AD_____

Award Number: W81XWH-05-1-0592

TITLE: PSMA-Targeted Nano-Conjugates as Dual-Modality (MRI/PET) Imaging Probes for the Noninvasive Detection of Prostate Cancer

PRINCIPAL INVESTIGATOR: Xiankai Sun, Ph.D.

CONTRACTING ORGANIZATION: The University of Texas Southwestern
Medical Center at Dallas
Dallas, TX 75390-9058

REPORT DATE: October 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-10-2006	Annual	15 Sep 2005 – 14 Sep 2006

4. TITLE AND SUBTITLE

PSMA-Targeted Nano-Conjugates as Dual-Modality (MRI/PET) Imaging Probes for the Noninvasive Detection of Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0592

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Xiankai Sun, Ph.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Texas Southwestern
Medical Center at Dallas
Dallas, TX 75390-9058

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
Original contains colored plates: ALL DTIC reproductions will be in black and white.

14. ABSTRACT

The goal of this project is to develop dual modality imaging probes for the detection of prostate cancer by doping radioisotopes to iron oxide nanoparticle, so that the sensitivity and specificity of prostate cancer diagnosis could be significantly improved. In the first year, we successfully developed standard procedures to reproducibly prepare dextran-coated iron oxide nanoparticles with desired particle size (DLS:7.5 – 35 nm) and size distribution (+/-5 nm) among the reaction condition variables, we found that the iron concentration plays the most important role in the nanoparticle formation. The high stability of the prepared particles was exhibited by a series of DLS measurements showing negligible aggregation or disintegration out to 10 days in water. By slightly modifying the synthetic conditions, we have doped three radioisotopes into the iron oxide core of the dextran-coated nanoparticles at high incorporation rates (>50%). The radioisotope-doped nano-platforms will be use for the development of MRI/PET amd MRI/SPECT dual modality imaging probes in the following two years.

15. SUBJECT TERMS
Prostate Cancer

16. SECURITY CLASSIFICATION OF:				18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	11	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Introduction

The goal of this project was set to explore a new approach that will combine the advantages of MRI and PET for the diagnostic imaging and staging of prostate cancer. We propose to dope positron-emitting isotopes to superparamagnetic iron oxide nanoparticle to make nanosized dual MRI/PET probes for the detection of prostate cancer by multi-modality (anatomical MRI plus functional PET) molecular imaging approaches, so that the sensitivity and specificity of prostate cancer diagnosis could be significantly improved. To realize the goal, two objectives were specified for this project: **Objective I**. Preparation/characterization of $^{77/74}$As-doped iron oxide nanoparticles and construction of PSMA-targeted nano-conjugates; and **Objective II**. Evaluation of the PSMA-targeted nano-conjugates in prostate cancer xenograft mouse models via conventional biodistribution and small animal MRI/PET imaging methods.

Body

In the statement of work (SOW), the focus of our first year work was on part of **Objective I**: Preparation and characterization of $^{77/74}$As-doped iron oxide nanoparticles. Specifically,

Months 0 – 12:
Milestone 1: Establish protocols of making dextran-coated iron oxide nanoparticles and obtain optimal conditions to control the size of nanoparticles in range of 20 – 40 nm.

Months 6 – 15:

Milestone 2: Establish protocols of making dextran-coated $^{77/74}$As-doped iron oxide nanoparticles to obtain optimal conditions to control the incorporation ratio of $^{77/74}$As into the iron oxide core.

Months 9 – 18:
Milestone: Establish protocols to construct PSMA-targeted nano-conjugates. Four such nano-conjugates are anticipated (two nano-conjugates with sizes of 25 nm and 35 nm per targeting molecule).

Research Progress in the First Year:

Milestone 1 (complete): Now we have established standard procedures to reproducibly prepare dextran-coated iron oxide nanoparticles with desired particle size by simply varying the concentrations of ferric and ferrous chloride solutions (Table 1). As the DLS measurement indicated, the dextran-coated iron oxide nanoparticles exhibited negligible aggregation or disintegration in aqueous solutions out to 10 days at pH from 12 to 7.

Table 1. Dextran-coated iron oxide nanoparticle size ($[Fe^{2+}]/[Fe^{3+}] = 1:2$).

$[Fe^{2+}]$ (mM)	34.0	17.0	4.80	1.63	1.04	0.93	0.10
DLS (nm)	35.4 ± 5.0	28.6 ± 4.6	20.5 ± 9.7	19.7 ± 4.4	14.6 ± 7.6	12.9 ± 5.5	7.5 ± 2.6

* DLS: Dynamic light scattering measurement.

