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14. ABSTRACT

Glioblastoma (GBM) is the most common and aggressive brain cancer. Even with the highest first-year cost (> $120,000) under standard-of-care treatments, the prognosis for GBM patients is dismal. Therefore, it is of great clinical significance to develop novel therapeutic approaches to improve GBM treatment efficacy. Alpha particle radiation therapy with high linear energy transfer (80 keV/μm) has a potent therapeutic effect independent of dose rate, cell cycle, and oxygen concentration. A single alpha-particle track can result in lethal DNA double-strand breaks. Astatine-211 (^{211}At) is an attractive alpha emitter for alpha particle radiation therapy because it has the advantages of an optimal half-life (7.2 h) and no long-lived decay "daughter" radionuclides thus avoiding toxicity from daughter radionuclide redistribution. However, traditional ^{211}At radiolabeling methods focusing on At-C chemical bonds have the challenges of having a complicated radiolabeling process and low conjugation efficiency. In this study, we develop targeted gold nanoparticles as a novel ^{211}At delivery nanoplatform for alpha particle radiation therapy. In the past year, we have synthesized gold nanoparticles with different sizes and tested their radiolabeling performance. Experimental results demonstrated that the synthesized AuNPs could achieve high radiolabeling efficiency by simply mixing ^{211}At radioisotopes and AuNPs in an aqueous solution at room temperature for a short time. Quantum chemistry calculations have been performed to investigate the bonding mechanism of the At-Au chemical bond. A sigma chemical bond was identified and the valence state for At and Au was determined to be -1 and +1, respectively. Targeting ligands, c(RGDfK) and angiopep-2 were conjugated to the AuNPs for brain cancer targeting. In vivo studies were performed to investigate ^{211}At-loaded AuNP's biodistribution after intravenous administration. We have been working on in vitro and in vivo studies to demonstrate the developed novel alpha particle radiation therapy with AuNPs can be exploited to generate improved therapeutic effect to treat GBM using murine animal models.

15. SUBJECT TERMS
Brain Cancer, Gold nanoparticles (AuNPs), Astatine-211 (^{211}At), Alpha particle radiation therapy.

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Glioblastoma (GBM) is one of the most common and aggressive brain cancer with more than 10,000 newly diagnosed patients in the United States each year. The median survival is only 15 months even after aggressive treatments including surgery, chemotherapy, and radiation therapy. There is a clear and urgent need to develop novel therapeutic approaches for effective GBM treatment. Alpha particle radiation therapy has the promise to improve brain cancer treatment with its potent cytotoxicity from high linear energy transfer. Among the different available alpha-particle emitters, astatine-211 (^{211}At) has the advantage of optimal half-life (7.2 h) and no confounding radioactive daughters. This project is aimed to develop targeted gold nanoparticles as a novel ^{211}At delivery platform to treat brain cancer using murine animal models.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Brain Cancer, Glioblastoma (GBM), Alpha particle radiation therapy, Astatine-211 (^{211}At), Gold nanoparticles (AuNPs).

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major goal 1: Develop AuNPs for At-211 alpha particle radiation therapy.

Milestone 1.1: Obtain AuNPs with different sizes for radiolabeling. (Target date: Dec 14th, 2019; Completed on Dec 1st, 2019).

Milestone 1.2: Get optimized AuNPs with high radiolabeling efficiency. (Target date: May 14th, 2020; Completed on April 30th, 2020).

Major goal 2: Functionalize AuNPs for brain cancer targeting and treatment.

Milestone 2.1: Functionalize AuNPs with both c(RGDfK) and angiopep-2 ligands. (Target date: May 14th, 2020; Completed on May 1st, 2020)

Milestone 2.2: Characterize AuNP's properties (Target date: May 14th, 2020; 80% completed).

Milestone 2.3: Demonstrate developed AuNPs can target and treat brain cancer cells with in vitro test (Target date: August 14th, 2020; 20% completed).

Major goal 3: Evaluate brain cancer targeting, pharmacokinetics, and therapeutic effect of developed AuNPs using murine animal models.

