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TITLE:

Characterization of Acute Exposure to Toxic Metals in Military Environments and Personnel

PRINCIPAL INVESTIGATOR:

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CONTRACTING ORGANIZATION: Vanderbilt University
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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Military personnel in modern conflicts are exposed to toxic metals from embedded fragments generated by explosive devices and inhaled near burn pits. Metal levels among veterans are centrally monitored, but to truly establish the impact of toxic metals on military personnel's health it is necessary to establish the peak exposure. No device exists to simultaneously detect and quantify multiple toxic metals in biological and environmental samples near the time of peak exposure. We hypothesize that a zinc oxide nanowire substrate decorated with gold nanoparticles and decorated with chelating ligands can sensitively detect toxic metals via surface-enhanced Raman spectroscopy (SERS). A patterned bed of ZnO nanowires will be grown and decorated with gold nanoparticles. Crown ethers identified based on selectivity for multiple toxic metals will functionalize the nanostructure. Sensitivity will be measured by parallel Raman studies of each selected ion coordinated with each crown ether, with and without plasmonic enhancement. Specificity will be assessed by SERS of each toxic metal in the presence of distractors. Selectivity of the crown ligands will be assessed by spectral analysis in the presence of distractors. Spectral analysis will also identify the optimal crown ethers for simultaneous, multiplexed sensing of the chosen toxic metals. Sensors in this optimized configuration will be fabricated and experimentally validated against predicted performance.

15. SUBJECT TERMS
Embedded fragments, toxic metals, burn pits

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TABLE OF CONTENTS

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

Military personnel in modern conflicts are exposed to toxic metals from embedded fragments generated by explosive devices and inhaled near burn pits. To truly establish the impact of toxic metals on military personnel's health it is necessary to establish the peak exposure, often during deployment in areas of conflict, in order to establish future health risk as well as identify those most needing of early therapeutic monitoring or interventional strategies to mitigate potential health risk. Contemporary methods to assess biologic and environmental metals exposure are limited, being either unsuitable for field analysis or limited to single-analyte detection. No device exists to simultaneously detect and quantify multiple toxic metals in biological and environmental samples near the time of peak exposure in the field. The goal of this project is to design a portable sensor based on a zinc oxide (ZnO) nanowire substrate decorated with gold (Au) nanoparticles and decorated with chelating ligands can sensitively detect toxic metals via surface-enhanced Raman spectroscopy (SERS). The first part of this study is the fabrication and functionalization of the sensing substrate. A patterned bed of ZnO nanowires will be grown and decorated with gold nanoparticles. Crown ethers will be amended to present sulfhydryl group(s) to enable strong association with the gold nanoparticles. Crown ethers will be selected for, and experimentally confirmed to possess selectivity for uranyl, cadmium, aluminum, and lead. These toxic metals represent among the most important in health outcomes from embedded fragments and serve as the proof-of-principle for future expansion of this approach. At each step in the fabrication process, the substrate will be characterized by multiple optical and spectroscopic techniques. Detection sensitivity will be measured by parallel Raman studies of each selected ion coordinated with each crown ether, with and without plasmonic enhancement. Detection specificity will be assessed by SERS of each toxic metal in the presence of other ions and distractors. Selectivity of the crown ligands will be assessed by spectral analysis in the presence of other metal ions and distractors. Spectral analysis will also identify the optimal crown ethers for simultaneous, multiplexed sensing of the four initial toxic metals. Sensors in this optimized configuration will be fabricated and experimentally validated against predicted performance.

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

Crown Ethers; Zinc Oxide; Nanowires; Silver; Nanoparticles; Surface-Enhanced; Raman Spectroscopy; Microfluidics; Toxic Metals; Detection; Portable; Multiplexed; Trace; Polydimethylsiloxane; Chelation; Quantification

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: Design and fabricate an advanced sensor for toxic metals based on zinc oxide nanowires decorated with nanoscale gold and functionalized with metal capture chemistry.**
    - Subtask 1: Prepare a mask using electron beam lithography to spatially localize deposition of the seed layer necessary to support the growth of zinc oxide nanowires.
    - Subtask 2: Prepare zinc nanowire forests by hydrothermal growth from previously discussed seed layer.
    - Subtask 3: Decorate zinc oxide nanowires with nanoscale gold by electron beam deposition.

- Subtask 4: Assess zinc oxide nanowires with and without gold decoration using scanning electron microscopy (including atomic compositional information by EDX), photoluminescence, and UV-Vis spectrophotometry.

- Subtask 5: Functionalize gold nanoparticles using cyclic polyethers (crown ethers) of various sizes and geometries. Initial crown ethers will be dibenzo functionalized, which are commercially available from 15-crown-5 (15 atoms in the crown, five of which are oxygen) through 30- crown-10. Cyclic polyethers will amended to functionalize the cyclic polyether to the gold nanoparticles.

- Subtask 6: Assess cyclic polyether localization to the gold-decorated zinc oxide nanowires using scanning electron microscopy (including atomic compositional information by EDX) and infrared spectroscopy.