Concentration, pH, temperature, and reaction times were evaluated as factors to control the nanoparticle formation. It appears that only the concentration significantly affects the final particle size. As show in Table 1, the particle size decreases as the iron concentration decreases, indicating that the particle size can be well controlled by simply varying the iron concentration. It is noteworthy that the size distribution of the dextran coated nanoparticle was quite narrow (+/-

5 nm) when the concentration of Fe^{2+} was 34.0 mM, 17.0 mM, 1.63 mM, or 0.10 mM. The freshly prepared nanoparticles (DLS: 28.6 ± 4.6 nm) were quite stable out to 10 days (DLS: 29.4 ± 13 nm). This indicates that the aggregation of the synthesized particle was negligible.

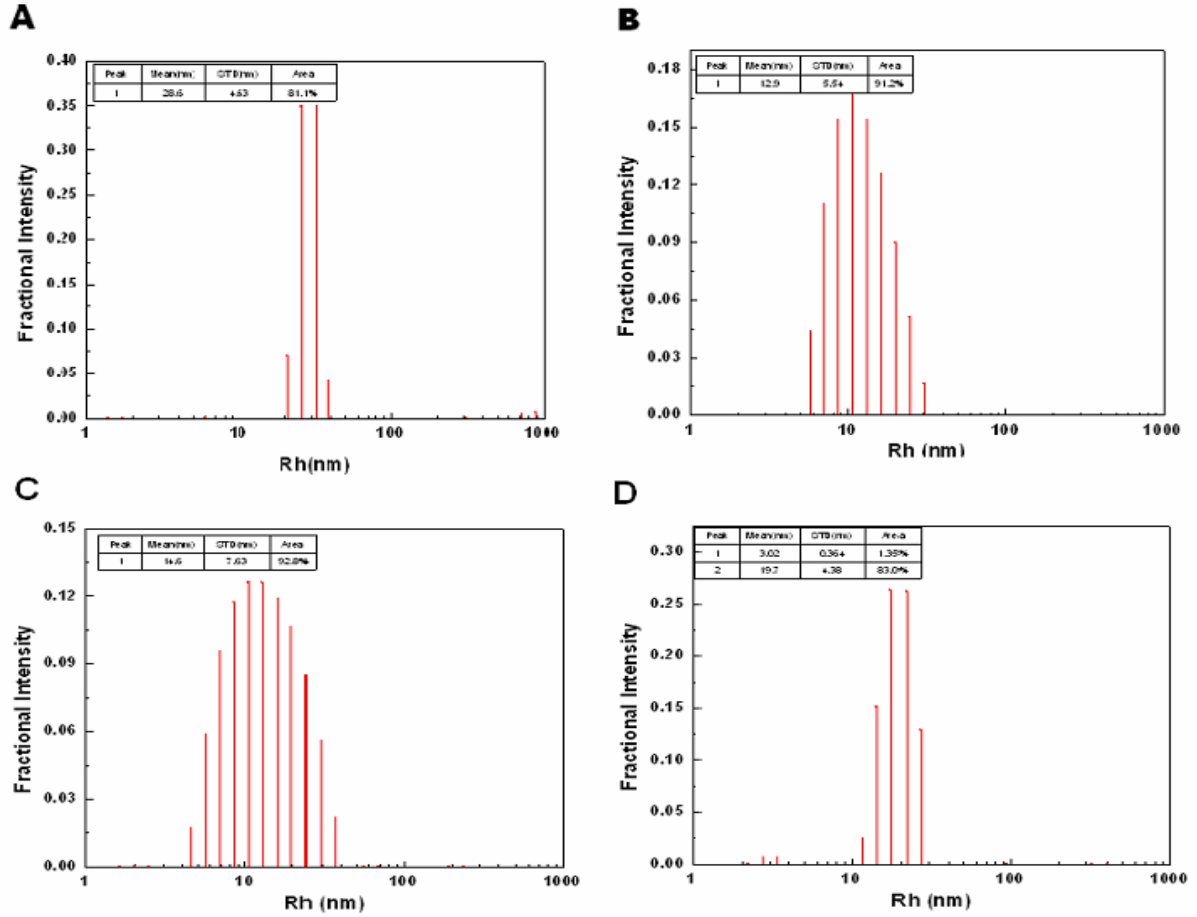

Fig.1. Typical DLS results of dextran coated iron oxide nanoparticles with the Rh of (A) 28.6 ± 4.6 nm; (B) 12.9 ± 5.5 nm; (C) 14.6 ± 7.6 nm; and (D) 19.7 ± 4.4 nm.

