AWARD NUMBER: W81XWH-17-1-0529

TITLE: Development of Diagnostic Tools for Metastatic Melanoma via Imaging of Heparanase Activity

PRINCIPAL INVESTIGATOR: Lina Cui

CONTRACTING ORGANIZATION: University of Florida

REPORT DATE: SEPTEMBER 2021

TYPE OF REPORT: Annual report

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
SEPTEMBER 2021	Annual Report	9/1/2020 – 8/31/2021

4. TITLE AND SUBTITLE

Development of Diagnostic Tools for Metastatic Melanoma via Imaging of Heparanase Activity

5a. CONTRACT NUMBER
W81XWH-17-1-0529

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Lina Cui, Kelton Schleyer, Zixin Chen

E-Mail:

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

DIVISION OF SPONSORED
RESEARCH 1523 UNION RD RM 207
GAINESVILLE FL 32611-1941

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Imaging the activity of heparanase is a promising approach to visualizing aggressive melanoma. However, current detection methods of heparanase activity lack specificity and sensitivity and no probe currently exists for in vivo imaging. In this research period, we have successfully developed key chemical groups to make the heparanase activatable probes. The synthesis of the final PET tracer is ongoing. We have successfully produced human recombinant heparanase using mammalian cells to facilitate the examination of our synthetic molecular probes. We have also screened through various human melanoma cell lines to identify the cell lines with the highest heparanase expression level. When we have efficient heparanase probes in hand, we will evaluate the effects of heparanase activity on oncogenic properties of melanoma cell lines, and on cell migration and invasion.

15. SUBJECT TERMS

Metastasis, heparanase, melanoma, PET, imaging

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	16	19b. TELEPHONE NUMBER *(include area code)*

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

Imaging the activity of heparanase is a promising approach to visualizing aggressive melanoma. However, current detection methods of heparanase activity lack specificity and sensitivity and are limited to *in vitro* detection. In this research period, we examined the precursor of the PET tracer for heparanase in vitro and improved the chemistry. The evaluation of the PET probe *in vitro* (at Moffitt Cancer Center) has been delayed due to the loss of personnel specialized in radiochemistry and the difficulties in hiring the new staff at Moffitt after COVID shutdown. We hope to have the personnel at Moffitt in position before the closure of this award.

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

Metastasis, heparanase, melanoma, PET, imaging

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 Task 1.1. Development of heparanase probes
Major Task 1.2. Validation using recombinant heparanase
Major Task 1.3. Evaluation in cultured melanoma cells
Major Task 1.4. Effects on oncogenic properties of melanoma cell lines
Major Task 1.5. Effects on cell migration and invasion

Major Task 2.1 In vivo imaging of heparanase activity in melanoma models with different heparanase expression
Major Task 2.2 Imaging in aggressive tumor model
Major Task 2.3 Imaging in models with induced alterations of heparanase
Major Task 2.4 Imaging in metastatic models

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.

This report contains our effort from 9/1/2020 to 8/31/2021. However, several lab members departed the program due to COVID from July to November 2020, which slowed down the progress of the project significantly. Please see more details in **Section 5**. However, we were able to make the following progress:

Major Task 1.1-1.2. Previously we reported the synthesis of the precursors for the heparanase PET probes and their control (unactivatable) (**Figure 1**).