- **Major Task 2: Characterize sensor performance for the detection of toxic metals alone, in combination, and in the presence of known distractors present in biological and environmental samples.**

  - Subtask 1: Measure the sensitivity of advanced sensors designed in Major Task 1 for detection of toxic metals with known military relevance. Selected toxic metal of interest will be measured over a range of relevant concentrations in the absence of other metals or potential sensing distractors in laboratory-scale Raman spectrometers, with appropriate parallel control studies.

  - Subtask 2: Characterize the surface enhanced Raman signal amplification created by the interactions among cyclic polyether resonance and the gold nanoparticle surface, as enabled by careful quantitative calibrations and Raman spectroscopy measurements of cyclic polyethers and toxic metals in the absence of advanced sensors. Design a portable instrument based on solid state electronics for the multiplexed sensing of toxic metals at relevant concentrations.

  - Subtask 3: Measure the specificity of advanced sensors for detection of toxic metals with known military relevance from explosive and burn pit exposures. Each toxic metal of interest will be measured over a range of relevant concentrations in the presence of other metals or distractors. These studies will be carried out in laboratory-scale Raman spectrometers with careful attention to quantitative characterization.

  - Subtask 4: Computationally characterize the sensing of each toxic metal of interest in the presence and absence of other metals and sensing distractors. Select cyclic polyethers to provide optimal sensitivity and selectivity for each of the toxic metals of interest in a mixed sample over a relevant range of concentrations.

  - Subtask 5: Prepare multiple advanced sensors on a single quartz substrate with each sensor functionalized with a different cyclic polyether as determined from Subtask 4.

  - Subtask 6: Measure the specificity and selectivity of advanced sensors for detection of toxic metals with known military relevance from explosive and burn pit exposures. Each toxic metal of interest will be measured over a range of relevant concentrations in the presence of other metals or potential sensing distractors. These studies will be carried out in laboratory-scale Raman spectrometers with careful attention to quantitative characterization. Actual performance to be compared to performance predicted in Subtask 4.

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.*

Major Task 1, Subtask 1

We designed and 3D-printed two masks shown in Figure 1. The mask shown in (a) was designed to spatially localize ZnO seed layer deposition to two spots in the central portion of the proposed microchannel. The mask shown in (b) was designed to spatially localize metal nanoparticle deposition to one of these spots. This was done in order to compare SERS with non-SERS measurements. Figure 2 demonstrates through UV-Vis absorption spectra acquired of seeded and non-seeded substrates after hydrothermal nanowire growth that patterning the ZnO seed layer is sufficient to pattern subsequent ZnO nanowire growth. The band-edge shoulder at sub-400 nm wavelengths present in the spectrum acquired of the seeded substrate indicates crystalline ZnO consistent with the presence of nanowires while the lack thereof in the spectrum acquired of the non-seeded substrate indicates a lack of nanowire growth through the absence of crystalline ZnO. This subtask is complete.

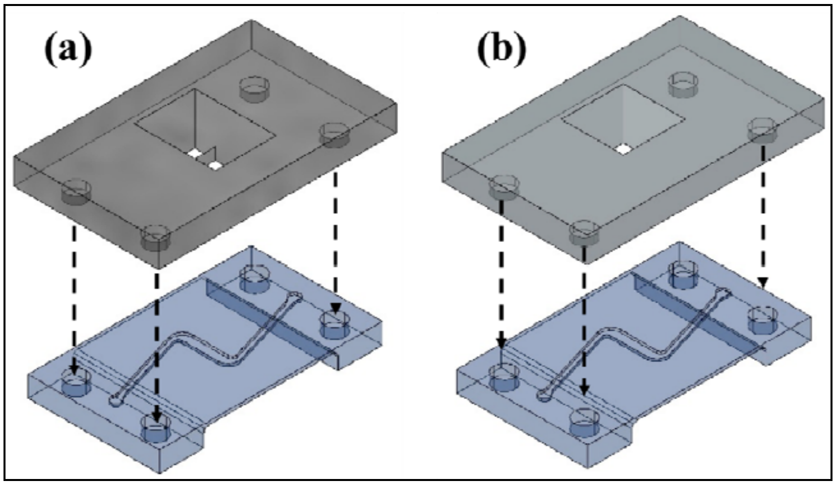

Figure 1: AutoCAD designs for masks to pattern deposition of (a) ZnO seed and (b) metal nanoparticles

