

BIOGRAPHICAL SKETCH

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NAME Anna Moroni		POSITION TITLE Professor of Physiology	
eRA COMMONS USER NAME (credential, e.g., agency login)			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)</i>			
INSTITUTION AND LOCATION	DEGREE (if applicable)	MM/YY	FIELD OF STUDY
University of Milan	B.A.	09/1986	Plant physiology
University of Toronto		07/1988	Plant membrane transport
Columbia University, NYC		12/1989	Photobiology
University of Milan	Ph.D.	05/1992	Cellular and Molecular biology
University of Milan	Postdoctoral	12/1994	Plant membrane transport
University of Milan	Postdoctoral	12/1998	Cardiac electrophysiology

A. Personal Statement

We run on electricity, from the heart beat that ensures life to the sight that reads this sentence, each function of our organism is driven by tiny electrical currents flowing through ion channels. My interest is to describe how channel proteins are regulated and to find adequate therapeutical treatments for neurological and cardiac diseases. We combine structural and functional approaches to describe the of ligands, second messengers and signaling lipids control channel activity, and to discover druggable pockets to mimic or prevent it. In the cardiac pacemaker HCN4 channels, we have dissected the mechanism of gating by cAMP (Saponaro et al. PNAS 2014; Porro et al, ELife, 2019; Porro et al, Nature Comm. 2024) and identified a modulatory binding pocket for cyclic di-nucleotides (Lolicato et al., Nat Chem Biol, 2014). We have obtained the structure of HCN4 by single particle cryoEM with and without cAMP bound and in complex with the drug ivabradine (Saponaro et al, Mol. Cell 2021; Nature Comm, 2024). We have further isolated nanobodies that modify the properties of the channel and shown their potential use in pathologic bradycardia and epilepsy (Sharifzadeh et al., ms in preparation).

Ongoing and recently completed project**Current research grants-**

EU- ERC Synergy MORONI 2024-2029

NeuroSonoGene: A sonogenetic brain-machine interface for neurosciences and visual restoration-
Role: Partner (2.140.000 as PI of Milan Unit)

PRIN 2022 MORONI 2022-2025

Structure-based design of isoform-specific HCN blockers for treatment of cardiac and neuronal diseases –

Role: Coordinator (Capofila) (125.000€ as PI of Milan Unit)

Telethon research project GGP20021 MORONI 2021-2024

Molecular characterization of early infantile epileptic encephalopathy (EIEE) related HCN1 mutations: advancing therapeutics and treatment"

Role: PI (240.000,00€)

ERC-2020- Proof of Concept 966841 MORONI 2021-2023

TICK: Temperature-induced K⁺ channels

Role: PI (150.000,00€)

Recently Completed research grants-

ERC-2019- Proof of Concept 875129 MORONI 2020-2022

BREAK: Blue light remote analgesia with K⁺ channels

Role: PI (150.000,00€)

ERC-2015-AdG 6695078 MORONI 2016-2022

NoMAGIC: Noninvasive Manipulation of Gating in Ion Channels

Role: PI (2.400.000,00€)

Relevant publications for the proposed research

1. Saponaro A. et al., "Structural determinants of ivabradine block of the open pore of HCN4". **Proc Natl Acad Sci U S A**. 2024 Jul 2;121(27):e2402259121. doi: 10.1073/pnas.2402259121.
2. Saponaro A. et al., "Gating movements and ion permeation in HCN4 pacemaker channels". **Mol Cell**. 2021 81(14):2929-2943.e6. doi: 10.1016/j.molcel.2021.05.033.
3. Porro A, Saponaro A, Gasparri F, Bauer D, Gross C, Pisoni M, Abbandonato G, Hamacher K, Santoro B, Thiel G, Moroni A. The HCN domain couples voltage gating and cAMP response in hyperpolarization-activated cyclic nucleotide-gated channels. **Elife**. 2019 Nov 26;8:e49672
4. Porro A, Saponaro A, Castelli R, Introini B, Hafez Alkotob A, Ranjbari G, Enke U, Kusch J, Benndorf K, Santoro B, DiFrancesco D, Thiel G, Moroni A. A high affinity switch for cAMP in the HCN pacemaker channels. **Nature Commun**. 2024 Jan 29;15(1):843. doi: 10.1038/s41467-024-45136-y.
5. Lolicato M., Bucchi A., Arrigoni C., Zucca S., Nardini M., Schroeder I., Simmons K., Aquila M., DiFrancesco D., Bolognesi M., Schwede F., Kashin D., Fishwick C.W.G, Johnson A.P., Thiel G., Moroni A. (2014) Cyclic dinucleotides bind the C-linker of HCN4 to control channel cAMP responsiveness. **Nature Chem Biol**. 10(6):457-62.

