Data in Brief 30 (2020) 105424

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

High-speed single molecule imaging datasets of membrane proteins in rat basophilic leukemia cells

Hanieh Mazloom-Farsibaf[a], William K. Kanagy[b], Diane S. Lidke[b,c], Keith A. Lidke[a,c,*]

[a] Department of Physics and Astronomy, University of New Mexico, Albuquerque, NM, United States
[b] Department of Pathology, University of New Mexico, Albuquerque, NM, United States
[c] Comprehensive Cancer Center, University of New Mexico, Albuquerque, NM, United States

ARTICLE INFO

Article history:
Received 22 January 2020
Revised 5 March 2020
Accepted 6 March 2020
Available online 6 April 2020

Keywords:
Single-particle tracking
Membrane proteins
Actin
Fluorescence microscopy

ABSTRACT

A high-speed fluorescence microscope operating at a 490 Hz frame rate was used to image two different membrane proteins- the high-affinity IgE receptor FcεRI, a transmembrane protein, and an outer-leaflet GPI-anchored protein. The IgE receptor was imaged via IgE labeled with Janelia Fluor 646 and the GPI-anchored protein was imaged using a GPI-GFP fusion protein and an ATTO 647 N labeled anti-GFP nanobody. Data was collected for both proteins in untreated cells and cells that had actin stabilized by phalloidin. This dataset can be used for development and testing of single-particle tracking methods on experimental data and to explore the hypothesis that the actin cytoskeleton may affect the movement of membrane proteins.

* Corresponding author.
E-mail address: klidke@unm.edu (K.A. Lidke).

https://doi.org/10.1016/j.dib.2020.105424

Specifications table

Subject	Biophysics
Specific subject area	Single-particle tracking
Type of data	Time series of images
How data were acquired	Fluorescence Microscopy
Data format	Raw
Parameters for data collection	638 nm laser excitation under total internal reflectance conditions. Camera exposure time of 2 ms giving a 490 Hz frame rate.
Description of data collection	Membrane proteins were labelled with organic fluorescent molecules and imaged at high-speed with a fluorescence light microscope. FcεRI was labelled with Janelia Fluor 646 conjugated IgE, whereas a transfected GFP-GPI-anchored fusion protein was imaged using an anti-GFP ATTO 647 N conjugated nanobody. Both proteins were imagined with and without phalloidin treatment to the cells.
Data source location	Institution: The University of New Mexico City/Town/Region: Albuquerque, NM Country: US
Data accessibility	Repository name: Mendeley Data Data identification number: doi:10.17632/d5z3gt7xs9.2 Direct URL to data: data.mendeley.com/datasets/d5z3gt7xs9/2

Value of the data

- These data are useful for exploring the lateral dynamics of membrane proteins, which can be used to infer membrane architecture. A comparison of the four permutations of transmembrane and outer leaflet protein with untreated and phalloidin treated cells can be used to test the hypothesis that the actin cytoskeleton may restrict lateral motion of membrane proteins.
- Researchers studying membrane protein organization and dynamics and those developing single particle tracking methods may benefit from this data.
- The performance of the two different organic dyes for high-speed imaging can be evaluated.
- The high-speed data can be used to test various diffusion models that predict different behavior at different time scales.

1. Data description

The shared data is a collection of image time series from high-speed imaging of membrane proteins. The data was collected using a custom-assembled fluorescence microscope based around an Olympus IX71 microscope base, a 1. 49 NA oil objective and an iXon 860 EMCCD camera. The high-affinity IgE receptor, FcεRI, a transmembrane protein was labeled with Janelia Flour 646. GFP-GPI-anchored fusion protein, an outer-leaflet protein, was labelled using an anti-GFP ATTO 647 N conjugated nanobody. Data was collected in rat basophilic leukemia (RBL-2H3) cells. Two experimental conditions were employed to test the effects of the actin cytoskeleton on membrane protein dynamics. In one condition, the proteins were imaged in untreated RBL-2H3 cells. In another condition, cells were treated with phalloidin, which is a heptapeptide for stabilizing the actin filaments (F-actin) [1]. Membrane proteins were imaged at 2 ms exposure giving 490 Hz frame rate.

