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## Design, Synthesis and Relaxivity Behaviour of mRNA Targeting Contrast Agents

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**Introduction:** Magnetic Resonance Imaging (MRI) is one of the most important diagnostic tools available in medicine. The specificity and sensitivity of MRI can be further enhanced by the introduction of contrast agents (CAs). As many clinically valuable targets reside inside the cell membrane, the development of efficient intracellular targeted MR contrast agent is required. Potential intracellular targets would be DNA, mRNA or protein/enzymes. The prerequisite for the intracellular targeting is not only the efficient delivery of probes inside the cell but also the colocalization with the target.
The objective of the present study is to design and synthesize efficient intracellular MR contrast agents [1] which include three functional domains (i) Gd based MR reporter part (ii) antisense PNA to target mRNA (iii) cell penetrating peptide (CPP) or cholesterol as the delivery agent. The antisense PNA can hybridize uniquely to the complementary mRNA and provides cell specific targeting for cells containing the mRNA. Thus, the expression of the corresponding gene can be visualized non invasively by in vivo MR imaging.
**Methods:** The conjugates were synthesized by continuous solid phase synthesis. Cellular uptake was confirmed by fluorescence microscopy, spectroscopy and MRI of labeled cells.
**Results:** CPP conjugated mRNA targeting CAs were taken up efficiently into cells by an exclusively endosomal mechanism. A subtoxic labeling concentration at low micromolar range was sufficient to enhance significant MR imaging contrast. Cell free binding assays proved a specific interaction with a synthetic target. However, because of the vesicular entrapment, it can be expected that there would be a lack of specific interaction between CA and mRNA located in the cytosol. In order to overcome this problem, cholesterol conjugated CAs are designed and synthesized [2]. The idea behind cholesterol coupling was from the already published report [3] where covalent conjugates of cholesterol and siRNAs were facilitating cellular import and were able to silence protein expression effectively. Initial results have shown that these agents were delivered more efficiently than CPP conjugated CAs. Unfortunately, they were also entrapped in vesicles. Both types of CA were able to enhance contrast in labeled target containing as well as non-targeted parent cells. However, there was not efficient colocalization and specific interaction of CA and target mRNA achievable to be exploited for MR imaging purposes.
**Conclusion:** CPP or lipid coupled CAs are internalized efficiently into cells. However, vesicular entrapment prevented sufficient specific interaction between CA and mRNA. Further modifications are required to achieve the release from endosomes or a direct uptake into the cytosol.
**References:**
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[3] C. Wolfrum et al., Nature biotechnology, 25(2007) 1149