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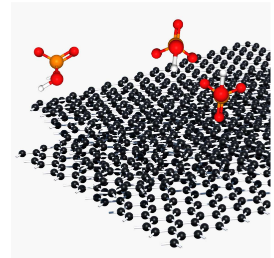
Fabrication of dip-and-read orthophosphate chemosensor based on laser inscribed graphene



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We use this protocol and it's working

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Disclaimer

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Abstract

This protocol describes the fabrication of a LIG dip-and-read orthophosphate sensor based on a 2 dimensional sorbent structure (poly[diallydimethylammonium] chloride, a.k.a, PolyDADAMAC) grafted on graphene oxide (GO) as recognition element. The ortho-P sensor uses electrochemical impedance spectroscopy (EIS) as a transduction technique, and laser-induced graphene electrodes as a transducer platform. The process requires approximately 5 hours (from sensor fabrication to testing)

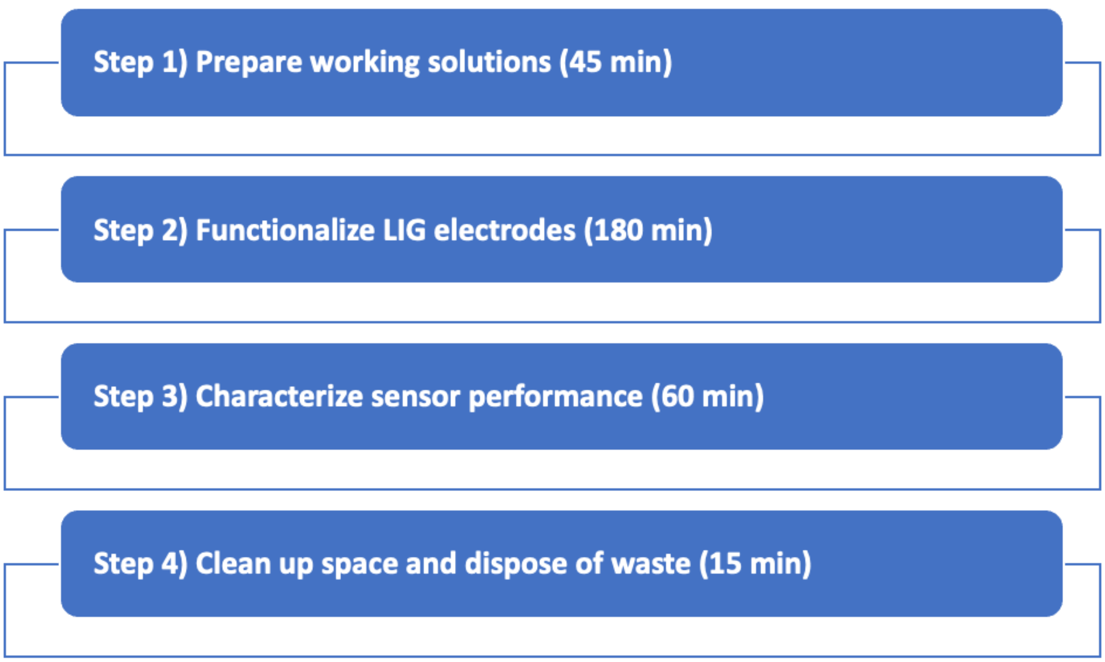

Figure 1. Process flow for fabrication of a LIG dip-and-read orthophosphate sensor.

Image Attribution

Image created in Blender.

Guidelines

1. Be sure to wear appropriate safety PPE throughout (lab coat, gloves, eyewear).
2. Electronic or physical lab notebook may be used throughout

Materials

MATERIALS

- LIG-chip (see protocols for fabrication of chip)
- GO-PDDA solution
- Phosphate standard solution (KH_2PO_4 in H_2O 1000 mg/l PO_4) (**link** to MSDS)
- Nitrate standard solution ($NaNO_3$ in H_2O 1000 mg/l NO_3) (**link** to MSDS)
- Chloride standard solution (NaCl in H_2O 1000 mg/l Cl) (**link** to MSDS)
- Sulfate standard solution (Na_2SO_4 in H_2O 1000 mg/l SO_4) (**link** to MSDS)
- Isotonic sodium chloride/sodium bicarbonate (NaCl/NaHCO3) solution (or other P-free buffer of choice) (**link** to MSDS)
- Potassium ferrocyanide/ferricyanide see details in protocol by Tang et al (**Link**)
- Glassware as noted

HARDWARE

- CO_2 laser (VLS2.30DT)
- MultiPalmSens4 potentiostat
- Vortex mixer
-

Safety warnings

SAFETY

General

- Lab coat, gloves, and closed-toed shoes are mandatory

Disposal

- All solutions should be discarded in appropriated waste container and not disposed of in the sink.
- Spent GO-PDDA solutions should be collected in the satellite area and disposed following local environmental health and safety guidelines
- Used LIG sensors should be collected as solid waste and disposed following local environmental health and safety guidelines

ADA COMPLIANCE

The following guidance is summarized from Perry and Baum 1 where relevant to this protocol.

