New Frontiers in Research Fund
Fonds Nouvelles frontières en recherche

Application - New Frontiers in Research Fund - Exploration 2019

Application ID: NFRFE-2019-00684 **Administering organization:** University of Alberta

Applicant: Serpe, Michael **Funding opportunity:** New Frontiers in Research Fund - Exploration

Title: Nanomaterial-Enhanced Positron-Emission Tomography Imaging and Targeted Drug Delivery

Application Details

Language of the application:

English

Does your proposal involve Indigenous research as defined by SSHRC?

No

List of Participants

Participant	Primary Affiliation
Serpe, Michael - Nominated Principal Investigator Professor University of Alberta, Chemistry Canada	Professor University of Alberta, Chemistry Canada
Schirrmacher, Ralf - Co-Principal Investigator Professor University of Alberta, Oncology Canada	Professor University of Alberta, Oncology Canada

Collaborators

Name	Position	Organization and Department

Canada

Fields of Research

Primary	Code	Group (Discipline)	Class	Field
●	RDF2110108	Nano-technology	Nano-technology	Nanomaterials
●	RDF3020218	Clinical medicine	Clinical sciences	Radiology, nuclear medicine and medical imaging
	RDF2060109	Materials engineering	Materials engineering	Polymers and plastics

Keywords

Au nanoparticles

Positron emission tomography imaging

Enhanced imaging resolution

Stimuli-responsive polymers

Targeted and triggered delivery

Summary of Proposal

Positron Emission Tomography (PET) is among the most sophisticated in vivo imaging modalities using radioactive isotopes that decay by positron emission. PET is non-invasive, painless, and hence patient friendly; provides full-depth images of the human body featuring dynamic space and time resolution of injected radio-labeled diagnostics (e.g. for cancer and neuro-imaging); and offers unprecedented detection sensitivity. PET radionuclides attached to bio-molecules or nano-materials (e.g., nanogels) follow the biodistribution of these compounds for disease staging, therapy, and/or biodistribution mapping. The latter feature is important to follow the in vivo dispersal of radio-labeled nanogels intended to deliver a therapeutic payload encapsulated within the nanogel structure to a tumor location. If loaded nanogels are injected into a cancer patient without the ability to understand their biodistribution (and accumulation near tumor sites), then an adequate assessment of their time-dependent therapeutic delivery cannot be obtained, thus hampering drug development. Only when the accumulation is high enough should the therapeutic payload be released by external stimulation/manipulation (e.g. radiation induced heating).

Our multidisciplinary team aims to develop radiolabeling protocols to chemically introduce fluorine-18 to chemotherapeutic-loaded nanogels by a simple one-step labeling procedure based on three non-canonical labeling methodologies: 1) the SiFA strategy which was developed by the Schirrmacher group and has now found its first human clinical application for in vivo tumor imaging (First-in-human 18F-SiFAlin-TATE PET/CT for NET imaging and theranostics, IMAGE OF THE MONTH, Ihan, H. et al Eur J Nucl Med Mol Imaging (2019); 2) the BF3 isotopic exchange labeling strategy (Perrin et al); and 3) the Al[18F]F chelation chemistry (Goldenberg et al). These labeling strategies will be applied to introduce the isotope 18F into the nanogel structure to follow their bio-distribution in vivo in a cancer animal model using small animal micro PET imaging. Nanogels will be used to "hold" the therapeutic payload, as they have a large free volume that can be used as a reservoir. The kinetics of the loaded/un-loaded nanogels' bio-distribution within tumor bearing animals will be accurately assessed by acquiring temporal dynamic PET images to prove that 18F-labeled nanogels can serve as delivery shuttles for tumor therapy.

Suggested Reviewers

Name	Position	Organization and Department	Areas of Expertise
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

Reviewer Exclusions

Name	Organization and Department

Proposed Budget

Direct Costs Year 1	Direct Costs Year 2	Total Direct Costs
$100,000	$100,000	$200,000
Indirect Costs Year 1	**Indirect Costs Year 2**	**Total Indirect Costs**
$25,000	$25,000	$50,000
Year 1 Total	**Year 2 Total**	**Total**
$125,000	$125,000	$250,000

GBA+

Is it appropriate to integrate GBA+ and SGBA considerations into the proposed research?

No

Justification

GBA+ is used to assess how diversity (e.g., sex, gender, ethnic) affects research outcomes or impacts of research findings. This proposal describes a nanotechnology-based approach to improve positron emission tomography image resolution, and thus its diagnostic potential. This phase of the project requires extensive lab work, with no patient interactions. Therefore, we believe no GBA+ is required. Although, when publishing, all results will be open access so to reach the most people possible.

Certifications, Licenses and Permits

Certification Requirements

Which of the following does the proposed research involve?

- Animals
- Biohazards
- Human Subjects
- Human pluripotent stem cells
- ✓ None of the above

What level of physical containment is required?

My proposed research does not require physical containment

Is this a clinical trial?

No

Does your proposed research require an exemption from Health Canada?

No

Environmental Assessment

Will any phase of the proposed research take place on federal lands in Canada, other than lands under the administration and control of the Commissioner of Yukon, the Northwest Territories or Nunavut, as interpreted in section 2(1) of the Canadian Environmental Assessment Act, 2012 (CEAA 2012)?

No

Will any phase of the proposed research take place outdoors and outside of Canada?

No

Will the grant permit a designated project, as listed in the CEAA 2012 Regulations Designating Physical Activities, to be carried out in whole or in part?

No

Will any phase of the proposed research depend on a designated project, as listed in the Regulations Designating Physical Activities, being carried out by a third party?

No

Equity, Diversity and Inclusion (EDI)

Analysis of Context

Provide a short description of the EDI context of your team

We are devoted to ensuring that HQP leave our team as well-rounded scientist and members of society armed with a deep knowledge of their respective research fields and an appreciation of how their skill can be used to improve human health outcomes. HQP training (professional development, and lab-based skills) are of utmost importance, and we will encourage team HQP to pursue diverse training experiences, which will serve to bolster HQP excellence, leading to higher quality research, and a dynamic learning and training atmosphere; this all results in more career opportunities and improved HQP satisfaction. The team promotes EDI by: 1) Using gender neutral pronouns in correspondences; 2) Making pronouns explicit in email and on social media; 3) Actively accentuating "standout" adjectives when describing HQP strengths in reference letters; 4) Making it explicit to HQP/others that we are EDI advocates; 5) Working on advocacy projects with HQP to explore EDI obstacles in STEM, and openly discussing the importance of EDI; 6) Selecting team event times that are sensitive to those with families (i.e., during normal work hours) and/or religious constraints (e.g., outside of prayer time for Muslims); and 7) Ensuring alcohol/meat free options are available at team events. These are just some concrete examples of the EDI context on the team, and we are always working on ways to diversify our teams and spread the importance of EDI in STEM.

