Angewandte
Communications

Luminescence

DOI: 10.1002/anie.201200887

pH-Responsive Luminescent Lanthanide-Functionalized Gold Nanoparticles with “On–Off” Ytterbium Switchable Near-Infrared Emission**

Laura K. Truman, Steve Comby, and Thorfinnur Gunnlaugsson**

Dedicated to Sigrún Ingibjög Gísladóttir on the occasion of her 80th birthday

Functionalized gold nanoparticles (AuNPs) have attracted great interest in biological applications, particularly as sensing systems because of their known stability, size- and shape-dependent properties, and biocompatibility.[1] The combination of AuNPs and lanthanides for the purpose of luminescent sensing has been recently investigated,[2] the first example of such in aqueous solution being a Eu^{III}-cyclen-conjugated AuNP system developed for the purpose of sensing phosphate anions.[3] Recently these systems have been further developed to exploit the interaction with the protein bovine serum albumin (BSA) for potential biomedical sensing and imaging.[4] The lanthanides possess many highly desirable photophysical properties that are ideal for biological applications. These include narrow linelike emission bands, and most notably, long-lived excited states, which enables the use of time-resolved detection.[5] However, since f-f transitions are characterized by oscillator strengths that are too weak to yield an efficient excitation path,[6] it is necessary to populate their excited state through sensitizing antennae. Lanthanides such as Nd^{III} and Yb^{III} ions emit in the near-infrared (NIR)-range, within the emission window of 840–1400 nm,[7] and as such, are particularly attractive for probing biological interactions, because of the increased tissue transparency and reduced interferences from both light scattering and biological matter autofluorescence.[8] Moreover, unlike the sensitization of ions such as Eu^{III} and Tb^{III}, the excited states of the NIR lanthanides can be populated by using visibly absorbing antennae, such as organic chromophores[9] or d-metal complexes.[10] However, to the best of our knowledge, the unique combination of AuNPs and NIR-emitting lanthanides has not yet been explored to date. Herein, we present the synthesis and application of 1·Yb, a cationic macrocyclic Yb^{III} cyclen conjugate, possessing three acetamide arms and an alkyl thiol group, Figure 1, enabling surface functionalization of AuNPs, and the generation of the NIR-emitting AuNP-1·Yb in the presence of appropriate antennae.

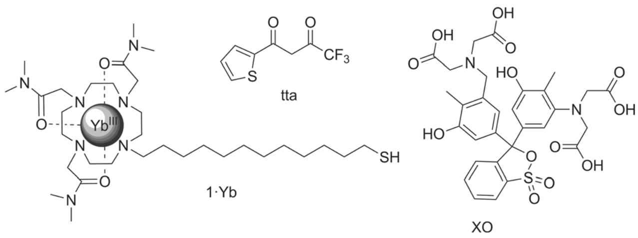

Figure 1. Complex 1·Yb, antennae tta, and xylenol orange (XO).

We also demonstrate that the NIR emission from AuNP-1·Yb can be achieved upon excitation at 580 nm, using the antenna xylenol orange (XO), and that the Yb^{III} emission of the resulting ternary complex AuNP-1·Yb-XO is highly pH-sensitive, allowing for the reversible pH-dependent switching of the Yb^{III} NIR emission.

The synthesis of ligand 1 has been previously reported[3] and the corresponding Yb^{III} complex 1·Yb was formed by microwave irradiation of 1 in methanol for 40 minutes in the presence of one equivalent of $Yb(CF_3SO_3)_3$, which gave 1·Yb in 74 % yield after precipitation from diethyl ether (see the Supporting Information). The synthesis of the surface-functionalized AuNP-1·Yb was achieved using a modified two-phase Brust method;[11] where TOAB-stabilized AuNPs in toluene were transferred into aqueous phase by direct ligand exchange using 1·Yb, without the need for intermediate DMAP-stabilized AuNPs (see Figure 2 a). The addition of $NaBH_4$ (1.5 equiv) to this mixture ensured that no oxidation of the S–H bond occurred. Complete transfer of the AuNP-

