NEWSLETTER 12 July 2021

Cari Colleghi ed Amici della Sezione AIC-BMM,

vi proponiamo un aggiornamento sulle attività della Sezione prima della pausa estiva.

Dal 7 al 9 Giugno si è svolto il **2nd AIC-BMM Meeting** in modalità web. L'evento ha confermato l'ottima propensione dei soci a partecipare illustrare i propri studi, scambiare idee e promuovere interazioni. Il nuovo format, che prevedeva il contributo di un rappresentante di Società affini alla nostra, quali SCI, SIB, SILS, BITS, DCMB, SIBPA ha favorito nuove interazioni e collaborazioni. Nell'ambito della conferenza si è anche discusso delle opportunità di accesso da parte dei gruppi italiani a infrastrutture che promuovano l'approccio integrato di varie competenze, essenziale per affrontare le sfide più avanzate dell'attuale Biologia Strutturale. Un report della conferenza è fornito in Allegato 1.

L'1 e il 2 Luglio si è svolto il congresso della Società di Bioinformatica (BITS), ancora in modalità web. Una delle sessioni è stata dedicata allo studio delle proprietà strutturali e funzionali delle proteine ed ha visto la partecipazione di alcuni soci AIC-BMM con affiliazione BITS. La moderazione della sessione è stata affidata dal presidente della BITS (Luciano Milanesi) ad un socio AIC-BMM. Il report dell'evento è fornito in Allegato 2.

Cogliamo l'occasione per ricordarvi i prossimi eventi di interesse per la sezione, riportati in ordine cronologico:

- **Scuola AIC** dal titolo "Fundamentals of Crystallography – the theory behind crystal structure solution" che si terrà in maniera virtuale dal 31 Agosto al 4 Settembre. La scuola sarà di indubbio interesse per i giovani biocristallografi.

- **Congresso AIC Parma**: il prossimo congresso AIC che si terrà in modalità online dal 6 all'8 settembre 2021, e in presenza a Parma nella giornata del 9 settembre (consegna premi AIC e Assemblea dei Soci). Tutte le informazioni sono sul sito http://www.cristallografia.org/congresso2021/

Auguriamo di trascorrere delle buone vacanze, che possano rigenerarci e prepararci ai prossimi impegni.

Il Coordinamento

Stefano Mangani
Rocco Caliandro
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Allegato 1:

The **2nd Meeting of the Biological Macromolecules Section** of the Italian Crystallographic Association has taken place as an on-line event, hosted by the University of Siena (http://www.congressi.unisi.it/icameeting/), from 7 to 9 June 2021. The meeting has been organized in five sessions dedicated to relevant topics of today's structural biology. Every session has been structured by combining contributions by outstanding invited speakers, selected speakers from submitted abstracts and, importantly, by representatives from Italian scientific societies closely related to AIC. Namely, Michele Cianci (Società Italiana di Luce di Sincrotrone), Sebastiano Pasqualato (Società Italiana di Biofisica Pura ed Applicata), Anna Marabotti (Società Italiana di Bioinformatica), Roberta Pierattelli (Società Chimica Italiana -Divisione di Chimica dei Sistemi Biologici).
About 100 scientists from Italy, Europe and abroad participated to the meeting providing a stimulating forum for international and cross-discipline convergence towards frontier aspects of structural biology.

Monday, June 7th, 2021

Morning session I - *Protein small molecule interactions – Structure Based Drug Design*

The first session of the 2nd AIC – BMM meeting was chaired by Prof. Cecilia Pozzi and was centered on the application of structural techniques to investigate the interaction between macromolecular protein targets and small ligands. The first invited lecture was delivered by Dr Alice Douangamath, Senior Beamline Scientist responsible for the XChem facility and its academic user program at the Diamond Light Source. Dr Douangamath presented the XChem platform operating at Diamond, reporting the impressive work done by her research team on the application of the Fragment-Based Drug Discovery to identify new chemical entities that can be developed into potent drug candidates targeting SARS-CoV-2 virus proteases. This topic has a key importance for the structural biology community indeed it resulted in a rich and wide discussion at the end of her talk, pointing out the strong interests of various BMM members in using the Diamond XChem facility.

