

OPTICAL NANOSENSOR PARTICLES FOR DETECTION OF METABOLITES IN LIVING CELLS

Anne Marie Scharff-Poulsen, Hong Gu, Kristoffer Almdal
Biosystems Department and Polymer Department
RISØ National Laboratory
Frederiksborgvej 399, P.O.Box 49, DK-4000 Roskilde, Denmark
E-mail: anne.marie.scharff@risoe.dk

KEY WORDS: Nanosensor, living cells, intracellular pH, protoplasts, fluorescence ratio imaging microscopy (FRIM)

BACKGROUND: 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.

Optical polymer-embedded nanosensors have recently been developed and inserted into mammalian cells [1]. These nanosensors consist of fluorescent reporter dyes embedded in a polymer matrix along with a reference dye, and the nanosensors have been used for ratiometric determination of a range of metabolites in mammalian cells. Such sensors have, however, not yet been used for *in vivo* imaging in plant cells.

RESULTS: A prototype ratiometric pH nanosensor, which contains a pH sensitive fluorescent dye as well as a pH insensitive reference dye, has been synthesized. The dyes are embedded in a polymer matrix, which consists of porous, highly crosslinked polyacrylamide. Microemulsion polymerisation is used for synthesis of particles with defined diameters in the range 20-30 nm.

BY2 tobacco protoplasts (plant cells without cell wall) are used as the first model system in order to facilitate the insertion of nanosensors. The nanosensor particles are inserted by gene gun bombardment into living protoplasts. 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.

PERSPECTIVES: The design of the particle matrix is essential for the performance of the dyes, and methods for synthesizing particles with different exterior and interior matrices will be explored. We are currently investigating methods for covalent bonding of sensor moieties to the polymer backbone in order to overcome problems of dyes leaching out of the particles.

The reporter molecules included in our nanosensors have so far been organic, fluorescent dyes, however, protein based sensing systems will also be explored. Sensors based on bacterial periplasmic binding proteins fused to two variants of green fluorescent protein (GFP) have been developed [2]. Technologies for embedding of these proteins in nanosensor particles are being developed with the aim of inserting multisensing nanosensors into living cells.

REFERENCES:

- [1] H.A. Clark, M. Hoyer, M.A. Philbert, and R. Kopelman, "Optical Nanosensors for Chemical Analysis inside Single Living Cells" *Anal. Chem.*, **71**, 4831-4836 (1999)
- [2] M. Fehr, W.B. Frommer, and S. Lalonde, "Visualization of Maltose Uptake in Living Yeast Cells by Fluorescent Nanosensors" *PNAS*, **99**, 9846-9851 (2002)