Final Scientific Report

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Title of the project: *Neodymium-based core@shell UCNPs decorated with phosphonate-based ruthenium complexes: towards NIR-activatable ruthenium anticancer prodrugs*

Period: 30/07/2016 to 20/08/2016

Introduction

Photo-activated chemotherapy (PACT) has been attracting attention as a potential novel anticancer treatment. The ability to photo-activate non-toxic metal-based anticancer prodrug with high spatial and temporal resolution in and around a localized tumour should potentially reduce their side effects and increase the treatment efficacy. Ruthenium thioether complexes are being studied at Leiden University as drug candidates for oxygen-independent Photo-Activatable ChemoTherapy (PACT). These complexes can be photo-activated using blue light. The poor permeability of human tissue (< 1mm) towards blue light poses a serious limitation with respect to their use in preclinical and clinical trials. To improve the efficiency of photo-activation in human tissue, Dr Natile (CNR-ICMATE) and Dr S. Bonnet (Leiden Institute of Chemistry) have recently started to exploit the possibility to combine Ru-complexes with Ln-doped upconverting nanoparticles, UCNPs, acting both as photon upconverter and as drug delivery system. In this way, in fact, blue light-absorbing Ru-complexes can be activated by NIR-light.

Objective

The aim of the STM was to design and develop an anticancer drug delivery system based on core@shell UCNPs, absorbing at 800 nm and emitting blue light in order to activate Ru-complexes in the phototherapeutic window.

Work plan

	1st WEEK		2nd WEEK		3th WEEK	
Photochemical characterization of as prepared UCNPs						
Synthesis, Characterization of Ru-complexes						
Decoration of UCNPs with Ru-complex						

Results

In Fig.1 the Transmission Electron Microscopy (TEM) images of $NaYF_4$:Yb,Tm core, $NaYF_4$:Yb,Tm@$NaYF_4$:Nd core@shell, and $NaYF_4$:Yb,Tm@$NaYF_4$:Nd@$NaYF_4$ core@shell@shell UCNPs prepared in Padova are reported. The successful shell coating was confirmed by the increase in particle size.

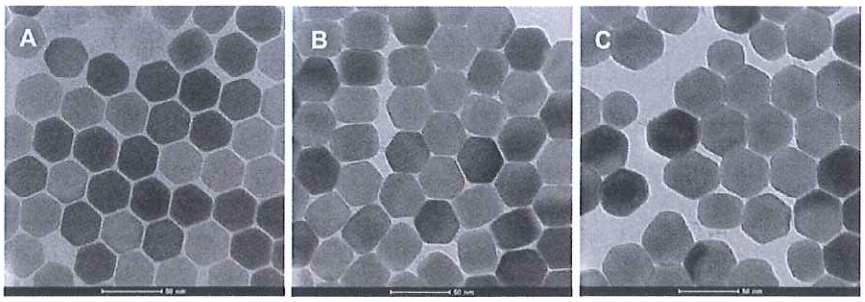

Figure 1. TEM images of (A) $NaYF_4$:Yb,Tm core (ø 33.9 nm), (B) $NaYF_4$:Yb,Tm@$NaYF_4$:Nd core@shell (ø 36.1 nm) and (C) $NaYF_4$:Yb,Tm@$NaYF_4$:Nd@$NaYF_4$ core@shell@shell UCNPs (ø 39.2 nm).

During the stay in Leiden the photochemical characterization of the above monodispersed UCNPs was carried out irradiating both with a 980 nm and 800 nm continuous wave lasers (Fig. 2). The presence of Nd ions in the first shell increases the cross section of UCNPs at 800 nm, while the outermost inert shell minimizes the surface quenching (Fig.2b). Under excitation at 980 nm (Fig. 2a) only an enhancement of the blue upconversion emission is evident: the presence of the two outermost shell layers have only the effect to minimize the surface quenching.