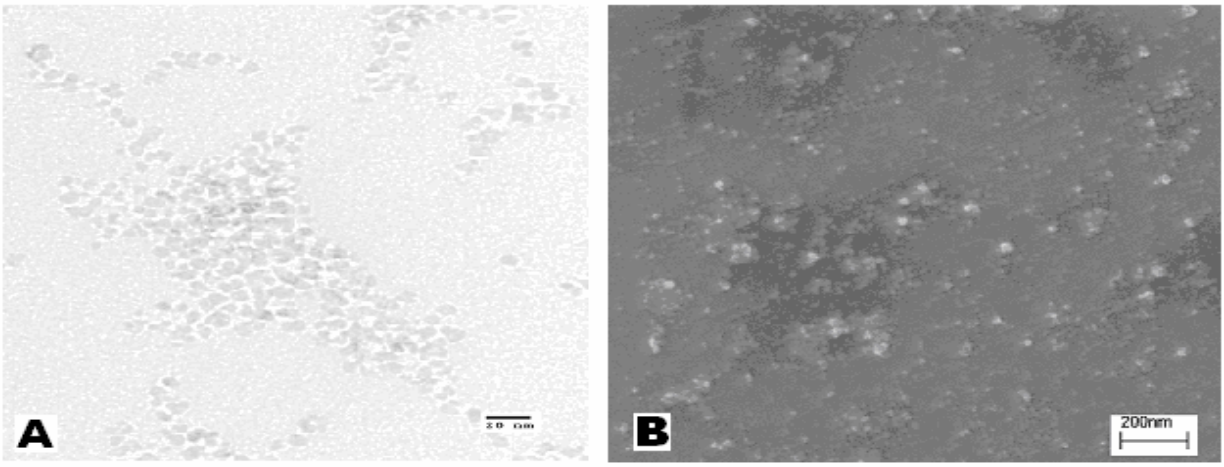

Fig. 2.TEM (A) and SEM micrographs (A) of the as-synthesized dextran coated iron oxide nanoparticles.

The morphology of the prepared nanoparticle was also evaluated by Scanning electron microscope (SEM), transmission electron microscopy (TEM), and atomic force microscope (AFM). The nanoparticle sample (DLS: 28.6 ± 4.6 nm) measured by DLS were used for the TEM (Fig.2A) and SEM (Fig. 2B), which show the solid particle size was much smaller (< 10 nm) than the DLS measurement. This is reasonable because the dextran coat of the nanoparticle is swollen and branched out in water. As shown in the AFM images (Fig.3), the dextran-coated nanoparticles appear flat (ϕ 55.0 ± 8.0 nm) on the MICA substrate with a height of 5.75 ± 0.68 based on 28 samples of the dextran coated iron oxide nanoparticle (DLS: 28.6 ± 4.6 nm).

