

# 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 bfunctiOnal eNzyme with phospholipase C-gamma (LIGATION)**  
Analysis of the biochemical and kinetic parameters of PFKFB3 phosphatase activity on PLC $\gamma$ , 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 bfunctiOnal 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 S.r.l.

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

**in collaboration with Takis S.r.l. – 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.l. 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 interaction	Spoken production
<b>English</b>	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, Linux, AR-decon, EMReady.
- Microsoft programs / Good knowledge of software for graphical representation of data (PRISM GRAPHPAD, Kaleidagraph) / FlowJo (FACS data analysis)

**Publications**

- **Novel Humanized Anti-HER3 Antibodies: Structural Characterization and Therapeutic Activity**  
Muzi, A.; Arriga, R.; **Bulfaro, G.**, et al.  
Antibodies, vol. 14, no. 4, p. 84, 2025, doi: 10.3390/antib14040084.
- **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>.

**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 site-directed mutagenesis

### Courses

- Introduction to Computational Antibody Engineering, Bioluminate, Schrödinger
- 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) (**invited 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**)

Driving licence            B    2025-2035

Roma    09/04/2026