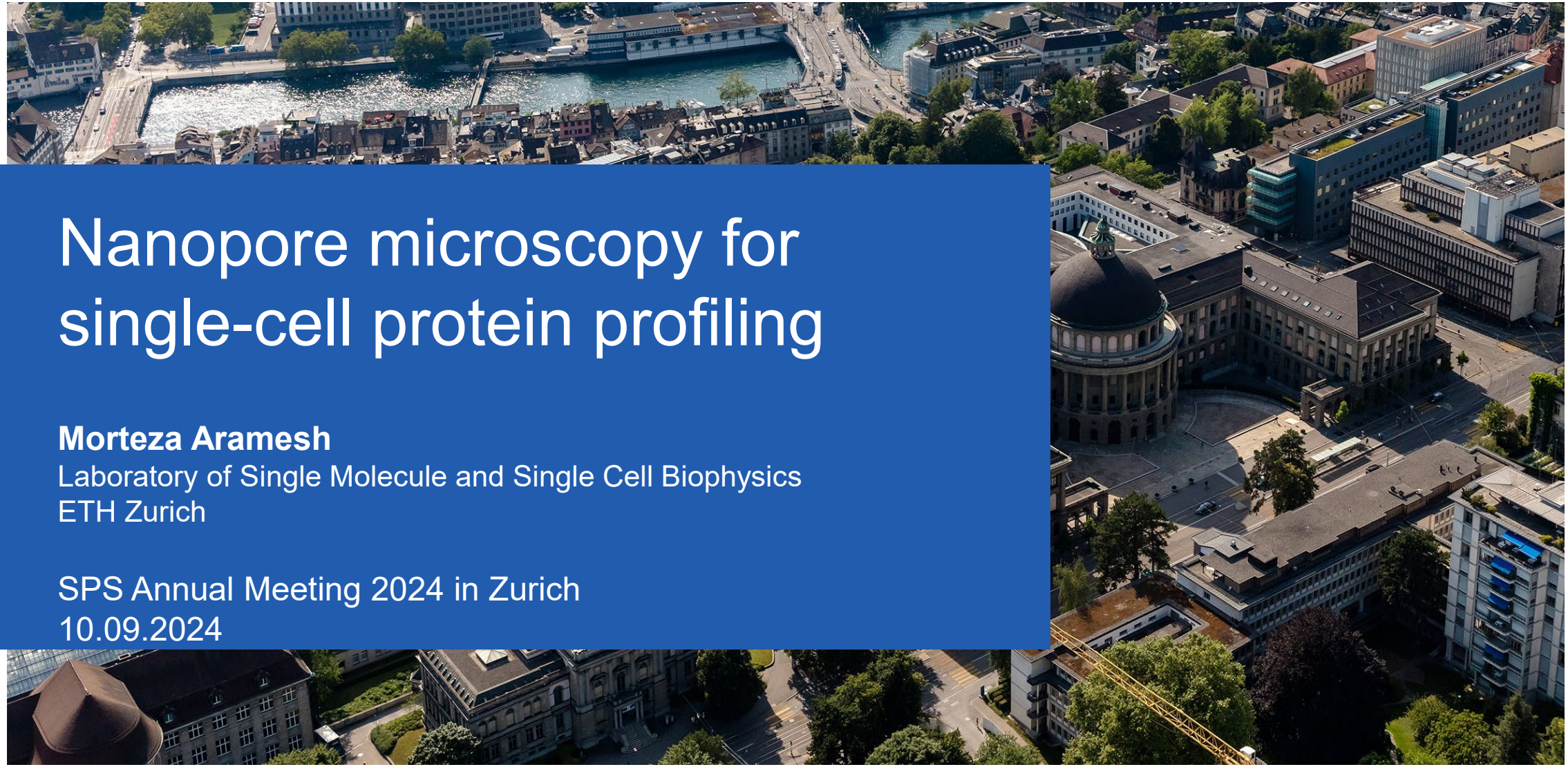
ETH zürich

Laboratory of Single Molecule and Single Cell Biophysics



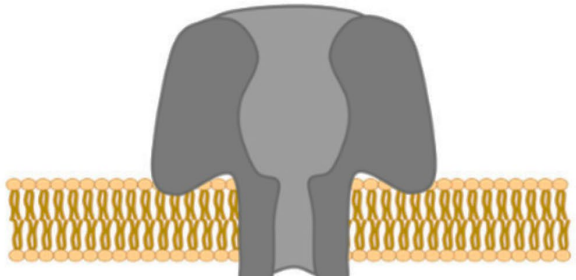
Nanopore microscopy for single-cell protein profiling

Morteza Aramesh
Laboratory of Single Molecule and Single Cell Biophysics
ETH Zurich

SPS Annual Meeting 2024 in Zurich
10.09.2024

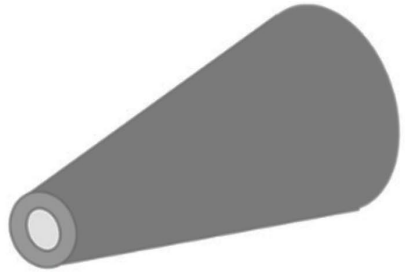
Nanopore Sensors

Protein-based nanopores



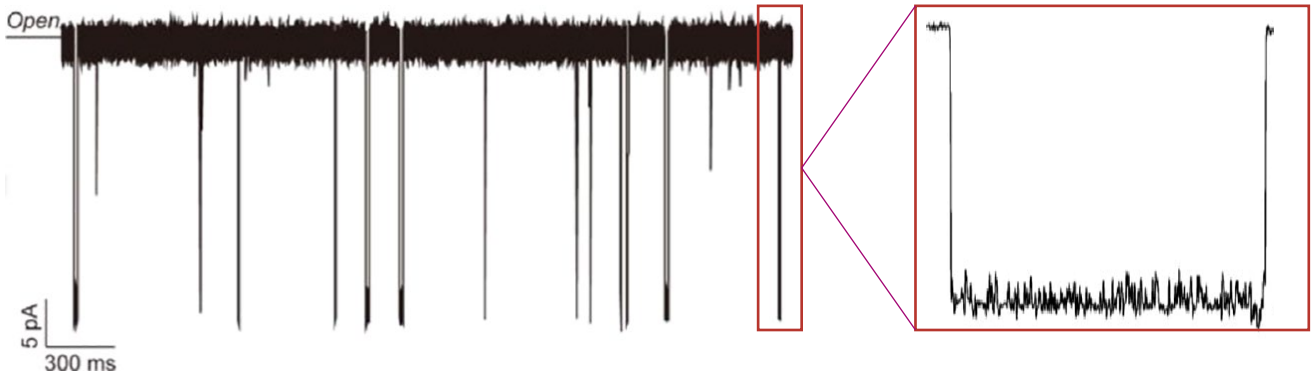
Solid-state nanopores

Thin-film membrane

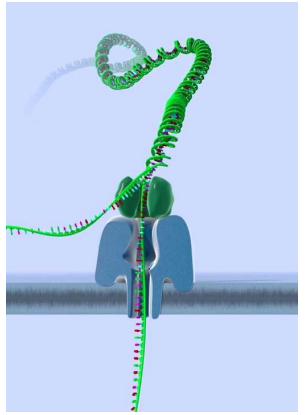


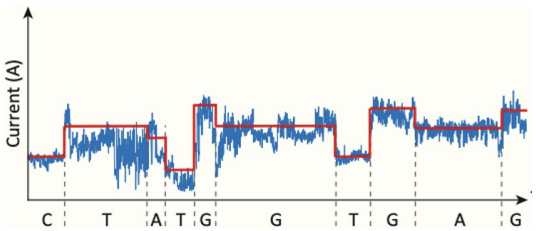
Glass nanopipettes

Ionic current measurement



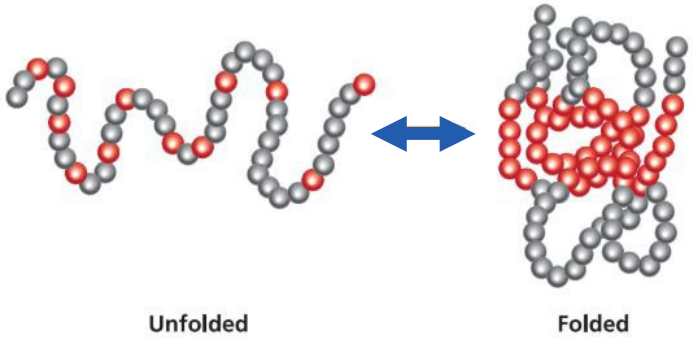
Oxford Nanopore for DNA Seq





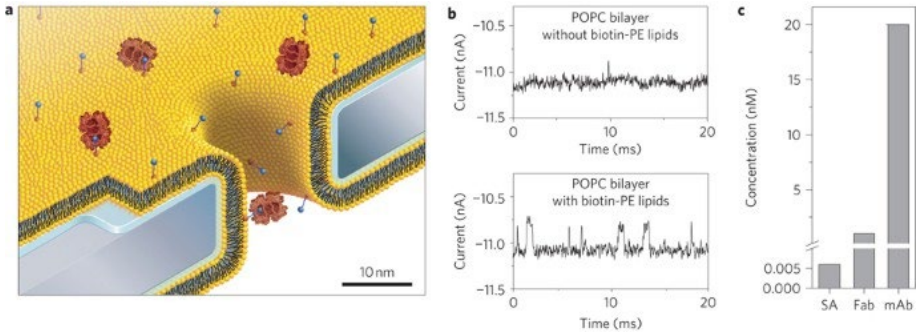
Nanopore Sensors for Protein Sensing

Protein Configuration/Charge Distribution



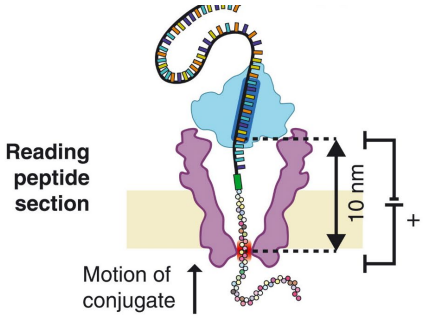
- Size
- Charge
- Stability
- Activity
- Number of amino acids

