

BIOSKETCH

NAME: Maria Carmela Bonaccorsi di Patti

POSITION TITLE: Associate Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Rome La Sapienza	Laurea in Scienze Biologiche	11.1989	Biological sciences
University of Rome La Sapienza	PhD in Biochemistry	10.1993	Biochemistry of ceruloplasmin

A. Personal Statement

I am a biochemist with a strong interest in the field of iron biology. During my career I have gained in-depth knowledge of CP and its peculiarities (CP was the subject of both my degree thesis and my PhD thesis about 30 years ago) and of how to handle such peculiarities. I have gradually expanded my expertise from classical biochemistry techniques of protein purification and analysis to application of recombinant DNA techniques for mutagenesis and heterologous expression of proteins, up to more advanced biophysical approaches for the study of protein structure and function. My field of work has widened from the structural and functional analysis of multicopper ferroxidases to iron permeases in humans and yeast.

Collaborations with internationally recognized experts on CP and FPN are firmly established and stretch back to over 15 years ago, leading to the finding that ferroxidase activity is required for the stability of the iron exporter FPN at the cell surface, providing an explanation for the brain iron overload phenotype of aceruloplasminemia patients. A large set of aceruloplasminemia missense CP mutants was characterized and a dominant mutant was identified that impaired the cellular copper loading machinery.

The expertise and the internationally recognized competence on CP and FPN are evident from my track record on these subjects and from the list of relevant publications.

Since 2000 I have been supervisor of over 30 students (undergraduate and PhD) for their degree thesis work and I have taught biochemistry-related courses to Biology students since 1998.

B. Positions, Scientific Appointments, and Honors

2020-present: associate professor, Dept. Biochemical Sciences University of Rome La Sapienza

1993-2020: researcher, Dept. Biochemical Sciences University of Rome La Sapienza

2017-present: member of the International Society for the study of iron in biology and medicine (BioIron)

2017-present: member of the Association of Resources for Biophysical Research in Europe (ARBRE)

C. Contributions to Science

Starting from the beginning of my career, the most significant contributions to science are related to work on CP and FPN, on the yeast ferroxidase Fet3 and iron permease Ftr1, and more recently on the yeast iron-responsive transcription factor Fep1.

After a series of studies on the spectroscopic properties of the protein and the finding that human CP is oxidatively modified during aging, the major contribution to science is the discovery that ferroxidase activity is required for the stability of FPN at the cell surface. Other relevant contributions include the characterization of a set of aceruloplasminemia CP missense mutants and the finding that the R701W mutant was dominant over the wild type protein causing relocalization of ATP7B, the protein required for loading of copper on the ferroxidase. Further work identified the role of five basic residues found in five surface-exposed loops in copper incorporation in CP, providing clues on the molecular mechanism of copper loading

in multicopper oxidases. More recently, a system for production of recombinant human CP in a modified glycoengineered yeast has been set up, allowing to obtain highly pure biologically active protein.

- Bonaccorsi di Patti MC, Cutone A, Nemčovič M, Pakanová Z, Baráth P, Musci G (2021) Production of recombinant human ceruloplasmin: improvements and perspectives. *Int. J. Mol. Sci.* 22, 8228.
- Maio N, Polticelli F, De Francesco G, Rizzo G, Bonaccorsi di Patti MC, Musci G (2010) Role of external loops of human ceruloplasmin in copper loading by ATP7B and Ccc2p. *J. Biol. Chem.* 285, 20507-20513.
- Bonaccorsi di Patti MC, Maio N, Rizzo G, De Francesco G, Persichini T, Colasanti M, Polticelli F, Musci G (2009) Dominant mutants of ceruloplasmin impair the copper loading machinery in aceruloplasminemia. *J. Biol. Chem.* 284, 4545-4554.
- De Domenico I, Ward DM, Bonaccorsi di Patti MC, Jeong SY, David S, Musci G Kaplan J (2007) Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin. *EMBO J.* 26, 2823-2831.
- Musci G, Bonaccorsi di Patti MC, Fagiolo U, Calabrese L (1993) Age-related changes in human ceruloplasmin. Evidence for oxidative modifications. *J. Biol. Chem.* 268, 13388-13395.

Regarding FPN, the most important results are the first identification of residues critical for binding of iron, on the basis of modeling studies. The role of D39 and D325 in metal binding was confirmed ten years later when the cryoEM structure of human FPN was published. Further studies on pathogenic FPN mutants allowed more detailed understanding of genotype/phenotype correlations. More recently, the use of amber suppression technology has been applied to genetically incorporate a fluorescent probe in specific positions of FPN enabling the investigation of conformational changes using fluorescence spectroscopy. This approach holds great promise for the study of the alternating access mechanism of FPN and advancing our understanding of the molecular basis of iron transport.