The session continued with five selected talks. The first was given by Dr Theo Battista, from the Sapienza University of Rome. The talk delivered by Dr Battista focused on the application of structural biology to identity new anti-trypanosomatidal drugs targeting trypanothione reductase (TR). Promising TR-inhibitors were shown during the talk, reporting also preliminary studies aimed at the application of this target for a wider campaign through the XChem platform, an interesting perspective that stimulated the discussion on this topic. The second talk was given by Dr Giovanni Bisello, form the University of Verona, who reported his results on the investigation of Human Aromatic L-Amino Acid Decarboxylase (AADC). Dr Bisello described a series of X-ray crystal structures of AADC in its native and inhibitor-bound state, showing the effects induced by pH variations. The third talk was by Dr Marta Ferraroni, from the University of Florence, reporting the results obtained by her research team on the identification of novel inhibitors of *Schistosoma mansoni* carbonic anhydrase, a key target for the treatment of the parasite diseases due to this worm. Important results were obtained through these studies, leading to the identification of a number of selenoureido derivatives, having superior selectivity for the parasite enzyme with respect to the human counterpart. The fourth talk was given by Dr Francesca Paoletti, from the National Institute of Chemistry (Ljubljana – SLO), reporting important results on the identification of endogenous modulators of the neurotrophin signaling. Through an integrative structural biology approach, Dr Paoletti and her research team were able to unveil for the first time the binding cartography of ATP to the human Nerve Growth Factor (NGF), a key neurotrophin family member playing a crucial role in the activation of the immune and endocrine systems and in the pain pathway. The fifth talk was by Prof Simona Fermani, from the University of Bologna, reporting her interesting results on plant glycolytic glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Structural and functional investigations performed on this target revealed that plant GAPDH is primed to form globular insoluble aggregates by a specific cysteine-based redox modification, namely the S-glutathionylation. All selected talks had a strong impact on the participants, stimulating a wide and rich discussion at the end of each of them.

The first session was closed by a second invited lecture delivered by Prof. Michele Cianci. In his talk, Prof. Cianci provided to the participants a perspective on the synergy between SILS and AIC-BMM stimulating a rich discussion on the potential of collaborations between this societies, both playing a prominent role for the development of the Italian structural biology community. Indeed, among the members of both societies there are prestigious experts, willing to open new collaborations to spread their knowledge on key structural

techniques. Prof. Cianci proposed to establish an E-mail discussion board among Italian structural biologists dedicated to exchange experiences, information, answer questions etc. for educational and research purposes. The discussion on this interesting perspective was continued during the Meeting.

Afternoon Session II – *Macromolecular complexes – Protein-protein – Protein-nucleic acid interactions*

The afternoon session in the first day of the 2nd AIC-BMM congress was chaired by Dr. Sebastiano Pasqualato and proposed talks focused on the structural characterisation of proteins involved in macromolecular complexes of several important biological processes, and the intervention of the representative of the Società Italiana di Biofisica Pura e Applicata (SIBPA).

