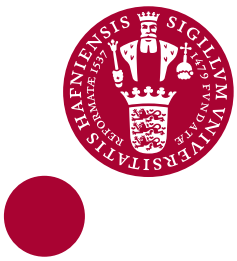

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Nanosensors for pH measurements in plants

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Introduction

Increasing focus on the role of pH signaling and proton flow in different plant physiological processes rises a demand for expansion of the repertoire of protein-based pH sensors with respect to pKa values and working ranges for *in vivo*, non-invasive pH measurements in different cellular compartments. We are working with the development and characterization of a new pH sensor for pH measurements in the apoplast. The sensor consists of a pH sensitive green fluorescent protein, GFP, fused to the less pH sensitive red fluorescent protein, mRFP1[1)]. This makes the sensor usefull for ratiometric measurements *in vivo*, which can be carried out without concern of sensor distribution and concentration.

Characterization of a new GFP based nanosensor for pH measurements in plants

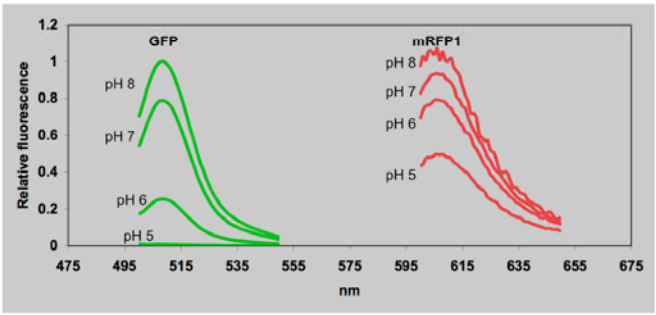

Figure 1
GFP fluorescence is more sensitive to high pH than mRFP1 fluorescence
Spectral properties of GFP (green) and mRFP1 (red) at different pH values. Low pH quenches GFP fluorescence to a much greater extend than mRFP1 fluorescence, making the latter a useful reference in ratiometric pH measurements.

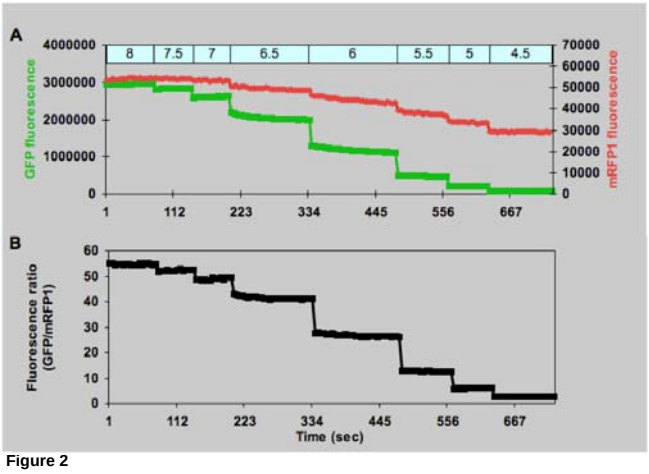

Figure 2
***In vitro* calibration of the sensor**
A buffered solution containing the GFP-mRFP1 sensor was stepwise acidified as indicated in the top bar by addition of small amounts of 1M HCl. Fluorescence was measured on a spectrofluorometer. **A**: Stepwise acidification of a buffered solution containing the GFP-mRFP1 pH-sensor show how the GFP (green; ex 488; em 510) and mRFP1 (red; ex 584; em 607) fluorescence changes differently in response to changes in pH. **B**: Fluorescence ratio (GFP/mRFP1) of the measurement in A.

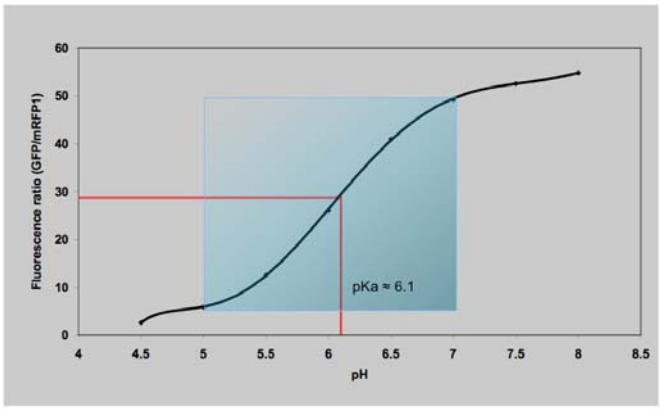

Figure 3
GFP-mRFP1 titration curve
Last datapoint for each pH value in figure 2b was used to generate a titration curve. A nonlinear fit to the datapoints was made and the pKa value determined as indicated. The blue square indicates the working pH range for the sensor.

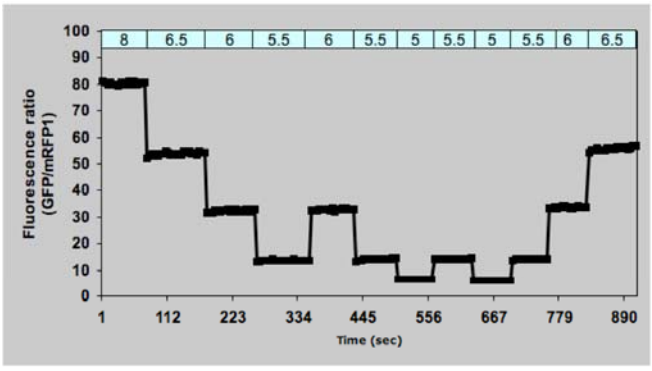

Figure 4
GFP-mRFP1 is stable for *in vitro* measurements within the working range of the sensor
The pH of a buffered solution containing the GFP-mRFP1 sensor was stepwise changed as indicated in the top bar by addition of small amounts of 1M HCl and NaOH. Fluorescence was measured on a spectrofluorometer.

Applications

This GFP-mRFP1 pH sensor is usefull within the pH range of 5 to 7 with a pKa value of approx. 6.1. With the plant apoplast beeing acidic relative to the cytosol this sensor is ideal for measurements of pH and proton fluxes in this extracellular compartment. With the appropriate promoter and/or targeting signal, a tissue or the entire plant body can be transformed to synthesize the sensor itself, avoiding any problematic loading procedures. Alternatively the protein sensor can be infiltrated into plant tissues. Using GFP variants originating from two different organisms (GFP: *A. victoria*; mRFP1: *Discosoma sp.*) should enhance expression by minimizing silencing.

References
1. Campbell, R. E. et al. A monomeric red fluorescent protein. PNAS 99, 7877-7882 (2002).