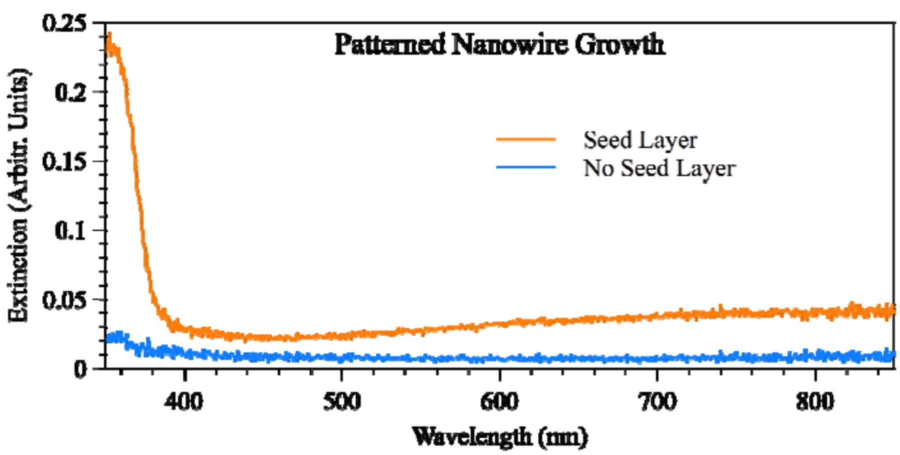

Figure 2: UV-Vis absorption spectra of ZnO-seeded and unseeded substrates following ZnO nanowire growth

Major Task 1, Subtask 2

While nanowires have not yet been grown in a PDMS microchannel, nanowire forests have been grown successfully on PDMS substrates. 100-nm ZnO seed layers were deposited on both thick (~1 mm) and thin (<0.2 mm) PDMS substrates, from which highly crystalline ZnO nanowires were grown successfully, as shown by SEM and photoluminescence (PL) under Subtask 4. This indicates that nanowire growth inside a microchannel is possible. ZnO nanowire growth inside a PDMS microchannel is scheduled for Q1 of 2020.

Major Task 1, Subtask 3

Decorate zinc oxide nanowires with nanoscale gold by electron beam deposition.

After ZnO nanowires were successfully grown on PDMS, silver nanoparticles were deposited onto the sides of the nanowires via electron beam deposition. Silver was chosen to form nanoparticles rather than gold because silver nanoparticles have a plasmon resonance in a 400-600 nm range of wavelengths, while gold nanoparticles' plasmon resonance is in the range of 600-800 nm. The Raman systems available for this research are better-suited for use with green lasers rather than red or NIR lasers. Gold can later be substituted for silver, should it prove necessary. The presence of Ag nanoparticles was demonstrated by both SEM images pre- and post-deposition, as well as by the presence of plasmon-based light absorption demonstrated by UV-Vis spectra acquired pre- and post-deposition. SEM and UV-Vis are both presented in Subtask 4. Ag nanoparticle deposition on nanowires grown inside a PDMS microchannel is scheduled for Q1 of 2020.

Major Task 1, Subtask 4

As stated in Subtask 2, ZnO nanowires were assessed using PL (Figure 3) and SEM (Figure 4). PL spectra of nanowires to remain bare and nanowires to be decorated with Ag nanoparticles were acquired. The PL peak at ~385 nm in Figure 3 results from the wide direct-bandgap nature of crystalline ZnO and indicates the presence of highly crystalline ZnO nanowires. Furthermore, a lack of photoluminescent emission in the visible region indicates a lack of defects in the ZnO crystal structure. The SEM images of Figure 4 indicate that this highly crystalline ZnO indeed takes the form of nanowires. They also confirm the single crystal nature of the nanowires since they have a hexagonal cross-section consistent with the wurtzite crystal structure of ZnO.

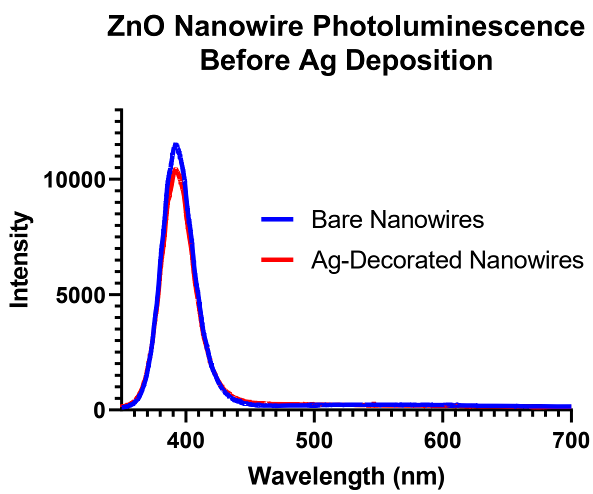

Figure 3: Photoluminescent spectra of ZnO nanowires, demonstrating high crystallinity and few defects

