Effects of Oncogenic Mutations and DNA Response Elements on the Binding of p53 to p53-binding Protein 2 (53BP2)*

Henning Tidow1, Dmitry B. Veprintsev, Stefan M. V. Freund, and Alan R. Fersht2
From the Centre for Protein Engineering, Medical Research Council, Hills Road, Cambridge CB2 2QH, United Kingdom

The tumor suppressor p53 is frequently mutated in human cancers. Upon activation it can induce cell cycle arrest or apoptosis. ASPP2 can specifically stimulate the apoptotic function of p53 but not cell cycle arrest, but the mechanism of enhancing the activation of pro-apoptotic genes over cell cycle arrest genes remains unknown. In this study, we analyzed the binding of 53BP2 (p53-binding protein 2, the C-terminal domain of ASPP2) to p53 core domain and various mutants using biophysical techniques. We found that several p53 core domain mutations (R181E, G245S, R249S, R273H) have different effects on the binding of DNA response elements and 53BP2. Further, we investigated the existence of a ternary complex consisting of 53BP2, p53, and DNA response elements to gain insight into the specific pro-apoptotic activation of p53. We found that binding of 53BP2 and DNA to p53 is mutually exclusive in the case of GADD45, p21, Bax, and PIG3. Both pro-apoptotic and non-apoptotic response elements were competed off p53 by 53BP2 with no indication of a ternary complex.

The tumor suppressor p53 is a tetrameric multidomain transcription factor that functions to maintain the genomic integrity of the cell (1). In response to cellular stresses like DNA damage, aberrant growth signals, UV irradiation, and hypoxia, p53-mediated transcriptional activation leads to DNA repair, cell cycle arrest, or apoptosis (2–4).

In about 50% of all human cancers, p53 is inactivated directly as a result of mutations in the p53 gene (5). On the basis of the crystal structure of human p53 core domain bound to consensus DNA (6), common cancer mutations can be classified as “structural” mutations that cause structural perturbations within the core domain or a loss of stability and “contact” mutations directly affecting DNA binding by the loss of a contacting residue (7). A third class of p53 mutations is proposed for those mutants that retain the capacity to bind DNA but are no longer able to interact with important cofactors (8).

It is still poorly understood why some cells undergo apoptosis upon p53 activation, whereas others respond with cell cycle arrest. Important factors seem to be the degree of DNA damage, cell type, and the presence of survival factors, but the exact mechanism remains unknown (9, 10). Recently, a family of proteins termed ASPP was identified that specifically stimulates the apoptotic function of p53 but not cell cycle arrest (11, 12). Full-length ASPP2 interacts with p53 and increases its ability to induce apoptosis when it is artificially expressed in cells (11). Further, the apoptotic function of p53 in response to chemotherapeutic drugs like cisplatin is inhibited when the expression of intrinsic ASPP23 is prevented. However, the mechanism of enhancing the activation of pro-apoptotic genes over cell cycle arrest genes is still unknown.

53BP2 (p53-binding protein 2) is the C-terminal domain of ASPP2. Shortly after 53BP2 was initially discovered in a yeast two-hybrid screen (13), the crystal structure of a complex of 53BP2 and p53 core domain has been solved (14). Unlike most other p53-interacting proteins that bind the N or C terminus of p53, 53BP2 interacts with the core domain of p53 (15, 16). 53BP2 contains four ankyrin repeats and an SH3 domain. The C-terminal ankyrin repeat and the SH3 domain bind to an evolutionarily conserved region within the p53 core domain that is frequently mutated in human cancers and overlaps with its DNA-binding region (14).

In this study, we used biophysical techniques to characterize the interaction between 53BP2 and p53 core domain to determine the effects of common cancer mutations in p53 on binding to cofactors that further determine the fate of cells after p53 activation. These results are discussed in comparison with mutational effects on DNA binding. We found mutants of p53 that do not bind 53BP2 even though they bind DNA. This might explain their effects in causing cancer by disrupting an important pro-apoptotic pathway.

