 doi:10.20944/preprints202208.0084.v1

Nanoparticles as contrast agents for preclinical and clinical bioimaging

Deependra K. Ban[1,*]

Affiliation:
Keck Graduate Institute, Claremont, CA, 91711, USA

Keywords: Nanomaterial, optical imaging, MRI imaging, nanoparticles

Abstract

Visualization of deep biological structures in human and animal bodies is not possible through the naked eye due to the scattering of visible light by tissues in tolerable intensities. Different types of imaging modalities based on electromagnetic and pressure waves have been developed that help us image deep biological tissues with varying resolution and contrast. Some of the most widely used modalities are X-ray imaging, ultrasound imaging, MRI imaging, fluorescence imaging, and photoacoustic imaging. Although these techniques have significantly helped the advancement of our understanding of deep biological tissues and functions, they often require the use of exogenous contrast agents to improve their image quality for better investigation. Nanoparticle-based contrast agents have captivated scientists because of multiple advantages associated with them such as their excellent photophysical and chemical properties, ability to be precisely delivered at the target, and superlative tunability. This article is aimed to give a brief outlook on the recent state of art advances in the usage of nanoparticles for preclinical and clinical bioimaging through fluorescence, photoacoustic, and MRI imaging modalities.

Introduction

At the nanoscale (i.e., size of 1 – 100 nm in at least one dimension), matter attains unique photophysical, chemical, and biological properties, thus making possible a range of biological applications including bioimaging, drug delivery, and therapy.[1,2] Nanostructures that are nanoscale in all three dimensions are known as nanoparticles.[3] Nanoparticles are used in multiple imaging modalities such as fluorescence imaging, photoacoustic imaging, magnetic resonance imaging (MRI), and radionuclide imaging. This article is targeted toward MRI imaging, fluorescence imaging, and photoacoustic imaging.

 doi:10.20944/preprints202208.0084.v1

The use of nanoparticles as contrast agents in optical bioimaging has increased exponentially in the last two decades because of several advantages associated with them such as ease of syntheses, tuneability, functionalization, excellent photophysical and chemical properties, biocompatibility, etc.[4] Different types of optical modalities such as optical coherence tomography, Raman imaging, fluorescence imaging, second harmonic generation, and photoacoustic imaging have been shown to perform bioimaging with the aid of engineered nanoparticles.[4–7] Engineered nanoparticles of different types such as organic nanoparticles (e.g., carbon dots), inorganic nanoparticles (e.g., gold nanoparticles), and biological nanoparticles (e.g., protein nanoparticles) have found usage in bioimaging through different optical imaging modalities owing to their unique interaction profiles with light at different shapes, sizes, chemical composition, and environments.[8–10] Although multiple research works have been published on the use of standalone nanoparticles for bioimaging, conjugates of nanoparticles with different types of biological molecules such as proteins, DNA, and cellular lipids have provided them with interesting photophysical and biological properties that have been useful in diseases diagnosis and therapy with the aid of optical interaction.[11–13] Increasing studies are being performed at a very fast rate on potential applications of nanoparticles in optical bioimaging; however, they have not been adopted clinically. Nonetheless, in preclinical imaging, nanoparticles have contributed immensely to understanding drug delivery, disease diagnosis, and treatment, cancer-related research, biotherapeutic analysis, etc.[9,14] Out of several imaging modalities suitable for optical imaging, fluorescence, and photoacoustic imaging modalities are front runners to benefit from the use of nanoparticles for preclinical and clinical biological applications.

Currently, radionuclide imaging and MRI imaging are the only major imaging modalities where nanoparticles have gained approval for clinical use for disease diagnoses.[15] Out of these two techniques, only MRI imaging has been briefly discussed in this article.

