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Multi-Functionality in Theranostic Nanoparticles: is more Always Better?

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Since the launch of the National Nanotechnology Initiative (NNI) in 2000, the pace of innovation in nanotechnology related areas worldwide has reached unprecedented levels. In the US alone the NNI umbrella covers the individual and/or cooperative nanotechnology centered activities of 26 different Federal Agencies. The 2013 federal budget for NNI is reported to be $1.8 billion [1].

With the advent of the ability to "construct" nanoparticles/nanocrystals (NCs) with atomic level precision, the terms "nanomaterials" and "nanotechnology" have realized their true meaning [1-13]. The diverse variety of research and application possibilities soared. Today we cannot imagine our lives without the direct and/or indirect benefits of one or another "nanomaterial" product. They have become an integral part of our daily lives, and the field is still in an expansion mode.

During this time, building from bottom-up has been a key, attractive, and successful avenue for generating functionally-diverse and molecularly-precise constructions, with high fidelity and better control of desired properties. The journey began with modestly functionalized "nanoplatforms" with only one or two functionalities and today we are talking about "theranostics", where the number and type of the functionalities have multiplied as well as diversified [14-19], Figure 1 illustrates just a few of the components that have been attached to theranostic nanoparticles. A more comprehensive list is given in Table 1.

Photodynamic Therapy (PDT) is a long-established approach to generate Reactive Oxygen Species (ROS) using photosensitizers (PS) and visible light. To circumvent some issues related with PDT (such as limited light penetration, lack of high enough target selectivity for PS molecules, and problematic skin phototoxicity), "dynamic" nanoplatform constructs have been developed. These new PDT paradigms can include infrared active dyes or very short-pulsed infrared lasers combined with two-photon absorbing PS and/or prodrug molecules. They may also include tumor-site recognition functionalities providing the ability to concentrate better in tumors and they are likely to also possess tumor-imaging capabilities. Progressively, these precision engineered constructs have become sophisticated built from bottom-up "dynamic" nanoplatforms Figure 2.

These nanocontructs have evolved to an extent that they can be injected into the blood stream, circulate harmlessly, and upon encountering the targets they recognize have the capability to zoom in and accumulate in the "problematic area" as shown in Figure 3. All this is achievable through selective molecular-targeting and recognition capabilities, and finally, when appropriate laser irradiation activates the chromophores, they deliver the designated application benefit(s) by killing cancer cells with locally generated ROS. Figure 4 shows how the wide variety of nanoparticles may assist PDT to move from "bench to bedside" or in this case "from mouse to man".

Over recent years, the great variety of nanoparticles as drug delivery vehicles in anticancer therapeutics has been gaining speed. Due to the facts that they exhibit unique pharmacokinetics (including minimal renal filtration, which in turn extends circulation time in the blood, depending on the surface functionalization characteristics [20]),

Therapeutics	Imaging
PDT[57,58]	Fluorescence [59,60]
Photothermal (plasmonic) [37,39]	Phosphorescence [61,62]
Alternating magnetic [63,64]	Photoacoustic [65,66]
Static magnetic (rheological) [77]	Radiolabeled [67,68]
Radioactive [69]	PET [70,71]
X-ray activated [72]	SPECT [71,73]
	Magnetic [74,75]
	MRI relaxation (Gd) [76]

Table 1: List of functionalities used in theranostic nanoparticles.

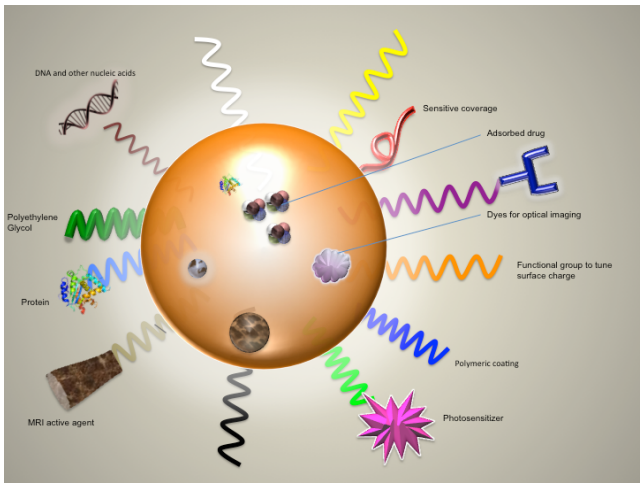

Figure 1: Nanoplatforms with theranostic functionalities: Fe_2O_3, magnetic NPs, photosensitizer, appropriate polymeric coating, surface-charge tuning groups, optical imaging dyes, targeting agents, adsorbed/bonded drugs, sensitive coverage, DNA and other nucleic acids, polyethylene glycol, proteins, MRI active agents, etc.