Milestone 3.0: Get animal protocol approved by Duke IACUC and DOD ACURO. (Target date: Nov 14th, 2019; Completed on Mar 2nd, 2020)

Milestone 3.1: Get in vivo biodistribution and pharmacokinetic properties of AuNPs. (Target date: Nov 14th, 2020; 30% completed)

Major goal 3.2: Obtain MTD of ^{211}At-AuNPs. (Targeted date: November 14th, 2020; 0% completed)

Major goal 3.3: Determine the therapeutic effect of the developed ^{211}At alpha-particle radiotherapy with AuNPs and prepare a manuscript for publication. (Targeted date: February 14th, 2020; 0% completed)

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Aim 1. Synthesize, optimize and evaluate AuNPs (< 5 nm) for ^{211}At and ^{124}I labeling

Major task 1: Synthesize AuNPs with size between 1 and 5 nm

We synthesized different gold nanoparticles for ^{211}At and ^{124}I labeling. Transmission electron microscopy and dynamic light scattering were used to characterize the synthesized 3 nm AuNPs (Fig. 1A) and 12 nm AuNPs (Fig 1B). 3 nm AuNPs were synthesized by reducing $HAuCl_4$ with $NaBH_4$ in the presence of SH-PEG_6 and SH-PEG_8-COOH in the ice-cooled water solution. The synthesized AuNPs were measured to have a similar hydrodynamic size as that of 13 KDa protein (Fig. 1C) using size exclusion chromatography. The 12 nm AuNPs were synthesized by reducing $HAuCl_4$ with sodium citrate in the boiling water. The obtained AuNPs in different sizes were used for the following radiolabeling studies.

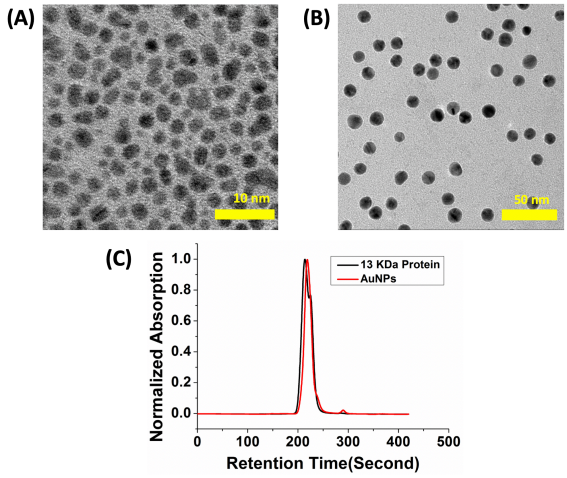

Fig 1. TEM image for the synthesized 3 nm AuNPs (A) and 12 nm AuNPs (B). Size exclusion chromatography for the synthesized 3 nm AuNPs (C). 13 KDa protein was used as a standard.

Major task 2: Evaluate ^{211}At and ^{124}I labeling on AuNPs with different size

^{211}At radioisotopes were produced using CS-30 cyclotron at Duke University. The ^{211}At radioisotopes generated on the bismuth target were collected by using a dry distillation method. The obtained ^{211}At radioisotopes in methanol were used for the following studies.

First, we used 3 nm AuNPs to test radiolabeling efficiency with ^{211}At in different oxidation states (-1, 0, and +1). After half-hour incubation, the radiolabeling efficiency was determined to be 97.6%, 96.4% and 89.2% for ^{211}At at -1, 0, and +1 oxidation state, respectively. We performed quantum chemistry calculations to investigate the bonding mechanism and found the At-Au bond contained 19% 5P:z atomic orbital, 36% 6S atomic orbital of At, and 44% 3D:z^2 atomic orbital of Au (Fig. 2). The valence state for At and Au was determined to be -1 and +1 respectively.

