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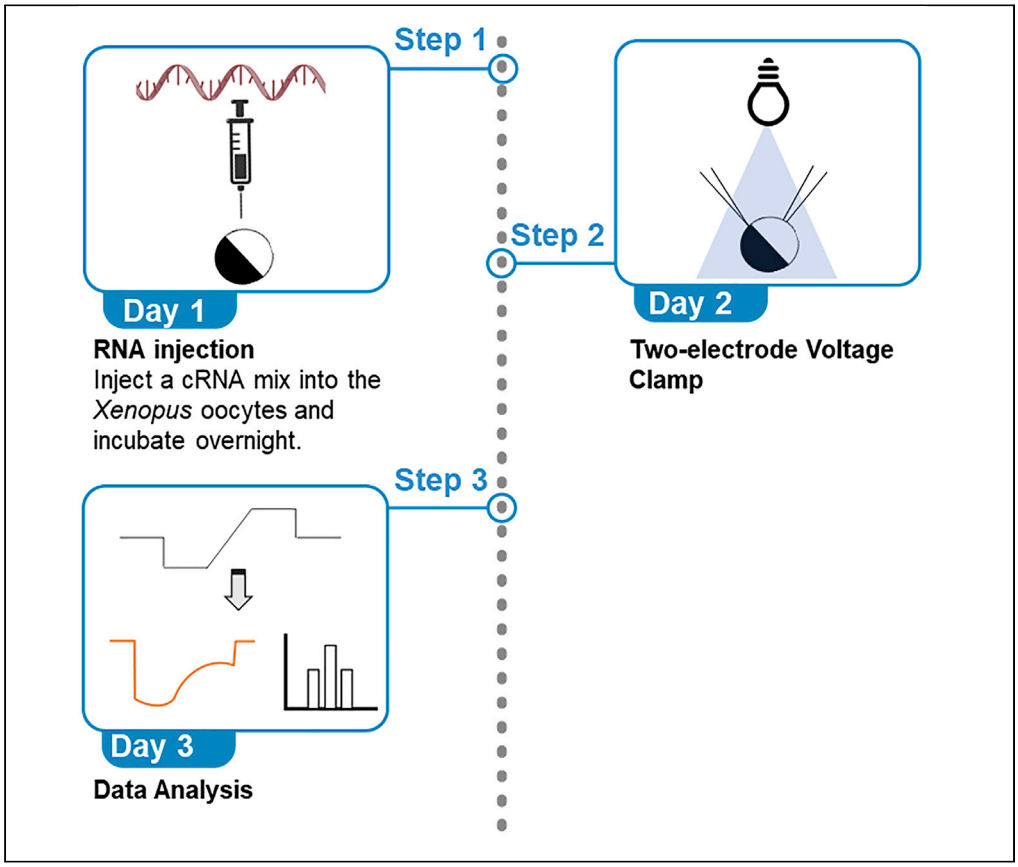
Protocol

Optogenetic dephosphorylation of phosphatidylinositol 4,5 bisphosphate in *Xenopus laevis* oocytes



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Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Kirin D. Gada, Yu Xu, Brenda T. Winn, Meghan Masotti, Takeharu Kawano, Heikki Vaananen, Leigh D. Plant

l.plant@northeastern.edu

Highlights

This protocol describes optogenetic dephosphorylation of PI(4,5)P_2 in *Xenopus* oocytes

Steps to microinject a cRNA mix into oocytes

Blue light illumination to induce rapid dephosphorylation of PI(4,5)P_2

Simultaneous two-electrode voltage clamp (TEVC) recording to assess ion channel responses

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Optogenetic dephosphorylation of phosphatidylinositol 4,5 bisphosphate in *Xenopus laevis* oocytes

Kirin D. Gada,[1] Yu Xu,[1] Brenda T. Winn,[1] Meghan Masotti,[3] Takeharu Kawano,[1] Heikki Vaananen,[1] and Leigh D. Plant[1,2,4,5,*]

[1]Department of Pharmaceutical Sciences, Bouve College of Health Sciences, Northeastern University, Boston, MA 02115, USA

[2]Center for Drug Discovery, Northeastern University, Boston, MA 02115, USA

[3]Northwestern University, Evanston, IL 60208, USA

[4]Technical contact: l.plant@northeastern.edu

[5]Lead contact

*Correspondence: l.plant@northeastern.edu
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SUMMARY

Here, we present a protocol for optogenetic dephosphorylation of the phosphoinositide PI(4,5)P_2 at the plasma membrane of *Xenopus laevis* oocytes. We first describe the co-injection of oocytes with cRNAs encoding (1) a light-activated PI(4,5)P_2 5-phosphatase fusion protein, (2) its dimerization partner fused to the plasma membrane, and (3) the potassium channel reporter for PI(4,5)P_2 dephosphorylation. We then detail blue light illumination to induce PI(4,5)P_2 dephosphorylation, combined with simultaneous two-electrode voltage clamp electrophysiological recording to assess potassium channel current responses.

