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The Mechanism of Hsp90 Regulation by the Protein Kinase-Specific Cochaperone p50^{cdc37}

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Summary

Recruitment of protein kinase clients to the Hsp90 chaperone involves the cochaperone p50^{cdc37} acting as a scaffold, binding protein kinases via its N-terminal domain and Hsp90 via its C-terminal region. p50^{cdc37} also has a regulatory activity, arresting Hsp90's ATPase cycle during client-protein loading. We have localized the binding site for p50^{cdc37} to the N-terminal nucleotide binding domain of Hsp90 and determined the crystal structure of the Hsp90–p50^{cdc37} core complex. Dimeric p50^{cdc37} binds to surfaces of the Hsp90 N-domain implicated in ATP-dependent N-terminal dimerization and association with the middle segment of the chaperone. This interaction fixes the lid segment in an open conformation, inserts an arginine side chain into the ATP binding pocket to disable catalysis, and prevents *trans*-activating interaction of the N domains.

Introduction

Hsp90 is a molecular chaperone with an essential role in eukaryotes in activation and maintenance of a wide range of regulatory and signaling proteins (Pearl and Prodromou, 2002; Picard, 2002; Pratt and Toft, 2003). Client protein activation by Hsp90 requires its ATPase activity (Panaretou et al., 1998; Obermann et al., 1998), which is coupled to a conformational cycle involving opening and closing of a "molecular clamp" via transient ATP-dependent association of the N-terminal domains in the Hsp90 dimer (Prodromou et al., 2000; Chadli et al., 2000). Small molecules such as geldanamycin and radicicol, which are competitive inhibitors of ATP binding to the N-terminal nucleotide binding domain, (Stebbins et al., 1997; Prodromou et al., 1997a; Roe et al., 1999), prevent closure of the clamp and disrupt client protein activation (Whitesell et al., 1994; Schulte et al., 1995; Chavany et al., 1996).

The largest coherent class of proteins amongst Hsp90s structurally and functionally varied clientele are the protein kinases. Known protein kinase clients in mammalian cells include: PKB/Akt (Sato et al., 2000; Basso et al., 2002; Fontana et al., 2002), PDK1 (Fujita et al., 2002), LKB1 (Boudeau et al., 2003), Raf-1 (Stancato et al., 1993; Schulte et al., 1995; Grammatikakis et al., 1999), ErbB2 (Xu et al., 2001), Bcr-Abl (An et al., 2000), Src-family kinases (Xu and Lindquist, 1993; Xu et al., 1999; Bijlmakers and Marsh, 2000), Aurora B (Lange et al., 2002), and the cyclin-dependent kinases Cdk4, Cdk6, and Cdk9 (Stepanova et al., 1996; Mahony et al., 1998; O'Keeffe et al., 2000). The involvement of Hsp90 in the activity of so many potentially oncogenic protein kinases has engendered considerable interest in Hsp90 as a target for cancer chemotherapy (reviewed in Neckers, 2002; Maloney and Workman, 2002; Blagosklonny, 2002).

Activation of Hsp90-dependent client proteins proceeds through an ordered sequence of complexes, linked to the ATPase cycle, and involving a variety of cochaperones (reviewed in Pearl and Prodromou, 2002). Hsp90-dependent protein kinases in mammalian cells are particularly associated with a 50 kDa protein (Brugge, 1986), whose N-terminal region has strong homology to the equivalent region of Cdc37p from budding yeast (Perdew et al., 1997; Stepanova et al., 1996). Functional dissection of this protein, p50^{cdc37}, identified a protein kinase binding domain at the N terminus (Grammatikakis et al., 1999), while the remainder of the protein was found to interact with Hsp90 (Shao et al., 2001). Thus, p50^{cdc37} acts as an adaptor or scaffold, facilitating protein kinase interaction with the Hsp90 molecular chaperone (Silverstein et al., 1998). Binding to p50^{cdc37} is highly specific, and otherwise closely related protein kinases may differ substantially in their interaction. Thus, Cdk4 associates with p50^{cdc37}, whereas Cdk2 does not (Dai et al., 1996; Stepanova et al., 1996). Similarly, the MAP-kinase MOK binds p50^{cdc37}, while ERK, JNK, and p38 do not (Miyata et al., 2001). While the majority of Hsp90-dependent protein kinases so far identified are also dependent on p50^{cdc37} (Hunter and Poon, 1997), the converse may not always be true and for some kinases interaction with p50^{cdc37} alone may be sufficient to afford a degree of stabilization (Tatebe and Shiozaki, 2003; Lee et al., 2002).

We have previously shown that p50^{cdc37} is not a passive scaffold, but is an active Hsp90 cochaperone participating in regulation of the Hsp90 chaperone cycle (Siligardi et al., 2002). Thus, like the TPR-domain cochaperone

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Hop/Sti1 (Prodromou et al., 1999; Richter et al., 2003), p50^{cdc37} is able to arrest the ATPase cycle to facilitate client protein loading. This property resides in the C-terminal part of the molecule and is retained, although to a lesser degree, in the yeast Cdc37p, whose C terminus has only low homology to its mammalian homologs. In order to define the nature of the functional interaction between p50^{cdc37} and Hsp90, we have mapped the p50^{cdc37} binding region in Hsp90 to the N-terminal nucleotide binding domain and determined the crystal structure of the core Hsp90-p50^{cdc37} complex. The structure reveals the mechanism by which p50^{cdc37} arrests the ATPase cycle of Hsp90, locking the "lid" of the nucleotide binding pocket in an open conformation, and holding the "jaws" of the Hsp90 molecular clamp apart to facilitate client protein loading.

Results and Discussion

Localizing the Hsp90-p50^{cdc37} Interaction

Previous studies of cochaperone interactions with Hsp90 had shown mutually exclusive binding of p50^{cdc37} and TPR-domain cochaperones such as Hop/Sti1 (Owens-Grillo et al., 1996), which has been generally interpreted in terms of competitive binding by p50^{cdc37} at or close to the known TPR binding site at the C terminus of Hsp90. However, there was some ambiguity in this assignment, as although p50^{cdc37} binding was competed by full-length TPR-domain cochaperones, an isolated TPR-domain could bind simultaneously with p50^{cdc37} (Silverstein et al., 1998). To resolve this question, we analyzed the interaction between p50^{cdc37} or a C-terminal segment of p50^{cdc37} (C-p50^{cdc37}: residues 138–378) previously shown to be sufficient for Hsp90 binding (Shao et al., 2001; Siligardi et al., 2002), and a set of yeast Hsp90 constructs, using isothermal titration calorimetry (ITC) (Figure 1). As expected from previous studies using difference CD (Siligardi et al., 2002), full-length p50^{cdc37} and C-p50^{cdc37} bound to full-length Hsp90 with very similar affinities (Kd = 1.46 μM, and 1.32 μM, respectively). However, no significant heats of interaction were observed with any Hsp90 construct lacking the N-terminal nucleotide binding domain, regardless of the presence or absence of the C-terminal constitutive dimerization domain. Consistent with these observations the isolated nucleotide binding domain bound to C-p50^{cdc37} with comparable affinity to full-length Hsp90 (Kd = 1.80 μM), identifying it unambiguously as the primary binding site for p50^{cdc37}. Although Hsp90s are extremely highly conserved especially in the N-terminal domain, we were concerned to verify that this interaction was not an artifact of yeast Hsp90 binding to human p50^{cdc37}. In confirmation of this, we found that the N-terminal domain of human Hsp90α bound to C-p50^{cdc37} with comparable affinity (Kd = 3.37 μM). In all cases, p50^{cdc37} constructs and Hsp90 constructs formed complexes with 1:1 stoichiometry.

On the basis of this observation, crystallization screens were conducted with equimolar mixtures of C-p50^{cdc37} and various Hsp90 N-terminal domain constructs. Diffraction quality crystals were obtained in several conditions using human C-p50^{cdc37} and yeast Hsp90(1–208). The structure was phased by molecular replacement using the known structure of the yeast Hsp90 N-terminal domain (N-Hsp90) (Prodromou et al., 1997a, 1997b) as a search model. The structure of C-p50^{cdc37} was determined from difference Fourier maps and the structure of the complex refined at 2.4 Å (see Experimental Procedures).

Structure of C-p50^{cdc37}

The C-terminal segment of p50^{cdc37} is entirely helical in structure, consisting of a large 6-helix bundle at the N-terminal end of the visible structure (residues 148–245) connected to a small 3-helix bundle (292–347) via a long single helix (246–286). This small helical bundle is less well ordered than the rest of C-p50^{cdc37} and no interpretable electron density is present for residues 309–315, which connect the first and second helices of this domain. The large helical domain has an unusual topology, with helices 2–5 forming a right-handed orthogonal 4-helix bundle, while helix 6 and the proximal part of the long connecting helix form an α-arch that wraps around the surface of helix 5 (Figure 2A). Comparison of C-p50^{cdc37} with libraries of known protein folds yielded no structure of comparable topology (Pearl et al., 2000; Murzin et al., 1995). The sequence of this region is reasonably conserved in vertebrates becoming more diverged in invertebrates and fungi (Figure 2B). Nonetheless, optimal sequence threading (Jones et al., 1992) of the highly diverged *S. cerevisiae* Cdc37p sequence unambiguously recognizes the large globular domain of p50^{cdc37} as the best fit, suggesting that despite sequence variation the overall fold is universally conserved.

