CV of Mario Milani De Mayo De Mari

WORK

From February 2012: I am the ***PI of the structure-based drug discovery group*** at CNR – Biophysics Institute (Milano session).
From January 2021: I am senior researcher at CNR.
From February 2009: Researcher at CNR – Biophysics Institute – Milano section.
April 2005 - February 2009: Temporary position as researcher at CNR c/o University of Milano, Dep. of Biomolecular Science and Biotechnology.
March 2003 - May 2003: I have been working in the microbiology group of Prof. Paolo Visca in Spallanzani Hospital, Rome.
July 1999 - April 2005: PhD and postdoc research experiences in the protein crystallography group of Prof. Martino Bolognesi (Advanced Biotechnology Center, Genoa).
June 1998 - December 1998: INFM (National Institute of Physics of Matter) fellowship, University "Roma Tre".
October 1996 - May 1998: undergraduate research experience in experimental surface physics with Prof. G. Stefani, "Roma 3" University, Rome.

TEACHING

September 2021 ***Honorary Associate Professor of Biophysics*** (Division of Medicine, University College London)
September 2006 - October 2014: ***Adj. Professor of Biophysics*** (Dept. of Physics, University of Milan).
September 2003 - September 2005: ***Adj. Professor of Biochemical Physics*** (Dep. of Physics, University of Genoa, Italy).

EDUCATION AND TRAINING

February 2002: PhD in Physics at Genoa University. The title of PhD Thesis: "X-ray diffraction and molecular dynamics studies on truncated hemoglobin-N from Mycobacterium tuberculosis". Tutors: [redacted].
May 1998: Degree in Physics, University "La Sapienza", Rome, Italy. Studies mainly oriented to atomic, molecular and solid-state physics. Final mark 110/110. Title of the Experimental Thesis: "On the scattering mechanism of electrons interacting with surfaces in specular reflection geometry". Tutor: [redacted].

RESEARCH SECTOR

I am an expert in ***protein structure and in structure-function-inhibition relationship***. During my graduate studies I acquired a strong background in matter physics and in experimental techniques involving electron-surface interactions. Starting from my PhD I gained extensive competences in protein expression and purification and in structural biology (X-ray crystallography, SAXS and CryoEM), biophysics and biochemistry, mainly focused on structural and dynamical determinants of protein activity. Finally, I have a wide experience in structure-based drug identification, design and optimization aided by *in silico* docking and molecular dynamics simulations.

SCIENTIFIC INTERESTS

After the initial experiences in matter physics and electron-surface interaction (Ruocco

et al., 1999), my early research was focused on the structure of a new class of bacterial proteins called truncated hemoglobins (TrHb). TrHbs displays a short version of the classical globin fold and inside their structural core the heme prostetic group is surrounded by atypical amino acids and it is linked to the protein external surface by a peculiar system of cavities. Beyond X-ray crystallography I have used molecular dynamics simulations to check the permeability and plasticity of such cavities in the presence of small ligands (main publications: Milani et al., 2001; Milani et al., 2003; Milani et al., 2004).

The interest for bacterial proteins proceeded with the study of a two-component bacterial system in a quasi-active conformation (Milani et al., 2005) and, more recently, with the study of essential bacterial proteins to find new antibiotics (Uruburu et al., 2019).

I am involved in the study of the structure of several enzymes from RNA viruses (mostly flaviviruses) to better understand the activity of essential viral proteins like methyltransferase (Milani et al., 2009), helicase (Mastrangelo et al., 2007) and polymerase (Milani et al., 2009; Mastrangelo et al., & Milani, 2012). The structures of such proteins often provided useful information for the identification of potential binding sites for inhibitors (in silico docking), characterized by binding experiments, point mutations and co-crystallization experiments. Among other powerful inhibitors, I was able to identify the drug ivermectin (currently in clinical use as antiparasitic drug) as a potent inhibitor of flavivirus helicases (Mastrangelo et al., & Milani, 2012). The new ivermectin function as antiviral drug is now investigated in many different clinical trials.

