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TITLE: A Spectroscopic Approach to Overcome the Barriers of Early Familial Hypercholesterolemia Diagnosis

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14. ABSTRACT
In diagnosing and treating familial hypercholesterolemia (FH) knowing both the "good" (HDL) and "bad" (LDL) cholesterol levels is extremely important as it allows a person to reduce the risk of heart disease and stroke. We propose to develop novel optical spectroscopy-based methods to cover the gap in FH diagnosis through use of surface-enhanced Raman spectroscopy (SERS). Our innovation is to use "near infrared" light (just outside the visible spectrum) and gold nanorods to measure LDL cholesterol more accurately and directly. We hypothesize that SERS spectra interpreted with quantum chemistry calculations and combined with the unique surface chemistry of gold nanorods will enable two critical advances in cholesterol detection for FH and other diseases. To test our hypothesis, we will 1) identify experimentally observable Raman vibrational modes for cholesterol detection and 2) test sensitivity of cholesterol detection in serum and in tissue. Major findings include that time dependent density-functional theory (TDDFT) calculations for the Raman vibrations of cholesterol show the presence of spectral bands of cholesterol's tetracyclic rings which provide information on local chemical properties and experimentally, a SERS signal is detectable from surfactant coated nanorods when placed onto skin, which could enable new non-invasive tissue analysis methods for disease diagnosis.

15. SUBJECT TERMS
Familial Hypercholesterolemia, diagnostic tools, screening, spectroscopy, non-invasive, low-density lipoprotein (LDL), high-density lipoprotein (HDL)

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1. INTRODUCTION

The scope of the research is to develop novel optical spectroscopy-based methods to cover the gap in familial hypercholesterolemia (FH) diagnosis through use of surface-enhanced Raman spectroscopy (SERS). FH results in excessively high levels of plasma cholesterol, xanthomas, premature atherosclerosis, and can lead to early death if untreated. Although current prevalence of the heterozygous form of FH is estimated to be around in 1 in 250 individuals in the general population, it is estimated that only 10% of the FH population is diagnosed and adequately treated, leading to a significant gap. Most individuals are unaware of their FH condition, as it often presents as symptomless, such that diagnosis is frequently done late in life or after the first cardiovascular event. We intend to use to Raman spectroscopy to establish the unique, vibrational modes of cholesterol and developed a real-time, non-invasive cholesterol meter that does not require any enzymatic reactions, like a standard lipid panel. We will carry out time-dependent density functional theory (TDDFT) calculations of cholesterol Raman modes and compare with experimental Raman spectra of cholesterol powder. We will then combine cholesterol with synthetic phospholipids to form solution-phase vesicles made up of phospholipid and cholesterol bilayers and record spectra from a more realistic sample. Coupling of these vesicles with gold nanorods will provide strong signal enhancements (ie SERS). Measuring the rate at which cholesterol from HDL and LDL particles transfer to gold nanorods in serum and using the kinetics, we will differentiate between these sources of cholesterol. Finally, a lipid-nanorod formulation will be applied to the surfaces of tissues to provide a new assay of non-bloodstream cholesterol, which could serve as an early indicator of xanthomas associated with FH.

2. KEYWORDS

Familial Hypercholesterolemia, diagnostic tools, screening, spectroscopy, non-invasive, low-density lipoprotein (LDL), high-density lipoprotein (HDL)

3. ACCOMPLISHMENTS

What were the major goals of the project?

Major Task 1 Perform time-dependent density-functional theory (TDDFT) calculations to identify Raman modes specific to the fused ring structure of sterols and prepare Institutional Animal Care and Use Committee (IACUC) protocol. (Months 1-4)
Major task 1 was completed 100%

Major Task 2 Experimentally obtain the Raman spectra of cholesterol powder. (Months 1-2)
Major task 2 was completed 100%

Major Task 3 Experimentally obtain the Raman spectra of cholesterol in synthetic lipid solutions. (Months 2-4)
Major task 3 was completed 100%

Major Task 4 Experimentally obtain the SERS spectra of cholesterol in synthetic lipids on gold nanorods. (Months 4-6)
Major task 4 was completed 100%

Major Task 5 To measure the peak intensity of cholesterol on the phospholipid-coated gold nanorods. (Months 7-10)
Major task 5 was completed 50%

Major Task 6 To determine the rate at which cholesterol from HDL and LDL particles transfer to gold nanorods in serum and use the kinetics to differentiate between these sources of cholesterol. (Months 10-14)
Major task 6 was completed 0%.

Major Task 7 To measure the SERS signal from the interaction of surfactant or lipid-stabilized gold nanorods within tissue. (Months 15-24)
Major task 7 was completed 10%

What was accomplished under these goals?

Specific Aim 1. To identify experimentally observable Raman vibrational modes for cholesterol detection.

Major Task 1 Perform time dependent density-functional theory (TDDFT) calculations to identify modes specific to the fused ring structure of sterols and prepare Institutional Animal Care and Use Committee (IACUC) protocol. (Months 1-4)

The subtasks achieved were: 1) Use the Amsterdam DFT commercial quantum chemistry package to run TDDFT calculations, modify parameters (numerical accuracy, conformer comparison, hydrogen bonding), assign peaks (Figure 1), 2) Initiate IACUC protocol, and 3) Obtain DoD ACURO (Animal Care and Use Review Office) approval.

TDDFT calculations were performed on four different conformational structures of cholesterol (named Cholesterol-0, Cholesterol-1, Cholesterol-7, Cholesterol-8) which were generated to have different dihedral angles in the iso-octyl chain. We identified the conformer that created the most accurate spectrum in comparison with an experimental powder spectrum, as we previously showed for anthraquinones[1] and flavonoids[2]. As expected, it was a conformer with an extended chain (Cholesterol-8). The full TDDFT calculated spectrum of Cholesterol-8 is displayed in Figure 1 (green), and the corresponding powder spectrum in black.[3] The numbers above each peak in the spectra are assigned to a vibration of the molecule, indicating that we can make 23 peak assignments. We present the vibration maps of four peaks (10, 13, 16, and 23) below the spectra shown in Figure 1. Spectra and vibration maps of all assigned peaks for all four conformers are shown in the Appendix Spectra and Vibration Maps.

