Towards comprehensive coverage of Bromodomain family for drug screening and discovery

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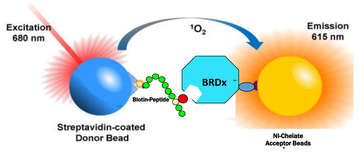
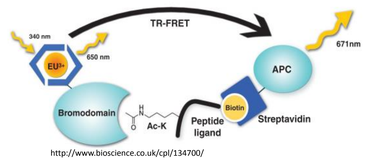
Epigenetic regulation of gene expression is a highly dynamic and reversible process essential to normal cellular function. However, it also contributes to human diseases, such as cancer and inflammation. Protein families that participate in epigenetic regulation include *writers*, which covalently modify chromatin; *readers*, which recognize chromatin modifications; and *erasers*, which remove modifications. A large volume of research in the field over the past decade has shown that many epigenetic proteins are potential druggable targets.

Bromodomains, which belong to the *readers* category, recognise acetylated lysine residues on histones and other proteins. Several potent, selective and cellularly active bromodomain compounds have recently been identified, increasing appreciation of the functional importance and therapeutic potential of this family. At RBC we are working towards the complete coverage of the bromodomain family. We both provide purified proteins and develop various types of assays to facilitate screening, drug discovery and validation of hits.

Available assay formats for bromodomains:

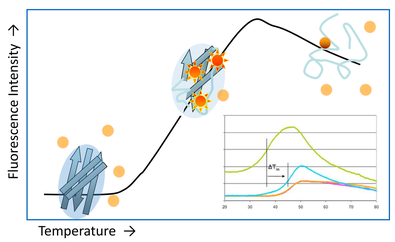


AlphaScreen Assay: Laser excitation of Donor beads (substrate specific) produce short-lived singlet oxygen molecules which can generate an amplified chemiluminescent signal from the Acceptor beads (tag specific). Interaction between protein of interest and a substrate enables singlet oxygen transfer. Assay is highly sensitive, homogenious and requires no washing steps.

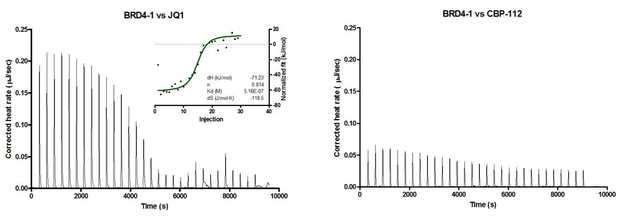


http://www.bioscience.co.uk/cpl/134700/

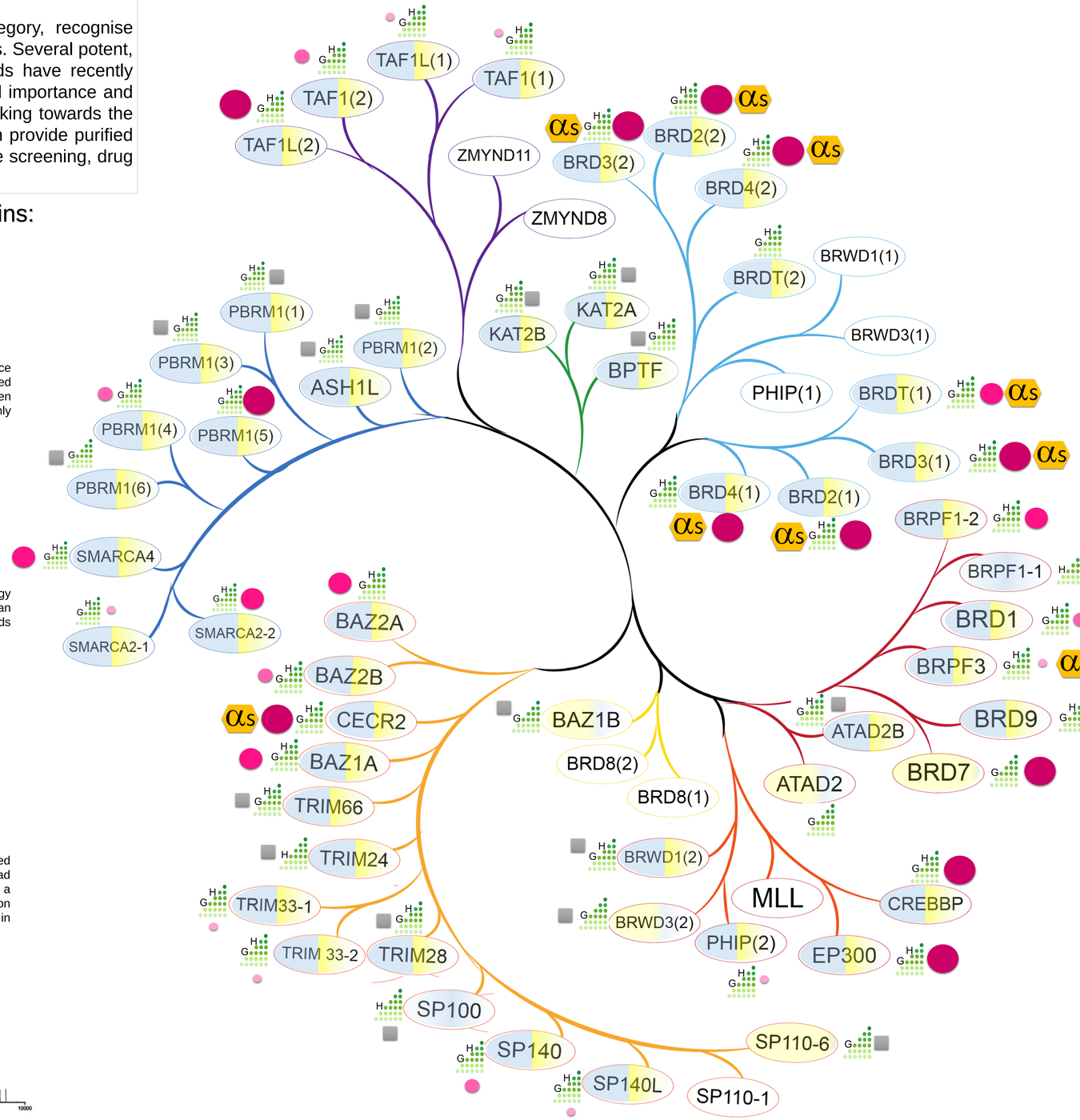
TR-FRET Assay: interaction between protein and substrate is detected by energy transfer between two fluorophores, which also results in dual emissions. The signal can be expressed as a ratio that helps to reduce fluorescent interference from compounds and other assay components.