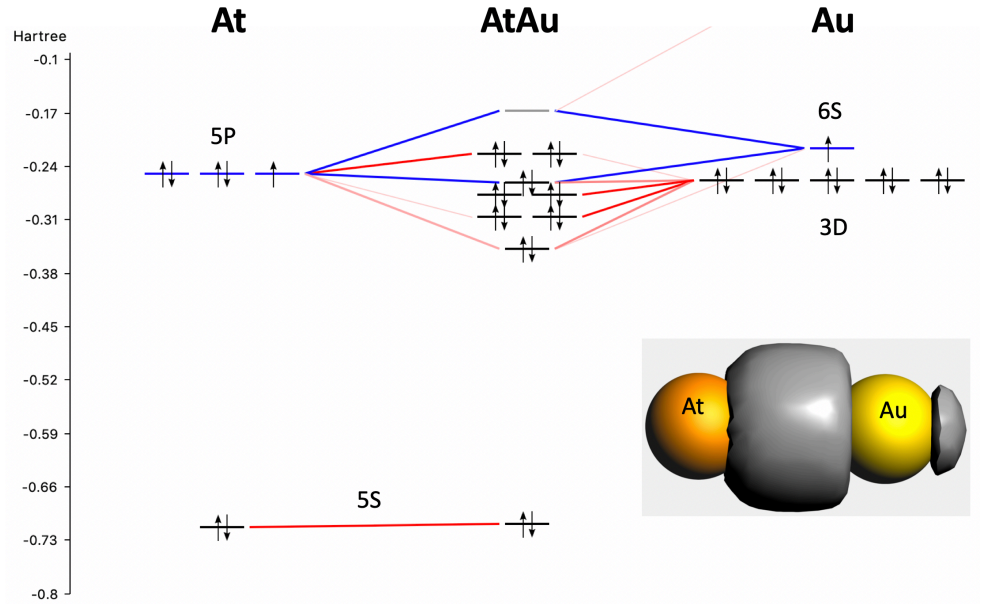

Fig. 2. Bonding mechanism analysis for At-Au chemical bond. Amsterdam Density Functional (ADF) software was used to perform quantum chemistry calculations.

Second, we used 3 nm AuNPs to test how incubation time and Au mass affect ^{211}At (-1 state) radiolabeling efficiency. The Au mass for the test was 200 µg in 200 µl solution. The radiolabeling efficiency at room temperature was measured to be 94%, 97%, and 98% for 1 min, 5 min, and 30 min incubation time, respectively. After that, we tested how Au mass affects ^{211}At radiolabeling efficiency with 5 min incubation at room temperature. The measured efficiency is 97% (200 µg), 90% (20 µg), 85% (2 µg), 76% (200 ng), 58% (20 ng), 10% (2 ng), respectively. Experiment results demonstrated that the synthesized 3 nm AuNPs could reach high radiolabeling efficiency by simply incubating AuNPs with At-211 for 5 minutes at room temperature.

Third, we performed ^{211}At stability test in different challenge conditions. The ^{211}At radioactivity remaining on AuNPs was measured to be 96% (smashed liver suspension), 98% (murine serum), and 99% (phosphate-buffered saline) after 3 hours incubation at 37 °C. Phantom studies demonstrated that the synthesized 3 nm AuNPs could load ^{211}At with high stability. 12 nm AuNPs had a similar performance for ^{211}At radiolabeling and stability tests.

We also tested 3 nm AuNP's radiolabeling performance for iodine radioisotopes (^{131}I). The radiolabeling efficiency was measured to be 99% (200 µg Au, 5 min incubation at room temperature) and 71% (2 µg Au, 5 min incubation at room temperature). Density functional theory calculation was performed to investigate the bonding mechanism of the Au-I chemical bond. We found the Au-I bond contained 19% 4P:z atomic orbital of I and 36% 6S atomic orbital and 44% 3D:z^2 atomic orbital of Au (Fig. 2). The oxidation state for I and Au was determined to be -1 and +1 respectively. The bonding mechanism of Au-I is similar to that of Au-At.