For complete details on the use and execution of this protocol, please refer to Xu et al. (2022).[1]

BEFORE YOU BEGIN

The oculocerebrorenal syndrome of Lowe is a disorder caused by the deficiency of a PI(4,5)P_2 5-phosphatase, OCRL-1. This protocol describes the specific steps for optogenetic dephosphorylation of PI(4,5)P_2 in *Xenopus laevis* oocytes by the inositol 5-phosphatase domain of OCRL fused to the plant protein cryptochrome 2 (CRY2-5-ptase$_{OCRL}$). As described in Idevall-Hagren et al.,[2] when CRY2-5-ptase$_{OCRL}$ is photoactivated by 458–490 nm (blue) light, it dimerizes with its protein partner CIBN, which is fused to the plasma membrane via a CAAX domain (CIBN-CAAX). Therefore, illuminating cells that express these proteins with blue-light initiates near instantaneous recruitment of CRY2-5-ptaseOCRL to the plasma membrane, where it rapidly dephosphorylates PI(4,5)P_2, generating PI(4)P. Here, we employ the PI(4,5)P_2-dependent inward rectifying potassium channel Kir2.1 (also called IRK1) as a reporter for PI(4,5)P_2 dephosphorylation. All Kir channels are intrinsically regulated by PI(4,5)P_2 and require the phospholipid to maintain their activity. Kir2.1 channels pass robust currents in *Xenopus* oocytes and multiple studies have shown that Kir2.1 currents decrease rapidly in response to acute PI(4,5)P_2 dephosphorylation or depletion.[3] In Xu et al.,[1] we demonstrated robust, reproducible loss of Kir2.1 function following activation of CRY2-5-ptase$_{OCRL}$.

Optogenetic dephosphorylation of PI(4,5)P_2 is a versatile system that can be activated in a non-invasive, spatiotemporally precise manner and is compatible with other existing functional assays

that can be performed in *Xenopus* oocytes. Here, we describe the steps required to perform this experiment in concert with two electrode voltage-clamp (TEVC) recordings of Kir2.1 currents. However, a plethora of ion channels can be studied in *Xenopus* oocytes opening this assay for a range of applications in fields as diverse as physiology, biochemistry, pharmacology, and genetics.

Institutional permissions

The *Xenopus* oocytes used for these studies were surgically extracted from *Xenopus laevis* frogs in accord with an IACUC protocol at Northeastern University. Acquire permissions from the relevant committee at your institution before performing all equivalent procedures.

> ***Note:*** For labs that routinely employ two-electrode voltage clamp experiments to study ion channels in *Xenopus* oocytes, it is reasonable to use their own established in-lab protocol for oocyte extraction and dissociation.

Oocyte dissociation

Timing: 3–4 h

1. Survival surgeries for oocyte extraction from *Xenopus laevis* frogs are performed in accord with an IACUC protocol at Northeastern University. We routinely prepare oocytes from two frogs, one ovary per animal and prepare each separately in case the oocytes from one animal are low quality. For each ovary:
   a. Place the tissue (~5 mL) into a 100 mm petri dish at room temperature.
   b. Using tweezers and scissors, carefully dissect the ovary into 2–3 mm sized pieces.
   c. Rinse the tissue with 20–30 mL of Oocyte Ringer 2(-) solution [OR2(-)] 2–3 times.