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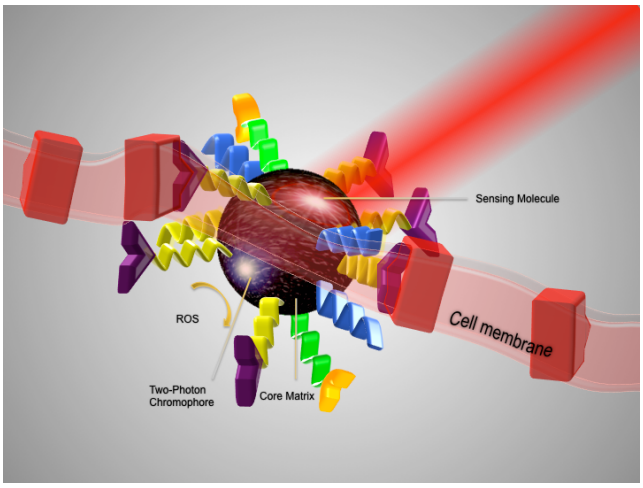

Figure 2: Dynamic nanoplatforms for PDT functionalized with molecular targeting moieties, two-photon chromophores, and sensing molecules all engineered into a core matrix.

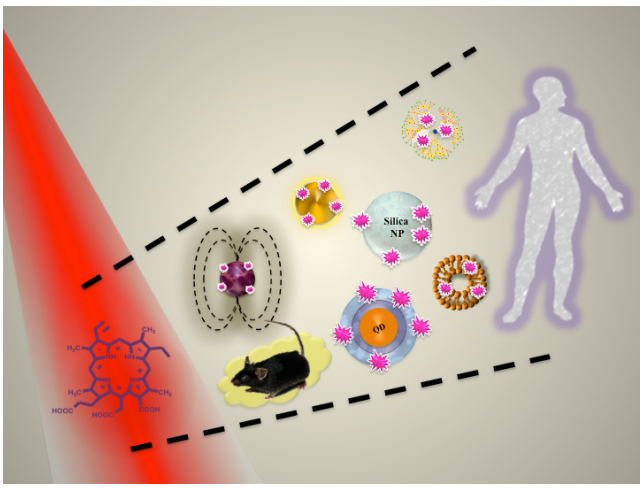

Figure 3: A wide range of lipid, polymer, ceramic, magnetic and other functionalized nanoparticles have been used in PDT that can improve photosensitizer delivery, together with fullerenes, carbon nanotubes and quantum dots, and raises the question of whether nanotechnology will truly improve the translation of PDT from mouse to man.

they have high surface to volume ratios (that enables modification with various functional groups), and possess the possibility to attach tailored functionalities together have made these nanoparticles enormously attractive for medical applications. Nanoparticle-constructs with capabilities to, simultaneously, target, allow real time imaging, and deliver therapeutics has become a reality, with plenty of room for further developments. The ultimate goal is to have the abilities of imaging and monitoring the diseased tissue; delivering prodrugs/drugs with enhanced spatial specificity; and a long-term hope of gaining the ability to finely tune the therapeutic time and dosage, and thus individualize medicine, as opposed to adopting a 'one size fits all' approach. This ultimate goal trajectory in itself moves the field of medicine toward an era of more effective and personalized treatment approaches.

To produce theranostic nanoparticles, various therapeutic strategies, such as nucleic acid delivery, chemotherapy, hyperthermia (photothermal ablation), photodynamic, and radiation therapy have been combined with one or more imaging functionalities in both *in vitro* and *in vivo* studies. Therapeutic delivery vehicles can be decorated with different imaging probes, such as MRI contrast agents (T1 and T2 agents), fluorescent markers (organic dyes and inorganic quantum dots), and nuclear imaging agents (PET/ SPECT/CT agents) in order to facilitate their imaging and, in doing so, we gain information about the trafficking pathway, kinetics of delivery, and therapeutic efficacy. Thus, creative approaches have being developed for the two major classes in clinical treatment of a disease: diagnosis and therapy via advancements and possibilities offered by nanotechnology.