Fluorescence imaging

Fluorescence imaging has found usage in a wide range of preclinical and clinical applications including disease diagnoses, imaging of physiological structures, functional neuronal imaging, drug delivery, studying pharmacokinetics, etc.[16–19] Whenever a matter or a molecule absorbs light of a particular frequency, it goes from a ground state to an excited state and then comes back to

 doi:10.20944/preprints202208.0084.v1

the ground state either by radiative or non-radiative decay (Figure 1). The radiative decay in the process is known as fluorescence.[20] By engineering nanoparticles with different chemical compositions, sizes, and shapes, they can be made fluorescent. Quantum dots are one of the most widely used nanoparticles for preclinical bioimaging.[21] For modern chemistry, quantum dots syntheses, purification, and characterization has become a trivial task, which has led to the production of quantum dots with a wide variety of photophysical properties. However, the best performing quantum dots are made of heavy metals such as cadmium, lead, etc., which have raised serious toxicity issues for bioimaging.[22] In recent years, new types of quantum dots are being developed from relatively safer materials such as zinc, gold, silver, etc.[23] However, for metal-based nanoparticles there have been concerns around poor biodegradability or inefficient clearance from the body, which may lead to chronic toxicity.[24,25] Several works have been performed to develop biocompatible/biodegradable metal-based nanoparticles, and limited but promising success has been achieved in large animals such as monkeys.[26,27] Apart from metals, carbon-based nanoparticles such as carbon dots have shown promising results *in vivo* fluorescence imaging particularly for sensing studies such as pH sensing.[28,29] Carbon dots are relatively inert to the immune system and was subjected to renal clearance as reported by multiple studies.[29]

Polymeric nanoparticles doped with small molecule dyes have generated encouraging results in preclinical imaging of tumors and other functional studies such as sensing pH.[30] Given the ease of biodegradability of polymers and small molecules, the nanoparticles made from these materials have a greater chance than metallic or purely carbon-based nanoparticles to be approved for clinical use. ICG is an FDA-approved dye that is utilized to image lymphatic vessels in lymph nodes in humans, while liposomes are lipid-based nanoparticles that are clinically used to deliver drugs.[31,32] Although ICG has been highly useful to image lymphatic systems with high contrast, they suffer from rapid clearance and biodegrade more readily. ICG-loaded liposomes have been shown to overcome these problems in preclinical fluorescence imaging and are one of the most promising candidates to be used for clinical bioimaging.[33,34]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 August 2022 doi:10.20944/preprints202208.0084.v1

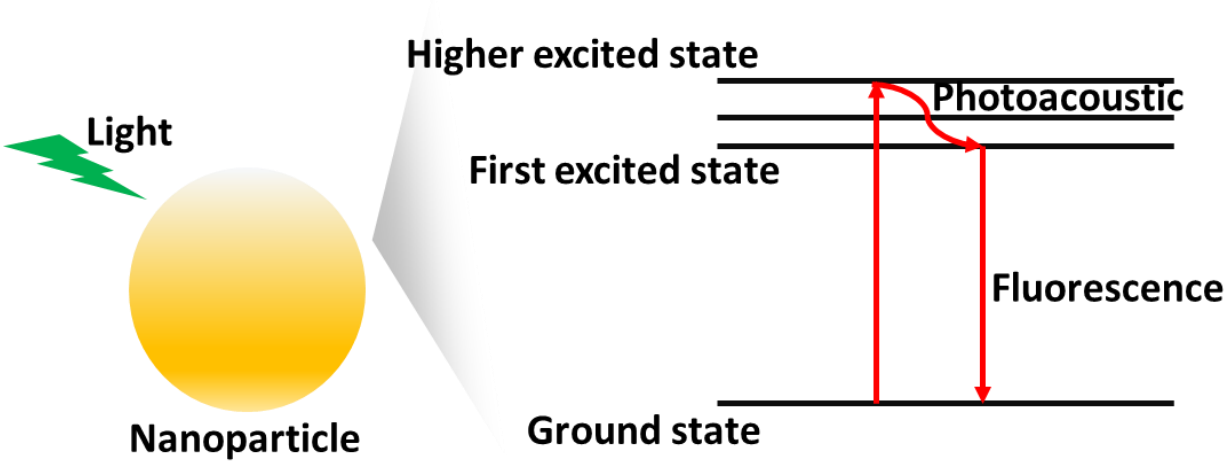

Figure 1: A nanoparticle excites from a ground state to an upper excited state upon being irradiated with light. From the upper excited state, it relaxes to the first excited state non-radiatively releasing heat, which is detected as a photoacoustic signal. From the first excited state, it relaxes to the ground state radiatively emitting fluorescence.

