

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Giuseppe, Orsomando

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Associate Professor of Biochemistry, Polytechnic University of Marche, Italy

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Start Date	Completion Date	FIELD OF STUDY
University of Camerino (UNICAM), Italy	Laurea	11/1987	07/1992	Biology
University of Camerino (UNICAM), Italy	Ph.D.	11/1992	07/1997	Protein Biology
University of Ancona (UNIVPM), Italy	Postdoctoral	02/1998	01/2000	Enzymology and Biochemistry

A. Personal Statement

I am an Associate professor of Biochemistry working at the Department of Odontostomatologic and Specialized Clinical Sciences (DISCO) at the Polytechnic University of Marche (UNIVPM) in Ancona. I've been actively engaged in research since my graduation in 1992, with a focus primarily on the biochemistry of proteins, enzymes, and their ligands. Over the years, my skills have evolved from traditional techniques such as protein purification and characterization to modern methods based on recombinant DNA technology. I have published peer-reviewed articles on NAD metabolism and enzymology [1-6], heterologous protein expression and characterization [7-10], enzyme assays [11, 12], analysis of *in vivo* metabolites [13-15], and metabolic profiling [16]. My current laboratory at UNIVPM is equipped for cloning, production, and structural analysis of recombinant proteins from bacteria and yeast. It is staffed by five colleagues or staff members, along with graduate and undergraduate students who develop their thesis work annually.

In recent years, I've been collaborating with an expert in programmed axon death (PAXD), Prof. Michael P. Coleman at the University of Cambridge (<https://neuroscience.cam.ac.uk/member/mcoleman/>), and his group. Our collaboration began in 2011, and since then, we have jointly secured funding from UK research councils and partly from the multinational company AstraZeneca, resulting in 15 publications (see references [16-30]), all highly relevant to the current project. The main grants supporting this collaboration are:

2022/2026 - Wellcome Trust Collaborative Award 220906/Z/20/Z

Coleman (PI), Role: Research Unit co-PI (60000 pounds - collaboration agreement G104817)

Project: Preventable axon degeneration in human disease

2019/2021 - BBSRC Industrial Partnership Award BB/S009582/1

Coleman (PI), Role: Research Unit co-PI (96000 pounds - collaboration agreement G100252)

Project: The regulation of axon degeneration by SARM1

2016/2019 - Medical Research Council MR/N004582/1

Coleman (PI), Role: Research Unit co-PI (40417 pounds - collaboration agreement RG82756-10440)

Project: Variability in Human Axon Survival

The current application logically builds on my prior work, and I believe I possess the necessary expertise, leadership, training, motivation, and experience to successfully carry out the proposed research project. I am fully aware of the importance of developing a realistic research plan, timeline, and budget, as well as maintaining frequent communication among project members and sharing data openly. In the past, I

have successfully managed other projects as PI or co-Investigator (e.g. staffing, budgeting, and both progress and final reporting), funded by my university or the Italian MUR, collaborating with other researchers, and publishing peer-reviewed articles based on the data obtained.

Citations:

