# C-11 radiolabeling of iron-oxide nanoparticles for dual-modality PET/MR imaging

Ramesh Sharma[†‡*], Youwen Xu[†], Sung Won Kim[†], Michael J. Schueller[†], David Alexoff[†], S. David Smith[†], Wei Wang[‖], and David Schlyer[†*]

[†] Brookhaven National Laboratory, Upton, NY 11973
[‡] Gordon Battelle Memorial Institute, Columbus, OH 43201
[‖] Oak Ridge National Laboratory, Oak Ridge, TN 37831
[*] Corresponding authors: ramesharma26@gmail.com, schlyer@bnl.gov

## Supporting Information

### (1) Synthesis, surface functionalization and purification of magnetic iron-oxide nanoparticles

Deionized $H_2O$ (resistance ~18 MΩ·cm) was used throughout the experiments, and all reactions were performed under $N_2$ atmosphere or in closed containers to protect samples from oxidation. Superparamagnetic $Fe_3O_4$ nanoparticles (SPIO NPs) were prepared by a chemical co-precipitation method[1] at room temperature (23 ºC). Typically, 0.2 M $FeCl_3$ (anhydrate, EM Science) and 0.1 M $FeSO_4·7H_2O$ (J. T. Baker) were mixed in 100 mL $H_2O$ under $N_2$. Then 10 mL of 29.5 wt% $NH_3·H_2O$ (J. T. Baker) was injected into the mixture with vigorous stirring. Upon adding the $NH_3·H_2O$, the color of the mixture turned from brown to black immediately, implying formation of iron hydroxide. After stirring overnight for dehydration, the deep brown $Fe_3O_4$ NPs were collected by magnet and washed three times with deionized water.

The $Fe_3O_4$ NPs were surface-modified with –COOH or –$NH_2$ groups through covalent bonding. To functionalize the surface with –COOH groups, the $Fe_3O_4$ nanoparticles were dispersed in a 250 mL mixture of ethanol and $H_2O$ (volume ratio 20: 1) at pH~8.5 adjusted by $NH_3·H_2O$, and then 2 mL of N(trimethoxysilylpropyl) ethylenediamine-triacetic acid-trisodium salt (35% in water, Gelest) as a silane coupling agent was added drop by drop while stirring. After 12 hours, the reaction mixture was adjusted to pH ~3.5 by HCl, and the reaction was terminated after 12 hours. The -COOH functionalized $Fe_3O_4$ NPs were collected by a bar magnet and repeatedly washed with deionized water five times. In the washing process, particles were re-dispersed by a vortex mixer and separated by a magnet so that ionic and nonmagnetic impurities could be removed. To functionalize the surface of $Fe_3O_4$ NPs with –$NH_2$ groups, the same protocol was repeated using a different silane agent, 3-aminopropyltriethoxysilane (APTES), (99+%, Gelest), which contains a –$NH_2$ functional group.

Surface charge properties were evaluated by zeta potential measurements with the dynamic light scattering method (Brookhaven ZetaPlus analyzer). The measurements show zeta potentials of -33.6 ± 2.7 mV for unmodified NPs, 34.1 ± 3.7mV for $–NH_2$ modified NPs and -46.6 ± 3.1 mV for –COOH modified NPs in water.

