Nanoparticles for neuroscientific imaging applications

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Abstract

Imaging is an important part of neuroscience research, and tremendous progress has been made in recent years. However, the standard techniques of MRI, fluorescence microscopy and radiology are facing certain limitations that the advent of nanotechnology seeks to resolve. In this review a range of nanoparticles are presented and examples are given as to how the nanoparticles can improve existing imaging techniques or offer entirely new possibilities for imaging in neuroscience. Prime areas of usage include signal strengthening, multi-modal imaging, higher biostability and targeting possibilities. The advantages of the many available functionalization groups as well as drawbacks related to challenging production and toxicology are also discussed.

1 Introduction

From elucidating higher order brain functions to investigating single neurons, imaging is one of the key methods to gain information in neuroscience research. The goals can range from investigating basic neurobiology to dealing with complex neuropathologies such as Alzheimer's disease.

For *in vivo* neuroscience research MRI[1] is most widely used, but SPECT, PET and CT are also used for certain applications. Fluorescence microscopy is the most used technique for *in vitro* research on neurons, often through more advanced implementations such as CLSM or TIRF. Electron microscopy is sometimes used if high resolution structure and localization is needed.

These techniques are constantly being improved. Advanced MRI is relying more and more on contrast agents, but classical gadolinium based agents are frequently challenging to target and toxicity is always a concern. For better imaging more powerful contrast agents are also needed [1]. Advanced fluorescent imaging using classical organic conjugated dyes suffers from photobleaching effects, low signal-to-noise ratios and cross-talk between fluorophores due to wide emission spectra [2]. This makes some tasks such as single particle tracking, deep-tissue imaging and colocalization of many different labels challenging. Combining contrast agents for multi-modal imaging, such as a fluorophore and a gadolinium conjugate for fluorescence and MRI imaging, relies on challenging chemical modification and is not particularly effective, although this would aid many forms of research [3]. Targeting the contrast agents to areas of interest is challenging, especially in the case of brain imaging where the blood-brain barrier must be crossed.

Nanoparticles are showing great promise for both *in vivo* and *in vitro* neuroimaging. The following sections will investigate the use of different types of nanoparticles for neuroimaging applications, and show how intelligent design of nanoparticles can address the issues of classical techniques outlined above. There must be no doubt that imaging is not the only application for nanoparticles. Much of the research on nanoparticles is related to making the nanoparticles function as both imaging and therapeutic agents in the field of "theranostics". However, this is beyond the scope of this review.

2 Nanoparticles

The ability to characterize and manipulate structures on the nanometer scale, the same level of organization as nature itself typically uses, is showing its promise in the rapidly expanding field of nanotechnology [4]. Nanoparticles are small nanoengineered

[1] Abbreviations used: MRI - Magnetic resonance imaging; SPECT - Single photon emission computed tomography; PET - positron emission tomography; CT - (X-ray) Computed tomography; CLSM - Confocal laser scanning microscopy; TIRF - total internal reflection microscopy; AMPA - a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, a neurotransmitter; SPIO - Superparamagnetic iron oxide particles; NGF - Nerve growth factor; PFCE - perfluoro-15-crown-5-ether; PBCA - polymeric n-butyl-2-cyanoacrylate

particles ranging in size from about 1-100 nm [5]. They can be composed of metals, semi-conductors, ceramics, polymers or a combination of these. Although often spherical, they can be designed in many shapes if this aids the function. The main strength of nanoparticles is how they can be relatively easily tailored by combining the properties of many functional materials and molecules into single entities.

Nanoparticles are used in many applications where specific targeting of contrast agents, multi-modal imaging, a stronger signal and long term stability is needed [5, 6, 7, 8, 9, 10, 11, 12]. The nanoparticles are often coated with proteins, aptamers or antibodies to target specific compounds, or coated with other molecules to limit toxicity or enhance lipophilic or hydrophilic distribution properties, or functionalized in many other ways [13]. Neuroimaging applications range from tracking of neural stem cells in live brains using MRI [14, 15, 16], measuring chemical changes in brain areas [17], detecting specific markers for disease *in vivo* [18], or tracking the diffusion of single neurotransmittor receptors [19].