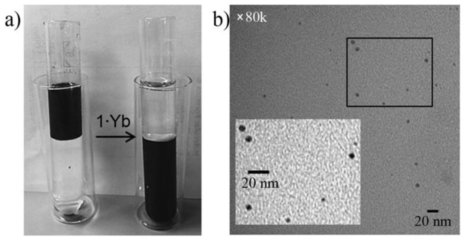

Figure 2. a) Complete transfer of AuNPs from toluene into aqueous layer with 1·Yb, giving AuNP-1·Yb. b) TEM image of AuNP-1·Yb.

---

[*] L. K. Truman, Dr. S. Comby, Prof. T. Gunnlaugsson
School of Chemistry, Centre for Synthesis and Chemical Biology
and Trinity Biomedical Sciences Institute
Trinity College Dublin, College Green, Dublin 2 (Ireland)
E-mail: combys7@gmail.com
gunnlaut@tcd.ie

[**] We thank the Science Foundation Ireland (SFI 2010 PI and RFP 2009 awards), the Irish Research Council for Science, Engineering and Technology (IRCSET Postgraduate Studentship, L.K.T.) and the Swiss National Science Foundation grant PA00P2_131444 (S.C.) for financial support.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201200887.

1·Yb into water was achieved within 12 h and purification from the excess free 1·Yb was achieved by size exclusion chromatography (see Figure S2a in the Supporting Information), using an aqueous NaCl (0.05 M) solution as the eluent. The resulting AuNP-1·Yb, stable for several months in aqueous solution, were characterized by UV/Vis absorption spectroscopy, where the characteristic gold surface plasmon resonance (SPR) band was found to be red-shifted from 517 to 523 nm (Figure S2b), as well as using TEM, the latter showing spherical nanoparticles with an average core diameter of 8 nm and no evidence of aggregation as shown in Figure 2b. The hydrodynamic diameter of the AuNP-1·Yb in solution was determined to be about 12 nm using dynamic light scattering (DLS; Figure S3), which corresponds to a homogeneous covering of the gold surface by the 1·Yb complex as observed for the Eu^{III} analogue.[4]

1·Yb was designed as a coordinatively unsaturated complex, with the presence of two metal-bound water molecules in aqueous solution. The complex also lacks the necessary sensitizing antenna, which makes 1·Yb and consequently AuNP-1·Yb, nonluminescent. However, the displacement of any metal-bound water molecules by a suitable coordinating antenna will make these systems coordinatively saturated, causing a "switch on" of the lanthanide emission upon excitation of the antenna.[3,4,12] In this work, the organic dye xylenol orange, XO, was selected as the antenna as it possesses strong absorption in the visible range, while having previously been shown to form stable luminescent coordination complexes with NIR-emitting lanthanides.[7a,9e] Addition of the XO antenna to a solution of 1·Yb (10 μM) in pH 7.4 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer resulted in the appearance of the characteristic Yb^{III} emission in the 900–1200 nm range upon excitation into XO absorption band at 580 nm, confirming the formation of a ternary assembly between 1·Yb and XO (Figure 3). Sensitization of the NIR emission by XO was ascertained by the excitation spectrum which closely matched the absorption spectrum.

Unlike our previously published articles,[3,4] XO possesses four carboxylic groups, enabling its binding to the Yb^{III} center through several different binding modes. Having demonstrated the formation of 1:1 luminescent ternary complexes using 1·Eu and nta (nta = 4,4,4-trifluoro-1-naphthalen-2-yl)butane-1,3-dione), the 4,4,4-trifluoro-1-(thiophen-2-yl)butane-1,3-dione (tta) β-diketone, that has been shown to be suitable for the sensitization of NIR-emitting lanthanides,[7a] was chosen as an antenna to further characterize 1·Yb and the corresponding functionalized AuNPs as well as compare their behavior in aqueous solution with their visible emitting Eu^{III} counterparts studied recently in our group. The formation of a 1:1 ternary complex, 1·Yb-tta, was confirmed by plotting the changes in the Yb^{III} $^2F_{5/2}\rightarrow{}^2F_{7/2}$ transition at 985 nm as a function of added amount of antenna (Figure 4, black full circles), which showed no significant variation of the NIR emission after addition of one equivalent of tta.