Further, the existence of a ternary complex consisting of 53BP2, p53, and different response elements was investigated to gain insight into the specific pro-apoptotic activation of p53. We found that binding of 53BP2 and DNA to p53 is mutually exclusive for pro-apoptotic and non-apoptotic response elements, i.e. they compete with each other. The magnitude of competition is modulated by differences in the binding affinity of p53 to these response elements with no indication of a ternary complex.

EXPERIMENTAL PROCEDURES

Molecular Cloning—The gene encoding for amino acids 891–1128 of human 53BP2 was extracted from a pGEX-GST-

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
1 Supported by a fellowship from the Boehringer Ingelheim Fonds.
2 To whom correspondence should be addressed. Tel.: 44-1223-402136; Fax: 44-1223-402140; E-mail: arf25@cam.ac.uk.

© 2006 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in the U.S.A.
53BP2-(682–1128) plasmid (courtesy of Dr. Xin Lu, LICR-UCL, London) and subcloned into a pRSET-derived plasmid containing an N-terminal fusion of His<sub>6</sub> lipoamyl domain, and TEV protease cleavage site (courtesy of M. D. Allen, CPE, Cambridge). The construction of the plasmids pRSET-p53C and pET24a-FLTV-flp53 has been described previously (17, 18). For full-length p53 and unstable “hot-spot” mutants of p53C, respectively, we used plasmids expressing the superstable mutant of p53 containing the following mutations in the core domain: M133L/V203A/N239Y/N268D (19, 20).

**Protein Expression and Purification—**53BP2 was expressed in *Escherichia coli* BL21 at 20 °C for 12 h and purified using standard His tag purification protocols followed by TEV protease digestion, a second nickel affinity chromatography step to separate the His-lipoamyl-TEV tag, and a final gel filtration step. 10% glycerol (v/v) was used throughout the purification to reduce aggregation. The protein was kept in storage buffer (25 mM sodium phosphate, pH 7.2, 150 mM NaCl, 5 mM DTT) and flash-frozen in liquid nitrogen. Wild-type p53C, T-p53C mutants, and full-length T-p53 were expressed and purified as described previously (17–19). Isotopically, <sup>15</sup>N-labeled p53C was expressed in M9 minimal medium supplemented with vitamin mix and 1 g/liter <sup>15</sup>N<sub>H</sub><sub>4</sub>Cl as sole nitrogen source.

**Analytical Ultracentrifugation (AUC)—**Equilibrium sedimentation experiments were performed with a Beckman XL-I Ultracentrifuge equipped with a Ti-60 rotor and 6-sector cells at 11,000, 15,000, 20,000, and 24,000 rpm at 10 °C. Data were collected at 280 nm for unlabeled protein and at 494 nm for fluorescein-labeled DNA or protein. To assess the oligomeric state of 53BP2, we used a 12 μM sample. When testing the interaction between 53BP2 and p53C, the concentrations were 8 μM each. For binding of 53BP2 and/or p53C to various fluorescein-labeled DNA response elements (21) (sequences and annealing procedure are described below), the protein concentrations were 50 μM (p53C) and 100 μM (53BP2), respectively; the concentration of fluorescein-labeled DNA was 5 μM. Buffer conditions were 25 mM sodium phosphate (pH 7.2), 150 mM NaCl, and 1 mM DTT. Sample volume was 110 μl. Samples were considered to be at equilibrium as judged by a comparison of the several scans of each speed. Data were processed and analyzed using Ultraspin software and KaleidaGraph (Abelbeck Software, Reading, PA) for graph plotting.

**NMR—**All NMR spectra were acquired on a Bruker DRX-600 spectrometer equipped with CryoProbe<sup>TM</sup> and single-axis gradients. For binding studies, HSQC spectra were acquired in a 25 mM sodium phosphate buffer (pH 7.2), 150 mM NaCl, 5 mM DTT at 298 K using standard Bruker pulse sequences (22) with concentrations of 53BP2 and uniformly labeled, perdeuterated <sup>15</sup>N<sub>2</sub><sup>1</sup>H<sub>2</sub>-p53C of 180 and 100 μM, respectively. The data were processed in Felix98 (MSI Biosystems), and figures were generated using NMR View (23). Effects of binding were analyzed using the assignment of p53C (17, 24) and mapped onto the structure using PyMOL (25).