In the following sections several types of nanoparticles will be introduced and some examples of uses will be given. Be aware that the categories are only rough guides since most nanoparticles are functionalized, modified or combined with other materials to enhance their properties.

2.1 Gold nanoparticles

Some of the simplest nanoparticles to produce and use are the single-crystal gold nanoparticles [20]. The gold nanoparticles have a surface plasmon resonance frequency that varies with the size of the particle. Close to resonance frequency the particles absorb and scatter light very effectively, and they are also effective electron scatterers. The absorbed photons are mostly converted into heat, which is an effect used in photothermal tracking (see below). Colloidal gold, which is often synonymous with gold nanoparticles, has been in use for decades [21]. The traditional uses include conjugation to antibodies so specific targets can be visualized by electron microscopy, but new uses are continuously emerging.

One recent example of innovative use of gold nanoparticles is reported by Lasne *et. al.* [22]. They demonstrate how gold nanoparticles can be used as an alternative to fluorescence labeling to track protein diffusion in cells, specifically AMPA receptors, the most abundant receptors in the central nervous system. Cells were illuminated by laser light near the gold nanoparticle plasmon frequency, causing heating of the nanoparticles. This was used to measure small changes in the refractive index in the area around the nanoparticle, allowing accurate pinpointing and tracking of the AMPA receptors to which they were bound.

Another example of gold nanoparticles in neuroimaging is reported by Zhang *et. al.* [23]. By growing neurons on a nanopatterned template containing an array of gold nanoparticles, they observed the shift in the plasmon resonance frequency caused by the electric field of neurons when action potentials were fired. This allowed direct visualization of action potential propagation at a resolution of individual cultured neurons. By functionalizing gold nanoparticles so they cross the blood brain barrier this could be extended to *in vivo* recordings, although this has not yet been done.

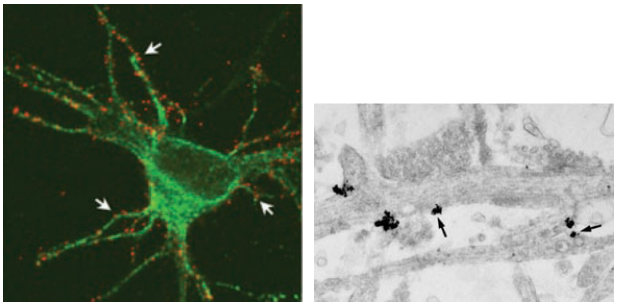
Although organic fluorophores and, more recently, quantum dots have been used for similuar purposes, gold nanoparticles have some advantages. They are not subject to photobleaching, are very stable and display low cytotoxicity [20]. This shows the versatility and sometimes surprising properties of the widely used gold nanoparticles.

2.2 Quantum dots

Quantum dots are semiconductor crystals typically 5-20 nm in diameter displaying powerful fluorescent properties. They are a type of nanoparticle gaining widespread use in neurocellular imaging [19, 24, 25, 26, 27, 28, 29, 30]. Primarily used due to their high photostability, broad absorption and narrow emission spectra and a blinking property that can be used to track individual quantum dots, they can also be functionalized to target proteins and other cellular structures. In addition, they function as contrast agents for electron microscopy, enabling colocalization studies.

In a pioneering 2003 study, Dahan *et. al.* tracked individual glycine receptors in cultured neurons using quantum dots [19]. They were tracked for long periods of time (20 minutes), which was possible due to the high photostability and the intermittent blinking property of the quantum dots, as illustrated in Figure 1a. Later the same quantum dots were imaged using electron microscopy, confirming the findings of the fluorescent microscopy, as illustrated in Figure 1b.

Although often used as "passive" labels, quantum dots can be functionalized to act as active agents as well. In a 2005 study Vu *et. al.* used quantum dots



(a) (b)

Figure 1: (a) Glycine receptors in cultured spinal chord neurons are labelled by quantum dots (red) which allows visualization and tracking of single receptors. (b) Transmission electron micrograph of the cultured neurons, confirming the position of quantum dot-labelled receptors with high resolution. Images from reference [19].

bound to NGF to target tyrosine kinase A receptors (TrkA) [29]. NGF, a high affinity ligand to TrkA, activates the tyrosin kinase A cascade when bound. The NGF retained biological activity when bound to the quantum dots, so in addition to being able to monitor the initial distribution of TrkA, the quantum dots could be monitored over time (4.5 days) in response to the evoked signal. The advantage of quantum dots in this case is the long-term stability and the ability to track single receptors.

