ESRF	TopBP1 resolution refinement trials	MX-1092
ID23-2	from 8th of April 2010 to 9th of April 2010	
1shift	Local contact: Sean Mcsweeney	

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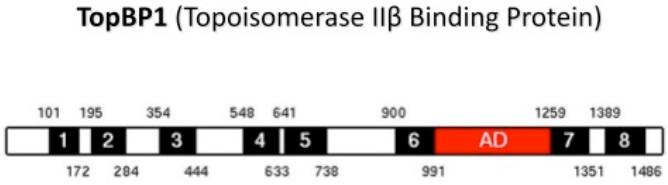
Report:

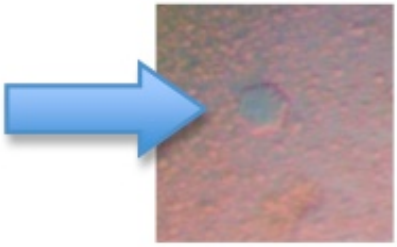
We aimed to solve the crystal structure of various regions of the human Topoisomerase II! Binding Protein (TopBP1) in order to better understand its role in the DNA damage response. TopBP1 has eight BRCT domains that are implicated in binding to phosphorylated proteins involved in DNA damage response (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 réplication 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).

Topoisomerase II! Binding Protein (TopBP1), *BRCT domains highlighted in black, activation domain highlighted in red*



After one month of incubation at 4 degrees Celsius we began to observe small crystals on the order of 20 to 30 microns in size in some of our vapour-diffusion screening conditions. We have since then brought a few of these small crystals to the ESRF during our visits for other experiments and have determined that they are indeed protein, but that they only diffract to a maximum resolution of 8 angstroms.

Image of hexagonal TopBP1 crystal, approximately 30 microns



Since our initial observations of these TopBP1 crystals, we have screened and optimized conditions using various techniques, including micro-seeding and streak-seeding. We have obtained slightly larger crystals, and optimized conditions with various additives and pH changes have yielded more small crystals as well.

Unfortunately, with the new batch of crystals, we didn't get any diffraction data, we didn't have real crystals. We will try for another time beam to obtain TopBP1 crystals that we obtained for the previous experiments (MX-1032 and MX-896) with better quality.

However, we brought as well some p53-DNA crystals that refers to a former experiment, MX-832. Using other DNA oligos, We were able to collect data allowing us to obtain a resolution of 3.3 angstroms. Thanks to the microbeam that we used (ID23-2), we hope having the same beamline for our next trip to the ESRF (proposal MX-1148) where we would like to bring as well as rev7 crystals, some of our new batch of p53 protein crystals and p53-DNA crystals.

References

Yamane K, Kawabata M, Tsuruo T. *A DNA-topoisomerase-II-binding protein with eight repeating regions similar to DNA-repair enzymes and to a cell-cycle regulator.* Eur J Biochem. 1997 Dec 15;250(3):794-9.

Delacroix S, Wagner JM, Kobayashi M, Yamamoto K, Karnitz LM. *The Rad9-Hus1-Rad1 (9-1-1) clamp activates checkpoint signaling via TopBP1*. Genes Dev. 2007 Jun

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Yamane K, Wu X, Chen J. *A DNA damage-regulated BRCT-containing protein, TopBP1, is required for cell survival.* Mol Cell Biol. 2002 Jan;22(2):555-66

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