In the crystals, C-p50^{cdc37} forms a homodimeric interaction with a symmetry related molecule (Figures 3A, 3B, and 3C). The core of the interface involves close packing of the main chain and side chains of Gln 247 and Tyr 248, in the first turn of the long connecting helix, with their equivalents in the other monomer. This hydrophobic core interaction is reinforced by ion-pairs between Lys 240 and Asp 245 from separate monomers, a carboxyl-carboxylate interaction between the side chains of Asp 294 from each monomer, and a network of hydrogen bonding involving the side chains of Arg 246 and Glu 250 from one monomer with Glu 221 and Glu 255 from the other (Figure 3D). Previous studies showed that full-length p50^{cdc37} is a dimer in solution with a Kd $\approx$ 5–10 μM (Siligardi et al., 2002). The isolated C-terminal domain of p50^{cdc37} retains the ability to dimerize but with reduced affinity (Kd $\approx$ 170 μM). However, at the concentrations used in crystallization experiments and in the presence of the molecular crowding agents used to promote crystallization, C-p50^{cdc37} would be predominantly dimeric so that the dimer observed in the crystals is likely to be authentic. The relatively small surface area buried in that interface (560 Å^2) would be consistent with the measured affinity.

Hsp90-p50^{cdc37} Interactions

The Hsp90 N-terminal nucleotide binding domain binds to the large helical domain of C-p50^{cdc37}, with helices 2, 3, and 5 from C-p50^{cdc37} packing against the surface of the lid segment in N-Hsp90 (residues 100–121), which is thought to close over bound ATP (Prodromou et al., 2000) (Figure 4A). The core of the interaction involves a relatively flat hydrophobic patch formed by the side

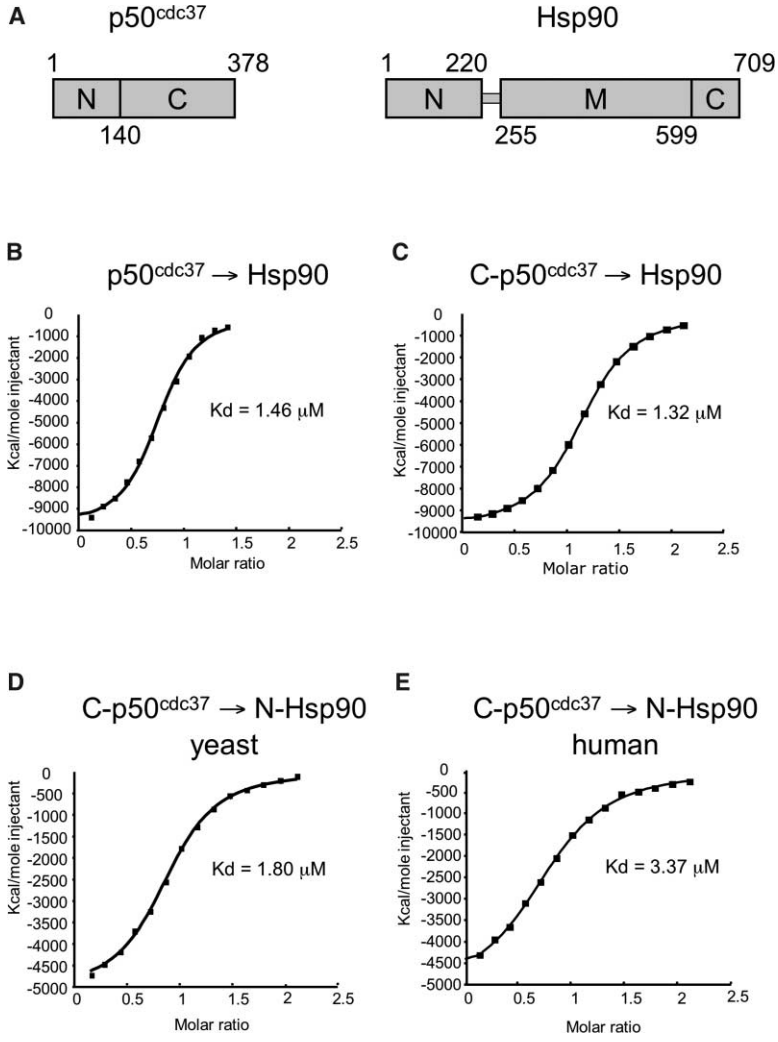

Figure 1. Localization of Hsp90-C-p50 Interaction

(A) Schematic of the domain structure of $p50^{cdc37}$ (left) and Hsp90 (right)

(B) Isothermal titration calorimetry (ITC) of full-length human $p50^{cdc37}$ injected into full-length yeast Hsp90. The proteins interact with Kd = 1.46 μM, with ≈ 1:1 stoichiometry, consistent with previous observations (Siligardi et al., 2002).

(C) ITC of a C-terminal construct of $p50^{cdc37}$ (residues 138–378: C-$p50^{cdc37}$) binding to full-length yeast Hsp90. The affinity of the interaction is very similar to that of the full-length proteins, confirming the previous observation that Hsp90 binding was a function of C-$p50^{cdc37}$.

(D) ITC of C-$p50^{cdc37}$ binding to the isolated nucleotide binding domain of Hsp90 (1–220: N-Hsp90). The affinity of the interaction is very close to that of the full-length protein, identifying N-Hsp90 as the primary binding site for $p50^{cdc37}$.

(E) As (D), but with the N-terminal nucleotide binding domain of human Hsp90α which has 69% sequence identity to the yeast equivalent. The similar affinity demonstrates unequivocally that the interaction between C-$p50^{cdc37}$ and N-Hsp90 is authentic and universal.

chains of Met 164, Leu 165, Ala 204, and Leu 205 of $p50^{cdc37}$, which pack against a similar patch formed by the side chains of Ala 103, Ala 107, Ala110, Gly 111, Ala 112, Met 116, and Phe 120 of Hsp90. The central hydrophobic interface is reinforced by a network of polar interactions including several intermolecular side chain to main chain hydrogen bonds. The most substantial part of this network involves Gln 119 of Hsp90 whose main chain oxygen is hydrogen bonded to the side chain of Gln 208 of $p50^{cdc37}$, while its side chain hydrogen bonds to the main chain carbonyl of Asp 120 and the main chain nitrogens of Arg 166 and Arg 167 of $p50^{cdc37}$ (Figure 4B). The interaction of Hsp90 and $p50^{cdc37}$ buries ≈1056 Å^2 of molecular surface. Although the Hsp90 N-terminal domain used in this study derives from yeast, all the residues in contact with $p50^{cdc37}$ are identical to those in both of the human cytoplasmic Hsp90 isoforms, with the exception of Ser 109 on the edge of the lid, which hydrogen bonds to the side chain of $p50^{cdc37}$ Lys 202—an interaction available to the glutamine found at this position in human Hsp90s.

We have previously described a mutation engineered in the surface of the lid segment, A107N, which activates the inherent ATPase of Hsp90 probably by favoring lid closure and N-terminal association on binding of ATP (Prodromou et al., 2000). As Ala 107 is intimately involved in the close-packed hydrophobic interface with $p50^{cdc37}$ observed in these crystals, its mutation to a larger hydrophilic residue would be expected to have a very substantial impact if that is indeed the functional interface between the two proteins. We therefore analyzed the effect of the A107N mutation on interaction of Hsp90 with $p50^{cdc37}$ in solution. In marked contrast to the wild-type, the ATPase activity of the Hsp90-A107N mutant was completely resistant to inhibition by $p50^{cdc37}$ (Figure 4C). We also examined the sensitivity of a second Hsp90 mutant, T22I, which like A107N has a substantially enhanced inherent ATPase activity (Prodromou et al., 2000), but unlike Ala 107, Thr 22 is not involved in the observed interface with $p50^{cdc37}$. In this case, the ATPase activity was fully sensitive to inhibition by $p50^{cdc37}$, showing that the desensitization produced by the A107N mutation is specific and not a general property of hyperactive Hsp90 mutants. Consistent with its effect on ATPase inhibition, and in contrast to wild-type, no significant heat was generated when $p50^{cdc37}$ was titrated into Hsp90-A107N in ITC experiments (Figure 4D).

