BIOGRAPHICAL SKETCH

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NAME: Mohamed Trebak, Ph.D.

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

POSITION TITLE: Professor, Department of Cellular and Molecular Physiology
The Pennsylvania State University College of Medicine

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
Université de Liège, Liège, Belgium	M.Sc.	06/1994	Biochemistry
Université de Liège, Liège, Belgium	Ph.D.	10/1998	Biochemistry
The Wistar Institute, Philadelphia, PA	Postdoc.	11/2000	Biochemistry
National Institutes of Health (NIEHS/NIH), NC	Postdoc.	12/2006	Biophysics

A. Personal Statement

The overall goal of my laboratory is to investigate the activation mechanisms of ion channels and their contribution to pathophysiology using animal models of disease. Our work is centered on the role of STIM/Orai Ca^{2+} channels and Transient Receptor Potential (TRP) cation channels in the processes of survival, proliferation and migration in cancer and pathological remodeling of airways and vessels. Our *in vivo* studies are complemented by the use of a variety of molecular, biochemical, biophysical and imaging techniques to gain mechanistic insights into regulation of these ion channels by second messengers and protein kinases. As a postdoctoral fellow in The Wistar Institute of Philadelphia, I have determined, using protein chemical and biophysical approaches, the oligomeric state of a membrane protein associated with colon cancer. During a second postdoctoral training at the NIH, I gained expertise in advanced imaging and ion channel biophysics, by which I have determined the activation mechanisms of canonical TRP channels and their regulation by protein phosphorylation and second messengers such as diacylglycerol and polyphosphoinositides. As an independent investigator, I have combined my prior expertise in cell biology, protein chemistry and biophysics to the study of native STIM/Orai and TRP channels and their contribution to cancer, vascular and lung diseases.

I joined the faculty at PSU on March, 2015. I have over 20 years of experience studying calcium signaling and ion channel regulation in immune and vascular cells. Below are few papers from my group that document our expertise:

- Zhang W, Halligan KE, Zhang X, Bisaillon JM, Gonzalez-Cobos JC, Hu G, Vincent PA, Zhou J, Singer HA, Matrougui K & **Trebak M. 2011**. Orai1-mediated I_{CRAC} is essential for neointima formation after vascular injury. ***Circulation Research****;109: 534-42.* **(highlighted in issue & cover article)**
- Shinde AV, Motiani RK, Zhang X, Abdullaev IF, Adam AP, Gonzalez-Cobos JC, Matrougui K, Vincent PA & **Trebak M**. **2013**. STIM1 controls endothelial barrier function independently of Orai1 and Ca^{2+} entry. ***Science Signaling***. 6(267):ra18. (**highlighted in perspective & cover article; Science magazine Editors' choice**)
- González-Cobos JC, Zhang X, Zhang W, Ruhle B, Motiani RK, Schindl R, Muik M, Bisaillon JM, Shinde AV, Fahrner M, Singer HA, Matrougui K, Barroso M, Romanin C & **Trebak M**. **2013**. Store-Independent Orai1/3 channels Activated by Intracrine LeukotrineC_4: Role in Neointimal Hyperplasia. ***Circulation Research***. 112(7):1013-25. **(highlighted in editorial, in this issue & cover article)**

- Desai PN, Zhang X, Wu S, Janoshazi A, Bolimuntha S, Putney JW and **Trebak M. 2015.** Multiple Types of Calcium Channels Arising from Alternative Translation Initiation of Orai1 Message. ***Science Signaling.*** *In Press*

B. Positions and Honors

Professional Experience

- 1992-1994: M.Sc. Research Scholar, Université de Liège School of Medicine, Dept. Pathology, Liège, Belgium (Mentor: Michel Moutschen, MD) ***Summa cum laude***
- 1994-1998: Ph.D. Research Scholar, Université de Liège School of Medicine, Dept. Pathology, Liège, Belgium (Mentor: Jacques Boniver, MD) ***Summa cum laude***
- 1999-2000: NIH Postdoctoral Fellow, Structural Biology program, The Wistar Institute, Philadelphia, PA (Mentor: David W. Speicher, Ph.D.)
- 11/2000-12/2006: IRTA Postdoctoral Fellow, Calcium Regulation Section, Laboratory of Signal Transduction, NIEHS/NIH, NC (Mentor: James W. Putney, Jr. Ph.D.)
- 2007-2010: Assistant Professor, Center for Cardiovascular Sciences, Albany Medical College, Albany, NY
- 2010-2015: Associate Professor, Center for Cardiovascular Sciences, Albany Medical College, Albany, NY
- 2012-2015: Associate Professor, State University of New York College of Nanoscale Science and Engineering (SUNY CNSE) Albany, NY
- 03/2015-Present: Professor (with tenure), Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, Hershey, PA