Team Composition and Recruitment Process

Provide the best practices implemented

Awareness is key, and our team is well aware of GBA+ how unconscious bias can negatively impact hiring. All team members will be required to take at least two EDI-related courses that I will recommend, and proof of taking the courses must be provided

Provide the relevance, approach and expected impact

Equity, Diversity, & Inclusion (EDI) has been shown to improve team performance in corporations, and labs. A 2010 study in Science showed the "collective intelligence" of diverse teams is greater than the "sum of the intelligence" of the individuals (i.e., diverse teams exhibit synergy). Despite EDI benefits, a gender/ethnicity disparity exists in STEM. For gender, the department of chemistry at the University of Alberta has 14% of our faculty that identify as female, while our graduate program has 40% identifying as female. Our department's hiring practices have changed significantly in recent years to attempt to improve the situation, but it is a process that will take many years to rectify as senior professor's retire, and new faculty are brought in. In contrast, graduates from our team's respective groups are closer to gender parity, e.g., ~50% M.Sc. and Ph.D. group graduates identify as female; 26% of current group HQP identify as female. We actively take steps to increase group diversity. When "hiring" HQP we keep in mind that unconscious/explicit bias exists, recognize biases, and actively identify ways to mitigate bias. In an online course by the Govt. of Canada's Canada Research Chairs program, we learned techniques to mitigate bias, e.g., stereotype replacement and perspective taking (we learned other techniques in an online Gender-based Analysis Plus (GBA+) course). To promote this thinking (and raise awareness) in our group, we will require all of our team HQP (regardless of if they are part of this project or not) to take the two online courses above.

Training and Development Opportunities

Provide the best practices implemented

Team members will receive EDI training through the Canadian Centre for Diversity and Inclusion (CCDI), which offers both online as well as in person training opportunities. Equitable access to present research at conferences will be ensured.

Provide the relevance, approach and expected impact

Our groups have strong traditions in educating future leaders (numerous past highly qualified personnel (HQP) now hold a faculty position themselves, and other HQP have been hired to industry positions with hiring decisions), so having all team members trained in EDI will have a positive snowballing impact on future career and research opportunities for underrepresented groups. A policy will be developed to identify EDI training needs, for example through having team members identify knowledge and awareness gaps, through unconscious bias tests etc. Current group members are from underrepresented groups (e.g., visible minorities, from diverse cultural and religious backgrounds), and their past and present experiences in academia in general, and our groups in particular would be valuable in assessing EDI training needs, should they be interested to share. Other resources are peer-reviewed publications and data from the University of Alberta's HR Department. The University of Alberta regularly offers training in EDI matters, and has furthermore formed an institutional partnership with the CCDI, which offers various training methodologies. Weekly group meetings already contain a regular topic discussion on safety measures, and we will include regular EDI discussions as a post-training recap and regular feature to avoid the EDI training being perceived as a "tick-the-box" exercise only.
Due to grant support we have been able to allow our group's students equal and ample opportunity to attend conferences, even if they did not present their research. This may not always be possible in the future, though, so that we will implement a database that will capture all career opportunities (conference presentations, networking opportunities and mentoring opportunities) for all students through their education stages once they become members of our groups. This will allow us to identify gaps in opportunities, and will allow us to focus grant support on students who have had fewer opportunities.

Inclusion

Provide the best practices implemented

We will increase the accommodations for team members for taking part in family, religious and cultural practises, and will designate an EDI champion in our team.

Provide the relevance, approach and expected impact

In the past, we have always been generous in accommodating our team members' obligations such as attendance at religious and cultural obligations during working hours, granting leave for taking care of family obligations (such as acting as translators for family members in various errands, attending children's functions etc), but this was on an ad-hoc basis that might not have been taken advantage of by all team members equally. We will therefore establish a formal policy outlining the process and the rights and obligations of all team members, and will ensure that all team members are aware of this policy. Part of this policy will outline the options team members have for taking days off for their various obligations and ways and means to keep this fair and equitable (e.g. by working during typically Canadian/Christian holidays, taking time in lieu etc). Another part of the policy will deal with other available resources, such as prayer rooms etc. In the past, a religious member of our team made informal use of a rarely used meeting room for prayers during working hours, and this could be formalized by booking the room formally for such purposes. This policy will be drafted by our newly designated EDI champion. As discussed in the section about Training and Development opportunities, the University of Alberta offers good opportunities to train individuals and groups in EDI issues, and our EDI champion, after having undergone the training themselves, will be assisting with policy drafting, ensuring that EDI related matters are a regular part of our team meetings, and that all team members understand the importance of EDI.

Eligibility Profile for Michael Serpe - Nominated Principal Investigator

Are you currently a nominated principal investigator, co-principal investigator or co-applicant on a **separate application** or an **active grant** for New Frontiers in Research Fund - Exploration?
No

Independent Research Appointment

As of 31/03/2020, will you be an independent researcher at your primary affiliation as defined by the New Frontiers in Research Fund - Exploration?
Yes

Primary Affiliation

Primary Affiliation

University of Alberta - Professor

Early Career Researcher (ECR)

Are you an early career researcher as defined by the New Frontiers in Research Fund?
No

Eligibility Profile for Ralf Schirrmacher - Co-Principal Investigator

Are you currently a nominated principal investigator, co-principal investigator or co-applicant on a **separate application** or an **active grant** for New Frontiers in Research Fund - Exploration?
No

Independent Research Appointment

As of 31/03/2020, will you be an independent researcher as defined by the New Frontiers in Research Fund - Exploration?

Yes

Affiliation

As of 31/03/2020, will your independent research appointment be with a federal, provincial, territorial or municipal government department or a for-profit organization?

