PERSONAL INFORMATION Giovanni Bulfaro

PROFILE OVERVIEW

Postdoctoral researcher with expertise in the structural and functional characterization of proteins of biotechnological and biomedical relevance. Skilled in expressing and purifying recombinant proteins using both prokaryotic (E. coli) and eukaryotic (HEK 293, CHO) systems, from construct design and optimization of expression protocols to advanced purification and biophysical characterization techniques, including calorimetry and affinity assays. Proficient in the structural analysis of proteins leveraging cutting-edge methodologies such as X-ray crystallography and cryo-electron microscopy, including autonomous data collection and processing at major synchrotron facilities.

Highly adaptable and detail-oriented professional with strong organizational and communication skills, honed through interdisciplinary research experiences and international collaborations, including work in competitive and multicultural settings. Demonstrates a proactive and collaborative approach to teamwork, fostering a positive and productive work environment while ensuring efficient project execution. Thrives under pressure, consistently meeting deadlines and delivering high-quality results. Independent, reliable, and committed to advancing scientific understanding through innovative research.

WORK EXPERIENCE

05/2024 - present Post-doctoral fellowship

"La Sapienza" University Department of biochemical Sciences "A.Rossi Fanelli", Via Aldo Moro 5 **PRIN 2022 CUP B53D23015930006**

Project: Unraveling The moonLightinG Activity of the PFKFB blfunctiOnal eNzyme with phospholipase C-gamma (LIGATION) Analysis of the biochemical and kinetic parameters of PFKFB3 phosphatase activity on PLCγ, with the aim of understanding the molecular mechanisms underlying their functional interaction.

05/2021 - 04/2024 PhD (Summa cum Laude) (La Sapienza University-Takis S.r.l.)

Project: BIOSTRUCT-FARM - Biologia Strutturale per lo sviluppo sinergistico di Farmaci innovativi

"La Sapienza" University

Department of biochemical Sciences "A.Rossi Fanelli", Via Aldo Moro 5

Structural characterization of antigen-antibody complexes via X-ray diffraction and Cryo-EM. This includes the full workflow: protein crystallization, grids preparation, autonomous synchrotron X-ray diffraction and cryo-EM data collection (ELETTRA and ESRF synchrotrons) and data analysis (CCP4 suite, CryoSPARC software, UCSF Chimera, Chimera X, Alphafold).

Takis S.r.l.

Via di Castel Romano, 100, 00128 Castel Romano RM

Expression and purification of proteins and monoclonal antibodies in eukaryotic and prokaryotic systems, affinity assays performed with Octet-BLI technology, advanced chromatographic methods for biophysical characterization and protein purification; protein stability evaluation by calorimetry assays (Nanotemper)

05/2023 - 07/2023 Internship (Columbia University – Prof. Shapiro's Lab - New York, USA) European Union's H2020-MSCA-RISE-2018 Research and Innovation program under the Marie Skłodowska-Curie (grant number GA.823780)

Cryo-EM training: samples vitrification using vitrobot (thermofisher scientific), clipping grids, quality evaluation of vitrified grids, Cryo-EM data analysis with CryoSPARC software

10/2018 - 01/2021 Internship (La Sapienza University) for Bachelor's and Master's degrees

- Phenotypic and functional characterization studies on NK cells: cytofluorimetry (FACS Canto BD), RTq-PCR, antibody-dependent stimulation in plate and through co-cultures, cytotoxicity assays, confocal microscopy.

- The role of mast cells in colorectal cancer: mice bones handling, differentiation of stem cells from the bone marrow into mast cells (for in vitro experiments), processing of the colon tissue of mice for tumor masses isolation.

EDUCATION AND TRAINING

05/2024 – Post-doctoral fellowship

"La Sapienza" University Department of biochemical Sciences "A.Rossi Fanelli", Via Aldo Moro 5 **PRIN 2022 CUP B53D23015930006**

Project: Unraveling The moonLightinG AcTivity of the PFKFB blfunctiOnal eNzyme with phospholipase C-gamma (LIGATION)

Aim of the project: investigate the phosphatase activity of PFKFB3 kinase against the protein phospholipase C-gamma.