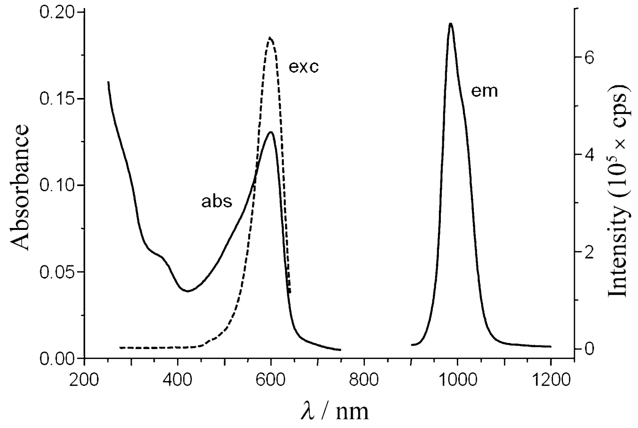

Figure 3. Absorption (abs), emission (em; λ_{ex} = 580 nm), and excitation (exc; λ_{an} = 985 nm) spectra of 1·Yb-XO in HEPES buffered solution (cps = counts per second).

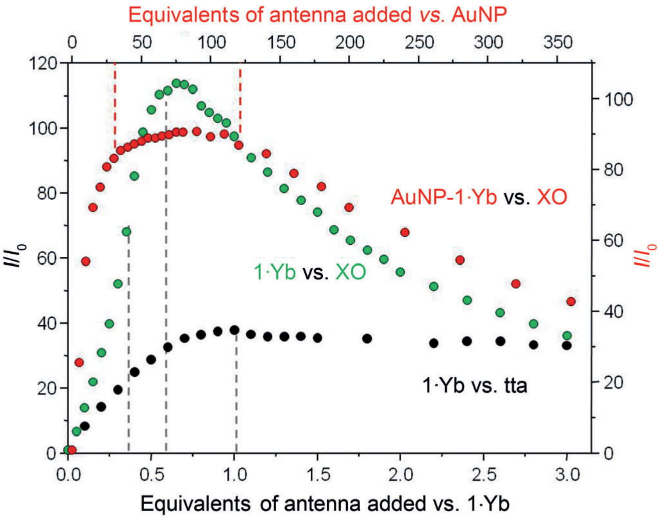

Figure 4. Changes in the NIR emission intensity (*I*) at 985 nm upon addition of tta to 1·Yb (black full circles) and XO to 1·Yb (green full circles) or to AuNP-1·Yb (red full circles, top-right scale).

Similar titrations were carried out using AuNP-1·Yb (0.1 μM) in a pH 7.4 buffered HEPES solution. The changes in the Yb^{III} NIR emission demonstrated that a plateau was reached after the addition of about 120 equivalents, indicating that 120 (± 20) 1·Yb complexes were covalently conjugated to each AuNP (Figure S4). This is in agreement with our previous results obtained for AuNP-1·Eu using the nta antenna.[4] However, it has to be stressed that tta was not used for further studies because of a photostability issue encountered under the experimental conditions of these measurements (Figure S5).

