BIOGRAPHICAL SKETCH

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NAME: **KARATEKIN, Erdem**

eRA COMMONS USER NAME (credential, e.g., agency login): **EKARATEKIN**

POSITION TITLE: Associate Professor of Cellular and Molecular Physiology (with Tenure)

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Louisville, Louisville, Kentucky	B.S. (Highest Honors)	05/1994	Chemical Engineering
Columbia University, New York, New York	Ph.D. (Distinction)	10/1999	Chemistry, soft matter
Curie Institute, Paris, France	Postdoctoral	12/2002	Membrane biophysics

A. Personal Statement

My lab aims to understand how mechanical information is transmitted within the cell membrane, in the form of membrane tension and flow. For this, we have developed concepts and approaches during >25 years of research on membrane phenomena, from artificial lipid bilayers to bacteria and neurons, including a diverse range of methods such as optical and electrical recordings, laser tweezers, micromechanical measurements, quantitative image analysis, and novel biochemical reconstitutions. They are fostered by strategic collaborations with experts at Yale and elsewhere.

My past and current interests span mechanisms of exocytosis, membrane fusion and fission, dynamics of exocytic fusion pores, and cell membrane tension propagation and membrane flow phenomena. We use laser tweezers and micromanipulation for cell membrane tension and flow measurements, and electrochemical, electrophysiological, and imaging methods to study single exo- and endocytosis events in neuroendocrine cells and neurons. Through these studies, we have realized that membrane *dynamics*, i.e. how the membrane flows in response to tension gradients, is critical for exo-endocytosis coupling, membrane fission, and likely many other membrane phenomena. First, different cell types display a surprising range of tension and flow dynamics, with nearly all cells lacking long-range propagation of membrane tension perturbations. Two exceptions we found are neurons and B-cells, which have extremely rapid tension dynamics, likely reflecting evolutionary specializations. Second, we found that the very different vesicle membrane recycling routes in different cell types may be explained by their very different cell membrane flow resistance: slow membrane flow imposes local recycling, while rapid membrane flow allows release and retrieval sites to be uncoupled. The former occurs in neuroendocrine cells, and the latter in the giant presynaptic terminal of the goldfish retinal bipolar neuron. Third, we showed that the cell membrane tension varies with exo-endocytosis activity in the retinal bipolar neuron presynaptic terminal. This suggests a decrease in membrane tension due to membrane addition may be a signal for endocytosis, even when endocytosis sites are far away from release sites. Fourth, the rapid tension dynamics stop when a B-cell encounters antigen. Fifth, membrane tension gradients and the membrane flows they drive are essential for membrane fission in bacteria during endospore formation.

Plasma membrane (PM)-cytoskeleton attachment is clearly important for cell membrane tension propagation and flow, but what are the most important linkers, and what determines their density? The spatial organization of the linkers should influence membrane flow resistance, but there are no measurements relating the two. Is the linker distribution dynamically regulated by lipid metabolism, and/or PM organization? How is membrane tension propagation rapidly switched off in B-cells upon antigen encounter? We will address these basic questions. Since all cells communicate with their environment through their cell membrane and all receive mechanical inputs, knowing how mechanical information is transmitted within the cell membrane will likely have very broad impact, including helping us understand (1) why different cell types display vastly different membrane tension dynamics; (2) how the cell knows when, where and how much compensatory endocytosis

to perform following stimulated exocytosis; (3) how B-cell signaling is related to cell membrane tension and flow dynamics.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2003-11 CNRS Chargé de Recherche/Investigator (permanent post, 1st class from 1/2007, long-term leave since 2011), *Saints-Pères Paris Institute for the Neurosciences (SPPIN – CNRS / Université Paris Cité UMR 8003).*
2008-11 Visiting Research Scientist, Cell Biology, School of Medicine, Yale University, New Haven, CT (on leave from CNRS). With James E. Rothman.
2012-18 Assistant Professor, Department of Cellular and Molecular Physiology, School of Medicine, Yale University, New Haven, CT.
2012- Member, Nanobiology Institute, Yale University West Campus, West Haven, CT
2015-18 Assistant Professor, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT. (Secondary appointment).
2018-22 Associate Professor on term, Department of Cellular and Molecular Physiology, School of Medicine, Yale University, New Haven, CT.
2018-22 Associate Professor on term, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT. (Secondary appointment).
2022- Wu Tsai Institute, Yale University, New Haven, CT.
2022- Associate Professor with tenure, Department of Cellular and Molecular Physiology, School of Medicine, Yale University, New Haven, CT.
2022- Associate Professor with tenure, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT. (Secondary appointment).

