
BIOGRAPHICAL SKETCH

NAME: Andrea Ilari

POSITION TITLE: Director of Research at the IBPM CNR

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Roma "La Sapienza"	Master degree in Chemistry	12/1993	Chemistry
University of Roma "La Sapienza"	PhD in Biophysics	05/1998	Biophysics
Dep. of Genetics and Microbiology ,University of Pavia		12/1998	Structural Biology
"Centre National de la Recherche scientifique"- Laboratoire de Cristallographie et Cristallisation des Macromolécules Biologiques		12/1999	Structural Biology
Research training in "Crystallization of hemoproteins in presence and absence of oxygen", Prof. W. E. Royer, Medical Center, University of Massachusetts, Worcester, USA		07/1997	Structural Biology

A. Personal Statement

I am a Director of Research at the Institute of Molecular Biology and Pathology (IBPM) of CNR. I have contributed to the scientific output of IBPM with over 100 publications in biochemistry and structural biology (110 documents; H-index 41; 5118 citations; Source: SCOPUS). I have been responsible for 18 scientific projects and have participated in 14 additional projects. I collaborate with researchers from both industrial entities, such as IRBM Science Park, and prestigious Italian and international scientific institutions, including various Departments of Sapienza University, the Istituto Superiore di Sanità (ISS), the University of Massachusetts Medical Center, and the Department of Genetics and Microbiology at the University of Pavia. I serve as a reviewer for numerous international scientific journals and recently became Editor-in-Chief of the section "Theoretical Modeling, Structure, Prediction and Design" of *Frontiers in Chemical Biology*. I have supervised multiple master's and doctoral students and have taught various courses in Medicine and Dentistry at Sapienza University and in Advanced Biotechnology at the University of Teramo. I am also a member of the teaching staff for the PhD in Biochemistry at Sapienza University. I possess extensive expertise in structural biology and biochemistry and currently lead the Biocrystal Facility, which focuses on protein crystallization, protein-protein interactions, and ligand binding studies. The facility, empowered by the PNRR ITACA.SB project, will be essential for the success of the SIGMA-HD project. It is equipped with cutting-edge instrumentation, including a crystallization robot ORYX8 with an LCP module for S1R crystallization, a Cryo-EM cluster node for processing Cryo-EM data and solving S1R cryo-EM structures, a Mass Photometer to study the polymerization/depolymerization of S1R

and its complex formation with BiP, and a Surface Plasmon Resonance apparatus (OCTET-SF3) to measure the interaction of S1R with ligands.

In recent years, I have focused on two main research areas: Structure-Based Drug Design and Structure-Function relationships:

1. Different approaches to targeting Trypanothione Reductase to discover new broad-spectrum trypanocidal drugs.
2. The structure-function relationship of the Sigma-1 Receptor (S1R).

In the first research area, I solved the X-ray structures of Leishmania enzymes involved in trypanothione metabolism and used structure-based drug design to develop lead compounds for new drugs against Leishmaniasis. Recently, I was funded to design PROTACs targeting trypanothione reductase, a key enzyme in Leishmania's redox metabolism, through the MUR-funded project FISIR2019_03796 PROLEISH.

Regarding the second area, I collaborated with the C.S.S. Mendel Institute and Hospital "Casa Sollievo della Sofferenza" on the effect of S1R agonists on neuroprotection in Huntington's Disease models. This project was financed by the Ministry of Health (RF 2016-02364123; RARE in rarity: "Advanced in vivo and in vitro technologies to study Juvenile Huntington's Disease neuronal connectivity and its relationship with clinical and genetic factors"). Thanks to this research, I discovered an efficient S1R agonist: Iloperidone.

I am also involved as a representative of LIRH (Lega Italiana Ricerca Huntington) in the Huntington's Disease Coalition for Patient Engagement (HD-COPE), a globally recognized program organized by leading HD patient advocacy organizations. This initiative gives families affected by Huntington's disease a direct voice in HD clinical research.

B. Positions, Scientific Appointments, and Honors

1: Positions

From 2023 – Director of Research at the Institute of Molecular Biology and Pathology of the Italian National Research Council (IBPM-CNR), Rome Italy

2007 – 2023 First researcher (senior scientist) at the Institute of Molecular Biology and Pathology of the Italian National Research Council (IBPM-CNR), Rome Italy

2001-2007 Researcher at the IBPM-CNR

2: Fellowships

1999-2001 Research fellowship: "Crystallization of macromolecules in microgravity", at the "Istituto di Biologia e Patologia Molecolari", Centro Nazionale delle Ricerche (Italian National Research Council) in collaboration with the ASI (Italian Space Agency).

