Stability of p53 Homologs

Tobias Brandt1,2*1, Joel L. Kaar1,2*1, Alan R. Fersht1,2*, Dmitry B. Veprintsev1,2,*2c

1 MRC Centre for Protein Engineering, Cambridge, United Kingdom, 2 MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

Abstract

Most proteins have not evolved for maximal thermal stability. Some are only marginally stable, as for example, the DNA-binding domains of p53 and its homologs, whose kinetic and thermodynamic stabilities are strongly correlated. Here, we applied high-throughput methods using a real-time PCR thermocycler to study the stability of several full-length orthologs and paralogs of the p53 family of transcription factors, which have diverse functions, ranging from tumour suppression to control of developmental processes. From isothermal denaturation fluorimetry and differential scanning fluorimetry, we found that full-length proteins showed the same correlation between kinetic and thermodynamic stability as their isolated DNA-binding domains. The stabilities of the full-length p53 orthologs were marginal and correlated with the temperature of their organism, paralleling the stability of the isolated DNA-binding domains. Additionally, the paralogs p63 and p73 were significantly more stable and long-lived than p53. The short half-life of p53 orthologs and the greater persistence of the paralogs may be biologically relevant.

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* E-mail: arf25@cam.ac.uk (ARF); dmitry.veprintsev@psi.ch (DBV)
* Current address: Max Planck Institute of Biophysics, Frankfurt am Main, Germany
* Current address: Department of Chemical and Biological Engineering, University of Colorado, Boulder, Colorado, United States of America
* Current address: Laboratory of Biomolecular Research, Paul Scherrer Institut, Switzerland and Department of Biology, Zürich, Switzerland

Introduction

The role of stability in the evolution of proteins is intriguing. Many enzymes from thermophilic organisms are less active than those from mesophilic organisms when assayed at mesophilic temperatures. On the other hand, early protein engineering studies showed that the stability of proteins can be increased by mutation without losing activity. It is generally assumed that the stability of proteins is evolutionarily adapted to the temperature of their environment. For proteins that have evolved to be unstable, instability offers a mechanism by which cells can tightly control intracellular protein concentrations, which can be crucial for cell survival [1,2,3,4,5].

Members of the p53 family of transcription factors have fundamental but distinct roles. p53 is at the centre of a tumour suppressor network whereas p63 and p73 have mainly developmental functions [6,7]. Protein stability plays a major role in regulating cellular levels of p53. Several lines of evidence suggest that p53 may have evolved to be relatively unstable with its low melting temperature of about 45°C [8,9,10,11,12]. Correspondingly, many tumours are linked to the malfunction of p53 caused by destabilising mutations [13,14].

The in vitro stability of the DNA-binding domain (DBD) has been extensively characterised for p53 [10], several p53 orthologs [15] as well as for human p63 and p73 [8,16,17]. Thermal and chemical denaturation studies show that the thermodynamic stability of the p53 DBD, and as a result the ability to bind DNA, is severely compromised by a significant fraction of the tumour-associated mutations [10,11]. Further, the rate of denaturation of the human p53 DBD at 37°C is correlated to the thermal stability of several mutants studied [18], a correlation also observed for DBDs of several orthologs of p53 [15]. Protein engineering studies have shown that the stability of p53DBD can be increased by mutation [19,20,21] and is, therefore, not constrained by sequence. The thermodynamic stability of the DBD of p53 orthologs also correlates with the body temperature for higher animals and especially for mammals, but not for arthropods or nematodes [15]. In contrast to p53, isolated DBDs of p63 and p73 are significantly more stable than p53 [8,16,17]. Taken together, existing data suggest that stability of p53DBD is evolutionarily fine-tuned to ensure that p53 is just stable enough to function at human body temperature.

