A Biologically Inspired Membrane Tension Sensor for *In Vivo* Force Imaging

A cell's ability to detect and respond to external forces is central to many physiological processes including development, cardiac remodeling, and immune signaling, and also in disease contexts such as tumor growth and cancer metastasis. At the membrane, mechanical forces generate a tension that cells can sense through Piezo proteins, a family of mechanosensitive ion channels (Figure 1A). In response to increased tension, these channels undergo a large conformational change, which opens a transmembrane pore and causes an influx of cations (Figure 1B). While such biological tension-sensing mechanisms are commonplace and often essential for proper cell function, it has not been possible for researchers to directly measure the membrane tension that cells sense. Without the ability to quantify membrane tension, it has remained challenging to determine how forces drive biological function. There is thus a need for new technologies to directly measure membrane tension.

Here, we propose to engineer a membrane tension sensor with fluorescent readout for use in living cells. Our sensor design exploits the natural mechanism of tension sensing by the Piezo protein: We will construct the sensor by crosslinking a force-responsive DNA structure to a chemically modified Piezo protein (Figure 1C, left panel). When the Piezo protein undergoes a tension-triggered conformational change, the ensuing extension ruptures the DNA structure and exposes a specific single-stranded DNA sequence. Exposure of this single-stranded sequence can be detected and visualized in a microscope through binding of a fluorescent DNA oligo ("imager strand") with a sequence complementary to the exposed single-stranded DNA (Figure 1C, right panel). We will acquire these measurements in live cell samples by immersing the cells in a pool of imager strands, using a well-established imaging protocol known as DNA PAINT. In this manner, cells (and locations on cells) that experience increased membrane tension will light up as bright spots under a microscope. Crucially, we can design tension sensors with different detection thresholds by altering the sensors' DNA sequence, and we can multiplex the readout from diverse sensors by using different imager strands labeled with different colors.

We will use our novel sensors to reveal the spatial distribution of membrane tension in cultured cells and in intact tissue under varying amounts of strain. We will also investigate how tissue defects (for instance due to injury) alter force patterns, and at what point these altered patterns lead to pathological remodeling and disease. We envision our fluorescent membrane tension sensors to become a standard tool in the field of mechanobiology.

