BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

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 studying 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 first structure of myosin at the beginning of the power stroke. While my interest on myosins continues to this day (we are now studying Myo19), when I started my first independent position at BBRI (Boston) in 1998 the main focus of my laboratory turned to actin and actin-binding proteins. In 2006, I moved to the Department of Physiology at the University of Pennsylvania. Here, my laboratory is also part of the Pennsylvania Muscle Institute (PMI), a world-class institution dedicated to the study of the cytoskeleton. At UPenn we have collaborations with several other lab, including Michael Ostap, Erika Holzbaur, Tatyana Svitkina and Robert Heuckeroth (CHOP). The collaboration with the Heuckeroth's lab has been particularly rewarding, as it bridges my interests in actin biochemistry with the urgent necessities of real-life patients, namely the study of the mechanisms of visceral myopathy in children, caused by mutations in alpha smooth muscle actin.

Our work aims to correlate structure and function by using a broad range of experimental approaches. Structural biology, including x-ray crystallography and cryo-EM, are major tools in the lab. We use these methods to obtain atomic-level information about cytoskeletal proteins and their complexes, and use a host of other approaches, including cell and molecular biology, bioinformatics, biophysical and biochemical methods (ITC, MALS, FRET, TIRF, SAXS) to correlate this knowledge with the physiological activities of proteins *in vitro* and in cells.

A key component of our mission is to train the next generation of scientists and educators. My lab has trained over 40 postdocs and students. The lab was initially located at the BBRI (Boston), a non-teaching institution, where lab trainees were primarily postdoctoral fellows. It was only after the move to UPenn that the lab became heavily invested in graduate and undergraduate training. Among previous lab trainees, 9 are now professors (or equivalent) in five different countries USA (Silvia Jansen and David Kast), Belgium (Frederic Kerff and Mohammed Terrak), France (Francois Ferron), South Korea (Sung Haeng Lee, Suk Namgoong and InGyun Lee), and UAE (Saif Alqassim), and several are pursuing successful careers in the pharmaceutical industry (Ludovic Otterbein, David Chereau, Adam Zwolak, Bengi Turegun and Austin Zimmet). Current lab trainees include 5 students: Peter Carman, Rachel Ceron, Fred Fregoso, Elana Baltrusaitis and Nicholas Palmer.

Four recent publications that highlight the lab's focus and experience

a. Rebowski G, Boczkowska M, Drazic A, Ree R, Goris M, Arnesen T, Dominguez R. Mechanism of actin N-terminal acetylation. ***Sci Adv*** 2020, **6**(15). DOI: 10.1126/sciadv.aay8793. PMC7141826

b. Zimmet A, Van Eeuwen T, Boczkowska M, Rebowski G, Murakami K, Dominguez R. Cryo-EM Structure of NPF-Bound Human Arp2/3 Complex and Activation Mechanism. ***Sci Adv*** 2020, **6**(23). DOI: 10.1126/sciadv.aaz7651. PMC7274804

c. Lee IG, Cason SE, Alqassim SS, Holzbaur ELF, Dominguez R. A tunable LIC1-adaptor interaction modulates dynein activity in a cargo-specific manner. ***Nat Commun*** 2020, **11**:5695. DOI: 10.1038/s41467-020-19538-7. PMC7655957

d. Baker RW, Reimer JM, Carman PJ, Turegun B, Arakawa T, Dominguez R, Leschziner AE. Structural insights into assembly and function of the RSC chromatin remodeling complex. ***Nat Struct Mol Biol*** 2021, **28**:71-80. DOI: 10.1038/s41594-020-00528-8. PMC7855068

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, Biomedical Research Core Facilities 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)
2020 MSFC, RM1 Study Section, ad-hoc Member
2021 Co-chair, BMB Graduate Group Admissions Committee
2021 Member, of UPenn POWER Cluster Hire Joint Search Committee