> ***Note:*** This step removes residual debris from the extraction surgery and ensures that the clumps of tissue that remain, are very small.

   d. Add the oocyte prep to a 50 mL conical tube and add fresh OR2(-) to the 10 mL mark.
   e. Weigh and add in 5–6 mg Collagenase Type 7 enzyme per tube.
   f. Place the tubes into a mechanical tube rotator for 1–1.5 h at room temperature, until the oocytes are dissociated into singular eggs and the follicular membrane is digested.
   g. Pour off the collagenase/OR2(-) liquid and wash 2–3 times with OR2(-).
   h. Add fresh OR2(-) to 15 mL mark.
   i. Rotate the tubes for another 20–30 min at room temperature and wash 4–6 times with OR2(-) or until the liquid is clear once swirled.
   j. Now wash once with OR2(+).
   k. Add OR2(+) to petri dish and pour in the oocytes.
   l. Place in an oocyte incubator at +17°C.

cRNA transcription

Timing: 2–3 days

2. Linearize plasmid DNAs with appropriate restriction enzymes.

> ***Note:*** *In vitro* RNA transcription for 5′capped plasmid constructs that will be microinjected into oocytes is performed using the mMESSAGE mMACHINE T7 Transcription Kit (Thermo Fisher Scientific) according to the manufacturer's protocol (can be found here Under user guide).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
NaCl	Fisher	S271
HEPES	Oakwood Chemical	047861
KCl	Fisher	P217
NaOH	Fisher	S318
$MgCl_2.6H_2O$	Fisher	M33
$CaCl_2.2H_2O$	Fisher	C79
$BaCl_2$	Fisher	B34
Collagenase Type 7	Worthington Biochemical	LS005332
RNAse-free water	Ambion	AM9920
1% Penicillin/Streptomycin	Cytiva	SV30010
Agarose	Thermo Fisher	17850
Critical commercial assays		
mMESSAGE mMACHINE™ T7 Transcription Kit	Thermo Fisher Scientific	Cat# AM1344
Experimental models: Organisms/strains		
Xenopus Laevis frog oocytes	Xenopus1 Corp.	N/A
Recombinant DNA		
CIBN-CAAX pMax	This paper; Xu et al.[1]	N/A
CRY2-5-ptase$_{OCRL}$ pMax	This paper; Xu et al.[1]	N/A
Rat Kir2.1 pMax	This paper; Xu et al.[1]	N/A
Software and algorithms		
WinWCP (v5 or newer)	University of Strathclyde	https://spider.science.strath.ac.uk/sipbs/software_ses.htm
GraphPad PRISM (v9 or newer)	Dotmatics	www.graphpad.com
Clampfit (v10 or higher)	Molecular Devices	https://www.moleculardevices.com/products/axon-patch-clamp-system/acquisition-and-analysis-software/pclamp-software-suite
Other		
Injection needles	Drummond Scientific	7 in #3-000-203-g/XL
Borosilicate glass pipettes	World Precision Instruments	TW100F-3
Projected Capacitive Touchscreen Optical Power and Energy Meter Console	Thor Labs	PM400
LED	Luminus Devices	PT-54-TE
Nanoject II	Drummond Scientific	3-000-204
GeneClamp 500 or Warner OC-725C	Molecular Devices (Geneclamp) Warner Instruments (OC-725C)	N/A
Micropipette puller	Sutter Instrument	P-97

MATERIALS AND EQUIPMENT

Oocyte Ringer's 2 (-) Solution

Reagent	Final concentration (mM)	Amount (g)
NaCl	85	4.96
HEPES	5	1.19
KCl	5	0.37
NaOH	5	0.2
$MgCl_2.6H_2O$	1	0.20
Total	**N/A**	**1 L in H_2O**

pH 7.4 with NaOH.
Check osmolarity = 200 mOsm Store at +17°C for 2 months.

Oocyte Ringer's 2 (+) Solution		
Reagent	Final concentration (mM)	Amount (mL)
OR2 (-)	–	988.2
1M $CaCl_2.2H_2O$	1.8	1.8
Pen/Strep	1%	10
Total	**N/A**	**1L**
pH 7.4 with NaOH.		
Check osmolarity = 200 mOsm.		
Store at +17°C for 2 months.		

Recording Pipette filling solution		
Reagent	Final concentration	Amount (g)
KCl	3M	6.7
Agarose	1.4 %	0.42
Total	**N/A**	**30 mL in H_2O**
Store filled electrodes at +4°C, in a humid environment for 2 months.		

Low K^+ (ND96)		
Reagent	Final concentration (mM)	Amount (g)
NaCl	96	5.61
HEPES	5	1.19
KCl	2	0.15
$CaCl_2.2H_2O$	1.8	0.26
$MgCl_2.6H_2O$	1	0.20
Total	**N/A**	**1 L in H_2O**
pH 7.4 with NaOH.		
Store at +4°C for 1–2 months.		