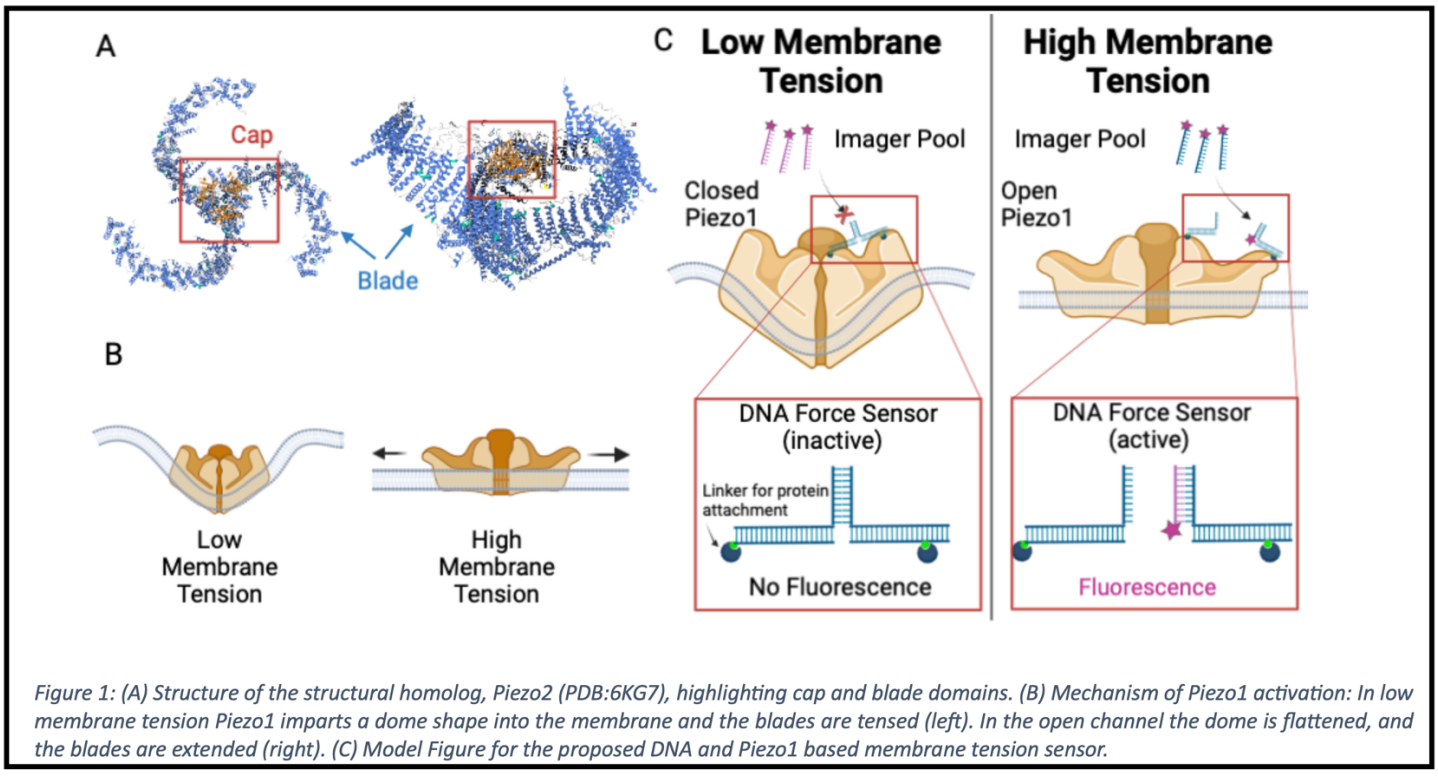

Figure 1: (A) Structure of the structural homolog, Piezo2 (PDB:6KG7), highlighting cap and blade domains. (B) Mechanism of Piezo1 activation: In low membrane tension Piezo1 imparts a dome shape into the membrane and the blades are tensed (left). In the open channel the dome is flattened, and the blades are extended (right). (C) Model Figure for the proposed DNA and Piezo1 based membrane tension sensor.

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PROFESSIONAL APPOINTMENTS

Assistant Professor, 2021-present
Salk Institute for Biological Studies, La Jolla, CA

Assistant Adjunct Professor, Department of Molecular Biology, Division of Biological Sciences, 2021-present
University of California San Diego, La Jolla, CA

Postdoctoral Fellow, Department of Chemistry and Chemical Biology, Department of Physics, 2013-2020
Harvard University, Cambridge, MA
Advisor: Xiaowei Zhuang, Professor of Chemistry and Chemical Biology, Professor of Physics
Research focus: Development of DNA self-assembly methods for single molecule imaging

EDUCATION

Ph.D. in Biochemistry and Molecular Biophysics, *with distinction*, 2012
Columbia University, New York, NY
Advisor: Julio M. Fernandez, Professor of Biological Sciences
Thesis: Mechanochemical methods for single molecule biochemistry

B.S./M.Sc. in Engineering Physics, 2005
Royal Institute of Technology (KTH), Stockholm, Sweden
Thesis research at European Organization for Nuclear Research (CERN), Meyrin, Switzerland
Advisors: Lars-Erik Berg (Professor, KTH), Valentin N. Fedosseev (Senior scientist, CERN)
Thesis: Operation and development of a Resonant Ionization Laser Ion Source

PUBLICATIONS

SELECTED PUBLICATIONS

Rotation tracking of genome-processing enzymes using DNA origami rotors
Kosuri P*, Altheimer BD*, Dai M, Yin P, Zhuang X (**co-first authors*)
Nature 572:136-40 (2019)

S-glutathionylation of cryptic cysteines enhances titin elasticity by blocking protein folding
Alegre-Cebollada J*, Kosuri P*, Giganti D, Eckels E, Rivas-Pardo JA, Hamdani N, Warren CM, Solaro RJ, Linke WA, Fernandez JM (**co-first authors*)
Cell 156:1235-46 (2014) *cover story*

Protein folding drives disulfide formation
Kosuri P, Alegre-Cebollada J, Feng J, Kaplan A, Ingles-Prieto A, Badilla C, Stockwell BR, Sanchez-Ruiz JM, Holmgren A, Fernandez JM
Cell 151:794-806 (2012)