No

Choose the affiliation associated with your eligible independent research appointment
University of Alberta - Professor

Early Career Researcher (ECR)

Are you an early career researcher as defined by the New Frontiers in Research Fund?
No

Supporting Documents

Document Name	Stage	Status
Description of the proposed research project (maximum 1 page)	Letter of Intent	Attached
Explanation of high risk (maximum 1 page)	Letter of Intent	Attached
Potential for high reward (maximum 1 page)	Letter of Intent	Attached
Interdisciplinarity / Fit to program (maximum 1 page)	Letter of Intent	Attached
Literature references - LOI (maximum 1 page)	Letter of Intent	Attached
Biographical information about the research team (maximum 3 pages)	Application	Attached
Research proposal (maximum 4 pages)	Application	Attached
Budget justification (maximum 1 page)	Application	Attached
Literature references - Application (maximum 3 pages)	Application	Attached

Brief synopsis. Our multidisciplinary team aims to develop a unique nanomaterial that will yield positron emission tomography (PET) images with unmatched resolution combined with the ability to be triggered to kill cancer cells with the aim of reduce the size of tumours and ultimate tumour disintegration. This will be accomplished by making the nanomaterial triggerable via an external stimulus that can allow the release of therapeutic small molecules to targeted sites, and/or via hyperthermia treatment from external heating of the nanomaterial. PET is one of the most sophisticated in vivo imaging modalities available that uses radioactive isotopes that decay by positron emission(1). PET is non-invasive, painless, and hence patient friendly; provides full-depth images of the human body featuring dynamic space and time resolution of injected radio-labeled diagnostics (e.g. for cancer and neuro-imaging); and offers unprecedented detection sensitivity(2). While this is the case, the resolution of PET needs to be improved to spatially resolve small metastases for improved cancer diagnoses(3). If this project is successful, it will greatly improve our ability to diagnose cancer and treatment. Ultimately, this will allow PET to achieve what no other single technique is capable of. **Research Goal**. PET's resolution is determined by the path length of the traveling positron in various tissues and in turn is dependent on its initial positron energy. The higher the positron's energy, the longer its travel path, which decreases image resolution. Here, we aim to enhance the resolution by incorporating various PET imaging radio-nuclides (18F, 68Ga, 64Cu, etc) and metal-based nanoparticles of variable sizes into the structure of hydrogel nanoparticles and microparticles (called nanogels and microgels, respectively), which will bring all species into close confinement. In brief, nano/microgels are highly porous, water-swollen polymer networks that have been shown to act as reservoirs for containing small molecules (e.g., chemotherapeutic agents(4)) and nanomaterials (e.g., metal nanoparticles(5)). Our strategy will consequently enhance the resolution of peptide and protein-based PET imaging agents conjugated to the nano/microgels by reducing the path length of the traveling positron through improved attenuation of the positron's kinetic energy in the metal nanoparticle environment. The attenuation of the positron occurs in soft body tissue leading to a travel length of a few millimeters for the most common PET isotopes, and thus results in a blurred PET image for isotopes that have higher positron energies. However, in a metal-rich environment, formed by the metal-nanomaterial within the micro/nanogel structure proposed here, we hypothesize that the positron attenuation will be significantly potentiated by stronger total inelastic and multiple Coulomb elastic scattering. Importantly, using the ability of the PET imaging agent-modified nano/microgels to target specific regions of the body (e.g., tumour sites) we can achieve enhanced image resolution with the ability to kill cancer cells in tumours. This will be done by exploiting the ability of metal nanoparticles to heat up significantly when exposed to certain frequencies of electromagnetic radiation. Thus, the heat generated can be used as a form of hyperthermia treatment. The heat can also trigger the nano/microgels to release their small molecule payload(6). Ultimately, we aim to introduce a paradigm shift from common PET tracer applications to resolution enhanced metal nanoparticle attenuated PET (ReM-PET) obtaining a complex but highly tunable platform for novel PET tracer development based on optimized resolution and nano/micromaterial enhanced accumulation (e.g. tumor targeting through the enhanced permeability and retention (EPR) effect and/or biomolecular targeting). This, combined with the ability to kill cancer cells/tumours makes our approach a gamechanger. **Challenges**. The challenge is to populate the inside of the nano/microgel evenly and densely with metal nanoparticles to ensure the full engulfment of the radio-isotopes for maximum positron attenuation. Furthermore, the gels' surface must be derivatized for bio-conjugation with peptides and proteins. A major challenge will be to incorporate the radio-isotopes into the gel cavity alongside with the metal nanoparticles. **Benefits**. This new generation of PET imaging agents has the potential to re-shape the landscape of modern PET diagnostic imaging, initiating a paradigm shift and creating new IP and revenue from clinical applications. It would significantly contribute to the applicability of more varied isotopes in PET imaging, allowing the use of longer-lived isotopes for slower physiological processes that have been hampered so far by less desirable decay characteristics of these isotopes.

The fields of nanoscience and medical imaging are very diverse, and significant advances in these areas require teams to come together with unique skills that are acquired through years of practical and research experience. As this project is about merging expertise in medicine, nanoscience, and materials science and engineering, there is not a fit within just NSERC or CIHR. Although, the advances that can be made by finding this project will allow completely novel research to be done, and ground-breaking advances made, which is what we believe to be the purpose of this program. Specifically, the introduction of enhanced metal nanoparticle attenuated PET (ReM-PET) capitalises on a completely new approach to physically improve the resolution of diagnostic PET imaging(10) while taking advantage of targeted delivery mechanisms based on the EPR effect(8) and/or peptide/protein targeting ligands(9, 10). The current paradigm in PET imaging is based on the assumption that the obtainable resolution of a PET camera is dictated by the used radionuclide. Using current radiotracers, nuclides such as 18F(11) with a low positron energy will always provide PET images of higher resolution and quality than radionuclides with a significantly higher energy such as 68Ga(12) or 64Cu(13). This belief is currently unchallenged. Our approach will radically change the performance of PET diagnostic imaging by unitizing the performance of PET isotopes through normalisation of traveling path length within the metal nanoparticle nano/microgel confined space. The traveling path length is causally connected to the positron energy of various nuclides and has a profound impact when looking at soft tissues. Our hypothesis is that the metal nanoparticle environment normalizes the traveling path length by strong inelastic and elastic scattering. All PET radionuclides integrated into our ReM-PET building blocks would basically display the same resolution in a PET image. However, this strongly depends on how well the metal nanoparticles surround the radionuclides within the nano/microgel confined environment. The development of easily applicable labeling and loading protocols will be a difficult task. To make these ReM-PET radio-tracers attractive for clinical transfer, the introduction of the nano/microgel content has to be one step and performed in a kit like manner. Past clinical translational work in PET imaging has demonstrated that only those PET imaging agents that can be synthesized by simple standardized procedures are successful in the clinical market. In addition to solving the problem of labeling/loading the nano/microgels, the outer surface of the nano/microgel has to be prepared for further bio-conjugation with targeting vectors. These targeting vectors are highly specific peptides and proteins binding to the surfaces of certain cancers or target inflammatory processes in the human body. Some of them are very insensitive to chemical modification but others are challenging to modify while retaining their high binding affinity to the target. When conjugated to a “large” nanogel/microgel structure, it is not unlikely that the targeting vector might lose its binding affinity to the targeting receptor. A vast amount of empirical work has to be allocated to address this problem and it is uncertain if some of the most prominent targets in PET clinical imaging can be successfully transferred to ReM-PET principle. This research program bears the potential to further our fundamental knowledge in PET diagnostic imaging, nano-material development and bio-conjugation by challenging the current resolution barrier of PET imaging while maintaining high target selectivity. Resolution enhancement will in turn reveal even the smallest metastases in cancer imaging significantly improving clinical diagnosis. PET resolution has not yet been considered to be alterable by chemical means and the combination of nano-material research aimed at changing the physical resolution of PET is unique and has to the best of our knowledge not been proposed so far. The development of a novel technique to realize a complex chemical entity such as radio-labeled metal nanoparticle-filled nano/microgels and their bio-conjugation to prominent targeting vectors will significantly change the landscape of modern PET imaging if successful.

