



**Loredano Pollegioni**  
UNIVERSITY OF INSUBRIA



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## Contact data

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## Biography

Loredano Pollegioni graduated in Biological Sciences at the University of Milan. He had a post-doc position at the lab of Prof. V. Massey (University of Michigan, USA) and at the lab of Prof. S. Ghisla (Universitat Konstanz, Germany).

He has been Technical Manager (from 1989), and Associate Researcher (from 1996) in the III Faculty of Biology of University of Milan since 1998, when it became the University of Insubria, where he was appointed Associate Professor of Biochemistry in November 2000. He was the winner of a comparative evaluation procedure for a fellowship as Full Professor in Biochemistry, and he was appointed by the School of Sciences in Varese in March of 2005.

He organized the School on Rapid Kinetics Techniques (in 2003) and on Enzyme Engineering (in 2005) for the Italian Society of Biochemistry.

## Qualifications and awards

Prof. Pollegioni is a member of the Italian Society of Biochemistry and Molecular Biology, of the European Federation of Biotechnology (Applied Biocatalysis) and of the American Society for Biochemistry and Molecular Biology. He is a referee for a number of international journals of the Biochemistry, Molecular and Structural Biology, and Biotechnology field.

## Research interests

In the scientific career of Prof. Loredano Pollegioni there are many international collaborations and more than 60 papers on international peer-review journals were produced over the years. The scientific career of Prof. Loredano Pollegioni focused, as a general topic, on the structure-function relationships in enzymatic proteins, as FAD-dependent oxidases (namely D-amino acid oxidase, cholesterol oxidase and, recently, glycine oxidase). The functional and structural properties of the enzyme D-amino acid oxidase from microorganisms, a model protein in enzyme biochemistry, has been investigated by using a multidisciplinary methodological approach: kinetic studies (steady state and pre-steady state kinetics), reaction mechanism studies (substrate specificity, chemical modification and site-directed mutagenesis), structural studies (X-ray crystallography, limited proteolysis), flavin reactivity and protein stability on heating and

denaturing agents (solvents, detergents, pH). Prof. Pollegioni has acquired a wide experience (as it is shown by the published papers) on the fundamental aspects of functional and structural properties of flavooxidases and, more recently, on the physiological role of the covalent link of the flavin coenzyme.

This experience has been also exploited in the field of protein biotechnology. D-amino acid oxidase (as well as acylases and proteases) has been employed as biocatalyst (D-amino acid oxidase has been used for the production of  $\beta$ -lactam antibiotics and as a pro-drug converter in cancer gene-therapy). Prof. Pollegioni significantly participated to develop a two-step bioconversion system, in which DAAO has been immobilised on a solid matrix, for the production of  $\beta$ -lactam antibiotics, as well as for the production of  $\alpha$ -keto acids. The same enzymatic reaction has been used for the production of a prototype biosensor for the analytical determination of D-amino acids in food.

In the last five years, prof. Pollegioni specialised in the evolution of enzymatic activities by using rational (site-directed mutagenesis) and directed evolution (random and site-saturation mutagenesis) methods. These investigations allowed the production of a new enzymatic activity in the bioconversion of cephalosporin C, and a new D-amino acid oxidase activity which is able to oxidise both neutral and acidic D-amino acids.

## Teaching experience and appointments

Teaching activities:

Biochemistry (Biology and Biotechnology Degrees)

Drug Design (Structural Biochemistry part) (Industrial Biotechnology and Biocatalysis, and Biology Applied to Biomedical Research Degrees)

Industrial Biochemistry (Biotechnology Degree)

Proteins and Cellular Processes (Biology Degree)

Cellular Biochemistry (Biology Applied to Biomedical Research Degree)

Regulation of Metabolic Processes (Biology Degree)

Institutional Position: Acting Chairman of the Degree in Biotechnology

## Representative publications

1) L. POLLEGIONI, S. GHISLA and M.S. PILONE (1992) Studies on the active center of *Rhodotorula gracilis* D-amino acid oxidase and comparison with pig kidney enzyme, *Biochem. J.* 286, 389-394

2) L. POLLEGIONI, B. LANGKAU, W. TISCHER, S. GHISLA and M.S. PILONE (1993) Kinetic mechanism of D-amino acid oxidases from *Rhodotorula gracilis* and *Trigonopsis variabilis*, *J. Biol. Chem.* 268, 13850-13857

