

Jul 08, 2024

vGlut1-pH imaging experiment analysis

 [Science Advances](#)

 In 1 collection

DOI

<https://dx.doi.org/10.17504/protocols.io.bp2l6275zgqe/v1>

Alexandros C Kokotos^{1,2}, Tim Ryan^{1,2}

¹Department of Biochemistry, Weill Cornell Medicine, New York, NY 10065, USA;

²Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, Maryland 20815, USA



Alexandros C Kokotos

Weill Cornell Medicine

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.bp2l6275zgqe/v1>

Protocol Citation: Alexandros C Kokotos, Tim Ryan 2024. vGlut1-pH imaging experiment analysis. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bp2l6275zgqe/v1>

**Manuscript citation:**

Phosphoglycerate kinase is a central leverage point in Parkinson's Disease driven neuronal metabolic deficits

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: July 08, 2024

Last Modified: July 08, 2024

Protocol Integer ID: 103024

Keywords: ASAPCRN, vGlut1-pH, data analysis, SV recycling, pHluorin, genetic sensor, image analysis, ph imaging experiment analysis this protocol, ph imaging experiment analysis, ph from primary neuron, imaging experiment, using vglut1, primary neuron, ph

Funders Acknowledgements:

ASAP

Grant ID: 000580

Abstract

This protocol describes how to analyze imaging experiments using vGlut1-pH from primary neurons.

Materials

Software

Microsoft Excel (<https://www.microsoft.com/en-us/microsoft-365/excel>, version 2308, RRID:SCR_016137)

GraphPad Prism (<http://www.graphpad.com>, version 10.0.2, RRID:SCR_002798)

ImageJ (NIH, US, <https://imagej.net>, version 1.52p, RRID:SCR_003070)

Time Series Analyzer plug-in (<https://imagej.net/ij/plugins/time-series.html>, version 3.0)

vGlut1-pH image analysis

- 1 Open the image stacks to be analyzed with ImageJ by drag and drop.
- 2 Identify nerve terminals that responded to electrical stimulation, by observing their fluorescence increasing in sync with the timing of the action potential train.
- 3 Place ROIs on the responding terminals using the Time Series Analyzer plug-in. We usually use ROIs of width and height 6 pixels each, with the pixel size being 400 nm in our images.
- 4 Get the average pixel intensity of each ROI and each image using the "Get Average" function. Save the produced csv results as well as the ROIs selection.
- 5 Similarly place background ROIs surrounding the nerve terminals identified. Save the pixel intensity values and background ROIs selection.
- 6 Analyze the NH_4Cl images in the exact same way, using the previously saved ROIs. In case the nerve terminals have slightly moved, the ROIs can be readjusted using the "Recenter" function.

vGlut1-pH data analysis

- 7 Open the produced csv files in Microsoft Excel.
- 8 For each time point, subtract the average background fluorescence values from the average nerve terminal vGlut1-pH fluorescence values.
- 9 Calculate the ΔF values, by subtracting the initial fluorescence values before the action potential train from all values, so that the first produced values will now be around 0.
- 10 The data can now be further normalized. To normalize against the total sensor fluorescence, revealed by the 50 mM NH_4Cl Tyrodes solution, analyze the NH_4Cl image stack as described above and calculate the maximal fluorescence change. Dividing all fluorescence values with the calculated ΔF_{NH_4} , will yield the $\Delta F_{\text{APs}}/\Delta F_{\text{NH}_4}$ normalization of the data-set.
Note: It is advised to use the initial F_0 when calculating the ΔF_{NH_4} , since manipulations that slow or block synaptic vesicle recycling will also increase the fluorescence of the

sensor at the end of the experiment.

- 11 The data-set can also be normalized internally to the fluorescence peak during the action potential train. This allows to specifically look at synaptic vesicle endocytic kinetics and is unaffected by changes in exocytosis. First, calculate the ΔF_{MAX} by identifying the maximal fluorescence values around the end of the action potential train. For 10 Hz trains, we locate the F_{MAX} at a 2 second window around the end of the action potential train. Dividing all fluorescence values with the calculated ΔF_{MAX} , will yield the $\Delta F_{APs}/\Delta F_{MAX}$ normalization of the data-set.
- 12 To calculate the endocytic efficiency, the leftover sensor fluorescence can be calculated for all single cell data, at a specified time-point. To minimize variability due to noise, we usually use a small time window around the chosen time point of approximately 2 seconds.
- 13 All produced time trace data and subsequent quantifications can be plotted and presented in Graphpad Prism.