BIOGRAPHICAL SKETCH

NAME: Michael Lin

eRA COMMONS USER NAME: LIN.MICHAEL

POSITION TITLE: Associate Professor of Neurobiology and Bioengineering

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Harvard University, Cambridge, Massachusetts	A.B. *summa cum laude*	06/1994	Biochemistry
Harvard Medical School, Boston, Massachusetts	Ph.D.	06/2002	Biological and Biomedical Sciences
UCLA School of Medicine, Los Angeles, California	M.D.	06/2004	Medicine
UCSD School of Medicine, La Jolla, California	postdoctoral	05/2009	Pharmacology and Neuroscience

A. Personal Statement

My research interests are at the interface of protein chemistry, protein engineering, and genetics. I received training with Dr. Michael Greenberg on the biochemistry of signal transduction enzymes, and with Dr. Roger Tsien on fluorescent protein chemistry and engineering. My laboratory specializes in applying structure-based protein engineering to improve fluorescent protein reporters for deep-tissue imaging in animals, and to design sensors of signal transduction and electrical activity in living cells.

My laboratory has special expertise in developing new imaging methods for studying neurobiology. We developed the ASAP-family of green fluorescent voltage indicators, which produce the largest fluorescence responses of genetically encoded voltage indicators characterized to date while tracking action potentials with sub-millisecond time resolution. We have used ASAP-family indicators together with high-speed two-photon scanning methods to visualize voltage responses in the brains of flies and mice, in work reported in *Nature Neuroscience* in 2014, *Cell* in 2016, and *Cell* in 2019. We have also developed bright bioluminescent reporters of calcium that enable non-invasive activity imaging, in work reported in *Nature Chemical Biology* in 2019. Another method is the visualization of activity-induced proteins in healthy and disease neurons using the drug-stabilized TimeSTAMP tag, in work published in *PNAS* in 2019. We have also developed improved fluorescent proteins for FRET-based reporting, including bright green, red, and far-red fluorescent proteins, in work reported in *Nature Methods* and *Nature Biotechnology*. I have disseminated my experience in fluorescent protein chemistry and fluorescent reporter development by writing reviews of voltage indicators and other genetically encoded neuronal activity indicators.

My lab also has a long-standing interest in applying protein engineering to detecting and controlling cell signals. For example, we have developed a novel photoregulatory domain from a fluorescent protein and used it to control a variety of protein activities in living cells in work reported in *Science* in 2013 and 2017. In addition, we pioneered the use of sequence-specific viral proteases to detect and control protein activity in living cells, beginning with a 2008 paper in *PNAS*, and continuing with studies published in 2015 in *Nature Chemical Biology* and 2018 in *Nature Methods*. Since 2010 we have been developing the RASER system, using NS3 to specifically rewire oncogenic signals that drive breast cancer to therapeutic outputs. RASER is the first treatment that is limited to cancerous states via intracellular signaling and thus highly novel. It is also highly effective, as we demonstrated it was superior to standard of care in specificity and efficacy on breast cancer cells, in work published in *Science* in 2019. We are excited to use the RASER system as the basis for engineering viruses with exquisite specificity for diseased cancer cells and the ability to amplify in tumors, with the goal of more complete and lasting treatments for breast cancer.

Selected Research Support

NIH/NINDS 1RM1NS132981 (co-PIs: Yuste, Basu, Nedivi, Segev, Lichtman, Lin) 07/01/2023 – 06/30/2028
"Reexamining the role of dendrites in neuronal function".
Major Goals: (1) Improve sensitivity and control subcellular localization of negatively tuned GEVIs for imaging dendritic and synaptic potentials, (2) Functional probing of dendritic responses of *Wfs1* neurons *in vivo,* (3) Anatomical and synaptic mapping of *Wfs1* neuron dendritic trees, (4) Computational modeling of *Wfs1* pyramidal cells, and (5) Multidimensional structure-activity datasets of *Wfs1* pyramidal cells.

