

Submission ID #: 69903

Scriptwriter Name: Sri Lakshmi Priya

Project Page Link: <https://review.jove.com/account/file-uploader?src=21320463>

**Title: Single-Channel Electrophysiology Using a Miniaturized Montal–Mueller Planar Lipid Bilayer System for Recording a Synthetic K2P Channel**

**Authors and Affiliations:**

Deborah Musso<sup>1</sup>, Alessandro Porro<sup>2</sup>, Cristina Arrigoni<sup>1</sup>, Federico Thei<sup>2</sup>, Marco Lolicato<sup>1</sup>

<sup>1</sup>Department of Molecular Medicine, University of Pavia

<sup>2</sup>Elements srl, Viale Europa 596

**Corresponding Authors:**

|                |                                      |
|----------------|--------------------------------------|
| Deborah Musso  | deborah.musso01@universitadipavia.it |
| Marco Lolicato | marco.lolicato@unipv.it              |

**Email Addresses for All Authors:**

|                   |                                      |
|-------------------|--------------------------------------|
| Deborah Musso     | deborah.musso01@universitadipavia.it |
| Alessandro Porro  | aporro@elements-ic.com               |
| Cristina Arrigoni | cristina.arrigoni01@unipv.it         |
| Federico Thei     | fthei@elements-ic.com                |
| Marco Lolicato    | marco.lolicato@unipv.it              |

## **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**
- 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. **NO**

### **Current Protocol Length**

Number of Steps: 10

Number of Shots: 37

# Interviews

---

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

## INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Marco Lolicato:** Our research focuses on understanding how ion channels and transporters function using integrative structural biology and electrophysiological techniques.
  - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Deborah Musso:** Conventional bilayer setups require complex instrumentation and manual assembly, whereas this is a miniaturized, ready-to-use platform integrating a compact amplifier.
  - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

## CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Marco Lolicato:** This protocol allows researchers to measure purified ion channels using a miniaturized electrophysiological system applicable to any channel.
  - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Deborah Musso:** The important consideration is ensuring a proper electrical connection between BLMchipMM and the amplifier, avoiding chlorination of silver wire ends.
  - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Are there any additional methods or analyses that can be performed following this procedure?~~

1.5. **Deborah Musso:** Additionally, following this procedure, researchers can investigate how potential modulators alter ion transport through the reconstituted ion channel.

1.5.1. 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.***

# Protocol

---

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

NOTE: SCREEN shot timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator Pallavi Sharma for queries regarding lab media.

## 2. Preparation of the Electrophysiology Setup

**Demonstrator:** Deborah Musso

- 2.1. To begin, prepare the electrophysiology setup by performing instrument calibration [1] and preparing the silver or silver chloride electrodes [2].
  - 2.1.1. WIDE: Shot of the electrophysiology setup on the laboratory bench and the talent performing instrument calibration. ***Videographer:** Please obtain CU shot as well for this shot.*
  - 2.1.2. Talent arranging the silver or silver chloride electrodes beside the electrophysiology setup.
- 2.2. Insert the unchlorinated terminal ends of the two silver wires into the printed circuit board or PCB (P-C-B) sockets [1]. Place the chlorinated ends of the wires into the fluidic channels drilled in the polymethyl methacrylate OR PMMA (P-M-M-A) component [2]. Gently rotate the black lipid membrane chip or BLMchipMM (B-L-M-chip-M-M) and mount it onto the PCB by fitting the bottom tooth of the PMMA recording chamber into the dedicated slot [3]. Ensure the wire reaches the end of the fluidic channels [4].
  - 2.2.1. WIDE: Talent inserting the unchlorinated ends of the silver wires into the PCB sockets.
  - 2.2.2. Talent placing the chlorinated wire ends into the fluidic channels of the PMMA component.
  - 2.2.3. Talent rotating the BLMchipMM and mounting it onto the PCB by fitting the bottom tooth of the PMMA recording chamber into the dedicated slot
  - 2.2.4. Close-up shot of the wires reaching the end of the fluidic channels.
- 2.3. Prepare 100 microliters of a 1% volume per volume hexadecane solution in 100% n-hexane (N-hexane) for septum aperture pre-treatment [1]. Resuspend 1,2-diphytanoyl-sn-glycero-3-phosphocholine or DPhPC (D-P-H-P-C) lipids in 100% n-pentane (N-pentane) to a final concentration of 15 milligrams per milliliter [2]. Prepare the electrolyte recording solution at room temperature by mixing 3 milliliters of 1 molar potassium chloride stock solution with 150 microliters of 1 molar HEPES stock solution

[3]. Add double-distilled water to bring the final volume to 15 milliliters and store the solution at 4 degrees Celsius for up to 1 week [4].

