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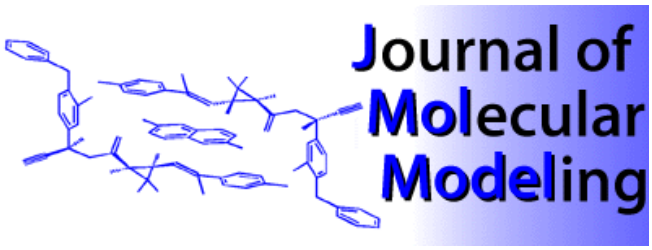

VRML in Cancer Research: Local Changes in Binding Properties of *Wild Type* and Mutated p53 Tumor Suppressor Protein[$]

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Abstract

The inactivation of the p53 tumor suppressor function by single missense point mutations is found in almost half of human tumors. Most p53 mutation hotspots are at the DNA binding interface, shown in the three-dimensional (3D) structure of a p53-DNA complex crystallized by Pavletich and coworkers [1]. We have investigated the influence of mutations on the predicted specific DNA binding capacities of p53 by using molecular modeling to compare biochemical properties of *wild type* and mutated p53 complexed to DNA. Changes in local properties e.g. electrostatic potential or hydrophilic/lipophilic properties, combined with the steric interferences, lead to a loss of specific binding and presumably disables the tumor suppressor function.
The 3D-structures combined with molecular biochemical properties of the *wild type* and the mutated p53-DNA complex can be transferred by the use of the Virtual Reality Modeling Language (VRML). Special tools e.g. 'space buttons' allow users the interactive exploration of structures, properties, and additional information via internet.

Keywords: P53-DNA interface, Mutation hotspots, VRML, 1D + 3D structure analysis

P53 tumor suppressor protein-DNA complex: 1D-structure + 3D-structure analysis

The p53 mutation data base has been developed by M. Hollstein and coworkers [2] and lists tumor specific mutations found in more than 50% of human cancers. Until Jan.

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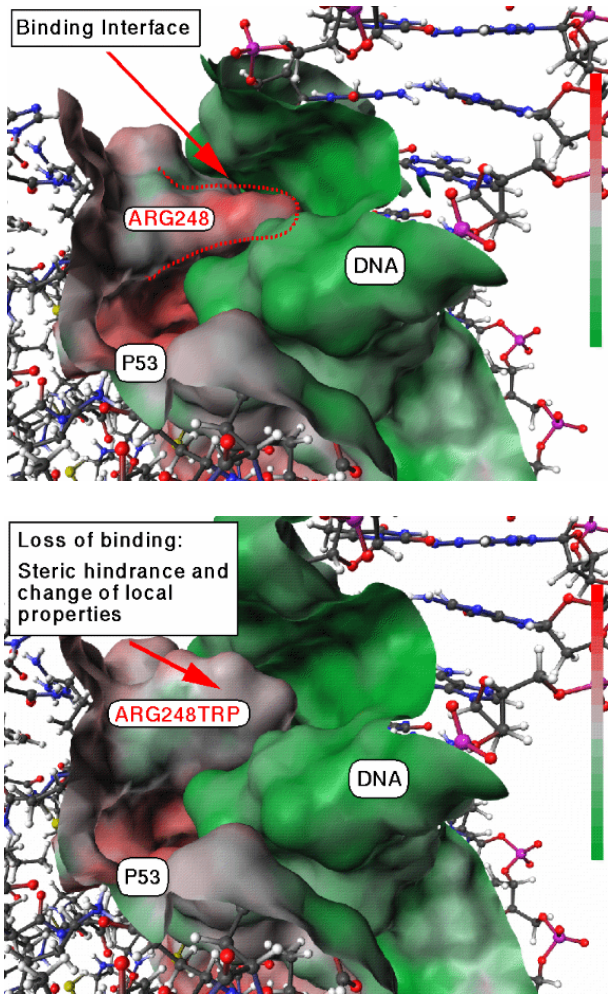

