The **4th Meeting of the Biological Macromolecules Section** of the Italian Crystallographic Association was held in the traditional location of the "Centro Studi Ricerca e Formazione CISL" in San Domenico, from 5 to 6 June 2023. The meeting has been organized in four sessions dedicated to relevant topics of today's structural biology. Every session has been structured by combining contributions by outstanding invited speakers, selected speakers among submitted abstracts and flash poster presentations.
Notably, the majority of the 40 participants were young researchers indicating the vitality of our Section and the excellent quality of research, as testified by their presentations.
The meeting has provided a stimulating exchange of information and debate. Indeed, older and younger participants mixed together well in a warm environment and discussions prolonged over the meals and coffee breaks, mainly about scientific subjects, but also on friendly conversations allowing to establish personal relationships. Below is a summary of the Meeting Sections.

Monday, June 5th, 2023

Afternoon session I (*protein-ligand interactions*)

The first session of the 4rd AIC – BMM meeting was chaired by **Massimiliano Perduca** and was centered on protein-ligand interactions. The first invited lecture was delivered by **Claudia Binda**, Associate Professor at the University of Pavia, who presented her important studies about human monoamino oxidases (MAOs), responsible for oxidative deamination of monoamines such as serotonin, adrenaline and dopamine, but also of exogenous aromatic amines derived from food and medications. In her talk, Prof. Binda showed as MAO inhibitors are employed to cure CNS pathologies such as Parkinson Disease and depression. Moreover, since MAOA is emerging as relevant player in cardiac aging, its inhibition could open interesting scenarios to cure cardiac pathologies.

The second talk was given by **Federica Gabriele** from University of L'Aquila who described the X-ray crystal structure of *Cryptosporidium parvum* (Cp) thioredoxin reductase (TrxR), Cp is the causative agent of cryptosporidiosis, one of the most common causes of diarrheal disease worldwide. Dr. Gabriele showed us that the inhibition of thioredoxin reductase a key enzyme for the protozoan survival in the human host could be a good strategy to cure this disease. The X-ray structure of TrxR in complex with auranofin has revealed the molecular basis of the enzyme inhibition by this drug.

The third talk was delivered by **Giusy Tassone** from the University of Siena who presented a molecule repurposing study to investigate the inhibitory properties of a library of amino-thiadiazole derivatives against the human enzyme glutaminyl cyclase (hQC), a zinc-dependent enzyme involved in the neurological disorders like Alzheimer and Huntington diseases. These molecules were formerly developed in the Siena lab to target the parasite enzyme, *Trypanosoma brucei* pteridine reductase 1 (*Tb*PTR1). A set of twenty-four amino-thiadizole compounds have been selected from this library and tested towards hQC, leading to the identification of three inhibitors having K_i values in the high nM range. X-ray crystallographic studies have allowed to establish the structure-activity relationship of these inhibitors and provided clues for further development of this class of molecules.

The fourth talk was given by **Luca Broggini** from the IRCCS Policlinico San Donato, San Donato Milanese, who presented a work on the use of llama-derived nanobodies (Nbs) directed to H3, an amyloidogenic immunoglobulin light chain obtained from patient affected by systemic immunoglobulin light chain (AL) amyloidosis with severe cardiac involvement. Such pathology is caused by conversion of immunoglobulin light chains (LC) from their soluble dimeric functional states into highly organized amyloid fibrils. The project stems from the idea of stabilizing Ig LC to prevent the unfolding preceding fibril formation.
The study demonstrated that Nbs stabilize H3 native fold and that they bind to their target with affinities in the nanomolar range. The crystal structures of H3-Nb complexes show that Nbs form large interaction surfaces with H3 monomeric variable (V_L) domains, stabilizing a partially monomeric LC conformation. *In vivo* experiments on *C. elegans* larinx demonstrated that Nbs complexed to H3 abolish LC toxicity. This work represents an important contribution to expand the toolbox available to fight the disease.

The fifth talk was given by **Cécile Exertier**, from the Institute of Molecular Biology and Pathology of the CNR (Italian National Research Council) who presented a study on fragment-to-lead optimization to design potent new trypanothione reductase inhibitors to tackle Leishmaniases. Dr. Exertier has been studying the inhibition mechanisms of Trypanothione Reductase, a key enzyme in the redox metabolism of *Leishmania* the causative agent of Leishmaniasis. Through the fragment-based screening at Diamond Light Source, she identified new TR low-affinity ligands. These ligands were used to synthesize new and potent lead compounds able to target TR, thereby killing the *Leishmania* parasites.

Antonella Costanzo from the University of Rome, La Sapienza, gave the last talk of the session, presenting a structural study about cytochrome P450 from *Streptomyces antibioticus* CYP107D1 (OleP) and its triple variant F84Q/S240A/v291G. OleP is a relevant enzyme for biotechnological applications as it is able to act on non-physiological substrates leading to products of pharmaceutical interest. The crystallographic studies, flanked by MD and equilibrium binding assays on the complexes of wild-type OleP and of its triple mutant, in complex with the substrate lithocholic acid (LCA) presented in this talk, provide the rationale to explain the changes in regio- and stereo-selectivity of the mutant with respect to the wild-type enzyme. This study opens the way to rational engineering of OleP in order to obtain useful products from a variety of substrates.