A 2009 study by Zhang *et. al.* [30] showed an innovative use of quantum dots to study vesicle fusion dynamics at nerve terminals. Synaptic vesicles were loaded with single quantum dots by moderate electrical stimulation. Due to the high signal-to-noise ratio and blinking property, individual quantum dots could be identified, and followed over many use-reuse cycles of the vesicles. The quantum dots also showed a pH-dependent luminosity, which allowed the determination of the relative rates of two types of synaptic fusion under different stimuli to the nerve cells. To identify active synapses an organic fluorophore was used, which shows that in many applications quantum dots are an enhancement rather than a substitution for classical techniques in imaging studies.

2.3 Superparamagnetic iron oxide nanoparticles

SPIO nanoparticles are so small that they display no net ferromagnetism, yet they show a very high magnetic susceptibility, influencing the nearby magnetic environment, allowing them to be used as strong MRI contrast agents. In a 2009 study Calzi *et. al.* [31] demonstrated how SPIO nanoparticles of 4 nm were taken up by stem cells after incubation, displayed no cytotoxicity and gave high MRI contrast. This labeling allowed the accurate tracking of a small number of stem cells after injection *in vivo*, the focus of much research in neuroregeneration studies [32].

Functionalization allows the SPIO nanoparticles to be targeted, demonstrated by e.g. Liu *et. al.* in a 2009 study [17]. They showed that DNA-linked SPIO nanoparticles could be used to identify differential gene expression due to amphetamine exposure with high reliability using MRI.

SPIO nanoparticles can also be used in combination with other nanoparticles for multimodal imaging, as shown by Lee *et. al.* in a 2006 study [33]. They designed 30 nm silica nanoparticles with embedded rhodamine, an organic fluorophore, and chemically linked them to SPIO nanoparticles as shown in Figure 2. These combined nanoparticles were further functionalized with antibodies against a certain protein, allowing identification of regions of these cells using MRI, and finding the specific localization of the nanoparticles using fluorescence microscopy.

2.4 Polymeric nanoparticles

Polymers are typically more biocompatible than heavy metals and are therefore used as coatings to passivate nanoparticles. However, nanoparticles can also be made entirely by polymers, avoiding potential heavy metal toxicity. These are often less costly and easier to synthesize, although controlling the size can be a challenge [34].

In a 2008 study, Ruiz-Cabello *et. al.* developed an efficient ^{19}F MRI contrast agent using nanoparticles of PFCE [15]. The nanoparticles were incubated with neural stem cells and internalized without the use of transfection agents. After injection into the mouse brain the cells could be easily tracked *in vivo* for 2 weeks. Since there is no natural ^{19}F signal in the brain, small numbers of cells could be tracked and visualized using these nanoparticles.

Although nanoparticles mainly are used in optical and MRI techniques for neuroimaging applications, a 2009 study by Kulkarni *et. al.* showed the use of a two-component nanoparticle for SPECT imaging [35]. They functionalized PCBA nanoparticles with 125I-clioquinol, a radio-labeled drug that has high affinity for the plaques that identify Alzheimer's disease. These nanoparticles crossed the blood-brain barrier easier than the free drug, and allowed

high contrast SPECT visualization of the areas with plaque.