3) L. POLLEGIONI, K. FUKUI and V. MASSEY (1994) Studies on the kinetic mechanism of pig kidney D-amino acid oxidase by site directed mutagenesis of tyrosine 224 and tyrosine 228 (1994) *J. Biol. Chem.*

- 4) L. POLLEGIONI, F. CECILIANI, B. CURTI, S. RONCHI and M.S. PILONE Studies on the structural and functional aspects of *Rhodotorula gracilis* D-amino acid oxidase by limited trypsinolysis (1995) *Biochem. J.* 310, 577-583
- 5) L. POLLEGIONI, W. BLODIG and S. GHISLA On the mechanism of D-amino acid oxidase. Structure/linear free energy correlations and deuterium kinetic isotope effects using p-substituted phenylglycines (1997) *J. Biol. Chem.* 272, 4924-4934
- 6) G. GADDA, G. WELS, L. POLLEGIONI, S. ZUCHELLI, D. AMBROSIUS, M.S. PILONE and S. GHISLA Characterization of cholesterol oxidase from *Streptomyces hygrosopicus* and *Brevibacterium sterolicum* (1997) *Eur. J. Biochem.* 250, 369-376
- 7) S. CAMPANER, L. POLLEGIONI, B.D. ROSS and M.S. PILONE Limited proteolysis and site directed mutagenesis revealed the origin of microheterogeneity of *Rhodotorula gracilis* D-amino acid oxidase (1998) *Biochem. J.* 330, 615-621
- 8) L.D. STEGMAN, H. ZHENG, E.R. NEAL, O. BEN-YOSEPH, L. POLLEGIONI, M.S. PILONE and B.D. ROSS Induction of cytotoxic oxidative stress by D-alanine in brain tumor cells expressing *Rhodotorula gracilis* D-amino acid oxidase: a cancer gene therapy strategy (1998) *Human Gene Therapy* 9, 185-193
- 9) L. POLLEGIONI, G. WELS, S. GHISLA and M.S. PILONE Kinetic mechanism of cholesterol oxidase from *Streptomyces hygrosopicus* and *Brevibacterium sterolicum* (1999) *Eur. J. Biochem.* 264, 140-151
- 10) C.M. HARRIS, G. MOLLA, M.S. PILONE and L. POLLEGIONI Studies on the reaction mechanism of *Rhodotorula gracilis* D-amino acid oxidase: Role of the highly conserved Tyr223 on substrate binding and catalysis (1999) *J. Biol. Chem.* 274, 36233-36240
- 11) G. MOLLA, D. PORRINI, V. JOB, L. MOTTERAN, C. VEGEZZI, S. CAMPANER, M.S. PILONE and L. POLLEGIONI Role of Arginine285 at the active site of *Rhodotorula gracilis* D-amino acid oxidase. A site-directed mutagenesis study (2000) *J. Biol. Chem.* 275, 24715-24721
- 12) S. UMHAU, L. POLLEGIONI, G. MOLLA, K. DIEDERICHS, W. WELTE, M.S. PILONE and S. GHISLA The X-ray structure of D-amino acid oxidase at very high resolution identifies the chemical mechanism of flavin dependent substrate dehydrogenation (2000) *Proc. Natl. Acad. Sci. USA* 97, 12463-12468
- 13) L. POLLEGIONI, D. Porrini, G. Molla and M. S. Pilone Redox potentials and their pH dependence of D-amino acid oxidase of *Rhodotorula gracilis* and *Trigonopsis variabilis* (2000) *Eur. J. Biochem.* 267, 6624-6632
- 14) L. MOTTERAN, M. S. PILONE, G. MOLLA, S. GHISLA and L. POLLEGIONI Cholesterol oxidase from *Brevibacterium sterolicum*:

covalent flavinylation and redox properties relationships (2001) J. Biol. Chem. 276, 18024-18030

15) C.M. HARRIS, S. GHISLA and L. POLLEGIONI pH and kinetic effects in D-amino acid oxidase catalysis. Evidence for a concerted mechanism in substrate dehydrogenation via hydride transfer (2001) Eur. J. Biochem. 268, 5504-5520

