

Dr. Maria Romano



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PROFESSIONAL EXPERIENCE

- IBB-CNR (Italy)** Institute of Biostructure and Bioimaging of the National Research Council, Naples
Duration 30/09/2019 – to date
Position Researcher
Principal activities Identification and characterization of targets responsible for infective pathologies, with regards to host-pathogen interaction. The research activities in the protein engineering lab include structural and functional studies, by combining structural biology and molecular biology with biochemical and biophysical techniques.
- Imperial College London (UK)** Imperial College of London, Department of Life Sciences
Membrane Protein Lab at RCaH, Harwell Campus, Oxfordshire
Duration 05/09/2016 – 29/09/2019
Position Postdoctoral Research Associate
Principal activities Functional and structural characterization of membrane proteins, such as ABC-transporters and outer membrane proteins, involved in the transport of drugs across the membranes. The results highlighted the state of the art of self-immunity mechanisms as well as antibiotic-resistance.
- IBB-CNR (Italy)** Institute of Biostructures and Bioimaging of the Italian Research Council, Naples
Duration 25/06/15 - 24/06/16
Position Research Fellowship “Euro-BioImaging: European Research Infrastructure for Imaging Technologies in Biological and Biomedical Sciences”
Principal activities Structural and functional characterization of new biomarkers. The activities included optimization of recombinant proteins production for high-throughput crystallization screening and *in vitro* functional analyses.
- IBB-CNR (Italy)** Institute of Biostructures and Bioimaging of the Italian Research Council, Naples
Duration 06/06/13 - 05/06/15
Position Research Fellowship “PON01_01426 - Study for the development, characterization and efficacy of a new recombinant factor IX highly active for the treatment of hemophilia B”
Principal activities Structural characterization of new recombinant factor IX expressed in bacteria, including circular dichroism analysis and high throughput crystallization screening.
- IBB-CNR (Italy)** Institute of Biostructures and Bioimaging of the Italian Research Council, Naples
Duration 15/05/2012– 14/05/2013
Position Research Fellowship “PRIN09 - Structural and functional studies of proteins important for the pathogenicity of *Mycobacterium tuberculosis*”
Principal activities Structural and functional characterization key enzymes involved in the *M. tuberculosis* infection. The activity included cloning, expression and purification for structural and functional analyses.

EDUCATION

- Ph.D.** University of Campania Luigi Vanvitelli, Italy (2011-2014)
Cellular and Molecular Biotechnology Award date: 15/12/2014
Thesis title: “Structural and functional characterization of proteins involved in important infective pathologies”, supervisor: Dr Rita Berisio
The PhD project was mainly focused on the structural and functional characterization of RNase AS (Ribonuclease A-specific), a key virulence factor of *M. tuberculosis* infection. The research activities included the fully characterization of the ribonuclease activity by *in vitro* functional assays, and structural characterization its three dimensional structure using X-ray crystallography (Romano et. al 2014).
- M.Sc.** University of Sannio, Italy (2009-2011)
Genetic Sciences and Technologies Award date: 23/02/2012, Score: 110/110 cum laude
Department of Science and Technology (DST)
Thesis Title: “Cytoplasmic localization of Cdk inhibitor p27^{kip1} and its implication in tumor progression”, supervisor: Prof. Giuseppe Viglietto
For my Master’s project at the Oncology lab of the Biogem Consortium, I gained excellent skills in eukaryotic cell cultures, western blot and biochemical assay *in vivo* and *in vitro*, with the aim to investigate the effect of the cytoplasmic localization of Cdk inhibitor p27^{kip1} in tumor progression.
- B.Sc.** University of Sannio, Italy (2006-2009)
Biotechnology Department of Science and Technology (DST)
Award date: 21/11/2009, Score: 110/110 cum laude
Thesis Title: “Preparation and characterization of important biological macromolecules for *M. tuberculosis* pathogenicity”, supervisor: Prof. Giuseppe Graziano
The undergraduate project aimed to express, purify and crystallize RipA, a protein involved in the *M. tuberculosis* cell division and resuscitation.

SKILLS AND EXPERTICES

Recombinant protein production (soluble proteins and membrane proteins)

- Cloning strategy design (expression vectors: pETM, pET, pBAD, pWALDO GFP);
- Small and large scale expression in bacteria (*Escherichia coli*) and in yeast (*Saccharomyces cerevisiae*);
- Protein purification using chromatographic methods (affinity chromatography, size-exclusion chromatography, ion-exchange chromatography).