05/2021-04/2024 PhD course in Biochemistry Summa cum laude 8 EQF

"La Sapienza" University – Takis Š.r.l. Department of biochemical Sciences "A.Rossi Fanelli", Via Aldo Moro 5 <u>in collaboration with Takis S.r.l.</u> – bando regione Lazio POR Lazio FSE 2014/2020

Project: BIOSTRUCT-FARM - Biologia Strutturale per lo sviluppo sinergistico di Farmaci innovativi

Aim of the project: investigate the structural interaction between monoclonal antibodies produced by Takis S.r.I. and the tumor marker ErbB3, in order to identify the structural determinants of the epitope recognition and the cellular effects relevant to cancer therapy. DNA transfection (expifectamine), proteins production (E.Coli, HEK 293, CHO cell lines), protein purification and characterization (IMAC, Size-exclusion chromatography, SDS-PAGE, WB), cell cultures, ELISA assays, X-ray crystallography (hanging/sitting drop techniques, macro-/micro-seeding, automated crystallization of proteins using Oryx4 robot for crystal screening), Cryo-EM.

11/2018 - 01/2021 Master's Degree in Medical Biotechnology (biomolecular curriculum) 110/110 cum laude 7 EQF

"La Sapienza" University

RNA extraction (Total RNA Mini Kit by Geneaid Biotech Ltd), RNA quantization (NanoDrop 2000c by Thermo Fisher Scientific), RTq-PCR, 7AAD probe cytotoxicity experiments, confocal microscopy experiments (fixation cells on slide and permeabilization for intracellular labeling using monoclonal antibodies), stimulation in antibody-dependent plates, use of software for statistical analysis (Prism-GraphPad).

10/2015 - 12/2018 Bachelor's degree in Biotechnology 110/110 cum laude 6 EQF

"La Sapienza" University

Extraction of white blood cells from peripheral blood (Ficoll), autonomous management of primary cell cultures and cell lines, direct and indirect labeling using monoclonal antibodies, use of the flow cytometer (FACS Canto by BD Biosciences), use of software for the analysis of flow cytometric data (FlowJo).

PERSONAL SKILLS

Mother tongue(s) Italian

Other language(s)

	Listening	Reading	Spoken ir	teraction	Spoken production
English	B2	B2	B2	B2	B2

Job-related skills

- Recombinant protein expression and purification (E.Coli, HEK 293, CHO cell lines, IMAC)
- Protein characterization by SDS-PAGE, WB, Size-exclusion chromatography, Nanotemper, BLI
- Protein crystallization (crystal screening, hanging/sitting drop techniques, macro-/micro-seeding, Oryx4 robot for crystal screening, data analysis with CCP4 suite program, Chimera X, UCSF chimera, Phenix software package)
- Cryo-EM (samples vitrification using vitrobot, clipping grids, data analysis with CryoSPARC software)

Digital skills

- AlphaFold, ChimeraX, USCF Chimera, CCP4, Phenix, CryoSPARC, bioluminate.
- Microsoft programs / Good knowledge of software for graphical representation of data (PRISM GRAPHPAD, Kaleidagraph) / FlowJo (FACS data analysis)

Publications

- 11573/1665426 2023 Effect of salts on the conformational dynamics of the Cytochrome P450 OleP DE SCISCIO, MARIA LAURA; NARDI, ALESSANDRO NICOLA; PARISI, GIACOMO; BULFARO, GIOVANNI; COSTANZO, ANTONELLA; GUGOLE, ELENA; FREDA, IDA; SAVINO, CARMELINDA; VALLONE, BEATRICE; MONTEMIGLIO, LINDA CELESTE; D'ABRAMO, MARCO journal: MOLECULES (Basel: MDPI Berlin: Springer, 1996-) pp. - - issn: 1420-3049 - wos: WOS:000927697700001 (0) - scopus: 2-s2.0-85146781706 (0)
- Binding of steroid substrates reveals the key to the productive transition of the cytochrome P450 OleP
 Antonella Costanzo; Francesca Fata; Ida Freda; Elena Gugole; Giovanni Bulfaro; Matteo Di Renzo; Luca Barbizzi; Cécile Exertier; Giacomo Parisi; Beatrice Vallone; Carmelinda Savino; Linda Celeste Montemiglio
 Journal : Structure, Volume 32, Issue 9, 2024, Pages 1465-1476.e3, ISSN 0969-2126, https://doi.org/10.1016/j.str.2024.06.005.
- Structural determinants of the inverted regioselectivity of the P450 OleP triple mutantF84Q/S240A/V291G toward lithocholic acid. *In preparation.* Costanzo A, Fata F, Freda I, Bulfaro G, Di Renzo M, Barbizzi L, Exertier C, Lardieri A, Demitri N, Vallone B, Savino C, Montemiglio LC.
- Preclinical activities and pharmacological study of humanized anti-ErbB3 antibodies In preparation.

