest in the pathogenesis of HIV infection. Therefore, we investigated the complex formation between Tat and the tetramerization domain of p53 using molecular docking and molecular dynamics simulations. We believe that the results presented in this manuscript provide valuable insights for the development of novel therapeutic agents targeting the p53/Tat interaction.

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) is the causative agent of acquired immunodeficiency syndrome (AIDS). During disease progression, vital immune cells, including CD4+ T cells (helper T cells), macrophages, and dendritic cells (which present antigens to T cells)—are infected by HIV. Consequently, CD4+ T cell numbers decrease, ultimately leading to host cell death. When CD4+ T cells fall below a critical threshold, cell-mediated immunity is lost, leaving the body susceptible to opportunistic infections, resulting in AIDS [1].

Interacting with the immune system, HIV encodes several accessory proteins, each playing a distinct role. Among these, HIV-1 Tat (transactivator of transcription) is particularly crucial in the viral life cycle [2]. Synthesized early during infection, Tat is secreted from infected T cells, which circulate in the bloodstream [3]. It is then taken up by uninfected cells, where it localizes to the nucleus and binds

the long terminal repeat (LTR) TAR element on viral RNA, enhancing transcription of all HIV-1 genes [4,5].

Tat is not limited to this primary function; it is associated with various pathological effects. For instance, it inhibits phagocytosis in macrophages by binding phosphoinositol-4,5-biphosphate (PI4,5P2) on cell membranes [6], alters potassium channel activity in cardiomyocytes [7], triggers neurodegeneration [8], disrupts the blood-brain barrier [9], and induces apoptosis in T cells [10]. Overall, Tat performs multiple tasks both intracellularly and extracellularly and has thus earned the reputation as the principal viral toxin of HIV-1 [11].

How can a single protein carry out so many seemingly unrelated biological functions? This functional diversity arises because Tat is an intrinsically disordered protein, lacking well-defined secondary structures [12–14]. Its structural plasticity allows it to adopt multiple conformations in solution, enabling interactions with a broad repertoire of proteins and receptors in both infected and uninfected cells [15]. Among these, a prominent interactor is p53, a cellular tumor suppressor [16].

In cells, p53 forms a homotetrameric transcription factor that regulates numerous genes, controlling downstream processes such as glycolysis [17], autophagy [18], oxidative stress [19], angiogenesis [20], and more. Moreover, p53 coordinates cellular responses to various stresses and regulates cell cycle progression and apoptosis, thereby playing a critical role in the survival or death of abnormal cells [21]. Since p53 plays a central role in regulating apoptosis and cell fate, it has become a common target not only of HIV but also of many other viruses, which employ distinct mechanisms to suppress or hijack its activity.

2. Background

The Tat–p53 interaction is particularly intriguing because their functions appear contradictory: p53 promotes apoptosis, whereas Tat aims to ensure HIV survival. It has been suggested that their interaction may involve a switching mechanism. In the early phase of infection, p53 binds Tat and presumably inhibits it [22]. As Tat accumulates in the cellular milieu, it suppresses p53 expression without direct physical interaction. This crosstalk may contribute to HIV-1 replication or cellular transformation [23]. Therefore, understanding this interaction at a molecular level is crucial for HIV research.

Despite its significance, studies on the molecular details of Tat–p53 interactions remain limited. Early evidence from yeast two-hybrid systems confirmed a specific interaction between these proteins [24]. More recent work demonstrated that p53 binds Tat through its tetramerization domain. Using peptide mapping and fluorescence anisotropy, this study revealed that the tetramerization domain of p53 specifically interacts with Tat residues 1–35 and 47–57 [25]. While these findings are important, a detailed atomistic investigation is necessary to interpret experimental results fully, given the biological implications. Motivated by this, we investigated Tat interactions with the p53 tetramerization domain and their thermodynamics using molecular docking and MD simulations.

3. Experimental

3.1. Docking Studies

In this study, Tat (PDB code: 1K5K, model 1) and p53 (PDB code: 2J0Z) were docked through four different web servers: ZDOCK [26], FRODOCK [27], HawkDock [28] and HPEPDOCK [29]. By visual inspection with VMD [30], ten top ranked complex structures from each server were compared with those from other servers and consensus binding poses were identified.

