FINAL SCRIPT: APPROVED FOR FILMING

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Title: BRET-Based G protein Biosensors for Measuring G Protein-Coupled Receptor Activity in Live Cells

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length
Number of Steps: 19
Number of Shots: 43

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

1.1. **Marie-Lise Jobin:** We are interested in understanding key questions in GPCR signaling that would allow us to identify new GPCR targets and ligand biased signaling.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What technologies are currently used to advance research in your field?

1.2. **Marie-Lise Jobin:** Biomolecular and biochemical methods, cryo-EM, NMR, and molecular dynamic simulations, are used to understand the regulation of GPCR by ligands with different efficacy or bias.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

What advantage does your protocol offer compared to other techniques?

1.3. **Adeline Coeugnet:** G-CASE BRET sensors are tricistronic constructions that allow us to measure precisely specific G-protein activation in real time and in live cells.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

How will your findings advance research in your field?

1.4. **Adeline Coeugnet:** Discovery of biased ligand with high throughput methods could open pharmacological studies for specific drug screening with improved efficacy.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please ensure that all testimonial shots are captured in a wide-angle format, while also maintaining sufficient headspace, given that the final videos will be rendered in a 1:1 aspect ratio.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.5. **Cœugnet Adeline, PhD student**: (authors will present their testimonial statements live)

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.6. **Cœugnet Adeline, PhD student**: (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

1. Seeding Well Plate with HEK293 Cells

Demonstrator: Adeline Cœugnet

1.1. To begin, pour approximately 6 milliliters of sterile-filtered 0.01% Poly-L-lysine solution into a multichannel reservoir **[1]**. Use a multichannel pipette to dispense 60 microliters of this solution into each well of a 96-well white microplate **[2]**. Incubate the microplate in the dark for 20 minutes **[3]**.

1.1.1. WIDE: Talent pouring Poly-L-lysine solution into a multichannel reservoir.

1.1.2. Talent using a multichannel pipette to fill each well of a 96-well plate with the solution.

1.1.3. Talent placing the 96-well plate inside a dark drawer or cabinet.

1.2. After incubation, aspirate the Poly-L-lysine solution from each well using a multichannel pipette **[1]** and transfer it to a 15-milliliter tube for storage at 4 degrees Celsius **[2]**. Wash the plate three times with DPBS *(D-P-B-S)* solution using a multichannel pipette **[3]**.

1.2.1. Talent aspirating the PLL from the wells.

1.2.2. Talent transferring it into a labeled 15-milliliter tube.

1.2.3. Talent washing the wells three times using DPBS and a multichannel pipette.

1.3. For cell preparation and plating, discard the medium in the flask containing the HEK293 *(H-E-K-Two-Ninety-Three)* cells **[1].** Then rinse the cells with 2 milliliters of DPBS solution **[2].**

1.3.1. Talent removing the old medium.

1.3.2. Talent adding 2 mL DPBS to the cells then discarding supernatant.

1.4. Now pipette 0.5 milliliter of trypsin to the flask **[1].** Incubate the suspension for 3 to 5 minutes at 37 degrees Celsius to detach the cells **[2]**.

1.4.1. Talent adding trypsin to the flask.

1.4.2. Talent placing the flask in an incubator.

1.5. Neutralize the trypsin with 4.5 milliliters of DMEM *(D-M-E-M)* **[1]**. Pipette the suspension up and down repeatedly to fully detach and resuspend the cells **[2]**.

1.5.1. Talent adding DMEM to the flask.

1.5.2. Talent pipetting the medium to resuspend the cells.

1.6. Transfer the cell suspension to a 15-milliliter tube **[1].** Then centrifuge at 1000 *g* for 5 minutes **[2].** Resuspend the cell pellet in 2 milliliters of DMEM, after discarding the supernatant **[3]**.