Canada has been at the forefront of radiopharmaceutical research and radionuclide supply for decades. For many years Canada was one of the world leaders in the production of molybdenum-99(14) (^{99}Mo/^{99m}Tc generators for diagnostic single-photon emission computerized tomography (SPECT) imaging) and iodine-131 (for radiotherapy). It is in danger however of losing its dominant role as a result of the shutdown of the Chalk River radionuclide production facility. Moreover, PET imaging is becoming more popular and will eventually replace common SPECT tracers in many areas. Radiotherapy will finally play a leading role in cancer therapy, especially when more endo-radiotherapeutics become available through new platform developments. Our research proposal aims to supply the future needs of this constantly growing market by providing simple but efficient tools to create new radiodiagnostics and radiotheranostics (combining therapeutic and diagnostic radionuclides on the same platform) by improving the resolution of the PET images through our ReM-PET idea. The health benefits for Canadians (and worldwide) will be substantial since the whole project is aimed at improving the health care options of patients suffering from serious and common diseases such as cancer, neurodegenerative diseases, and other diseases detectable by or treatable with radiation. Improved PET image resolution naturally translates into a higher degree of disease detection precision. Through resolution enhancement, we will be able to more precisely pinpoint changes in diseases and significantly enhance diagnostic accuracy. A second reward is the increase of highly qualified personnel in nuclear- and radiopharmaceutical science. Canada itself is currently unable to satisfy the increasing demand for nuclear- and radiopharmaceutical chemists and has to attract highly qualified personnel from other countries. Despite a long and successful history in radiochemistry research, the current output of Canadian PhD students who obtain a degree in nuclear- and radiochemistry is insufficient to support the growing number of academic as well as industry job opportunities. During the phase of ReM-PET development, we will train PhD students, post-doctoral fellows as well as MSc students to prepare Canada's growing theranostic market for the future. This strategy will also ensure that all trainees make the important connection between the scientific aspects of the project and the commercialization opportunities originating from this research. The realization of the practical and commercial value of their research contribution will accelerate the data acquisition by focusing on a goal-oriented outcome. All major research facilities in Canada have started seminal and ground-breaking research into new PET diagnostics and the production of novel therapeutic radionuclides. The chemical platforms needed to leverage these programs have yet to be developed although great efforts are currently being made to prepare these new radionuclides and tracers for the market. Our proposed research project will also strengthen Canada's commercial footing in the global market through the development of a new and more sophisticated line of radionuclide-based products. This will naturally translate into an increased number of available positions and increased revenue. To reinstate Canada's traditional role as a world leader of novel radiopharmaceuticals and founder of new paradigm shifting discoveries, the field of radiopharmacy and clinical in vivo imaging and radio-therapy will be significantly advanced. If successful, the establishment of ReM-PET will spark new research directions and foster clinical improvements in modern precision medicine.

As highlighted extensively in the above sections, we believe the proposed development of ReM-PET perfectly fits the program's mandate of pushing the boundaries of existing technology, possibly initiating a paradigm shift from current PET imaging technology and associated theranostic applications of nanomaterial-based PET/theranostics towards an unprecedented level of accuracy and precision. The proposal combines knowledge and expertise from general chemistry, nanochemistry, radiochemistry, material science and in vivo imaging and is aimed at reaching the following main goals (1) the development of new imaging technology through resolution enhancement of currently available PET cameras, (2) introduction of a radionuclide-based platform for peptide and protein-based PET probes, and (3) the application of new combinatorial nano-materials. These three trendsetting scientific pillars provide an extraordinary basis for new developments by combining advancements in basic science to the benefit of medical methodology implementation. Only interdisciplinary connectivity will lead to a constructive academic as well as commercially viable outcome of the proposed research. The imaging group has a long-standing track record in tracer development for PET imaging and is internationally recognized as being among the leaders in the field. The team recently introduced new technology into the clinic that shows the commitment to bench-to-bedside translational research. The team's trendsetting non-canonical 18F-radiochemistry has now entered diagnostic cancer imaging. Thirteen cancer patients were imaged with a novel 18F-labeled peptide surpassing the quality of the current 68Ga-gold standard for imaging. The other team has a long history of developing polymer-based materials and nanomaterials, for example, for immobilizing reactive groups and nanomaterials in nanogels and microgels. All the research in their group is done for myriad applications, and this project is no exception.

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Background and Expertise: The research team assembled here is uniquely in a position to capitalize on our joint expertise in polymer/nanogel synthesis and nuclear chemistry/radiochemistry for medical imaging applications. The PI and co-PI have >40 years of combined experience in our respective areas, with our research teams well-armed with the knowledge to make this project a success by allowing all team members to work together in a collaborative setting focused on problem solving. Both research teams have the tools and group knowledge/expertise in place to get research results as soon as funding becomes available. Specifically, the PI's group has a deep knowledge of nanogel and metal nanoparticle synthesis/characterization and modification of their chemistry to give the desired materials. The group is equipped with lab equipment and hood space to begin synthesis of the nanogels right away. We have the tools, expertise, and knowledge in place to characterize the synthesized nanogels, metal nanoparticles, and the hybrid materials that we will generate from them. For example, the lab has a scanning electron microscope (SEM), atomic force microscope (AFM), differential interference contrast (DIC) and fluorescence microscope, and access to high resolution SEM and transmission electron microscopy (TEM), dynamic light scattering (DLS), and Fourier-transform infrared spectroscopy (FTIR) that are more than enough to characterize the materials. We also have the synthetic organic chemistry knowledge and characterization tools to generate nanogels with myriad chemistries that will be used for radioisotope labelling (e.g., nuclear magnetic resonance spectroscopy (NMR) and various types of liquid chromatography-mass spectrometry (LC-MS)). Once the nanogels are generated, they will be given to highly qualified personnel (HQP) in the co-PI's lab for radioisotope labelling and assessment of their ability to improve PET imaging resolution.