3.2. MD Simulations

The MD simulation files were prepared through CharmmGUI [31] and subsequently, the simulations were run for 1000 ns using NAMD (version 2.14) [32]. For each Tat-p53 model structure three replica simulations were carried out. Electrostatic interactions were calculated using the particle-mesh Ewald method with a grid spacing of 1 Å [33]. Simulations were performed with a time step of 2 fs, with all interactions calculated at every time step. The temperature and pressure were kept at 303.15K and 1 atm, respectively. Atomic coordinates were recorded at every 20 ps.

The stability of the complexes was investigated with RMSD analysis using the VMD program. In this analysis, simulation trajectory is aligned on p53 initial structure, and then RMSD calculation was performed for the Tat protein to see the stability of the Tat binding. A python library PyContact is used to extract information about the atomistic interactions formed between p53 and Tat [34]. Hydrogen bond analysis between p53 and Tat was performed using VMD program with default parameters (3Å for Donor-Acceptor distance and 20 for Angle Cutoff degree).

3.3. MM/PBSA Analysis

To estimate the binding affinities, the end-point approaches, such as MM/PBSA have been widely used. To conduct MM/PBSA calculations, a VMD plugin named Calculation of Free Energy (CaFE) were used with default parameters [35]. MM/PBSA calculations were performed for the last 200 ns of the simulation trajectories.

4. Present Study

In investigating the interaction between Tat and the tetramerization domain of p53, our approach initiates with the docking of the two proteins utilizing three docking servers (see experimental section for comprehensive details). Subsequently, the best ten models generated by each server are scrutinized in comparison to those produced by the other servers, according to the position of Tat relative to that of p53. Following this comparative analysis, models exhibiting similarities are filtered, thereby reducing the total count of models to four. The names of the models, along with the servers they originated from, are given in Table 1 and these complexes are shown in Figure 1.

Table 1. (Left) The names of the results from protein servers and (right) their respective names in the manuscript.

Server Output	Name in this article
Model-1 from HPEPDOCK	model_HDOCK
Ligand-1 from FRODOCK	model1_FRODOCK
Ligand-6 from FRODOCK	model2_FRODOCK
Complex-3 from ZDOCK	model_ZDOCK

Next, we performed three 1 μs long three replica simulations to investigate the stability of the predicted complex structures. In some of the simulations, Tat remained stable in its initial position, whereas in some other simulations the position of Tat has changed considerably. Through MM/PBSA analysis, it is observed that the binding energies of 10 out of the 12 models fell within the range of approximately -1 kcal/mol to -15 kcal/mol. However, there are two exceptions, namely replica 1 and 2 of model_ZDOCK (refer to Table 2). It should be noted that the binding energy of replica 2 is positive, indicating a thermodynamically unfavorable binding pose. Conversely, that of replica 1 is found to be -46.6 kcal/mol, suggesting this to be the most favorable one among all the considered replicas. Figure 2 presents snapshots for the conformation of p53 and Tat at the beginning and at the end of the simulations.

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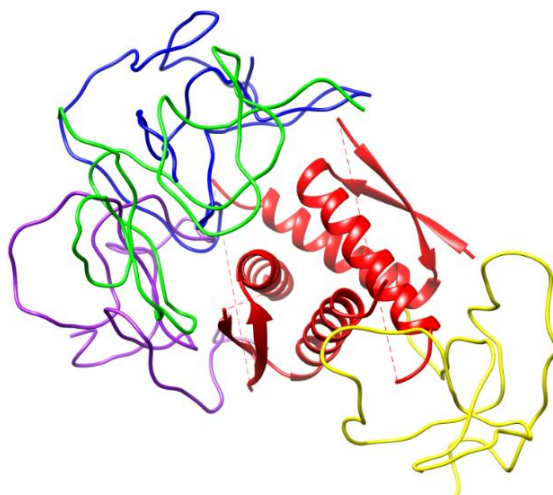
Figure 1. Models, which bind to p53 in different directions, were obtained from three different webservers (Purple: model2_FRODOCK, green: model_ZDOCK, blue: model1_FRODOCK, and yellow: model_HDOCK).

Table 2. Results of MM/PBSA calculations (kcal/mol) obtained from CaFE for each complex and each replica simulation.