All raw datasets that are part of this shared data collection are listed in Table 1. The raw data with file names starting with “IgE Untreated” are image time series of fluorescently labeled IgE in untreated RBL-2H3 cells. The raw data with file names starting with “IgE Phalloidin” are image time series of fluorescently labeled IgE in phalloidin treated RBL-2H3 cells. The raw data with file names starting with “GPI Untreated” are image time series of fluorescently labeled GPI-anchored proteins in untreated RBL-2H3 cells. The raw data with file names starting with “GPI Phalloidin” are image time series of fluorescently labeled GPI-anchored proteins in phalloidin

Table 1
The experimental conditions for each shared dataset.

Filename	Membrane protein	Cell condition	Number of frames
IgE Untreated 1	IgE receptor	Untreated cell	3000
IgE Untreated 2	IgE receptor	Untreated cell	3000
IgE Untreated 3	IgE receptor	Untreated cell	3000
IgE Phalloidin 1	IgE receptor	Phalloidin-treated cell	3000
IgE Phalloidin 2	IgE receptor	Phalloidin-treated cell	2500
IgE Phalloidin 3	IgE receptor	Phalloidin-treated cell	2500
GPI Untreated 1	GFP-GPI-anchored fusion protein	Untreated cell	3000
GPI Untreated 2	GFP-GPI-anchored fusion protein	Untreated cell	3000
GPI Phalloidin 1	GFP-GPI-anchored fusion protein	Phalloidin-treated cell	3000
GPI Phalloidin 2	GFP-GPI-anchored fusion protein	Phalloidin-treated cell	3000
GPI Phalloidin 3	GFP-GPI-anchored fusion protein	Phalloidin-treated cell	3000

treated RBL-2H3 cells. The numeral in each file name enumerates a separate experiment and data collection under identical conditions.

2. Experimental design, materials, and methods

2.1. Cell lines and reagents

2.1.1. Transmembrane protein, FcεRI protein

RBL-2H3 cells [2,3] were plated MEM supplemented with 10% heat-inactivated fetal bovine serum, 5 U/ml penicillin, 0.05 mg/ml streptomycin, and 2 mM L-glutamine on a 25 mm coverslips (# 1.5) in a 6-well plated, at 37 °C and 5% carbon dioxide. After 24 h of growing adherent monolayer, the coverslips were then imaged using a 25 mm coverslip Attofluor cell chamber (ThermoFisher, A7816). Just before imaging cells were labeled with the IgE conjugated to Janelia Fluor® 646 (Tocris Bioscience, Cat. No. 6148) [4] for 15 min at room temperature. To achieve desired labeling densities for single-particle tracking, the labeled IgE was diluted in live-cell imaging buffer at a final concentration of 150–250 pM. Live-cell imaging buffer consisted of 0.9X solution of Hanks Balanced Salt Solution (HBSS) (Invitrogen, SKU#14,065- 056), 10 mM 4-(2-hydroxyethyl)−1-piperazineethanesulfonic acid (HEPES), 5 mM Glucose, 0.7 mM $MgSO_4$, 0.13% $NaHCO_3$ in ddH_2O (HANKS) buffer. For phalloidin-treatment, the cells were pre-extracted using 0.05% Saponin (Sigma, CAS No. 8047–15–2) in cytoskeleton-preserving buffer, 80 mM PIPES pH 7.2, 10 mM EGTA, 4 mM MgCl2 (PEM), and with a 1 pM of Phalloidin-ATTO 488 (Sigma, Cat No. 49,409) incubation for the final 20 s. The samples were then washed gently 6x with the HANKS buffer and labeled with the IgE conjugated to Janelia Fluor® 646 as descibed above.

2.1.2. Extracellular leaflet protein, GPI anchored protein

RBL-2H3 cells were transiently transfected to express a chimeric membrane-anchored GFP-GPI fusion protein. Transient transfections were completed using product recommended procedures with the Amaxa™ Nucleofector™ system (Program T-20), Lonza Solution L, and 3 µg of plasmid DNA. Fluorescence microscopy imaging was used to confirm correct membrane localization and sufficient expression of the GFP-GPI protein. After transfection, cells were immediately plated in (#1) 8-well Lab-Tek (Nunc) chambers (ThermoFisher Scientific) at a single-cell density (5×10^5 cells per well at the time of imaging). Samples were then incubated at 37 °C at 5% carbon dioxide for 24–48 h before imaging. Immediately before imaging cells were maintained at 37 °C and washed 4x with warm HANKS buffer and imaged in HANKS buffer.

