Project for PhD thesis for Sept 2016

Activatable 3-component probes that respond to specific enzyme activity

Contact : jens.hasserodt@ens-lyon.fr – 04 72 72 83 94

Laboratoire de Chimie, Equipe de Chimie Bio-Organique, Université de Lyon – ENS

In order to understand the biological mechanisms underlying certain pathologies, it is of prime importance to detect and precisely localize a particular enzyme activity within a live cell or organism. The habitually employed molecular probes suffer from a number of weaknesses, including signal diffusion away from the enzyme locus,[1] or the formation of a fluorochrome with a small Stokes' shift that allows autofluorescence of live tissue[2] to severely interfere with detection sensitivity. In order to meet these challenges, we have designed 3-component probes[3] that comprise :

1. a masked ESIPT fluorochrome (Excited State Intramolecular Proton Transfer), insoluble under physiological conditions,[3] thus allowing for effective **tagging** of cells hosting active target enzyme (Figure 2),
2. an enzyme-susceptible trigger group that renders the probe **specific**,
3. an auto-immolative smart spacer that makes the probe operate in the off→ON mode, thus conferring **high sensitivity** onto the detection/imaging process.

Our present results are highly encouraging[4,5] and constitute an essential proof-of-concept (figure 2). However, our present and future research focusses on the design and synthesis of probes that target important enzyme biomarkers of disease while simultaneously homing in on a second selection criterion. Three patents have already been filed (ENSL/CNRS) on this project. The pursuit of these goals entails further molecular design and the elaboration of short and efficient chemical syntheses. Collaborations with two companies are underway. Contacts with a number of biology laboratories in France, the U.S., Brazil, Japan and China bode well for significant expansion of our technology.

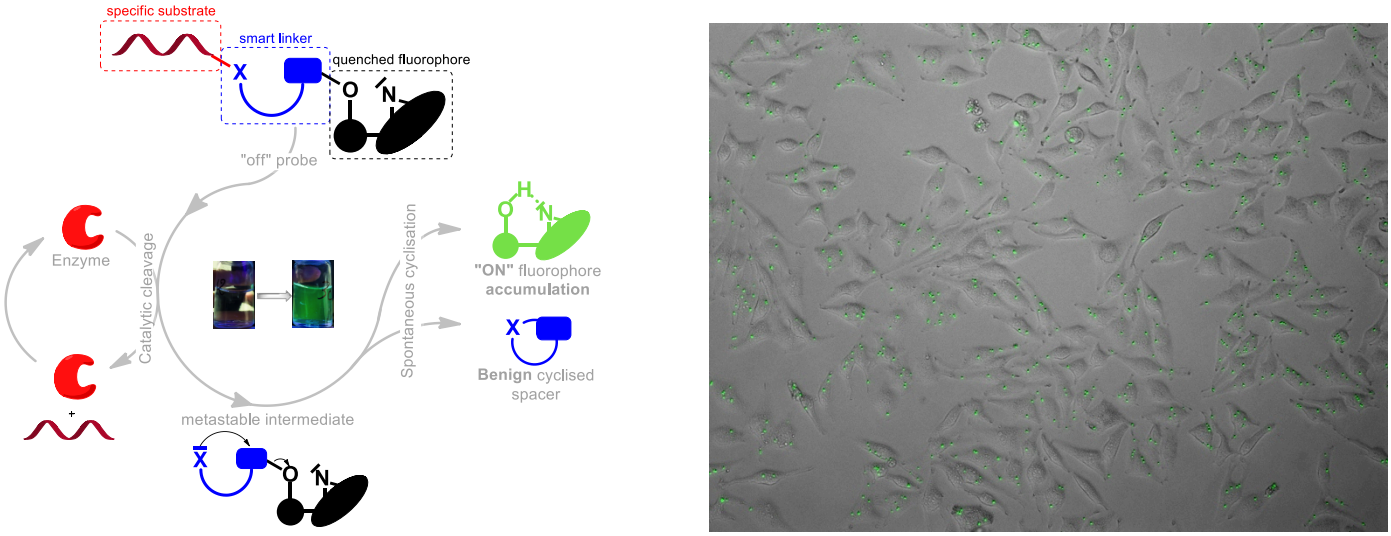

Figure 1 – Probes' mode of action (left) & live cells that fluorescently labelled themselves via their own enzyme activity

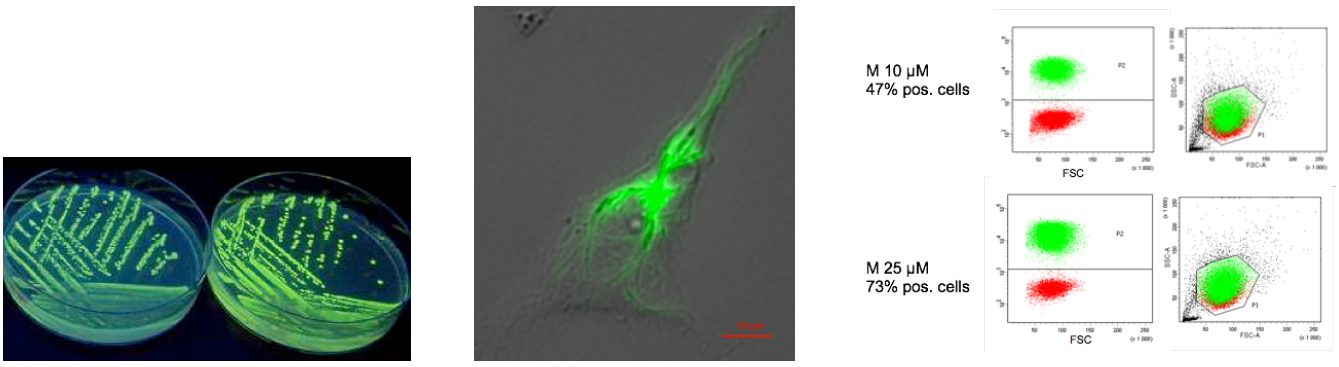

Figure 2 – bacterial colony assay & mysterious labelling patterns found in selected cells & labelling efficiency tests via FACS

1. V. B. Paragas, J. A. Kramer, C. Fox, R. P. Haugland, V. L. Singer, *J. Microsc.* **2002**, *206*, 106–19.
2. E. Boonacker, C. J. F. Van Noorden, *J. Histochem. Cytochem.* **2001**, *49*, 1473–1486.
3. (a) Waibel, M.; Zhang, X. ; Hasserodt J. , *Synthesis* **2009**, 318-324.
   (b) Zhang, X. ; Waibel, M. ; Hasserodt J. *Chem. Eur. J.* **2010**, *16*, 792-795
   (c) O. Thorn-Seshold, M. Vargas-Sanchez, S. McKeon, J. Hasserodt, *Chem. Commun.* **2012**, *48*, 6253–6255.
4. M. Prost, L. Canaple, J. Samarut, J. Hasserodt, *ChemBioChem* **2014**, *15*, 1413–7.
5. M. Prost, J. Hasserodt, *Chem. Comm.* **2014**, *50*, 14896-14899.