As demonstrated above, XO was also able to populate the $^2F_{5/2}$ excited state of 1·Yb and as expected from the binding potential of such antenna, the titration of 1·Yb with XO (see Figure S6) gave a different binding isotherm to that seen for the tta titration (Figure 4, green full circles). Nonlinear least squares analysis of the data using SPECFIT (Figure S7) showed the successive formation of 3:1, 2:1, and 1:1 1·Yb/XO species with $\log\beta$ of 19.7 ± 0.1, 13.48 ± 0.07, and 7.02 ± 0.05, respectively. It is also apparent from Figure 4 that the 1:1 species, mainly formed at higher equivalents of XO, is less emissive than the multimetallic ones. Similarly, AuNP-1·Yb (0.1 μM) was titrated with XO in a pH 7.4 buffered HEPES solution (Figure S8). As before, the formation of a self-assembly between AuNP-1·Yb and XO, resulted in large luminescence enhancements in the NIR region, signifying the formation of AuNP-1·Yb-XO. The binding isotherm for this titration is shown in Figure 4 (red full circles), demonstrating a rapid emission enhancement within the addition of about 30(±10) equivalents of XO before levelling off and finally decreasing after addition of 120 equivalents. Given that we had determined that about 120 1·Yb complexes were adsorbed to each AuNP, it is possible to postulate that XO binds dominantly to the Yb^{III} complexes in a 3:1 or 4:1 (1·Yb/XO) ratio. The latter species, not observed in the study of the complex alone, could possibly be explained by the closer proximity of the 1·Yb complexes once attached to the AuNP surface relative to their proximity in solution. These results clearly demonstrate that NIR emission from functional AuNPs can be obtained, upon formation of a ternary complex between AuNP-1·Yb and a suitable antenna. To the best of our knowledge, these are the first examples of such NIR-emitting functional AuNPs. Moreover, the functionalization of the AuNP by 1·Yb gives rise to a highly stable system after the formation of AuNP-1·Yb-XO, the latter assembly being found to be stable over a period of several weeks, with no measurable photodegradation being observed.

To quantify the ability of the XO antenna to sensitize the NIR-emitting Yb^{III} ion, the emission quantum yields for 1·Yb-XO and AuNP-1·Yb-XO were determined upon excitation at 580 nm in water and were found to be 0.20 ± 0.03 % and 0.036 ± 0.005 % for 1·Yb-XO and AuNP-1·Yb-XO, respectively. The value for 1·Yb-XO is in good agreement with literature data,[7] particularly in aqueous solution, where the presence of proximate O−H oscillators often induces considerable quenching for ions having a small energy gap. In contrast, the value for AuNP-1·Yb-XO is five-fold smaller, demonstrating as expected some quenching by the gold surface.

XO is also known as a pH indicator, where in pH 7.4 solution the chromophore absorbs at 580 nm, being blue-shifted to 435 nm at pH 4.5, with striking purple to yellow color changes (Figure S9). With this in mind, pH titrations of AuNP-1·Yb-XO, formed using AuNP-1·Yb (0.1 μM) and 30 equivalents of XO, were carried out over the pH range of 2 to 11 in 0.1 M NaCl solution, whereby the changes in both the absorption and the NIR emission of AuNP-1·Yb-XO were monitored. The overall changes in the UV/Vis absorption mirrored that seen for XO alone. However, as shown in Figure 5 there are clear differences in the pH profiles, where in the case of AuNP-1·Yb-XO the main changes occurred within the pH range of 4–6. This is most likely because of the fact that upon binding to Yb^{III}, there are fewer protonation sites available in XO. This was confirmed by carrying out the same titration on 1·Yb-XO, which showed similar pH-dependent changes than for AuNP-1·Yb-XO (Figure 5 and Figure S10).

Next, we evaluated the changes in the Yb^{III} NIR emission of AuNP-1·Yb-XO as a function of the pH value.[13] The behavior observed clearly demonstrates that the emission from AuNP-1·Yb-XO is highly pH dependent as for the 1·Yb-XO system (Figure 6 and Figure S11), with the Yb^{III} emission being "switched off" in acidic media between pH 2–4, independent of the excitation wavelength owing to the dissociation of the ternary complex, and "switched on" above pH 4. Furthermore, the Yb^{III} emission remains relatively constant from pH 6–10, being slightly enhanced above pH 10.

