Implementing *in vivo* FRET-FLIM in living plant roots reveals cell type-specific protein interactions

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Abstract:
Protein complex formation has been extensively studied using Förster Resonance Energy Transfer (FRET) measured by Fluorescence Lifetime Imaging Microscopy (FLIM). However, direct visualization of differential nuclear complexes controlling target gene expression has been challenging. Here we use *in vivo* FRET-FLIM to reveal spatial distribution of protein interactions in relation to cell fate specification at different developmental stages (Figure 1 and 2, [1]

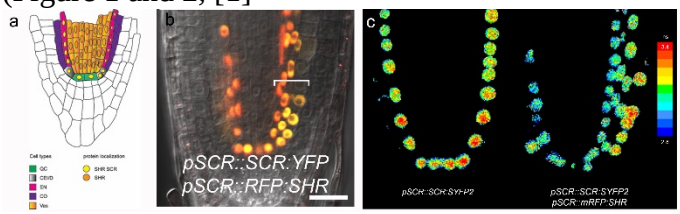

Figure 1: In vivo FRET-FLIM in living Arabidopsis roots, a, Schematic representation of Arabidopsis root. B, Confocal image of root coexpressing the nuclear stem regulators pSCR::SCR:SYFP2 and pSCR:mRFP::SHR. c Fluorescence lifetime heatmaps of roots expressing donor only (left panel). When expressed alone, the donor signal exhibited a long lifetime depicted by the reddish pseudo-color on the heatmap. Upon SHR:RFP co-expression (right panel) a "blue-shift indicates lifetime reduction

By optimizing the labeling conditions to detect FRET-FLIM in living plant tissues, we show that, three fully functional fluorescently tagged cell fate regulators establish specific and complementary cell type interactions at native expression levels. We provide new information on the dynamic redistribution of nuclear protein complex configurations in different developmental stages.

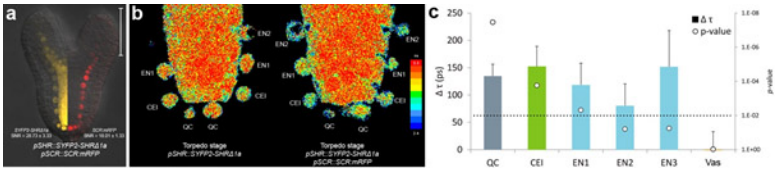

Figure 2*: In vivo* FRET-FLIM of SHR-SCR in embryos.a Arabidopsis embryo co-expressing donor (Yellow) and acceptor (red). b, Heatmaps of fluorescence lifetime in donor-only and sample embryo. (c) Quantification of lifetime change (Δτ) in single cells

In addition, we reveal that cell type-specific *in vivo* FRET-FLIM distributions reflect conformational changes of these complexes to differentially regulate target genes and specify distinct cell fates within living plant roots

References:

[1] Long, Y., Stahl, Y., Weidtkamp-Peters, S., Postma, M., Zhou, W., Goedhart, J., Gadella, TWG Jr, Simon, R., Scheres, B & Blilou, I. 2017. In vivo FRET-FLIM reveals cell type-specific protein interactions in Arabidopsis roots. Nature, 548: 97–102.