- 2.3.1. Close-up shot of the talent pipetting hexadecane into a microcentrifuge tube containing n-hexane
- 2.3.2. Talent resuspending DPhPC lipids in n-pentane.
- 2.3.3. Talent mixing potassium chloride and HEPES stock into a bottle.
- 2.3.4. Talent adding double-distilled water to the recording solution.

### **3. Formation of the Planar Lipid Bilayer Membrane**

3.1. Using a pipette tip, pre-treat the septum aperture with 2 microliters of 1% volume per volume hexadecane solution in n-hexane on each side [1]. Allow the solvent to evaporate for 5 minutes at 23 to 25 degrees Celsius and 45 to 50% relative humidity [2]. Insert the assembled BLMchipMM into the amplifier [3]. Connect the amplifier to the computer via a USB cable, then open the acquisition software [4]. Set the current range to 2 nanoamperes and activate the resistance-capacitance estimation tool to monitor the membrane capacitance and resistance [5].

- 3.1.1. Talent applying hexadecane solution to both sides of the septum aperture using a pipette tip.
- 3.1.2. Close-up shot of the septum aperture during solvent evaporation.
- 3.1.3. Talent inserting the assembled BLMchipMM into the amplifier.
- 3.1.4. Talent connecting the amplifier to the computer with a USB cable and opening the acquisition software.
- 3.1.5. SCREEN: SCREEN-3.1.5.mp4: 00:02-00:12

*Videographer: Please film the computer screen as a back up*

3.2. Using a 0.2 micrometer syringe filter, filter the recording solution [1]. Fill each compartment with 30 microliters of recording solution up to the lower margin of the aperture without wetting the surrounding region [2]. Spread approximately 2.5 microliters of the lipid solution dropwise onto the aqueous surface in each compartment [3], then allow the solvent to evaporate for 2 minutes [4]. Place a pipette tip at the bottom of one compartment at approximately a 90-degree angle, add approximately 80 microliters of recording solution, and repeat for the second compartment [5].

- 3.2.1. Talent filtering the recording solution using a syringe filter.

- 3.2.2. Close-up shot of the talent filling both compartments with recording solution up to the aperture lower margin.
- 3.2.3. Talent adding lipid solution dropwise onto the aqueous surface in each compartment.
- 3.2.4. Close-up shot of the compartments during solvent evaporation.
- 3.2.5. Talent adding recording solution into the compartments using a pipette tip positioned at a 90 degree angle.
- 3.3. Close the Faraday cage lid and monitor the current signal [1]. Apply a triangular voltage waveform of 100 millivolts at 10 hertz to assess membrane capacitance [2]. Apply voltage steps in 20-millivolt increments, up to plus or minus 200 millivolts, to assess membrane resistance [3]. Monitor the current and noise values using a 5-kilohertz sampling rate and 2.5-kilohertz low-pass filtering [4].
  - 3.3.1. Shot of the talent closing the Faraday cage lid beside the electrophysiology setup.
  - 3.3.2. SCREEN: SCREEN-3.3.2.mp4; 00:00-00:20
  - 3.3.3. SCREEN: SCREEN-3.3.3-(From-20-to-200-mV).mp4; 00:17-00:20, 03:10-03:20
  - 3.3.4. SCREEN: SCREEN-3.3.4.mp4; 00:02-00:19

#### **4. Recording ancTREK1 Ion Channel Activity**

- 4.1. Prepare the ancTREK1 (*anc-trek-one*) expression construct using cloning and transformation methods [1]. Express ancTREK1 in 293F (*two-ninety-three-F*) cells using a vector containing GFP and His8 (*His-eight*) tags [2].
  - 4.1.1. Shot of the talent arranging cloning reagents on the laboratory bench beside a thermal cycler and agar plates.
  - 4.1.2. Shot of suspension 293F cell culture flasks shaking while the talent adds the transfection mixture dropwise.
- 4.2. Solubilize membrane proteins using detergent-containing lysis buffer [1]. Purify the ancTREK1 protein using nickel-nitrilotriacetic (*nickel-nitrilo-tri-acetic*) acid and anti-GFP affinity purification methods [2]. Concentrate and store the purified ancTREK1 protein at minus 80 degrees Celsius [3].
  - 4.2.1. Talent adding detergent-containing lysis buffer to the cell pellet.
  - 4.2.2. Talent loading the protein sample onto a gravity purification column and collecting the elution fractions in tubes.
  - 4.2.3. Talent placing the microcentrifuge tube containing the concentrated purified

protein sample into cold storage.

