Curriculum Vitae

Stefano Benini

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Abilitazione Scientifica Nazionale alle funzioni di professore universitario di seconda fascia nel Settore Concorsuale 05/E1 BIOCHIMICA GENERALE (SSD BIO/10, 04/10/2022 - 04/10/2034)

Abilitazione Scientifica Nazionale alle funzioni di professore universitario di seconda fascia nel Settore CHEM-05/A - Chimica organica (11/12/2013 -11/12/2022 14/03/2025-14/03/2037),

Languages

- Italian mother tongue
- English C1 (City and Guilds 2012)
- Deutsch B2 (Goethe 2014)

Role:

Ricercatore a tempo indeterminato (CHEM-05/A / CHIM06, 03/C1) presso la Libera Università di Bolzano (unibz), Facoltà di Scienze e Tecnologie-Faculty of Agricultural, Environmental and Food Sciences.

- Insegnamento del corso di 5 crediti "Fondamenti di Chimica" per la laurea triennale in Agricoltura sostenibile e Gestione forestale in Ambiente montano (L25) (2025-)
- Insegnamento del corso di 3 crediti "Organic Chemistry" per la laurea triennale in Scienze alimentari ed enogastronomiche (L26) (2025-)
- Insegnamento del corso di 8 crediti "Fondamenti di Chimica" per la laurea triennale in Scienze Agrarie, degli Alimenti e dell'Ambiente montano (L25) (2019-2024)
- Insegnamento del corso di 6 crediti "Chimica Organica" per la laurea triennale in Scienze Agrarie ed Agroambientali (L25) (dal 2009 al 2019)

Academic Studies

- 2001 PhD in Chemistry, University of York (UK)/ EMBL Hamburg (DE)
- 1991 Master in Scienze Agrarie (Laurea quinquennale) 108/110, Università di Bologna

Work experience

- 2007-2009 Protein crystallographer. Pharmaceutical company AstraZeneca plc.(United Kingdom) protein structure determination, supervision of technicians and graduate students.
- 2002-2007 Postdoctoral research fellow. York Structural Biology Laboratory, Department of Chemistry, University of York (United Kingdom), Research activities, gene cloning, protein purification and characterization, structure determination. Supervision of a technician and graduate students.

- 2000-2002 Postdoctoral research fellow. International Centre for Genetic Engineering and Biotechnology (ICGEB) Trieste (Italy). Research activities, gene cloning, protein purification and characterization protein structure determination, supervision of graduate students.
- 1996-2000 Predoctoral research fellow. European Molecular Biology Laboratory (EMBL) Hamburg (Germany). Research activities, protein purification and characterization, protein structure determination
- 1992-1996 Assistente alla ricerca. Istituto di Chimica Agraria, Università di Bologna (Italy). Purification and characterization of proteins from plants and bacteria using spectroscopic techniques (UV-Vis, NMR) under the supervision of students and graduate students. Analysis of wine distillates using SNIF-NMR to quantify deuterium isotopic substitution and identify any illegal sugar additions during must fermentation as part of the fraud prevention service of the Ministry of Agriculture and Forestry.

Visiting scientist

- April 2003 Pasteur Institute, Paris, cloning *Mycobacterium tuberculosis* genes with the "GATEWAY" recombination system, hosted by Dr Pedro Alzari.
- November-December 1996 European Molecula Biology Laboratory (EMBL), Hamburg, crystallization of bacterial proteins, hosted by Professor Keith Wilson and Dr Wojciech Rypniewski.
- February 1993 Universität des Saarlandes, Fachrichtung Mikrobiologie, Saarbrucken, purification of urease hosted by Professor Dr Heinrich Kaltwasser.