## (2) Synthesis, surface functionalization and purification of $SiO_2$ nanoparticles

Silica NPs were synthesized by the Stöber method[2] of hydrolyzing tetraethoxysilane (TEOS) with ammonia as a catalyst in microemulsion media.[3] The microemulsion was first prepared by mixing 25.0 g Igepal CO-520 (Aldrich), 210 mL cyclohexane (EM Science), 3.3 mL $H_2O$ and 2.5 mL 29.5% $NH_3{\cdot}H_2O$ under magnetic stirring, and then 9.3 mL TEOS (≥99%, Aldrich) was added in the microemulsion under vigorous stirring. Hydrolysis and condensation of the TEOS monomers catalyzed by ammonia allow for formation of $SiO_2$ NPs. The reaction was continued for 24 hours to allow for completion to produce ~1g $SiO_2$ NPs. Surface functionalization of $SiO_2$ NPs with $–NH_2$ functional groups was carried out by dropwise addition of 10 mL silane coupling agent, APTES (10% in cyclohexane), to the microemulsion. Upon completion of the reaction in 24 hours, the microemulsions were broken by adding isopropanol, and the functionalized NPs were separated by centrifuge. The NPs were purified by washing with acetone, ethanol and water by re-dispersion-centrifugation cycles. Finally, the NPs were redispersed in water and transferred into a dialysis tube (SnakeSkin Pleated Dialysis Tubing, 3500 MWCO, Thermo Scientific) and then dialyzed in deionized water (resistivity ~18.2 MΩ·cm) to remove soluble impurities until the electrical conductivity was <2 µS.

Surface charge properties of the synthesized NPs were examined by dynamic light-scattering measurements (Brookhaven ZetaPlus analyzer). The measurements show zeta potentials of -35.4 ± 2.3 mV for unmodified and 34.8 ± 3.7 mV for $–NH_2$ modified silica NPs.

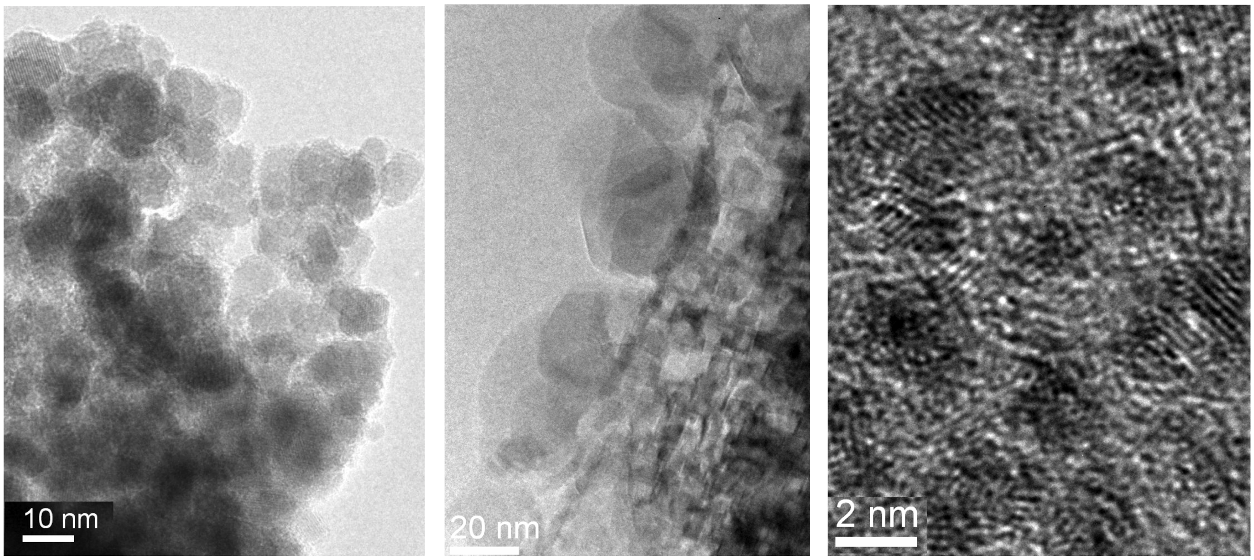

**Figure S1|** The TEM images of iron-oxide NPs (16 nm, Left), silica NPs (32 nm, Middle) and platinum NPs (2.5 nm, Right) used in C-11 labeling experiments.