ADDITIONAL PUBLICATIONS

Tetra-gel enables superior accuracy in combined super-resolution imaging and expansion microscopy
Lee H, Yu CC, Boyden ES, Zhuang X, Kosuri P
Scientific Reports 11:16944 (2021)

Work done by titin protein folding assists muscle contraction
Rivas-Pardo JA, Eckels EC, Popa I, Kosuri P, Linke WA, Fernandez JM
Cell Reports 14:1339-1347 (2016)

Predicting readmission of heart failure patients using automated follow-up calls
Inouye S, Bouras V, Shouldis E, Johnstone A, Silverzweig Z, Kosuri P* (**corresponding author*)
BMC Medical Informatics and Decision Making 15:22 (2015)

Picomolar amyloid-β peptides enhance spontaneous astrocyte calcium transients
Lee L, Kosuri P, Arancio O
Journal of Alzheimer's Disease 38:49-62 (2014)

Force dependency of biochemical reactions measured by single-molecule force-clamp spectroscopy
Popa I*, Kosuri P*, Alegre-Cebollada J, Garcia-Manyes S, Fernandez JM (**co-first authors*)
Nature Protocols 8:1261-76 (2013)

Direct observation of disulfide isomerization in a single protein
Alegre-Cebollada J, Kosuri P, Rivas-Pardo JA, Fernandez JM
Nature Chemistry 3:882-7 (2011)

Protease power strokes force proteins to unfold
Alegre-Cebollada J, Kosuri P, Fernandez JM
Cell 145:339-40 (2011) *preview*

Single-molecule paleoenzymology probes the chemistry of resurrected enzymes
Perez-Jimenez R, Ingles-Prieto A, Zhao Z, Sanchez-Romero I, Alegre-Cebollada J, Kosuri P, Garcia-Manyes S, Kappock TJ, Tanokura M, Holmgren A, Sanchez-Ruiz JM, Gaucher EA, Fernandez JM
Nature Structural & Molecular Biology 18:592-6 (2011)

Single-molecule force spectroscopy approach to enzymatic catalysis
Alegre-Cebollada J, Perez-Jimenez R, Kosuri P, Fernandez JM
Journal of Biological Chemistry 285:18961-6 (2010)

Kalman filter estimates of the contour length of an unfolding protein in single-molecule force spectroscopy experiments
Fernandez VI, Kosuri P, Parot P, Fernandez JM
Review of Scientific Instruments 80:113104 (2009)

Partially folded equilibrium intermediate of the villin headpiece HP67 defined by 13C relaxation dispersion
O'Connell NE, Grey MJ, Tang Y, Kosuri P, Miloushev VZ, Raleigh DP, Palmer AG
Journal of Biomolecular NMR 45:85-98 (2009)

Diversity of chemical mechanisms in thioredoxin catalysis revealed by single-molecule force spectroscopy
Perez-Jimenez R, Li J, Kosuri P, Berne BJ, Fernandez JM
Nature Structural & Molecular Biology 16:890-6 (2009)

Force-clamp spectroscopy detects residue co-evolution in enzyme catalysis
Perez-Jimenez R, Wiita AP, Rodriguez-Larrea D, Kosuri P, Gavira JA, Sanchez-Ruiz JM, Fernandez JM
Journal of Biological Chemistry 283:27121-9 (2008)

Coupling of ribosomal L1 stalk and tRNA dynamics during translation elongation
Fei J, Kosuri P, MacDougall DD, Gonzalez RL
Molecular Cell 30:348-59 (2008)

Development of a RILIS ionisation scheme for gold at ISOLDE, CERN
Marsh BA, Fedosseev VN, Kosuri P
Hyperfine Interactions 171:109-16 (2006)

PATENTS

Force-clamp spectrometer with functionalized cantilever tip, US 9,880,088 (Licensed to: *Luigs & Neumann GmbH*)
Fernandez JM, Perez-Jimenez R, Kosuri P

Ancestral proteins, EP 2,593,472 (Licensed to: *Evolgene Genomics SL*)
Fernandez JM, Perez-Jimenez R, Gaucher E, Kosuri P