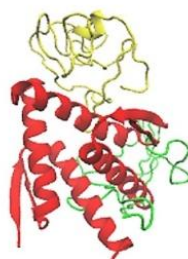| Model	model_ZDOCK	model1_FRODOCK	model_HDOCK	model2_FRODOCK
Replica - 1	-46.3	-13.3	-8.0	-1.7
Replica - 2	3.5	-10.4	-10.2	-2.6
Replica - 3	-1.7	-6.8	-13.3	-9.1
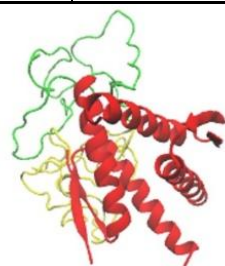
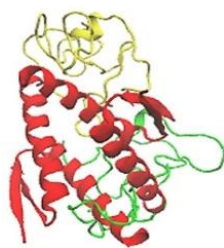-13.3 kcal/mol   -10.4 kcal/mol   -6.8 kcal/mol
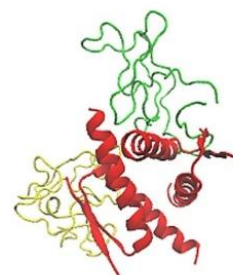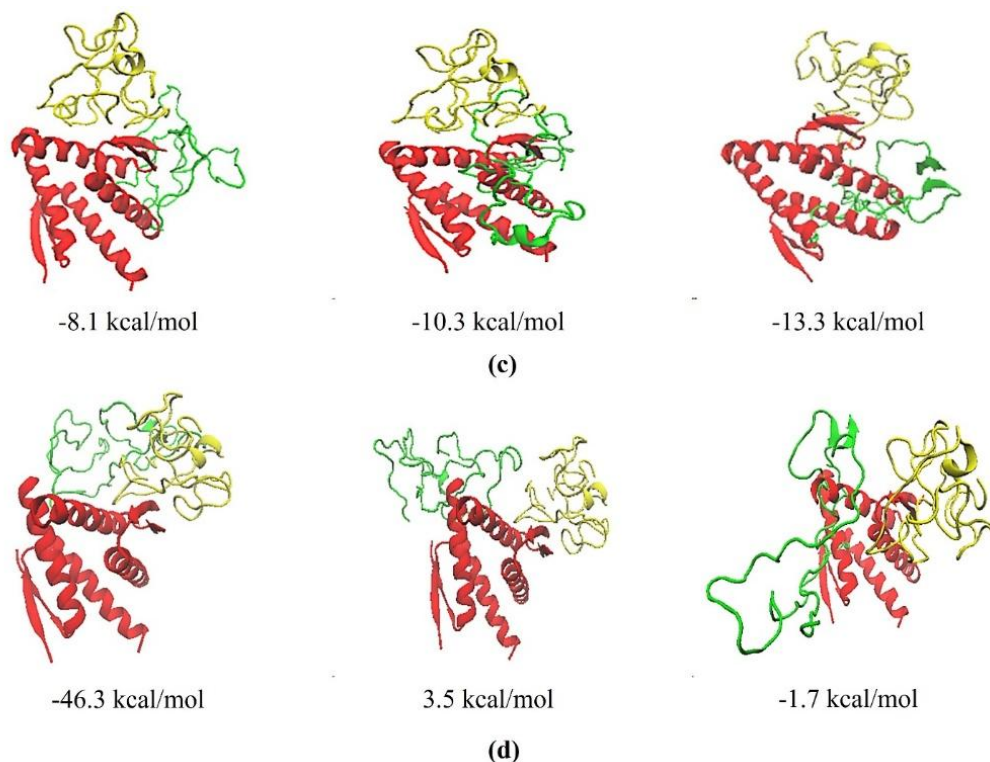
(a)

-1.8 kcal/mol   -2.6 kcal/mol   -9.2 kcal/mol

(b)

(Figure 2)

Figure 2. Snapshots for four complexes at the beginning and at the end of the 1 μs simulation and their MM/PBSA energy calculation results. Red: p53, yellow: Tat protein at the beginning of the simulation, green: Tat at the end of the replica-1, replica-2, and replica-3. (a) Model1_FRODOCK, (b) model2_FRODOCK, (c) model_HDOCK, (d) model_ZDOCK.