NIH/NINDS 1R01NS123681-01 (PI: Lin) 06/01/2021 – 05/31/2024
"The power of positivity: a novel class of voltage indicators for high-fidelity brain activity imaging".
Major goals: (1) High-content combinatorial screening of improved voltage indicators based on ASAP4, (2) quantitative validation by 1-photon and 2-photon microscopy *in vivo*, and (3) developemnt of an ultrahigh-throughput method for further screening of voltage indicators.

NIH/NIAID U19AI171421-8526 (PI: Glenn, subproject PI: Lin) 05/01/2022 – 04/31/2025
"Development of outpatient antiviral cocktails against SARS-CoV-2 and other potential pandemic RNA viruses: Development of selective and potent protease inhibitors for corona and other pandemic viruses".
Major Goals: (1) optimizing the therapeutic index (TI) and pharmacokinetic (PK) profile of SARSCoV2 Mpro inhibitors, and define their therapeutic potential; (2) generalizing our methods to other coronaviruses and key togaviruses, and study our inhibitors' mechanisms of action.

Wallace Coulter Foundation (PI: Lin) 05/16/2021 – 05/15/2022
"Potent and selective protease inhibitors for treatment of coronavirus disease".
Major goal: Optimizing boceprevir-based inhibitors of SARSCoV2 for oral administration.

University Hospitals Harrington Discovery Institute 07/01/2022 – 06/30/2024
"Potent and selective protease inhibitors for treatment of COVID-19 and future coronavirus diseases"
Major goal: Optimization and testing of tripeptideSARSCoV2 protease inhibitors.

NIH/NINDS 1R21NS122055-01 (PI: Lin) 05/01/2021 – 10/31/2022
"Bioluminescent indicators for noninvasive imaging of acetylcholine release".
Major goals: (1) creating a bioluminescent ACh indicator based on a bacterial binding domain for ACh; and (2) creating a bioluminescent ACh indicator based on a muscarinic G-protein-coupled receptor.

NIH/NINDS 1U01NS103464 (PI: Lin) 08/01/2017 – 07/30/2020
"Bringing laser focus to voltage imaging: Enhanced indicators and advanced scanning methods for two-photon recording of dense networks in vivo"
Major goals: (1) develop red-emitting, subcellularly localized, and ratiometric voltage indicators; (2) develop random-access multi-photon microscopy with motion correction and enhanced throughput; (3) record voltage from hundreds of neurons *in vivo* with millisecond precision.

NIH/NIMH 1RF1MH114105 (PI: Lin) 08/08/2017 – 08/07/2020
"Revealing circuit control of neuronal excitation with next-generation voltage indicators"
Major goals: (1) generate brighter and more responsive variants of the voltage indicators ASAP2s and Ace-mNeonGreen; (2) validate voltage indicators for reporting contributions of specific inputs to subthreshold and action potential responses in fly neurons in vivo; (3) test indicator performance under two-photon excitation in striatal spiny projection neurons in acute brain slices and in living mice.

NIH 5DP1GM111003-02 (PI: Lin) 10/1/2013 – 07/31/2018
"Optogenetics For All: A General Method for Optical Control of Protein Activity"
Major goals: (1) to characterize the fluorescent light-inducible protein architecture; (2) to extend the phenomenon of light-induced dissociation of fluorescent protein domains to target proteins with various topologies; (3) to determine whether fluorescent light-induced proteins can be used to control neuronal development in living animals.

B. Positions and Honors

Positions

- 1992-1994: Undergraduate research assistant, Laboratory of Dr. Fotis Kafatos, Dept. of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts
- 1994-1996: Medical student, Laboratory of S. Larry Zipursky, Dept. of Biological Chemistry, UCLA School of Medicine, Los Angeles, California
- 1996-2002: Graduate research associate, Laboratory of Dr. Michael E. dGreenberg, Division of Neuroscience, Children's Hospital, Boston, Massachusetts
- 2002-2004: Medical student, UCLA School of Medicine, Los Angeles, California
- 2004-2009: Postdoctoral research fellow, Laboratory of Dr. Roger Y. Tsien, Dept. of Pharmacology and Howard Hughes Medical Institute, University of California at San Diego, La Jolla, California
- 2009-2017: Assistant Professor of Pediatrics and Bioengineering and, by courtesy, Chemical and Systems Biology, Stanford University School of Medicine, Stanford, California
- 2017-current: Associate Professor of Neurobiology and Bioengineering and, by courtesy, Chemical and Systems Biology, Stanford University School of Medicine, Stanford, California

