New Reagents for Detecting Free Radicals and Oxidative Stress

Journal:	*Organic & Biomolecular Chemistry*
Manuscript ID:	OB-REV-06-2014-001172.R1
Article Type:	Review Article
Date Submitted by the Author:	14-Jul-2014

Complete List of Authors:	Barzegar Amiri Olia, Mina; The University of Melbourne, School of Chemistry Taylor, Michelle; The University of New England, Division of Chemistry Schiesser, Carl; The University of Melbourne, School of Chemistry

Journal Name

RSCPublishing

ARTICLE

New Reagents for Detecting Free Radicals and Oxidative Stress.

Cite this: DOI: 10.1039/x0xx00000x

Mina Barzegar Amiri Olia,[a,b] Carl H. Schiesser*[a,b] and Michelle K. Taylor,[a,c]

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Free radicals and oxidative stress play important roles in the deterioration of materials, and free radicals are important intermediates in many biological processes. The ability to detect these reactive species is a key step on the road to their understanding and ultimate control. This short review highlights recent progress in the development of reagents for the detection of free radicals and reactive oxygen species with broad application to materials science as well as biology.

Introduction

Free radicals and oxidative stress play significant roles in the deterioration of materials that range from polymers through to biomolecules and have been implicated in many diseases. The ability to readily detect free radicals and oxidative stress is therefore a crucial endeavour and the development of reagents, sensors and devices to achieve that end has become the focus of many research groups around the world.

Several reviews have been written on this topic over the past few years.[1-3] The purpose of this short review article is to showcase recent progress toward the development of reagents for the detection of free radicals and oxidative stress since the most recent reviews in the field. It is not intended to be comprehensive, rather to provide a "flavour" for this burgeoning field. It is deliberately limited to recent developments in methods for the detection of nitric oxide, an increasingly important biologically-relevant molecule,[1] to free radicals and oxidative stress in general through the emerging and maturing field of nitroxide (aminyloxyl) radical chemistry,[2] and through the use of novel metal-based techniques.[3] While the chemistry discussed in this review is relevant to a wide cross-section of applications, this article will generally limit these applications to those of biological relevance.

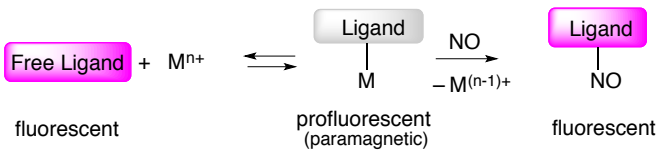
Detection of nitric oxide

Nitric oxide (NO) is a well-established ubiquitous signalling molecule.[4] It is involved in a variety of physiological and pathological pathways and is a key vertebrate messenger that acts in multiple mechanisms.[4]

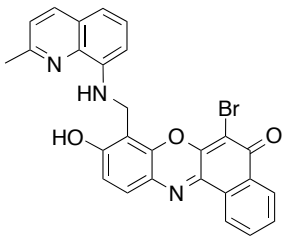
There has been considerable recent effort directed toward further understanding the role that nitric oxide plays in biology and the design and development of molecular probes is crucial to achieve that end. A variety of quantification methods have been developed to date,[5] and these include chemiluminescence,[6] colourimetry,[7] electron spin resonance (ESR),[8] electrochemistry[9] and fluorimetry.[10] Among these techniques, the fluorescence technique has distinct advantages over the remaining because of high sensitivity, convenience and high spatiotemporal resolution.

Approaches for the detection of nitric oxide often rely on transition metal complexes that are either paramagnetic and quench the fluorescence of a bound fluorophore, or make use of the heavy atom effect to achieve the same outcome. In both cases, upon reaction with NO, the metal is released and fluorescence is restored as depicted in Scheme 1. This chemistry effectively provides an "on/off sensor" for the presence of NO and can readily be applied to biological systems.

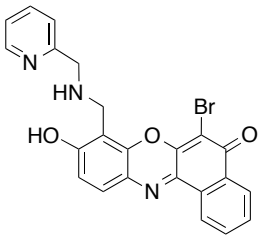
Lippard and coworkers recently reported a number of probes based on this principle; examples include the benzoresorufin probes **1, 2**.[11] These probes are the latest in a series that have been developed over a number of years.[12]



Scheme 1.

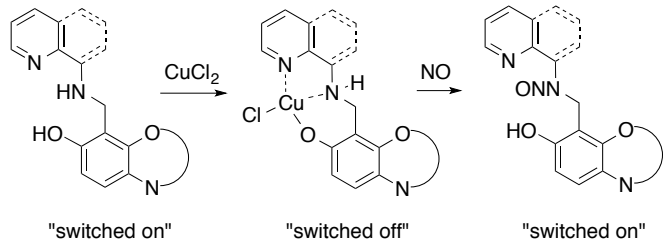


1



2

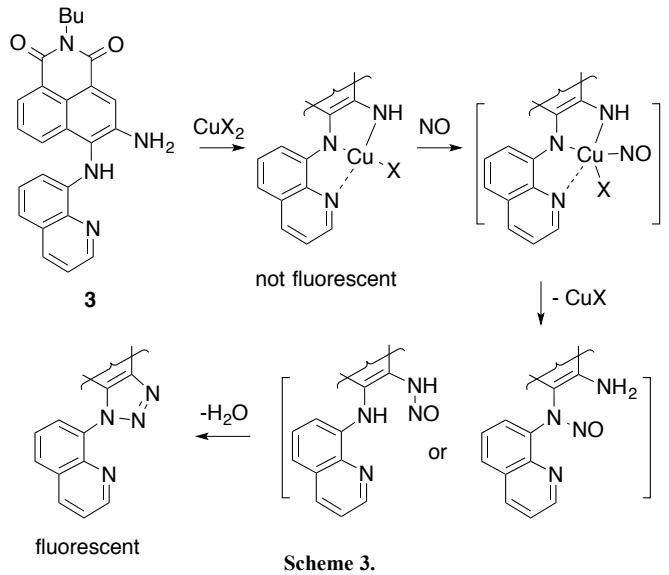
The fluorescence of these probes is effectively quenched by the addition of $CuCl_2$ and reactivated by the subsequent reaction with nitric oxide (Scheme 2).



Scheme 2.

Qian and coworkers reported a copper-promoted NO probe **3** based on the *o*-phenylenediamine structure.[13] While based on a similar principle to the probes developed by Lippard, the mechanism of action is different (Scheme 3).