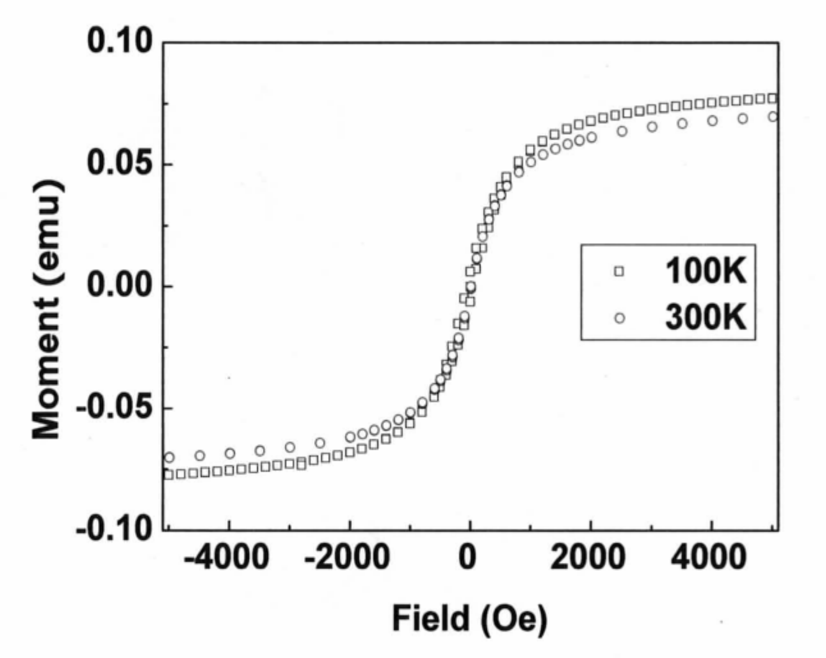

**Figure S2|** The magnetization curves of iron-oxide NPs acquired at 100 K (squares) and 300 K (circles) indicate the superparamagnetic behavior for these particles.