1999 Research fellowship : "Structural dynamics of nitrite reductase". In collaboration with the "Centre National de la Recherche scientifique"- Laboratoire de Cristallographie et Cristallisation des Macromolécules Biologiques – Marseille (France).

1998 Post-doctoral fellowship: "Research on resistance-related proteins of Mycobacteria", Dep. of Genetics and Microbiology ,University of Pavia, Italy

3: Institutional responsibilities

From 2024 . member of the the Scientific Council of the Department of Biomedical Sciences of CNR

From 2020 – Scientific Advisor of the Biological Macromolecules Section of the AIC (Italian Crystallography Association)

From 2015 – Member of the Commission appointed on 16 March 2015 with provision number 17, for the participation of the CNR in the IUCr (International Union of Crystallography).

2015 - Member of the scientific committee of LIRH (Lega Italiana Ricerca Huntington and related diseases)

2015 – 2020 Member of the Institute Council of the Institute of Molecular Biology and Pathology of the CNR.

From 2015 Principal Investigator of Biocrystal facility (<https://biocrystalfacility.it/it/facility-di-biocrystallografia/>)

From 2014 Member of the committee of Ph.D In biochemistry, Dep. Of Biochemical Sciences, Sapienza University of Rome

4: Editorial Board membership

From 2023 - Specialty Chief Editor of Theoretical Modeling, Structure Prediction & Design (specialty section of Frontiers in Chemical Biology)

5: Teaching activities

2021-2026 Lecturer of the course STRUCTURES AND INTERACTIONS BETWEEN BIOMOLECULES of the integrated course STRUCTURAL BIOLOGY FOR DRUG DESIGN of the COURSE OF STUDY IN ADVANCED BIOTECHNOLOGIES - LM-9, Faculty of Biosciences, University of Teramo

C. Contributions to Science

1: Studies on structure-function relationships in cage-like proteins involved in mechanism of defence from oxidative damage

Since the early years of my scientific career I have been concerned with defence mechanisms against oxidative damage. In particular, in 2000 I began studying a class of proteins produced by bacteria in starvation conditions that protect DNA from oxidative damage: Dps (DNA binding proteins from starved cells). I solved the X-ray structure of the Dps of *Listeria innocua* in 2000, the work reporting the structural analysis of the Dps of *Listeria innocua* (1) has aroused considerable interest in the scientific community and has been cited for this 220 times, as it is a paradigmatic example of how the structure allows us to understand the protein function. In particular, the structure of the protein made it possible to discover that Dps are able to oxidize and incorporate iron into the internal cavity and the oxidation of iron occurs thanks to a ferroxidase center present at the interface of two subunits. These studies were financed by the CNR with the Young Researchers project (2000-2002). Subsequent work on the Dps of other bacteria has made it possible to understand that ferroxidase activity is one of the mechanisms protecting DNA from oxidative damage, since through this activity the hydrogen peroxide produced during oxidative metabolism is consumed (2). Structural studies have made it possible to understand that the protein is made up of 12 identical subunits assembled with 23 symmetry to form a hollow sphere in which iron (up to 500 ions) is stored in microcrystalline form (3). Dps is therefore capable of forming ferric oxyhydroxide nanoparticles and this property is very important from a biotechnological point of view. Both Dps and Ferritins (cage-like proteins homologous to Dps but which form hollow spheres made up of 24 subunits) are capable of synthesizing nanoparticles of other metals. The mechanism by which ferritins synthesize silver nanoparticles was described by my studies on the ferritin of *Pyrococcus furiosus*, published in JACS (4) and (cited 100 times).

References

1. Ilari, A., Stefanini, S., Tsernoglou, D., and Chiancone E., "The dodecameric ferritin from *L. innocua* contains a novel intersubunit iron-binding site", *Nature Struct. Biol.*, 7, 38-43, (2000).
2. Ceci P., Ilari A., Falvo E., Chiancone E., "The Dps protein of *Agrobacterium tumefaciens* does not bind to DNA but protects it toward oxidative cleavage: X-ray crystal structure, iron binding, and hydroxyl-radical scavenging properties." *J. Biol. Chem.*, 278, 20319-26, (2003).
3. Ilari A., Ceci P., Ferrari D., Rossi G.L., Chiancone E., "Iron incorporation into *Escherichia coli* Dps gives rise to a ferritin-like microcrystalline core" *J. Biol. Chem.*, 277, 37619-23, (2003).
4. Kasyutich O., Ilari A., Fiorillo A., Tatchev D., Hoell A., Ceci P. Silver ion incorporation and nanoparticle formation inside the cavity of *Pyrococcus furiosus* ferritin: structural and size-distribution analyses. *J. Am. Chem. Soc.*, 2010, 132(10):3621-7.