**Isothermal Titrination Calorimetry (ITC)—**The thermodynamic parameters were determined using an isothermal titration calorimeter (VP-ITC, MicroCal, Inc.) as described previously (26, 27) at 20 °C in a 25 mM sodium phosphate buffer (pH 7.2), 150 mM NaCl, and 1 mM DTT. For binding of 53BP2 to wild-type p53C and various p53C mutants, ~ 200 μM p53C was titrated into the sample cell containing 10 μM 53BP2. Injection steps were 10 μl (first injection, 3 μl) with 250-s spacing. Baseline corrections were performed by titrating protein into sample buffer. Further data evaluation was done using the MicroCal Origin<sup>TM</sup> program.

**Fluorescence Anisotropy Titration—**Measurements were performed on a PerkinElmer LS55 luminescence spectrometer equipped with a Hamilton Microlab titrator controlled by laboratory software. Fluorescence anisotropy was measured with excitation at 480 nm and emission at 530 nm and slit width of 14 and 18 nm, respectively. Reactions were carried out at 16 and 37 °C in 25 mM sodium phosphate buffer (pH 7.2), 150 mM NaCl, and 5 mM DTT. Titrations were performed as described previously (28, 29). In the binding experiments, 200 μM p53C was titrated into 10 nm fluorescein-labeled DNA constructs. Dissociation constants were obtained by fitting anisotropy data to equations corresponding to a one-site binding model with

$$K_d = (K_1 \times K_2 \times K_3 \times K_4)^{-1}$$

In the competition binding assay, a stock solution of 150–300 μM 53BP2 was titrated into 1 ml of preincubated mixture of 5–10 μM p53C or 200 nm full-length...
**Binding of p53 to 53BP2**

T-p53 and 10 nM of fluorescein-labeled DNA. Oligonucleotides used for fluorescence anisotropy were labeled only on the 5'-end of the forward strand with fluorescein (represented by Fl in the sequences) to avoid energy transfer between fluorophores. The following sequences were used for the different response elements (30) (only forward strand shown, reverse strand accordingly): GADD45 response element: 5'-Fl-GTACAGGCTCTGGTGGG-3' /p21 response element: 5'-Fl-ATCAGGAACATGTCCCAACATGTTGAGCTC-3' /Bax response element: 5'-Fl-TCACAAGTTAGACAGCTGACGGTGCCAGC-3' /PIG response element: 5'-Fl-GCACCCAGCTGCCCACCCATGCTCAAGAT-3'. The oligonucleotides were annealed by heating to 95 °C for 10 min followed by gradually cooling to 4 °C over 4 h.

**RESULTS AND DISCUSSION**

**Characterization of Binding of 53BP2 to p53 Core Domain**—

We characterized the binding of 53BP2 to p53C in solution using different biophysical techniques. Analytical ultracentrifugation is a suitable technique to determine the mass of a protein or a complex under native conditions. 53BP2 is monomeric in solution. The complex p53C-53BP2 had an M<sub>r</sub> of 52,000 ± 1100 corresponding to a 1:1 stoichiometry in solution (data not shown).

NMR spectroscopy is able to provide information about the binding surface as well as conformational changes at a residue-specific level. The HSQC spectrum of p53C revealed that several peaks disappeared because of intermediate exchange upon binding to 53BP2 (Fig. 1A). Most of the affected residues are localized in the binding surface that is present in the crystal structure (14). In addition, residues within loop 1, which shows conformational flexibility in the solution structure (24), and the C-terminal helix of p53C were also affected (Fig. 1B).

We further analyzed the magnitude of binding by ITC. 53BP2 binds to p53C in a 1:1 ratio with a K<sub>d</sub> of 2.2 μM (Fig. 2A). This value is in the range of other proteins binding to p53 core domain, like 53BP1-BRCT (31), Rad51 (32), or HIF-1α (33, 34).

