BIOGRAPHICAL SKETCH

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NAME: Nikolaos M. Tsoukias

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

POSITION TITLE: Associate Professor

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
National Technical University, Athens, Greece	Diploma	11/1994	Chemical Engineering
University of California, Irvine	M.S.	06/1997	Chem.& Biochem. Eng
University of California, Irvine	Ph.D	06/1999	Engineering
University of California, Irvine	Post. Doc.	06/2000	Biomed. Engineering
Johns Hopkins University, Baltimore	Post. Doc.	06/2003	Biomed. Engineering

A. Personal Statement

I have a longstanding interest in the application of engineering methodologies to the analysis of physiological function and processes. My primary research focus is on vascular physiology with emphasis on the regulation of microcirculatory tone. My early work as a doctoral student with Professor Steve George (University of California, Irvine) was on Nitric Oxide biotransport. By combining mathematical modeling and experiments, we were able to explain the dynamics of the exhaled NO concentration and provide recommendations for its use as a diagnostic test of lung function. As a postdoctoral fellow, I trained with Professor Aleksander Popel (Johns Hopkins) and developed detailed models of Nitric Oxide, blood flow and Oxygen transport in three dimensional vascular networks. As an independent investigator, I have continued to rely on the parallel development of computational models and experiments for my investigations. I have developed and presented some of the first detailed models of Ca^{2+} dynamics and electrophysiology in vascular cells. This allowed us a unique quantitative perspective of microcirculatory signaling. Our models have proven valuable for elucidating experimental observations, particularly as pertaining to intercellular communication and electrical properties of blood vessels, and more recently to localized subcellular Ca^{2+} signaling events. Previous studies on vasoreactivity and Ca^{2+} dynamics highlight the benefits of mathematical modeling in the analysis of complex signaling mechanisms. My research focus has shifted over the last few years to the cerebral microcirculation as a result of collaborating activities with Professor Mark Nelson and his group at University of Vermont. This interaction led to significant progress in multiscale modeling of brain blood flow control and in formulating groundbreaking hypotheses on neurovascular coupling, setting the stage for a bottom-up approach in the interpretation of BOLD fMRI imaging of brain function.

In summary, my past contributions have focused on mathematical modeling of cardiovascular and respiratory function and particularly on multiscale models that bridge the gap between cell-level signaling and macroscale functional responses. My expertise span from cell electrophysiology, calcium dynamics and cellular signaling to vascular biomechanics, hemodynamics and biotransport and will allow me to review a broad range of applications that utilize quantitative approaches for the analysis of pathophysiological systems and process.

a. Moshkforoush A, B Ashenagar, O Harraz, F Dabertrand, T Longden, MT Nelson and **NM Tsoukias**. The capillary Kir channel as sensor and amplifier of neuronal signals: modeling insights on K+-mediated neurovascular communication. *Proceedings of the National Academy of Sciences, USA.* Accepted, 2020.

b. A Moshkforoush, Ashenagar B, **NM Tsoukias**, Alevriadou BR. Modeling the role of endoplasmic reticulum-mitochondria microdomains in calcium dynamics. *Scientific Reports*. Nov 19;9(1):17072, 2019. PMCID: PMC6864103.
c. Koide M, Moshkforoush A, **NM Tsoukias,** Hill-Eubanks DC, Wellman GC, Nelson MT, Dabertrand F. The yin and yang of KV channels in cerebral small vessel pathologies. *Microcirculation*. 25(1), 2018. PMID: 29247493
d. Kapela A, Behringer EJ, Segal SS., and **NM Tsoukias**. Biophysical properties of microvascular endothelium: Requirements for initiating and conducting electrical signals. *Microcirculation* 25(2), 2018. PMID: 29117630.