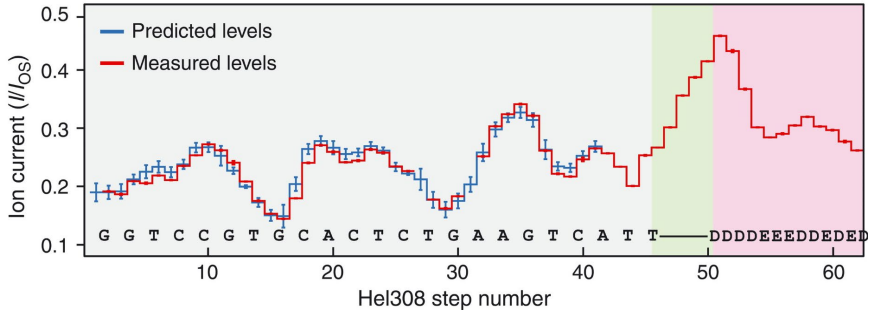
Solid-state nanopore



https://doi.org/10.1038/nnano.2011.12

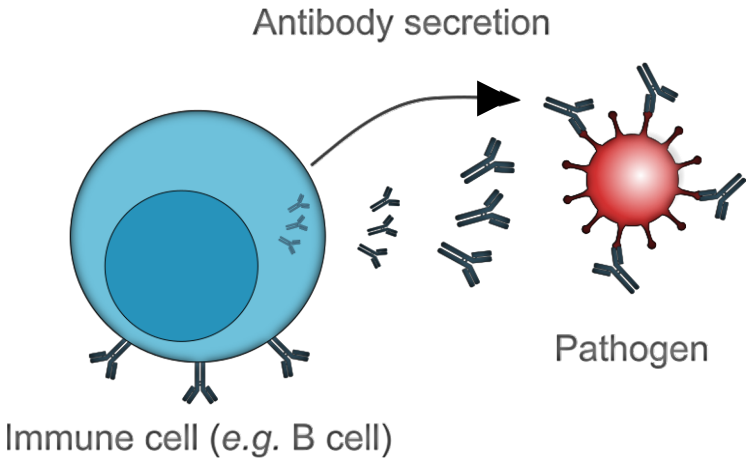
Protein-based nanopore





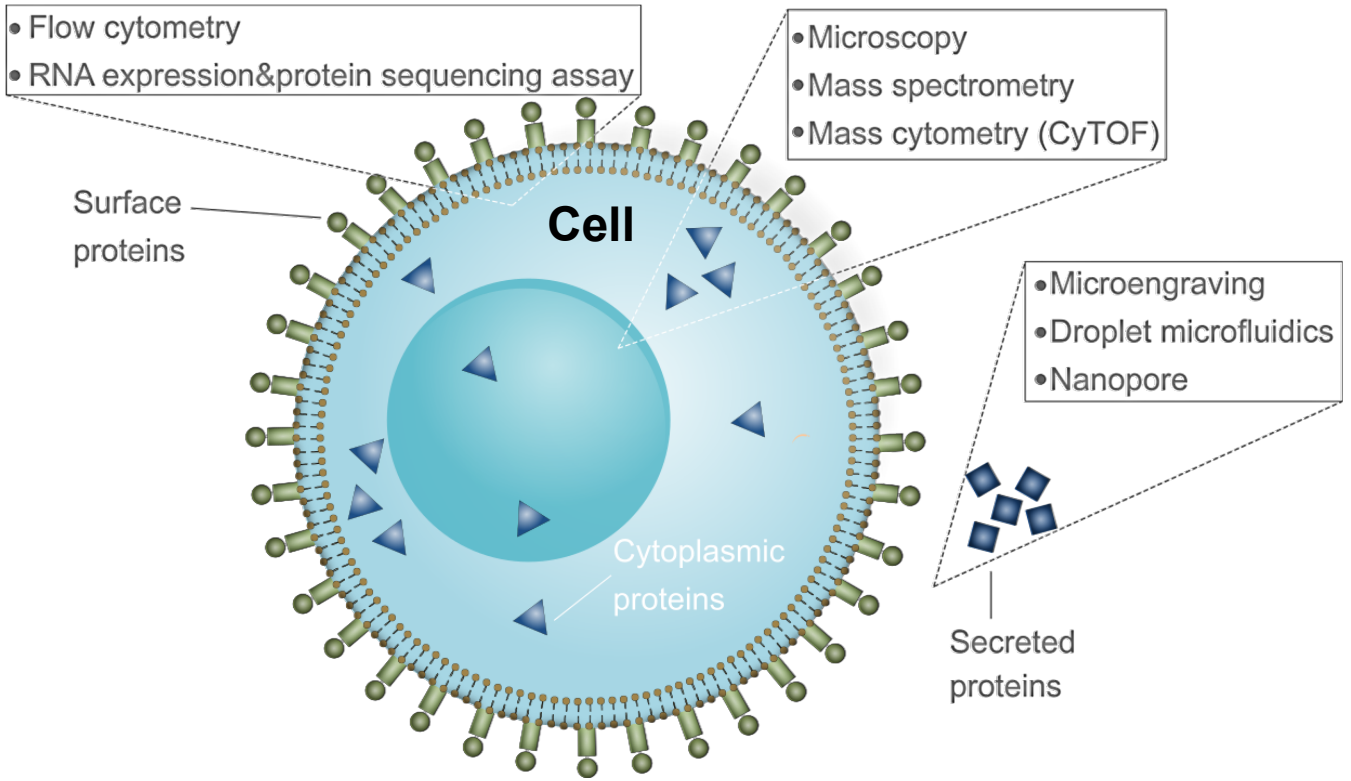
DOI: 10.1126/science.abl438

Protein Profiling at the Single-Cell Level



Protein production and secretion:

- Cell type and state
- Cell heterogeneity
- Cellular environment
- Diseases
- Medications
- Age
- …



Challenges:

- Low quantities of proteins
- Abundant variety
- Small volume handling
- Live cell monitoring

Nanopore sensors for single-cell proteome profiling ?

Interfacial nanopore

Common Challenges:

- Low quantities of proteins
- Abundant variety
- Small volume handling
- Live cell monitoring

+

Specific Challenges:

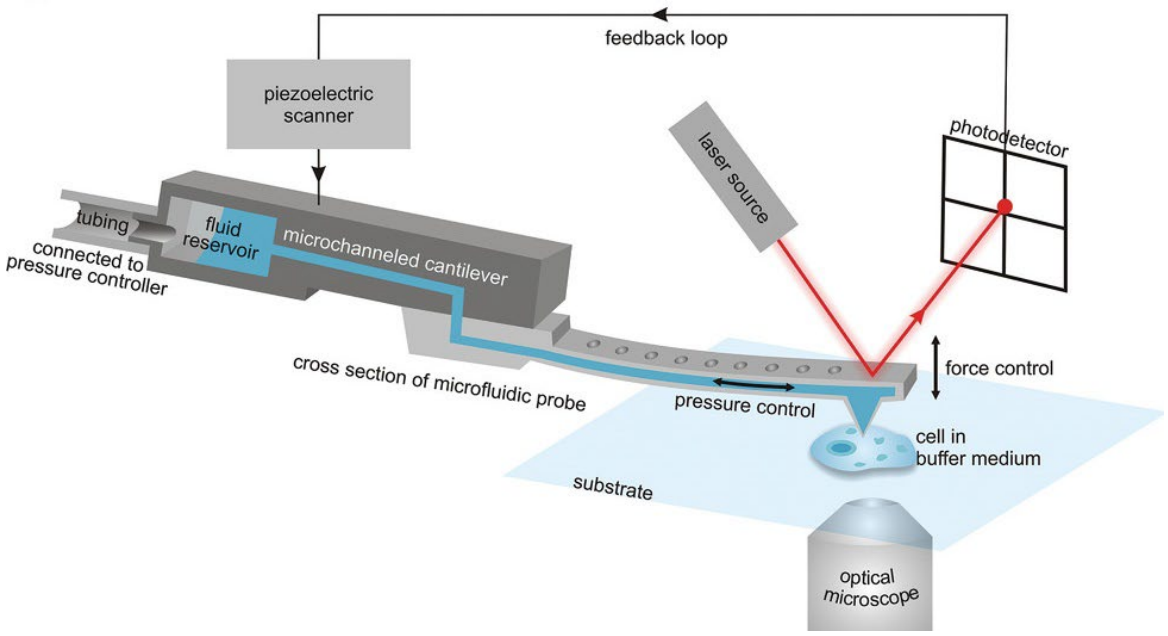
- Lack of specificity
- Pore clogging
- Pore size dependence
- Pore stability
- Range of proteins

