RED/FAR-RED OPTOGENETICS TOOLS FOR SYSTEMS MICROSCOPY

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Abstract: Over the last decade, traditional biochemical techniques have provided invaluable insights about the topology of biochemical networks, their dynamic behaviour and their correlation with cell fate [1]. However, state-of-the art biochemical tools – being applied to bulk measurements of proteins – cannot adequately quantify fast, asynchronous and heterogeneous biochemical signalling underlying cellular decisions [2]. Live cell fluorescence microscopy of individual cells expressing biosensors permits to overcome this limitation; however the number of sensors that can be read-out simultaneously is constrained by the broad emission spectra of fluorescent proteins. Recent developments in Optogenetics, now also permit us to manipulate biochemical activity with genetically encodable probes.

Here we describe the development of tools for a Systems Microscopy platform that allow multiplexing a number of FRET-based biosensors (see Figure 1). We have developed novel FRET pairs, including the most red-shifted FRET pair that allows the efficient use of the full visible spectra. Moreover, the red/far-red spectral band can be used for the active control of biochemical network nodes with Optogenetics tools. Using a plant Phytochrome-based system, we demonstrate the control of oncogene signalling with light.

The integration of tools for quantitative parallel biochemical imaging and light-induced perturbation is likely to impact how biochemical (signalling or metabolic) networks are understood. Our developments represent a significant step forward in this direction.

References