Specialty area: Educational Course Molecular Imaging

Speaker Name: Silvio Aime (silvio.aime@unito.it)

Highlights:

- Determinants of the contrast in MR images
- Paramagnetic Gd-based Contrast Agents
- Systems with enhanced sensitivity

Talk Title: **Basics of Relaxation & Agents**

Target audience: Radiologists and Biologists interested to acquire the basic knowledge on the origin of the contrast in MR images and how contrast agents are designed and tested.

Outcome/Objectives: To provide the attendees with the basic principles on how the contrast is generated in a MR image and on how chemists design contrast agents for MRI applications

Purpose: To get a better understanding of the relationships between contrast enhancement, relaxivity, biodistribution and safety of Mri contrast agents.

Contrast in an MR image is determined by a complex interplay of factors that are both intrinsic to the tissue examined and of instrumental nature. The most important intrinsic factors are the proton density and the longitudinal (T_1) and transverse (T_2) relaxation times of tissue water protons. The instrumental factors, such as type of sequence used, are exploited to enhance the contrast based on the difference in T_1 or T_2. The T_1-weighted sequence, for example, is used to enhance the contrast based on differences in T_1, where the tissue with the shorter T_1 will show a more intense (usually brighter) signal in the image. On the contrary, in a T_2-weighted sequence, there is a loss of signal in the regions with short T_2 (darker in the image). The success of these techniques has been determined by the fortunate coincidence that the relaxation times are related to the biochemical environment of a given tissue, and that they are modified in the presence of a pathological process.

Contrast in MR images can be further enhanced with the administration of suitable contrast agents (CAs). The presence of the CA causes a great increase in the water proton relaxation rate, thus adding further physiological information to the already extraordinary anatomical resolution usually obtained without the CA. Therefore, contrast media are routinely used in several protocols and are particularly useful to evaluate organ perfusion, and any abnormality in the blood-brain barrier and in renal clearance. Nowadays, CAs are used in ca. 35% of MR diagnostic assays. Unlike CAs used in nuclear medicine, MRI CAs are not directly visualized

in the image. Only their effects are observed: contrast is affected by the variation that the CA causes on water proton relaxation times, and consequently, on the intensity of the NMR signal. Generally, the purpose is to reduce T_1 in order to obtain an intense signal in shorter times and a better signal-to-noise ratio with the acquisition of a higher number of measurements. CAs that decrease either T_1 or T_2 are called positive, whereas those that mainly affect T_2 are called negative. Since unpaired electrons are able to reduce markedly T_1 and T_2, the search for positive CAs is mainly oriented towards paramagnetic compounds, particularly towards paramagnetic metal complexes. The paramagnetic metal ions most extensively studied have been either in the transition metals or in the lanthanide series.

Gd(III)-based Contrast Agents

As far as lanthanides are concerned, the attention is essentially focused on Gd(III) ion both for its high paramagnetism (seven unpaired electrons) and for its favorable properties in terms of electronic relaxation.[1] This metal does not possess any physiological function in mammals, and its administration as free ion is strongly toxic even at low doses (LD_{50} 0.4 mmol/Kg)[2]. For this reason, it is necessary to use ligands that form very stable chelates.[3, 4] The high affinity shown by Gd(III) ions towards some polyaminocarboxylic acids, either cyclic or linear, has been exploited to form complexes endowed with very high stability (up to log K_{ML}> 20). The first CA approved for clinical use, Gd-DTPA (Magnevist®, Bayer Healthcare, Germany), in more than twenty-five years of clinical use has been administered to many millions of patients (see Figure 1). Other Gd(III)-based CAs similar to Magnevist have been marketed, namely Gd-DOTA (Dotarem®, Guerbet, France), Gd-DTPA-BMA (Omniscan®, GE Healthcare, USA) and Gd-HPDO3A (Prohance®, Bracco Imaging, Italy).[5] These CAs have very similar pharmacokinetic properties because they distribute in the extracellular fluid and are eliminated via glomerular filtration. They are particularly useful to delineate lesions in the blood-brain barrier. Other commercial systems include Gd-EOB-DTPA (Eovist®, Bayer Healthcare, Germany) and Gd-BOPTA (Multihance®, Bracco Imaging, Italy).[6, 7] They are Gd-DTPA derivatives endowed with an enhanced lipophilicity owing to the introduction of an aromatic substituent on the ligand surface.