Honors, Awards and Achievements

- Chair, Gordon Research Conference "*Calcium Signaling*", 2019 (Vice-Chair, 2017)
- Chair: International Meeting on Ion Channel signaling Mechanisms, 2011
- City of Paris *Mayor's Office* Visiting Professor, Université Pierre et Marie Curie, Paris 6, 2012
- Keynote lecture; Annual meeting of the Spanish scientific network HERACLES, 2011
- Plenary lectures; Gordon Research Conference, FASEB Summer Conference, Experimental Biology Meeting, Biophysical Society Meeting, 2010-2016
- Dr. Helen H. Molinari Graduate Encouragement and Merit Award, Albany Medical College NY, 2010
- Editorial Board: Journal of Biological Chemistry, 1013-2018
- Editorial Board: Molecular & Cellular Oncology , 2014-present
- Editorial Board: Cell Calcium, 2014-2019
- Associate Editor: Calcium Signaling, 2014-present
- Academic Editor: PLOS ONE, 2013-present
- Editorial Board: Frontiers in Ion Channel Pharmacology and Channelopathies , 2010-present
- Managing Editor: Frontiers in Biosciences, 2010
- Editorial Board: Pflügers Archives, 2008-present
- Member: Remote Evaluator Panel; Belgian Science Fund (FNRS), 2010-present
- Member: American Heart Association: Cell Transport 2 study section 2009-present
- NIH Hypertension and Microcirculation (MH) Study Section, 2014, 2015
- NIH Molecular and Integrative Signal Transduction (MIST) Study Section, 2014, 2015
- NIH SEP 2015/01 ZES1 LWJ-D (ME)1. Mitochondrial and Nuclear Induced Cross-Talk Perturbations in Response to Environmental Insults, 2014
- *Ad hoc* reviewer for: Austrian, Czech Republic, German, Israel, Singapore, Qatar, Turkey and United Kingdom (MRC, UK Cancer research & Wellcome Trust) science foundations; PA DOH; FL DOH; American Cancer Society and US National Science Foundation
- Wiggers Fellowship Travel award NY, 2007
- NIH Transition to Independent Position award, 2006
- Gordon Research Conference travel award, Oxford, United Kingdom, 2005
- NIH Fellows Award for Research Excellence, 2004
- Bruxelles-Lambert Bank scholarship Belgium, 1997-1998
- Anticancer Center Biomedical research scholarship Belgium, 1995-1997
- Henri Jacquemin Immunology travel award Belgium, 1997
- Léon Frédéricq Foundation award Belgium, 1996

C. Contributions to Science

(1) Defining the activation and regulation mechanisms of Canonical Transient Receptor Potential (TRPC) channels: In my postdoctoral work with Jim Putney at NIEHS/NIH, we pioneered early understanding of the mechanisms of activation and regulation of TRPC channels and established these channels as receptor-activated cation channels. We were first to show that endogenously produced diacylglycerol (DAG), through the action of Phospholipase C, was the signal necessary for human TRPC3 activation, independently of inositol1,4,5-trisphosphate (IP_3), IP_3 receptors, store depletion and G proteins. We subsequently discovered a novel mode of negative regulation of TRPC3 channels by PKC-mediated phosphorylation and identified a conserved site (Serine712) as the major mediator of PKC action on TRPC3. We have delineated the mechanisms of regulation of TRPC5, TRPC6 and TRPC7 channels by calcium and polyphosphoinositides. We also provided the first direct evidence that the apparent activation of TRPC3 channels by store depletion reported by many investigators is an artifact resulting from compromised endoplasmic reticulum calcium buffering caused by SERCA inhibitors. Our fundamental studies have helped establish TRPC channels as second messenger-activated nonselective cation channels and paved the way for numerous research groups exploring the role of TRPC channels in various physiological systems such as brain, heart and vessels.