2: Sorcin: a protein involved in calcium mediated signal transduction and multidrug resistance

Another topic I have dealt with is calcium-mediated signal transduction. In particular, since the first years of my activity I have been dealing with the structure-activity relationship in Sorcin (Soluble Resistance related Calcium binding protein), a protein that is overexpressed in various tumor lines resistant to chemotherapy and which is involved both in calcium-mediated signal transduction than in "multidrug resistance". In 2002 I solved the high resolution structure of the SCBD (Sorcin Calcium Binding Domain) (5) which demonstrated that Sorcin belongs to the PEF family (Penta-EF Hands calcium binding proteins) and that the calcium binding with determines a conformational variation that exposes hydrophobic surfaces which make it capable of interacting with its molecular partners. Subsequent studies in which I participated have highlighted that sorcin is able to interact with a series of calcium channels: the ryanodine receptor (RYR2), the sodium calcium exchanger (NCX1), the voltage-dependent calcium channel (LTCC), and the sarcoplasmic and endoplasmic calcium ATPase (SERCA), thus regulating calcium homeostasis. Thanks to the resolution of the structures of calcium-bound and apo human sorcin, we have discovered the molecular mechanism by which the conformational variation induced by calcium promotes the interaction with its molecular partners (6). The importance of Sorcin in preventing stress of the endoplasmic reticulum and regulating the flow of calcium between ER and mitochondria through the MAM (mitochondria-associated membrane) is of fundamental importance, even in neurodegenerative diseases such as HD, PD, AD. In fact, in cellular models of these diseases sorcin is overexpressed to counteract metabolic

imbalances caused by endoplasmic reticulum stress as I demonstrated together with my collaborators (7). Finally, I contributed to demonstrating with my research that sorcin is overexpressed in many types of cancer and is significantly upregulated in cell lines resistant to different chemotherapeutics (MultiDrug Resistant). Thanks to my contribution it was discovered that sorcin is able to bind doxorubicin and other chemotherapeutics and is therefore expressed in large quantities in MDR cancer cells (8).

References

5. Ilari A., Johnson K. A., Nastopoulos V., Verzili D., Zamparelli C., Colotti G., Tsernoglou D. and Chiancone E., "The crystal structure of the sorcin calcium binding domain provides a model of Ca²⁺-dependent processes in the full-length protein", *J. Mol. Biol.*, 317, 447-458, (2002).
6. Ilari A, Fiorillo A, Poser E, Lalioti VS, Sundell GN, Ivarsson Y, Genovese I, Colotti G. Structural basis of Sorcin-mediated calcium-dependent signal transduction. *Sci Rep.* 2015 Nov 18;5:16828. doi: 10.1038/srep16828.
7. Genovese I, Giamogante F, Barazzuol L, Battista T, Fiorillo A, Vicario M, D'Alessandro G, Cipriani R, Limatola C, Rossi D, Sorrentino V, Poser E, Mosca L, Squitieri F, Perluigi M, Arena A, van Petegem F, Tito C, Fazi F, Giorgi C, Cali T, Ilari A, Colotti G. Sorcin is an early marker of neurodegeneration, Ca²⁺ dysregulation and endoplasmic reticulum stress associated to neurodegenerative diseases. *Cell Death Dis.* 2020 Oct 15;11(10):861.
8. Genovese I, Fiorillo A, Ilari A, Masciarelli S, Fazi F, Colotti G. Binding of doxorubicin to Sorcin impairs cell death and increases drug resistance in cancer cells. *Cell Death Dis.* 2017 Jul 20;8(7):e2950.

3: Targeting the trypanothione metabolism enzymes good target to find new drugs against Leishmaniasis

Since 2008 I have become interested in Leishmaniasis, a disease transmitted by sandflies which affects populations in tropical and subtropical areas in America, Asia and Africa but is also spreading in southern Europe. It is a disease that if left untreated can lead to death and in fact kills 60,000 people a year. Leishmaniasis is a neglected disease because it affects the poorest part of the world's population.