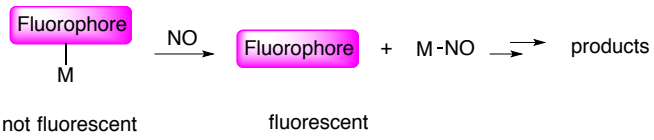
Another series of direct-detection probes is based on transition metal complexes with coordinated fluorophores that are released upon reaction with NO (Scheme 4). While there appears to be no recent work reported, the example of Lippard is included for completeness (Scheme 5).[14]



3

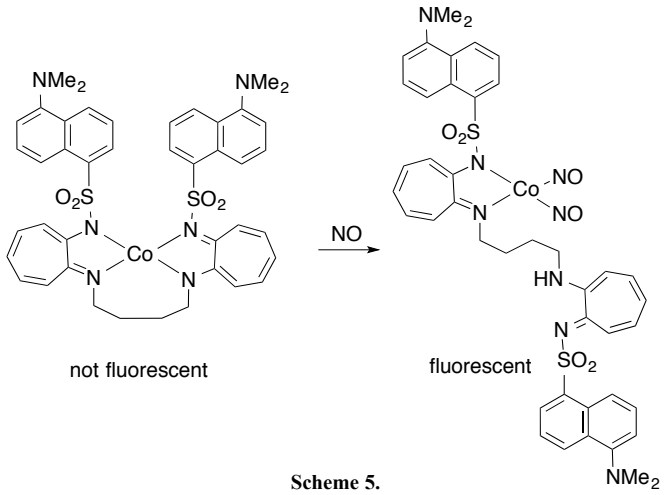
Scheme 3.

In 2009, Strano and coworkers demonstrated that a diaminophenyl-functionalised dextran polymer enables the rapid and direct response of single-wall nanotubes (SWNTs) to nitric oxide.[15] Since this "early" discovery, this group went on to prepare fluorescent SWNT-based sensors comprised of

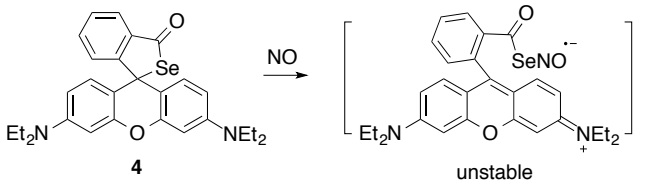


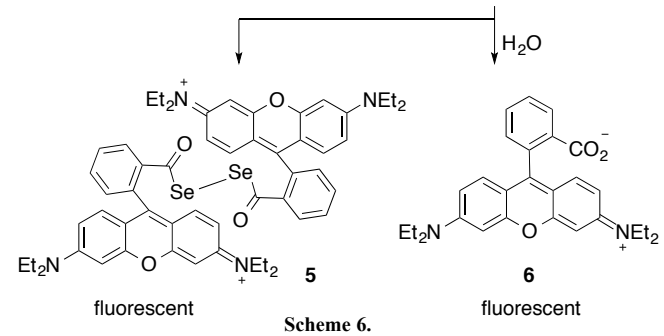
Scheme 4.

single-stranded d(AT)15 DNA oligonucleotide-encased nanotubes (AT15-SWNT).[16] The distinguishing feature of these structures is that they exhibit an intense fluorescence signal at near-infrared wavelengths (900 – 1400 nm) and the DNA sequence in AT15-SWNTs is able to detect NO selectively when compared with other polymer-SWNT complexes.



Scheme 5.



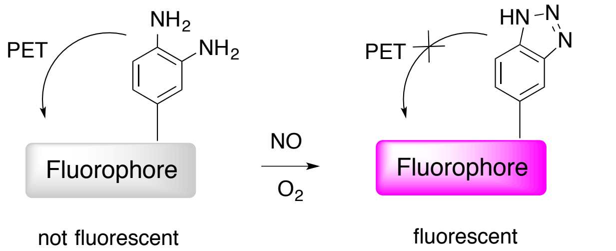


Scheme 6.

Ma and coworkers introduced a new strategy for the detection of nitric oxide that relies on the interaction of NO with selenium.[17] Spiroselenide **4** is based on rhodamine B and is weakly fluorescent. In the presence of NO, however, the selenium atom undergoes homolytic attack with subsequent ring opening to produce two possible fluorescent compounds **5** or **6**, depending on reaction conditions (Scheme 6).

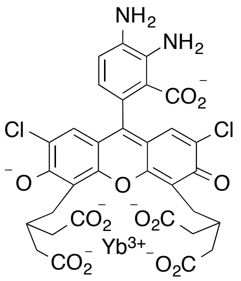
Other methods for the detection of NO rely on achieving fluorescence through photo-induced electron transfer (PET) or fluorescence resonance energy transfer (FRET) chemistry after

reaction of a suitable moiety with NO within the probe, often an *o*-phenylenediamine group.[18] The general principle of this technique for a PET promoted probe is shown in Scheme 7. Luminescent lanthanide complexes such as **7** incorporating Yb^{3+} or Nd^{3+} appear to be particularly effective, especially since they possess long luminescent lifetimes (ms) and have large Stokes shifts (> 300 nm).[19]

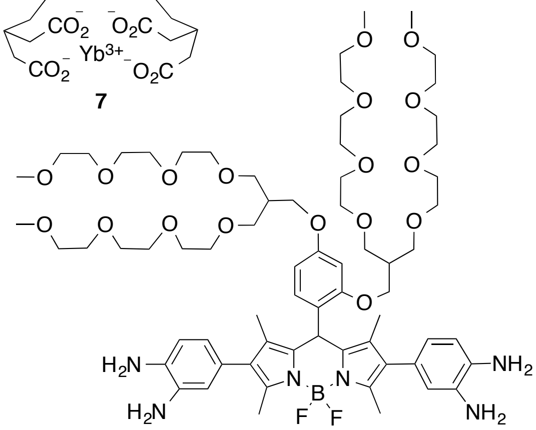


Scheme 7.

Recently, probes based on BODIPY (4,4'-difluoro-4-bora-3a,4a-diaza-s-indacene) have also been popular choices for the detection of NO.[3] For example, Liu reported a series of probes with emissions ranging from green to infrared with **8** being a typical water-soluble version.[20] Similarly, Wang and coworkers reported a B,O-chelated BODIPY analogue, BOBP **9** that fluoresces in the near infrared (654 nm) with high quantum yield.[21]

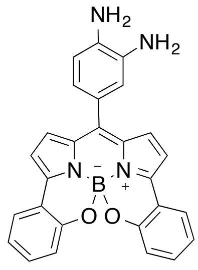


7

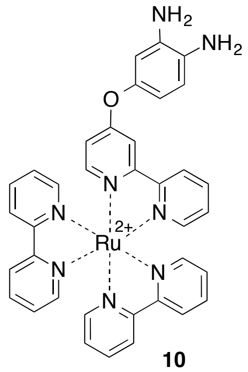


8

An increasingly important class of luminescent sensing materials contain transition metals such as ruthenium, rhenium and iridium.[22] Among their desirable properties are intense visible excitations and emissions, high photo, thermal and chemical stabilities, and very low cytotoxicity which makes them ideal for biological application.[23] An example of this class of compound is **10**, a ruthenium complex reported by Yuan.[24] Complex **10** is essentially non fluorescent and shows a remarkable enhancement in luminescent properties upon reaction with nitric oxide under aerobic conditions; **10** was also shown not to react with other reactive oxygen or nitrogen species.

