

Curriculum Vitae Nicolò Angeleri

Name: Nicolò Angeleri

Education/training

| INSTITUTION AND LOCATION | DEGREE | Start Date | Completion Date | FIELD OF STUDY |
|---------------------------------|-------------|------------|-----------------|--------------------------------------|
| I.S. Sobrero, Casale Monferrato | High school | 09/2015 | 06/2020 | Liceo Scientifico, Scienze applicate |
| University of Pavia, Pavia | BS | 10/2020 | 07/2023 | Biotechnology |
| University of Pavia, Pavia | MS | 10/2023 | 07/2025 | Molecular Biology and Genetics |

A. Personal Statement

I have been a curious person for as long as I can remember. Initially driven by enthusiastic reading about marine life, then deeply interested in the chemical, biochemical and structural elements of life. Through my undergraduate and graduate research experiences I have focused on fields related to molecular biology and structural biochemistry, gaining expertise in a range of experimental and computational techniques such as protein purification and crystallography but also computational structural analysis and bioinformatics tools. My academic journey also helped me to acquire important team working skills and learn efficient communication with colleagues.

What drives me is the opportunity to explore biological systems at the molecular level and to contribute to solving complex scientific problems. I thrive in multidisciplinary environments where collaboration across fields leads to deeper insights and innovative outcomes. My academic path has taught me not only how to be technical rigorous but also trained my ability to be persistent and adaptable.

Through this PhD program, I aim to advance my research skills and broaden my scientific perspective. I am particularly drawn to the program's interdisciplinary approach, which aligns with my belief that complex biological questions benefit from diverse scientific viewpoints. I hope to bring my analytical mindset, collaborative spirit, and commitment to scientific growth to your research environment.

B. Positions, Previous experiences, and Honors

Positions and previous experiences

| From – to | Description |
|-------------------|--|
| 02/2022 – 07/2022 | Part time at University of Pavia at the Earth Science department under the guidance of Michela Comensoli. |
| 02/2023 – 07/2023 | Bachelor thesis performed at the laboratory of Biochemistry of the platelets. PI: Professor Gianni Guidetti. Thesis Title: "Analysis of Platelet Activation Induced by CPG Oligonucleotides in Wild-Type and CD93 Knockout Mouse Models" |
| 02/2025 | Participation to Erasmus+ BIP in redox biology in health and disease at the University of Coimbra, Barcelona. |
| 09/2023 – 07/2025 | Master thesis at the laboratory of Structural Biology. PI: Professor Andrea Mattevi. Thesis Title: "Structural and biochemical analysis of SorC and SorD: flavin-dependent enzymes involved in sorbicillinoid production" |

Honors

| Date | Description |
|---------|--|
| 09/2018 | "Young RM Ambassador" by RM@School at the International Science Fair 2018 at Wetsus (Leeuwarden, NL) |
| 03/2020 | Winner of "I Giovani e le Scienze" 2020, FAST (Milano, IT) |

C. Contributions to Science

1. High School Research:

Participation to the project "Prussian Blue: A simple pigment turned into the colour of recycling" presented at the International Science Fair 2018 from RM@School at Wetsus (Leeuwarden, NL). In collaboration with the CNR in Milan, we chemically synthesized Prussian blue, a well-known pigment with potential for absorbing heavy metal ions such as cesium and thallium. We qualitatively tested its metal-absorption capacity with other metals (cobalt, copper, and manganese) using a simple chromatographic setup, where Prussian blue acted as the stationary phase and the metal solutions as the mobile phase. The filtered solutions were then tested by adding hexacyanoferrate, which forms colored complexes with these metals. The absence of color indicated that the pigment successfully retained the metal ions, effectively removing them from the solution. This simple experiment highlights the potential of Prussian blue as a didactic tool to introduce students to water purification and recycling concepts, as well as underlining a promising future in industrial applications, such as wastewater treatment in the metallurgy sector.

