

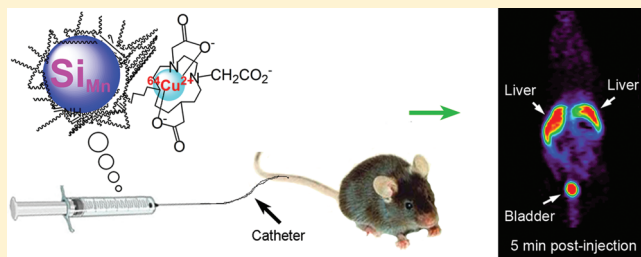
PET Imaging and Biodistribution of Silicon Quantum Dots in Mice

Chuqiao Tu,[†] Xuchu Ma,[‡] Adrian House,[†] Susan M. Kauzlarich,^{*,‡} and Angelique Y. Louie^{*,†}[†]Department of Biomedical Engineering and [‡]Department of Chemistry, University of California, Davis, One Shields Avenue, Davis, California 95616, United States

S Supporting Information

ABSTRACT: Investigation of nanomaterial disposition and fate in the body is critical before such material can be translated into clinical application. Herein a new macrocyclic ligand-⁶⁴Cu²⁺ complex was synthesized and used to label dextran-coated silicon quantum dots (QD), with an average hydrodynamic diameter of 15.1 ± 7.6 nm. The chelate showed exceptional stability, demonstrated by no loss radiolabel under a ligand competition reaction with EDTA. The QDs' biodistribution in mice was quantitatively evaluated by *in vivo* positron emission tomography (PET) imaging and *ex vivo* gamma counting. Results showed that they were excreted via renal filtration shortly postinjection and also accumulated in the liver.

KEYWORDS: Biodistribution, positron emission tomography, imaging, silicon, quantum dot



Over the past decade, quantum dots (QDs) have been touted as promising agents for *in vivo* biomedical imaging due to their desirable properties of high quantum yield, resistance to photobleaching, narrow emission peak, and tunable emission wavelength.^{1–3} Investigation of nanomaterial disposition and fate in the body, i.e. biodistribution and plasma clearance, is critical before such material can be translated into clinical application. The biodistribution of group II–VI QDs has been investigated in rodents via both fluorescence and radioactivity-based techniques.^{1,4–6} However, to date, such studies have not been reported for recently emerged, fluorescent silicon QDs, which are expected to be an ideal candidate for many biological applications because of their biocompatibility.^{7–11}

Taking advantage of the QD's intrinsic luminescence properties, optical imaging techniques can be used to investigate QDs *in vitro* and offer the benefits of noninvasiveness, high sensitivity, and relatively low cost. However, for *in vivo* use, tissue absorption and scattering of light impair excitation of QDs in deeper lying tissues, and the fluorescence signal detected from deeper structures is significantly attenuated as a function of tissue depth, which makes optical tracking and quantification of QDs in living systems difficult.⁶ For example, there are studies showing that current fluorescence imaging systems significantly underestimate QD mass amounts in living animals, especially in deep tissues, and therefore are not robust enough to accurately measure *in vivo* biodistribution.^{4,12}

Positron emission tomography (PET) is a noninvasive, highly sensitive nuclear imaging technique that can produce three-dimensional images.^{13–15} We have used PET to track the biodistribution of a wide array of imaging probes. The results demonstrated that this noninvasive imaging technique is capable of providing a robust and reliable measure of *in vivo* probe