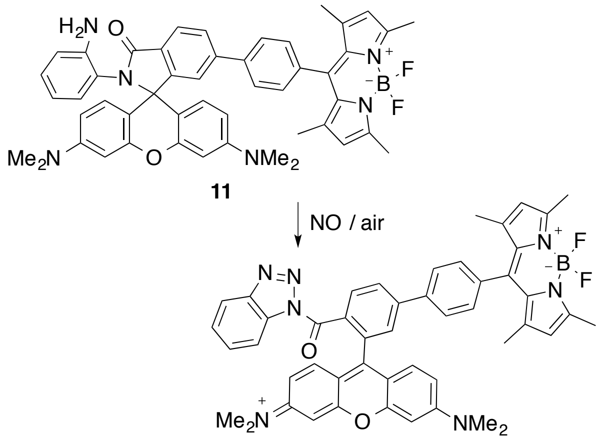


9



10

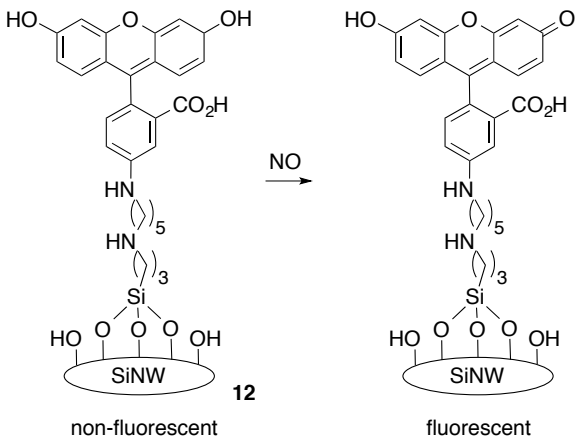
More recently a new generation of NO sensitive reagents based on ratiometric analysis have been developed. Ratiometric probes are small molecule reagents that normally function in a mixture of water and an organic solvent, and that have dual emission wavelengths.[25] One of these emissions serves as the control and is present in the reagent itself, while the other gets "switched on" through reaction with nitric oxide. In this manner, a concentration-independent outcome is possible. An example is the BODIPY-like reagent **11** (Scheme 8) in which a second emission becomes clearly evident after reaction with NO under aerobic conditions.[26]



Scheme 8.

An effective strategy for improving the selectivity and the stability of the fluorophore required for the detection of NO is through immobilisation of the reactive molecules into a matrix. Shi and coworkers developed such a sensor (**12**) by covalently immobilising reduced fluorescein molecules onto the surface of silicon nanowires (SiNWs).[27] This probe not only proved to exhibit high sensitivity and selectivity for NO, but also responded rapidly to NO released from liver extract exhibiting a linear relationship between fluorescence intensity and nitric oxide concentration (Scheme 9).

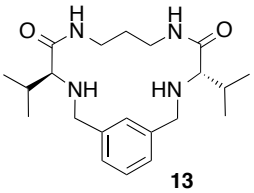
"Carbon dots" (CDs) are a new class of fluorescent nanoparticle that have attracted growing interest in recent years



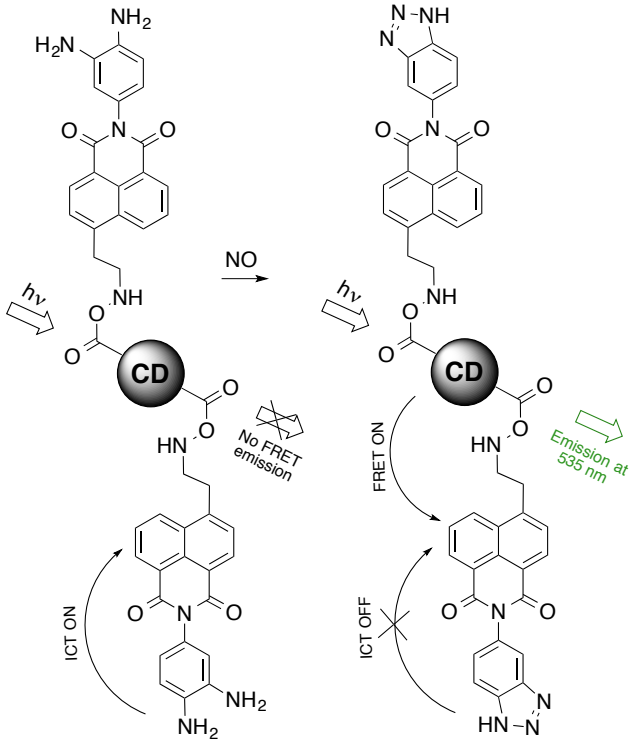
Scheme 9.

because of their alluring physical, chemical and photoluminescent properties.[28] They are reported to have excellent water solubility, biocompatibility, cell membrane permeability, tunable surface functionality, as well as excellent photostability.[28] Recently Wu et al. reported a CD-based nanosensor for the ratiometric determination of nitric oxide (Scheme 10).[29]

The past decade has also seen the development of fluorescent organogels for the detection of NO. This is an important development because of potential applications that include optoelectronic devices and sensors.[30] One of the most reliable strategies for the preparation of fluorescent organogels involves the use of hybrid organic/inorganic materials. Examples include nanoparticle-gel composites based on the interaction of metal nanoparticles with self-assembled fibrillar networks, and hybrid materials that use intrinsically fluorescent semiconductor nanoparticles (quantum dots, QDs).[31,32] For example, Luis and coworkers described the preparation of a new type of soft material that comprises an organogel and a fluorescent CdSe/ZnS QD that is sensitive to nitric oxide. Pseudo-peptidic macrocycle **13** was used to prepare the hybrid organogel which exhibited excellent optical transparency.[33] The resultant material proved to be sensitive to NO concentrations that ranged from 0.05 to 0.50 %vol. Remarkably, this organogel was reported to remain in tact after exposure to nitric oxide. While far from a practical application, this system can be considered to be proof-of-concept for new soft material sensing devices.



13

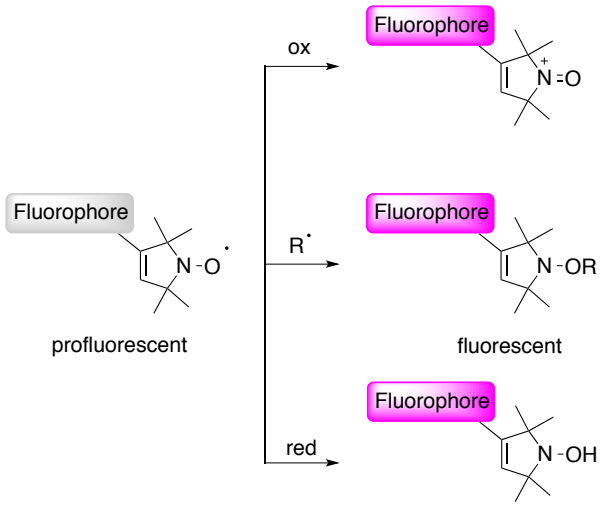


Scheme 10.