Honors

- Roger Tsien Award for Excellence in Chemical Biology, World Molecular Imaging Society, 2019
- NIH Pioneer Award, 2013
- Rising Star Award, Biomedical Engineering Society, Cellular and Molecular Bioengineering Group, 2013
- Damon Runyon-Rachleff Innovation Award, November 2012
- Rita Allen Scholar Award, 2011
- Young Investigator Award, Alliance for Cancer Gene Therapy, 2010
- Burroughs Wellcome Career Award for Medical Scientists, 2007
- University of Illinois at Urbana Champagne Young Excellence in Engineering Seminar, 2006
- Jane Coffin Childs Memorial Fund for Medical Research Fellowship, 2005
- United States Department of Defense Graduate Fellowship, 1996
- NIH Medical Scientist Training Program Scholarship, 1994
- Harvard University Phi Beta Kappa, 1994

Other Experience, Professional Memberships, and Certifications

- 2019, 2021: Co-organizer, Cajal Advanced Neuroscience Training course on Biosensors and Actuators in Cellular and Systems Neuroscience
- 2021: Organizer committee member, World Molecular Imaging Congress
- 2011-current: Member, American Society for Biochemistry and Molecular Biology
- 2008-current: Member, Biophysical Society
- 2008-current: Member, American Society for Cell Biology
- 2006-current: Member, Society for Neuroscience
- USMLE Step I and II certified

C. Contribution to Science

1. A major contribution of my research with wide impacts in multiple fields of biology has been the engineering of fluorescent reporters to enable dynamic real-time imaging of biochemical processes in cells and animals. Most notably, we have developed the ASAP family of genetically encoded voltage indicators that are fast cofactor-free brightly fluorescent and highly responsive. ASAP-family voltage indicators are also equally highly responsive in both one-photon and two-photon imaging. They are now widely used the field of neuroscience for recording spiking patterns in genetically defined neuronal networks and visualizing subcellular and subthreshold voltage dynamics. On the opposite end fo the temporal spectrum, my lab has also created a the Fucci4 indicator system for reporting of all four cell cycle stages, including a reliable S-G2 transition indicator. Fucci4 is now widely used to investigate how genetic and environmental perturbations alter the cell cycle.

Evans SW*, Shi DQ*, Chavarha M*, Plitt MH, Taxidis J, Madruga B, Fan JL, Hwang FJ, van Keulen SC, Suomivuori CM, Pang MM, Su S, Lee S, Hao YA, Zhang G, Jiang D, Pradhan L, Roth RH, Liu Y, Dorian CC, Reese AL, Negrean A, Losonczy A, Makinson CD, Wang S, Clandinin TR, Dror RO, Ding JB, Ji N, Golshani P, Giocomo LM, Bi GQ, **Lin MZ**. A positively tuned voltage indicator for extended electrical recordings in the brain. ***Nature Methods*** **2023**, 20:1104. PMCID: PMC10627146. *Equal contributions.

Villette V*, Chavarha C*, Dimov IK, Bradley J, Pradhan L, Mathieu B, Evans SW, Chamberland S, Shi D, Yang R, Kim BB, Ayon A, Jalil A, St-Pierre F, Schnitzer MJ, Bi G, Toth K, Ding J, Dieudonné S, **Lin MZ.** Ultrafast two-photon imaging of a high-gain voltage indicator in awake behaving mice. ***Cell*** **2019**, 179:1590. PMCID: PMC6941988. *equal contribution.

St-Pierre F, Marshall JD, Yang Y, Gong Y, Schnitzer MJ, **Lin MZ**. High-fidelity optical reporting of neuronal electrical activity with an ultrafast fluorescent voltage sensor. ***Nature Neuroscience*** **2014**, 17:884. PMCID: PMC4494739.

