

BIOGRAPHICAL SKETCH

NAME		POSITION TITLE	
Pollegioni Loredano, Professor		Full Professor of Biochemistry, Dept. Biotechnology and Life Sciences, University of Insubria (Varese, Italy)	
EDUCATION/TRAINING			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
University of Milan (Italy)	5 years B.S.	1986 (honors)	Biology
Italian Society of Biochemistry (Italy)	Specialist	1987	Protein Science
University of Michigan (USA)	Postdoctoral training	2003-2004	Enzymology
University of Tokushima (Japan)	Visiting Professor	2005	Molecular Enzymology

Professional positions:

1987-1991	Researcher and Technical Assistant at the University of Milano (Italy)
1991-1993	Visiting Researcher in Molecular Enzymology, University of Konstanz (Germany)
1993-1994	Post-Doc in Enzymology at the University of Michigan (USA)
1994-1995	Research Fellow at the University of Milano (Italy)
1996-2000	Assistant Professor at the III Faculty of Science – University of Milano (Italy)
2000-2005	Associate Professor of Biochemistry, University of Insubria, Varese (Italy)
2003 – present	Adviser for various pharmaceutical companies (Antibioticos SpA, Farmaopera SpA)
2005 – present	Full Professor of Biochemistry, University of Insubria, Varese (Italy)
2005 – 2009	Head of the Degree in Biotechnology, University of Insubria, Varese (Italy)
2008 – 2010	President of the Research Center in Biotechnology for Human Health
2009 – 2015	Director of the Research Center The Protein Factory – Politecnico of Milano, ICRM CNR Milano and University of Insubria
2011-2012	Head of the Degree in Biotechnology, University of Insubria, Varese (Italy)
2013-2017	Coordinator of the PhD Course in Biotechnology, Biosciences and Surgical Technology
2015-2018	ViceDirector of the research center The Protein Factory
2017-present	Coordinator of the PhD Course in Life Sciences and Biotechnology (Uninsubria)
2017-2018	Head of the Course in Biological Sciences (Uninsubria)
2017-present	Member of the board of Fondazione Istituto Insubrico Ricerca per la Vita
2015-present	Member of the regional Cluster Green Chemistry and of the Cluster Life Sciences
2018-present	Member of Publication Committee of FEBS, European Federation of Biochemical Societies

Memberships:

1998-present	Member of the Italian Society of Biochemistry and Molecular Biology (SIB)
2000	Organizer of the National Symposium "Proteine 2000"
2001 – 2015	Member of the American Society for Biochemistry and Molecular Biology (ASBMB)
2005 – 2015	Member of the European Federation of Biotechnology
2004	Organizer of the National School on Rapid Kinetics Techniques
2005	Organizer of the National School on Enzyme Engineering
2006 – present	Board Member of the Italian Association on Biocatalysis (AIBB)
2012 - 2014	Responsible of the Enzymology group of Italian Society of Biochemistry
2015	Organizer of the International School on Enzyme Discovery
2017	Organizer of the International Congress on D-amino acids IDAR2017

2015 – present Treasurer of the Italian Society of Biochemistry

He acts as referee for a number of international journals of the Biochemistry, Molecular and Structural Biology, and Biotechnology field. He is member of the Editorial Board of FEBS Journal (from 2008), BBA Proteins and Proteomics, Frontiers in Molecular Sciences.

Scientific interests:

The scientific career of Loredano Pollegioni focused, as a general topic, on the structure-function relationships in enzymatic proteins, as FAD-dependent oxidases. These enzymes have been investigated by using a multidisciplinary methodological approach: kinetic studies (steady state and pre-steady state kinetics), reaction mechanism studies, structural studies, flavin reactivity and protein stability (on heating and denaturing agents) studies. This experience has been largely exploited in the field of protein biotechnology: flavooxidases, acylases and proteases have been employed as biocatalysts and as a pro-drug converter in cancer gene-therapy. In the last years, the research activity of prof. Pollegioni focused on the evolution of enzymatic activities by using rational (site-directed mutagenesis) and directed evolution (random and site-saturation mutagenesis) methods, and in the investigation of human enzymes involved in pathologies and in important biochemical processes (DAAO, pLG72, proline oxidase: all related to schizophrenia susceptibility). These investigations allowed the production of new enzymatic activities and the characterization of human flavoenzymes. The DAAO enzymatic reaction has been also used for the production of a prototype biosensor for the analytical determination of D-amino acids (and recently to develop a microbiosensor for the determination of D-serine in the brain) and for the production of α -keto acids.