- **Trebak M**, Bird GS, McKay RR, Putney JW, Jr. **2002**. Comparison of human TRPC3 channels in receptor-activated and store-operated modes. Differential sensitivity to channel blockers suggests fundamental differences in channel composition. ***J. Biol. Chem.*** 277: 21617-23.
- **Trebak M**, St JBG, McKay RR, Birnbaumer L, Putney JW, Jr. **2003**. Signaling mechanism for receptor-activated canonical transient receptor potential 3 (TRPC3) channels**.** ***J. Biol. Chem***. 278: 16244-52.
- **Trebak M**, Vazquez G., Bird GSJ, and Putney JW, Jr. **2003**. The TRPC3/6/7 subfamily of cation channels. ***Cell Calcium****.* 33: 451-461
- **Trebak M**, Hempel N, Wedel BJ, Smyth JT, Bird GS, Putney JW, Jr. **2005**. Negative regulation of TRPC3 channels by protein kinase C-mediated phosphorylation of serine 712. ***Mol Pharmacol*** . 67: 558-63.

(2) Identification of STIM1 and Orai1 as important players in endothelial cell function: STIM1 is a crucial sensor of Ca^{2+} within the endoplasmic reticulum (ER) and is a major mediator of inter-organelle communication within cells, allowing direct communication between ER and plasma membrane ion channels, including Orai1 store-operated Ca^{2+} (SOC) channels. My laboratory was first to establish STIM1 and Orai1 as essential components of SOC channels activated by thrombin and vascular endothelial growth factor (VEGF) in primary endothelial cells and discovered a role for Ca^{2+} gradients mediated by STIM1 and Orai1 in driving endothelial proliferation at the G2/M phase. Our studies have revealed the central role that STIM1 proteins play in the control of endothelial barrier function, whereby STIM1 mediates the activation of RhoA to facilitate the increase in endothelial permeability in response to GPCR agonists. We have generated vascular cell-specific STIM1 knockout mice and discovered a differential role for STIM1 in endothelia versus smooth muscle in the regulation of vascular reactivity *in vivo*.

- Abdullaev IF, Bisaillon JM, Potier M, Gonzalez JC, Motiani, RK and **Trebak M**. **2008**. Stim1 and Orai1 Mediate CRAC Currents and Store-Operated Calcium Entry important for Endothelial Cell proliferation. ***Circulation Research*****.**103:1289-1299. (**Circ. Res. highly cited**)
- **Trebak M**. **2009**. STIM1/Orai1, I_{CRAC}, and endothelial SOC. ***Circulation Research***. 104(9):e56-7.
- Shinde AV, Motiani RK, Zhang X, Abdullaev IF, Adam AP, Gonzalez-Cobos JC, Matrougui K, Vincent PA & **Trebak M**. **2013**. STIM1 controls endothelial barrier function independently of Orai1 and Ca^{2+} entry. ***Science Signaling.*** 6(267):ra18. (**highlighted in perspective & cover article; Science magazine Editors' choice**)
- Stolwijk JA, Matrougui K, Renken C, and **Trebak M**. **2014**. Practical Guidelines for the Study of Endothelial Barrier Function in Response to GPCR Agonists using Electric Cell-Substrate Impedance Sensing. ***Pflügers Archives.*** *In Press*

(3) Establishing STIM and Orai up-regulation in smooth muscle as an essential determinant in vascular and airway remodeling: My laboratory was first to measure and characterize at the molecular level in vascular and airway smooth muscle cells the archetypical SOC current, Calcium Release-Activated Calcium (CRAC) current. We showed that STIM1 and Orai1 are selectively upregulated in the fibroproliferative smooth muscle phenotype (also called synthetic) that is the hallmark of many vascular diseases such as atherosclerosis and hypertension. We have characterized the calcium entry pathways activated during agonist-mediated smooth muscle migration and showed STIM1 and Orai isoforms are upregulated in medial and

neointimal smooth muscle during vascular injury of rat carotid arteries where they couple to specific downstream pathways and transcriptional activities. We have also established using *in vivo* animal models that STIM1, Orai1 and Orai3 are potential targets for therapy of vascular occlusive diseases.

