

## BIOGRAPHICAL SKETCH

NAME: Maria Antonietta Vanoni

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

POSITION TITLE: Full Professor of Biochemistry

### EDUCATION/TRAINING

| INSTITUTION AND LOCATION          | DEGREE<br>(if applicable) | Completion Date<br>MM/YYYY | FIELD OF STUDY       |
|-----------------------------------|---------------------------|----------------------------|----------------------|
| Liceo R. Oppenheimer              | Baccalaureat              | 07/1976                    | Science              |
| Universita' degli Studi di Milano | Laurea                    | 10/1980                    | Biology/Biochemistry |

### A. Personal Statement

After earning a degree in Biological Sciences (Biochemistry) at the University of Milano (1980), MAV was postdoctoral associate with Prof. Rowena G. Matthews at the Biophysics Research Division and Department of Biochemistry of the University of Michigan (Ann Arbor, MI, USA, 1980-1983), Researcher (Assistant Professor, University of Milano, 1983-2000), Associate Professor (University of Insubria, 2000-2002, and University of Milano, 2002-2003). Since 2003, MAV is Professor of Biochemistry at the Department of Biosciences (DBS) of the University of Milano UNIMI).

As highlighted in the next section, research work is curiosity-driven and the focus is on structure-function relations of enzymes, especially – but not only – flavoenzymes of interest for fundamental science as well as their applications.

Each system has been (is being) studied by combining equilibrium and time-resolved spectroscopies, steady-state and pre-steady-state kinetics, protein engineering and structural approaches in the home lab or through *ad hoc* collaborations. The latter led, over time to gain (often hands-on) experience on biophysical (EPR, NMR) and computational (classical molecular dynamics and *ab initio* simulations of enzyme-catalyzed reactions) work, beside structural approaches (MX, SAXS and cryoEM).

A common theme deals with the mechanisms of regulation and self-regulation of activity in several of the enzymes, especially glutamate synthases and human MICAL1.

The expertise in mechanistic studies of flavoenzymes has been exploited to help colleagues to dissect their systems within collaborations on, e.g.:

- Ferredoxin NAD(P) reductases and the Apoptosis Inducing Factor with Prof. Alessandro Aliverti, (DBS) (see e.g.: Sorrentino L, et al. (2015) Key Role of the Adenylate Moiety and Integrity of the Adenylate-Binding Site for the NAD(+)/H Binding to Mitochondrial Apoptosis-Inducing Factor. *Biochemistry* 54:6996-70);
- (histone) lysine demethylase with Prof. A Mattevi (Pavia) (Forneris F et al. (2005) Human histone demethylase LSD1 reads the histone code. *J Biol Chem.* 280:41360-5);
- monoamine oxidase A with Prof. Claudia Binda and Andrea Mattevi (Pavia) (Iacovino LG et al. (2020) Rational Redesign of Monoamine Oxidase A into a Dehydrogenase to Probe ROS in Cardiac Aging. *ACS Chem Biol.* 15:1795-1800).

Expertize in enzymology also led to collaborations with physicists, structural or cell biologists to study, e.g.:

- the folding mechanism of HIV1 protease with Profs. Broglia, Tiana (Physics Department, UNIMI) (Rösner HI et al. (2022) The denatured state of HIV-1 protease under native conditions. *Proteins* 90:96-109; Rösner HI et al. (2017) Cold Denaturation of the HIV-1 Protease Monomer. *Biochemistry.* 56:1029-1032);
- the role of glutamine synthetase in Huntington's disease with Prof. Paola Bellosta (now University of Trento) (Vitali T et al (2023) Quantitation of Glutamine Synthetase 1 Activity in *Drosophila melanogaster*. *Methods Mol Biol.* 2675:237-260; Vernizzi L, et al. (2020) Glutamine Synthetase 1

Increases Autophagy Lysosomal Degradation of Mutant Huntingtin Aggregates in Neurons, Ameliorating Motility in a Drosophila Model for Huntington's Disease. Cells. 9:196);

MAV has been engaged in *ad hoc* collaborations with private companies in order to develop robust specific enzymatic assays on systems of biotechnological and biomedical applications (see below).