In recent years he worked on the evolution and application of ligninolytic enzymes for production of chemicals.

He has been responsible of 15 grants from national (MIUR, Regione Lombardia, Fondazione Cariplo, etc.) and International (FP7) agencies, as well as companies (Antibioticos, Flamma, Sepracore, etc).

Prof. Loredano Pollegioni is author of more than 185 peer-review publications on main international journals in the field of Biochemistry, Molecular and Structural Biology and Biotechnology (and of 2 patents; 250 communications to national and international congresses). IF total: > 750; Citations: > 7950; H factor: 49; i10-index: 137.

He has been the organizer of national and international meetings and schools on biochemistry and protein biotechnology.

Relator or co-relator of 92 thesis (Bachelor and Master degree) and 13 PhD-thesis.

Selected peer-reviewed publications (in chronological order)

1. 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.
2. 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. 269, 31666-31673.
3. 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.
4. 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.
5. 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.
6. 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.

7. 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.
8. 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 (2000) *J. Biol. Chem.*, 275: 24715-24721.
9. 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, 12463-14638.
10. L. MOTTERAN, PILONE M.S., MOLLA G., GHISLA S. and L. POLLEGIONI Cholesterol oxidase from *Brevibacterium sterolicum*. The relationship between covalent flavinylation and redox properties (2001) *J. Biol. Chem.*, 276, 18024-18030.
11. 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.
12. 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.
13. SACCHI S., LORENZI S., MOLLA G., PILONE M.S., ROSSETTI C, POLLEGIONI L. Engineering the substrate specificity of D-amino-acid oxidase (2002) *J. Biol. Chem.*, 277, 27510-27516.
14. POLLEGIONI L, DIEDERICHS K., MOLLA G., UMHAU S., WELTE W, GHISLA S, PILONE M.S. Yeast D-amino acid oxidase: structural basis of its catalytic properties (2002) *J. Mol. Biol.* 324, 535-546.
15. L. POLLEGIONI, STEFANIA IAMETTI, D. FESSAS, L. CALDINELLI, L. PIUBELLI, A. BARBIROLI, M. S. PILONE, F. BONOMI Contribution of the dimeric state to the thermal stability of the flavoprotein D-amino acid oxidase (2003) *Protein Science* 12, 1018-1029.
16. L. CALDINELLI, S. IAMETTI, A. BARBIROLI, F. BONOMI, P. FERRANTI, M. S. PILONE, L. POLLEGIONI Unfolding of the peroxisomal flavoprotein D-amino acid oxidase (2004) *J. Biol. Chem.* 279, 28426-28434.
17. MORTL M, DIEDERICHS K, WELTE W, MOLLA G, MOTTERAN L, ANDRIOLO G, PILONE MS, POLLEGIONI L. Structure-function correlation in glycine oxidase from *bacillus subtilis*. (2004) *J Biol Chem.*, 279: 29718-29727.
18. CALDINELLI L, 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 (2005) *J Biol Chem.* 280: 22572-22581.
19. J-P. MOTHET, L. POLLEGIONI, G. OUANOONO, D. CHERROUB, P. FOSSIER, 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.
20. MOLLA G, BERNASCONI M, SACCHI S, PILONE MS, POLLEGIONI L. Expression in *Escherichia coli* and in vitro refolding of the human protein pLG72. *Protein Expr Purif.* (2006) 46: 150-155.
21. POLLEGIONI L, 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) 14: 3064-3076.
22. MOLLA G, SACCHI S, BERNASCONI M, PILONE MS, FUKUI K, POLLEGIONI L. Characterization of human D-amino acid oxidase. *FEBS Lett.* (2006) 580: 2358-2364.
23. A. PANATIER, D. T. THEODOSIS, J-P. MOTHET, B. TOUQUET, L. POLLEGIONI, D. A. POULAIN, S.H.R. OLIET Glia-Derived d-Serine Controls NMDA Receptor Activity and Synaptic Memory. (2006) *Cell* 125, 775-784.
24. POLLEGIONI L., PIUBELLI L., SACCHI S., PILONE M.S., MOLLA G. Physiological functions of D-amino acid oxidases: from yeast to humans. *Cell Mol Life Sci.* (2007) 64, 1373-1394.
25. SACCHI S, BERNASCONI M, MARTINEAU M, MOTHET JP, RUZZENE M, PILONE MS, POLLEGIONI L, MOLLA G. pLG72 modulates intracellular D-serine levels through its interaction with D-amino acid oxidase: Effect on schizophrenia susceptibility. *J Biol Chem.* 2008 Jun 10. [Epub ahead of print]
26. PERNOT P, MOTHET JP, SCHUVAILO O, SOLDATKIN A, POLLEGIONI L, PILONE M, ADELIN MT, CESPUGLIO R, MARINESCO S. Characterization of a yeast D-amino acid oxidase microbiosensor for D-serine detection in the central nervous system. *Anal Chem.* (2008) 80(5):1589-97.
27. POLLEGIONI L, SACCHI S Metabolism of the neuromodulator D-serine. *Cell Mol Life Sci.* (2010) 19:1500-1512