**Investigating the Binding of p53 Hot-spot Mutations to 53BP2**—

Most mutations that are observed in human cancers map to the DNA-binding core domain (5). The binding of several of these point mutants including the most frequently occurring hot-spot mutants to 53BP2 was investigated using ITC (Fig. 2). For some mutants, a superstable quadruple mutant (T-p53 = M133L/V203A/N239Y/N268D) with almost identical structure and DNA bind-
ing activity was used as a framework to increase stability (7, 19). T-p53C-V143A, T-p53C-F270L, p53C-R273H, and T-p53C-R282W bind 53BP2 with an affinity similar to wild-type p53C or T-p53C, respectively (Table 1). T-p53C-V143A and T-p53C-F270L are structural mutants that simply create a cavity within the hydrophobic core. Although the presence of these mutations decreases the thermodynamic stability, it has no major influence on either binding to DNA or 53BP2.

The crystal structure of T-p53C-R282W revealed that loop L1 is disordered in this mutant. The retention of binding of T-p53C-R282W to 53BP2 suggests that loop L1, which has an important yet modular role in apoptotic signaling (35), is not crucial for binding to 53BP2.

53BP2 bound tightly to p53C-R273H, although it is a contact mutant with substantially reduced DNA binding (20).

### TABLE 1

| p53C variant binding to 53BP2 | $K_d$ (μM) | Mutation region | DNA binding | Ref. |
|------------------------------|------------|-----------------|-------------|------|
| Wild-type p53C               | 2.2 ± 0.2  | Zinc region     | +           | 6    |
| p53C-R181E                   | No binding | DNA-binding region | +           | 47   |
| p53C-R249S                   | No binding | DNA-binding region | +           | 20   |
| p53C-R249S/H168R             | No binding | DNA-binding region | +           | 20   |
| T-p53C                       | 1.7 ± 0.4  | β-Sandwich      | +           | 47   |
| T-p53C-V143A                 | 2.8 ± 1.0  | β-Sandwich      | +           | 47   |
| T-p53C-R175H                 | No binding | DNA-binding region | –           | 47   |
| T-p53C-G245S                 | No binding | DNA-binding region | +           | 47   |
| T-p53C-F270L                 | 5.2 ± 1.2  | DNA-binding region | +           | 47   |
| T-p53C-F270L                 | 2.4 ± 1.0  | DNA-binding region | +           | 47   |

Measurements were performed in a 25 mM sodium phosphate buffer (pH 7.2), 150 mM NaCl, 1 mM DTT at 20 °C. The detection limit for the $K_d$ was estimated to be around 40 μM.

Binding of p53C mutants to GADD45 or consensus DNA at 20 °C compared with wild-type binding (47).

T-p53C = superstable quadruple mutant (M133L/V203A/N239Y/N268D) with almost identical structure and DNA-binding activity (7, 19).

Not determined.

Unpublished.

![Figure 3](https://example.com/figure3.png)

**FIGURE 3.** Binding of p53C to various DNA sequences examined by fluorescence anisotropy. Binding of p53C to GADD45 (A), p21 (B), PIG3 (C), and Bax (D) was measured at 16 °C in 25 mM sodium phosphate buffer (pH 7.2), 150 mM NaCl, 5 mM DTT. The continuous line in each plot corresponds to a single-site binding model fit.
Presumably, although Arg273 makes a crucial DNA contact (6), it is located at the edge of the 53BP2-binding interface (14) and makes only minor electrostatic contributions to the binding.

Arg249 is located in the centers of both the DNA- and 53BP2-binding interfaces. The structural mutation R249S disrupts a network of interactions that stabilize the L3 loop in a conformation enabling Arg248 to make DNA contacts (20). As Arg249 also directly interacted with the SH3 domain of 53BP2, it was not surprising that the structural mutant p53C-R249S did not detectably bind to 53BP2 (Fig. 2C and Table 1). Interestingly, the double mutant T-p53C-R249S/H168R does bind to 53BP2 with Kd = 5.2 μM (3-fold weaker than T-p53C), and thus this double mutation, which is identified as a “second-site suppressor” in vivo (36, 37) reverts not only defects in DNA binding but also in binding to 53BP2 (Fig. 2C and Table 1). The recently solved crystal structure of T-p53C-R249S/H168R revealed that the structural changes caused by the cancer mutations H168R and R249S are reverted if both mutations are present in the same molecule (20). In this case, the guanidinium group of Arg168 superimposes very well with its corresponding position in Arg249 in wild-type p53. The fact that binding of the second-site suppressor mutant T-p53C-R249S/H168R to 53BP2 is 3-fold weaker than T-p53C, despite full restoration of DNA binding, highlights the importance of Arg249 in binding to 53BP2.