The side chain of Arg 167 from $p50^{cdc37}$ points down

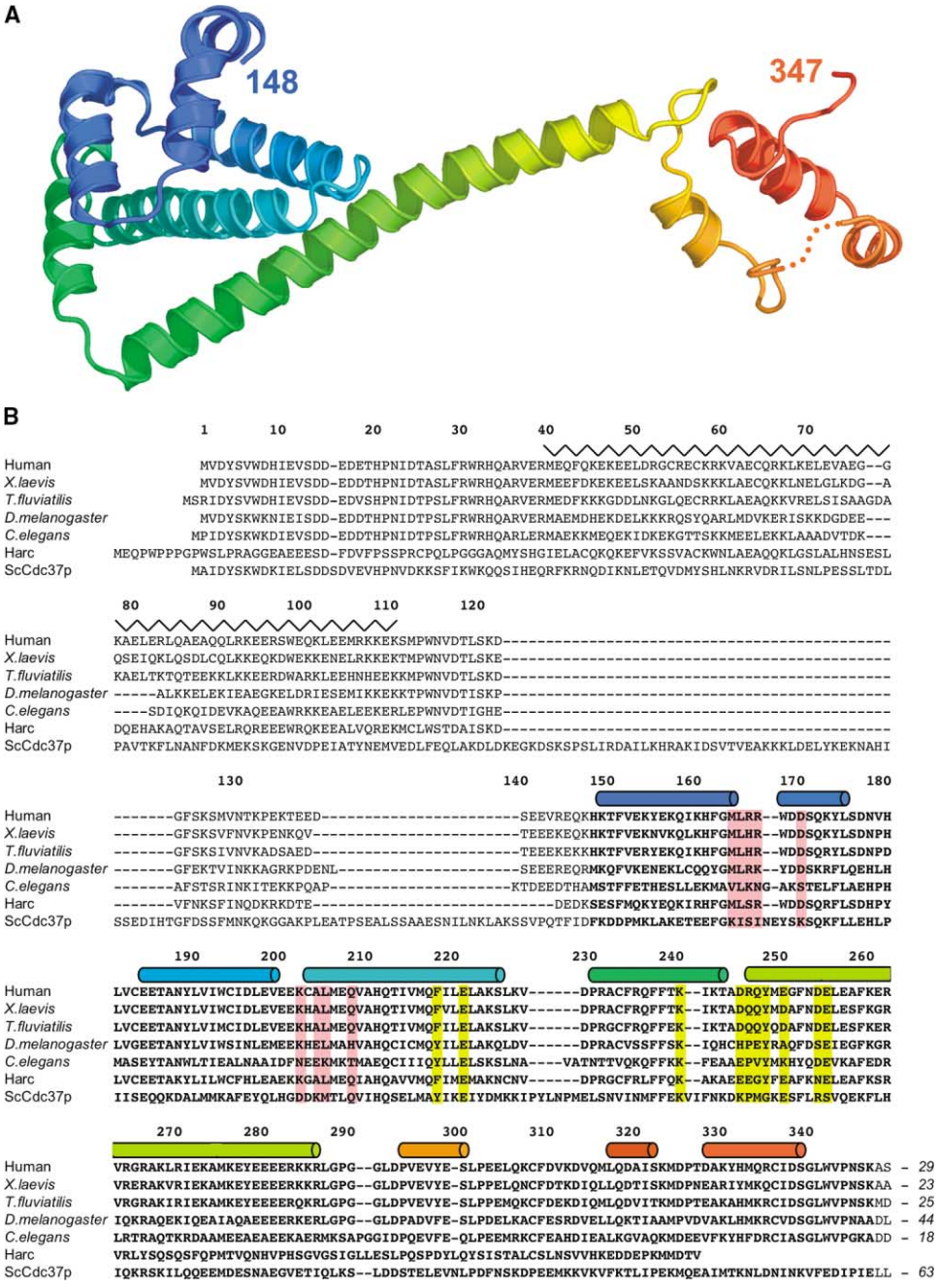

Figure 2. Structure of C-p50^{cdc37}

(A) Secondary structure cartoon of C-p50^{cdc37} "rainbow" colored from the visible N terminus at residue 148 (blue) to the visible C terminus at 347 (red). The disordered polypeptide chain connecting residues 308 and 316 is indicated by a dotted line.

(B) Alignment of the sequence and structure of human p50^{cdc37} with the amino acid sequences of representative p50^{cdc37} homologs from amphibians (*Xenopus laevis*), fish (*Tetraodon fluviatilis*–pufferfish), insects (*Drosophila melanogaster*), and worms (*Caenorhabditis elegans*); human Harc (Hsp90-associating relative of Cdc37- (Scholz et al., 2001) and *Saccharomyces cerevisiae* Cdc37p. Sequences in the region delineated by the C-p50^{cdc37} structure described here are shown in bold-face type and the observed secondary structural elements are shown above the sequence. The yeast Cdc37p sequence beyond residue 120 is extremely diverged with several substantial relative insertions and cannot be aligned reliably. The alignment shown here is based on optimal sequence threading (Jones et al., 1992) of the Cdc37p sequence on the C-p50^{cdc37} structure and may be more accurate than a purely sequence-based alignment, but should be regarded with suspicion. The "zigzag" over the N-terminal protein kinase binding region of the p50^{cdc37} sequence indicates a predicted ~70 residue coiled-coil segment. Residues involved in C-p50^{cdc37} dimerization are highlighted in yellow; those involved in interaction with Hsp90 are highlighted in pink.

into the mouth of the nucleotide binding pocket in the Hsp90 N-terminal domain and hydrogen bonds to the carboxyl side chain of Glu 33 (Figure 5A). This residue is believed to act as the general base in the chaperone's ATPase reaction (Prodromou et al., 1997a; Panaretou et al., 1998; Obermann et al., 1998), and the guanidinium

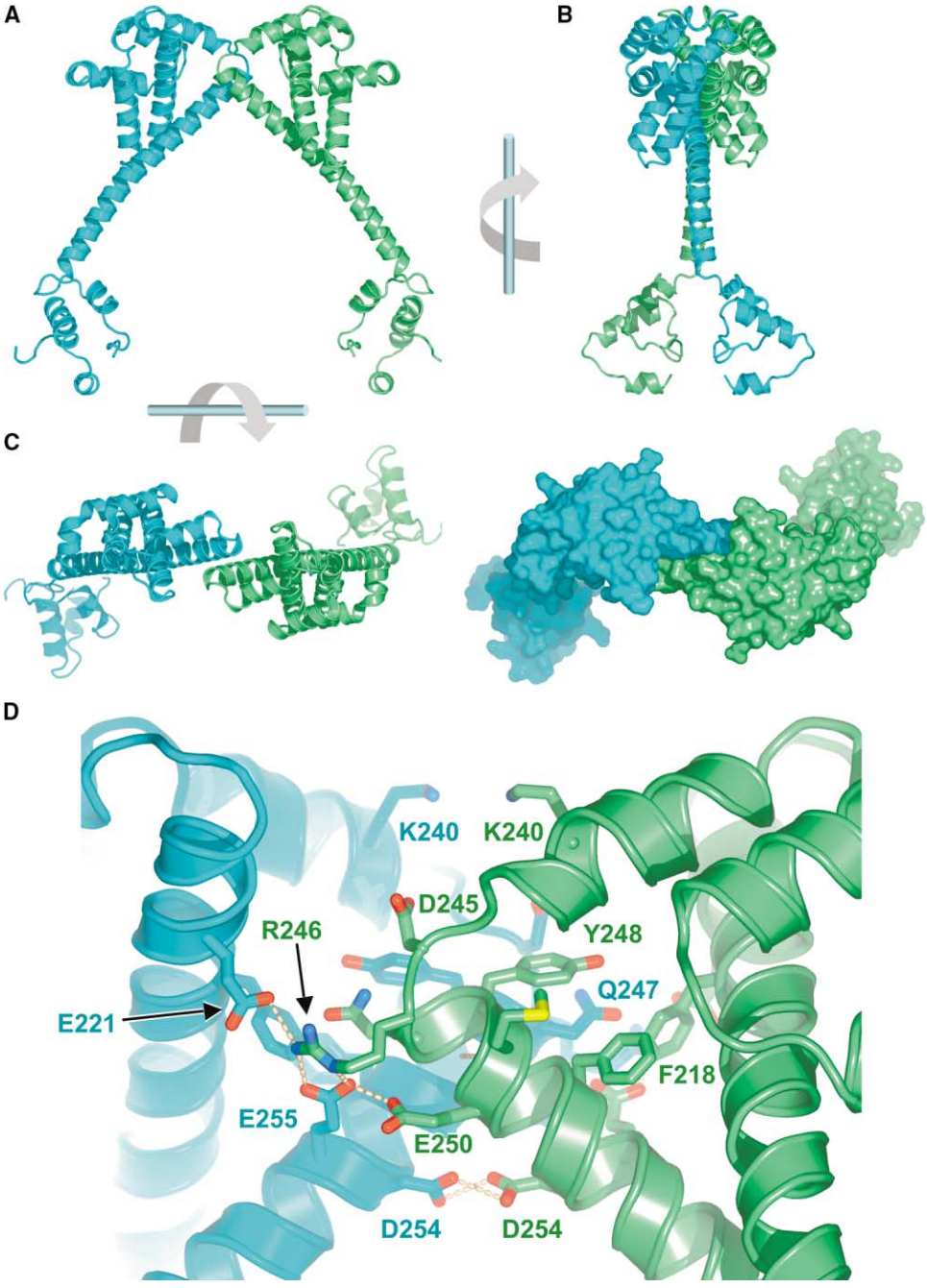

Figure 3. C-p50^{cdc37} Dimer

(A) Secondary structure cartoon of C-p50^{cdc37} dimer (one monomer is colored cyan, the other green).