Photoacoustic imaging

The photoacoustic effect was first discovered in the 19th century by the legendary Alexander Graham Bell using a photophone; however, the innovations and applications in the field only took off in the last two decades.[35–37] In the photoacoustic effect, thermoelastic absorption of light results in pressure waves that can be sensed by a transducer (Figure 1). Bioimaging is the most widely used application of photoacoustic effect and it has gained success in both preclinical and clinical imaging right from imaging anatomical structures, studying drugs, imaging tumors, imaging cellular structures, imaging neuronal function, and histology.[38–43] One of the significant advantages of photoacoustic imaging over fluorescence imaging is that it has a higher resolution to depth ratio thus allowing imaging of deeper biological structures at a greater resolution.[36] Given that different biomolecules have unique chemical compositions and light absorption fingerprints, photoacoustic imaging has been employed to image a wide range of biological structures from DNA to lipids to proteins.[36,44,45] In preclinical and clinical imaging, blood has been used as the primary endogenous contrast agent for photoacoustic imaging because the hemoglobin in blood is present in a very high concentration of 1 – 2 mM.[46] Although endogenous contrast agents have greatly benefited the use of photoacoustic imaging, exogenous contrast agents can significantly enhance the contrast-to-noise ratio and enable the study of several biological events. The use of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 August 2022 doi:10.20944/preprints202208.0084.v1

exogenous contrast agents.[47,48] Multiple varieties of nano-agents have been developed and utilized for photoacoustic bioimaging; however, until now, all the photoacoustic imaging of nanomaterial-based contrast agents has been reported in preclinical studies only. Gold nanoparticles are the most utilized contrast agent for photoacoustic imaging due to their excellent surface plasmon resonance effect, which allows tuning of its absorption spectra from visible to NIR-II range.[47] Gold nanoparticles of various shapes and sizes have been synthesized which allowed imaging not only in the visible light range (450 nm – 600 nm) but also in the NIR-I (600 nm – 1000 nm) and NIR-II (1000 nm – 1300 nm) light range thus allowing deeper imaging of biological tissues including the brain.[11,49–51] Several types of multifunctional nanoparticles have been prepared by functionalizing gold with polymers, proteins, and other metals that have been useful for photoacoustic imaging.[52–54] Apart from gold other transition metal nanoparticles based on cobalt, gadolinium, copper, molybdenum, etc. have been used to image tumors in preclinical studies.[55–57] Besides metal, small molecules such as pyropheophorbide-a embedded liposomes have also been used to image tumors in mice using photoacoustic imaging with high contrast to noise ratio.[58]

Given that both the fluorescence and photoacoustic imaging modalities use light to excite the sample (i.e., nanoparticle in the context of this article), any nanoparticle that receives FDA approval for clinical bioimaging, can be used by both modalities. Both modalities have their advantages and disadvantages and can be used independently or combined to study biological events. For example, although photoacoustic proves a better resolution in deep tissues, fluorescence provides higher sensitivity to molecular probes.[59] Several studies have been reported with dual imaging capabilities of both fluorescence and photoacoustic thus harnessing the advantages of both modalities.[60–62]

MRI imaging

MRI imaging is one of the most widely used techniques in clinical imaging as it offers high contrast of soft tissue with a high resolution and excellent depth penetration.[63] MRI imaging works on the principle of longitudinal and transverse relaxations of nuclear spins of water protons after absorbing the radiofrequency energy.[64] The longitudinal relaxation (spin-lattice) time is characterized as T1 and transverse relaxation (spin-spin) time is characterized as T2.[64]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 August 2022 doi:10.20944/preprints202208.0084.v1

Endogenous contrast from tissue is produced due to the difference in T1 and T2 times (T1>> T2) of body water proton density after absorbing radio frequency energy. Although MRI gives high contrast images of soft tissues including the brain through the skull, contrast agents are often utilized to increase the signal intensity during disease diagnoses.[65] Nanoparticles work by reducing the T1 and T2 times, which increases the signal intensity. Different types of nanoparticles work by different mechanisms in reducing T1 and T2 relaxation times and are called either T1 contrast agents or T2 contrast agents.