## **B. Positions, Scientific Appointments, and Honors**

### POSITIONS

- |                |                                                                                                                                                     |
|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|
| 2003 – present | Professor of Biochemistry, University of Milano, Milano, Italy                                                                                      |
| 2002- 2003     | Associate Professor of Biochemistry, University of Milano, Milano, Italy                                                                            |
| 2000 - 2002    | Associate Professor of Biochemistry, Università dell' Insubria, Como, Italy                                                                         |
| 1992 – 2000    | Lecturer, Biological Chemistry, School of Chemistry, Università dell' Insubria (formerly, Como Branch, University of Milano) Como.                  |
| 1983 - 2000    | Ricercatore (Assistant Professor), Department of General Physiology and Biochemistry, University of Milano, Milano.                                 |
| 1981- 1983     | Post-doctoral Research Associate, Biophysics Research Division and Department of Biological Chemistry, University of Michigan, Ann Arbor, MI (USA). |
| 1990 - 1997    | Visiting Scientist, Emory University, School of Medicine, Atlanta, (Several 1-2 months stays)                                                       |
| 1989           | Visiting Scientist, University of Michigan, Ann Arbor and University of Washington, Seattle (on a leave of absence, 2 months)                       |
| 1988           | Visiting Scientist, Institut Pasteur, Paris (F, 2 months)                                                                                           |
| 1987           | Visiting Assistant Professor, University of Michigan, Ann Arbor, MI (USA) (on a leave of absence, 2 months)                                         |
| 1985           | Visiting Scientist, Albert Einstein College of Medicine, Yeshiva University, Bronx, NY (USA) (on a leave of absence, 3 months)                      |

### Professionally relevant activities:

- |                |                                                                                                                                                                                                                                                                                                                                                                                                                               |
|----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1986 - present | Member of the Organising Committee of National and International Conferences                                                                                                                                                                                                                                                                                                                                                  |
| 1990 – present | Invited Speaker at several institutions and conferences mainly abroad.                                                                                                                                                                                                                                                                                                                                                        |
| 1990 – present | Reviewer for Archives Biochemistry and Biophysics (Member of Editorial Advisory Board since 2002); Biochimica Biophysica Acta; Biochemistry; FEBS Journal (previously European Journal of Biochemistry, Member of Editorial Advisory Board 2000 - 2008); FEMS Microbiology Letters; FEBS Letters; Protein Sciences; Journal of Biological Chemistry; Journal of Bacteriology; Scientific Reports; Frontiers (Editor); others. |
| 1996 – present | Member of PhD thesis Committees in Italy and, especially, abroad; member of selection and/or promotion committees of faculty in Italy and abroad.                                                                                                                                                                                                                                                                             |
| 1999 -present: | <i>Ad hoc</i> reviewer Fund for Scientific Research - Flanders, Belgium, External Reviewer (1999); European Commission, Brussels, Belgium (2001- ); Ministero della Ricerca Scientifica e tecnologica/Ministero dell'Università', Rome, Italy (2005- ); University of Milano, Verona, Insubria, Rome; Dutch National Science Foundation (2010); Wellcome Trust (2010); French National Research Agency (2021)                 |
| 2004-present   | Lecturer and Discussion Leader of several EMBO Courses or EMBO Global Lectures on "Solution scattering from biological macromolecules" and "Integrative structural biology" organized by the EMBL Outstation-Hamburg                                                                                                                                                                                                          |
| 2006-2019      | Funder and co-organiser of the biannual "Trends in Enzymology" Conference, converted in 2019 into an Advanced School. The activity has been interrupted due to the COVID pandemics.                                                                                                                                                                                                                                           |
| 2008-present:  | Component of the Priority evaluation Committee for the EMBL-Hamburg beamlines at DESY-PETRA III synchrotron, which also serves as advisory committee to the staff devoted to service to the user's community.                                                                                                                                                                                                                 |

### HONORS

- 2007 - Premio Marotta - Accademia dei XL, Rome.

## C. Contributions to Science

Only key findings, and selected publications (since 2015) are cited.

