A robust genetically encoded sensor for quantitative intracellular calcium imaging

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An impressive number of genetically encoded probes that are based on GFP and GFP-like proteins have been developed. Such sensors can be used to detect concentrations of small molecules and molecular activities.
Ideally, the sensor readout is directly linked to the concentration of a compound or the activity of a protein of interest. However, most sensors rely on intensity changes of the fluorescent protein(s) which hinders quantification. Fluorescence intensity is influenced by many factors, including sensor concentration, cell thickness, microscope properties and settings (such as exposure time, illumination intensity and the transmission efficiency of the equipment).
Here we focus on the development of a new class of genetically encoded sensors that enables a robust quantitative read-out. This class of sensors is based on single circular permutated fluorescent proteins that exhibit a change in fluorescent lifetime. I will present the development and application of the first sensor, that shows a robust lifetime change depending on intracellular calcium concentrations. Moreover, I show that it is less sensitive to environmental factors than existing sensors.

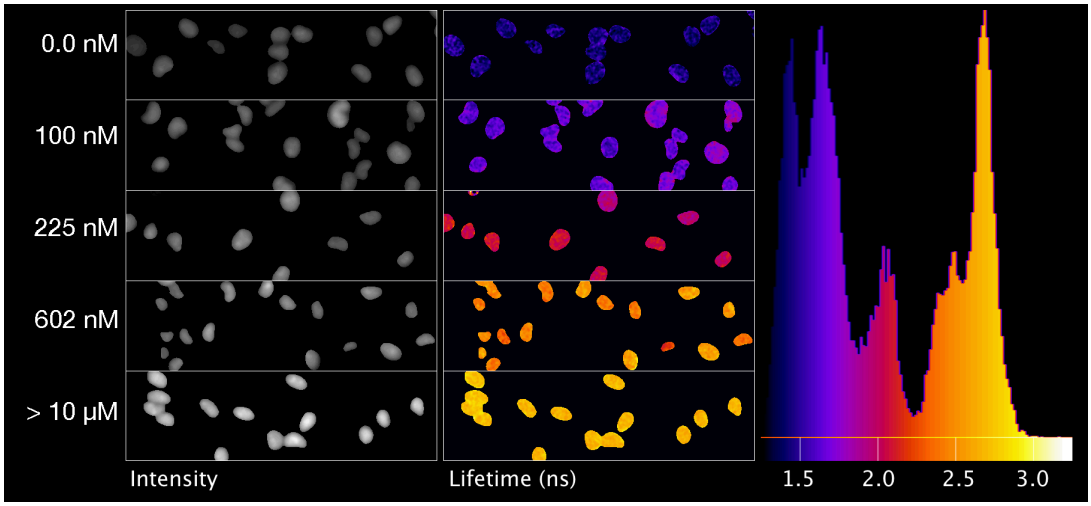

Figure 1: The sensor reports different lifetimes for a range of calcium in HeLa cells. The sensor is targeted to the nucleus of the cells.