October 24, 2018

Application Ref. IT12688

**RE: Mitacs Accelerate Proposal**

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|---|---|
| **Project Title:** | **Development and validation of BRET-based biosensors for drug candidate profiling** |
| **Internship Supervisor(s):** | **Michel Bouvier, Terry Hébert, Stéphane Laporte, Richard Leduc, Graciela Pineyro** |
| **Intern(s):** | **Nourhen Mnasr**<br>**Iness Charfi**<br>**Interns to be determined** |
| **Department:** | **Biochemistry, Pharmacology, Medicine, Pharmacology-Physiology, Psychiatry** |
| **Institution:** | **Université de Montréal**<br>**McGill University**<br>**Research Institute of the McGill University Health Centre**<br>**Université de Sherbrooke** |
| **Partner Organization:** | **Domain Therapeutics NA** |

Dear Dr. Bouvier, Dr. Hébert, Dr. Laporte, Dr. Leduc, Dr. Pineyro,

Your application to Mitacs Accelerate has successfully passed research review. Your **research project** has been approved for a total grant of **$419,999.90** that will be delivered through **eligible internships**. Your internship should not start prior to confirmation of eligibility as outlined below:

1. Mitacs must receive partner funds before each internship start date to pay the intern(s) on time;
2. Project leads must confirm intern name(s) and start date(s). No costs for an internship can be incurred prior to the research project approval and the receipt of partner funds for the internship;
3. To identify a new intern, the Intern Profile form (including CV and memorandum) must be submitted to Mitacs before the applicable internship's start date and obtain Mitacs approval

All awards are subject to ongoing program eligibility, continuation of funding by the partner organization(s), and continuation of funding by our government partners. Mitacs gratefully acknowledges the Government of Québec through the Ministère de l'Économie, de la Science et de l'Innovation (MESI) and the Government of Canada for their support of Mitacs Accelerate.

Welcome to Mitacs Accelerate. With this program, interns can apply their specialized expertise while companies gain a competitive advantage. We look forward to the success of your project.

Please feel free to contact Rebekah Thompson at rthompson@mitacs.ca or at 514-840-1235 ext. 4442 if you require further information.

Sincerely,

Olga Stachova
Chief Operating Officer


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Enclosures:

- Appendix A: Referee comments
- Appendix B: Terms and Conditions of the Award
- Attachment: New Intern Profile form (for newly identified interns or replacement interns)
- Attachment: Intern Onboarding Information

## Appendix A: Referee Comments

Your proposal has undergone external review. We received four review reports and the comments are in general very positive. The reviewers are supportive of your research rationale, objectives and methodological approach. The proposal presents a clear research question, an innovative idea that will benefit the research field and the partner organization and an excellent opportunity for the interns to acquire new skills and tackle an important industry-oriented research problem. You may wish to consider Reviewer 3 and Reviewer 4's comments and suggestions about the methods and future steps that may be helpful for your project (see Reviewer 3 -question #6 and Reviewer 4 - questions #4 & 6).

The comments are provided below for your information. We wish the applicants success in their research.

### Reviewer 1

1) Mitacs Accelerate supports research-based internships. Does the proposed project qualify as research in its discipline?

Yes. The proposed investigation is directed toward the development and deployment of BRET-based biosensors that report on events at different stages in signalling processes. They are intended for use by the applicants and the wider community in studies that address basic questions of receptor-mediated signalling, especially signalling via G protein-coupled receptors (GPCRs). The importance of the issues to be addressed is self-evident.

Questions of particular interest will include the tendency of the signalling profile exhibited by a ligand or group of ligands to be preserved from one cell-type to another, the behaviour of biosensors in different subcellular environments, the signalling profiles of ligands to specific receptors (e.g., angiotensin 1 receptor, cannabinoid 1 receptor) in specific cells (e.g., vascular smooth muscle cells, glutaminergic and gabaergic neuronal cells) and changes in the signalling profile that occur at different stages of cellular differentiation. Tests will be conducted in primary cells of various types and in induced pluripotent stem cells (iIPSCs); the sensors will be introduced by means of CRISPR-based editing of the genome, lentiviruses and adeno-related viruses. The proposed studies are based largely on the 70-odd sensors developed to date, but one of the projects involves a new departure in which a biosensor has been attached to a Transient Receptor Potential ion channel (i.e., TRP3).