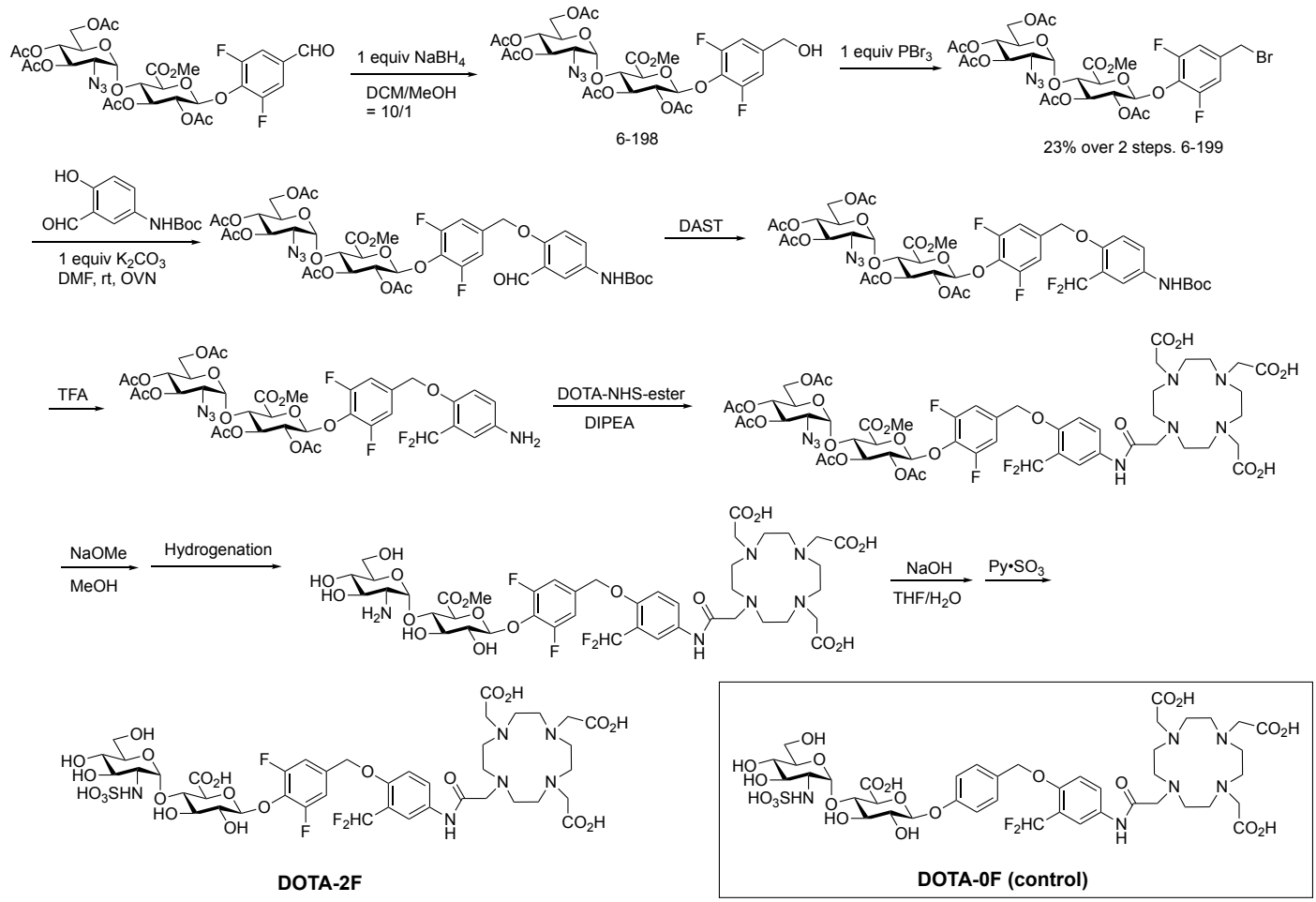


Figure 1. *Optimized chemical synthetic approach to achieve the precursor (before radiolabeling) of the PET tracer for heparanase* ***DOTA-2F*** *and the unactivable control* ***DOTA-0F****.*

We have learned from our previously reported work that in order for the probe to be activated by heparanase, sufficient electron withdrawing at the aglycon side is needed (similar to HADP) (Liu, Schleyer, et al, Chemical Science, 2021). In our original design for the heparanase PET probes, we synthesized the molecule DOTA-2F with a linker containing 2 fluorine (2F) atoms (**Figure 1**). In our parallel study, we found that 2F atoms could not provide sufficient electronegativity to enable the activation by heparanase (**Figure 2**, compound **JL7-189**), while addition of two more fluorine atoms provided sufficient probe activation (**Figure 2**, compound **JL8-12**). This is consistent with what we observed for the PET probes: when we evaluated the heparanase PET probes (DOTA-2F) using recombinant human heparanase, they were not able to be activated by heparanase enzyme.

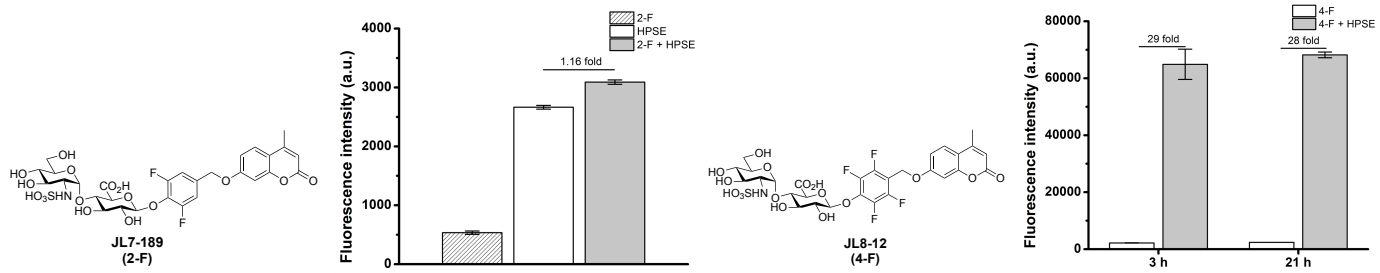


Figure 2. *Evaluation of the linker chemistry. Left: probes with 2F linker could not be activated. Right: probes with 4F linker could be activated.*