After running MD simulations and subsequent molecular analysis, we obtained important insights into the interactions between the tetramerization domain of p53 and Tat, with particular emphasis on identifying the binding regions within both proteins. In the research conducted by Gabizon and colleagues, two specific domains within Tat were proposed to be directly involved in complexation: Tat(47–57), also known as the basic patch, and Tat(1–31) [25]. These two regions exhibit contrasting amino acid compositions. Specifically, Tat(47–57), characterized by a high abundance of lysine and arginine residues, carries a net charge of +8, whereas Tat(1–31), primarily composed of cysteine residues and a hydrophobic core, carries a total charge of only +2. For p53, residues 326–355 within the tetramerization domain were identified as directly engaging with Tat. Gabizon and colleagues further noted that the initial interaction between these two proteins involves long-range electrostatic forces, supporting the involvement of the highly charged Tat(47–57) domain. Subsequently, this interaction is fine-tuned by additional contacts of lower ionic character, suggesting that Tat(1–31) becomes the main region interacting with p53 (for further details, see the original paper). The binding site within p53 was reported to span residues Glu326 to Arg342.

To determine which domains of Tat and p53 interact, we analyzed the last 200 ns of replica 1 of model_ZDOCK, which exhibited the highest binding energy, using the PyContact tool. In this replica, we observed that both the N-terminal region and the basic patch of Tat bind to p53, consistent with the findings of Gabizon et al. The interactions identified include: (i) hydrogen bonds between Tyr32–Thr329, Tyr32–Gln331, Cys34–Thr329, and (ii) salt bridges between Arg49–Glu343, Arg52–Glu339, Lys50–Glu343 (see Table 3). Notably, the C-terminal domain of Tat contributed only minimally to binding with p53, with a single residue, Glu87, forming a salt bridge with Lys351. Collectively, our results indicate that the interaction between the two proteins primarily involves the basic patch (residues 47–57) and the N-terminal region of Tat (residues 1–40). These findings partially align with previous observations suggesting that Tat residues 73–86 may also participate in complex formation with p53 [23].

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Table 3. PyContact analysis from model_ZDOCK's replica 1 in the last 200 ns.

p53	resid	Tat	resid	interaction_type	mean_score	median_score	percentage
Thr	329	Tyr	32	Hbond	9.571	9.751	93.3
Glu	343	Lys	50	Saltbr	5.484	5.682	84.905
Glu	343	Arg	49	Saltbr	4.041	4.103	72.865
Glu	339	Arg	52	Saltbr	5.704	7.392	72.685
Gln	331	Tyr	32	Hbond	2.380	2.453	58.505
Arg	335	Glu	9	Saltbr	5.573	6.654	54.175
Glu	326	Lys	28	Saltbr	2.944	3.621	50.93
Glu	336	Lys	51	Saltbr	3.280	3.436	50.375
Arg	335	Pro	10	Hbond	1.496	0.000	35.815
Thr	329	Cys	34	Hbond	2.244	2.260	29.93
Lys	351	Glu	87	Saltbr	1.550	0.000	23.845
Arg	342	Phe	38	Hbond	1.198	0.118	21.265
Glu	336	Tyr	47	Hbond	2.146	1.369	19.28
Gly	356	Lys	86	Hbond	2.099	0.000	18.83
Glu	336	Arg	52	Saltbr	1.426	0.000	18.27
Glu	339	Lys	41	Saltbr	0.850	0.000	13.915
Arg	335	Asp	80	Saltbr	1.169	0.000	13.535
Glu	339	Lys	50	Saltbr	2.113	1.001	12.36
Thr	329	Hsd	33	Hbond	3.798	3.596	11.95
Glu	326	Lys	24	Saltbr	0.553	0.000	10.975
Gly	356	Arg	56	Hbond	0.714	0.000	10.24

Additional evidence for this scenario is provided by model_ZDOCK's replica 2, where the binding thermodynamics are unfavorable. In this case, Tat binds to p53 in a conformation distinct from that observed in replica 1, which was extensively analyzed in the previous paragraph. PyContact analysis of the last 200 ns of this replica indicates that residues near the C-terminus of Tat, as well as some within the basic patch, are involved in binding with p53 (Table S1). Key hydrogen bonds observed here include Glu71–Glu326, Gln63–Asp352, and Arg57–Glu339. Taken together, these results stand in sharp contrast to those from replica 1, where the N-terminal of Tat does not participate in binding. This suggests that both the N-terminal region and the basic patch of Tat must be involved to achieve a thermodynamically favorable interaction with p53.