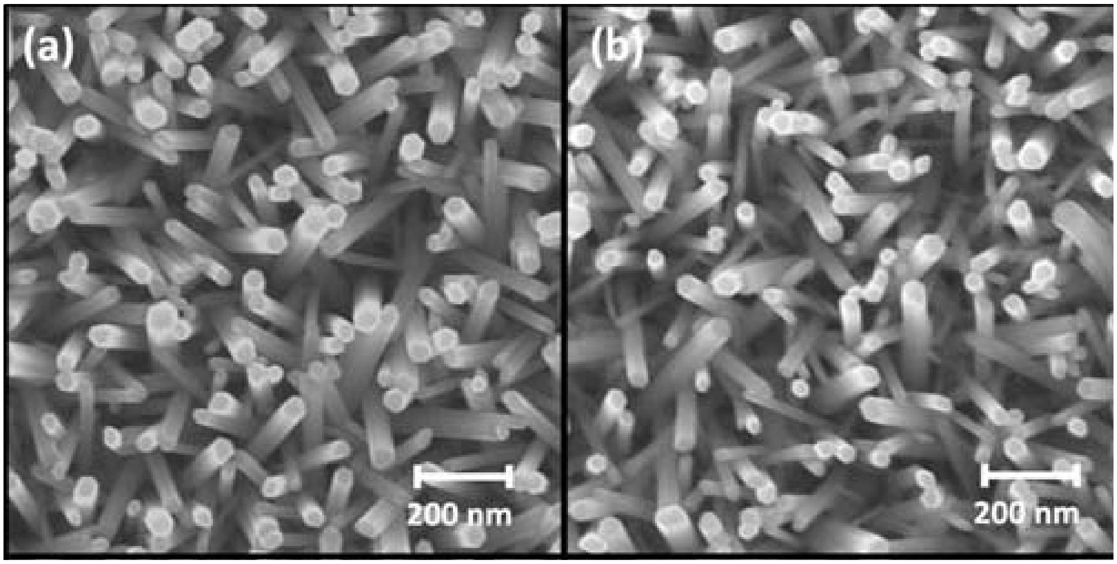

Figure 4: SEM images of ZnO nanowires, confirming single crystal nanowire formation

As stated in Subtask 3, nanoparticle decoration was assessed using SEM imaging (Figure 5) and UV-Vis spectrophotometry (Figure 6). SEM images taken before and after nanoparticle deposition demonstrate the presence of nanoparticles on the sides of the nanowires. UV-Vis spectra acquired before and after nanoparticle deposition show the presence of a plasmon absorption peak centered at ~450 nm, consistent with Ag nanoparticle formation. Assessment of Ag-decorated ZnO nanowires inside a PDMS microchannel will be performed after nanostructure fabrication scheduled in Q1 of 2020.

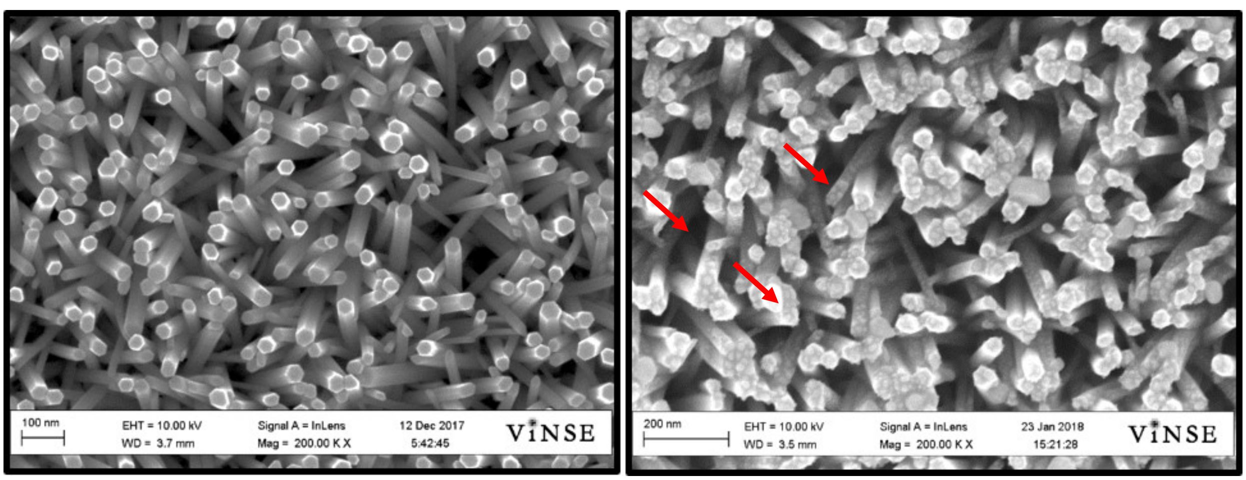

Figure 5:SEM Images of ZnO nanowires before and after nanoparticle deposition, showing the presence of Ag nanoparticles on the sides of the nanowires. Some of these nanoparticles are pointed out for clarity.