We did a computational study of the linkers for these probe molecules (**Figure 3**) and found the probe with no F (0-F) has the shortest/strongest C-O bond (glycosidic bond, in red), while the one with 4F has the longest and weakest C-O bond, similar to the glycosidic bond of HADP. This explains why the probe with 4F can be activated, as it offers a lowered energy barrier for the enzymatic activation reaction.

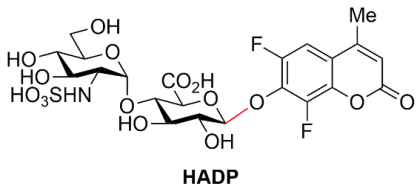
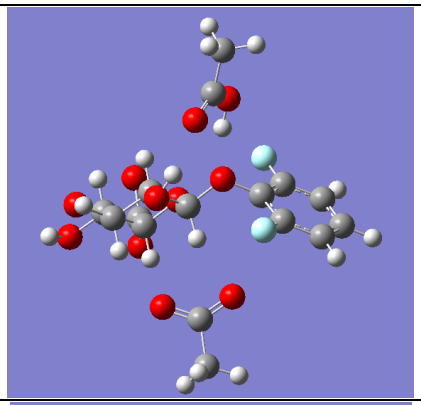
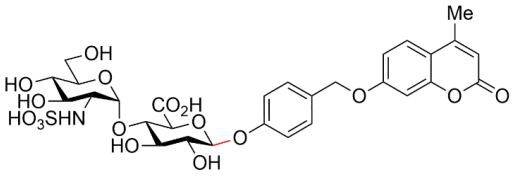
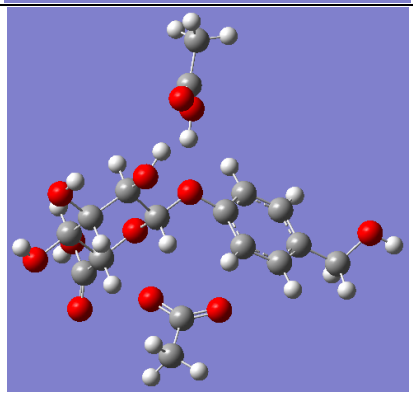
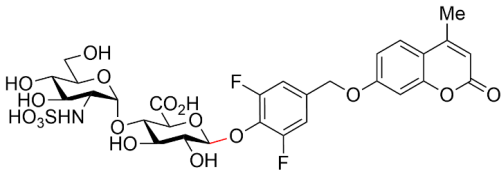
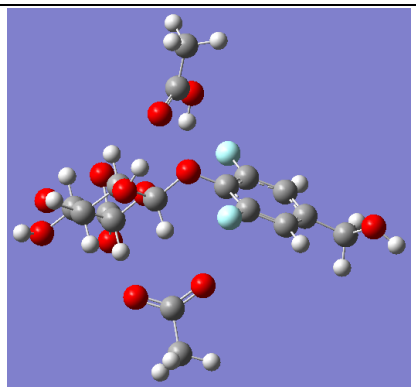
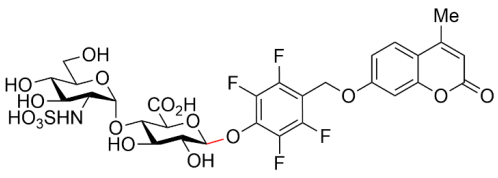
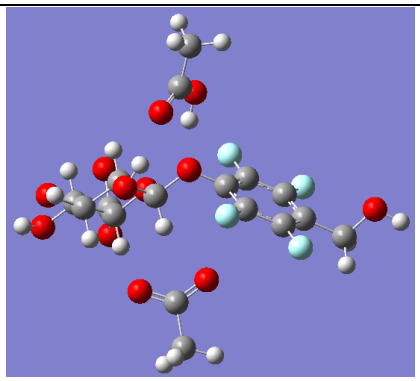
Compounds		*R*(C-O) / Å
 **HADP**		1.44093
 **0-F**		1.42012
 **2-F**		1.44037
 **4-F**		1.44370

Figure 3. *Computational calculation of the C-O glycosidic bond of the heparanase probe molecules. A longer bond means a weaker bond, which allows the enzyme to process/activate the molecule. HADP is the first activatable small molecular probe for heparanase developed in our lab during the grant period (Chemical Science 2021).*

Charge calculations of the carbon and oxygen of the glycosidic bond were performed (Table I). Similarly, 4-F compound shares similar charge values as HADP, our activatable positive control.