High K^+ (ND96K)		
Reagent	Final concentration (mM)	Amount (g)
NaCl	2	0.12
HEPES	5	1.19
KCl	96	7.16
$CaCl_2.2H_2O$	1.8	0.26
$MgCl_2.6H_2O$	1	0.20
Total	**N/A**	**1 L in H_2O**
pH 7.4 with KOH.		
Store at +4°C for 1–2 months.		

1 M $BaCl_2$		
Reagent	Final concentration (M)	Amount (g)
$BaCl_2$	1	10.41
Total	**N/A**	**50 mL in H_2O**
Store at +4°C for 1–2 months.		
10 mM Ba^{2+} in ND96K.		
To make 50 mL, add 500 µL of 1 M $BaCl_2$ to 49.5 mL of ND96K.		
Store at +4°C for 1–2 months.		

LED light source

Optogenetic activation can be accomplished using an LED light source with an appropriate wavelength and suitable power output. We typically use a 460 nm LED (Luminus PT-54-TE, or equivalent) but a broad range of LEDs between 445–490 nm are also suitable. In all cases, drive the LED with a DC power supply recommended by the LED manufacturer. The LED should be focused on the oocytes using a collimating lens (ThorLabs) until the output of the LED at the level of the oocyte recording chamber is 5 mW/cm^2. This is measured using an optical power and energy meter console (ThorLabs). An appropriate system can also be produced using a commercial flashlight where the LED is pre-mounted in a unit with an appropriate lens and a focusing bezel. Multiple flashlight options are available from a range of high street vendors and are typically cataloged as 'tactical'.

Micropipette puller

Pipettes were pulled using a P-97 micropipette puller (Sutter Instruments) and cut to 10–15 μm in diameter. Similar micropipette pullers can be obtained from other manufactures, for example Narishige (PC-100).

Note: The program we use has the following parameters –

Pressure = 300

Heat = 569

Pull = 10

Velocity = 120

Time = 200

Microinjection apparatus

Microinjection of oocytes is performed using a Nanoject injector from Drummond Scientific. The injector is mounted onto a retort stand at a 45° angle such that the injection needle is pointing downwards. The injector tip is visualized using a dissecting microscope head (any commercial brand), as described below.

STEP-BY-STEP METHOD DETAILS

Microinjection of oocytes with transcribed cRNA

Timing: Day 1 [1–2 h]

The cRNA for each construct that is to be expressed in the oocytes is injected along with appropriate control groups.

1. Select oocytes that are in stage IV or V of maturation.
2. Mix 20 ng each of CIBN-CAAX, CRY2-5-ptase$_{OCRL}$ and rat Kir2.1 RNA in a tube brought up to a final volume of 1 μL with RNAse-free water.

   Note: This recipe allows 20 oocytes to be injected with 50 nL each containing 1ng of each construct.

3. Pull needles for microinjection to an internal diameter of 12–18 microns from glass capillaries.
4. Prefill the injection needle with mineral oil and then fit it onto the injection needle.

   Note: This ensures that there is no air-gap between the needle and the injection mix, allowing accurate volumes to be dispensed with each injection.

5. Pipette the RNA onto a piece of parafilm placed on the sample stage of the injection set-up.
6. Using a dissecting microscope head to visualize the working field, aspirate the RNA drop into the injection needle by holding down the 'fill' button.
7. Pipette oocytes onto a grid filled with OR2(+) and inject each oocyte with 50 nL of the RNA mixture.
8. Following injection, incubate the oocytes for 1–2 days at +17°C in OR2(+) supplemented with 1% Penicillin/Streptomycin in a petri dish.

Note: Cover the petri dish with foil to decrease the exposure to ambient light.

Two-electrode voltage clamp (TEVC)

Timing: Day 2 [3–4 h]

Injected oocytes are evaluated in electrophysiology experiments with blue light illumination to assess the response of expressed proteins to $PI(4,5)P_2$ dephosphorylation.

9. A day prior to the experiment, pull borosilicate glass pipettes (thin-walled glass 1.0 OD/ 0.7 I.D).
   a. Cut pipettes to 10–15 μM in diameter at the tip.
   b. Prefill the recording pipettes with filling solution containing 3 M KCl and 1.5% agarose.