1) General building codes for laboratory

- Minimum 2 Exits for labs ≥500 sf (150 sm) net area.
- Minimum 2 Exits for labs using chemical fume hoods or glove box
- Minimum 2 Exits for labs using flammable and combustible: liquids, gases, cryogenics, dusts and solids.
- Minimum 2 Exits for labs using oxidizers, unstable reactives, water reactives, organic peroxides, highly toxics, corrosives.

2) Egress for wheelchair 360° Turn is 1.5 m (5 ft) clearance. Wheelchair clearance must be provided for:

- Both sides of Exit and Entry doors to
- Emergency Eyewash & Safety Shower
- In front of wall benches, sinks, equipment
- In front of chemical fume hoods
- At chalk/marker board
- Between benches
- Aisles that lead to Primary Exits, back to front
- Aisles that allow passage side to side in lab

3) Standard accommodations for use of chemical hood or other exhaust air containment systems

- knee space obstructions
- adjustable work surface height
- accessible receptacles and alarm control

Common equipment

- Where visual inspection is utilized, alternative technologies should be listed as optional (colorimeters, spectro-radiometers, etc.)

References
1.Perry, J. & Baum, J. Assessing the Laboratory Environment. in Accessibility in the Laboratory vol. 1272 3–25 (American Chemical Society, 2018).

Ethics statement

N/A

Before start

Other protocols required

- LIG fabrication (**Link**)
  LIG 3-electrode plug-and-play chip (**Link**)
- Cyclic voltammetry using ferrocyanide/ferricyanide couple (**Link**)

Step 1) Prepare working solutions

45m

1 Prepare Solution 1: 1 mg/mL GO-PDDA solution

- Prepare a working solution for GO-PDDA at 1 mg/mL by diluting the stock solution (5 mg/mL) in DI water.
- Agitate using a vortex mixer until the solution is dispersed

2 Prepare Solution 2: 2X Sodium chloride/sodium bicarbonate ($NaCl/NaHCO_3$) solution

- Add two packets of buffer (isotonic buffer pouches) to 240 mL of DI water.
- Agitate with vortex mixer until dissolved

3 Prepare Solution 3: 100 mg/L phosphate, nitrate, sulfate, chloride salt solutions

- Dilute standard solutions of phosphate, nitrate, sulfate and chloride (1000 mg/L) in DI water to get a final concentration of 100 mg/L for each ion separately.
- Working solutions may be prepared in 1.5 mL microcentrifuge tubes if desired

Step 2) Functionalize LIG electrodes

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- Follow LIG fabrication protocol (**Link**)
- Next, prepare 3-electrode plug-and-play chip using the published protocol (**Link**)
- After fabricating LIG electrodes, follow CV protocol (**Link**)
- Follow the quality control (QC) guideline in Qian et al (**Link**) to select triplicates

Note

- To select triplicates, make sure to plot the selected voltammograms of electrodes grouped in a cluster by Qian et al (**Link**). This approach will guarantee that you are selecting electrodes that overlap in their voltammograms. See **Figure 1A-C** for expected results

- Pipette 10 mL of GO-PDDA working solution (1 mg/mL) onto the LIG working electrode. Be careful that the solution does not coat reference and/or counter electrodes.
- Allow the GO-PDDA working solution to dry at room temperature for at least 20 minutes.
- Repeat these steps two more times; pipette 10 mL of GO-PDDA solution and then dry at room temperature.

Note

Gently rinse the surface of the working electrode to remove any loose material from the surface.

- After LIG functionalization, run an additional CV test in ferro/ferricyanide solution (**Link**) to confirm surface coating is stable. See **Figure 1D** for expected results

- If available, use a stereo microscope to image the surface and inspect for homogenous coating