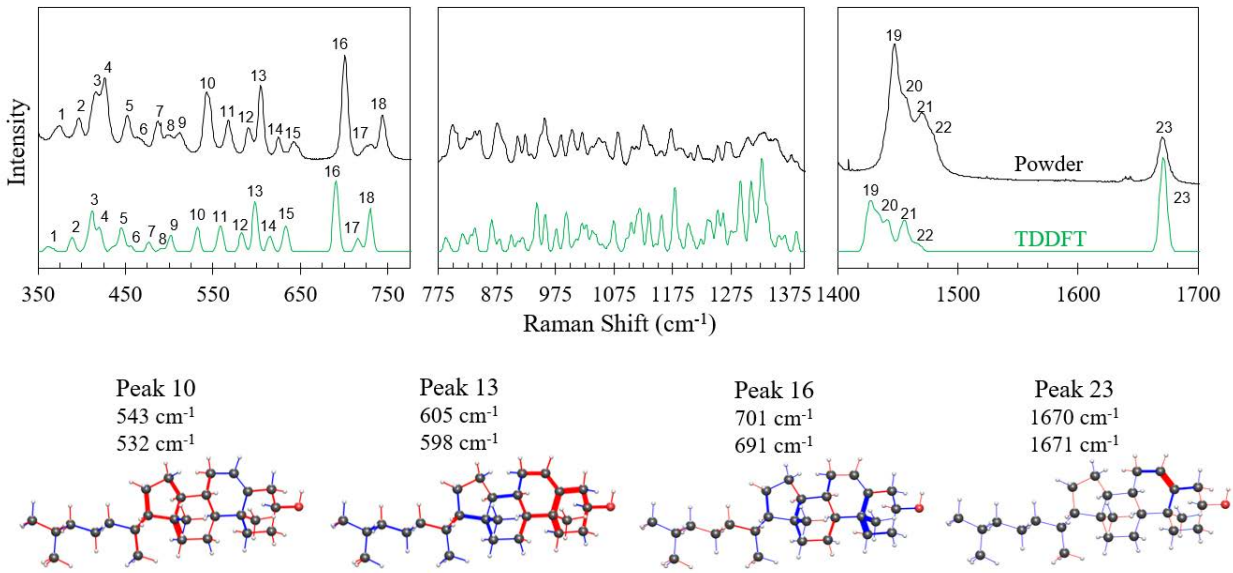


Figure 1. TDDFT calculations and peak assignments to identify modes specific to the fused ring structure of sterols. The calculated (green) and experimental (black) Raman spectra of cholesterol. Individual peaks that match based on position and relative amplitude are labeled. Vibration maps are displayed for four modes with their experimental (top) and calculated (bottom) Raman shifts. In the maps, each bond's thickness indicates its stretching amplitude, and the red/blue color indicates the relative phase.

The IACUC protocol for this study entitled “2021 DoD PRMRP Discovery Familial Hypercholesterol” (ID IS00006547) was approved by the The Methodist Hospital Research Institute IACUC on 11/16/2021; IACUC approval expires 11/15/2024.

DoD ACURO approval was obtained as of 12/16/2021 for the use of mice and pigs and will remain so until modification, expiration or cancellation.

Major Task 2 Experimentally obtain the Raman spectra of cholesterol powder. (Months 1-2)

The subtasks achieved were: 1) Acquire cholesterol power from the commercial vendor (Sigma-Aldrich) and determine acquisition time for reproducible Raman spectra with low background and 2) Identify the peak assignments and vibrational modes.

Raman spectra were recorded from cholesterol powder. We determined optimal signal was obtained for acquisition times of 20 minutes for each of the four spectral windows. The powder spectra are plotted in Figure 1 in Major task 1 (black spectra in each figure indicate powder spectra). The plots were used to complete subtask 2 of Major task 1, where 23 experimental peaks were assigned to Raman modes calculated by TDDFT.

Major Task 3 Experimentally obtain the Raman spectra of cholesterol in synthetic lipid solutions. (Months 2-4)

The subtasks achieved were: 1) Prepare stable solutions of large multilamellar vesicles made up of synthetic phospholipid and cholesterol bilayers and obtain Raman spectra (Figures 2-4) and 2) Identify the peak assignments and vibrational modes present from the cholesterol and/or lipids solutions (Figure 4).

To identify the spectral peaks in a more biologically relevent sample, solution-phase phospholipid vesicles that contain 25 mol-% cholesterol were created by the following protocol: stable lipid solutions were prepared by first obtaining powdered cholesterol from Sigma-Aldrich (>99% purity). For the phospholipid component, 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) (>99% purity) was obtained from Avanti Polar Lipids in chloroform solution. A phospholipid-cholesterol solution with a 3:1 molar ratio of phospholipids to cholesterol was prepared. The lipids were dried under gentle argon flow for ~45 min, then placed in a turbopumped vacuum chamber for ~2 hours until the pressure dropped below 3 x 10^{-6} Torr. The solutions were then hydrated with deionized water to obtain a 10 mg/mL phospholipid concentration. The resulting solutions were mixed and sonicated for ~20 min. This removes the lipids from the glass container wall forming a milky white multilamellar vesicle (MLV) solution (Figure 2 left side).

Although not included as an initial subtask we also performed stable solutions of small unilamellar vesicles (SUVs). This process utilized sonication to transform the MLVs into SUVs turning the solution clearer with a bluish tint, displayed in Figure 2 (right side). Aqueous solutions were stored in a refrigerator and used for measurements within 2 days. The advantages of preparing and using stable SUVs over MLVs include: 1) The SUV are optically clearer which reduces the extinction with gives better Raman signal, 2) the SUVs have a more consistent structure resulting in sharper Raman peaks, and 3) the SUVs more efficiently transfer lipid material to the nanorods.

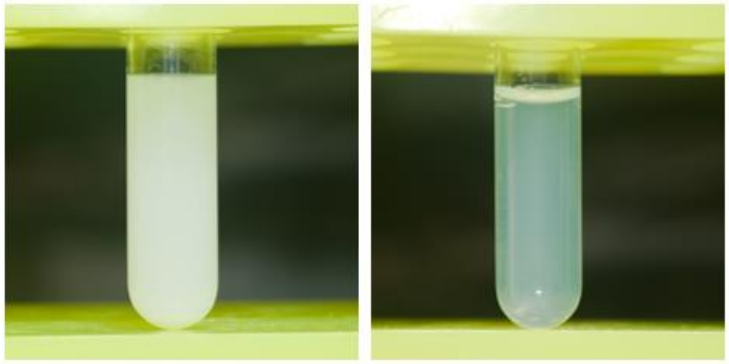


Figure 2. Photographs of solution-phase MLV (left) and SUV (right) lipid vesicles. The less turbid nature of the SUV solution is indicative of the smaller vesicle size.

Dynamic light scattering (DLS) was carried out on MLV sample with and without cholesterol to confirm the lipid vesicle size was not adversely affected by the presence of cholesterol. The results, displayed in Figure 3, show that there is no effect on the size distribution or the appearance of the MLV solution.

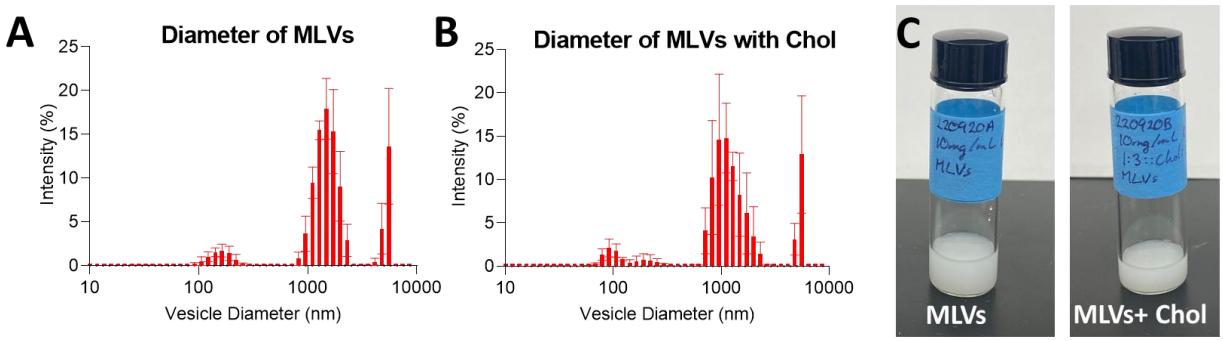


Figure 3. Size distribution histogram for vesicle diameter of (A) large multilamellar vesicles (MLV) and (B) MLVs with cholesterol obtained from Dynamic Light Scattering (DLS) measurements and (C) photos of the lipid vesicles without and with cholesterol.

