Nanometer sized fluorescent gel particles for detection of metabolites in living cells

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Nanometer sized fluorescent gel particles for detection of metabolites in living cells.

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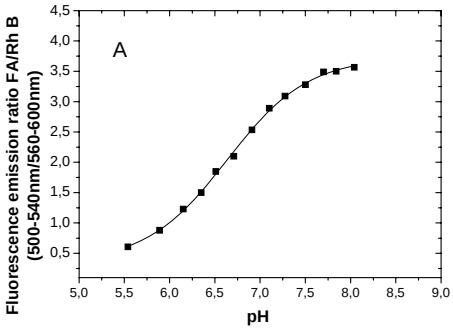
Particles with embedded fluorescent dyes have potential as nanosensors for the detection of spatially and time resolved measurements of metabolite concentrations in living cells. The quantification of the potential analyte can be based on either ratiometric detection of fluorescence from two dyes or on a reversible analyte concentration-dependent change in Fluorescence Resonance Energy Transfer (FRET) efficiency. For ratiometric detection the dyes are chosen such that the fluorescence of one dye is a function of an analyte concentration whereas the fluorescence of the other dye is independent of variations in the medium. For FRET based detection one molecule containing two fluorophores which have an analyte concentration dependent conformation is needed. International collaborators[1] have developed methods that allow metabolite binding proteins from various bacteria to be inserted between fluorescent proteins where it serves like a hinge (FLIP-proteins). This configuration makes the FRET signal dependent on the concentration of the targeted metabolite. As an example maltose sensitive FLIP-proteins with a range of sensitivities have been developed. The present contribution focuses on incorporation of such ratiometric and FRET based system in hydrogel particles.

In the Figure is shown the pH-response of hydrogel based (acryl amide) nanosensors particles with both a reference and reporter dye for pH covalently bonded to the interior of the particle. These particles function as ratiometric pH-sensors. The acrylamide gel particles were synthesized in water in oil (hexane) inverse micelles. Prepared nanoparticles were characterized with a variety of methods including dynamic light scattering, size exclusion chromatography and small angle X-ray scattering. The particle sizes obtained are in the 20-80 nm range.

The contribution will focus on the challenges involved in utilising inverse microemulsions for synthesis in as well as in utilising such particles as nanosensors.



A pH calibration curve of the pH sensing nanoparticles. Particles were suspended (1mg/mL) in 10mM phosphate buffer at different pH values. The fluoresceinamine (FA) and N-acrylamide-N'-rhodamine B thiourea (Rh B) dyes were effectively excited at 488nm and 543nm, respectively. The ratio between the fluorescence emission intensities of FA (500-540nm) and Rh B (560-600nm) was plotted against pH of the solution.

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