Participation to the project "C.A.E.S.A.R.: Chromium Aquo-ions Estraction with Saponite And Recovery" in collaboration with Università del Piemonte Orientale (UPO, Alessandria IT), presented at the science fair "I Giovani e le Scienze" 2020 at Milano. The research focused on the use of a synthetic lamellar clay (Na-SAP-20) produced via hydrothermal synthesis to remove Cr(III) ions from aqueous solutions. The project focused on addressing heavy metal pollution using cost-effective and sustainable materials. The absorption capacity of the synthetic clay was evaluated through UV-vis spectrophotometry and NMR, and compared with a commercial natural clay (MMT K10). Results demonstrated significantly higher efficiency of Na-SAP-20 in capturing chromium ions, confirming its potential for use in environmental remediation. The study emphasized green chemistry principles, including potential material reuse and ion recovery.

2. Undergraduate Research:

Bachelor thesis regarding platelet activation under CpG-ODN stimulation.

Platelets are small, anucleate blood cells essential for hemostasis and thrombosis, but they also participate in immune responses by recognizing pathogen-associated molecules (PAMPs) and damage signals (DAMPs). Among these are unmethylated CpG DNA fragments, common in bacterial DNA, which strongly activate the innate immune system. My Bachelor thesis focuses on the glycoprotein CD93 on platelets. Evidence shows that CD93 can bind DNA and synthetic CpG oligonucleotides (CpG-ODNs), suggesting a role in immune signaling. We investigated whether the receptor CD93 influences platelet activation and aggregation in response to CpG-ODNs, using wild-type and CD93 knockout mouse platelets. Platelet aggregation was measured using Born's turbidimetric method and protein activation was assessed via immunoblotting. Results show that CpG-ODNs can induce aggregation even without CD93, indicating it is not essential for this function. However, the absence of CD93 significantly reduced phosphorylation of key signaling proteins, suggesting that CD93 plays a crucial role in specific platelet activation pathways. This indicates a potential function of CD93 in the immune activity of platelets. This receptor could be involved in the release of antimicrobial molecules, such as reactive oxygen species and complement proteins.

3. Graduate Research:

Master thesis on FAD-dependent enzymes, SorC and SorD, involved in sorbicillinoid biosynthesis.

Flavoproteins are a class of enzymes that require a flavin cofactor, either flavin adenine dinucleotide (FAD) or flavine mononucleotide (FMN), to carry out a wide variety of chemical transformations. Within this class, oxidases and monooxygenases are able to react with molecular oxygen as a critical step in their catalytic cycle. These enzymes can perform a diverse range of activities and are often involved in biosynthetic pathways for the production and modification of small molecules, such as sorbicillinoids. Sorbicillinoids are a family of secondary metabolites produced by a few fungal genera that have gained scientific interest for their biological potential as radical scavengers and anticancer agents. The biosynthetic pathway of sorbicillinoids has only been recently elucidated and revolves around the production and modifications of sorbicillin, the primary monomer used to produce more complex sorbicillinoids. Sorbicillin is enzymatically modified by two flavin-dependent enzymes: SorC, a monooxygenase, and SorD, an oxidase. SorC catalyzes the oxidative dearomatization of sorbicillin to produce sorbicillinol. This intermediate is exported into the extracellular matrix by a (MFS)-type transporter called SorT, where it reacts with water to form hydroxyl-sorbicillinol. SorD oxidizes this molecule to produce oxosorbicillinol, a key precursor for the assembly of more complex sorbicillinoids. My master thesis explores the biochemical properties and structural characteristics of SorC and SorD to better understand their roles in this biosynthetic pathway.

