The tumor suppressor p53, a 393-amino acid transcription factor, induces cell cycle arrest and apoptosis in response to genotoxic stress. Its inactivation via the mutation of its gene is a key step in tumor progression, and tetramer formation is critical for p53 post-translational modification and its ability to activate or repress the transcription of target genes vital in inhibiting tumor growth. About 50% of human tumors have TP53 gene mutations; most are missense ones that presumably lower the tumor suppressor activity of p53. In this study, we explored the effects of known tumor-derived missense mutations on the stability and oligomeric structure of p53; our comprehensive, quantitative analyses encompassed the tetramerization domain peptides representing 49 such substitutions in humans. Their effects on tetrameric structure were broad, and the stability of the mutant peptides varied widely (ΔT_m = 4.8 ~ −46.8 °C). Because formation of a tetrameric structure is critical for protein-protein interactions, DNA binding, and the post-translational modification of p53, a small destabilization of the tetrameric structure could result in dysfunction of tumor suppressor activity. We suggest that the threshold for loss of tumor suppressor activity in terms of the disruption of the tetrameric structure of p53 could be extremely low. However, other properties of the tetramerization domain, such as electrostatic surface potential and its ability to bind partner proteins, also may be important.

Genome instability and DNA breakage are the hallmarks of cancer cells that arise in response to the activation of oncogenes through point mutations, gene amplifications, or gene translocations (1, 2). Counterbalancing the effects of oncogenes are tumor suppressor proteins, the most important of which is p53, a transcription factor that modulates cell cycle arrest, senescence, apoptosis, and DNA repair largely via the direct or indirect induction or repression of hundreds of genes (3).

The p53 tumor suppressor monomer is a 393-amino acid protein with five domains: an N-terminal transactivation domain (amino acids 1 – 42); a proline-rich domain (amino acids 61 – 92); a central site-specific DNA-binding domain (amino acids 101 – 300); a tetramerization domain (TD, amino acids 326 – 356); and a C-terminal basic domain (amino acids 364 – 393). Several stressors, including DNA damage, activate p53 partly through multiple post-translational modifications modulating its activity and stability (4). However, wild-type p53 acts as a transcription factor only when it binds site-specific DNA response elements as a tetramer (5). Furthermore, a number of the post-translational modifications that are believed to be important regulators of p53 activity depend on its quaternary structure (6 – 11). The p53 protein also exhibits transcription-independent apoptogenesis, possibly contributing to its role in tumor suppression, which is mediated through its interaction with BCL2 family members, including Bak. The efficient targeting to and oligomerization of Bak in the mitochondrial membrane reportedly depends on p53 oligomerization (12). Thus, tetramer formation by p53 is crucial to its tumor suppressive activity.

About half of human tumors carry inactivating mutations in the TP53 gene (13, 14). Unlike other tumor suppressor genes, such as RB1, APC, BRCAl, and CDKN2A, which are inactivated primarily by deletion or nonsense mutations, 74% of TP53 tumor-derived mutations are point mutations that change a single amino acid. More than 95% of these missense mutations occur in the DNA-binding domain; they fall into two main categories, commonly termed DNA contact and conformational mutations. In contrast, ~17% of germ line p53 mutations in people with Li-Fraumeni syndrome and Li-Fraumeni-like syndromes affect amino acids in the TD, even though its activity is only that of a DNA binding domain, whereas ~80% of germ line mutations affect DNA-binding domain residues, viz., six times as long as the TD (14). This finding implies that germ line mutations exist at similar frequencies in the tetramerization and DNA-binding domains, and both are essential for p53-mediated tumor suppressor activity.

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The on-line version of this article (available at http://www.jbc.org) contains supplemental “Experimental Procedures,” Table S1, Figs. S1 – S6, and an additional reference.