The principle co-PI will be responsible for all radiolabeling procedures, the phantom measurements and the images generation and interpretation. Necessary knowledge such as radionuclide production, radionuclide workup, radiolabeling and quality-control of radio-labeled materials are the co-PI's main expertise. Radiation safety and student education are traditionally well executed in the co-PIs lab to ensure a safe work environment for students and HQP. Furthermore, the co-PI is an internationally recognized leader in the field of ^{18}F labeling and the development of PET imaging probes for brain- and cancer imaging and therefore well capable of handling a research program of high complexity including various radionuclides. After the principle investigator synthesized the nano-materials, the principle co-PI will receive the material and start the incorporation of the radio-isotopes. The quality control and labeling efficiency will be performed at the co-PI's radiochemistry labs at one of the most modern and well- equipped radiochemistry facilities in Canada. Instrumentation such as a cyclotron for radionuclide production, lead shielded hot cells for performing the labeling as well as radio-HPLC for quality control are at the co-PIs disposal. After the successful validation of the synthetic procedures, the labeled materials will be placed into various phantoms and evaluated as to their improved image resolution in comparison to non-conjugated radio-isotopes.

PI's Training Environment: I am devoted to ensuring that HQP leave the group well-rounded scientist and members of society armed with a deep knowledge of materials science/chemistry and appreciation of how these skill can be used to solve some of society's problems. I regularly discuss career/life goals with HQP, providing advice as needed. I provide HQP every opportunity (time/funds) to pursue training opportunities. I believe (and let HQP know) that individual HQP successes, and diverse training experiences, bolsters HQP excellence in the group. I believe this yields high quality research/publications, improved funding, and a dynamic learning and training atmosphere; this all results in more career opportunities and improved HQP satisfaction.

Our research environment will offer HQP associated with this project training in materials synthesis and characterization in world class facilities. HQP will learn presentation skills, e.g., in weekly meetings 1-2 HQP present their research (and recent literature), where we rigorously

question the presenter to identify any issues and new directions; fact-based, respectful/collegial discussion is emphasized. I frequently have 1:1 meetings with HQP to discuss research progress. I use all aspects of research to teach HQP, e.g., with manuscript writing. In my group, manuscript writing is an iterative process -- HQP provide drafts, and I provide feedback until we submit the manuscript. I explain the submission process, share reviewer comments, craft responses with HQP, and explain the resubmission.

In summary, I will continue to build an environment where scientific curiosities can be explored and open thought and communication encouraged. This is my contribution to developing highly skilled and responsible HQP to feed into the industrial/academic/govt. work force. This is an environment that HQP associated with this project will benefit from.

Co-PI's Training Environment: As a nuclear chemist and radiochemist the principal co-applicant feels a strong obligation towards research, teaching and education of under-graduate as well as graduate students and postdoctoral fellows in the field of applied radiochemistry. During the co-PIs career, the co-PI was strongly involved in teaching nuclear and radiochemistry had the opportunity to supervise MSc theses as well as PhD theses. Teaching has always been more of a privilege than a duty for him. Having the opportunity to help a future generation develop scientific thinking and reasoning has made the co-PI realize how influential a good tutor can be for the lives and careers of his students, and for the developments in science. Radiochemists are **extremely rare** and the co-PI considers it of utmost importance to provide interested students with a comprehensive knowledge of radio-chemistry, radiopharmacy and molecular imaging using Positron Emission Tomography (PET). Many students are deterred at first when they hear that the co-PIs discipline actually involves working with radioactive isotopes and it was always important to lower the level of anxiety by clearly describing the practical aspects of radiation protection, a necessity in radiochemistry which is of undisputable importance. The well-being of students always was and always has to be a priority. The handling of hazardous materials such as radioactive isotopes requires certain basic skills such as being responsible, planning ahead, showing determination and acting reliably. Learning these important requirements contributes to the overall development of students. These skills cannot be acquired in the radiation safety courses that are offered by various institutions, they have to be learned through practical hands-on experience. This helps students to assess risks correctly, plan techniques and put them to the test and learn from their first-hand experiences. Overall, it encourages an analytical process development.

The co-PIs ultimate goal is not only to provide students with a sound knowledge in radiochemistry and in vivo imaging with PET, but also to enable them to critically reflect scientific publications and discover their strengths and limitations. The interdisciplinary aspect of the co-PIs research topic encourages students to broaden their knowledge base of related sciences and helps them see the necessity of in-depth study of pure and applied sciences. The co-PI will continue to use all available experience over the last 20 years to provide students with a top education, in depth knowledge and hands on experience in radiochemistry, radiopharmacology and related fields of research.

Introduction: Positron emission tomography (PET) is a non-invasive, painless, and hence patient friendly imaging modality that provides full-depth images of the human body with unprecedented dynamic space and time resolution[1]. PET has revolutionized medical imaging and diagnostics, which has led to dramatically improved health outcomes for those with cancer, neurological, and other diseases[2]. PET imaging utilizes radiotracers (i.e., positron emitting radioisotopes like ^{11}C, ^{18}F and ^{124}I or radiometals such as ^{68}Ga and ^{64}Cu attached to bioactive compounds) that bind to specific biological receptors/enzymes on structures that need to be imaged, and/or form an integral part of various metabolic pathways[3-5]. As a result of the localization of these radiotracers in very close proximity (or attached to) "regions-of-interest", images can be collected and interpreted to diagnose and monitor disease states. Images are generated by a series of detectors in a series of rings surrounding a specimen (e.g., a human body) receiving a signal when two annihilation gamma rays (energy of 511 keV) strike two detectors at the same time; the two gamma rays are a product of a positron emitted from a radiotracer annihilating with an electron of the surrounding media. While PET is a revolutionary tool, the positron can travel a few millimeters away from its point of origin which limits the resolution of the technique (illustrated schematically in **Figure 1(a)**). To illustrate the underlying concept, positrons stemming from ^{18}F or ^{86}Y have an E_{max} (maximum kinetic positron energy) of 635 keV and 3.1 MeV, respectively, and exhibit mean traveling ranges within organic tissue (e.g., the human body) of ca. 0.6 and 7 mm, respectively. PET images obtained with ^{86}Y are blurry compared to images using ^{18}F, and much harder to interpret compared to other radionuclides with lower positron emission energies, which can lead to the image analysis expert (i.e., nuclear medicine expert) missing small features (e.g., cancer metastases) and ultimately misdiagnosing. On the other hand, ^{18}F has a relatively short half-life of 110 min, which is incompatible with biological processes that require long radiotracer residence times in the blood compartment and slow receptor binding kinetics (e.g. labeled full antibodies) whereas ^{86}Y has a much more appropriate half-life to image longer biological processes. Therefore, if the PET imaging resolution of an ^{86}Y labeled radiotracer could be enhanced by physicochemical attenuation of the high energy positrons, the resulting PET images would be less blurry and biological processes requiring longer imaging protocols could be investigated with high image resolution/accuracy matching (or even surpassing) the image quality of ^{18}F labeled radiotracers.

