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Bimodal intracellular contrast agent based on the sugar moiety targeting beta-galactosidase

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Molecular-genetic imaging is a rapidly growing field aimed at providing vital information about the distribution and level of gene expression in the living specimens over time. The common approaches to evaluate the efficiency of genetic manipulation *in vivo* usually involve the visualization of products formed by the expression of a reporter gene introduced together with the therapeutic gene of interest. Such reporter genes encode proteins, cell surface receptors or enzymes, which can be detected directly (fluorescent proteins (e.g. GFP)) or *via* interactions with compatible imaging probes. Although MRI has proved its excellent diagnostic capability as a routinely used tool in clinical practice, still a diminutive progress has been made in development of MR reporter genes and corresponding contrast agents [1]. Thus, highly specific and sensitive probes are required in order to achieve noninvasive imaging of gene expression in *vivo* by the means of MR imaging. The intracellular localization of many molecular targets inspired us to design a contrast agent which should accumulate inside the targeted cell by a specific interaction with a cytoplasmic constituent. Our model probe is based on a sugar moiety serving as enzymatically cleavable linker between transport part and MR probe targeting the intracellular enzyme beta-galactosidase. This enzyme is expressed by bacterial *LacZ* gene, a frequently used reporter gene. Bimodal contrast agents containing gadolinium chelator DOTA and fluorescent part (FITC) for combined MR/optical imaging were synthesized. The cellular uptake and subcellular localization of the obtained probe were evaluated by fluorescence spectroscopy and microscopy in a transgenic rat glioma cell line C6/LacZ (containing target: β-galactosidase) and compared with the parent C6 cells (without target). The cell studies showed that synthesized CA could enter efficiently into the cells in a concentration dependent manner from 5 to 20 µM without inducing significant toxicity. Concentration and time dependency of uptake were investigated. The ability of synthesized CA agents to increase intracellular relaxation rates of water protons was confirmed by *in vitro* MR cell studies. MR images of labeled cells showed significant contrast enhancement as compared to control cells.

[1] A. A. Gilad, P. T. Winnard Jr, P.C. M. van Zijl, J. W. M. Bulte, *NMR Biomed.,* **2007**; 20: 275–290.