After the initial training in the laboratory of Prof. Rowena G. Matthews (University of Michigan, 1980-1983, 1987, 1989) where MAV carried out kinetic and mechanistic studies on mammalian methylenetetrahydrofolate reductase (MTHFR) and on its allosteric regulation by adenosylmethionine, the main research interests have been:

**1. Mammalian D-amino acid oxidase (DAAO).** DAAO is a flavoenzyme that catalyzes the oxidation of D- $\alpha$ -amino acids with concomitant production of the corresponding  $\alpha$ -ketoacid, ammonia and hydrogen peroxide. In spite of the fact that it has been studied for over 9 decades, and it has been a model enzyme for the understanding of the mechanism of action of other flavin-dependent enzymes, several questions remained unanswered until recently, and some still are. With Prof. Curti (Milano), Mattevi (Pavia) and Fois (Computational Chemistry, Università dell'Insubria@Como), MAV contributed to the determination of the first three-dimensional structure of the enzyme, which, together with pioneering *ab initio* computational studies of the reaction mechanism, set the decades long question of the chemical mechanism of the reaction that we showed to proceed by hydride transfer rather than through a carbanion intermediate. These results have been of great value in the field of mechanistic flavoenzymology, in general, and for biotechnological and biomedical applications of DAAO (e.g. semisynthesis of cephalosporins, removal of unnatural synthetic D-isomers of amino acids from racemic mixtures) and, mostly, the design of drugs of potential use in neurological disorders (e.g.: schizophrenia)

Familiarity with D- and L-amino acid oxidases recently led to contribute to the characterization of members of the novel class of the ubiquitous and highly conserved imine detoxifying enzymes of the RidA family, the physiological role of which is still poorly understood, (with Prof. Laura Popolo and Stefano Ricagno). Indeed the (short-lived) imine substrate of RidA is produced by D- and L-amino acid oxidases. Our kinetic approach is complementing spectroscopic (CD) and structural (MX) work aimed to understand the substrate specificity and exceptional structural stability of native RidA forms and their engineered variants.

- Rizzi G, et al (2024) Site-directed mutagenesis reveals the interplay between stability, structure, and enzymatic activity in RidA from *Capra hircus*. *Protein Sci.* 33:e5036.
- Visentin C et al. (2022) *Apis mellifera* RidA, a novel member of the canonical YigF/YER057c/UK114 imine deiminase superfamily of enzymes pre-empting metabolic damage. *Biochem Biophys Res Commun.* 616:70-75.
- Digiovanni S et al. (2021) Using D- and L-Amino Acid Oxidases to Generate the Imino Acid Substrate to Measure the Activity of the Novel Rid (Enamine/Imine Deaminase) Class of Enzymes. *Methods Mol Biol.* 2280:199-218.
- Digiovanni S et al. (2020) Two novel fish paralogs provide insights into the Rid family of imine deaminases active in pre-empting enamine/imine metabolic damage. *Sci Rep.* 10:10135.
- Degani G. et al. (2018) Imine Deaminase Activity and Conformational Stability of UK114, the Mammalian Member of the Rid Protein Family Active in Amino Acid Metabolism. *Int J Mol Sci.* 19:945.

**2. Glutamate synthase (GltS).** GltS are complex iron-sulfur flavoenzymes, which participate with glutamine synthetase to ammonia assimilation processes in bacteria, microorganisms, plants and lower animals. The properties of the bacterial NADPH-dependent GltS and plant ferredoxin-dependent form have been studied in the laboratory by means of site-directed mutagenesis, kinetics, spectroscopies (UV-Vis; NMR and EPR with Prof. D. Edmondson, Atlanta), computational (with V. Coiro, Rome) and structural biology [MX with A. Mattevi (Pavia), SAXS with D. Svergun (EMBL@Hamburg), cryoEM with N. Boisset (Paris) and later P. Swuec (DBS/UNIMI)] leading to the current model of the enzyme.

GltS is an amidotransferase that transfers the glutamine amide group from glutamine to 2-oxoglutarate in a reaction that is subject to a tight mechanism of control and coordination of the partial activities that take place at the three catalytic sites of the enzyme. NADPH binds to the small ( $\beta$ ) subunit (~50 kDa) of GltS and reduces the FAD coenzyme; electrons are transferred to the FMN coenzyme at the synthase site on the large ( $\alpha$ ) subunit (~150 kDa) through two low

potential 4Fe/4S clusters and one 3Fe/4S center. Reduced FMN converts the 2-iminoglutarate formed upon addition of  $\alpha$ -ketoglutarate and ammonia to L-glutamate. Ammonia derives from L-glutamine hydrolysis at the glutaminase site through the 30 Å-long intramolecular ammonia tunnel (on the  $\alpha$  subunit). The activities of the glutaminase and synthase across the tunnel are strictly controlled, so that no wasteful hydrolysis of glutamine takes place in the absence of  $\alpha$ -ketoglutarate and reducing equivalents at the synthase site.

