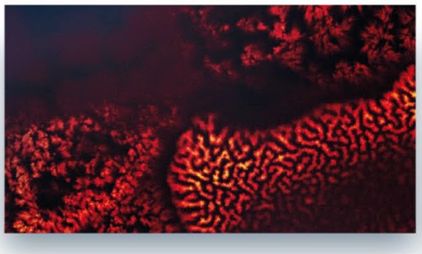


Actin in microvilli of Caco2 cells. Prof. Dorothee Günzel & Roman Mannweiler

abberior

Science beyond barriers

abberior Instruments GmbH

Hans-Adolf-Krebs-Weg 1
37077 Göttingen, Germany
+49 551 9995 4010

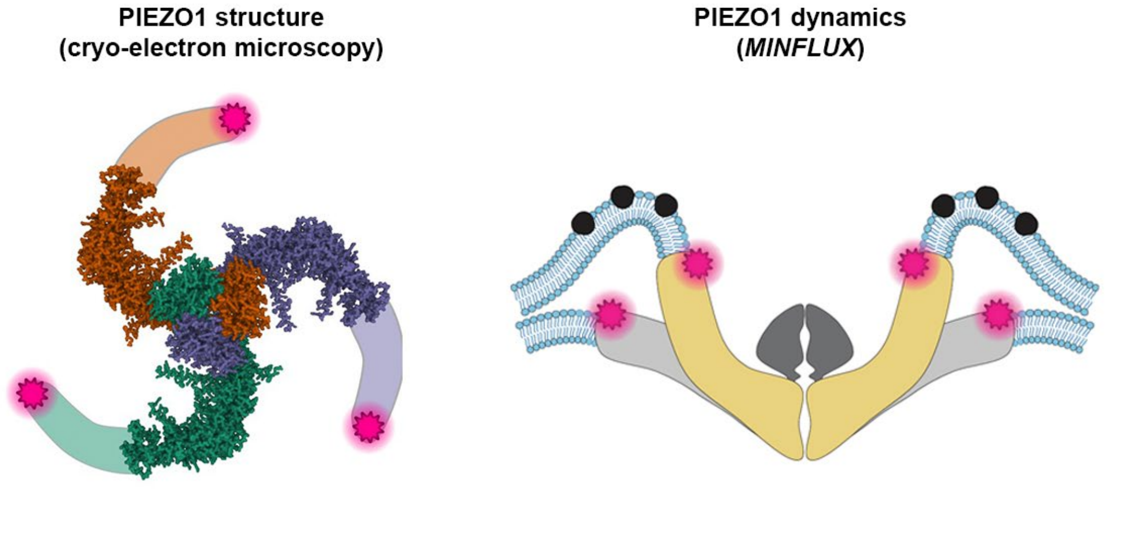
www.abberior.rocks
Media inquiries: f.koepper@abberior.rocks

August 17, 2023

MINFLUX unravels the structural dynamics of PIEZO1 ion channels in living cells

New study shows microscopy technique's potential

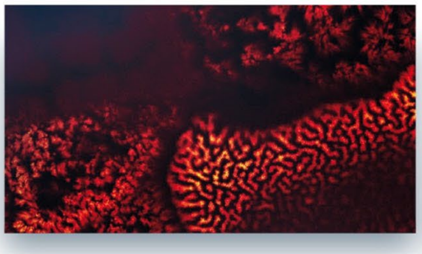
In the current issue of *Nature*[1], 2021 Nobel laureate and neuroscience professor Ardem Patapoutian, PhD, and his team at Scripps Research (La Jolla, US), unravel the conformational changes, with single nanometer resolution, of PIEZO1 ion channels in response to mechanical stimuli.



adapted from Mulhall et al., *Nature* 2023, DOI: 10.1038/s41586-023-06427-4

Available protein structural models of PIEZO1 lack information on the flexible outer parts of the blades (top view, left). By labeling the blades' tips with a fluorescent dye, scientists were now able to determine their position and movement by MINFLUX microscopy (cross-section, right).

PIEZOs are a family of ion channels that adapt their shape and permeability in response to mechanical stimuli. PIEZOs consist of three identical protein subunits that form a central pore with three blades of transmembrane domains extending outwards and upwards, resembling a propeller. It has been proposed that these blades sense changes in membrane tension and gate the channel


Actin in microvilli of Caco2 cells. Prof. Dorothee Günzel & Roman Mannweiler

in response. Patapoutian received the 2021 Nobel Prize in Physiology or Medicine for his PIEZO discoveries.

Classical methods reach limits

Structural models of PIEZO1, developed via cryo-electron microscopy, have provided molecular details about the pore and the proximal part of its blades, but these models are incomplete and lack the distal one-third of the blades. Furthermore, cryo-electron microscopy – and related techniques such as crystallography and nuclear magnetic resonance spectroscopy – all require the protein of interest to be highly purified, and therefore struggle to provide contextual information about how proteins interact with their native physiological environment. These techniques also rely on extensive averaging to assemble molecular models due to their low signal-to-noise ratio and often fail to resolve distinct conformational states of individual proteins.

