

# **<sup>19</sup>F MRI of trifluoroacetic acid encapsulated into liposomes**

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## **Introduction:**

Due to the lack of MR visible fluorine in living organisms there is no background <sup>19</sup>F-signal in MR images. Hence, a detected signal doubtlessly originates from previously injected fluorinated contrast agents, which makes them highly interesting for molecular imaging. In particular, liposomes are excellent carrier particles which can be filled with a variety of contrast media and functionalized by coupling them to specific antibodies. Kimura et al. (2004) have shown that perfluorooctanoic acid encapsulated into 1µm sized liposomes can be used successfully for MR imaging.

## **Materials and Methods:**

Small liposomes (EPC3/Chol – 55/45 mol%, 50-150 nm in diameter) were fabricated in a 300 mM and a 1 M trifluoroacetic acid (TFA) solution. By ultracentrifugation the liposomes were separated from TFA which was not encapsulated. The 1 M TFA liposomes were examined by NMR spectroscopy, while the 300 mM TFA liposomes were used for MR imaging. All MR experiments were performed on a 9.4 T Bruker BioSpin Scanner in conjunction with a linear RF resonator (72 mm in diameter) tunable to <sup>1</sup>H and <sup>19</sup>F.

## **Results:**

The amount and the leakage of encapsulated TFA was investigated. To separate the liposomes from the TFA-solution we performed three steps of ultracentrifugation and collected the resulting supernatant each time. Figure 1 shows spectra of the liposomes (bottom) and of the third supernatant (middle). These spectra were acquired using a non-localized singlepulse sequence (20 kHz bandwidth, 16384 data points, 32 averages, 2.5s repetition time, 35° flip angle). The SNR was calculated by integrating the spectral feature within the dashed lines in Fig. 1 and dividing it by a noise estimation (taken from outside the dashed lines). Since there is almost no fluorine signal left in the supernatant we can conclude that the <sup>19</sup>F signal in the bottom spectrum originates from fluorine which is encapsulated within the liposomes, but not from free TFA.

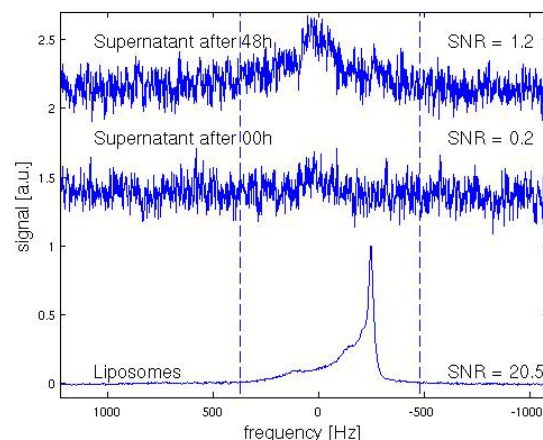


Fig. 1: Spectra of the liposomes after washing (bottom) and the corresponding supernatant of the last ultracentrifugation step (middle). The top spectrum shows the supernatant after a further ultracentrifugation step 2 days later. The dashed lines indicate the spectral region used for SNR determination.

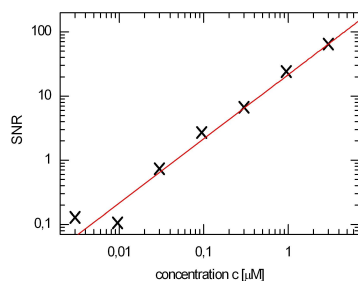


Fig. 2: Determination of the detection limit. The SNR is plotted for different concentrations of the liposomes. The red line shows the theoretical SNR normalised to the highest concentration.

However, a fourth step of ultracentrifugation was performed 2 days later. The spectrum of the corresponding supernatant shows a marginal fluorine signal, indicating that some of the TFA (<6%) has diffused out of the liposomes or that some liposomes broke down.

We have determined the detection threshold by taking spectra of different concentrations of liposomes (Fig. 2). These spectra were acquired using a non-localized singlepulse sequence (10 kHz bandwidth, 16384 data points, 128 averages, 3 s repetition time, 30° flip angle). We could detect signal from a 0.03 µM sample in a 1.75 mL tube. Thus, our detection threshold is on the order of 10<sup>13</sup> liposomes. Having in mind that each liposome is filled with 1M TFA solution this results in 10<sup>19</sup> fluorine atoms.

A first image of our liposomes was acquired using a TrueFisp sequence with TE=0.736 ms, TR= 67.57 ms, flip angle 30° and 8192 averages. With a slice thickness of 10 mm and an in-plane resolution of 0.125cm/pix we got an image with SNR~7.5 within ~9min scan time.

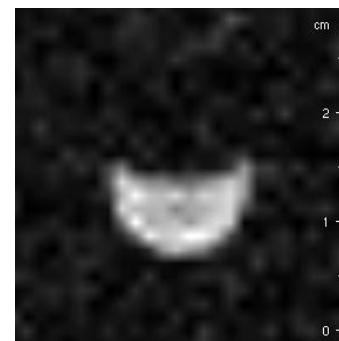


Fig. 3: A TrueFisp image acquired within ~9min. The slice thickness is 10mm and the resolution is 0.125cm/pix. The resulting SNR is about 7.4.

## **Discussion:**

We have demonstrated that liposomes filled with TFA can be detected by MRI and are stable at least on a timescale of about 2 days in vitro. Thus, TFA liposomes are promising for in vivo imaging, particularly with respect to molecular imaging applications. Detection limits given in literature are generally on the order of 10<sup>17</sup> - 10<sup>18</sup> fluorine atoms per voxel (e.g. Srinivas et al. 2007). Our detection limit of 10<sup>13</sup> liposomes (10<sup>19</sup> fluorine atoms) may be lowered by encapsulating molecules with a higher fluorine content than provided by TFA. Furthermore the volume of the RF resonator used in this study is about 3000 times as large as the volume of our samples, resulting in a very low volume filling factor. Thus, the detection limit can easily be lowered by using smaller surface coils for data reception.

## **References:**

Kimura et al., Magn Reson Imaging 2004; 22(6): 855-860  
Srinivas et al., Magn Reson Med 2007; 58(4): 725-734

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