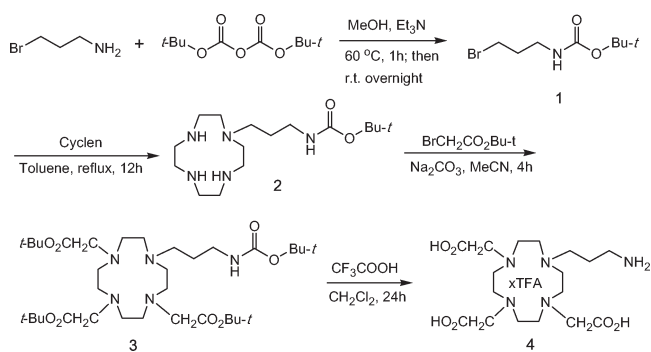
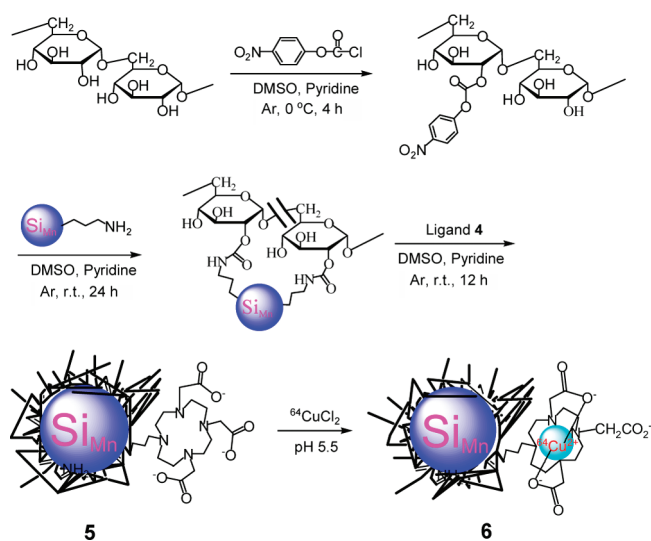
distribution.^{16–18} PET has been employed to study biodistribution in living animals of group II–VI CdSe QDs radiolabeled with a positron-emitting tracer.^{4,19} Herein, we report the evaluation of Si QDs biodistribution in mice using ⁶⁴Cu labeled Si QDs for both *in vivo* PET imaging and gamma counting of *ex vivo* tissues.

Silicon QDs were synthesized from the precursor sodium silicide through a solution-phase reduction, as previously reported, except that Si_{Mn} QDs (Si_{Mn} QDs = 1% manganese doped Si QDs) were coated with neutral dextran instead of anionic dextran sulfate.¹¹ The dextran sulfate coated Si QDs are targeted to macrophages while the dextran coated Si QDs are nonspecific nanoparticles. That is, they do not interact specifically with any type of cells and therefore report the clearance of Si QDs from the body mainly based on the particle size.^{11,20} DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) is a frequently used ligand for chelation of ⁶⁴Cu ion that forms a complex of high thermodynamic stability (DOTA-Cu log *K* = 20–28).^{4,21–24} However, in practice we found that attachment of commercially available *p*-SCN-Bn-DOTA to the particles using traditional bioconjugation methods was synthetically difficult, probably due to the steric hindrance caused by the short and rigid arm of SCN-Bn.¹⁷ Therefore, we synthesized a new bifunctional DO3A (1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid) derivative **4** that has a more flexible functional arm for chelation of ⁶⁴Cu. The conjugation of ligand **4** with the dextran coating of Si_{Mn} QDs was realized by polarity inversion of dextran hydroxyl groups.

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Scheme 1. Synthesis of Ligand 4 for ^{64}Cu ChelationScheme 2. Synthesis of ^{64}Cu -DO3A Conjugated Dextran Si_{Mn} QDs 6

The synthetic route for ligand 4 is shown in Scheme 1. Di-*tert*-butyl dicarbonate and 3-bromopropylamine hydrobromide were stirred in methanol in the presence of triethylamine to afford compound 1 in 79% yield. After cyclen was monoalkylated with 1, the resultant compound 2 was further alkylated with 3 equiv of *tert*-butyl bromoacetate to produce compound 3.^{25,26} Compound 3 was hydrolyzed with trifluoroacetic acid in dichloromethane to give the free ligand 4 as an adduct of trifluoroacetic acid to free base, which was confirmed by ^1H and ^{13}C NMR and the strong absorption at -74.25 ppm in ^{19}F NMR of 4.

The polarity of dextran was partially inverted by esterification with 4-nitrophenyl chloroformate and 4-(dimethylamino)pyridine (DMAP) (catalyst) in DMSO/pyridine.²⁷ The following nucleophilic addition–elimination reactions of polar, partially inverted dextran with propylamine capped Si_{Mn} QDs and ligand 4 generated DO3A conjugated, dextran coated Si_{Mn} QDs 5,¹¹ as shown in Scheme 2. The average hydrodynamic diameter of the purified particle 5 was 15.1 ± 7.6 nm. Particle 5 contained 1.5% silicon. Its emission peak was at 440 nm and the intensity of emission was maximal for excitation at 360 nm, similar to our previous results for the unconjugated particles.¹¹ The results support that particle 5 is dextran coated silicon QDs, which is

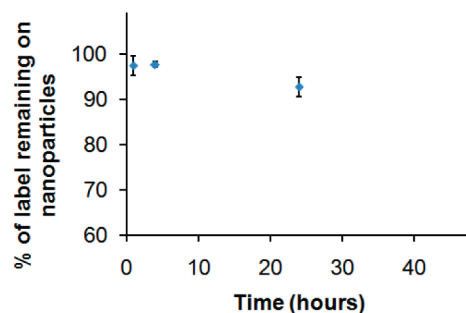


Figure 1. Stability of QDs 6- ^{64}Cu in pH 5.5 sodium acetate–acetic acid buffer solution over the 48 h time period.