B. Positions and Honors

Positions and Employment:

1996-1999 Research assistant, Department of Chemical and Biochemical Engineering, University of California, Irvine
1999-2000 Postdoctoral fellow, Department of Chemical and Biochemical Engineering, University of California, Irvine
2000-2003 Research Fellow, Department of Biomedical Engineering, Johns Hopkins University, Baltimore.
2003-2009 Assistant Professor, Department of Biomedical Engineering, Florida International University.
2015-2018 Associate Professor, Department of Chemical Engineering, National Technical University, Athens.
2009- Associate Professor, Department of Biomedical Engineering, Florida International University.

Other Experience and Professional Memberships:

2003-present Member, American Physiological Society
2004-present Member, American Heart Association
2004-present Member, Biomedical Engineering Society
2006-present Member, Microcirculation Society
20010-present Editorial Board: Microcirculation

Honors and Awards:

2006 Arthur Guyton Award for Excellence in Integrative Physiology, American Physiological Society

C. Contributions to Science

Exhaled NO.

My earlier work investigates the dynamics of Nitric Oxide in the exhaled breath and its potential as a diagnostic test of lung function. In the late 90's we were one of the two groups who observed that the concentration of NO in the exhaled breath depends on the expiratory flow rate. This discovery prompted the American Thoracic Society to issue recommendations for the standardization of exhaled NO monitoring. Our theoretical models explained this phenomenon and provided a method to analyze and to partition the exhaled NO levels into an alveolar and airway origin. Our proposed method of analysis has been widely used in the literature and in clinical studies over the last twenty years.

a. **Tsoukias, N. M**., Z. Tannous, A. F. Wilson and S. C. George. Single Exhalation Profiles of NO and CO2 in Humans: Effect of Dynamically Changing Flow Rate. *Journal of Applied Physiology* 85(2):642-652, 1998. PMID: 9688743
b. **Tsoukias, N. M**. and S. C. George. A Two-Compartment Model of Pulmonary Nitric Oxide Exchange Dynamics. *Journal of Applied Physiology* 85(2):653-666, 1998. PMID: 9688744
c. **Tsoukias, N. M**. and S. C. George. A Single Breath technique with variable flow rate to characterize Nitric Oxide Exchange Dynamics in the Lungs. *Journal of Applied Physiology* 91(1):477-487, 2001. PMID: 11408466
d. George S.C. and **N.M. Tsoukias**. An apparatus and method for the estimation of flow independent parameters which characterize the relevant features of Nitric Oxide production and exchange in the human lungs. (WO 01/82782; US 6,866,637).

Nitric Oxide Biotransport.
Despite significant prior work on the vasoreactive role of NO, the factors that regulate NO bioavailability in the vasculature have not been elucidated. For example it is not known how NO escapes scavenging by the erythrocytic hemoglobin and what NO levels can be maintained in the vascular wall to induce vasodilation (reported/predicted NO concentrations range from pico to micro molar). Through mathematical modeling we have been able to quantify some of these interactions and to make physiological relevant predictions. We have shown for example, that NO activity can be regulated by the frequency of the underlying Ca^{2+} events. We have tested experimental approaches for assessing NO in biological tissues and highlighted the limitations of existing fluorescent markers and we have elucidated the kinetics of NO preservation through the formation of Nitrosothiols. Our combined theoretical and experimental methodology has provided insights for the fate of NO in the vasculature.

a. **N.M. Tsoukias**, M. Kavdia and A.S. Popel. A theoretical model of nitric oxide transport in arterioles: frequency vs amplitude dependent control of cGMP formation. *American Journal of Physiology* 286(3):H1043-56, 2004. PMID: 14592938
b. K. Madrasi, T. Gadkari, M. S. Joshi and **N.M.Tsoukias**. Glutathiyl Radical as an Intermediate in the Glutathione Nitrosation. *Free Radic Biol Med*; 53(10):1968-76, 2012. PMCID: PMC3494776
c. Namin S., S. Nofallah, M. Joshi and **N.M. Tsoukias**. Kinetic analysis of Diaminofluorescein activation by NO: Toward calibration of a NO-sensitive fluorescence probe. *Nitric Oxide*;28:39-46, 2013. PMCID: PMC3544979
d. Gadkari T. V., N. Cortes, **N M. Tsoukias**, and M. S. Joshi. Agmatine Induced NO Dependent Rat Mesenteric Artery Relaxation and its Impairment in Salt-Sensitive Hypertension. *Nitric Oxide*, 35C:65-71, 2013. PMCID: PMC3844099