B. Positions, Scientific appointments and honors

Positions and scientific appointments

Positions and Employment

1999-2005 Researcher (Plant physiology) Dept. of Biology, University of Milan, Italy
2006-2011 Associate Professor (Plant physiology), Dept. of Biology, University of Milan, Italy
2011-2023 Professor (Plant physiology), Dept. of Biosciences, University of Milan, Italy
2016-2017 Visiting Professor, Dept Physiol & Cell Biophys, Columbia University NYC, USA
From 2023 Professor (Physiology), Dept. of Biosciences, University of Milan, Italy

Commission of trust

2010-2015 Editorial Board Member, Journal of Biological Chemistry
2015- HHMI-Janelia Research Campus Graduate research review panel
2018- Editorial Board Member, Journal of General Physiology
2022 International Scientific Advisory Board, Evaluation of the National Institute of Chemistry
 Ljubljana
2023 ERC evaluation panel **LS1**, Consolidator Grant (ERC-2023-CoG)

Other Experience

2008-2012 Council member, Italian Society of Plant Physiology
2010-2016 Council member, It. Soc. of Pure and Applied Biophysics
2017-2022 Council member, Biophysical Society, USA
2022 Nomination Committee and Program Committee member, Biophysical
 Society, USA
2018-2021 National Scientific Qualification (ASN) committee member

Honors and Awards

2006 Baccarini Melandri Award from the Italian Society of Plant Physiology
2006 Invited News and Views: Nature Chem. Biol. 2 (11), 572-573, 2006
2013 Women in science, IUPAB, International Union for Pure and Applied Biophysics
2015 Voices of Chemical Biology, Nature Chem Biol, 10th Anniversary special issue
2016 Schaefer Award for excellence in human physiology research, Columbia University, NYC
2016 Recipient of an ERC Advanced grant
2018 Author file - Nature Methods <https://doi.org/10.1038/s41592-018-0192-y>
2020 Profiles in Biophysics, Biophysical society Bulletin, January 2020 newsletter
 (<https://www.biophysics.org/bps-bulletin/january-2020->)
2022 Elected Member of the Academia Europaea
2023 Elected Member of Istituto Lombardo
2023 Aspen Institute award for scientific collaboration between Italy and USA
2024 Recipient of an ERC Synergy Grant
2024 Premio nazionale " Milano cultura 2023 " Ada Burrone
2024 Elected EMBO Member

C. Contributions to Science

1. Viral K⁺ channels

Back in 2000, I contributed to discover a class of K⁺ channels in viruses and dedicated myself to understand the basic principle of operation of these miniature channels, an ideal model system to study the properties of the channel pore. I have identified a synergistic interaction between K⁺ channels and aquaporins that are co-expressed during viral infection and contributed to identify a targeting signal for mitochondria in viral K⁺ channels.

A. Plugge B, Gazzarrini S, Nelson M, Cerana R, Van Etten JL, Derst C, DiFrancesco D, **Moroni A**, Thiel G. (2000) A new potassium channel protein encoded by Chlorella virus PBCV-1. *Science*, Volume 287, 1641-1644.

B. Kang, M., **Moroni, A.**, Gazzarrini, S., DiFrancesco, D., Thiel, G., Severino, M., and Van Etten, J.L. (2004) Small potassium ion channel proteins encoded by chlorella viruses. *PNAS*, 101,15,5318-5324.

C. Gazzarrini, S., Kang, M., Epimashko, S., Van Etten, J.L., Dainty J., Thiel, G., **Moroni, A.** (2006) Chlorella Virus MT325 Encodes Water and Potassium Channels that Interact Synergistically. *Proc Natl Acad Sci U S A*, 103, 5355-5360.

D. Balss, J., Papatheodorou. P., Mehmel, M., Baumeister, D., Hertel, B., Delaroque, N., Chatelain, F.C., Minor, D.L. Jr, Van Etten, J.L., Rassow, J., **Moroni, A.**, Thiel, G. (2008) Transmembrane domain length of viral K⁺ channels is a signal for mitochondria targeting. *Proc Natl Acad Sci U S A*. 105(34):12313-8.

2. Structural properties of HCN channels

An ongoing research line in my laboratory is centered on the structural properties of HCN channels and on their regulatory mechanisms related to the cytosolic C-terminal portion of the channel including the CNBD. We have shown the different tetramerization behavior of HCN1, compared to HCN2 and HCN4. Moreover, we have discovered a binding pocket for cyclic-di-nucleotides in the C-linker of HCN4 and found a compound (BPU) that prevents cAMP regulation of the channel. To my knowledge, this is the first and, so far, the only subtype-specific compound that acts on HCN channels. Another interest of my lab has been the molecular explanation of the interaction between HCN CNBD and the regulatory subunit TRIP8b. This work has been done in collaboration with the lab of Steven Siegelbaum at Columbia U., in particular with Bina Santoro. By using NMR spectroscopy, we were able to solve the first apo structure of HCN CNBD and we mapped the interactions with TRIP8b. We have used this knowledge to identify a minimal binding peptide, TRIPnano, that prevents cAMP binding to all HCN isoforms (H1, H2 and H4) and constitutes a powerful tool for the control of the native current. If. More recently, we obtained, by cryoEM, the structure of HCN4 in the open and closed pore conformation and with the drug ivabradine bound.