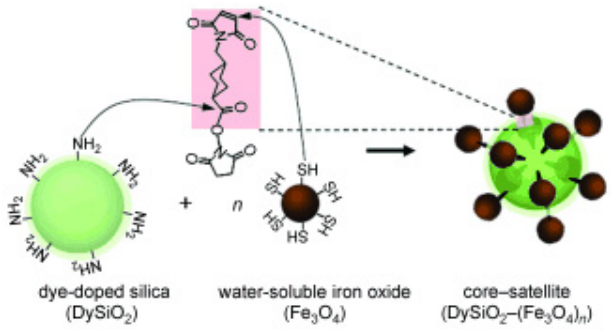
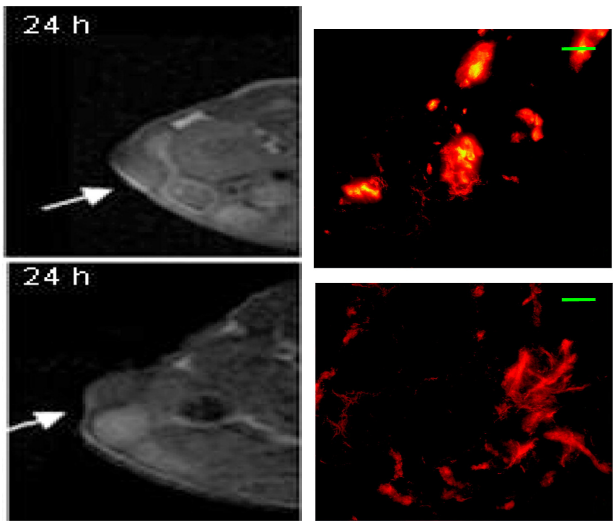


Figure 2: Making multimodal nanoparticles by combining SPIO nanoparticles for MRI contrast and silica nanoparticles with embedded rhodamine for fluorescent microscopy. Images from reference [33].



(a) **(b)**

Figure 3: (a) MRI and (b) fluorescence images of mouse tumor tissue marked by liposomes. The two top images were acquired using targeted liposomes as contrast agents, while the images below are of untargeted liposomes with the same contrast agents. In (a) the tumors are marked by the arrows. Both liposomes contain both Gd-based MRI contrast agents and the fluorophore rhodamine for subsequent fluorescent imaging. Scale bars in (b) are 50μm. Images from reference [36].

2.5 Lipid nanoparticles

Lipid-based nanoparticles are a wide class of nanoparticles that use small micelles or liposomes as delivery vehicles for a wide variety of substances [37]. Contrary to the examples above, the nanoparticles themselves are not what makes the imaging possible. Rather, that is done by the classical or nanoparticle-based contrast agents that are encapsulated within the lipids. Encapsulating contrast agents into lipid capsules can increase the effectiveness of the contrast agent, and the liposomes can be targeted and tailored to react to e.g. differences in pH or chemical signals. This allows imaging of such distributions *in vivo* with MRI, and molecules such as fluorophores can be included for multi-modal imaging [38].

In a 2009 study, Kamaly *et. al.* used liposomes to label and image tumors in mice with both MRI and fluorescent microscopy, as shown in Figure 3 [36]. Although the tumors they imaged were not brain tumors, the principles illustrate the general idea of multifunctional liposome nanoparticles. The liposomes were made so they incorporated gadolinium-based contrast agents and rhodamine, a fluorophore. These accumulated somewhat in the tumor, as the two lower images of Figure 3 show. If the liposomes additionally were functionalized with folate they were targeted to a larger degree as shown both in the MRI and fluorescence images (two top images of Figure 3). This is because folate binds to folate receptors which are over-expressed in the tumor cells. In addition, localization of the gadolinium inside the liposomes increased the signal-to-noise ratio. Naturally, further research includes encapsulating drugs to treat tumors as well as image them, without causing toxic effects from the gadolinium.

3 Issues regarding nanoparticles

The use of nanoparticles goes hand in hand with the production of nanoparticles. Reliable and affordable production methods for advanced nanoparticles are needed before they can be used for research and especially clinical purposes. This is one factor that limits widespread use, and even design of nanoparticles that conceptually seem very useful can be challenging in practice. Without efficient production facilities prices for nanoparticles are high, which limits use outside proof-of-concept research, although this is continuously improving [39].

Another main issue regarding nanoparticles, and nanotechnology in general, is that of safety. Neurons are sensitive cells, and the neurotoxicology of

nanoparticles depends on many different factors such as size, elemental composition and surface functionalization [40, 41]. These factors need to be accounted for in the use of nanoparticles for live diagnostics, but also in research to avoid influencing results. In addition the blood-brain barrier and cellular defense mechanisms provides formidable hindrances to any compound that wishes to influence and enter neurons. Accumulation of nanoparticles and long-term exposure effects are also areas of much discussion, which limits many potential *in vivo* applications.