Functional and structural protein characterization *in solution*:

- Sample characterization by SDS-PAGE and Western blot;
- Structural characterization in solution by CD (Circular Dichroism) and MALS (Multiple Angle Light Scattering);
- Detergent screening for membrane proteins for structural (FSEC: Fluorescence size exclusion chromatography) and functional analyses;
- Membrane proteins reconstitution in liposome (proteo-liposome) for functional analysis;
- Biochemical analyses for *in vitro* interaction such as ITC (Isothermal Titration Calorimetry) and MST (Microscale thermophoresis). Enzymatic characterization by spectrophotometric assays, zymograms, and RP-HPLC (Reverse Phase-High Performance Liquid Chromatography).

Structural characterization of proteins:

- Protein crystallization using vapour diffusion techniques (hanging drop e sitting drop);
- Automated high-throughput protein crystallization screening at nanoliter scale (Hamilton STARlet NanoJet 8+1, TTP Labtech Mosquito)
- Crystals optimization using additive screening e detergent screening (Hampton Research e Molecular Dimensions) an nanoliter and microliter scale.
- Data collection using GDA software (i24, i03, i04 beamlines - Diamond light source synchrotron facility). Plate screening (tray mode on i24 beamline, VMXi: high-flux *in situ* macromolecular crystallography beamline).
- Data analyses (Mosfilm software and ISPyB - Information System for Protein Crystallography Beamline) for data collection strategy and data analyses. Molecular Replacement and refinement using CCP4 and Phenix program suites.

RESEARCH ACTIVITIES

- Publications
- **Romano M.**, Ruggiero A., Squeglia F., Berisio R. “An engineered stable mini-protein to plug SARS-CoV-2 Spikes” Pre-print on BioRxiv 2020 doi: <https://doi.org/10.1101/2020.04.29.067728>
 - **Romano M.**, Ruggiero A., Squeglia F., Maga G. and Berisio R. “A Structural View of SARS-CoV-2 RNA Replication Machinery: RNA Synthesis, Proofreading and Final Capping” *Cells* 2020, 9, 1267. doi: 10.3390/cells9051267. (review)
 - Wong J., **Romano M.**, Kerry L., Kwong H., Low W., Brett S., Clements A., Beis K., Frankel G. “OmpK36-mediated Carbapenem resistance attenuates ST258 *Klebsiella pneumoniae* in vivo” *Nat Commun.* 2019 Sep 2;10(1):3957. doi: 10.1038/s41467-019-11756-y.
 - Calvanese L., Squeglia F., **Romano M.**, D'Auria G., Falcigno L., Berisio R. “Structural and dynamic studies provide insights into specificity and allosteric regulation of ribonuclease AS, a key enzyme in mycobacteria virulence” *J Biomol Struct Dyn.* 2019 Jul 22:1-13. doi: 10.1080/07391102.2019.1643786.
 - Wang L., Bateman B., Zanetti-Domingues L.C., Moores AN., Astbury S., Spindloe C., Darrow MC., **Romano M.**, Needham SR., Beis K., Rolfe DJ., Clarke DT., Martin-Fernandez ML. “Solid immersion microscopy images cells under cryogenic conditions with 12nm resolution” *Commun. Biol.* 2019 Feb 21;2:74. doi: 10.1038/s42003-019-0317-6. eCollection 2019.
 - Husada F., Bountra K., Tassis K., de Boer M., **Romano M.**, Rebuffat S., Beis K., Cordes T. “Conformational dynamics of the ABC transporter McjD seen by single-molecule FRET” *EMBO J.* 2018 Nov 2;37(21). pii: e100056. doi: 10.15252/embj.2018100056. Epub 2018 Sep 20.
 - **Romano M.**, Fusco G., Choudhury H.G., Mehmood S., Robinson C.V., Zirah S., Hegemann J.D., Lescop E., Marahiel M.A., Rebuffat S., De Simone A., Beis K. “Structural Basis for Natural Product Selection and Export by Bacterial ABC Transporters” *ACS Chem Biol.* 2018 Jun 15;13(6):1598-1609. doi: 10.1021/acscchembio.8b00226. Epub 2018 May 18.
 - Squeglia F., **Romano M.**, Ruggiero A., Berisio R. “Molecular Players in Tuberculosis Drug Development: Another Break in the Cell Wall” *Curr Med Chem.* 2017 Nov 24;24(36):3954-3969. doi: 10.2174/0929867324666170208150016. (review)
 - Majkowska-Skrobek G., Łątka A., Berisio R., Maciejewska B., Squeglia F., **Romano M.**, Lavigne R., Struve C., Drulis-Kawa Z. “Capsule-Targeting Depolymerase, Derived from *Klebsiella* KP36 Phage, as a Tool for the Development of Anti-Virulent Strategy” *Viruses.* 2016 Dec 1;8(12). pii: E324.
 - Dennehy R, **Romano M.**, Ruggiero A, Mohamed YF, Dignam SL, Mujica Troncoso C, Callaghan M, Valvano MA, Berisio R, McClean S. “The *Burkholderia cenocepacia* peptidoglycan- associated lipoprotein is involved in epithelial cell attachment and elicitation of inflammation” *Cell Microbiol.* 2017 May;19(5). doi: 10.1111/cmi.12691. Epub 2016 Nov 25 (**co-first**).
 - Ruggiero A, Squeglia F, **Romano M.**, Vitagliano L, De Simone A, Berisio R. “Structure and dynamics of the multi-domain resuscitation promoting factor RpfB from *Mycobacterium tuberculosis*” *J Biomol Struct Dyn.* 2017 May;35(6):1322-1330. doi: 10.1080/07391102.2016.1182947. Epub 2016 Jul 15.
 - Ruggiero A, Squeglia F, **Romano M.**, Berisio R. “Use of Hydrolytic Enzymes for Algal Biomass Treatment” *Current Biotechnology* Volume 5, Issue 4, 2016 doi: 10.2174/2211550105666160518143432 (review)
 - Casey W., Spink N., Cia F., Collins C., **Romano M.**, Berisio R., Gregory J. Bancroft & McClean S. “Identification of an OmpW homologue in *Burkholderia pseudomallei*, a protective vaccine antigen against melioidosis”, *Vaccine.* 2016. doi:10.1016/j.vaccine.2016.03.088
 - Ruggiero A., Squeglia F., **Romano M.**, Vitagliano L., De Simone A., Berisio R. “The structure of Resuscitation promoting factor B from *M. tuberculosis* reveals