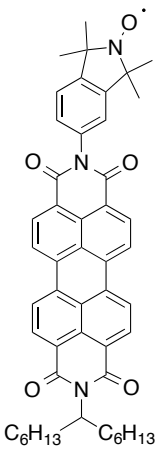
General Detection of Free Radicals and Oxidative Stress

(1) Aminyloxyl radicals (nitroxides)

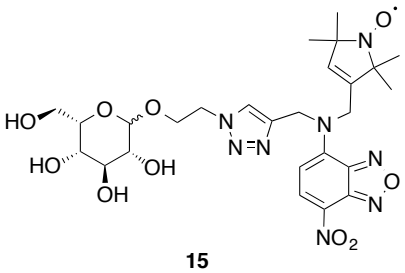
The use of profluorescent nitroxides to detect free radicals and oxidative stress is a well-established area of research. A recent review by Bottle and coworkers describes the basic principles and provides several key examples.[2] A critical element of this technology is the paramagnetic nature of the probe which, like the metal-based reagents described above, is in a "switched off" (profluorescent) state until the unpaired



Scheme 11.

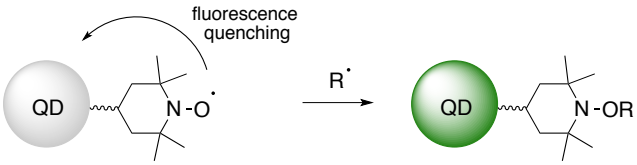


14



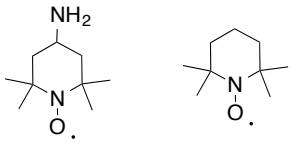
15

electron is quenched either through coupling with another radical species, or through redox chemistry (Scheme 11).[2] As a consequence, profluorescent nitroxides are excellent, sensitive probes for the detection of oxidative change and free radicals in a wide range of applications ranging from materials through to biology. Recent molecular probes based on this technology include the perylene-substituted nitroxide **14** that was used to explore the thermal degradation of polyester resins,[34] and the water-soluble system **15** developed for the detection of radicals and oxidative stress in biofilms.[35] Other probes have been developed that effectively monitor mitochondrial redox reactions.[36,37]



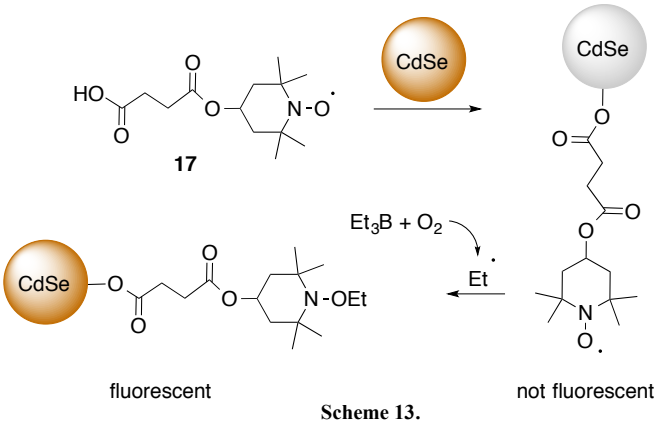
Scheme 12.

There has been some considerable interest in expanding the structures of these probes to include non-organic fluorophores. In that regard, quantum dots and carbon dots appear to provide excellent alternative fluorescence sources. The use of QDs in organic chemistry was reviewed in 2009.[38] Unlike the mechanism responsible for fluorescence quenching in conventional (organic) fluorophores,[2] stable radicals such as nitroxides effectively quench the fluorescence of QDs through a mechanism involving electron transfer from the nitroxide moiety to the tethered nanoparticle. Importantly, fluorescence is restored upon reaction with a radical (Scheme 12).



16 TEMPO

Scaiano demonstrated that 4-amino-TEMPO (**16**) was effective at quenching CdSe QDs, whereas TEMPO itself

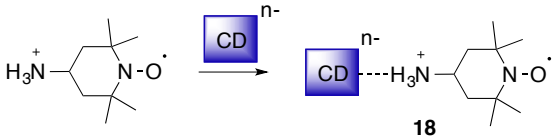


Scheme 13.

proved to be significantly less effective, presumably because of the superior ability of **16** to bind to the surface of the QD.[39]

Braslau showed that carboxylic acid derivatives of TEMPO, such as **17** are effective quenchers of 3.7nm (orange) CdSe QD fluorescence emission.[40] Addition of Et_3B and air to the quenched QD solution results in the restoration of strong fluorescence after 60 minutes (Scheme 13). A few years later Tang and co-workers described the use of CdTe QDs functionalised by **16** for the detection of nonprotein thiols based on electron transfer.[41]

Recently, Guo et al. reported that nitroxide radicals **16** can also quench the fluorescence of blue CDs. Although the mechanism of fluorescence of these nanoparticles is not fully understood, it is believed that quenching in these particles also occurs through an electron transfer process.[42] An important difference between this work and the QD work described above is that the "CD@TEMPO" conjugates **18** are formed through electrostatic interactions between the ammonium salt of **16** and the negatively charged blue CD (Scheme 14).[42] These conjugates are "promising bimodal responsive receptors" that have off-on fluorescence and on-off ESR signalling for the detection of antioxidants and carbon-centred radicals.[42]



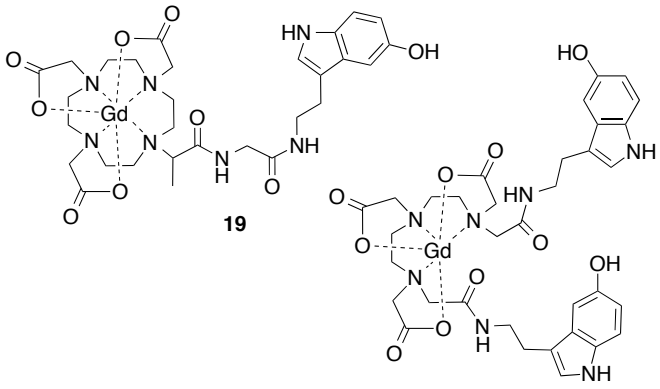
Scheme 14.

(2) MRI contrast agents.

The vast majority of sensors for the detection of reactive oxygen species (ROS) rely on fluorescence or luminescence responses (*vide supra*). While many of these techniques offer advantages in selectivity, sensitivity and spacial resolution, a significant limitation can be the relatively short wavelength of radiation required to excite fluorescence, resulting in poor tissue penetration impeding their use in *in vivo* applications.[43-48] In contrast, magnetic resonance imaging (MRI) is a widely used *in vivo* imaging technique providing high spacial resolution and soft tissue information. Contrast in MRI is largely dependent

on differences in the relaxation times of protons in water molecules and contrast agents (CAs) are often employed to improve image quality through interations with water molecules that alter relaxation times. Metal ions with multiple unpaired electrons are at the core of the majority of CAs, with Gd(III) the most widely used, however Mn(II) is an alternative that has received considerable attention.[48-51]

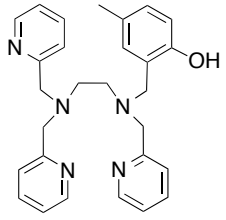
Over the years, significant advances have been made in the design of functional MRI contrast agents whose relaxivity or mobility is altered in response to local physiological conditions such as pH, O_2 pressure and enzyme concentration.[49,52,53] Although very few contrast agents directly responsive to ROS have been developed to date, interest in redox responsive probes and non-invasive methods of imaging the inflammatory process are emerging areas.[48,51,54-65]