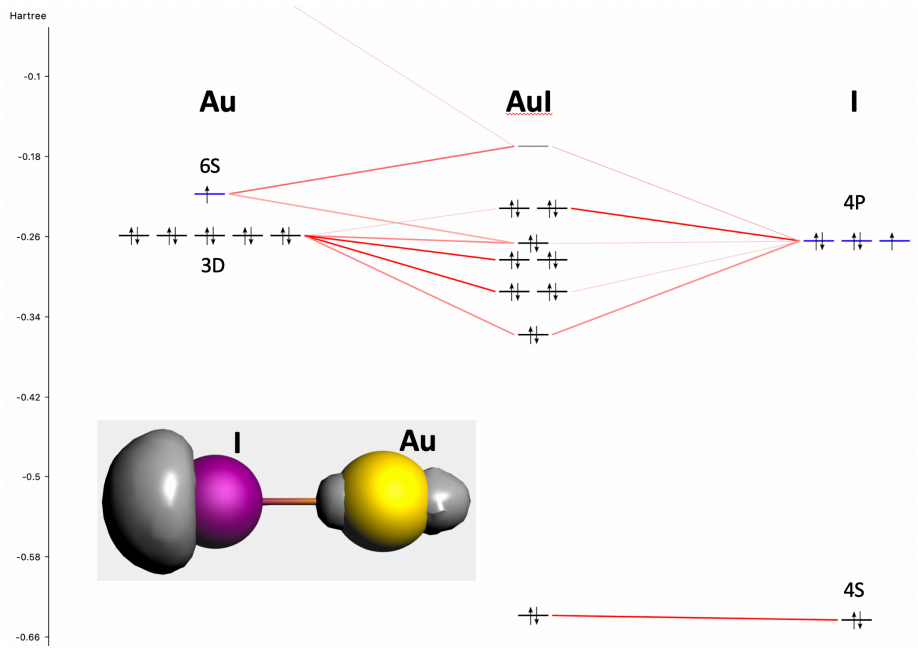

Fig. 3. Bonding mechanism analysis for At-I chemical bond. Amsterdam Density Functional (ADF) software was used to perform quantum chemistry calculations.

Aim 2. Conjugate AuNPs with tumor-homing peptides for brain cancer-targeting and perform *in vitro* tests to demonstrate the developed nanoagent can target and treat brain cancer.

Major task 1: Functionalize AuNPs with brain cancer-targeting ligands and peptides for blood-brain-barrier penetration.

We conjugated c(RGDfK) peptide to 3 nm AuNPs by using the Amine-NHS chemistry method. The 3 nm AuNPs had carboxylic acid functional groups at the end of SH-PEG_8 chains. The carboxylic acid group was converted to N-hydroxysuccinimide (NHS) ester by using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and Sulfo-NHS. After that, the free amine group in the c(RGDfK) side chain was used to react with NHS ester for conjugation. In addition, we also functionalized 3 nm AuNPs with angiopep-2. The custom-synthesized peptide had a cystine on the end, which was used to bind to AuNPs. Angiopep-2 peptides could help AuNPs penetrate blood-brain-barrier by low-density lipoprotein receptor-mediated transcytosis. We have developed AuNPs functionalized with both c(RGDfK) and angiopep-2 peptides.

Major task 2: Characterize synthesized AuNPs and quantify ligands number per AuNP nanoparticle.

We characterized AuNPs with transmission electron tomography, dynamic light scattering, and size exclusion chromatography (Fig. 1.). We also used inductively coupled plasma mass spectrometry (ICP-MS) to measure the gold mass of synthesized AuNPs. And we are currently using liquid chromatography-mass spectrometry (LC-MS) to quantify the targeting ligand number per AuNPs. The studies for targeting ligand quantification were delayed due to COVID-19.

Major task 3: Perform *in vitro* evaluation of the binding behavior and cytotoxicity of targeted ^{211}At-AuNPs using the U87MG brain cancer cell line from ATCC.

We used U87MG brain cancer cells to perform in vitro cell binding studies for c(RGDfK)-functionalized AuNPs. c(RGDfK) peptide has a high binding affinity of 2 nM to αVβ3 receptors overexpressed on brain cancer and its neovasculature cells. Preliminary in vitro cell binding study with radiolabeled AuNPs showed that c(RGDfK) functionalized AuNPs could bind to the U87MG brain cancer cells overexpressing αVβ3 receptors. In vitro binding assay and toxicity assay studies were delayed due to COVID-19. We are currently working on this major task and should be able to finish it in the next report period.

Aim 3: Evaluate *in vivo* brain cancer targeting, pharmacokinetics, and therapeutic effect of targeted AuNPs labeled with both ^{211}At and ^{124}I.

Major task 1: Investigate brain cancer uptake, biodistribution, and pharmacokinetics of targeted AuNPs by microPET/CT imaging and using the GBM brain tumor model with U87MG brain cancer cells from ATCC.

We performed in vivo biodistribution studies for the synthesized 3 nm AuNPs and 12 nm AuNPs. For 3 nm AuNPs, we did in vivo biodistribution study for both ^{211}At and ^{131}I radiolabeling 1h, 4h, and 24h after intravenous (IV) injection through the tail vein. For ^{211}At radiolabeled 3 nm AuNPs (**Table 1**), high radioactivity was found in the urine 1 h after IV injection. Bladder and stomach were organs with high uptake. Thyroid had moderate uptake which changed from 5.32 %ID/g at 1h to 9.44 %ID/g at 24h. The biodistribution results might indicate ^{211}At radioisotopes leached out of 3 nm AuNPs and got cleared quickly by urine. We plan to use ICP-MS to determine whether it is ^{211}At-labeled AuNPs or leached ^{211}At found in the urine.