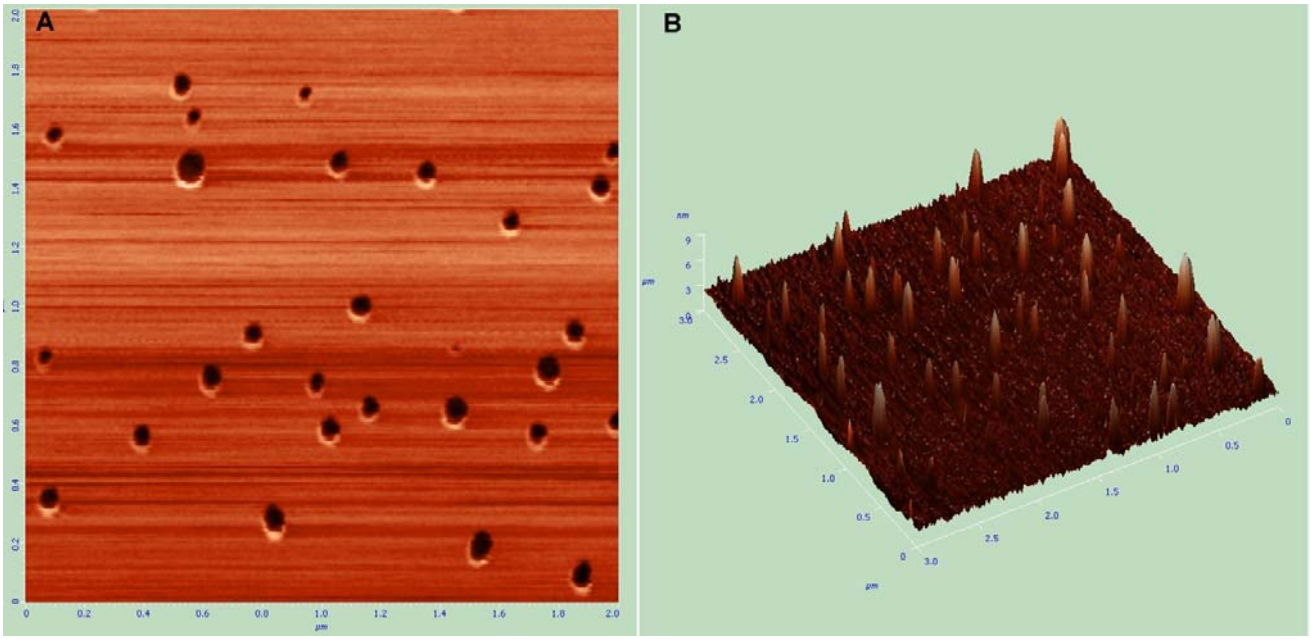

Fig.3. (A) The AFM images of dextran coated iron oxide nanoparticles on MICA substrate (image size: 2 μm × 2 μm), and (B) its corresponding 3-D image (3 μm × 3 μm × 9 nm).

Milestone 2 (complete):

In this project, only arsenic radionuclides were initially proposed to be doped into the nanoparticle core so that the nuclear imaging could be enabled. Actually metal radioisotopes with reasonably long half-lives can be also used for this purpose. Considering the common availability of ^{64}Cu and ^{177}Lu, we tried to incorporate these two isotopes to the iron oxide core of the dextran-coated nanoparticles besides arsenic isotopes. The decay characteristics of ^{64}Cu and ^{177}Lu are summarized in Table 2.

Table 2. Decay Characteristics of ^{64}Cu and ^{177}Lu.

Isotope	$t_{1/2}$	β^- MeV (%)	β^+ MeV (%)	EC (%)	γ MeV (%)
^{64}Cu	12.7 h	0.578 (39%)	0.653 (17.4%)	41%	1.35 (0.6%) 0.511 (38.6%)
^{177}Lu	6.71 d	0.497 (78.7%)			0.208 (11.0%) 0.113 (6.4%)

Copper PET radiopharmaceuticals are emerging as promising imaging and therapeutic agents. There are four copper radionuclides that can be used as PET imaging agents (^{64}Cu, ^{62}Cu, ^{61}Cu and ^{60}Cu). Among them, ^{64}Cu has diverse applications in PET imaging and radiotherapy

cancer therapy(*1-10*) due to the ability to produce it in high yield and specific activity on a small biomedical cyclotrons (*7, 11*) and its decay characteristics. Radiolanthanides are typical γ and β^--emitting isotopes that have been clinically used for diagnostic and therapeutic radiopharmaceuticals due to their disparate nuclear properties and similar chemical behavior (*12-19*). The low energy β^- of ^{177}Lu has shown promise for the palliative treatment of bone metastases, and its γ emissions can be used for diagnostic imaging and dosimetry of radiotherapy (*15-17*). In addition, several lanthanide radionuclides including ^{177}Lu are commercially available from the MURR center (Columbia, Missouri) on weekly basis. An additional reason for choosing ^{177}Lu, a promising therapeutic radionuclide, is that it emits γ photons with 208 keV that is ideal for our in-house small animal SPECT (single photon emission computed tomography) system with an energy window from 70 – 300 keV (*20, 21*), by which the imaging role of ^{177}Lu-labeled dual modality imaging probes will be validated.

Beside ^{77}As, the isotope proposed in this project, we have also successfully doped both ^{64}Cu and ^{177}Lu to the iron oxide core of the dextran-coated nanoparticles at reasonably higher incorporation rates (^{77}As: 58.0% after 3-h reaction; ^{177}Lu: 88.1% after 2-h reaction at carrier-added condition; ^{64}Cu: 50.6% after 1-h reaction) during the nanoparticle formation (Table 3).