Ca^{2+} dynamics and inter-cellular signaling in the vasculature.
Despite more than 50 years of modeling of the heart, there is very little progress in the modeling of the vasculature. We have devoted most of our research efforts over the last decade to bridge this gap. My group has constructed the most detailed models of Ca^{2+} dynamics and electrophysiology in vascular cells. These cellular models have been utilized by us and others as the basis for theoretical investigations of vascular function. The models have been particularly useful in investigations of intercellular communication in the microcirculation. Our studies have revealed how myoendothelial communication contributes to the regulation of vessel tone and how signals are conducted upstream the vascular network to coordinate network blood flow and to match supply to local metabolic demand. These past efforts provide a significant benefit for analysis of vasoactive regulation in the brain vasculature outlined in this proposal.

a. Silva H.S., A. Kapela and **N.M. Tsoukias**. A mathematical model of plasma membrane electrophysiology and calcium dynamics in vascular endothelial cells. *American Journal of Physiology* 293(1):C277-93*,* 2007. PMID: 17459942
b. Kapela A., A. Bezerianos, and **N.M. Tsoukias**. A mathematical model of Ca^{2+} dynamics in rat mesenteric smooth muscle: agonist and NO stimulation. *Journal of Theoretical Biology* 253: 238-60, 2008. PMID: 18423672
c. Kapela A., A. Bezerianos and **N.M. Tsoukias**. A mathematical model of vasoreactivity in rat mesenteric arterioles: I Myoendothelial communication. *Microcirculation* 16(8):694-713, 2009. PMCID: PMC3547604
d. Kapela A., Parikh J. and **N.M. Tsoukias**. Multiple factors influence calcium synchronization in vasomotion. *Biophysical Journal* 102(2):211-20, 2012. PMCID: PMC3260665

Localized signaling events.
We have extended our cellular models to account for localized (subcellular) signaling events. In collaboration with experimentalist we have investigated distinct Ca^{2+} events observed near the myoendothelial projections (i.e. wavelets, pulsars). A combined modeling/experimental approach enabled us to examine the dynamics of these localized Ca^{2+} events and their role in the regulation of vessel tone. Recently, TRPV4-induced Ca^{2+} sparklets discovered and experimentally characterized by our collaborators in this proposal, were analyzed by our mathematical models. Simulations showed that the stochastic opening of a cluster of TRPV4 channels lead to the observed quantal Ca^{2+} events in the experiments. The theoretical study makes predictions for the local Ca^{2+}

concentration levels and the contribution of TRPV4 channels to vessel hyperpolarization-relaxation. Simulations revealed the physiological relevance of TRPV4 channel cooperativity in enhancing Ca^{2+} spread, recruitment of K_{Ca} channels and relaxation.

a. A. Moshkforoush, Ashenagar B, **NM Tsoukias**, Alevriadou BR. Modeling the role of endoplasmic reticulum-mitochondria microdomains in calcium dynamics. *Sci Rep*. Nov 19;9(1):17072, 2019. PMCID: PMC6864103.
b. S. Nagaraja, A. Kapela, C. Tran, D. Welsh and **N.M. Tsoukias**. Role of microprojections in myoendothelial feedback-a theoretical study. *Journal of Physiol*, 591(11): 2795-2812, 2013. PMCID: PMC3690687
c. J. Parikh, A. Kapela, and **N.M. Tsoukias**. Stochastic model of a TRPV4 Calcium sparklet: Effect of bursting and cooperativity on endothelial-derived hyperpolarization. *Biophysical Journal* 108(6):1566-76, 2015. PMCID: PMC4375679
d. J. Parikh, Kapela A. and **N.M. Tsoukias**. Can endothelial Hemoglobin alpha regulate Nitric Oxide vasodilatory signaling? *American Journal of Physiology* 312(4):H854-H866, 2017. PMCID: PMC5407155