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2 The abbreviation used is: TD, tetramerization domain.
The p53TD consists of a β-strand (Glu<sup>326</sup>–Arg<sup>333</sup>), a tight turn (Gly<sup>334</sup>), and an α-helix (Arg<sup>335</sup>–Gly<sup>356</sup>) (15). The structure of the TD was determined by NMR spectroscopy (16) and x-ray crystallography (17). Two monomers form a dimer through their antiparallel β-sheets and α-helices, and two dimers become a tetramer through the formation of an unusual four-helix bundle. Ala-scanning of p53TD revealed that 9 hydrophobic residues constitute critical determinants of its stability and oligomerization status (18). An earlier study of tumor-derived mutants R337H, R337C, or L344P from patients with Li-Fraumeni-like syndrome revealed a propensity for dramatic destabilization; the presence of the R337H mutation entailed pH-dependent instability of the mutant p53 tetramer (19, 20). Leu<sup>334</sup> occurs in the α-helix, and after introducing a helix-breaking proline (L344P), p53 could not form tetramers. R337C forms dimers and tetramers at low temperature; however, even though its tetrameric structure is destabilized significantly at physiological temperatures, it is only partially inactivated in several functional assays (21, 22). The p53 proteins with these mutations, as with other p53TD mutations (e.g. L330H, R337L, R342P, E349D, and G334V), exhibit an overall decrease in DNA binding and transactivation activity (23, 24).

Because the p53 tetramer is in equilibrium with the monomer, the protein concentration of p53 will affect its oligomeric status (18, 25). In unstressed normal cells, p53 is maintained at low levels by continuous ubiquitylation and subsequent degradation by the 26 S proteasome (26). DNA damage-induced phosphorylation of N-terminal residues of p53, and of Mdm2, a ubiquitin protein ligase, inhibits its binding to the latter and enables p53 stabilization and accumulation (4). A high concentration of p53 shifts the monomer-tetramer equilibrium toward the tetramer state, thereby promoting increased DNA binding, interactions with proteins important for p53 activation and function, and heightening post-translational modifications that activate p53. Past research used only semiquantitative analyses to assess the effects of mutations on the oligomeric structure and transcriptional activity of p53 (27–29). Although this research determined the oligomeric status of the mutant p53 protein by cross-linking (28) or by fluorescence intensity distribution analysis (29), the abundance of p53 protein was not controlled; thus, a destabilized mutant might show wild-type stability under high concentrations of mutant p53.

Because of the difficulty of constructing numerous cell lines with TD mutant p53 in which the p53 concentration can be carefully regulated, in this study, we quantitatively analyzed the oligomeric structure and stability of TD peptides from the reported cancer-associated TD mutants of p53. Surprisingly, the abilities of these mutants to form tetramers spanned a broad, almost continuous distribution. Although mutants that changed the domain core drastically prevented tetramer formation and/or folding as reported previously, the effects of many mutants were much more subtle. Nevertheless, even for mutants that slightly destabilized tetramer formation, at an endogenous concentration of p53, the fraction of tetramer decreased significantly. Our data further suggested that additional studies of the biochemical and biophysical properties of the TD may be required to explain why some p53 TD mutations are cancer-associated.

**EXPERIMENTAL PROCEDURES**

**Peptide Synthesis and Purification**—WT and mutant p53 TD peptides, comprising residues 319–358 of the extended TD, were synthesized as described previously (30). We measured peptide concentrations spectrophotometrically using an extinction coefficient for mutant p53TD peptides, ε<sub>280</sub> = 1280 m<sup>2</sup> cm<sup>−1</sup>, corresponding to a single tyrosine; for G334W and G356W, ε<sub>280</sub> = 6800 m<sup>2</sup> cm<sup>−1</sup>, corresponding to a single tyrosine and a tryptophan. Because the peptides Y327D, Y327H, and Y327S have no Tyr or Trp, peptide concentrations were determined by the BCA method (Thermo Fisher Scientific) using a WT peptide as the standard.

**Gel Filtration Chromatography**—We resolved the WT and mutant p53TD peptides using a Superdex 75 PC 3.2/30 (GE Healthcare) with a Precision Column Holder (GE Healthcare) in 50 mM phosphate buffer, pH 7.5, 100 mM NaCl (30). Peptide concentrations were 100 μM. The flow rate was 0.1 ml/min at 15 °C, and we monitored the effluent at 280 nm. Each peak was quantified by calculating the peak area using IGOR software (Wavemetrics).