Figure 1. *The molecular electrostatic potential (red=positive, green=negative) is visualized on the solvent accessible surface of the p53-DNA binding interface. Arg248 of the wild type p53 (above, left-hand side) binds to the minor groove of the DNA. The p53 mutant Arg248Trp (below, left-hand side) shows steric interferences as well as a neutral electrostatic potential leading to a loss of specific binding properties.*

'97, more than 6000 entries have been stored in this data base, which is maintained at the International Agency for Research on Cancer (IARC) [3] and is accessible through the European Bioinformatics Institute (EBI, ftp://ftp.ebi.ac.uk/pub/databases/p53/). Co-crystallization of the p53 core domain with target DNA by N. Pavletich and coworkers [1] allows investigations on the influence of mutations on DNA binding and protein functions.

Molecular Properties

We have analyzed the *wild type* and mutated p53 tumor suppressor protein-DNA complex using the molecular modeling package SYBYL/MOLCAD [4]. The amino acid substitutions at the mutation hotspots were performed with the molecular dynamics simulation program CHARMM [5]. The investigations of molecular properties are based on Connolly's concept of molecular surfaces [6]. These *solvent accessible surfaces* show the three-dimensional size and topography of the molecules. Additionally the surfaces are used as maps for a color coded representation of local molecular properties such as hydrophilicity/lipophilicity or electrostatic potential.

The electrostatic potential in solution was calculated by using a finite difference algorithm to solve the Poisson-Boltzmann equation [7]. A smoothing scheme for the dielectric values of the solvent and the solute was introduced to receive a better approximation of the transition from the molecular interior to the solvent [8]. The local charge distribution of the contact region between p53 and DNA should be oppositely charged to maximize Coulomb attraction (see Figure 1).

In order to determine local hydrophilic/lipophilic properties we used a method, which is based on hydrophobic atomic partial values [9]. As a new feature our structure analysis incorporates charged amino acid fragments and accounts for their hydrophobic partial values [10]. After a connectivity analysis to characterize the atoms in their individual structural environment, the local hydrophilic and lipophilic values were calculated from the atomic partial values with respect to the *solvent accessible surface*. By using a special weighting procedure [11], it is guaranteed, that only those atomic fragments that are in close neighborhood to a surface point contribute significantly to the surface value of hydrophobicity (see Figure 2).

Local hydrophobic parts of molecules should be in close contact for an effective hydrophobic interaction and also to avoid unfavorable solvent interactions. Both methods are implemented in the protein properties software of the Darmstadt group.

P53 *wild type* structure differs from mutated structure

The analysis of the crystal structure of the p53 protein-DNA complex has shown that the five most prominent mutation hotspots are located either in the zinc binding region or in the protein-DNA interface [1]. As a result of our comparative investigations point mutations at hotspots found in the p53 data base are related to changes in local biochemical properties leading to a loss of specific binding to DNA. As an example the electrostatic potential and local hydrophobicity of the 'contact mutant' Arg248Trp are calculated and visualized on the molecular surfaces of the *wild type* and the mutated p53 structure (see Figure 1 for the electrostatic potential and Figure 2 for local hydrophilic/lipophilic properties). Steric interferences as well as the neutral electrostatic potential and the local hydrophobic properties of the Arg248Trp mutant are different from the surrounding DNA interface in the minor groove leading to intermolecular re-