The studies presented in this session have provided an interesting picture of how structural biology studies can be used in the design of new lead compounds and how they can allow us to understand the functioning mechanisms of macromolecular complexes.

Afternoon session II (*vaccines and neurodegenerative disorders*)

The second session of the 4th AIC – BMM meeting was chaired by **Massimo Degano** and focused on structural approaches to vaccine development.

The keynote lecture was given by **Rita Berisio** from the Institute of Biostructure and Bioimaging of the CNR of Naples. Rita presented an overview of the ongoing work in her lab as part of the Marie Skłodowska-Curie Action BactiVax (Anti-Bacterial Innovative Vaccines). The projects aim to develop new subunit vaccines against bacterial pathogens, including *Klebsiella pneumoniae, Enterococcus faecium, Staphylococcus aureus, and M. tuberculosis*. The talk gave an insightful perspective on current effort in structural vaccinology.

The second talk was delivered by **Flavia Squeglia**, from the same CNR institute, who reported on the structural characterization of the *K. pneumoniae* Spike depolymerases. These enzymes assist in the disruption of the external capsule polysaccharide, a sugar-based capsule used by *K. pneumoniae* microbes to evade the immune response. Crystallographic structures of Spike depolymerases from several phages revealed the molecular basis for their activity, which was confirmed by functional studies carried out with separation-of-function mutants. Additionally, Flavia used the structural information from her crystallographic studies to engineer smaller chimeric depolymerases for innovative antibacterial treatments.

The third contribution was from **Giovanni Barra**, representing the Università degli Studi della Campania Luigi Vanvitelli of Caserta. Giovanni presented a structure-based approach to identify immunogenic regions of the chaperone-like protein HtpGMtb from *Mycobacterium tuberculosis* for the development of new vaccines. His studies revealed that HtpGMtb is a dimeric protein interacting with nucleotides, and its most promising immunoreacting region is located in the C-terminus. Importantly, his predictions were validated by immunological assays in mice, demonstrating the effectiveness of the structural vaccinology approach he pursued.

The last talk was given by **Giovanni Bisello** from University of Verona, who presented an important study about human aromatic amino acid decarboxylase (AADC), responsible for the synthesis of the essential neurotransmitters dopamine and serotonin and its variants associated with AADC deficiency, a rare autosomal recessive disease that leads to neurotransmitter imbalance. Bisello and coworkers

collected X-ray diffraction data on three AADC variants present in three homozygous AADC deficiency patients thereby defining the molecular basis for the pathologies linked to the mutations.

After the flash-talks associated with the posters, we had a contribution by **Monica Zoppè**, from the Institute of Biophysics of the CNR of Milan, who presented the development of 3D printing methods to generate three-dimensional soft models of protein complexes in silicone. These fascinating techniques allow for a more faithful representation of protein flexibility and conformational rearrangements that proteins can undergo within multi-subunit assemblies.

Overall, the studies presented in this session provided an interesting overview of forefront structure-based approaches currently used for the development of new subunit vaccines, with lively discussions and participation from the audience.

Tuesday, June 6th, 2023

Morning session I – *Combined techniques*

The third session of the 4nd AIC – BMM meeting was chaired by **Cecilia Pozzi** and it was centered on the combined application of different techniques to characterize the structure of single proteins and complexes. The invited lecture was delivered by **Massimo Degano**, project leader at the Università Vita-Slute San Raffaele (Milan). He presented a study about the human ClpP protease, an enzyme involved in mitochondrial proteostasis, which is overexpressed in multiple myeloma and leukemia cells. Its knockdown enhances death of myeloma cell lines in culture, while having limited activity on the electron transport chain. The ClpP structure was determined by X-ray diffraction at 2.5 Å resolution in absence of ligands and by cryo-EM at 2.0 Å resolution in the presence of a covalent inhibitor, which caused a conformational rearrangement of its quaternary structure. Degano showed that even without crystallographic tools such as difference Fourier and omit maps it is possible to investigate chemical modifications of the ligand in cryo-EM maps.

The session continued with four selected talks. The first was given by **Gabriele Giachin**, from the University of Padua. He delivered a talk focused on integrative approaches to understand the complexity of a mitochondrial energy converting machinery. A macromolecular assembly formed by the mitochondrial Complex I and ACAD9, which acts as enzyme in the β-oxidation of fatty acids in mitochondria, was structurally characterized by using cell-based assays, SAXS, EPR and cryo-EM. Notably this latter technique was used to determine from the same images the structural model of Complex I, ACAD9 and the complex formed by the two proteins.

The second talk was given by **Enrico Ravera**, form the University of Firenze, who reported about an integrative view on bioinspired silica-lysozyme composites. Here, the protein promotes the formation of condensed silica microparticles and techniques such as X-ray crystallography, MAS-NMR, SAXS, SEM and EPR spectroscopy were used for understanding the interaction between the protein and the precursor and the fate of the protein after the reaction.