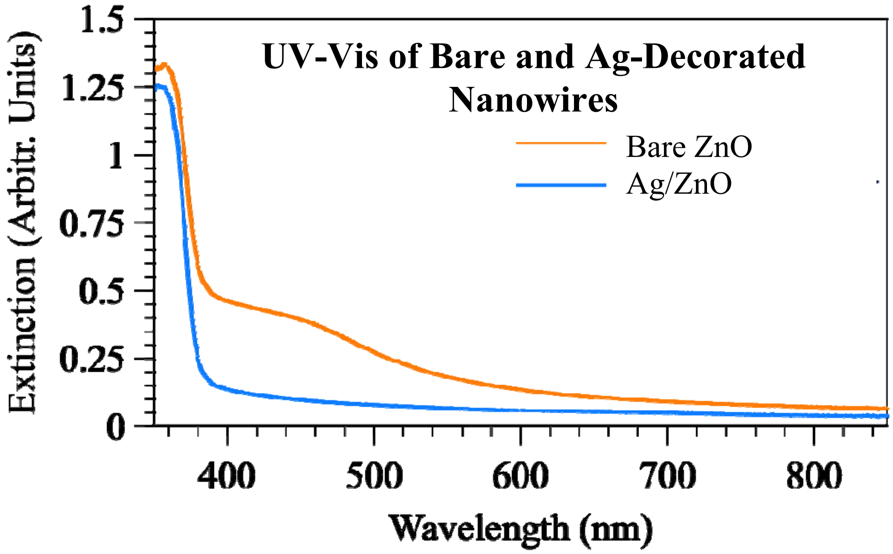

Figure 6: Extinction spectra of ZnO nanowires before and after Ag nanoparticle deposition

Major Task 1, Subtask 5

Work is ongoing to successfully functionalize silver-decorated ZnO nanowires with 4’-aminobenzo-18-crown-6 ether. Amine groups have a high affinity for silver, so it should be just a matter of having the right solvent. If we cannot find success functionalizing silver nanoparticles with aminated crown ethers, we can switch from silver to gold nanoparticles, for which amines have a stronger affinity. Additionally, we could start using thiolated crown ethers, because thiols have a higher affinity than amines for both silver and gold. Functionalization of Ag-decorated ZnO nanowires inside a PDMS microchannel with aminated crown ethers including 4’-aminobenzo-18-crown-6 is scheduled for Q1 of 2020.

Major Task 1, Subtask 6

The assessment of crown ether localization in this subtask is dependent on the completion of Subtask 5. And is scheduled for Q1 of 2020.

Major Task 2, Subtask 1

This subtask is dependent upon completion of Major Task 1. The nanowire-based sensor must be completed before true sensitivity of the sensor can be determined. However, work has been done toward using fluorescence as an additional sensitivity measure separate from SERS. Sarfo et. al. showed that 4’-aminobenzo-18-crown-6 has a broad fluorescence peak in the range of 300-400 nm when excited at 295 nm[1]. When chelating metal ions, this fluorescence is quenched. We have had some difficulty replicating their results, but preliminary evidence shown in Figure 7 demonstrates that while the fluorescent peak of the crown ether is quenched in the presence of concentrations of arsenic and molybdenum ions equimolar to the crown ether concentration, it is not completely quenched. This indicates that fluorescence can be used as a check upon measuring SERS sensitivity. Completion of this subtask is scheduled for Q2 of 2020.

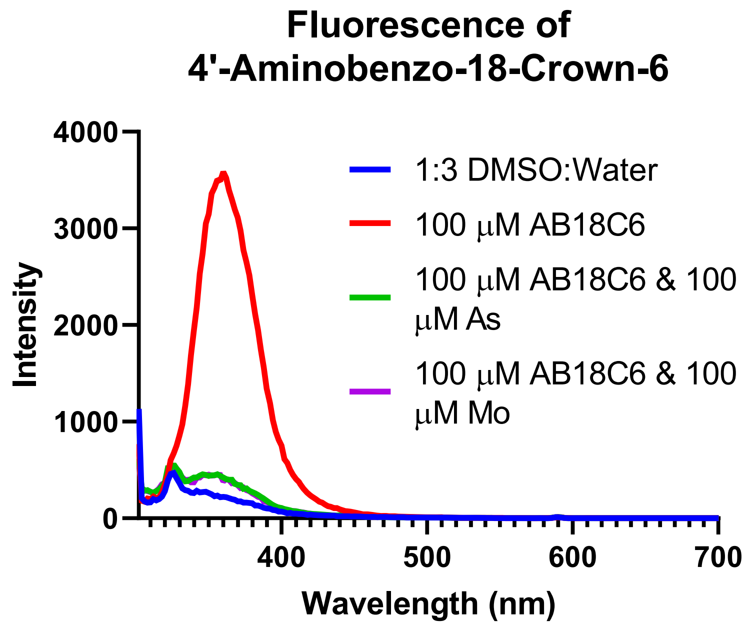

Figure 7: Fluorescence of 4'-aminobenzo-18-crown-6 with and without arsenic and molybdenum ions in a 1:3 DMSO:Water solution.

Major Task 1, Subtask 2

This subtask is dependent upon completion of Major Task 1. However, it is important to determine whether acquiring Raman spectra through PDMS won't significantly interfere with the acquisition of crown ether spectra. To this end, Raman spectra were acquired of 25-µM crystal violet through Ag-decorated ZnO nanowires on PDMS. Two different thicknesses of PDMS were used. The "thick" PDMS was about ~1.0 mm while the "thin" PDMS was <0.2 mm. As shown in Figure 8, Raman spectra of crystal violet through the thick PDMS showed barely any enhancement, while spectra through the thin PDMS showed enhancement comparable to our previous work, which acquired SERS spectra of crystal violet through fused silica[2]. This indicates that as long as the thickness of the microchannel wall through which spectra are acquired is controlled, enhanced Raman spectra can be acquired. Completion of this subtask is scheduled for Q2 of 2020.