Leishmania enters the host's blood as a promastigote following the bite of the sandfly and is phagocytosed by macrophages where it differentiates into an amastigote and multiplies. The reactive oxygen species produced by macrophages are neutralized by the parasite thanks to trypanothione, a molecule present in large quantities in the protozoan. The trypanothione metabolism enzymes are therefore essential enzymes for the survival of Leishmania and trypanosomatids in general and, being absent in humans, they are good targets for finding new "lead compounds" against leishmaniasis. The first contribution I made in this field of research concerned the solution of the crystal structure of Trypanothione Reductase (TR) and the other enzymes of the trypanothione metabolism (9,10). At the same time, I studied the mechanism of action of antimonials, the most used drugs against this disease, and I discovered that antimony (III), the active ingredient, is able to bind to two catalytic cysteines of TR, thus blocking the reduction of trypanothione and therefore its activation (10). In the following years I then concentrated on "Structure-Based Drug design" (SBDD) and thanks to the collaboration with various groups of pharmaceutical chemists from public and private institutions, I contributed to the design of inhibitors capable of inhibiting TR at a submicromolar concentration and therefore killing the parasite without being toxic to humans (11, 12).

References

9. Fiorillo A, Colotti G, Boffi A, Baiocco P, Ilari A. The crystal structures of the trypanothione reductase couple unveil the structural determinants of leishmania detoxification pathway. *PLoS Negl Trop Dis.* 2012, 6(8):e1781
10. Baiocco P, Colotti G, Franceschini S, Ilari A. "Molecular basis of antimony treatment in leishmaniasis." *J Med Chem.*, 2009; 52(8):2603-12
11. Ilari A, Genovese I, Fiorillo F, Battista T, De Ionna I, Fiorillo A, Colotti G. Toward a Drug Against All Kinetoplastids: From LeishBox to Specific and Potent Trypanothione Reductase Inhibitors. *Mol Pharm.* 2018 Aug 6;15(8):3069-3078. doi: 10.1021/acs.molpharmaceut.8b00185.
12. Turcano L, Torrente E, Missineo A, Andreini M, Gramiccia M, Di Muccio T, Genovese I, Fiorillo A, Harper S, Bresciani A, Colotti G, Ilari A. Identification and binding mode of a novel Leishmania Trypanothione reductase inhibitor from high throughput screening. *PLoS Negl Trop Dis.* 2018 Nov 26;12(11):e0006969
13. Exertier C, Salerno A, Antonelli L, Fiorillo A, Ocello R, Seghetti F, Caciolla J, Uliassi E, Masetti M, Fiorentino E, Orsini S, Di Muccio T, Ilari A, Bolognesi ML. Fragment Merging, Growing, and Linking Identify New Trypanothione Reductase Inhibitors for Leishmaniasis. *J Med Chem.* 2024 Jan 11;67(1):402-419.

4: Structure-function relationship in proteins involved in molecular mechanism linked to Huntington Disease.

Lately I have begun to deal with the molecular mechanisms linked to Huntington's disease (HD). Huntington's disease is a rare autosomal dominant genetic disease caused by the mutation of the Huntingtin gene where an expansion of the poly-CAG tract (which corresponds to a polyglutamine tract) occurs. The mutated protein (mHtt) (which contains a stretch of polyglutamines greater than 35 units) causes imbalances in energy metabolism and calcium metabolism in neuronal cells. The toxicity of mHtt is increased by the sumoylation process which makes the protein soluble and makes it capable of migrating into the nucleus and therefore binding to different targets, thus altering cellular metabolism. Sumoylation is catalyzed by the Rhes protein (Ras Homolog Enriched in Striatum). Thanks to the studies conducted by me and my collaborators it was possible to create a model of Rhes (Ras Homolog Enriched in Striatum) and hypothesize the mHtt sumoylation mechanism which could be useful for the design of peptides that inhibit this process (14). Another interesting protein for its involvement in

HD and other neurodegenerative diseases is the sigma1 receptor, an endoplasmic receptor involved in repair mechanisms (Unfolding Protein Response), in the regulation of autophagy and in the regulation of calcium flows from the endoplasmic reticulum to the mitochondria. Our studies funded by the Ministry of Health (finalized research 2016, RF 2016-02364123) have contributed to revealing the working mechanisms of S1R. We found that S1R interacts with Sorcin and this may be one of the mechanisms by which the protein controls calcium fluxes from the ER to the mitochondrion via the MAMs (7). Since the activation of S1R has as its ultimate consequence the protection of the neuron from damage caused by protein aggregation, my studies have focused on the search for new S1R agonists through SBDD. In particular, applying the concept of "Drug repurposing" we carried out the VS of the "FDA-approved drugs" on the structure of S1R by selecting some molecules which through cell biology assays we have demonstrated to be receptor agonists (15). Among them the most promising selected S1R agonist appear to be loperidone an atypical antipsychotics. Finally, our studies contributed at identifying the compounds with steroid scaffold as preferred ligands for S1R and at discovering how the ligands may reach the binding site i.e through the lipidic membrane. (16).