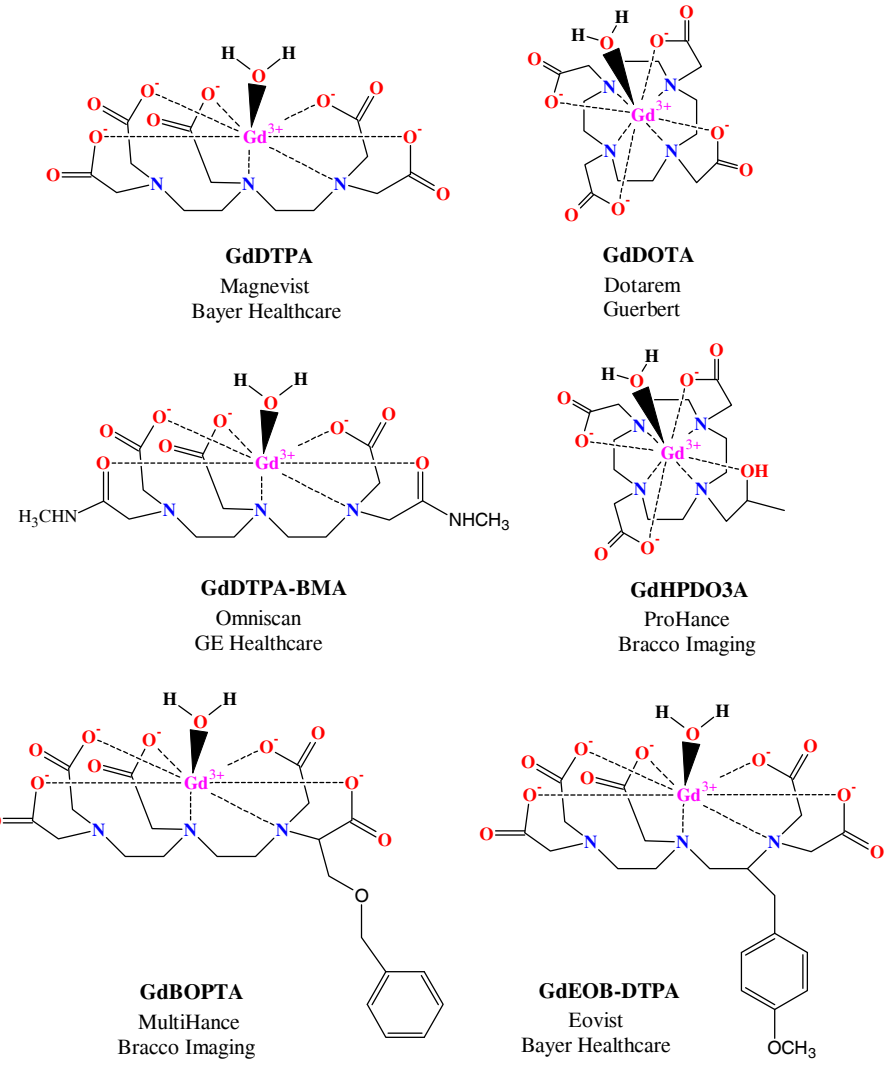

Figure 1

Determinants of the Relaxivity of Gd(III) Complexes

An MRI CA should be endowed with high thermodynamic and kinetic stability, and have at least one water molecule coordinated to the metal ion in fast exchange with the bulk water. This would permit to influence strongly the relaxation process of all protons present in the solvent in which the CA is dissolved. The Gd(III) chelate efficiency is commonly estimated in vitro through the measure of its relaxivity (r_{1p}), that for CAs as Magnevist, Dotarem, Prohance and Omniscan is around 3.4-3.5 $mM^{-1}s^{-1}$ (at 20 MHz and 37°C). The observed longitudinal relaxation rate (R_1^{obs}) of the water protons in an aqueous solution containing a paramagnetic complex is the sum of three contributions: (i) a diamagnetic one, whose value corresponds to a proton relaxation rate measured in the presence of a diamagnetic (La, Lu, Y) complex of the same ligand; (ii) a

paramagnetic one, relative to the exchange of water molecules from the inner coordination sphere of the metal ion with bulk water (R_{1p}^{is}); and (iii) a paramagnetic one relative to the contribution of water molecules that diffuse in the external coordination sphere of the paramagnetic center (R_{1p}^{os}).[8] Sometimes also a fourth paramagnetic contribution is taken into account that is due to the presence of mobile protons or water molecules (normally bound to the chelate through hydrogen bonds) in the second coordination sphere of the metal ion.[9]