At the same tune, in recent years many researchers have been focused on development of new synthetic- nonviral vehicles as potentially nontoxic and less immunostimulatory carriers [21,22]. These vehicles can be based on synthetic polymers, dendrimers, liposomes, cell-penetrating peptides, and inorganic nanoparticles. Moreover, developments in generating successful delivery vehicle combined with specific DNA labeling capabilities allowed the use of more sophisticated imaging techniques, such as time-lapse microscopy, Fluorescence Resonance Energy Transfer (FRET), Fluorescence Correlation Spectroscopy (FCS) [23-25], and single-molecule fluorescence spectroscopy [25]; techniques that can provide more precise information regarding polyplex trafficking, detachment, and thus, leading to development of better imaging and delivery systems. More importantly, with advancements in our imaging capabilities, researchers have realized that labeling polymers with "classical" fluorescent types of labels can change the physiochemical properties of these polymers. With the help of FCS it was found that the cationic di-block polyethyleneglycol–polyethyleneimine (PEG-PEI) copolymers lose their ability to bind to nucleic acids when labeled with a fluorescent cyanine dye Cy5 [26].

With the achievements in synthesizing quantum size confined nanoparticles known as quantum dots (QDs) [2,4-7,10,11] the nanoplatform concept has gained new meaning and span. QDs paired with fluorescent organic dyes (as FRET pairs) have been used in monitoring polyplex trafficking *in vitro* [15]. The intracellular dissociation of polymer-pDNA complexes was monitored via QD-FRET in HEK293 cells to monitor the unpackaging of chitosan-pDNA complexes. In the study streptavidin-functionalized, Cy5-labeled chitosan was conjugated to QD-labeled pDNA via biotin-streptavidin binding [27]. To address possible toxicity issues regarding the use of QDs for medical applications an *in vivo* study with murine animals was performed. In the study InAs/InP/ZnSe QDs were injected

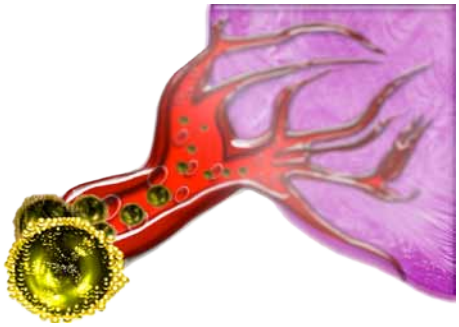

Figure 4: The concept of long-circulating nanoparticles with tumor localizing ligands attached being injected and left to selectively accumulate in tumors by a combination of active and passive (enhanced permeability and retention effect) targeting.

intravenously and showed significant renal clearance; confirmed both by Near-Infrared (NIR) fluorescence imaging and urine sample assessments [28]. Nie and colleagues reported an *in vivo* cancer targeting and imaging with semiconductor QDs where a triblock copolymer of co-polybutylacrylate-polyethylacrylate-polymethacrylic acid with hydrophobic hydrocarbon side chains was used to modify QDs and to which a Prostate-Specific Membrane Antigen (PSMA) targeting antibody was conjugated as well. When administered to prostate cancer bearing mice they showed that the QDs accumulated in the tumor area. This accumulation was attributed to both Enhanced Permeability and Retention (EPR) effect and specific antibody–antigen interaction specific to the tumor site [29].

In our study we used engineered nanoplatforms built from bottom-up with CdSe/ZnS core/shell QDs and double fuctionalization: PEGylation for the EPR effect and cyclic RGD-peptide functionalization for $\alpha_v\beta_3$ integrin specific recognition ($\alpha_v\beta_3$ integrin being overexpressed during angiogenesis). As a negative control for $\alpha_v\beta_3$ integrin specificity, cyclic RAD-peptide functionalization was employed. *In-vitro* studies were done with human umbilical vein endothelial cells (HUVECs), while the *in-vivo* studies were performed with male C57/BL6 mice carrying syngeneic murine melanoma cells. As seen in Figure 5a RGD functionalized nanoplatforms were target specific for the $\alpha_v\beta_3$ integrins and homed to the tumor site (24 hrs after being injected at the mice tail) whereas the RAD functionalized nanoplatforms did not show that effect. Confocal images in y-z axis (Figure 5b) clearly show the uptake of the RGD-functionalized nanoplatforms by the HUVECs (lower panel), whereas the RAD-functionalized nanoplatforms do not show that effect.

Along the same lines, to achieve simultaneously imaging, site-specific homing, and therapeutics delivery effects, a nanoplatform with the QDs, aptamers (Apt), and doxorubicin (DOX) elements was prepared in the study by Bagalkot et al. [30], where they used 10-base RNA aptamer coupled to the QDs as a bio-vector- targeting Prostate-Specific Membrane Antigen (PSMA). After homing to the tumor site DOX payload gradually got released from the nanoplatform, thus initiating the therapeutic functions and recovering the QDs fluorescence [30].