The third talk was by **Michele Tiberi**, from the University of Milan, reporting an integrated structural biology approach to unravel the molecular interplay between transcription factors (TFs). Here, the focus was on the low molecular weight TF/DNA complexes, which are still challenging for cryo-EM characterizations. Combined investigations were focused on the Nuclear Factor Y (NF-Y) and its interplay with other TFs. SAXS and cryo-EM techniques were used to characterize the interaction of NF-Y with a E-box protein, having a molecular weight of only 75 kDa. Noteworthy, the cryo-EM structure was determined at 4.3 Å resolution by using 8 mM CHAPS detergent to eliminate preferential orientation effects.

The fourth talk was given by **Francesco Rinaldi**, from the Italian Institute of Technology, Genoa, reporting important results on the interaction between two proteins: BRCA2, composed of eight BRC repeats, and RAD51, involved in cell repair pathways for DNA lesions. Negative staining TEM combined with SEC revealed that the fourth BRC repeat (BRC4) erodes RAD51 fibrils from their

termini and does not attack the fibril at random positions. SAXS combined with Alphafold2 modeling allowed to investigate the RAD51-BRC4 complex, leading to hypothesize that BRC4 binding triggers a conformational rearrangement on the hRAD51 N-terminal domain from a more ordered to an intrinsically disordered state.

The talks of this section supplied an overview of state-of-the-art techniques that can be used in synergy to gain structural information on protein complexes, protein-protein interactions and conformational changes triggered by ligand binding.

Morning Session II

The final session of the Meeting was chaired by **Rita Berisio** from the Istituto di Biostrutture e Bioimmagini of the CNR who introduced five talks dedicated to crystal preparation and molecular mechanisms.
Cecilia Pozzi, from the University of Siena, presented a study about the C-type lectin-like receptor CD93 as target for antiangiogenic and antioncogenic therapies. Binding of CD93 to Multimerin-2 is involved in endothelial cell migration and tissue remodelling. The crystal structure determination of the sushi domain of CD93 revealed the arrangement of the protein as antiparallel dimer and molecular modelling suggests that this dimer is the functional state to bind Multimerin-2 so promoting the signal to cell migration and remodelling. Interestingly, the structure of CD93 sushi domain could be solved by molecular replacement using a structural model provided by AlphaFold.

Sharda Bharti from the Free University of Bolzano illustrated her results on the structural and functional characterization of proteins involved in siderophore-mediated iron uptake in *Erwinia amylovora*, the causal agent of fire blight disease of rosaceous plants, such as apple or pear trees. Siderophore-assisted iron uptake requires at least an outer membrane receptor (FhuA and FoxR in *E. amylovora*), a periplasmic binding protein (FhuD), an ABC cassette type receptor and a siderophore utilization protein (ViuB). Bharti presented protocols to produce the ViuB protein and initial trials for its crystallization.

Anna Giovanna Sciancalepore from the Institute of Crystallography of the CNR reported her recent results about the characterization of DyPB enzyme from *Rhodococcus jostii* and of its role in aflatoxin removal. DyPB is a heme containing type B peroxidase, providing new insights into the toxin degradation process. Although the binding of Mn(II) ions to DyPB was proposed to have a role in aflatoxin degradation, this study casts doubts about such function.

Silvia Fanti from the University of Bologna presented her interesting results on the investigation of Bovine Serum Albumin (BSA)-nanoparticles as innovative heterogeneous nucleant for protein crystallization. The scope of the project is to search for new materials able induce the nucleation of protein crystals. The novel idea is to explore the ability of nanomaterials exposing protein portions on the surface to induce protein nucleation. For this purpose, Bovine Serum Albumin nanoparticles (BSA-NPs) of different sizes have been prepared, purified and tested as nucleating agents against model proteins (BSA, thaumatin and lysozyme). The results show that larger BSA-NPs are the more effective, indicating a relationship between exposed protein surface area and nucleating power.

The final lecture was given by **Paola Storici** from the Elettra Sincrotrone Trieste who provided a thorough overview of the past and present experiences at the Elettra Protein Facility and, mainly, on the future updates of the Facility in terms of new instrumentation and capabilities from protein

expression, purification, QC and crystallization. In this respect, Paola has asked the contribution from our community to suggest which kind of updates will be mostly desirable in order to prioritize the intervention on the Facility.

The Meeting was concluded by the announcement by Stefano Mangani of the two prizes given by the Scientific Committee to the best oral presentations by young crystallographers consisting in a certificate and of one-year inscription fee to the Italian Crystallographic Association. The prizes were awarded to: **Giovanni Bisello** (on the left) and **Luca Broggini** (on the right, in the photo below with Stefano Mangani)

This was followed by the announcement of the two prizes given by the Scientific Committee to the best posters by young crystallographers consisting in a certificate. The prizes were awarded to:
Flavia Catalano (on the left) and **Sonia Ragusa (**on the right, in the photo below with Stefano Mangani)

A final remark about the extremely good quality of the presented scientific work was done by the Committee, with the wish to meet again next year with intact will to pursue excellence in our science.