28. PEDOTTI M, ROSINI E, MOLLA G, MOSCHETTI T, SAVINO C, VALLONE B, POLLEGIONI L. Glyphosate resistance by engineering the flavoenzyme glycine oxidase. *J Biol Chem.* (2009) 284:36415-23
29. CALDINELLI L., MOLLA G., SACCHI S., PILONE M.S., POLLEGIONI L. Relevance of weak flavin binding in human D-amino acid oxidase. *Protein Sci.* (2009) 18, 801-810.
30. SAAM J, ROSINI E, MOLLA G, SCHULTEN K, POLLEGIONI L, GHISLA S. 2010 O₂-reactivity of flavoproteins: dynamic access of dioxygen to the active site and role of a H⁺ relay system in D-amino acid oxidase. *J Biol Chem.* 285(32):24439-46.
31. CALDINELLI L, MOLLA G, BRACCI L, LELLI B, PILERI S, P CAPPELLETTI, SACCHI S, POLLEGIONI L. 2010 Effect of ligand binding on human D-amino acid oxidase: implications for the development of new drugs for schizophrenia treatment. *Protein Science*, 19: 1500-1512.
32. POLLEGIONI L, MOLLA G. 2011 New biotech applications from evolved D-amino acid oxidases. *Trends Biotechnol.* 29(6):276-283.
33. POLLEGIONI L, SCHONBRUNN E, SIEHL D. 2011 Molecular basis of glyphosate resistance - different approaches through protein engineering. *FEBS J.* 278, 2753-2766.
34. SACCHI S, CAPPELLETTI P, GIOVANNARDI S, POLLEGIONI L. 2011 Evidence for the interaction of D-amino acid oxidase with pLG72 in a glial cell line. *Mol Cell Neurosci.* 48, 20-28.
35. VOLONTE F, PIUBELLI L, POLLEGIONI L. 2011 Optimizing HIV-1 protease production in *Escherichia coli* as fusion protein. *Microb Cell Fact.* 10:53.
36. FRATTINI LF, PIUBELLI L, SACCHI S, MOLLA G, POLLEGIONI L. 2011 Is rat an appropriate animal model to study the involvement of D-serine catabolism in schizophrenia? Insights from characterization of D-amino acid oxidase. *FEBS J.* 278:4362-73.
37. GHISLA S, POLLEGIONI L, MOLLA G. 2011 Revisitation of the β Cl-Elimination Reaction of D-Amino Acid Oxidase: NEW INTERPRETATION OF THE REACTION THAT SPARKED FLAVOPROTEIN DEHYDROGENATION MECHANISMS. *J Biol Chem.* 286(47):40987-98.
38. FOSSAT P, TURPIN FR, SACCHI S, DULONG J, SHI T, RIVET JM, SWEEDLER JV, POLLEGIONI L, MILLAN MJ, OLIET SH, MOTHET JP. 2012 Glial D-serine gates NMDA receptors at excitatory synapses in prefrontal cortex. *Cereb Cortex.* 22 (3), pp. 595-606