Table I. Mulliken charges on C and O atoms of compounds.

Compounds	Mulliken charges	
	δ_C	δ_O
HADP	0.049778	-0.294861
0-F	-0.297077	-0.454127
2-F	0.115701	-0.271543
4-F	0.086134	-0.277514

The enzymatic activation of probes 2-F and 4-F was evaluated and confirmed using HPLC (**Figure 4**). Activation of the probes by heparanase releases a 7-MU molecule which emits fluorescent light. 2-F probe did not emit any fluorescent light when incubated with heparanase, and the retention time on the HPLC column was the same as that without enzyme incubation. On the other hand, the 4-F probe molecule, when incubated with heparanase, yielded strong fluorescence from 7-MU, and the retention time of the enzymatic reaction product matched that of the free 7-MU, suggesting the efficient and complete activation of the 4-F probe by human heparanase.

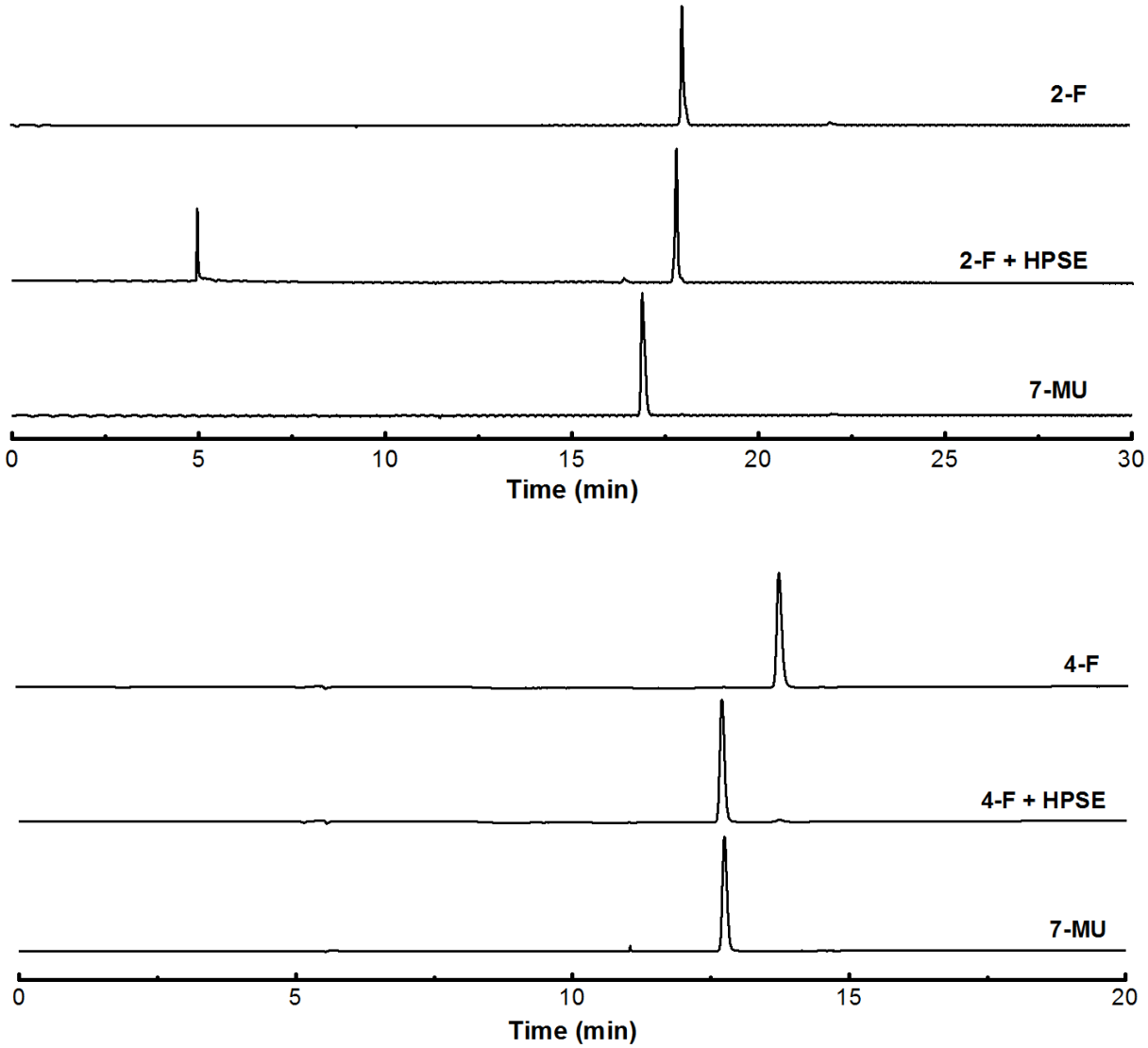