4 Conclusions and future outlooks

Imaging, especially MRI and fluorescence imaging, provides an important source of information in neuroscience research, but existing techniques face some challenges. Gold nanoparticles, quantum dots, superparamagnetic iron oxide nanoparticles, polymer nanoparticles and lipid nanoparticles have all been shown to hold great promise for a wide range of neuroimaging applications. In some applications nanoparticles offer powerful novel approaches, but in many areas nanoparticles facilitate combinations or provide enhancements to existing techniques. The ability to functionalize nanoparticles with many types of molecules allows tailoring of beneficial properties. However, advanced nanoparticles require advanced production schemes, which often are costly, and toxicology effects must be evaluated before use.

A vast majority of the articles cited in this review are published in 2006 or later, and many of them are purely proof-of-concept articles, albeit containing promising ideas for future work. On the other hand, many of the nanoparticles described are commercially available, but often in relatively unfunctionalized forms compared to some of the examples [12]. Advances in fabrication technologies, characterization of new nanomaterials as well as novel uses of existing nanomaterials will drive the use of nanoparticles for neuroimaging forward. There will be a continuous transition from proof-of-concept research to clinical application, with safety, bulk production schemes and economical aspects being surmountable challenges on the way.

References

[1] Matthew N Rhyner, Andrew M Smith, Xiaohu Gao, Hui Mao, Lily Yang, and Shuming Nie. Quantum dots and multifunctional nanoparticles: new contrast agents for tumor imaging. *Nanomed*, 1(2):209–217, Aug 2006.

[2] W. C. Chan and S. Nie. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science*, 281(5385):2016–2018, Sep 1998.

[3] L. Frullano and T.J. Meade. Multimodal mri contrast agents. *Journal of Biological Inorganic Chemistry*, 12(7):939–949, 2007.

[4] Dr. Angela Hullmann. The economic development of nanotechnology - an indicators based analysis. European Commission, DG Research, Unit Nano S&T - Convergent Science and Technologies, November 2006.

[5] JM Provenzale and GA Silva. Uses of nanoparticles for central nervous system imaging and therapy. *American Journal of Neuroradiology*, 30(7):1293, 2009.

[6] Willem J M Mulder, Gustav J Strijkers, Geralda A F van Tilborg, Arjan W Griffioen, and Klaas Nicolay. Lipid-based nanoparticles for contrast-enhanced mri and molecular imaging. *NMR Biomed*, 19(1):142–164, Feb 2006.

[7] Jean-Luc Bridot, Anne-Charlotte Faure, Sophie Laurent, Charlotte Rivire, Claire Billotey, Bassem Hiba, Marc Janier, Vronique Josserand, Jean-Luc Coll, Luce Vander Elst, Robert Muller, Stphane Roux, Pascal Perriat, and Olivier Tillement. Hybrid gadolinium oxide nanoparticles: multimodal contrast agents for in vivo imaging. *J Am Chem Soc*, 129(16):5076–5084, Apr 2007.

[8] Peter Babinec and Melnia Babincov. Towards multimodal nanoparticle labels for molecular imaging of biological processes. *Med Hypotheses*, 69(3):703–704, 2007.

[9] Dhananjay Thakur, Shuang Deng, Thierno Baldet, and Jessica O Winter. ph sensitive cds-iron oxide fluorescent-magnetic nanocomposites. *Nanotechnology*, 20(48):485601, Dec 2009.

[10] Jinhao Gao, Hongwei Gu, and Bing Xu. Multifunctional magnetic nanoparticles: design, synthesis, and biomedical applications. *Acc Chem Res*, 42(8):1097–1107, Aug 2009.

[11] R. Koole, W.J.M. Mulder, M.M. van Schooneveld, G.J. Strijkers, A. Meijerink, and K. Nicolay. Magnetic quantum dots for multimodal imaging. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, 1(5), 2009.

[12] Jaeyun Kim, Yuanzhe Piao, and Taeghwan Hyeon. Multifunctional nanostructured materials for multimodal imaging, and simultaneous imaging and therapy. *Chem Soc Rev*, 38(2):372–390, Feb 2009.

[13] Eugenii Katz and Itamar Willner. Integrated nanoparticle-biomolecule hybrid systems: synthesis, properties, and applications. *Angew Chem Int Ed Engl*, 43(45):6042–6108, Nov 2004.