Table 1. In vivo biodistribution of ^{211}At-loaded 3 nm AuNPs 1h, 4h, and 24h after intravenous injection. Sm. Int. is short for small intestine and Lg. Int. is short for large intestine. %ID/g is defined as the percent injected dose per gram tissue. SD, standard deviation (n=4).

^{211}At-3nm AuNPs	1h		4h		24h	
%ID/g	Mean	SD	Mean	SD	Mean	SD
Liver	1.39	0.05	1.56	0.22	0.51	0.15
Spleen	2.91	0.63	3.96	1.59	0.44	0.42
Lung	9.41	3.70	7.06	1.33	0.67	0.33
Heart	1.61	0.48	1.82	0.20	0.15	0.15
Kidney	1.99	0.25	1.76	0.30	0.22	0.08
Bladder	29.02	15.52	4.33	2.24	0.15	0.47
Stomach	21.46	9.68	27.95	14.00	2.72	1.59
Sm. Int.	2.17	0.24	1.58	0.32	0.18	0.03
Lg. Int.	1.09	0.14	0.95	0.23	0.15	0.09
Thyroid	5.32	2.11	5.73	1.13	9.44	4.03
Blood	1.23	0.06	0.98	0.22	0.08	0.02
Urine	262.27	195.02	73.23	69.55	3.29	2.11
Skin	2.15	1.23	1.87	0.56	0.32	0.04
Brain	0.19	0.05	0.17	0.06	0.02	0.08

For ^{131}I-loaded 3 nm AuNPs (**Table 2**), high radioactivity was also found in the urine 1h after IV injection. The stomach, bladder, and thyroid were organs with high radioactivity. The biodistribution study results could indicate that ^{131}I radioisotopes leached out of 3 nm AuNPs after IV injection. We will determine whether it was ^{131}I-labeled AuNPs or leached ^{131}I found in the urine by using ICP-MS. ^{131}I is expected to have a similar radiolabeling performance with AuNPs as that of ^{124}I.

Table 2. In vivo biodistribution of ^{131}I-loaded 3 nm AuNPs 1h, 4h, and 24h after intravenous injection. Sm. Int. is short for small intestine and Lg. Int. is short for large intestine. %ID/g is defined as the percent injected dose per gram tissue. SD, standard deviation (n=4).

^{131}I-3nm AuNPs	1h		4h		24h	
%ID/g	Mean	SD	Mean	SD	Mean	SD
Liver	1.55	0.14	0.94	0.41	0.03	0.01
Spleen	1.35	0.27	0.62	0.28	0.00	0.01
Lung	2.77	0.74	1.41	0.47	0.01	0.01
Heart	1.36	0.41	0.83	0.37	0.00	0.01
Kidney	1.97	0.57	1.22	0.57	0.02	0.01
Bladder	12.01	7.78	2.13	1.34	0.00	0.04
Stomach	48.03	26.32	30.54	20.94	0.30	0.14
Sm. Int.	4.50	1.46	1.56	0.73	0.02	0.01
Lg. Int.	1.57	0.20	2.33	1.20	0.02	0.01
Thyroid	27.78	14.89	16.88	6.85	5.57	3.81
Blood	3.76	1.20	2.08	0.77	0.02	0.01
Urine	104.54	64.11	48.77	35.42	1.29	1.25
Skin	2.59	1.11	1.43	0.47	0.10	0.03
Brain	0.16	0.05	0.08	0.04	0.00	0.00

In addition to 3 nm AuNPs, we also performed in vivo biodistribution study for ^{211}At-labeled 12 nm AuNPs. As shown in the **Table 3**, high radioactivity was found in blood but not urine 1h after IV injection. The radioactivity in blood decreased from 44.22 %ID/g at 1h to 16.33 %ID/g at 24h. Both thyroid and stomach had low radioactivity. The biodistribution results showed that ^{211}At was stable on 12 nm AuNPs after IV injection. In addition, ^{211}At-labeled 12 nm AuNPs were found not to be cleared out of the body by the kidney and had a long blood circulation time.

We developed a murine intracranial brain tumor model with U87MG brain cancer cells. We will use the developed intracranial brain tumor model for PET/CT scan to investigate the biodistribution and pharmacokinetics of the targeted AuNPs for ^{211}At delivery. The PET/CT study was delayed due to the COVID-19 pandemic.