1.6.1. Talent transferring the suspension to a centrifuge tube.

1.6.2. Talent placing the tube in the centrifuge.

1.6.3. Talent removing supernatant and gently resuspending the pellet with DMEM.

1.7. Pipette 40 microliters of the resuspended cells on a Malassez counting chamber **[1].** Now, count the cells using a Malassez counting chamber **[2]**. Prepare 10 milliliters of the cell suspension at a concentration of 300,000 cells per milliliter **[3]**.

Added shot: 1.7.0.Preparation of the Malassez counting chamber.

1.7.1. Talent counting cells using a Malassez counting chamber.

1.7.2. Talent preparing cell suspension in a tube.

1.8. Pour this suspension into a multichannel reservoir **[1].** Then use a multichannel pipette to seed each well of the 96-well plate with 100 microliters of the solution **[2-TXT]**. Incubate the microplate at 37 degrees Celsius with 5% carbon dioxide for approximately 24 hours **[3]**.

1.8.1. Shot of the suspension being poured into a multichannel reservoir.

1.8.2. Talent seeding the microplate with a multichannel pipette. **TXT: Final density: 30,000 cells/well**

AUTHOR'S NOTE: Cut the video after 3 or 4 columns are already filled

1.8.3. Talent placing the seeded plate in a humidified incubator.

2. Plasmid transfection and Data Acquisition

2.1. For transfection, transfer 10 micrograms of plasmid DNA and 20 microliters of Lipofectamine into separate tubes, each containing 500 microliters of Opti-MEM *(Opti-Mem)* medium **[1]**.

2.1.1. Talent pipetting DNA and Lipofectamine into separate tubes of Opti-MEM and placing them on the bench for incubation.

2.2. After a 5-minute incubation at room temperature, combine the solutions into a single tube **[1].** Mix gently to obtain a 1-milliliter transfection mixture and incubate **[2-TXT]**.

2.2.1. Talent mixing the DNA and Lipofectamine solutions into one tube and gently

pipetting to mix. **TXT: Incubation: RT, 20 min**
AUTHOR'S NOTE: Combine 2.2.1 and 2.2.2

~~2.2.2. Talent gently pipetting to mix. **TXT: Incubation: RT, 20 min**~~

2.3. Using a multichannel pipette, add 10 microliters of the transfection mixture dropwise into each well of the 96-well plate **[1]**. Gently rock the plate back and forth to ensure uniform distribution **[2]**. Then incubate the plate in a humidified incubator at 37 degrees Celsius with 5% carbon dioxide for 24 hours **[3]**.

2.3.1. Talent pipetting the transfection mixture dropwise into the wells.

2.3.2. Talent gently rocking the microplate in a crosswise motion.

2.3.3. Talent placing the plate in the incubator and closing the door.

2.4. The next day, prepare a ligand serial dilution of 10 microliters in the appropriate solubilization solvent, centered around the dissociation constant of the ligand **[1]**. Store the serial dilution at minus 20 degrees Celsius **[2]**.

2.4.1. Talent preparing serial dilution of ligand in small tubes.

2.4.2. Talent labeling and placing tubes into a minus 20 degrees Celsius freezer.

2.5. For each concentration and the vehicle control, perform a 1 in 100 dilution by adding 1.3 microliters of the stock to 128.7 microliters of HBSS *(H-B-S-S)* **[1]**. Then prepare 980 microliters of Furimazine *(Fury-mah-zeen)* at 1 in 100 dilution by adding 9.8 microliters of stock to 970.2 microliters of HBSS **[2]**.

2.5.1. Talent pipetting precise volumes of ligand dilutions and HBSS into new tubes.

2.5.2. Talent pipetting Furimazine stock and HBSS into a microcentrifuge tube and mixing.

2.6. To perform plate reading, aspirate the medium from the first 4 columns of the 96-well plate **[1]**. Rinse each well with 100 microliters of HBSS **[2]**, then add 80 microliters of HBSS to each well **[3]**. Then stick a white sticker on the underside of the plate **[4].**

2.6.1. Talent removing the medium from the designated columns using a multichannel pipette.

2.6.2. Talent rinsing each well with100 µL HBSS using a multichannel pipette.

2.6.3. Talent adding 80 microliters of HBSS to each well.

Added shot 2.6.4: Talent adding a white sticker on the underside of the plate.