[14] E. Sykova and P. Jendelova. In vivo tracking of stem cells in brain and spinal cord injury. *Neurotrauma: New Insights Into Pathology and Treatment*, page 367, 2007.

[15] Jess Ruiz-Cabello, Piotr Walczak, Dorota A Kedziorek, Vadappuram P Chacko, Anna H Schmieder, Samuel A Wickline, Gregory M Lanza, and Jeff W M Bulte. In vivo "hot spot" mr imaging of neural stem cells using fluorinated nanoparticles. *Magn Reson Med*, 60(6):1506–1511, Dec 2008.

[16] E.S.M. Lee, J. Chan, B. Shuter, L.G. Tan, M.S.K. Chong, D.L. Ramachandra, G.S. Dawe, J. Ding, S.H. Teoh, O. Beuf, et al. Microgel iron oxide nanoparticles for tracking human fetal mesenchymal stem cells through magnetic resonance imaging. *Stem Cells*, 27(8):1921, 2009.

[17] Christina H Liu, Jia Q Ren, Jinsheng Yang, Charng ming Liu, Joseph B Mandeville, Bruce R Rosen, Pradeep G Bhide, Yuchio Yanagawa, and Philip K Liu. Dna-based mri probes for specific detection of chronic exposure to amphetamine in living brains. *J Neurosci*, 29(34):10663–10670, Aug 2009.

[18] Y.Z. Wadghiri, E.M. Sigurdsson, M. Sadowski, J.I. Elliott, Y. Li, H. Scholtzova, C.Y. Tang, G. Aguinaldo, M. Pappolla, K. Duff, et al. Detection of alzheimer's amyloid in transgenic mice using magnetic resonance microimaging. *Magnetic Resonance in Medicine*, 50(2):293–302, 2003.

[19] Maxime Dahan, Sabine Lvi, Camilla Luccardini, Philippe Rostaing, Batrice Riveau, and Antoine Triller. Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking. *Science*, 302(5644):442–445, Oct 2003.

[20] R.A. Sperling, P.R. Gil, F. Zhang, M. Zanella, and W.J. Parak. Biological applications of gold nanoparticles. *Chemical Society Reviews*, 37(9):1896–1908, 2008.

[21] WP Faulk and GM Taylor. An immunocolloid method for the electron microscope. *Immunochemistry*, 8(11):1081, 1971.

[22] David Lasne, Gerhard A Blab, Stphane Berciaud, Martin Heine, Laurent Groc, Daniel Choquet, Laurent Cognet, and Brahim Lounis. Single nanoparticle photothermal tracking (snapt) of 5-nm gold beads in live cells. *Biophys J*, 91(12):4598–4604, Dec 2006.

[23] Jiayi Zhang, Tolga Atay, and Arto V Nurmikko. Optical detection of brain cell activity using plasmonic gold nanoparticles. *Nano Lett*, 9(2):519–524, Feb 2009.

[24] Smita Pathak, Elizabeth Cao, Marie C Davidson, Sungho Jin, and Gabriel A Silva. Quantum dot applications to neuroscience: new tools for probing neurons and glia. *J Neurosci*, 26(7):1893–1895, Feb 2006.

[25] L. Groc, M. Lafourcade, M. Heine, M. Renner, V. Racine, J.B. Sibarita, B. Lounis, D. Choquet, and L. Cognet. Surface trafficking of neurotransmitter receptor: comparison between single-molecule/quantum dot strategies. *Journal of Neuroscience*, 27(46):12433, 2007.

[26] B. Cui, C. Wu, L. Chen, A. Ramirez, E.L. Bearer, W.P. Li, W.C. Mobley, and S. Chu. One at a time, live tracking of ngf axonal transport using quantum dots. *Proceedings of the National Academy of Sciences*, 104(34):13666, 2007.

[27] C. Bouzigues, M. Morel, A. Triller, and M. Dahan. Asymmetric redistribution of gaba receptors during gaba gradient sensing by nerve growth cones analyzed by single quantum dot imaging. *Proceedings of the National Academy of Sciences*, 104(27):11251, 2007.

[28] M. Howarth, W. Liu, S. Puthenveetil, Y. Zheng, L.F. Marshall, M.M. Schmidt, K.D. Wittrup, M.G. Bawendi, and A.Y. Ting. Monovalent, reduced-size quantum dots for imaging receptors on living cells. *Nature methods*, 5(5):397, 2008.