To identify the peak assignments and vibrational modes present from the cholesterol and/or lipids solutions we loaded the samples in 1x1 mm glass capillaries and actively pumped the sample throughout the measurement to avoid settling and heating. Spectra were acquired for cholesterol in lipid vesicles using acquisition times that varied from 1-5 hours for each of the four spectral regions, totally approximately 8 hours overall (Figure 4). 21 experimental peaks in solution (cholesterol in lipid samples) of the 23 powder cholesterol peaks (Figure 1) were assigned.

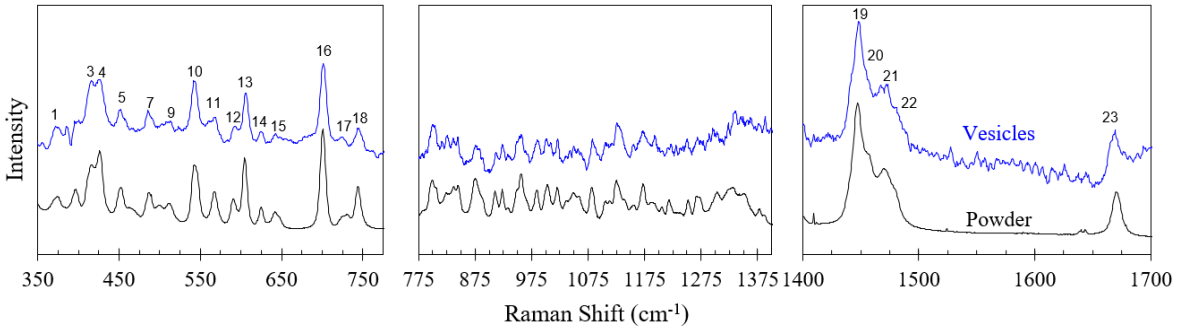


Figure 4. A comparison of the measured Raman spectrum of cholesterol in phospholipid vesicles (blue) and as a microcrystalline powder (black). Peaks in the vesicle spectrum that were identified in Figure 1 are labelled.

Major Task 4 Experimentally obtain the SERS spectra of cholesterol in synthetic lipids on gold nanorods. (Months 4-6)

The subtasks achieved were: 1) Prepare stable gold nanorod solutions with a surface coating of synthetic phospholipid layers containing cholesterol and obtain SERS spectra (See Figure 5A, B) and 2) Identify enhancement and peak assignments and vibrational modes present from the cholesterol and/or lipids solutions on the gold nanorods (Figure 5C, D).

Nanorods were synthesized in cetyltrimethylammonium bromide (CTAB) surfactant.[4] They were pelleted via centrifugation, the CTAB solution was removed, and the nanorods were

resuspended in the lipid SUV solution described above. Under these conditions, lipids displace CTAB at the nanorod surface.[4] This process of putting lipids on nanorods was carried out both for pure phospholipids (DOPC) and phospholipids with cholesterol. Figure 5A confirms the rod geometry and Figure 5B displays the resulting SERS spectra. We observe one of the phospholipid peaks (labelled PC) and 5 of the peaks from the powder cholesterol spectra (3, 4, 10, 13, and 16), indicating that cholesterol remains within the lipid bilayer. The signal is enhanced by the nanorods surface (Figure 5C, D) as seen by the increasing signal intensity with increasing nanorod concentration.

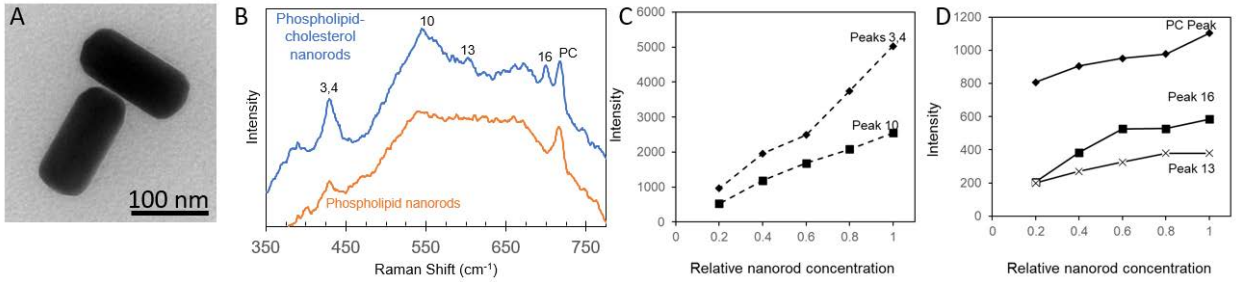


Figure 5. (A) TEM image of gold nanorods encapsulated with lipids, (B) SERS spectrum of nanorods encapsulated with lipids (orange) and nanorods with phospholipids + cholesterol (blue). PC = phosphocholine. Peaks assignments are indicative of cholesterol. (C,D) Peak intensities with varying nanorod concentration indicate the SERS intensity.

Major Task 5 To measure the peak intensity of cholesterol on the phospholipid coated gold nanorods. (Months 7-10)

The subtasks achieved were: 1) Prepare stable gold nanorod solutions with a surface coating of synthetic phospholipid layers containing cholesterol and obtain SERS spectra (See Figure 5 above), 2a) Collect blood samples (n=8 from pigs, 30 ml/animal and n=8 from mice, 1 ml/animal) from other studies upon euthanasia or alternatively purchase blood samples from a commercial source (Animal Technologies, Inc). (**although not treated by ultracentrifugation or spiked with cholesterol in lipid vesicles), and 3) Obtain background Raman spectra and identify strong peaks in spectral region of interest (200 – 1000 cm^{-1}), strength of the Raman background, and fluorescence (See Figure 6).

The subtasks that were not yet achieved were: 2b) Collect blood samples (n=8 from pigs, 30 ml/animal and n=8 from mice, 1 ml/animal) from other studies upon euthanasia or alternatively purchase blood samples from a commercial source (Animal Technologies, Inc). Treat by ultracentrifugation to remove HDL-C and LDL-C particles. Spike with known concentrations of cholesterol in lipid vesicles and 4) Add gold nanorods to treated blood samples and record and analyze SERS measurements.

We first tested the serum that could still have HDL and LDL particles present with surfactant-coated gold nanorods (rather than lipids) as a control to be able to identify what type of signal and background would be present. The surfactants used were CTAB and sodium dodecyl sulfate (SDS) which in future preparations would be displaced by the phospholipid DOPC. The investigators have already made progress on the use of SDS for nanorod stabilization.[5,6] Figure 6 shows some peaks in the raw serum (black spectrum). Even though the serum samples were clear without evidence of hemolysis, serum bands were detected from 600-650 cm^{-1}, 800-850

cm^{-1}, and 1000-1200 cm^{-1}. We anticipate that these peaks will also appear as background signal in spectra where we add phospholipid nanorods. Unfortunately, no new peaks appear from the nanorod spiked samples indicating the lack of SERS.