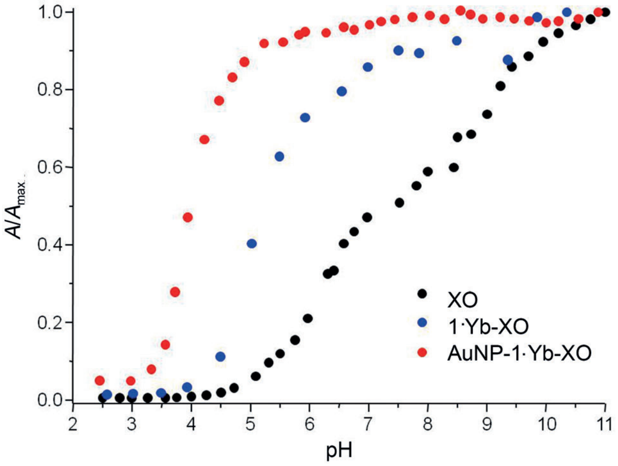

Figure 5. Effect of the pH in the range of 2 to 11 on the absorbance at 580 nm of XO, 1·Yb-XO, and AuNP-1·Yb-XO.

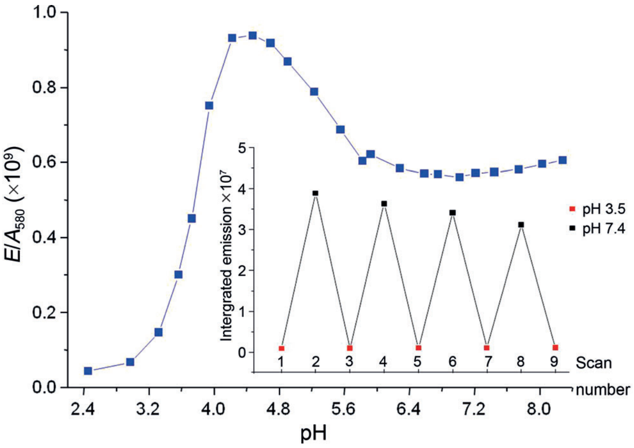

Figure 6. Changes in the Yb^{III}-integrated emission corrected by the absorbance at the excitation wavelength (E/A_{580}) of AuNP-1·Yb-XO between pH 2–8. Inset: "on–off" NIR behavior between pH 3.5 and 7.4.

By plotting the changes in the NIR emission at 985 nm as a function of the pH value (Figure 6), a pH emission profile that mirrors that seen in the UV/Vis absorption spectra, shown in Figure 5, was observed. However, this is again different to that seen for the "free" XO, which tends to confirm a direct coordination of the XO antenna to the Yb^{III} center, and that such coordination shifts the pK_a values of the antenna to more acidic regions. This would suggest that the antenna does not dissociate from AuNP-1·Yb-XO, within the pH window of 4 to 7, and that the observed "on–off" switching in Figure 6, is directly because of the ability of the antenna to populate the Yb^{III} $^2F_{5/2}$ excited state.

Having established that the absorption spectrum was fully recovered when the pH was modulated between pH 3.5 and 7.4, the reversibility of the "on–off" NIR emission within this pH window was demonstrated over several cycles, as shown in the inset in Figure 6, indicating that the formation of the ternary complex is fully reversible both in solution and on the surface of the modified AuNPs. Consequently, relying on the fact that the Yb^{III} NIR emission could be repeatedly switched "on–off" as a function of the pH value for several cycles, AuNP-1·Yb-XO can function as a supramolecular NIR luminescent pH switch for imaging pH changes in a similar pH range as previously reported for NIR pH-activable probes or QD-NIR organic dye systems.[14]