The session opened with the presentation from the invited speaker prof. Filippo Mancia, of Columbia University, New York, who described recent work aimed at the structural characterization of the complex between palmitoleated Wnt8a and its receptor WLS. Prof. Mancia beautifully described the work carried out to isolate the complex from mammalian cells, and to solve its structure by cryo-electron microscopy, taking advantage of lipid-filled nanodiscs. The structure showed a GPCR fold for WTS, and clearly depicted how the palmitoleate moiety of Wnt8a is hosted in a WLS hydrophobic cavity, protruding to insert into the lipid bilayer, whilst glycans mediate the interaction between the two proteins.
Two talks from PhD candidates followed: one from Ludovica Lopresti, University of Siena, who described the structural insights of the YAP/TEAD4 complex, two downstream effectors of the Hippo pathway, and the other from Cristina Renna, European Institute of Oncology, who discussed about the role of the protein NuMA as a mitotic dynein/dynactin adaptor, presenting the crystal structures of the N-terminal domain of NuMA and its interaction with dynein.
Pietro Roversi, who recently joined the Institute of Agricultural Biology and Biotechnology of the CNR in Milan, then presented its work in progress towards the structural elucidation of key components of the ER Associated Degradation (ERAD) glycoprotein misfolding checkpoint, namely the *Chaetomium thermophilum* ERAD checkpoint complex, the heterodimer formed by the EDEM and PDI proteins.
After the coffee break, three PhD students reported on their studies: Vincenzo Taibi of the FIRC Institute of Molecular Oncology in Milan described his efforts towards the structural characterization of a complex between the HECT domain of the Nedd4 ubiquitin ligase and a ubiquitin trimer; Sharon Spizzichino of the La Sapienza University in Rome presented the ongoing cryo-EM analysis of serine hydroxymethyltransferase (SHMT) and insights in its interaction with RNA; finally Marta Semrau from the Elettra Synchrotron in Trieste talked about her studies on the characterization of the PTG/PP1 complex, key player of the glycogen metabolism in neurons, describing the structure of a portion of PTG in complex with cyclodextrins and the complex of PP1 with two regions of PTG.
The session was closed by prof. Francesco Spinozzi, of the Università Politecnica delle Marche, representative of the Società Italiana di Biofisica Pura e Applicata (SIBPA), who illustrated the activity of SIPBA in terms of conferences, scholarships and scientific contributions.

Tuesday, 8th June 2021
Morning Session III – *Structures of Membrane proteins - Macromolecular Complexes - CryoEM*

The Third Session of the AIC-BBM meeting, chaired by Dr. Linda Celeste Montemiglio, has been focused on macromolecular systems whose high level of structural complexity has required the use of single particle cryo-electron microscopy (cryo-EM) to obtain structural information at near to atomic resolution. More in details, the session has been dedicated to membrane proteins and large macromolecular complexes involved in human pathologies.

The session started with the talk entitled "*Interaction of Huntingtin and HTT-HAP40: what we know (little) and what we don't know (much)"* given by Professor Stefan Kochanek, from the University of Ulm, Germany. He presented his outstanding work on Huntingtin (HTT), the protein whose mutation leads to the neurodegenerative disorder known as Huntington's disease. The cryo-EM structure of HTT obtained in complex with the HTT Associated Protein 40 (HSP40), solved by Prof Kochanek and his group, revealed the overall architecture of the protein, providing a molecular description of a crucial system that for years has remained elusive given the difficulties in obtaining a homogeneous and stable sample amenable for structural

studies. Further investigations of the interaction between HTT and HSP40 provided evidence for its evolutionary conservation, showing that the two proteins started co-evolving at the root of eukaryotes and serve each other to properly fold and possibly function.

There then followed three selected talks from abstracts.

The first one has been held by Professor Stefano Ricagno, from the University of Milan, that presented the "*Structural bases of Light Chain (LC) Amyloidosis: the role of the native and the fibrillar states"*. He showed how single particle cryo-EM allowed shedding light on the molecular structures of cardiac LC amyloid fibrils directly extracted from patients with severe amyloid cardiomyopathy, and how changes in their overall shape are patient-specific.
The second selected talk has been given by Dr Alessandro Grinzato, from ESRF, Grenoble, France, and the University of Padua, Italy. He presented the "*Cryo-EM structure of tetanus neurotoxin in complex with two monoclonal antibodies that interfere with toxin binding and translocation into neurons"*, that revealed how two selected human antibodies, endowed with high neutralizing activities and considered promising candidates for prophylaxis and therapy of human tetanus, are able to interfere with binding and translocation of the neurotoxin into neurons. His talk has been also awarded by the Prize for Best Young Crystallographer presentation.
The third selected talk entitled "*Cryo-EM analysis of membrane NAPE-PLD associated to nanodiscs"* was given by Dr Gianpiero Garau, from the Center IIT@NEST of the Scuola Normale Superiore of Pisa. Thanks to single particle cryo-EM and the usage of a specific nanodisc technology, Dr Garau and his group unveiled the molecular determinants that drive the association of the enzyme to membranes, understanding the role of cofactors of bile acids that bind the enzyme at the membrane interface activating it.
The second part of the Session started with the invited speaker Professor Martino Bolognesi, from the University of Milan, Italy. In his talk entitled "*Mapping gating structural transitions and ion permeation in the HCN4 pacemaker channel"*, he showed the cryo-EM structures of the hyperpolarization-activated cyclic nucleotide (HCN) 4, a member of the voltage-gated potassium channel (Kv) superfamily, in its ligand free form and in complex with cAMP. Prof. Bolognesi and its collaborators observed how, upon cAMP binding, the channel undergoes a large conformational change that is propaedeutic to the current passage, providing further understanding of the mechanism of HCN channel gating, cyclic nucleotide-dependent modulation, and ion permeation.