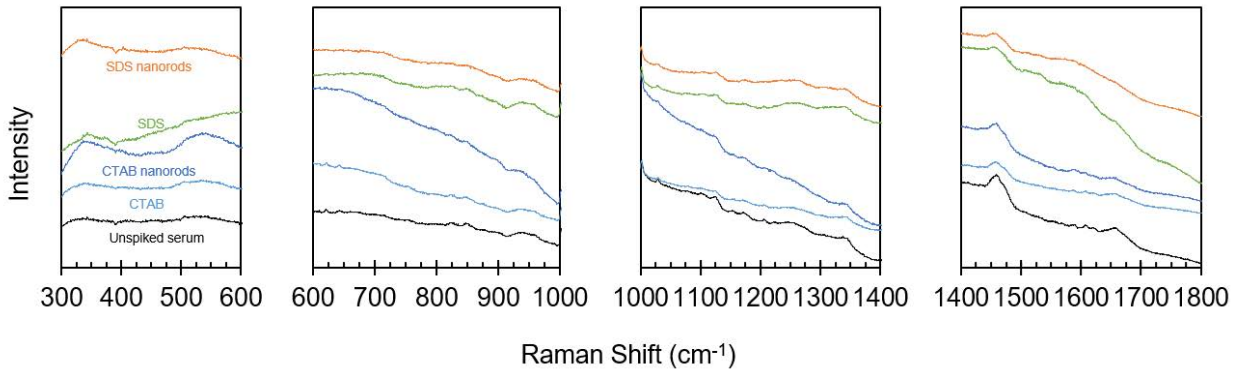


Figure 6. Raman and SERS spectra of unspiked rodent serum (black), serum with CTAB (light blue), serum with CTAB encapsulated nanorods encapsulated (dark blue), serum with SDS (green), and serum with SDS encapsulated nanorods (orange).

To overcome this limitation, future studies are planned to: 1) proceed with the original intention to use phospholipids instead of surfactants, 2) greatly increase the ratio of nanorods to serum (as opposed to the presented data where rods were spiked into the serum), and 3) control for incubation times by testing times ranging from 10 minutes to 24 hrs. We do still intend to try to remove HDL and LDL from the serum prior to adding the nanorods to see how the spectrum differs. We also still intend to spike cholesterol back into the serum prior to adding the nanorods to see if we can observe a dose-dependent effect of cholesterol in serum as seen in pure vesicles (as seen in Figure 5 C, D).

Major Task 6 To determine the rate at which cholesterol from HDL and LDL particles transfer to gold nanorods in serum and use the kinetics to differentiate between these sources of cholesterol. (Months 10-14)

The subtasks that were not yet achieved were: 1) Measure the growth of cholesterol SERS peak over time after mixing lipid coated nanorods with serum. Fit kinetic models to identify and quantify specific rates for LDL-C and HDL-C. 2) Calibrate the LDL-C and HDL-C rate measurements for sensing with spiked serum measurements.

These subtasks were not yet achieved due to the lack of cholesterol SERS peaks identifiable in serum under the conditions tested so far. Integration time will be extended to try to overcome weak signal. The transfer concept was tested with cholesterol in pure phospholipid vesicles (See Figure 7). As cholesterol transfers from vesicles to the nanorod surface, the cholesterol SERS signal increases with time.

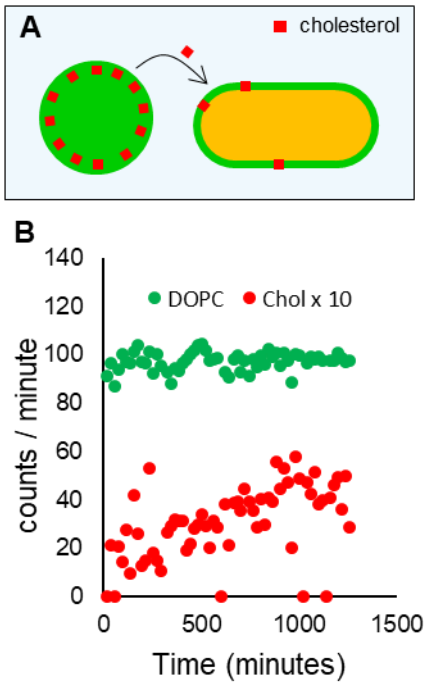


Figure 7. Cholesterol transfer from synthetic vesicles to gold nanorod surfaces. (A) A schematic of the process. (B) Preliminary data based on the time dependence of the intensities of the phospholipid headgroup peak at 718 cm^{-1} and the cholesterol peak at 701 cm^{-1}.

Major Task 7 To measure the SERS signal from the interaction of surfactant or lipid-stabilized gold nanorods within tissue. (Months 15-24)

The subtasks achieved were: 1) Collect tissue samples (liver, heart, adipose, dermal – ~1g/sample/mouse and ~50g/sample/pig) from animals (n=8 from pigs and n=8 from mice) on other studies upon euthanasia or alternatively purchase tissues from a commercial source (Animal Technologies, Inc) and apply lipid-nanorod formulations. Monitor the SERS signals.

The subtasks that were not yet achieved were: 2) Statistical analyses, interpretations, report writing, and 3) Prepare manuscript for publication and submit abstract for conference presentation of results.

To observe the effects of nanorods we spiked areas of shaved rodent skin with 2 uls of 10 mM surfactant solution (control) and 2 uls of highly concentrated nanorods (~10 OD at the plasmon peak) in 10 mM surfactant solution. The skin was then scanned across its surface with a raman probe to obtain Raman and SERS spectra. Scanning was performed using a motorized system with an integration time of ~2 minutes per spectrum.

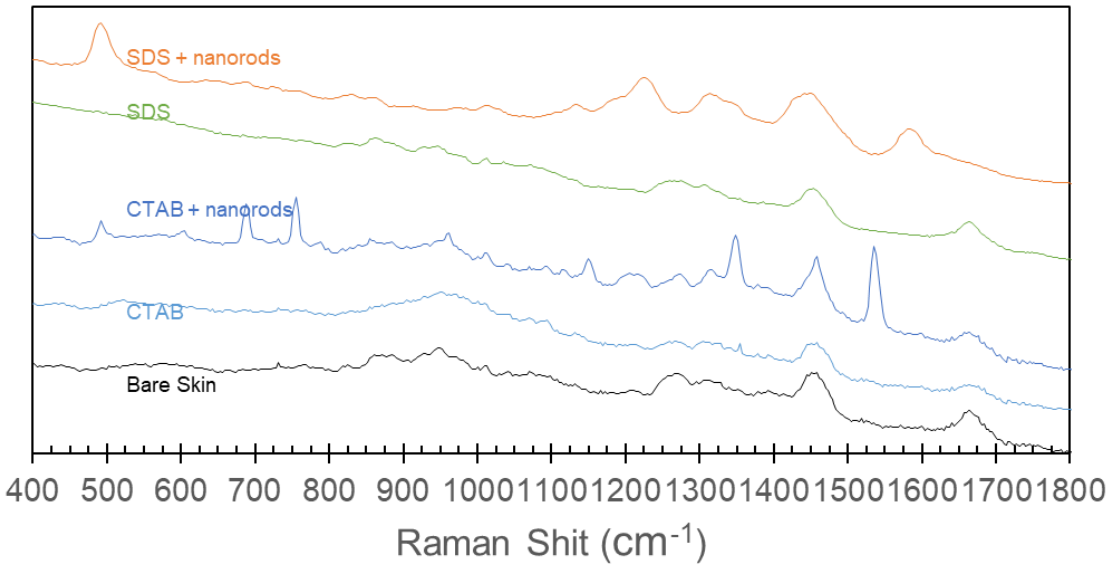


Figure 8. Raman and SERS spectra of unspiked rodent skin (black), skin with CTAB (light blue), skin with CTAB encapsulated nanorods encapsulated (dark blue), skin with SDS (green), and skin with SDS encapsulated nanorods (orange).