Research activity

In 2009 I established the Bioorganic chemistry and Bio-Crystallography laboratory (B_2Cl) to study the structure and function of enzymes by protein crystallography and biochemical and molecular biology techniques

I coordinate a research group dedicated to the study of proteins and enzymes of bacterial, fungal, and plant origin. These proteins are produced in the laboratory using molecular biology techniques and expression in *Escherichia coli*. The laboratory is equipped with instruments that allow us to begin with gene cloning and proceed to the expression, purification, biochemical characterization, and crystallization of the corresponding proteins. Protein purification is performed using an Äkta purifier and chromatography columns, while a crystallization robot speeds up the screening of initial conditions. The crystallized proteins are then transported to synchrotrons (ELETTRA, Trieste (IT), DIAMOND, Didcot (UK), PETRAIII, EMBL Hamburg (DE)) to collect diffraction data, which are then processed to derive the 3D structure in our laboratory in Bolzano. The biochemical and functional characterization of proteins and enzymes is performed both in the Bolzano laboratory and through international collaborations (see list of collaborators and list of publications).

Research projects

- • Biomolecular characterization of proteins from Erwinia amylovora, the bacterium responsible for apple and pear fire blight, a disease of strategic importance for the economy of fruit cultivation worldwide. The research focuses on the study of a series of pathogenicity factors, including proteins involved in the biosynthesis of the exopolysaccharide amylovoran and the enzymes responsible for the biosynthesis of the siderophore desferrioxamine E (DFO-E, a small molecule for iron transport). We were the first group in the world to characterize in vitro and obtain the structures of all the enzymes in the DFO-E biosynthetic pathway (a lysine decarboxylase, DfoJ, a cadaverine monooxygenase DfoA, and a desferrioxamine synthetase DfoC, Salomone-Stagni et al. 2018).

- Study of carbohydrate-active enzymes with potential biotechnological applications. Their products can be used as food additives or precursors in chemoenzymatic reactions. Recent results include the structure of the enzyme levansucrase, an enzyme that, starting from the hydrolysis of sucrose, is then able to produce fructooligosaccharides (FOS). The structure of this enzyme was obtained both in native form and in complex with the products of sucrose hydrolysis (glucose and fructose), in complex with levanbiose (a fructose dimer formed from the hydrolysis of sucrose and subsequent dimerization of the hydrolysis product in the crystal) and butanetriol. See list of publications.

- Study of the biosynthetic pathway of the siderophore triacetylfusarinine C (TAFC) produced by the human pathogen Aspergillus fumigatus. The aim of this research project, in collaboration with Professor Haas of the Medical University of Innsbruck and Professor Sheref Mansy of the University of Alberta/University of Trento, is to study the enzymes responsible for the biosynthesis of the fungal siderophore TAFC for the biotechnological production of modified siderophores for use in medical diagnostics and therapy.

- Study of the enzymes involved in the biosynthesis of dihydrochalcones, compounds produced by apples that have beneficial effects on health. The first result obtained is the enzymatic characterization of an "NADPH-dependent double bond reductase" and the obtaining of its structure at a resolution of 1.5 Å via X-ray crystallography in the native form without a cofactor, in complex with a cofactor, and with a cofactor and ligand.

Funding obtained as principal investigator and coordinator

- 2020 Coordinator Stefano Benini, collaborators: Professor Benedetto Ruperti University of Padova, Professor Francesco Musiani, Università di Bologna Libera Università di Bolzano “Discovering Apple Quality Markers for a Sustainable Conservation in Dynamic Controlled Atmosphere (DAMaSCo)” 66.822 €
- 2019 Coordinator Stefano Benini, partners: Professor Hubertus Haas, Medical University of Innsbruck and Professor Sheref Mansy, University of Alberta/Università di Trento. Euregio Science Fund, Interregional Project Network IPN 95
  Siderophore mediated iron uptake in *Erwinia amylovora* and *Aspergillus fumigatus* (SupErA) 330.100 € (unibz 99.813 €, Medical University of Innsbruck 103.887 €, University of Trento 126.400 €)
- 2018 Principal investigator, Libera Università di Bolzano
  Towards the understanding of *Erwinia amylovora* exopolysaccharide biosynthesis (TowEr) 65.000 €
- 2017 Principal investigator (coordinator Stefan Martens FEM-IASMA, Partner, Professor Hermann Stuppner University of Innsbruck) Euregio Science Fund, Interregional Project Network IPN55
  Exploring the potential of apple dihydrochalcones ExPoApple2 106.050 €
- 2016 Principal investigator, Libera Università di Bolzano
  Investigating the Molecular Properties of AmsI from *Erwinia amylovora*. Control of fire blight by AmsI inhibition, towards a sustainable phytosanitary strategy (IMPACTS) 38000 €
- 2014 Principal investigator, Libera Università di Bolzano
  Molecular engineering of the structural and catalytic properties of levansucrase (Mescal) 72000 €
- 2012 Principal investigator, Fondazione Libera Università di Bolzano
  Galactose and glucuronic Acid Metabolism in *Erwinia spp.* (GAMEs) 29600 €
- 2012 Principal investigator, Libera Università di Bolzano
  Galactose and glucuronic Acid Metabolism in *Erwinia spp.* (GAMEs) 31822 €
- 2011 Principal investigator, Fondazione Libera Università di Bolzano
  A Structural genomics approach for the study of *Erwinia amylovora* virulence and pathogenesis 29600 €

