

## CV MARCO ORLANDO

### Personal information

### Positions

- 1<sup>st</sup> October 2021 to present** Adjunct Professor, responsible for the frontal and laboratory lessons of the module "Data Science" (3 cfu) of the course "Biostatistics and Data Science" (<https://www.uninsubria.it/ugov/degrecourse/155227>, 6 cfu) during the academic year 2021/2022 at the Department of Biotechnologies and Life Sciences of the University of Insubria, Italy.
- 1<sup>st</sup> September 2020 to present** Post-Doc Research fellow at the laboratory The Protein Factory 2.0 at the Department of Biotechnologies and Life Sciences of the University of Insubria, Italy (Lab head: prof. Loredano Pollegioni, Supervisor: prof. Gianluca Molla). I am working on two projects: i. PET hydrolyzing enzymes, both in wet lab (enzyme production, kinetic analysis on model substrates and biophysical analysis by circular dichroism) and *in silico* (conservation, docking and dynamic modelling analyses). ii. Optimization of protocols for lipids and proteins extraction from Black Soldier Fly (*Hermetia illucens*) biomass reared on organic waste. My aim in these projects is to participate in improving biotechnological process for the reuse of wastes (plastics or biomasses) within a circular economy framework.
- 1<sup>st</sup> January 2020 to 31<sup>st</sup> August 2020** Post-Doc Research scholarship at the laboratory of protein engineering and industrial enzymology at the Department of Biotechnologies and Biosciences of the University of Milano-Bicocca (Lab head and supervisor: prof. Marina Lotti). I worked with lysozyme enzymes that have a relatively high activity at low temperature, by employing and comparing experimental and computational approaches. My aim in this project was to study the cold-related properties of enzymes by biochemical and biophysical approaches in comparison to their molecular evolution and predicted *in silico* features from models and simulations.
- 1<sup>st</sup> November 2016 to 31<sup>st</sup> October 2019** PhD student in Biology and Biotechnology at the Department of Biotechnologies and Biosciences of the University of Milano-Bicocca, Milan

(Italy), in the laboratory of protein engineering and industrial enzymology (Lab head and supervisor: prof. Marina Lotti). Qualification obtained on 31<sup>st</sup> January 2020 by discussing the thesis with the title “Biochemical and biophysical analysis of two Antarctic lysozyme endolysins and *in silico* exploration of glycoside hydrolase 19 sequence space”.

**October 2018 to  
January 2019**

Adjunct Professor, responsible for the laboratory lessons of the course “Structures and Molecular Interactions” held by Prof. Luca De Gioia, during the academic year 2018/2019 at the Department of Biotechnologies and Biosciences of the University of Milano-Bicocca, Milan (Italy).

## Education & training

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**May 2019 to  
5<sup>th</sup> July 2019**

Visiting PhD student in the laboratory of Juergen Pleiss at the Institute of Biochemistry and Technical Biochemistry of Stuttgart (Germany). I worked on a joint project on setting-up a new SQL-based database of a glycoside hydrolase family (<https://gh19ed.biocatnet.de/>). Bioinformatics tools have been used for analyzing the sequence space, the structures, and study protein evolution; previous information from experimental literature was integrated. The aims of the project were building a new, extended, family classification, finding sequence patterns for predicting activity specificity and detecting evolutionary trends in structural elements.

**21<sup>st</sup> October to  
22<sup>nd</sup> November 2018**

Visiting PhD student in the Innovation Unit at the Elvysys microfluidics innovation center, Paris (France). I got an update on the possible applications of droplet-based microfluidics technique and I had the opportunity to observe and operate with fluid flows under a microscope in presence of experts in the field of droplet-based microfluidics.

**25<sup>th</sup> - 27<sup>th</sup> - 29<sup>th</sup> June 2018**

Course “Fundamentals of Biostatistics” organized by “Scuola di Dottorato” of the University of Milano-Bicocca, Milan (Italy).

**4<sup>th</sup> to 8<sup>th</sup> September 2017**

Summer school “Towards a Bio-based Economy: science innovation, economics, education” at the University of Milano-Bicocca, Milan (Italy).