(B) As (A) but rotated around the vertical.

(C) As (A) but rotated about the horizontal. The dimer is shown as molecular surfaces on the right. The C-p50^{cdc37} dimer exists in solution with a Kd $\approx$ 170 μM and dimer formation buries $\sim$560 Å^2 of molecular surface.

(D) Details of the interactions in the C-p50^{cdc37} dimer interface. Hydrogen bonds are indicated by broken rods connecting interacting atoms.

group of Arg 167 occupies the position expected for the attacking nucleophilic water. Arg 167 does not penetrate deeply enough into the pocket to obstruct binding of nucleotides or drugs such as geldanamycin (Figure 5B). This is consistent with previous experimental observations that binding of p50^{cdc37} does not displace geldanamycin from Hsp90 (Siligardi et al., 2002) and that geldanamycin does not disrupt Hsp90-p50^{cdc37} complexes (Shao et al., 2001). Nonetheless, the presence of the side chain of Arg 167 does alter the electronic and chemical environment at the top of the pocket, so that Hsp90 inhibitors that impinge upon this part of the pocket could be tailored to bind selectively to, or be selectively excluded from, Hsp90-p50^{cdc37} complexes.

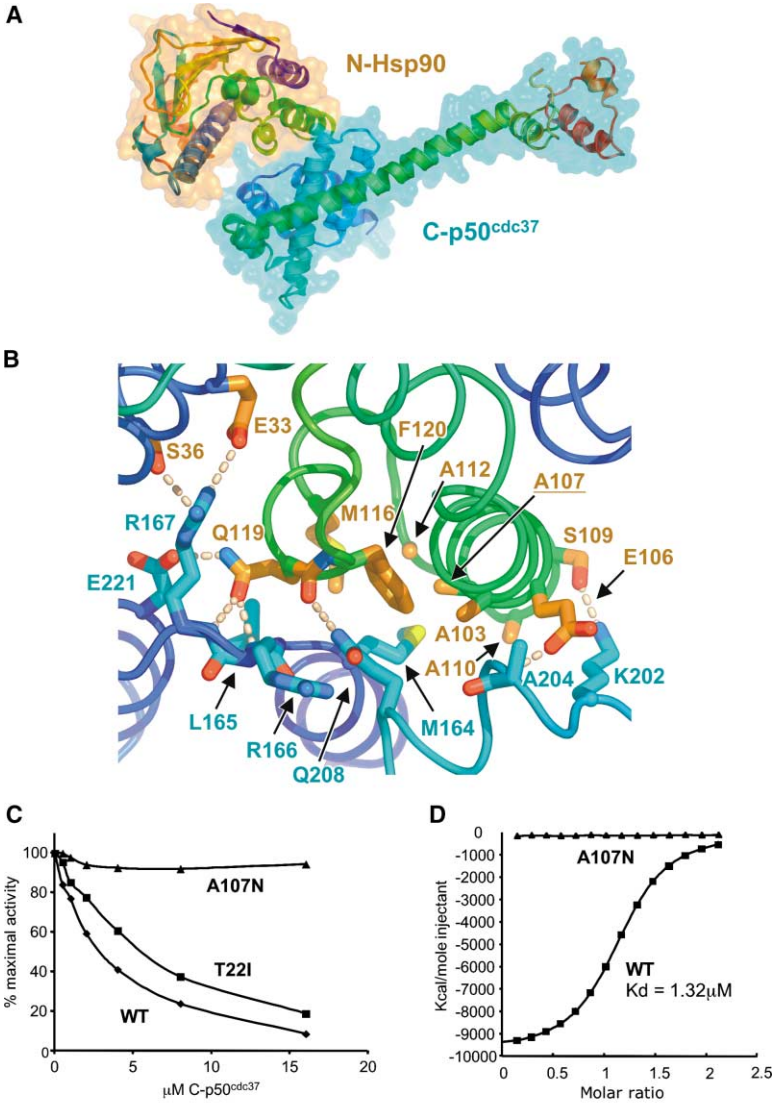

Figure 4. Hsp90-p50 Interaction

(A) Secondary structure cartoon of the N-Hsp90-C-p50^{cdc37} complex (rainbow colored as in Figure 2A) superimposed on the molecular surfaces of the two proteins (C-p50^{cdc37} cyan, N-Hsp90 gold).

(B) Details of the interactions between N-Hsp90 (gold side chain carbons and labels) and C-p50^{cdc37} (cyan side chain carbons and labels). The interface primarily involves Hsp90 residues exposed on the face of the lid segment (residues 100–121) in free N-Hsp90 structures, and residues from the loop between the first two helices and the beginning of the third helix, in the C-p50^{cdc37} structure. Ala 107 (underlined) from Hsp90 is fully exposed in free N-Hsp90 structures, and its mutation to asparagine has previously been shown to enhance the ATPase activity of Hsp90 (Prodromou et al., 2000) by favoring lid closure on ATP binding, and promoting N-terminal association in the Hsp90 dimer. In the complex Ala 107 is buried in the core of the hydrophobic interface with C-p50^{cdc37}, which would be completely disrupted by the presence of larger hydrophilic residues such as asparagine.

(C) ATPase activity of wild-type and mutant Hsp90s (see Experimental Procedures), in the presence of increasing amounts of C-p50^{cdc37}. Compared to wild-type, the A107N mutant Hsp90 is effectively resistant to ATPase arrest by C-p50^{cdc37}. A second hyperactive Hsp90 mutant T22I retains full sensitivity to C-p50^{cdc37}.

(D) ITC of C-p50^{cdc37} binding to wild-type or A107N Hsp90. While the wild-type chaperone interacts with micromolar affinity, there is no detectable binding to the A107N mutant, consistent with the disruption of the interface this mutation would cause.

Binding of p50^{cdc37} elicits some small but potentially significant conformational changes in the N-terminal domain of Hsp90 compared to the structures of the free or nucleotide bound domain. The lid segment, which provides the majority of the interaction with p50^{cdc37}, moves away from the rest of the domain, hinging at glycines 100 and 121, with the tip of the lid moving by more than 3 Å compared to the structures in the absence of p50^{cdc37} (PDB codes 1AH6, 1AMW) (Figure 5C). Accompanying the lid movement, is a substantial restructuring of the backbone between Leu 93 and Gly 100 which takes on an α-helical conformation, extending the helix from 101–109 in the free structure by nearly two turns at its N terminus and the helix from 86–92 by a turn at its C terminus. This change in backbone conformation causes substantial movement in the side chains of Ile 96, Ala 97, and particularly Lys 98 whose side chain ϵ-amino group is flipped by $>$12Å away from the ATP binding pocket, where it interacts with the β-phosphate of bound nucleotides (Prodromou et al., 1997a), and out into the solvent (Figure 5D). This segment of the polypeptide chain displays two alternative conformations in human Hsp90 N-terminal domain structures (Stebbins et al., 1997), however this helical conformation observed in the presence of p50^{cdc37} is substantially different from either of those.

Architecture of the Hsp90-p50^{cdc37} Complex

Within the crystals the C-p50^{cdc37} dimer sits between two symmetry related Hsp90 N-terminal domains, which are orientated so that the open mouths of their nucleotide binding pockets are facing each other, but $>$40Å apart. The large globular domain of the C-p50^{cdc37} molecules forming the dimer, and the two bound Hsp90 N-terminal domains lie in a plane, with the long helix and small C-terminal helical bundle from each of the C-p50^{cdc37} molecules projecting $>$50 Å from one face of the plane (Figures 6A and 6B). Hsp90 functions as a dimer, with a high-affinity constitutive dimerization interface provided by the C-terminal regions (Nemoto et al., 1995) and an ATP-dependent interface involving the N-terminal domains (Prodromou et al., 2000; Chadli et al., 2000; Weg-