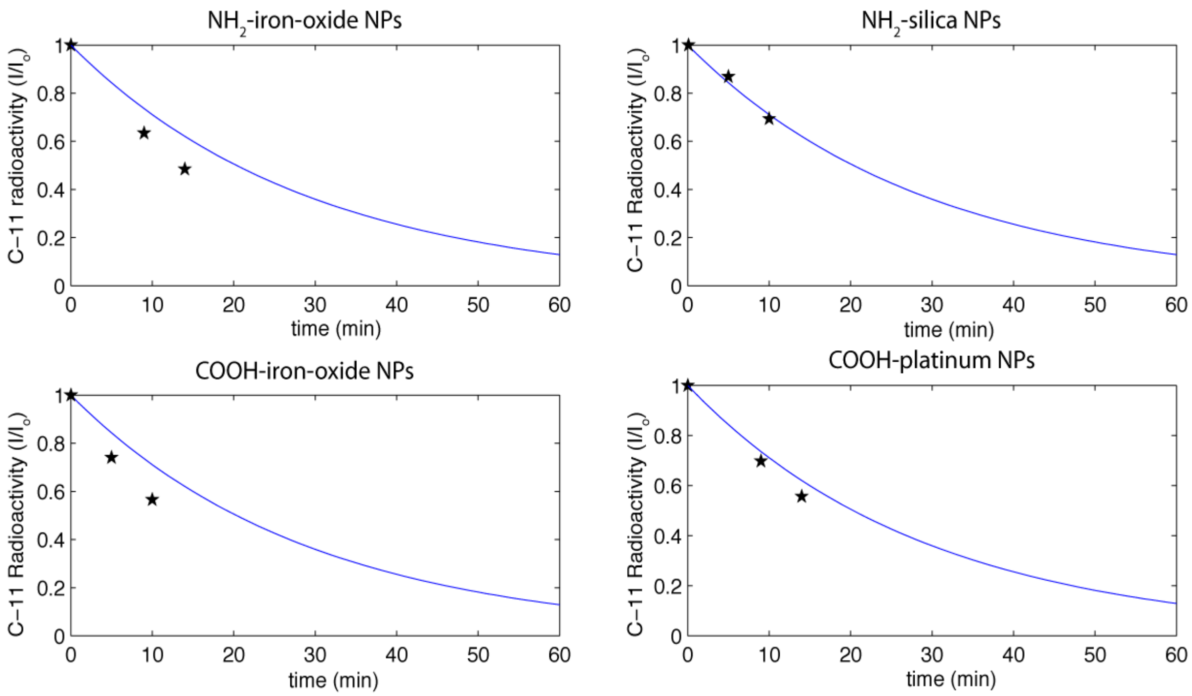

**Figure S3|** The radioactivity measurements in the C-11 labeled NPs during the course of purifications. The 3 corresponding data points (★) obtained after second, third and fourth washings, respectively, are plotted for 4 different types of NPs. The solid line is the theoretical radioactivity curve for C-11 ($t_{1/2}$ = 20.4 min). The experimental values (normalized to a value corresponding to the second wash) approximately match the theoretical values, confirming most of the C-11 labeling does not come off of the particles during the multiple cycles of washings.

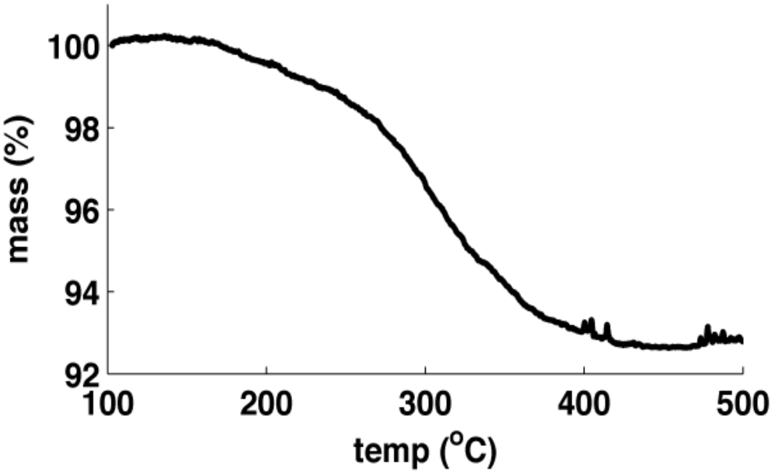

**Figure S4|** The TGA measurement of COOH-functionalized SPIO NPs shows the ligand composition in the dry sample is < 8% by mass.

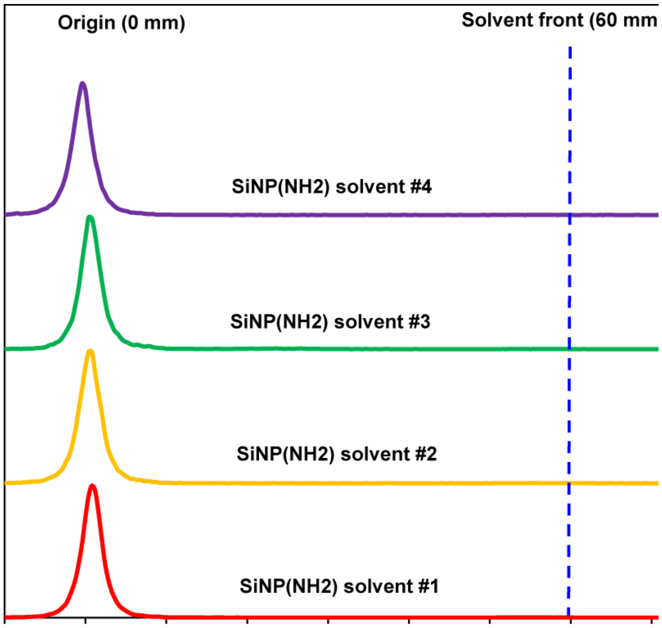

**Figure S5|** Radio-TLC chromatograms of $^{11}$C-labeled $SiO_2$-$NH_2$ NPs show the radioactive decay is present primarily at the origin. The TLC solvent systems were (1) hexane, (2) mixture of hexane and ethyl acetate (volume ratio 1:1), (3) water and (4) acetonitrile.