**4<sup>th</sup> July 2017**

Seminar lesson and laboratory “A field trip in Metagenomics” organized by the PhD course in “Tecnologie Convergenti per I Sistemi Biomolecolari” of the University of Milano-Bicocca, Milan (Italy)

**October 2013 to March  
2016**

Master’s degree in Evolutionary Biology, 110/110 cum laude, at the University of Padova, Padova (Italy).

During my thesis internship, I spent a year in the laboratory of

Evolutionary Biology of Arthropods at the Department of Biology (University of Padova, Italy).

I was trained in the use of stereo- and light- microscopes for describing morphological characters of arthropods, and combine them with computational analysis of nucleotide sequences of nuclear and mitochondrial molecular markers to delimit species by integrative taxonomy.

**October 2010 to July 2013** Bachelor's degree in Biology, 110/110 cum laude, at the University of Padova, Padova (Italy).

## Research interests

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Computational tools are playing a major role in support of experimental evidence, especially when dealing with the study of mechanisms underlying biological complexity, which is the results of non-addictive interactions between genes, proteins, their structures up to phenotypes and entire communities.

I am interested in the applications of bioinformatics tools in improving the biomedical research and the human economy, by understanding the evolving dynamics of metabolic pathways and protein interactions associated with human diseases, and by predicting the best candidate enzymes to choose and which modifications to introduce for evolving them towards efficient and selective biocatalysts in industrial processes.

Although I am more oriented towards bioinformatic tasks, I have been working in a lab environment since years, enjoying the contact with multi-disciplinary point of views. From this experience, I learned to make an efficient integration of different expertise to approach biological issues and identify worth to be addressed scientific questions.

## Personal skills

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**Mother tongue** Italian.

**Other languages** English; C1 level of CEFR in understanding (listening/reading), writing and expression (spoken interaction/spoken production).

**Software** Deep knowledge of Linux-based operating systems (Ubuntu distributions) and bash shell. Good use of Python 3.x programming language for scripting. Advanced user of Microsoft Office and Origin suites for documents and plots production in a Microsoft Windows environment. Advanced use of computational tools for predicting the effect of point mutations on protein activity or/and stability (i. e., FoldX, ROSETTA package). Advanced use of tools for genomic data annotation

and function prediction, for building multi-alignment of homologue DNA/RNA/protein sequences (i.e., Mafft, T-coffee, Clustal-X, Muscle), tools for trimming unreliable aligned positions (i.e., Aliscore, ZORRO, Guidance 2). Expertise in studying the molecular evolution of genetic and protein data, through phylogenetic packages (TNT, PhyML, RAxML, MrBayes, Beast, Bali-phy) and statistical tests of model adequacy. Expertise in DNA-based species delimitation tools and DNA barcoding methods. Basic use of Mesquite and R package for the implementation of some functions of the tools mentioned above. Advanced user of Modeller package for homology modelling of proteins and good user of recent deep-learning approaches for structure prediction (AlphaFOLD, trROSETTA, RoseTTAFold). Advanced user of software for protein structure visualization (Pymol, Visual Molecular Dynamics, Chimera). Advanced user of GROMACS package for preparing and performing/analyzing molecular dynamic simulations of protein systems in water solvent with ions at constant pH and temperature. Good knowledge of Statistica Statsoft for descriptive presentation of data and statistical analysis of quantitative and meristic morphological data. Basic knowledge of Microsoft SQL Server for storing and annotating biological sequence data from public databases, and of Cytoscape platform for biological networks visualization. Basic use of QGIS for plotting geo-referenced points on a map.

#### **Lab expertise**

Taking morphometric measures and work with multi-stacks photos captured at stereo- and light-microscopes. Molecular biology for obtaining a recombinant clone in a plasmid expressed in *E. coli*. Biochemical techniques for the purification and the functional characterization of enzymes, like Immobilized Metal Affinity Chromatography, and enzymatic assays for detecting reaction rates in different conditions. Use of circular dichroism and tryptophan intrinsic fluorescence for biophysical characterization of proteins. Growth inhibition plate assays. Soxhlet extraction by petroleum ether. Chemical methods for the extraction of proteins applied for insect biomasses (TCA precipitation, pH-dependent solubilization and precipitation, Osborne fractionation).