The inner sphere contribution is directly proportional to the molar concentration of the paramagnetic complex, to the number of water molecules coordinated to the paramagnetic center, q, and inversely proportional to the sum of the mean residence lifetime, τ_M, of the coordinated water protons and their relaxation time, T_{1M}. This latter parameter is directly correlated to the sixth power of the distance between the metal center and the coordinated water protons and depends on the molecular reorientational time, τ_R, of the chelate, on the electronic relaxation times, T_{iE} (i=1,2), of the unpaired electrons of the metal (which depend on the applied magnetic field strength) and on the observed frequency itself. The outer sphere contribution depends on T_{iE}, on the distance of the maximum approach between the solvent and the paramagnetic solute, on the relative diffusion coefficients and, again, on the magnetic field strength. The dependence of R_{1p}^{is} and R_{1p}^{os} on the magnetic field is very important, because, from the analysis of the magnetic field dependence it is possible to assess the principal parameters characterizing the relaxivity of a Gd(III) chelate. This information can be obtained through an NMR instrument in which the magnetic field is changed (field-cycling relaxometer) to obtain the measure of R_1 over a wide range of frequencies (typically 0.01-80 MHz). At 0.5-1.5 T R_1 is generally determined by the τ_R of the chelate so that high molecular weight systems display a higher relaxivity. A quantitative analysis of R_1 dependence on the different structural and dynamic parameters shows that, for systems with long τ_R, the maximum attainable R_1 values can be achieved through the optimization of τ_M and T_{1M}.[8]

On this basis, much attention has been devoted to the design of systems characterized by long τ_R values. In principle, this task can be tackled either by designing high molecular weight systems or by pursuing the non covalent interaction of small-sized, properly functionalized Gd(III) complexes with endogenous macromolecules. In macromolecular systems the relaxation induced by paramagnetic species usually displays

remarkable changes, primarily related to the increase of the molecular reorientational time τ_R on going from the free to the bound form, which results in a marked increase of the inner sphere R_{1P}^{IS} term. For the supramolecular protein-Gd(III) complexes adduct, from the measurement of the relaxivity enhancement, it is possible to assess the affinity (and the number of binding sites) between the interacting partners.[10]

As anticipated above, high relaxivities can be attained by means of an elongation of the molecular reorientational time τ_R, i.e., dealing with slowly moving paramagnetic systems. Over the years, this prompted a number of studies on the interaction of Gd(III) complexes with proteins and other macromolecular substrates. While avoiding the use of covalent conjugates (i.e., systems based on Gd(III) chelates covalently bound to macromolecules, such as Gd-DTPA-HSA, whose metabolic fate may be problematic[11]), research activities have been addressed to design Gd(III) chelates bearing on their surface suitable functionalities that promote the reversible binding to a target-protein.[12]

Human serum albumin (HSA) has been by far the most investigated protein for binding Gd(III) chelates. Besides the attainment of high relaxivities, a high binding affinity to HSA enables the Gd(III) chelate to have a long intravascular retention time which is the property required for a good blood pool agent for MR angiography. Moreover, the presence of a good binding to HSA is useful to carry out dynamic contrast enhanced MRI (DCE-MRI) studies aimed at assessing changes in vascular permeability.[13] In blood, HSA has a concentration of about 0.6 mM and its main physiological role deals with the transport of a huge number of substrates.[14, 15] For many of them, the binding region has been identified on the basis of extensive competitive assays. The availability of the solid state X-ray crystal structure of HSA, combined to molecular modeling procedures, allows to get more insight into the structural details of the binding interaction and the corresponding relaxivity enhancement.[16-18] The information gained from the studies of the interaction of the various substrates to HSA has been very important to address the design of Gd(III) based blood pool agents (Figure 2).[16, 19, 20]

