BIOGRAPHICAL SKETCH

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NAME: Kirchhausen, Tomas

eRA COMMONS USER NAME: kirchhausen

POSITION TITLE: Professor of Cell Biology and Springer Family Professor of Pediatrics, Harvard Medical School and Senior Investigator, Program in Cellular and Molecular Medicine, Boston Children's Hospital

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
Universidad Peruana Cayetano Heredia, Peru	B.S.	04/1972	Biology
Instituto Venezolano de Investigaciones Cientificas, Venezuela	M.S.	06/1975	Biophysics
Universidad Peruana Cayetano Heredia, Peru	M.S.	06/1975	Biophysics
Instituto Venezolano de Investigaciones Cientificas, Venezuela	Ph.D.	06/1977	Biophysics and Physiology
Harvard University	M.A. (HON)	12/1999	Cell Biology

A. Personal Statement

Successful and productive research projects in structural biology, cell biology, chemical genetics, live cell and single-molecule fluorescence microscopy imaging, have enabled strong track record in advising pre-doctoral and post-doctoral trainees. Experience in teaching and organizing international, graduate-level courses in different countries. Principal participant since 1997 in biennial membrane-biology EMBO course (Cargese). Chair/co-chair of two Gordon Research Conferences. Leader (and co-founder) for 7 years of the Harvard-Portugal program, the longest lasting international collaborative educational and research program at HMS.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2016- Visiting Scientist at Janelia Research Campus, HHMI
2013- Springer Family Professor of Pediatrics, Harvard Medical School
2012- Professor, Department of Pediatrics, Harvard Medical School
2012- Senior Investigator, Program in Cellular and Molecular Medicine (PCMM) at Boston Children's Hospital
2009-2015 HMS Director, Harvard-Portugal Program in Translational Research and Medical Education
2007-2012 Executive Faculty Committee, Immune Disease Institute
2006-2012 Chair IT Committee, Immune Disease Institute
1999-2012 Senior Investigator, Immune Disease Institute, (formerly CBR Inst. for Biomedical Research)
1999- Professor, Department of Cell Biology, Harvard Medical School
1993-1999 Associate Professor, Department of Cell Biology, Harvard Medical School
1992-1999 Investigator, The Center for Blood Research, Inc.
1991-1993 Associate Professor of Anatomy and Cellular Biology, Harvard Medical School
1986-1991 Assistant Professor of Anatomy and Cellular Biology, Harvard Medical School
1984-1985 Assistant Head Tutor, Biochemical Sciences, Harvard University
1982-1985 Research Associate in Biochemistry, Harvard University
1981-1982 Postdoctoral, Harvard University, Department of Biochemistry and Molecular Biology
1980- Tutor in Biochemical Sciences, Harvard University

Honors

2020	IUBMB Jubilee Lecture, Lanzen Peru 2020
2018	EMBO Global Lecturer, India
2017	EMBO Keynote Lecture, FEBS – EMBO Advance Lecture Course in Molecular Architecture, Dynamics and Function of Biomembranes
2017-	Honorary Member, Associazione di Biologia Cellulare e del Differenziamento, Italy
2016-	Honorary Professor, University of Hong Kong
2016-	Academia Nacional de Ciencias (Peru), Académico Correspondiente
2015	EMBO Keynote Lecture, Systems Biology of Infection
2015-	Netherlands Society for Biochemistry and Molecular Biology: Speaker of the year
2014-	SMB Jose Laguna Lecture for Outstanding Basic Research in Dynamics of Endocytosis
2014-	Associated Member EMBO
2013-	Doctor Honoris Causa, Universidad Ricardo Palma, Peru
2013-	Springer Family Professor of Pediatrics, Harvard Medical School
2012	John Cebra Endowed Lecture, Dynamics and Endocytosis. Marine Biological Laboratory, Woods Hole
2008-	AAAS Fellow
2002-	Honorary Professor, Universidad Peruana Cayetano Heredia, Peru
1986-1991	Established Investigator Award, American Heart Association
1982-1984	Research Fellow, Charles A. King Trust

Other Experience and Professional Memberships

2004-2012	Ad-hoc member of NIH Study Sections CD-4 and ZRG1
2001-	Member, Advisory Committee, Imaging Facility, Dept. of Cell Biology, Harvard Med. School
1999-2003	Member, NIH Study Section, CDF-2
1996-1999	Ad-hoc member of NIH Study Section, CB-1

C. Contributions to Science

1. Molecular architecture of clathrin coats and mechanism of uncoating (1981-) [1] [2] [3] [4]

Through studies extending over three decades, we defined the structure and interactions of clathrin and many of its associated proteins and the assembly and uncoating mechanisms for clathrin coats. Highlights: clathrin heavy-chain sequence -- at the time, the longest protein sequence derived by cDNA cloning; high-resolution crystal structures of a large N-terminal fragment of clathrin and its complex with a "clathrin box" peptide, of the core of the AP-1 adaptor complex, and of a complex between Dishevelled and the μ2 subunit of the AP-2 adaptor complex; subnanometer structure of a clathrin coat (22MDa) by electron cryomicroscopy (cryoEM), yielding a complete molecular model for clathrin at 8 Å resolution; cryoEM structure of a clathrin coat with bound auxilin and Hsc70 and subsequent *in vitro* and *in vivo* single-molecule total internal reflection fluorescence (TIRF) microscopy studies of uncoating.