Honors and Awards

1994 Metro Conference Student Athlete of the Year, Metro Athletic Conference, USA.
1994-99 Faculty Fellowship, Columbia University, New York.
1999 Hammett Award, “for excellence in studies and research toward the Ph.D.”, Columbia University, New York.
1999 “Distinction” for dissertation work, awarded to a small fraction of dissertations university-wide, Columbia U.
2000-01 Association pour la Recherche sur le Cancer (ARC) Post-doctoral Fellowship.
2001-02 Fondation Pour la Recherche Médicale (FRM) Post-doctoral Fellowship.
2002 European Molecular Biology Organization (EMBO) long term fellowship.
2013-15 Kavli Neuroscience Scholar Award, Kavli Foundation.
2021 LabEx Cell(n)Scale International Chair (Institut Curie, Paris).

Review panels

2025 **NIH reviewer** (ad hoc) for the Cell Structure and Function Panel 1 (CSF1) member conflict panel, ZRG1-CDB-A(02)M

C. Contributions to Science

1. Soft matter physics and chemistry of free radical polymerization. During my doctoral work advised jointly by experimentalist Nicholas J. Turro and theoretician Ben O’Shaughnessy, I invented new methods to study mechanisms of free radical polymerization (FRP), the process used to produce the majority of synthetic polymeric materials. In FRP, a free radical created by photolysis or heat attacks surrounding monomer, covalently attaching itself to it and transferring the unpaired, highly reactive electron to the newly added monomer. Thus, the radical group is always at the end of a growing chain. Growth ends when two reactive chain-ends meet, or when the radical is transferred to a monomer without addition. The length distribution of the reactive chains is the fundamental quantity that determines the properties of the end-product, yet due the transient nature and low concentrations of reactive chains, this measurement had never been achieved previously. We showed that the growing chains could be rapidly capped with fluorescent labels by creating a large excess of stable, fluorescently labeled free radicals using an intense laser pulse to photolyse “photoinhibitor” compounds. The labeled, quenched free radical chains could subsequently be analyzed using

standard methods. In addition, I developed a method to follow the chain-addition reactions in real time. This relied on photochemically creating free radicals with unpaired electrons whose spin distribution was far from equilibrium (spin polarized radicals) which could be detected using time-resolved electron paramagnetic resonance spectroscopy (EPR). Spin polarization is maintained during a few monomer addition reactions, each addition transferring the unpaired electron to the growing end of the chain, and the EPR spectra reporting on each addition event. (* indicates corresponding author)

a. E. Karatekin, B. O'Shaughnessy, and N. J. Turro*, *"Time Resolved EPR: A Novel Method for Studying Living Chains"*, Macromolecules, **31**, 7992-7995 (1998).
b. E. Karatekin, M. S. Landis, G. Lem, B. O'Shaughnessy*, and N. J. Turro, *"Photocopying Living Chains. 1. Steady-State"*, Macromolecules, **34**, 8187-8201 (2001).
c. E. Karatekin, M. S. Landis, G. Lem, B. O'Shaughnessy*, and N. J. Turro, *"Photocopying Living Chains. 2. Time-Dependent Measurements"*, Macromolecules, **34**, 8202-8215 (2001).

2. Transient pores in lipid bilayers. As a post-doc with Françoise Brochard-Wyart at the Curie Institute, I studied dynamics of transient pores in giant liposomes (10-50 μm diameter). When liposomes are filled with a highly viscous solution there is little leakage of contents during rapid dilation of a pore until the tiny line tension, the energy per unit length for creating a bilayer edge, is balanced by the membrane tension that relaxes as the pore grows. This allows pores to reach sizes that are as large as half the liposome radius, allowing direct visualization of pore dynamics. Pore closure is limited by the slow leakage of contents that maintains the quasi-balance between the line and membrane tensions. Pore closure dynamics allow quantification of line tension, whose precise measurement had been a challenge because lipid bilayers do not like having edges. I showed how pore dynamics could be modulated by manipulating the line tension. We drew an analogy between adsorption of edge-active compounds that partitioned between the bulk phase and pore edges, and surface-active compounds that partition between a bulk phase and a surface, measuring a one-dimensional Langmuir edge-adsorption isotherm (line tension as a function of bulk concentration of edge-actants). In addition, I showed that lowering the line tension facilitated fusion between protein-free giant liposomes. This marked my entry into the field of membrane fusion which has been a major focus ever since.

