DETECTION OF MATRIX METALLOPROTEINASES USING AN "ON/OFF" ^{19}F MR PROBE

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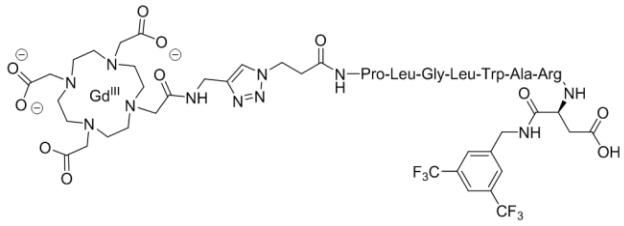


Figure 1: Chemical structure of MMP sensor: Chelated gadolinium is linked via a peptide sequence to a fluorinated amino acid; the linker is cleaved upon contact with specific MMP. Here, the peptide sequence is a generic substrate for MMPs.

Target audience – Researchers interested in MR chemical sensors, particularly in monitoring enzyme activity and other molecular targets.

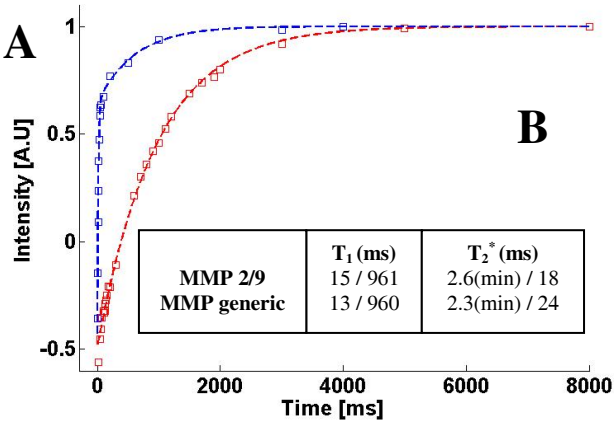
Purpose – Matrix Metalloproteinases (MMPs) are a group of endopeptidases which play key roles in the re-modelling of the extra cellular matrix in a range of disease pathologies. In particular, MMPs have been shown to be up-regulated in certain neurodegenerative diseases [1], although there is currently no non-invasive method for which to measure this. Our goal is to develop a fluorinated MRI sensor which can detect MMPs in solution (non-invasively) and develop this towards an *in vivo* application.

Methods – A novel ^{19}F MR sensor has been developed which links a gadolinium chelate to a fluorinated amino acid via an MMP specific substrate; to date we have developed a generic MMP and an MMP 2 and 9 (2/9) substrate sensor. In the uncleaved state, the paramagnetic influence of the gadolinium will cause short relaxation times and a broad fluorine signal. If the linker is cleaved by the specific MMP, then the distance between the gadolinium is increased, causing a change in relaxation time; this "on/off" principle was first demonstrated for fluorine MR sensors by Mizukami et al [2]. Two samples were prepared with 600 µL of 100 µM of each MMP sensor and 5 µL of MMP 9 (10 mg / ml) was then added. Following MMP addition, a Bruker 600 MHz spectrometer was used to measure a 2-D time course in which a single scan (TR = 5 s, NS = 32, Sweep width = 50 kHz, Acq. time ~ 2 m 30 s) was repeated for a period of around 13 hours. A further inversion recovery experiment was performed for both sensors, before and after MMP addition, to investigate relaxation properties.

Results - A clear change is present in the fluorine signal intensity after MMP addition; a 3.5-fold increase is found, as show in Fig. 3. The T_1 relaxation time (and T_2^* time) also increase after the enzyme is added (see Fig. 2); T_1 values change by around a factor of 60, with a ten-fold increase in T_2^*. Figure 3 B is a 3-D plot demonstrating the change in spectral shape over time; the effect upon the fluorine signal intensity following enzymatic cleavage was modelled using Michelis-Menton type equations. Sensor specificity was also verified using mass spectroscopy for a sample combination of the MMP 2/9 with MMP 12; no cleavage products were found.

Discussion – Using a synthesised ^{19}F sensor, we have demonstrated an observable contrast mechanism following MMP detection. Since a signal is observed with spectroscopy, a higher sensor concentration (~ mM) will be detectable with MRI on an imaging platform. Similar T_1 /T_2^* relaxation times are found for both MMP sensors, implying that other enzyme substrates would exhibit a similar relaxation difference. Our findings are in agreement with a recent paper by Yue et al. which showed a similar order of magnitude change in T_1 relaxation [3].

Conclusion – We have presented preliminary data for two sensors with different peptide specific sequences; both sensors show changes in the MR signal after contact with MMPs. Monitoring of MMP levels could give an important biomarker of the first stages of disease progression in neurodegenerative diseases and future work will aim to apply this "on/off" mechanism *in vivo* to an animal disease model which over expresses MMPs.



	T_1 (ms)	T_2^* (ms)
MMP 2/9	15 / 961	2.6(min) / 18
MMP generic	13 / 960	2.3(min) / 24

Figure 2: Relaxation differences between pre and post cleavage states for sensors: (A) T_1 inversion recovery experiment showing data points for pre (blue) and post (red) cleavage after MMP 9 addition for the MMP 2/9 sensor (fitted curve is used to find T_1 value.); **(B)** Table showing relaxation properties for the MMP 2/9 sensor ; data is of the pre MMP addition/ post MMP addition form.

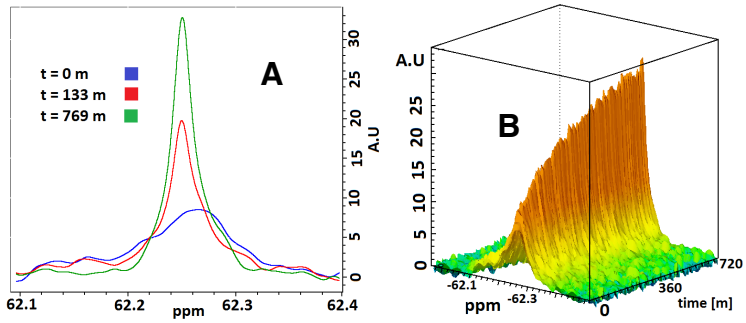


Figure 3: Signal intensity time course for generic MMP sensor after MMP 9 addition: (A) 1-D fluorine spectrum showing the narrowing and increase of the peak intensity over time; **(B)** 3-D intensity plot where the exponential nature of the intensity increase, due to the enzymatic cleavage, is visible.

References 1. McClain J, Phillips L, Fillmore H. Neurosci Lett, 2009;460(1):27-31 **2.** Mizukami S, Takikawa R, Fuminori S, et al. *J. Am. Chem. Soc.,* 2008;130 (3):794–795 **3.** Yue X , Wang Z, Zhu L, et al., *Mol. Pharmaceutics*, 2014;11 (11): 4208-4217