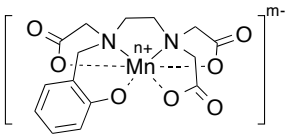
19

20

One contrast agent that has been used successfully in numerous studies does so by responding to the presence of Myeloperoxidase (MPO), a key biomarker for inflammation, and hydrogen peroxide.[54] Initial studies focused on **19** in which the active site was covalently linked to serotonin.[55] The phenolic moiety acts as a substrate for the enzyme and are oxidised to the corresponding radicals that can either oligomerise or bind to matrix proteins effectively increasing the size of the CA, decreasing rotational correlation times and increasing relaxivity. Further development led to the Gd(III) probe **20** which has been shown to increase MRI signals in the presence of the enzyme and hydrogen peroxide, effectively enabling the non-invasive tracking of active MPO in tissues.[56]



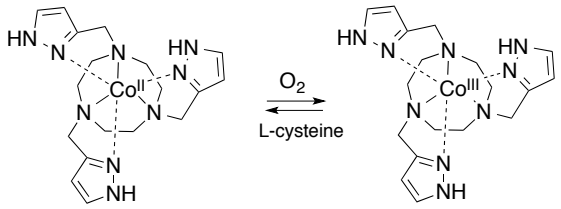
21



22

The bis-serotonin derivative **20** proved to have low toxicity and has been used to track the inflammatory response in stroke, myocardial ischemia, atherosclerotic plaques and vasculitis.[56-59]

Yu and co-workers recently reported the synthesis of a CA in which the redox-active Hptp1 ligand **21** was complexed to Mn(II). The isolated $[Mn(Hptp1)(MeCN)]^{2+}$ MRI contrast agent proved to be responsive to H_2O_2 and exploited a similar phenolic coupling reaction to that of **20** but was independent of MPO.[48]



23 MRI-active

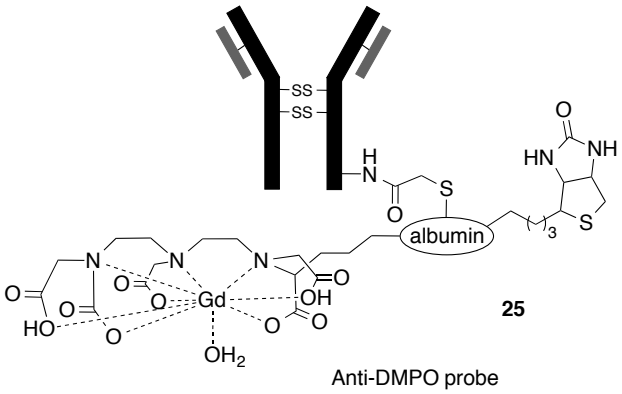
24 MRI-silent

Scheme 15.

In contrast to the approaches described above that rely on the redox activity of the ligand, there are also examples of probes that exploit the redox activity of the metal centre. Redox responsive agents of this type require the redox potential of the metal centre to be matched to that of the biological process being investigated. In addition, a significant change in relaxivity is also required, and ligands must be designed to accommodate the metal in several oxidation states. While Lanthanides such as europium appear to be well suited to this chemistry, the difficulties in stabilising and controlling the redox potentials of these metals has resulted in a shift of interest toward more traditional transition metals.

Loving et al. reported recently a manganese-based active MRI probe based on a reversible Mn(II)/Mn(III) couple that exploits the difference in relaxivity between Mn(II) (high relaxivity) and Mn(III) (low relaxivity).[51] The hexadentate hydroxybenzylethylenediamine triacetic acid (HBET) ligand was found to stabilise both Mn(II) and Mn(III) providing complexes **22** with biologically relevant redox potentials and accompanying significant changes in relaxivity.

One of the limitations of the Mn(II)/Mn(III) couple is that large differences in relaxivities between oxidation states would not ordinarily be expected at clinically relevant frequencies. This prompted Morrow to investigate the use of a paramagnetic/diamagnetic Co(II)/Co(III) couple **23**/**24** possessing the TPT ligand that enables the switch between MRI-active and MRI-silent states (Scheme 15). Complex **23** proved to be redox tuneable in the required biological range.[60] The use of the TPT ligand that possesses highly exchangeable pyrazole NH protons enable the expoitation of the efficiency of

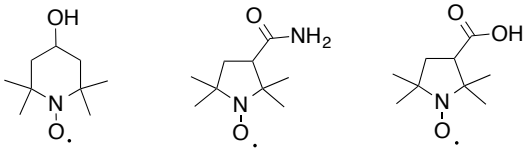


25 Anti-DMPO probe

Co(II) as a chemical shift agent.[60]

Very recently, Towner and coworkers combined immuno-spin trapping with MRI in order to detect *in vivo* trapped radicals in the spinal cords of $SOD1^{G93A}$-transgenic mice, a model for amyotrophic lateral sclerosis (ALS).[65] Using DMPO (5,5-dimethyl-1-pyrrolidine-N-oxide) to scavenge radicals, the anti-DMPO probe **25** was effectively used to detect DMPO-radical adducts through a significant sustained increase in MRI signal intensities.

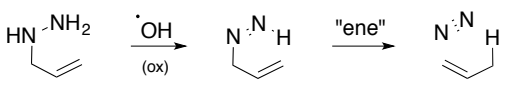
Nitroxides (aminyloxyl radicals) have proven to be effective contrast agents for use with MRI and have found to be useful for the probing of the intracellular redox status of tumours.[66-68] For example, Hyodo and co-workers showed that cell-permeable nitroxides such as TEMPOL and 3-carbamoyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl **26** showed faster decay in tumour tissue when compared to the cell-impermeable 3-carboxy-2,2,5,5-tetramethylpyrrolidine-1-oxyl **27**.[67] In a further example, Ustimi and co-workers were able to use isotopically-labelled nitroxides to simultaneously monitor both oxidation and reduction processed in cells through nOe-enhanced MRI imaging.[68]



(3) Ultrasound contrast agents.

Ultrasound is a commonly used inexpensive medical imaging technique that utilises the reflection of echoes from tissues with different acoustic impedances to provide real-time images. The use of microbubble based ultrasound contrast agents (UCAs), alongside significant advances in technology, has resulted in the widespread use of contrast-enhanced ultrasound in diagnostic imaging. Early microbubble contrast enhanced ultrasound involved the introduction of a continuous stream of short-lived gas microbubbles into the bloodstream, however modern methods employ preformed microbubbles consisting of a gas enclosed within a lipid, protein or polymer shell. While the majority of the clinical applications focus on blood pool, echocardiology and hepatology, a rapidly growing area of research is in the development of site directed UCAs that incorporate targeting ligands on the surface of the shell. These ligands, including monoclonal antibodies, peptides and biomolecules specific to particular cell surface receptors are selected to respond to specific biomarkers/cell surface receptors and result in site-specific accumulation of the UCAs.[69-72]