As shown in Table 2, the binding energies of the remaining replicas range from –1 to –14 kcal/mol, indicating moderate-to-strong interactions. Initially, we hypothesized that the number of hydrogen bonds formed between Tat and p53 would directly dictate binding energy, with more hydrogen bonds leading to stronger binding. However, hydrogen bond analyses revealed that the number of hydrogen bonds was consistently similar across replicas, suggesting that hydrogen bonding alone does not account for the observed variation in binding energies (Figures 4, S1–S3). Therefore, more detailed structural analyses will be required to draw definitive conclusions.
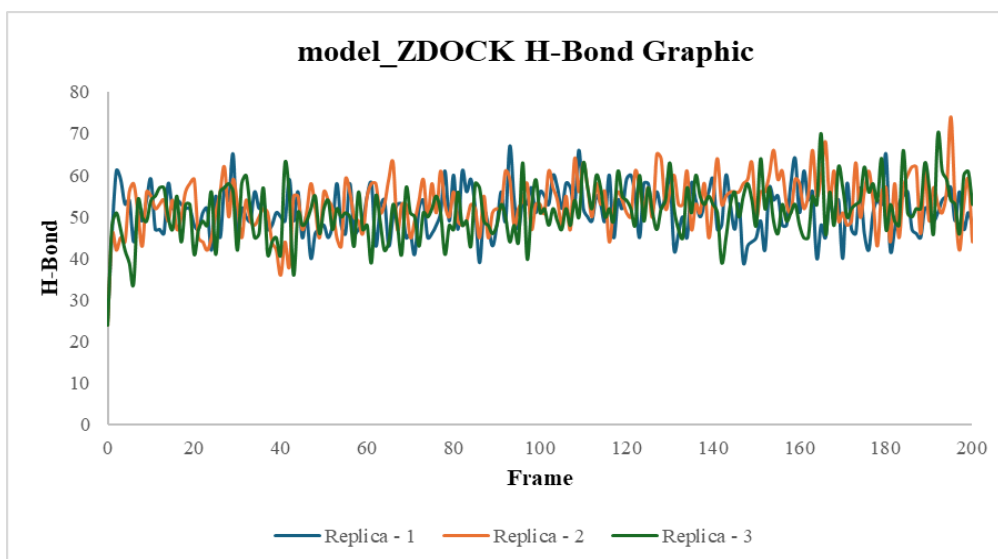
Figure 4. The numbers of hydrogen bonds between p53 and Tat in model_ZDOCK in the last 200 ns.

The last finding pertains to the binding conformation of p53. In this context, Gabizon and colleagues reported that the negatively charged residues Glu343, Glu346, and Glu349 within the p53(326–355) region are proposed to mediate the initial contact between the two proteins. This is consistent with the high positive charge of the Tat(47–57) region. Analysis of model_ZDOCK's replica 1 further confirmed the presence of hydrogen bonds and salt bridges between p53(Glu343) and Tat(Arg49) during the last 200 ns of simulation. However, Glu346 and Glu349 did not appear to contribute significantly to complex formation in our model.

Next, we compare our theoretical findings with the general body of experimental results reported in the literature. Our findings indicate that the N-terminus of Tat, particularly the basic patch spanning residues 49–57, plays a critical role in its interaction with p53. In contrast, the C-terminal region of Tat also engages in interactions with the p53 tetramerization domain, although these are noticeably weaker compared to those observed in other regions. When compared with previous studies, our results are consistent with the genetic analysis by Longo *et al.*, [24] who reported an interaction between Tat(49–57) and p53. Similarly, the more comprehensive experimental work by Gabizon *et al.*, [25] employing fluorescence anisotropy, demonstrated that Tat(1–35) and Tat(49–57) peptides bind to the tetramerization domain of p53, whereas other C-terminal fragments such as Tat(30–49), Tat(56–76), Tat(65–80), and Tat(73–86) do not exhibit detectable binding. This suggests that Tat binding to the tetramerization domain of p53 is mainly driven by N-terminal, and the basic patch of Tat. In the respect, our results mostly align with this observation.

An intriguing aspect of our results, however, is that certain amino acids within the C-terminal region of Tat appear capable of establishing weak contacts with the p53 tetramerization domain. This suggests that the C-terminus may not be entirely dispensable and could contribute partially to binding (Table 3). Therefore, the Tat–p53 interaction may represent a more nuanced and dynamic interplay than one strictly limited to discrete motifs or domains. In conclusion, our findings are most consistent with the results of Gabizon *et al.* among the limited experimental studies available, and in certain aspects, they provide complementary insights into their observations.

