The number of new cases of cancer worldwide is expected to hit 25 million by the year 2040, with low- and middle-income countries bearing the brunt of the rise. In 2018, there were an approximated 18.1 million fresh cases of cancer worldwide. Because of its complexity, only specialist hospitals can implement cutting-edge clinical management, and cancer care is unevenly dispersed throughout nations.

The tumour suppressor p53 protein is the predominant cell responder to a variety of stress signals, including hypoxia, oncogene activation, reactive oxygen species (ROS), DNA damage, etc. When p53 is activated, it causes several different cellular reactions, such as arresting the cell cycle in order to preserve genetic integrity or senescence, apoptosis, or ferroptosis to destroy irreparable cells. Be a result, p53 is referred to as the "Guardian of the Genome" in order to stop the accumulation of the oncogenic mutations which cause malignant tumours.

p53 failure can contribute to the development or spread of a variety of human malignancies and give them malignant traits include alterations in cellular differentiation, genetic instability, as well as enhanced metastatic potential. Missense mutations as well as deletions that affect the protein's ability to regulate transcription typically cause *TP53* to be inactivated in majority of the human solid tumours. However, in instances where mutations in p53 are less common, binding of MDM2 (Murine Double Minute 2) may also impair p53's ability to function. The p53-binding protein MDM2 controls the stability as well as cellular localization of p53. As a result of this interaction, p53 is degraded by the proteasome and p53-mediated transcriptional activity is inhibited.

MDM2, which is the predominant negative regulator of p53, binds to p53 via many sites of interaction, among which only one site of interaction has been studied well. The well-studied or the primary site of interaction is between the N-Terminal Domain (NTD) of MDM2 and Transactivation Domain 1 (TAD1) of p53. However, the structural characterization of the p53-MDM2 complex at the atomistic level and the mechanism of binding/unbinding of the p53-MDM2 complex still remain unclear. Therefore, we demonstrate here the probable binding (unbinding) pathway of Transactivation Domain 1 (TAD1) of p53 during the formation (dissociation) of the p53-MDM2 complex in terms of free energy as a function of reaction coordinate from the potential of mean force (PMF) study using two different force fields: ff99SB and ff99SB-ILDN. From the PMF plot, we noticed the PMF to have a minimum value at a p53-MDM2 separation of 12 Å, with a dissociation energy of 30 kcal mol^{-1}. We also analyzed the conformational dynamics and stability of p53 as a function of its distance of separation from MDM2. The secondary

structure content (helix and turns) in p53 was found to vary with its distance of separation from MDM2. The p53-MDM2 complex structure with lowest potential energy was isolated from the ensemble at the reaction co-ordinate corresponding to the minimum PMF value and subjected to molecular dynamics (MD) simulation to identify the interface surface area, interacting residues at the interface and the stability of the complex. The simulation results highlights the importance of hydrogen bonds and the salt bridge between Lys94 of MDM2 and Glu17 of p53 in the stability of the p53-MDM2 complex. We also carried out the Binding Free Energy (BFE) calculations and the Per Residue Energy Decomposition (PRED) analyses of the interface residues of the p53-MDM2 complex. We found the binding affinity between MDM2 and p53 is indeed high ($\Delta G_{bind}/\Delta G_{binding}$ = -7.29 kcal mol^{-1} from MM-PBSA and $\Delta G_{bind}/\Delta G_{binding}$ = -53.29 kcal mol^{-1} from MM-GBSA). The total binding energy obtained using MM-PBSA method was noticed to be closer to the experimental values (-6.4 to -9.0 kcal mol^{-1}). The p53-MDM2 complex binding profile was observed to follow the same trend even in the duplicate simulation run and also in the simulation carried out with different force field. We found Lys51, Leu54, Tyr100, and Tyr104 from MDM2 and the residues Phe19, Trp23, and Leu26 from p53 provide the highest energy contributions for the p53-MDM2 interaction.