39. Papouin T, Ladépêche L, Ruel J, Sacchi S, Labasque M, Hanini M, Groc L, Pollegioni L, Mothet JP, Oliet SH. Synaptic and Extrasynaptic NMDA Receptors Are Gated by Different Endogenous Coagonists. *Cell.* 2012 150(3):633-46.
40. CURCIO L, PODDA MV, LEONE L, PIACENTINI R, MASTRODONATO A, CAPPELLETTI P, SACCHI S, POLLEGIONI L, GRASSI C, D'ASCENZO M. Reduced D-serine levels in the nucleus accumbens of cocaine-treated rats hinder the induction of NMDA receptor-dependent synaptic plasticity. *Brain.* 2013 136(Pt 4):1216-30.
41. Li Y, SACCHI S, POLLEGIONI L, BASU AC, COYLE JT, BOLSHAKOV VY. Identity of endogenous NMDAR glycine site agonist in amygdala is determined by synaptic activity level. *Nat Commun.* 2013 4:1760.
42. LE BAIL M, MARTINEAU M, SACCHI S, YATSENKO N, RADZISHEVSKY I, CONROD S, AIT OUARES K, WOLOSKER H, POLLEGIONI L, BILLARD JM, MOTHET JP. Identity of the NMDA receptor coagonist is synapse specific and developmentally regulated in the hippocampus. *Proc Natl Acad Sci U S A.* 2015 Jan 13;112(2):E204-13.
43. POLLEGIONI L, TONIN F, ROSINI E. Lignin-degrading enzymes. *FEBS J.* 2015 Apr;282(7):1190-213
44. Tessaro, D., Pollegioni, L., Piubelli, L., Darrigo, P., Servi, S. Systems biocatalysis: An artificial metabolism for interconversion of functional groups *ACS Catalysis* 5(3), pp. 1604-1608
45. Conti, G., Pollegioni, L., Rosini, E. One-pot conversion of cephalosporin C by using an optimized two-enzyme process *Catalysis Science and Technology* 5 (3), pp. 1854-1863
46. Pollegioni L, Tonin F, Rosini E. Lignin-degrading enzymes. *FEBS J.* 2015 282(7):1190-213.
47. Punzo D, Errico F, Cristino L, Sacchi S, Keller S, Belardo C, Luongo L, Nuzzo T, Imperatore R, Florio E, De Novellis V, Affinito O, Migliarini S, Maddaloni G, Sisalli MJ, Pasqualetti M, Pollegioni L, Maione S, Chiariotti L, Usiello A. Age-related changes in D-aspartate oxidase promoter methylation control extracellular D-aspartate levels and prevent precocious cell death during brain aging. *J Neurosci.* 2016 36(10):3064-78.
48. Luks L, Maier MY, Sacchi S, Pollegioni L, Dietrich DR. Understanding renal nuclear protein accumulation: an in vitro approach to explain an in vivo phenomenon. *Arch Toxicol.* 2017 91: 427-437