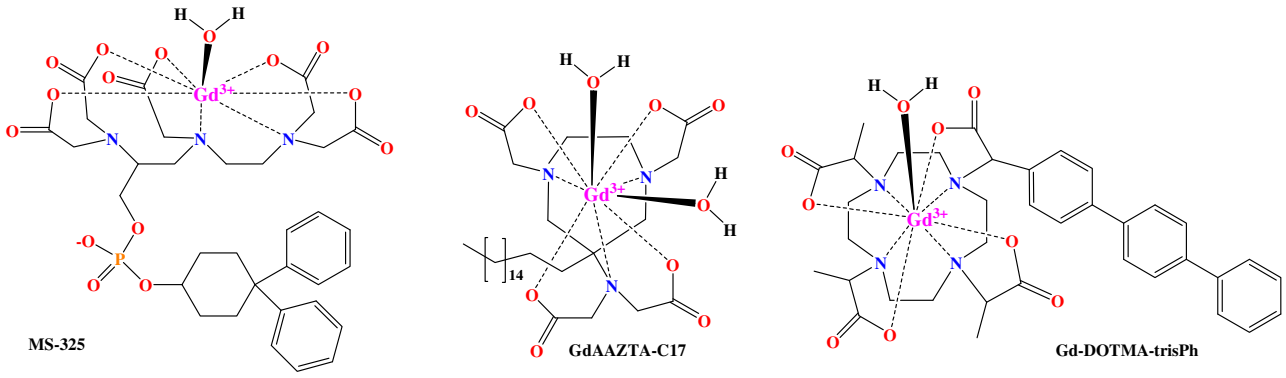

Figure 2

Gd(III)-based Contrast Agents with q>1

As underlined by the theory of paramagnetic relaxation, the number of bound water molecules has a strong effect on the relaxivity of Gd(III) complexes. A straightforward approach to increase the inner-sphere relaxivity can be pursued through the increase of the hydration number q, resulting in an increase of this contribution at any field. The use of hepta- or hexadentate ligands would, in principle, result in Gd(III) complexes with 2- and 3-coordinated water molecules, respectively, but the decrease of the ligand coordination number is likely to be accompanied by a decrease of their thermodynamic stability and an increase of their toxicity. Furthermore, systems with q = 2 may suffer a "quenching" effect upon interacting with endogenous anions or with proteins, as donor atoms from lactate or Asp or Glu residues may replace the coordinated watermolecules.[21] The commercial Gd(III)-based CAs have q=1 but some stable Gd(III) chelates containing two inner sphere water molecules have been identified and are currently under intense scrutiny.

A novel Gd(III) chelate with the heptadentate AAZTA ligand (AAZTA: 6-amino-6-methylperhydro-1,4-diazepinetetraacetic acid; see Figure 3) has been recently characterized.[22] AAZTA is readily obtained in high yields and its Gd(III) complex displays interesting properties to be considered the prototype of a new class of enhanced MRI agents. It is characterized by a quite high relaxivity value (7.1mM^{-1} s^{-1} at 20MHz and 298 K), a relatively fast exchange rate of the coordinated water molecules (τ_M = 90 ns at 298 K), a high thermodynamic stability in aqueous solution and a nearly complete inertness towards the coordination of bidentate endogenous anions.[23] Another interesting class is represented by Gd-HOPO complexes developed by Raymond and co-workers. HOPO ligands (see Figure 3) are based on 4-carboxyamido-3,2-

hydroxypyridinone chelating units and act as heptadentate ligands towards Gd(III) thus leaving two water molecules in the inner coordination sphere.[24, 25] The peculiar coordinating geometry of Gd-HOPO complexes does not allow an easy replacement of the two water molecules by other ligands. Moreover, the exchange rate of the coordinated water molecules is in the range of the optimal values as well as the electronic relaxation appears to be slow enough to allow the attainment of very high relaxivities.[26]