The principal investigators are the same as those in an application which I reviewed in 2015. All have contributed to developments in relevant fields over many years, and the proposed studies are a continuation of that work. Those studies have contributed substantially to our understanding of GPCR-mediated signalling, particularly the

phenomenon of biased signalling and the related concept of a 'signalling profile': that is, the notion that a ligand elicits a mix of responses that depends or may depend upon the environment. They also have advanced and popularized the use of signalling technologies based on bioluminescence resonance energy transfer (BRET) and BRET-based sensors. The work therefore has been and will continue to be of interest both for its scientific implications and for its industrial and commercial applications. Commercialization of the biosensors is the stated aim of the industrial partner, Domain Therapeutics, NA.

2) Is the project appropriate for the academic degree level of the intern?

Yes, so far as can be ascertained. Two of the five interns are identified in the application (i.e., Nourhen Mnasri and Iness Charfi), and both have doctoral degrees. In one case the doctoral training was in the laboratory of one of the principal investigators, where the questions and techniques were essentially the same as those in the proposed investigation. In the other case, the training was in another laboratory at the University of Montréal, and the focus was on signalling via the epidermal growth factor receptor. The named interns therefore are highly qualified to undertake the work proposed in the application. Given the number of people associated with the principal investigators in Canada and abroad, it can be expected that similarly qualified interns will be identified and engaged for the three remaining positions.

3) Are the objectives clear?

Yes. Each of the five sub-themes has a specific aim that is to be tackled in a specific manner. Although there is much overlap in terms of the technology, especially BRET, and the overarching scientific questions, the individual projects are nonetheless distinct and could proceed independently.

4) Is the methodology appropriate to achieve the objectives?

Yes. The methodologies are the objectives in large measure, and the scientific objectives have evolved in large measure from the development and the application of the methodologies. The principal investigators have ready access to all of the facilities and techniques that are required and appropriate for the proposed studies. They also are better placed than most to deal with unforeseen complications and challenges.

5) Is the timeline realistic?

Yes. Based on the rather brief description of the project that is possible in the application, it seems unlikely that there will be major surprises. The various procedures are routine in the various laboratories. Most if not all will be familiar to one of the named interns. Many will be familiar to the other named intern, who has related experience. One can expect that 24 months will be sufficient to complete the work.

6) Other comments and suggestions for the applicants. For example, is there prior work that should be considered by the researchers?

None.

**Reviewer 2**

1) Mitacs Accelerate supports research-based internships. Does the proposed project qualify as research in its discipline?

Absolutely. This is state of the art research into development of novel methodologies to permit understanding of intracellular signaling pathways coupled to G protein coupled receptors in a spatio-temporal manner. Importantly, the influence of cell phenotype is being considered.

2) Is the project appropriate for the academic degree level of the intern?

Yes. The applicant team has outstanding experience in training post-doctoral fellows and the nature of the research proposed will ensure that these lucky fellows will receive excellent training.

3) Are the objectives clear?

Yes. Each project has a clearly defined set of research objectives and deliverables.

4) Is the methodology appropriate to achieve the objectives?

The methodology proposed is not only appropriate but is pushing the boundaries to move the field forward. The proposed methodology is sorely needed.

5) Is the timeline realistic?

Yes. And the experience of the research team combined with the fact that they have worked well together in the past reinforces that the goals can be met within the timeframe.

6) Other comments and suggestions for the applicants. For example, is there prior work that should be considered by the researchers?

The applicants are world-renowned leaders in the field of functional selectivity/biased agonism. They are fully aware of the status of the field and have not missed anything as far as I can tell.

**Reviewer 3**

1) Mitacs Accelerate supports research-based internships. Does the proposed project qualify as research in its discipline?

This proposal builds on the previous MITACS program that developed new biosensors for measuring multiple signaling events in living cells in order to enhance drug screening for biased ligands. The rationale is that this will result in more efficient identification of compounds with greater efficacy, but lacking undesirable effects. These BRET-based biosensors and analytical tools have been transferred to the private partner Domain Therapeutics for use in drug screening programs. The current proposal will continue to develop new BRET-biosensors for signaling to effectors and in subcellular compartments, and will establish new cellular models as more physiological systems to conduct BRET screening. In addition to providing valuable industrial tools, these will provide new insights into GPCR signaling. Particularly exciting would be the human IPSCs incorporating biosensors, allow for drug screening in a physiologically relevant human cell model.

This project includes 5 postdoctoral fellows doing 6 units each: TBD1 (Bouvier), Nourhen Mnasri (Hebert), TBD2 (Laporte), TBD3 (Leduc), Iness Charfi (Pineyro).

The proposal is research that will provide new information on GPCR signalling to downstream effectors, signalling of internalized receptors, and develop tools for further understanding of GPCR signalling in series of physiological cell models. In particular incorporation of BRET sensors into human IPSCs is a new area of research that could lead to high innovative therapeutic approaches.