Table 3. Incorporation rates of radioisotopes into dextran-coated iron oxide nanoparticle (DLS: 15.1 ± 10.6 nm).

Radioisotope		Incorporation rates		
		1-hour	2-hour	3-hour
Arsenic-77				58.0%
Lutetium-177	NCA	73.4%	73.5%	73.9%
	CA		88.1%	86.8%
Copper-64		50.6%	46.6%	

* NCA: non-carrier-added; CA: carrier-added.

Milestone 3 (Months 9 – 18): Establish protocols to construct PSMA-targeted nano-conjugates. Four such nano-conjugates are anticipated (two nano-conjugates with sizes of 25 nm and 35 nm per targeting molecule).

We are about to start the work for this Milestone. This work was a little delayed because extra time was needed in the beginning to get students and my laboratory staff familiar with the proposed procedures.

Key Research Accomplishments

1. Reproducible dextran-coated nanoparticle preparation
2. Methods of controlling the particle size (7.5 – 35 nm) and size distribution (+/- 5 nm)
3. The characterization results of dextran-coated nanoparticles via DLS, SEM, TEM, and AFM methods.
4. Stability of the prepared dextran-coated nanoparticle (no aggregation out to 10 days)
5. Success in the incorporation of three radioisotopes into the iron oxide core of the dextran-coated nanoparticles (^{77}As: 58.0%; ^{177}Lu: 88.1% ; ^{64}Cu: 50.6%) for MRI/PET (^{74}As and

^{64}Cu doped iron oxide nano-platforms) and MRI/SPECT (^{177}Lu doped iron oxide nano-platform) dual modality imaging probe development.

Reportable Outcomes

Manuscripts and abstracts are to be considered in the 2nd and 3rd year.

Conclusions

We have successfully established standard procedures to reproducibly prepare dextran-coated iron oxide nanoparticles with desired particle size (DLS: 7.5 – 35 nm) and size distribution (+/- 5 nm). In addition to the DLS characterization, the morphology of the prepared nanoparticles was also evaluated by SEM, TEM, and AFM methods. Among the reaction condition variables, the iron concentration plays the most important role in the formation of the dextran-coated nanoparticles. Determined by the DLS measurements, negligible aggregation or disintegration of the nanoparticles was observed in water out to 10 days, indicating the high stability of the prepared particles. By slightly modifying the synthetic conditions, we have doped three radioisotopes into the iron oxide core of the dextran-coated nanoparticles at high incorporation rates (> 50%) under non-carrier-added and carrier-added conditions. The radioisotope-doped iron oxide nanoparticles will be used for the development of MRI/PET (^{74}As and ^{64}Cu doped) and MRI/SPECT (^{177}Lu doped) dual modality imaging probes in the following two years.

Materials and Methods

1. Materials

All chemicals were of reagent grade and used as received. Ferric chloride hexahydrate ($FeCl_3 \bullet 6H_2O$, >99.0%), ferrous chloride tetrahydrate ($FeCl_2 \bullet 4H_2O$, >99.0%), sodium hydroxide, and dextran (Average MW, 64 – 76 KDa) were purchase from Sigma- Aldrich (St. Louis, MO). Ammonium hydroxide water solution (puriss. wt. 29.46%) was purchased from Fisher. Dialysis tubing (MWCO 100 KDa) was purchased from Spectrum Laboratories (Rancho Dominguez, CA). Acrodisc Syringe Filter was purchased from Pall Life Sciences (New York 11548 U.S.A). The aqueous solutions were prepared in Milli-Q water (resistivity: 18.2 MQ cm) deoxygenated by bubbling with N_2 gas for 30min prior to use. Arsenic-77 was purchased from the University of Texas at Austin, ^{177}Lu from the University of Missouri-Columbia, and ^{64}Cu from MDS Nordion (Canada). Centricons (YM-100: MWCO 100 KDa) were purchased from Millipore (MA 01821, USA). Dialysis tubing (MWCO 100 KDa) was purchased from Spectrum Laboratories (Rancho Dominguez, CA). Super sharp diamond-like carbon (DLC) tips and Highly-oriented Pyrolytic Graphite (HOPG), GRAS/1.5 mm thick substrate were purchased from Nanotech-America (Allen, TX 75002). Mica Grade V-1 15mm x 15 mm x 0.15 mm substrate was purchased from SPI Supplies and Structure Probe, Inc (West Chester, PA 19380).