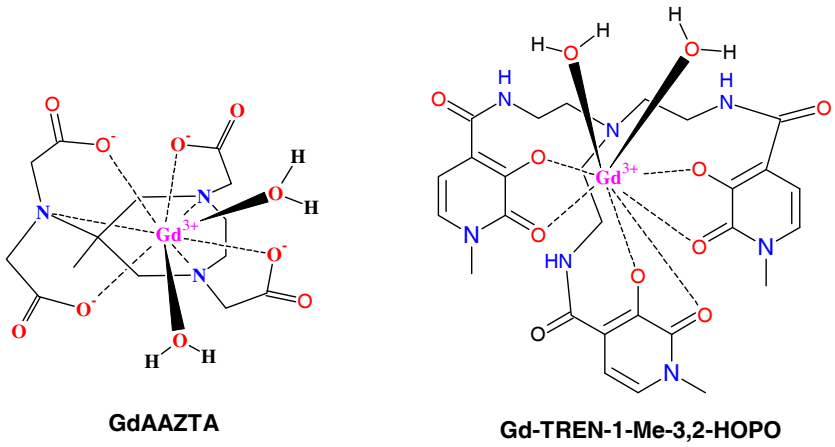

Figure 3

Nanosized Carriers

In recent years a considerable amount of systems containing a high payload of Gd(III) chelates have been reported. Most of these systems have been endowed with specific cell receptor targeting capabilities. They consist of a different kind of nanocarriers, such as micelles, liposomes, dendrimers, coupled to biological vectors such as peptides, proteins, lipoproteins, monoclonal antibodies and viral capsids.[27, 28] In general, these systems share an enhanced relaxivity and longer excretion lifetimes. Gd(III) complexes can be loaded either inside the nanovesicular carriers (like liposomes or apoferritin),[29, 30] or they can be loaded onto the outer surface of the system through covalent linkages (e.g. dendrimers) or being one of the components of the hydrophobic aggregations (micelles, liposomes, solid lipid nanoparticles).[16, 31] When properly designed, these nanosized probes may show several advantages(being minimal the level of modification introduced by the loading of the paramagnetic complexes) such as the absence of immunological reactions, limited uptake by macrophages and optimal biodistribution properties. From the relaxometric point of view, the higher efficiency of these systems is related to the restricted rotational mobility (long τ_R) of the paramagnetic

complex upon binding to the nanocarrier, even though for supramolecular adducts the presence of a long spacer may decouple the fast motion of the complex and the slow tumbling of the carrier, resulting in a reduced relaxivity. For nanovesicular carriers, where the Gd(III) complexes are encapsulated in the inner aqueous cavity, one can observe either an increase of the relaxivity (e.g. apoferritin, due to the contribution arising from the internal mobile protons of the protein)or a decrease due to the "quenching" effect, as in the case of liposomes, characterized by low water permeability. The latter disadvantage can be exploited for the visualization of drug delivery/release processes, upon the "lighting-up" of contrast when the vesicle is destroyed.

Concerning the exploitation of natural nanosized carriers, a nice example is represented by apoferritin, a protein devoted to the storage of iron in cells. Ferritin consists of 24 proteins that self-assemble by means of saline and hydrogen bonds to yield a spherical aggregate containing the iron core, displaying 10 channels for communicating between the inner and outer compartments. A method has been reported to replace the iron core in the inner cavity of ferritin with up to 8–10 Gd-HPDO3A molecules per apoferritin. Very interestingly, the relaxivity of Gd-HPDO3A entrapped in the apoferritin cavity shows a relaxivity that, at 20 MHz, is ca. 20 times higher than that of the free Gd-HPDO3A complex.[30] The presence of a high number of ferritin transporters on hepatocytes allows to the Gd-loaded apoferritin system to be quickly taken up by liver. The synthesis of a Gd-loaded apoferritin derivative containing biotinylated residues on its surface, coupled with the use of a targeting peptide recognized by avidin, has allowed the MRI visualization of the overexpression of NCAM (neural cell adhesion molecule) epitopes in neo-formed tumor endothelia.[32]

References

1. P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem Rev*, 1999, **99**, 2293-2352.
2. M. F. Tweedle, G. T. Gaughan, J. Hagan, P. W. Wedeking, P. Sibley, L. J. Wilson and D. W. Lee, *Int J Rad Appl Instrum B*, 1988, **15**, 31-36.
3. E. Brucher and A. D. Sherry, *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, First edn., Wiley, Chirchester, 2001.
4. J.-C. G. Bünzli and G. R. Choppin, *Lanthanide probes in life, chemical, and earth sciences : theory and practice*, First edn., Elsevier, Amsterdam, 1989.
5. H.-J. Weinmann, A. Mühler and B. Radüchel, *Methods in Biomedical Magnetic Resonance Imaging and Spectroscopy*, 1 edition edn., Wiley, 2000.
6. F. Uggeri, S. Aime, P. L. Anelli, M. Botta, M. Brocchetta, C. d. Haeen, G. Ermondi, M. Grandi and P. Paoli, *Inorg Chem*, 1995, **34**, 10.
7. H. Schmitt-Willich, M. Brehm, C. L. Ewers, G. Michl, A. Muller-Fahrnow, O. Petrov, J. Platzek, B. Raduchel and D. Sulzle, *Inorg Chem*, 1999, **38**, 1134-1144.

