BIOGRAPHICAL SKETCH

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NAME: Dominguez, Roberto

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

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date	FIELD OF STUDY
Faculty of Physics, Odessa Mechnikov National University, former USSR	B.S., M.S.	09/1982 – 06/1987	Theoretical Physics and Mathematics
Pasteur Institute & University of Paris-Sud, France	Ph.D.	07/1993 – 03/1996	Protein Crystallography and Biochemistry
Rosenstiel Center, Brandeis University, MA	PostDoctoral Fellow	03/1996 – 02/1998	Structural Biology of Molecular Motors

A. Personal Statement

My laboratory has had a long-standing interest in understanding the proteins that control actin cytoskeleton and membrane dynamics, and the signaling pathways that regulate their activities. These proteins control a myriad of cellular functions, including cell locomotion, intracellular transport, and endo/exocytosis. Dysfunction of cytoskeletal components is linked to devastating human diseases, such as cancer, immune, musculoskeletal and neurodegenerative disorders.

My interest in the cytoskeleton started during my postdoctoral years with Carolyn Cohen at Brandeis University, where I determined the structure of myosin at the beginning of the power stroke. While I have continued making contributions to the myosin field to this day, when I started my first independent position at BBRI (Boston) in 1998 the main focus of my laboratory turned to actin and actin-associated proteins. In 2006, I moved to the Department of Physiology at the University of Pennsylvania, a powerhouse in the cytoskeleton. My laboratory is also part of the Pennsylvania Muscle Institute (PMI), a world-class organization dedicated to the study of the cytoskeleton. The move to UPenn has had a major impact in my research, as I developed new collaborations and adopted a more interdisciplinary research approach. Our work aims to correlate structure and function, for which we use a broad spectrum of experimental approaches. A major research tool in the group is x-ray crystallography, producing atomic 'snapshots' that provide a wealth of information. We gain additional knowledge about the physiological activities of cytoskeletal components using a host of other approaches, including cell and molecular biology, bioinformatics, biophysical and biochemical methods (ITC, MALS, SAXS, FRET, TIRF, cryo-EM).

A key component of our mission is to prepare the next generation of scientists and educators, by actively participating in training of students and postdocs. Seven alumni members of my laboratory are now independent investigators (Frederic Kerff and Mohammed Terrak in Belgium; Francois Ferron in France; Sung Haeng Lee and Suk Namgoong in Korea; Silvia Jansen and David Kast at WashU).

Four recent publications that highlight our focus and experience

a. Rao JN, Madasu Y, Dominguez R. Mechanism of actin filament pointed-end capping by tropomodulin. *Science* (2014) **345**:463-467

b. Drazic A, Aksnes H, Marie M, Boczkowska M, Varland S, Timmerman E, Foyn H, Glomnes N, Rebowski G, Impens F, Gevaert K, Dominguez R, Arnesen T. Naa80 is actin's N-terminal acetyltransferase and regulates cytoskeleton assembly and cell motility. *PNAS* (2018) doi:10.1073/pnas.1718336115

c. Lee IG, Olenick MA, Boczkowska M, Franzini-Armstrong C, Holzbaur EL, Dominguez R. A Conserved Interaction of the Dynein Light Intermediate Chain with Dynein-Dynactin Effectors Necessary for Processivity. *Nat Commun* (2018) **9**:986 doi: 10.1038/s41467-018-03412-8

d. Kast DJ, Dominguez R. Mechanism of IRSp53 inhibition by 14-3-3. *Nat Commun* (2019) **10**:483 doi: 10.1038/s41467-019-08317-8

B. Positions and Honors

Positions and Employment

1987-1989 Scientist, Center for Genetic Engineering and Biotechnology, Havana, Cuba
1989-1991 Pre-doctoral Trainee, University of Liége, Belgium (group of Dr. O Dideberg)
1992-1993 Pre-doctoral Trainee, EMBL, Heidelberg, Germany (group of Dr. D Suck)
1993-1996 PhD Student, Pasteur Institute & Paris-Sud University, Paris, France (group of Dr. PM Alzari)
1996-1998 Postdoctoral Fellow, Rosenstiel Center, Brandeis U., MA (group of Dr. C Cohen)
1998-2001 Scientist (Assistant Prof), Boston Biomedical Research Institute, Watertown, MA
2001-2006 Principal Scientist (Associate Prof), Boston Biomedical Research Institute, Watertown, MA
2006-2010 Associate Professor, U. of Pennsylvania, Perelman School of Medicine, Philadelphia, PA
2010-present Professor of Physiology, U. of Pennsylvania, Perelman School of Medicine, Philadelphia, PA

Other Experience and Professional Memberships

1998- Member, Biophysical Society
2006- Member, American Society for Cell Biology
2006-2010 Member, NIH Study Section MSFC
2008-2014 Member, Editorial Board of the *Biophysical Journal*
2009-present Associate Editor, *Cytoskeleton*
2015-present Member, Editorial Board of the *Journal of Muscle Research and Cell Motility*
2016 NIGMS Council Meeting ad-hoc Member
2014-present Member Bridge Funding Advisory Committee, Perelman School of Medicine
2017-present Member Cores Advisory Committee, Perelman School of Medicine
2018-present Member Limited Applications Committee, Perelman School of Medicine
2018-present Member Faculty Mentoring Committee, Dr. Shae Padrick (Drexel U. College of Medicine)
2019-present Member Scientific Advisory Board of TroBio Therapeutics Ltd (Australia)

