

## ParaCEST: imaging pH with high $\mu_{\text{eff}}$ Ln(III) complexes on a 1T permanent magnet.

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### Purpose:

Chemical Exchange Saturation Transfer<sup>1</sup> (CEST) contrast agents have attracted interest in recent years because of their unique “frequency encoding” properties. So far, the potential of CEST agents has been investigated mainly at high magnetic fields (> 3T). The aim of this work is to seek for CEST agents and procedures for applications at lower field strengths. To generate CEST contrast, the agent has to own mobile proton pools in slow/intermediate exchange, on the NMR time scale, with bulk water ( $\Delta\omega > k_{\text{ex}}$ ). Paramagnetic molecules (PARACEST) characterized by large  $\Delta\omega$  values appear the most suitable systems for low field applications. The PARACEST complexes investigated in this work are very stable, neutral chelates of different lanthanide ions ( $\text{Ln}^{\text{III}}$ -HPDO3A, figure 1)<sup>2</sup>, screened for the saturation transfer (ST) ability of their -OH ligand protons at the  $B_0$  value of 1T. Moreover, their pH and temperature responsiveness *in vitro* or *in vivo* has been assessed.

### Methods:

Measurements were carried out on the Icon scanner (Bruker) equipped with a 1T permanent magnet (Aspect).

The investigated molecules are  $\text{Dy}^{\text{III}}$ -,  $\text{Yb}^{\text{III}}$ - and  $\text{Tm}^{\text{III}}$ -HPDO3A. The Z-spectra are reported in Fig.2. ST effects were calculated as the ratio between “on” and “off” resonance saturation,

$\text{ST}\% = 100 \cdot (I_{\text{off}} - I_{\text{on}}) / I_{\text{off}}$ . The ratiometric curves were obtained according to the procedure reported in ref.3 using a

phantom of 20mM concentration solutions at different pH values. The RARE sequence parameters were as follows: Presaturation length (bp): 1500 ms, RF power: 24  $\mu\text{T}$ , RARE factor: 32, TR: 7000 ms (5000 ms *in vivo*), TE: 6ms, Centric encoding, Matrix size: 32x32, Isotropic FOV: 55mm, Slice thickness: 3 mm, NA: 4. *In vivo* pH measurements were performed in the bladder and kidneys of two Lewis rats with an injected dose of 1.6 mmol/kg ( $\text{Tm}^{\text{III}}$ -HPDO3A). The calibration curve was considered in its interval of linearity between pH 6.58 and 8.06 ( $y = 2,4113x - 14,372$ ).

### Results:

All the investigated complexes showed the presence of at least one mobile proton pool possessing a chemical shift separation from the bulk water ( $\Delta\omega$ ) larger than the exchange rate between the two peaks. Among the three candidates,  $\text{Tm}^{\text{III}}$ -HPDO3A showed two distinguishable peaks in the Z spectrum at 1T (figure 2), and it has been selected for further studies as pH and temperature responsive probe. The temperature is easily assessed by the chemical shift separation of OH protons (from the Z-spectrum). The pH was determined by setting up a ratiometric procedure based on the comparison of the ST% measured for the two OH resonances whose exchange rate is differently catalyzed by the solution pH (figure 3). For the *in vivo* measurements the ratiometric curve obtained at 34°C was used. Results of pH in the bladder were 7.30 (rat 1, collected urine: 7.19) (figure 4) and 6.66 (rat 2, collected urine: 6.21), respectively. Results in the kidney yielded pH 6.70 (rat 1) and pH 6.56 (rat 2), respectively.

### Discussion:

This work demonstrates that contrast agent mediated CEST analyses can be performed at field strengths of 1T. Among PARACEST,  $\text{Tm}$ -HPDO3A appears a very interesting candidate as it displays two highly shifted mobile proton pools that allow its use as temperature and pH responsive agent. The access to the herein reported ratiometric approach makes the pH measurement independent from the knowledge of the local concentration of the agent.

### Conclusion:

The principle of ratiometric analysis in CEST experiments is well established nowadays, and could meet some clinical needs for non-invasive imaging of tissue physiopathology variations (pH, T, enzymatic activity...). The use of cheap, safe and versatile low field instrumentation will further expand the scope of CEST techniques.

### References:

1. Ward KM, Aletras AH, Balaban RS. *J. Magn. Reson.*, **1999**, 143, 79–87.
2. Aime S, Barge A, Delli Castelli D, Fedeli F, Mortillaro A, Nielsen FU, Terreno E. *Magn. Reson. Med.*, **2002**, 47(4), 639-48.
3. Delli Castelli D, Terreno E, Aime S. *Angew. Chemie Int.Ed.*, **2011**, 50, 1798-1800.

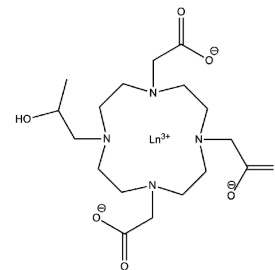


Figure 1: A generic lanthanide HPDO3A chelate.

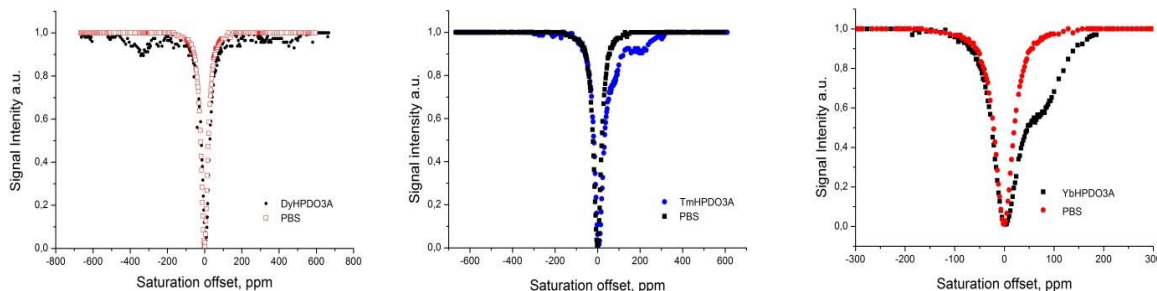


Figure 2: Z spectra of three  $\text{Ln}^{\text{III}}$ -HPDO3A compounds acquired at 1T.

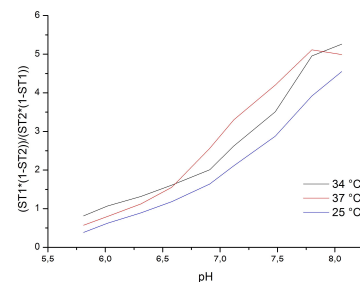


Figure 3: ratiometric curves of  $\text{Tm}$ -HPDO3A containing solutions at different temperatures.

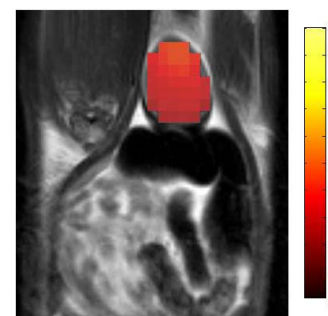


Figure 4: pH mapping inside a rat's bladder.