Supplementary Figure 1. Fourier Shell Correlation (FSC) of the final p53 3D reconstruction, plotted as a function of spatial frequency (blue curve), together with the 3σ threshold curve (orange line). The vertical axis indicates the value of the Fourier shell correlation coefficient versus the resolution in 1/Å represented on the horizontal axis. The dashed red lines indicate the frequency level at which the correlation correspond to 0.5.
Supplementary Figure 2 Stereo view of potential contact between a core-node and an N/C node involves three conservative loops (LN, L4 and L11) of the core domain opposite to the DNA binding surface. Ct, N1 and N2 indicate positions of the carboxy- and amino-terminal helices respectively.

The interface between N/C node and the core node involves three loops on the side opposite to the sequence-specific DNA-binding surface of p53. The N-terminal loop (LN) with residues Tyr103, Ser106 and Tyr107 upstream of the β-strand S1 (Cho et al., 1994), the L4 loop (residues Ser149, Pro151, Pro152, Pro153) between strands S3 and S4 and the L11 loop (residues Asp259, Ser260 and Se261) between strands S9 and S10 of the core domain, are all facing the N/C node and thus having a potential role in core-N/C nodes interaction. These amino acids are highly conserved among p53 proteins including the stretch of three prolines that belong to the mutation hot-spot region A′ which encompass codons 151-159, and mutations in which were reported for numerous human tumours1. It appears that the turn within the 323-363 region of p53 and the upstream protein sequence are positioned appropriately to make a contact with some of these loops. This position is consistent with the reported peptide array data demonstrating that the peptide spanning core domain residues 105-126 interacts with the C-terminal peptide (residues 321-344), derived from the oligomerisation domain2.

Supplementary Figure 3. Biacore analysis of intramolecular interaction between p53 domains. Different domains of p53 fused with GST, representing the N-terminus (residues 1-100: Nt), the core (100-309), and the C-terminus (320-393: Ct), together with GST as a control, were separately immobilised to the four different flow cells on a CM5 sensor chip. The GST-N(1-100)-terminal p53 protein was then injected as analyte over the serially linked ligand surfaces. The representative sensorgram indicates that binding was only observed to the surface of an immobilised C terminus of p53. Curves representing core and N-terminal domains and GST are essentially identical and overlap. RU – resonance units.

To test interaction between N and C terminal domains of p53 that is predicted by our model we employed the Surface Plasmon Resonance (SPR) technique (BIAcore technology). The representative sensorgrams were adjusted to the same baseline level at an interval prior to the start of sample injection and are shown here without background subtraction. A background "bulk-effect" is present for the entire injection interval (0-360 s), caused by a different refraction of the sample solution compared to the running buffer of the Biacore. The specific binding response of the analyte N-terminus domain to the immobilized C-terminus domain of p53 is indicated by the pink curve, which rises above the signal profile of the bulk effect, and owing to a slow dissociation, does not reach back to the baseline level. The binding ability of the chip with regard to the ligand solutions was estimated by 0.5 µg/ml and 1 µ/ml GST solutions (which in both cases gave 325 RU for all lanes). The only positive interaction observed was between ligand C-terminus vs analyte N-terminus, thus demonstrating that these two regions of p53 are capable of intramolecular interaction within p53 tetramer.
Surface plasmon resonance based screen for intra-molecular interactions of p53

All SPR experiments were carried out at 25° C using a BIAcore 2000 system (BIAcore, Uppsala, Sweden). A new sensor chip (CM5) was activated according to the standard amine coupling procedure and loaded with anti-GST antibody for a covalent binding. Chip was prepared by repeated injections of 30 µl of 2 µM GST (followed by a regeneration step) until stable and repeatable binding of GST was observed. These steps were performed in a running buffer containing 10 mM HEPES pH 7.4, 150 mM NaCl, 1 mM DTT, 0.5% Triton X100, as was the rest of the experiment. Ligands were loaded to the sensor chip to give a relatively equal surface density, in the following order: core domain (2 µg/ml) on lane 2, N-terminus (2 µg/ml) on lane 3, C-terminus (2 µg/ml) on lane 4, and finally, GST (56 µg/ml) in a high concentration onto lane 1 to provide a reference surface. This same GST injection was additionally allowed to pass via flow cells 2, 3 and 4 to ensure the saturation of possible free GST binding sites. This injection did not result in any changes of ligand binding levels, observed from the various ligand surfaces, thus indicating a total saturation of GST binding sites on the anti-GST antibody linker. Blocking of non-specific binding sites was additionally achieved by the injection of 30 µl of 0.1 mg/ml BSA prior to sample injection. At this point the chip was ready for an experiment and the analyte solution (N-terminus) was injected (30 µl) over all lanes. Regeneration was arranged with 5 µl Glycine, 10 mM, pH 2.2, 5 µl at 30 µl/min flow rate.
Supplementary Figure 4. Two core domains of a dimer bound to a DNA molecule composed from two p53 recognition sites (1tup). The dimeric pair bound to DNA is shown from different angles. The actual sites of contacts between the core domain and DNA are shown in blue. Zn atoms are represented as cyan spheres. The core domains are coloured similarly to that in Figure 6A and B.
Supplementary Figure 5. Full length p53 protein has been treated with increasing amounts of thermolysin (100, 200 and 400 ng). The protein is quickly degraded by thermolysin, lanes 1-3. The cleavage is however accelerated in presence of ADP (lanes 4-6) and slowed down in the presence of ATP-γS (lanes 7-9), suggesting that ADP promotes the open conformation and ATP-γS closed conformation of p53. Fragments of p53 were detected with PAb240 antibody against core domain.

p53 protein is a flexible molecule. We have analysed the stability of p53 protein in presence of different small natural chemicals, and have shown that the stability of the p53-DNA complex can be modulated by the presence of ATP and/or ADP (Okorokov and Milner, 1999). Subsequent biochemical analysis demonstrated that p53 bound to ATP-γS (slow-hydrolysable analogue of ATP), is trapped in the state that appears to be a closed latent conformation which is more resistant to protease treatment (lanes 7-9). In contrast, when bound to ADP, p53 exhibits an open conformation, which resembles the active form of the transcription factor and is maximally exposed to proteolytic attack. We used ATP-γS to trap p53 into the closed latent state and to minimise its flexibility, thus to maximise the amount of particles in homogeneous conformation.