One of the secondary sites of interaction is between the central Acidic Domain (AD) of MDM2 and the core DNA Binding Domain (DBD) of p53. This interaction is crucial for the ubiquitination of p53, and this interaction has not been studied in detail yet. We have studied the conformational dynamics and stability of the p53(DBD)-MDM2(AD) complex using molecular dynamics (MD) simulation. We have also determined the protein-protein interaction (PPI) profile for the complex. The interface area involved in the interaction were found to be 1119 $Å^2$ and 1056 $Å^2$ for p53(DBD) and MDM2(AD) respectively. The MD simulation results highlight the significance of salt bridges and hydrogen bonds in the stability of the p53(DBD)−MDM2(AD) complex. We also carried out the binding free energy as well as per-residue energy decomposition analyses for the complex. A good binding affinity between p53 (DBD) and MDM2 (AD) was found (-17.22 kcal mol^{-1}).

Another site of interaction was found to be between the N-Terminal Lid present in the MDM2(NTD) and the C-Terminal Domain (CTD) of p53. But this interaction has not been studied in detail yet. And also how this interaction might affect the primary interaction between MDM2(NTD) and p53(TAD1) is yet to be explored. we have studied the conformational dynamics and stability of the N-Terminal Domain of MDM2 with the

lid, and the p53(CTD)-MDM2(NTD) complex using molecular dynamics (MD) simulation. It was found that the N-Terminal Domain of MDM2 with the lid remains in closed conformation throughout the simulation, while the N-Terminal lid in the p53(CTD)-MDM2(NTD) complex gets displaced throughout the simulation and the initial closed conformation of the p53(CTD)-MDM2(NTD) complex gets shifted to an open conformation at the end of the simulation. We then docked the p53 Transactivation Domain 1 (TAD1) with the lowest energy structure of the p53(CTD)-MDM2(NTD) complex, and it was found that the p53 TAD1 fits exactly into the N-Terminal binding cavity of MDM2.

Recently a photoactivatable MDM2 inhibitor, a Photoremovable Protecting Group (PPG) in complex with idasanutlin has been reported to exert no functional effect on cellular outgrowth but allows for the selective, non-invasive activation of antitumor properties due to the release of active MDM2 inhibitor idasanutlin (RG7388) from the complex upon irradiation with 400 nm light. In this study, using molecular docking and Molecular Dynamics (MD) simulations, we have investigated the interaction of (i) PPG-idasanutlin complex and (ii) the active inhibitor idasanutlin with MDM2 at the molecular level. We noticed that the PPG-idasanutlin complex fails to fit into the binding cavity of MDM2. But the active inhibitor idasanutlin when it is free from PPG was found to fit perfectly into the binding cavity of MDM2. From the Dictionary of Secondary Structure of Proteins (DSSP) analysis, we found that the number of α-helices, which aid in the stability of protein, were found to be more in the MDM2-idasanutlin complex rather than in the MDM2-PPG-idasanutlin complex. Using the PDBsum server, we have compared the interaction profiles of MDM2-PPG-idasanutlin, MDM2-idasnautlin and MDM2-p53 complexes. From the interaction profile, we found the active inhibitor, idasanutlin free from PPG to bind to the region in MDM2 where p53 prefers to bind.

Idasanutlin is a well-studied small molecule, the antagonist of MDM2 with potential antineoplastic activity. Nevertheless, the highly significant information pertaining to the free energy profile, intermediates, and the association of receptor and ligand components in the MDM2-idasanutlin complex remains unclear. Here we have studied the free energy profile of the MDM2-idasanutlin complex in terms of the Potential of Mean Force (PMF) method. We have used the PMF method coupled with umbrella sampling simulations to generate the free energy profile for the association of N-Terminal Domain (NTD) of MDM2 and idasanutlin along with a specific reaction coordinate for identifying transition states, intermediates as well as the relative stabilities of the