Gadolinium-based nanoparticles are the most widely used T1 contrast agents and about 10 different such contrast agents have received FDA approval for clinical use.[65] Gadolinium is paramagnetic and it helps produce highly intense signals in T1 weighted images. Free gadolinium ions are known to be toxic and so they are chelated to large organic molecules to enable renal clearance without causing any acute toxicity.[66] Multiple varieties of gadolinium-based nanoparticles have been developed with different functionalities such as enhanced targeting and retention at the target site, better clearance, and higher signal.[67] Apart from gadolinium, other ions such as Mn^{2+} and Fe^{3+} have also been used as T1 contrast agents.[68,69]

Ferromagnetic or superparamagnetic iron-oxide-based nanoparticles have been used as T2 MRI contrast agents for over three decades.[70] Iron(II, III) oxide and iron (III) oxide are the most widely used T2 contrast agents because they are chemically stable and produce high signals.[65,71] Since T2-based contrast works by enhancing the negative background signals, the targeting of T2-based nanoparticle contrast agents is significantly driven by their sizes. [72] One of the major drawbacks of iron-oxide nanoparticles as T2 contrast agents is that they tend to create black holes in the images that prevent effective evaluation. Calibration of nanoparticles is very important for them to work with the highest efficiency. Overall, it can be concluded that nanoparticles have greatly aided in increasing the efficiency of MRI imaging; however, there are several limitations associated with them that hinder their significant and wide use.

Conclusion

Nanoparticles can be extremely useful to enhance the capabilities of optical and MRI bioimaging; however, being approved for clinical use, they need to overcome all the acute/chronic toxicity issues concerning them. Nanoparticles for optical imaging have not yet received FDA approval

 doi:10.20944/preprints202208.0084.v1

for clinical use but they have aided significantly our understanding of different physiological processes, drug delivery, drug discovery, different types of diseases, etc. in preclinical models. In MRI imaging, nanoparticles have been a great boon in early diagnoses of different types of tumors. Although there have concerns around acute and chronic toxicity but with the recent advancements in the field of nanotechnology which enables precise targeting as well as clearance, it is only a matter of time before nanoparticles will be used regularly in clinics for biomedical imaging.

References

1. Khan, I., Saeed, K. & Khan, I. Nanoparticles: Properties, applications and toxicities. *Arab. J. Chem.* **12**, 908–931 (2019).
2. Mitchell, M. J. *et al.* Engineering precision nanoparticles for drug delivery. *Nat. Rev. Drug Discov.* **20**, 101–124 (2021).
3. Murthy, S. K. Nanoparticles in modern medicine: state of the art and future challenges. *Int. J. Nanomedicine* **2**, 129–141 (2007).
4. Sun, G. *et al.* Bright fluorescent nanoparticles for developing potential optical imaging contrast agents. *Nanoscale* **2**, 548–558 (2010).
5. Weber, J., Beard, P. C. & Bohndiek, S. E. Contrast agents for molecular photoacoustic imaging. *Nat. Methods* **13**, 639–650 (2016).
6. Dempsey, W. P., Fraser, S. E. & Pantazis, P. SHG nanoprobes: Advancing harmonic imaging in biology. *BioEssays* **34**, 351–360 (2012).
7. Kang, J. W., So, P. T. C., Dasari, R. R. & Lim, D.-K. High Resolution Live Cell Raman Imaging Using Subcellular Organelle-Targeting SERS-Sensitive Gold Nanoparticles with Highly Narrow Intra-Nanogap. *Nano Lett.* **15**, 1766–1772 (2015).
8. Linden, S., Kuhl, J. & Giessen, H. Controlling the Interaction between Light and Gold Nanoparticles: Selective Suppression of Extinction. *Phys. Rev. Lett.* **86**, 4688–4691 (2001).
9. Hong, H., Gao, T. & Cai, W. Molecular imaging with single-walled carbon nanotubes. *Nano Today* **4**, 252–261 (2009).
10. Maley, A. M., Lu, G. J., Shapiro, M. G. & Corn, R. M. Characterizing Single Polymeric and Protein Nanoparticles with Surface Plasmon Resonance Imaging Measurements. *ACS Nano* **11**, 7447–7456 (2017).
11. Khadria, A. & Paul, S. Formation of Stable Cruciform Assembly of Gold Nanoparticles from Cannabis indica Leaves. *Appl. Nano* **3**, 143–148 (2022).

