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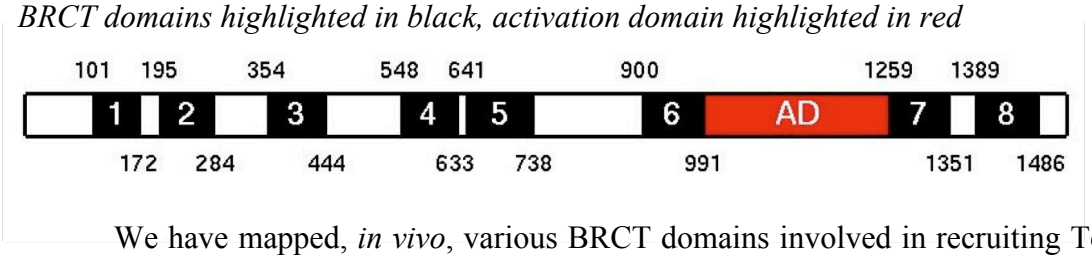
Application for beam time at ESRF – Experimental Method

This document should consist of a maximum of two A4 pages with a minimal font size of 12 pt.

Aims of the experiment and scientific background

We aim 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



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.

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 previous visits for other experiments and have determined that they are indeed protein, and currently that they diffract to a resolution of 4.5 angstroms.

Image of hexagonal TopBP1 crystal,
Approximately 30 microns

Experimental method

The experimental method will be identical to that of our previous visits to the ESRF. This is a basic crystallographic experiment using native protein crystals cryo-prepared in loops and transported to the ESRF in liquid nitrogen. We plan to use the automatic sample changer if available; all of our samples will be mounted in a SPINE standard CryoLoop (Hampton Research, CA, USA) as recommended by the ESRF. All samples will be removed from the ESRF and returned with us at the end of our shift.

Results expected

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.

We expect that this new batch of crystals will be slightly better than the first in terms of resolution and overall size. Furthermore, we are trying different methods of cryopreservation in attempts to obtain crystals that give better than 4.5 angstrom resolution. We expect to collect complete datasets from these new TopBP1 crystals, hoping that they will be in the resolution range of 2-3 angstroms. Despite their relatively small size, on the order of 100 to 300 microns, we hope that by using the micro-focus beamline will give us the chance to collect complete datasets 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.