**Thermal Denaturation by CD Spectroscopy**—For our CD measurements, we employed a Jasco-805 spectropolarimeter using a 1-mm path length quartz cell. CD spectra were recorded in 50 mM sodium phosphate buffer containing 100 mM NaCl, pH 7.5. For our thermal denaturation studies, spectra were recorded at discrete temperatures from 4 to 96 °C with a scan rate of 1 °C/min; ellipticity was measured at 222 nm for the p53TD solutions (10 μM monomer in 50 mM phosphate buffer, pH 7.5). We fitted the unfolding process of the p53TD peptide to a two-state transition model wherein the native tetramer directly converts to an unfolded monomer, as described previously (18, 25). The thermodynamic parameters of the peptides were determined by calculation with the functions described by Mateu and Fersht (18). We determined the T<sub>m</sub> and ∆H<sub>m</sub>/T<sub>m</sub> by fitting the fraction of monomer; we estimated the K<sub>d</sub> value of the tetramer–monomer transition from K<sub>d</sub> = (1 − K<sub>n</sub>)/2) (31, 32). For dimer mutants, we used K<sub>d</sub> = K<sub>n</sub>−1. The oligomeric states at 37 °C against the peptide concentration were assessed via the K<sub>n</sub> value.

**Structural Modeling of p53TD Mutants**—The three-dimensional coordinates of p53TD wild-type (Protein Data Bank code 3SAK) were used as a template. Homology modeling of mutants was performed with Modeler software (33).

**RESULTS**

**Oligomerization State of Mutant p53 Tetramerization Domains**—Forty-nine distinct mutations in human cancers occur in 24 of the 31 residues that comprise the p53 core TD (amino acids 326–356) (Fig. 1). We synthesized WT and mutant p53TD peptides corresponding to residues 319–358 and analyzed their oligomeric state and thermodynamic stability; we quantified this state from the peak areas corresponding to a monomer and tetramer during gel-filtration chromatography (Table 1 and supplemental Fig. S1). WT and most mutant peptides eluted as tetramers, but five mutants, L330P, L330R,
R337P, L342P, and L344P, eluted as a single peak contemporaneously with the monomer mutant L330A. Interestingly, three mutants (F341C, L344R, and A347T) eluted between the tetramer and monomer fractions. Accordingly, five mutants, L330R/P, R337P, L342P, and L344P, exist as monomers, three mutants, F341C, L344R, and A347T as dimers, and the others as tetramers under our conditions. Moreover, some mutants, such as L330H, R337C, and L348S, contained a lower tetramer fraction (45.1, 54.8, and 40.5%, respectively), and part of these peptides were chromatographed as monomers or dimers under these same conditions. After heating and subsequent cooling, WT and mutants except for G334V, G334W, and Met340. Four cancer-associated mutants had amino acids changed (T329I, Q331H, Q331R, or G356A; Table 1 and Fig. 2) such that tetramer stability actually increased. We noted a good correlation ($r^2 = 0.64$) between the fraction of oligomers and the $T_m$ value of the mutants observed by CD (supplemental Fig. S3), indicating that these thermodynamic parameters corresponded to the tetrameric state of the p53 peptides.

**Modeling of Mutant p53TDs**—Mutations that changed some solvent-exposed p53TD amino acid residues had little or no significant effect on the thermal stability of the tetramers. To elucidate why these mutations occur in human cancers, we modeled the TD of each mutant (supplemental Fig. S4), finding that changes in some solvent-exposed residues altered the calculated electrostatic potential on the surface of the p53TD. This was especially so for E339K, E339Q, E343G, E346A, and Q354K. We suggest that these changes might influence either the interdomain or the intermolecular interactions with binding partners that thereby could account for their selection as cancer mutants.