2) Is the project appropriate for the academic degree level of the intern?

The project provides an excellent training opportunity for interns to develop new approaches of BRET technology and engineer new cell lines to apply to drug screening. The new technologies to be developed are based on currently well-established BRET methods that have been pioneered in the Bouvier, Hebert, and other applicants' laboratories. In addition, a major limitation in the field of biased signalling has been that most characterization is done in HEK tumor cells, rather than more physiological cells. This problem will be addressed by developing new cells including primary and IPSC-derived cells that incorportate the biosensors in their genome for stable propagation. These techniques are cut-edge, yet entirely appropriate for interns given the expertise of the applicants in developing BRET technology, and in cell culture approaches. The preliminary data indicate that all of the techniques are in hand, including developing primary or IPSC containing the BRET biosensors, and responding to ligands concentration- and ligand-dependently. Hence all proposed projects are appropriate for training. In addition, the applicants will benefit from working in a dynamic and world-leading GPCR research group, including 5 post-doctoral fellows, 5 excellent academic directors and an industry-based research director.

3) Are the objectives clear?

The program has 3 main objectives, and each of the PIs is clearly identified and the roles/projects they will lead are well defined.

1. Developing new BRET-based biosensors for signaling to effectors (e.g., TRPs, Leduc); this is an important goal, to begin to address receptor signaling beyond G protein or beta-arrestins to specific effectors. These effectors are likely to vary between different cell types, hence progress in this area of BRET screening will be very important.

2. Developing new BRET-based biosensors for subcellular signaling (Hebert). This idea of targeting biosensors to different organelles to sense signaling within different cellular compartments is important and novel, and should be highly feasible. Ligands that target internal receptor pools will be screened for.

3. Engineering BRET into primary and IPSC-derived cells (Bouvier, Hebert, Laporte, Leduc, Pineyro). This project is the major part of the proposal and has high translational potential, going beyond HEK cells to physiological cell systems. The BRET biosensors will be knocked in using Cas9-CRISPR system or using adeno- or lentivirus, and preliminary data show that there is sufficient expression for biosensor screen. Each lab will target different cell types including cardiomyocytes (Bouvier), VSMCs (Laporte), hepatocytes (Leduc), and IPSC-derived neurons (Pineyro). Developing an efficient system for BRET in physiologically relevant cells would be a major advantage in drug screening.

4) Is the methodology appropriate to achieve the objectives?

As presented above, the methodology is highly appropriate and feasibility is high given the extensive expertise of the team researchers and interns and the strong preliminary data presented for each project. Each objective has a logical flow with reagent set-up (expression and function of biosensors), validation of cell types, and screening activities (ligand specificity, responses) clearly defined using appropriate established and new approaches. Some details will need verification, such as which receptors are most functional in different cell types, which signaling pathways, etc. However, these variables are easily checked and will not impede progress on the proposal.

5) Is the timeline realistic?

For each of the main objectives a clear timeline is presented in 2-month blocks over 2 years, with reagent set-up, validation, and screening activities clearly defined. The timeline is appropriate and feasible and supported by convincing preliminary data for each aim and investigator. Given the expertise and support of the group, the facilities available, and the quality of the researchers, it is likely that the the proposed studies will be completed successfully.

6) Other comments and suggestions for the applicants. For example, is there prior work that should be considered by the researchers?

Although beyond the scope of the present proposal, the applicants should be thinking of next steps. For example, it will be important to continue to develop further new biosensors to interrogate downstream signalling, which is likely to vary the most in different cell types: it is likely that GPCR coupling to G-proteins and beta-arrestin may be ubiquitous (although ligand dependent), while coupling to ion channels and MAPK may vary depending on what isoforms are expressed. Second, one exciting application of the IPSC approach would be to implant these in animals to allow for in vivo BRET analysis of signalling, or to generate transgenic or knock-in animals for these purposes. This would allow for in vivo screens of drug action, and of signalling, a further step in enhancing drug screens and research tools.

**Reviewer 4**

1) Mitacs Accelerate supports research-based internships. Does the proposed project qualify as research in its discipline?

G protein-coupled receptors (GPCRs) is the largest family of membrane proteins. It was recently recognized that GPCRs can engage multiple signaling effectors in response to pathway-specific ligands (a.k.a biased signaling). This concept of biased signaling is highly

important to identify new GPCR-targeted drugs with limited side effects. The project will develop and test BRET-based biosensors that allow to monitor the functional selectivity of GPCR ligands. The objectives of the proposal are to engineer BRET-based biosensors and validate their use in human iPSC-derived cells (cardiomyocytes, hepatocytes, VSMC, neurons). The proposal will advance our understanding of GPCR biased signaling and therefore develop better drugs that present better efficacy and less adverse effects.