a. E. Karatekin, O. Sandre, H. Guitouni, N. Borghi, P.-H. Puech, and F. Brochard-Wyart*, *"Cascades of Transient Pores in Giant Vesicles: Line Tension and Transport"*, Biophys. J., **84**, 1734-1749 (2003).
b. P.-H. Puech, N. Borghi, E. Karatekin, and F. Brochard-Wyart*, *"Line Thermodynamics: Adsorption at a Membrane Edge"*, Phys. Rev. Lett., 90, 128304 (2003).
c. N. Borghi, F. Brochard-Wyart, E. Karatekin, and I. Derényi, *"Giant vesicles: Transient pores and tube extraction"*. Proc. Int. School of Physics "Enrico Fermi". Course CLV, F. Mallamace and H. E. Stanley (Eds.); IOS Press, Amsterdam (2004).

3. Secretory granule motions and calcium-triggered exocytosis. As an investigator of the of the Centre National de la Recherche Scientifique (CNRS), in the Laboratory of Cellular and Molecular Secretion (CNRS UPR1929, Institut de Biologie Physico-Chimique, Paris), I developed hardware and analysis tools to study calcium-regulated exocytosis in human neuroendocrine cells. Using a home-built total internal reflection fluorescence (TIRF) microscope, we described the 3-dimensional transient motions of secretory granules, and correlated motion types with underlying mechanisms. Shortly before exocytosis secretory granules make a step-wise approach toward the plasma membrane, a "priming" event required for fusion competence. Further, we demonstrated how fusion of a granule with the ghost of one previously fused with the plasma membrane (sequential fusion) could be differentiated from transient, "kiss-and-run" fusion events using TIRF microscopy and showed that sequential fusion occurs more frequently than previously appreciated.

a. S. Huet, E. Karatekin*, V. S. Tran, I. Fanget, S. Cribier and J.-P. Henry*, *"Analysis of transient behavior in complex trajectories: application to secretory vesicle dynamics."* Biophys. J. **91**, 3542-3559 (2006).
b. V. S. Tran, S. Huet, I. Fanget, S. Cribier, J. P. Henry and E. Karatekin*, *"Characterization of sequential exocytosis in a human neuroendocrine cell line using evanescent wave microscopy and `virtual trajectory' analysis"*, Eur. Biophys. J., **37**, 55-69 (2007).
c. E. Karatekin*, S. Tran, S. Huet, I. Fanget, S. Cribier and J. P. Henry*. *"A 20 nm step toward the cell membrane preceding exocytosis may correspond to docking of tethered granules."* Biophys. J., **94**, 2891-2905 (2008).

4. Mechanisms of SNARE-mediated fusion. Still in Paris, I developed a novel microfluidic-based assay to detect docking and fusion of *single* v-SNARE reconstituted small unilamellar vesicles (v-SUVs) with t-SNARE bearing planar bilayers supported on a soft, hydrophilic polymer cushion (t-SBLs). To learn the biochemistry and reconstitution of SNARE proteins I collaborated initially with Michael Seagar (Neurobiology of Ionic Channels and the Synapse, INSERM, Marseille) and later, during an extended leave from the CNRS, with

James E. Rothman (Cell Biology, Yale University). While at Yale, I improved the assay to achieve single-molecule resolution and developed software tools for analysis, in collaboration with D. Vavylonis (Lehigh U.). The assay showed that 5-10 SNARE complexes are required for efficient fusion, 100-200 ms after docking.