In summary, we have developed the first example of a NIR-emitting lanthanide-based AuNP system, AuNP-1·Yb-XO. While the functionalization of the AuNPs by 1·Yb results in some quenching of the NIR emission, the latter remains sizeable in aqueous solution and in contrast to 1·Yb, requires less loading of the emitting system. Moreover, the choice of the antenna, that is, XO, allowed for long-wavelength excitation of the emitting system, up to 600 nm, which, coupled with the intense NIR emission in solution, is a particularly interesting feature for potential biomedical applications. We further demonstrate that the Yb^{III} emission arising from AuNP-1·Yb-XO can be switched "on–off" in a reversible manner over several cycles as a function of the pH value, enabling AuNP-1·Yb-XO to function as a supramolecular NIR switch. We are currently in the process of exploring the potential applications of this unique system in greater detail.

Received: February 1, 2012
Revised: July 26, 2012

Keywords: gold · lanthanides · luminescence · nanoparticles · self-assembly

---

[1] a) L. Dykman, N. Khlebtsov, *Chem. Soc. Rev.* **2012**, *41*, 2256; b) M. Montalti, N. Zaccheroni, L. Prodi, N. O'Reilly, S. L. James, *J. Am. Chem. Soc.* **2007**, *129*, 2418; c) Z. Wang, L. Ma, *Coord. Chem. Rev.* **2009**, *253*, 1607; d) E. Boisselier, D. Astruc, *Chem. Soc. Rev.* **2009**, *38*, 1759; e) O. R. Miranda, X. Li, L. Garcia-Gonzalez, Z.-J. Zhu, B. Yan, U. H. F. Bunz, V. M. Rotello, *J. Am. Chem. Soc.* **2011**, *133*, 9650.

[2] a) B. I. Ipe, K. Yoosaf, K. G. Thomas, *J. Am. Chem. Soc.* **2006**, *128*, 1907; b) D. J. Lewis, T. M. Day, J. V. MacPherson, Z. Pikramenou, *Chem. Commun.* **2006**, 1433; c) A. C. Savage, Z. Pikramenou, *Chem. Commun.* **2011**, *47*, 6431.

[3] a) J. Massue, S. J. Quinn, T. Gunnlaugsson, *J. Am. Chem. Soc.* **2008**, *130*, 6900; b) C. S. Bonnet, J. Massue, S. J. Quinn, T. Gunnlaugsson, *Org. Biomol. Chem.* **2009**, *7*, 3074.

[4] S. Comby, T. Gunnlaugsson, *ACS Nano* **2011**, *5*, 7184.

[5] a) C. P. Montgomery, B. S. Murray, E. J. New, R. Pal, D. Parker, *Acc. Chem. Res.* **2009**, *42*, 925; b) C. M. G. dos Santos, A. J. Harte, S. J. Quinn, T. Gunnlaugsson, *Coord. Chem. Rev.* **2008**, *252*, 2512; c) J.-C. G. Bünzli, *Chem. Rev.* **2010**, *110*, 2729; d) J. Hovinen, P. M. Guy, *Bioconjugate Chem.* **2009**, *20*, 404; e) J. Xu, T. M. Corneillie, E. G. Moore, G.-L. Law, N. G. Butlin, K. N. Raymond, *J. Am. Chem. Soc.* **2011**, *133*, 19900.

[6] J.-C. G. Bünzli, C. Piguet, *Chem. Soc. Rev.* **2005**, *34*, 1048.

[7] a) S. Comby, J.-C. G. Bünzli, *Handbook on the Physics and Chemistry of Rare Earths*, *Vol. 37*, Elsevier B. V., Amsterdam, **2007**, p. 217; b) J. C. G. Bünzli, S. V. Eliseeva, *J. Rare Earths* **2010**, *28*, 824; c) M. Andrews, J. E. Jones, L. P. Harding, S. J. A. Pope, *Chem. Commun.* **2011**, *47*, 206; d) S. Shinoda, H. Tsukube, *Analyst* **2011**, *136*, 431; e) S. Comby, D. Imbert, A.-S. Chauvin, J.-C. G. Bünzli, *Inorg. Chem.* **2006**, *45*, 732; f) J. Samuel, G. Tallec, P. Cherns, W. L. Ling, O. Raccurt, O. Poncelet, D. Imbert, M. Mazzanti, *Chem. Commun.* **2010**, *46*, 2647; g) H. Uh, S. Petoud, *C. R. Chim.* **2010**, *13*, 668.