We also found two mutants that did not detectably bind to 53BP2 even though they retained a weak DNA-binding affinity. p53C-G245S as well as the stabilized variant T-p53C-G245S did not bind to 53BP2 (Fig. 2B and Table 1). Because Gly245 does not directly interact with 53BP2, this finding is somehow surprising and might be explained by an unfavorable destabilization of loop L3 that disrupts the p53C-53BP2 interaction.
fact, the crystal structure of T-p53C-G245S revealed small but distinct changes within the L3 loop.4

The short H1 helix of p53C forms the main interface with 53BP2. By mutating Arg181→Glu within this helix, binding of p53C to 53BP2 is disrupted (Table 1). There are several p53 mutants with mutations at residue Arg181. They lack apoptosis-inducing activity, although they retain DNA binding activity in vivo (11, 38–40). Although the H1 helix is not directly involved in DNA binding, it provides an additional core domain-core domain dimerization interface (41–44), and mutations in this region reveal weaker DNA binding than wild-type p53 (18).5

The fact that these mutants did not bind to 53BP2 despite retaining DNA-binding capacity could relate them to a third class of p53 mutants (8) alongside contact and structural mutations. Their effect in promoting cancer might be caused by disrupting the binding to ASPP2 as a cofactor within an important pro-apoptotic pathway.

53BP2 Competes off p53 from Both Pro-apoptotic and Non-apoptotic DNA Response Elements—To investigate the selective up-regulation of pro-apoptotic response elements, we used fluorescence anisotropy to analyze the competition between 53BP2 and different response elements for binding to p53C and full-length p53. GADD45 and p21 were used as examples of non-apoptotic genes, whereas Bax and PIG3 were used as pro-apoptotic response elements. First, we measured the binding of p53C to these fluorescently labeled response elements. The binding of p53C to GADD45 ($K_d = 7.5 \mu M$) and p21 ($K_d = 7.1 \mu M$) is significantly tighter than its binding to the pro-apoptotic response elements PIG3 ($K_d = 15 \mu M$) and Bax ($K_d = 48 \mu M$) (Fig. 3). AUC equilibrium sedimentation revealed that p53C bound to the response elements in a 4:1 complex. As 53BP2 binds to p53C with $K_d = 2.2 \mu M$, and the binding surface on p53C shows significant overlap with the one in the p53C-DNA complex (14), we tested whether competition occurs between 53BP2 and DNA response elements bound to p53 (Fig. 4).

We found that binding of 53BP2 to p53C competes off both pro-apoptotic and non-apoptotic response elements. For the non-apoptotic response elements GADD45 and p21, this effect is most obviously detectable by fluorescence anisotropy. On adding 53BP2 to p53C, preincubated with the appropriate response element, the anisotropy decreased to the value of the free DNA (Fig. 4A), consistent with their overlapping binding sites on p53C.

As the binding of p53C to the pro-apoptotic response elements Bax and PIG3 is much weaker compared with GADD45 and p21, competition experiments with these response elements by fluorescence anisotropy showed a decrease in anisotropy followed by a linear increase (Fig. 4B). The smaller decrease in anisotropy is because of the fact that at the starting point only a small fraction of p53C is bound to DNA. The linear increase at high titrant concentration results from an increase in viscosity and could not be explained by binding of 53BP2 to the response element as tested in a control experiment (Fig. 5).

---

5 S. Rutledge and A. R. Fersht, unpublished results.
We confirmed the competition between 53BP2 and pro-apoptotic response elements for binding to p53C by analytical ultracentrifugation. The equilibrium sedimentation profile of fluorescein-labeled Bax with p53C fitted to a double exponential (=2 component) model with \( M_t = 118,200 \pm 2400 \) for the predominant species, indicating a 4:1 stoichiometry in binding of p53C to Bax (Fig. 6A). On adding 53BP2 to p53C bound to Bax, the sedimentation of the labeled component revealed a single component of \( M_t = 17,200 \pm 1900 \), corresponding to the molecular weight of the unbound response element. A comparison of the sedimentation profiles of Bax alone and Bax + p53C + 53BP2 showed virtually no difference in curvature. Both profiles fitted to a single-exponential (single component) function with \( M_t = 18,600 \pm 600 \) (Fig. 6B). This indicates that 53BP2 is able to compete off pro-apoptotic response elements from p53C and excludes the existence of a ternary complex.