Figure 4. *Evaluation of the linker chemistry via HPLC. Top: probes with 2F linker could not be activated. Bottom: probes with 4F linker could be activated.*

We therefore revised our PET probe structure to the 4-F linker (**Figure 5**).

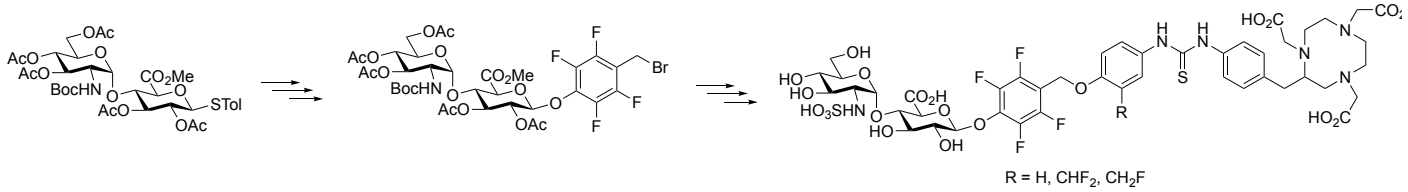
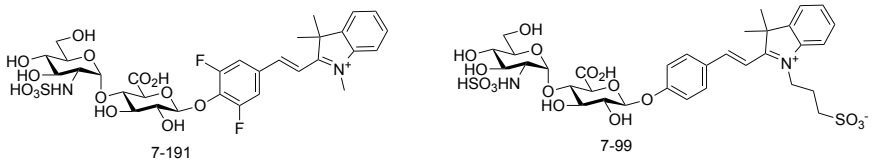
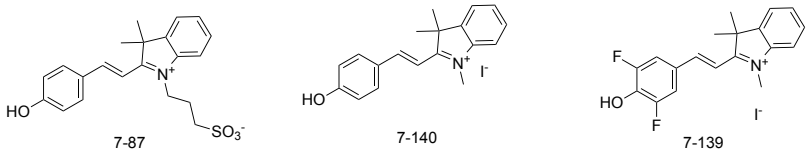


Figure 5. Brief synthetic scheme of the optimized probe structures with a 4F linker.

Major Task 1.3-1.5. Evaluation in cultured melanoma cells

During this report period, the cell work was impacted significantly by the COVID shutdown. During July-Nov 2020, several of my lab personnel, including one maintaining the cell lines, left my research group. This led to a severe contamination due to lack of maintenance of the cell culture room where we perform our cell work. We spent several months to decontaminate the lab space, and reobtained clean cell lines.





