BRET-BASED BIOSENSORS - LIGAND-BIASED SIGNALLING AT GPCRS

<table>
<thead>
<tr>
<th>THERAPEUTIC AREA</th>
<th>Various</th>
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<td>INDICATION</td>
<td>Various</td>
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<tr>
<td>OBJECTIVE</td>
<td>To develop cell-based tools to screen for pharmacoactive molecules that bind to GPCRs and to define ligand-biased signalling signatures</td>
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<td>TYPE OF PROJECT</td>
<td>Drug discovery tool</td>
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<td>TYPE OF TARGET</td>
<td>G Protein-Coupled Receptors (GPCR desensitization and G protein-independent signalling)</td>
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<td>IP STATUS</td>
<td>US patent №. 7,932,080 granted Apr 26, 2011</td>
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<td>Canadian patent application №. 2,607,015</td>
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<td>US patent application №. US20090298162</td>
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<tr>
<td>PRINCIPAL INVESTIGATOR</td>
<td>Michel Bouvier</td>
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<td>TYPE OF PARTNERSHIP SOUGHT</td>
<td>Non-exclusive licensing</td>
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COMPETITIVE ADVANTAGES
- Signalling signature profiles in homogeneous assay format
- Wide coverage of GPCR signalling pathways
- Clustering of ligands based on activity profiles
- Applicable to established cell lines or primary cultures
- Amenable to HTS, hit-to-lead and lead-optimization

Scientific Background and Rationale
The technology relates to biosensors that are based on bioluminescence resonance energy transfer (BRET) which allow the direct, real-time examination of GPCR activation as well as activity of downstream effector molecules. In these BRET-based biosensors, the bioluminescent donor Renilla luciferase (Luc) or a mutant form thereof and the fluorescent acceptor is yellow fluorescent protein (YFP), green fluorescent protein (GFP) or a variant thereof.

GPCRs relay the information provided by numerous hormones and neurotransmitters into intracellular signalling pathways, primarily through their coupling to heterotrimeric G proteins although other signalling events can occur independently of G-protein involvement, as depicted in Figure 1 below. Fig. 1a) shows the effector pathways engaged by GPCRs and the type of activity that they can regulate. Fig. 1b) highlights the concept of ligand-biased efficacy wherein 3 different ligands can induce and/or stabilize different receptor conformations that will each promote distinct relative efficacies toward different effector systems.

Adapted from Galandrin S et al., Oligny-Longpré G. and Bouvier M. TRENDS in Pharm. Sc. 2007.

A more thorough knowledge of ligand-biased signalling efficacy is of growing importance in the drug discovery field. This concept highlights the importance of going beyond the conventional active/inactive receptor state upon ligand binding and explores the downstream
effectors activated by such ligands on a same receptor. Two ligands acting on the same receptor could elicit different signalling pathways, one with therapeutic benefit and the other with adverse effects. We have thus developed a panel of novel BRET-based biosensors (unimolecular and bimolecular) and methods to monitor the signalling landscape of GPCR signalling. This panel covers most of the proximal signalling events such as beta-arrestins and G-protein activation (subunit specificity), classical second messengers such as Ca2+, cAMP and DAG, and more distal events such as Merlin’s, PKC’s and ERK’s activation. The panel also encompasses GRK2 & 3, potassium channels, the cytoskeletal protein PTEN and the small G protein Ras. The corresponding lentiviral constructs of these biosensors, along with stable cell lines, are also available.

Applications/Deliverables
1. Newly developed individual biosensors monitoring specific signalling pathways
2. Multiplexing modes and conditions to monitor multiple pathways simultaneously
3. Stable cell lines and viral vectors expressing/encoding biosensors for generation of cell signalling arrays
4. Procedures/software/algorithms to accurately quantify and represent signalling signatures of compounds based on different outputs measured
5. Signalling signatures as correlated with beneficial therapeutic properties and with reported side effects of well-established drugs
6. Validated signalling signatures as predictors of biological activities in pre-clinical models.

IP Status
1. US patent № 7,932,080 filed May 4, 2005 and granted April 26, 2011 (Title: Double brilliance beta-arrestin: a biosensor for monitoring the activity of receptors and signalling molecules, and method of using same)
2. Corresponding Canadian patent application № CA 2,607,015 (pending)
3. US Continuation in part of US patent № 7,932,080, filed Apr 22, 2011 - US2011/02755134 (Title: Arrestin Biosensor)
4. US patent application № US 20090298162 filed Feb 16, 2016 (Title: Biosensors for monitoring receptor-mediated G-protein activation)

Lead Scientist
Michel Bouvier, Ph.D.
• Head of the Molecular Pharmacology Laboratory, IRIC
• Director, Groupe de recherche universitaire sur le médicament, UdeM
• Full Professor, Dept of Biochemistry, Faculty of Medicine, UdeM
• Chief Executive Officer, IRICoR

During his postdoctoral training at Duke University, Dr Bouvier started to focus his studies on the molecular pharmacology of G-protein coupled receptors (GPCR), the drug targets of more than half of all medications prescribed today. He studied post-translational modifications of GPCRs and participated in several major discoveries that were scientific firsts: the expression of GPCRs in a heterologous mammalian cell system, the discovery of the palmitylation of a GPCR, and the discovery of phosphorylation sites responsible for the desensitization of GPCRs. Back to UdeM, Dr Bouvier became Director of the Department of Biochemistry in 1997, joined IRIC as Head of the Molecular Pharmacology Laboratory Since 2001, he holds the Canada Research Chair in Signal Transduction and Molecular Pharmacology. He has recently received the Adrien-Pouliot Award for his collaborative work with France by the Acfas in 2011 and in 2012, he was honored with the NRC Research Press Senior Investigator award. Dr Bouvier and his colleagues made the first demonstration of ligand-independent spontaneous activity of wild-type GPCRs and discovered inverse agonism for this class of receptors. These findings then led to the demonstration of the pluri-dimensionality of signalling efficacy. Dr Bouvier and his group also pioneered the development of assays to monitor protein-protein interactions within signal transduction pathways, the bioluminescence resonance energy transfer (BRET) technology that allows studying the dynamics of real time signalling in living cells. Dr Bouvier has also developed the concept of pharmacological chaperones, a new drug class directed to the treatment of many genetic diseases.