# A NOVEL GENERATION OF IMPROVED LIPOCEST MRI AGENTS WITH HIGHLY SHIFTED INTRALIPOSOMAL WATER PROTONS

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*Purpose*

To optimise the properties of the highly-sensitive paramagnetic MRI-CEST agents based on the use of liposomes (LIPOCEST).

*Introduction*

The advent of the Molecular Imaging era prompts the search for innovative imaging probes in order to set up novel procedures for pursuing early diagnosis and efficient follow-up of therapeutic treatments. Among MRI agents, those dubbed CEST (from Chemical Exchange Saturation Transfer) have the unique property of yielding a "frequency-encoded" contrast that may allow, analogously to what usually done with optical imaging probes, the visualisation of different agents in the same region.[1,2] Within the class of CEST agents, LIPOCESTs display the highest sensitivity (sub-nanomolar scale) owing to the extremely high number of mobile intraliposomal water protons, properly shifted by the presence of an encapsulated paramagnetic Ln(III)-based shift reagent (SR), that can be selectively saturated.[3]

*Results and Discussion*

In addition to the sensitivity, a very important characteristic of CEST agents is the range of the resonance frequency values of their mobile protons. For the first generation of LIPOCESTs, this interval is rather small (from –4 to 4 ppm with respect to the resonance of bulk water depending on the magnetic anisotropy of the SR) being limited by the concentration of the encapsulated SR that is mainly controlled by osmotic effects.

To enhance the chemical shift of the intraliposomal water protons, several routes have been pursued, including:

- the encapsulation of neutral multimeric SRs for increasing the maximum amount of encapsulated SR
- the exploitation of the chemical shift contribution arising from bulk magnetic susceptibility effect by inducing an osmotic shrinkage of the liposomes (Figure 1). This effect is proportional to the paramagnetism of the encapsulated Ln(III) ion and it depends on the shape and orientation of the liposomes with respect to the external magnetic field.
- the incorporation of amphiphilic SRs in the liposome membrane in order to: i) increase ( at least for the SR units pointing inwards) the concentration of the SR in the intraliposomal cavity, and ii) to influence the orientation of the shrinked liposomes with respect to the field.

It will be shown that the combination of these strategies may enhance the window of the accessible saturation frequencies of LIPOCESTs of almost one order of magnitude (Figure 2), thus making possible, for the first time, the MRI visualisation of different LIPOCEST probes in the same region of interest.

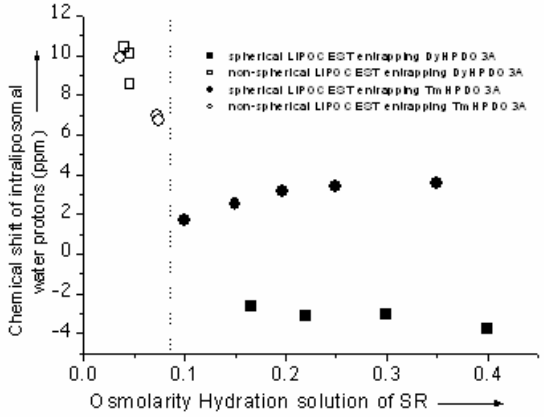


**Figure 1**: Chemical shift (298K) of intralinosomal water protons (referred to the bulk water) as a function of the osmolarity of the SR solution used for hydrating the thin lipidic film (lipidic film composition DPPC/DSPE-PEG 95:5 molar ratio, total lipid amount 20 mg).

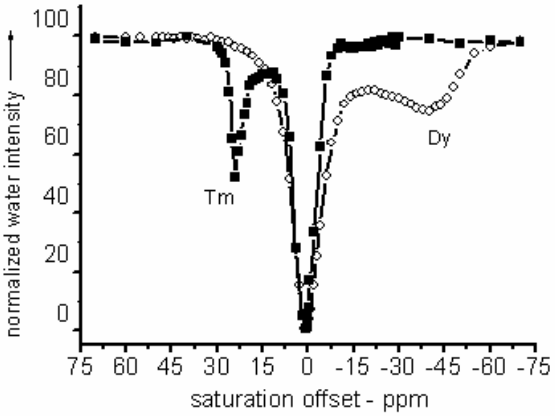


**Figure 2**: Z-spectra (7 T, 312 K, irradiation: single 2 s long block pulse, intensity 6 μT) of two osmotically shrinked LIPOCESTs encapsulating an hydrophylic SR (Tm-hpdo3a - filled square, or Dy-hpdo3a - open circle) and incorporating an amphiphilic SR, (Tm- or Dy-based, respectively) in the liposome membrane (lipidic film composition DPPC/DSPE-PEG/SR 75:5:20 molar ratio, total lipid amount 20 mg).

*References*

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