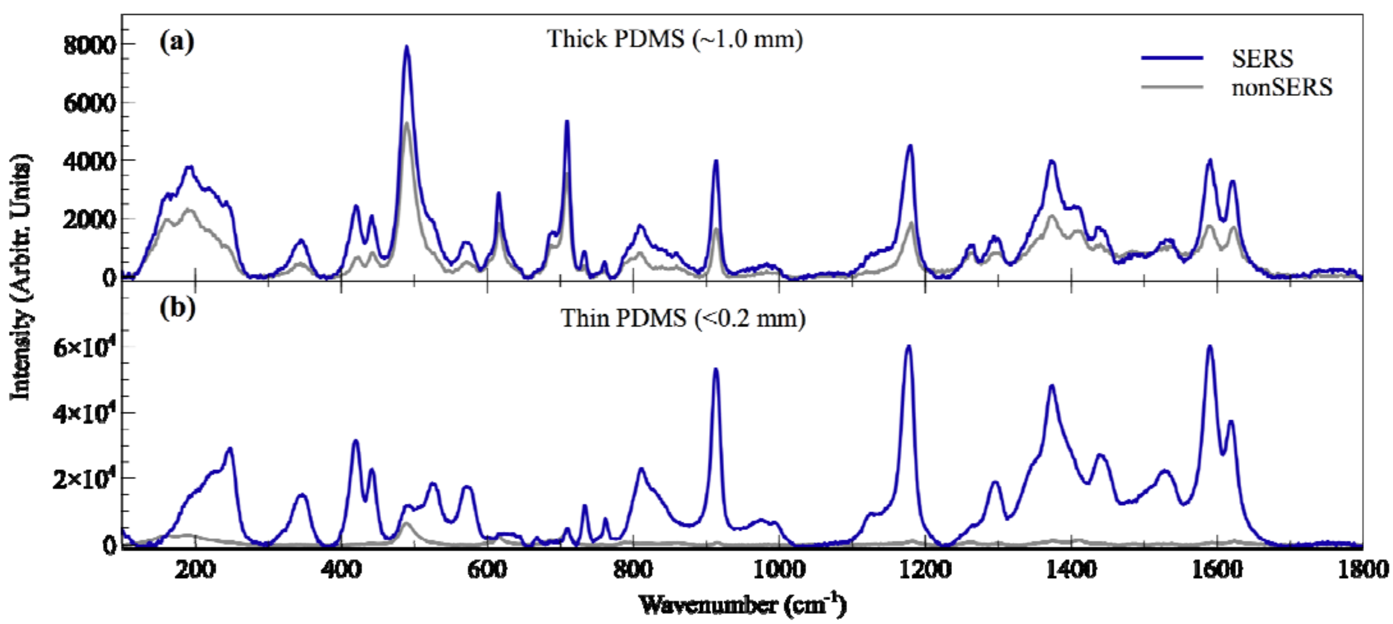

Figure 8: Raman spectra of 25 µM crystal violet through (a) thick PDMS and (b) thin PDMS

Major Task 2, Subtask 3

This subtask is dependent upon completion of Major Task 1. However, the specificity of the crown ethers can be explored using UV-Vis spectrophotometry. Sarfo et. al. demonstrated that 4’-aminobenzo-18-crown-6 has an extinction peak around 300 nm[1]. When the crown ether chelates a metal, this peak is reduced and a peak appears at about 280 nm. An optical extinction-based study was performed of the interactions between 4’-aminobenoz-18-crown-6 and a group of metal ions including metals on the Veteran’s Association toxic metals biomonitoring panel, other toxic or potentially toxic metals, metals commonly found in the human body, and metals incorporated into the proposed biosensor. In total, this sweep included 22 metals and was performed in solutions of 1:1 DMSO:water and 1:3 DMSO:water (as performed by Sarfo et. al.[1]). For the 1:1 DMSO:water solution (Figure 9), there was clear interactions between the crown ether and As, Hg, Al, Mo, Li, and W. For the 1:3 DMSO:water solution (Figure 10), Sarfo et. al. reported clear chelating of Pb, and none of Ba, Ca, Cd, Co, Cu, Hg, K, Li, Na, and Ni[1]. However, we noted no discernable chelating of Pb. We did see chelating of several other metals including Mo, As, U, W, Cr, and Fe. This subtask is scheduled for completion in Q2 of 2020.

