ABSTRACT

Covalent Post Translational Modifications (PTMs) of histone proteins are introduced and removed by the 'writer' and 'eraser' enzymes, respectively. These modifications are recognized by highly specific binding domains, the so-called 'reader' proteins, which are thought to mediate epigenetic signalling. Misregulation of these proteins often leads to abnormal gene expression patterns that are more frequently linked to human diseases.

In comparison to writers and erasers, readers have been less intensively pursued so far as therapeutic targets, especially with regard to methylation. To date, different biochemical and biophysical techniques are reported to assess the binding of new putative modulators of methyl readers. Nevertheless, given the intrinsic limitations each technique encompasses, reliable data can be obtained using a combination of methods.

Thus, this project is focused on the development of a versatile screening platform for the identification of small-molecule ligands of methylation reader proteins. Among different reader domains, the attention was focused on the Tudor domains of PHF20, Tudor domains of Spindlin1 and the Chromodomain of MRG15. After optimizing the conditions of expression and purification for these proteins, the screening platform was applied to all proteins in order to screen a library of compounds synthetized in Epigenetic Medicinal Chemistry Laboratory (EMCL).

This platform is composed by biophysical techniques such as nanoDSF, MST and SPR while the only biochemical technique used was the AlphaLISA. Comparing the results obtained from each technique it was possible to identify true hits.