1. Kirchhausen, T., Owen, D. & Harrison, S. C. Molecular structure, function, and dynamics of clathrin-mediated membrane traffic. Cold Spring Harb Perspect Biol 6, a016725 (2014). PMID: 2478982. PMC3996469.
2. Fotin, A. et al. Molecular model for a complete clathrin lattice from electron cryomicroscopy. Nature 432, 573–579 (2004).
3. Xing, Y. et al. Structure of clathrin coat with bound Hsc70 and auxilin: mechanism of Hsc70-facilitated disassembly. EMBO J 29, 655–665 (2010). PMCID: PMC2830701.
4. Böcking, T., Aguet, F., Harrison, S. C. & Kirchhausen, T. Single-molecule analysis of a molecular disassemblase reveals the mechanism of Hsc70-driven clathrin uncoating. Nat Struct Mol Biol. 18, 295–301 (2011). PMCID: PMC3056279.

2. Live-cell imaging (2004-) [5] [6] [7] [8]

Building on the biochemical and structural discoveries outlined above, we analyzed mechanisms of coated-vesicle formation in living cells, applying emerging technologies in fluorescence microscopy and live-cell imaging. Our use of spinning disc confocal microscopy led to the following description of molecular events in clathrin-mediated endocytosis: coated pits nucleate at the plasma membrane and grow by steady addition of

clathrin triskelions; Hsc70-mediated uncoating follows promptly upon dynamin-induced membrane scission; arrival of auxilin, the clathrin specific, J-domain co-chaperone for Hsc70, determines the timing of this event; clathrin assembly ordinarily provides the principal driving force for membrane invagination, but at high membrane tension or with very elongated cargo, actin polymerization is also required. When our TIRF microscopy technology had reached the level of single-molecule counting, we showed that coated-pit initiation proceeds by coordinated arrival of clathrin and the AP2 adaptor complex, the latter recruited by interaction with PtdIns (3,4)P2, and that accessory proteins are then essential for sustained growth, and that a single rung of a dynamin tube is sufficient for coated pit neck scission. Our most recent findings that follow our keen and long standing interests on studies focusing on mechanisms of cell host / virus interactions revealed that inhibiting the PIKfyve kinase through two small-molecule related antivirals are potently prevent Zaire ebolavirus and SARS-CoV-2 infection.

5. Ehrlich, M. et al. Endocytosis by random initiation and stabilization of clathrin-coated pits. 118, 591–605 (2004); Massol, R. H., Boll, W., Griffin, A. M. & Kirchhausen, T. A burst of auxilin recruitment determines the onset of clathrin-coated vesicle uncoating. Proc Natl Acad Sci USA 103, 10265–10270 (2006).
6. Cocucci, E., Aguet, F., Boulant, S. & Kirchhausen, T. The first five seconds in the life of a clathrin-coated pit. Cell. 150, 495–507 (2012). PMCID: PMC3413093.
7. Cocucci, E., Gaudin, R. & Kirchhausen, T. Dynamin recruitment and membrane scission at the neck of a clathrin-coated pit. Mol Biol Cell 25, 3595–3609 (2014). PMCID: PMC4230619.
8. Kang, Y.-L. et al. Inhibition of PIKfyve kinase prevents infection by Zaire ebolavirus and SARS-CoV-2. Proceedings of the National Academy of Sciences 15, 202007837 (2020). PMC7263545.