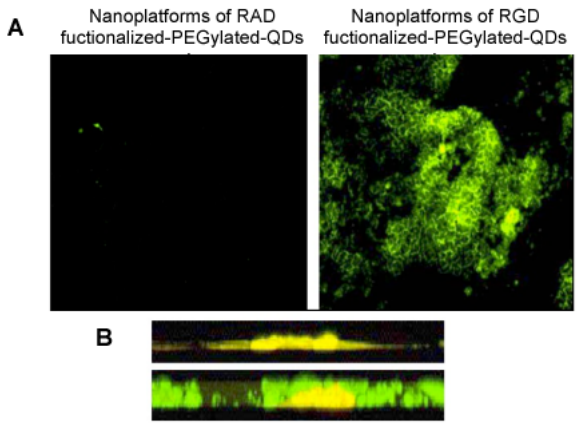

Figure 5: a) Epifluorescence imaging of doubly functionalized RGD-PEGylation-QDs (showing $\alpha_v\beta_3$ integrin targeting due to RGD peptide) and RAD-PEGylation-QD nanoplatforms (lacking the integrin targeting due to the scrambled RAD peptide)
b) Confocal images in the y-z axis, showing uptake of RGD-PEGylated-QDs nanoplatforms by the HUVECs (the lower panel), and the lack of uptake in the case of RAD-PEGylated-QD nanoplatforms (upper panel). The yellow color (as a pseudocolor) shows the propidium iodide labeled nuclei and the green color is due to QDs fluorescence.

In various studies QDs were used either as photosensitizers themselves or as carriers of drug/prodrug moieties [31-33]. QDs photosensitizer activity can happen, since the particles can be activated by light, and then, can transfer the triplet state energy to the nearby oxygen molecules, causing cell damage [34]. Tsay et al. [35] showed the generation of Reactive Oxygen Intermediates (ROI), through Nitroblue Tetrazolium (NBT) assay in QDs modified with streptavidin and conjugated with biotinylated pDNA. When these modified QDs were photoactivated, it was shown that they can generate ROI through, thus eliciting damage to purine and pyrimidine bases [35]. Samia et al. [34] have studied the interaction between CdSe QDs with a silicon phthalocyanine (Pc4) PDT photosensitizer. They found that the QDs could be used to sensitize the PDT agent through (FRET) mechanism, or interact directly with molecular oxygen via a Triplet Energy-Transfer process (TET), thus generating reactive singlet oxygen species that can be used for PDT cancer therapy [34]. Rakovich et al. [36], studied photodynamic properties of CdTe QDs and methylene blue hybrid photosensitizer. *In vitro* studies on HepG2 and HeLa cancerous cells showed effective cell kill by this hybrid system [36]. Thus, it is safe to say that QDs have proven to have a wide range of biomedical applications.

In case of the gold nanoparticles, with their strong surface plasmon absorption properties, it is a completely different ball game. Coupled with their stability, biosafety, and easy tunability, the unique opportunities that gold nanoparticles (Au NPs) offer in the medical imaging areas (such as computed tomography (CT), photoacoustics and Surface-Enhanced Raman Spectroscopy (SERS)) can easily be appreciated. Just changing the shape of Au NPs, from spherical to rod-like, can push the absorption wavelength to the near-IR (NIR) region (650-900 nm). This suggests specific roles for Au NPs as probes in photoacoustic imaging or as mediators in photothermal therapeutics [37,38]. In order to achieve desirable NIR-active capability, attainable by playing with the shape of pure gold NPs, researchers developed a nanoparticle with cheaper silica core coated with a gold shell layer [39]. In various studies Au NPs were functionalized to carry various drug (paclitaxel (PTX) [40,41]) and cytokine (tumor necrosis factor (TNF) [42,43]) molecules. In various studies many different ways of anchoring pharmaceutics, other than the well known thiol–Au association, have been investigated; high PDT efficient Pc4 was adsorbed on PEGylated Au NPs and similarly on Zn-Pc [44]. Further functionalization, using amphiphilic-block-copolymer- coated Au NP, for tumor targeting and drug delivery were formulated [45]. Not only pure gold NPs, but also metallic core/shell NPs with iron oxide cores and gold shells, have been explored to provide combined benefits of imaging and therapeutic delivery. For instance, Au NPs with iron oxide cores (magnetite (Fe_2O_4) or maghemite (γ-Fe2O4)) are been used for imaging via MRI [15].

