# Frequency-Encoded MRI-CEST Agents Based on Paramagnetic Liposomes/RBC Aggregates

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**Purpose:** CEST-MRI agents have been under intense scrutiny for applications in molecular imaging and in MRI guided drug delivery. They allow to generate a frequency-encoded contrast so giving the opportunity to be used for Multicolor Imaging and as responsive agents. The intrinsic low sensitivity has prompted researchers to develop new systems in which the number of equivalent mobile protons was high, so increasing the detection threshold (*e.g.* nanosystems, lipoCEST) [(1,2)]. In particular, recently it has been reported that RBCs loaded with paramagnetic shift reagent (e.g. Dy-HPDO3A) can act efficiently as CEST probe with a sensitivity in the fM–pM range[(3)]. It is shown that erythroCEST agents can be obtained by inducing the BSM effect from the external side of the RBCs; this task has been addressed by anchoring cationic liposomes loaded with paramagnetic complexes to the negatively charged molecules present on the outer side of the RBCs membrane (*Fig.1, left*).

**Methods:** Cationic paramagnetic liposomes were prepared by following the lipidic thin film hydration method (DPPC/ DOTAP/ cholesterol, 75/15/10 molar ratio) and hydrated at 55°C with LnHPDO3A (where Ln= Dy or Eu) solutions. They were mixed with RBCs in PBS buffer for 10 min at 4°C to obtain Liposomes/RBCs aggregates and used for MR images acquisition. "True" ErythroCEST have been prepared *via* the cellular internalization of Dy-HPDO3A by applying the hypotonic swelling procedure as previously reported [(3)]. MR images of the samples were recorded at 7T on a Bruker Avance 300 Spectrometer equipped with microimaging probe. Z-spectra were acquired by using a RARE spin echo sequence (TE=3ms, TR=5s, 63x64 acquisition matrix, FOV of 3 cm and slice thickness of 1mm). The sequence was preceded by a saturation scheme consisting in a continuous rectangular wave pulse 2s long with a RF pulse of 3μT. For *in vivo* experiments, eight weeks female Balb/C mice were subcutaneously inoculated with $5x10^5$ TSA murine breast cancer cells. RBCs taken from mice were incubated with Dy-liposomes to obtain Dy-liposomes/RBCs aggregates that were i.v. injected in the mouse tail vein. MR images of the tumour region were acquired before and after the administration of labelled RBCs.

**Results:** Fig.1 reports the Z-spectra of RBCs/Dy-liposomes aggregates (*red*) and of control specimens (*black, RBCs* with diamagnetic liposomes). In the case of RBCs/Dy-liposomes aggregates, two peaks are present at +4.8 ppm and–4.2 ppm, respectively. The negative peak is assigned to the water inside the liposome (shift determined by the dipolar contribution generated by Dy-HPDO3A). The positive chemical shift centered at +4.8 ppm is ascribable to intracellular water shifted by the changes in magnetic susceptibility due to the paramagnetic Dy-liposomes anchored on the RBCs'outer surface. Furthermore, it has been noticed that the chemical shift of the cytoplasmatic water protons is affected by the amount and typology of paramagnetic liposomes anchored on the surface (kind of metal complex and its content, size of liposomes etc…). As a proof of concept on their potential "theranostic" role, the LipoCEST/RBC aggregates have been i.v. administered to a TSA breast cancer bearing mouse and CEST maps have been acquired. At t=0, the LipoCEST/RBCs distribution reports about the extent of vascularization in the tumor (mean CEST% in the entire tumor is ca. 9.5%). The CEST effect due to the Dy-liposomes/RBCs aggregates decreases and after 1h the mean CEST% (as mearured at 3.2 ppm) is ca. 2%. This is due to the desassembling of liposomes from RBCs membrane. Conversely, the LipoCEST water signal at – 4.2 ppm does not change so much and at = 1 h its CEST% effect is still good (ca. 6%). This observation suggests that at t= 1 h more than half of the LipoCEST have been detached from RBCs and likely are extravasated in the tumor extracellular matrix.

**Conclusions:** Dy-liposomes/RBCs aggregates yield two CEST pools represented by liposomal water protons (*LipoCEST*) and cytoplasmatic water protons (*ErythroCEST*). The proposed approach of anchoring paramagnetic liposomes on the external surface of cells can represent either i) a new way to label cells, ii) a route to improve the circulation lifetime of the liposomes and iii) a CEST procedure to assess the disassembly of the Liposomes/RBCs aggregates and accumulation of the liposomes in the ROI.

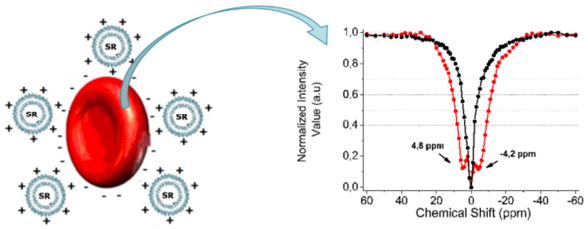


Figure 1. (*left*) Interaction of paramagnetic liposomes (Dy-HPDO3A-loaded liposomes) with RBCs; (*right*) Z-spectra of control RBCs (*black*) and Dy-Liposomes/RBCs aggregates ( *red*).

**References:** 1) Delli Castelli D et al. NMR Biomed. 2013, 26, 839-49. 2) Ferrauto G et al. Nanoscale. 2014, 6, 9604-7. 3) Ferrauto G et al. J. Am. Chem. Soc. 2014, 136, 638–41.