3. Frontier optical-imaging modalities using LLSM (2015-2017) [9] [10] [11] [12]
After initial use at Janelia Research Campus of LLSM to resolve the dorsal from the ventral surfaces of thin lamellipodial protrusions, we built a second generation LLSM with guidance from Betzig and team at Janelia Research Campus; it has been in continuous use since July, 2014. Our published work with this instrument has included: reexamination of the clathrin-coat assembly dynamics over the entire surfaces of 45 cells and ~250,000 AP-2 traces that required MATLAB software development resulting in the generation of 3D cmeAnalysis (clathrin-mediated endocytosis analysis (with Francois Aguet and Gaudenz Danuser); full-cell imaging measurements of cell surface area and volume throughout the cell cycle, both for single cells in culture and for cells in the eye of a developing zebrafish embryo; studies of lipid-droplet maturation (with Walter and Farese); studies of assembly mechanisms in the ESCRT pathway in yeast (with David Teis). We provide direct evidence (with Janet Shaw) that the intracellular location of mitochondria shapes subcellular energy gradients. Finally, we developed a new generation of phosphoinositide sensors and showed that a cascade of molecular conversions, made possible by the separation of a clathrin coated vesicle from its parent membrane, can label membrane-traffic intermediates and determine their destinations. In this work, we used LLSM to visualize the sensors across the entire cellular volume, showing that a cascade of molecular signals, two of which are accumulation of PtdIns (3)P or PtdIns(4)P and PtdIns (3,4)P2 during uncoating, may bring about arrival of auxilin and Rab5GTPases, respectively.

9. Kural, C. et al. Dynamics of intracellular clathrin/AP1- and clathrin/AP3-containing carriers. Cell Rep 2, 1111–1119 (2012). PMC4472015.
10. Aguet, F. et al. Membrane dynamics of dividing cells imaged by lattice light-sheet microscopy. Mol Biol Cell (2016). doi:10.1091/mbc.E16-03-0164. PMC5221578.
11. Adell, M. A. Y. et al. Recruitment dynamics of ESCRT-III and Vps4 to endosomes and implications for reverse membrane budding. Elife 6, e31652 (2017). PMC5665648.
12. He, K. et al. Dynamics of phosphoinositide conversion in clathrin-mediated endocytic traffic. Nature 552, 410–414 (2017). PMC6263037.

4. Frontier optical-imaging modalities using AO-LLSM and FIB-SEM (2016-) [13] [14] [15] [16]
Since August 2016, we have collaborated closely with Eric Betzig in developing AO-LLSM and using it to visualize live, multicellular structures (organoids, worms, plants and most extensively, zebrafish embryos). A collaborative study with Sean Megason led to the unexpected discovery of a physical relief valve in the endolymphatic sac, a dead-end epithelial tube connected to the inner ear: Megason. The most recent study is a comprehensive collaboration with Betzig to test AO-LLSM, particularly focused on visualizing developmental processes in zebrafish embryos. Examples include: quantitative tracking of all endocytic coated pits and vesicles in muscle and brain cells (the dynamics are faster in brain, and the coats are smaller); visualization and quantification of migrating lymphocytes and human cancer cells; measurements of cell area and volume and organelle distribution during cell division; tracing of axonal growth in the hindbrain. Through our ongoing

collaboration with Betzig and Herald Hess, we contributed to the nanoscale 3D visualization of endosomes combining low temperature super-resolution fluorescence microscopy of high-pressure frozen cells coupled with focused ion beam scanning electron microscopy (FIB-SEM).

These applications demonstrate the advantage of LLSM and AO-LLSM and how they set a new standard for imaging membrane dynamics in single cells and multicellular assemblies.

13. Liu, T.-L. et al. Observing the cell in its native state: Imaging subcellular dynamics in multicellular organisms. Science 360, eaaq1392 (2018). PMC6040645.
14. Hoffman, D. P. et al. Correlative three-dimensional super-resolution and block-face electron microscopy of whole vitreously frozen cells. Science 367, eaaz5357 (2020). PMC7339343.
15. Swinburne, I. A. et al. Lamellar projections in the endolymphatic sac act as a relief valve to regulate inner ear pressure. Elife 7, 2837 (2018). PMC6008045.
16. Gao, R. et al. Cortical column and whole-brain imaging with molecular contrast and nanoscale resolution. Science 363, eaau8302 (2019). PMC6481610.