5. Conclusion

The p53 tumor suppressor is a pivotal transcription factor with essential roles in cell cycle regulation, apoptosis, and differentiation. Its tetramerization domain is central to function, controlling the conformational balance required for DNA binding and protein–protein interactions [36]. This structural feature not only underscores the importance of p53 in maintaining cellular integrity but also explains why viruses frequently evolve mechanisms to interfere with this domain.

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Reactivation of p53 in infected cells triggers apoptosis, thereby limiting viral replication. To counter this, viruses have evolved mechanisms to suppress p53 activity and prolong host cell survival. For example, influenza A virus–induced activation of p53 enhances apoptosis and restricts replication [37]. High-risk human papillomaviruses (HPVs) employ the E6 protein to recruit E6AP ligase, leading to p53 degradation [38]. Adenovirus type 5 proteins E1B-55K and E4orf6 bind to the C-terminal region of p53, disrupting DNA binding, oligomerization, and nuclear localization, while repressing its transcriptional activity [39]. Similarly, Kaposi's sarcoma-associated herpesvirus (KSHV) viral interferon regulatory factors (vIRFs) interfere with the DNA-binding and tetramerization domains of p53, blocking transcriptional and apoptotic functions [40].

HIV is not an exception in this regard. The HIV-1 Tat protein binds to the tetramerization domain of p53, thereby inhibiting its function and preventing apoptosis of infected cells [41]. In addition to blocking apoptosis, it has also been suggested that the Tat–p53 interaction may enhance viral transcriptional activity, reflecting the fact that both proteins function as nuclear transcription factors [42]. Consequently, interactions between viral proteins such as Tat and the tetramerization domain of p53 are of critical pharmacological significance, and targeting this mechanism may provide a promising avenue for the development of novel antiviral agents.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/organic-communications

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References

[1] Deeks, S. G.; Overbaugh, J.; Phillips, A.; Buchbinder, S. HIV infection. *Nat. Rev. Dis. Primers* **2015**, *1*(*1*), 1-22.

[2] Bagashev, A.; Sawaya, B. E. Roles and functions of HIV-1 Tat protein in the CNS: an overview. *Virol. J.* **2013**, *10*(*1*), 1-20.

[3] Clark, E.; Nava, B.; Caputi, M. Tat is a multifunctional viral protein that modulates cellular gene expression and functions. *Oncotarget* **2017**, *8*(*16*), 27569.

[4] Ali, A.; Mishra, R.; Kaur, H.; Banerjea, A. C. HIV-1 Tat: An update on transcriptional and non-transcriptional functions. *Biochimie* **2021**, *190*, 24-35.

[5] Chiozzini, C.; Toschi, E. HIV-1 TAT and immune dysregulation in AIDS pathogenesis: a therapeutic target. *Curr. Drug Targets* **2016**, *17*(*1*), 33-45.

[6] Debaisieux, S.; Lachambre, S.; Gross, A.; Mettling, C.; Besteiro, S.; Yezid, H.; Henaff, D.; Chopard, C.; Mesnard, J.-M.; Beaumelle, B. HIV-1 Tat inhibits phagocytosis by preventing the recruitment of Cdc42 to the phagocytic cup. *Nat. Commun.* **2015**, *6*(*1*), 1-12.

[7] Es-Salah-Lamoureux, Z.; Jouni, M.; Malak, O. A.; Belbachir, N.; Al Sayed, Z. R.; Gandon-Renard, M.; Lamirault, G.; Gauthier, C.; Baró, I.; Charpentier, F.; Zibara, K.; Lemarchand, P.; Beaumelle, B.; Gaborit, N.; Loussouarn, G. HIV-Tat induces a decrease in IKr and IKs via reduction in phosphatidylinositol-(4,5)-bisphosphate availability. *J. Mol. Cell. Cardiol.* **2016**, *99*, 1-13.

[8]   Nath, A.; Psooy, K.; Martin, C.; Knudsen, B.; Magnuson, D. S.; Haughey, N.; Geiger, J. D. Identification of a human immunodeficiency virus type 1 Tat epitope that is neuroexcitatory and neurotoxic. *J. Virol.* **1996**, *70*(*3*), 1475-1480.