2.7. Insert the 96-well plate into the plate reader to begin recording the cpVenus173 *(C-P-Venus-One-Seventy-Three)* emission spectrum **[1]**. Set the monochromator to 535/30 *(Five-thirty-five-by-thirty)* nanometers and measure luminescence emission between 500 and 600 nanometers with 2-nanometer resolution **[2]**.

2.7.1. Talent loading the microplate into the plate reader.

2.7.2. SCREEN: 68463_3.7.2.mp4. 00:26-00:35

2.8. Record the Nluc *(N-luke)* emission intensity by setting the monochromator to 450/40 *(Four-fifty-by-forty)* nanometers and scanning between 400 and 600 nanometers with 5-nanometer resolution **[1]**.

2.8.1. SCREEN: 68463_3.8.1.mp4 00:05-00:21, 00:53-01:06

2.9. Remove the plate from the reader **[1].** Then add 10 microliters of the prepared Furimazine solution to each well using a multichannel pipette **[2-TXT]**.

2.9.1. Shot of the plate being removed from the instrument.

2.9.2. Talent pipetting Furimazine solution into the wells with a multichannel pipette.
TXT: Record the G protein luminescence spectra again

2.10. To record G protein basal BRET *(brett)* signal, measure both Nluc and cpVenus173 emissions using the 450/40-nanometer and 535/30-nanometer monochromators, before adding GPCR ligands **[1-TXT]**.

2.10.1. SCREEN: 68463_3.10.1.mp4 00:18-00:41
TXT: Measure 3 cycles of 60 s, 0.30 s interval

2.11. Then remove the plate and add 10 microliters of the prepared GPCR ligand dilutions to the wells of one column using a multichannel pipette **[1]**. Reinsert the plate into the reader **[2]** to record ligand-induced BRET by measuring Nluc and cpVenus173 emissions with the same monochromator settings **[3-TXT]**.

2.11.1. Talent adding ligand dilutions to the appropriate wells using a multichannel pipette.

2.11.2. Talent loading the plate back into the reader.

2.11.3. SCREEN: 68463_3.11.3.mp4 00:19- 00:39
TXT: Measure 16 cycles of 60 s, 0.30 s interval

2.12. For data analysis, plot the emission intensity of each well against the wavelength to visualize the control spectra **[1]**. Then calculate the BRET ratio for each well using the three basal readings **[2]**. Calculate the BRET ratio for each well for all ligand-induced

readings across the 16 measurement cycles **[3]**.

2.12.1. SCREEN: 68463_3.12.1.mp4 00:18-00:39, 00:55-00:56

2.12.2. SCREEN: 68463_3.12.2-3.12.3.mp4. 00:14-00:34

2.12.3. SCREEN: 68463_3.12.2-3.12.3.mp4. 02:30-02:49

Results

3. Results

3.1. A concentration-dependent decrease in Δ *(delta)* BRET values was observed in HEK 293T cells expressing β *(beta)* 2-adrenergic receptors upon isoproterenol stimulation, reaching a plateau around 5 minutes **[1]**. No significant ΔBRET change was observed in HEK 293T cells transfected with pcDNA3.1 *(P-C-D-N-A-Three-Point-One)* vector upon isoproterenol stimulation **[2]**.

3.1.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the curves between 0 to 5 min.*

3.1.2. LAB MEDIA: Figure 3B.

3.2. Sigmoidal dose-response curves showed an EC_{50} *(E-C-fifty)* of approximately 9.4 nanomolar for isoproterenol in β2-adrenergic receptor-expressing cells **[1]**. ΔBRET values measured at 15 minutes post-stimulation with 10 micromolar isoproterenol were significantly lower in β2-adrenergic receptor-expressing cells compared to pcDNA control cells **[2]**.

