



PhD Thesis Proposal Form China Scholarship Council (CSC)

CHEMISTRY – CHEMICAL BIOLOGY

Thesis title:

**Enzyme-responsive probes releasing a fluorescent precipitate for
cytometric high-throughput screening and live-cell imaging**

▪ Laboratory name : **Laboratoire de Chimie**

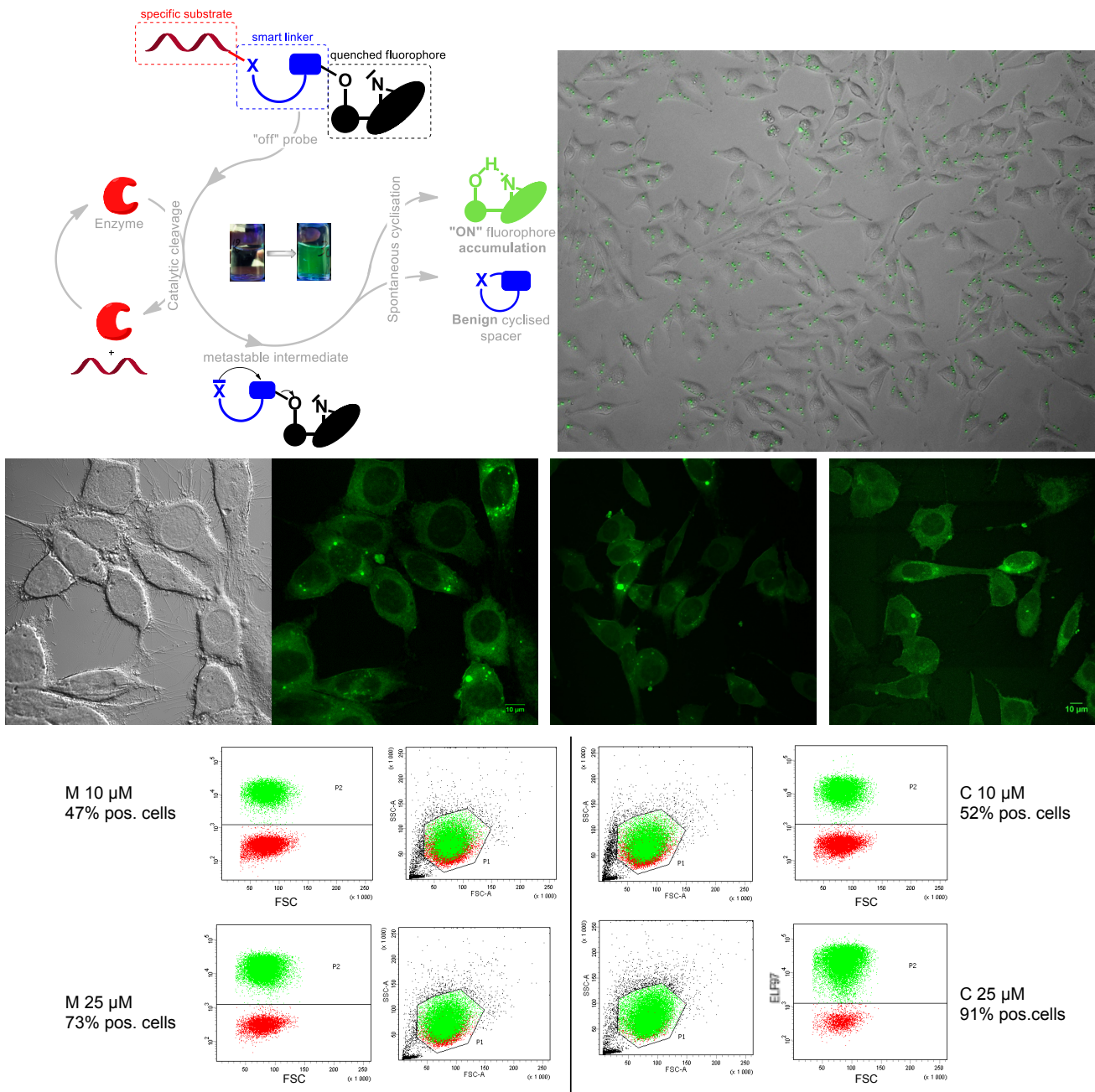
▪ PhD supervisor (contact person):