Recent studies have exploited the high sensitivity of microbubble UCAs to detect ROS at clinically relevant concentrations.[73,74] The detection of ROS cannot be achieved through the use of conventional ligand targeted UCAs and as such these methods are designed such that the ROS generate microbubbles *in situ* in response to the presence of target substrates via chemical reactions. Pioneered by Perng,[73] liposome encapsulated allylhydrazine was used to generate gas bubbles, detectable by ultrasound imaging, in the presence of free radicals, both *in vitro* and *in vivo*.[73] In the example shown in Scheme 16, oxidation of allyl hydrazine by hydroxyl radicals affords both gaseous nitrogen and propene. The ability of these liposome-encapsulated allylhydrazines to detect ROS *in vivo* was demonstrated in mice using a lipopolysaccharide induced inflammatory response.[73]

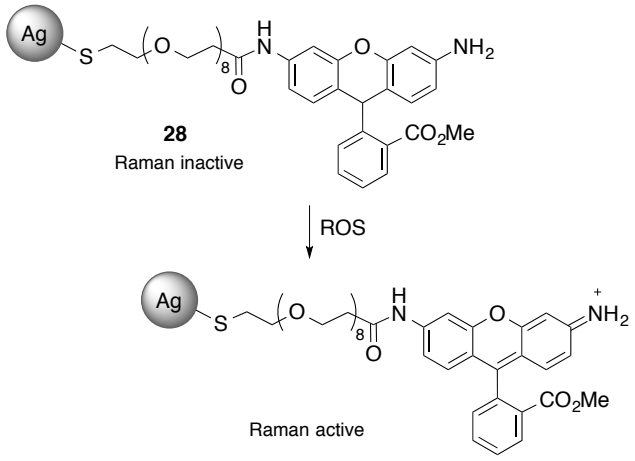


Scheme 16.

An alternative approach for the detection of ROS by ultrasound was inspired by the ability of tubular micromotor converters (MMCs) to use H_2O_2 as a fuel for propulsion.[74] These conical structures posses an inner coating of either platinum or a dual layer of catalase over gold that catalyses the conversion of H_2O_2 to O_2; the bubbles produced providing propulsion and a trail of microbubbles. Olson and co-workers successfully demonstrated the ability of ultrasound to detect the microbubbles generated from Pt coated tubular MMCs, showing that this technique has potential to be used in ultrasound imaging. As the motion of these converters is not important for imaging, spherical nanospheres consisting of concentric shells of catalase and poly(sodium styrenesulfonate) were selected as alternatives. These triple layer nanospheres were subsequently shown to generate oxygen microbubbles that could be detected by ultrasound imaging to detect H_2O_2 in *ex vivo* studies using activated neutrophils as well as in an *in vivo* model of abscess in rats.[74]

Recently Prussian blue nanoparticles (PBNs) have also been reported as potential ROS imaging agents. Significantly, these particles have the ability to detect ROS in both ultrasound as well as MRI imaging.[75] These PBNs are stabilised by a biocompatible poly(vinylpyrrolidone) polymer coating that ensures particle dispersion, aids solubility, and can be used to control particle size.[76] These PBNs possess catalase-like abilities and were found to catalyse the breakdown of H_2O_2 resulting in the formation of microbubbles detectable by ultrasound imaging in a similar manner to that reported by Olson.[74]

The potential of PBNs in MRI has been previously discerned; the relaxivity of these particles arises through five unpaired electrons per (Fe^{2+}–CN–Fe^{3+}) unit and the fact that some of the Fe^{3+} centres are accessible to water enhances T_1 relaxation.[77] This recent work showed an increased T_1 image enhancement in the presence of both ROS and PBN compared to PBN alone demonstrating that both the PBN and the paramagnetic O_2 microbubbles assist T_1 relaxation and, as a consequence, the MRI enhancement is also ROS responsive. In recent years there has been a surge of interest in multimodal imaging technology that combines complimentary abilities of different techniques.[78] The ability of these probes to act as contrast agents for the detection of ROS using both ultrasound and MRI makes them particularly attractive.



Scheme 17.

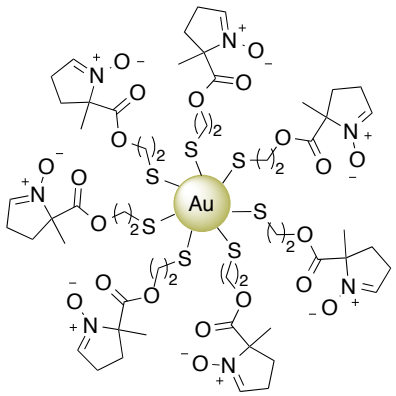
(4) Nanoparticle-based sensors

The use of nanoparticles in sensors and imaging is a rapidly growing area of interest. Several nanoparticle-based approaches for the direct detection of reactive oxygen species have already been mentioned (*vide supra*), and nanoparticle and nanotube approaches to ROS sensing were reviewed recently by Hempel and Uusitalo.[79]

Recent advances of note include the ROS responsive surface enhanced Raman spectroscopy (SERS) probe **28** developed by Zhang,[80] as well as the enhanced sensing ability of nanoparticle mounted spin traps to detect radicals reported by Liu.[81]

The Raman inactive dihydrorodhamine dye in **28** can be oxidised by a variety of ROS, including hydroxyl radicals, hydrogen peroxide, singlet oxygen, and hypochlorite to give a Raman active species; subsequent "lighting up" of the SERS probe occurs through the surface enhancement of silver nanoparticles (Scheme 17). The probe (**28**) was shown to be biocompatible, and was effective in detecting ROS in living cells at levels as low as femtomolar, and to enable the direct observation of ROS distribution in cells, including endogenously generated oxygen-centred radicals, through high special resolution Raman imaging.[80]

The use of spin traps such as nitrones for the ESR detection of radicals is a well-established technique,[82] however, the low trapping efficiency and need for high concentrations of spin trap presents significant problems in relation to their use in biological systems. Liu and coworkers were able to successfully link the 2-(ethoxycarbonyl)-2-methyl-3,4-dihydro-2H-pyrrole 1-oxide (EMPO) spin trap to gold nanoparticles, effectively exploiting the higher reaction rates of self assembled monolayers of organic molecules, compared to individual molecules, to increase their reaction rates with free radicals.[81] The probe **27** exhibited increased stability as well as an increased bimolecular rate constant for reaction with hydroxyl radicals when compared to free EMPO and shows great promise as a probe for detecting free radicals in biological systems.



29

Acknowledgements

We thank the Australian Research Council through the Centres of Excellence Scheme for financial support.

Notes and references

[a]ARC Centre of Excellence for Free Radical Chemistry and Biotechnology, Australia
[b] School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Victoria, 3010, Australia.
[c] School of Science and Technology, University of New England, Armidale, NSW, 2351, Australia.