The mechanistic and structural work on GltS shed light on the several features of the reaction highlighted above, which are also relevant for the understanding of how other amidotransferase and flavin and iron-sulfur centers-dependent enzymes work.

For example, dihydropyrimidine dehydrogenase (DPD), which we have studied in collaboration with Drs. Schnackerz (Wuerzburg) and Hagen (Wageningen), shares with GltS and few other enzymes a domain (whose prototype is the  $\beta$  subunit of GltS) carrying the NADPH oxidizing site, the FAD coenzyme and two unusual low potential 4Fe-4S clusters, as well as a complex intramolecular electron transfer pathway with a mechanism of (self) control of the activities taking place at distant catalytic subsites, which involves both binding of the substrates and the redox state of the cofactors.

Interestingly, we have shown that GltS contains a native [3Fe-4S]<sup>0,+1</sup> cluster, which participates in the redox process, demonstrating for the first time that this type of clusters do exist in nature as physiological redox centers, and are not purification artifacts.

Furthermore, it was with GltS that a novel energy conservation mechanism, now called "Flavin-based Electron bifurcation", was initially proposed. The latter was, however, ruled out by structural work by small angle-X-ray scattering (with Dr. Dmitri Svergun, EMBL-Hamburg, since 2003) combined with cryoEM (first with Dr. Nicolas Boisset, Paris VI (2008) and, more recently, with Dr. Paolo Swec at UNIMI), but the mechanism has now been proven in several bacterial systems. It should be noted that in 2008 only a handful of structures beside GltS had been solved at a resolution of less than 1 nm by cryoEM.

- Swec P, Chaves-Sanjuan A, Camilloni C, **Vanoni MA**, Bolognesi M. (2019) Cryo-EM Structures of *Azospirillum brasilense* Glutamate Synthase in Its Oligomeric Assemblies. *J Mol Biol.* 2019;43:4523-4526.

- **Vanoni MA**. (2021) Iron-sulfur flavoenzymes: the added value of making the most ancient redox cofactors and the versatile flavins work together. *Open Biol.* 11:210010.

3. Human MICAL-1 belongs to the class of multidomain, mainly cytosolic, MICAL enzymes that participate in the control of actin cytoskeleton dynamics by catalyzing a H<sub>2</sub>O<sub>2</sub>-producing NADPH oxidase reaction. The latter is catalyzed by the N-terminal FAD-containing domain (MO) that is followed by domains that are believed to be implicated in the tight control of the, otherwise potentially harmful, catalytic activity.

MICALs are being implicated in a growing number of processes (e.g.: axon growth/steering, cell migration, completion of cell division, control of gene expression). MICAL1 role has recently been reinforced by its direct implication in disease (epithelial-mesenchymal transition of metastatic cells, generation of oxidative stress in cancer cells, hereditary epilepsy forms).

Using an *in vitro* approach on isolated enzyme forms, which complements work of others mainly based on genetic and cell biology approaches, the work in MAV lab is focusing on the understanding of how precisely MICAL1 catalyzes *the unprecedented NADPH-dependent F-actin depolymerization reaction*, which is the *mechanism of autoinhibition* of MICAL, and *how proteins and (signaling) metabolites control its activation state*. Our data revealed that, contrary to findings of others, MICAL catalytic domain always functions as H<sub>2</sub>O<sub>2</sub>-producing NADPH-oxidase, and that it is the *in situ* (enhanced) production of H<sub>2</sub>O<sub>2</sub> that is responsible of oxidation of actin residues, which weakens the filament and promotes its dissociation. Mechanistically, solvent viscosity effects revealed the presence of a partially rate determining conformational change preceding FAD reduction by NADPH. Such step is tentatively identified with the repositioning of Trp400, which acts as a gate interfering with productive binding of the NADPH nicotinamide ring. The approach may have broad application in flavoenzymes of different classes that, however, share with MICAL's catalytic (MO) domain similar "gating" residues.