The nanoparticle characterizations were done with the WyattQELS dynamic light scattering (DLS) and the Leo 1530 Scanning Electron Microscope (SEM) at University of Dallas, the Jeol 1200 Transmission Electron Microscope (TEM) and NT-MDT SOLVER PRO Atomic Force Microscope (AFM) at UT Southwestern Medical Center.

2. Synthesis of dextran coated iron oxide nanoparticles

Dextran coated iron oxide nanoparticles were prepared via the coprecipitation of Fe^{3+} and Fe^{2+} from their aqueous solution by the addition of a base solution. Briefly, dextran (MW: 64 – 76

KDa) was oxidized with 3N NaOH at 80°C, followed by neutralization to pH 7. The $FeCl_3 \bullet 6H_2O$ and $FeCl_2 \bullet 4H_2O$ salts at an appropriate molar ratio were dissolved in deoxygenated Milli-Q water, giving a clear light yellow solution with the concentration of Fe^{3+}or Fe^{2+} in range of 0.02 mM to 35 mM. To the Fe^{3+}/ Fe^{2+} solution, 0.1 ~ 1.5 g of the NaOH-oxidized dextran was added. The resulting solution (pH ~ 3) was stirred at a speed of 600 rpm under a nitrogen flow at 4 L per minute (LPM) for 15 min. Then the nitrogen flow rate was decreased to 3 LPM for the following procedures. In a 10 min period, 1 ~ 5 mL of 5.9% ammonia solution was added dropwise, during which the color of the reaction mixture changed from light yellow to light green to brownish green. At the end of the ammonia addition, the pH was 7 - 8. The pH of the solution was further adjusted to above 11 with 1N NaOH for the crystallization of iron oxide and the subsequent dextran coating to occur. The solution was then neutralized to pH 7 by drop-wise addition of 1N HCl, which was kept stirring until the particle solution was sampled. The sampled solution was loaded to a centricon filter tube (MWCO: 100 KDa) and washed with three portions of 2 mL of milli-Q water, or to a dialysis tube (MWCO: 100 KDa) and dialyzed extensively against 1 L of milli-Q water two times (12 h each) to remove nonreacted ions, dextran, or free radioisotopes.

3. Incorporation of radioisotopes into dextran coated iron oxide nanoparticles

3.1 Incorporation of ^{77}As. To a reaction flask, 9 mL of mixture composed of 6.5 mg (0.024 mmol) $FeCl_3 \bullet 6H_2O$ and 2.1 mg (0.011 mmol) $FeCl_2 \bullet 4H_2O$ was added firstly, and then 108 mg oxidized dextran was added following by 900 μL of ^{77}As (~ 6 μCi in 1N HCl). The resulting mixture was then stirred under nitrogen gas with a speed of 600 rpm. And 2 mL of 29.46% ammonia was added drop wise. The reaction was incubated for 3 h at room temperature. The following procedures were the same as described above. The purified product and the one without dialysis were sampled and counted by a gamma-counter. The incorporation rate of ^{77}As into iron oxide was about 58%.

3.2 Incorporation of ^{177}Lu. To incorporate ^{177}Lu into dextran coated iron oxide nanoparticles, a lower concentration reaction mixture was prepared in order to enhance the incorporation efficiency. Briefly, 5.5 mg of $FeCl_3 \bullet 6H_2O$ and 1.8 mg of $FeCl_2 \bullet 4H_2O$ were first dissolved in 50 mL deoxygenated water and then 2.5 mL of above mixture was taken and diluted to 10 mL with water, to which 123 mg dextran and about 500 μCi of ^{177}Lu in 0.05 N HCl was added. Then 1 mL of 5.88% ammonia solution was added drop wise into the reaction flask. The following procedures were the same as described above. At cetain time points (1 h, 2 h, and 3 h), 1 mL of the sample was collected and washed via YM-100 centricon three times with 2 mL of milli-Q water (the centrifuge condition: 5,000 rpm, 30 min, 4°C). And then all the purified samples together with those not purified (standard) were counted by gamma-counter. The incorporation rate of ^{177}Lu under this condition was 73%. Under a carrier-added condition (molar Fe: Lu = 1000: 1), the incorporation rate increased to 88%.

