

EUROPEAN SYNCHROTRON RADIATION FACILITY

ESRF User Office

BP 220, F-38043 GRENOBLE CEDEX, France

Delivery address: 6 rue Jules Horowitz, 38043 GRENOBLE, France

tel: +33 (0)4 7688 2552; fax: +33 (0)4 7688 2020; email: useroff@esrf.fr; web:

<http://www.esrf.fr>



	Human <u>multidomain</u> p53 in complex with non specific DNA	MX-1656
ID23-1	15th of May 2014	
1shift	Local contact: <u>POUDEVIGNE</u> Emilie	

Experimental Report

Aim of the experiment and specific background :

We aim to solve the co-crystal structure of the human transcription factor containing both the p53 DNA-binding and oligomerization domains in complex with semi-specific DNA. We have already determined the structure of the co-crystal structure of p53 in complex with specific consensus DNA as well as in complex with one of its natural response element (*CDKN1A* (*p21*)) (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 its apo form and in complex with DNA (4,5,6).

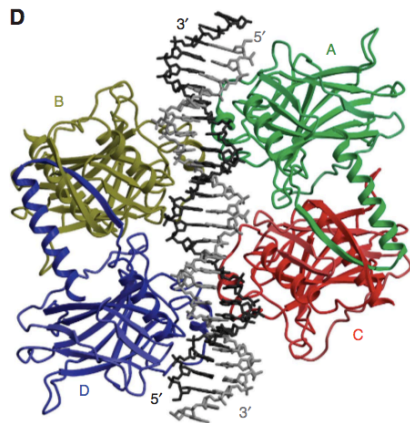


Figure 1. Structure of p53 in complex with DNA

Experimental method :

We purified a stable p53 containing both the DNA-binding and oligomerization domains. 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).

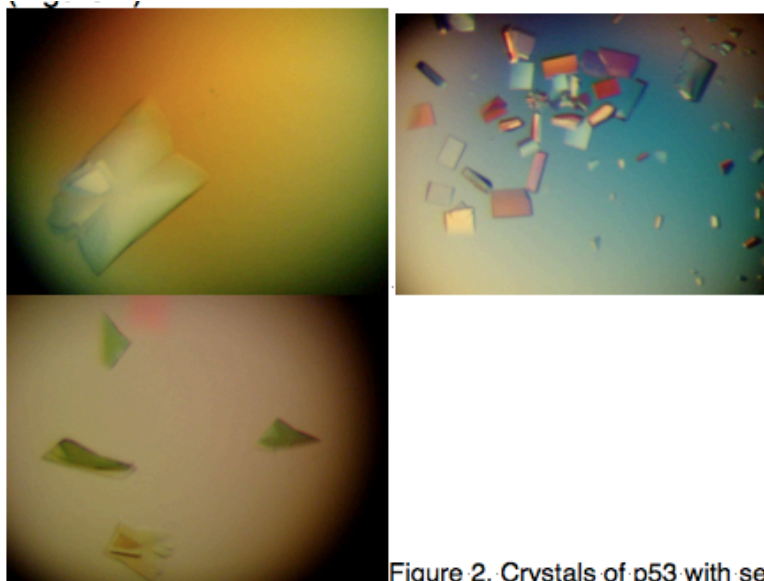


Figure 2. Crystals of p53 with semi-specific DNA

Results:

We previously had crystals of p53 in complex with semi-specific DNA (7). However, although the resolution was good (about 3.5-4.0 Angström), it was not as good as we expected and impossible to analyze. This time, we had better resolution (2.9-3.2 Angström). However, after about a month of analysis and refinement we are not confident about the structure we obtained. We need to grow more crystals.

References :

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