CTAB + nanorods peaks	SDS + nanorods peaks
492 cm^{-1}	492 cm^{-1}
605 cm^{-1}	
687 cm^{-1}	
755 cm^{-1}	
	1138 cm^{-1}
1150 cm^{-1}	
	1187 cm^{-1}
1202 cm^{-1}	
1226 cm^{-1}	1224 cm^{-1}
1315 cm^{-1}	1313 cm^{-1}
1349 cm^{-1}	1346 cm^{-1}
	1447 cm^{-1}
1456 cm^{-1}	
1535 cm^{-1}	
	1585 cm^{-1}

Table 1. SERS peaks that appear on rodent skin spiked with CTAB encapsulated rods and SDS encapsualted rods.

Importantly, peaks appear with the addition of nanorods with both surfactants (see Figure 8 and table 1 for a list of peaks). Some peaks are present with both surfactants (e.g. 492 cm^{-1}), while some peaks are only present for a single surfactant (e.g. 687 cm^{-1}). This suggests we can control what biomolecules we are detecting by changing surfactants. The reproducibility and molecular sources of these peaks is being further investigated.

References.

1. Simeral, Mathieu L.; Hafner, J. H. The Raman Active Vibrational Modes of Anthraquinones. *Astrobiology* 2022, 22 (11), 1–11.
2. Uyeki, C. M.; Pacheco, C. M.; Simeral, M. L.; Hafner, J. H. The Raman Active Vibrations of Flavone and Quercetin: The Impact of Conformers and Hydrogen Bonding on Fingerprint Modes*. J. Phys. Chem. A* under revision.
3. Simeral, M. L.; Demers, S. M. E.; Sheth, K.; Hafner, J. H. The Fingerprint Raman Modes of Cholesterol: Spectral Markers for Hydrogen Bonding and Iso-Octyl Chain Conformation. *J. Am. Chem. Soc.* submitted.
4. Matthews, J. R.; Payne, C. M.; Hafner, J. H. Analysis of Phospholipid Bilayers on Gold Nanorods by Plasmon Resonance Sensing and Surface-Enhanced Raman Scattering. *Langmuir* 2015, 31 (36), 9893–9900. https://doi.org/10.1021/acs.langmuir.5b01203.
5. Terracciano, R.; Zhang, A.; Butler, E. B.; Demarchi, D.; Hafner, J. H.; Grattoni, A.; Filgueira, C. S. Effects of Surface Protein Adsorption on the Distribution and Retention of Intratumorally Administered Gold Nanoparticles. *Pharmaceutics* 2021, 13 (2), 216. https://doi.org/10.3390/pharmaceutics13020216.
6. Terracciano, R.; Zhang, A.; Simeral, M. L.; Demarchi, D.; Hafner, J. H.; Filgueira, C. S. Improvements in Gold Nanorod Biocompatibility with Sodium Dodecyl Sulfate Stabilization. J. Nanotheranostics 2021, 2 (3), 157–173. https://doi.org/10.3390/jnt2030010.

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

A manuscript is in preparation.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

While studying the calculated Raman vibrations of cholesterol, spectral bands were found among the complex fingerprint vibrations of cholesterol's tetracyclic rings that provide information on local chemical properties. The intensities of peaks 3 and 4 vary with hydrogen bonding at cholesterol's hydroxyl group, and the spacing and intensities of peaks 10-15 are sensitive to two dihedral angles in the iso-octyl chain.[3] This suggests that Raman spectroscopy can contribute much more to biomolecular structure than previously thought, given the use of TDDFT to identify vibrational motions. Also these peak identifications would help with biomedical detection, since cholesterol is dysregulated in a myriad of diseases. This work demonstrates the power of quantum chemical methods if appropriately applied to biomedical problems.

Also importantly, we found that the spectral set up allowed for sensitivity high enough to be able to detect SERS signal from surfactant coated nanorods when placed onto skin tissue. We also have enough throughput to be able to scan across different areas of tissue in rapid succession. Furthermore, we find different SERS peaks with different nanorods surface chemistries suggesting we can selectively detect substances in tissue. This could enable new non-invasive tissue analysis methods for disease diagnosis and progression monitoring.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

The SERS signal of cholesterol transfer was very slow and weak (See Figure 7), even at pure lipid solutions and serum will be even more challenging considering we saw no SERS peaks at our first attempts. Moving forward, we will 1) add increased nanorod concentration and decreased Serum concentration for a cleaner sample, 2) purify the serum of LDL and HDL particles by ultracentrifugation, and 3) spike the serum with a known concentration of cholesterol until we see a SERS signal. This process will tell us the realistic cholesterol concentration and transfer kinetics we can detect in a natural sample.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

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

Publications, conference papers, and presentations

Journal publications.

1. Uyeki, C. M.; Pacheco, C. M.; Simeral, M. L.; Hafner, J. H. The Raman Active Vibrations of Flavone and Quercetin: The Impact of Conformers and Hydrogen Bonding on Fingerprint Modes. *J. Phys. Chem. A* under revision.
2. Simeral, M. L.; Demers, S. M. E.; Sheth, K.; Hafner, J. H. The Fingerprint Raman Modes of Cholesterol: Spectral Markers for Hydrogen Bonding and Iso-Octyl Chain Conformation. *J. Am. Chem. Soc.* submitted.

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers, and presentations.

1. Fallon BC, Carcamo-Bahena Y, Simeral ML, Royal ALR, Hafner JH, Filgueira CS A Spectroscopy-Based Non-Enzymatic Method to Detect Cholesterol for Familial Hypercholesterolemia, HMRI Nanomedicine Department Symposium, Houston, TX December 5th, 2022. Poster Presentation.
2. Carcamo-Bahena Y, Simeral M, Hafner J, Filgueira CS Coupling Raman Spectroscopy with Thin Layer Chromatography to Investigate Lipid Membranes. 2022 ACS Fall Meeting “Sustainability in a Changing World”, August 21-25th, 2022. Poster Presentation.
3. Carcamo-Bahena Y, Simeral M, Hafner J, Filgueira CS A Spectroscopy-based Non-enzymatic Method to Detect Cholesterol for Early Familial Hypercholesterolemia Screening. George and Angelina Kostas Research Center for Cardiovascular Nanomedicine Annual International Meeting, October 24, 2022. Oral/Blitz Talk Presentation. *Third place presentation winner.

References.

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Site 1: Houston Methodist Research Institute

Name:	**Carly Filgueira**
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-3246-303X
Nearest person month worked:	1.2
Contribution to Project:	Dr. Filgueira obtained IACUC and ACURO approval, obtained the cholesterol powder, assisted in peak assignment identification of powder cholesterol, cholesterol solutions and lipid solutions, and cholesterol and/or lipids solutions on gold nanorods, collected blood and tissue samples from animals, spiked known concentrations of cholesterol in lipid vesicles, and added gold nanorods to treated blood and tissue samples.

Name:	**Yareli Carcamo-Bahena**
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	0000-0001-9998-9696
Nearest person month worked:	2.4
Contribution to Project:	Ms. Carcamo assisted with performing dynamic light scattering on the vesicles, collecting blood and tissue samples from animals, performing lipid extractions, and ultracentrifugation.

Site 2: Rice University

Name:	**Jason Hafner**
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-6943-4232
Nearest person month worked:	0.25
Contribution to Project:	Dr. Hafner performed the time dependent density-functional theory (TDDFT) calculations, identified the peak assignments and vibrational modes of powder cholesterol, cholesterol solutions and lipid solutions, and cholesterol and/or lipids solutions on gold nanorods, prepared the stable gold nanorod solutions with phospholipid layers with and without cholesterol, and measured the growth of cholesterol SERS peak over time after mixing lipid coated nanorods with serum.