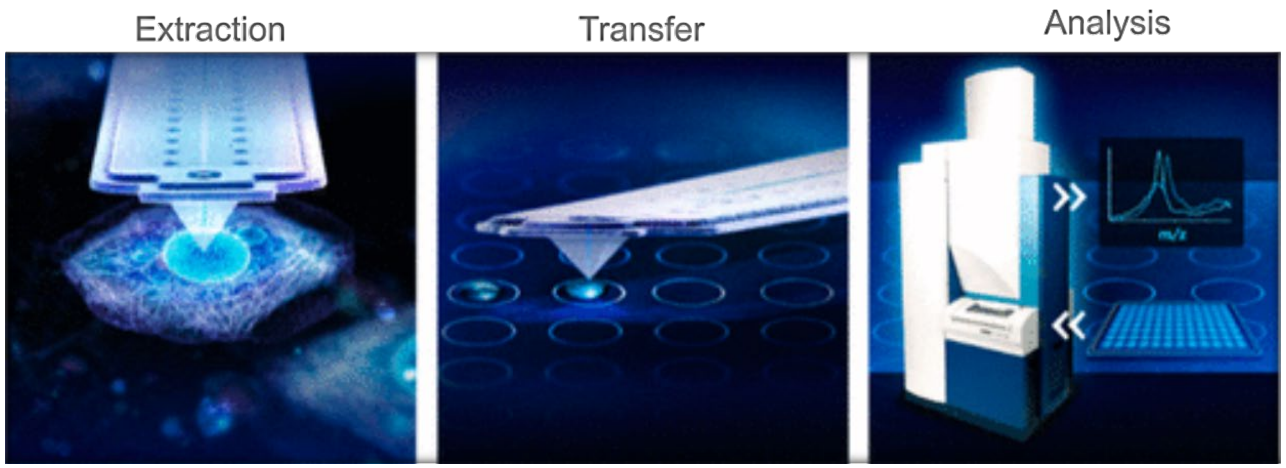
nanopipettes

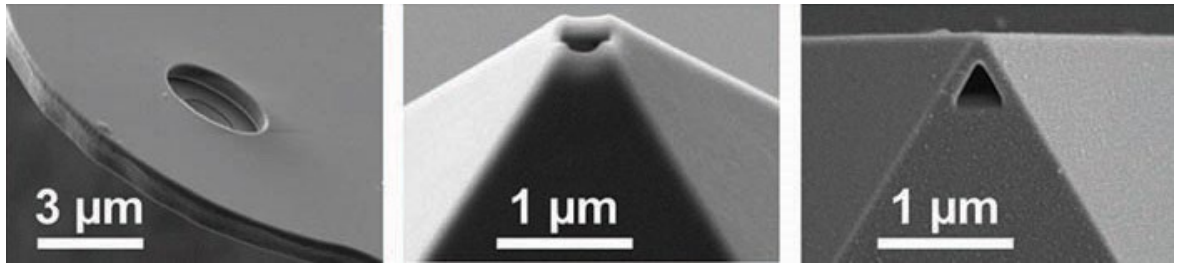
Choosing of the nanopore

Protein-based nanopores vs **Solid-state nanopores**

Studying Single Cells with FluidFM



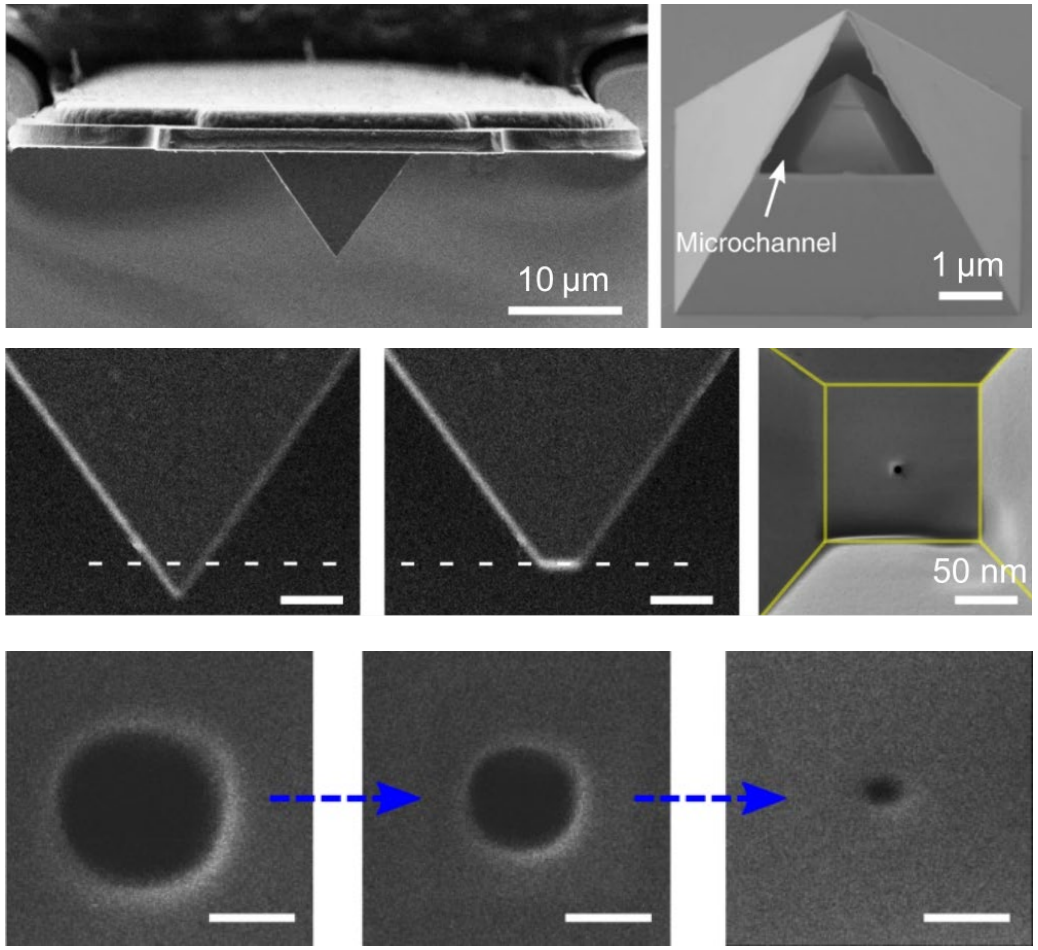


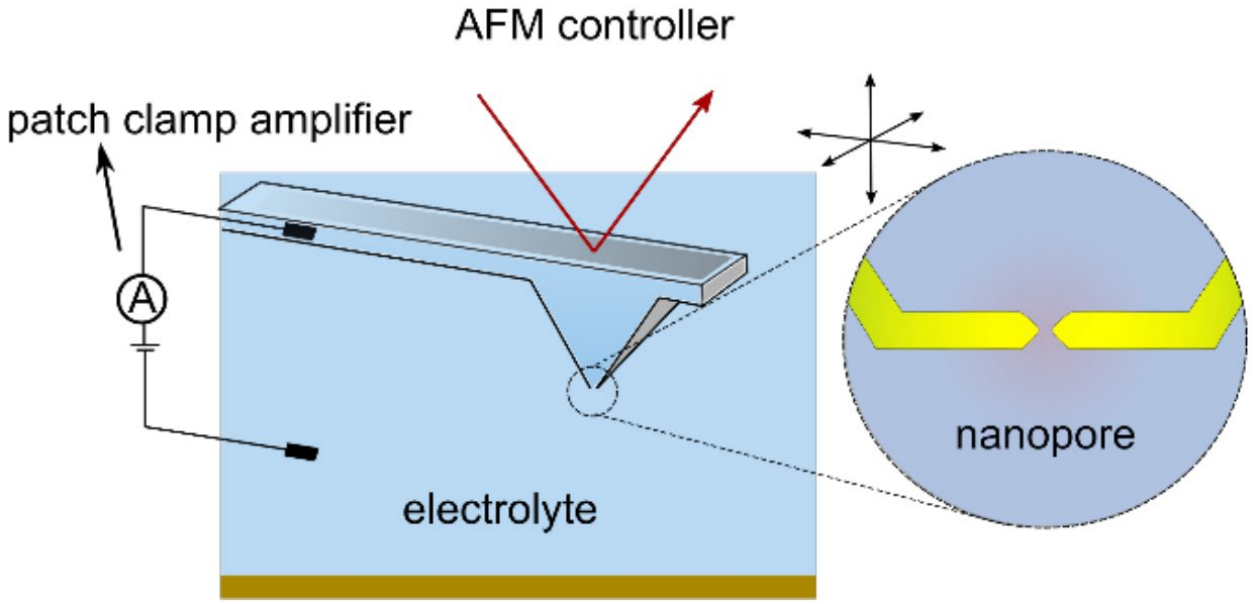


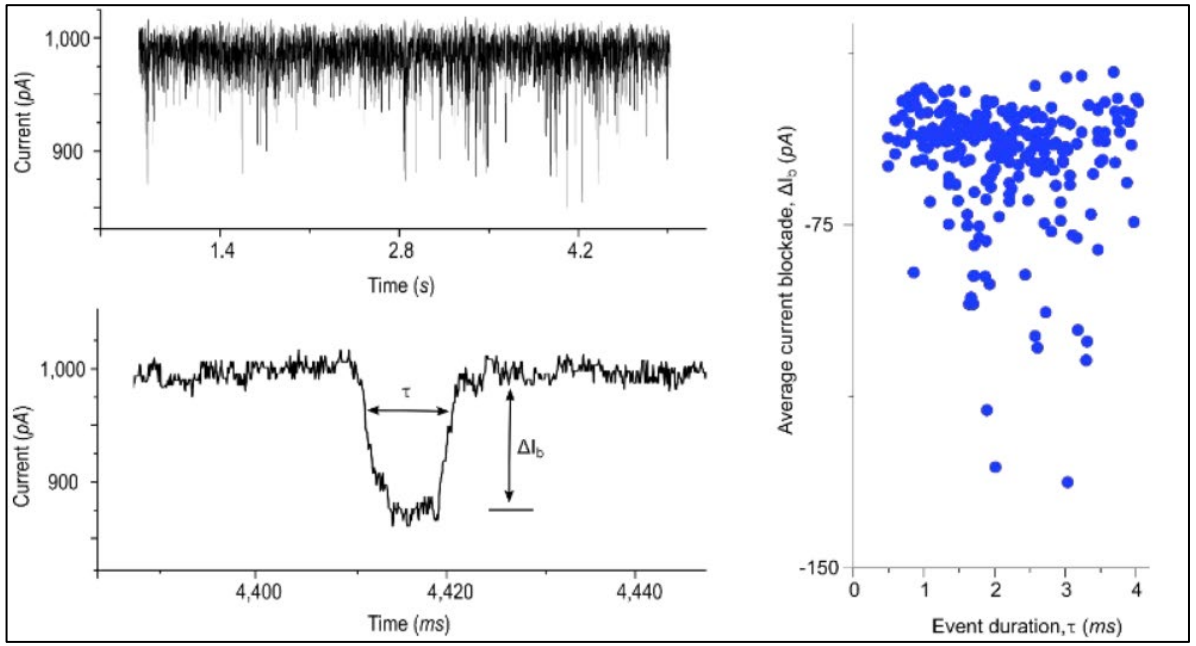
Courtesy: cytosurge

