Abstract

The identification of natural products (NPs) target proteins is pivotal to understand their mechanism of action, in order to develop molecular probes and/or potential drugs. In the last 15 years, affinity chromatography-coupled to mass spectrometry (AP-MS) has been the top-choice technique in the Drug Target Deconvolution field, having brought brilliant results in the *targetome* profiling of a multitude of bioactive compounds.

Unfortunately, since a chemical modification of the molecule to be investigated is mandatory, AP-MS is not suitable for compounds that do not exhibit properly reactive structural features.

Furthermore, in the absence of information on the structure-activity relationships (SAR) of the analyte (e.g. in the case of natural products, often isolated in a too low amount for this investigation), the covalent modification would leave some concerns about whether or not the molecule original bioactivity is retained.

In this scenario, my PhD project was devoted to the development of an alternative Functional Proteomics Drug Target Deconvolution platform, based on two complementary *label-free* methods avoiding any chemical modification of the molecules for a proteome-wide profiling of their *targetome*.

Indeed, this platform takes advantage of both Drug Affinity Responsive Target Stability (DARTS), useful to identify the target protein(s) of a bioactive molecule, and targeted Limited Proteolysis coupled to Multiple Reaction Monitoring Mass Spectrometry (t-LiP-MRM), for the characterization of the interaction features between a protein and its ligand, enlightening the protein(s) region(s) directly or distally influenced by the NP binding.

After preliminary optimization steps, this *label-free* platform allowed the *interactome* characterization of three structurally different marine metabolites: the norcembranoid 5-epi-

Sinuleptolide interacting with actins, as also validated by the AP-MS approach, the sulfated bis-steroid Crellastatin A interacting with poly [ADP ribose] polymerase 1 and the acetogenin BrACG, interacting with both the cytosolic and mitochondrial Hsp70 isoforms.

Moreover, the DARTS results were validated through Western Blotting, while the t-LiP-MRM evidences were corroborated by Molecular Docking, and i*n vitro*/*in cell* activity assays were also performed, to assess the bioactivities of the marine metabolites.