INVITED SEMINAR TALKS (SELECTED)

Foundations of Nanoscience, Snowbird, UT, 2023

Johns Hopkins University School of Medicine, Dept. of Molecular Biology and Genetics, Baltimore, MD, 2023

UC Irvine, Department of Developmental and Cell Biology, Irvine, CA, 2023

Aspen Center for Physics: Single Molecule Biophysics Meeting, Aspen, CO, 2023

Karolinska Institutet, Department of Medical Biochemistry and Biophysics, Stockholm, Sweden, 2022

Okanagan Biophysics Conference, University of British Columbia, Kelowna, BC, 2022

Frontiers in Biophysics Conference, Simon Fraser University, Vancouver, BC, 2022 (*keynote*)

Swiss Society of Biomaterials & Regenerative Medicine, ETH Zürich, Switzerland, 2022 (*keynote*)

Boston Protein Design and Modeling Seminar Series, Harvard Medical School, Boston, MA, 2021

Genetics, Bioinformatics and Systems Biology Colloquium, UC San Diego, San Diego, CA, 2020

Biophysical Society Annual Meeting, San Francisco, CA, 2018 (*session co-chair*)

Physics of Living Systems, Harvard University, Cambridge, MA, 2014

Bauer Forum, Center for Systems Biology, Harvard University, Cambridge, MA, 2014

The New York Academy of Sciences, New York, NY, 2012

TEACHING & ADVISING EXPERIENCE

Chromatin Structure & Dynamics BGGN 283 / BIMM 194 (Undergraduate and Graduate Level), UC San Diego

Cellular Physiology of Disease (Undergraduate and Graduate Level), Columbia University

Molecular Biophysics (Graduate Level), Columbia University

Experimental Biophysics (Graduate Level), Tel Aviv University, Israel

AWARDS

Beckman Young Investigator Award

Titus M. Coan Prize for Excellence in Basic Research

Columbia University Distinction Award for doctoral defense

Columbia Technology Ventures Validation Fund Award

Henrik Göransson Sandviken Foundation Scholarship

Fulbright Scholarship

OTHER SERVICE & EXPERIENCE

COMMITTEES

- Program Director, Physical Cell Biology, Biophysical Society, 2022-2023
- Elected Representative, Academic Council, Salk Institute for Biological Studies, 2022-present
- Director, Engagement & Wellbeing Initiative, Salk Institute for Biological Studies, 2021-present
- Board Member, Harvard University Institutional Review Board (IRB), 2018-2020
- President, Graduate Student Organization, Columbia University Medical Center, 2007-2008

EDUCATIONAL OUTREACH

- Group Leader, Mentor, Harvard Health Professions Recruitment & Exposure Program (HPREP), 2013-2015

OTHER PROFESSIONAL APPOINTMENTS

- InSITE Fellow, Startup & Venture Capital fellowship at Columbia Business School, 2011-2015
- Research Fellow, Columbia Technology Ventures, Technology Transfer, 2010-2013

MEMBERSHIP IN PROFESSIONAL SOCIETIES

- American Heart Association (AHA), 2021-present
- International Society for Nanoscale Science, Computation and Engineering (ISNSCE), 2018-present
- Biophysical Society (BPS), 2008-present

GRANT REVIEWER (SELECTED)

- German Research Foundation (DFG)
- City University of New York (CUNY)
- University of Wisconsin-Milwaukee (UWM)

RESEARCH ARTICLE REVIEWER (SELECTED)

- Nature
- Nature Physics

TRAINEES

- Postdoctoral fellows:
    - Yuening Liu, PhD
    - Abigail Neininger, PhD
- Doctoral students:
    - Amanda Wacker (Biological Sciences)
    - Ryan Fantasia (Biological Sciences)
    - Jocelyn Olvera (Biological Sciences; co-mentored by Dmitry Lyumkis)
- Master's student:
    - Julia Rune (Visiting student from KTH Royal Institute of Engineering, Sweden)
- Undergraduate students:
    - Jerry Wu (Bioengineering)
    - Annabelle Coles (Bioinformatics)
- Research Assistant:
    - Lauren Takiguchi