[8] a) R. Weissleder, V. Ntziachristos, *Nat. Med.* **2003**, *9*, 123.

[9] a) Y. Zhong, L. Si, H. He, A. G. Sykes, *Dalton Trans.* **2011**, *40*, 11389; b) N. M. Shavaleev, R. Scopelliti, F. Gumy, J. C. G. Bünzli, *Inorg. Chem.* **2009**, *48*, 2908; c) A. Bodi, K. E. Borbas, J. I. Bruce, *Dalton Trans.* **2007**, 4352; d) R. F. Ziessel, G. Ulrich, L. Charbonnière, D. Imbert, R. Scopelliti, J.-C. G. Bünzli, *Chem. Eur. J.* **2006**, *12*, 5060; e) Y. Korovin, N. Rusakova, *J. Fluoresc.* **2002**, *12*, 159; f) M. H. V. Werts, R. H. Woundenberg, P. G. Emmerink, R. Van Gassel, J. W. Hofstraat, J. W. Verhoeven, *Angew. Chem.* **2000**, *112*, 4716; *Angew. Chem. Int. Ed.* **2000**, *39*, 4542.

[10] a) S. Faulkner, L. S. Natrajan, W. S. Perry, D. Sykes, *Dalton Trans.* **2009**, 3890; b) M. D. Ward, *Coord. Chem. Rev.* **2007**, *251*, 1663; c) W. S. Perry, S. J. A. Pope, C. Allain, B. J. Coe, A. M. Kenwright, S. Faulkner, *Dalton Trans.* **2010**, *39*, 10974; d) L. Aboshyan-Sorgho, C. Besnard, P. Pattison, K. R. Kittilstved, A. Aebischer, J. C. G. Bünzli, A. Hauser, C. Piguet, *Angew. Chem.* **2011**, *123*, 4194; *Angew. Chem. Int. Ed.* **2011**, *50*, 4108; e) A. M. Nonat, C. Allain, S. Faulkner, T. Gunnlaugsson, *Inorg. Chem.* **2010**, *49*, 8449; f) A. M. Nonat, S. J. Quinn, T. Gunnlaugsson, *Inorg. Chem.* **2009**, *48*, 4646; g) E. Di Piazza, L. Norel, K. Costuas, A. Bourdolle, O. Maury, S. Rigaut, *J. Am. Chem. Soc.* **2011**, *133*, 6174.

[11] M. Brust, M. Walker, D. Bethell, D. J. Schifrin, R. Whyman, *J. Chem. Soc. Chem. Commun.* **1994**, 801.

[12] J. P. Leonard, C. M. G. dos Santos, S. E. Plush, T. McCabe, T. Gunnlaugsson, *Chem. Commun.* **2007**, 129.

[13] a) T. Gunnlaugsson, A. J. Harte, J. P. Leonard, K. Senegal, *J. Am. Chem. Soc.* **2003**, *125*, 12062; b) T. Gunnlaugsson, D. Parker, *Chem. Commun.* **1998**, 511.

[14] a) J. Murtagh, D. O. Frimannsson, D. F. O'Shea, *Org. Lett.* **2009**, *11*, 5386; b) H. Lee, W. Akers, K. Bhushan, S. Bloch, G. Sudlow, R. Tang, S. Achilefu, *Bioconjugate Chem.* **2011**, *22*, 777; c) R. Tang, H. Lee, S. Achilefu, *J. Am. Chem. Soc.* **2012**, *134*, 4545.