For PIEZO1, this means that the exact conformation of its blades, how they perceive mechanical force, and subsequently influence channel activity are unknown.

In the new study, first author and Scripps Research postdoctoral fellow, Eric Mulhall, PhD, and team applied the cutting-edge microscopy technique *MINFLUX* to tackle these questions. *MINFLUX* is a super-resolution light microscopy technique first published in 2016 by 2014 Chemistry Nobel laureate Stefan Hell.[2] It offers unprecedented spatial and temporal resolution – on the order of ~1 nanometer and ~100 microseconds, respectively – even in living cells, making it possible to localize and track individual molecules, in their native state, in real time and three dimensions.[3]

Monitoring PIEZO1 changing conformation with MINFLUX

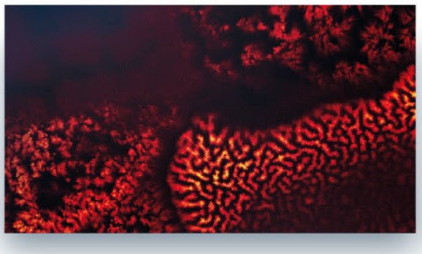
The researchers combined MINFLUX with novel algorithms to investigate individual PIEZO1 molecules in cells with single nanometer resolution. Using a commercial *MINFLUX* microscope manufactured by *abberior*, they monitored how the blades of individual PIEZO1 molecules changed confirmation in their physiological context. By fluorescently labeling each blade at its most distal position – the propeller's tips, so to speak – they were able to identify their exact position with nanometer precision and measure the distance between the blades. This in turn provided information about the protein's conformational state. The scientists found that the blades are significantly expanded at rest due to the bending force exerted by the plasma membrane and that their conformational flexibility increased with distance to the central channel.

MINFLUX inventor Hell says: "This outstanding study from Ardem Patapoutian's lab is exactly what I always dreamed *MINFLUX* would end up enabling: making groundbreaking physiological discoveries."

As the measurements were performed directly in cells, the researchers were also able to observe the effects of a physiological stimulus. When exerting a hypo-osmotic shock, they witnessed how the blades stretched in response to an increased membrane tension and correlated this movement with channel activity.

To directly observe how PIEZO1 changes conformation and reacts to physiological changes in a living cell would not have been possible without *MINFLUX*.

Mulhall emphasizes: "This work provides a foundation for understanding how PIEZO1 is activated in a cellular context, and for the structural analysis of membrane proteins in their native environment."


Actin in microvilli of Caco2 cells. Prof. Dorothee Günzel & Roman Mannweiler

Citations

[1] Mulhall, E.M., Gharpure, A., Lee, R.M., Dubin, A.E., Aaron, J.S., Marshall, K.L., Spencer, K.R., Reiche, M.A., Henderson, S.C., Chew, T-L., Patapoutian, A.: Direct Observation of the Conformational States of PIEZO1. *Nature*, August 16, 2023, DOI: 10.1038/s41586-023-06427-4

[2] Balzarotti, F., Eilers, Y., Gwosch, K.C., Gynnå, A.H., Westphal, V., Stefani, F.D., Elf, J., Hell, S.W.: Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. *Science* 355 (2016), DOI: 10.1126/science.aak9913

[3] Schmidt, R., Weihs, T., Wurm, C.A. et al. MINFLUX nanometer-scale 3D imaging and microsecond-range tracking on a common fluorescence microscope. *Nat Commun* 12 (2021), DOI: 10.1038/s41467-021-21652-z

Contact

Ardem Patapoutian, PhD
Presidential Endowed Chair in Neurobiology
Professor, Department of Neuroscience
Investigator, Howard Hughes Medical Institute
Scripps Research
DNC 202
10550 N Torrey Pines Road
La Jolla, CA 92037, USA
ardem@scripps.edu

Scripps Research Communications Office
press@scripps.edu

Frederik Köpper, PhD
Communication and Marketing
abberior Instruments GmbH
Hans-Adolf-Krebs-Weg 1
37077 Göttingen, Germany
+49 (0) 551 99954 184
f.koepper@abberior.rocks

About *abberior*

abberior Instruments GmbH was founded as a spin-off of Nobel laureate Prof. Stefan W. Hell's research group at the Max Planck Institute for Multidisciplinary Sciences in Göttingen, Germany. Besides Stefan Hell, all founding members and decision makers are senior scientists and have shaped the field of optical super resolution over decades. *abberior* is a leading innovator, developer and manufacturer of modern superresolution STED and proprietary *MINFLUX* microscopes, which allow unique applications in cell and molecular biology. Numerous awards, including the TOP100 Innovation Award 2021, the Innovation Award of German Science and the Focus Growth Champion 2019, underline the success.