3.3 Incorporation of ^{64}Cu. A mixture with 11.2 mg of $FeCl_3 \bullet 6H_2O$ and 3.8 mg of $FeCl_2 \bullet 4H_2O$ in 50 mL deoxygenated water was prepared, out of which 2.5 mL was taken and diluted to 10 mL with water. To the resulting solution, 123 mg of oxidized dextran and 1.1 mCi of ^{64}Cu were added following by the dropwise addition of 1 mL of 5.9% ammonia solution. The following procedures were the same as described above. Samples was collected at 1 h and 2.5 h. The incorporation rate was 51%.

4. Stability of the dextran-coated nanoparticles

For the stability study, the sample with a hydrodynamic radius of 28.6 ± 4.6 nm was chosen. After synthesis, the sample without purification was kept at RT in a sealed scintillation vial. Ten days later, the sample was examined by DLS.

5. Characterization of dextran coated iron oxide nanoparticles

5.1 Particle size and size distribution. Size and size distribution of the dextran coated iron oxide nanoparticles were measured by Dynamic Light Scattering (WyattQELS). The samples were prepared by adding a small amount (20 μL) of the nanoparticle solution to a scintillation vial, and then diluting it with de-ionized water until the DLS counter rate was within the measurement range.

5.2 Morphology of the dextran-coated nanoparticles.

TEM and SEM: The morphology and size of the as-synthesized dextran-coated iron oxide nanoparticles were examined with a Jeol 1200 Transmission Electron Microscope at an acceleration voltage of 120 kV and a Leo 1530 Scanning Electron Microscope. Samples were prepared by transferring 2 μL of the much diluted dextran coated iron oxide solution to a TEM copper grid or a SEM silica wafer, and then allowed to dry at room temperature. The resulting coating was washed by water (5 x 1 mL), and allowed to air dry at RT.

AFM: The dextran coated iron oxide nanoparticles were also examined by AFM before and after purification with samples prepared on two different substrates, MICA and HOPG. Samples were prepared by transferring 2 μL of the diluted dextran coated iron oxide solution to the freshly cleaved mica or graphite surface, while both MICA and HOPG substrates were sated on a strong magnet. For the MICA substrate, the samples were allowed air dry; while for the HOPG substrate, the samples were dried in a nitrogen chamber.

Reference:

(1) Anderson, C. J., Dehdashti, F., Cutler, P. D., Schwarz, S. W., Laforest, R., Bass, L. A., Lewis, J. S., and McCarthy, D. W. (2001) ^{64}Cu-TETA-Octreotide as a PET imaging agent for patients with neuroendocrine tumors. *J. Nucl. Med. 42*, 213-221.

(2) Anderson, C. J., Jones, L. A., Bass, L. A., Sherman, E. L., McCarthy, D. W., Cutler, P. D., Lanahan, M. V., Cristel, M. E., Lewis, J. S., and Schwarz, S. W. (1998) Radiotherapy, toxicity and dosimetry of copper-64-TETA-octreotide in tumor-bearing rats. *J. Nucl. Med. 39*, 1944-1951.

(3) Anderson, C. J., Rocque, P. A., Weinheimer, C. J., and Welch, M. J. (1993) Evaluation of copper-labeled bifunctional chelate-albumin conjugates for blood pool imaging. *Nucl. Med. Biol. 20*, 461-467.

(4) Anderson, C. J., and Welch, M. J. (1999) Radiometal-labeled agents (non-technetium) for diagnostic imaging. *Chem. Rev. 99*, 2219-2234.

(5) Connett, J. M., Anderson, C. J., Guo, L. W., Schwarz, S. W., Zinn, K. R., Rogers, B. E., Siegel, B. A., Philpott, G. W., and Welch, M. J. (1996) Radioimmunotherapy with a Cu-64-labeled monoclonal antibody: A comparison with Cu-67. *Proc. Natl. Acad. Sci. USA 93*, 6814-6818.

(6) Lewis, J. S., R., L. M., Cutler, P. D., Srinivasan, A., Schmidt, M. A., Schwarz, S. W., Morris, M. M., Miller, J. P., and Anderson, C. J. (1999) Radiotherapy and dosimetry of ^{64}Cu-TETA-Tyr3-octreotate in a somatostatin receptor-positive, tumor-bearing rat model. *Clin. Cancer Res. 5*, 3608-3616.

(7) McCarthy, D. W., Shefer, R. E., Klinkowstein, R. E., Bass, L. A., Margenau, W. H., Cutler, C. S., Anderson, C. J., and Welch, M. J. (1997) The efficient production of high specific activity Cu-64 using a biomedical cyclotron. *Nucl. Med. Biol. 24*, 35-43.