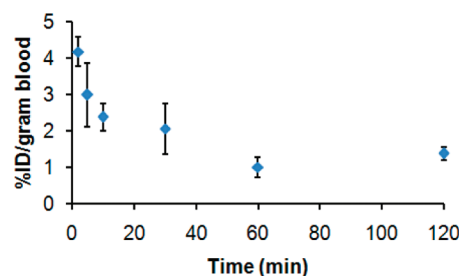


Figure 2. Blood clearance of ^{64}Cu -DO3A conjugated dextran Si_{Mn} QDs 6 in mice ($n = 3$).

important because the dextran alone can form different sizes of nanoparticles.²⁸ DO3A conjugated Si_{Mn} QDs 5 were metalated with $^{64}\text{CuCl}_2$ in pH 5.5 acetate buffer solution followed by the extraction of possible uncoordinated free copper ions by applying excess ethylenediaminetetraacetic acid (EDTA). After purification by centrifuge filtration, the radiolabeling yield was found to be 78%.

To confirm that ^{64}Cu ions remain attached to the QDs 6 during imaging and verify that distribution is not due to free dissociated copper, the kinetic stability of QDs 6- ^{64}Cu was tested by subjecting QDs 6- ^{64}Cu to a competitive ligand exchange with EDTA, which is known to have a high affinity for Cu ($\log K_{\text{Cu-EDTA}} = 18.7$),²⁹ over the 48-h time period. Figure 1 shows that, over time, the QDs 6- ^{64}Cu in pH 5.5 sodium acetate–acetic acid buffer solution do not lose radiolabel, confirming the exceptional stability of the chelator.

The clearance of Si_{Mn} QDs 6 from the blood circulation is rapid, as shown in Figure 2. By 2 min post tail-vein injection, 95.7%ID/g (percentage of injected dose per gram blood or tissue) has already been cleared out from the bloodstream. The rapid clearance continued until 10 min postinjection, when $<2.5\%$ ID/g was left in the bloodstream. Thereafter, the clearance slowed and $\sim 1.5\%$ ID/g remained in the blood 120 min post-injection. The fast blood clearance of Si_{Mn} QDs 6 is similar to that of polyethylene glycol coated CdSe QDs (12 or 21 nm, respectively) measured by PET imaging and gamma counting in mice, which were also cleared in minutes,⁴ but differs from reports of polymer-coated QDs or dextran-coated iron oxide nanoparticles measured by fluorescence imaging, which had similar hydrodynamic diameters to Si_{Mn} QDs 6 but much longer blood circulation times of up to a few hours.^{1,6,16}

The concentrations of particles radiolabeled with positron-emitting tracer can be determined semiquantitatively from the PET images and used to analyze the biodistribution of Si_{Mn} QDs

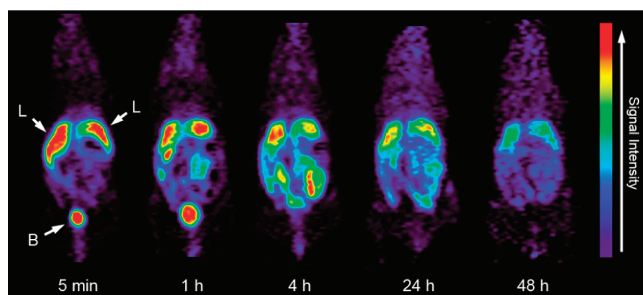


Figure 3. *In vivo* PET images of mice ($n = 4$) at 5 min, 1 h, 4 h, 24 h, and 48 h postinjection of ^{64}Cu -DO3A conjugated dextran Si_{Mn} QDs **6**: L, liver; B, bladder.

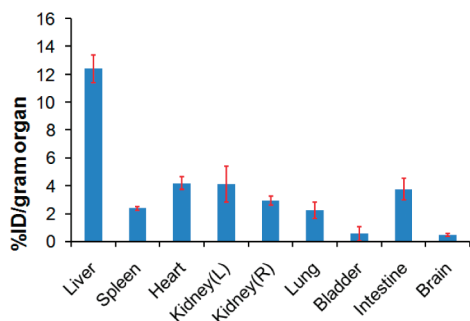


Figure 4. *Ex vivo* biodistribution of ^{64}Cu -DO3A conjugated dextran Si_{Mn} QDs **6** in mice. Mice ($n = 4$) were sacrificed 48 h post intravenous injection. Organs were harvested and measured by well gamma counting.