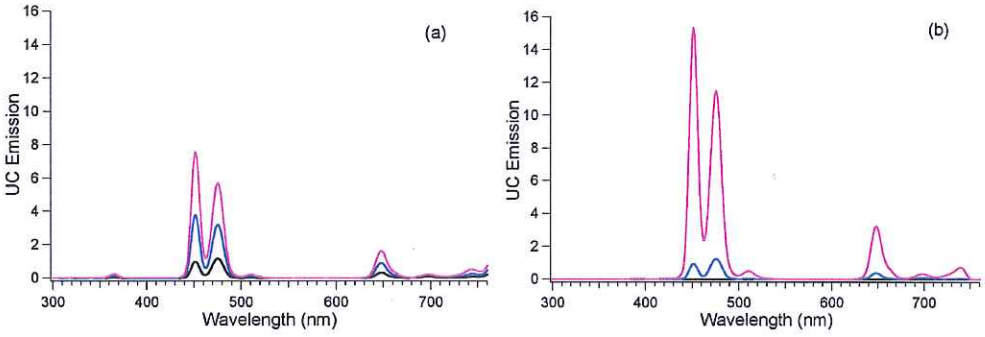

Figure 2. Upconversion emission spectra of (black line) $NaYF_4$:Yb,Tm core, (blue line) $NaYF_4$:Yb,Tm@$NaYF_4$:Nd core-shell and (magenta line) $NaYF_4$:Yb,Tm@$NaYF_4$:Nd@$NaYF_4$ core@shell@shell UCNPs dispersed in toluene (1 mg/ml) under excitation at (a) 980 nm and (b) 800 nm (2.00 W, 46 W/cm^2)

At the same time also the synthesis of two Ru-complexes were carried out (first and second week). For this purpose, the well-known *cis*-[$Ru(bpy)_2Cl_2$] scaffold was used, and the chloride ligands were replaced with one of two novel bidentate ligands, acquiring complexes of the following structure: *cis*-$[Ru(bpy)_2(L)]^{2+}$ (Fig. 3). These ligands were designed to both contain a total of four binding sites (two for coordination to the Ru ion, and two for attaching the Ru complex to the UCNP). The UCNP binding sites consisted of either carboxylate

or phosphonate moieties, both of which are known to bind well to the UCNP surface. The successful synthesis of the abovementioned complexes was confirmed by mass spectrometry and NMR spectroscopy. Irradiation of the complex in water with blue light leads to expulsion of one of the ligands, producing a bis-aqua species, Fig.3, as indicated by a colour change from pale orange to red-brown and a corresponding shift in the UV/VIS absorption spectrum.

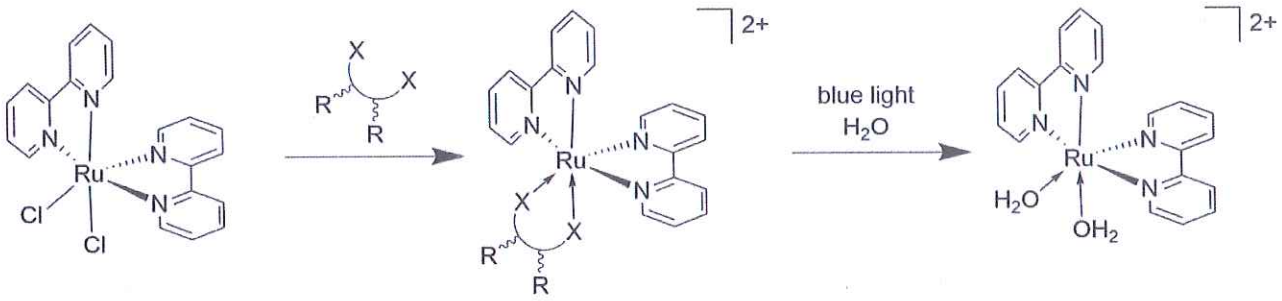

Figure 3. Scheme depicting the synthesis and visible light activation of the Ru complexes used in the study.

A two steps ligand exchange procedure was optimized to substitute the oleate capping ligands on the surface of the as-prepared nanoparticles with carboxylic groups on the Ru-complex. In the first step tetrafluoroborate anions were employed to replace the oleate, thus stabilizing the UCNPs in various hydrophilic media without aggregation or precipitation as revealed by TEM image. Successively, by a second ligand exchange the obtained hydrophilic UCNPs were further functionalized with the carboxylic based Ru-complex and transferred in water media. The successful decoration of the UCNPs surface was evidenced first by the orange colour of the conjugate system (the as prepared UCNPs are white) and then by UV-vis spectroscopic analysis of the conjugate system dispersed in water, which shows the absorption profile of the complex. Due to time restraints, the decoration of UCNPs with phosphonate-based ligands has not been performed. Anyway the same optimized procedure will be exploited in the near future for the phosphonate Ru-complex.

Conclusions and future work

The short-term mobility program has allowed setting up both a protocol to prepare a Ru-complex with a carboxylic or phosphonate moieties, which have high affinity for the UCNP surface, and a procedure to functionalize the UCNPs surface with the new Ru-complexes. The next step will be to evaluate the efficiency of NIR light to activate the conjugate system.

Foreseen joint publications

The STM has contributed to strengthen the scientific collaboration between Dr Natile and Dr Bonnet. The two groups will continue the collaboration on this field.

A proper acknowledgement to the STM program will be given in publication/communication prepared jointly by the two research teams.

Padova 18/10/2016