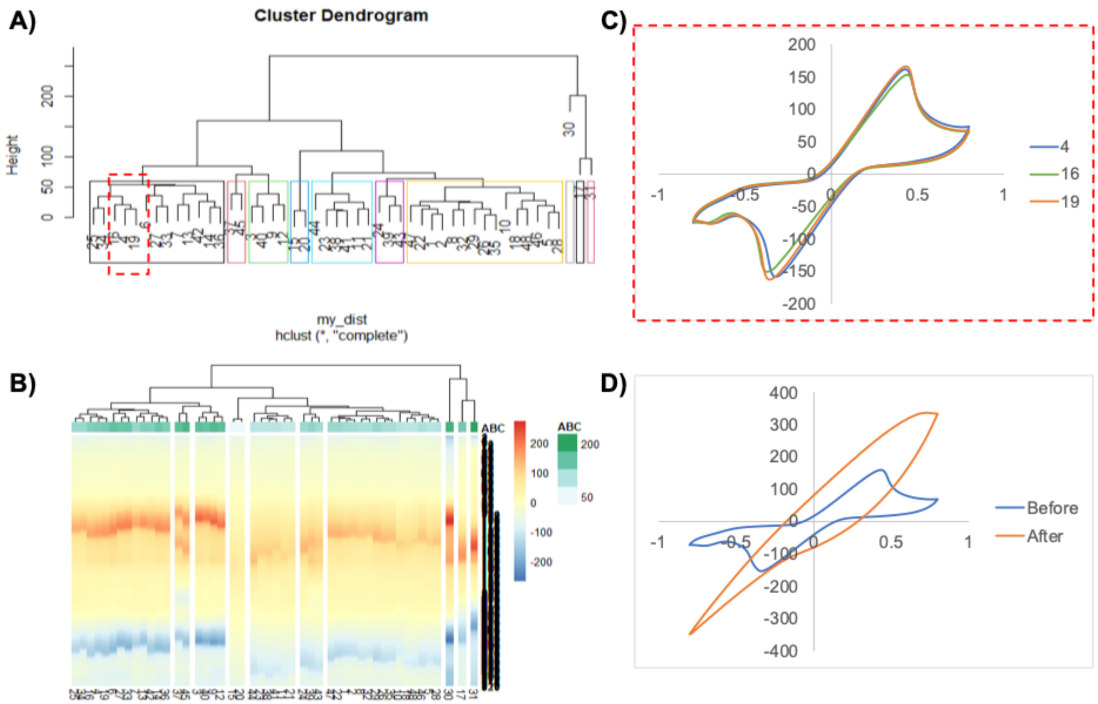

Figure 1. Orthophosphate sensor development. **A)** and **B)** showing triplicate selection using R program developed by Qian et al (2024); **C)** Triplicates selected from the cluster of LIG electrodes based on similar electrochemical properties (area between curve and peak current); **D)** Voltammogram of a selected replicate before and after functionalization with GO-PDDA solution.

Step 3) Characterize sensor performance

5

- Add 15 mL of 2X Sodium chloride/sodium bicarbonate (NaCl/$NaHCO_3$) solution to an electrochemical cell.
- Immerse LIG-GO-PDDA sensor into the solution.

Note

If possible, analyze multiple electrodes (e.g., MultiPalmSens4 potentiostat allows running three electrodes simultaneously).

- Conduct Run EIS for baseline measurements following the settings below:

Note

Equilibration time: 3 s
Scan type: fixed DC potential
Edc: 0.45 V
Eac: +/- 0.01 V
Frequency type: scan
n frequencies: 61
max frequency: 10,000 Hz
min frequency: 0.01 Hz

- Record the raw data of baseline measurements for each replicate in an Excel file (**Template**).
- Titrate stock solution (e.g., 100 mg/L of phosphate, nitrate, sulfate or chloride salt). An example calibration experiment is shown in the Table below

Analyte final concentration (mg/L) in 15 mL solution	Volume of analyte working solution at 100 mg/L to add (in ml)
0.01	1.5
0.2	30
0.4	30
0.6	30
0.8	30
1	30

Table 1. Example of calibration experiment for stepwise titration of stock solution (same solution method)

- After analyte injection, mix the buffer solution with a pipette, allow the solution to sit for one minute, and then run EIS measurements for each concentration.
- Record the raw data of different analyte concentrations for each replicate in an Excel file (**Template**).

Step 4) Clean up space and dispose of waste

15m

6
- Dispose of used chemicals according to the lab safety plan.

- Wash all shot glass cups with mild detergent and warm water.

Protocol references

Casso-Hartmann, L., G.A.M. Moreira, Y. Tang, D. Vanegas, E.S. McLamore (2024) Fabrication of laser inscribed graphene (LIG) 3-electrode plug-and-play chip, Protocol I/O. DOI: 10.17504/protocols.io.dm6gpze1dlzp/v1.

Tang, Y., L. Casso-Hartmann, D. Bahamon-Pinzon, G. Moreira, D.C. Vanegas, E.S. McLamore (2023). Electrochemical analysis of laser-inscribed graphene electrodes using cyclic voltammetry (ferri/ferrocyanide redox couple). Protocol I/O, DOI: 10.17504/protocols.io.4r3l27q7jg1y/v1.

E.S. McLamore, D. Vanegas, D. Bahamon-Pinzon, K. McCourt, Y. Tang (2021). Fabrication of laser inscribed graphene electrodes (Universal Laser System). Protocol I/O, DOI: 10.17504/protocols.io.byc4psyw.