# Gadolinium- and dysprosium-encapsulated single-walled ultra-short carbon nanotubes as intracellular agents for high field MR microscopy at 11.75 and 21.1 T

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**Introduction:** Single-walled carbon nanotubes (SWCNT) have gained interest in recent years for their biocompatibility and multifunctional applications, such as drug delivery [1,2]. Ultra-short SWCNT (US-tubes) have demonstrated the capability to encapsulate gadolinium ions ($Gd^{3+}$) and have been successfully used to label murine macrophages for cellular imaging using MRI [3]. In contrast to Gd, which displays decreased relaxation rates at high magnetic fields, ionic dysprosium ($Dy^{3+}$) shows the opposite trends above 3 T. The hollow interior and carbon surface may present additional benefits for lanthanide-doped US-tubes at higher magnetic fields due to nanoscale confinement and favorable water exchange. This study evaluates Gd- and Dy-doped US-tubes at 11.75 and 21.1 T both in solution and with a murine microglial cell line (Bv2).

**Methods:** Gd- and Dy-doped US-tubes were synthesized by immersing homogenized US-tubes in aqueous $GdCl_3$ and $DyCl_3$ solutions, respectively, followed by bath sonication and multiple washings with deionized water. A biocompatible solution was made with a 1.0% (w/v) Pluronic F108 solution using a probe sonicator. The suspension was centrifuged, and the supernatant was collected and dialyzed against running water to remove any excess surfactant [3,4]. MRI was performed on 11.75- and 21.1-T vertical widebore magnets equipped with microimaging gradients (Bruker-BioSpin, Billerica, MA). For solution experiments, doped US-tubes were serial diluted from stock solution using deionized water at four concentrations to measure $r_1$ and $r_2$ relaxivity. For *in vitro* cell experiments, a rat Bv2 microglia cell line was used following methods outline previously [3, 5]. Bv2 cells were transfected for 12 h with doped US-tubes at a final lanthanide concentration of 17 μM. Cells were washed three times to remove US-tubes that were not internalized or were adherent to the cell surface. Following harvest, 150,000 cells were mixed with an equal volume of 2% agarose solution and set into a 10-mm NMR tube. Dy- and Gd-doped US-tubes were imaged together with cells exposed only to empty US-tubes (no lanthanide) and with unlabeled cells as controls. For $T_1$ and $T_2$ relaxation measurements, single slice 2D spin-echo (SE) sequences were used with TR and TE times varied, respectively. In addition, a 3D gradient recalled echo (GRE) sequence was acquired at 50-μm isotropic resolution and TE/TR = 7.5/150 ms.

**Table 1: Relaxation measurements performed in stock solution**

| Ln-doped US-tubes | $T_1$ (ms) | | $T_2$ (ms) | |
|---|---|---|---|---|
| | 11.75 T | 21.1 T | 11.75 T | 21.1 T |
| Gd (47 mM) | 952.3 | 1174.9 | 38.2 | 16.7 |
| Dy (59 mM) | 2296.7 | 2183.5 | 41.2 | 15.4 |
| Empty (No Ln ) | 2856.2 | 2703.3 | 95.2 | 59.9 |

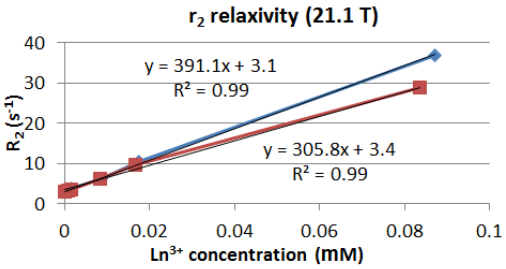


**Fig 1:** $r_2$ of Dy- (♦) and Gd- (■) US-tubes at 21.1 T. Relaxivity slope & goodness of fit are shown for each lanthanide plot.

**Results:** Comparing the effect of increased field strength in solution (Table 1), Gd-US-tubes show an overall shorter $T_1$ that increases at 21.1 T. Dy-US-tubes, on the other hand, shows a decrease in $T_1$ value consistent with theoretical expectations [6,7]. For $T_2$, both Dy and Gd show a reduction in the relaxation times at the higher field with a slight benefit for the Dy. Figure 1 shows that Dy-US-tubes has a larger $r_2$ relaxivity at 21.1 T (28% higher than Gd-US-tubes). For $r_1$ relaxivity (data not shown), Gd-US-tubes still dominates with an $r_1 = 8.31\ mM^{-1}s^{-1}$ compared to $0.89\ mM^{-1}s^{-1}$ for the Dy-US-tubes. When the doped SWCT are incorporated into Bv2 cells, $T_1$ contrast is quenched while $T_2$ and $T_2$* are the dominating contrast mechanisms (Fig 2). For cells, Dy-US-tubes are the more effective intracellular contrast agent with a 9% shorter $T_2$ and a much larger susceptibility effect with a 58% shorter $T_2$*.

**Discussion:** Relaxation for Dy- and Gd-doped US-tubes at these two fields appears to follow general trends expected for these lanthanides. $T_1$ contrast is not seen for either Ln once the doped US-tubes are incorporated into cells. This effect is likely due to $T_1$ quenching from the low surface-to-volume ratio and limited water access across endosomal membranes [6]. As such, the expected favorable water access implemented by the cylindrically shaped carbon nanotubes appears not to have affected the intracellular $T_1$ contrast. $T_2$ and in particular $T_2$* contrast is the dominant contrast mechanism for intracellular US-tubes and compares quite favorably to other Dy conjugated contrast agents both in solution and in cells at similar concentrations [6].

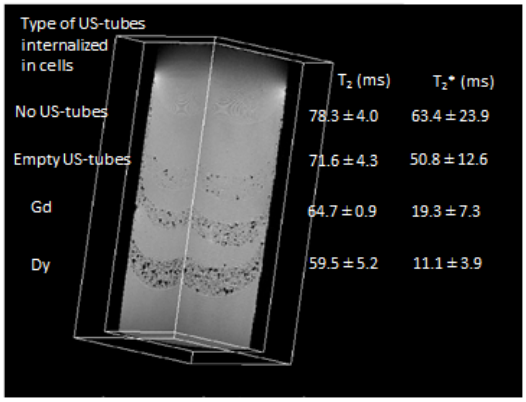


**Fig 2:** 3D GRE at 21.1 T showing increased contrast for Gd- and Dy-US-tubes

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