1. Magni, G., et al., *Enzymology of NAD⁺ homeostasis in man*. Cell Mol Life Sci, 2004. **61**(1): p. 19-34.
2. Magni, G., et al., *Structure and function of nicotinamide mononucleotide adenylyltransferase*. Curr Med Chem, 2004. **11**(7): p. 873-85.
3. Magni, G., G. Orsomando, and N. Raffaelli, *Structural and functional properties of NAD kinase, a key enzyme in NADP biosynthesis*. Mini Rev Med Chem, 2006. **6**(7): p. 739-46.
4. Magni, G., et al., *Enzymology of mammalian NAD metabolism in health and disease*. Front Biosci, 2008. **13**: p. 6135-54.
5. Magni, G., et al., *NAD(P) biosynthesis enzymes as potential targets for selective drug design*. Curr Med Chem, 2009. **16**(11): p. 1372-90.
6. Ruggieri, S., et al., *Regulation of NAD biosynthetic enzymes modulates NAD-sensing processes to shape mammalian cell physiology under varying biological cues*. Biochim Biophys Acta, 2015. **1854**(9): p. 1138-49.
7. Orsomando, G., et al., *PcF protein from Phytophthora cactorum and its recombinant homologue elicit phenylalanine ammonia lyase activation in tomato*. Cell Mol Life Sci, 2003. **60**(7): p. 1470-6.
8. Orsomando, G., et al., *Phytotoxic protein PcF, purification, characterization, and cDNA sequencing of a novel hydroxyproline-containing factor secreted by the strawberry pathogen Phytophthora cactorum*. J Biol Chem, 2001. **276**(24): p. 21578-84.
9. Nicastro, G., et al., *Solution structure of the phytotoxic protein PcF: the first characterized member of the Phytophthora PcF toxin family*. Protein Sci, 2009. **18**(8): p. 1786-91.
10. Orsomando, G., et al., *Comparative structural and functional characterization of putative protein effectors belonging to the PcF toxin family from Phytophthora spp*. Protein Sci, 2011. **20**(12): p. 2047-59.
11. Zamporlini, F., et al., *Novel assay for simultaneous measurement of pyridine mononucleotides synthesizing activities allows dissection of the NAD⁺ biosynthetic machinery in mammalian cells*. FEBS Journal, 2014. **281**(22): p. 5104-5119.
12. Orsomando, G., et al., *Plant gamma-glutamyl hydrolases and folate polyglutamates: characterization, compartmentation, and co-occurrence in vacuoles*. J Biol Chem, 2005. **280**(32): p. 28877-84.
13. Amici, A., et al., *Synthesis and Degradation of Adenosine 5'-Tetraphosphate by Nicotinamide and Nicotinate Phosphoribosyltransferases*. Cell Chem Biol, 2017. **24**(5): p. 553-564 e4.
14. Orsomando, G., et al., *Evidence for folate-salvage reactions in plants*. Plant J, 2006. **46**(3): p. 426-35.
15. Akhtar, T.A., et al., *A central role for gamma-glutamyl hydrolases in plant folate homeostasis*. Plant J, 2010. **64**(2): p. 256-66.
16. Mori, V., et al., *Metabolic profiling of alternative NAD biosynthetic routes in mouse tissues*. PLoS One, 2014. **9**(11): p. e113939.
17. Conforti, L., et al., *Reducing expression of NAD⁺ synthesizing enzyme NMNAT1 does not affect the rate of Wallerian degeneration*. Febs J, 2011. **278**(15): p. 2666-79.
18. Gilley, J., et al., *Absence of SARM1 rescues development and survival of NMNAT2-deficient axons*. Cell Rep, 2015. **10**(12): p. 1974-81.
19. Di Stefano, M., et al., *A rise in NAD precursor nicotinamide mononucleotide (NMN) after injury promotes axon degeneration*. Cell Death Differ, 2015. **22**(5): p. 731-42.
20. Di Stefano, M., et al., *NMN Deamidase Delays Wallerian Degeneration and Rescues Axonal Defects Caused by NMNAT2 Deficiency In Vivo*. Curr Biol, 2017. **27**(6): p. 784-794.
21. Loreto, A., et al., *Neurotoxin-mediated potent activation of the axon degeneration regulator SARM1*. Elife, 2021. **10**.

22. Huppke, P., et al., *Homozygous NMNAT2 mutation in sisters with polyneuropathy and erythromelalgia*. Exp Neurol, 2019. **320**: p. 112958.
23. Lukacs, M., et al., *Severe biallelic loss-of-function mutations in nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2) in two fetuses with fetal akinesia deformation sequence*. Exp Neurol, 2019. **320**: p. 112961.
24. Loreto, A., et al., *Mitochondrial impairment activates the Wallerian pathway through depletion of NMNAT2 leading to SARM1-dependent axon degeneration*. Neurobiol Dis, 2020. **134**: p. 104678.
25. Carpi, F.M., et al., *Simultaneous quantification of nicotinamide mononucleotide and related pyridine compounds in mouse tissues by UHPLC-MS/MS*. Separation Science Plus, 2018. **1**(1): p. 22-30.
26. Angeletti, C., et al., *SARM1 is a multi-functional NAD(P)ase with prominent base exchange activity, all regulated by multiple physiologically relevant NAD metabolites*. iScience, 2022. **25**(2): p. 103812.
27. Antoniou, C., et al., *Chronically Low NMNAT2 Expression Causes Sub-lethal SARM1 Activation and Altered Response to Nicotinamide Riboside in Axons*. Mol Neurobiol, 2025. **62**(3): p. 3903-3917.
28. Cirilli, I., et al., *Adaptation of a Commercial NAD⁺ Quantification Kit to Assay the Base-Exchange Activity and Substrate Preferences of SARM1*. Molecules, 2024. **29**(4).
29. Llobet Rosell, A., et al., *The NAD⁺ precursor NMN activates dSarm to trigger axon degeneration in Drosophila*. Elife, 2022. **11**.
30. Orsomando, G., et al., *Simultaneous single-sample determination of NMNAT isozyme activities in mouse tissues*. PLoS One, 2012. **7**(12): p. e53271.