- Potier M, Gonzalez JC, Motiani RK, Abdullaev IF, Bisaillon JM, Singer HA and **Trebak M**. **2009**. Evidence for STIM1- and Orai1-dependent Store-Operated Calcium Influx through I_{CRAC} in Vascular Smooth Muscle cells. ***FASEB Journal***. 23:2425-37 (**FASEB J highly cited)**
- Bisaillon JM, Motiani RK, Gonzalez JC, Potier M, Halligan KE, Alzawahra W, Singer HA, Jourd'heuil D, and **Trebak M**. **2010**. Essential Role for STIM1- and Orai1-Mediated Calcium Influx in PDGF-Induced Vascular Smooth Muscle Migration. ***Am J Physiol Cell Physiol.*** 298(5): C993-1005. **(AJP highly cited)**
- Zhang W, Halligan KE, Zhang X, Bisaillon JM, Gonzalez-Cobos JC, Hu G, Vincent PA, Zhou J, Singer HA, Matrougui K & **Trebak M. 2011**. Orai1-mediated I_{CRAC} is essential for neointima formation after vascular injury. ***Circulation Research.****109: 534-42.* **(highlighted in issue & cover article)**
- González-Cobos JC, Zhang X, Zhang W, Ruhle B, Motiani RK, Schindl R, Muik M, Bisaillon JM, Shinde AV, Fahrner M, Singer HA, Matrougui K, Barroso M, Romanin C & **Trebak M**. **2013**. Store-Independent Orai1/3 channels Activated by Intracrine LeukotrieneC_4: Role in Neointimal Hyperplasia. ***Circulation Research***. 112(7):1013-25. **(highlighted in editorial, in issue & cover article)**

(4) Discovery of a novel store-independent Ca^{2+} selective channel in smooth muscle encoded by two exclusively mammalian proteins: Orai3 and an extended variant of Orai1, termed Orai1α: My laboratory discovered a novel native Ca^{2+} selective channel in synthetic smooth muscle cells (not present in quiescent smooth muscle) that is contributed by subunits of both Orai3 and the long form of Orai1, Orai1α. This novel channel is activated by pro-proliferative agonists in a store-independent manner and couples to Akt activation. We mechanistically linked the activation of this novel channel to the production of leukotrieneC_4 through metabolism of arachidonic acid. We further showed that targeted *in vivo* knockdown of leukotrieneC_4 Synthase reverses neointimal hyperplasia and vascular occlusion in injured rat carotid arteries.

- Zhang X, González-Cobos JC, Schindl R, Muik M, Ruhle B, Motiani RK, Bisaillon JM, Zhang W, Fahrner M, Barroso M, Matrougui K, Romanin C & **Trebak M**. **2013**. Mechanisms of STIM1 activation of store-independent LRC Channels. ***Mol. Cell Biol.*** 33: 3715-23. **(Highlighted in *Mol. Cell Biol.* Spotlight)**
- Zhang X, Zhang W, González-Cobos JC, Jardin I, Romanin C, Matrougui M and **Trebak M**. **2014**. Complex Functions for STIM1 in the Activation of Store-Independent Orai1/3 Channels. ***J. Gen. Physiology***. 143: 345-59. **(Highlighted in *J. Gen. Physiol.* Video summary)**
- Zhang W, Zhang X, Stolwijk JA, Matrougui K and **Trebak M**. **2015**. LeukotrieneC_4 Synthase, a Critical Enzyme in the Activation of Store-Independent Orai1/Orai3 Channels, is required for Neointimal Hyperplasia. ***J. Biol.Chem.*** 290(8):5015-27.
- Desai PN, Zhang X, Wu S, Janoshazi A, Bolimuntha S, Putney JW and **Trebak M. 2015.** Multiple Types of Calcium Channels Arising from Alternative Translation Initiation of Orai1 Message. ***Science Signaling.*** *In Press*

(5) Characterizing the role of Orai1 and Orai3 channels in tumorigenesis and the regulation of Orai3 by Estrogen Receptorα (ERα) in breast cancer: By analyzing a large number of estrogen receptor positive (ER^+) and negative (ER^-) breast cancer cell lines, we have identified exclusive expression patterns of STIM/Orai and TRPC isoforms in these two different types of breast cancers. My laboratory established that ER^+ breast cancer cells use mainly Orai3 channels for store-operated calcium entry in response to growth factors while ER^- cells mediate calcium entry through Orai1 channels. We showed that while Orai1 is required for ER^- cell migration, Orai3 acts as pro-migratory in ER^+ cells and showed that Orai3 expression is regulated by Estrogen receptorα. We demonstrated that Orai3 is required for breast tumor development in immune compromised mice thus establishing Orai3 as a potential specific target for therapy of ER^+ breast tumors. Furthermore, in collaboration with Dr. Alexander Mongin, we demonstrated the upregulation of Orai1 channels (but not Orai3) in a large set of patient-derived glioblastomas and established Orai1 as an important contributor to glioblastoma invasion.