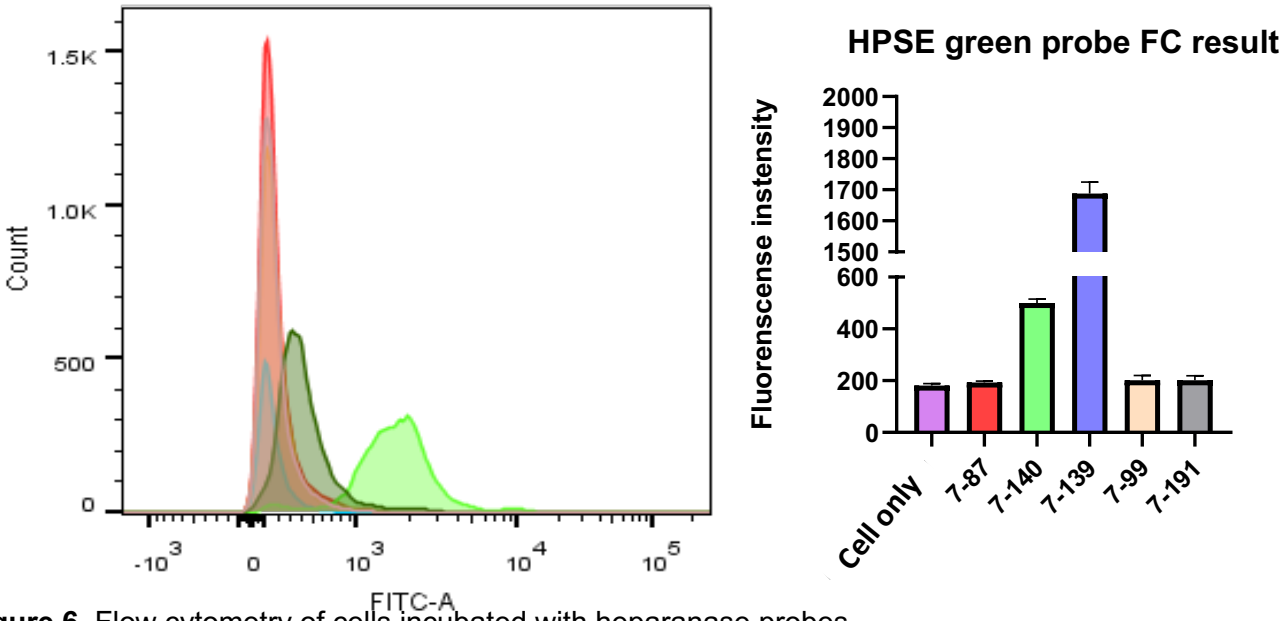


Figure 6. Flow cytometry of cells incubated with heparanase probes.

Nonetheless, we evaluated our probes (fluorescent derivatives) in melanoma cells (**Figure 6**), and the initial result suggested these probes work preferably in the extracellular matrix, where active heparanase is located. Therefore, we will be continuously working on new in vitro culture systems to evaluate our probes.

Major Tasks 2.1-2.4 *In vivo* imaging of heparanase activity in tumor models

Protocol for PET imaging at Moffitt Cancer Center has been approved, although there was interruption during COVID-19 shutdown. However, the hiring of the personnel to carry out the proposed radiolabeling and PET scanning is still ongoing.

In summary, we have successfully optimized and analyzed the structures for the heparanase activatable PET probes. We have confirmed the structures that can be activated by heparanase. The radiochemistry and PET scanning will be carried out immediately when the lab staff in hired at Moffitt Cancer Center.

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.

My career goal as a cancer researcher is to establish myself as independent researcher/expert in diagnostic imaging of cancers including melanoma using novel chemistries and new molecules. This Career Development Award will help me gain knowledge and experience in melanoma biology and diagnosis. My past and current mentors are specialized in interest in preclinical studies and translation of novel cancer diagnostics and therapeutics to clinical applications. And they have been guiding me through project design, choice of tumor cell lines in the first year of the project. I also consult Dr. Berwick, expert in melanoma, who has been helping me plan the clinical relevance/management part of the experiments, and she will also provide consultation/ collaborative support of melanoma biology, choices of cell lines and establishment of animal models. My mentors and Dr. Berwick have been helping me develop skills in melanoma research and helping me build connections and solicit new collaborations. My mentors have introduced me to be an AACR member; I and my students/postdocs have presented at the conferences to report the progress of our ongoing projects. We also presented at the ACS annual meeting, UF Health Cancer Center symposium, UF College of Pharmacy Research Day, and UF Center for Natural Products, Drug Discovery and Development (CNPD3). I also gave talks at other institutions.

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.

During the last report period, I joined the programs of Florida Comprehensive Cancer Research Training Opportunities for Outstanding Leaders (RETOOL) Program and University Multicultural Mentor Program (UMMP) as a mentor to share our research projects with undergraduate students who are interested in research.

Since October 2019, I have become a mentor of the UF Undergraduate Research Program Scholars (URPS) program, which is offered to the top 5% of the entering freshmen class and prepares the scholars for undergraduate research. My undergrad trainees are students from the Departments of Chemistry, Chemical Engineering, and Biomedical Engineering, in regular, pre-pharm or pre-med track.

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

- We continue the evaluation of the molecular probes in cell culture.
- We will evaluate the effects of heparanase activity on oncogenic properties of melanoma cell lines, and on cell migration and invasion.
- We will continue developing melanoma animal models and start evaluating the probes in Task 2.1.
- We will also present our work in conferences and invited seminars so that more researchers will be aware of our work.

