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OPTICAL NANOSENSOR PARTICLES FOR DETECTION OF pH IN LIVING CELLS

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The understanding of cellular metabolism is limited by the lack of tools for measuring of metabolite levels in living cells with high spatial and temporal resolution. It is therefore relevant to develop new and versatile methods for non-destructive metabolite imaging in order to describe dynamical biological processes in intact systems.

We have developed a new type of intensely fluorescence-labelled nanosensor particles for the sensitive and robust detection of pH. A pH sensing dye and a reference dye has been covalently linked to a porous, highly crosslinked polyacrylamide matrix by microemulsion copolymerization. The dual dye approach permits ratiometric measurements, which has the advantage that factors such as excitation source fluctuations and sensor concentration will not affect the measurement. The sensors are capable of reflecting pH in the physiologically relevant range from pH 5.8 to pH 7.2. The polyacrylamide NPs have defined diameters around 50 nm, are hydrophilic and can be stably dispersed in water.

Human keratinocytes (HaCat) and suspension cultured tobacco cells (BY2) are used as model systems. The nanosensor particles are microinjected into the living cells. Fluorescence responses are visualized by confocal laser scanning microscopy, and responses from the pH sensitive dye and the reference dye permit intracellular pH measurements by fluorescence ratio imaging microscopy.