- Motiani RK, Abdullaev IF, and **Trebak M**. **2010**. A novel native store-operated calcium channel encoded by Orai3: selective requirement of Orai3 versus Orai1 in estrogen receptor-positive versus estrogen receptor-negative breast cancer cells. ***J. Biol. Chem.*** 285: 19173-83. (**JBC most cited**)
- Motiani RK, Zhang X, Harmon KE, Keller RS, Matrougui K, Bennett JA, and **Trebak M**. **2013**. Orai3 is an Estrogen Receptor α-Regulated Ca^{2+} Channel that Promotes Tumorigenesis. ***FASEB J.*** 27: 63-75.

- Motiani RK, Hyzinski-Garcia M, Zhang X, Henkel M, Abdullaev IF, Kuo Y-H, Matrougui K, Mongin AA, and **Trebak M**. **2013**. Orai1-mediated I_{CRAC} is essential for glioblastoma invasion. ***Pflügers Archives***. 465: 1249-60.
- Motiani RK, Stolwijk JA, Newton RL, Zhang X, and **Trebak M**. **2013**. Emerging roles of Orai3 in pathophysiology. ***Channels***. 7(5) 392-401

(6) Complete List of Published Work in My Bibliography (Total of 91 publications):

http://www.ncbi.nlm.nih.gov/myncbi/mohamed.trebak.1/bibliography/40846103/public/?sort=date&direction=ascending

D. Research Support

1R01 HL123364 Mohamed Trebak (PI) 06/01/2014-05/31/2018
NIH, NHLBI
*"*LeukotrieneC_4 and STIM/Orai channels in airway smooth muscle remodeling*"*
The objectives of this proposal are to study the mechanisms of Ca^{2+} entry in airway smooth muscle and the role of Orai3 and STIM1 in airway hyperresponsiveness in murine models of asthma

14GRNT18880008 Mohamed Trebak (PI) 01/02/2014-01/01/2017
American Heart Association (AHA)
"Leukotriene-regulated Ca^{2+} channels in vascular remodeling"
The aims of this proposal is to study the role of Leukotriene-regulated Ca^{2+} channels in vascular smooth muscle migration and their contribution to neointimal hyperplasia in animal models of restenosis

1R01 HL097111 Mohamed Trebak (PI) 02/01/2009-11/30/2015 (**NCE**)
NIH, NHLBI
"Receptor-regulated Ca^{2+} Entry Pathways in Smooth Muscle"
The goals of this proposal is to study the mechanisms of regulation of store-operated Orai1 channels and TRPC6 channels in vascular smooth muscle and their contributions to vascular remodeling

NPRP 7 - 542 - 3 - 145 Mohamed Trebak (PI) 02/01/2016-01/31/2019
Qatar National Research Fund (QNRF)
"Interplay between Ca^{2+} release and Ca^{2+} entry pathways in hypertension"
The aim of this proposal is determine the temporal changes in expression and function of isoforms of the IP3 receptors and Orai Ca2+ entry channels and their interdependence in two different animal models of chronic hypertension

NSF1455544 Mohamed Trebak (Co-PI; 10%)
National Science Foundation (NSF) 06/01/2015-07/14/2018
"The NANAPHID: A novel aphid-like nanosensor network for real-time measurements of carbohydrates in live plant tissue"
The aims of this proposal are develop and test a new nanosensor for carbohydrate measurements in trees.

R21 AG11587820 Mohamed Trebak & Jean S. Hulot (Co-PIs) 03/01/2016-02/28/2018
NIH, NIA. **8th percentile; pending council review**
"Genes and miRNAs controlled by ORAI3 in cardiovascular remodeling"
The goal of this proposal is to determine the gene and miRNA nettworks specifically controlled by Orai3 in Cardiac and vascular remodeling

Completed Research Support

Applied Biophysics Inc. Mohamed Trebak (PI) 08/15/2012-12/31/2014
"TRPC channels and endothelial barrier function"
The aims of this grant is to determine the role of TRP channels in endothelial barrier function using *in vitro* impedance measurements and establish better high throughput methods with the ECIS apparatus

American Heart Association Medical Student Research fellowship
Brian Ruhle (PI); Mohamed Trebak (Mentor) 07/01/2012-06/30/2013
"Activation mechanisms of TRPC6 channels in Vascular Smooth muscle"
The goal of this fellowship is to study the diacylglycerol-activated TRPC6 channel in vascular smooth muscle and its regulation by STIM1.