Full-length proteins of the p53 family contain additional structured domains such as the tetramerisation domain or, in the case of the β-isomers of p63 and p73, a sterile-alpha motif domain. Intrinsically disordered domains, flanking the DBD at its amino and carboxy termini, may also affect overall protein stability [22]. These additional domains are far less conserved than the DBDs and thus may affect stability differently. It is, therefore, important to study the stability of full-length proteins as well as stability of DBDs in the context of the full-length proteins.

Here, we examined the thermodynamic and kinetic stability of full-length proteins of the p53 family using differential scanning fluorimetry (DSF) and isothermal denaturation fluorimetry (ITDF). Specifically, we have compared human p53 with orthologous p53 from evolutionarily close and distant organisms as well as with its paralogs p63 and p73. DSF and ITDF involve monitoring the unfolding of a protein in the presence of an exogenous fluorescent dye reporter, which fluoresces strongly upon binding to hydrophobic sites of a protein. In DSF, protein unfolding is followed while the temperature is continuously...
increased [23,24]. Conversely, in ITDF, the unfolding of a protein is followed at constant temperature over time, yielding kinetic data and the protein’s half-life (\( t_{1/2} \)) [25,26,27]. A high-throughput, real-time PCR thermocycler with very low demands on protein amounts and excellent temperature control was used for DSF and ITDF, permitting high accuracy melting temperature and half-life measurements.

**Results and Discussion**

**Evolution of Protein Stability within the p53 Family**

The domain structure of the p53 family is shown in Figure S1. To study the stability of the p53 family, we expressed and purified full-length p53 from frog (\( X. \) laevis, Xlp53), fruit fly (\( D. \) melanogaster, Dmp53), mouse (\( M. \) musculus, Mmp53), and zebrafish (\( D. \) rerio, Drp53). We also expressed and purified full-length human p53 (Hsp53) as well as a super-stable variant of full-length human p53 (QM-Hsp53) [21]. In addition to full-length p53, isolated core domains of wild-type and stable human p53 (Hsp53DBD and QM-Hsp53DBD, residues 94–312) and human p53 deletion constructs missing either the C-terminal domain (p63DBD, residues 115–351) and the p73 core domain (p73DBD, residues 109–312).

**Kinetics of denaturation.** The process of denaturation and subsequent aggregation of the p53DBD is described by scheme 1 [18,28,29]:

\[
p53 \xrightarrow{k_1} p53' \xrightarrow{k_2} p53'' \xrightarrow{\text{fast polymerisation}} \text{aggregation competent}
\]

The denaturation and aggregation of the human protein follows simple sequential first-order kinetics but under certain conditions \( k_1 \) is rate determining and \( k_2 \) is not observed when monitored by probes that effectively measure loss of native protein although methods that monitor aggregate formation conform to lag kinetics. The simplest kinetics for the exponential phase follow equation 1, being composed of \( k_{obs} \) and a linear drift term from the subsequent aggregation process.

\[
F = F_0 + Ae^{-k_{obs}t} + Bt
\]

For several members of the p53 family, we measured the kinetic stability using ITDF. A typical denaturation curve is shown in Figure 1A. The apparent exponential increase in fluorescence is directly proportional to the increase in denatured species. In order to measure accurately the unfolding kinetics, the exponential phase was followed for at least five half-lives and was analysed in isolation from non-exponential intensity changes due to equilibration or precipitation.

In most cases data were fitted to a single-exponential model (equation 1) to yield the unfolding rate constant \( k_{obs} \). However, in the case of mammalian full-length p53 family members (Hs53, QM-Hp53 and Mmp53), we detected the additional, faster unfolding event \( (k_2) \), which at certain temperatures required a double-exponential model (equation 2).

\[
F = F_0 + A_1e^{-k_1t} + A_2e^{-k_2t}
\]

In both models, \( F \) denotes the measured fluorescence, \( F_0 \) the intrinsic fluorescence of the sample as measured at the start of the analysis, \( A \) the amplitude of fluorescence change, and \( k \) the observed rate constant of denaturation. In traces with two unfolding events, the second transition was modelled either as a slow transition, using a linear drift term \( (Bt) \), or as a second exponential term. \( t_{1/2} \) was calculated from \( t_{1/2} = \frac{0.6931}{k} \). As \( k_1 \) and \( k_2 \) are interchangeable in equation 2, one cannot automatically assign the faster phase to \( k_1 \) and the slower to \( k_2 \) in scheme 1, or vice versa.