a. E. Karatekin*, J. Di Giovanni, C. Iborra, J. Coleman, B. O'Shaughnessy, M. Seagar, and J. E. Rothman*, *"A fast, single-vesicle fusion assay mimics physiological SNARE requirements."* Proc. Natl. Acad. Sci. USA, **107**, 3517–3521 (2010).
b. M. B. Smith, E. Karatekin, A. Gohlke, H. Mizuno, N. Watanabe, D. Vavylonis*, *"Interactive, computer-assisted tracking of speckle trajectories in fluorescence microscopy: application to actin polymerization and membrane fusion."* Biophys. J., **101**, 1794-1804 (2011).
c. E. Karatekin*, J. E. Rothman, *"Fusion of single proteoliposomes with planar, cushioned bilayers in microfluidic flow cells"* Nature Protocols, **7**, 903-920 (2012).
d. W. Xu, B. Nathwani, C. Lin, J. Wang, E. Karatekin, F. Pincet, W. Shih*, J. E. Rothman*, *"A Programmable DNA Origami Platform to Organize SNAREs for Membrane Fusion"*, J. Am.Chem. Soc., 138,4439-4447 (2016).

5. Dynamics of single fusion pores. After I started my laboratory in the Dept. of Cellular and Molecular Physiology at Yale, we focused our attention on the dynamics of the fusion pore – the initial, nm-sized connection between two fusing membranes. In every system where the pore could be detected with sufficient sensitivity and time-resolution, from protein-free fusion of artificial lipid bilayers to viral fusion to exocytosis, it was found that the pore is a metastable structure. It can fluctuate in size, open and close repeatedly (flicker), and either dilate or shut irreversibly. Fusion pore properties determine the size and the amount of cargo released, and the time course of release, which affect downstream processes. Despite its universal nature and importance, molecular mechanisms governing pore nucleation and dynamics were poorly understood, mainly due to a lack of biochemically defined assays with single-pore sensitivity and sub-ms time resolution. We developed such assays and discovered how cholesterol, SNARE transmembrane domains, molecular crowding, and the neuronal calcium sensor Synaptotagmin-1 affect pore flickering, lifetimes and sizes.

a. ● Z. Wu, S. M. Auclair, O. Bello, W. Vennekate, N. Dudzinski, S. Krishnakumar, and E. Karatekin*, *"Nanodisc-cell fusion: control of fusion pore nucleation and lifetimes by SNARE protein transmembrane domains"*, Sci. Rep., **6**, 27287; doi: 10.1038/srep27287 (2016).
Here we established a novel assay to probe single, nm-sized fusion pores with biochemically defined composition with sub-ms time resolution using methods adapted from single ion-channel recordings and employing fusion of SNARE-reconstituted nanodiscs with engineered cells expressing the complementary SNARE proteins on their surfaces with a flipped topology.
b. ● B. S. Stratton, Z. Wu, J. M. Warner, G. Wei, E. Karatekin*****, and Ben O'Shaughnessy*, *"Cholesterol Increases the Openness of SNARE-Mediated Flickering Fusion Pores"* Biophys. J. **110**, 1538–1550 (2016).
c. ●● Z. Wu, O. Bello, S. Thiyagarajan, S. M. Auclair, W. Vennekate, S. S. Krishnakumar, B. O'Shaughnessy, and E. Karatekin*, *"Dilation of fusion pores by crowding of SNARE proteins"*, *eLife*; 6:e22964 doi: 10.7554/eLife.22964 (2017).
Using the single-pore nanodisc-cell fusion assay, this work demonstrates that only 2-3 SNARE complexes are sufficient for opening a fusion pore, but more than a dozen are required to dilate it efficiently from its small preferred radius (~1 nm). Using quantitative modeling, molecular crowding is identified as a key mechanism driving pore dilation.
d. ● Z. Wu, N. Dharan, Z.A. McDargh, S. Thiyagarajan, B. O'Shaughnessy*, E. Karatekin*, "*The neuronal calcium sensor Synaptotagmin-1 and SNARE proteins cooperate to dilate fusion pores"*. eLife **10**:e68215 doi: 10.7554/eLife.68215 (2021).
This manuscript shows how the major neuronal calcium-sensor, Synaptotagmin-1, dilates fusion pores in a SNARE, calcium, and PI(4,5)P_2-dependent manner. Combining previous structural data and our experimental and modeling results, we suggest Synaptotagmin-1 uses the post-fusion SNARE complex as a calcium-dependent mechanical lever to dilate fusion pores.