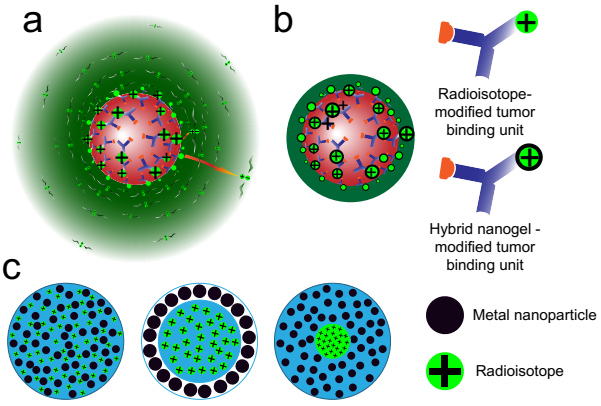

Fig. 1. (a) In "normal" PET imaging, a radioisotope-modified binding unit specifically binds to a "region-of-interest" to be imaged (central red sphere). Images are generated when positrons annihilate with an electron in the surrounding medium generating two gamma rays that are detected and images constructed from those events. The further the positron travels from the imaging region, the lower the resolution of the image. This is illustrated in the green gradient around the imaging area. Many positrons are annihilated near the imaging area (dense green), although many travel far away from the imaging region to decrease resolution (lighter green). (b) Nanogels with (c) various compositions/distribution of radioisotope and metal nanoparticles will be used to decrease (or eliminate) the distance the positron travels before generating gamma rays (illustrated as dense green region around the imaging area), thus dramatically increasing PET image resolution.

In this proposal, we aim to dramatically improve the resolution of PET imaging by combining 10-20 nm diameter metal nanoparticles and radioisotopes in 200-300 nm diameter hydrogel polymer particles (nanogels) that can be modified with biomolecular targeting agents that will allow the radioisotopes to be localized on the feature to be imaged (exactly as in normal PET imaging). However, in this case, we hypothesize that the electron-rich metal nanoparticles present in the nanogel matrix, in close proximity of the radioisotopes, will serve to dramatically limit the distance that the positron can travel from the "region-of-interest" before their annihilation, dramatically improving the image resolution. This concept is illustrated in **Figure 1(b)**; we hypothesize that these materials will allow images acquired using ^{68}Ga (Emax=1.92 MeV) to be similar or better in resolution compared to ^{18}F (low Emax=0.635 MeV). Ultimately, if these hybrid, multicomponent nanomaterials can be generated, and they behave as predicted, they will allow never before seen PET image resolution to be achieved. The improved resolution will allow increasingly small features to be imaged, e.g., metastatic cancer sites,

which will dramatically improve health outcomes for patients. Metastatic cancer is a result of cancer cells sloughing off from a primary tumor and circulating in the body until they get "lodged" at a specific site where another tumor can grow, and serve as a nucleus for further metastasis. It is estimated that metastasis is responsible for 90% of all cancer-related deaths[6]. Therefore, if metastasis can be detected early, localized treatment can be initiated to treat and destroy the metastatic site, preventing further metastasis and dramatically improving cancer treatment and follow-up diagnostic outcomes. Importantly, the nanomaterials that we propose have the very interesting property of generating heat when excited with electromagnetic radiation of specific frequencies (e.g., radiofrequencies),[7] and we will assess the ability of these particles to heat up sufficiently to kill cancer cells via photothermal heating. The concept of heating cancer cells to kill them is a promising approach to cancer treatment, and is referred to as hyperthermic treatment.[8] Furthermore, we will utilize the heat to trigger the release of chemotherapeutic agents to tumor sites as well (see below).[9, 10] The proposed research is multidisciplinary, and incorporates concepts from many different fields (medicine/medical imaging, radiochemistry, nanotechnology, polymer chemistry, biochemistry), to yield a technology that can allow PET imaging to be used to its full capacity. Due to the high risk of the project, and the lack of preliminary data to support our claims here, this research is impossible to get funded by NSERC and CIHR independently. Therefore, this opportunity is an idea catalyst to develop this promising technology.

Project Summary: The ultimate goal of this project is to develop 200-300 nm diameter polymer-based nanogels infused with 10-20 nm diameter metal nanoparticles, and radioisotopes (^{18}F and ^{68}Ga initially) that will be capable of achieving the maximum PET image resolution. As mentioned above, this paradigm shifting new imaging technology will be made possible because the electron-rich metal nanoparticles will allow positron annihilation closer to the region-of-interest (ideally without traveling any distance), thus allowing higher resolution images to be acquired regardless of the radioisotope used[11]; we call the technique **R**esolution **e**nhanced **M**etal nanoparticle attenuated PET (ReM-PET). We hypothesize that this will be possible based on previous results that demonstrated positron annihilation via their interaction with conduction electrons in metals[12, 13]. Specifically, when an energetic positron (e.g. from ^{68}Ga or ^{86}Y) is introduced into a metal lattice, the positron is annihilated via thermalization within a few picoseconds by a succession of ionizing collisions, plasmon and electron-hole excitations, and phonon interactions (among other possibilities). Hence, our ReM-PET building blocks will serve as radio-imaging tags for peptide and protein-based PET imaging agents providing unprecedented image quality by annulling the current physical limitations of PET imaging due to unwanted positron traveling within biological tissue.