For all the proteins studied, we collected kinetic stability data at several temperatures. This allowed the use of Arrhenius plots (Figure 1B) to calculate the temperature at which \( t_{1/2} \) is 15 min (\( T_{T_{1/2}} \), Table 1). This time was, for all the proteins studied, well...
within the linear range of the Arrhenius plots covered by the measurements.

To confirm the accuracy of ITDF measurements, denaturation kinetics were also measured by monitoring intrinsic protein fluorescence. Specifically, the fluorescence of tyrosine residues of QM-Hsp53 was followed using a cuvette-based fluorimeter (Figure 2A). This method utilises the decrease in fluorescence intensity of tyrosine residues within the p53DBD upon unfolding [18]. The resulting Arrhenius plot was very similar to the one obtained by ITDF (Figure S2), suggesting that increased fluorescence of the exogenous fluorescent dye reporter Sypro Orange reflects the loss of native protein.

Similar to the isolated DBD, full-length Hsp53 was significantly less stable than QM-Hsp53. For Hsp53 and QM-Hsp53, the rate constants determined for the slower unfolding event (k1) were very similar to those obtained for their respective isolated DBDs.

In the analysis of the full-length proteins, the second, faster event was resolved at lower temperatures. In order to attribute it to an unfolding process of p53, we analysed the concentration event was resolved at lower temperatures. In order to attribute it to an unfolding process of p53, we analysed the concentration event at lower temperatures, whereas the kinetics of unfolding of QM-Hsp53, Figure 2A) did not exhibit a second unfolding event at lower temperatures, whereas the kinetics of unfolding of the DBD (k1) were very similar in these constructs (Table 1). In contrast, QM-p53NCT, which included the acidic N-terminal domain, showed this second, faster unfolding event but with much smaller amplitude than for the full-length protein. The slope of the Arrhenius plot (Figures 1B and S2) was similar for both unfolding events, suggesting similar activation energies. A possible explanation for these observations is that the acidic N-terminus of p53 interacts transiently and unspecifically with the basic DBD and C-terminal domains of other p53 molecules [22]. As the concentration of protein in these experiments (15–60 µM) was much higher than the dissociation constant of the p53 tetramer (ca. 20 nM) [30], such interactions are likely to involve different tetramers. It remains to be seen if this observation has physiological relevance.

Additionally, the precipitation of protein aggregates, characterised by a drop in fluorescence intensity, depended on the p53 construct used. Earlier studies have shown that the p53DBD readily aggregates under different denaturing conditions, thereby making the process of denaturation irreversible [10,18,28,31,32]. Here, aggregation occurred earlier for constructs without the N-terminal domain (QM-Hsp53DBD, QM-Hsp53CT and QM-Hsp53CTC), but was not detected within the time frame examined for QM-p53NCT and full-length p53 (Hsp53 and QM-Hsp53, Figure 2B). This suggests that the N-terminal domain is also important in preventing p53 aggregation.

The p53 paralogs ΔNp63β, p73DBD and especially ΔNp63γ were kinetically more stable than Hsp53. Interestingly, only very low critical concentrations of small aggregates triggered precipitation after unfolding for all paralogs. For p63DBD this effect prevented unfolding analysis. However, despite this, it was possible to ascertain that the protein was unstable (t1/2<60 s) at 57°C, but stable below 45°C. Additionally, in contrast to Hsp53, the p73DBD was kinetically less stable than Hsp53...
naturally occurring isoforms. Finally, we found that the kinetic stability of TAp73b is almost identical to that of DNp73b (see Table 1). However, no precipitation was observed within the time frame examined for TAp73b, the only paralog analysed with a full-length N-terminus. Similarly to p53, this suggests an involvement of the N-terminal TA-domain in aggregation prevention.