- 2011 Principal investigator, Provincia Autonoma di Bolzano

La Genomica Strutturale per lo Studio della Virulenza e Patogenesi di *Erwinia amylovora* 179600 €

- 2010 Principal investigator, Libera Università di Bolzano
  Erwinia amylovora's Secretome (EraSe). Structural and functional study of the secreted proteins of *E. amylovora*, the causative agent of fire blight 42800 €
- 2009 Principal investigator, Facoltà di Scienze e Tecnologie, Libera Università di Bolzano
  Molecular basis of chemicals and odorants recognition in the insect model organism *Tribolium castaneum* by structural biology of chemoreceptor proteins 4692 €

Collaboration:

1. Dr Michele Cianci Università Politecnica delle Marche.
2. Dr Rob Meijers European Molecular Biology Laboratory, Hamburg, Germany.
3. Professor Lukasz Berlicki Laboratory of Bioorganic Chemistry Faculty of Chemistry, Wroclaw University of Technology, Wroclaw, Poland.
4. Dr Francesco Musiani Dipartimento di Farmacia e Biotecnologie Università di Bologna
5. Professor Stefano Ciurli Dipartimento di Farmacia e Biotecnologie Università di Bologna
6. Dr Mickael Malnoy Fondazione E. Mach IASMA S. Michele all'adige Trento, Italy.
7. Professor Robert Field, Department of Biological Chemistry, John Innes Centre Norwich Research Park UK.
8. Professor Richard J. Morris, Department of Computational and Systems Biology, John Innes Centre Norwich Research Park UK.
9. Professor Youfu Zhao, Department of Crop Sciences University of Illinois at Urbana-Champaign (USA).
10. Professor Andreas Peil, Julius Kuehn-Institute, Federal Research Centre for Cultivated Plants, Dresden (DE),
11. Dr Martin Walsh Diamond Light Source, Harwell Science and Innovation Campus, United Kingdom.
12. Dr Nicola Demitri Elettra-Sincrotrone Trieste, Italy.
13. Dr Stefan Martens Research and Innovation Center, Fondazione Edmund Mach, S. Michele all'Adige, Italy. Collaboratore in ExpoApple2
14. Professor Hermann Stuppner Institute of Pharmacy/Pharmacognosy, Center for Chemistry and Biomedicine University of Innsbruck. Collaboratore in ExpoApple2
15. Professor Hubertus Haas Division of Molecular Biology/Biocenter, Medical University of Innsbruck, Collaboratore in SupErA
16. Professor Sheref Mansy Centre for Integrative Biology – CIBIO, University of Trento, Collaboratore in SupErA
17. Professor Oliver Spadiut Integrated Bioprocess Development, Biochemical Engineering. TU Wien
18. Dr Thanapon Charoenwongpaiboon Department of Chemistry, Faculty of Science, Silpakorn University, Nakhon Pathom, Thailand;

Supervisor of postdocs:

1. Dr Thanalai Poonsiri 2020- project SupErA
2. Dr Ivan Polsinelli 2018-2021 projects: IMPACTS/TowEr
3. Dr Rosanna Caliandro 2018-2021 project: ExpoApple2 (Euregio, Interregional Project Network).
4. Dr Luigimaria Borruso 2016-2017 project: Bioinformatics analysis of *Erwinia amylovora* strains genome sequences (BioinfEa) (unibz).
5. Dr Jochen Würges, 2014 project: La Genomica Strutturale per lo Studio della Virulenza e Patogenesi di *Erwinia amylovora* (provincia autonoma Bolzano).
6. Dr Mirco Toccafondi, 2013-2015 project: Galactose and glucuronic Acid Metabolism in *Erwinia spp.* (GAMEs, Libera Università di Bolzano e Fondazione Libera Università di Bolzano).

7. Dr Marco Salomone-Stagni, 2012 -2014 project: La Genomica Strutturale per lo Studio della Virulenza e Patogenesi di *Erwinia amylovora* (provincia autonoma Bolzano e Fondazione Libera Università di Bolzano). 2015- 2017 project: Mescal (unibz)
8. Dr Lorenzo Caputi, 2012-2013 project: La Genomica Strutturale per lo Studio della Virulenza e Patogenesi di *Erwinia amylovora* provincia autonoma Bolzano.
9. Dr Daniela De Luchi, 2011-2012 project: EraSe

Supervisor of PhD students:

1. Lavinia Carlini (Italy) "PhD Programme in Mountain Environment and Agriculture" (2020-) PhD Thesis title:"Biosynthetic pathway of the exopolysaccharide amylovoran in the plant pathogen *Erwinia amylovora"*
2. Bharti Sharda (India) "PhD Programme in Mountain Environment and Agriculture" (2020-) PhD Thesis title:"Iron uptake and metabolism in the plant pathogen *Erwinia amylovora"*
3. Luca Invernizzi (Italy) "PhD Programme in Mountain Environment and Agriculture" (2019-) PhD Thesis title:"Siderophore mediated iron uptake in the human pathogen *Aspergillus fumigatus* and in the plant pathogen *Erwinia amylovora"*
4. Ivan Polsinelli (Italy) "PhD Programme in Mountain Environment and Agriculture" (2016-2018) PhD Thesis title:"Iron uptake and levan biosynthesis in *Erwinia amylovora*"
5. Joseph Dale Bartho (New Zealand) "PhD Programme in Mountain Environment and Agriculture" (2013-2016), PhD Thesis title:" Structural investigation of pathogenicity associated proteins from *Erwinia amylovora*"
6.

Supervisor of unibz Bachelor student

1. Marilena Feller, "Cloning and expression of genes from the phytopathogen *Erwinia amylovora*". Voto finale 110/110 *cum laude* (2020)

Supervision of visiting students:

1. Paulina Kosikowska (Laboratory of Bioorganic Chemistry Faculty of Chemistry, Wroclaw University of Technology, Wroclaw, Poland) settembre 2011, cristallizzazione di ureasi da *Sporosarcina pasteurii.*
2. Katarzyna Macegoniuk (Laboratory of Bioorganic Chemistry Faculty of Chemistry, Wroclaw University of Technology, Wroclaw, Poland) ottobre 2013 purificazione e cristallizzazione di urease di *Sporosarcina pasteurii.*
3. Luca Mazzei (Laboratorio di Chimica Bioinorganica, Università di Bologna) cristallizzazione di NikR from Helicobacter pylorii e cristallizzazione di urease di *Sporosarcina pasteurii* in complesso con inibitori (2013-2014).
4. Simone Albani inhibition study on "tyrosine protein phosphatase AmsI"