Name:	**Mathieu Simeral**
Project Role:	Graduate Student
Nearest person month worked:	6
Contribution to Project:	Mr. Simeral assisted with monitoring Raman background and SERS signals, determined the acquisition time for reproducible Raman spectra of cholesterol with low background, preparing stable solutions of large multilamellar vesicles made up of synthetic phospholipid and cholesterol and preparing stable gold nanorod solutions with synthetic phospholipids.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes, attached are the changes to the active support for Dr. Filgueira and Dr. Hafner.

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

Not applicable

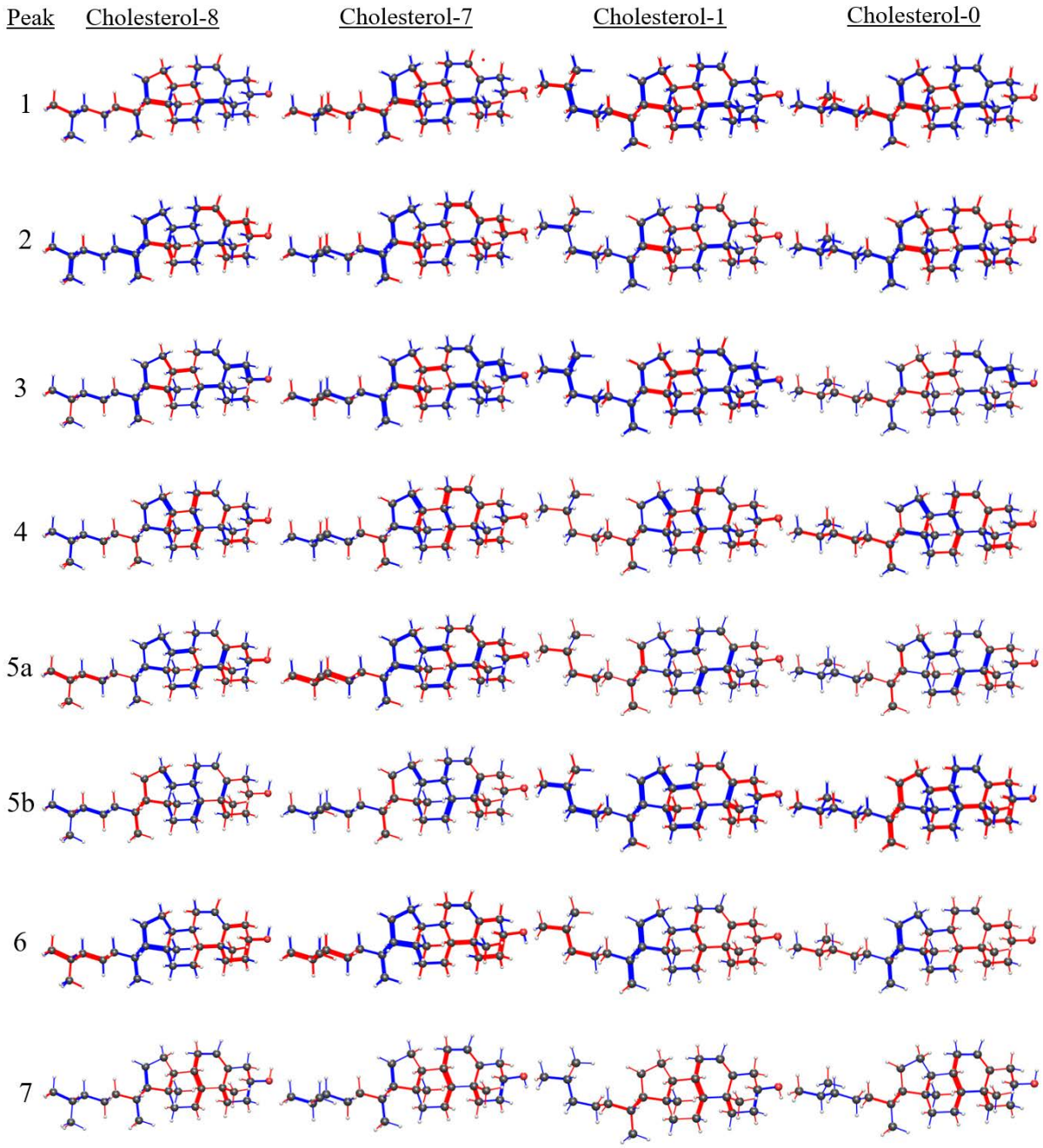
9. APPENDICES

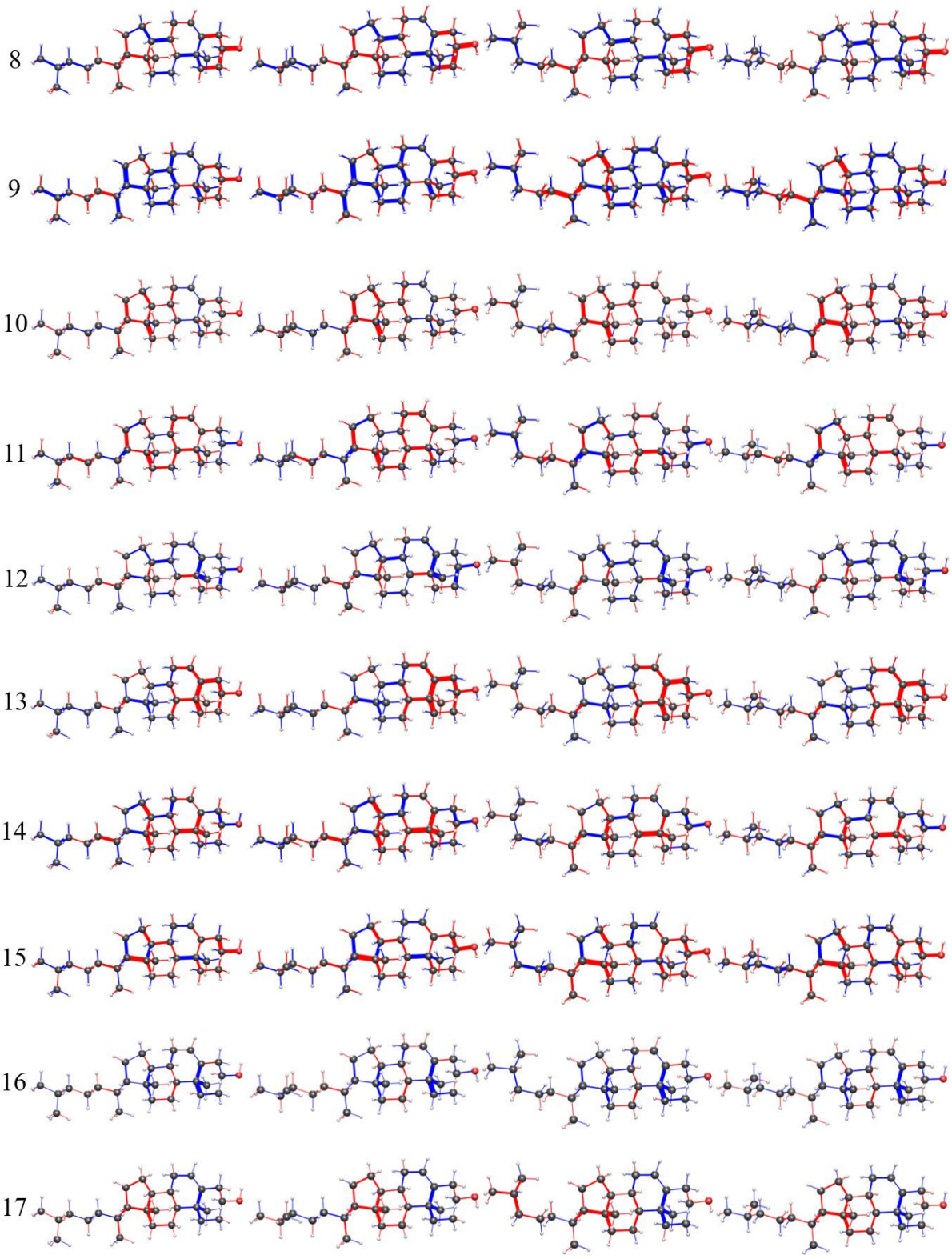
Spectra and Vibration Maps

Changes to ongoing research support

Spectra and Vibration Maps

Below are the vibration maps for all identified vibrational modes (1 – 23) for each of the four conformers described (Chol-0, -1, -7, and -8). The widths of the bonds indicate stretching amplitudes and the colors indicate relative phase. Note that each map is normalized to the largest vibration for that map, so comparisons of the vibration amplitude between modes or conformers cannot be made. Also, since these maps show bond stretching, they do not illustrate CH_2 or CH_3 deformations. Peaks 19-22 are therefore not representative of the motion that creates the Raman peaks in the 1400-1500 cm^{-1} window. When two maps are shown (a/b), the calculations found two modes at essentially the same wavenumber shift.