Note: We find that 'agarose-cushioned' recording pipettes facilitate stability in longer experiments by reducing KCl leakage into the oocyte.[4]

10. Set-up for TEVC by priming the perfusion apparatus.
    a. Flush the perfusion tubes with warm water.
    b. Add low-K (96 mM Na^+), high-K (96 mM K^+) and 10 mM $BaCl_2$ made up in high-K to separate reservoirs in the perfusion set-up.
    c. Prime their flow to attain a flow rate of 1–2 drops per second.
    d. Adjust the bath aspiration to obtain a quick exchange rate of the bath solution with the perfusate.
    e. Orient the blue light apparatus toward the oocyte recording chamber.
11. To perform a two-electrode voltage-clamp experiment, open the recording program WinWCP and create a -80 to +80 mV ramp protocol (Figure 1) by navigating from **Setup** > **Stimulus/recording protocol editor**.
    Stimulus/recording protocol editor:
    a. On the left panel set stimulus type to 'voltage' and the holding potential to 0 mV.
    b. Under the 'Recording' tab, change the recording duration to **360 ms**, with a stimulus repeat period of 1 s and no. of records set to 10000.
    c. Under the '**Stimulus**' tab, drag AO waveforms **step** (Ⅱ), **ramp** (/) and **step** (Ⅱ) from the toolbox into the boxes under the '**protocol**' section.
    d. Amend the details for each part of the protocol as shown in Figure 1.
    e. Save this protocol in a folder and close the protocol editor.
    f. From the overhead menu, navigate to **record > record to disk** and select the folder in which the protocol is saved and then select the protocol in the protocol drop-down menu.
12. Place the pre-filled glass pipettes over the silver electrode wires.
    a. Lower the pipettes into the bath solution and compensate the pipette junction potential by turning the appropriate amplifier knobs corresponding to each electrode.
    b. Check the resistance of the electrodes on the recording amplifier, this should fall in the range of ~0.2–1 MΩ.
13. Place an oocyte into the recording chamber and insert both recording pipettes into the oocyte under low-K^+ perfusion.
14. Create a new data file in WinWCP (**file > new data file**) and name the file.

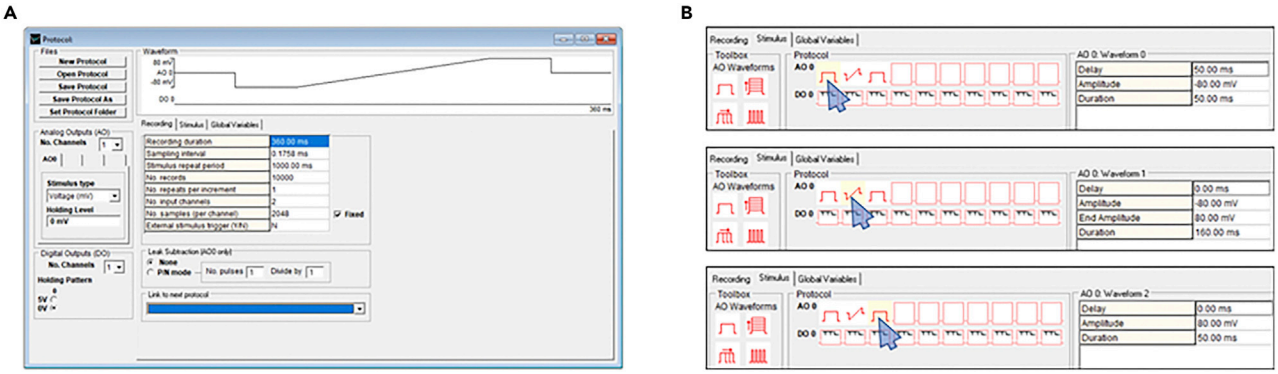

Figure 1. WinWCP screenshots to create a voltage-ramp recording protocol

(A) Recording details entered in each field.

(B) Waveform details entered by dragging hold and ramp waveforms into relevant boxes. Blue arrows indicate the box that needs to be clicked to enter details of that waveform to the right of the window.

15. Hit 'record'.
16. After the current stabilizes under low-K^+, switch the perfusion to high-K^+ and wait for the increase in current to stabilize.
17. After current in high-K^+ has stabilized, turn-on the blue LED to activate the phosphatase.
18. After the current decay pursuant to blue-light activation reaches steady-state, switch the perfusion to 10 mM Ba^{2+} in high-K.

Note: Barium is a blocker of Kir2.1 channels and removes any residual Kir2.1 channel current from the remaining signal. The residual current after barium block corresponds to non-specific 'leak' current in the system that can then be subtracted during subsequent data analysis.

19. Allow the current following barium block to stabilize to a steady state level before ending the recording.
20. To begin recording from the next oocyte, start again from step 7.