endpoints. We also have determined the binding characteristics and interacting residues at the interface of the MDM2-idasanutlin complex from the Binding Free Energy (BFE) and Per Residue Energy Decomposition (PRED) analyses. The PMF minima for the MDM2-idasanutlin complex was observed at a center of mass (CoM) distance of separation of 11 Å with dissociation energy of 17.5 kcal mol^{-1}. As a function of the distance of separation of MDM2 from idasanutlin, we also studied the conformational dynamics as well as stability of the NTD of MDM2. We found that there is indeed a high binding affinity between MDM2 and idasanutlin ($\Delta G_{binding}$ = -3.19 kcal mol^{-1}). We found that in MDM2, the residues MET54, VAL67, and LEU58 provide the highest energy input for the interaction between MDM2 and idasanutlin.

Recently, a small molecule with the chemical name 2-(2-(2-methoxyethoxy)ethoxy)ethyl-(29S,3R,49S,59R)-5'-((4-carbamoyl-2-methoxyphenyl)carbamoyl)-6-chloro-4'-(3-chloro-2-fluorophenyl)-29-neopentyl-2-oxospiro[indoline-3,39-pyrrolidine]-19-carboxylate (also called XR-2) was found to bind to the N-Terminal Domain (NTD) of MDM2, resulting in reactivation of functioning of p53 molecules. In this study, we have studied the interaction profile as well as the structural dynamics of MDM2-XR-2 complex using molecular dynamics simulation. It was found that XR-2 remained intact within the N-Terminal binding cavity of MDM2 throughout the simulation. We also carried out the binding free energy as well as per residue energy decomposition analyses for the MDM2-XR-2 complex. The binding affinity was found to be good ($\Delta G_{binding}$ = -16.26 kcal mol^{-1}), and residues PHE55, ILE61, VAL93 and LEU58 of MDM2 provide the highest energy contributions for the interaction between MDM2 and XR-2.

There are also some p53-MDM2 interaction inhibitors which bind to p53 instead of MDM2. Two of the examples are Epigallocatechin gallate (EGCG) and Reactivation of p53 and Induction of Tumour Cell Apoptosis (RITA).

Recently, EGCG was found to bind to the N-Terminal Domain (NTD) of p53 molecule, causing the p53 molecules to resume their normal function. In this study, we have studied the interaction profile as well as the structural dynamics of p53(NTD)-EGCG complex using molecular dynamics simulation. It was found that the molecule EGCG remains intact with the TAD1 of p53(NTD) throughout the simulation.. We also carried out the binding free energy as well as per residue energy decomposition analyses for the p53(NTD)-EGCG complex. The binding affinity was found to be good ($\Delta G_{binding}$ = -9.79 kcal mol^{-1}), and residues GLU28, MET40 and LYS24 of p53 provide the highest

energy contributions for the interaction between p53 and EGCG.

RITA (reactivation of p53 and induction of tumour cell apoptosis), was given the name by Issaeva *et al*., in 2004. It is a small molecule inhibitor that prevents the interaction between p53 and MDM2, and it both increases the amount of wild-type p53 and restores its function. But the interaction profile as well as structural dynamics of the p53(NTD)-RITA complex has not been studied in details at the molecular level. We have studied the structural dynamics and the interaction profile of p53(NTD)-RITA complex using molecular dynamics simulation. It was found that the molecule RITA innitially binds to the residues 33–37 of human p53. But throughout the course of the simulation, the small molecule RITA gets displaced and then gets bound to the Transactivation Domain (TAD1) of p53(NTD). We also carried out the binding free energy as well as per residue energy decomposition analyses for the p53(NTD)-RITA complex. We found a good binding affinity between the TAD1 of p53(NTD) and RITA ($\Delta G_{binding}$ = -2.31 kcal mol^{-1}). The residues TRP23, GLU28, LEU26 and LEU14 of p53, which are present in the TAD1 of p53(NTD) provide the highest energy contributions for the interaction between p53(NTD) and RITA.