unexpected ubiquitin-like domains”, *Biochim Biophys Acta*. 2016 Feb;1860(2):445-51. Epub 2015 Nov 5.

- **Romano M.**, Squeglia F., Berisio R. “Structure and function of RNase AS: A novel virulence factor from *Mycobacterium tuberculosis*”, *Curr Med Chem*. 2015;22(14):1745-56. (review)
- Squeglia F., Ruggiero A., **Romano M.**, Vitagliano L. and Berisio R. “Mutational and structural study of RipA, a key enzyme for *Mycobacterium tuberculosis* cell division: evidence for the L- to D-inversion of configuration of the catalytic cysteine”, *Acta Crystallogr D Biol Crystallogr*. 2014 Sep;70(Pt 9):2295-300. Epub 2014 Aug 29.
- **Romano M.**, van de Weerd R., Brouwer F.C.C., Roviello G., Lacroix R., Vandembroucke-Grauls C.M.J.E, Sparrius M., Brink-van Stempvoort G., Maaskant J.J., van der Sar A.M., Appelmelk B.J., Geurtsen J.J., Berisio R. “Structure and function of RNase AS, a polyadenylate- specific exoribonuclease affecting mycobacterial virulence *in vivo*” - *Structure*. 2014 May 6;22(5):719-30. doi: 10.1016/j.str.2014.01.014. Epub 2014 Apr 3.
- Squeglia F., Bachert B., **Romano M.**, Lukomski S., Berisio R. “Crystallization and preliminary X-ray crystallographic analysis of the variable domain of Scl2.3, a streptococcal collagen-like protein from invasive M3-type *Streptococcus pyogenes*”, *Acta Crystallogr Sect F Struct Biol Cryst Commun*. 2013 Sep;69(Pt 9):1023-5.
- Squeglia F, **Romano M**, Ruggiero A, Vitagliano L, De Simone A, Berisio R. “Carbohydrate Recognition by RpfB from *Mycobacterium tuberculosis* Unveiled by Crystallographic and Molecular Dynamics Analyses”, *Biophys J.*, 2013 Jun 4;104(11):2530-9.

Research project
Responsibility

Short Term Scientific Mission – COST BM 1003 “Functional characterisation of key proteins in *Burkholderia cepacia* infection”, 26 May 2014 – 27 June 2014 - Centre of Microbial Host Interactions - ITT Dublin, Ireland

The project, funded by the European Union, aimed at biochemical and structural study of proteins involved in *Burkholderia cepacia*, an opportunistic pathogen particularly dangerous for cystic fibrosis (CF) patients.