Further, we confirmed the competition between 53BP2 and both pro-apoptotic and non-apoptotic response elements with full-length p53 at physiological temperature (Fig. 4C). All tested response elements bound tightly to full-length p53 and were displaced upon addition of 53BP2 as indicated by the decrease in fluorescence anisotropy. In summary, these results indicate that p53 can either bind DNA response elements or 53BP2, but not both simultaneously, as DNA is displaced from p53 when 53BP2 is present.

Several models exist to explain how cells choose between apoptosis and cell cycle arrest (45, 46). Supporting a variant of the promoter-specificity model, it was postulated that ASPP2 might act as a cofactor by subtly altering the ability of p53 to bind low-affinity pro-apoptotic response elements and thus might act as a cofactor by subtly altering the ability of p53 to promote apoptosis and cell cycle arrest (45, 46). Supporting a variant of 53BP2 is present.

Acknowledgments—We thank Drs. M. Fernandez-Fernandez and A. Joerger for helpful discussions; Drs. H. C. Ang, A. Joerger, S. Mayer, and S. Rutledge for plasmids and proteins; C. Blair for TEV protease; and Dr. C. Johnson for advice and help on the experimental setup.

REFERENCES
1. Vogelstein, B., Lane, D., and Levine, A. J. (2000) Nature 408, 307–310
2. Levine, A. J. (1997) Cell 88, 323–331
3. Ryan, K. M., Phillips, A. C., and Vousden, K. H. (2001) Curr. Opin. Cell Biol. 13, 332–337
4. Vousden, K. H., and Lu, X. (2002) Nat. Rev. Cancer 2, 594–604
5. Hainaut, P., and Hollstein, M. (2000) Adv. Cancer Res. 77, 81–137
6. Cho, Y., Gorina, S., Jeffrey, P. D., and Pavletich, N. P. (1994) Science 265, 346–355
7. Joerger, A. C., Allen, M. D., and Fersht, A. R. (2004) J. Biol. Chem. 279, 1291–1296
8. Slee, E. A., and Lu, X. (2003) Toxical Lett. (Shannon) 139, 81–87
9. Bates, S., and Vousden, K. H. (1999) Cell. Mol. Life Sci. 55, 28–37
10. Vousden, K. H. (2000) Cell 103, 691–694
11. Samuels-Lev, Y., O’Connor, D. J., Bergamaschi, D., Trigante, G., Hsieh, J. K., Zhong, S., Campargue, I., Naumovski, L., Crook, T., and Lu, X. (2001) Mol. Cell 8, 781–794
12. Lane, D. (2001) Nature 414, 25–27
13. Ishiwabu, K., Bartel, P. L., Li, B., Marraccino, R., and Fields, S. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 6098–6102
14. Gorina, S., and Pavletich, N. P. (1996) Science 274, 1001–1005
15. Lu, X. (2005) Curr. Opin. Genet. Dev. 15, 27–33
16. Trigante, G., and Lu, X. (2006) Nat. Rev. Cancer 6, 217–226
17. Wong, K. B., DeDecker, B. S., Freund, S. M., Proctor, M. R., Bycroft, M., and Fersht, A. R. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 8438–8442
18. Veprintsev, D. B., Freund, S. M., Andreeva, A., Rutledge, S. E., Tidow, H., Canadillas, J. M., Blair, C. M., and Fersht, A. R. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 2115–2119
19. Nikolova, P. V., Henckel, J., Lane, D. P., and Fersht, A. R. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 14675–14680
20. Joerger, A. C., Ang, H. C., Veprintsev, D. B., Blair, C. M., and Fersht, A. R. (2005) J. Biol. Chem. 280, 16030–16037