Guillaume-Gentil et al. *Nature* 2023
Guillaume-Gentil et al. *Cell* 2017

Making nanopores on FluidFM Cantilevers

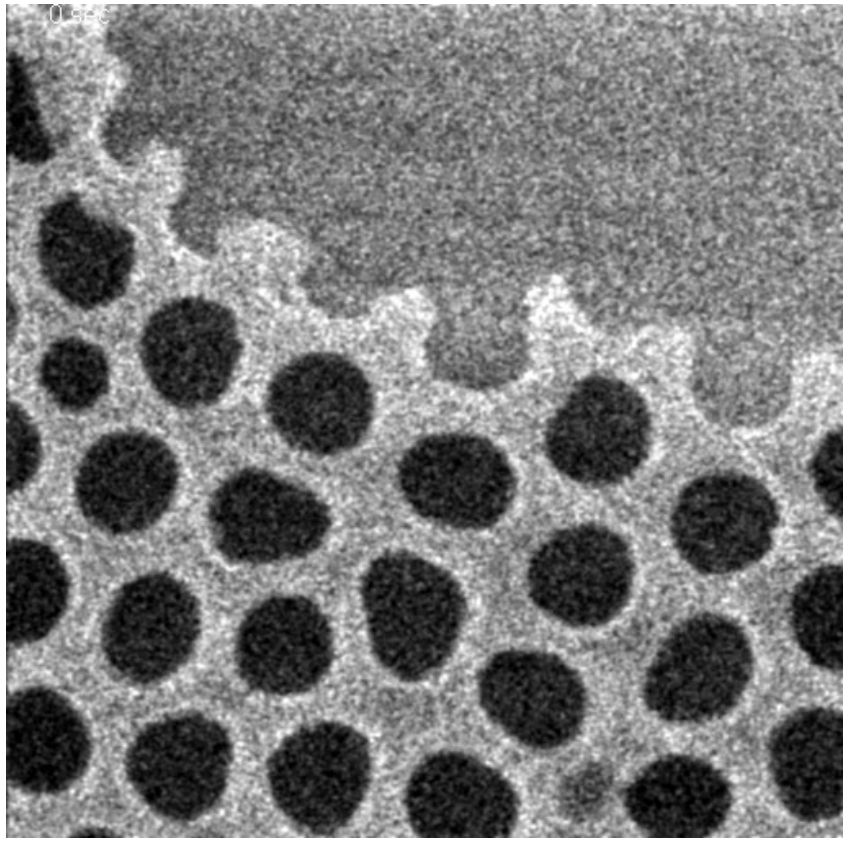


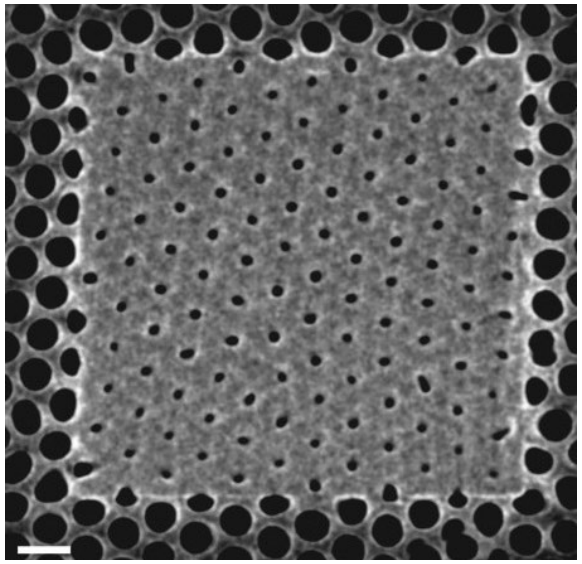


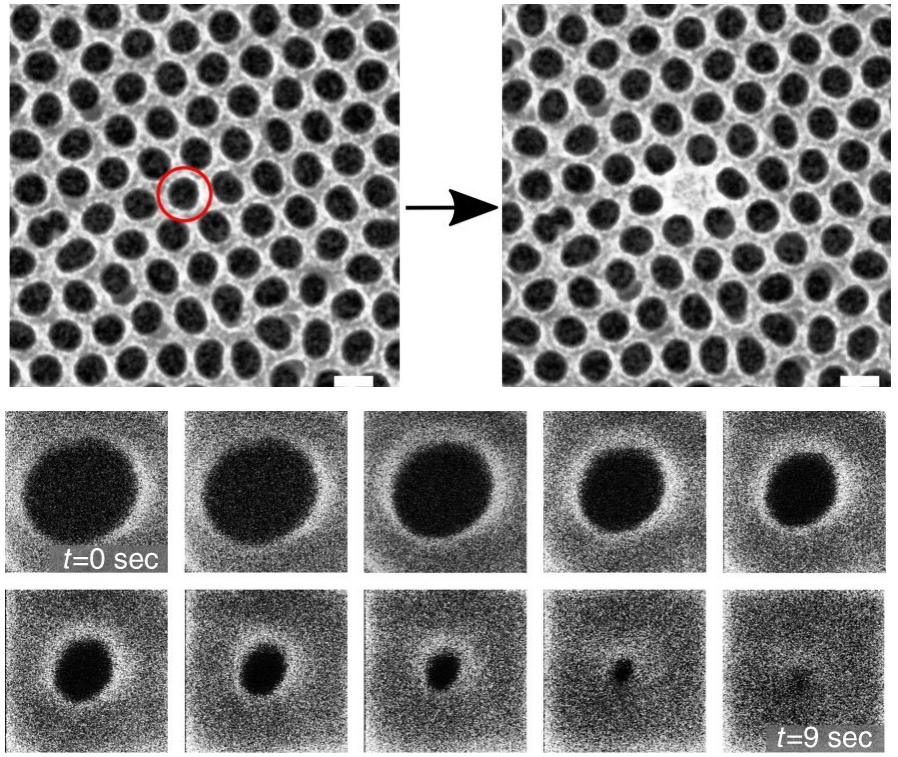


Aramesh and Vörös et al. *Nature Nanotech* 2019
https://www.nature.com/articles/s41565-019-0493-z

