Lanthanide Luminescence: Applications in Biotechnology

Nitin Neelakantan Literature Seminar November 14th, 2013

The lanthanides, also know as 'rare-earth metals', consist of the 4f-block elements (La to Lu). They share many similarities with one another, such as ionic radius and oxidation state (III). They have very unique luminescent properties that can be utilized in a wide variety of applications such as plasma displays, liquid crystals and lasers.[1-3] Perhaps most characteristic of these properties are f-f transitions, which are Laporte forbidden. Due to various relaxation mechanisms such as vibronic coupling, these transitions still occur, albeit with very weak extinction coefficients (~1 mol^{-1} cm^{-1}). On the other hand, their forbidden nature leads to a distinctly long lifetime on the order of milliseconds, as well as sharp emission bands, both of which are highly desirable in the field of biotechnology. In order to efficiently promote luminescence, a photosensitizer that can transfer energy to the lanthanide is necessary.[4]

A good photosensitizer, or 'antenna', should have favorable energy overlap with the lanthanide (Ln) in question, and be designed to prevent deactivation processes that would otherwise quench fluorescence. In aqueous media, the obvious culprit is ligation by water molecules, which would de-activate the excited state via O-H stretches. As a consequence, the photosensitizer should have a very high binding affinity for Ln, as to render the complex resistant to hydrolysis. Unsurprisingly, many photosensitizers are organic polydentate chromophores.

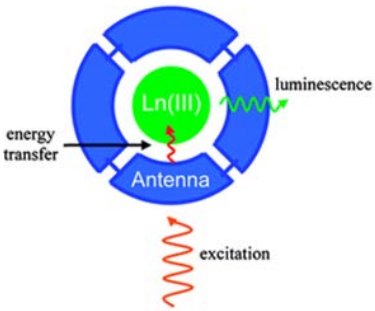

Figure 1 A diagram of the sensitization process[5]

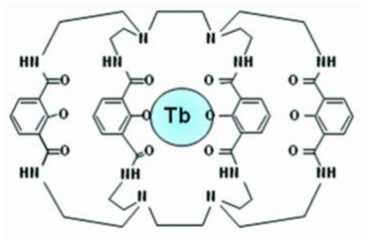

Figure 2 Lumi-4-Tb[6]

In 2009, Raymond, et. al. tested a variety of possible chromophores. Their goal was to design a complex that could be used in homogenous time-resolved fluoroassay (HTRF).[5] Since the lifetime of a Ln-complex is far longer than that of biological autofluorescence, time resolution can more easily separate signal from noise. Initial results showed a quantum yield of 61% for a Tb-hydroxyisophthalamide complex, which led to the development of a commercially used product, Lumi-4-Tb (Fig 2).

Sensitizers are not limited to organic ligands; even the lanthanides themselves can support metastable energy levels and dramatically increase luminescence intensity. For instance, Yb^{3+} can effectively sensitize an adjacent Er^{3+} ion. When this occurs, a photon is absorbed by each ion and each can be promoted to the E1 state described in Fig. 3. Through non-radiative energy-transfer, one ion is promoted to E2, while the other relaxes to the ground state. Since E2 is at a higher energy than the absorbed light, this results in a large anti-Stokes shift, in a process known as energy transfer upconversion (ETU).[4]

The advantage in using ETU is most evident when considering that Yb-sensitized Er efficiently converts near-infrared (NIR) light to visible light. Most biological tissue is permeable to IR light, thus upconverting nanoparticles (UCNP) offer a high-resolution *in vivo* imaging technique with good depth penetration and almost no background fluorescence. Recently, Liu, et. al. demonstrated the application of this method in cancer detection by synthesizing Yb/Er doped $NaGdF_4$ nanocrystals.[7] After functionalization with polyethylene glycol (PEG) for water-solubility and a cancer-specific antibody for active targeting, tumors with a diameter as small as 2 millimeters were successfully imaged using an IR laser.