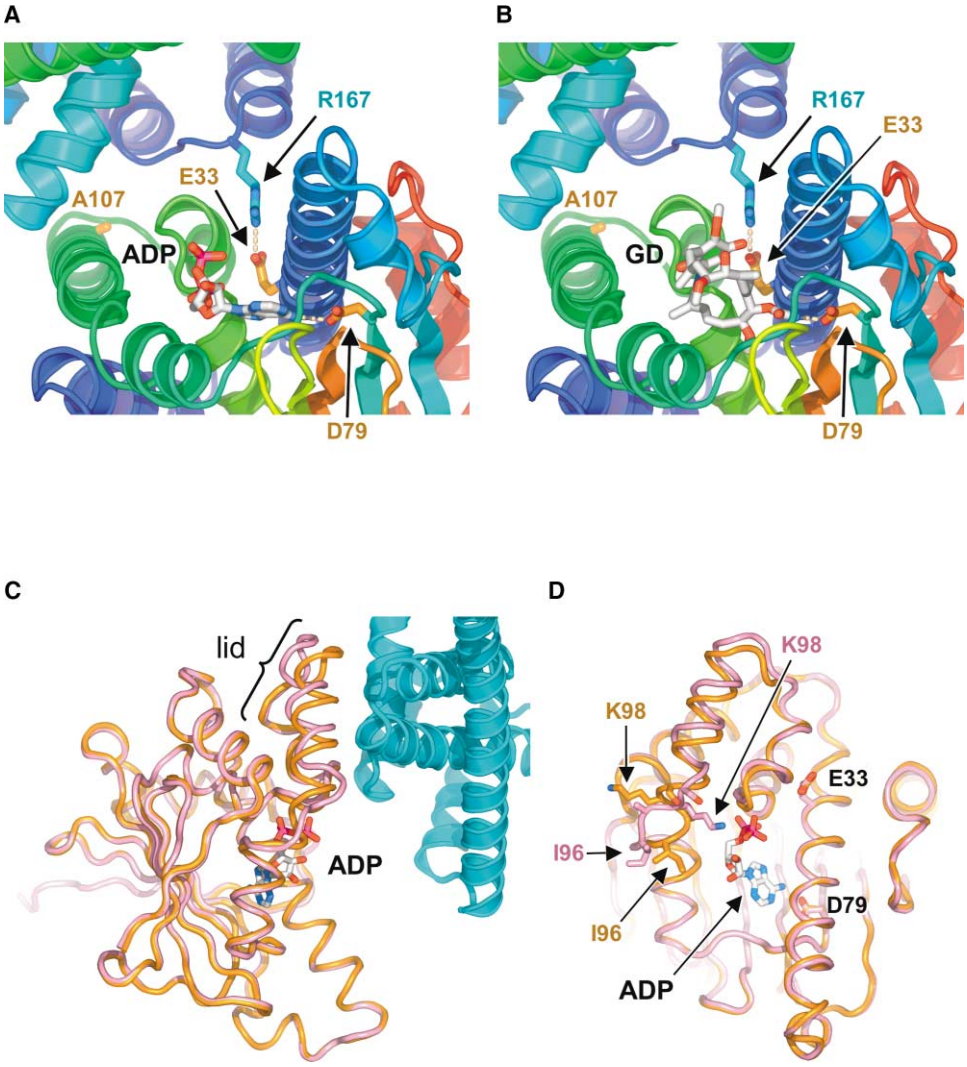

Figure 5. Pocket and Lid Interactions

(A) Model of ADP bound to N-Hsp90 in the presence of C-p50^{cdc37}, generated by superimposing N-Hsp90 from the previously described N-Hsp90-ADP complex (Prodromou et al., 1997a) onto the N-Hsp90 in this complex. Arg 167 of C-p50^{cdc37} inserts its side chain into the mouth of the nucleotide binding pocket of Hsp90, hydrogen bonding with the carboxylate side chain of the catalytic residue Glu 33, and with its guanidinium head group occupying the position expected for the attacking water in the ATPase reaction. The key adenine binding residue, Asp 79, is indicated.

(B) Model of geldanamycin (GD) bound to N-Hsp90 in the presence of C-p50^{cdc37}, generated by superimposing N-Hsp90 from the previously described N-Hsp90-GD complex (Roe et al., 1999) onto the N-Hsp90 in this complex. Consistent with biochemical observations (Siligardi et al., 2002; Shao et al., 2001) the presence of Arg 167 would not be expected to interfere with geldanamycin binding, however it would certainly alter the chemical environment of the mouth of the pocket, and in vivo could selectively limit or enhance binding of larger Hsp90 inhibitors.

(C) Superimposition of N-Hsp90 structure in the p50^{cdc37} complex (gold Cα trace) on the N-Hsp90 structure from the ADP-complex. Binding of C-p50^{cdc37} (cyan cartoon) lifts the lid away from the body of the structure, and causes a rearrangement of residues 96–99.

(D) As (C) but viewed toward the nucleotide binding pocket. Lifting of the lid imposes a helical conformation on residues 96–99, causing the tip of the side chain of Lys 98 to move by $>$12 Å.

ele et al., 2003). Hsp90 and p50^{cdc37} interact as dimers with 1:1 molar stoichiometry, and part of the p50^{cdc37} dimerization interface is provided by the Hsp90 binding C-terminal region of p50^{cdc37} present in the structure described here (Siligardi et al., 2002). All the interactions observed between Hsp90 and p50^{cdc37} and within the p50^{cdc37} dimer are provided by the large globular domain of the C-p50^{cdc37} structure, consistent with the observation in yeast Cdc37p that regions C-terminal to this are dispensable for normal function in vivo (Lee et al., 2002). Taken together, all these data suggest that the complex of the N-Hsp90 and C-p50^{cdc37} domains described here is a true representation of the interaction of these regions of the two molecules within the full $(Hsp90)_2$-$(p50^{cdc37})_2$ heterotetramer complex.

In Hsp90, the N-terminal domain is followed by a middle segment, consisting of two $\alpha\beta\alpha$ domains connected by a short helical supercoil (Meyer et al., 2003), which is directly implicated in interactions with several client proteins, including protein kinases (Sato et al., 2000;

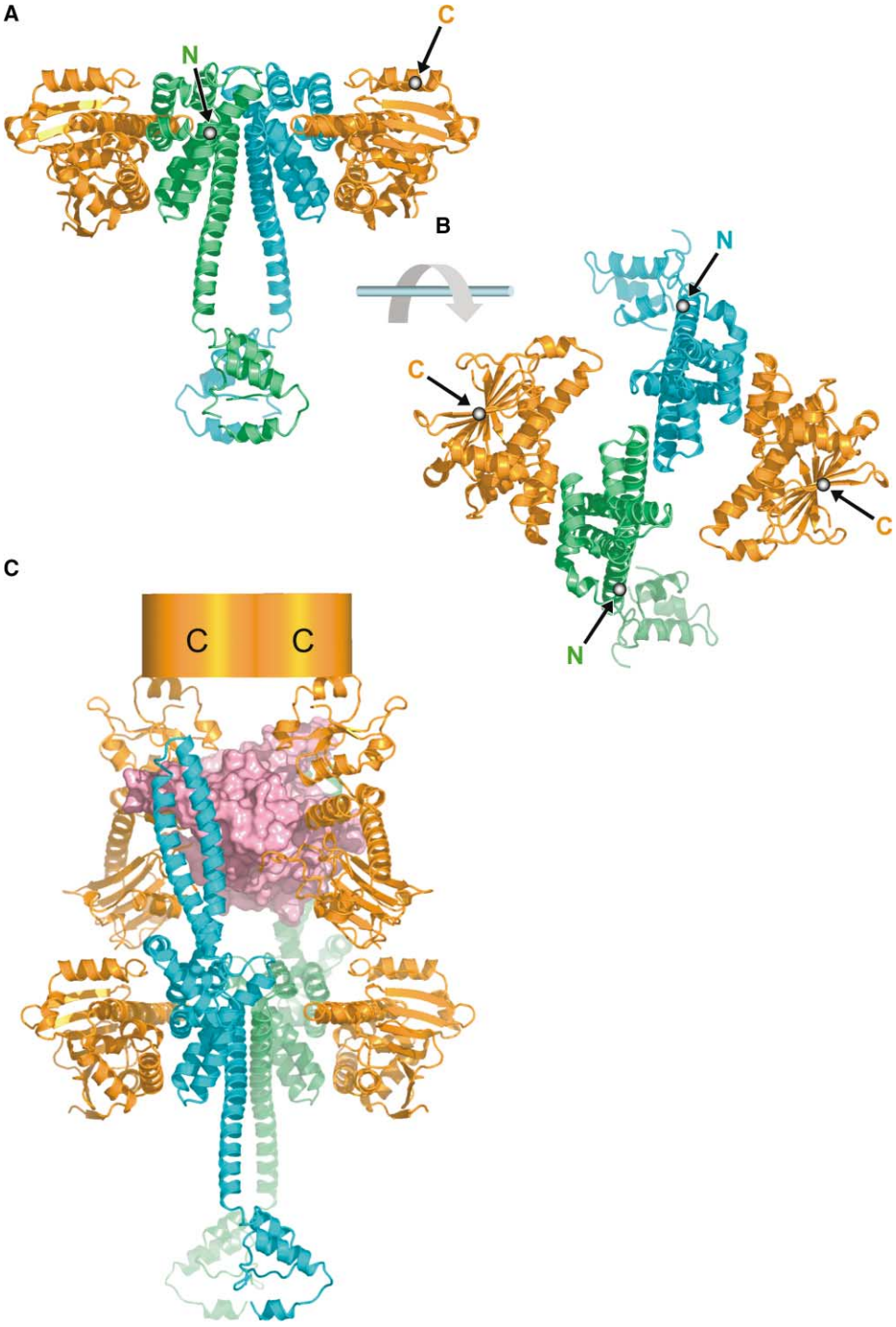

Figure 6. Architecture of the Hsp90-p50^{cdc37} Complex

(A) Secondary structure cartoon of the (N-Hsp90)$_2$-(C-p50^{cdc37})$_2$ complex. The crystal asymmetric unit contains one N-Hsp90-C-p50^{cdc37} heterodimer, and the complete heterotetramer is formed by the homodimerization of two crystallographically related C-p50^{cdc37} molecules (cyan/green). N-Hsp90 is shown in gold. The positions of the N terminus of C-p50^{cdc37} and the C terminus of N-Hsp90 are indicated.