Shrinking nanopores with ion irradiation

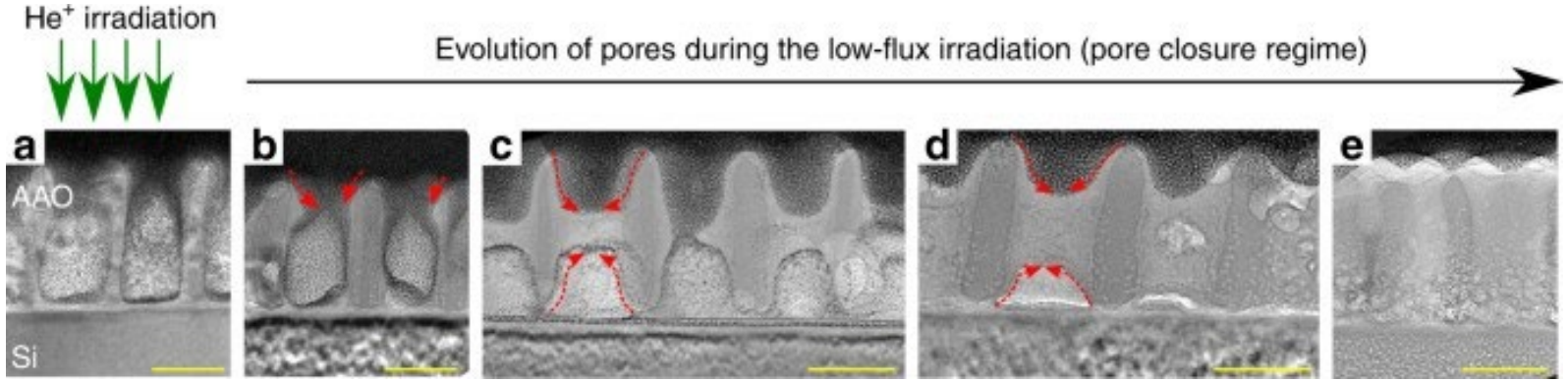




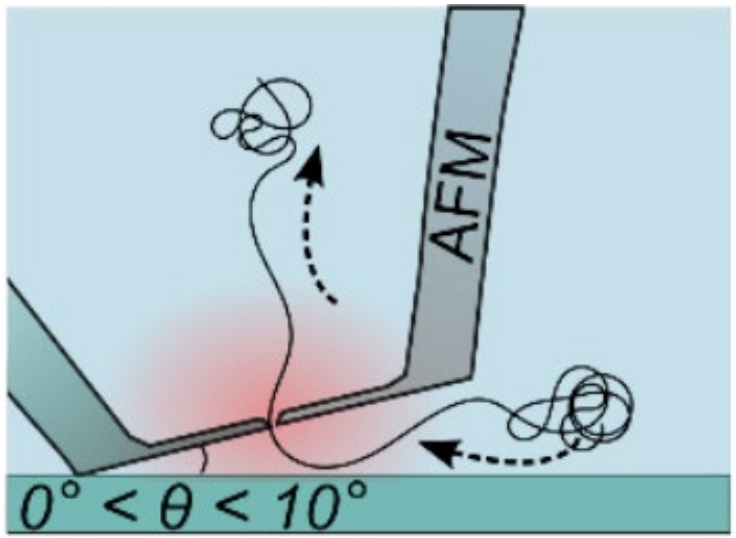


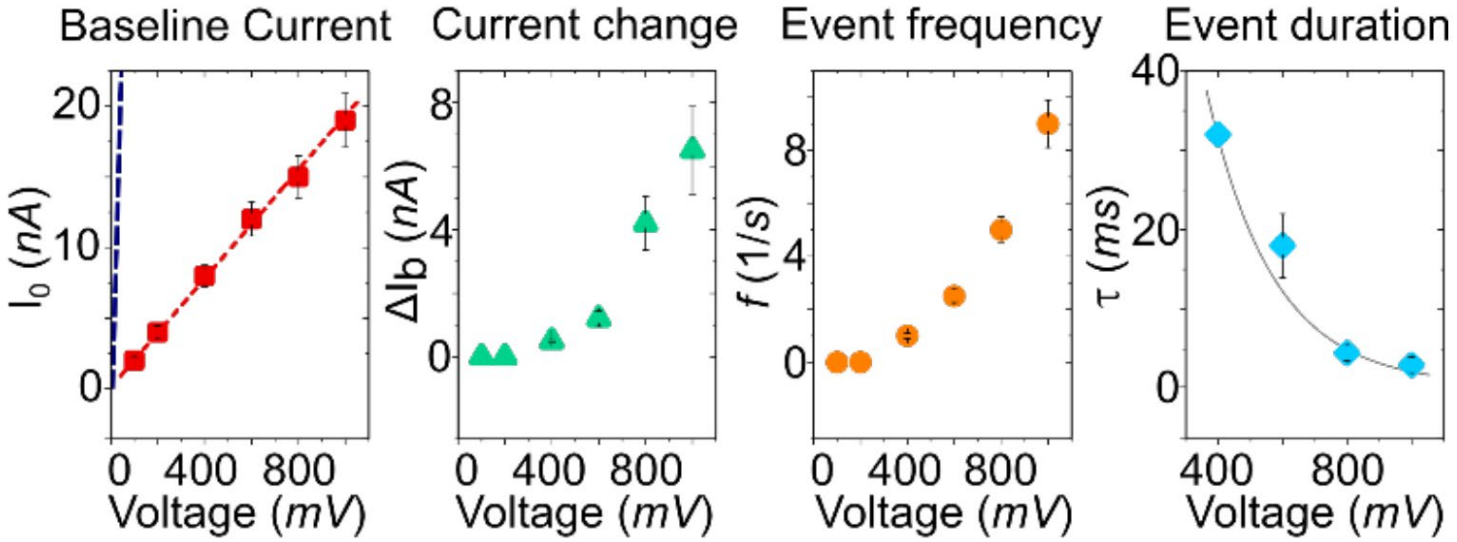
Aramesh et al. *Nat. Commun. 2018*

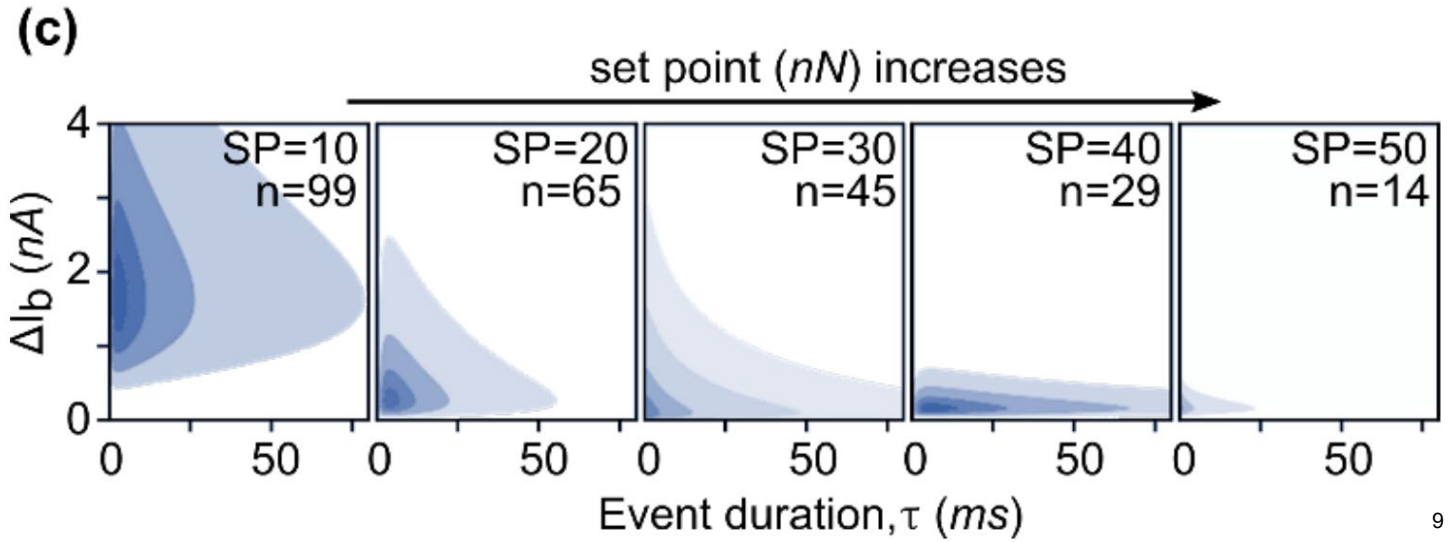
doi: 10.1038/s41467-018-03316-7



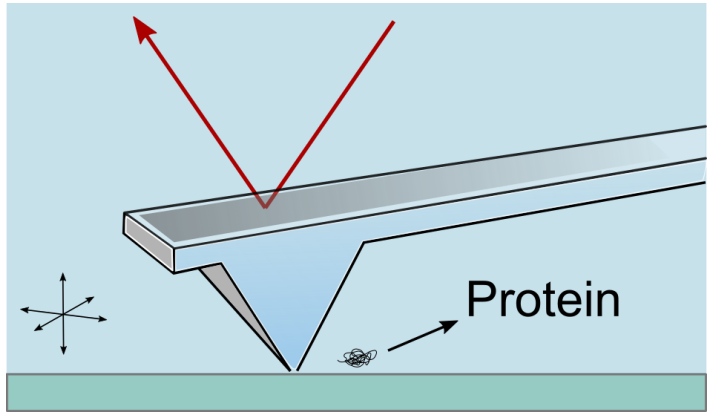
