A Chemoproteomic Assay for the Analysis of Bromodomain Inhibitors

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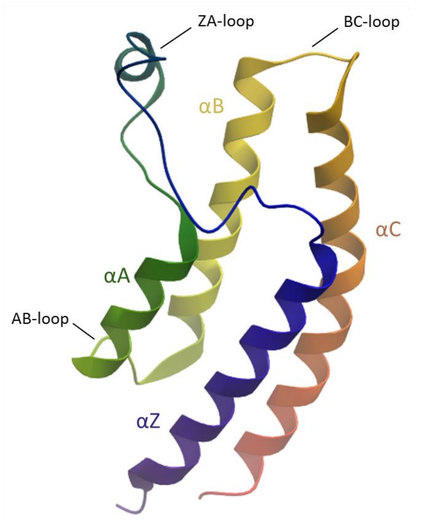
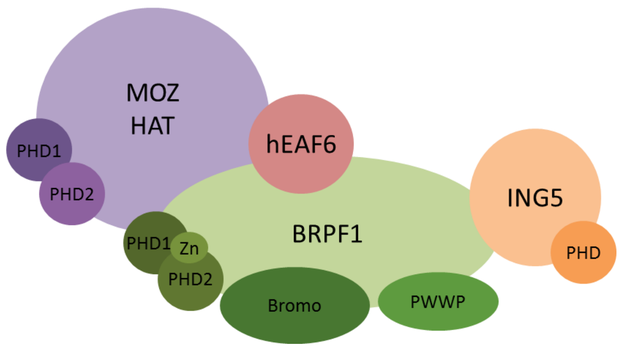
Bromodomains



The Bromodomain of BRPF1B (PDB-4LC2).

- Bromodomains act as specific readers of acetylated lysine (Kac) and mediate the recruitment of proteins to macromolecular complexes.
- Bromodomains have a low sequence homology but a conserved overall fold: 4 α-helices linked by variable loop regions, which form the docking site.
- Bromodomain containing proteins are implicated in a large variety of diseases; hence, potent bromodomain inhibitors are sought after.
- A method for monitoring the inhibition of bromodomains is required.
- Focussing on BRPF1B, a pulldown assay is under development to measure the binding of inhibitors to BRPF1B within cells using LCMS/MS.

BRPF1B & the MOZ HAT complex

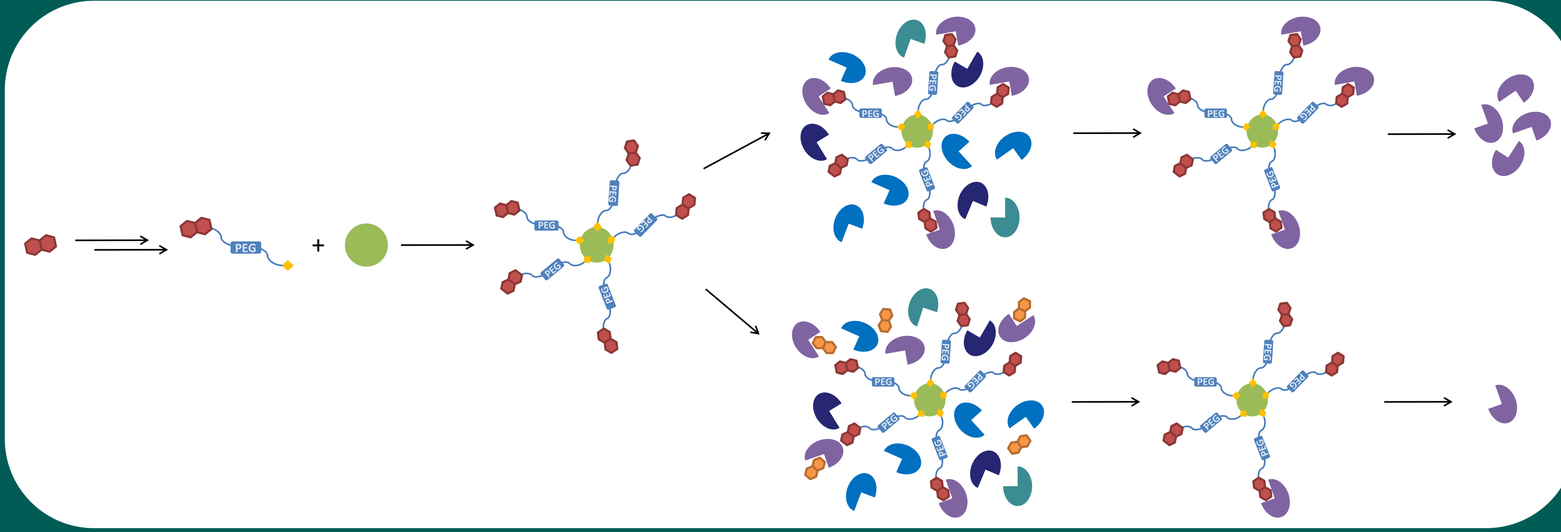


Schematic representation of the MOZ HAT complex.