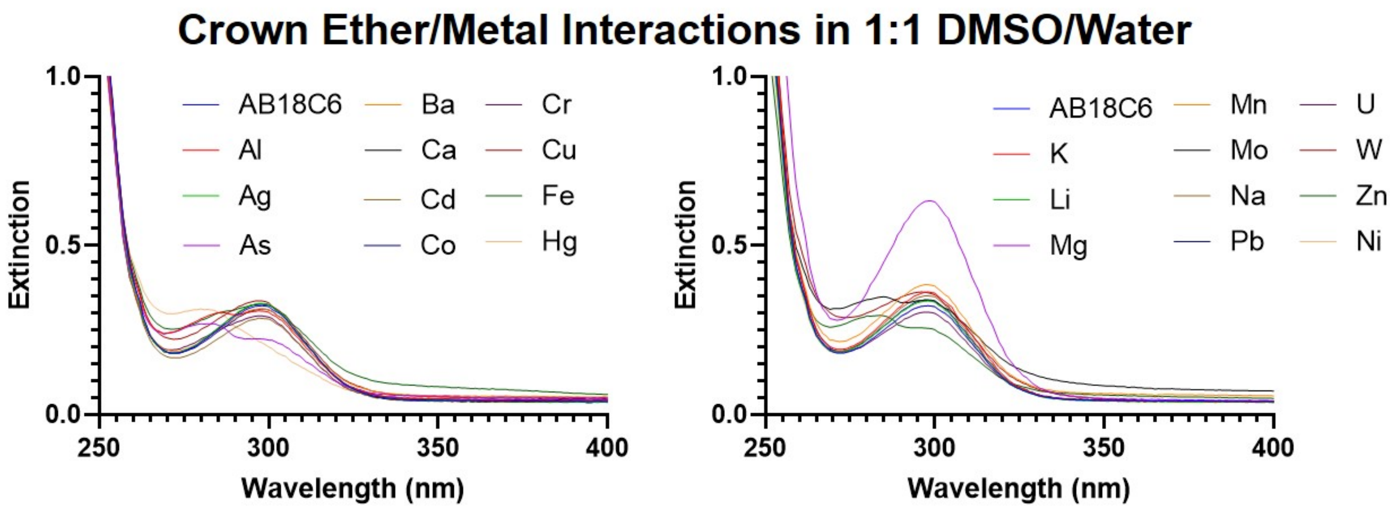

Figure 9: UV-Vis spectra of interactions between 4’-aminobenzo-18-crown-6 and 22 different metals in a 1:1 DMSO:water solution.

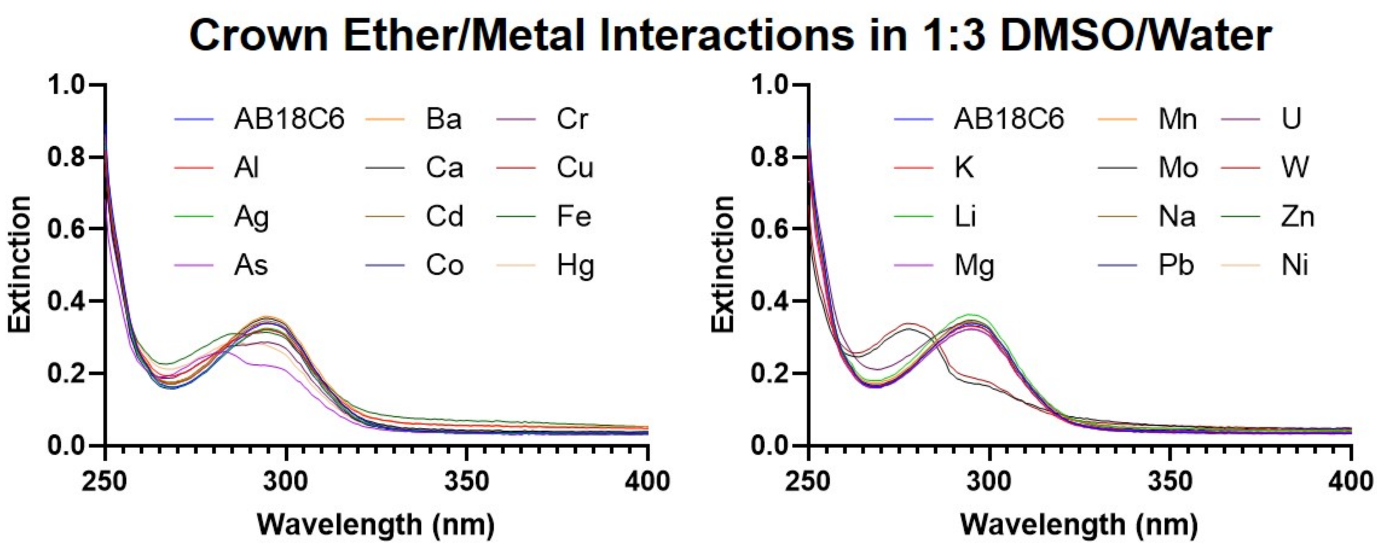

Figure 10: UV-Vis spectra of interactions between 4’-aminobenzo-18-crown-6 and 22 different metals in a 1:1 DMSO:water solution.

<u>Major Task 2, Subtask 4</u>
Completion of this subtask relies on the completion of Major Task 2, Subtasks 2 and 3, to gather the information necessary for training an algorithm to identify chelated metals. Completion of this subtask is scheduled for Q2 of 2020.

<u>Major Task 2, Subtask 5</u>
Completion of this subtask requires completion of Major Task 2, Subtasks 2 and 3, and is scheduled for completion in Q2 of 2020.

<u>Major Task 2, Subtask 6</u>
Completion of this subtask requires completion of Major Task 2, Subtask 4, and is scheduled for completion in Q2 of 2020.