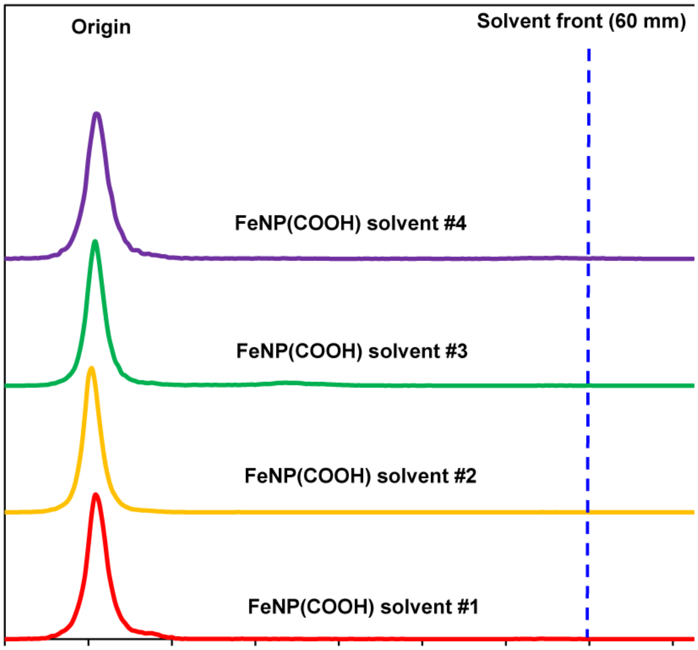

**Figure S6|** Radio-TLC chromatograms of $^{11}$C-labeled iron-oxide-COOH NPs show the radioactive decay is present primarily at the origin. The TLC solvent systems were (1) hexane, (2) mixture of hexane and ethyl acetate (volume ratio 1:1), (3) water and (4) acetonitrile.

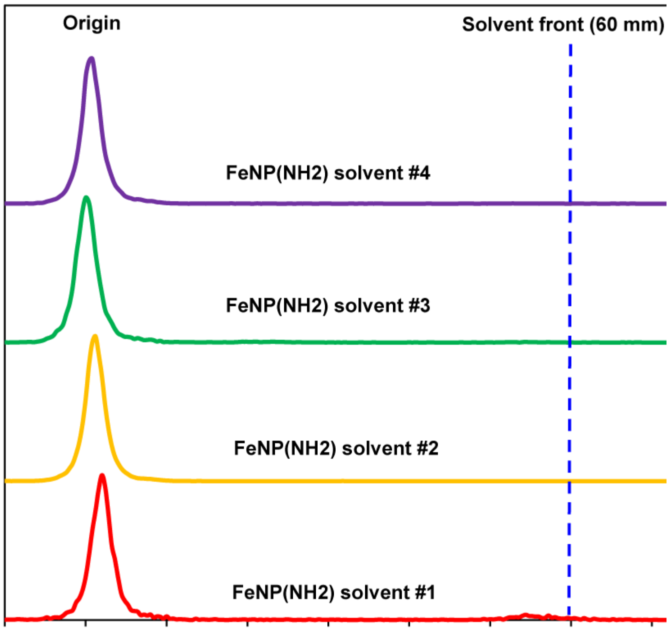

**Figure S7|** Radio-TLC chromatograms of $^{11}$C-labeled iron-oxide-$NH_2$ NPs show the radioactive decay is present primarily at the origin. The TLC solvent systems were (1) hexane, (2) mixture of hexane and ethyl acetate (volume ratio 1:1), (3) water and (4) acetonitrile.