49. Polcari D., S. C. Perry, L. Pollegioni, M. Geissler, J. Mauzeroll Localized detection of D-serine using an enzymatic amperometric biosensor and scanning electrochemical microscopy. *ChemElectroChem*. 2017, 4, 920-926
50. Molla G, Melis R, Pollegioni L. Breaking the mirror: L-Amino acid deaminase, a novel stereoselective biocatalyst. *Biotechnol Adv*. 2017 Nov 1;35(6):657-668. doi:10.1016/j.biotechadv.2017.07.011

Patents

POLLEGIONI L., PILONE M., MOLLA G., CUCCHETTI E., VERGA R., CABRI W. and ANTIBIOTICOS SPA. US2005158818: Enzymes with cephalosporin C acylase activity obtained by site-directed, random or "site-saturation" mutagenesis of a native sequence optimised for expression in *E. coli*.

MARINESCO S., PERNOT P., MOTHET JP., CESPUGLIO R., SCHUVAILO O., SOLDATKIN A., POLLEGIONI L., PILONE M. EP06291523.6-1223 Microsensor for detection of D-amino acids.

Protein Data Bank submissions:

ID	Title	Release Date
5FJM	Structure of L-Amino acid deaminase from <i>Proteus myxofaciens</i>	4/6/2016
5FJN	Structure of L-Amino acid deaminase from <i>Proteus myxofaciens</i> in complex with anthranilate	4/6/2016
1C0I	CRYSTAL STRUCTURE OF D-AMINO ACID OXIDASE IN COMPLEX WITH TWO ANTHRANYLATE MOLECULES	2/27/2002
1C0K	CRYSTAL STRUCTURE ANALYSIS OF D-AMINO ACID OXIDASE IN COMPLEX WITH L-LACTATE	11/22/2000
1C0L	D-AMINO ACID OXIDASE: STRUCTURE OF SUBSTRATE COMPLEXES AT VERY HIGH RESOLUTION REVEAL THE CHEMICAL REACTION MECHANISM OF FLAVIN DEHYDROGENATION	11/22/2000
1C0P	D-AMINO ACID OXIDASE IN COMPLEX WITH D-ALANINE AND A PARTIALLY OCCUPIED BIATOMIC SPECIES	11/22/2000
1RYI	STRUCTURE OF GLYCINE OXIDASE WITH BOUND INHIBITOR GLYCOLATE	2/22/2005
3IF9	Crystal structure of Glycine Oxidase G51S/A54R/H244A mutant in complex with inhibitor glycolate	10/27/2009

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4CNJ	L-Aminoacetone oxidase from Streptococcus oligofermentans belongs to a new 3-domain family of bacterial flavoproteins	10/15/2014
4CNK	L-Aminoacetone oxidase from Streptococcus oligofermentans belongs to a new 3-domain family of bacterial flavoproteins	10/15/2014
4HSR	Crystal Structure of a class III engineered cephalosporin acylase	2/27/2013
4HST	Crystal structure of a double mutant of a class III engineered cephalosporin acylase	2/27/2013
2I0K	Cholesterol Oxidase from Brevibacterium sterolicum- His121Ala Mutant	12/11/2007
4QFC	Co-crystal structure of compound 3 (4-hydroxy-6-[2-(7-hydroxy-2-oxo-4-phenyl-2h-chromen-6-yl)ethyl]pyridazin-3(2h)-one) and FAD bound to human DAAO at 2.4Å	7/16/2014
4QFD	Co-crystal structure of compound 2 (3-(7-hydroxy-2-oxo-4-phenyl-2H-chromen-6-yl)propanoic acid) and FAD bound to human DAAO at 2.85Å	7/16/2014
3ZNN	IN VITRO AND IN VIVO INHIBITION OF HUMAN D-AMINO ACID OXIDASE: REGULATION OF D-SERINE CONCENTRATION IN THE BRAIN	5/15/2013
3ZNO	IN VITRO AND IN VIVO INHIBITION OF HUMAN D-AMINO ACID OXIDASE: REGULATION OF D-SERINE CONCENTRATION IN THE BRAIN	5/15/2013
3ZNP	IN VITRO AND IN VIVO INHIBITION OF HUMAN D-AMINO ACID OXIDASE: REGULATION OF D-SERINE CONCENTRATION IN THE BRAIN	5/15/2013
3ZNQ	IN VITRO AND IN VIVO INHIBITION OF HUMAN D-AMINO ACID OXIDASE: REGULATION OF D-SERINE CONCENTRATION IN THE BRAIN	5/15/2013