Labeling and tracking of the GFP-GPI fusion protein were completed by incubating cells with an anti-GFP nanobody conjugated to ATTO 647 N (Chromotek, Cat No. gba647n-10) for 20 min. The Nanobody was diluted in HANKS buffer at a final concentration of 0.001 µg/ml. Samples were washed once with the HANKS buffer before single-particle imaging, used for eventual

tracking. For phalloidin-treatment, the cells were pre-extracted by using 0.05% Saponin (Sigma, CAS No. 8047–15–2) in cytoskeleton-preserving buffer, 80 mM PIPES pH 7.2, 10 mM EGTA, 4 mM MgCl2 (PEM), and in the presence of 1 pM of Phalloidin-Alexa 568 (ThermoFisher, Cat No. A12380) for 20 s. Before imaging, the sample was washed gently 6x with HANKS buffer and labeled with the anti-GFP nanobody conjugated to ATTO 647 N, as described above.

2.2. Live-cell imaging conditions

The sample was mounted on the stage of the microscope with a custom-designed chamber holder. The sample was kept in HANKS buffer during the live-cell imaging. The imaging system was built on an inverted microscope (IX71, Olympus America Inc.). An *xyz* piezo stage (Mad City Labs, NanoLPS100) mounted on a *x-y* manual stage was installed on the microscope for cell positioning. A 638 nm laser (Laser diode, L638P200, Thorlabs) was mounted in a temperature controlled laser diode mount (ThorLabs, LDM9T/M) which was controlled by a laser diode current controller (ThorLabs, TLD001). The laser was coupled into single-mode fiber and the output was focused onto the back focal plane of the 1.49 NA objective lens (UAPON 100XOTIRF, Olympus America Inc.). Emission was collected through a single-edge standard epi–fluorescence dichroic beamsplitter (FF624-Di01, Semrock) and a single-band bandpass filter (FF01-692/40–25, Semrock) on an iXon 860 EMCCD camera (Andor Technologies, iXon DU-860E-CS0-#BV). Calibration of the EMCCD to extract the effective photon number and offset gave gain at 13 $\frac{e^-}{ADU}$ and offset of 90 ADU. The image size was 128×128 pixels with a pixel size of 0.1185 µm. All the instruments were controlled by custom-written software in MATLAB (MathWorks Inc.) [5]. Imaging was performed with TIRF illumination with an excitation Intensity of ~1 kW/cm^2 with samples at room temperature. Images were acquired at 2 ms exposure time for a total of 6000 frames. The first 2500 or 3000 frames are given in the datasets as indicated in Table 1.

Acknowledgments

This work was supported by the National Institutes of Health grants 1R01GM109888-01, R35 GM126934, and P50GM085273. We also thank Shayna Lucero for assistance with cell culture.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] P. Dancker, I. Löw, W. Hasselbach, T. Wieland, Interaction of actin with phalloidin: polymerization and stabilization of F-actin, Biochimica et Biophysica Acta (BBA)-Protein Struct. 400 (2) (1975) 407–414, doi:10.1016/0005-2795(75)90196-8.
[2] H. Metzger, G. Alcaraz, R. Hohman, J.P. Kinet, V. Pribluda, R. Quarto, The receptor with high affinity for immunoglobulin e, Annu. Rev. Immunol. 4 (1986) 419–470, doi:10.1016/0005-2795(75)90196-8.
[3] B.S. Wilson, J.R. Pfeiffer, J.M. Oliver, Observing fcεri signaling from the inside of the mast cell membrane, J. Cell Biol. 149 (2000) 1131–1142, doi:10.1083/jcb.149.5.1131.
[4] S.L. Schwartz, C. Cleyrat, M.J. Olah, P.K. Relich, G.K. Phillips, W.S. Hlavacek, K.A. Lidke, B.S. Wilson, D.S. Lidke, Differential mast cell outcomes are sensitive to fcεri-syk binding kinetics, Mol. Biol. Cell 28 (23) (2017) 3397–3414.
[5] Lidke Lab, Matlab Instrument Control Software, 2018. https://github.com/LidkeLab/matlab-instrument-control.