DNA sensing near the surface

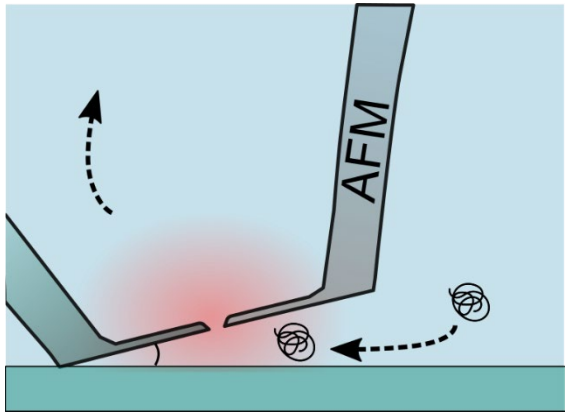


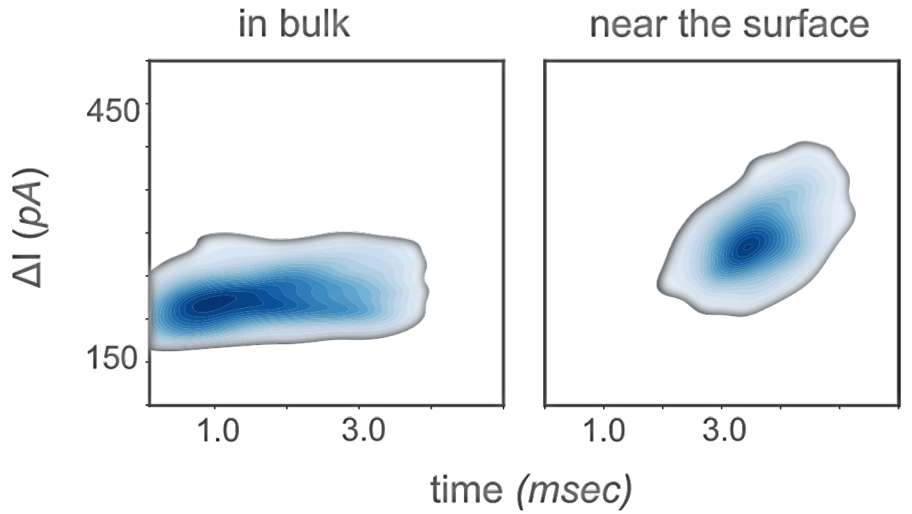




Protein detection – *in bulk vs. near the surface*



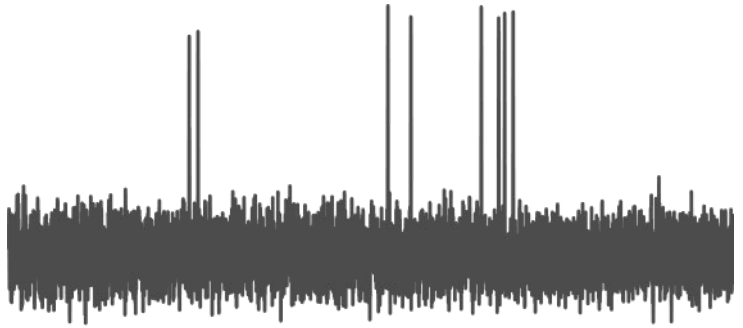




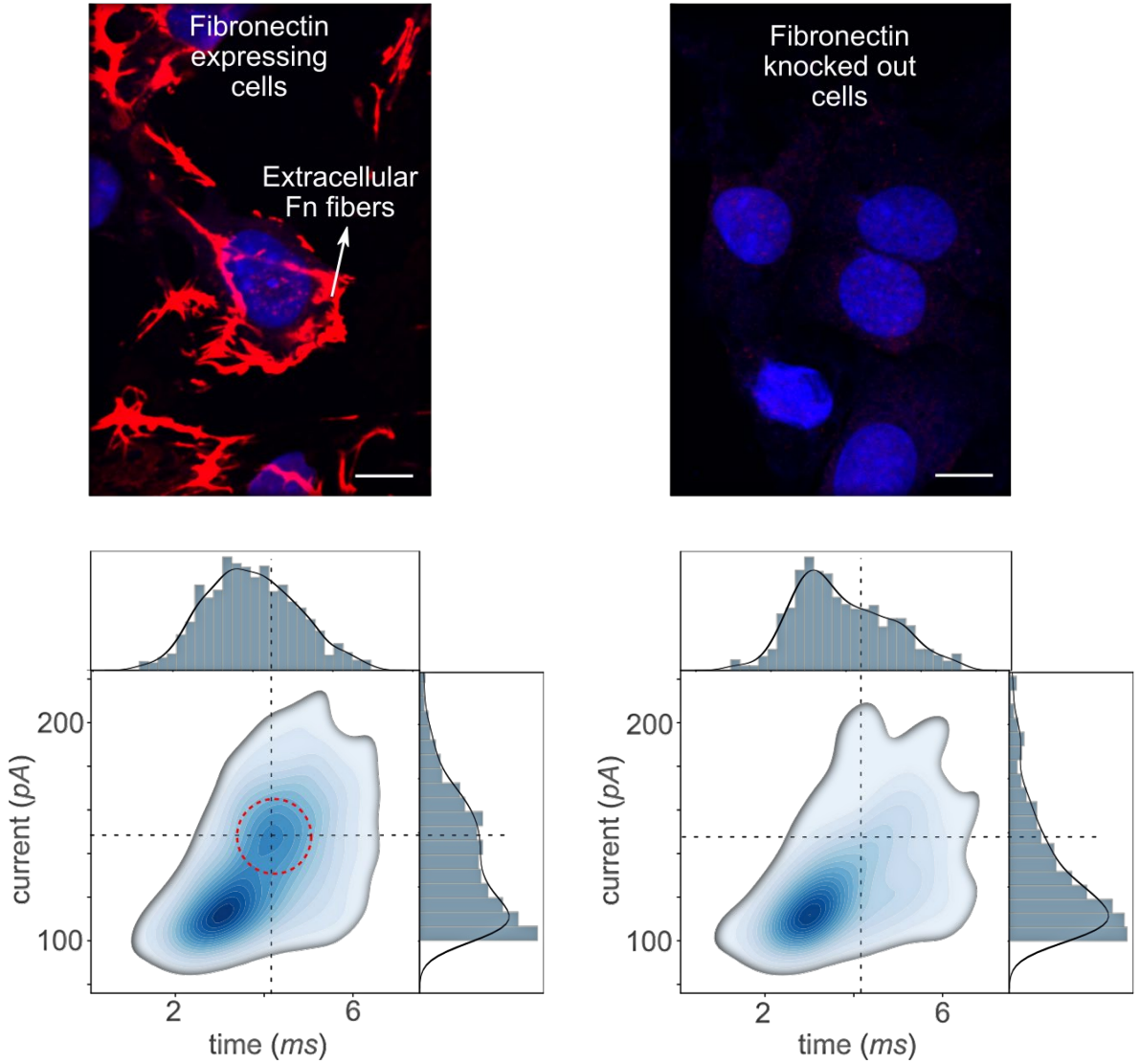
in bulk

near the surface

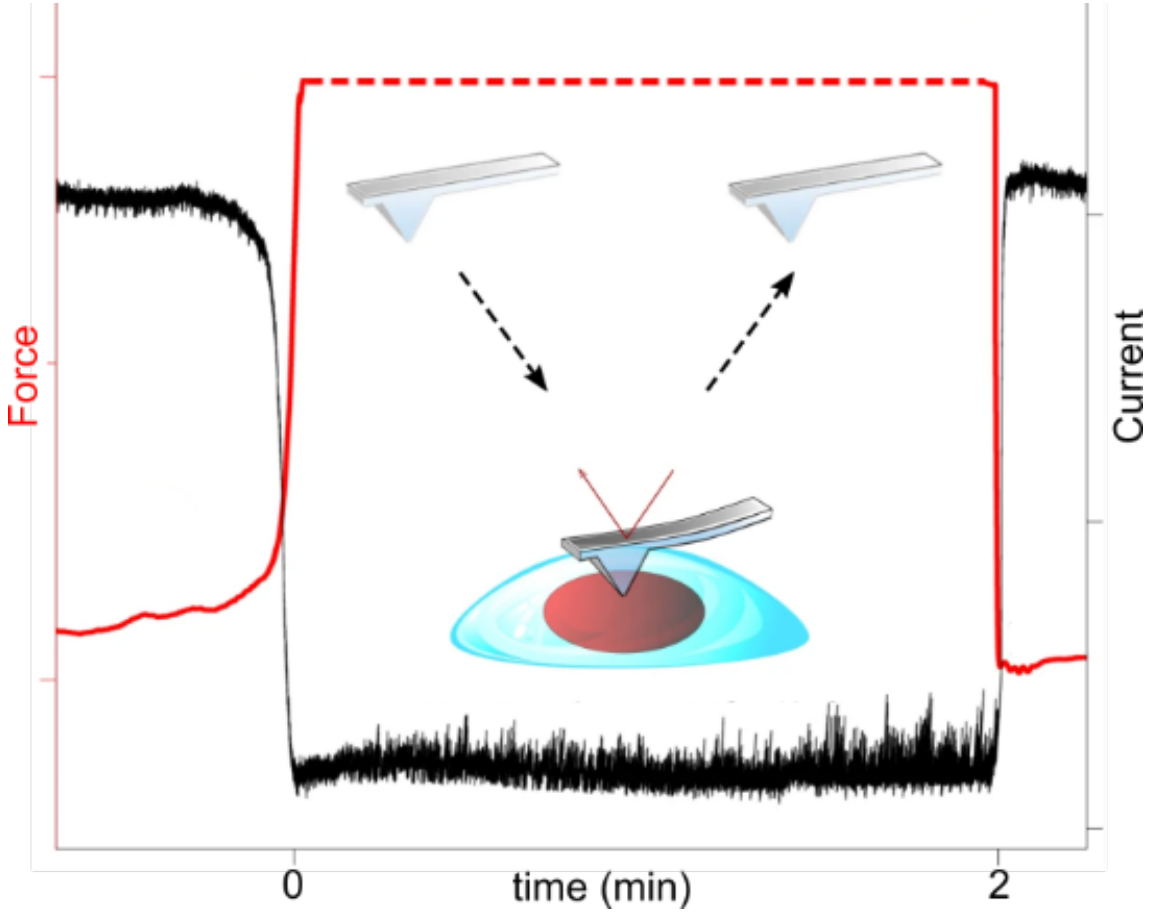
Fn
(600 mV)