Muzi A., Arriga R., Bulfaro G., Fata F., Costanzo A., D'Acunto E., Principato E., Chiarini E., Cappelletti M., Ferrara F., Bucci F., Montemiglio L. C., Savino C., Marra E., Ciliberto G, Aurisicchio L., Vallone B., Roscilli G.

Fundings

- December 2024: coordinator and principal investigator of the instruct-ERIC access proposal fundend by instruct-ERIC for the project "Structural Characterization of hAb1 and hAb2 Monoclonal Antibodies Targeting ErbB3 for Cancer Therapy" (**PID**: 36005).

Scientific participation in research project

- Progetti per Avvio alla Ricerca - Tipo 1 AR123188B0A0B47D: Redirecting P450 OleP regioselectivity toward C7 of testosterone by rational sitedirected mutagenesis

Courses

- Introduction to Computational Antibody Engineering
- Vitrojet Workshop Imaging center, EMBL, Heidelberg (Germany)-27-28 march 2023
- NATIONAL LEGISLATION AND ETHICS LEVEL 1, MODULES 1 AND 2, DM AUGUST 5, 2021 7.5 Continuing Medical Education (CME) Credits
- BIOLOGY AND MANAGEMENT OF LABORATORY ANIMALS, MODULES 3.1, 4, 5, 6.1, 7. DM AUGUST 5, 2021, RODENTS AND LAGOMORPHS 19.5 CME Credits
- Modules 3.2-6.2-8 training and refresher course for the protection of laboratory animals in scientific research: 10/23-10/27/2023; 26 CME Credits
- GENe to Structure International School (AIC School 2024) "In-silico Structural Biology", Bari, 28-31/10/2024

Congress participation

- 4th Joint AIC-SILS Conference Trieste, 12-15 September 2022 (participant)
- INTEGRATED STRUCTURAL BIOLOGY APPROACH FOR MEMBRANE PROTEIN RESEARCH 3-4 MARCH 2022 Sapienza University of Rome (European Union's H2020-MSCA-RISE-2018 Research and Innovation program under the Marie Skłodowska-Curie Grant Agreement No. 823780) (participant)
- PROMETEUS INTERNAL WORKSHOPS ON KNOWLEDGE ACQUIRED AND KNOWLEDGE RETURN PHASE 6 December 2023 Online workshop (European Union's H2020-MSCA-RISE-2018 Research and Innovation program under the Marie Skłodowska-Curie Grant Agreement No. 823780) (speaker)
- FLAGSHIP PROJECT 7 MID-TERM PLENARY WORKSHOP ROME TECHNOPOLE Advanced and automated innovation labs for diagnostic and therapeutic biopharma solutions ROMA TRE UNIVERSITY (Aula Magna, via Ostiense 133, Roma) - JUNE 13, 2024 (speaker)
- ROME TECHNOPOLE SPOKE 6 INTERNATIONAL YOUNG RESEARCHER WORKSHOP 28 June 2024 La Ginestra Room - Dept. of Chemistry Cannizzaro Building - Sapienza University of Rome (speaker)
- Nanoinnovation international conference 9-13 September 2024, Faculty of Civil and Industrial Engineering Sapienza University of Rome Young innovation session Cryo-TEM (speaker)
- AICS2024 workshop "In-Silico Structural Biology" 28-31 October 2024, Area di Ricerca CNR Bari (BA), https://school2024.cristallografia.org/

Driving licence B 2015-2025

Data 10/04/2025