- Name: **Jens HASSERODT**
- Position: **Professor**
- E-mail: **jens.hasserodt@ens-lyon.fr**
- Phone number: **+33 472728394**

▪ Thesis proposal (max 1500 words):

The domain of bioanalytics and chemical biology has seen some significant efforts in the design of chemical probes that allow for the detection/depiction of a target enzyme ACTIVITY in live cells or animals. However, a fundamental challenge consists in the retention of any physical signal thus generated by action of this enzyme activity on the probe. Indeed, the transformed probe molecule will show comparable speed of diffusion as seen for the intact probe. During the incubation delay, transformed probes thus diffuse away from the site where the enzyme resides. In order to avoid this, a rare solution was proposed by incorporating in enzyme-responsive probes a unit that, upon its release by the enzyme activity, AGGREGATES and thereby simultaneously loses its diffusion properties and starts to fluoresce brighter (Ben Zhong Tang et al., University of Hongkong).

We at the ENS Lyon, are the group that has proposed another strategy, that of releasing a unit from the enzyme-responsive probe that PRECIPITATES, thereby becoming intensely fluorescent. The unit is called ELF97 alcohol and is rare in its property to be extremely insoluble while only becoming fluorescent once precipitated. However, when incorporated into the intact responsive probe, the resulting construct can be made perfectly water-soluble. It took us quite a few years (references 1a-c, 2) to find a molecular architecture that shows highly satisfactory robustness of the probe against spontaneous degradation and the concomitant generation of a false positive signal, absolutely prohibitive for robust life sciences applications.



With our technology sufficiently mature, we have now engaged in projects reaching from (a) high-throughput screening via cytometry (or bubble microfluidics) of cell libraries hosting libraries of mutant enzymes over (b) identifying cancer cells to (c) developing a new reporter gene technology.

In this context, the current PhD thesis proposal aims at the expansion of our line of enzyme-responsive probes to new enzyme activities with relevance for the identification of cancer cells or of a new reporter gene candidate. The proposal also comprises a chapter on the exploration of new molecular constructs for the precipitating unit to show even lower solubility and/or a different color than our current champion. A third aspect concerns the aim to equip the responsive probes with A SECOND biological selection criterium (beyond the enzyme selectivity). This would potentially permit better fidelity in the identification of particular cell types. Such an extra molecular unit on the probe will mostly be a known ligand for a particular cell-surface receptor so as to concentrate the probe in tissue rich in this cell type. But targeting ANOTHER enzyme activity as the second selection criterium is also an option (reference 3).

Work plan Initially, the newly arrived PhD student will be trained in his new field by reproducing already established well-working protocols of his host group. A period of about six months will thus be devoted to his training on known chemistries and on the use of our lab equipment. He will then

start his research activity by exploring the latest and most pertinent hypotheses of his host group. As part of an ongoing collaboration with our partner from the State Key Lab of Chemosensing at Hunan University, the student's progress will be co-supervised by Prof. Zhang Xiaobing, Deputy Director of the Key Lab, who is perfectly aware of the fundamental goals in this research field as well as the technological limitations that need to be overcome.

Qualities of the host institution The Ecole Normale Supérieure de Lyon (ENS de Lyon) is one of France's foremost research and teaching organizations. The ENSL recruits her own students via a stringent entry exam solely at the level of the 3rd university year. As such, the successful candidate is guaranteed a stimulating elite atmosphere of highly accomplished students around him. He will benefit from the particular attention placed by the ENSL administration on the hosting of international students, including training in French culture and language and regular meetings with the community of foreign students from all over the world. A significant community of Chinese researchers (including one faculty member, Pr. Wei Dong, theoretical chemist) and Chinese PhD students will ease his social integration.