Bajar BT*, Lam AJ*, Badiee R, Oh YH, Chu J, Zhou XX, Kim N, Kim BB, Chung M, Yabonovitch AL, Cruz BF, Kulalert K, Tao JJ, Meyer T, Su XD, **Lin MZ**. Fluorescent indicators for simultaneous reporting of all four cell cycle phases. ***Nature Methods*** **2016,** 13:989. PMCID: PMC5548384.

2. Another of my major contributions has been to advance the capabilities of bioluminescence imaging, which has vast unexplored potential for translational research through the combination of optical and molecular imaging but which has been constrained for the past few decades by inherent catalytic and wavelengths limits of luciferase enzymes. We have pioneered the adaptation of the highly catalytic NanoLuc luciferase for in vivo anatomical and activity imaging. We described the first comparison of NanoLuc and FLuc for in vivo mouse imaging (Chu et al. listed below), then later collaborated with Promega to develop improved substrates for NanoLuc-based reporters for body imaging (Su et al., 2020 Nature Methods, not listed here) and brain imaging (Su et al. listsed below). We also created the first calcium-modulated bioluminescent indicator (CaMBI) capable of visualizing organ-wide calcium signaling in mice completely non-invasively, through the body wall, without surgery (Oh et al. below). Finally we created a series of kinase-modulated bioluminescent indicators (KiMBIs) for non-invasive real-time optical reporting of drug pharmacodynamics in tumors and tissues in living mice (Wu et al. below).

Wu Y, Walker JR, Ning L, Westberg M, Monje M, Kirkland TA, **Lin MZ***, Su Y*. Kinase-modulated bioluminescent indicators enable noninvasive imaging of drug activity in the brain. ***ACS Central Science*** **2023**, 9:719. PMCID: PMC10141594. *Co-corresponding authors.

Su Y†, Walker JR†, Hall M, Klein M, Wu X, Encell L, Casey K, Liu LX, Hong G, **Lin MZ***, Kirkland TA*. An optimized bioluminescent substrate for non-invasive anatomical and activity imaging in the brain. ***Nature Chemical Biology*** **2023**, 19:731. PMCID: PMC10229426. †Equal contributions. *Co-corresponding authors.

Oh Y, Park Y, Cho JH, Wu H, Paulk NK, Liu LX, Kim N, Kay MA, Wu JC, Lin MZ. An orange calcium-modulated bioluminescent indicator for non-invasive activity imaging. ***Nat Chem Biol. 2019*** May;15(5):433-436. PMCID: PMC6563924.

Chu J, Oh YH, Sens A, Ataie N, Dana H, Macklin J, Laviv T, Welf ES, Dean KM, Zhang F, Kim BB, Tang CT, Hu M, Baird MA, Davidson MW, Fioka F, Kay M, Fiolka R, Yasuda R, Kim DS, Ng H-L, **Lin MZ**. A bright cyan-excitable orange fluorescent protein enables dual-emission microscopy and highly sensitive bioluminescence imaging *in vivo*. ***Nature Biotechnology*** **2016,** 34:760. PMCID PMC4942401.

3. Another of my major contributions has been developing generalizable and robust methods for creating single-chain optically controlled proteins, a longstanding challenge in the fields of chemical and optical biology. Through the reengineering of a green fluorescent protein from a purely fluorescent function to acquire photoallosteric features, we generated photodissociable dimeric fluorescent proteins, the only cofactor-free proteins with molecular interactions regulated by visible light. These pdFPs reassociate upon illumination wih another wavelength of visible light, and self-report their oligomerization state via fluorescence, giving them a total of three useful capabilities not seen in natural LOV and CRY domains. We then pioneered a generalizable single-chain architecture for photocontrol of protein activity. We have applied our photoswitchable proteins to discover fast feedback pathways in kinase pathways and investigate temporal functions of proteins in the nervous system.

Westberg M†, Song D†, Duong V, Fernandez D, Huang P-S, **Lin MZ**. Photoswitchable binders enable temporal dissection of endogenous protein function. *In review. Preprint at* https://doi.org/10.1101/2023.09.14.557687. †*Equal contributions.*

Zhou XX, Fan L, Zhou P, Shen K, **Lin MZ.** Optical control of cell signaling by single-chain photoswitchable kinases. ***Science*** **2017**, 355:836. PMCID: PMC5589340.