3.2.1. LAB MEDIA: Figure 3C. *Video editor: Emphasize the orange curve*

3.2.2. LAB MEDIA: Figure 3D. *Video editor: Highlight the orange dots*

3.3. A concentration-dependent decrease in ΔBRET was observed in HEK CB1 *(H-E-K-C-B-One)* cells stimulated with WIN-55,212-2, *(Win-Fifty-Five-Two-One-Two-Two)* stabilizing around 5 minutes **[1]**. HEK wild-type cells did not show significant ΔBRET changes upon WIN-55,212-2 stimulation, confirming specificity of CB1 receptor activation **[2]**.

3.3.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the blue curves in the HEK CB1 panel, between 0 to 5 min*

3.3.2. LAB MEDIA: Figure 4B.

3.4. WIN-55,212-2 induced a sigmoidal dose-response in HEK CB1 cells, with an EC_{50} of approximately 112 nanomolar **[1]**. ΔBRET values at 15 minutes post-stimulation with 10 micromolar WIN-55,212-2 were significantly lower in HEK CB1 cells compared to HEK wild-type cells **[2]**.

3.4.1. LAB MEDIA: Figure 4C. *Video editor: Emphasize the blue curve*

3.4.2. LAB MEDIA: Figure 4D. *Video editor: Highlight the blue dots clustered*

Pronunciation Guide:

BRET
Pronunciation link: https://www.merriam-webster.com/dictionary/BRET

IPA: /brɛt/
Phonetic Spelling: bret

G protein
Pronunciation link: https://www.merriam-webster.com/dictionary/G%20protein

IPA: /ˈdʒiː ˌproʊˈtiːn/
Phonetic Spelling: jee proh·teen

Biosensors
Pronunciation link: https://www.merriam-webster.com/dictionary/biosensor

IPA: /ˈbaɪoʊˌsɛnsər/
Phonetic Spelling: bye·oh·sen·ser

G protein-coupled receptor
Pronunciation link: https://www.merriam-webster.com/dictionary/G%20protein-coupled%20receptor

IPA: /ˈdʒiː ˌproʊˈtiːn ˈkʌpəld rɪˈsɛptər/
Phonetic Spelling: jee proh·teen kuh·puhld ri·sep·ter

GPCR
Pronunciation link: https://www.merriam-webster.com/dictionary/GPCR

IPA: /ˌdʒiː ˌpiː ˌsiː ˈɑːr/
Phonetic Spelling: jee pee see ar

Cryo-EM
Pronunciation link: https://www.merriam-webster.com/dictionary/cryo-electron%20microscopy

IPA: /ˈkraɪoʊ iˌlɛkˈtrɑːn maɪˈkrɑːskəpi/
Phonetic Spelling: krye·oh ee·lek·tron my·krah·skuh·pee

NMR
Pronunciation link: https://www.merriam-webster.com/dictionary/NMR

IPA: /ˌɛn ˌɛm ˈɑːr/
Phonetic Spelling: en em ar

Tricistronic
Pronunciation link: https://www.merriam-webster.com/dictionary/tricistronic

IPA: /ˌtraɪsɪˈstrɑːnɪk/
Phonetic Spelling: try·sis·tron·ik

Poly-L-lysine
Pronunciation link: https://www.merriam-webster.com/dictionary/polylysine

IPA: /ˌpɑːliˈlaɪsiːn/
Phonetic Spelling: pah·lee·lye·seen

HEK293
Pronunciation link: https://www.howtopronounce.com/hek-293

IPA: /hɛk tuː naɪn ˌθriː/
Phonetic Spelling: heck two·nine·three

DPBS
Pronunciation link: https://www.howtopronounce.com/dpbs

IPA: /ˌdiː piː biː ˈɛs/
Phonetic Spelling: dee pee bee ess

Trypsin
Pronunciation link: https://www.merriam-webster.com/dictionary/trypsin

IPA: /ˈtrɪpsɪn/
Phonetic Spelling: trip·sin

DMEM
Pronunciation link: https://www.howtopronounce.com/dmem

IPA: /ˌdiː ɛm iː ˈɛm/
Phonetic Spelling: dee em ee em

Malassez
Pronunciation link: https://www.howtopronounce.com/malassez

IPA: /ˌmæləˈzeɪ/

Phonetic Spelling: mal·uh·zay

Lipofectamine
Pronunciation link: https://www.merriam-webster.com/dictionary/Lipofectamine