SorC was heterologously expressed in *E. coli* and successfully purified using affinity and size exclusion chromatography. The protein was crystallized and its structure solved by X-ray diffraction in both its apo form and in complex with sorbicillin. In the substrate-bound structure, sorbicillin adopts a catalytically relevant binding pose, stabilized within a narrow active site pocket. Biochemical assays demonstrated that SorC functions optimally at pH 8, due to the required substrate deprotonation, and can use both NADPH and NADH as electron donors. The structure-guided identification of Glu245 as catalytic base was validated through mutagenesis experiments. Substitution of Glu245 with alanine, glutamine, or aspartate nearly abolished enzyme activity. For these mutants, minimal activity was still detected, likely due to the pKa of sorbicillin (determined to be ~7.3), which allows for partial activity under slightly basic pH conditions. Nevertheless, Glu245 was shown to be critical, as its presence enhanced catalytic efficiency by a factor of 40. Additional mutants targeting residues involved in FAD stabilization were also studied and resulted in inactive enzymes, underlining their importance. Other single-point mutations of active site residues resulted in reduced but not abolished activity, indicating these residues cooperate for optimal substrate positioning and catalysis.

Regarding SorD, initial attempts to produce the enzyme in recombinant *E. coli* were unsuccessful. In collaboration with the University of Leiden (NL), the oxidase was instead produced in *Aspergillus niger*. Crystals of SorD were obtained and the solved structure revealed a covalent bond between a histidine residue and the FAD cofactor. A lower-resolution structure of SorD bound to sorbicillin was also obtained. Although the physiological substrate of SorD is hydroxyl-sorbicillinol, sorbicillin was used as a structural mimic in cocrystallization experiments in order to hypothesize the catalytic mechanism of SorD.

In summary, this work provides a detailed structural and biochemical analysis of the flavoenzymes SorC and SorD, highlighting their precise roles in the biosynthesis of sorbicillinoids. The findings offer valuable insights into enzyme-substrate interactions, catalytic mechanisms, and the broader potential of flavoprotein-mediated biocatalysis in natural product biosynthesis.

D. Research Outputs

1. • **Peer-Reviewed Journal Publications:** Structural and Mechanistic Characterization of the Flavin-Dependent Monooxygenase and Oxidase Involved in Sorbicillinoid Biosynthesis. Tjallinks G., Angeleri N., Nguyen Q., Mannucci B., Arentshorst M., Visser J., Ram A. F. J., Fraaije M. W., Mattevi A. (2025) *ACS Chemical Biology* 20(3).

2. • **Conference Presentations:** Angeleri, N., Caprioglio, C., Merlo, G., Piccone, S., Poncina, M., Zeppa, A., *Prussian Blue: A simple pigment turned into the color of recycling* [poster]. International Science Fair 2018 at Wetsus, Leeuwarden, Netherlands. September 2018.

E. Skills and Expertise (Technical and Linguistic Competencies)

1. Laboratory and Experimental Techniques:

Strong expertise in DNA-based techniques, including PCR, site-directed mutagenesis (e.g., QuickChange and Golden-Gate), bacterial transformation, DNA extraction, and agarose gel electrophoresis. Skills in protein expression and purification using chromatographic methods and Äkta pure™ systems. Deep knowledge of protein crystallization and structure determination procedures. Experience in protein analysis techniques such as SDS-PAGE, western blotting, spectrophotometry, oximetry, mass photometry, and thermal stability assays (ThermoFAD and Tycho). Basic familiarity with microscale electrophoresis and mass spectrometry sample preparation.

2. Software and IT Tools:

Proficient in the Windows operating system and Microsoft Office suite (Word, PowerPoint, Excel, Publisher). Deep knowledge of structural biology software, including CCP4/CCP4i, Coot, PyMOL, ChimeraX, and Avogadro. Experience with scientific software such as AutoDock Vina, SnapGene and Graphpad. Familiar with basic HTML programming. Additionally, skilled in the use of online scientific databases (UniProt and PDB) and bioinformatics tools (BLAST, AlphaFold and Neurosnap) for protein sequence analysis. Skilled in literature research platforms (Google Scholar and Pubmed) and reference managers (Mendeley).

3. Languages:

1. **Italian:** Native speaker
2. **English:** C1 level

Data 20/08/2025

Firma