Active investigation of iron oxide nanoparticles (IONPs) as nanoplatforms on which active construct probes containing multiple imaging motifs are the ambitious goal to load drugs into these nanosystems to achieve all-in-one theranostic agents that possess capabilities, including diagnostic imaging, drug delivery, and therapeutic monitoring are been the thrust in the biomedical research [46,47]. IONPs having magnetic properties allow them to accumulate together up on an external magnetic field. This property has made it utilize as a targeting mechanism to improve its drug delivery efficacy. One such study, when patients with metastatic breast cancer were infused with epirubicin- loaded IONPs and after that a magnetic field

was established around the tumor. The magnetic field successfully directed ferrofluid to the tumor to induce tumor regression [19]. Recently feridex particles are been approved by FDA for the detection of liver and spleen lesions, and also the analog Combidex has entered into phase III clinical trials for lymph node imaging [19]. Further due to high temperature decomposition, IONPs can itself play dual role for imaging/therapy, because of its potential in hyperthermia it has emerged as a useful strategy in nanoparticle preparation for theranostics application. Besides its wide application as a theranostics material, still more work needs to be done to expound the interaction of such combinational therapy in both *in vitro* and *in vivo* applications.

A class of nanostructures that has attracted interest in the past two decades for biomedical applications is the nano-carbon allotropes: including fullerenes, carbon nanoparticles, Carbon Nanotubes (CNTs), graphene, and nano-diamonds [48-50]. Nano-carbons are classified into sp^2 and sp^3-carbon nanomaterials based on their bonding structures and as such they possess inherent optical properties such as fluorescence and photoacoustic emission that makes them a useful contrast agent in optical imaging and sensing [51,52]. PEGylation of CNTs by both covalent and non-covalent methods are much less toxic *in vitro* and *in vivo*, which make them to be preferred for biomedical application [53].

Fullerenes have shown potential application in several different cancer therapeutic approaches such as photodynamic therapy, photothermal treatment, radiotherapy and chemotherapy. Fullerenes can also acts as novel contrast agents in Magnetic Resonance (MR) imaging [54]. Similarly graphene has risen considerable interest for biomedical applications [55]. Unique physical and surface features are a key player in the field of nanomedicine. Besides loading drug molecules, nanoparticles such as IONPs or AuNPs can be easily bound to CNTs to further enrich the nanoplatform. But still their non-biodegradability and toxicity remains to be a great concern in the area of biomedical applications [56].

With the modern trend of steadily increasing complexity and a race to produce the theranostic agent with the greatest number of different functionalities, we must ask ourselves if there is a practical (as well as a theoretical) limit? As the constructs getting more and more sophisticated, and the number of functionalities increases, we must realize that there must be a limit set before they start cross-talking, quenching each other, interfering with each other in some other fashion, or impeding the expected/ sought after effects. Thus, at what point will the "law of diminishing returns" take over and become a dominant factor? These are some of the questions that must be answered by the proponents of the mantra "the more, the merrier" with regard to theranostic multi-functionalization.