The murine ortholog of Hsp53, Mmp53, also displayed two unfolding events. Both rate constants were very similar to those of Hsp53. Dmp53, Drp53 and Xlp53 were kinetically less stable than mammalian full-length p53, but their propensity to denature at or around ambient temperature significantly hampered accurate stability measurements. Drp53 and Xlp53 in particular were unstable at 32°C, which, due to temperature control limitations, was the lowest temperature at which stability could be measured.

Specific and non-specific binding prolongs p53 half-life. After initial stability measurements, the effect of specific and non-specific binding partners that modulate the kinetic stability of p53 in vivo, thereby contributing to longer protein activity, was examined. It has been shown previously that the thermodynamic stability of Hsp53DBD is increased by the addition of ligands like heparin [11] and drug-like small molecules [33]. Additionally, Hsp53DBD and Hsp53 have been shown to be more stable under pressure denaturation conditions if bound to the specific p53 DNA response element [34]. Furthermore, the protein stability in vitro is affected by the buffer composition.

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**Table 2.** Kinetic stability of QM-Hsp53. Effect of ligands and protein concentration.

| T/°C | c/lM ligand | t1/2/s | n | SD/s |
|------|-------------|--------|---|------|
| 45   | 15 –        | 390    | 4 | 16   |
| 15   | 2 mg/mL heparin | 780 | 4 | 28   |
| 15   | 5 μM DNA    | 990    | 4 | 46   |
| 38   | 15 –        | 4800   | 3 | 74   |
| 30   | –           | 3700   | 4 | 127  |
| 60   | –           | 2400   | 4 | 115  |

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The addition of heparin (2 mg/mL) approximately doubled the half-life of QM-Hsp53 (Table 2, Figure 3A). Even more pronounced was the stabilisation of QM-Hsp53 upon addition of 5 μM DNA, containing a p53 response element (Kd = 120 nM [35]). In contrast to free QM-Hsp53 at 45°C, the data for DNA-bound protein were best described by a double-exponential model, indicating a significant stabilisation of full-length p53 upon binding of DNA. Taken together, our results confirm that the kinetic stability of full-length p53 can be extended by protein-ligand interactions.

Additionally, we determined the effect of the buffer additives glycerol and sodium chloride on the kinetic stability of QM-Hsp53 (Figure 3B). In the presence of 10% glycerol, QM-Hsp53 was stabilised by about 20%. Similarly, increasing sodium chloride concentration lengthened the t1/2 of QM-Hsp53. The small differences induced by non-specific additives can be reliably detected using ITDF. Identification of optimal buffer conditions is of importance for protein formulations, such as, among others, vaccines and biological therapeutics.