Zhou XX, Chung HK, **Lin MZ.** Optical control of protein activity by fluorescent protein domains. ***Science*** **2013**,

4. Another of my major contributions has been improving the photophysics of fluorescent proteins to make them more robust tools for the community of biologists at large. For example, my lab developed optimal fluorescent protein pairs for FRET and dual-lifetime imaging (Lam et al., Chu et al., and Laviv et al. below), the brightest monomeric far-red fluorescent protein with superior performance for non-invasive longitudinal imaging of stem cell differentiation in living mice (Chu et al. below), and uniquely bright cyan-excitable orange and red fluorescent proteins.

Laviv T*, Kim BB*, Chu J, **Lin MZ**, Yasuda R. Simultaneous dual-color fluorescence lifetime imaging with novel red-shifted fluorophores. ***Nature Methods*** **2016,** 13:989*.* doi:10.1038/nmeth.4045. PMID 27798609. PMCID PMC5322478. *co-first authors.

Bajar BT, Wang ES, Lam AJ, Kim BB, Jacobs CL, Howe ES, Davidson MW, **Lin MZ***, Chu J*. Improving brightness and photostability of green and red fluorescent proteins for live cell imaging and FRET reporting. ***Scientific Reports*** **2016,** 6:20889*.* PMCID: PMC4754705. *corresponding authors.

Chu J, Haynes RD, Corbel SY, Li P, González-González E, Burg JS, Ataie NJ, Lam AJ, Cranfill PJ, Baird MA, Davidson MW, Ng HL, Garcia KC, Contag CH, Shen K, Blau HM, **Lin MZ**. Non-invasive intravital imaging of cellular differentiation with a bright red-excitable fluorescent protein. ***Nature Methods.*** **2014** May;11(5):572-8. PMCID: PMC4008650.

Lam AJ, St-Pierre F, Gong Y, Marshall JD, Cranfill PJ, Baird MA, McKeown MR, Wiedenmann J, Davidson MW, Schnitzer MJ, Tsien RY, **Lin MZ**. . Improving FRET dynamic range with bright green and red fluorescent proteins. ***Nature Methods*** **2012,** 9:1005-12. PMCID: PMC3461113.

5. Another major contribution of my research with wide impact in synthetic biology has been the application of highly sequence-specific proteases from RNA viruses for labeling and controlling proteins in living cells. These include time-specific tag for the age measurement of proteins (TimeSTAMP), small-molecule-assisted shutoff (SMASh) tags for drug-induced shutoff of protein synthesis, and stabilizable protein linkages (StaPLs) for drug-induced preservation of new copies of proteins of interest. Most recently we have pioneered the specific detection and rewiring of oncogenic signaling using viral proteases as molecular integrators in the rewiring of aberrant signaling to effector release (RASER) concept.

Chung HK, Zou X, Bajar BT, Brand BR, Huo Y, Alcudia JF, Ferrell JE, **Lin MZ.** Rewiring aberrant cancer signaling to therapeutic effector release with a synthetic two-component system. ***Science*** **2019***,* 364:eaat6982. PMCID: PMC7053279.

Yang Y, Geng Y, Ning N, Kim HJ, Jeon NL, Lau A, Chen L, **Lin MZ**. mTOR pathway inhibition restores PSD95 induction in neurons lacking Fragile X mental retardation protein. ***Proceedings of the National Academy of Sciences*** **2019,** 11:12007. PMCID: PMC6575583.

Jacobs CL, Badiee RK, **Lin MZ.** StaPLs: versatile genetically encoded modules for engineering drug-inducible proteins. 2018. ***Nature Methods*** **2018,** 15:523. PMCID: PMC6456726.

Chung HK, Jacobs CL, Huo Y, Yang J, Krumm SA, Plemper RK, Tsien RY, **Lin MZ**. Tunable and reversible drug control of protein production via a self-excising degron. ***Nature Chemical Biology*** **2015,** 11:713-720. PMCID PMC4543534.

A complete list of published work is at https://www.ncbi.nlm.nih.gov/myncbi/michael.lin.1/bibliography/public/