21. Friedler, A., Veprintsev, D. B., Freund, S. M., von Glos, K. I., and Fersht, A. R. (2005) Structure (Camb.) 13, 629–636
22. Mori, S., Abeygunawardana, C., Johnson, M. O., and van Zijl, P. C. (1995) J. Magn. Reson. 108, 94–98
23. Johnson, B. A., and Blevins, R. A. (1994) J. Biol. Chem. 269, 603–614
24. Canadillas, J. M., Tidow, H., Freund, S. M., Rutherford, T. J., Ang, H. C., and Fersht, A. R. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 2109–2114
25. Delano, W. L. (2002) The PyMOL Molecular Graphics System, DeLano Scientific, San Carlos, CA
26. Wiseman, T., Willistion, S., Brandts, J. F., and Lin, L. N. (1989) Anal. Biochem. 179, 131–137
27. Ladbury, J. E., and Chowdary, B. Z. (1996) Cell 81, 791–801
28. Weinberg, R. L., Veprintsev, D. B., and Fersht, A. R. (2004) J. Biol. Chem. 321, 1145–1159
29. Fernandez-Fernandez, M. R., Veprintsev, D. B., and Fersht, A. R. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 4735–4740
30. Qian, H., Wang, T., Naumovski, L., Lopez, C. D., and Brachmann, R. K. (2002) Oncogene 21, 7901–7911
31. Eklad, C. M., Friedler, A., Veprintsev, D., Weinberg, R. L., and Itzhaki, L. S. (2004) Protein Sci. 13, 617–625
32. Friedler, A., Veprintsev, D. B., Rutherford, T., von Glos, K. I., and Fersht, A. R. (2005) J. Biol. Chem. 280, 8051–8059
33. Hansson, L. O., Friedler, A., Freund, S., Rudiger, S., and Fersht, A. R. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 10305–10309
34. Sanchez-Puig, N., Veprintsev, D. B., and Fersht, A. R. (2005) Mol. Cell 17, 11–21
35. Zupnick, A., and Prives, C. (2006) J. Biol. Chem. 281, 20464–20473
36. Brachmann, R. K., Yu, K., Eby, Y., Pavletich, N. P., and Boeke, J. D. (1998) EMBO J. 17, 1847–1859
37. Baroni, T. E., Wang, T., Qian, H., DeDecker, B. S., Freund, S. M., Proctor, M. R., Bycroft, M., and Fersht, A. R. (1999) Proc. Natl. Acad. Sci. U. S. A. 101, 4930–4935
38. Crook, T., Marston, N. J., Sara, E. A., and Vousden, K. H. (1994) Cell 79, 817–827
39. Friedlander, P., Haupt, Y., Prives, C., and Oren, M. (1996) Mol. Cell. Biol. 16, 4961–4971
40. Smith, P. D., Crossland, S., Parker, G., Osip, P., Brooks, L., Waller, J., Philp, E., Crompton, M. R., Gusterson, B. A., Allday, M. J., and Crook, T. (1999) Oncogene 18, 2451–2459
41. Klein, C., Planker, E., Diercks, T., Kessler, H., Kunkele, K. P., Lang, K., Hansen, S., and Schwaiger, M. (2001) J. Biol. Chem. 276, 49020–49027
42. Rippin, T. M., Freund, S. M., Veprintsev, D. B., and Fersht, A. R. (2002) J. Mol. Biol. 319, 351–358
43. Dehner, A., Klein, C., Hansen, S., Muller, L., Buchner, J., Schwaiger, M., and Kessler, H. (2005) Angew. Chem. Int. Ed. Engl. 44, 5247–5251
44. Kitayner, M., Rozenberg, H., Kessler, N., Rabinovich, D., Shaulov, L., Haran, T. E., and Shakked, Z. (2006) Mol. Cell 22, 741–753
45. Saller, E., Tom, E., Brunori, M., Otter, M., Estreicher, A., Mack, D. H., and Iggo, R. (1999) EMBO J. 18, 4424–4437
46. Ludwig, R. L., Bates, S., and Vousden, K. H. (1996) Mol. Cell. Biol. 16, 4952–4960
47. Bullock, A. N., Henckel, J., and Fersht, A. R. (2000) Oncogene 19, 1245–1256
48. Ang, H. C., Joerger, A. C., Mayer, S., and Fersht, A. R. (2006) J. Biol. Chem. 281, 21934–21941