(B) As (A) but viewed from the opposite side to the long helical protrusions of C-p50^{cdc37}. The N termini of both C-p50^{cdc37} molecules and the C termini of both N-Hsp90s are on this face.

(C) Hypothetical model of a complete Hsp90-p50^{cdc37}-client protein kinase complex. The bottom of the structure is the observed (N-Hsp90)$_2$-(C-p50^{cdc37})$_2$ heterotetramer, described here. The known crystal structure of the middle domain of Hsp90 (Meyer et al., 2003) has been positioned so that its N terminus is within 8 Å of the C terminus of N-Hsp90. The structure of the C-terminal constitutive dimerization domain of Hsp90 has not yet been described and is represented by a pair of cylinders. Although the structure of the N-terminal domain of p50^{cdc37} is also unknown, it predicts strongly as a substantial coiled-coil, and in this model is represented by a coiled-coil of comparable length, from seryl-tRNA synthetase (PDB code 1SET), docked to connect with the N terminus of the observed C-p50^{cdc37}. Finally, the structure of a known client

Fontana et al., 2002). From the position of the C terminus of the Hsp90 N-domain in the complex described here, the middle and C-terminal segments in the intact Hsp90 dimer would have to be on the opposite side of the plane (see above) to the projecting helical segments of p50^{cdc37}. The structure of the N-terminal kinase binding domain of p50^{cdc37} is as yet unknown, although structure prediction suggests it has a substantial coiled-coil content. From a similar consideration of the position of the N terminus of C-p50^{cdc37}, whatever its structure the N-terminal domain is also likely to be directed in the opposite direction to the helical protrusions and in the same direction as the middle segment of Hsp90, suggesting an overall head-to-tail interaction between Hsp90 and p50^{cdc37}. This arrangement would allow simultaneous interaction of a client protein kinase with the middle segment of Hsp90, and with the N-terminal domain of p50^{cdc37} (Figure 6C).

Mechanism of Hsp90 ATPase Arrest by p50^{cdc37}

The combination of the constitutive C-terminal interface and the ATP-dependent association of the N-terminal domains generates a molecular clamp, whose opening and closing is coupled to the ATPase activity of the chaperone (Prodromou et al., 2000). Although it has not yet been observed directly, substantial evidence from biochemical studies (Prodromou et al., 2000; Richter et al., 2001, 2002) and analogy with related dimeric GHKL-family ATPases (Dutta and Inouye, 2000) suggests that the N-terminal domains in the Hsp90 dimer must come into direct contact for ATP hydrolysis to take place. Dimerization of the N-terminal domains within the dimer is believed to be driven by self-association of a hydrophobic surface, exposed when the lid segment closes over bound ATP (Prodromou et al., 1997a, 2000), and reinforced by interactions involving the first strand of the N-domain (Richter et al., 2002). However, dimerization of the N-terminal domains while necessary is not sufficient, and the middle segment of Hsp90 from each monomer must contribute additional catalytic residue(s), for ATP-hydrolysis to occur (Soti et al., 2002; Meyer et al., 2003).

From the complex described here, it is evident that p50^{cdc37} achieves its inhibitory effect on the Hsp90 ATPase cycle by interfering with several key points in the chain of events that permits ATP-hydrolysis. Firstly, p50^{cdc37} chemically disables nucleophilic attack on the β-γ phosphoester bond of ATP by inserting the side chain of Arg 167 into the mouth of the nucleotide binding pocket, where it hydrogen bonds to and neutralizes the general base Glu 33, occupying the probable site of the attacking water molecule. While this certainly contributes to inhibition, it cannot be essential, as Arg 167 is not fully conserved, being lysine in flies, asparagine in worms, and isoleucine in yeast Cdc37p, which is nonetheless able to inhibit Hsp90 ATPase activity (Siligardi et al., 2002), albeit with lower affinity than the mammalian homologs. Secondly, p50^{cdc37} binds to the open face of the lid segment in the N-domain of Hsp90, simultaneously preventing its closure over bound ATP and blocking access by the middle segment that provides the "missing" catalytic residue(s) needed to orientate and polarize the γ-phosphate. Thirdly, the p50^{cdc37} dimer sits between the two N-domains of the Hsp90 clamp, holding them open like a brace and preventing their *trans*-activating interaction.

An ability to arrest the Hsp90 ATPase cycle is shared by p50^{cdc37} and Hop/Sti1 (Prodromou et al., 1999; Siligardi et al., 2002), both of which are "early" cochaperones involved in recruitment of client proteins to the Hsp90 machinery. How Hop/Sti1 inhibits Hsp90 is not yet described. p50^{cdc37} holds Hsp90 in an "open" conformation in which the putative binding surfaces in the middle segment of the chaperone (Fontana et al., 2002; Sato et al., 2000; Meyer et al., 2003) would be accessible to a client protein kinase bound to the N-terminal domain of p50^{cdc37}. Progress from this arrested "loading" phase through the ATPase cycle would absolutely depend on ejection of the p50^{cdc37} C terminus from between the jaws of the Hsp90 clamp to allow it to close. However, p50^{cdc37} could remain persistently associated with the cycling Hsp90 complex via the interaction of its N terminus with the bound client protein kinase. The rate with which the arrested Hsp90-p50^{cdc37} complex converts to a cycling complex will undoubtedly be affected by other cochaperones such as Aha1, immunophilins, and p23/Sba1, whose binding influences the Hsp90 ATPase cycle (Panaretou et al., 2002; Lotz et al., 2003; Young and Hartl, 2000). However, it is also likely to depend on the nature and affinity of the interaction of the client protein itself with Hsp90, communicated via the N-terminal kinase binding domain of p50^{cdc37} to its inhibitory C terminus.

Experimental Procedures

Expression and Purification of Proteins

Native and mutant yeast Hsp90 constructs, or human p50^{cdc37} constructs, were inserted with N-terminal His_6-tag into pRSETA and expressed in *E. coli* BL21(DE3) pLysS as previously described (Prodromou et al., 2000; Siligardi et al., 2002; Meyer et al., 2003). In all cases, expressed proteins were purified by metal affinity chromatography on Talon resin, ion-exchange on Q-Sepharose, and size-exclusion chromatography on a Superdex 75 PG or Sephacryl 400 HR column equilibrated in 20 mM Tris [pH 7.5], 150 mM NaCl, 1 mM EDTA, and 0.5 mM DTT (buffer A). Fractions containing pure cleaved proteins were dialyzed against buffer A and finally concentrated using 5K Vivaspin concentrators. Purified human Hsp90α N-terminal domain was a kind gift from Dr. Adam Collier, Vernalis, Cambridge, United Kingdom.

Isothermal Titration Calorimetry and ATPase Assays

Heats of interaction between Hsp90 and p50^{cdc37} constructs were measured on a MSC system (Microcal Inc.). 15 aliquots of 20 μl C-p50^{cdc37} (250 μM) were injected into 1.458 ml of 25 μM Hsp90 construct at 30°C in 40 mM Tris [pH 8.0] containing 1 mM EDTA and 5 mM NaCl. For the full-length proteins, p50^{cdc37} was injected

protein kinase, Cdk6 (PDB code 1BI7) (pink molecular surface) was docked between the two Hsp90 middle segments and the modeled p50^{cdc37} N-terminal coiled-coil, so as to minimize steric clashes while contacting residues from the middle segment implicated in interaction with client proteins (Sato et al., 2000; Fontana et al., 2002; Meyer et al., 2003). While this model is certainly inaccurate in many aspects, it provides a useful synthesis of a great deal of fragmentary data.

Table 1. Crystallographic Statistics

Data Collection	*overall (outer shell)*
Resolution limit (Å)	2.3
Observations	26462
Completeness (%)	96.4 (96.4)
Multiplicity	3.6
R_{sym}	0.063 (0.284)
I/σI	15.2 (4.0)
Refinement	
Resolution range (Å)	72.5–2.3
No. reflections	25125
No. of protein atoms	3414
No. of solvent atoms	176
R_{cryst}	0.185
R_{free} (5% data)	0.241
$<B>$ (Å^2)	38.1
Rmsd bond lengths (Å)	0.030
Rmsd bond angles (°)	2.075

at 324 μM into Hsp90 at 32.4 μM. Heats of dilution were determined in a separate experiment by diluting protein in buffer, and the corrected data were fitted using a nonlinear least square curve-fitting algorithm (Microcal Origin) with three floating variables: stoichiometry, binding constant, and change of enthalpy of interaction.

Hsp90 ATPase activity and its inhibition by p50^{cdc37} were measured using an enzyme-linked assay as previously described (Panaretou et al., 1998; Prodromou et al., 1999; Siligardi et al., 2002). Wild-type and mutant Hsp90s were used at 2 μM, and p50^{cdc37} constructs in a range from 0.5–16 μM. All data were corrected for geldanamycin-resistant background as previously described.