The Session closed with "*Cryo-electron microscopy"* structure "*of Campylobacter jejuni serine protease HtrA"* presented by the third invited speaker, Prof Giuseppe Zanotti, from the University of Padua, Italy. The serine protease HtrA is an important bacterial virulence factor secreted by *C. jejuni*, the pathogen that causes gastroenteritis. Prof. Zanotti showed the dodecameric architecture of the enzyme, which is composed by a flexible tetramer of trimers, explaining how the protein rearranges upon substrate binding, and providing the first molecular description of the system that in the near future may allow the development of therapeutically relevant inhibitors.

Round Table – *New facilities for structural biology in Italy and Europe*

The round table chaired by Dr. Andrea Ilari, was organized with the purpose to illustrate to the Italian community the main features of the new facilities and the modality of access. The discussion was focussed on Cryo-electron microscopy, which represents the new frontier for the Italian structural biology community.

In the first part of the round table, representatives from different facilities were invited to briefly illustrate them.

The first speaker was Prof. Roberta Pierattelli from University of Florence who presented the platform "Instruct Italia" promoting and fostering an integrated approach, at the national level, providing access to X-ray crystallography, NMR, Cryo-EM as well as protein expression and crystallization. Then, we heard from Dr. Giancarlo Tria who has spoken about the Cryo-EM facility in Florence, followed by Dr. Antonio Chaves who gave us an update about the Milano's Cryo-EM facility and Dr. Paolo Swuec who presented us the Human Technopole facility that is still under construction. Finally, the last speaker was Dr. Andrea Alfieri from the Pavia University where a 200 kV Cryo-Transmission Electron Microscope (Cryo-TEM) has been recently installed.

After these brief presentations, a discussion was opened by Prof. Martino Bolognesi about the future of the Cryo-EM facilities. Major concerns emerged from the following debate.

At the moment, four new facilities are operating or will be operating by the end of this year. All the facilities are concentrated in the north part of the country. What many people underlined in the discussion, is that the facilities have high costs of maintenance and that the Italian structural biology community is quite small. For these reasons, it is important to gather all efforts to raise funds to provide access to such facilities though national public and private agencies and to enlarge the Cryo-EM user community in order to guarantee the maintenance of the instrumentation.

A consequent aspect of paramount relevance is the training of newcomers. Courses and schools should be activated to teach at any level, from undergraduate to doctorate students, the basics of biocrystallography and cryo-electron microscopy. In this respect the AIC-BMM committee, together with the BMM section members, might have a pivotal role for promoting such activities.

Afternoon Session IV – *Computational Methods – Structural Bioinformatics*

The session IV of the 2nd AIC-BMM meeting was chaired by Dr. Benny Danilo Belviso and included four talks focused on new *computational methods and approaches devoted to structural biology*.

The first invited speaker was Dr. Anna Marbotti from Dept. Chemistry and Biology of the University of Salerno. In her talk, which was titled "Prediction of protein structure: The ultimate solution lies Deep (in) Mind", she showed the operation mode of the machine learning approach and how machine learning can be applied in protein structure prediction. The talk was focused on AlphaFold software, an artificial intelligence program developed by Google's DeepMind which performs predictions of protein structure. Dr. Marbotti showed how the prediction of distances between pairs of amino acids and the angles between chemical bonds connecting such amino acids are exploited by AlphaFold for structural prediction and gave details about the results reached by the program in the CASP competition. Finally, the prediction of five SARS-CoV-2 targets made by AlfaFold have also been shown.