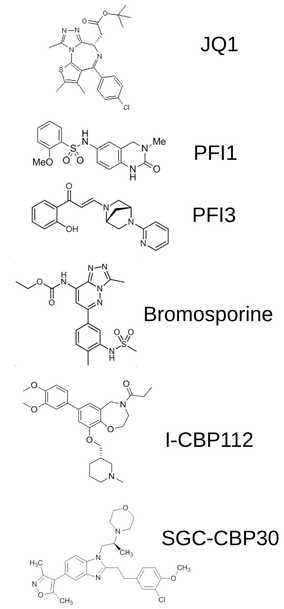
DSF- differential scanning fluorimetry - protein thermal stability assay. Heat-induced protein denaturation exposes hydrophobic surfaces that interact with the dye and lead to the increase of fluorescent signal. The fluorescence profile is analyzed to obtain a melting temperature (Tm), represented by an inflection point of the curve. Interaction between the protein and a ligand increases protein stability that leads to the increase in Tm.

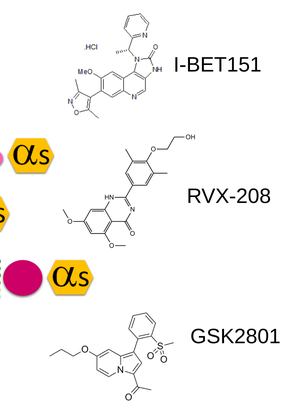


Isothermal Titration Calorimetry (ITC) – commonly used technique to characterize binding interactions. ITC is label free, determines ligand binding constants (K_a or K_d), binding stoichiometry and contribution of non-covalent forces responsible for binding.

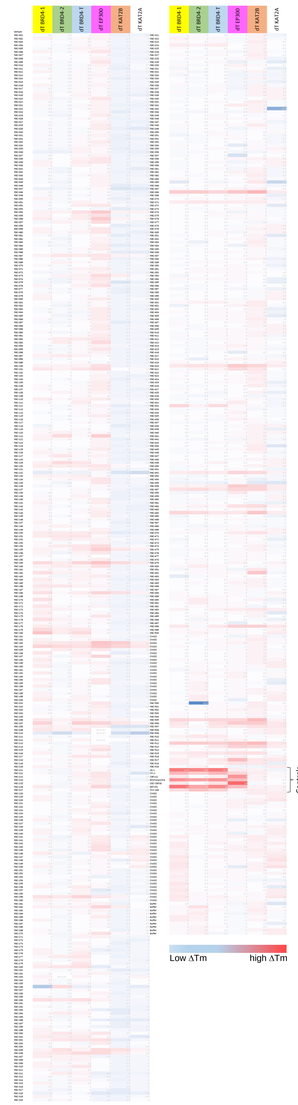


Control compounds





Library screening



TAG	Purified protein	Available for sale
6xHis-		
GST-		
Both versions		

αs Alpha screen assay available

ΔT_m^{obs} (°C) >6 4-6 2-4 <2 Good melt, no compound