A novel recombinant pH probe for in vivo pH measurements in *Aspergillus niger*

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Accurate measurement of intracellular pH in unperturbed cells is fraught with difficulties. We have developed a recombinant pH-probe, RaVe_C, from a class of pH-sensitive GFP’s. RaVe_C has a ratiometric dual excitation and displays reversible emission ratio changes in the range from pH 5.5 to pH 7.8. With a pK\(_a\) of 7.0 RaVe_C is ideal for monitoring pH-changes in the cytosol. *In vivo* calibration using nigericin proved that the RaVe_C probe is highly pH-sensitive in living fungal hyphae, therefore RaVe_C can successfully be used as a non-invasive genetically encoded intracellular sensor. We have analysed the performance of this probe *in vivo* using confocal laser scanning microscopy and addressed the following questions: (1) Does the pH homeostat respond to changes in extracellular pH and pharmacological treatments? (2) Does the deletion of a vacuolar Ca-ATP-ase influence the pH homeostasis?