▪ Publications of the laboratory in the field (max 5):

1. (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.
2. M. Prost, L. Canaple, J. Samarut, J. Hasserodt, *ChemBioChem* **2014**, 15, 1413–7.
3. M. Prost, J. Hasserodt, *Chem. Comm.* **2014**, 50, 14896-14899.
4. 4 patents

Joint Phd (cotutelle) : NO
▪ Co-directed PhD : YES

In case of a Co-directed or a Joint PhD, please detail :

- Partner University name: HUNAN UNIVERSITY
- Laboratory name and web site: Laboratory name and web site: State Key Laboratory of Chemo/Bio-sensing and chemometrics; <http://cbse.hnu.edu.cn/>
- PhD co-director (contact person):

Name: Xiaobing ZHANG

Position: Professor and Deputy-Director

E-mail: xbzhang@hnu.edu.cn

Phone number: 0086-731-88821894

- Provisional duration and timetable of the PhD student stays at the ENS de Lyon

Full initiation of the PhD student in his project as well as his successful integration in the ways of the host laboratory require his presence for the full first year at ENS Lyon. The student will send his annual report prepared for the Lyon Graduate School of Chemistry to Prof. ZHANG Xiaobing at Hunan University for review. At the end of his 1st PhD year, a visit of the PhD student and J.H. to Hunan University will be organized. The goal of this visit is to initiate specific sub-projects within the student's PhD project where synergies between the two partner laboratories can be found. This will be repeated at the end of the 2nd PhD year. A 3rd stay at the partner laboratory may be organized depending on the progress of the sub-projects that the student has made. Prof. ZHANG will be a member of the student's defense jury. The student's supervisor of his MSc. thesis will also be invited to join as a jury member.

- If previous collaborations with the Chinese codirector/university, please detail:

2003-04 (1-year postdoctoral fellowship to X. Z. by French Ministry of Research for collaboration with J.H. at ENS Lyon).

2007: 1 week visit by J.H. to Hunan University (and ECNU/Shanghai)

2009: Visiting professorship fellowship for 3 months awarded to X.Z. by ENS Lyon

2014: J.H. invited as plenary speaker to the 6th International Symposium on Bioanalysis, Biomedical Engineering and Nanotechnology, organized by Hunan University (May 29- June 2)

2014: Visiting professorship fellowship for 1 month (august) awarded to X.Z. by ENS Lyon

2015: J.H. and X.Z. participate in the PhD jury of Jinping WANG, Prosfer student, at ECNU in Shanghai (April 20, 2015). Subsequent visit of J.H. to State Key Lab Changsha + research lecture.

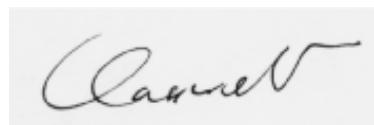
2016: J.H. invited as speaker to the 7th International Symposium on Bioanalysis, Biomedical Engineering and Nanotechnology, organized by Hunan University (May 27- 29).

- Interest of the Joint Phd for the French codirector, for his/her laboratory, for ENS de Lyon

This precious contact with a vibrant enterprise, that is the State Key Lab at Hunan University, has already given J.H. the occasion to present his research conducted at ENS Lyon to a high-profile international audience on two occasions, the ISBBN, that is organized with a more than significant investment by the Chinese authorities every other year (2014, 2016). Among others, the editor of one of world's most important chemistry journals (Peter Stang, JACS), as well as the director of the chemistry dpt of Stanford (Richard Zare), and many other chemist luminaries, essentially from the U.S., are invited speakers. The competencies of the Key lab are widespread and fairly complementary to the research activities at ENS. Mutual training of PhD students at both sites is an attractive perspective in order to perpetuate this privileged contact.

Date : Dec 14, 2017

Signature of the PhD director



Name and signature of the Laboratory director



Dr. Chantal ANDRAUD

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