6. Dynamics of cell membranes. We showed that the cell membrane tension propagates at vastly different speeds in different eukaryotic cell types, and that properties of the associated membrane flows impose strong constraints on the spatiotemporal coupling of exo- and endocytosis. We discovered a new membrane fission mechanism in bacteria that uses the same physical principle as membrane fission in some clathrin-independent endocytosis, requiring tension-driven lipid flow between two membrane compartments, and oligomerization of a protein at their boundary that impairs flow and leads to a runaway tension increase, causing membrane fission through lysis. In collaborative work, we found that PLSCR1 prevents SARS-CoV-2 infection by inhibiting membrane fusion. Current work is aimed at understanding how cell membrane flows impact cellular processes, in particular exo-endocytosis coupling.

a. ●●● C. Gomis Perez, N.R. Dudzinski, M. Rouches, A. Landajuela, B. Machta, D. Zenisek, E. Karatekin. *"*Rapid propagation of membrane tension at retinal bipolar neuron presynaptic terminals.*"* Sci Adv. **8**:eabl4411. doi: 10.1126/sciadv.abl4411 (2022). PMID: 34985955.
This work suggested that exo-endocytosis coupling may be regulated by cell membrane tension and flow dynamics, probed using optical tweezers force measurements of membrane tethers pulled from a giant neuronal presynaptic terminal and a neuroendocrine cell.

b. A. Landajuela*, M. Braun, C.D.A. Rodrigues, A. Martínez-Calvo, T. Doan, F. Horenkamp, A. Andronicos, V. Shteyn, N.D. Williams, C. Lin, N.S. Wingreen, D.Z. Rudner, E. Karatekin*, *"FisB relies on homo-oligomerization and lipid binding to catalyze membrane fission in bacteria."* PLoS Biol., **19**:e3001314. doi: 10.1371/journal.pbio.3001314. (2021). PMID: 34185788
This work showed that in bacteria, membrane fission relies on the recruitment of a FisB protein membrane scaffold at the fission site, but the scaffold by itself cannot mediate fission, nor remodel membranes.

c. ●● A. Landajuela†*, M. Braun†*, A. Martínez-Calvo, C. D. A. Rodrigues, C. Gomis Perez, T. Doan, D. Z. Rudner, N. S. Wingreen, and E. Karatekin*, (†equal contribution; *co-corresponding authors), *"Membrane fission during bacterial spore development requires cellular inflation driven by DNA translocation"*, Curr. Biol., 32:4186-4200.e8. doi: 10.1016/j.cub.2022.08.014. (2022) PMID: 36041438
This work was the first description of a membrane fission mechanism in bacteria. The mechanism relies on synergy between a membrane tension gradient that mobilizes membrane flow and a protein scaffold at the fission site that impairs flow due to high protein-membrane friction.

d. D. Xu, W. Jiang, L. Wu, R.G. Gaudet, E.S. Park, M. Su, S.K. Cheppali, N.R. Cheemarla, P. Kumar, P.D. Uchil, J.R. Grover, E.F. Foxman, C.M. Brown, P.J. Stansfeld, J. Bewersdorf , W. Mothes, E. Karatekin, C.B. Wilen, J.D. MacMicking*. *"PLSCR1 is a cell-autonomous defence factor against SARS-CoV-2 infection."* Nature, **619:**819–827. https://doi.org/10.1038/s41586-023-06322-y (2023) PMID: 37438530

e. ●● M. Tsemperouli†, S.K. Cheppali†, F. Rivera Molina, D. Chetrit, A. Landajuela, D. Toomre, E. Karatekin*, "*Vesicle docking and fusion pore modulation by the neuronal calcium sensor Synaptotagmin-1"*, († equal contribution). Biophy. J. 124(11):1798–1814. PMID: 39719826 DOI: 10.1016/j.bpj.2024.12.023 (2025).
We identified mutations in Synaptotagmin-1 that induce vesicle docking defects and make fusion pores expand faster in a human neuroendocrine cell line, using electron microscopy and electrical detection of serotonin release from single fusion events. In follow up work in preparation, we showed the mechanism to be due to lower membrane-Syt1 friction in the mutants.

Complete List of Published Work in MyBibliography:
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