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 lab at Brandeis University I determined structures of smooth muscle myosin with bound ADP•Pi and ATP analogs. These 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 lab continued the work on myosin motors, including revealing the unusual structural properties of the long lever arm of myosin V. More recently, in collaboration with the Ostap lab at Penn, we have determined structures of myosin-I. The lab has now embarked on the structural-functional study of myosin XIX, involved in mitochondria dynamics. 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, Rebowski G, Lu RC, Grabarek Z, Dominguez R. Structure of the light chain-binding domain of myosin V. ***PNAS*** 2005, **102**:12718-12723. PMC1200277

c. 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. PMC3926069

d. Mentes A, Huehn A, Liu X, Zwolak A, Dominguez R, Shuman H, Ostap EM, Sindelar CV. High-resolution cryo-EM structures of actin-bound myosin states reveal the mechanism of myosin force sensing. ***PNAS*** (2018) **115**:1292-1297. PMC5819444

2. *Structural biology of actin and actin-binding proteins*: As an independent investigator at BBRI, the main focus of my lab became the actin cytoskeleton. At the time, the structure of actin had only been determined in complexes 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 myriad of cellular functions, this was considered a major limitation. We solved the problem by covalently modifying actin at Cys-374 with the fluorescent dye TMR, allowing for the first crystallization of monomeric actin in both the ADP and ATP states. These structures showed for the first time 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. Through the years, our lab has been at the forefront of actin structural biology, determining several ground-braking structures, including those of ternary complexes of actin with gelsolin and tropomodulin and with profilin and actin's N-terminal acetyl transferase NAA80, which we discovered in collaboration with the Arnesen lab. We have also studied countless actin-binding proteins (Ena/VASP, CARMIL, etc.), proteins involved in pathogen infection that highjack the actin cytoskeleton (toxofilin, Sca2, VopL), and dynein-dynactin regulatory proteins (BICD2, HOOK, CRACR2a). Our work is characterized by its attention to protein function and its multidisciplinary nature, extending from cellular to structural biology and making extensive use of biophysical approaches.

a. Otterbein LR, Graceffa P, Dominguez R. The crystal structure of uncomplexed actin in the ADP state. ***Science*** 2001, **293**:616-618

b. 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. ***Nat Commun*** 2013, **4**:2523. DOI:10.1038/ncomms3523. PMC3796438

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. PMC5841405

d. Rebowski G, Boczkowska M, Drazic A, Ree R, Goris M, Arnesen T, Dominguez R. Mechanism of actin N-terminal acetylation. ***Sci Adv*** 2020, **6**(15). DOI: 10.1126/sciadv.aay8793. PMC7141826

3. *Study of the muscle cytoskeleton*. In addition to our myosin work (described above), our laboratory has had a long-standing interest in understanding the mechanisms that regulate muscle contraction. One example is 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, composed of three subunits: the catalytic subunit PP1, the regulatory subunit MYPT1, and a small subunit of unknown function (M20). Contrary to protein kinases that tend to be substrate-specific, PP1 is ubiquitous, and its activity is regulated through a combinatorial mechanism

whereby PP1 forms complexes with myriad regulatory subunits (>200) that control its substrate specificity and activity in time and space. Our structure of PP1-MYPT1 was the first structure ever determined of a PP1-regulatory subunit complex. Therefore, the protein phosphatase field has relied on this structure for inspiration to understand the role of regulatory subunits in PP1 function. Several pharmaceutical companies are also using this structure to design PP1 inhibitors to be used in therapies to treat diseases such as cancer. We have also studied other muscle regulatory components, including tropomodulin, tropomyosin and leiomodin.

a. Terrak M, Kerff F, Langsetmo K, Tao T, Dominguez R. Structural Basis of Protein Phosphatase 1 Regulation. ***Nature*** 2004, **429**:780-784
b. Rao JN, Madasu Y, Dominguez R. Mechanism of actin filament pointed-end capping by tropomodulin. ***Science*** 215, **345**:463-467. PMC4367809
c. Boczkowska M, Rebowski G, Kremneva E, Lappalainen P, Dominguez R. How Leiomodin and Tropomodulin use a common fold for different actin assembly functions. ***Nat Commun*** 2015, **6**:8314. DOI: 10.1038/ncomms9314. PMC4571291
d. Kumari R, Jiu Y, Carman PJ, Tojkander S, Kogan K, Varjosalo M, Gunning P, Dominguez R, Lappalainen P. Tropomodulins control the balance between protrusive and contractile structures by stabilizing actin-tropomyosin filaments. ***Curr Biol*** 2020, **30**:767-778. PMC7065974