#### **Congresses**

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##### **4<sup>th</sup> to 7<sup>th</sup> September 2019**

16<sup>th</sup> International Conference on Computational Intelligence methods for Bioinformatics and Biostatistics, held at the University of Bergamo, Bergamo (Italy).

##### **1<sup>st</sup> to 4<sup>th</sup> September 2019**

8<sup>th</sup> Congress of the "Italian Society of Evolutionary Biology" (SIBE or ISEB) at the "Orto Botanico" at the University of Padova, Padova (Italy).

- 25<sup>th</sup> to 27<sup>th</sup> August 2019** Meeting "Perspectives in bioinformatics: data mining and scale bridging" in Söllerhaus, Hirschegg (Austria).
- 3<sup>rd</sup> to 5<sup>th</sup> July 2019** Workshop "Nature as a teacher: big data, complex systems, new chemistry", held at the *Tagungshotel* campus.guest of the University of Stuttgart, Stuttgart (Germany).
- 14<sup>th</sup> - 15<sup>th</sup> February 2019** Workshop "Understanding complexity in life sciences" at the Department of Earth and Environmental Sciences, University of Milano-Bicocca, Milan (Italy).
- 16<sup>th</sup> to 20<sup>th</sup> September 2018** 12<sup>th</sup> International Congress of "Extremophiles" at Hotel Continental Ischia, Ischia, Naples (Italy).
- 28<sup>th</sup> to 30<sup>th</sup> May 2018** Congress "Proteine 2018" held in the "Silos di Ponente" of "Polo di Santa Marta", University of Verona, Verona (Italy).
- 28<sup>th</sup> to 31<sup>st</sup> August 2017** 7<sup>th</sup> Congress of the "Italian Society of Evolutionary Biology" (SIBE or ISEB) at the Department of Science of the University of Roma Tre, Rome (Italy). I received an update on the major topics and methods currently applied in evolutionary research activities, and the new techniques for teaching biological disciplines.
- 4<sup>th</sup> April 2017** Conference "Digital Revolution: come cambierà la nostra vita", held at the University of Milano-Bicocca. It is a special edition of the "13<sup>th</sup> world conference Science and Society: science for a better life".
- 31<sup>st</sup> August to 3<sup>rd</sup> September 2015** 6<sup>th</sup> Congress of the "Italian Society of Evolutionary Biology" (SIBE or ISEB) at the University of Bologna, Bologna (Italy).

## Meeting

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### Contributions

- 1<sup>st</sup> to 4<sup>th</sup> September 2019** (Poster) Orlando M., Buchholz P. C. F., Lotti M., Pleiss J., Diversity and evolution of glycoside hydrolases family 19 revised by sequence space mining. 8<sup>th</sup> Congress of the "Italian Society of Evolutionary Biology" at Padova (Italy).
- 25<sup>th</sup> to 27<sup>th</sup> August 2019** (Oral communication) Orlando M., Introduction to "FuncLib": next-generation automatic design of multipoint mutations at enzyme active sites. Meeting "Perspectives in bioinformatics: data mining and scale bridging" at Hirschegg (Austria).

- 3<sup>rd</sup> to 5<sup>th</sup> July 2019** (Poster and flash presentation) Orlando M., Buchholz P. C. F., Lotti M., Pleiss J., Diversity and evolution of glycoside hydrolases family 19 revised by sequence space mining. Workshop “Nature as a teacher: big data, complex systems, new chemistry” at Stuttgart (Germany).
- 16<sup>th</sup> to 20<sup>th</sup> September 2018** (Poster) Orlando M., Pucciarelli S., Lotti M., Biochemical and sequence signatures of cold-activity in Antarctic glycoside hydrolase enzymes. 12<sup>th</sup> International Congress of “Extremophiles” at Ischia, Naples (Italy).
- 20<sup>th</sup> to 22<sup>nd</sup> September 2018** (Oral communication) Lotti M., Brocca S., Mangiagalli M., Pischedda A., Orlando M., Maione S., De Pascale D., Pucciarelli S., Nardini M., Braslavsky I., “Cold Biotechnology”: enzymes and anti-freeze proteins from Antarctic organisms. 59<sup>th</sup> Congress Italian Society of Biochemistry and Molecular Biology at Caserta (Italy).
- 28<sup>th</sup> to 30<sup>th</sup> May 2018** (Poster) Orlando M., Maione S., Mangiagalli M., Pischedda A., Valtorta R., Mondo D., Pennati M., Brocca S., Pucciarelli S., Lotti M., Biochemical and structural features of enzymes from an Antarctic metagenome. Proteine 2018 at Verona (Italy).
- 1<sup>st</sup> to 4<sup>th</sup> July 2018** (Oral communication) Lotti M., Brocca S., Mangiagalli M., Pischedda A., Orlando M., Maione S., De Pascale D., Pucciarelli S., Nardini M., Braslavsky I., “Enzymes and ice binding proteins from Antarctic organisms”. 18<sup>th</sup> European Congress on Biotechnology at Geneva (Switzerland).
- 30<sup>th</sup> August to 2<sup>nd</sup> September 2016** Oral presentation of my Master’s thesis work at the first national joint congress of “Società Italiana di Ecologia”- “Unione Zoologica Italiana”- “Società Italiana di Biogeografia” (SITE-UZI-SIB) at the University of Milano-Bicocca, Milan (Italy).