Multiscale modeling of tissue perfusion and oxygenation.
We have previously developed and presented the first detailed model of tissue perfusion and O_2 delivery in three-dimensional vascular networks of skeletal muscle and we utilized this model to answer questions relating to angiogenesis or the design of hemoglobin-based blood-substitutes. Over the last decade we focused on modeling underlying mechanisms of blood flow control. At the cell level we presented models of Ca^{2+} dynamics and electrophysiology in vascular cells of mesenteric vessels. At the vessel level we examined vasoactive signaling and the biophysical determinants of ascending vasodilation in skeletal muscle. The long term goal, as outlined in this proposal, is to capitalize on these previous mathematical approaches and develop a multiscale framework for linking tissue perfusion and oxygenation to the underlying molecular/subcellular mechanisms in health and in disease.

a. **N.M Tsoukias**, D. Goldman, A. Vadapalli, R. N. Pittman and A. S. Popel. A computational model of oxygen delivery by hemoglobin-based oxygen carriers in three dimensional microvascular networks. *Journal of Theoretical Biology* 248(4):657-74, 2007. PMCID: PMC2741314
b. Kapela A, **N.M Tsoukias**. Multi-Scale FEM Modeling of Vascular Tone: From Membrane Currents to Vessel Mechanics. *IEEE Trans Biomed Eng*. 58(12):3456-59, 2011.
c. Kapela A. and **N.M Tsoukias**. A mathematical model of vasoreactivity in rat mesenteric arterioles: II Conducted vasoreactivity. *American Journal of Physiology* 298(1):H52-65, 2010. PMCID: PMC2806131
d. Kapela A, Behringer E.J., Segal S.S., and **N.M Tsoukias**. Biophysical properties of microvascular endothelium: Requirements for initiating and conducting electrical signals. *Microcirculation* 25(2), 2018. PMID: 29117630

Complete List of Published Work in My Bibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/nikolaos.tsoukias.1/bibliography/40295009/public/?sort=date&direction=descending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support:

R01NS110656 Nelson (PI) 04/01/19-04/01/24
Ion channel dysfunction in small vessel disease of the brain
This project aims to elucidate channel-mediated defects in small vessel disease of the brain.
Role: Co-Investigator

R35 HL140027 Nelson (PI) 01/01/19-11/31/25
Capillaries as a sensory web that controls cerebral blood flow in health and disease
This comprehensive research Program extends the research in 1R01 HL131181 on the regulation of cerebral blood flow.
Role: Co-Investigator

R01HL136636 Dabertrand (PI) 2/01/17-1/31/22
Capillary control of cerebral blood flow, and its disruption in small vessel disease.
The overall goals of this proposal are to understand the role of calcium signaling in functional hyperemia, its regulation by the extracellular matrix proteins, and its subsequent suppression in the CADASIL syndrome.
Role: Co-Investigator

<u>Research Support completed During the Last Three Years:</u>

R15HL121778 Tsoukias (PI) 08/01/14-07/31/18
National Institutes of Health.
Integrative modeling to link vascular phenotype to gene expression.
The goal of this grant is to investigate ion channel expression level control in resistance vessels in normotensive and hypertensive rats through mathematical modeling and experimentation.
Role: PI

R01HL131181 Nelson (PI) 2/01/16-12/31/18
K+ sensing & electrical signaling by Kir channels in brain vasculature.
The major goals of this project are to examine the mechanisms underlying neurovascular coupling and the electrical and calcium signaling properties of brain endothelium.
Role: Co-Investigator