6 in living mice over time. As shown in Figure 3, the main sites for accumulation of **6** are the urinary bladder and the liver after 5 min and 1 h post tail-vein injection. After 4 h, 24 h, and 48 h post-injection, Si_{Mn} QDs **6** were found to mainly accumulate in the liver. Only a weak signal was seen in the bladder after 4 h post-injection.

The absolute quantification of biodistribution and deposition of Si_{Mn} QDs **6** in living mice was obtained via gamma counting of *ex vivo* tissues. The four mice used for PET imaging were sacrificed after 48-h PET scan, and organs were harvested and analyzed quantitatively by a gamma-counter detector. The mean and SD of %ID/g have been corrected for natural decay of ^{64}Cu . Liver was found to be the major organ where Si_{Mn} QDs **6** accumulated (Figure 4). In detail, the liver accumulated $12.4 \pm 1.0\%$ ID/g of Si_{Mn} QDs **6**, whereas the left and right kidneys took up $4.1 \pm 1.3\%$ ID/g and $3.0 \pm 0.3\%$ ID/g of **6**, respectively. Heart ($4.2 \pm 0.4\%$ ID/g), intestine ($3.8 \pm 0.8\%$ ID/g), spleen ($2.4 \pm 0.1\%$ ID/g), and lung ($2.2 \pm 0.6\%$ ID/g) also showed localization of Si_{Mn} QDs **6**, and a small amount of **6** was found in the bladder ($0.6 \pm 0.5\%$ ID/g) and brain ($0.5 \pm 0.1\%$ ID/g).

Among many factors that may affect the biodistribution of systemically administered QDs in the body, the size of nanoparticles is critical.^{30–33} Immediately upon exposure to blood, QDs may be quickly adsorbed by opsonins and in turn flagged for phagocytosis, an obligate response of the immune system when encountering a nanoparticulate foreign body.^{30,34} Small nanoparticles (<7 nm) may be rapidly and efficiently eliminated via renal filtration with excretion into the urine, while larger particles are taken up nonspecifically by the reticuloendothelial system (RES) and end up in the liver, spleen, and lymphatic system,

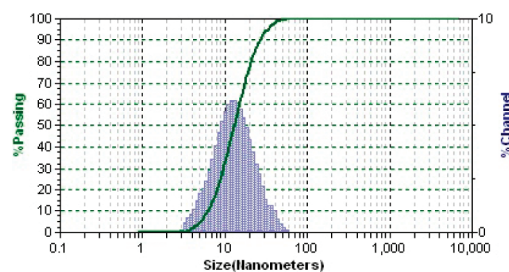


Figure 5. Hydrodynamic diameter of DO3A conjugated dextran Si_{Mn} QDs **5** measured by dynamic light scattering (DLS).

where they are excreted into the biliary system after hepatobiliary (HB) processing and enter the gall bladder and intestine.^{20,30,35–37} Our observations for biodistribution are consistent with expectations based on size (Figure 5). We conjecture that the fraction of Si_{Mn} QDs **6** with small hydrodynamic diameter was rapidly excreted from the body through renal filtration and urinary bladder, while the fraction of Si_{Mn} QDs **6** with larger hydrodynamic diameter was taken up by the RES and accumulated in the liver.

In conclusion, these results present the first analysis of *in vivo* biodistribution for Si QDs radiolabeled with a positron-emitting tracer. Knowledge of detailed biodistribution of QDs in living animals is of paramount importance before diagnostic or therapeutic application in the clinic is possible.^{4,6,32} The present study shows rapid clearance of dextran coated Si QDs from mouse bloodstream, and the QDs were excreted from the body via both renal filtration and urinary bladder, and accumulated in the liver, which may provide useful information for the future design of new QDs and nanoparticles for biomedical applications.

■ ASSOCIATED CONTENT

S Supporting Information. Synthetic procedures and characterization of compounds **1–4** and nanoparticles **5** and **6**, details of the stability measurements of ^{64}Cu labeled dextran Si_{Mn} QDs, and *in vivo* PET imaging and *ex vivo* gamma counting procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail addresses: S.M.K., smkauzlarich@ucdavis.edu; A.Y.L., aylouie@ucdavis.edu.

Author Contributions

C.Q.T. developed Si QDs coating synthesis, executed ^{64}Cu radiolabeling and *in vivo* PET imaging, and wrote manuscript. X.C.M. synthesized Si cores. A.H. handled animals. S.M.K. and A.Y.L. conceived of design, syntheses, experiments and edited manuscript.

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