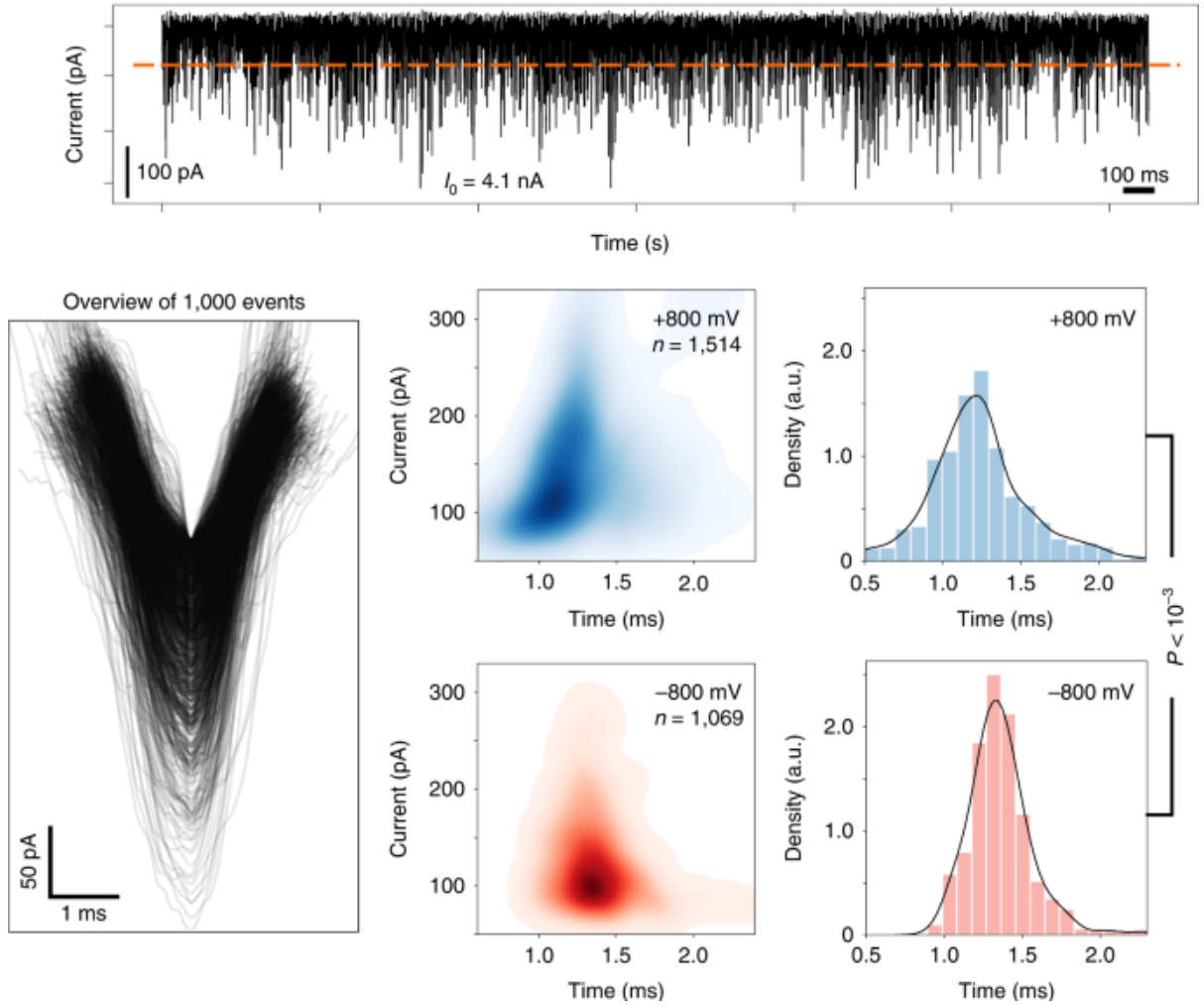


Single cell profiling – *statistical discrimination*

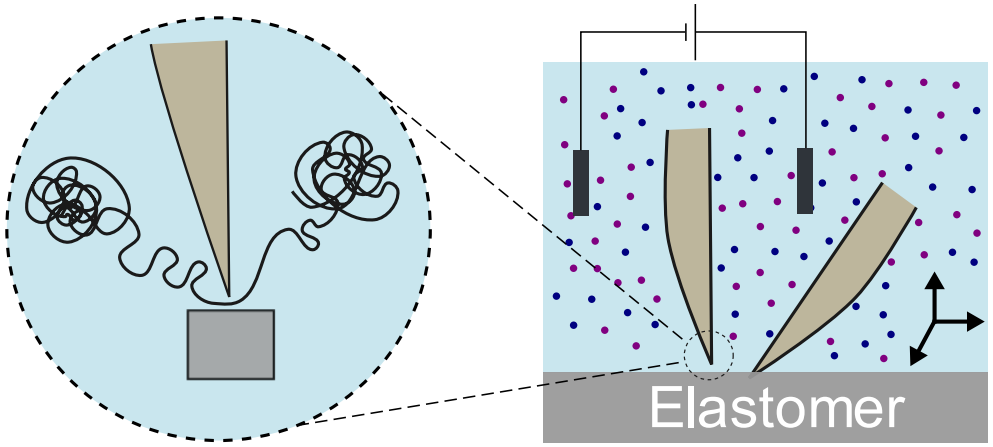


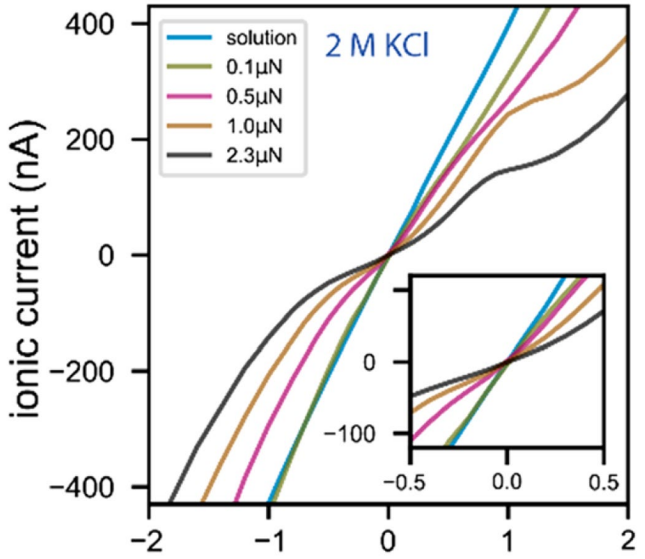
Intracellular content of the cell





Interfacial Nanopores – in situ nanopore formation at the interface

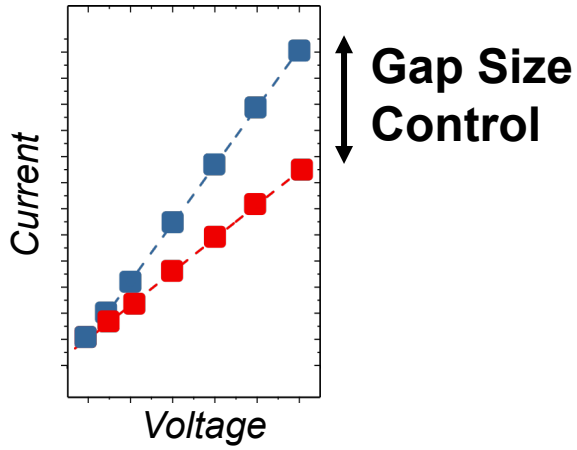


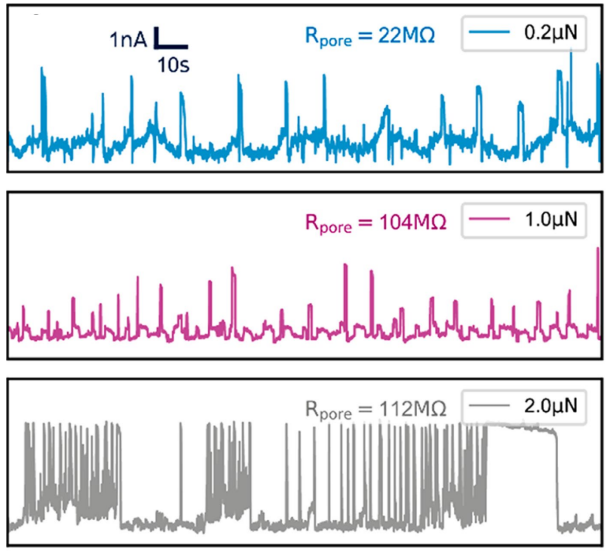


On demand adjustment of Pore size: <1 nm – 300 nm

Clog-free

Gap resistance

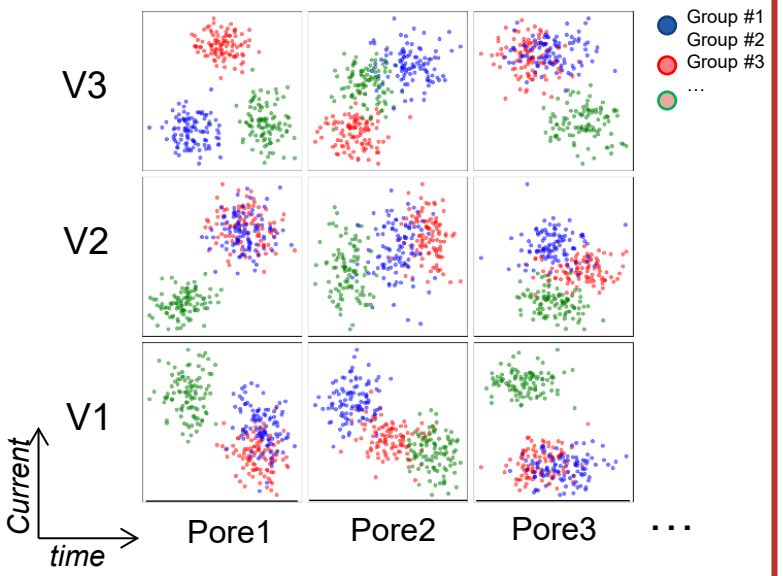




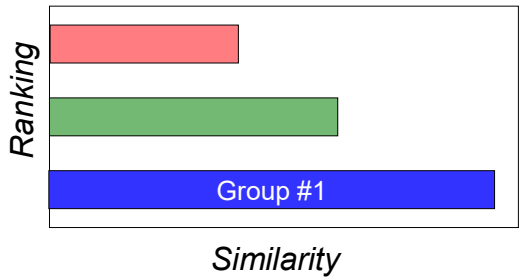
Interfacial Nanopores for Single-Cells Protein Profiling

Proteome profiling

by systematic control of pore size&voltage

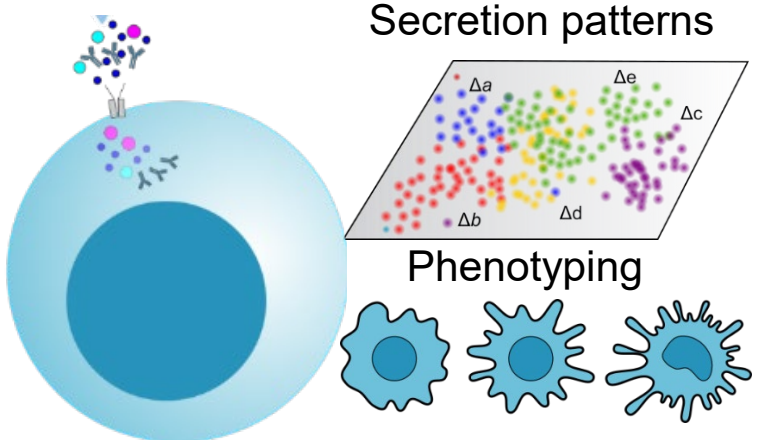


Cluster affiliation of a protein



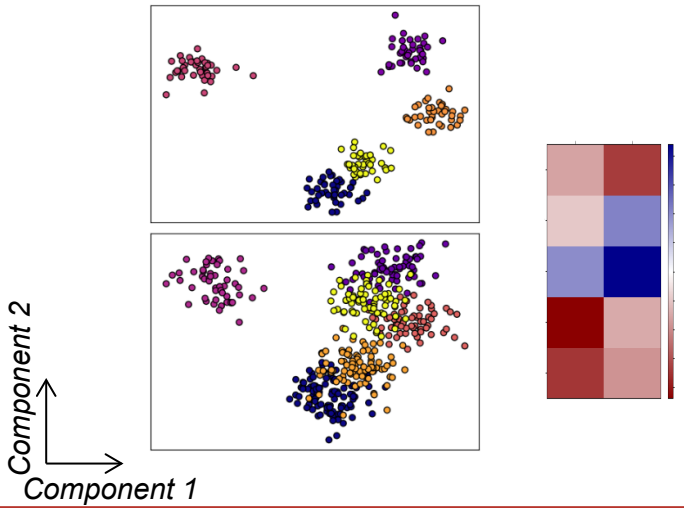
Single Cell Proteome