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References

1. http:/www.nano.gov/
2. Lee CW, Yang SH, Hsu CS, Meng HF (2009) Improved efficiencies in organic light emitting diodes made with CdSe/ZnS quantum dots and a semiconducting polymer. J Nanosci Nanotechnol 9: 2076-2080.
3. Alivisatos AP (1997) Scaling law for structural metastability in semiconductor nanocrystals. Berichte der Bunsengesellschaft fur physikaliche Chemie 101: 1573-1577.
4. Peng X, Schlamp MC, Kadavanich AV, Alivisatos AP (1997) Epitaxial growth of highly luminescent cdse/cds core/shell nanocrystals with photostability and electronic accessibility. J Am Chem Soc 119: 7019-7029.
5. Hines MA, Guyot-Sionnest P (1996) Synthesis and characterization of strongly luminescing ZnS-Capped CdSe nanocrystals. J Phys Chem 100: 468-471.
6. Katari JEB, Colvin VL, AP Alivisatos (1994) X-ray photoelectron spectroscopy of CdSe nanocrystals with applications to studies of the nanocrystal surface. J Phys Chem 98: 4109-4117.
7. Colvin VL, Schlamp MC, Alivisatos AP (1994) Light-emitting diodes made from cadmium selenide nanocrystals and a semiconducting polymer. Nature. 370: 354-357.
8. Weller H (1993) Colloidal semiconductor Q-particles: chemistry in the transition region between solid state and molecules. Angew Chemie Int Ed Engl 32: 41-53.
9. Murray CB, Norris DJ, Bawendi MG (1993) Synthesis and characterization of nearly monodisperse CdE (E = sulfur, selenium, tellurium) semiconductor nanocrystallites. J Am ChemSoc 115: 8706-8715.
10. Colvin VL, Goldstein AN, Alivisatos AP (1992) Semiconductor nanocrystals covalently bound to metal surfaces with self-assembled monolayers. J AmChem Soc 114: 5221-5230.
11. Wang Y (1991) Three-dimensionally confined diluted magnetic semiconductor clusters: Zn1,âíxMnxS. Solid State Commun 77: 33-38.
12. Bawendi MG, Wilson WL, Rothberg L, Carroll PJ, Jedju TM, et al. (1990) Electronic structure and photoexcited-carrier dynamics in nanometer-size CdSe clusters. Phys Rev Lett 65: 1623-1626.
13. Reed MA (1993) Quantum Dots, in Quantum Dots. Scientific American 118.
14. Bhaskar S, Tian F, Stoeger T, Kreyling W, de la Fuente JM, et al. (2010) Multifunctional Nanocarriers for diagnostics, drug delivery and targeted treatment across blood-brain barrier: perspectives on tracking and neuroimaging. Part Fibre Toxicol 7: 3.
15. Kelkar SS, Reineke TM (2011) Theranostics: combining imaging and therapy. Bioconjug Chem 22: 1879-1903.
16. Mura S, Couvreur P (2012) Nanotheranostics for personalized medicine. Adv Drug Deliv Rev.
17. Xie, J, Liu G, Eden HS, Ai H, Chen X (2011) Surface-engineered magnetic nanoparticle platforms for cancer imaging and therapy. Acc Chem Res 44: 883-892.
18. Ma X, Zhao Y, Liang XJ (2011) Theranostic nanoparticles engineered for clinic and pharmaceutics. Acc Chem Res 44: 1114-1122.
19. Xie J, Lee S, Chen X (2010) Nanoparticle-based theranostic agents. Adv Drug Deliv Rev 62: 1064-1079.
20. Alexis F, Pridgen E, Molnar LK, Farokhzad OC (2008) Factors affecting the clearance and biodistribution of polymeric nanoparticles. Mol Pharm 5: 505-515.
21. Niidome T, Huang L (2002) Gene therapy progress and prospects: nonviral vectors. Gene Ther 9: 1647-1652.
22. Akhtar S (2006) Non-viral cancer gene therapy: beyond delivery. Gene Ther 13: 739-740.
23. Matsumoto Y, Itaka K, Yamasoba T, Kataoka K (2009) Intranuclear fluorescence resonance energy transfer analysis of plasmid DNA decondensation from nonviral gene carriers. J Gene Med 11: 615-623.
24. Schwille P (2001) Fluorescence correlation spectroscopy and its potential for intracellular applications. Cell Biochem Biophys 34: 383-408.
25. Sahoo H, Schwille P (2011) FRET and FCS--friends or foes? Chemphyschem 12: 532-541.
26. Remaut K, Lucas B, Raemdonck K, Braeckmans K, Demeester J, et al. (2007) Can we better understand the intracellular behavior of DNA nanoparticles by fluorescence correlation spectroscopy? J Control Release 121: 49-63.
27. Ho YP, Chen HH, Leong KW, Wang TH (2006) Evaluating the intracellular stability and unpacking of DNA nanocomplexes by quantum dots-FRET. J Control Release 116: 83-89.
28. Xie R, Chen K, Chen X, Peng X (2008) InAs/InP/ZnSe Core/Shell/Shell quantum dots as near-infrared emitters: bright, narrow-band, non-cadmium containing, and biocompatible. Nano Res 1: 457-464.

29. Gao X, Cui Y, Levenson RM, Chung LW, Nie S (2004) *In vivo* cancer targeting and imaging with semiconductor quantum dots. Nat Biotechnol 22: 969-976.

30. Bagalkot V, Zhang L, Levy-Nissenbaum E, Jon S, Kantoff PW, et al. (2007) Quantum dot-aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on bi-fluorescence resonance energy transfer. Nano Lett 7: 3065-3070.

31. Willard DM, Van Orden A (2003) Quantum dots: Resonant energy-transfer sensor. Nat Mater 2: 575-576.

32. Bakalova R, Ohba H, Zhelev Z, Ishikawa M, Baba Y (2004) Quantum dots as photosensitizers? Nat Biotechnol 22: 1360-1361.