My interest in protein with prosthetic groups was addressed to different flavoproteins involved in human diseases (Milani et al., 2007; Milani et al., 2011).

Another broad research interest regards proteins regulating cell apoptosis and in particular I participated to the structure-based optimization of small molecules (called SMAC mimetics) that are being developed as anticancer compounds (Cossu et al., 2009; Mastrangelo et al., & Milani 2015; Cossu et al., 2019).

I am currently involved in the study of different calcium binding proteins responsible of human genetic diseases like gelsolin amyloidosis and cone dystrophy (Boni et al., 2016; Giorgino et al., 2019: Boni et al., 2020). I am also involved in the study of human proteins related to different diseases like stroke (protein ASIC1a, Gornati et al., submitted) and ALS (retromer complex; Muzio et al., 2020). Even in this case understanding the protein structure and dynamics can help in unraveling the molecular basis of the diseases and to find new therapeutical options. Finally, during 2020 I started the *in silico* study of different protein from CoV2 arriving at the identification of new promising inhibitors (https://doi.org/10.1101/2020.11.12.379958).

SELECTED CONFERENCES

2007 August, invited speaker ECM-24, Marrakech. "Structure of flaviviral enzymes: helicase and methyltransferase".

2008 November, invited speaker at 7th ICAV, Beijing. Cina. "Structural based inhibition of flavivirus replication enzymes: Helicase and Methyltransferase".

2010 May, Lecture at SIAS Symposium, Hamburg.

2011 March, 55th Biophysical Society Meeting. Poster "Flaviviral helicases: structure, function, inhibition and dynamics".

2011, May, 24th ICAR Sofia, Poster title "Targeting the flavivirus helicase" (Award for the best poster in the young investigator section)

2011, Biophysical Society Meeting, Baltimore: "Flaviviral helicases: structure, function, inhibition and dynamics".

2012 Biophysical Society Meeting, San Diego: "Structure based inhibition of the calicivirus

RdRp"
2012 25th ICAR- Sapporo, Japan, "Structure-based inhibition of norovirus RNA-dependent RNA-polymerases".
2013 26th ICAR-San Francisco, "Structure of norovirus RNA-dependent RNA-polymerase in complex with three naphthalene derivates inhibitors".
2015, 28th ICAR-Rome, "Targeting the flavivirus polymerase: a new class of non-nucleoside inhibitors mimicking the stacking interaction of two RNA bases".
2016, 29th ICAR- San Diego, "Targeting RNA dependent RNA polymerase: the discovery of a new class of potent compounds against flaviviruses".
2017, Drug Discovery and Therapy World Congress 2017, Boston, invited speaker: "The difficult path toward the knowledge: an ongoing story on new class of Dengue virus inhibitors".
2020 The 18th Asian Chemical Congress, invited speaker: "IAPs Inhibition in Cancer Therapy".

MAIN AWARDED RESEARCH GRANTS
2012-2013 Grant from 60° Pharmaceuticals, LLC "Ivermectin-mediated dengue virus inhibition". Role: Principal Investigator.
2013-2016 Italian Projects PRIN: NOXSS (X-ray Single Shots of Nano Objects): structural determination of nano objects using the X-ray pulses from the European Free Electron Laser sources. Role: Partner Coordinator.
2016-2021 grant Telethon: Cone dystrophies and retinal degeneration from protein structures to biological networks. Toward the design of therapeutic molecules. Role: Partner Coordinator.
2018-2020 grant Instruct (https://www.structuralbiology.eu/) "Structural analysis of the interaction between retinal guanylyl cyclase 1 (GC1) and guanylate cyclase activating protein 1 (GCAP1)". Role Principal Investigator.
2020-2023 grant ARISLA. Project TRAILER: Therapeutic effects of retromer stabilization in Amyotrophic Lateral Sclerosis. Role: Partner Coordinator.

Editorial activity
Associated Editor for Frontiers in Molecular Biosciences. Associated editor for the International Journal of Molecular Sciences. Editorial board membership: Antiviral Chemistry and Chemotherapy; Journal of Antimicrobial Agents and Chemotherapy.