12. Yang, J. *et al.* Gold/alpha-lactalbumin nanoprobes for the imaging and treatment of breast cancer. *Nat. Biomed. Eng.* **4**, 686–703 (2020).
13. Yu, M., Zhou, C., Liu, J., Hankins, J. D. & Zheng, J. Luminescent Gold Nanoparticles with pH-Dependent Membrane Adsorption. *J. Am. Chem. Soc.* **133**, 11014–11017 (2011).
14. Han, X., Xu, K., Taratula, O. & Farsad, K. Applications of nanoparticles in biomedical imaging. *Nanoscale* **11**, 799–819 (2019).
15. Thakor, A. S. *et al.* Clinically Approved Nanoparticle Imaging Agents. *J. Nucl. Med.* **57**, 1833 (2016).
16. Khadria, A. Tools to measure membrane potential of neurons. *Biomed. J.* (2022). doi:10.1016/j.bj.2022.05.007
17. Qi, S. *et al.* The bright future of nanotechnology in lymphatic system imaging and imaging-guided surgery. *J. Nanobiotechnology* **20**, 24 (2022).
18. Priem, B., Tian, C., Tang, J., Zhao, Y. & Mulder, W. J. M. Fluorescent nanoparticles for the accurate detection of drug delivery. *Expert Opin. Drug Deliv.* **12**, 1881–1894 (2015).
19. Yang, Z. *et al.* Pharmacokinetics and biodistribution of near-infrared fluorescence polymeric nanoparticles. *Nanotechnology* **20**, 165101 (2009).
20. Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*. (Springer, 2007).
21. Ballou, B., Lagerholm, B. C., Ernst, L. A., Bruchez, M. P. & Waggoner, A. S. Noninvasive Imaging of Quantum Dots in Mice. *Bioconjug. Chem.* **15**, 79–86 (2004).
22. Pelley, J. L., Daar, A. S. & Saner, M. A. State of Academic Knowledge on Toxicity and Biological Fate of Quantum Dots. *Toxicol. Sci.* **112**, 276–296 (2009).
23. Gil, H. M. *et al.* NIR-quantum dots in biomedical imaging and their future. *iScience* **24**, 102189 (2021).
24. Soo Choi, H. *et al.* Renal clearance of quantum dots. *Nat. Biotechnol.* **25**, 1165–1170 (2007).
25. Longmire, M., Choyke, P. L. & Kobayashi, H. Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats. *Nanomedicine* **3**, 703–717 (2008).
26. Chiarelli, P. A. *et al.* Nanoparticle Biokinetics in Mice and Nonhuman Primates. *ACS Nano* **11**, 9514–9524 (2017).
27. Liao, J. *et al.* Broadening the biocompatibility of gold nanorods from rat to Macaca fascicularis: advancing clinical potential. *J. Nanobiotechnology* **19**, 195 (2021).
28. Yang, S.-T. *et al.* Carbon Dots for Optical Imaging in Vivo. *J. Am. Chem. Soc.* **131**, 11308–11309 (2009).
29. Luo, P. G. *et al.* Carbon “quantum” dots for optical bioimaging. *J. Mater. Chem. B*

1, 2116–2127 (2013).