- BRPF1B acts as a bridging subunit of the monocytic leukaemia zinc finger (MOZ) histone acetyltransferase (HAT) complex.
- BRPF1B contains multiple sequence motifs including:
  - a double PHD and zinc finger (PZP)
  - a bromodomain
  - a PWWP domain
  - two protein interaction domains
- Inactivation of BRPF1B is lethal in embryonic mice.[1]
- The BRPF1B bromodomain preferentially binds to H2AK5ac, H4K12ac, H3K14ac histone ligands.[2]
- Translocation of MOZ HAT is associated with a subtype of acute myeloid leukaemia (AML) and poor prognosis.
- AML accounts for 15% of childhood and 80% of adult leukaemia cases.[3]

1. You *et al.* (2014). *Epigenetics.* 9(6):860-872
2. Poplawski *et al.* (2014). *J. Mol. Biol.* 426: 1661-1676
3. Carlson, *J. Cell. Phys.* 229(11)1571-1574

Pulldown Assay



Scheme 1: The isolation of BRPF1B from cell lysate. A biotinylated analogue of a compound known to be a specific inhibitor of BRPF1B (red) was synthesised. This compound can be immobilised on magnetic streptavidin beads (green). The immobilised compound can be incubated with cell lysate, washed to remove other proteins and the BRPF1B eluted (top). If the immobilised compound is incubated with lysate in the presence of an inhibitor of BRPF1B (orange), less BRPF1B will be captured (bottom). The amount of BRPF1B can be quantified using LCMS/MS.

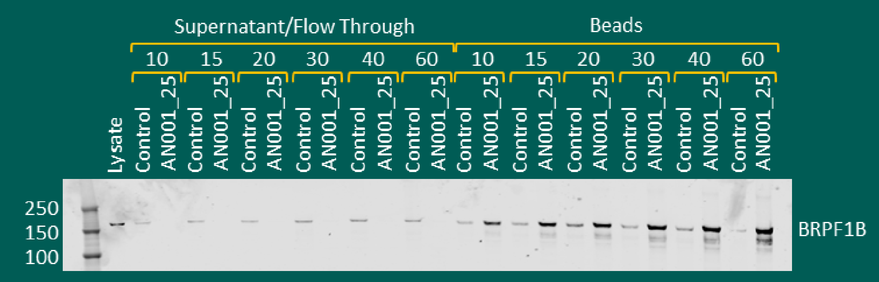
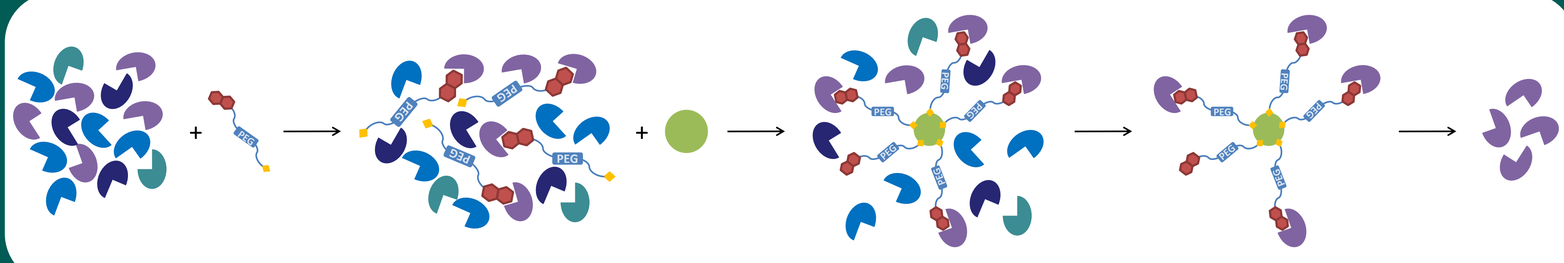


Figure 1: Western blot for the pulldown of FLAG-BRPF1B where different concentrations of HEK293 cell lysate transfected with FLAG-BRPF1B were incubated with immobilised biotinylated compound (AN001_25) or control (no compound immobilised). Concentration dependence is observed.