[9]   Banks, W. A.; Robinson, S. M.; Nath, A. Permeability of the blood–brain barrier to HIV-1 Tat. *Exp. Neurol.* **2005**, *193*(*1*), 218-227.

[10]   Goldstein, G. HIV-1 Tat protein as a potential AIDS vaccine. *Nat. Med.* **1996**, *2*(*9*), 960–964.

[11]   Rayne, F.; Debaisieux, S.; Yezid, H.; Lin, Y. L.; Mettling, C.; Konate, K.; Chazal, N.; Arold, S. T.; Pugnière, M.; Sanchez, F.; Bonhoure, A.; Briant, L.; Loret, E.; Roy, C.; Beaumelle, B. Phosphatidylinositol-(4,5)-bisphosphate enables efficient secretion of HIV-1 Tat by infected T-cells. *EMBO J.* **2010**, *29*(*8*), 1348-1362.

[12]   Kunihara, T.; Hayashi, Y.; Arai, M. Conformational diversity in the intrinsically disordered HIV-1 Tat protein induced by zinc and pH. *Biochem. Biophys. Res. Commun.* **2019**, *509*(*2*), 564-569.

[13]   Bayer, P.; Kraft, M.; Ejchart, A.; Westendorp, M.; Frank, R.; Rösch, P. Structural studies of HIV-1 Tat protein. *J. Mol. Biol.* **1995**, *247*(*4*), 529-535.

[14]   Shojania, S.; O'Neil, J. D. HIV-1 Tat is a natively unfolded protein: the solution conformation and dynamics of reduced HIV-1 Tat-(1–72) by NMR spectroscopy. *J. Biol. Chem.* **2006**, *281*(*13*), 8347-8356.

[15]   Xue, B.; Mizianty, M. J.; Kurgan, L.; Uversky, V. N. Protein intrinsic disorder as a flexible armor and a weapon of HIV-1. *Cell. Mol. Life Sci.* **2012**, *69*(*8*), 1211-1259.

[16]   Chandra, K.; Maes, M.; Friedler, A. Interactions of HIV-1 proteins as targets for developing anti-HIV-1 peptides. *Future Med. Chem.* **2015**, *7*(*8*), 1055-1077.

[17]   Matoba, S., Kang, J. G., Patino, W. D., Wragg, A., Boehm, M., Gavrilova, O., Hurley, P. J., Bunz, F., Hwang, P. M. p53 regulates mitochondrial respiration. *Science* **2006**, *312*(*5780*), 1650-1653.

[18]   Crighton, D.; Wilkinson, S.; O'Prey, J.; Syed, N.; Smith, P.; Harrison, P. R.; Gasco, M.; Garrone, O.; Crook, T.; Ryan, K. M. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. *Cell* **2006**, *126*(*1*), 121-134.

[19]   Bensaad, K.; Vousden, K. H. Savior and slayer: the two faces of p53. *Nat. Med.* **2005**, *11*(*12*), 1278-1279.

[20]   Teodoro, J. G.; Parker, A. E.; Zhu, X.; Green, M. R. p53-mediated inhibition of angiogenesis through up-regulation of a collagen prolyl hydroxylase. *Science* **2006**, *313*(*5789*), 968-971.

[21]   Feroz, W.; Sheikh, A. M. A. Exploring the multiple roles of guardian of the genome: p53. *Egypt. J. Med. Hum. Genet.* **2020**, *21*(*1*), 1-23.

[22]   Li, C. J.; Wang, C.; Friedman, D. J.; Pardee, A. B. Reciprocal modulations between p53 and Tat of human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*(*12*), 5461-5464.

[23]   Ariumi, Y.; Kaida, A.; Hatanaka, M.; Shimotohno, K. Functional cross-talk of HIV-1 Tat with p53 through its C-terminal domain. *Biochem. Biophys. Res. Commun.* **2001**, *287*(*2*), 556-561.

[24]   Longo, F.; Marchetti, M. A.; Castagnoli, L.; Battaglia, P. A.; Gigliani, F. A novel approach to protein-protein interaction: complex formation between the p53 tumor suppressor and the HIV Tat proteins. *Biochem. Biophys. Res. Commun.* **1995**, *206*(*1*), 326-334.