Crystallization, Data Collection, and Structure Determination

After initial screens to identify promising conditions, diffraction quality crystals of the complex were grown from a mixture of N-Hsp90 and C-p50 at a final concentration of 0.5 mM and 0.4 mM respectively, in a solution containing 12% polyethylene glycol 4000, 16% isopropanol, and 100 mM sodium citrate, [pH 6.0]. Crystal drops were set up using the hanging-drop vapor diffusion method, initially at 4°C for 48 hr and then transferred to 14°C. Complex crystals were cryoprotected in crystallization buffer with the addition of 25% glycerol and were flash-cooled in liquid nitrogen. Crystals belonged to space group $P3_121$ (or its enantiomorph) with unit cell dimensions a = 83.76 Å, c = 148.45 Å. Analysis of the Matthews coefficient suggests a solvent content of 60%, with one molecule each of N-Hsp90 and C-p50 in the asymmetric unit. Diffraction data were collected on beamline ID14.4 at the ESRF Grenoble, and images processed and data reduced using MOSFLM (Leslie, 1995) and SCALA (CCP4, 1994). A clear molecular replacement solution in $P3_121$ was found using the program Molrep (Vagin and Teplyakov, 2000), with the structure of the N-terminal domain of yeast Hsp90 as a search model (PDB code 1AMW). The top solution gave a correlation coefficient of 0.363 and an R factor of 0.515 for one N-terminal Hsp90 molecule in the asymmetric unit. The electron density map generated from the molecular replacement solution was of a high quality and density corresponding to the C-p50 component of the complex was clearly visible. ARP-wARP (Lamzin and Wilson, 1997) was used initially for autotracing of the main chain polypeptide and subsequent building was performed manually in O (Jones et al., 1991). Refmac (Murshudov et al., 1997) was used for structure refinement. 100% of residues in the Hsp90 fragment were in the most favored or allowed region of the Ramachandran plot, and 98.3% for the p50^{cdc37} fragment. Statistics for the crystallographic data collection and refinement are given in Table 1. All molecular graphics images were generated using PyMol (DeLano, 2002).

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References

An, W.G., Schulte, T.W., and Neckers, L.M. (2000). The heat shock protein 90 antagonist geldanamycin alters chaperone association with p210bcr-abl and v-src proteins before their degradation by the proteasome. Cell Growth Differ. *11*, 355–360.

Basso, A.D., Solit, D.B., Chiosis, G., Giri, B., Tsichlis, P., and Rosen, N. (2002). Akt forms an intracellular complex with heat shock protein 90 (Hsp90) and Cdc37 and is destabilized by inhibitors of Hsp90 function. J. Biol. Chem. *277*, 39858–39866.

Bijlmakers, M.J., and Marsh, M. (2000). Hsp90 is essential for the synthesis and subsequent membrane association, but not the maintenance, of the Src-kinase p56(lck). Mol. Biol. Cell *11*, 1585–1595.

Blagosklonny, M.V. (2002). Hsp-90-associated oncoproteins: multiple targets of geldanamycin and its analogs. Leukemia *16*, 455–462.

Boudeau, J., Deak, M., Lawlor, M.A., Morrice, N.A., and Alessi, D.R. (2003). Heat-shock protein 90 and Cdc37 interact with LKB1 and regulate its stability. Biochem. J. *370*, 849–857.

Brugge, J.S. (1986). Interaction of the Rous sarcoma virus protein pp60v-src with the cellular proteins pp50 and pp90. Curr. Top. Microbiol. Immunol. *123*, 1–22.

CCP4 (Collaborative Computational Project 4) (1994). The CCP4 suite: programs for protein crystallography. Acta Crystallogr. D Biol. Crystallogr. *50*, 760–763.

Chadli, A., Bouhouche, I., Sullivan, W., Stensgard, B., McMahon, N., Catelli, M.G., and Toft, D.O. (2000). Dimerization and N-terminal domain proximity underlie the function of the molecular chaperone heat shock protein 90. Proc. Natl. Acad. Sci. USA *97*, 12524–12529.

Chavany, C., Mimnaugh, E., Miller, P., Bitton, R., Nguyen, P., Trepel, J., Whitesell, L., Schnur, R., Moyer, J., and Neckers, L. (1996). p185erbB2 binds to GRP94 in vivo. Dissociation of the p185erbB2/GRP94 heterocomplex by benzoquinone ansamycins precedes depletion of p185erbB2. J. Biol. Chem. *271*, 4974–4977.

Dai, K., Kobayashi, R., and Beach, D. (1996). Physical interaction of mammalian CDC37 with CDK4. J. Biol. Chem. *271*, 22030–22034.

DeLano, W.L. (2002). The PyMOL Molecular Graphics System. (San Carlos, CA: DeLano Scientific).

Dutta, R., and Inouye, M. (2000). GHKL, an emergent ATPase/kinase superfamily. Trends Biochem. Sci. *25*, 24–28.

Fontana, J., Fulton, D., Chen, Y., Fairchild, T.A., McCabe, T.J., Fujita, N., Tsuruo, T., and Sessa, W.C. (2002). Domain mapping studies reveal that the M domain of hsp90 serves as a molecular scaffold to regulate Akt-dependent phosphorylation of endothelial nitric oxide synthase and NO release. Circ. Res. *90*, 866–873.

Fujita, N., Sato, S., Ishida, A., and Tsuruo, T. (2002). Involvement of Hsp90 in signaling and stability of 3- phosphoinositide-dependent kinase-1. J. Biol. Chem. *277*, 10346–10353.

Grammatikakis, N., Lin, J.-H., Grammatikakis, A., Tsichlis, P.N., and Cochran, B.H. (1999). p50cdc37 acting in concert with Hsp90 is required for Raf-1 function. Mol. Cell. Biol. *19*, 1661–1672.

Hunter, T., and Poon, R.Y.C. (1997). Cdc37: A protein kinase chaperone? Trends Cell Biol. *7*, 157–161.

Jones, T.A., Zou, J.-Y., Cowan, S.W., and Kjeldgaard, M. (1991). Improved methods for building protein models in electron density maps and the location of errors in these models. Acta Crystallogr. *A47*, 110–119.

Jones, D.T., Taylor, W.R., and Thornton, J.M. (1992). A new approach to protein fold recognition. Nature *358*, 86–89.

Lamzin, V.S., and Wilson, K.S. (1997). Automated refinement for protein crystallography. Methods Enzymol. *277*, 269–305.

Lange, B.M.H., Rebollo, E., Herold, A., and Gonzalez, C. (2002). Cdc37 is essential for chromosome segregation and cytokinesis in higher eukaryotes. EMBO J. *21*, 5364–5374.

Lee, P., Rao, J., Fliss, A., Yang, E., Garrett, S., and Caplan, A.J. (2002). The Cdc37 protein kinase-binding domain is sufficient for protein kinase activity and cell viability. J. Cell Biol. *159*, 1051–1059.

Leslie, A.G.W. (1995). MOSFLM users guide. (Cambridge, UK: MRC Laboratory of Molecular Biology).

Lotz, G.P., Lin, H., Harst, A., and Obermann, W.M.J. (2003). Aha1 binds to the middle domain of Hsp90, contributes to client protein activation, and stimulates the ATPase activity of the molecular chaperone. J. Biol. Chem. *278*, 17228–17235.

Mahony, D., Parry, D.A., and Lees, E. (1998). Active cdk6 complexes are predominantly nuclear and represent only a minority of the cdk6 in T cells. Oncogene *16*, 603–611.

Maloney, A., and Workman, P. (2002). HSP90 as a new therapeutic target for cancer therapy: the story unfolds. Expert Opin. Biol. Ther. *2*, 3–24.

Meyer, P., Prodromou, C., Hu, B., Vaughan, C., Roe, S.M., Panaretou, B., Piper, P.W., and Pearl, L.H. (2003). Structural and functional analysis of the middle segment of Hsp90: implications for ATP hydrolysis and client-protein and co-chaperone interactions. Mol. Cell *11*, 647–658.

Miyata, Y., Ikawa, Y., Shibuya, M., and Nishida, E. (2001). Specific association of a set of molecular chaperones including HSP90 and Cdc37 with MOK, a member of the mitogen-activated protein kinase superfamily. J. Biol. Chem. *276*, 21841–21848.

Murshudov, G.N., Vagin, A.A., and Dodson, E.J. (1997). Refinement of macromolecular structures by the maximum-likelihood method. Acta Crystallogr. *D53*, 240–255.

Murzin, A.G., Brenner, S.E., Hubbard, T., and Chothia, C. (1995). SCOP: a structural classification of proteins database for the investigation of sequences and structures. J. Mol. Biol. *247*, 536–540.

Neckers, L. (2002). Hsp90 inhibitors as novel cancer chemotherapeutic agents. Trends Mol. Med. *8*, S55–S61.

Nemoto, T., Ohara-Nemoto, Y., Ota, M., Takagi, T., and Yokoyama, K. (1995). Mechanism of dimer formation of the 90-kDa heat-shock protein. Eur. J. Biochem. *233*, 1–8.