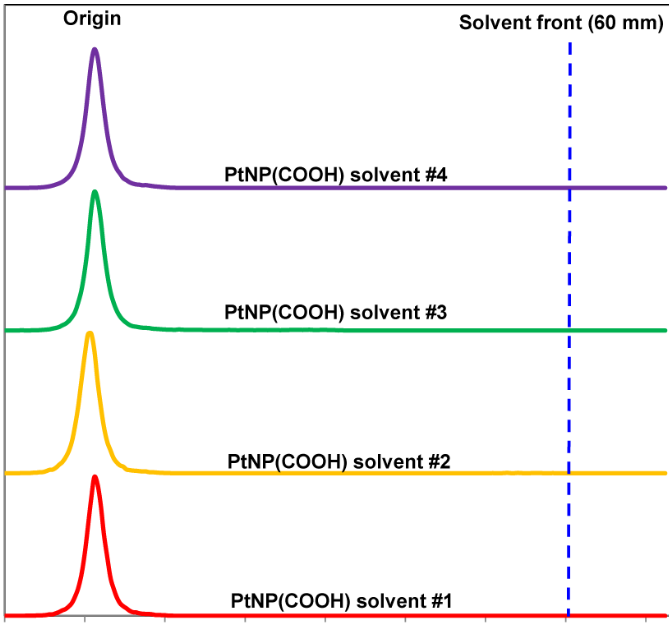

**Figure S8|** Radio-TLC chromatograms of $^{11}$C-labeled platinum-COOH NPs show the radioactive decay is present primarily at the origin. The TLC solvent systems were (1) hexane, (2) mixture of hexane and ethyl acetate (volume ratio 1:1), (3) water and (4) acetonitrile.

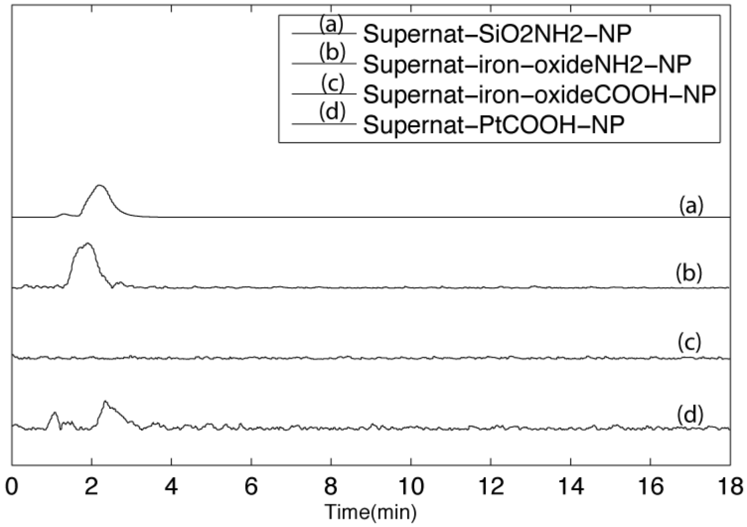

**Figure S9|** The radio-HPLC chromatograms of supernatant solutions obtained from the final washes of $^{11}$C-labeled NPs. The small peak positioned around 2 min is residual [$^{11}$C]CH$_3$I or [$^{11}$C]CH$_3$OH byproduct in the reaction mixture. The amount of radioactivity in the supernatant solution compared to $^{11}$C-labeled NPs was 10% or less, determined independently through radioactive decay measurements.

## References:

1. A.-H. Lu, E. L. Salabas and F. Schüth, *Angewandte Chemie International Edition*, 2007, **46**, 1222-1244.

2. W. Stöber, A. Fink and E. Bohn, *Journal of Colloid and Interface Science*, 1968, **26**, 62-69.

3. W. Wang and S. A. Asher, *Journal of the American Chemical Society*, 2001, **123**, 12528-12535.