doi:10.1017/S1431927622004809
Microsc. Microanal. 28 (Suppl 1), 2022

Uncovering Tumor Suppressor P53 Dynamics Using Microprocessor Materials

Maria J. Solares[1,3,4], GM Jonaid[2,3,4], Deborah F. Kelly[3,4*]

1. Molecular, Cellular, and Integrative Biosciences Graduate Program, Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA, USA.
2. Bioinformatics and Genomics Graduate Program, Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA, USA.
3. Department of Biomedical Engineering, Pennsylvania State University, University Park, PA, USA.
4. Center for Structural Oncology, Pennsylvania State University, University Park, PA, USA.
*Corresponding author: dfk5267@psu.edu

Widely regarded as the "guardian of the genome," tumor suppressor protein TP53 (p53) is heavily implicated in cancer [1]. Although it has essential roles in cell cycle arrest and DNA, there is poor representation of p53's structural information across the field [2]. In broad terms, p53 can be divided into three major regions: N-terminal domain (NTD), DNA-binding domain (DBD), and the C-terminal domain (CTD) [Fig 1A]. Only the DBD, which is the least flexible region of the protein, has been resolved via X-ray crystallography methods. The NTD and the CTD, on the other hand, are regions too disorganized and flexible to be fully resolved through X-ray crystallography or NMR. However, by overlooking the other 50% of its sequence, valuable information has been lost. This has caused major opposing theories to arise from the lack of data.

Although cryo-electron microscopy (EM) can resolve structures of flexible macromolecules, a few limitations arise. Historically, researchers have been relying on recombinant p53 constructs to observe their structure as the yield and purity of p53 isolated from cancer cells does not meet the technique's standards. Several important insights have been made through these models. Yet, these proteins are devoid of important molecular signals linked to cancer disease like ubiquitination. There is an immense need for a high-resolution, full-length p53 model.

We adapted silicon nitride-based microprocessor chips, or microchips, to resolve the first full-length structure of p53. These microchips are well known for their pristine flat surfaces and reliable ability to perform well under extreme temperature conditions. By pairing microchips with an added layer of nickel-NTA and a highly reproducible extraction and enrichment technique, we can increase the capture of monomers and oligomers for better reconstructions [3]. Frozen p53 samples from glioblastoma cells were imaged with a Talos F200C TEM operating at 200 kV. Images were collected using a CETA CMOS camera at low dose conditions (<5 electrons/A^2/pixel) and at ~92,000x magnification.

Single particle imaging processing using RELION [4] were able to produce full-length dimers (~4.2 Å) [Fig 1B]. Full length models of p53 monomers was produced using I-TASSER Protein Fold Recognition Server [5], and two were easily accommodated by the electron density map using PHENIX and ISOLDE capabilities [6,7]. Interestingly, there was no density available that would correspond to DNA leading us to believe that we had resolved an "inactive state" dimer. By observing the interface of inactive p53 dimer and comparing it to an active or DNA-bound dimer, we were able to identify the residues that could mediate the protein-protein interaction [Fig 1C]. There are potential residues that may be ubiquitinated to block MDM2 binding, a well-known regulatory partner, to sequester p53 in the cell or acetylated to initiate apoptotic pathways. Further, several "hot spot" mutations fall within or are adjacent

to this interface region, which may be a key therapeutic target in the future. With these insights, we were able to reconstruct a possible model to explain the conformational changes p53 dimers follow as they transition from an inactive to an active DNA-binding state [Fig 2].

The cure to cancer lies in the intersection of materials science, structural biology, and biochemical techniques. By adapting advances in all these fields, our current work showcases how these snapshots of biology can help untangle decade's worth of questions. It is paramount to decipher the conformational states as well as the molecular modifications of p53 to understand how important pathways are deregulated in cancers. These insights may be able to help facilitate the development in novel chemotherapeutics.