Email: carlhs@unimelb.edu.au; Fax: +61 3 9347 8189; Tel: +61 3 8344 2432

* Corresponding author

1. N. Kumar, V. Bhalla and M. Kumar, *Coord. Chem. Rev.*, 2013, **257**, 2335–2347.
2. J. P. Blinco, K. E. Fairfull-Smith, B. J. Morrow and S. E. Bottle, *Aust. J. Chem*, 2011, **64**, 373–389.
3. A. Loudet, K. Burgess, *Chem. Rev.*, 2007, **107**, 4891–4932.
4. *Nitric Oxide: Biology and Pathobiology*, L. J. Ignarro (Ed.), Academic Press, San Diego, CA, 2000.
5. Y. M. Yang, Q. Zhao, W. Feng and F. Y. Li, *Chem. Rev.*, 2013, **113**, 192–270.
6. J. F. Brien, B. E. McLaughlin, K. Nakatsu and G. S. Marks, *Method. Enzymol.*, 1996, **268**, 83–92.
7. F. O. Brown, N. J. Finnerty, F. B. Bolger, J. Millar and J. P. Lowry, *Anal. Bioanal. Chem.*, 2005, **381**, 964–971.
8. Y. Katayama, N. Soh and M. Maeda, *ChemPhysChem*, 2001, **2**, 655–661.
9. F. Bedioui and N. Villeneuve, *Electroanal.*, 2003, **15**, 5–18.
10. E. W. Miller and C. J. Chang, *Curr. Opin. Chem. Biol.*, 2007, **11**, 620–625.
11. U.-F. Apfel, D. Buccella, J. J. Wilson and S. J. Lippard, *Inorg. Chem.*, 2013, **52**, 3285–3294.

12. For example see: M. H. Lim, B. A. Wong, W. H. Pitcock Jr., D. Mokshagundam, M.-H. Baik and S. J. Lippard, *J. Am. Chem. Soc.*, 2006, **128**, 14364–14373.
13. X. Sun, Y. Xu, W. Zhu, C. He, L. Xu, Y. Yang and X. Qian, *Anal. Methods,* 2012, **4**, 919–922.
14. K. J. Franz, N. Singh and S. J. Lippard, *Angew. Chem. Int. Ed.*, 2000, **39**, 2120–2122.
15. J.-H. Kim, D. A. Heller, H. Jin, P. W. Barone, C. Song, J. Zhang, L. J. Trudel, G. N. Wogan, S. R. Tannenbaum and M. S. Strano, *Nature Chem.*, 2009, **1**, 473–481.
16. J. Zhang, A. A. Boghossian, P. W. Barone, A. Rwei, J.-H. Kim, D. Lin, D. A. Heller, A. J. Hilmer, N. Nair, N. F. Reuel and M. S. Strano, *J. Am. Chem. Soc.*, 2011, **133**, 567–581.
17. C. Sun, W. Shi, Y. Song, W. Chen and H. Ma, *Chem. Commun.,* 2011, **47**, 8638–8640.
18. For example see: H. Kojima, Y. Urano, K. Kikuchi, T. Higuchi, Y. Hirata and T. Nagano, *Angew. Chem. Int. Ed.*, 1999, **38**, 3209–3212.
19. T. Terai, Y. Urano, S. Izumi, H. Kojima and T. Nagano, *Chem. Commun.*, 2012, **48**, 2840–2842.
20. G. K. Vegesna, S. R. Sripathi, J. Zhang, S. Zhu, W. He, F. T. Luo, W. J. Janhg, M. Frost and H. Liu, *Appl. Mater. Interfaces*, 2013, **5**, 4107–4112.
21. J.-B. Chen, H.-X. Zhang, X.-F. Guo, H. Wang and H.-S. Zhang, *Anal. Chim. Acta*, 2013, **800**, 77–86.
22. K. K.-W. Lo, K. H.-K. Tsang, K.-S. Tse, C.-K. Chung, T. K.-M. Lee, K. Y. Zhang, W.-K. Hui, C.-K. Li, J. S.-Y. Lau, D. C.-M. Ng and N. Zhu, *Coord. Chem. Rev.*, 2007, **251**, 2292–2310.
23. O. Zava, S. M. Zakeeruddin, C. Danelon, H. Vogel, M. Grätzel and P. J. Dyson, *ChemBioChem*, 2009, **10**, 1796–1800.
24. R. Zhang, Z. Ye, G. Wang, W. Zhang and J. Yuan, *Chem. Eur. J.*, 2010, **16**, 6884–6891.
25. For example see: D. Savateeva, D. Melnikau, V. Lesnyak, N. Gaponik and Y. P. Rakovich, *J. Mater. Chem.*, 2012, **22**, 10816–10820.
26. H. Yu, L. Jin, Y. Dai, H. Li and Y. Xiao, *New. J. Chem.*, 2013, **37**, 1688–1691.
27. R. Miao, L. Mu, H. Zhang, H. Xu, G. She, P. Wang and W. Shi, *J. Mater. Chem.*, 2012, **22**, 3348–3353.
28. K.-T. Yong, I. Roy, M. T. Swihart and P. N. Prasad, *J. Mater. Chem.,* 2009, **19**, 4655–4672.
29. C. Yu, Y. Wu, F. Zeng and S. Wu, *J. Mater. Chem. B*, 2013, **1**, 4152–4159.
30. *Optical Sensors. Industrial, Environmental and Diagnostic Applications*, R. Narayanaswamy and O. S. Wolfbeis (Eds.), Springer-Verlag, Berlin, 2004.
31. S. Roy and A. Banerjee, *Soft Matter*, 2011, **7**, 5300–5308.
32. C. B. Murray, C. R. Kagan and M. G. Bawendi, *Annu. Rev. Mater. Sci.,* 2000, **30**, 545–610.
33. P. D. Wadhavane, M. A. Izquierdo, F. Galindo, M. I. Burguete and S. V. Luis, *Soft Matter,* 2012, **8**, 4373–4381.
34. P. D. Sylvester, H. E. Ryan, C. D. Smith, A. S. Micallef, C. H Schiesser and U. Wille, *Poly. Degrad. Stabil.*, 2013, **98**, 2054–2062.
35. S.-A. Alexander and C. H. Schiesser, unpublished.
36. S. Hirosawa, S. Arai and S. Takeoka, *Chem. Commun.*, 2012, **48**, 4845–4847.
37. B. J. Morrow, D. J. Keddie, N. Gueven, M. F. Lavin and S. E. Bottle, *Free Rad. Biol. Med.*, 2010, **40**, 67–76.
38. R. E. Galian and M. de la Guardia, *Trends Anal. Chem.*, 2009, **28**, 279–291.
39. V. Maurel, M. Laferrière, P. Billone, R. Godin and J. C. Scaiano, *J. Phys. Chem. B*, 2006, **110**, 16353–16358.
40. C. Tansakul, E. Lilie, E. D. Walter, F. Rivera III, A. Wolcott, J. Z. Zhang, G. L. Millhauser and R. Braslau, *J. Phys. Chem. C*, 2010, **114**, 7793–7805.
41. K. Xu, H. Chen, H. Wang, J. Tian, J. Li, Q. Li, N. Li and B. Tang, *Biosens. Bioelectron.*, 2011, **26**, 4632–4636.
42. F. Lin, D. Pei, W. He, Z. Huang, Y. Huang and X. Guo, *J. Mater. Chem.*, 2012, **22**, 11801–11807.
43. T. Guo, L. Cui, J. Shen, R. Wang, W. Zhu, Y.Xu and X. Qian, *Chem. Commun.*, 2013, **49**, 1862–1864.
44. D. Oushiki, H. Kojima, T. Terai, M. Arita, K. Hanaoka, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2010, **132**, 2795–2801.
45. B. Kalyanaraman, V. Darley-Usmar, K. J. Davies, P. A. Dennery, H. J. Forman, M. B. Grisha, G. E. Mann, K. Moore, L. J. Roberts II and H. Ischiropoulos, *Free Radic. Biol. Med.*, 2012, **52**, 1–6.
46. N. Soh, *Anal. Bioanal. Chem.*, 2006, **386**, 532–543.
47. H. Y. Anh, K. E. Fairfull-Smith, B. J. Morrow, V. Lussini, B. Kim, M. V. Bondar, S. E. Bottle and K. D. Belfield, *J. Am. Chem. Soc.,* 2012, **134**, 4721–4730.
48. M. Yu, R. J. Beyers, J. D. Gorden, J. N. Cross and C. R. Goldsmith, *Inorg. Chem.*, 2012, **51**, 9153–9155.
49. S. Aime, M. Botta, E. Gianolio and E. Terreno, *Angew. Chem. Int. Ed.*, 2000, **39**, 747–750.
50. D. Pan, A. H. Schmieder, S. A. Wickline and G. M. Lanza, *Tetrahedron*, 2011, **67**, 8431–8444.
51. G. S. Loving, S. Mukherjee and P. Caravan, *J. Am. Chem. Soc.* 2013, **135**, 4620–4623.
52. R. A. Moats, S. E. Fraser and T. J. Meade, *Angew. Chem. Int. Ed.,* 1997, **36**, 726–728.
53. B. Yoo and M. D. Pagel, *Front. Biosci.*, 2008, **13**, 1733–1752.
54. M. Querol, J. W. Chen and A. Bogdanov Jr., *Org. Biomol. Chem.* 2006, **4**, 1887–1895.
55. J. W. Chen, W. Pham, R. Weissleder and A. Bogdanov Jr., *Magn. Reson. Med.*, 2004, **52**, 1021–1028.
56. M. Querol, J. W. Chen, R. Weissleder and A. Bogdanov Jr., *Org. Lett.*, 2005, **7**, 1719–1722.
57. M. O. Breckwoldt, J. W. Chen., L. Stangenberger, E. Aikawa, E. Rodriguez, S. Qiu, M. A. Moskowitz and R. Weissleder, *PNAS*, 2008, **105**, 18584–18589.
58. J. A. Ronald, J. W. Chen, Y. Chen, A. M. Hamilton, E. Rodriguez, F. Reynolds, R. A. Hegele, K. A. Rogers, M. Querol, A. Bogdanov, R. Weissleder and B. K. Rutt, *Circulation*, 2009, **120**, 592–599.
59. H. S. Su, M. Nahrendorf, P. Panizzi, M. O. Breckwoldt, E. Rodriguez, Y. Iwamoto, E. Aikawa, R. Weissleder and J. W. Chen, *Radiology*, 2012, **262**, 181–190.
60. P. B. Tsitovich, J. A. Spernyak and J. R. Morrow, *Angew. Chem. Int. Ed.*, 2013, **52**, 13997–14000.
61. S. J. Ratnakar, S. Viswanathan, Z. Kovacs, A. K. Jindal, K. N. Green and A. D. Sherry, *J. Am. Chem. Soc.*, 2012, **134**, 5798–5800.
62. C.Tu, R. Nagao and A. Y. Luoie, *Angew. Chem. Int. Ed.*, 2009, **48**, 6547–6551.