**Correlation between Kinetic and Thermodynamic Stability**

Mutants of Hsp53DBD exhibit a correlation between kinetic and thermodynamic stability [18]. To determine if there is such a correlation among full-length p53 family members that contain considerable amino acid differences, we measured apparent Tm values (Table 1) by following the unfolding process using DSF (Figure 4A, B). Strictly speaking, p53 and its homologs denature irreversibly and the measured apparent Tm varies with the heating rate. However, if the heating rate is sufficiently fast, the measured Tm approximates to its true value because equilibration, which in turn is fast compared with the heating rate, is faster than the irreversible process [33]. We used DSC experiments as controls and found that results of DSF and DSC were in good agreement (Figure 4C and D). The thermal stability of p53 orthologs mirrored their kinetic stability. The Tm of Hsp53 and Mmp53 were very similar and the thermodynamic stability of the remaining p53 orthologs (Dmp53, Drp53 and Xlp53) was significantly lower. In contrast to Hsp53, the full-length proteins Dmp53 and Xlp53 were 4–5°C less stable than their respective, isolated DBDs.
The stability data of several full-length p53 orthologs supported a model that p53 has evolved to have only marginal thermodynamically and kinetically stable under its different organismal environmental conditions. The lower the body temperature (for homeothermic species) or optimal development temperature (for poikilothermic species) of an organism, the less stable is the p53 of this organism (Table 1, Figure 5). A similar trend has been reported for the isolated DBDs of homeothermic species, but was not as pronounced for those of poikilothermic species [15]. We conclude that the less conserved N-and C-terminal domains of Dmp53 and to a lesser extent also of Drp53 and Xlp53 might have evolved to destabilise the protein and adapt to the pressure of lower environmental temperatures, while the stability of the DBD changed to a lesser degree. Further, the middle between melting temperature and environmental temperature was found to be slightly smaller in organisms that regulate their body temperature and environmental temperature was found to be thermodynamically more stable than p53, making them less susceptible to thermal denaturation and unfolding, which is beneficial to their involvement in long-term developmental processes. In addition, deletion of p63 and p73 leads to lethality or strong developmental defects [37,38]. In contrast to p53, p63 and p73 may, therefore, need to be thermodynamically “protected” from destabilising mutations.

Generally, the thermodynamic stability ($T_m$) measured for the p53 family members that were studied, correlated well with their kinetic stability ($T_{15}$, unfolding of the DBD) (Figure 6). However, the correlation was not perfectly linear. Interestingly, the DBDs of full-length Hsp53 and QM-Hsp53 were kinetically slightly less stable, but thermodynamically more stable than their respective isolated DBDs. Noticeably, the p63 isoforms studied and Dmp53 were kinetically relatively less stable than Hsp53, which was evident from the larger difference between their respective $T_m$ and $T_{15}$ values.

Conclusions

Using a combined approach of ITDF and DSF we obtained a comprehensive picture of the stability of full-length proteins from the p53 family. Most significantly, ITDF and DSF allowed the study of protein unfolding processes, including that of full-length multi-domain proteins. In addition, due to the minimal sample amount requirements of these methods, it was possible to determine the stability of a range of proteins, which could not be expressed in sufficient quantity for analysis by classical methods. The parameters obtained by ITDF and DSF correlated very well. In addition, DSF and ITDF can be used for characterisation of the effects of additives such as small molecules on the protein stability of the p53 family. As an example, we show that stabilisation of the DNA-binding domain by optimisation of buffer conditions or by binding of a specific ligand (cognate DNA oligonucleotide) prolongs the half-life of the full-length human p53.

Interestingly, we found that, despite strong sequence conservation, the stability of the proteins studied varied considerably. Reflecting the stability of the isolated DNA-binding domains, the stability of the full-length p53 orthologs were found to be marginal and constrained in a very narrow range. As for the DNA-binding domain of homeothermic organisms [15], protein stability correlated with the respective temperature of their host environment for both poikilothermic and homeothermic organisms (Figure 5). In most cases, the stability of the full-length protein was the same as of its DNA-binding domain. Full-length proteins, similar to their isolated DNA-binding domains, also showed the same correlation between kinetic and thermodynamic stability, suggesting that the DNA-binding domain controls their stability.