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).

The newly developed heparanase PET probes in this project will be the first PET probes targeting heparanase. They are translatable to clinical applications as diagnostics for melanoma and other types of cancers after evaluation in animal models. In addition, the findings we have in this reporting period will extend the understanding of the chemistry of heparanase activation process. This can guide the development of not only heparanase probes but also other types of molecular probes. The heparanase molecular probes developed in this project will be a unique tool for researchers in not only heparanase research but also the broad cancer research area.

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.

Since heparanase is implicated in other diseases such as various types of inflammation, the molecules we develop in this project can have potential impact in these fields. Also, while developing the chemistry, we have been using model systems targeting beta-galactosidase, which is over expressed in aging or stressed tissues; these model molecules will be very important for the study of senescence as well as therapy response monitoring.

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.*

At the final stage of this award, if everything works as designed, the product can be patented for technology transfer to industry or it can lead to a start-up company. We have one patent related to this work. At this reporting period, there is nothing to report.

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.*

Nothing to report at this stage.

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:*

Nothing to report.

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.

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.

The delay in the reporting period is primary caused by the loss of several lab personnel. We have hired new people to replace them. However, the hiring of the researcher at Moffitt Cancer Center delays the animal work significantly. We hope someone will be hired shortly to finish the animal work before the closure of the award.

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.

Additional cell lines and animals were purchased due to the interruption caused by COVID.

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

n/a

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

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).*

J. Liu(p), K.A. Schleyer(g), T.L. Bryan(p), C. Xie, G. Seabra, Y. Xu, A. Kafle(g), C. Cui, Y. Wang(p), K. Yin, B. Fetrow, P. Henderson, P. Fatland, J. Liu, C. Li, Hua Guo, and **L. Cui**. Ultrasensitive small molecule fluorogenic probe for human heparanase. Chemical Science, 2021.

Jun Liu(p), Xiaowei Ma(p), Ying Wang(p), Chao Cui, Philip R. Deenik(g), and Lina Cui. 2020. A Self-Immobilizing NIR Probe for in vivo Imaging Cellular Senescence. *J Med Chem 2021 (Accepted)*

K.A. Schleyer, J. Liu, H. Guo and L. Cui*, A self-immolative linker for heparanase activatable probes, bioRxiv, 2021. doi: https://doi.org/10.1101/2021.06.15.448502

G. Zeng, W. Zhao, Z. Chen, A. Jimenez Ybargollin, X. Li, F.-S. Liang* and L. Cui*, Epigenetic control of heparanase expression through CRISPR/dCas9, bioRxiv.

P. Zhang, X. Ma, C. Cui, Y. Wang, R. Ghosh, N.L. Matteson, V. Perea, K. Sheehan, D.T. Cramb, C.-C. Ling* and L. Cui*, Easily accessible sub-30-nm capsules for drug formulation, bioRxiv.

K.A. Schleyer, Z. Chen, J. Rao and L. Cui*, Molecular imaging probes: design strategies and mechanisms for imaging contrast. Chem. Soc. Rev. 2021 (Submitted)

J. Liu, Z. Chen, C. Cui, A.L. Sigler, and L. Cui*, A Versatile Linker for Self-Immobilizing Probes Via Quinone Methide Chemistry. bioRxiv, 2021. doi: https://doi.org/10.1101/2021.06.14.448363

K.A. Schleyer and L. Cui*, Molecular probes for selective detection of cysteine cathepsins, Organic & Biomolecular Chemistry, 2021,19, 6182-6205 (Invited contribution).
Cover Feature: https://pubs.rsc.org/en/content/articlepdf/2021/ob/d1ob90107a

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).*

Nothing to report.

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.*

Invited Talks:

1.Targeting an emerging therapeutic biomarker, heparanase. July 6-9, 2021, Materials for Humanity, Singapore.