30. Chen, M. & Yin, M. Design and development of fluorescent nanostructures for bioimaging. *Prog. Polym. Sci.* **39**, 365–395 (2014).
31. Mihara, M. *et al.* Indocyanine Green (ICG) Lymphography Is Superior to Lymphoscintigraphy for Diagnostic Imaging of Early Lymphedema of the Upper Limbs. *PLoS One* **7**, e38182 (2012).
32. Akbarzadeh, A. *et al.* Liposome: classification, preparation, and applications. *Nanoscale Res. Lett.* **8**, 102 (2013).
33. Bhavane, R., Starosolski, Z., Stupin, I., Ghaghada, K. B. & Annapragada, A. NIR-II fluorescence imaging using indocyanine green nanoparticles. *Sci. Rep.* **8**, 14455 (2018).
34. Cheung, C. C. L. *et al.* Liposome-Templated Indocyanine Green J- Aggregates for *In Vivo* Near-Infrared Imaging and Stable Photothermal Heating. *Nanotheranostics* **4**, 91–106 (2020).
35. Bell, A. G. On the production and reproduction of sound by light. *Am. J. Sci.* **s3-20**, 305 LP – 324 (1880).
36. Wang, L. V & Hu, S. Photoacoustic tomography: In vivo imaging from organelles to organs. *Science (80-.).* **335**, 1458–1462 (2012).
37. Wang, L. V & Yao, J. A practical guide to photoacoustic tomography in the life sciences. *Nat. Methods* **13**, 627–638 (2016).
38. Lin, L. *et al.* Single-breath-hold photoacoustic computed tomography of the breast. *Nat. Commun.* **9**, 2352 (2018).
39. Khadria, A. *et al.* Photoacoustic imaging reveals mechanisms of rapid-acting insulin formulations dynamics at the injection site. *Mol. Metab.* **62**, 101522 (2022).
40. Gottschalk, S. *et al.* Rapid volumetric optoacoustic imaging of neural dynamics across the mouse brain. *Nat. Biomed. Eng.* **3**, 392–401 (2019).
41. Pleitez, M. A. *et al.* Label-free metabolic imaging by mid-infrared optoacoustic microscopy in living cells. *Nat. Biotechnol.* **38**, 293–296 (2020).
42. Wong, T. T. W. *et al.* Fast label-free multilayered histology-like imaging of human breast cancer by photoacoustic microscopy. *Sci. Adv.* **3**, e1602168 (2022).
43. Khadria, A. *et al.* Long-duration and non-invasive photoacoustic imaging of multiple anatomical structures in a live mouse using a single contrast agent. *bioRxiv* (2022). doi:10.1101/2022.05.17.491718
44. Shi, J. *et al.* High-resolution, high-contrast mid-infrared imaging of fresh biological samples with ultraviolet-localized photoacoustic microscopy. *Nat. Photonics* **13**, 609–615 (2019).
45. Wong, T. T. W. *et al.* Label-free automated three-dimensional imaging of whole organs by microtomy-assisted photoacoustic microscopy. *Nat. Commun.* **8**, 1386 (2017).

