Bimodal Intracellular Nanoparticles Based on Quantum Dot Technology for High Field MR Microscopy at 21.1 T

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Introduction: Numerous efforts have been made to utilize intracellular contrast agents, but these studies tend to focus on the application of iron-oxide or gadolinium-based particles. This focus has been motivated by the high sensitivity of these agents and the well known chemistry involved in their fabrication. However, these particles may not be as beneficial at higher fields (>7 T). Other paramagnetic lanthanides, including dysprosium, ytterbium and thulium, may offer improved performance at higher fields. For these rare earth ions, relaxivity is not intended to decrease as dramatically as gadolinium with increasing magnetic fields (in some cases, relaxivity is expected to increase), and there exists the possibility of utilizing Paramagnetic Chemical Exchange Saturation Transfer (PARACEST) contrast[1,2].
In this study, we have modified fluorescent quantum dot nanoparticles with lanthanides to produce multi-modal agents that provide both MRI and optical contrast. This report details evaluations of these intracellular contrast agents in an *in vitro* intracellular model at 21.1 T (900 MHz), the highest field available for MR microscopy.

Background: MRI contrast agents that are available commercially are limited in their effectiveness at high magnetic fields. For example, the effectiveness of Gd-based agents drops drastically with field strength[3]. Meanwhile, other lanthanides (*e.g.* Dy) improve at higher fields due to different relaxation mechanisms. Preliminary studies on commercial agents and quantum dots (Qdots) demonstrate this improvement for fields between 4.7 and 17.6 T. However, these novel high field agents need to be manipulated to increase their contrast efficiency for bimodal intracellular operation. Our objective is to synthesize and characterize contrast agents that can be used to label the intracellular space of mammalian cell lines, ultimately with the intent of implantation.

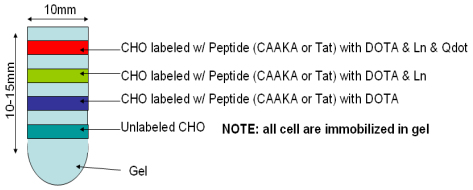


Fig 1 – Cellular phantom layout within a 10-mm NMR tube

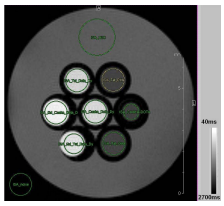
Methods: A DOTA-succimidyl ester complex (EDC, Sulfo-NHS, DOTA, 4 C for 30 min) was reacted 1:1 with a 5-amino acid peptide, CAAKA[4,5]. Each peptide-DOTA sample was purified by reverse-phase HPLC and subsequently lyophilized. The peptide-DOTA complex and a 1.5-mole excess of $Dy(NO_3)_3$ was dissolved in 0.1-M glycine/HCl buffer (pH=3.5) and heated to 80 C for 3 hours[6]. The sample was desalted to remove free Dy^{3+} prior to coupling the peptide-DOTA-Dy^{3+} to the InP/ZnS Qdot by biphasic ligand exchange. The Qdot-complex was transfected into a Chinese Hamster Ovary (CHO) cell sample using a lipofectamine2000 (Invitrogen) for CAAKA. The cells were harvested after a 24-hour incubation, mixed with an equal volume of 2% agarose solution, and set into a 10-mm NMR-tube for MR microscopy at 21.1 T. Note: Extra control layers (as indicated in Fig 1) have been added to illustrate the effect of each component of the nanoparticle.

All data have been acquired with a 105-mm, 21.1-T 900-MHz ultra-widebore (UWB) vertical magnet constructed at the NHMFL and equipped with a Bruker Advance console and Micro2.5 gradients. A 10-mm Bruker RF birdcage coil was used. The following sequence parameters were common to all quantitative acquisitions: Matrix=128x128, BW=75 kHz, FOV=1.9x1.0 cm, Slice Thickness=0.5 mm. For T_1 and T_2 measurements, a single-slice 2D spin-echo (SE) sequence was used with TR and TE parameters varied, respectively. For T_2* measurements, TEs were varied in repeated acquisitions of a single-slice 2D gradient-recalled echo (GRE) sequence (TR = 5 s). High resolution 3D GRE images were acquired with the following parameters: TE/TR=5/150ms, BW=55 kHz, Matrix=440x190x190, FOV=22x9.5x9.5 mm, Resolution=50-µm isotropic.

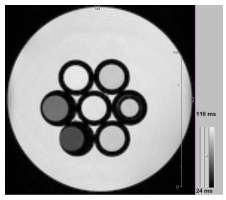
Results:

Qdot Agents in Aqueous Solutions: CAAKA binding peptide

	T_1 [ms]	T_2 [ms]	T_2* [ms]
CAAKA-DOTA	2712.5 ± 53.5	115.6 ± 0.43	24.2 ± 3.4
CAAKA-DOTA-Dy	491.9 ± 2.14	85.0 ± 0.40	61.2 ± 3.73
CAAKA-DOTA-Dy-Qdot	60.5 ± 1.15	32.6 ± 0.25	43.1 ± 0.82



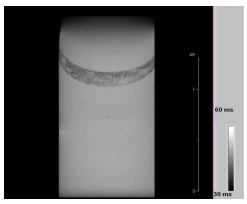
Left: T_1-weighted image of contrast agent solutions.



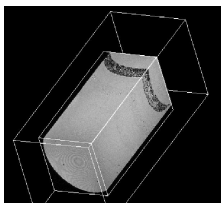
Left: T_2-weighted image of contrast agent solutions

CHO cells labeled with CAAKA-DOTA-Dy-Qdots

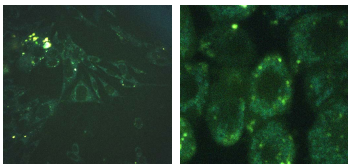
	T_1 [ms]	T_2 [ms]	T_2* [ms]
CHO cells	3067.5 ± 204.4	71.4 ± 0.9	41.9 ± 2.6
CHO-CAAKA-DOTA	2922.8 ± 189.4	63.7 ± 0.9	27.5 ± 1.9
CHO-CAAKA-DOTA-Dy	2866.2 ± 175.8	59.8 ± 1.0	42.0 ± 2.2
CHO-CAAKA-DOTA-Dy-Qdot	3274.5 ± 519.2	29.9 ± 2.5	9.8 ± 1.6
Qdot-CAAKA	3648.9 ± 598.1	68.0 ± 0.9	30.2 ± 0.8
Agarose	2688.7 ± 85.8	60.2 ± 0.9	43.3 ± 3.7



Left: T_2-weighted image of labeled CHO cells with layers stratified according to Fig 1



3D GRE of labeled CHO cells @ 50-µm isotropic resolution



Confocal spectral images of CAAKA-DOTA-Dy-Qdots Loaded in CHO cells (2 samples). The images were taken using 404-nm laser excitation and 40x (left) and 60X (right) magnification. The InP/ZnS emission is approximately 560 nm.

Conclusions: We have been able to configure a bimodal contrast agent with both MRI and confocal properties. The CAAKA–DOTA-Ln^{3+}-Qdot displays strong MRI contrast enhancement for all relaxation mechanisms but the strongest contrast for intracellular loading is seen with T2/T2* weighting. As yet, T1 enhancement, which is desirable for *in vitro* and *vivo* agents at high fields, has not been achieved in our studies. 3D volume renderings display strong T2*-contrast from CAAKA-DOTA-Dy-Qdots. Other lanthanides (like Yb^{3+} and Eu^{3+}) conjugated to Qdots should be capable of generating ParaCEST contrast, an avenue of investigation that will be evaluated in future studies.

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