Table 3. In vivo biodistribution of ^{211}At-loaded 12 nm AuNPs 1h, 4h, and 24 h after intravenous injection. Sm. Int. is short for the small intestine and Lg. Int. is short for the large intestine. %ID/g is defined as the percent injected dose per gram tissue. SD, standard deviation (n=3).

^{211}At-12 nm AuNPs	1 h		4 h		24 h	
%ID/g	mean	SD	mean	SD	mean	SD
Liver	9.79	1.43	10.86	2.09	12.27	1.08
Spleen	4.83	1.70	7.44	0.47	16.92	3.45
Lung	17.54	3.24	15.18	2.23	6.23	2.91
Heart	9.82	1.87	9.44	2.13	5.50	0.94
Kidney	9.45	1.07	9.13	1.13	5.84	0.32
Bladder	2.21	0.39	2.54	0.96	2.90	0.66
Stomach	4.96	1.03	4.83	0.86	2.53	0.38
Sm. Int.	2.23	0.40	2.37	0.56	1.51	0.18
Lg. Int.	0.73	0.19	0.94	0.19	0.72	0.20
Thyroid	5.14	1.09	5.41	0.74	2.84	0.82
Blood	44.22	6.22	41.21	4.89	16.33	0.80
Urine	1.53	0.65	2.52	2.18	2.79	0.72
Tumor	0.00	0.00	0.00	0.00	0.00	0.00
Skin	2.15	0.50	2.86	0.74	3.81	0.79
Brain	1.51	0.21	1.25	0.26	0.64	0.03

Major task 2: Determine the maximum tolerated dose (MTD) of ^{211}At-AuNPs.

We are currently working on this major task with the target date of November 14th, 2020.

Major task 3: Determine therapeutic response for targeted AuNPs with both ^{211}At and ^{124}I labeling using GBM murine animal model with nude mice and U87MG cell line.

We will work on this major task with the target date of February 14, 2021.

Summary

In summary, we have developed targeted AuNPs with high ^{211}At loading capability for alpha particle radiation therapy to treat brain cancer. We will continue working on in vitro and in vivo studies to demonstrate the developed AuNPs with ^{211}At can target brain cancer and generate therapeutic benefit using murine animal models.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

In this past year, the Horizon Award has provided me precious opportunities to take training as a radiochemist on ^{211}At production, processing, purification, and radiolabeling. I have also learned how to perform in vitro cell culture, binding affinity assay, and cytotoxicity assay. In addition, I have learned how to perform in vivo pharmacokinetics and biodistribution studies. I have met my research mentors, Professor Tuan Vo-Dinh and Professor Michael Zalutsky, weekly to discuss project progress and received their professional comments and suggestions. Besides, I attended a virtual webinar-style meeting held by the National Isotope Development Center (NIDC) of the U.S. Department of Energy (DOE) focusing on ^{211}At generation and biomedical applications. I plan to attend the conference Pacifichem 2021 for the symposium entitled "Advancements in the Chemistry of Targeted Alpha Therapy" to learn the latest advancements in alpha particle radiation therapy using ^{211}At and other alpha emitters.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

I submitted an abstract for the conference Pacifichem 2020 with the title of "Gold nanoparticles as a novel delivery strategy for targeted alpha therapy" to disseminate the results to communities of interest. However, due to the COVID-19 pandemic, the conference was postponed to December 16-21, 2021. I will submit a new abstract in 2021.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next reporting period, for the nanoparticle development, I plan to further optimize formulation to obtain renal clearable metallic nanoparticles with high ^{211}At in vivo stability. For the in vitro studies, I will finish in vitro binding affinity study and cytotoxicity study. For the in vivo studies, I will determine the maximum tolerable dose (MTD), perform PET/CT study to investigate pharmacokinetics and biodistribution, and perform a survival study to demonstrate the ^{211}At-loaded AuNPs can generate improved therapeutic effect to treat GBM using murine animal models.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

From this project, we demonstrated that AuNPs can be used as a novel delivery platform for ^{211}At alpha particle radiation therapy. The developed strategy has the advantages of the simple radiolabeling process and high conjugation efficiency. Furthermore, we have performed a theoretical study to investigate the bonding mechanism of the At-Au chemical bond. Study results could make an impact on the knowledge of Astatine radiochemistry and its biomedical applications, which have many unknown properties.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

We are first to explore gold nanoparticles as a novel ^{211}At carrier for in vivo cancer treatment. Results show that gold nanoparticles' formulation and properties could affect ^{211}At in vivo stability. The optimized gold nanoparticles with high ^{211}At binding affinity may serve as a novel delivery strategy for alpha particle radiation therapy to treat aggressive cancer.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