Dr. Nicole Balasco (selected speaker) from Institute of Biostructures and Bioimaging (Consiglio Nazionale delle Ricerche) gave a talk entitled "*Unraveling the determinants of the conformational preferences of amino acid residues: the role of the local geometry*". She showed the importance of sequence and of the local conformation states of the individual protein residues for protein structure prediction. Particularly, it has been shown that (dis)similarities of propensity scales, calculated by using Chou-Fasman like formula, between different regions of the Ramachandran plot are coupled with (dis)similarities in the local geometry of protein residues a finding that could have implication in protein prediction.

Dr. Gianluca Santoni from ESRF synchrotron (selected speaker) reported about Coronavirus Structural Task Force, how it works and which is the target of the project. Around 95% of the users who download protein structures from PDB are not experienced structural biologists and, thus, they are not trained to recognize easily errors in the deposited structures. Unavoidably, this affects processes such as drug design that bases on protein structure. This task force gathers 25 international structural biologists which offer their time and expertise to fix errors in structural models of proteins in the PDB or before deposition in the database. The current target of the project regards COVID19 related proteins but it is not excluded that, in the future, it can be applied also to proteins related to other diseases in order to give the best protein structure to drug designers.

The last part of the session was dedicated to the contribute of Prof. James Fraser from University of California, San Francisco (awarded of the 2020 IUCr Bragg Prize). His talk was titled "Targeting COVID-19 Viral Enzymes in an Evolving Landscape of Publishing and Peer Review" and it was about the identification of new inhibitors directed to SARS-CoV-2 main protease and, particularly, towards the NSP3 domain of the protease, which is one of less studied target among those of SARS-CoV-2. His lab made-up a fragment-soaking pipeline by which the electron density maps arising from multiple soaking experiments are averaged and subtracted to density of a single soaking experiment, a process that leads to a density map where the signal related to the binding event is enhanced. It has been shown how cryo-cooling, which is necessary for automation and crystal protection, increases conformational and orientation differences between datasets, thus affecting density map averaging process. Such method provided up to 230 potential ligands for the main protease that, in turn, have been linked/merged to produce final optimized molecules. The best inhibitor produced by this method show dissociation constant close to 5μM, suggesting that a strong inhibition is a way off still.

Wednesday, June 9th 2021
Session V – *Cool & hot structures – Difficult Cases – Disordered proteins*

The last session of the 2nd AIC-BMM congress, chaired by Dr. Rocco Caliandro, was devoted to challenging cases, which can be tackled by the synergistic use of latest discoveries in computational methods and different experimental techniques, such as MX, NMR and SAXS.
The first invited speaker was Dr. Rita Berisio from CNR-IBB, who gave an overview on how bacterial cell wall degrading enzymes can be used against challenging antimicrobial resistant infectious pathogens. By degrading the capsule polysaccharides (CPS) these enzymes can be used as antibacterials. Here the difficulty was in the poor diffraction of crystals formed by large flexible molecules, for example formed by the complex of an adhesin and a CPS degrading enzyme.
Among the speakers selected from abstract, Dr. Benny Danilo Belviso, from CNR-IC, explained how SAXS data have been used to structurally characterize the C-term domains of important epigenetic targets: the nuclear SET domain methyltransferases (NSD). Atomistic models derived by integrating molecular dynamics with SAXS data have been achieved for two constructs of NSD2 and NSD3, and compared with recently delivered cryoEM models of histone-NSD complexes. Dr. Gabriele Giahin, from University of Padova, presented a study on the human prion protein mutans, which were co-crystallized with a nanobody with anti-prion activity. The crystallographic study was complemented by NMR and molecular dynamics simulations.
The second invited speaker was Prof. Roberta Pierattelli from University of Florence, as representative of the Italian Chemical Society (SCI). She opened a window about the use of NMR to characterize flexible linkers between structured domains and intrinsic disordered proteins in terms of secondary structure propensity.
The last part of the session was dedicated to contributes from Trieste. Dr. Sonia Covaceuszach from CNR-IC showed how a combined evolutionary and structural approach allow to disclose the structural determinants essential for proneurotrophins biological functions. Dr. Elisa Costanzi from Elettra Sincrotrone Trieste presented the latest results of the Exscalate4Cov project. She showed a complex of the main protease of SARS-COV2 with myricetin, which could be used for drug repurposing, and further protein-ligand complexes that can be used for de-novo structure-based drug design. The last invited speaker was Dr. Annie Heroux, beamline scientist of the XRD2 beamline of Elettra. She showed the potentialities of the beamline and technical details about its use as remote or in person user.
At the end of the meeting, **the best young Crystallographer prize** was assigned, based on the quality of the presentation, the relevance of the results and on the discussion given by the speaker. The prize was awarded with equal merit to Alessandro Grinzato, Sharon Spizzichino and Elisa Costanzi. The prize consists in a Certificate and a free registration for one year to the AIC.
The session ended by the concluding remarks of the AIC-BMM congress, made by Prof. Stefano Mangani on behalf of the organizing commitee.