33. Yaghini E, Seifalian AM, MacRobert AJ (2009) Quantum dots and their potential biomedical applications in photosensitization for photodynamic therapy. Nanomedicine (Lond) 4: 353-363.

34. Samia AC, Chen X, Burda C (2003) Semiconductor quantum dots for photodynamic therapy. J Am Chem Soc 125: 15736-15737.

35. Tsay JM, Trzoss M, Shi L, Kong X, Selke M, et al. (2007) Singlet oxygen production by Peptide-coated quantum dot-photosensitizer conjugates. J Am Chem Soc 129: 6865-6871.

36. Rakovich A, Savateeva D, Rakovich T, Donegan JF, Rakovich YP, et al. (2010) CdTe quantum dot/dye hybrid system as photosensitizer for photodynamic therapy. Nanoscale Res Lett 5: 753-760.

37. Hu M, Chen J, Li ZY, Au L, Hartland GV, et al. (2006) Gold nanostructures: engineering their plasmonic properties for biomedical applications. Chem Soc Rev 35: 1084-1094.

38. Chen J, Saeki F, Wiley BJ, Cang H, Cobb MJ, et al. (2005) Gold nanocages: bioconjugation and their potential use as optical imaging contrast agents. Nano Lett 5: 473-477.

39. Jain PK, Huang X, El-Sayed IH, El-Sayed MA (2008) Noble metals on the nanoscale: optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. Acc Chem Res 41: 1578-1586.

40. Gibson JD, Khanal BP, Zubarev ER (2007) Paclitaxel-functionalized gold nanoparticles. J Am Chem Soc 129: 11653-11661.

41. Chen YH, Tsai CY, Huang PY, Chang MY, Cheng PC, et al. (2007) Methotrexate conjugated to gold nanoparticles inhibits tumor growth in a syngeneic lung tumor model. Mol Pharm 4: 713-722.

42. Paciotti GF, Myer L, Weinreich D, Goia D, Pavel N, et al. (2004) Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery. Drug Deliv 11: 169-183.

43. Powell AC, Paciotti GF, Libutti SK (2010) Colloidal gold: a novel nanoparticle for targeted cancer therapeutics. Methods Mol Biol 624: 375-84.

44. Cheng Y, C Samia A, Meyers JD, Panagopoulos I, Fei B, et al. (2008) Highly efficient drug delivery with gold nanoparticle vectors for in vivo photodynamic therapy of cancer. J Am Chem Soc 130: 10643-10647.

45. Prabaharan M, Grailer JJ, Pilla S, Steeber DA, Gong S (2009) Gold nanoparticles with a monolayer of doxorubicin-conjugated amphiphilic block copolymer for tumor-targeted drug delivery. Biomaterials 30: 6065-6075.

46. Lee S, Chen X (2009) Dual-modality probes for in vivo molecular imaging. Mol Imaging 8: 87-100.

47. Xie J, Huang J, Li X, Sun S, Chen X (2009) Iron oxide nanoparticle platform for biomedical applications. Curr Med Chem 16: 1278-1294.

48. Fabbro C, Ali-Boucetta H, Da Ros T, Kostarelos K, Bianco A, et al. (2012) Targeting carbon nanotubes against cancer. Chem Commun (Camb) 48: 3911-3926.

49. Prato M, Kostarelos K, Bianco A (2008) Functionalized carbon nanotubes in drug design and discovery. Acc Chem Res 41: 60-68.

50. Liu Z, Tabakman SM, Chen Z, Dai H (2009) Preparation of carbon nanotube bioconjugates for biomedical applications. Nat Protoc 4: 1372-1382.

51. Welsher K, Liu Z, Sherlock SP, Robinson JT, Chen Z, et al. (2009) A route to brightly fluorescent carbon nanotubes for near-infrared imaging in mice. Nat Nanotechnol 4: 773-780.

52. Wang X, Wang C, Cheng L, Lee ST, Liu Z (2012) Noble metal coated single-walled carbon nanotubes for applications in surface enhanced Raman scattering imaging and photothermal therapy. J Am Chem Soc 134: 7414-7422.

53. Yang ST, Luo J, Zhou Q, Wang H (2012) Pharmacokinetics, metabolism and toxicity of carbon nanotubes for biomedical purposes. Theranostics 2: 271-282.

54. Chen Z, Ma L, Liu Y, Chen C (2012) Applications of functionalized fullerenes in tumor theranostics. Theranostics 2: 238-250.