**Correlation between Stability of p53TD Peptides and That of Full-length p53 Protein**—We compared the stabilities of the tetrameric structures of the mutant p53TD peptides with the oligomeric state of full-length p53-EGFP fusion proteins carrying TD mutations; we employed fluorescence intensity distribution analysis, which measures the fluorescence intensity of EGFP-tagged p53 in a very small volume ($10^{-13}$ liter) of cell extract using confocal microscopy and yields a quantitative assessment of the fraction of protein oligomers in vivo at
physiologically relevant concentrations (29). The clear correlation (r^2 = 0.75) between the Tm measured here and the in vivo oligomerization state (supplemental Fig. S5) strongly suggests that our quantitative data on the tetrameric structure of p53 peptides can be extended to the full p53 protein.

**DISCUSSION**

Our study represents the first comprehensive, quantitative biophysical analysis of the oligomeric state and thermal stability of the 49 TD mutants identified in human cancers. Most mutant p53TD peptides formed a WT-like tetrameric structure with diminished stability (Fig. 2). However, tetrameric mutants with altered hydrophobic core residues (Phe328, Leu330, Arg337, Phe338, Phe341, Leu344, and Leu348), except I322V, exhibited dramatic reductions in stability and, in some cases, unfolding of the peptide (e.g. L330H/P/R, R337C/P, R342P, and L344P) as determined by CD measurements (supplemental Fig. S2). In particular, mutations that introduced proline in the α-helix devastated tetramer formation; some mutants could not form tetramers and existed as unfolded monomers (L330P/R, R337P, R342P, and L344P), or as folded dimers (F341C, L344R, and A347T). Indeed, our thermal denaturation study predicted that several TD mutants (e.g. L330H, R337C/H/L, F338I, F341C, L344R, and L348S) are thermally unstable at or near body temperature. These results are consistent with an alanine-scanning study of the p53TD that identified 9 key hydrophobic resides important for TD thermal stability and oligomerization (18).

In contrast to mutations that affect hydrophobic core residues, mutations that affect residues accessible to solvent were less destabilizing. The WT p53TD is thermally quite stable (Tm = 70 °C, Table 1) compared with the core DNA-binding domain (15); most mutations that affect TD domain residues, except as noted above, would not be expected to unfold the domain structure. Nevertheless, for most thermally stable TDs, the change in amino acids significantly altered the dissociation constant for tetramer formation (Table 1). Importantly, because tetramers are essential for DNA binding and activating transcription (9), and the p53 tetramer is in equilibrium with the monomer, the intranuclear p53 concentration is a critical factor in determining p53 function. In cultured, undamaged human embryonic skin fibroblasts (WS-1 cells), p53 abundance was 7.8 × 10^3 molecules/cell, whereas after DNA damage from neocarzinostatin, it rose 3-fold to 21.8 × 10^3 molecules/cell (34). The volume of the nucleus of a human fibroblast is 10^-12 liters (35). Correspondingly, the p53 concentration in the nucleus of normal, unstressed human cells is ~13 nM and increases to ~36 nM after DNA damage. At these concentrations, we can assess the fraction of tetramer for TD mutants at 37 °C (Fig. 3). Thus, we predict that 80% of the accumulated WT p53 protein (WT p53TD Kd = 10.2 nM) is in the tetrameric state following DNA damage. These values might be somewhat high as the skin fibro-

**TABLE 1**

Thermodynamic parameters for the mutant peptides

| No | mutant | Tm (°C) | ΔH (kcal/mol) | ΔG (kcal/mol) | Kd (nM) |
|----|--------|---------|---------------|---------------|---------|
| 1  | E326G  | 70.5    | -0.6          | 0.6           | 7.8     |
| 2  | V327H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 3  | V327H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 4  | Y327H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 5  | Y327H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 6  | F328H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 7  | F328H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 8  | V327H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 9  | Y327H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 10 | L330H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 11 | L330H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 12 | L330M  | 70.6    | -0.6          | 0.6           | 7.8     |
| 13 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 14 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 15 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 16 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 17 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 18 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 19 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 20 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 21 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 22 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 23 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 24 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 25 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 26 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 27 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 28 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 29 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |
| 30 | G334H  | 70.6    | -0.6          | 0.6           | 7.8     |

**FIGURE 2.** Thermal denaturation of WT and mutant p53TD peptides. Thermal denaturation of the peptides was analyzed by measuring the ellipticity at 222 nm for peptide solutions containing 10 μM peptide in 50 mM sodium phosphate, pH 7.5, 100 mM NaCl over the range of 4 to 96 °C, with a scan rate of 1 °C per minute.