PATENTS
2007, C. Scolastico, L. Manzoni, P. Seneci, L. Belvisi, D. Delia, M. Bolognesi, E. Mastrangelo, M. Milani, I. Motto, C. Drago (2007). EPO 7021843. "New SMAC mimetic compounds as apoptosis inducers".
2011, Milani, Mastrangelo, Bolognesi et al. "Avermectins and milbemycins for the treatment of flavivirus infections" (WO2011051159 (A1)).

PAPERS
From 1999 to 2021, I am coauthor of more than 90 scientific papers in peer-reviewed international journals. Total citations (google scholar): 3558, H index (google scholar): 34 (https://scholar.google.it/citations?user=dzPqpWIAAAAJ&hl=en&oi=ao).
SCOPUS author Id 35237674300; H-index: 31
ORCID ID 0000-0001-6098-3991
Web of Science ResearcherID B-6446-2015; H-index: 30

SELECTED PAPERS

“Combined in silico and in vitro approaches identified the antipsychotic drug lurasidone and the antiviral drug elbasvir as SARS-CoV2 and HCoV-OC43 inhibitors” **Mario Milani**, Manuela Donalisio, et al., Eloise Mastrangelo. Antiviral research 189, 105055

"Retromer stabilization results in neuroprotection in a model of Amyotrophic Lateral Sclerosis" Luca Muzio, Riccardo Sirtori, Davide Gornati, et al., **Mario Milani**, Pierfausto Seneci, Gianvito Martino. Nature communications 11 (1), 1-17 (2020).

“Targeting the BIR domains of inhibitor of apoptosis (IAP) proteins in cancer treatment”. F Cossu, **M Milani**, E Mastrangelo, D Lecis Computational and structural biotechnology journal 17, 142-150 (2019).

“Glycine Amidinotransferase (GATM), renal Fanconi syndrome, and kidney failure”. M Reichold, ED Klootwijk, J Reinders, EA Otto, **M Milani**, C Broeker, et al., Journal of the American Society of Nephrology 29 (7), 1849-1858 (2018).

"Targeting flavivirus RNA dependent RNA polymerase through a pyridobenzothiazole inhibitor" Delia Tarantino, Rolando Cannalire, Eloise Mastrangelo, Romina Croci, Gilles Querat, Maria Letizia Barreca, Martino Bolognesi, Giuseppe Manfroni, Violetta Cecchetti, **Mario Milani** Antiviral research 134, 226-235 (2016).

"Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug". E Mastrangelo, M Pezzullo, T De Burghgraeve, S Kaptein, B Pastorino, et al., & **Milani M.** Journal of Antimicrobial Chemotherapy 67 (8), 1884-1894, 247 (2012).

"Structure-based inhibition of Norovirus RNA-dependent RNA polymerases" E Mastrangelo, M Pezzullo, D Tarantino, R Petazzi, F Germani, D Kramer, et al., & **M Milani**. Journal of molecular biology 419 (3-4), 198-210 (2012).

"FAD-binding site and NADP reactivity in human renalase: a new enzyme involved in blood pressure regulation". **M Milani**, F Ciriello, S Baroni, V Pandini, G Canevari, M Bolognesi, et al., Journal of molecular biology 411 (2), 463-473 (2011).

“Flaviviral methyltransferase/RNA interaction: structural basis for enzyme inhibition”. **M Milani**, E Mastrangelo, M Bollati, et al., Antiviral research 83 (1), 28-34 (2009).

“An active-like structure in the unphosphorylated StyR response regulator suggests a phosphorylation-dependent allosteric activation mechanism” **M Milani**, L Leoni, G Rampioni, E Zennaro, P Ascenzi, M Bolognesi. Structure 13 (9), 1289-1297 (2005).

"Mycobacterium tuberculosis hemoglobin N displays a protein tunnel suited for O2 diffusion to the heme". **M Milani**, A Pesce, Y Ouellet, P Ascenzi, M Guertin, M Bolognesi. The EMBO journal 20 (15), 3902-3909 (2001).