MULTI-COLOUR FLIM FOR A SINGLE-CELL SYSTEMS BIOLOGY OF CELL FATE

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1. BACKGROUND

Cellular decisions—for instance, those inherent in cell fate determination (proliferation, differentiation, cell death), cell cycle regulation (commitment to the cell cycle, cell cycle checkpoints) or cell migration—are the outcome of the activity of complex biochemical networks processing information from the extracellular milieu and the intracellular compartments. Notably, most cellular decisions exhibit significant cell-to-cell variability caused by non-genetic determinants [1]: in the face of an identical stimulus, non-genetic heterogeneity can manifest itself as broad distributions in the timing at which individual cells respond to the stimulus (*e.g.*, apoptosis [2]) or as distinct choices of cell fates (*e.g.*, proliferation versus differentiation [3]). For instance, using live FRET microscopy, we have identified non-genetic heterogeneity in mitotic entry after genotoxic damage [4]. Therefore, data commonly used for systems biology approaches, often gathered by high-throughput methodologies such as genomics, transcriptomics and proteomics, need to be complemented by high quality biochemical data, possibly acquired at single cell resolution and capturing the dynamics of biochemical networks and phenotypes, in order to better resolve biochemical complexity at the cellular level.

2. MULTIPLEXED FLIM AND SYSTEMS MICROSCOPY

We have developed a number of technology platforms based on multi- or hyper- spectral FLIM and novel FRET pairs dedicated to biochemical multiplexing. With these techniques, we aim to perform perturbation analysis of biochemical networks in living cells and to correlate network topologies and their heterogeneity with cellular decisions. Here, we will describe the development and application of multi-colour FLIM for the simultaneous measurement of three FRET-based biosensors and, more specifically, for the description of cascades of biochemical events within the living cells, the study of cellular heterogeneity in biochemical networks and cellular decisions. The integration of these sensing platforms with Optogenetics [5] is now promising to enable the quantitative spatio-temporal control and read-out of biochemical reactions in the living cells, permitting a single-cell systems biology approach to the understanding of cellular decisions.

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