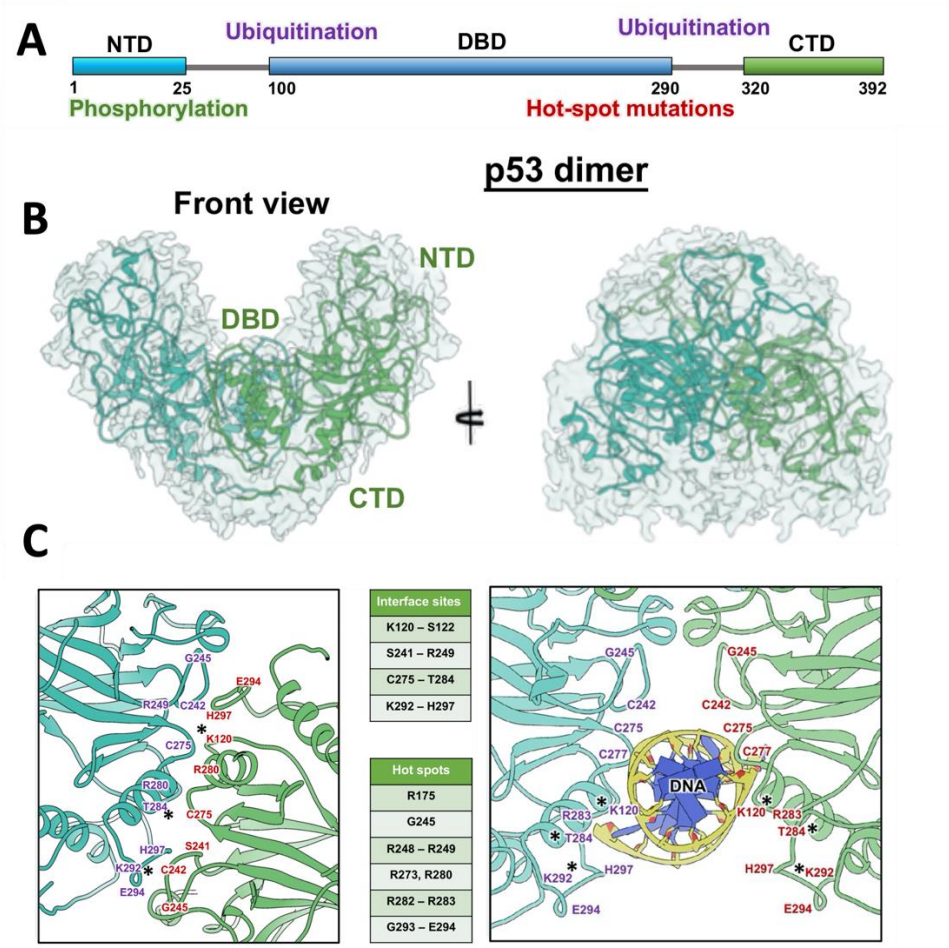

Figure 1: Full-length p53 dimers in an inactive state can shed light on important protein-protein interactions. (A) A schematic for the p53 primary structures highlighting the NTD, DBD, and CTD regions. Sites for phosphorylation as well as hot spot mutations are also indicated. **(B)** Rotational view of inactive p53 dimer (~4.2 Å). Two p53 monomers (green and cyan) were fit into the EM density map. **(C)** Magnified views of the of the dimer interface with and without DNA, highlighting residues involved in the protein-protein interaction or protein-DNA interaction. Tables include the interface residue regions and key hotspot mutations found in cancer.

https://doi.org/10.1017/S1431927622004809 Published online by Cambridge University Press

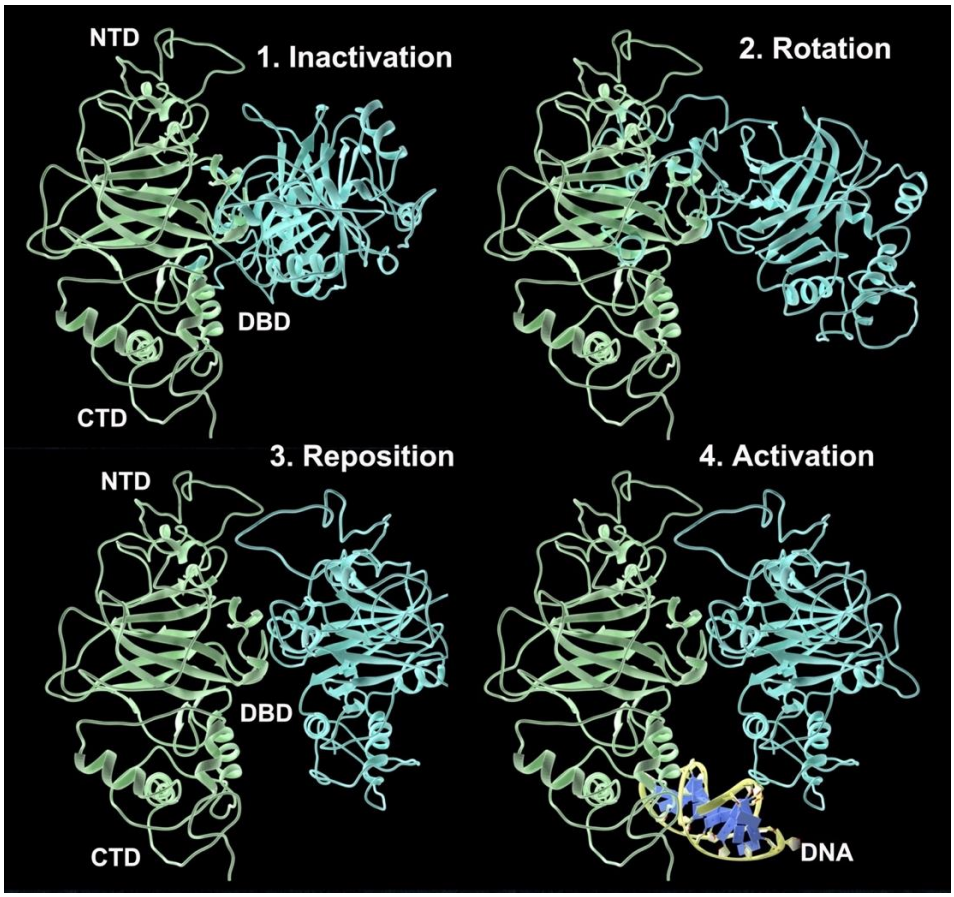

Figure 2: Proposed model of conformational transition stages from an inactive to active p53 dimer. Activation of p53 dimers may follow four stages: 1) inactivation, where monomers are blocking the DNA-binding site; 2) rotation, where one monomer rotates to expose the DNA-binding site; 3) reposition, where the monomer is repositioned for DNA binding; and 4) activation, where dimer is bound to DNA (yellow) and actively transcribing.

References:

[1] AC Joerger and AR Fersht, Cold Spring Harb Perspect Biol **2** (2010), p. a000919.
[2] AL Okorokov and EV Orlova, Current Opinion in Structural Biology **19** (2009), p. 197.
[3] MJ Solares et al., Anal. Chem. **92** (2020), p. 15558.
[4] SHW Scheres, J Struct Biol **180** (2012), p. 519.
[5] A Roy, A Kucukural and Y Zhang, Nat Protoc **5** (2010), p. 725.
[6] TI Croll, Acta Crystallogr D Struct Biol **74** (2018), p. 519.
[7] D Liebschner et al., Phenix. Acta Crystallogr D Struct Biol **75** (2019), 861.

https://doi.org/10.1017/S1431927622004809 Published online by Cambridge University Press