⚠ CRITICAL: Chloride the silver wires used for recording as well as bath electrodes by placing them in commercial bleach for ~5 min. Wires should then be rinsed in water and dried before they are used in experiments. Optimize the setting on the 'blue' LED to attain close to 5 mW/cm^2 of power for optimal recruitment of the phosphatase to the oocyte membrane. In our experience, it is not critical to orient the oocyte in a specific way (i.e., animal, or vegetal pole) to obtain robust, reproducible responses from Kir2.1 channels. However, other channels of interest might have a more specific distribution that requires optimization of oocyte placement relative to the incident light source. Please see the limitations section of this protocol for more information.

Data analysis

Timing: Day 3 [2–3 h]

21. Open the file in WinWCP.
22. Go to analysis > waveform measurement.
    a. Under the analysis tab, drag both olive colored cursors at the edges of the waveform window and place them over the section of the waveform corresponding to the -80 mV step in the voltage protocol, away from the capacitance spikes.
    b. Without changing any other window settings, click 'Do Analysis' on the left panel.

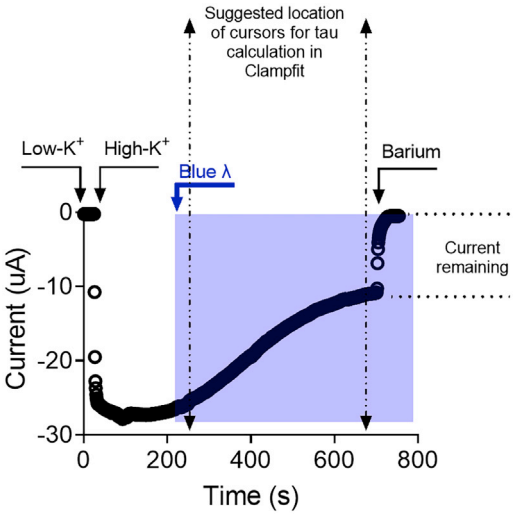

Figure 2. Expected time-course of Kir2.1 current obtained at the -80 mV phase of consecutive voltage ramps

Upon illumination with blue light, a contemporaneous decrease in Kir2.1 channel activity is initiated that corresponds to an inhibition of the current caused by dephosphorylation of $PI(4,5)P_2$. The inhibition reaches a plateau and leaves behind residual current that is blocked by 10 mM barium.

c. Under the tables tab, select only 'record' and 'average' and click 'add variable' to populate current values in the table.
d. Export data to a spreadsheet using edit > copy data.
e. To make the data compatible with Clampfit software (Molecular Devices), select file > export > (output format Axon ABF) OK. The file will be saved with a '*.abf' extension in the same folder as the source file.

⚠ CRITICAL: Update the WinWCP software to its latest version from the University of Strathclyde website.

EXPECTED OUTCOMES

Kir2.1 channels are inwardly rectifying, and currents obtained in TEVC recordings should be negative, signifying the inward flow of potassium ions under the recording conditions used. Blue light illumination should result in the activation of $5ptase_{OCRL}$ and the subsequent inhibition of Kir2.1 channel activity (Figure 2). This will result in a time-dependent decay in the magnitude of the Kir2.1 current.

QUANTIFICATION AND STATISTICAL ANALYSIS

1. Create time-course graphs from the exported data by plotting the current (average) on the y-axis and the time (record) on the x-axis (Figures 2 and 3).
2. Calculate average current for each manipulation at the steady state level, i.e., high-K^+ response is calculated by averaging the current obtained for five sweeps before blue light illumination. Calculate average currents for high-K^+, blue light and barium.
3. Subtract the current obtained after the barium block from all the other current averages to obtain the barium sensitive current for each recording.
4. To calculate %current reduction following blue light activation:
   a. Subtract the barium-sensitive current decay at steady-state: *I(blue)* from the barium-sensitive high-K+ current: *I(Basal)*.

$$\Delta I = I_{Basal} - I_{blue}.$$

   b. % Current reduction = $(\Delta I / I_{(Basal)}) \times 100$.