4. *Actin filament nucleation*. For the last 15 years, our laboratory has been at the forefront of the study of actin nucleators, with a specific focus on molecular mechanisms. Processes such as cell motility, intracellular trafficking, and the movement of several pathogens require rapid bursts of actin polymerization and depolymerization. Since 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. Our contributions in this area include 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. Recently, we have also determined crucial structures of Arp2/3 complex with N-WASP family nucleation-promoting factors bound using cryo-EM.

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. Boczkowska M, Rebowski G, Kast DJ, Dominguez R. Structural analysis of the transitional state of Arp2/3 complex activation by two actin-bound WCAs. ***Nat Commun*** 2014, **5**:3308. DOI: 10.1038/ncomms4308. PMC4364448
d. Zimmet A, Van Eeuwen T, Boczkowska M, Rebowski G, Murakami K, Dominguez R. Cryo-EM Structure of NPF-Bound Human Arp2/3 Complex and Activation Mechanism. ***Sci Adv*** 2020, **6**(23). DOI: 10.1126/sciadv.aaz7651. PMC7274804

5. *BAR domain proteins*. The study of BAR domain proteins that coordinate actin cytoskeleton and membrane dynamics under the control of signaling cascades is one area in which our lab has had a considerable impact during the last 10 years, and our focus in this area is expanding. We have studied the structures of several BAR domain proteins, including Missing-in-Metastasis (MIM), PinkBAR, IRSp53, and PICK1 as well as the mechanism by which they are regulated *in vitro* and in cells.

a. Pykäläinen A, Boczkowska M, Zhao H, Saarikangas J, Rebowski G, Jansen M, Hakala J, Koskela E, Peränen J, Vihinen H, Jokitalo E, Salminen M, Ikonen E, Dominguez R*, Lappalainen P*. Pinkbar is an epithelial-specific BAR domain protein that generates planar membrane structures. ***Nat Struct Mol Biol*** 2011, **18**:902-907. PMC3910087
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. ***Nat Struct Mol Biol*** 2014, **21**:413-422. PMC4091835

c. Kast DJ, Dominguez R. Mechanism of IRSp53 inhibition by 14-3-3. ***Nat Commun*** 2019, **10**:483. DOI: 10.1038/s41467-019-08317-8. PMC6351565

d. Kast DJ, Dominguez R. IRSp53 Coordinates AMPK and 14-3-3 Signaling to Regulate Filopodia Dynamics and Directed Cell Migration. ***Mol Biol Cell*** 2019, **30**:1285–1297. PMC6724608

My-NCBI Publications List: https://www.ncbi.nlm.nih.gov/myncbi/roberto.dominguez.1/bibliography/public/

D. Research Support

Ongoing Research Support

R01 GM073791 (Dominguez R) 3/1/19 – 2/28/23 3.0 CM
NIH/NIGMS
Structural Basis of Actin Cytoskeleton Dynamics

R01 MH087950 (Dominguez R) **This Grant, No-cost extension** 6/1/16 – 5/31/22 3.0 CM
NIH/NIMI
BAR Proteins Linking Membrane and Cytoskeleton Dynamics

RM1 GM136511 (MPI: Ostap EM, Holzbaur E, Lakadamyali M, Dominguez R) 5/1/20 – 4/30/25 3.0 CM
NIH/NIGMS
Integrative Mechanisms of Organelle Dynamics from the Atomic-to-Cellular Level

Pending Research Support

R01 DK128282 (Heuckeroth RO) 3/1/22 – 2/2/827
NIH/NIDDK
Dominguez, R (Co-Investigator) 1.0 CM
Biochemical and cellular mechanisms linking actin mutations to visceral myopathy

R01 MH087950 (Dominguez, R) **Renewal** 6/1/21 – 5/31/26 3.0 CM
NIH/NIMI
BAR Domain Proteins Linking Membrane and Cytoskeleton Dynamics in Neuronal Cells

OVERLAP

There is no scientific overlap between the aims of the projects