2. Structurally Defined Fluorogenic Probe Enables High-Throughput Screening of Inhibitors for Heparanase, April 2021, ACS National Meeting (Virtual)

3. Targeting an emerging oncogene biomarker, heparanase. Jan 15, 2021, UF Health Cancer Center, FL.

4. Molecular Imaging with Synthetic Glycans, Dec 10, 2020, ACS Carbohydrate/European Glycoscience Community, Webinar

5. Molecular Imaging with Synthetic Glycans, Nov 12, 2020, Middle Tennessee State University, TN.

6. Molecular Imaging with Synthetic Glycans, Nov 06, 2020, University of Calgary, AB, Canada.

7. Molecular Imaging with Synthetic Glycans, Oct 7, 2020, Northeastern University, Boston, MA.

Posters:

1. Expanding the toolkit of small molecule imaging probes for heparanase. Kelton Schleyer Dr. J. Liu; Z. Wang; Z. Rabinowitz; B. Fetrow; P. Fatland; A. Rollins; V. Wajsbrot; Prof. H. Guo, Prof. L. Cui. ACS Fall meeting, Aug 22-26, 2021.
2. Self-immobilizing Near-Infrared Probe for In Vivo Imaging of Senescence. Zixin Chen, Chao Cui, Jun Liu, Xiaowei Ma, and Lina Cui. ACS Fall meeting, Aug 22-26, 2021.
3. Epigenetic control of heparanase expression through CRISPR/dCas9. Guihua Zeng, Weiye Zhao, Zixin Chen, Alberto Jimenez Ybargollin, Xiaogang Li, Fu-Sen Liang and Lina Cui*, UF Drug Discovery Symposium, April 22-23, 2021 (Virtual).
4. Sub-30-nm capsules for drug delivery. Xiaowei Ma, Ping Zhang, Chao Cui, Ying Wang, Ramprasad Ghosh, Qifang Wang, Nathaniel L. Matteson, Valerie Perea, Kate Sheehan, David T. Cramb, Chang-Chun Ling and Lina Cui. UF Drug Discovery Symposium 2021 (Virtual).
5. Expanding the toolkit of small molecule imaging probes for heparanase. Kelton Schleyer Dr. J. Liu; Z. Wang; Z. Rabinowitz; B. Fetrow; P. Fatland; A. Rollins; V. Wajsbrot; Prof. H. Guo, Prof. L. Cui. UF Drug Discovery Symposium 2021 (Virtual). Oral.
6. Mapping the activities of heparanase with synthetic disaccharides. Zhishen Wang, Meijun Xiong, Rina Saksena, and Lina Cui*. UF Drug Discovery Symposium 2021 (Virtual). *(Poster Award.)*
7. Epigenetic control of heparanase expression through CRISPR/dCas9. Guihua Zeng, Weiye Zhao, Zixin Chen, Alberto Jimenez Ybargollin, Xiaogang Li, Fu-Sen Liang and Lina Cui*, 34th Annual UF College of Pharmacy Research Showcase, April 12-13, 2021 (Virtual).
8. Expanding the toolkit of small molecule imaging probes for heparanase. Kelton Schleyer Dr. J. Liu; Z. Wang; Z. Rabinowitz; B. Fetrow; P. Fatland; A. Rollins; V. Wajsbrot; Prof. H. Guo, Prof. L. Cui. 34th Annual UF College of Pharmacy Research Showcase 2021 (Virtual). *(Poster Award.)*
9. Mapping the activities of heparanase with synthetic disaccharides. Meijun Xiong, Zhishen Wang, Rina Saksena, and Lina Cui*. 34th Annual UF College of Pharmacy Research Showcase 2021 (Virtual).
10. Targeting an emerging oncogenic biomarker, heparanase. Lina Cui. American Association for Cancer Research Annual Meeting, April 10-15, 2021 (Virtual). Published in Cancer Research.
11. Selective fluorogenic probe for rapid detection of cathepsin L activity. Kelton A. Schleyer, Ben Fetrow, Peter Fatland, Jun Liu, Maya Chaaban, Biwu Ma, and Lina Cui. American Association for Cancer

- **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.

  www.linacui.org

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

  We have developed imaging probes for heparanase, and its activator cathepsin L. The chemical strategy developed in this project also led to several molecular probes for senescence.

- **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.

  Lina Cui, Jun Liu, and Kelton Schleyer(g). 2020. HEPARANASE COMPOUNDS AND METHODS OF USE. PCT/US2020/029627.

  L. Cui, and K. Schleyer(g). 2020. A Selective Fluorogenic Probe for Rapid Detection of Cathepsin L Activity. US62/971,558.

- **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.*

- Molecular reagents are developed, which can be made available to other research groups.
- Melanoma cell lines are available.
- DNA plasmids for heparanase production are available.
- Recombinant heparanase enzyme is available.

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".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Names of the main contributors in this report period:
Dr. Lina Cui (no change);
Dr. Zixin Chen (no change);
Kelton Schleyer (no change).

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.

Nothing to report

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

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

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.*