Obermann, W.M.J., Sondermann, H., Russo, A.A., Pavletich, N.P., and Hartl, F.U. (1998). *In vivo* function of Hsp90 is dependent on ATP binding and ATP hydrolysis. J. Cell Biol. *143*, 901–910.

O'Keeffe, B., Fong, Y., Chen, D., Zhou, S., and Zhou, Q. (2000). Requirement for a kinase-specific chaperone pathway in the production of a Cdk9/cyclin T1 heterodimer responsible for P-TEFb-mediated tat stimulation of HIV-1 transcription. J. Biol. Chem. *275*, 279–287.

Owens-Grillo, J.K., Czar, M.J., Hutchison, K.A., Hoffmann, K., Perdew, G.H., and Pratt, W.B. (1996). A model of protein targeting mediated by immunophilins and other proteins that bind to hsp90 via tetratricopeptide repeat domains. J. Biol. Chem. *271*, 13468–13475.

Panaretou, B., Prodromou, C., Roe, S.M., O'Brien, R., Ladbury, J.E., Piper, P.W., and Pearl, L.H. (1998). ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone *in vivo*. EMBO J. *17*, 4829–4836.

Panaretou, B., Siligardi, G., Meyer, P., Maloney, A., Sullivan, J.K., Singh, S., Millson, S.H., Clarke, P.A., Naaby-Hansen, S., Stein, R., et al. (2002). Activation of the ATPase activity of Hsp90 by the stress-regulated co-chaperone Aha1. Mol. Cell *10*, 1307–1318.

Pearl, L.H., and Prodromou, C. (2002). Structure, function and mechanism of the Hsp90 molecular chaperone. Adv Protein Chem. *59*, 157–185.

Pearl, F.M.G., Lee, D., Bray, J.E., Sillitoe, I., Todd, A.E., Harrison, A.P., Thornton, J.M., and Orengo, C.A. (2000). Assigning genomic sequences to CATH. Nucleic Acids Res. *28*, 277–282.

Perdew, G.H., Wiegand, H., VandenHeuvel, J.P., Mitchell, C., and Singh, S.S. (1997). A 50 kilodalton protein associated with raf and pp(60v-src) protein kinases is a mammalian homolog of the cell cycle control protein cdc37. Biochemistry *36*, 3600–3607.

Picard, D. (2002). Heat-shock protein *90*, a chaperone for folding and regulation. Cell. Mol. Life Sci. *59*, 1640–1648.

Pratt, W.B., and Toft, D.O. (2003). Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. Exp. Biol. Med. *228*, 111–133.

Prodromou, C., Roe, S.M., O'Brien, R., Ladbury, J.E., Piper, P.W., and Pearl, L.H. (1997a). Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. Cell *90*, 65–75.

Prodromou, C., Roe, S.M., Piper, P.W., and Pearl, L.H. (1997b). A molecular clamp in the crystal structure of the N-terminal domain of the yeast Hsp90 chaperone. Nat. Struct. Biol. *4*, 477–482.

Prodromou, C., Siligardi, G., O'Brien, R., Woolfson, D.N., Regan, L., Panaretou, B., Ladbury, J.E., Piper, P.W., and Pearl, L.H. (1999). Regulation of Hsp90 ATPase activity by tetratricopeptide repeat (TPR)-domain co-chaperones. EMBO J. *18*, 754–762.

Prodromou, C., Panaretou, B., Chohan, S., Siligardi, G., O'Brien, R., Ladbury, J.E., Roe, S.M., Piper, P.W., and Pearl, L.H. (2000). The ATPase cycle of Hsp90 drives a molecular 'clamp' via transient dimerization of the N-terminal domains. EMBO J. *19*, 4383–4392.

Richter, K., Muschler, P., Hainzl, O., and Buchner, J. (2001). Coordinated ATP hydrolysis by the Hsp90 dimer. J. Biol. Chem. *276*, 33689–33696.

Richter, K., Reinstein, J., and Buchner, J. (2002). N-terminal residues regulate the catalytic efficiency of the Hsp90 ATPase cycle. J. Biol. Chem. *277*, 44905–44910.

Richter, K., Muschler, P., Hainzl, O., Reinstein, J., and Buchner, J. (2003). Sti1 is a non-competitive inhibitor of the Hsp90 ATPase. J. Biol. Chem. *278*, 10328–10333.

Roe, S.M., Prodromou, C., O'Brien, R., Ladbury, J.E., Piper, P.W., and Pearl, L.H. (1999). The structural basis for inhibition of the Hsp90 molecular chaperone, by the anti-tumour antibiotics radicicol and geldanamycin. J. Med. Chem. *42*, 260–266.

Sato, S., Fujita, N., and Tsuruo, T. (2000). Modulation of akt kinase activity by binding to hsp90. Proc. Natl. Acad. Sci. USA *97*, 10832–10837.

Scholz, G.M., Cartledge, K., and Hall, N.E. (2001). Identification and characterization of Harc, a novel Hsp90-associating relative of Cdc37. J. Biol. Chem. *276*, 30971–30979.

Schulte, T.W., Blagosklonny, M.V., Ingui, C., and Neckers, L. (1995). Disruption of the Raf-1-Hsp90 molecular complex results in destabilization of Raf-1 and loss of Raf-1-Ras association. J. Biol. Chem. *270*, 24585–24588.

Shao, J., Grammatikakis, N., Scroggins, B.T., Uma, S., Huang, W.J., Chen, J.J., Hartson, S.D., and Matts, R.L. (2001). Hsp90 regulates p50(cdc27) function during the biogenesis of the active conformation of the heme-regulated eIF2 alpha kinase. J. Biol. Chem. *276*, 206–214.

Siligardi, G., Panaretou, B., Meyer, P., Singh, S., Woolfson, D.N., Piper, P.W., Pearl, L.H., and Prodromou, C. (2002). Regulation of Hsp90 ATPase activity by the co-chaperone Cdc37p/p50^{cdc37}. J. Biol. Chem. *277*, 20151–20159.

Silverstein, A.M., Grammatikakis, N., Cochran, B.H., Chinkers, M., and Pratt, W.B. (1998). P50(cdc37) binds directly to the catalytic domain of Raf as well as to a site on hsp90 that is topologically adjacent to the tetratricopeptide repeat binding site. J. Biol. Chem. *273*, 20090–20095.

Soti, C., Racz, A., and Csermely, P. (2002). A nucleotide-dependent molecular switch controls ATP binding at the C-terminal domain of Hsp90-N-terminal nucleotide binding unmasks a C-terminal binding pocket. J. Biol. Chem. *277*, 7066–7075.

Stancato, L.F., Chow, Y.-H., Hutchinson, K.A., Perdew, G.H., Jove, R., and Pratt, W.B. (1993). Raf exists in a native heterocomplex with Hsp90 and p50 that can be reconstituted in a cell-free system. J. Biol. Chem. *268*, 21711–21716.

Stebbins, C.E., Russo, A.A., Schneider, C., Rosen, N., Hartl, F.U., and Pavletich, N.P. (1997). Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. Cell *89*, 239–250.

Stepanova, L., Leng, X.H., Parker, S.B., and Harper, J.W. (1996). Mammalian p50(Cdc37) is a protein kinase-targeting subunit of Hsp90 that binds and stabilizes Cdk4. Genes Dev. *10*, 1491–1502.

Tatebe, H., and Shiozaki, K. (2003). Identification of Cdc37 as a novel regulator of the stress-responsive mitogen-activated protein kinase. Mol. Cell. Biol. *23*, 5132–5142.

Vagin, A., and Teplyakov, A. (2000). An approach to multi-copy search in molecular replacement. Acta Crystallogr. D Biol. Crystallogr. *56*, 1622–1624.

Wegele, H., Muschler, P., Bunck, M., Reinstein, J., and Buchner, J. (2003). Dissection of the contribution of individual domains to the ATPase mechanism of Hsp90. J. Biol. Chem. *278*, 39303–39310.

Whitesell, L., Mimnaugh, E.G., De Costa, B., Myers, C.E., and Neckers, L.M. (1994). Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. Proc. Natl. Acad. Sci. USA *91*, 8324–8328.

Xu, Y., and Lindquist, S. (1993). Heat-shock protein Hsp90 governs the activity of Pp60(V-Src) kinase. Proc. Natl. Acad. Sci. USA *90*, 7074–7078.

Xu, Y., Singer, M.A., and Lindquist, S. (1999). Maturation of the tyrosine kinase c-Src as a kinase and as a substrate depends on the molecular chaperone Hsp90. Proc. Natl. Acad. Sci. USA *96*, 109–114.

Xu, W., Mimnaugh, E., Rosser, M.F., Nicchitta, C., Marcu, M., Yarden, Y., and Neckers, L. (2001). Sensitivity of mature Erbb2 to geldanamycin is conferred by its kinase domain and is mediated by the chaperone protein Hsp90. J. Biol. Chem. *276*, 3702–3708.

Young, J.C., and Hartl, F.U. (2000). Polypeptide release by Hsp90 involves ATP hydrolysis and is enhanced by the co-chaperone p23. EMBO J. *19*, 5930–5940.

Accession Numbers

Coordinates and structure factors for the refined complex have been deposited in the Protein Databank with PDB code: 1US7.