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2. Kreutzberger, A.J.B., Sanyal, A., Ojha, R., Pyle, J.D., Vapalahti, O., Balistreri, G., and Kirchhausen, T. (2021). Synergistic block of SARS-CoV-2 infection by combined drug inhibition of the host entry factors PIKfyve kinase and TMPRSS2 protease. J Virol, JVI0097521. PMID: 34406858. [Pubmed in process].
3. Chou, Y.Y., Upadhyayula, S., Houser, J., He, K., Skillern, W., Scanavachi, G., Dang, S., Sanyal, A., Ohashi, K.G., Di Caprio, G., et al. (2021). Inherited nuclear pore substructures template post-mitotic pore assembly. Dev Cell. PMID: 34129835. PMCID: PMC8261643.
4. Kreutzberger AJB, Sanyal A, Ojha R, Pyle JD, Vapalahti O, Balistreri G, Kirchhausen T. 2021. Synergistic inhibition of two host factors that facilitate entry of Severe Acute Respiratory Syndrome Coronavirus 2. bioRxiv. PMID: 34100014 PMCID: PMC8183009.
5. Emperador-Melero J, Wong MY, Wang SSH, de Nola G, Nyitrai H, Kirchhausen T, Kaeser PS. 2021. PKC-phosphorylation of Liprin-alpha3 triggers phase separation and controls presynaptic active zone structure. Nat Commun 12:3057. PMID: 34031393 PMCID: PMC8144191.
6. Chen YC, et al. 2021. Activating Sphingosine-1-phospahte signaling in endothelial cells increases myosin light chain phosphorylation to decrease endothelial permeability thereby inhibiting cancer metastasis. Cancer Lett 506:107-119. PMID: 33600895. PMID: 33600895 PMCID: PMC8034284.
7. Zang R, et al. 2020. Cholesterol 25-hydroxylase suppresses SARS-CoV-2 replication by blocking membrane fusion. Proc Natl Acad Sci U S A 117:32105-32113. PMID: 33239446. PMID: 33239446 PMCID: PMC7749331.
8. Salman, M. M., Marsh, G., Kusters, I., Delince, M., Di Caprio, G., Upadhyayula, S., de Nola, G., Hunt, R., Ohashi, K. G., Gray, T., Shimizu, F., Sano, Y., Kanda, T., Obermeier, B. and Kirchhausen, T.,Secondary Salman, M. M., Marsh, G., Kusters, I., Delince, M., Di Caprio, G., Upadhyayula, S., de Nola, G., Hunt, R., Ohashi, K. G., Gray, T., Shimizu, F., Sano, Y., Kanda, T., Obermeier, B. and Kirchhausen, T.,2020. Design and Validation of a Human Brain Endothelial Microvessel-on-a-Chip Open Microfluidic Model Enabling Advanced Optical Imaging. Front Bioeng Biotechnol. 2020 Sep 28 8,573775. PMID: 33117784. PMCID: PMC7576009.
9. Ruochen Zang, James Brett Case, Maria Florencia Gomez Castro, Zhuoming Liu, Qiru Zeng, Haiyan Zhao, Juhee Son, Paul W. Rothlauf, Gaopeng Hou, Sayantan Bose, Xin Wang, Michael D. Vahey, Tomas Kirchhausen, Daved H. Fremont, Michael S. Diamond, Sean P.J. Whelan, Siyuan.Ding. Cholesterol 25-hydroxylase suppresses SARS-CoV-2 replication by blocking membrane fusion. bioRxiv 2020.06.08.141077; doi: https://doi.org/10.1101/2020.06.08.141077. PMID: 33239446. Epub 2020 Nov 25. [Preprint].
10. Mootaz Salman, Graham Marsh, Ilja Küsters, Matthieu Delincé, Giuseppe Di Caprio, Srigokul Upadhyayula, Giovanni de Nola, Ronan Hunt, Kazuka G. Ohashi, Fumitaka Shimizu, Yasuteru Sano, Takashi Kanda, Birgit Obermeier, Tom Kirchhausen. An in-vitro BBB-on-a-chip open model of human

blood-brain barrier enabling advanced optical imaging. bioRxiv 2020.06.30.175380; doi: https://doi.org/10.1101/2020.06.30.175380. [Preprint].

11. Shipley, Dani, Xu, Deister, Cui, Head, Sadegh, Fame, Shannon, Flores, Kishkovich, Jang, Klein, Goldey, He, Zhang, Holtzman, Kirchhausen, Wyart, Moore, Andermann and Lehtinen, 2020. Tracking Calcium Dynamics and Immune Surveillance at the Choroid Plexus Blood-Cerebrospinal Fluid Interface. Neuron S0896-6273(20)30655-3. PMID: 32961128. PMCID: PMC7847245.
12. Magupalli, Negro, Tian, Hauenstein, Di Caprio, Skillern, Deng, Orning, Alam, Maliga, Sharif, Hu, Evavold, Kagan, Schmidt, Fitzgerald, Kirchhausen, Li and Wu,2020. HDAC6 mediates an aggresome-like mechanism for NLRP3 and pyrin inflammasome activation. Science 369(6510):eaas8995. PMID: 32943500. PMCID: PMC7814939.
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15. Yuan-Lin Kang, Yi-Ying Chou, Paul W. Rothlauf, Zhuoming Liu, Silvia Piccinotti, Timothy K. Soh, David Cureton, James Brett Case, Rita E. Chen, Michael S. Diamond, Sean P. J. Whelan, Tom Kirchhausen. (2020). Inhibition of PIKfyve kinase prevents infection by EBOV and SARS-CoV-2. bioRxiv preprint doi: https://doi.org/10.1101/2020.04.21.053058. PMID: 32764148. PMC7263545.
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