[29] T.Q. Vu, R. Maddipati, T.A. Blute, B.J. Nehilla, L. Nusblat, and T.A. Desai. Peptide-conjugated quantum dots activate neuronal receptors and initiate downstream signaling of neurite growth. *Nano Letters*, 5(4):603–608, 2005.

[30] Q. Zhang, Y. Li, and R.W. Tsien. The dynamic control of kiss-and-run and vesicular reuse probed with single nanoparticles. *Science*, 323(5920):1448, 2009.

[31] Sergio Li Calzi, David L Kent, Kyung-Hee Chang, Kyle R Padgett, Aqeela Afzal, Saurav B Chandra, Sergio Caballero, Denis English, Wendy Garlington, Paul S Hiscott, Carl M Sheridan, Maria B Grant, and John R Forder. Labeling of stem cells with monocrystalline iron oxide for tracking and localization by magnetic resonance imaging. *Microvasc Res*, 78(1):132–139, Jun 2009.

[32] Gatan J-R Delcroix, Matthieu Jacquart, Laurent Lemaire, Laurence Sindji, Florence Franconi, Jean-Jacques Le Jeune, and Claudia N Montero-Menei. Mesenchymal and neural stem cells labeled with hedp-coated spio nanoparticles: in vitro characterization and migration potential in rat brain. *Brain Res*, 1255:18–31, Feb 2009.

[33] Jae-Hyun Lee, Young-Wook Jun, Soo-In Yeon, Jeon-Soo Shin, and Jinwoo Cheon. Dual-mode nanoparticle probes for high-performance magnetic resonance and fluorescence imaging of neuroblastoma. *Angew Chem Int Ed Engl*, 45(48):8160–8162, Dec 2006.

[34] Moritz C Baier, Johannes Huber, and Stefan Mecking. Fluorescent conjugated polymer nanoparticles by polymerization in miniemulsion. *J Am Chem Soc*, 131(40):14267–14273, Oct 2009.

[35] P.V. Kulkarni, C.A. Roney, P.P. Antich, F.J. Bonte, A.V. Raghu, and T.M. Aminabhavi. Quinoline-n-butylcyanoacrylate-based nanoparticles for brain targeting for the diagnosis of alzheimer's disease. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, 2009.

[36] Nazila Kamaly, Tammy Kalber, Maya Thanou, Jimmy D Bell, and Andrew D Miller. Folate receptor targeted bimodal liposomes for tumor magnetic resonance imaging. *Bioconjug Chem*, 20(4):648–655, Apr 2009.

[37] Willem J M Mulder, Gustav J Strijkers, Geralda A F van Tilborg, David P Cormode, Zahi A Fayad, and Klaas Nicolay. Nanoparticulate assemblies of amphiphiles and diagnostically active materials for multimodality imaging. *Acc Chem Res*, 42(7):904–914, Jul 2009.

[38] Sjoerd Hak, Honorius M H F Sanders, Prashant Agrawal, Sander Langereis, Holger Grll, Henk M Keizer, Francesca Arena, Enzo Terreno, Gustav J Strijkers, and Klaas Nicolay. A high relaxivity gd(iii)dota-dspe-based liposomal contrast agent for magnetic resonance imaging. *Eur J Pharm Biopharm*, 72(2):397–404, Jun 2009.

[39] M. RITTNER. Nanoparticles: What's now, what's next? *Chemical engineering progress*, 99(11):39–42, 2003.

[40] M. Tang, T. Xing, J. Zeng, H. Wang, C. Li, S. Yin, D. Yan, H. Deng, J. Liu, M. Wang, et al. Unmodified cdse quantum dots induce elevation of cytoplasmic calcium levels and impairment of functional properties of sodium channels in rat primary cultured hippocampal neurons. *Environmental Health Perspectives*, 116(7):915, 2008.

[41] A.O. Choi, S.J. Cho, J. Desbarats, J. Lovrić, and D. Maysinger. Quantum dot-induced cell death involves fas upregulation and lipid peroxidation in human neuroblastoma cells. *Journal of nanobiotechnology*, 5(1):1, 2007.