The scientific mission allowed to start a collaboration with the Dr. Siobhan Mc Clean, with follow-up results published on peer-review scientific journals.

Referee activity

Journal CCADD (Current Computer-Aided Drug Design) - Bentham Science

School and
International
Conferences

- 1st AIC-BMM Group Meeting – Fiesole (FI), 20-21 February 2020 (**Invited speaker/oral presentation**: “Structural basis for antibiotic resistance mediated by bacterial porins”)
- AIC International Crystallography School “Crystallographic Information Fiesta – CIFiesta” 29 August – 3 September 2019, University Federico II of Naples (Italy)
- Mechanism of membrane transport, Gordon Research Conference (GRC) Folding and Evolution in the Membrane Environment: New Ways of Understanding Transport Mechanisms, June 23rd – 28th, 2019, Colby-Sawyer College, New London (US) - **Poster presentation**
- Centre of Structural Biology 2019 Open Day - 31th May 2019 - Sir Alexander Fleming Building, South Kensington Campus, UK (**Invited speaker/oral presentation**: “Structural basis for antibiotic resistance mediated by bacterial porins”)
- Biochemical Society Conference “Structure and mechanism of membrane proteins”, 2nd-3rd August 2018, Maple House, Birmingham, UK. **Poster presentation**
- FEBS Special Meeting on ABC Proteins - ABC2018 “ATP-Binding Cassette (ABC) Proteins: From Multidrug Resistance to Genetic Disease”, 6th-12th March, 2018, Innsbruck, Austria

- CCP4 Study Weekend 2017 – 9th-11th January 2017, East Midlands Conference Centre (EMCC), University of Nottingham, UK
- MX Bag Training - Training sessions for user of the Macromolecular Crystallography (MX) beamlines, 22nd – 23rd February 2017 – Diamond Light Source, Research Complex at Harwell, Harwell, UK
- MRC Open Day: Public engagement in Demo session for undergraduate students “Hands on protein crystallisation: lysozyme crystallization” 20 June 2017 - Research Complex at Harwell, Harwell, UK (**Staff scientist for the crystallization session**)
- Centre for Structural Biology 2017 Open Day, 1th June 2017 - Sir Alexander Fleming Building, South Kensington Campus, UK
- AIC International School 2017 “Bridging the gap between Cryo-EM and crystallography”, 3rd-6th September 2017 - University of Pavia, Italy
- 15th Naples Workshop on Bioactive Peptide, 23rd-25th June 2016 – University of Naples “Federico II” and Consortium CIRPEB
- Indigo Workshop 2016 – “Molecular scenario behind bacterial infections”, 4-5 May 2016, Imperial College London, Kensington Campus, London, UK (**Invited speaker/oral presentation: “Structure and function of RNase AS, a novel virulence factor of *M. tuberculosis***)
- AIC European school of crystallography, "Reinforcing Foundations to build the second century of modern crystallography", 31/08/14 – 06/09/14. University of Pavia, Italy - **Scholarship awarded**
- Dublin Training School - COST Project BM1003, “Ion transport, airway liquid dynamics & host pathogen interactions in CF lung epithelia”, 10th-12th September 2014 – University of Dublin, Ireland - **Scholarship awarded**
- Autumn Training School – COST BM1003, “The advantages and limitations of methods used in bacteria identification and typing”, 23rd-25th September 2013 – University of Wroclaw, Poland – **Scholarship awarded**
- Winter Training School – COST BM 1003, “Microbial infection from the chemistry perspective: the bottom-up approach”, 23rd-25th January 2013 - University CEU San Pablo/CIB-CSIC, Madrid, Spain– **Scholarship awarded**
- Training School - COST BM1003, “Assessing bacterial Virulence: a COST Training School”, 5th -7th November 2012 – University of Tübingen, Germany – **Scholarship awarded**
- 13th Naples Workshop on Bioactive Peptide, 7th-10th June 2012 – University of Naples “Federico II” and Consortium CIRPEB – **Poster presentation**
- Workshop “Molecular determinants of bacterial diseases” COST BM1003, 4 June 2012 – University of Naples “Federico II” – **Poster presentation**
- “Course hands-on crystallization of proteins using high-throughput screening” 12th-14th December 2011 - Institute of Biostructures and Bioimaging of the Italian Research Council, Naples