**R342P, and L344P** as determined by CD measurements (supplemental Fig. S2). In particular, mutations that introduced proline in the α-helix devastated tetramer formation; some mutants could not form tetramers and existed as unfolded monomers (L330P/R, R337P, R342P, and L344P), or as folded dimers (F341C, L344R, and A347T). Indeed, our thermal denaturation study predicted that several TD mutants (e.g. L330H, R337C/H/L, F338I, F341C, L344R, and L348S) are thermally unstable at or near body temperature. These results are consistent with an alanine-scanning study of the p53TD that identified 9 key hydrophobic resides important for TD thermal stability and oligomerization (18).

In contrast to mutations that affect hydrophobic core residues, mutations that affect residues accessible to solvent were less destabilizing. The WT p53TD is thermally quite stable (Tm ~ 70 °C, Table 1) compared with the core DNA-binding domain (15); most mutations that affect TD domain residues, except as noted above, would not be expected to unfold the domain structure. Nevertheless, for most thermally stable TDs, the change in amino acids significantly altered the dissociation constant for tetramer formation (Table 1). Importantly, because tetramers are essential for DNA binding and activating transcription (9), and the p53 tetramer is in equilibrium with the monomer, the intranuclear p53 concentration is a critical factor in determining p53 function. In cultured, undamaged human embryonic skin fibroblasts (WS-1 cells), p53 abundance was 7.8 × 10^3 molecules/cell, whereas after DNA damage from neocarzinostatin, it rose ~3-fold to 21.8 × 10^3 molecules/cell (34). The volume of the nucleus of a human fibroblast is ~10^-12 liters (35). Correspondingly, the p53 concentration in the nucleus of normal, unstressed human cells is ~13 nM and increases to ~36 nM after DNA damage. At these concentrations, we can assess the fraction of tetramer for TD mutants at 37 °C (Fig. 3). Thus, we predict that ~80% of the accumulated WT p53 protein (WT p53TD Kd = 10.2 nM) is in the tetrameric state following DNA damage. These values might be somewhat high as the skin fibro-
that could affect protein interactions important for p53 function. Tetramer formation of p53 is essential for its interaction with human papillomavirus (HPV)-16 E2, c-Abl, and Mdm2 (9, 22). The binding affinity of p53 to MDM2 fell when p53 contained the mutation L344P or R337C found in Li-Fraumeni patients (22). c-Abl binds directly to the C-terminal basic domain of p53, and this interaction requires a tetramer. c-Abl may stabilize the tetrameric conformation, resulting in a more stable p53-DNA complex (40). In contrast, the interaction of caspase recruitment domain (ARC) with the p53TD inhibits tetramer formation and increases nuclear export (41). The binding of S100 family proteins depends on the oligomeric status of p53 and controls the balance between monomer and tetramer (42). Binding of the 14–3–3 protein to p53 enhances sequence–specific DNA binding by inducing p53 to form tetramers at lower concentrations (43).