- Amadei M, Niro A, Fullone MR, Miele R, Polticelli F, Musci G, Bonaccorsi di Patti MC (2023) Genetic incorporation of dansylalanine in human ferroportin to probe the alternating access mechanism of iron transport. *Int. J. Mol. Sci.* 24, 11919.
- Majore S, Bonaccorsi di Patti MC, Valiante M, Polticelli F, Cortese A, Di Bartolomeo S, De Bernardo C, De Muro M, Faienza F, Radio FC, Grammatico P, Musci G (2018) Characterization of three novel pathogenic SLC40A1 mutations and genotype/phenotype correlations in 7 Italian families with type 4 hereditary hemochromatosis. *Biochim. Biophys. Acta - Molecular basis of disease* 1864, 464-470.
- Bonaccorsi di Patti MC, Polticelli F, Cece G, Cutone A, Felici F, Persichini T, Musci G (2014) A structural model of human ferroportin and of its iron binding site. *FEBS J.* 281, 2851-2860.

The study of the ferroxidase-permease Fet3-Ftr1 system led to the first identification of residues essential for binding of iron by yeast ferroxidases. The initial in vitro studies on *Saccharomyces cerevisiae* Fet3 were complemented by in vivo mutational analyses.

Cloning and characterization of the Fet3 protein from the yeast *Pichia pastoris* showed that this ferroxidase has a much higher catalytic efficiency compared to *S. cerevisiae* Fet3. Further advances led to cloning of *PpFtr1* and to the first experimental demonstration of the physical association between Fet3 and Ftr1. This result was obtained by cross-linking of membrane suspensions, isolation of the ferroxidase-active cross-linked complex and Edman degradation to identify the interacting partners.

- Bonaccorsi di Patti MC, Miele R, Schininà ME, Barra D (2005) The yeast multicopper oxidase Fet3p and the iron permease Ftr1p physically interact. *Biochem. Biophys. Res. Commun.* 333, 432-437.
- Paronetto MP, Miele R, Maugliani A, Borro M, Bonaccorsi di Patti MC (2001) Cloning of *Pichia pastoris* Fet3: insights into the high affinity iron uptake system. *Arch. Biochem. Biophys.* 392, 162-167.
- Bonaccorsi di Patti MC, Paronetto MP, Dolci V, Felice MR, Lania A, Musci G (2001) Mutational analysis of the iron binding site of *Saccharomyces cerevisiae* ferroxidase Fet3. An in vivo study. *FEBS Lett.* 508, 475-478.

- Bonaccorsi di Patti MC, Felice MR, Camuti AP, Lania A, Musci G (2000) The essential role of Glu185 and Tyr354 residues in the ferroxidase activity of *Saccharomyces cerevisiae* Fet3. FEBS Lett. 472, 283-286.

Optical and resonance Raman spectroscopy have provided the first demonstration that a [2Fe-2S] cluster is bound by the iron-responsive GATA factor Fep1 of *Pichia pastoris*, in both anaerobic and aerobic conditions. Biophysical characterization by analytical ultracentrifugation sedimentation velocity (AUC SV), small-angle X-ray scattering (SAXS), differential scanning calorimetry (DSC) and fluorescence spectroscopy demonstrate that Fep1 is able to bind DNA independently of the presence of a stoichiometric [2Fe-2S] cluster. This finding suggests that the cluster is not essential for binding to DNA but it may play a role in recruiting other proteins required for regulation of transcription in response to changes in intracellular iron levels.

- Miele AE, Cervoni L, Le Roy A, Cutone A, Musci G, Ebel C, Bonaccorsi di Patti MC (2021) Biophysical characterization of the complex between the iron-responsive transcription factor Fep1 and DNA. Eur. Biophys. J. 50, 501-512.
- Cutone A, Howes BD, Miele AE, Miele R, Giorgi A, Battistoni A, Smulevich G, Musci G, Bonaccorsi di Patti MC (2016) *Pichia pastoris* Fep1 is a [2Fe-2S] protein with a Zn finger that displays an unusual oxygen-dependent role in cluster binding. Scientific Reports 6, 31872.
- Miele R, Barra D, Bonaccorsi di Patti MC (2007) A GATA-type transcription factor regulates expression of the high-affinity iron uptake system in the methylotrophic yeast *Pichia pastoris*. Arch. Biochem. Biophys. 465, 172-179.