Concluding Remarks
The p53 protein is a tumor suppressor that transcriptionally regulates target genes important in many different cellular processes such as cell cycle control, apoptosis, differentiation, senescence, DNA repair and recombination. p53 is among the most commonly mutated genes identified in human cancers. In an unstressed cell p53 is inactive and maintained at low levels due to targeted degradation. Following cellular stress such as DNA damage, hypoxia, and nucleotide depletion, p53 is transiently stabilized, accumulates in the nucleus and is activated as a transcription factor. Single point mutations in p53 DNA binding domain compromise with its native structure and mutant protein is often found to be thermodynamically destabilized to large extents. Structural perturbation and thermodynamic fluctuation makes the mutant protein to excessively accumulate in tumor cells and elicit oncogenic response. As already mentioned, since the crystal structure of the full-length protein is not yet fully understood, the consequences of point mutations in manipulating its structure and function is also an enigma for the researchers working with p53.

A general understanding of the structural organization involving disease-causing p53 mutations is helpful in correlating the loss of its tumor suppressor function and novel oncogenic gain of function. Therefore, the reactivation of mutant p53 with small molecules and stapled peptides needs severe computational study to explore the docking site on the surface of p53 and interacting bio-molecules such as MDM2/MDMX and DNA. Prediction of the accurate docking interface is believed to help in designing small molecules which will potentially interfere with p53-MDM2 interaction. In future, the extensive application of algorithm-based computational analysis supported with experimental studies is believed to produce appropriate findings to overcome the limitations of p53 reactivation in the way to develop novel cancer therapeutics.

Many p53 missense mutations possess dominant-negative activity and oncogenic gain of function. Report suggests that for structurally destabilized p53 mutants, these effects from mutant-induced coaggregation of wild-type p53 and its paralogs p63 and p73, thereby inducing a heat-shock response. Aggregation of mutant p53 arises from self-assembly of a conserved aggregation-nucleating sequence within the hydrophobic core of the DNA-binding domain, which becomes exposed after mutation. In addition, mutant p53 accumulates excessively in tumors and develops dominant-negative activity as well as
wild-type–independent gain-of-function effects that contribute to cancer development. Hence, whereas wild-type p53 is a tumor suppressor, cancer-associated mutations transform p53 into a potent oncogene.

Functional conversion of p53 from a tumor suppressor to an oncogene by structurally destabilized p53 mutations result from the increased aggregation propensity of these mutants, which is mainly achieved by exposing an aggregation-nucleating sequence stretch from the hydrophobic core of the DNA-binding domain. Exposing this aggregation-nucleation sequence by structural destabilization of the DNA-binding domain triggers coaggregation of wild-type p53 into cellular inclusions in cytosol, thereby abrogating wild-type activity and explaining the dominant-negative behaviour of the mutant. Understanding the connection between these disputes in p53 nuclear trafficking events of p53 conformational mutants and their biochemical consequences require a structural mechanism providing a specific mode of interaction. Notably, coaggregation is not only confined to wild-type p53. Indeed, it can be demonstrated that structurally destabilized p53 mutants also coaggregate with the p53 family members p63 and p73, thereby explaining its gain-of-function activity. Previous studies have shown that p53 traffics on microtubules and this transport occurs on dynein. Our study establishes that monomeric G-actin guides the p53 traffic toward the nucleus through direct physical association as soon as the cells are exposed to genotoxic stress. Histidine-tagged pull-down assay confirmed the direct physical association between p53 and G-actin; more importantly co-immunoprecipitation data further reveals the interaction is augmented in response to DNA damage. 3D reconstruction of consecutive optical sections has revealed a perinuclear pattern of intense colocalization between the proteins. Since in response to DNA damage, p53 molecules are stabilized and shuttle into the nucleus, G-actin interacts with p53 and take the responsibility of guiding the p53 molecules towards the nucleus. Constraint-based theoretical model of p53:G-actin complex has been derived to get an atomistic insight of this protein-protein association. With the help of three different algorithm-based protein-protein docking servers (ZDOCK 3.2, Schrödinger’s protein-protein docking module PIPER, and Cluspro 2.0), we have derived the same model for p53:G-actin. MD simulation reveals that p53 DNA-binding domain arrests very well the G-actin protein. Docking benchmark studies have been carried out for a known crystal structure, 1YCS (complex
between p53DBD and BP2), which validates the docking protocol we adopted. These array of findings clearly established that p53:G-actin complexation is a pre-requisite for nuclear translocation of tumor suppressor p53. Crucial amino acid contacts in p53:G-actin complex are also identified from the constraint-based docked model. Further analysis of the post-simulation model proposes that p53 interacts at the “target-binding cleft” of monomeric G-actin, where other actin binding proteins interact. Parallel to p53:G-actin complexation, we also tried to explore the probable reason behind why p53 specifically interacts with G-actin, not with the fibrous form of actin. Structural super-imposition of p53:G-actin model and actin:actin dimer model elaborates that the region of protomer-3 of G-actin participates in both p53:G-actin as well as actin:actin complexation. Since in F-actin fibre, the protomer-3 region of G-actin is engaged in formation of fibrous actin, p53 no longer can get the access to interact with it. Coimmunoprecipitation study using “hot-spot” p53 mutants suggested reduced G-actin association with cancer-associated p53 conformational mutants (R175H and R249S). Considering these findings, we hypothesized that point mutation in p53 structure, which diminishes p53:G-actin complexation, results in mutant p53 altered subcellular localization. Our model suggests p53Arg249 form polar-contact with Arg357 of G-actin, which upon mutation, destabilizes p53:G-actin interaction and results in cytoplasmic retention of p53R249S. According to the p53:G-actin hypothetical model, since p53Arg273 resides away from the most nearby residue (Glu152) of protein-protein interface, mutation from p53Arg273 into His273 is not supposed to interfere with p53:G-actin complexation. p53R273H forms a stable complex with G-actin and translocate into the nucleus. On the contrary, mutation at p53Arg249 into Serine, results in reduced p53:G-actin association. Superimposition of post-simulated coordinates indicate that in wild-type p53:G-actin complex, Arg249 (p53wt) forms a polar contact with Arg357 (G-actin). When mutated to Ser249, this crucial interaction is perturbed, whereas Arg357 forms another contact with p53Arg174. Hence the loss of Arg249-Arg357 interaction is compensated with the Arg174-Arg357 interaction. This alteration at the atomic level of p53:G-actin interaction can be hypothesized to be the reason for reduced degree of p53R249S:G-actin association leading to reduced p53R249S nuclear import.