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	Human p53 DNA-binding domain loop mutants	MX-I506
ID29	from 3rd of April 2013 to 4th of April 2013	
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Experimental Report

Aim of the experiment and specific background :

We aim to solve the co-crystal structure of the human transcription factor p53 DNA-binding domain dimer bound to DNA. We have already determined the structure of the co-crystal structure of that complex (Figure 1) (1-3). Our structure shows two p53 dimers, having in total four DNA binding domains, bound to double stranded DNA. We observed that the loop L1 that interacts with DNA has two different conformations, recessed and extended. We demonstrated that the p53-DNA binding occurs via an induced fit mechanism with a switch in the conformation that involves the loop L1 (2). We solved the structure of the loop mutant p53 in complex with DNA (4). We are now interested to determine the structure of the free mutant p53.

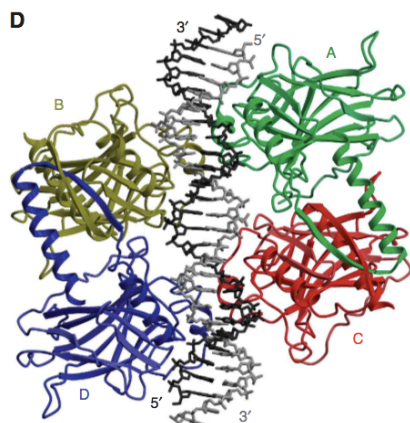


Figure 1. Structure of p53 in complex with DNA

Experimental method :

We purified a stable p53 containing the DNA binding domain as well as two mutations in the loop L1. We proceeded then to screen crystallographic conditions in order to obtain crystals. All experiments, from purification through crystal screening, were performed at 4 degrees Celsius to reduce precipitation and increase the chances of obtaining crystals.

We grew p53 crystals with different sizes in a range of 50 to 200 microns and obtained excellent resolution of 1.3-1.4 Ångströms (5, Figure 2). From these data we were able to solve the structure.

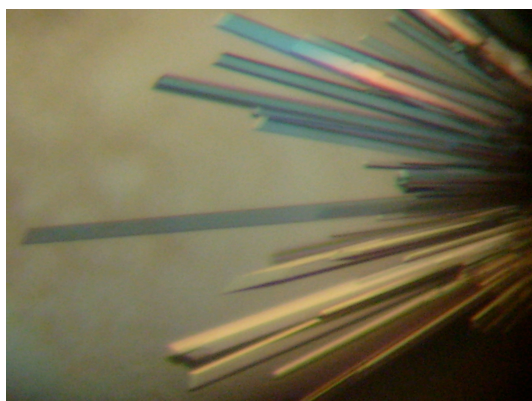


Figure 2. p53 DNA-binding domain loop

mutant

Results:

We grew crystals of the same p53 DNA-binding domain loop mutant but with other point mutations in order to know if the loop L1 can adopt others conformations. Unfortunately, we were not able to collect any data set, they were salt and no real protein crystals.

References :

1. MX-832 report
2. Petty TJ, Emamzadah S, Costantino L, Petkova I, Stavridi ES, Saven JG, Vauthey E, Halazonetis TD. *An induced fit mechanism regulates p53 DNA binding kinetics to confer sequence specificity*. EMBO J. 2011 Jun 1;30(11):2167-76
3. Emamzadah S, Tropia L, Halazonetis TD
Crystal structure of a multidomain human p53 tetramer bound to the natural CDKN1A (p21) p53-response element. Mol Cancer Res. 2011 Nov;9(11):1493-9
4. MX-1316 report
5. MX-1456 report