Allegato 2:

Partecipazione al congress BITS

Il socio Rocco Caliandro, membro del comitato di coordinamento della sessione BMM, ha partecipato come co-chair al meeting della società italiana di Bioinformatica (BITS), che si à svolto in maniera virtuale l'1 e il 2 Luglio 2021.

Un rinnovato impulso ad instaurare rapporti tra AIC e BITS è stato dato in occasione del secondo AIC-BMM meeting, svoltosi anch'esso in maniera viruale il dal 7 al 9 giugno 2021, durante il quale Anna Marabotti dell'Università di Salerno, in qualità di rappresentante della BITS, ha tenuto un intervento dal titolo "Prediction of protein structure: the ultimate solution lies Deep (in) Mind" nell'ambito del microsimposio "Computational methods – Structural Bioinformatics". L'intervento è stato molto apprezzato dai soci AIC, in quanto ha presentato in maniera didattica le strategie di implementazione delle reti neurali per la predizione della struttura delle proteine a partire dalla loro sequenza.

L'interazione tra AIC e BITS è proseguita con l'invito espresso dal presidente BITS Luciano Milanesi ad un socio AIC-BMM a partecipare alla sessione di bioinformatica strutturale del convegno BITS in qualità di co-chair.

Il programma del meeting BITS prevedeva le seguenti sessioni: Systems Biology, Gene regulation, transcriptomics and epigenomics, Algorithms for Bioinformatics and Biological Networks, Bioinformatics challenges in the SARS-Cov-2/COVID-19 pandemic, Multiomics and Single Cell Analysis, Miscellaneous, Protein structure and function. Gli aspetti strutturali sono stati affrontati solo nell'ultima sessione, nella quale si sono succeduti tre contributi selezionati da abstract tenuti da giovani ricercatori ed un talk su invito. Giulia Babbi, dell'Università di Bologna (gruppo di Rita Casadio) ha presentato una caratterizzazione fisico-chimica e funzionale su larga scala delle variazioni patogeniche a singolo residuo, Bernardina Scafuri dell'Università di Salerno (gruppo di Anna Marabotti) ha presentato un confronto tra predittori della stabilità termodinamica di mutazioni e Matteo Uggeri dall'Università di Genova ha presentato uno studio computazionale sull'interazione di una piccola molecola su una proteina coinvolta nella fibrosi cistica. Le presentazioni sono state seguite da discussioni stimolanti, alle quali hanno preso parte anche soci AIC (Luigi Vitagliano e Rocco Caliandro). Il talk su invito è stato tenuto da Josè Duarte, che ha celebrato il 50-imo anniversario del PDB con un excursus storico incentrato proprio sulla Cristallografia delle macromolecole biologiche.

Prima della conclusione, il meeting BITS ha previsto una discussione sugli aspetti didattici della Bioinformatica, nel quale vari professori universitari hanno confrontato i loro programmi di insegnamento e scambiato opinioni circa la possibilità di inquadrare meglio la Bioinformatica nell'ambito dei vari corsi universitari. In particolare, è stata avanzata l'ipotesi di formulare un nuovo settore scientifico-disciplinare incentrato sulla Bioinformatica, istanza che sarebbe stata supportata presso il ministero dalla società BITS tutta. Una discussione analoga in seno alla AIC potrebbe essere auspicabile.