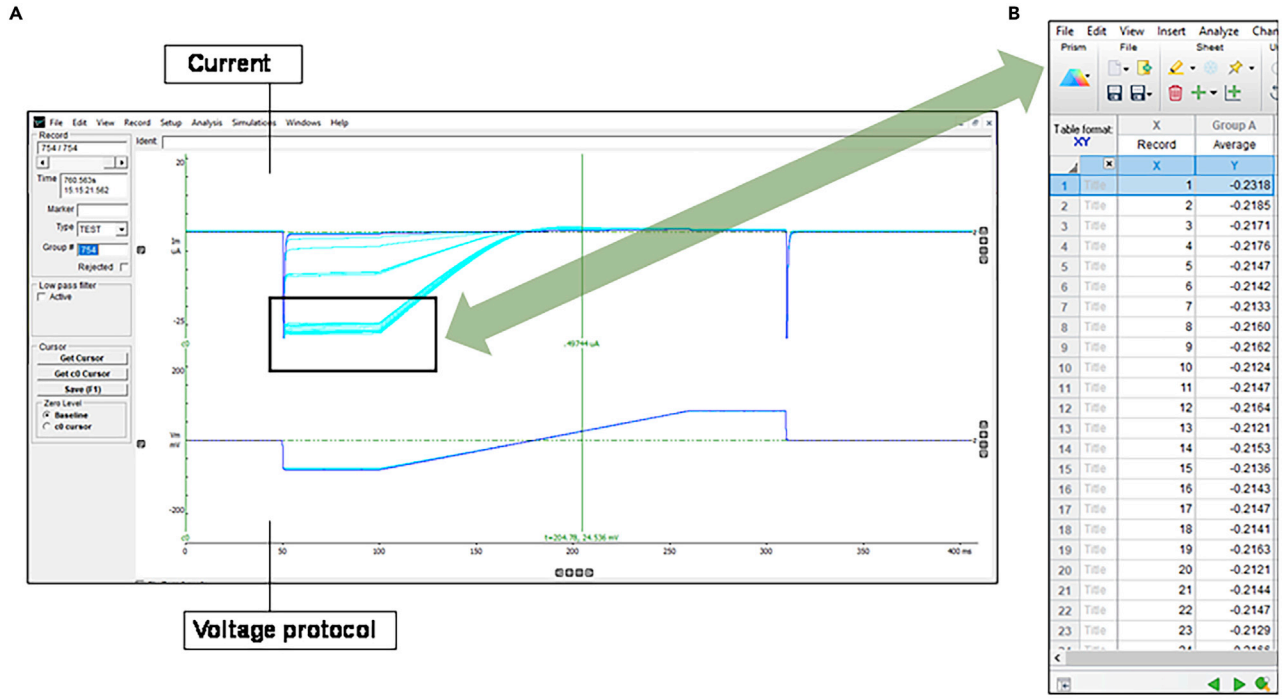

Figure 3. Raw current data from Kir2.1 channels

(A and B) Example of raw current data from Kir2.1 channels shown as the overall of all sweeps from a single time course study in WinWCP software, in (A), and after transferring the data values from the - 80 mV portion of each consecutive sweep to Graphpad Prism (B).

c. Average values of %current reduction across several oocyte recordings are reported as summary data. Typically, smaller values indicate stronger affinity for PI(4,5)P_2 and greater values indicate diminished PI(4,5)P_2 sensitivity.

5. To calculate the time-constant of inhibition (τ) following blue light activation:
   a. Open the .abf file in Clampfit.
   b. Drag cursors 1 & 2 over the current trace corresponding to the -80 mV step.
   c. Go to analyze > quickgraph > I-V…
      i. In the pop-up window, select 'user list' in the dropdown against the 'Epoch' field.
      ii. Minimize the I-V window that pops-up and go to window > results.
      iii. Select the data and click analyze > create graph, to display a time course.
   d. Use this graph for the pursuant analyses.
      i. Click on the 'select region' tool in toolbar to display two, vertical cursors.
      ii. Drag and place one cursor at the start of current inhibition on this graph and place the other cursor at a point at which current inhibition reached steady state.
      iii. Click the *fx* tool in the toolbar.
      iv. Select the Boltzmann, standard function with 'Number of terms' set to 1 for a one-phase decay fit.
      v. Click 'OK'.
   e. Navigate to the results window; window > results.
   f. The time constant of current decay is populated under the 'tau' column.
   g. Summary data are represented in the form of bar graphs containing τ values for several oocytes.
   h. A faster τ indicates less channel affinity for PI(4,5)P_2 compared to slower τ.

