

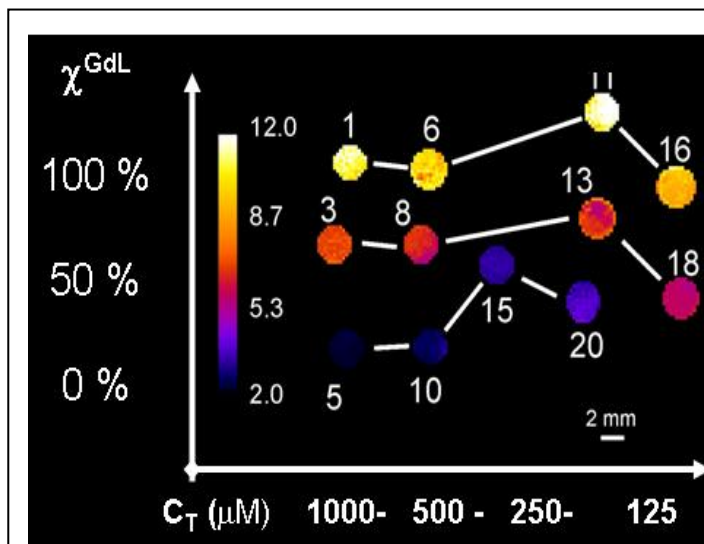
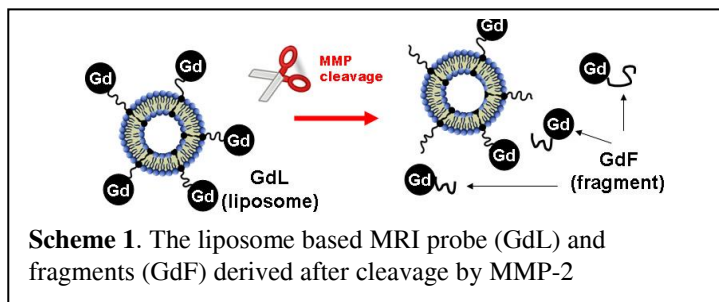
## A $R_{2p}/R_{1p}$ ratiometric approach with Gd containing liposomes for the imaging of Matrix Metalloproteinases by MRI

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The approach to molecular imaging of enzymes (including MMPs) by MRI typically relies upon imaging probes composed of an enzyme-cleavable moiety conjugated with a paramagnetic imaging reporter, such as a Gd(III) chelate.<sup>[1]</sup> Upon enzymatic processing, the probe is transformed into a fragment with an altered relaxivity, leading to a different capability to enhance contrast in MR images with respect to the parent species. However,

exact knowledge of the total concentration of Gd ( $C_T$ ) is essential to translate gadolinium contrast enhancement into the molar ratio of unprocessed *vs* processed forms, hence into *in vivo* enzyme activity maps. A viable solution to the concentration problem can be provided by the  $R_{2p}/R_{1p}$  ratiometric approach, which is based on the measurement of the ratio between the transverse and longitudinal paramagnetic contributions to the water proton relaxation rate, *i.e.*  $R_{2p}$  and  $R_{1p}$  (with  $R_i=1/T_i$ ,  $i=1,2$ ).<sup>[2]</sup> We have developed a new system for the ratiometric assessment of the activity of Matrix Metalloproteinases (MMPs), based on a MMP-2 cleavable peptide sequence conjugated with a Gd-DOTA chelate at the N-terminus and with an alkyl chain at the C-terminus. This amphiphilic probe has been inserted into the membrane of a stealth liposome, to obtain the paramagnetic probe GdL (Scheme 1). This probe can be cleaved by MMP-2 to release GdF, the free peptide fragment bearing the Gd-chelate. At a magnetic field strength of 7T, the transverse millimolar relaxivities of GdL and GdF are 15.6 and 5.6  $\text{mM}^{-1}\text{s}^{-1}$  respectively, while longitudinal relaxivity are very similar (5.9 and 5.6  $\text{mM}^{-1}\text{s}^{-1}$ ), making the GdL/GdF couple a sensitive ratiometric couple. Parametric  $R_{2p}/R_{1p}$  maps measured *in vitro* under a MRI setting showed image contrast which was responsive to the GdL-to-GdF ratio, but independent from total gadolinium concentration, and with a detection limit in the 50-120  $\mu\text{M}$  range. The proposed liposome-based MRI reporter is well amenable to combine imaging and therapy in an all-in-one system, eventually providing a theranostic approach to MMP-related pathologies, including cancer.



**Figure 1.**  $R_{2p}/R_{1p}$  parametric image of a 1% agar phantom of mixtures of GdL and GdF at four different total Gd concentration. Samples with the same  $C_T$  are connected with a line.

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[2] S. Aime et al. *J. Am. Chem. Soc.* **2006**, *128*, 11326–11327.