# Enzyme mediated MRI Probes: Design, Synthesis and Relaxivity behaviour of a beta-galactosidase reporter

**E. Gianolio[1], J. Bhagavath Singh[1], M. Aswendt[2], F. Arena[1], A. Barge[3], M. Hoehn[2], and S. Aime[1]**
[1]Chemistry IFM and Molecular Imaging Center, University of Torino, Torino, TO, Italy, [2]In-vivo-NMR Laboratory, Max Planck Institute for Neurological Research, Cologne, Germany, [3]Department of Drug Science and Technology, University of Torino, Torino, Italy

## Introduction

The efficiency in accumulating imaging reporters at the targeting sites is the major task in MRI for the visualization of biological processes at the cellular and molecular level.[1] One way to tackle this amplification route is to seek for the formation of self-assembled aggregates of Gd-chelates.[2] Bogdanov et al showed that melanin-like polymers can form when hydroxo-functionalized Gd-chelates are in the presence of the suitable enzymes (e.g. tyrosinase or myeloperoxidase).[3] Our goal is to exploit this approach in order to set up a MRI method to assess the expression of $\beta$-galactosidase ($\beta$-gal).

## Methods

Relaxometric methods have been used for the in vitro physico-chemical characterization of the Gd-based probe either through the measure of NMRD (Nuclear Magnetic Relaxation Dispersion) profiles over a frequency range from 0.01 to 70 MHz (Proton Larmor Frequency) and at a fixed frequency value of 20 MHz on Stelar Relaxometers (Mede – Italy). The efficiency of responsiveness to β-gal and tyrosinase enzymes has been assessed through relaxometric and spectrophotometric measures either in buffered water solutions and in cellular systems (B16F10 – murine melanoma cell line). MR Images of capillaries containing the cell pellets, were recorded on a Bruker Avance300 spectrometer operating at 7.1T equipped with a microimaging probe and on an Aspect spectrometer operating at 1T using a standard $T_1$ weighted multislice multiecho sequence.

## Results

A Gd-DOTA based probe containing a tyrosine –OH functionality protected by a galactose moiety (Gd-DOTAgal) has been synthesized and relaxometrically characterized. Upon cleavage of the galactose moiety (step activated by the presence of β-galactosidase) the tyrosine group becomes available for the tyrosinase activated melanin-like polymerization. It is well established that the relaxivity of Gd-complexes increases, in the field range 0.5-1.5T, if they are part of macromolecular systems as a consequence of the lengthening of their reorientational correlation time ($\tau_R$). We exploited this concept to prove that the tyrosinase enzyme acts on the Gd-DOTAgal complex, previously activated by β-galactosidase cleavage, fostering the formation of a melanin-like high relaxivity aggregate. The in vitro relaxivity of the target molecules with and without sugar has been investigated by $^1$H NMRD in the presence and the absence of enzymes. The enzymatic responsiveness has been assessed in vitro either in β-gal/tyrosinase containing water solutions and in B16F10 murine melanoma cell lysates as this cell line has been reported to possess high tyrosinase activity related to its natural melanin pigmentation. Finally, intracellular behaviour of the Gd-DOTAgal system has been followed by pursuing its internalization (by pynocitosis and electroporation) in B16F10 cells .

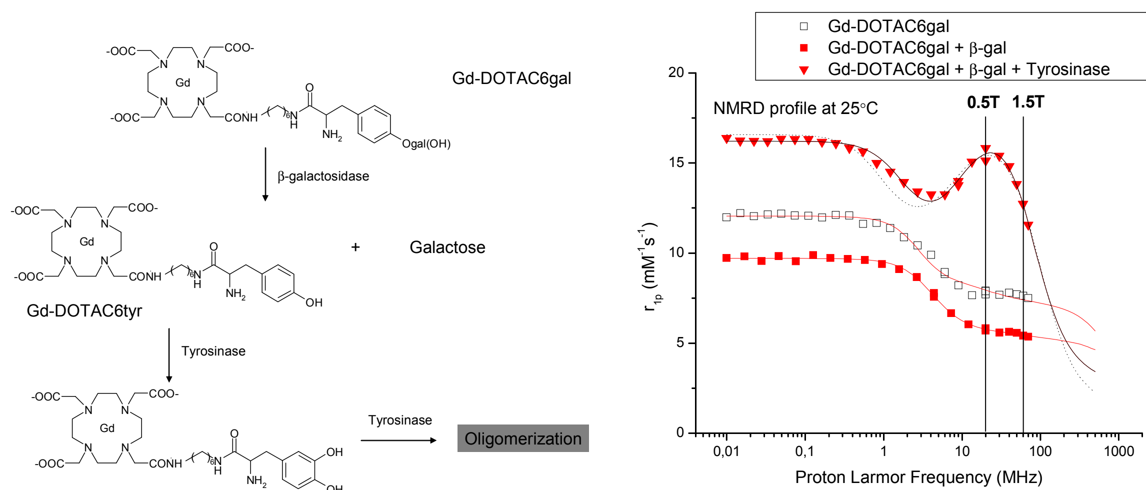


*Fig. 1. Steps of the $\beta$-gal/Tyr activated polymerization and 1/$T_1$ NMRD profiles of the different involved species in the presence and in the absence of tyrosinase and $\beta$-galactosidase enzymes registered at 25°C and neutral pH.*

## Conclusions

The Gd-DOTAgal derivative has been proven to be a good $\beta$-galactosidase expression reporter that results in a system that can undergo polymerization in the presence of Tyrosinase upon cleavage of the tyr-gal bond.

## References

[1] S.Aime, C.Cabella, S.Colombatto, S.G.Crich, E.Gianolio, F.Maggioni, *J.Magn.Reson. Imaging*, **16** (2002) 394-406
[2] S.Aime, M.Botta, M.Fasano, E.Terrano, *Chem.Soc.Rev*, **27** (1998) 19-29.
[3] M.Querol, W.C.John, A.A.Bogdanov, *Org.Biomol.Chem*, **4** (2006) 1887-1895.