2) Is the project appropriate for the academic degree level of the intern?

The proposal requires expertise in molecular biology and in vitro cell system assays. The two identified postdoctoral fellows that will carry on the project have extensive expertise to troubleshoot all aspects of the proposal including optimization of the biosensor probes, conditions of transfection/transduction and BRET readout.

3) Are the objectives clear?

The main objective of the project is to improve the physiological relevance of BRET-based approach for drug candidate profiling. To do so, the BRET-based biosensors will be expressed in primary cells as well as in iPSCs. Three strategies will be used to express the biosensors: genome editing, lentiviruses and adeno-associated viruses. Five postdocs are requested to conduct these experiments in different cell types: iPSCs stably expressing biosensors, cardiomyocytes, hepatocytes, glial cells, vascular smooth muscle cells and neurons.

The first postdoc in Dr. Bouvier's lab will examine the functional selectivity of the beta2-adrenergic (β2AR), the Apelin (APJ) and the formyl peptide (FPR) receptors in iPSCs-derived cardiomyocytes. He/She will use enhanced bystander BRET (ebBRET) sensors to assess the recruitment of a variety of signaling effectors at the cell surface as well as endosomal compartments.

The second postdoc (Dr. Nourhen Mnasri) in Dr. Hebert's lab, will determine the influence of cell context on biosensor activity and functional selectivity. He will generate human XCL1 iPSCs lines stably expressing plasma membrane (CAAX-rGFP), nuclear (EPACNLS) and endosomal (FYVE-rGFP) biosensors.

The third postdoc will test biosensors in primary vascular smooth muscle cells (VSMCs) and in VSMC-differentiated iPSCs. Using the angiotensin type 1 receptor prototype whose signaling signature to AngII biased ligands has been largely investigated by Laporte's team in HEK cell system, the intern will transduce PKC and Rho BRET sensors in primary VSMC and iPSCs-derived VSMCs. He will compare the signaling profile of 14 AngII analogs previously tested in HEK cells.

The 4th objective will establish BRET-based biosensors for TRP channels in different cell types. Specifically, the intern will test whether AT1R β-arrestin biased ligands can engage TRP channels in hepatocytes and astrocytes. This approach will be used to monitor intramolecular rearrangement of TRP channels as a surrogate of channel activation in response to arrestin-biased receptor ligands. Although this objective is innovative, it was unclear how BRET signal corresponds to channel activation, and particularly ionic current. And if it does, it was unclear in what this approach provides advantage compared to classical electrophysiological methods.

The last objective will test existing biosensors in primary neurons, assess receptor signaling profile across different stages of differentiation of iPSCs-derived neurons.

The data will be collected by Dr. Iness Charfi, who received training in the Pineyro's lab. Receptors tested will be the CB1 and MOR that are molecular targets of analgesics. The last aim is also highly relevant as it will examine how different cellular backgrounds (neuronal phenotype) influence time course of receptor signaling and effector coupling. Given that both CB1R and MOR are Gi/o negatively coupled to AC, the intern will monitor cAMP levels using EPAC biosensor.

Although one limitation is that neuronal background is likely influenced by the neuronal circuit it belongs to and the surrounding cellular environment (glial, vascular cells), this approach will at least answer whether neuronal phenotype associated with differentiation stages can impact analgesic response and desensitization. Finally, while GABAergic and Glutamatergic phenotype was assessed by immunostaining, evaluation of receptor function should be included.

4) Is the methodology appropriate to achieve the objectives?

Feasibility of the experiments is supported by track record of publication of the team on BRET-based biosensors as well as included preliminary data. Methods of biosensor expression, transduction and gene editing are appropriate. Demonstration that iPSC differentiation into various cell types has been established in the different labs could have been emphasized.

5) Is the timeline realistic?

Timeline of the project is doable. A number of BRET-based biosensors have been validated by the team for drug candidate profiling and already been licensed for their use in Canada and North America.

6) Other comments and suggestions for the applicants. For example, is there prior work that should be considered by the researchers?

Efforts to take into account the influence of the cellular environment on receptor signaling were recognized by the reviewer. I wonder if the next step will be to consider the extracellular microenvironement and the influence of the 3D structure of the tissue, thus requiring to test these BRET-based biosensors in organoids ?

## Appendix B: Mitacs Accelerate – Terms and Conditions of the Award

**Please read all conditions carefully**. Any questions or requests for clarification should be directed to your Grant Management Specialist listed in the letter above.