References

14. Carbo M, Brandi V, Pascarella G, Staid DS, Colotti G, Polticelli F, Ilari A, Morea V. Bioinformatics analysis of Ras homologue enriched in the striatum, a potential target for Huntington's disease therapy. *Int J Mol Med*. 2019 Dec;44(6):2223-2233.
15. Pascarella G, Antonelli L, Narzi D, Battista T, Fiorillo A, Colotti G, Guidoni L, Morea V, Ilari A. Investigation of the Entry Pathway and Molecular Nature of $\sigma 1$ Receptor Ligands. *Int J Mol Sci*. 2023 Mar 28;24(7):6367.
16. Pascarella G, Antonelli L, Narzi D, Battista T, Fiorillo A, Colotti G, Guidoni L, Morea V, Ilari A. Investigation of the Entry Pathway and Molecular Nature of $\sigma 1$ Receptor Ligands. *Int J Mol Sci*. 2023 Mar 28;24(7):6367.

5. 10 Relevant publications

1. # Exertier C, Salerno A, Antonelli L, Fiorillo A, Ocello R, Seghetti F, Caciolla J, Uliassi E, Masetti M, Fiorentino E, Orsini S, Di Muccio T, Ilari A, Bolognesi ML. Fragment Merging, Growing, and Linking Identify New Trypanothione Reductase Inhibitors for Leishmaniasis. *J Med Chem*. 2024 Jan 11;67(1):402-419
2. Pascarella G, Antonelli L, Narzi D, Battista T, Fiorillo A, Colotti G, Guidoni L, Morea V, Ilari A. Investigation of the Entry Pathway and Molecular Nature of $\sigma 1$ Receptor Ligands. *Int J Mol Sci*. 2023 Mar 28;24(7):6367.
3. # Battista T, Pascarella G, Staid DS, Colotti G, Rosati J, Fiorillo A, Casamassa A, Vescovi AL, Giabbai B, Semrau MS, Fanelli S, Storici P, Squitieri F, Morea V, Ilari A. Known Drugs Identified by Structure-Based Virtual Screening Are Able to Bind Sigma-1 Receptor and Increase Growth of Huntington Disease Patient-Derived Cells. *Int J Mol Sci*. 2021 Jan 28;22(3):1293
4. #Genovese I, Giamogante F, Barazzuol L, Battista T, Fiorillo A, Vicario M, D'Alessandro G, Cipriani R, Limatola C, Rossi D, Sorrentino V, Poser E, Mosca L, Squitieri F, Perluigi M, Arena A, van Petegem F, Tito C, Fazi F, Giorgi C, Calì T, Ilari A, Colotti G. Sorcin is an early marker of neurodegeneration, Ca^{2+} dysregulation and endoplasmic reticulum stress associated to neurodegenerative diseases. *Cell Death Dis*. 2020 Oct 15;11(10):861
5. # Ilari A, Fiorillo A, Poser E, Lalioti VS, Sundell GN, Ivarsson Y, Genovese I, Colotti G. Structural basis of Sorcin-mediated calcium-dependent signal transduction. *Sci Rep*. 2015 Nov 18;5:16828. doi: 10.1038/srep16828
6. # Fiorillo A, Colotti G, Boffi A, Baiocco P, Ilari A. The crystal structures of the trypanothione-trypanothione peroxidase couple unveil the structural determinants of leishmania detoxification pathway. *PLoS Negl Trop Dis*. 2012, 6(8):e1781
7. # Kasyutich O, Ilari A, Fiorillo A, Tatchev D, Hoell A, Ceci P. Silver ion incorporation and nanoparticle formation inside the cavity of Pyrococcus furiosus ferritin: structural and size-distribution analyses. *J. Am. Chem. Soc.*, 2010, 132(10):3621-7.
8. # Baiocco P, Colotti G, Franceschini S, Ilari A. "Molecular basis of antimony treatment in leishmaniasis." *J Med Chem.*, 2009; 52(8):2603-12
9. Ilari A., Johnson K. A., Nastopoulos V., Verzili D., Zamparelli C., Colotti G., Tsernoglou D. and Chiancone E., "The crystal structure of the sorcin calcium binding domain provides a model of Ca^{2+} -dependent processes in the full-length protein", *J. Mol. Biol.*, 317, 447-458, (2002).
10. Ilari, A., Stefanini, S., Tsernoglou, D., and Chiancone E., "The dodecameric ferritin from *L. innocua* contains a novel intersubunit iron-binding site", *Nature Struct. Biol.*, 7, 38-43, (2000).

A. Ilari is corresponding author