Activity-based probes targeting metalloproteinases with no photoactivation: toward the profiling of these enzymes in vivo?

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Biological systems often respond to environmental modifications by regulating enzymes activity through a complex array of post translational mechanisms, which results in a discrepancy between the enzymes abundance and their related activity. By selectively reacting with nucleophiles involved in the catalytic act, activity-based probes (ABPs) with an electrophilic "warhead" enable an unambiguous discrimination between the active enzyme and its inactive counterparts[1]. This approach has been successfully applied to the in vivo profiling of serine and cysteine proteases possessing a hydroxyl and a thiol catalytic nucleophiles, respectively. In the case of zinc-metalloproteinases that lack a canonical nucleophile involved in catalysis, this strategy is however not straightforward applicable and reactive chemical probes systematically incorporate a photo labile group. Upon UV irradiation, this chemical moiety promotes the formation of a covalent bond, yielding a covalent complex, that can be subsequently characterized through a wide set of analytical methods. Such affinity-based probes have been used for the detection of active metalloproteinases, including matrix-metalloproteases (MMPs), in fluids and tissue extracts but not in living animals where the photo-activation step is not feasible.

By exploiting ligand-guided chemistry, we recently identified a new generation of ABPs capable of covalently modifying matrix metalloproteases (MMPs) active site without any external trigger[2]. In the frame of this presentation, we will show how this approach has been expanded to a larger set of metalloproteases. We will also discuss the parameters that impact the probes selectivity as well as their labelling efficiency in complex proteomes and will present our preliminary results on the in vivo labeling of MMPs.

1. Mechanism-based profiling of enzyme families. Evans MJ, Cravatt BF. *Chem Rev*. **2006** Aug;106(8):3279-3011.
2. Ligand-Directed Modification of Active Matrix Metalloproteases: Activity-based Probes with no Photolabile Group. Kaminska M, Bruyat P, Malgorn C, Doladilhe M, Cassar-Lajeunesse E, Fruchart Gaillard C, De Souza M, Beau F, Thai R, Correia I, Galat A, Georgiadis D, Lequin O, Dive V, Bregant S, Devel L. Angew Chem Int Ed Engl. **2021** Aug 9;60(33):18272-18279.