A. Lolicato, M., Bucci, A., Arrigoni, C., Zucca, S., Nardini, M., Schroeder, I., Simmons, K., Aquila, M., DiFrancesco, D., Bolognesi, M., Schwede, F., Kashin, D., Fishwick, C.W.G, Johnson, A.P., Thiel, G., **Moroni, A.** (2014) Cyclic dinucleotides bind the C-linker of HCN4 to control channel cAMP responsiveness. *Nat Chem Biol*. 10(6):457-62.

B. Saponaro A, Pauleta SR, Cantini F, Matzapetakis M, Hammann C, Donadoni C, Hu L, Thiel G, Banci L, Santoro B, **Moroni A.** (2014) Structural basis for the mutual antagonism of cAMP and TRIP8b in regulating HCN channel function. *Proc Natl Acad Sci U S A* 111(40):14577-82.

C. Saponaro A, Bauer D, Giese MH, Swuoc P, Porro A, Gasparri F, Sharifzadeh AS, Chaves-Sanjuan A, Alberio L, Parisi G, Cerutti G, Clarke OB, Hamacher K, Colecraft HM, Mancina F, Hendrickson WA, Siegelbaum SA, DiFrancesco D, Bolognesi M, Thiel G, Santoro B, **Moroni A.** Gating movements and ion permeation in HCN4 pacemaker channels. *Mol Cell*. 2021 Jul 15;81(14):2929-2943.e6.

D. Saponaro A, Krumbach JH, Chaves-Sanjuan A, Sharifzadeh AS, Porro A, Castelli R, Hamacher K, Bolognesi M, DiFrancesco D, Clarke OB, Thiel G, **Moroni A.** Structural determinants of ivabradine block of the open pore of HCN4. *Proc Natl Acad Sci U S A*. 2024 Jul 2;121(27):e2402259121.

3. Synthetic channel engineering

In 2010 I decided to use our understanding of the gating mechanism of Kcv to engineer synthetic channels with new properties. Along this line, we created KvSynth1, a voltage-gated channel by fusing a voltage sensor protein of a phosphatase protein to the pore of Kcv; a ROS sensitive and a ROS insensitive Kcv channel; a calcium-calmodulin gated Kcv channel; and more recently a light-gated channel, BLINK1. During the sabbatical year at Columbia U. (2016-2017), in collaboration with Henry Colecraft we have developed a new version of this channel, BLINK2 with new kinetic properties allowing sustained (>30 min) inhibition of neuronal firing (Alberio et al., 2018).

A. Arrigoni C, Schroeder I, Romani G, Van Etten JL, Thiel G, **Moroni A.** (2013) The voltage-sensing domain of a phosphatase gates the pore of a potassium channel. *J Gen Physiol.* 141(3):389-95.

B. DiFrancesco ML, Gazzarrini S, Arrigoni C, Romani G, Thiel G, **Moroni A.** (2015) Engineering a Ca⁺⁺-sensitive (bio)sensor from the pore-module of a potassium channel. *Sensors (Basel)* 15(3):4913-24.

C. Cosentino C, Alberio L, Gazzarrini S, Aquila M, Romano E, Cermenati S, Zuccolini P, Petersen J, Beltrame M, Van Etten JL, Christie JM, Thiel G, **Moroni A.** (2015) Engineering of a light-gated potassium channel. *Science* 348(6235):707-10.

D. Alberio L, Locarno A, Saponaro A, Romano E, Bercier V, Albadri S, Simeoni F, Moleri S, Pelucchi S, Porro A, Marcello E, Barsotti N, Kukovetz K, Boender AJ, Contestabile A, Luo S, Moutal A, Ji Y, Romani G, Beltrame M, Del Bene F, Di Luca M, Khanna R, Colecraft HM, Pasqualetti M, Thiel G, Tonini R, **Moroni A.** (2018) A light-gated potassium channel for sustained neuronal inhibition. *Nat Methods.* 15(11):969-976.

A complete list of my publications (comprising about 130 papers in peer-reviewed journals) can be found at GoogleScholar:

<https://scholar.google.it/citations?user=PK42kwgAAAAJ&hl=it>