IPA: /ˌlaɪpoʊˈfɛktəmiːn/
Phonetic Spelling: lye·poh·fek·tuh·meen

Opti-MEM
Pronunciation link: https://www.howtopronounce.com/opti-mem

IPA: /ˈɑːptaɪ mɛm/
Phonetic Spelling: op·tye mem

Ligand
Pronunciation link: https://www.merriam-webster.com/dictionary/ligand

IPA: /ˈlɪgænd/
Phonetic Spelling: lig·and

Dissociation constant
Pronunciation link: https://www.merriam-webster.com/dictionary/dissociation%20constant

IPA: /dɪˌsoʊsiˈeɪʃən ˈkɑːnstənt/
Phonetic Spelling: dih·soh·see·ay·shun kon·stunt

HBSS
Pronunciation link: https://www.howtopronounce.com/hbss

IPA: /ˌeɪtʃ biː ɛs ɛs/
Phonetic Spelling: aych bee ess ess

Furimazine
Pronunciation link: https://www.howtopronounce.com/furimazine

IPA: /ˌfjʊrɪˈmæziːn/
Phonetic Spelling: fyur·ih·mah·zeen

cpVenus173
Pronunciation link: No confirmed link found
IPA: /siː piː ˈviːnəs wʌn ˈsɛvən tiː ˈθriː/
Phonetic Spelling: see pee vee·nuss one sev·en·tee three

Nluc
Pronunciation link: https://www.howtopronounce.com/nluc

IPA: /ˈɛn luːk/
Phonetic Spelling: en luke

Monochromator
Pronunciation link: https://www.merriam-webster.com/dictionary/monochromator

IPA: /ˌmɑːnoʊˈkroʊmeɪtər/
Phonetic Spelling: mon·oh·kroh·may·ter

Luminescence
Pronunciation link: https://www.merriam-webster.com/dictionary/luminescence

IPA: /ˌluːmɪˈnɛsəns/
Phonetic Spelling: loo·mih·nes·suhns

Isoproterenol
Pronunciation link: https://www.merriam-webster.com/dictionary/isoproterenol

IPA: /ˌaɪsoʊproʊˈtɛrənɔːl/
Phonetic Spelling: eye·soh·proh·ter·uh·nol

β2-adrenergic
Pronunciation link: https://www.merriam-webster.com/dictionary/adrenergic

IPA: /ˌbeɪtə tuː ˌædrəˈnɜːrdʒɪk/
Phonetic Spelling: bay·tuh two ad·ruh·ner·jik

pcDNA3.1
Pronunciation link: No confirmed link found
IPA: /ˌpiː siː diː ɛn eɪ θriː pɔɪnt wʌn/
Phonetic Spelling: pee see dee en ay three point one

EC50
Pronunciation link: https://www.merriam-webster.com/dictionary/EC50

IPA: /ˌiː siː ˈfɪfti/
Phonetic Spelling: ee see fif·tee

Nanomolar
Pronunciation link: https://www.merriam-webster.com/dictionary/nanomolar

IPA: /ˌnænoʊˈmoʊlər/
Phonetic Spelling: nan·oh·moh·ler

Micromolar
Pronunciation link: https://www.merriam-webster.com/dictionary/micromolar

IPA: /ˌmaɪkroʊˈmoʊlər/
Phonetic Spelling: my·kroh·moh·ler

Sigmoidal
Pronunciation link: https://www.merriam-webster.com/dictionary/sigmoidal

IPA: /sɪgˈmɔɪdəl/
Phonetic Spelling: sig·moy·duhl