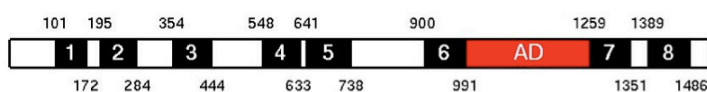
	TopBP1 resolution refinement trials	MX-1241
ID23-2	from 2nd of March 2011 to 3rd of March 2011	
I shift	Local contact: Dr. Ricardo FERRAZ LEAL	

EXPERIMENT REPORT

We aimed to solve the crystal structure of various regions of the human Topoisomerase II β Binding Protein (TopBP1) in order to better understand its role in the DNA damage response. TopBP1 has eight BRCT domains that are implicated in binding to phosphorylated proteins involved in DNA damage response (Yamane et al., 1997). BRCT domains 1 and 2 are involved in binding to the 9-1-1 complex in order to recruit TopBP1 to stalled replication forks (Delacroix, et al., 2007), while the 5th BRCT domain is essential for TopBP1 foci formation (Yamane et al., 2002). The activation domain (AD), located between BRCT domains 6 and 7, is required for ATR/ATRIP activation of the checkpoint response (Kumagai, et al., 2006) (Figure 1).

TopBP1

TopBP1 (Topoisomerase II β Binding Protein)



- **AD** is required for ATR/ATRIP activation for the checkpoint response (Kumagai, et al., 2006)

Figure 1. Topoisomerase II β Binding Protein. BRCT domains highlighted in black, activation domain in red.

We have mapped, *in vivo*, various BRCT domains involved in recruiting TopBP1 to sites of DNA damage, with certain domains implicated in recruitment to either DNA double strand breaks or stalled replication forks. We subsequently expressed various fragments of human TopBP1 containing the activation domain and BRCT domains in *E. coli*. Using classical ion-exchange and size-exclusion chromatography, we have purified and set up crystal screens for these various TopBP1 fragments. All experiments, from purification through crystal screening, were performed

at 4 degrees Celsius to reduce precipitation and increase the chances of obtaining crystals.

From one of our previous visit, we obtained a resolution in a range of 4-8 Ångströms. Were able to grow more TopBP1 crystals of about 50-100 microns (Figure 2) and tested them on the beam ID 23-2 (2nd of March 2011).

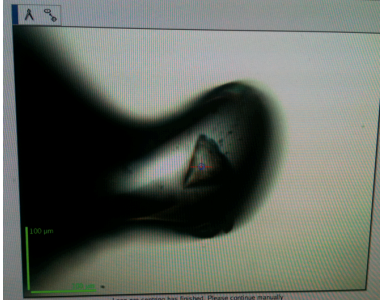


Figure 2. Example of new batch of TopBP1 crystals

We obtained a resolution of 3.2-3.4 Ångströms. We could collect complete data sets. We are now processing the data and hope that the data will be good enough to resolve the structure of the Activation Domain.

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