Title: **Evaluation of an Intracellular Contrast Agent Targeting Beta-Galactosidase for Cellular Labeling**

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Molecular imaging of cells or cellular processes can be obtained by targeting e.g. specific enzymes, receptors or mRNA. Aiming to track specific enzymes present in the cytosol an intracellular MR contrast agents (CA) targeting the enzyme b-galactosidase (b-gal) was synthesized as a model system. The conjugate was composed of four domains: a gadolinium loaded ligand 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra-acetate (DOTA), fluorescent dye FITC for optical imaging, an enzymatically cleavable “sensor unit” based on the enzyme targeting sugar moiety (*b*-D-galactopyranose), and a cell penetrating peptide (d-Tat_{57-49}) as delivery vector. In the presented approach the MR detectable part should be selectively accumulated in the target cells containing *b*-gal, due to the enzymatic cleavage of internalization vector (CPP) from the enzyme targeting sugar moiety. Cellular uptake of CA was confirmed by the fluorescence microscopy and spectroscopy in C6 glioma cells as well as in the transgenic C6/LacZ7 cell line containing the targeted *b*-gal. Fluorescence studies have shown that the obtained CA was efficiently internalized into both cell lines in a concentration dependent manner from 5µM to 30µM without inducing significant toxicity. However, no selective accumulation of CA was observed in the *b*-gal expressing cells. The vesicular localization of CA around the nucleus indicates to a predominantly endosomal uptake mechanism without any detectable release into the cytosol (Fig.1). Thus, this endosomal entrapment of CA might explain, that there is no selective accumulation of CA in targeted cells, because of the inability of the contrast agent inside the vesicles to interact with the targeted enzyme located in the cytosol. Nonetheless, the synthesized CA showed an excellent ability for intracellular delivery and might prove to be useful in the cellular labeling and tracking studies.

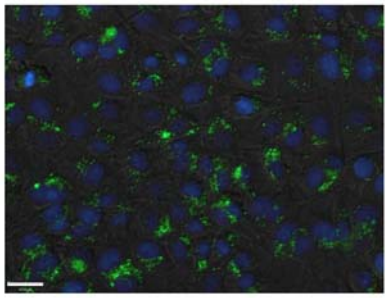

Fig. 1: Microscopic image of C6/LacZ7 cells after incubation with 30 µM of the β-gal targeted CA.
Overlay of phase contrast, fluorescence of nuclear stain H33342 (blue) and fluorescence of fluorescein in CA (green). Bar represents 20 µm.