Research goal (short-term): We propose to significantly improve the resolution of PET by placing various PET imaging radio-nuclides (starting with ^{18}F, ^{68}Ga, and continuing to ^{64}Cu and other nuclides) and metal-based nanoparticles (Ag, Au, Cu, and Fe) of various sizes and shapes into the structurally confined space of nanogels, which will bring all components into close proximity (see **Figure 1(c)**), effectively reducing the positron travel distance from the region-of-interest, and improving PET image resolution. We will generate highly porous, water-swollen, and biocompatible nanogels that have previously been shown to act as "reservoirs" for containing small molecules (e.g., chemotherapeutic agents)[9, 10, 14, 15] and nanomaterials (e.g., metal nanoparticles)[16-21] for the purpose of bringing radionuclides and metals close to one another. As a proof of concept, we will initially focus on incorporating two radioisotopes into the nanogels that are available at our institution and significantly differ in their positron energies, ^{68}Ga (E_{max} 1.92 MeV) and ^{18}F (E_{max} 0.635 MeV). It has been shown conclusively that the radioisotope with the lower positron energy results in PET images of superior quality and resolution[22]. For example, ^{18}F labeled peptides in comparison to their ^{68}Ga counterparts yield improved images of small metastasis[22, 23]. Either the unbound radioisotope or the radioisotope incorporated into nanogels composed of metal nanoparticles will be placed in uniform cylindrical phantoms[24], which are routinely used to normalize and calibrate PET scanners and to qualify scanners for quantitative imaging in a human clinical setting. Additionally, phantom scans can improve a PET-CT QC program in monitoring calibrations over time, identifying artifacts, and investigating resolution problems stemming from the nature of the radioisotope used. We will use a commercially available PET-CT phantom (available at our institution) that complies to the NEMA2012 standard for determining the difference in image resolution using the "free" radioisotope and the radioisotope confined in the metal nanoparticle-loaded nanogels in water and fluids matching various human body composition for CT

attenuation correction (e.g. lung and bone). This phantom is capable of simulating whole human body imaging using PET and camera-based coincidence imaging techniques. The phantom possesses six fillable spheres of varying diameter (10, 17, 22, 28, 37 mm respectively) enabling us to determine the coincidence count rate characteristics in different imaging scenarios such as brain and cardiac imaging, the relationship between true coincidences count rate and used radioactivity, determination of address errors caused by address pile up and the count loss correction scheme. Alternatively, we propose to use a 1 mm capillary tube, a Jaszczak phantom or a Derenzo phantom (all available at our institution) especially suitable for a high resolution animal PET scanner, which is also available at our institution. This will enable us to qualitatively and quantitatively evaluate ReM PET vs conventional PET and determine if our materials can indeed improve PET image resolution. **Research goal (long-term)**: After we successfully validate that ReM PET leads to enhanced PET image resolution using the above phantoms, we will bioconjugate tumor homing peptides to the surface of the nanogels (e.g. tyrosine-3-octreotate (TATE), bombesine or RGD peptides) and proteins (e.g. antibodies such as Ibritumomab tiuxetan (Zevalin) targeting the CD20 antigen in Non-Hodgkin Lymphoma). We will use a variety of bioconjugation strategies which have been proven successful in the past and validated by others[25]. For example, carbodiimide coupling is a convenient route to couple amine groups of biomolecules to carboxylic acids on nanogels.[26] These bioactive compounds are supposed to act as targeting vectors and transport vehicles for the nanogels to the tumor/metastasis site. In addition to the image enhancement due to the ReM PET principle, the gels will be loaded with cancer therapeutics (e.g., doxorubicin),[10] which can be released upon application of an external stimulus such as electromagnetic radiation. [7] This will not only heat the metal particles and induce hyperthermic treatment but also lead to the liberation of the therapeutic payload (chemotherapeutics). These bioconjugated nanogels will become true theranostics combining resolution enhanced cancer imaging with an improved therapeutic index. In addition to active targeting via biological vectors, we will exploit the enhanced permeability and retention (EPR) effect for tumor accumulation. This concept in addition to active targeting specific surface epitopes in cancer has the potential to become a valuable tool in tumor theranostics. **Experimental Plan (short term goals)**: We will introduce various metal nanoparticles into the nanogels to evaluate their attenuation efficiency for the emitted positrons present within the gel. The positron stopping power for individual metals is known[27]. However, in our opinion, this is not the only factor influencing an efficient annihilation within the metal cluster confinement of the nanogel. The size and the shape of the metal clusters will most likely influence their packing density and therefore directly alter the probability of positron-metal annihilation. Specifically, we will generate poly (N-isopropylacrylamide) (pNIPAm)-based nanogels with diameters of 200-300 nm using free-radical precipitation polymerization, as previously reported.[28, 29] We will then load the nanogel structures with ^{18}F and ^{68}Ga, as these are available at our institution and will allow us to confirm that the concept works as ^{18}F and ^{68}Ga have very different positron energies. In theory, ReM-PET will yield the same resolution for both radionuclides after incorporation. We will attempt numerous approaches to load these radioisotopes into the nanogels, e.g., we will attempt to load ^{18}F into the nanogels using weak Van der Waals interactions between the highly electronegative F and polarizable atoms in the monomer units that make up the nanogels. We will also attempt to utilize the interaction of F^- with positively charged monomers that can be incorporated into the nanogels (e.g., N-(3-Aminopropyl)methacrylamide hydrochloride). Finally, we will explore generating nanogels composed of propargyl acrylate, which contains an alkyne group that can be “clicked” (i.e., chemically attached) to azo-modified silicon-fluoride acceptor (SiFA) moieties[3, 22, 30], to yield ^{18}F-loaded nanogels. We will investigate the use of 1,4,7-Triazacyclononane-N,N’, N”-triacetic acid (NOTA) to incorporate ^{68}Ga into the nanogels; NOTA has been shown to very effectively bind Ga.[31] To accomplish this, we will synthesize 1-(1-Carboxy-3-carbo-tert-butoxypropyl)-4,7-(carbotert-butoxymethyl)-1,4,7-triazacyclononane ($NODAGA(tBu)_3$) following previously published protocols.[31] The $NODAGA(tBu)_3$ has a free carboxylic acid group that can be coupled to amine-modified nanogels (e.g., nanogels composed of N-(3-Aminopropyl)methacrylamide hydrochloride) via carbodiimide-based coupling chemistry.[26] Upon successful incorporation of $NODAGA(tBu)_3$ into the microgel (confirmed by infrared spectroscopy), the tBu groups will be removed utilizing deprotection chemistry to generate the carboxylic acid groups of NOTA.[32] In either case, the ability of the nanogels to load ^{18}F and ^{68}Ga will be done using radioactivity measurement of the isolated materials. We will also quantify the nanogel diameter and morphology