Secretion patterns



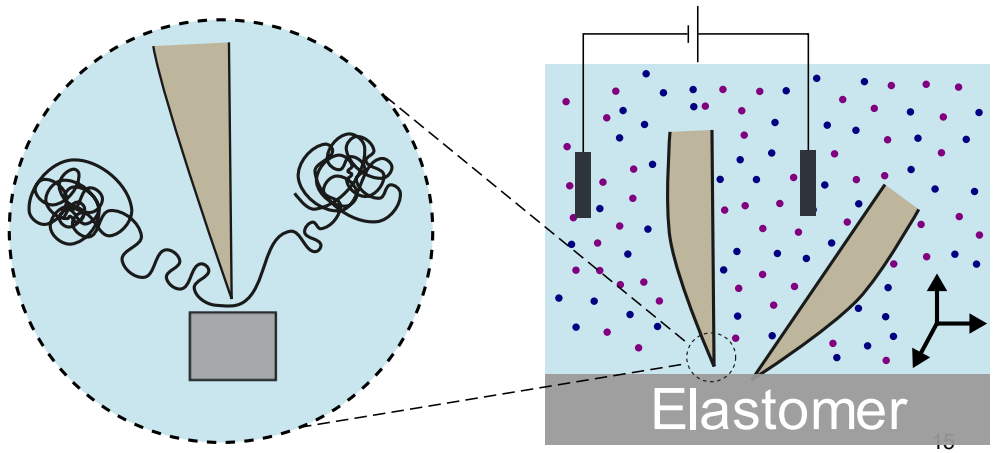
Secretion in Immune cells upon activation

activated vs non-activated B cells



Summary

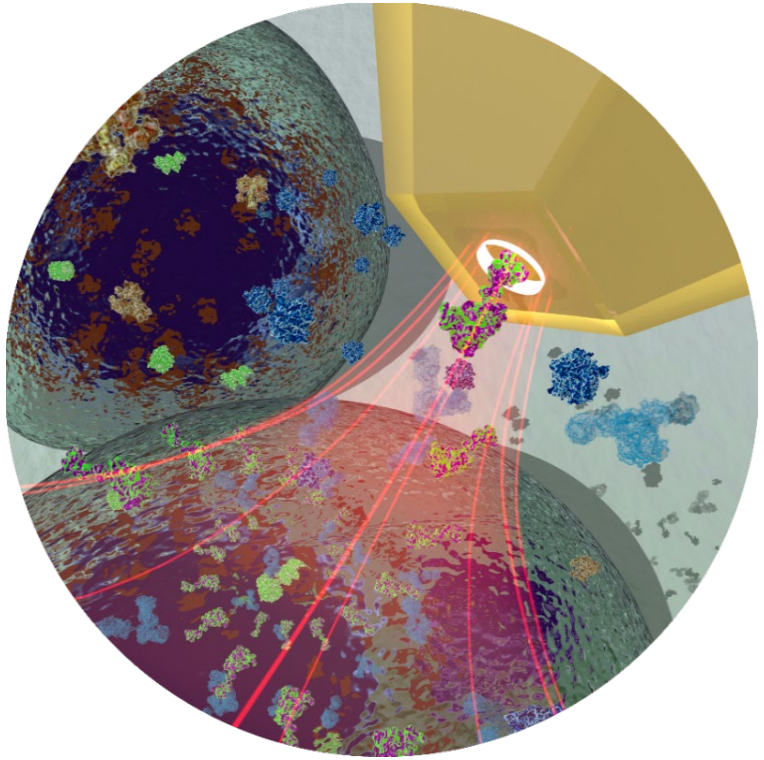
- Nanopores sensors show promise for protein sensing applications , despite several challenges (specificity, clogging, etc.)
- Nanopipettes (FluidFM or glass capillaries) as solid-state nanopore sensors for single-cell profiling/phenotyping
- Interfacial nanopores → adding a new dimension into nanopore analysis
- Road ahead → interfacial nanopores for single-cell secretome profiling



maramesh@ethz.ch
www.bmc.ethz.ch

BMCLab
Biophysics of Single-Molecules and Single-Cells

Thank you!



Acknowledgement:

Prof. Janos Vörös
Prof. Tomaso Zambelli
Dr. Til Schlotter

Open PhD positions!!

ETH zürich

erc