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.

Nothing to report.

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.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

There are multiple papers in the works. The first paper concerns the integration of the Ag-decorated ZnO nanowire substrate with microfluidics, and is scheduled to be submitted in Q1 of 2020. The second paper concerns the functionalization of the Ag-decorated ZnO nanowire substrate with 4'-aminobenzo-18-crown-6 and how the crown ether's Raman spectrum changes when it chelates different metals. This paper is scheduled to be submitted in Q2 of 2020.

4. **IMPACT:** *Describe 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).

Nothing to Report.

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.

Nothing to Report.

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.*

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

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.

Silver nanoparticles were deposited on the ZnO nanowires rather than gold, because silver nanoparticles have plasmon resonances in the 400-600 nm wavelength range, while gold nanoparticles have plasmon resonances in the 600-800 nm range. Preliminary studies suggest that the plasmon resonances of silver will interact with the crown ether/toxic metal chelation more favorably than for gold. Additionally, silver tends to better enhance Raman spectra than gold. The facilities available for this research are better suited for lasers in the former range rather than the latter and represent an approach that may have improved potential for translation.

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.

Difficulty has been encountered in replicating the results reported by Sarfo et. al. [1] Specifically, while chelation of multiple metal ions (such as molybdenum and arsenic) by 4'-aminobenzo-18-crown-6 has been observed, this crown ether has failed to chelate lead ions, even under the same conditions reported by Sarfo et. al. Additionally, Sarfo reported in quantification of lead concentration via fluorescence [1]. Specifically, they reported a fluorescence peak at ~590 nm when excited at 295 nm that increased with increasing lead concentration. While the fluorescence of 4'-aminobenzo-18-crown-6 in Figure 7 exhibits a broad fluorescence peak between 300 and 400 nm that gets quenched when chelating molybdenum and arsenic, a sharp peak at 590 nm has not been observed. We have engaged a number of additional experts to review our results and attempt to replicate the data presented in Sarfo. We have been unable to reconcile the differences among our results and those of Sarfo. Based on the quenching in Figure 7, however, it is expected that this broad fluorescence peak can be used to quantify chelation via peak fluorescence quenching. We will pursue this alternative that may provide suitable function during the next performance period.

In addition to our difficulties in replicating the results of Sarfo et. al., difficulty was encountered in ZnO nanowire growth. There came a point where nanowires stopped growing correctly. Instead of dense forests of nanowires as shown in Figures 4 & 5, nanowire growth was very sparse, as shown in Figure 11. After several weeks of troubleshooting, it was discovered that due to an aging quartz crystal microbalance in the electron beam deposition system being used, much less ZnO seed was being deposited than assumed. This resulted in replacement of the microbalance and nanowire growths performed since have reverted to a useful morphology. This problem is resolved and progress has resumed at expectation.

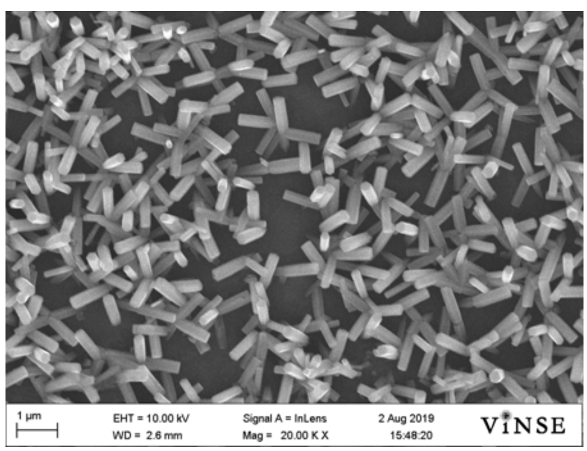

Figure 11: SEM image of sparse nanowire growth

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.

Nothing to report.

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

Nothing to report.

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).*

  Two manuscripts are in preparation, as noted earlier in this 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).*

  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.*

  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.

  Nothing to report.

- **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".

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.)

Name: Andrew Cook
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): ORCID ID: 0000-0002-2859-8804
Nearest person month worked: 12

Contribution to Project: Mr. Cook has performed all of the research reported here.
Funding Support: This award and the National Science Foundation

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.*

Fisk University provided facilities for this project.

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.*

References

[1] D. K. Sarfo, E. L. Izake, A. P. O'Mullane, and G. A. Ayoko, "Molecular recognition and detection of Pb(II) ions in water by aminobenzo-18-crown-6 immobilised onto a nanostructured SERS substrate," *Sens. Actuators B Chem.*, vol. 255, pp. 1945–1952, Feb. 2018, doi: 10.1016/j.snb.2017.08.223.

[2] A. L. Cook, C. S. Carson, C. E. Marvinney, T. D. Giorgio, and R. R. Mu, "Sensing trace levels of molecular species in solution via zinc oxide nanoprobe Raman spectroscopy: Zinc oxide nanoprobe Raman," *J. Raman Spectrosc.*, vol. 48, no. 8, pp. 1116–1121, Aug. 2017, doi: 10.1002/jrs.5180.