Honors

1989-1991 Fellow of the Societé Française de Belgique
1992-1992 Fellow of the German Academic Exchange Service (DAAD)
1998-2001 Basil O'Connor Scholar of the March of Dimes
1999-2001 American Heart Association, Grant-in-Aid Junior Investigator Award
2002-2005 Established Investigator of the American Heart Association
2010 Wenner-Gren Foundation Distinguished Lecturer (FEBS/EMBO meeting, Sweden)
2019 Appointed William Maul Measey Presidential Professor of Physiology

C. Contribution to Science

1. *Myosin structure-function*: As a postdoctoral fellow in Dr. Carolyn Cohen's laboratory at Brandeis University I determined structures of smooth muscle myosin with bound ADP•Pi and ATP analogs. The structures provided the first direct visualization of the pre-power stroke state, and conclusively demonstrated the swinging lever-arm hypothesis that is now featured in textbooks. As an independent investigator at BBRI, my laboratory continued the work on myosin motors, focusing on the unusual properties and structure of the long lever arm of myosin V. More recently, in collaboration with the Ostap laboratory at Penn, we determined the structure of myosin-Ib. These highly cited studies have had a deep impact in the field. Numerous groups have used our structures to design myosin mutations, position fluorescent probes, analyze EM maps, and test dynamic models of the myosin ATPase cycle.

a. Dominguez R, Freyzon Y, Trybus KM, Cohen C. Crystal structure of a vertebrate smooth muscle myosin motor domain and its complex with the essential light chain: visualization of the pre-power stroke state. *Cell* (1998) **94**:559-571
b. Terrak M, Wu G, Stafford WF, Lu RC, Dominguez R. Two distinct myosin light chain structures are induced by specific variations within the bound IQ motifs-functional implications. *EMBO J.* (2003) **22**:362-371

c. Terrak M, Rebowski G, Lu RC, Grabarek Z, Dominguez R. Structure of the light chain-binding domain of myosin V. *PNAS* (2005) **102**:12718-12723
d. Shuman H, Greenberg MJ, Zwolak A, Lin T, Sindelar CV, Dominguez R, Ostap EM. A vertebrate myosin-I structure reveals unique insights into myosin mechanochemical tuning. *PNAS* (2014) **111**:2116-2121

2. *Structure of monomeric actin*: As an independent investigator at BBRI, the main focus of my laboratory became the actin cytoskeleton. At the time, the structure of actin had only been determined in complex with three actin-binding proteins (DNase I, gelsolin and profilin). These proteins inhibit nucleotide hydrolysis, which had impeded visualization of the ADP state in actin. Because actin is the most abundant protein in mammalian cells, and a crucial player in a myriad of cellular functions, this was a considerable limitation. We solved the problem by covalently modifying actin at Cys-374 with the fluorescent dye TMR, allowing for the crystallization of monomeric ADP-actin. Subsequently, we used a nucleotide analog to also obtain the structure of monomeric ATP-actin. Together these two structures illustrate the changes that take place in the actin monomer upon nucleotide hydrolysis and γ-phosphate release. Numerous studies in the field have found inspiration in this original work that has been widely cited.

a. Otterbein LR, Graceffa P, Dominguez R. The crystal structure of uncomplexed actin in the ADP state. *Science* (2001) **293**:616-618
b. Graceffa P, Dominguez R. Crystal structure of monomeric actin in the ATP state – Structural basis of nucleotide-dependent actin dynamics. *J. Biol. Chem.* (2003) **278**:34172-34180

3. *Myosin phosphatase*. Our laboratory has had a long-standing interest in understanding the mechanisms that regulate muscle contraction, and the role of cytoskeletal components and myosin motors in this process. In addition to our work on myosins (described above), we became interested in the regulation of smooth muscle contraction by phosphorylation/dephosphorylation of the myosin regulatory light chain (RLC). Dephosphorylation, which results in muscle relaxation, is catalyzed by the myosin phosphatase, which is composed of three subunits: the catalytic subunit PP1, the regulatory subunit MYPT1, and a small subunit of unknown function (M20). The lack of structural information on the PP1-MYPT1 complex was only part of the problem. Indeed, contrary to protein kinases that tend to be more specialized, PP1 is ubiquitous, and its activity is regulated through a combinatorial mechanism whereby PP1 forms complexes with a myriad of regulatory subunits (>200) that control PP1 specificity and activity in time and space. Our structure of PP1-MYPT1 was the first structure ever determined of a PP1-regulatory subunit complex, thus offering a glance into the molecular mechanism for the so-called combinatorial control of PP1 function. To this day, there is only one other such structure – that of PP1-spinophilin determined recently. Thus, the entire protein phosphatase field has relied on our structure for inspiration to understand the role of regulatory subunits in PP1 function. Several biotechnology companies are also using this structure to design PP1 inhibitors to be used in therapies to treat diseases such as cancer.