8. E. Toth, L. Helm and A. E. Merbach, *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, First edn., Wiley, Chirchester, 2001.
9. M. Botta, *European Journal of Inorganic Chemistry*, 2000, 399-407.
10. R. A. Dwek, *Nuclear Magnetic Resonance in Biohemistry; Applications to Enzyme Systems*, First edn., Clarendon Press, Oxford, 1973.
11. U. Schmiedl, M. Ogan, H. Paajanen, M. Marotti, L. E. Crooks, A. C. Brito and R. C. Brasch, *Radiology*, 1987, **162**, 205-210.
12. S. Aime, M. Chiaussa, G. Digilio, E. Gianolio and E. Terreno, *J Biol Inorg Chem*, 1999, **4**, 766-774.
13. P. Farace, F. Merigo, S. Fiorini, E. Nicolato, S. Tambalo, A. Daducci, A. Degrassi, A. Sbarbati, D. Rubello and P. Marzola, *Eur J Radiol*, 2011, **78**, 52-59.
14. S. Curry, *Drug Metab Pharmacokinet*, 2009, **24**, 342-357.
15. J. Ghuman, P. A. Zunszain, I. Petitpas, A. A. Bhattacharya, M. Otagiri and S. Curry, *J Mol Biol*, 2005, **353**, 38-52.
16. E. Gianolio, G. B. Giovenzana, D. Longo, I. Longo, I. Menegotto and S. Aime, *Chemistry*, 2007, **13**, 5785-5797.
17. S. Avedano, L. Tei, A. Lombardi, G. B. Giovenzana, S. Aime, D. Longo and M. Botta, *Chem Commun (Camb)*, 2007, 4726-4728.
18. S. Aime, E. Gianolio, D. Longo, R. Pagliarin, C. Lovazzano and M. Sisti, *Chembiochem*, 2005, **6**, 818-820.
19. P. Caravan, N. J. Cloutier, M. T. Greenfield, S. A. McDermid, S. U. Dunham, J. W. Bulte, J. C. Amedio, Jr., R. J. Looby, R. M. Supkowski, W. D. Horrocks, Jr., T. J. McMurry and R. B. Lauffer, *J Am Chem Soc*, 2002, **124**, 3152-3162.
20. E. K. Yucel and R. B. Lauffer, *Seminars in Interventional Radiology*, 1998, **15**, 215-222.
21. S. Aime, E. Gianolio, E. Terreno, G. B. Giovenzana, R. Pagliarin, M. Sisti, G. Palmisano, M. Botta, M. P. Lowe and D. Parker, *J Biol Inorg Chem*, 2000, **5**, 488-497.
22. S. Aime, L. Calabi, C. Cavallotti, E. Gianolio, G. B. Giovenzana, P. Losi, A. Maiocchi, G. Palmisano and M. Sisti, *Inorg Chem*, 2004, **43**, 7588-7590.
23. Z. Baranyai, F. Uggeri, G. B. Giovenzana, A. Benyei, E. Brucher and S. Aime, *Chemistry*, 2009, **15**, 1696-1705.
24. S. M. Cohen, J. Xu, E. Radkov, K. N. Raymond, M. Botta, A. Barge and S. Aime, *Inorg Chem*, 2000, **39**, 5747-5756.
25. E. J. Werner, S. Avedano, M. Botta, B. P. Hay, E. G. Moore, S. Aime and K. N. Raymond, *J Am Chem Soc*, 2007, **129**, 1870-1871.
26. C. J. Jocher, E. G. Moore, J. Xu, S. Avedano, M. Botta, S. Aime and K. N. Raymond, *Inorg Chem*, 2007, **46**, 9182-9191.
27. W. Y. Huang and J. J. Davis, *Dalton Trans*, 2011, **40**, 6087-6103.
28. A. Louie, *Chem Rev*, 2010, **110**, 3146-3195.
29. D. A. Sipkins, D. A. Cheresh, M. R. Kazemi, L. M. Nevin, M. D. Bednarski and K. C. Li, *Nat Med*, 1998, **4**, 623-626.
30. S. Aime, L. Frullano and S. Geninatti Crich, *Angew Chem Int Ed Engl*, 2002, **41**, 1017-1019.
31. I. R. Corbin, H. Li, J. Chen, S. Lund-Katz, R. Zhou, J. D. Glickson and G. Zheng, *Neoplasia*, 2006, **8**, 488-498.
32. S. Geninatti Crich, B. Bussolati, L. Tei, C. Grange, G. Esposito, S. Lanzardo, G. Camussi and S. Aime, *Cancer Res*, 2006, **66**, 9196-9201.