The delivery nanoplatform developed in this project has the promise to make an impact by clinical translation to perform alpha particle radiation therapy with ^{211}At.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."
Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Results from this project could make an impact on society by improving public knowledge about cancer therapy. The targeted alpha particle radiation therapy is an emerging technology to improve cancer treatment efficacy.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Due to the COVID-19 pandemic, the research progress was delayed. We plan to speed up the research activities in the next reporting period. In addition, we may need to request an extension to finish this project.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

The expenditures were less than anticipated because the research progress was delayed by the COVID-19 pandemic.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Human subjects are not involved in this project.

Significant changes in use or care of vertebrate animals

There are no significant changes in use or care of vertebrate animals.

Significant changes in use of biohazards and/or select agents

There are no significant changes in use of biohazards or select agents.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal;*

volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

We submitted an abstract to the conference Pacifichem 2020. But due to COVID-19, the conference was postponed to 2021 and we will submit a new abstract.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared

From the research activities in this project, AuNPs were developed as a novel delivery nanoplatform for therapeutic alpha particle emitter, ^{211}At. We submitted an abstract to the conference Pacifichem 2020 to share the developed technology.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Yang Liu

Project Role: Principal Investigator

Researcher Identifier (ORCID ID): 0000-0003-3640-8852

Nearest person month worked: 12

Contribution to Project: Yang Liu is the PI for this project and has been actively involved in the whole project including targeted nanoparticles development, radiolabeling test, in vitro, and in vivo studies.

Funding Support: 100% from this award.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Yang Liu (PI)

No change.

Tuan Vo-Dinh (Key Personnel)

3 new grants become active:

1. Title: Integrated Acoustofluidic Plasmonic Molecular Diagnostic System for Detecting MicroRNA Biomarkers
PI: Vo-Dinh, Tuan
Start and End Dates: 1/1/2020-12/31/2023
Level of Effort: 0.11 academic month
Funding Agency: NIH, 1R01GM135486 - 01
Point of Contact: Maricela Trrujillo (NIGMS/NIH)
Level of Funding: $315,621/yr. Direct Costs

2. Title: Plasmonic nanoparticle-mediated immunotherapy to treat metastatic cancer
PI: Vo-Dinh, Tuan
Start and End Dates: 5/1/2019-1/31/2023
Level of Effort: 0.6 academic month
Funding Agency: NIH, 5R01EB028078 - 02
Point of Contact: Ruthann McAndrew, McAndrew, (NIH/NIBIB), ruthann.mcandrew@nih.gov
Level of Funding: $336,111/yr. Direct Costs

3. Title: Product Development of the Vertical Integrated Flow Assay System Technology (VERIFAST) for Multiplex Pathogens Detection
PI: Vo-Dinh, Tuan
Start and End date: 04/06/20– 04/19/23
Level of Effort: 1 summer month
Funding Agency: University of AZ/ NIH
Point of Contact: N/A
Level of Funding: $68,343/yr. Direct Costs

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

Generic Award Chart:

W81XWH1910684: Targeted Gold Nanoparticles (AuNPs) for Potent Alpha-Particle Radiotherapy of Brain Cancer

PI: Yang Liu, Duke University, NC
Budget: $240,041.00
Topic Area: FY 2018 DoD PRCRP, Horizon Award
Mechanism: W81XWH-18-PRCRP-HA

Research Area(s): 0805, 0808, 0817
Award Status: 15-Aug-2019 To 14-Feb-2021

Study Goals:

The overall objective of this research is to develop a novel image-guided ^{211}At radiotherapy with targeted AuNPs (<5 nm) for brain cancer treatment.

Specific Aims:

1. Synthesize, optimize and evaluate ultrasmall AuNPs (< 5 nm) for ^{211}At and ^{124}I labeling.
2. Conjugate AuNPs with tumor-homing peptides for brain cancer targeting and perform in vitro tests to demonstrate the developed nanoagent can target and treat brain cancer.
3. Evaluate in vivo brain cancer uptake, biodistribution, pharmacokinetics, and therapeutic effect of targeted AuNPs with both ^{211}At and ^{124}I labeling using a murine animal model.

Key Accomplishments and Outcomes:

Publications: none to date
Patents: none to date
Funding Obtained: none to date

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

Not applicable.