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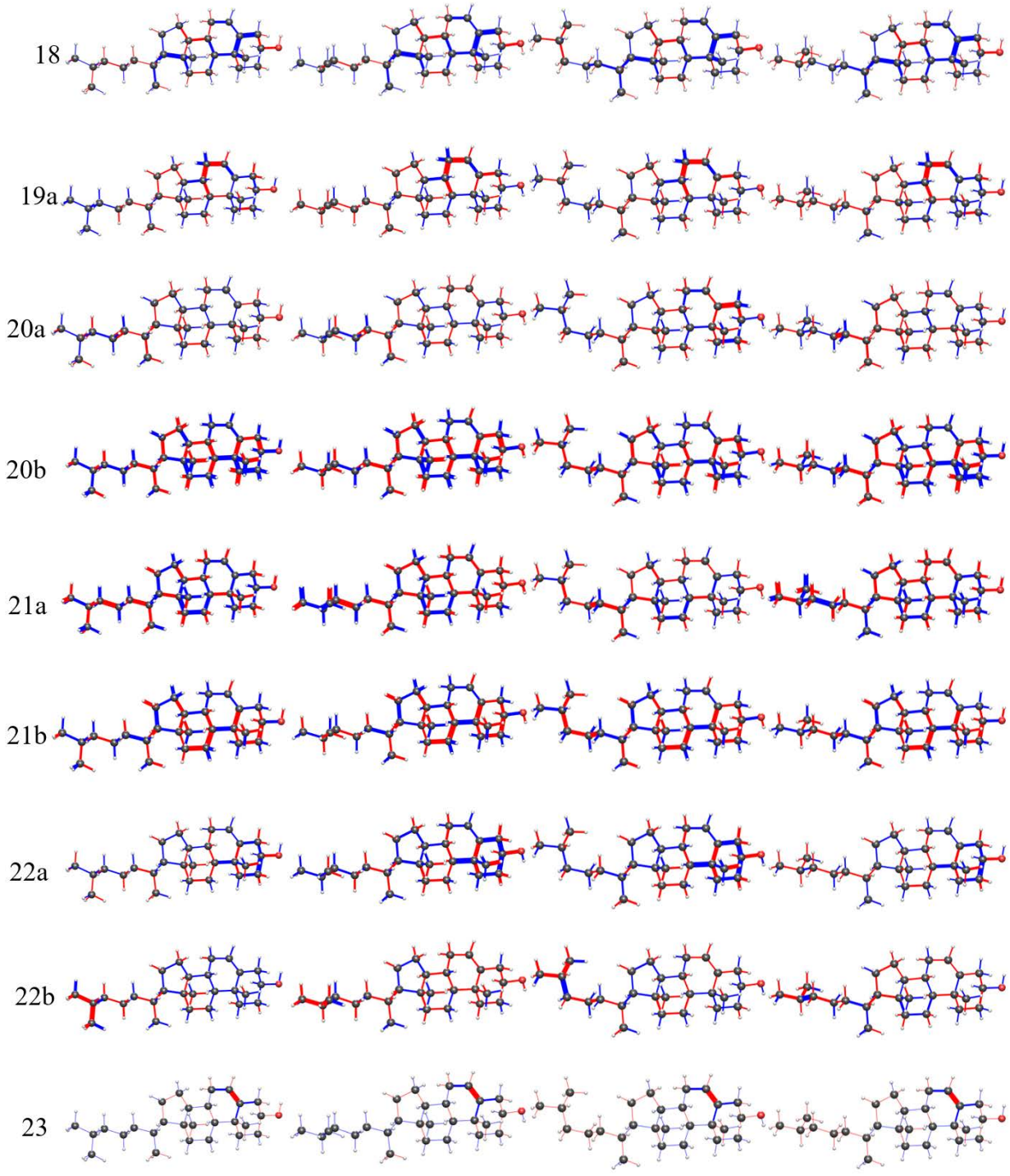
18
19a
20a
20b
21a
21b
22a
22b
23

FILGUEIRA, CARLY S. (Principal Investigator)

Ongoing Research Support

W81XWH2210007 (Filgueira) 12/1/2021 – 11/30/2023 1.8 calendar
DOD
Effects of Thyroid Hormone Metabolite Treatment for Postmenopausal Heart Repair
We propose to explore use of T3, the active form of TH, and its metabolite 3,3'-diiodothyronine (3,3′-T2) for post-myocardial infarction (MI) treatment in female rodents.
Specific Aims: 1) To evaluate the role of THs (T3, 3,3′-T2) post-MI using euthyroid and hypothyroid female rats and compare efficacy of salvage treatment. 2) To evaluate the role of THs (T3, 3,3′-T2) post-MI in OVX female rats with and without estrogen (E2) replacement therapy and compare efficacy of treatment.
Role: Principal Investigator
Point of Contact: Jodi Cardoza
Overlap: None

W81XWH2210002 (Filgueira) 1/1/2022 – 12/31/2023 1.2 calendar
DOD
A Spectroscopic Approach to Overcome the Barriers of Early Familial Hypercholesterolemia Diagnosis
Our goal at the end of the proposed project is to have developed a real-time, non-invasive cholesterol meter to improve early diagnosis of familial hypercholesterolemia and the implementation of diagnostic tools, including in the pediatric population.
Specific Aims: 1) To identify experimentally observable Raman vibrational modes for cholesterol detection. 2) To test sensitivity of cholesterol detection in serum and in tissue.
Role: Principal Investigator
Point of Contact: Michelle L. Cromwell
Overlap: None

