ESRF	**Experiment title:** Topoisomerase IIBeta Binding Protein [TopBP1] - Microcrystal Trial 1	**Experiment number:** **MX-896**
Beamline: ID23-2	**Date of experiment:** from: 27 April 2009 to: 28 April 2009	**Date of report:** 28 July, 2009
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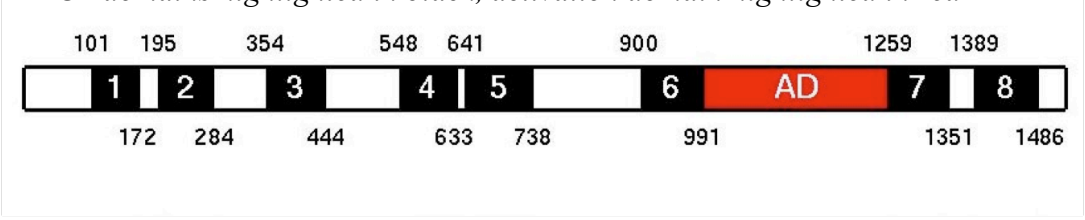
Report:

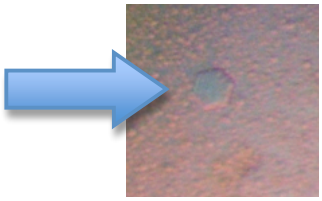
We aimed to solve the crystal structure of various regions of the human Topoisomerase IIβ Binding Protein (TopBP1) in order to better understand its role in the DNA damage response. TopBP1 has eight BRCT domains that are implicated in binding to phosphorylated proteins involved in DNA damage response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

Topoisomerase IIβ 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.

This new batch of crystals, however, was only marginally better than the first batch, in terms of resolution and overall size; it is difficult to index them with the few frames we collected. We were only able to collect data to 6 angstroms, and it was very weak. The small size of the crystals makes it difficult to properly cryo-preserve and mount in the loops. We also tried different methods of cryopreservation in attempts to obtain crystals that give better than 8 angstrom resolution. Although we did improve resolution marginally, we must continue to optimize crystal growth and cryo-preservation conditions. Despite their relatively small size, on the order of 30 to 80 microns, the micro-focus beamline ID23-2 gave us the chance to collect some data from these 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 15;21(12):1472-7.

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

Kumagai A, Lee J, Yoo HY, Dunphy WG. *TopBP1 activates the ATR-ATRIP complex*. Cell. 2006 Mar 10;124(5):943-55.