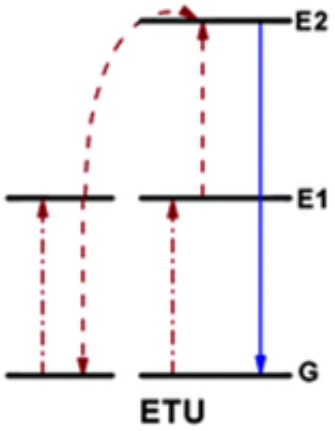

Figure 3 Mechanism of Energy transfer upconversion[4]

The Gd-shell of the nanocrystals offers a dual-modality in the diagnosis of cancer. Since Gd(III) is paramagnetic, it can be used as a contrast agent for T1 magnetic resonant imaging (MRI). Cancerous tissue has a different relaxation time from healthy tissue[8], so high contrast is of paramount importance. In this paper, the authors report that a nearly 30% increase in contrast was achieved compared to a commonly used contrast agent (Fig. 4).

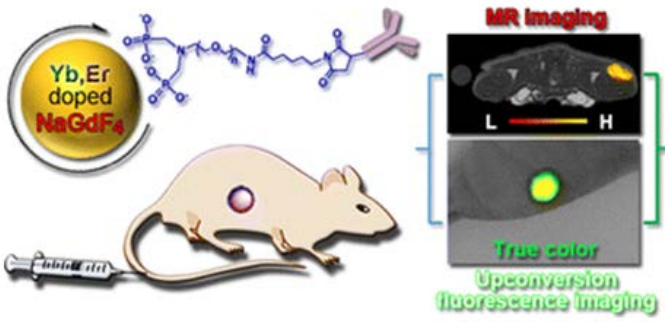

Figure 4 Schematic of experimental design, MR image with contrast and luminescence image of tumors[8]

By altering the surface functionality of the UCNP and attaching a sensitizer capable of generating reactive oxygen species (ROS), a third modality is possible: photodynamic therapy. This new class of materials, which can both detect and treat cancer, is referred to as 'theranostic' (therapeutic + diagnostic). Park, et. al. also synthesized the same nanoparticles as Liu, but with a core/shell structure and bound by a chromophore capable of producing cytotoxic singlet oxygen.[9] Without an anti-body for active targeting, the authors relied on a passive targeting effect known as enhanced permeability and retention.[10] The leaky and disorganized vasculature of carcinogenic cells allows for nanoparticles to accumulate therein. After the injection of UCNP, mice were successfully imaged via luminescence, magnetic resonance, and finally treated with IR laser irradiation. Compared to a saline control, and UCNP without IR light, the UCNP in conjunction with photodynamic therapy greatly inhibited the growth of glioma cells (Fig. 5).

In the newly burgeoning field of theranostics, lanthanides have received large interest as a possible source of materials that exhibit multi-modality. Although many advances have been made in recent years, Ln nanotechnology in this area is still in its infancy, having not yet been approved for clinical trials.[11]

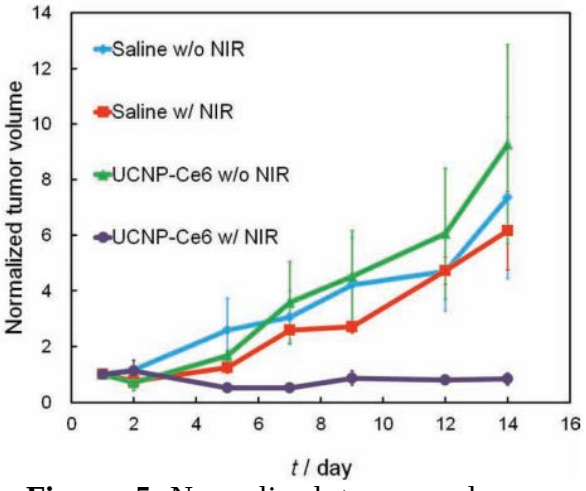

Figure 5 Normalized tumor volume vs. treatment time in days[9]

While results have been shown *in vivo*, future studies will need to determine the long-term toxicity of lanthanides[12] before they can be administered to humans. Ultimately, given the long lifetime luminescence, and ability to convert IR to visible light, lanthanide-based nanotechnology have extreme potential to be used in biomedicine in the years to come.

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