(8) Sun, X., Wuest, M., Kovacs, Z., Sherry, A. D., Motekaitis, R., Wang, Z., Martell, A. E., Welch, M. J., and Anderson, C. J. (2003) In Vivo Behavior of Copper-64-Labeled Methanephosphonate Tetraazamacrocyclic Ligands. *J. Biol. Inorg. Chem. 8*, 217-225.

(9) Wipke, B. T., Wang, Z., Kim, J., McCarthy, T. J., and Allen, P. M. (2002) Dynamic visualization of a joint-specific autoimmune response through positron emission tomography. *Nat. Immunol. 3*, 366-372.

(10) Zimmermann, K., Grunberg, J., Honer, M., Ametamey, S., Schubiger, P. A., and Novak-Hofer, I. (2003) Targeting of renal carcinoma with $^{67/64}$Cu-labeled anti-Li-CAM antibody chCE7: selection of copper ligands and PET imaging. *Nucl. Med. Biol. 30*, 417-427.

(11) McCarthy, D. W., Bigott, H. M., Perkins, T. A., Voller, T. F., and Welch, M. J. (2001) A research resource in radionuclide research. *J. Label. Compd. Radiopharm. 44*, S817-S819.

(12) Liu, S., Cheung, E., Ziegler, M. C., Rajopadhye, M., and Edwards, D. S. (2001) (90)Y and (177)Lu labeling of a DOTA-conjugated vitronectin receptor antagonist useful for tumor therapy. *Bioconjug Chem 12*, 559-68.

(13) Liu, S., Harris, T. D., Ellars, C. E., and Edwards, D. S. (2003) Anaerobic 90Y- and 177Lu-labeling of a DOTA-conjugated nonpeptide vitronectin receptor antagonist. *Bioconjug Chem 14*, 1030-7.

(14) Bayouth, J. E., Macey, D. J., Kasi, L. P., Garlich, J. R., McMillan, K., Dimopoulos, M. A., and Champlin, R. E. (1995) Pharmacokinetics, dosimetry and toxicity of holmium-166-DOTMP for bone marrow ablation in multiple myeloma. *J. Nucl. Med. 36*, 730-7.

(15) Chakraborty, S., Das, T., Unni, P. R., Sarma, H. D., Samuel, G., Banerjee, S., Venkatesh, M., Ramamoorthy, N., and Pillai, M. R. (2002) ^{177}Lu labelled polyaminophosphonates as potential agents for bone pain palliation. *Nucl Med Commun 23*, 67-74.

(16) Das, T., Chakraborty, S., Unni, P. R., Banerjee, S., Samuel, G., Sarma, H. D., Venkatesh, M., and Pillai, M. R. (2002) ^{177}Lu-labeled cyclic polyaminophosphonates as potential agents for bone pain palliation. *Appl Radiat Isot 57*, 177-84.

(17) Lewis, J. S., Wang, M., Laforest, R., Wang, F., Erion, J. L., Bugaj, J. E., Srinivasan, A., and Anderson, C. J. (2001) Toxicity and dosimetry of ^{177}Lu-DOTA-Y3-octreotate in a rat model. *Int J Cancer 94*, 873-7.

(18) Smith, C. J., Gali, H., Sieckman, G. L., Hayes, D. L., Owen, N. K., Mazuru, D. G., Volkert, W. A., and Hoffman, T. J. (2003) Radiochemical investigations of 177Lu-DOTA-8-Aoc-BBN[7-14]NH2: an in vitro/in vivo assessment of the targeting ability of this new radiopharmaceutical for PC-3 human prostate cancer cells. *Nucl Med Biol 30*, 101-9.

(19) Volkert, W. A., and Hoffman, T. J. (1999) Therapeutic radiopharmaceuticals. *Chem Rev 99*, 2269-92.

(20) Tsyganov, E., Antich, P., Parkey, R., Selunin, S., Slavine, N., and Soesbe, T. (2003) in *EEE Nuclear Science Symposium & MIC-2003 Conference Record. October 19-26.* pp M3-31, Portland, Oregon, USA.

(21) Tsyganov, E. N., Antich, P. P., Kulkarni, P. V., Mason, R. P., Parkey, R. W., Seliounine, S. Y., Shay, J. W., Soesbe, T. C., and Zinchenko, A. I. (2003) in *IEEE NSS/MIC conference.*, Rome, Italy.