before and after radioisotope loading (after the isotopes decayed) to confirm that the nanogels remain spherical, and do not undergo any unforeseen changes. Next, we will load various metal nanoparticles into (and potentially around) the resultant nanogel utilizing previously reported protocols,[17, 33-36])[16, 18-21] see **Figure 1(c)** for various configurations we will explore. For instance, it is well known that Ag and Au nanoparticles (NPs) can be generated inside nanogels using in-situ metal ion reduction.[17, 37] This approach will be attempted for generating Cu and Fe nanoparticles as well. It has also been shown that Au (and other metal) NPs can be generated with various shapes, which we will also investigate here.[38-40] For example, Murphy and coworkers have shown that Au nanorods can be generated by exploiting the use of surfactants to guide the growth.[39] Furthermore, Halas and coworkers have shown that metal nanoshells (e.g., Au and Cu) can be generated on nanoparticles.[33-35] Regardless, of how we do this, we will assess the metal nanoparticle distribution in the polymer nanogels to allow for structure-function relationships to be made. We will vary this concentration of metal nanoparticles in the nanogels, and assess the impact it has on function. After the successful preparation of the metal nanoparticle and radioisotope (^{18}F and ^{68}Ga)-loaded nanogels, they will be filled into a phantom (PET/CT phantom Biodex and Jaszczak phantom for the human scanner, 1 mm capillary tube and Derenzo phantom for the animal PET scanner) to investigate the concept of ReM PET using our available PET systems (human scanner and animal PET camera). Multiple performance characteristics of camera-based PET systems can be evaluated from a single scan of the phantom. We will investigate the influence of ReM PET materials on spatial resolution, scatter compensation, volume sensitivity and lesion detectability in direct comparison to the unaltered radionuclides dissolved in water or other solvents mimicking specific body compartments (e.g., lungs). We anticipate that the difference in scanner resolution will be especially prominent in the case of ^{68}Ga, which has a much higher positron energy than ^{18}F. Performance assessment will be done as previously described[41]. The phantom PET measurements will follow this general scheme: 1. Fill phantom with ~100MBq of Ga-68; 2. Place phantom in center of scanner's field-of-view; 3. Acquire scan for attenuation correction (CT); 4. Acquire PET scan for 30 min (three bed-positions (10 min per bed-position)); 5. Apply scatter and attenuation correction to raw data; 6. Reconstruct with filtered back-projection (NEMA Standard) and with preferred clinical protocol (OSEM with resolution recovery and time-of-flight). Both radionuclides ^{18}F and ^{68}Ga are available at our institution; ^{18}F can be produced on a daily basis by one of our two available cyclotrons and ^{68}Ga can be obtained anytime from our stationary ^{68}Ge/^{68}Ga generator system. **Experimental Plan (long term goals)**: After establishing the ReM PET platform, the surface of the metal nanoparticle and radioisotope (^{18}F and ^{68}Ga)-loaded nanogels will be modified with chemical linkers that provide "clickable" end groups for further external bioconjugation of biologically active peptides and proteins.[42] Especially tumor homing peptides such as TATE, bombesin, RGD and monoclonal antibodies lend themselves preferably to this intent. Bioconjugation techniques for this purpose are well established and will be evaluated as to their eligibility to surface modify the gels with targeting vectors[43, 44]. To further increase the theranostic efficiency of the ReM PET platform, we will load the inside of the gels with chemotherapeutics, as mentioned above, and evaluate the ability of the nanogel assemblies to release small molecules in response to electromagnetically-induced heating of the nanogels. Specifically, doxorubicin absorbs light in the visible wavelength range, and therefore we will utilize UV-vis to quantify the release behavior from the nanogels. **Major project milestones are detailed in the Activity Timeline.**

Activity Timeline	2 Year Project Term, each block is 6 months			
Synthesis and characterization of nanogels and metal nanoparticles	■			
Synthesize NOTA and incorporate into nanogels		■		
Load nanogels with radioisotopes and metal nanoparticles	■	■		
Phantom imaging to compare ^{18}F and ^{68}Ga ReM PET images to traditional images in water or other media	■	■		
Synthesis of clickable peptides and antibodies for bioconjugation to nanogel surface		■	■	
Bioconjugate peptides/antibodies to nanogel surface and load with radioisotope and metal nanoparticles		■	■	
Commencing phantom measurements of ReM PET bioconjugates			■	
Investigate chemotherapeutic potential of nanogels, i.e., doxorubicin release and hyperthermic treatment.				■

Total Requested Budget: $250,000

Personnel: (2 year total budget is $118,000) We will recruit 2 Ph.D. students for this project. One of the students will primarily work in the Serpe Group, while the other will primarily work in the Schirrmacher Group; although the two students will work very closely together to generate the proposed materials and performed the described imaging experiments. The Ph.D. student primarily in the Serpe Group will work on generating nanogels, and the radioisotope/metal nanoparticle-loaded nanogels. The Ph.D. student primarily in the Schirrmacher group will work with the Serpe Group Ph.D. student to develop the radioisotope chemistry for attachment to the nanogels, and be primarily responsible for the phantom PET studies and interfacing with the PET personnel who will perform the PET scans imaging experiments and image analysis details. Salaries for graduate students in the Department of Chemistry are $26k/year on average (salaries vary from year-to-year), therefore we request $52k/year for the two years for a **total of $104k** to pay salaries for these students to work on this project as research assistants. Additionally, we would like to train 2 undergraduate students on this project (1 each summer); we will pay each of these students $7k for their summer research, for a **total of $14k**.

User Fees: (**2 year total budget is $32,000)**Purchasing scanning time at the human PET/CT scanner: (200 CAD per hour), it has been estimated that this research will require approximately 20h of scanning time per year, for a **total of $4k/year**.

Purchasing scanning time at the animal PET/CT scanner: ($150 CAD per hour), it has been estimated that this research will require approximately 20h of scanning time per year, for a **total of $4k/year**.

To characterize the nanogels and metal nanoparticles that we generate, we will require the use of scanning and transmission electron microscopy (SEM/TEM) for multiple hours each year. X-ray photoelectron spectroscopy (XPS), X-ray diffraction, nuclear magnetic resonance spectroscopy (NMR), and mass spectrometry (MS) will also be needed to characterize the nanomaterials, and the molecular precursors that will be used to generate the nanogels. The TEM/SEM, and X-ray facilities charge $50/h, and I anticipate that we will need 100h/year combined SEM/TEM/X-ray time **($5k/year)**. In reference to MS and NMR costs, these tools are housed in the Department of Chemistry, which charges user fees for each of the services used, to a maximum of $3k/year for each service. Based on our department's low internal user fees to access this equipment, I anticipate that this project will require a total of $**3k/year** combined MS and NMR user fees.

Materials and supplies: (**2 year total budget is $50,000)**This project will require the purchase of radionuclides, various monomers for generating the nanogels, organic molecules for synthesizing the NOTA group that is able to be coupled to the nanogels for ^{68}Ga binding, metal ions/salts and metal ion reducing agents, proteins to couple to the nanogels for targeting applications, HPLC solvents, reagents for radio-synthesis, dry ice, liquid nitrogen, glassware, stir bars, gloves, pipets, pipet tips, needles, syringes, Petri dishes, etc. We expect that the materials and supplies costs will be **$25k/year.**

Indirect costs: $25k/year for a **total of $50,000**

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