IN VIVO MRI MULTICONTRAST KINETIC ANALYSIS OF THE UPTAKE AND INTRACELLULAR TRAFFICKING OF PARAMAGNETICALLY LABELED LIPOSOMES

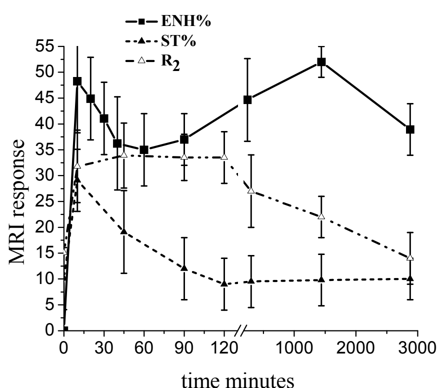
D. Delli Castelli[1], E. Terreno[1], W. Dastrù[1], E. Cittadino[1], F. Mainini[1], E. Torres[1], M. Spadaro[1], and S. Aime[1]
[1]University of Torino, Turin, Italy

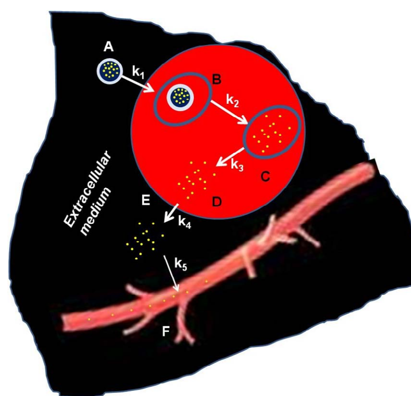
Purpose
To exploit the MR multicontrast ability of paramagnetic Lanthanide(III)-loaded nanovesicles to report about their uptake and intracellular trafficking directly *in vivo* in the tumor environment.

Introduction
Liposomes are supramolecular aggregates characterized by membranes made of naturally occurring or synthetic amphiphiles. Such vesicles are already used in the pharmaceutical field mainly as drug delivery systems. Despite their large use, there is still a lack of information about the interaction between the nanovesicles and the cells in tumor environments and their intracellular fate after the cellular uptake. Most of the information on this topic comes out from in vitro cellular studies that are not always reliable models for mimicking the in vivo system. The aim of this work is the visualisation of the metabolic pathway of these vesicles directly in vivo by means of MRI. Since MRI does not provide enough spatial resolution to directly observe events at subcellular level, we have developed a multicontrast analysis that provides indirect evidence about the uptake and the intracellular trafficking of the nanovesicles. The method relies on the peculiarity of nanovesicles encapsulating paramagnetic Ln(III)-based complexes that may act as T1, T2, and CEST agents. Upon injecting the liposomes, either stealth or pH sensitive, directly into the tumor mass and by acquiring T1, T2, and CEST MR-images, it is possible to follow the evolution of liposomes at the tumor region (macrophage/tumour uptake; intracellular de-assembly; washing out etc...). In order to account for the observed MRI data, a kinetic model able to describe the underlying biological processes has been developed. The fit of the data provides a rough estimate of the kinetic constants for each process considered in the model.

Methods
Non targeted, stealth or pH-sensitive, liposomes encapsulating paramagnetic lanthanide(III) complexes or carboxyfluorescein were prepared and *in vitro* characterized. The liposomes were locally injected in B16 melanoma tumor xenografted on C57 mice. The temporal evolution of T_1, T_2 and CEST MR contrast was followed at 7 T until 48 h post-injection. Tumors were excised and prepared for citofluorimetry analysis.

Results and Discussion
The plot reported below displays an example of the temporal evolution of T_1, T_2 and CEST contrast observed after intratumoral injection of stealth liposomes encapsulating Gd-HPDO3A (simultaneously acting as T_1 / T_2^* agent) or Tm-DOTMA (monitored as T_2^*/CEST agent), respectively.





$$\frac{d[A]}{dt} = -k_1[A]$$
$$\frac{d[B]}{dt} = k_1[A] - k_2[B]$$
$$\frac{d[C]}{dt} = k_2[B] - k_3[C]$$
$$\frac{d[D]}{dt} = k_3[C] - k_4[D]$$
$$\frac{d[E]}{dt} = k_4[D] - k_5[E]$$
$$\frac{d[F]}{dt} = k_5[E]$$

The differences in the kinetic behaviors may be interpreted in terms of a fast cellular uptake of the nanoprobe and their entrapment into endosomes. The endosomal confinement is detrimental for T_1 and CEST contrasts that are particularly sensitive to the presence of additional biological barriers between the paramagnetic agent and cytoplasm and extracellular compartments whose water content determines the detected water signal. Then, the release of the vesicle content, first inside the endosomes and then, possibly, to cytosol, determines a late-enhancement of T_1 contrast, whereas the CEST contrast disappears following the vesicle degradation. The T_2 contrast, dominated by magnetic susceptibility effects that are less dependent on the intracellular compartmentalization of the intact probe, is almost constant in the few hours after injection and starts to decrease following the vesicle degradation. The analysis revealed an unexpected fast cellular uptake both for the stealth and for the pH-sensitive vesicles, while the cytosolic release differs very much between the two membranes, being very slow for the pegylated liposomes and very fast for the pH sensitive ones, as expected. FACS analysis has been used to elucidate the role of Tumour Associated Macrophages in the uptake of liposomes. For both the liposomal formulations investigated TAM are the major consumers of nanovesicles, as expected. In conclusion MRI multicontrast analysis appears as a powerful tool for visualizing in vivo liposomes' intracellular trafficking and delivery process at the tumor site..