The p53TD from ~13 apparently cancer-associated mutants in eight residues, mostly in the α-helical region of the TD, only moderately affected, by ~5-fold, the $K_d$ of tetramer formation of E326G, T329L, E335L, E339K, E339Q, and E346A, and very slightly affected, by 2-fold, that of Q331H, Q331R, I332V, G356A, and G356W (Table 1). The apparently minimal effect of these changes is particularly surprising for mutations that affect Glu$^{326}$, Ile$^{332}$, Glu$^{339}$, and Glu$^{346}$, because these are among the 12 most highly evolutionarily conserved residues in the TD (9), and changes to conserved residues are deleterious to function. Thus, we questioned why mutations causing these changes exist among p53-associated cancer mutants. As Soussi et al. (44) noted, mistakes occur in the literature on p53, possibly due to errors in sequencing or PCR, so caution is needed about accepting mutants that have been reported in cancers only once or a few times; data on germ line mutants should be more reliable. Of the 13 mutants noted above, all but four (Q331H, Q331R, R342Q, and G356W) occur only once in the International Agency for Research on Cancer TP53 mutant database, and none have been reported as germ line mutations. Thus, some of these mutants may be false reports. Of the remaining four mutants (supplementary Fig. S4), two are clearly solvent-exposed residues that either change the surface charge (R342Q) or replace a small residue with one bearing a bulky, hydrophobic side chain at the surface (G356W) (supplementary Table S1). Although mutations affecting solvent exposed residues and altering the electrostatic potential of the surface of p53 were less thermally destabilizing than core mutants were, the change in surface charge potential might affect intraprotein interactions or the interaction of the TD with one or more of its many binding partners. R342Q represents such a change, and the G356W change might disrupt surface complementarity that could affect protein interactions important for p53 function. Many mutants with greatly changed $K_d$ values also in-
volve surface-exposed alterations in charge that affect the predicted electrostatic potential of the p53TD surface (supplementary Fig. S4). In the crystal structure, E349D, a change that moderately increases the $K_d$ (to 1450 nM) is implicated in crystal contacts and, therefore, probably is important for such interactions (45). Three mutants (G334V, G334W, and E346A) showed a $\beta$-dominant spectrum after heating and subsequent cooling (supplemental Fig. S2). We have previously reported that G334V peptide forms amyloid fibrils under physiological conditions of temperature and pH (46). Also, other p53 domains (the transactivation domain and the DNA-binding domain) have been shown to undergo aggregation (47–49). The aggregates of p53 domains might be correlated with cancer.

Residue Gln$^{331}$ is in the short $\beta$-sheet that forms part of the monomer–monomer interface, but Gln$^{331}$ is not involved in monomer–monomer interactions, and the change to either His, Arg, or even Pro had only minor effects on p53TD thermal stability (Table 1). A recent yeast-based assay for transcriptional activation revealed that almost any amino acid sufficed at this position (50). Thus, biophysical or biochemical measurements do not show why mutations that alter this residue appear among cancer-associated mutations. Nevertheless, we argue that even mild changes to the $K_d$ for tetramer formation or in p53 stability could significantly affect p53 function because of the sensitivity of tetramer formation to the $K_d$ and p53 concentration in the physiological range. Many previous studies involved a ~20-fold p53 overexpression (supplementary Fig. S6A) that would not reveal the detrimental effects of many p53TD mutations (supplementary Fig. S6B).

Analyses of SNPs in p53 and its pathway support our suggestion of the potential importance of the subtle effects of TD mutants on tetramer formation. The TP53 gene reportedly contains 19 exonic polymorphisms, among which researchers have validated four (R47S, R72P, V217M, and G360A). The P47S and R72P polymorphisms subtly alter expression of p53 transcriptional targets. Although controversial (51), molecular evidence suggests that both polymorphisms alter cancer risk (50–54). Additional evidence comes from SNPs in p53 pathways. Bond et al. (55), working on the most intensively studied T/G SNP at nucleotide 309 in the first intron of the MDM2 gene, demonstrated that the 309G variant is bound to 1450 nM) is implicated in crystal contacts and, therefore, probably is important for such interactions (45). Three mutants (G334V, G334W, and E346A) showed a $\beta$-dominant spectrum after heating and subsequent cooling (supplemental Fig. S2). We have previously reported that G334V peptide forms amyloid fibrils under physiological conditions of temperature and pH (46). Also, other p53 domains (the transactivation domain and the DNA-binding domain) have been shown to undergo aggregation (47–49). The aggregates of p53 domains might be correlated with cancer.

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