ESRF	Human p53 DNA-binding domain loop mutant	MX-1456
ID14-1	from 28th of September 2012 to 29th of September 2012	
Ishift	Local contact: ZANDER Ulrich	

Experiment 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 occures 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.

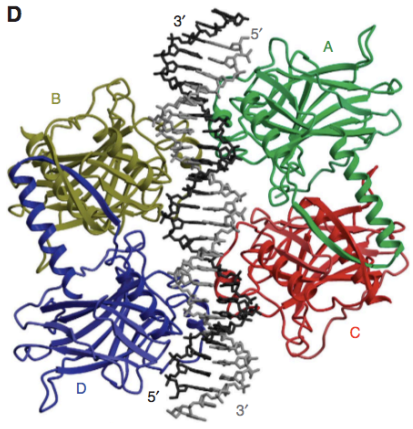

Figure 1. Structure of p53 in complex with DNA

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 obtained p53 crystals with different sizes in a range of 50 to 200 microns (Figure 2).

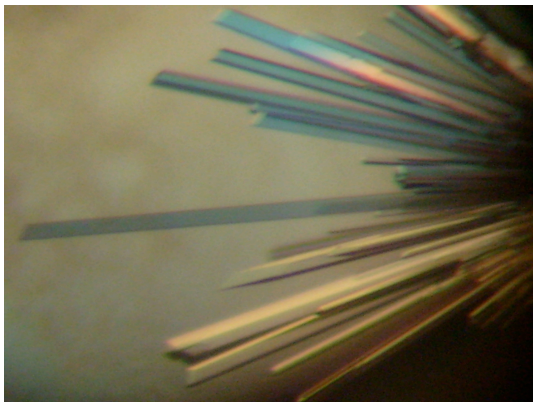

Figure 2. p53 DNA-binding domain loop mutant

Results:

We obtained excellent resolution of 1.3-1.4 Ångströms for these crystals and are very confident in resolving the structure of p53 DNA-binding domain loop mutant.

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