46. Wang, L. V. *Photoacoustic Imaging and Spectroscopy*. (CRC Press, 2009).
47. Li, W. & Chen, X. Gold nanoparticles for photoacoustic imaging. *Nanomedicine (Lond).* **10**, 299–320 (2015).
48. Fu, Q., Zhu, R., Song, J., Yang, H. & Chen, X. Photoacoustic Imaging: Contrast Agents and Their Biomedical Applications. *Adv. Mater.* **31**, 1805875 (2019).
49. Huang, P. *et al.* Biodegradable gold nanovesicles with an ultrastrong plasmonic coupling effect for photoacoustic imaging and photothermal therapy. *Angew. Chemie* **125**, 14208–14214 (2013).
50. Wang, Y. *et al.* Photoacoustic Tomography of a Nanoshell Contrast Agent in the in Vivo Rat Brain. *Nano Lett.* **4**, 1689–1692 (2004).
51. Chen, Y.-S., Zhao, Y., Yoon, S. J., Gambhir, S. S. & Emelianov, S. Miniature gold nanorods for photoacoustic molecular imaging in the second near-infrared optical window. *Nat. Nanotechnol.* **14**, 810 (2019).
52. Jin, Y., Jia, C., Huang, S.-W., O'Donnell, M. & Gao, X. Multifunctional nanoparticles as coupled contrast agents. *Nat. Commun.* **1**, 41 (2010).
53. Mantri, Y., Davidi, B., Lemaster, J. E., Hariri, A. & Jokerst, J. V. Iodide-doped precious metal nanoparticles: measuring oxidative stress in vivo via photoacoustic imaging. *Nanoscale* **12**, 10511–10520 (2020).
54. Liang, S. *et al.* CD44v6 Monoclonal Antibody-Conjugated Gold Nanostars for Targeted Photoacoustic Imaging and Plasmonic Photothermal Therapy of Gastric Cancer Stem-like Cells. *Theranostics* **5**, 970–984 (2015).
55. Yang, W. *et al.* Albumin-Bioinspired Gd:CuS Nanotheranostic Agent for In Vivo Photoacoustic/Magnetic Resonance Imaging-Guided Tumor-Targeted Photothermal Therapy. *ACS Nano* **10**, 10245–10257 (2016).
56. Song, X.-R. *et al.* Co9Se8 Nanoplates as a New Theranostic Platform for Photoacoustic/Magnetic Resonance Dual-Modal-Imaging-Guided Chemo-Photothermal Combination Therapy. *Adv. Mater.* **27**, 3285–3291 (2015).
57. Chen, J. *et al.* Single-Layer MoS2 Nanosheets with Amplified Photoacoustic Effect for Highly Sensitive Photoacoustic Imaging of Orthotopic Brain Tumors. *Adv. Funct. Mater.* **26**, 8715–8725 (2016).
58. Lovell, J. F. *et al.* Porphysome nanovesicles generated by porphyrin bilayers for use as multimodal biophotonic contrast agents. *Nat. Mater.* **10**, 324–332 (2011).
59. Wang, L. V *et al.* Combined Photoacoustic and Molecular Fluorescence Imaging In Vivo. in *2005 IEEE Engineering in Medicine and Biology 27th Annual Conference* 190–192 (2005). doi:10.1109/IEMBS.2005.1616374
60. Doan, V. H. M. *et al.* Fluorescence/photoacoustic imaging-guided nanomaterials for highly efficient cancer theragnostic agent. *Sci. Rep.* **11**, 15943 (2021).
61. Chen, T. *et al.* Dual activated NIR-II fluorescence and photoacoustic imaging-guided cancer chemo-radiotherapy using hybrid plasmonic-fluorescent assemblies.

Nano Res. **13**, 3268–3277 (2020).

62. Akers, W. J. *et al.* Noninvasive Photoacoustic and Fluorescence Sentinel Lymph Node Identification using Dye-Loaded Perfluorocarbon Nanoparticles. *ACS Nano* **5**, 173–182 (2011).
63. Grover, V. P. B. *et al.* Magnetic Resonance Imaging: Principles and Techniques: Lessons for Clinicians. *J. Clin. Exp. Hepatol.* **5**, 246–255 (2015).
64. Westbrook, C. & Talbot, J. *MRI in Practice*. (Wiley Publishing, 2018).
65. Estelrich, J., Sánchez-Martín, M. J. & Busquets, M. A. Nanoparticles in magnetic resonance imaging: from simple to dual contrast agents. *Int. J. Nanomedicine* **10**, 1727–1741 (2015).
66. Wiegers, C. B. *et al.* Evaluation of two new gadolinium chelates as contrast agents for MRI. *Magn. Reson. Imaging* **10**, 903–911 (1992).
67. Fatima, A. *et al.* Recent Advances in Gadolinium Based Contrast Agents for Bioimaging Applications. *Nanomaterials* **11**, (2021).
68. Miao, Y. *et al.* Fe3+@polyDOPA-b-polysarcosine, a T1-Weighted MRI Contrast Agent via Controlled NTA Polymerization. *ACS Macro Lett.* **7**, 693–698 (2018).
69. Pan, D., Schmieder, A. H., Wickline, S. A. & Lanza, G. M. Manganese-based MRI contrast agents: past, present, and future. *Tetrahedron* **67**, 8431–8444 (2011).
70. Yang, H. *et al.* Effects of iron oxide nanoparticles as T2-MRI contrast agents on reproductive system in male mice. *J. Nanobiotechnology* **20**, 98 (2022).
71. Filippousi, M. *et al.* Surfactant Effects on the Structural and Magnetic Properties of Iron Oxide Nanoparticles. *J. Phys. Chem. C* **118**, 16209–16217 (2014).
72. Bulte, J. W. M. & Kraitchman, D. L. Iron oxide MR contrast agents for molecular and cellular imaging. *NMR Biomed.* **17**, 484–499 (2004).

Conflict of interest: The author declares no conflict of interest.