Butler/**Filgueira** 9/1/2018-12/31/2022 0.24 calendar
Golfer's Against Cancer total
Nanoparticle Enhanced Radioimmunotherapy for Lung Cancer
Our goal is to intratumorally deliver gold nanoparticles and immunoadjuvants to significantly enhance radiotherapy and produce synergistic effects.
Our aims are to: 1) determine the dose dependent effects of irradiation coupled with gold nanoparticle treatment on lung cell tumor regression (measure tumor size, change in luminescence), 2) quantify the amount of gold nanoparticles required to achieve tumor regression, and 3) perform radiotherapy of the primary tumor in combination with immunoadjuvants (CD40 monoclonal antibody) to test for increased survival and immune-mediated regression of metastasis outside the radiation field, based on an abscopal effect.
Role: Co- Principal Investigator
Point of Contact: Tiffany Polk
Overlap: None

Butler/**Filgueira** 3/22/2019-12/31/2022 0.24 calendar
Golfer's Against Cancer total
Nanoparticle Induced Anti-tumor Immunity for Lung Cancer
Our goal is to improve cancer treatment and promote cancer immunity by inducing the abscopal effect in a more robust manner to generate a tumor-specific immune response using an antibody-gold nanoparticle construct.
Our aims are to: 1) develop an antibody-gold nanoparticle construct, 2) demonstrate with computed tomography (CT) imaging that our chemically modified nanoparticles distribute differently in the tumor environment than unmodified nanoparticles and monitor length of particle entrapment and clearance in a solid tumor, 3) determine the effects of treatment with irradiation and chemically modified nanoparticles (changes in tumor growth, immune activation, and prevalence of lung metatasis).

Role: Co- Principal Investigator
Point of Contact: Tiffany Polk
Overlap: None

(New)
Filgueira/Weiner 1/1/2022-12/31/2022 0.24 calendar
Houston Methodist Research Institute
Use of a Protaglandin Analog to Enhance Blood Flow and Tendon Regeneration in a Rabbit Model
Our goal is to show Remodulin can be administered locally in the knee to improve blood flow to the tendon and accelerate regeneration.
Specific Aims: 1) To optimize the 7T MR sequences for the patella tendon in a normal rabbit knee. 2) To induce tendon injury in rabbits and assess with contract enhanced 7T MR improvements due to repeated Remodulin administration.
Point of Contact:
Overlap: None

(New)
B2TRI (**Filgueira**) 4/1/2022 – 9/30/2022 0.24 calendar
Houston Methodist Research Institute
Design of a Multi-Lumen Syringe and Catheter System
Our goal is to develop several prototypes of a multi-lumen syringe and catheter for multi-component *in situ* administration of gelatinous materials.
Specific Aims: 1) Design and fabricate multi-lumen syringe and catheter. 2) Test deployment in *in vivo* model. Point of Contact: Fernando Cabrera
Overlap: None

(New)
EnMed Capstone (Moskow, **Filgueira**) 9/1/2022 – 8/31/2024 0.12 calendar
Houston Methodist Research Institute
Design of an intra-articular injection system to aid in osteoarthritis diagnosis and management
This project supports the Graduate Capstone project of Joshua Moskow to be conducted under the mentorship of Dr. Filgueira. Goal of the project is to design and test a protype of an injection system.
Point of Contact:
Overlap: None

Previous Research Support

(Completed)
The Provost TMC Collaborator Fund (Hafner) 7/01/2021 – 6/30/2022 0.24 calendar
Rice University for Dr. Hafner, no funds for Dr. Filgueira. An Optical Sensor for Lipophilic Biomarkers in Tissue
The major goal of this internal seed-funded project is to develop surface enhanced Raman scattering as a general platform for analysis of lipophilic biomarkers in tissue, with an emphasis on lipid chain saturation for intraoperative tumor margins.
Role: Co-Investigator

Filgueira/Hafner 03/01/2020-12/31/2021 0.24 calendar Houston Methodist Research Institute/Rice University
A Field-Deployable, Small Molecule Nanosensor with Specificity Based on Lipophilicity
The project will test a mobile chemical sensing platform that uses our recent advances in solution-phase surface-enhanced spectroscopy from lipid-coated gold nanoparticles.

Specific Aims: 1) Utilize a surface-enhanced Raman spectroscopy (SERS) platform of gold nanorods coated with lipid membranes to address reproducibility in defense and medical sensing applications, 2) Calculate specific fingerprint modes of the simulant methyl salicylate with time-dependent density functional theory
Role: Co-Principal Investigator

HAFNER, JASON H. (Principal Investigator)

Ongoing Research Support

Hafner 07/01/2017-06/01/2023 1.0 calendar
National Science Foundation total
Membrane Structure Analysis by Enhanced Raman Scattering
The major goal of this project is to use surface-enhanced Raman scattering to determine molecular structure in lipid membranes on gold nanorods. We aim to develop experimental methods and data analysis to solve membrane structures based on spectral ratios, and to solve for lipid, cholesterol, and dye structures in membranes. Finally, we will investigate effect of nanoparticle size.
Role: Principal Investigator
Point of Contact: Lin He, Program Officer, Division of Chemistry, National Science Foundation
Overlap: This project studies similar molecules, but for an entirely different purpose (structural biology).

Hafner/Filgueira 10/01/2021-09/01/2023 0.25 calendar
DOD CDMRP (to Hafner)
A Spectroscopic Approach to Overcome the Barriers of Early Familial Hypercholesterolemia Diagnosis
Our goal at the end of the proposed project is to have developed a real-time, non-invasive cholesterol meter to improve early diagnosis of familial hypercholesterolemia and the implementation of diagnostic tools, including in the pediatric population.
Specific Aims: 1) To identify experimentally observable Raman vibrational modes for cholesterol detection. 2) To test sensitivity of cholesterol detection in serum and in tissue.
Role: Co-Principal Investigator
Point of Contact: Michelle L. Cromwell
Overlap: None

Previous Support

(Completed)

Hafner 07/01/2021- 06/30/2022 0.0 calendar
Rice University TMC Collaborator Fund (to Hafner)
An Optical Sensor for Lipophilic Biomarkers in Tissue
The major goal of this internal seed-funded project is to develop surface-enhanced Raman scattering as a general platform for analysis of lipophilic biomarkers in tissue, with an emphasis on lipid chain saturation for intraoperative tumor margins.
Role: Co-Principal Investigator
Point of Contact: Rose Berridge

Hafner 05/01/2020- 04/30/2022 0.0 calendar
Rice University TMC Collaborator Fund
IDEA - Monitoring Molecular Interactions in Membranes
The major goal of this seed-funded project is to combine the tools of three labs at Rice (Hafner, Biswal, Marti) to study molecular interactions in lipid membranes.
Specific Aims: Detect fluorescent and pH sensitive surfactants in lipid membranes and determine their orientation. Detect exosomal membrane material.
Role: Co-Investigator
Point of Contact: Sharon Pepper, Office of the Provost, Rice University

Filgueira/**Hafner** 03/01/2020-12/31/2021 0.0 calendar
Houston Methodist Research Institute/Rice University

A Field-Deployable, Small Molecule Nanosensor with Specificity Based on Lipophilicity
The project will test a mobile chemical sensing platform that uses our recent advances in solution-phase surface-enhanced spectroscopy from lipid-coated gold nanoparticles.
Specific Aims: 1) Utilize a surface-enhanced Raman spectroscopy (SERS) platform of gold nanorods coated with lipid membranes to address reproducibility in defense and medical sensing applications, 2) Calculate specific fingerprint modes of the simulant methyl salicylate with time-dependent density functional theory
Role: Co-Principal Investigator
Point of Contact: Connie Green
Overlap: None.