55. Shen H, Zhang L, Liu M, Zhang Z (2012) Biomedical applications of graphene. Theranostics 2: 283-294.

56. Liu Z, XJ Liang (2012) Nano-carbons as theranostics. Theranostics 2: 235-237.

57. Huang P, Lin J, Wang X, Wang Z, Zhang C, et al. (2012) Light-triggered theranostics based on photosensitizer-conjugated carbon dots for simultaneous enhanced-fluorescence imaging and photodynamic therapy. Adv Mater 24: 5104-5110.

58. Sharma SK, Chiang LY, Hamblin MR (2011) Photodynamic therapy with fullerenes in vivo: reality or a dream? Nanomedicine (Lond) 6: 1813-1825.

59. Cao X, Lin W, Zheng K, He L (2012) A near-infrared fluorescent turn-on probe for fluorescence imaging of hydrogen sulfide in living cells based on thiolysis of dinitrophenyl ether. Chem Commun (Camb).

60. Wu-Zhang AX, Murphy AN, Bachman M, Newton AC (2012) Isozyme-specific interaction of protein kinase cdelta (PKCdelta) with mitochondria dissected using live cell fluorescence imaging. J Biol Chem.

61. Steunenberg P, Ruggi A, van den Berg NS, Buckle T, Kuil J, et al. (2012) Phosphorescence imaging of living cells with amino acid-functionalized tris(2-phenylpyridine)iridium(III) complexes. Inorg Chem 51: 2105-2114.

62. Wanek J, Blair NP, Shahidi M (2012) Outer retinal oxygen consumption of rat by phosphorescence lifetime imaging. Curr Eye Res 37: 132-137.

63. Yallapu MM, Othman SF, Curtis ET, Gupta BK, Jaggi M, et al. (2011) Multi-functional magnetic nanoparticles for magnetic resonance imaging and cancer therapy. Biomaterials 32: 1890-1905.

64. Xie J, Jon S (2012) Magnetic nanoparticle-based theranostics. Theranostics 2: 122-124.

65. Beard P (2011) Biomedical photoacoustic imaging. Interface Focus 1: 602-631.

66. Hu S, Wang LV (2010) Photoacoustic imaging and characterization of the microvasculature. J Biomed Opt 15: 011101.

67. Weinstein DA, Krisnangkura K, Mayfield MB, Gold MH (1980) Metabolism of radiolabeled beta-guaiacyl ether-linked lignin dimeric compounds by phanerochaete chrysosporium. Appl Environ Microbiol 39: 535-540.

68. Jalilian AR, Khoshdel MR, Garousi J, Yousefnia H, Hosseini MA, et al. (2009) Development of a radiolabeled beta-human chorionic gonadotropin. Acta Pharm 59: 421-429.

69. Hobbs RF, Song H, Watchman CJ, Bolch WE, Aksnes AK, et al. (2012) A bone marrow toxicity model for (2)(2)(3)Ra alpha-emitter radiopharmaceutical therapy. Phys Med Biol 57: 3207-3222.

70. Peñuelas I, Domínguez-Prado I, García-Velloso MJ, Martí-Climent JM, Rodríguez-Fraile M, et al. (2012) PET Tracers for Clinical Imaging of Breast Cancer. J Oncol 2012: 710561.

71. Oude Munnink TH, Nagengast WB, Brouwers AH, Schröder CP, Hospers GA, et al. (2009) Molecular imaging of breast cancer. Breast 3: S66-73.

72. Chen W, Zhang J (2006) Using nanoparticles to enable simultaneous radiation and photodynamic therapies for cancer treatment. J Nanosci Nanotechnol 6: 1159-1166.

73. Apostolova I, Wunder A, Dirnagl U, Michel R, Stemmer N, et al. (2012) Brain perfusion SPECT in the mouse: Normal pattern according to gender and age. Neuroimage.

74. Weber AL (1992) Magnetic resonance imaging and computed tomography of the internal auditory canal and cerebellopontine angle. Isr J Med Sci 28: 173-182.

75. Shenton ME, Hamoda HM, Schneiderman JS, Bouix S, Pasternak O, et al. (2012) A review of magnetic resonance imaging and diffusion tensor imaging findings in mild traumatic brain injury. Brain Imaging Behav 6: 137-192.

76. Garimella PD, Datta A, Romanini DW, Raymond KN, Francis MB (2011) Multivalent, high-relaxivity MRI contrast agents using rigid cysteine-reactive gadolinium complexes. J Am Chem Soc 133: 14704-14709.

77. Bouchlaka MN (2012) Mechanical disruption of tumors by iron particles and magnetic field application results in increased anti-tumor immune responses. PLoS ONE.