BRPF1B binds to the biotinylated compound (AN001_25) with a K_D of 1.7 μM (measured using OctetRed BLI). Despite its fast k_{off} binding of BRPF1B to the immobilised compound is readily observed (Figure 1).

Alternative Pulldown Assay



Scheme 2: An alternative method for the isolation of BRPF1B from cell lysate. A biotinylated analogue of a compound known to be a specific inhibitor of BRPF1B (red) can be incubated with cell lysate prior to the addition of magnetic streptavidin beads (green). After incubation with magnetic streptavidin beads, the beads can be washed to remove other proteins and the BRPF1B eluted. The amount of BRPF1B can be quantified using LCMS/MS.

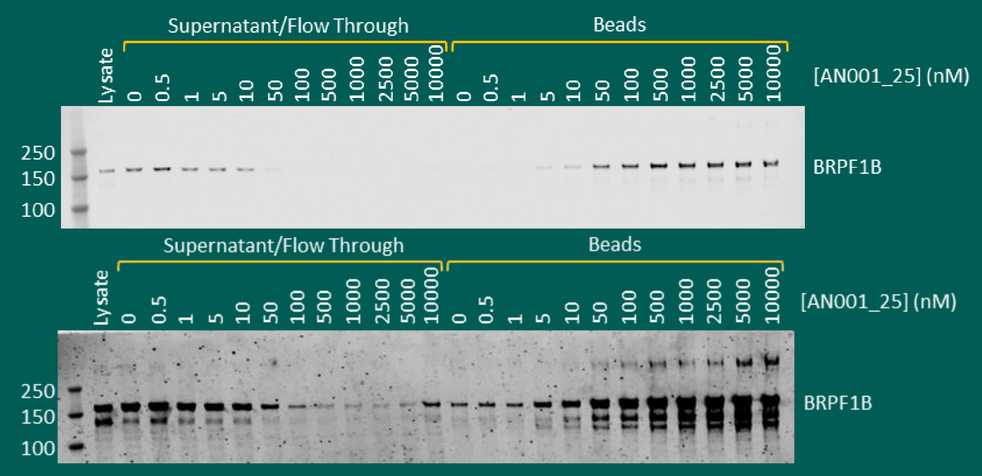


Figure 2: Western blot for the pulldown of FLAG-BRPF1B where HEK293 cell lysate transfected with FLAG-BRPF1B was incubated with different concentrations of biotinylated compound (AN001_25) for 10 min followed by a 1 min incubation with magnetic streptavidin beads (top). By overexposing the blot (bottom), it is possible to see a drop in protein flowthrough at 100 nM AN001_25.

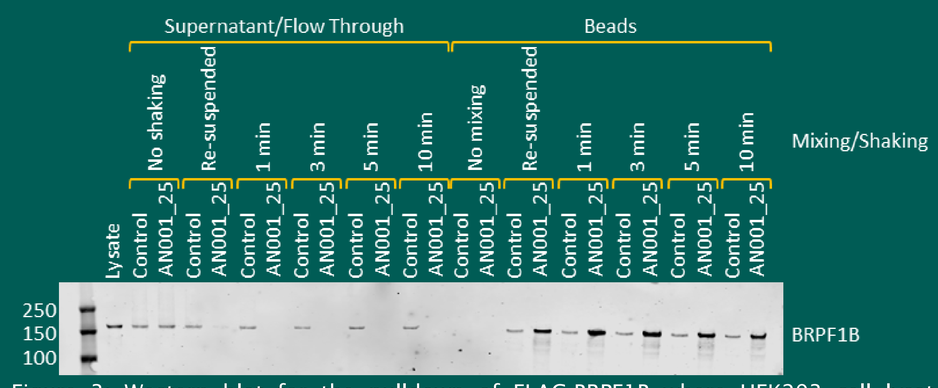


Figure 3: Western blot for the pulldown of FLAG-BRPF1B where HEK293 cell lysate transfected with FLAG-BRPF1B was incubated with 100 nM biotinylated compound (AN001_25) for 10 min followed by incubation with magnetic streptavidin beads for 0-10 min.

A potential problem is an effective local concentration/avidity of immobilised compound compared to free inhibitor. Therefore, an alternative method was also investigated.

Conditions for the pulldown assays have been optimised:

- Excess protein would cause inhibited protein not to be detected; i.e. all sites on beads would be occupied by the remaining free protein.
- Excess biotinylated compound competing with inhibitor may prevent inhibition being observed. 100 nM biotinylated compound has been chosen (Figure 2).
- Ample time for binding of the biotinylated compound to the beads without avidity being a problem is needed. 1 min was found to be sufficient (Figure 3).

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