## Tutoring activity

- July 2018** I supervised for a month Hüseyin Okan Soykam, a visiting Master Student from Yeditepe University. We worked on gene annotations and bioinformatic analysis on coding protein sequences.
- July-August 2020** I provided a 4-hours online course to four students of the Bachelor course in Biotechnologies at the University of Milano-Bicocca, on computational prediction of protein function, fast modelling and annotation of uncharacterized protein sequences (glycosyl hydrolase) from metagenomic open reading frames.

I supervised for 4 months a Bachelor student of the University of Insubria in studying the sequence space diversity and conservation of SSADH family within ALDH superfamily.

## Publications

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(Article) Orlando, M., Buchholz C. F., P., Lotti, M., Pleiss, J. (accepted for publication). The GH19 Engineering Database: sequence diversity, substrate scope, and evolution in glycoside hydrolase family 19. *Plos One*.

(Article) Orlando, M., Fortuna, S., Oloketuyi, S., Bajc, G., Goldenzweig, A., de Marco, A. (2021). CDR1 composition can affect nanobody recombinant expression yields. *Biomolecules*.

**DOI: 10.3390/biom11091362**

(Article) Ubbiali D., \*Orlando M., Kovačič M., Iacobucci C., Semrau M. S., Bajc G., Fortuna S., Ilc G., Medagli B., Oloketuyi S., Storici P., Sinz A., Grandori R., de Marco A. (2021). An anti-HER2 nanobody binds to its antigen HER2 via two independent paratopes. *International Journal of Biological Macromolecules*.

**DOI: 10.1016/j.ijbiomac.2021.04.032**

(Article) Orlando M., Pucciarelli S., Lotti M. (2021). Endolysins from Antarctic *Pseudomonas* Display Lysozyme Activity at Low Temperature. *Marine Drugs*.

**DOI: 10.3390/md18110579**

(Article) Mangiagalli M., Lapi M., Maione S., \*Orlando M., Brocca S., Pesce A., Barbiroli A., Camilloni C., Pucciarelli S., Lotti M., Nardini M. (2021). The co-existence of cold activity and thermal stability in an Antarctic GH42  $\beta$ -galactosidase relies on its hexameric quaternary arrangement. *The FEBS Journal*.

**DOI: 10.1111/febs.15354**

(Review) Mangiagalli M., Brocca S., \*Orlando M., & Lotti M. (2020). The "cold revolution". Present and future applications of cold-active enzymes and ice-binding proteins. *New Biotechnology*.

**DOI: 10.1016/j.nbt.2019.09.003**

(Article) Bonato L., \*Orlando M., Zapparoli M., Fusco G., & Bortolin F. (2017). New insights into Plutonium, the least known of the European large scolopenders (Chilopoda): occurrence, evolution and morphology.

*Zoological Journal of the Linnean Society.*

**DOI: 10.1093/zoolinnea/zlw026**

(Article) Bonato L., Bortolin F., Drago L., \*Orlando M., & Danyi L. (2017). Evolution of Strigamia centipedes (Chilopoda): a first molecular assessment of phylogeny and divergence times. *Zoologica Scripta*.

**DOI: 10.1111/zsc.12234**

24/10/2021

Marco Orlando