- 4.3. Dilute ancTREK1 protein in recording solution to approximately 2 micrograms per milliliter [1]. Add 1 microliter of diluted protein solution to the cis chamber near the septum aperture [2]. Close the Faraday cage lid before recording [3]. Apply voltage steps in plus or minus 20 millivolt increments up to plus or minus 120 millivolts [4]. Monitor for discrete current transitions indicating ion channel insertion events [5].
  - 4.3.1. Close-up shot of pipetting recording solution into the tube containing the ancTREK1 protein.
  - 4.3.2. Close-up shot of adding diluted protein solution into the cis chamber near the septum aperture.
  - 4.3.3. Shot of the talent closing the Faraday cage lid beside the electrophysiology setup.
  - 4.3.4. SCREEN: SCREEN-4.3.4--4.3.5-Protein-(From-20-to-120-mV).mp4: 00:02-00:05, 00:56-01:00
  - 4.3.5. SCREEN: SCREEN-4.3.4--4.3.5-Protein-(From-20-to-120-mV).mp4: 01:43-01:45, 01:51-01:54,

# Results

---

## 5. Results

- 5.1. The I–V (*i-v*) relationship showed linear behavior up to plus or minus 200 millivolts, with high membrane resistance in both 75 [1] and 100 micrometer apertures [2].
  - 5.1.1. LAB MEDIA: Figure 8A. *Video editor: Emphasize the yellow circle data points labeled as BLMchip 75um*
  - 5.1.2. LAB MEDIA: Figure 8A. *Video editor: Emphasize the green square data points labeled as BLMchip 100um*
- 5.2. RMS noise remained low, and membranes stayed stable for over 1 hour [1] without instability up to plus or minus 200 millivolts [2].
  - 5.2.1. LAB MEDIA: Figure 8B.
  - 5.2.2. LAB MEDIA: Figure 8A. *Video editor: Emphasize the green and yellow datapoints at 200 mV.*
- 5.3. Single-channel recordings revealed the characteristic flickering behavior of two-pore domain potassium or K<sub>2P</sub> (*K-two-P*) channels [1].
  - 5.3.1. LAB MEDIA: Figure 9A, 9B.
- 5.4. Current amplitude increased from 13.02 picoampere at 80 millivolt [1] to approximately 18.72 picoampere at minus 80 millivolt [2].
  - 5.4.1. LAB MEDIA: Figure 9C. *Video editor: Emphasize the tallest histogram peak in the graph labeled +80mV*
  - 5.4.2. LAB MEDIA: Figure 9C. *Video editor: Emphasize the tallest histogram peak in the graph labeled -80mV*
- 5.5. Measurable single-channel currents were observed at both positive [1] and negative potentials under symmetric potassium conditions [2].
  - 5.5.1. LAB MEDIA: Figure 9D. *Video editor: Emphasize the plotted data points on the right side of the graph.*
  - 5.5.2. LAB MEDIA: Figure 9D. *Video editor: Emphasize the plotted data points on the left side of the graph.*
- 5.6. ancTREK1 exhibited a higher normalized open probability than TREK1 (*trek-one*) measurements obtained with excised patch configurations [1].
  - 5.6.1. LAB MEDIA: Figure 10. *Video editor: Emphasize the data points on the right side of the graph from 0 to 120 mV.*

5.7. The NPo–voltage (*N-P-O-voltage*) relationship showed increased channel activity at positive potentials, reaching almost 0.78 at 80 millivolt [1] while decreasing to approximately 0.53 at minus 80 millivolt [2] and 0.28 at minus 120 millivolt [3].