B. Positions, Scientific Appointments and Honors

2023 - 2034	Habilitation ASN from the Italian MUR as Full Professor of Biochemistry
2023 - Present	Associate Professor (BIO/10 Biochemistry), UNIVPM, Ancona, Italy
2022 - Present	Editorial Board MOLECULES (ISSN 1420-3049), Section Chemical Biology
2018 - Present	Member, Ph.D. committee "Scienze Biomediche", UNIVPM, Italy
2017 - 2028	Habilitation ASN from the Italian MUR as Associate Professor of Biochemistry
2004 - 2006	Exchange Visitor Research Associate with Fellow from Andrew D. Hanson, C.V. Griffin, Sr. Eminent Scholar, Horticultural Sciences Department, University of Florida Gainesville, USA
2002 - 2008	Member, Ph.D. committee "Biotecnologie Biomediche", UNIVPM, Italy
2001 - 2002	Member, Ph.D. committee "Alimenti e Salute", UNIVPM, Italy
2000 - 2023	Assistant Professor (BIO/10 Biochemistry), UNIVPM, Italy
2000 - Present	Member, Italian Society of Biochemistry (SIB)
1998 - 2000	Fellow, Postdoctoral from the University of Ancona (UNIVPM, Italy)
1992 - 1996	Fellow, Ph.D. from the University of Camerino (UNICAM, Italy)

C. Contributions to Science

I am the co-author of 51 publications in peer-reviewed journals, 9 preprints on BioRxiv, and 41 abstracts from national and international meetings (including 14 in ISSN journals). A complete list of my published work in IRIS can be seen at <https://iris.univpm.it/cris/rp/rp02416>. Additionally, I serve as a reviewer upon request for scientific journals such as Biochemistry, Cell Chemical Biology, Food Chemistry, GeroScience, Molecules, Tetrahedron Letters, and others.

ORCID ID: [0000-0001-6640-097X](https://orcid.org/0000-0001-6640-097X); SCOPUS author ID: 6603108532
 total IF (ISI) 230; H-index (SCOPUS) 30; total citations (SCOPUS) 2650

When I began working at the Polytechnic University of Marche (UNIVPM) in Ancona in February 1998, my research focused on plant nutrition and host-pathogen interactions. This work led to the discovery of a new family of oomycete protein effectors, the *PcF Toxin Family*, which activates defense responses in certain plant cultivars. The characterization of these proteins has progressed up to the determination of their 3D structures (see references [7-10] above).

During my sabbatical leave at the University of Florida, from January 2004 to December 2006, I joined Andrew Hanson's laboratory, where I concentrated on plant metabolism, specifically on folate turnover and salvage in plants, with a focus on γ -glutamylhydrolase (GGH). I also contributed to uncovering unique folate salvaging reactions in plants. This research on folate resulted in two first-author peer-reviewed publications (see references [14-15] above).

Upon returning home, I continued to pursue my main research interest in NAD metabolism in mammals, both in health and disease. My interest in this area began at the University of Camerino in 1991, where I initially trained for two years as an undergraduate and then for four years as a Ph.D. student, working on NAD-related key enzymes –specifically their purification, assay, kinetics, and structure. Over the years, this work has led to reviews (see references [1-6] above) and original research articles on NAD-related enzymes such as NAMPT, NMNAT, NADS, and SARM1 (see references [13,16,26,30] above), where I served as the principal investigator or corresponding author.

In the last 15 years, my NAD enzymology expertise has been focused on axonal models of PAXD (Wallerian Degeneration) in collaboration with Prof. Michael P. Coleman at the University of Cambridge (UK). Our goal has been to uncover the molecular mechanisms underlying various PAXD-related neuronal disorders in humans. Together, we clarified the role of NMNAT2 in PAXD (see references [22-24,27] above) and, well before SARM1 was known to be involved, we first proposed NMN as a key initial trigger for PAXD (see references [19,20,29]. Additionally, we studied SARM1 (see references [18,21,24] above) and its regulation by pyridine metabolites, along with its involvement in base exchange reactions (transglycosylation) for the potential *in vivo* synthesis of physiologically relevant or PAXD-related NAD(P) analogues (see reference [26] above). This latter aspect of my research has resulted in two cited papers as corresponding author (see references [19,26] above) and is highly relevant to the proposed research project.