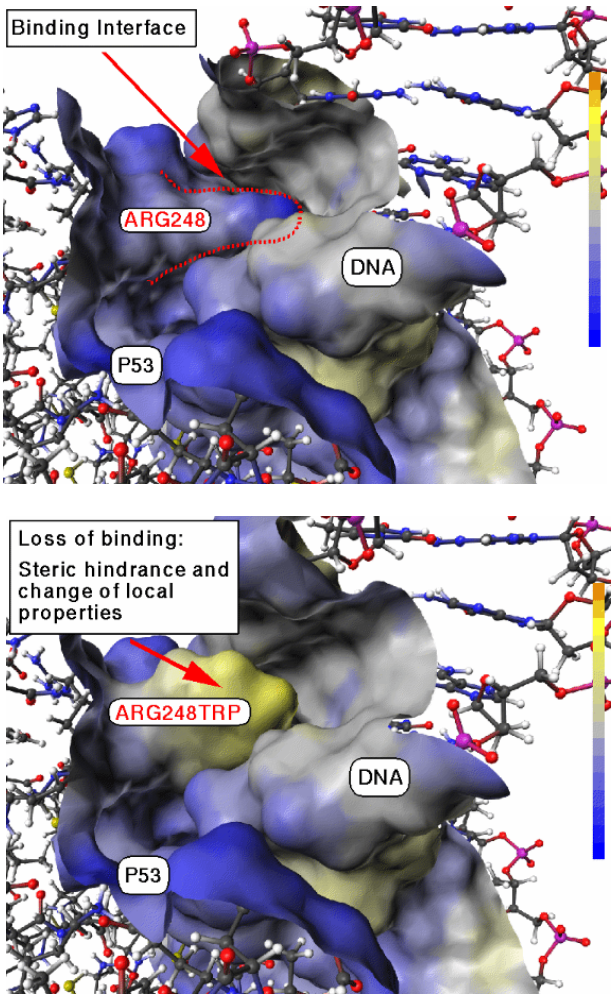

Figure 2. *The local hydrophilic (blue) and lipophilic (yellow) properties are visualized on the solvent accessible surface of the p53-DNA binding interface. Arg248 of the wild type p53 (above, left-hand side) binds to the minor groove of the DNA. The p53 mutant Arg248Trp (below, left-hand side) shows steric interferences as well as lipophilic properties leading to a loss of specific binding to DNA.*

jection (see right panel of Figure 1 for the electrostatic potential and the right panel of Figure 2 for the hydrophilic/lipophilic properties of the mutated p53). The most mutated amino acid, Arg273 (p53 mutation database version Jan. ´97), binds to the phosphate backbone of DNA between the minor groove binding contact and multiple bonds in the major groove. The corresponding 'contact mutant', Arg273His, loses direct binding contact to the DNA phosphate group. The Arg249Ser mutant - an example for a 'structural mutant' - prohibits the formation of binding of Arg248 into the minor groove, due to disruption of an intramolecular bond to Glu171 and local structural changes.

The differences in molecular properties of *wild type* and mutated p53 protein derived from a combination of 1D sequence information (p53 mutation database) and 3D structure data, give hints to a distortion of p53's specific bonds to DNA and this could presumably impair the tumor suppressor function.

VRML

The 3D-structures of the *wild type* and the mutated p53-DNA complex combined with its molecular biochemical properties are visualized by using the Virtual Reality Modeling Language (VRML) and are available as supplementary material in HTML/VRML file format. Additional information is presented on the WWW pages of the Darmstadt group (http://www.pc.chemie.th-darmstadt.de/vrml/p53dna) . To provide researchers with related information and to facilitate the usage, we have introduced special tools such as 'space buttons'. They allow users to interactively explore both the structures and the biochemical properties of the molecular complex. Additional text information is provided as well.

Conclusion

These examples from our ongoing investigations of the *wild type* and mutated p53-DNA complex demonstrate, that the concept of molecular surfaces is very useful to show local changes in topology and molecular interactions caused by single point mutations. It is also shown, that VRML provides an efficient tool to enable scientists to communicate world wide using complex biomolecular models such as the 3D structure of the p53-DNA complex.

Supplementary Material Available

The p53 tumor suppressor protein-DNA complex as well as the mutated p53 molecules with corresponding biochemical properties visualized on molecular surfaces are available as supplementary material. The 3D-structures can be inspected interactively by the use of standard HTML/VRML browsers.

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