16) V. JOB, L. MARCONE, M.S. PILONE and L. POLLEGIONI Glycine oxidase from *Bacillus subtilis*: characterization of a new flavoprotein (2002) J. Biol. Chem. 277, 6985-6993

17) V. JOB, M.S. PILONE and L. POLLEGIONI Overexpression of a recombinant wild-type and His-tagged *Bacillus subtilis* glycine oxidase in *Escherichia coli* (2002) Eur. J. Biochem. 269, 1456-1463

18) E. Ardini, B. Sporchia, L. POLLEGIONI, M. Modugno, C. Ghirelli, F. Castiglioni, E. Tagliabue and S. Ménard Identification of a novel function for 67 kDa laminin receptor: Increase in laminin degradation rate and release of motility-fragments (2002) Cancer Research 62, 1321-1325

19) S. SACCHI, S. LORENZI, G. MOLLA, M.S. PILONE, C. ROSSETTI and L. POLLEGIONI Engineering the substrate specificity of D-amino acid oxidase (2002) J. Biol. Chem. 277, 27510-27516

20) A. BOSELLI, S. SACCHI, V. JOB, M.S. PILONE and L. POLLEGIONI Role of tyrosine 238 in the active site of *Rhodotorula gracilis* D-amino acid oxidase. A site-directed mutagenesis study (2002) Eur. J. Biochem. 269, 4762-4771

21) L. POLLEGIONI, K. DIEDERICHS, G. MOLLA, W. WELTE, S. UMHAU, S. GHISLA and M.S. PILONE Yeast D-amino acid oxidase: structural basis of its catalytic properties (2002) J. Mol. Biol. 324, 535-546

22) G. MOLLA, L. MOTTERAN, V. JOB, M.S. PILONE and L. POLLEGIONI Kinetic mechanism of glycine oxidase from *Bacillus subtilis* (2003) Eur. J. Biochem. 270, 1474-1482

23) L. POLLEGIONI, Stefania Iametti, D. Fessas, L. Caldinelli, L. Piubelli, A. Barbiroli, M. S. Pilone and F. Bonomi Contribution of the dimeric state to the thermal stability of the flavoprotein D-amino acid oxidase (2003) Protein Science 12, 1018-1029

24) L. Piubelli, G. Molla, L. Caldinelli, M.S. Pilone and L. POLLEGIONI Dissection of the structural determinants involved in formation of the dimeric form of D-amino acid oxidase from *Rhodotorula gracilis*: role of the size of the  $\beta$ F5-  $\beta$ F6 loop (2003) Protein Engineering, 16, 1-7

25) M. Mörtl, K. Diederichs, W. Welte, G. Molla, L. Motteran, G. Andriolo, M. S. Pilone, and L. POLLEGIONI Structure-function correlation in glycine oxidase from *Bacillus subtilis* (2004) J. Biol. Chem. 279, 29718-29727

- 26) L. Caldinelli, S. Iametti, A. Barbiroli, F. Bonomi, P. Ferranti, M. S. Pilone, and L. POLLEGIONI Unfolding of the peroxisomal flavoprotein D-amino acid oxidase (2004) *J. Biol. Chem.* 279, 28426-28434
- 27) S. Sacchi, E. Rosini, G. Molla, M.S. Pilone Ghisla, S. and L. POLLEGIONI Modulation D-amino acid oxidase substrate specificity: production of an enzyme for analytical determination of all D-amino acids by directed evolution (2004) *Protein Engineering Design and Selection* 17, 517-525
- 28) J-P. Mothet, L. POLLEGIONI, G. Ouanouno, D. Cherroub, P. Fossier, and G Baux Glutamate receptor activation triggers a calcium-dependent and SNARE protein-dependent release of the gliotransmitter D-serine. *Proc Natl Acad Sci U S A.* (2005) 102, 5606-5611.
- 29) L. Caldinelli, Iametti S, Barbiroli A, Bonomi F, Fessas D, Molla G, Pilone MS, POLLEGIONI L. Dissecting the structural determinants of the stability of cholesterol oxidase containing covalently bound flavin. *J. Biol. Chem.* (2005) 280, 22572-22581.
- 30) L. POLLEGIONI, Lorenzi S, Rosini E, Marcone GL, Molla G, Verga R, Cabri W, Pilone MS. Evolution of an acylase active on cephalosporin C. *Protein Sci.* (2005) in press