### 1. General guidelines:

1.1. The average amount of partner interaction for each four-month internship across the project should be 50% (or minimum of 25% with sufficient justification in original application).

1.2. Interns may complete up to 2 internship segments (4-6 months each) during a Master's degree, and up to 6 during a PhD and Post-doctoral Fellows may complete a maximum of 6 internship segments.

1.3. The start date of each internship cannot predate the research approval of the project and the receipt of partner funds for the internship.

1.4. Awarded funds must be allocated according to the internship period(s) and stipend/salary allocations, which will be outlined on the Mitacs Award Letter.

1.5. Please report any substantial change (internship dates, project personnel, partner organizations, etc.) concerning your project *immediately* to your Mitacs Grant Management Specialist contact.

### 2. Funding:

2.1. Contribution funds from the partner organization must be paid to Mitacs prior to the internship start date to facilitate efficient payment process for interns.

2.2. Funding will not be released for un-named interns.

2.3. The stipend portion of the award must be spent as outlined in the award letter. *No extensions can be granted on stipend amounts*.

2.4. The research grant portion of the award must be spent by end date of the award**.** Any remaining research funds may be used by the academic supervisor to supplement the intern's stipend at the Academic Supervisor's discretion. Any funds not spent by this date must be returned to Mitacs unless an extension of the award is granted by Mitacs. To obtain an extension, the supervisor needs to contact Mitacs before the end date of the award, with justification of such a request.

2.5. In-kind contributions cannot be used towards matching Mitacs funds.

2.6. Inter-university transfers are not permitted.

2.7. To ensure that your project complies with the Mitacs university agreement and federal granting council policies, you must provide Mitacs with copies of all contracts that govern the research being performed using Mitacs funds.

### 3. Intern Stipends:

3.1. Each intern **must** receive a minimum $10,000 stipend/salary for each internship unit.

3.2. All stipend/salary funds must be paid over a period of no less than 4 months and no greater than 6 months per internship unit.

## 4. Research Funds:

4.1. The remaining funding may be used to cover expenses in support of the internship research and its related costs, as directed by Tri-Council. A detailed description of eligible expenses is available on the NSERC website at the following link:
http://www.nserc-crsng.gc.ca/Professors-Professeurs/FinancialAdminGuide-GuideAdminFinancier/FundsUse-UtilisationSubventions_eng.asp

4.2. Relevant research expenses incurred outside of the indicated funding period may be approved by Mitacs on a case-by-case basis.

## 5. Reporting:

5.1. If the award straddles fiscal years, the University will provide a separate Form 300 (statement of account) for each account by **May 31st** for each fiscal year until the account reaches a zero balance. The University will provide a Final Form 300 for each account **within 30 days of the end date of this award**. The Academic Supervisor and Finance Officer must sign the Form 300s and forward to Mitacs once finalized. *Please include the Application Reference number and Project Title on the Form 300 and submit to form300@mitacs.ca.*

5.2. Projects involving more than 50 internship units and spanning longer than 2 years are required to submit a progress report to Mitacs at the mid-point of the project. Please designate one academic supervisor to be the coordinator who is responsible for submitting the report to Mitacs. Failure to submit a satisfactory report may result in delay or cancellation of subsequent funding.

5.3. All parties are required to provide Mitacs with a final report and completed exit surveys at the end of the Mitacs Accelerate internship. Please find the links below:

Accelerate Final Report:
http://www.mitacs.ca/sites/default/files/uploads/page/mitacs_accelerate_final_report_mar2013.zip

Accelerate Intern Exit Survey:
http://sondages.outsidesoft.com/Engine/Default.aspx?surveyID=9eff01b6-316a-49c9-b257-83afff51e497&lang=EN

Accelerate Professor Exit Survey:
http://sondages.outsidesoft.com/Engine/Default.aspx?surveyID=023afce3-c581-4c97-9294-6e8f5e25b1ef&lang=EN

Accelerate Partner Exit Survey:
http://sondages.outsidesoft.com/Engine/Default.aspx?surveyID=f413d47d-b4f3-49a4-be2c-5e0a947bcafb&lang=EN

## 6. Acknowledgement of Mitacs:

6.1. All Mitacs-sponsored results must explicitly credit Mitacs. This includes work by professors, post-docs, interns, and research associates. Mitacs funding can be acknowledged in publications using the following statement: "This work was supported by Mitacs through the Mitacs Accelerate Program." All networking activities initiated by your project for which Mitacs funds are used must also credit Mitacs. For advertising purposes, the Mitacs logo is available in several formats for download from the website at www.mitacs.ca.