Master Project internship

Project title	**High throughput generation of fluorescent bioactive compounds for the discovery of new specific cellular probes**
Laboratory	Biomolecular Screening Facility (http://bsf.epfl.ch/)
Head of Lab **Assistants**	Dr Gerardo Turcatti, MER Dr Luc Reymond and Dr. Fabien Kuttler
Contact	gerardo.turcatti@epfl.ch (021 693 96 66) luc.reymond@epfl.ch (021 693 71 54) fabien.kuttler@epfl.ch (021 693 16 28)
Starting date	2020

Background

Chemical collections are valuable for discovering active chemical entities but they can also be assayed for generating fluorescent probes bearing a variety of chemical scaffolds, upon chemical linkage with fluorophore. These fluorescent molecules may have numerous and useful applications, i.e. tools as reporters of cellular events and their dynamics, subcellular localizations identification and tracking, all of them with expected applicability in image-based assays and screens with automated fluorescence microscopy.

Aim

One foreseen approach is to identify new live cell probes based on silicon rhodamine (SiR) fluorophore. [1, 2]. The aim here is to develop and optimize a miniaturized labeling chemistry to prepare a large number of SiR-conjugates and apply them to live and fixed cells. A cellular fluorescence read-out of the localization of the SiR fluorescence will be then used to identify staining patterns that are pertinent and non-random.

For labeling the chemicals, we will subject compounds from the collection of bioactive molecules containing primary and secondary aliphatic amines to reaction with a NHS ester derivative of SiR, followed by an inactivation step of the NHS ester that leads to a non-cell permeable compound. The process will be automated and miniaturized using an acoustic dispenser for reaching submicroliter reaction volumes allowing a cost efficient high throughput labeling method.

Selected probes will be synthesized in mg scale and subjected to fluorescence microscopy characterization through end point and time-lapse fluorescence measurements for monitoring probes internalization, localization and time-scale of the cellular events detected. In addition, ‘hits’ or selected labeled probes from primary screens will be profiled for their toxicity using a label free screening method based on digital holographic microscopy [3, 4].

This chemical biology project is centered on the automation of fluorescent labeling of bioactive probes and development of image-based cellular screens for detecting specific subcellular labeling. It is at the interface of several disciplines: process automation, chemistry, screening, analytical chemistry, high content screening and machine-learning-based deep

image analysis. It would involve the participation of most of the members of BSF-ACCESS and may result in the discovery of new probes useful as read-outs in cell-based assays for drug discovery programs and chemical biology studies

References

1. Lukinavicius, G., Umezawa, K., Olivier, N., Honigmann, A., Yang, G., Plass, T., Mueller, V., Reymond, L., Corrêa, I., Luo, Z.-G., Schultz, C., Lemke, E. A., Heppenstall, P., Eggeling, C., Manley, S., and Johnsson K., (2013) A Near-Infrared Fluorophore for Live-Cell Super-Resolution Microscopy of Cellular Proteins. *Nature Chemistry 5,* 132-139.

2. Lukinavicius, G., Reymond, L., D'Este, E., Masharina, A., Gottfert, F., Ta, H., Guther, A., Fournier, M., Rizzo, S., Waldmann, H., Blaukopf, C., Sommer, C., Gerlich, D. W., Arndt, H.-D., Hell, S. W., and Johnsson, K. (2014) Fluorogenic probes for live-cell imaging of the cytoskeleton, *Nature methods 11*, 731-733.

3. Kühn, J., Shaffer, E., Mena, J., Breton, B., Parent, J., Rappaz, B., Chambon, M., Emery, Y., Magistretti, P., Depeursinge, C., and Turcatti, G. (2013) Label-Free Cytotoxicity Screening Assay by Digital Holographic Microscopy, *ASSAY and Drug Development Technologies 11*, 101-107.

4. Rappaz, B., Breton, B., Shaffer, E., and Turcatti, G. (2014) Digital Holographic Microscopy: A Quantitative Label-Free Microscopy Technique for Phenotypic Screening, *Comb Chem High T Scr 17*, 80-88.