[25]   Gabizon, R.; Mor, M.; Rosenberg, M. M.; Britan, L.; Hayouka, Z.; Kotler, M.; Shalev, D. E.; Friedler, A. Using peptides to study the interaction between the p53 tetramerization domain and HIV-1 Tat. *Pept. Sci.* **2008**, *90*(*2*), 105-116.

[26]   Pierce, B. G.; Wiehe, K.; Hwang, H.; Kim, B. H.; Vreven, T., Weng, Z. ZDOCK server: interactive docking prediction of protein–protein complexes and symmetric multimers. *Bioinformatics* **2014**, *30*(*12*), 1771-1773.

[27]   Ramírez-Aportela, E.; López-Blanco, J. R.; Chacón, P. FRODOCK 2.0: fast protein–protein docking server. *Bioinformatics* **2016**, *32*(*15*), 2386-2388.

[28]   Weng, G., Wang, E., Wang, Z., Liu, H., Zhu, F., Li, D., Hou, T. HawkDock: a web server to predict and analyze the protein–protein complex based on computational docking and MM/GBSA. *Nucleic Acids Res.* **2019**, *47*(*W1*), W322-W330.

[29]   Zhou, P., Jin, B., Li, H., Huang, S. Y. HPEPDOCK: a web server for blind peptide–protein docking based on a hierarchical algorithm. *Nucleic Acids Res.* **2018**, *46*(*W1*), W443-W450.

[30]   Humphrey, W.; Dalke, A.; Schulten, K. VMD: visual molecular dynamics. *J. Mol. Graph.* **1996**, *14*(*1*), 33-38.

[31]   Jo, S.; Kim, T.; Iyer, V. G., Im, W. CHARMM-GUI: a web-based graphical user interface for CHARMM. *J. Comput. Chem.* **2008**, *29*(*11*), 1859-1865.

[32]   Phillips, J. C.; Braun, R.; Wang, W.; Gumbart, J.; Tajkhorshid, E.; Villa, E.; Chipot, C.; Skeel, R. D.; Kale, L.; Schulten, K. Scalable molecular dynamics with NAMD. *J. Comput. Chem.* **2005**, *26*, 1781-1802.

[33]   Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G. A smooth particle mesh Ewald method. *J. Chem. Phys.* **1995**, *103*(*19*), 8577-8593.

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[34]   Scheurer, M.; Rodenkirch, P.; Siggel, M.; Bernardi, R. C.; Schulten, K.; Tajkhorshid E.; Rudack, T. PyContact: Rapid, Customizable, and Visual Analysis of Noncovalent Interactions in MD Simulations. *Biophys. J.* **2018**, *114*(*3*), 577-583

[35]   Liu, H.; Hou, T. CaFE: a tool for binding affinity prediction using end-point free energy methods. *Bioinformatics* **2016**, *32*(*14*), 2216-2218.

[36]   Chene, P. The role of tetramerization in p53 function. *Oncogene* **2001**, *20*(*21*), 2611-2617.

[37]   Aloni-Grinstein, R.; Charni-Natan, M.; Solomon, H.; Rotter, V. p53 and the Viral Connection: Back into the Future. *Cancers* **2018,** *10*(*6*), 178.

[38]   Thomas, M.; Massimi, P.; Banks, L. HPV-18 E6 inhibits p53 DNA binding activity regardless of the oligomeric state of p53 or the exact p53 recognition sequence. *Oncogene* **1996**, *13*(*3*), 471-480.

[39]   Dobner, T.; Horikoshi, N.; Rubenwolf, S.; Shenk, T. Blockage by adenovirus E4orf6 of transcriptional activation by the p53 tumor suppressor. *Science* **1996**, *272*(*5267*), 1470-1473.

[40]   Seo, T.; Park, J.; Lee, D.; Kim, S.; Choe, J. Viral interferon regulatory factor 1 of Kaposi's sarcoma-associated herpesvirus binds to p53 and represses p53-dependent transcription and apoptosis. *J. Virol.* **2001**, *75*(*13*), 6193–6198.

[41]   Spotss, K. L. Interactions between HIV-1 Tat and tumor suppressor p53. *Master's Thesis. University of South Carolina*, **2016**.

[42]   Selivanova, G.; Wiman, K. G. p53: A Cell Cycle Regulator Activated by DNA Damage. *Adv. Cancer Res.* **1995**, *66*, 143-180.