Kinetic experiments and small-angle X-ray scattering measurements coupled to size exclusion chromatography (SEC-SAXS) with Dr. D. Svergun (EMBL@Hamburg) have been used to structurally characterize MICAL forms, and to determine MICAL1 interaction with the active form of the small GTPase Rab8. We confirmed that Rab8 indeed binds and activates full-length MICAL,

but not to forms lacking the C-terminal region (RBD from Rab binding domain). MICAL1 was found to form a 1:1 complex with Rab with a  $K_d$  in the  $\mu\text{M}$  range, while a 2:1 Rab/RBD stoichiometry with dissociation constants in the nM range were found by others with the isolated RBD. Based on the SEC-SAXS data, the first model of full-length autoinhibited MICAL has been proposed, as well as that of the Rab-activated form.

The experiments led to the recent (and ongoing) work aiming at the determination of a high resolution structure of MICAL by cryoEM (in collaboration with Dr. Antonio Chaves, and Diane Bonnet, Department of Biosciences and UNITECH, No LIMITS, UNIMI). The inspection of the MICAL1 models is guiding us towards a re-evaluation of the current model of its (self)regulation, and extension of the work to further clarify MICAL's mechanistic and regulatory properties. The expertise acquired in actin biochemistry and handling has been made available to Dr. Matteo de Rosa and co-workers (Institute of Biophysics, National Research Council) in order to allow them to study the effect of pathological mutations of gelsolin on its interaction with F-actin.

- Vitali T, Maffioli E, Tedeschi G, **Vanoni MA**. (2016) Properties and catalytic activities of MICAL1, the flavoenzyme involved in cytoskeleton dynamics, and modulation by its CH, LIM and C-terminal domains. Arch Biochem Biophys. 2016 Mar 1;593:24-37.

- **Vanoni MA**. (2017) Structure-function studies of MICAL, the unusual multidomain flavoenzyme involved in actin cytoskeleton dynamics. Arch Biochem Biophys, 632:118-141

- Esposito A, Ventura V, Petoukhov MV, Rai A, Svergun DI, **Vanoni MA**. (2018) Human MICAL1: Activation by the small GTPase Rab8 and small-angle X-ray scattering studies on the oligomerization state of MICAL1 and its complex with Rab8. Protein Sci. 2019; 28(1):150-166.

- de Rosa M, Barbiroli A, Boni F, Scalone E, Mattioni D, **Vanoni MA**, Patrone M, Bollati M, Mastrangelo E, Giorgino T, Milani M. (2020) The structure of N184K amyloidogenic variant of gelsolin highlights the role of the H-bond network for protein stability and aggregation properties. Eur Biophys J. 49:11-19.

4. **Human HDAC6 inhibitors**. Histone deacetylase 6 (HDAC6) is perhaps the most complex and least understood among HDACs because of the presence of two catalytic domains, the individual properties of which are still poorly understood. The degree of cross-talk between them is also poorly understood. The search of inhibitors that are highly selective for specific HDAC forms is an active field of research in academic and industrial settings.

Within a collaboration with a pharmaceutical company, MAV is contributing to set-up kinetic assays and spectroscopic methods suitable to characterize novel inhibitors. Interestingly, we found that a common – but too often disregarded - feature of even well-characterized HDAC inhibitors is their slow binding/slow release behavior. Therefore, inhibition studies in academic and industrial screenings for novel potential drugs need to take such “time factor” into account to provide sound information. In this context, MAV contributed to characterize a novel class of highly specific HDAC6 inhibitors that function as slow substrates, and lead to a tightly bound (inhibitory) intermediate.

- Cellupica E, Caprini G, Fossati G, Mirdita D, Cordella P, Marchini M, Rocchio I, Sandrone G, Stevenazzi A, Vergani B, Steinkühler C, **Vanoni MA**. (2023) The Importance of the "Time Factor" for the Evaluation of Inhibition Mechanisms: The Case of Selected HDAC6 Inhibitors. Biology (Basel). 12:1049.

- Cellupica E, Caprini G, Cordella P, Cukier C, Fossati G, Marchini M, Rocchio I, Sandrone G, **Vanoni MA**, Vergani B, Žrubek K, Stevenazzi A, Steinkühler C. (2023) Difluoromethyl-1,3,4-oxadiazoles are slow-binding substrate analog inhibitors of histone deacetylase 6 with unprecedented isotype selectivity. J Biol Chem. 299:102800.