While p53 from all analysed organisms appeared to have evolved to be marginally stable, p63 and p73 are significantly more stable and long-lived. It is interesting to note that unlike for the other proteins studied, the stability of several full-length isoforms of p63 was significantly decreased relative to the isolated

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**Figure 3. Kinetic stabilisation of p53.** A: Raw data for QM-p53 alone (black), with added heparin (red) and with added DNA (0.4 μM, blue; 5 μM, green). B: Comparison of half-lives of QM-p53 at different NaCl concentration without (black) and with 10% glycerol (red). doi:10.1371/journal.pone.0047889.g003

The stability data of several full-length p53 orthologs supported a model that p53 has evolved to have only marginal thermodynamically and kinetically stable under its different organismal environmental conditions. The lower the body temperature (for homeothermic species) or optimal development temperature (for poikilothermic species) of an organism, the less stable is the p53 of this organism (Table 1, Figure 5). A similar trend has been reported for the isolated DBDs of homeothermic species, but was not as pronounced for those of poikilothermic species [15]. We conclude that the less conserved N-and C-terminal domains of Dmp53 and to a lesser extent also of Drp53 and Xlp53 might have evolved to destabilise the protein and adapt to the pressure of lower environmental temperatures, while the stability of the DBD changed to a lesser degree. Further, the middle between melting temperature and environmental temperature was found to be slightly smaller in organisms that regulate their body temperature than in those that do not.

The α, β and γ isoforms of p63 studied, were about 10°C more stable than p53 but about 5°C less stable than the p63DBDBD in isolation in terms of $T_m$. Thus, for p63, analysis of the stability of the DBD alone is not representative of that of the full-length protein. Small differences in stability of p63 were due to the isoform-specific C-termini, with the β-isoform being the least and the γ-isoform being the most stable protein. All of the p73 isoforms studied were about 3–4°C more stable than Hsp53 and isoform-specific differences were small. For previously studied proteins QM-Hsp53, QM-Hsp53DBD and p63DBD [8,16], we found 1–2°C higher $T_m$ values, which was presumably due to the stabilising effect of the 10% glycerol in our experiments.

A possible reason for greater stability of p63 and p73 relative to p53 may lie in their respective cellular roles. As a tumour suppressor p53 may cause cell-cycle arrest or apoptosis. It is thought that the stability of p53 is compromised in order to enable tight control of its abundance in healthy cells [36]. In contrast, both p63 and p73 have evolved to be kinetically and thermodynamically more stable than p53, making them less susceptible to thermal denaturation and unfolding, which is beneficial to their involvement in long-term developmental processes. In addition, deletion of p63 and p73 leads to lethality or strong developmental defects [37,38]. In contrast to p53, p63 and p73 may, therefore, need to be thermodynamically “protected” from destabilising mutations.

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p63 DNA-binding domain. This difference suggests that other domains can contribute to the stability control. The different stability of the isoforms may be related to in vivo function.

In summary, full-length p53 family members behaved similarly to the isolated DNA-binding core domains in respect to their stability. This correlation emphasises that observations made for isolated core domains of p53 are also valid for the full-length proteins. Exceptions were observed for evolutionary more distant homologs such as human p63 or Dmp53, in which other domains contribute significantly to overall protein stability. However, in the case of human p53, the correlation reinforces the validity of the concept that stabilisation of the core domain of mutant p53 by specific ligands is a viable therapeutic strategy.

Materials and Methods

Gene Cloning

For human full-length p53 we used WT-Hsp53 protein and a super-stable mutant, which has four mutations in the core domain (QM-Hsp53): M133L/V203A/N239Y/N268D [21,39]. A plasmid encoding Mmp53 was kindly provided by Geoffrey Wahl. We amplified Dmp53 from a cDNA library kindly provided by Simon Bullock. Sequences encoding other studied proteins were amplified...
from clones provided by the MGC collection (distributed via Geneservice). For p63 isoforms, parts of the gene were amplified from a genomic DNA library (Geneservice). Additionally, we made a p73-DBD (109–312) and a p63DBD (115–351) construct. All inserts were cloned into a pET24a-HLTEV plasmid containing the N-terminal 6×His purification tag, a lipoyl domain [40] for improved solubility and a TEV-protease cleavage site.