63. B. Jagadish, G. P. Guntle, D. Zhao, V. Gokhale, T. J. Ozumerzifon, A. M. Ahad, E. A. Mash and N. Raghunand, *J. Med. Chem.*, 2012, **55**, 10378–10386.
64. P. B. Tsitovich, P. J. Burns, A. M. McKay and J. R. Morrow, *J. Inorg. Biochem.*, 2014, **133**, 143–154.
65. R. A. Towner, N. Smith, D. Saunders, F. Lupu, R. Silasi-Mansat, M. West, D. C. Ramirez, S. E. Gomez-Mejiba, M. G. Bonini, R. P. Mason, M. Ehrenshaft and K. Hensley, *Free Radic. Biol. Med.*, 2013, **63**, 351–360.
66. K.-I. Matsumoto, F. Hyodo, A. Matsumoto, A. P. Koretsky, A. L. Sowers, J. B. Mitchell and M. C. Krishna, *Clin. Cancer Res.*, 2006, **12**, 2455–2462.
67. F. Hyodo, K.-I. Matsumoto, A. Matsumoto, J. B. Mitchell and M. C. Krishna, *Cancer Res.*, 2006, **66**, 9921–9928.
68. H. Utsumi, K.-I. Yamada, K. Ichikawa, K. Sakai, Y. Kinoshita, S. Matsumoto and M. Nagai, *PNAS*, 2006, **103**, 1463–1468.
69. S. Unnikrishnan and A. L. Klibanov, *Am. J. Roentgenol.,* 2012, **199**, 292–299.
70. J. R. Lindner, *Nat. Drug. Discov.*, 2004, **3**, 527–533.
71. R. Gessner and P. A. Dayton, *Mol. Imaging*, 2010, **9**, 117-127.
72. G. L. ten Kate, S. C. H. van den Oord, E. J. G. Sijbrands, A. van der Lugt, N. de Jong, J. G. Bosch, A. F. W. van der Steen and A. F. L. Schinkel, *J. Vasc. Surg.*, 2013, **57**, 539–546.
73. J. K. Perng, S. Lee, K. Kundu, C. F. Caskey, S. F. Knight, S. Satir, K. W. Ferrara, W. R. Taylor, F. L. Degertekin, D. Sorescu and N. Murthy, *Ann. Biomed. Eng.*, 2012, **40**, 2059–2068.
74. E. S. Olson, J. Orozco, Z. Wu, C. D. Malone, B. Yi, W. Gao, M. Eghtedari, J. Wang and R. F. Mattrey, *Biomaterials*, 2013, **34**, 8918–8924.
75. F. Yang, S. Hu, Y. Zhang, X. Cai, Y. Huang, F. Wang, S. Wen, G. Teng and N. Gu, *Adv. Mater.*, 2012, **24**, 5205–5211.
76. T. Uemura and S. Kitagawa, *J. Am. Chem. Soc.*, 2003, **125**, 7814–7815.
77. M. Shokouhimehr, E. S. Soehnlen, J. Hao, M. Griswold, C. Flask, X. Fan, J. P. Basilion, S. Basu and S. D. Huang, *J. Mater. Chem.*, 2010, **20**, 5251–5259.
78. A. Louie, *Chem. Rev.* 2010, **110**, 3146–3195.
79. L. M. Uusitalo and N. Hempel, *Int. J. Mol. Sci.*, 2012, **13**, 10660–10679.
80. C. Jiang, R. Liu, G. Han and Z. Zhang, *Chem. Commun.*, 2013, **49**, 6647–6649.
81. L. Du, S. Huang, Q. Zhuang, H. Jia, A. Rockenbauer, Y. Liu, K. Liu and Y. Liu, *Nanoscale*, 2014, **6**, 1646–1652.
82. C. L. Hawkins and M. J. Davies, *BBA. Gen. Subjects*, 2014, **1840**, 708–721.