a. Terrak M, Kerff F, Langsetmo K, Tao T, Dominguez R. Structural Basis of Protein Phosphatase 1 Regulation. *Nature* (2004) **429**:780-784

4. *Actin filament nucleation*. Processes such as cell motility, intracellular trafficking, and the movement of several pathogens involve rapid bursts of actin polymerization/depolymerization. Because, the formation of new filaments is kinetically unfavorable, cells use actin filament nucleators to control the *de novo* formation of actin filaments in time and space. For the last 10 years, our laboratory has been at the forefront of the study of actin nucleators, with a specific focus on their structures and molecular mechanisms. This includes the discovery of Leiomodin, a muscle cell-specific nucleator, characterization of the molecular mechanism of Arp2/3 complex activation by members of the WASP family of NPFs, and dissection of the structure and function of the most common actin-binding domain in nucleation, the WH2 domain.

a. Chereau D, Kerff F, Graceffa P, Grabarek Z, Langsetmo K, Dominguez R. Actin-bound structures of Wiskott-Aldrich syndrome protein (WASP)-homology domain 2 and the implications for filament assembly. *PNAS* (2005) **102**:16644-16649

b. Chereau D, Boczkowska M, Skwarek-Maruszewska, A, Fujiwara I, Rebowski G, Hayes DB, Lappalainen P, Pollard TD, Dominguez R. Leiomodin is an actin filament nucleator in muscle cells. *Science* (2008) **320**:239-243
c. Namgoong S, Boczkowska M, Glista MJ, Rebowski G, Kovar DR, Dominguez R. Mechanism of actin filament nucleation by *Vibrio* VopL and implications for tandem W domain nucleation. *Nature Struct Mol Biol.* (2011) **18**:1060-1067
d. Boczkowska M, Rebowski G, Kast DJ, Dominguez R. Structural analysis of the transitional state of Arp2/3 complex activation by two actin-bound WCAs. *Nature Commun*. (2014) **5**:3308. doi: 10.1038/ncomms4308

5. *Actin-binding proteins and BAR domain proteins*. Two other areas in which our laboratory has had a major impact in the field is the structural and functional study of proteins that regulate actin dynamics in cells and BAR domain proteins that coordinate actin cytoskeleton and membrane dynamics under the control of signaling cascades. Some of our contributions include the structures of complexes of actin with vitamin D-binding protein, toxofilin, Ena/VASP and tropomodulin and the functional characterization of several BAR domain proteins, including Missing-in-Metastasis (MIM), PinkBAR, IRSp53, and PICK1.

a. Zwolak A, Yang C, Feeser EA, Ostap EM, Svitkina T, Dominguez R. CARMIL leading edge localization depends on a non-canonical PH domain and dimerization. *Nature Commun* (2013) **4**:2523. DOI:10.1038/ncomms3523
b. Kast DJ, Yang C, Disanza A, Boczkowska M, Madasu Y, Scita G, Svitkina T and Dominguez R. Mechanism of IRSp53 inhibition and combinatorial activation by Cdc42 and downstream effectors. *Nature Struct Mol Biol* (2014) **21**:413-422
c. Kast DJ, Zajac AL, Holzbaur EL, Ostap EM, Dominguez R. WHAMM directs the Arp2/3 complex to the ER for autophagosome biogenesis through and actin comet tail mechanism. *Curr Biol* (2015) **25**:1791-1797.
d. Lee IG, Olenick MA, Boczkowska M, Franzini-Armstrong C, Holzbaur EL, Dominguez R. A Conserved Interaction of the Dynein Light Intermediate Chain with Dynein-Dynactin Effectors Necessary for Processivity. *Nat Commun* (2018) **9**:986 doi: 10.1038/s41467-018-03412-8

My-NCBI Complete Publications List: https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/41144338

D. Research Support

Ongoing Research Support

R01 GM073791 (Dominguez, R) 03/01/2019 – 29/02/2023 (NCE) 3.0 CM
NIH/NIGMS Direct costs: $232,680
Structural Basis of Actin Cytoskeleton Dynamics

P01 GM087253 (Ostap, EM) NCE 09/01/2014 – 8/31/2019 3.6 CM
NIH/NIGMS Direct costs: $242,932
Cytoskeletal Motors And Scaffolds In Membrane Dynamics And Motility
Project-3 (Dominguez, R)
Cytoskeleton-Membrane Scaffolds in Organelle Morphogenesis and Motility

R01 MH087950 (Dominguez, R) 08/08/2016 – 05/31/2021 3.0 CM
NIH/NIMI Direct costs: $250,000
BAR Proteins Linking Membrane and Cytoskeleton Dynamics

R37 GM057247 (Ostap, EM) 12/01/2016-11/30/2020 0.6 CM (salary only)
NIH/NIGMS
Molecular Function of Myosin-I

Pending Research Support

RM1 GM136511-01 (Dominguez, R; Ostap, EM; Holzbaur, E; Lakadamyali, M) 4/1/2020- 3/31/2025
NIH/NIGMS