Oral presentations

1. Studying *Erwinia amylovora* biology by protein crystallography. 2nd International Symposium on Fire Blight of rosaceous plants (ISFB2019) Traverse city, Michigan, USA 17-21 June 2019. Stefano Benini.
2. Towards the understanding of iron uptake and siderophore biosynthesis in *Erwinia amylovora*. 1st International Symposium on Fire Blight of rosaceous plants (ISFB2016) Girona, Spain 5-8 July 2016. Ivan Polsinelli, Marco Salomone-Stagni, Joseph Dale Bartho and Stefano Benini
3. The structural biology of *Erwinia amylovora* desferrioxamine biosynthetic pathway at a glance. 1st International Symposium on Fire Blight of rosaceous plants (ISFB2016) Girona, Spain 5-8 July 2016. Marco Salomone-Stagni, Joseph Dale Bartho and Stefano Benini

4. Structural and functional characterization of enzymes involved in the biosynthesis of amylovoran. 1st International Workshop: Molecular Basis of Fire Blight, Bolzano 15/10/2014, Stefano Benini
5. Investigating the molecular basis of fire blight by structural and functional genomics of *Erwinia amylovora*. Thirteenth International Workshop on Fire Blight, Zurich Switzerland 02-05/07/2013, Stefano Benini
6. The crystal structure of *Mycobacterium tuberculosis* inorganic pyrophosphatase. International meeting on inorganic pyrophosphatases University of Birmingham June 28th -July 1th, 2004. Stefano Benini
7. A New Proposal for Urease Mechanism Based on High Resolution Structures of Complexes. International School of Crystallography 31st Course: Chemical Prospectives in Crystallography of Molecular Biology. Erice Sicily May 25th-June 4th 2000. Stefano Benini
8. Structure and Function Relationship of Urease from *Bacillus Pasteurii*. TMR Summer School "Structure and Function of Metalloproteins", Oeiras Portugal, September 7th-10th, 1998. Stefano Benini

Seminars held at national and international scientific institutions

1. Structural and functional characterization of enzymes and proteins involved in the pathogenicity of *Erwinia amylovora,* Kutateladze Institute of Pharmacochemistry, Tbilisi State Medical University Tbilisi Georgia Hosted by Professor Alexander Skhirtladze, Laboratory of Steroidal Compounds 07/12/2015.
2. New Insight into the Enzymatic Hydrolysis of Urea. ICGEB New Delhi India 15/01/2002.
3. Relazioni Struttura-Funzione da Studi Cristallografici con Luce di Sincrotrone di Ureasi da *Bacillus Pasteurii* Nativa ed Inibita. Universita' di Verona, Dipartimento Scientifico e Tecnologico, Verona Italy 02/06/1999.
4. Small or Big but With Synchrotron Radiation: Crystallographic Studies on Cytochrome c-553 and Urease from *Bacillus pasteurii.* Area Science Park, Synchrotron ELETTRA, Basovizza, Trieste Italy 28/05/1999.
5. Relazioni Struttura Funzione di Ureasi da *Bacillus Pasteurii* Nativa ed Inibita. Universita' di Ferrara, Dipartimento di Biochimica e Biologia Molecolare, Ferrara Italy 11/05/1999.
6. Why Urea Hydrolysis Costs Two Nickels. University of York, Chemistry Department York United Kingdom 23/11/1998.

Workshop organized

First International workshop: "Molecular Basis of Fire Blight" Bolzano 15.10.2014. Scientists from New Zealand, USA, Switzerland, Austria, Germany, England and Italy contributed with oral presentations. (funding from the project: La Genomica Strutturale per lo Studio della Virulenza e Patogenesi di *Erwinia amylovora,* provincia autonoma di Bolzano)

Editorial Board Member: *Scientific Reports* Nature publishing group

Guest editor special issues:

- 2019 "Carbohydrate-Active Enzymes: Structure, Activity and Reaction Products"

A special issue of International Journal of Molecular Sciences (IF 4.183) (ISSN 1422-0067)

- 2020 "Carbohydrate-Active Enzymes: Structure, Activity and Reaction Products 2020"

A special issue of International Journal of Molecular Sciences (IF 4.183) (ISSN 1422-0067)

- 2020 "Carbohydrate-Active Enzymes: Structure, Activity and Reaction Products 2020"

A special issue of Life (ISSN 2075-1729)