5.7.1. LAB MEDIA: Figure 10. *Video editor: Emphasize the plotted point at positive 80 millivolt.*

5.7.2. LAB MEDIA: Figure 10. *Video editor: Emphasize the plotted point at minus 80 millivolt.*

5.7.3. LAB MEDIA: Figure 10. *Video editor: Emphasize the plotted point at minus 120 millivolt.*

1. Electrophysiology  
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophysiology>  
IPA: /ɪˌlek.troʊˌfɪz.iˈɑː.lə.dʒi/  
Phonetic Spelling: ihˌlekˌtrohˌfizˌeeˌolˌuhˌjee
2. Silver chloride  
Pronunciation link: <https://www.merriam-webster.com/dictionary/silver%20chloride>  
IPA: /ˈsɪl.vər ˈklɔːr.aɪd/  
Phonetic Spelling: silˌver klorˌide
3. Unchlorinated  
Pronunciation link: No confirmed link found  
IPA: No verified IPA available  
Phonetic Spelling: unˌklorˌuhˌnayˌtid
4. Polymethyl methacrylate  
Pronunciation link: <https://www.merriam-webster.com/dictionary/polymethyl%20methacrylate>  
IPA: /ˌpɑː.liˈmeθ.əl məˈθæk.rə.leɪt/  
Phonetic Spelling: polˌeeˌmethˌuhl muhˌthakˌruhˌlayt
5. Hexadecane  
Pronunciation link: <https://www.merriam-webster.com/dictionary/hexadecane>  
IPA: /ˈhek.səˌdeɪn/  
Phonetic Spelling: hekˌsuhˌdayn
6. Diphytanoyl  
Pronunciation link: No confirmed link found  
IPA: No verified IPA available  
Phonetic Spelling: dyeˌfyˌtanˌoyl
7. Phosphocholine  
Pronunciation link: <https://www.merriam-webster.com/medical/phosphocholine>  
IPA: /ˌfɑːs.foʊˈkoʊ.liːn/  
Phonetic Spelling: fosˌfohˌkohˌleen
8. HEPES  
Pronunciation link: <https://www.howtopronounce.com/hepes>  
IPA: /ˈhep.ez/  
Phonetic Spelling: hepˌez
9. Electrolyte  
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrolyte>  
IPA: /ɪˌlek.troˌlaɪt/  
Phonetic Spelling: ihˌlekˌtruhˌlyte
10. Bilayer  
Pronunciation link: <https://www.merriam-webster.com/dictionary/bilayer>  
IPA: /ˈbaɪˌleɪ.ər/  
Phonetic Spelling: byeˌlayˌer
11. Capacitance  
Pronunciation link: <https://www.merriam-webster.com/dictionary/capacitance>  
IPA: /kəˈpæs.ə.təns/  
Phonetic Spelling: kuhˌpassˌuhˌtuhns

12. Faraday  
Pronunciation link: <https://www.merriam-webster.com/dictionary/Faraday>  
IPA: /'fer.ə.deɪ/  
Phonetic Spelling: fair·uh·day
13. Solubilize  
Pronunciation link: <https://www.merriam-webster.com/dictionary/solubilize>  
IPA: /sə'lu:.bəˌlaɪz/  
Phonetic Spelling: suh·loo·buh·lyze
14. Nitriiotriacetic  
Pronunciation link: <https://www.howtopronounce.com/nitriiotriacetic>  
IPA: /naɪˌtraɪˌlooˌtraɪ.ə'si:.tɪk/  
Phonetic Spelling: ny·try·loh·try·uh·see·tik
15. Affinity  
Pronunciation link: <https://www.merriam-webster.com/dictionary/affinity>  
IPA: /ə'fɪn.ə.ti/  
Phonetic Spelling: uh·fin·uh·tee
16. ancTREK1  
Pronunciation link: No confirmed link found  
IPA: No verified IPA available  
Phonetic Spelling: ank·trek·one
17. Transfection  
Pronunciation link: <https://www.merriam-webster.com/dictionary/transfection>  
IPA: /træn'sfekʃən/  
Phonetic Spelling: trans·fek·shuhn
18. Flickering  
Pronunciation link: <https://www.merriam-webster.com/dictionary/flicker>  
IPA: /'flɪk.ə.ɪŋ/  
Phonetic Spelling: flik·er·ing
19. Symmetric  
Pronunciation link: <https://www.merriam-webster.com/dictionary/symmetric>  
IPA: /sɪ'met.rɪk/  
Phonetic Spelling: sih·met·rik
20. Excised  
Pronunciation link: <https://www.merriam-webster.com/dictionary/excise>  
IPA: /ɪk'saɪzd/  
Phonetic Spelling: ik·syzd