LIMITATIONS

Successful utilization of this protocol is predicated on the use of healthy oocytes that can be employed to generate any standard TEVC data in the lab. For example, the currents under investigation

are of typical magnitude, the cells have a low background leak conductance, and these characteristics can be maintained for the duration of the optogenetic study. Issues impacting these parameters are somewhat variable depending on the ion channel under investigation. Users might need to consider that the distinct expression patterns of some ion channels might require a specific orientation of the oocyte relative to the photoactivating incident light to observe reproducible effects on the current. We have not found this to be necessary for Kir2.1 or Kir3 channels.

TROUBLESHOOTING

Problem 1

No apparent, or reduced activity of the CRY2-5-ptase$_{OCRL}$ system or no response to blue light or indistinguishable from current run-down.

Potential solution

- Check the power of the incident light from the blue LED at the level of the recording chamber using the power meter (adjust to 5 mW/cm^2) and avoid physical obstacles, such as wires and the recording pipettes, that could reduce the transmission of the light to the oocyte [related to step 10(e)].
- Increase the power of the blue LED or its proximity to the recording chamber [related to step 10(e)].
- Increase the amount of CIBN-CAAX, CRY2-5-ptase$_{OCRL}$ cRNA injected or decrease the amount of channel expressed in the oocyte [related to step 2].
- Consider if the oocytes have been pre-exposed to blue-light prior to the experiment. Cover the oocyte dish in foil and leave for 30–35 min before trying the experiment again. This should be adequate time for the CRY2-5-ptase$_{OCRL}$ to disengage from CIBN at the plasma membrane and for PI(4,5)P_2 to regenerate [related to step 8].
- Set up control experiments with Kir3.4 (GIRK4) to ensure that the optogenetic system is functional. Although Kir3 channels typically pass smaller currents that Kir2.1, Kir3.4 has a lower affinity for PI(4,5)P_2[5] and typically shows more rapid decrease in currents (faster time constant for current decay) following acute PI(4,5)P_2 dephosphorylation. This will help in determining whether the system is operational.
- Set up control experiments with Kir2.1 alone to check that it produces the expected levels of current in the absence of the CIBN-CAAX, CRY2-5-ptase$_{OCRL}$ cRNA. If so, consider remaking the CIBN-CAAX, CRY2-5-ptase$_{OCRL}$ cRNA and troubleshooting the current by injecting Kir2.1 with CIBN-CAAX and separately with CRY2-5-ptase$_{OCRL}$ in the absence of blue light stimulation [related to step 8].
- Check the orientation of the oocyte. Some channels show clear expression preferences on one pole (typically the animal, or pigmented pole) and the oocytes might be oriented so that this portion of the membrane does not receive direct or adequate illumination from the LED. In our hands, we have not found oocyte orientation to be an issue in our studies of Kir2.1 or Kir3.4. Typically, we use a clear acrylic recording chambers with a low profile which, when coupled with reflection in the system, results in illumination of a significant portion of the oocyte surface. Appropriate chambers can be purchased from Automate Scientific (Oocyte Perfusion Chamber), or Warner Instruments, or can be fabricated in the lab from a 35 mm petri dish. We have not found substantive differences in results between such chambers when studying Kir2.1.
- Standard commonplace troubleshooting factors include checking and titrating the procedures for obtaining and digesting the oocytes (e.g., collagenase treatment, see below), the composition, osmolarity and pH of the solutions used, and the temperature of the oocyte incubator. To this end, we typically produce and separately maintain oocytes from two frogs for each experimental trial to avoid issues that can arise from variability in animal health.
- New batches of collagenase can have different intrinsic activity and sometimes contain, additional contaminants that increase or decrease their overall enzymatic activity. When using a new batch of collagenase for the first time, it is important to monitor digestion of the oocytes with increased

frequency. This can be achieved by removing a sample of the oocytes every 10 min and inspecting the extent of digestion visually using a dissecting microscope. Where possible, it is preferred to order new collagenase in advance of running out of the current batch, this allows a 'head-to-head' comparison to make sure that the new batch performs according to expectation.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Leigh D. Plant: l.plant@northeastern.edu.

Materials availability

Plasmids generated in this study are available upon request from the lead contact.

Data and code availability

This study did not generate/analyze datasets/code.

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AUTHOR CONTRIBUTIONS

K.D.G. and B.T.W. contributed to writing and editing the manuscript. Y.X. performed the experiments that generated the raw data presented in this manuscript. M.M., T.K., and H.V. contributed to development and troubleshooting the technology used in this manuscript. L.D.P. contributed to conception of the project, editing the manuscript, and providing funding.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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