Protein Expression and Purification

Small scale expression screening was done as described before [30]. Large-scale expression and purification was carried out as described earlier [30,41,42]. All the proteins studied were overexpressed in E. coli BL21 or B834 cells (Novagen) at 18°C for 16–20 h and purified using standard Ni-affinity chromatography protocols. Subsequently, the N-terminal tags were cleaved off by TEV-protease digestion. For p53 orthologs, heparin affinity chromatography was then used. The final purification step was gel filtration chromatography using a Superdex 200 16/60 preparative gel filtration column (GE Healthcare) in 225 mM NaCl, 25 mM sodium phosphate pH 7.2, 10% glycerol and 5 mM DTT. Protein purity of >95% was determined by SDS-gel electrophoresis. Samples were flash frozen in liquid nitrogen and stored at −80°C until used.

Isothermal Denaturation Fluorimetry

The proteins studied were dialysed against buffer A (225 mM NaCl, 25 mM phosphate [pH 7.2], 10% glycerol, 5 mM DTT). Protein and water soluble ligands were introduced in buffer A, whereas SYPRO orange (5000×, Invitrogen) was introduced in DMSO. Final protein concentrations were 15 μM. SYPRO orange was diluted to a final dilution of 5×. Heparin (sodium salt from bovine intestinal mucosa, Fluka) was used at a final concentration of 2 mg/mL. We used palindromic, self-annealing DNA (GGACATGTCCGGACATGTCC, Eurogentec) at 0.4 and 5 μM.

Fluorescence was measured with a RotorGene 6000 qPCR machine (Qiagen) using an excitation wavelength of λex=460 nm and an emission wavelength of λem=610 nm. The samples were equilibrated for about 30 s, a process which can be followed because fluorescence itself is temperature dependent. Datasets were, therefore, trimmed at 60 s to cut any temperature equilibration-related artefacts and as soon as fluorescence dropped sharply due to precipitation. The data obtained had a very good signal to noise ratio, and temperature fluctuations during a run were found to be negligible. Reproducibility was good, reflected by a standard error of 6.8%, averaged over all data collected for the proteins studied. The fast acquisition of data points enabled us to reliably detect kinetic processes with half-lives down to about 100 s. Data analysis was done with ORIGIN software (Microcal) and laboratory-developed software DataFitter (http://www.mrc-lmb.cam.ac.uk/dbv/). Experiments were done at three temperatures at least and were repeated four times.

Differential Scanning Fluorimetry

All experiments were carried out in buffer A. Sample size was 20 μL and protein concentrations of 8–14 μM were used. For Dmp53, TAp73β and TAp73γ only 3–5 μM samples could be obtained. SYPRO orange fluorescent dye (5000×, Invitrogen) was added to protein solutions and was optimised for each protein to a final dilution of 5×–50×. Melting curves were measured between 25°C and 80°C with a scan rate of 270°C/h (comparable to DSC experiments) using a RotorGene 6000 qPCR machine (Qiagen) and were carried out in quadruplicates. Data analysis was done with manufacturer supplied software.

Differential Scanning Calorimetry

All experiments were carried out in buffer A. We used a VP-DSC (Microcal) instrument with an autoampler and heated the samples from 15 to 80°C at a scan rate of 125°C/h. These high
scanning rates unfold the protein faster than the rate of thermal denaturation in order to measure the apparent melting temperatures, T_m. 400 μL of 10 μM samples were used and experiments were repeated up to three times depending on the amount of protein available. Blank experiments with buffer were carried out as well and used as a baseline. Data analysis was done with ORIGIN software (Microcal) and the laboratory-developed software, DataFitter.

Supporting information

Figure S1  p53 family domain organisation. (TIF)

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Figure S2  Kinetic denaturation of p53. (TIF)

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Author Contributions

Conceived and designed the experiments: TB ARF DBV. Performed the experiments: TB JK. Analyzed the data: TB JK ARF DBV. Contributed reagents/materials/analysis tools: TB JK DBV. Wrote the paper: TB JK ARF DBV.

Stability of p53 Homologs