Joint Workshop IC- Kemijski inštitut

Report of Contributions

Contribution ID: **1** Type: **not specified**

Discussion and Conclusions

Wednesday, 21 June 2023 16:55 (5 minutes)

Speakers: Dr. Cinzia Giannini (IC-CNR) and Dr Barbara Tišler (the Assistant director for development and quality -Kemijski inštitut)

Contribution ID: **2** Type: **not specified**

Welcome and intro IC-CNR & KEMIJSKI INSTITUT

Wednesday, 21 June 2023 14:00 (15 minutes)

Presenters: Dr GIANNINI, Cinzia (Director IC-CNR); Dr TIŠLER, Barbara (the Assistant director for development and quality -Kemijski inštitut)

Session Classification: Session

Contribution ID: **3** Type: **not specified**

NMR-assisted studies of ligand-protein interactions in solution

Wednesday, 21 June 2023 14:15 (15 minutes)

Nuclear Magnetic Resonance (NMR) spectroscopy in solution can effectively recognize and characterize protein state heterogeneity and can simultaneously probe the structures and dynamics of proteins and their complexes with atomic resolution. It can also detect sparsely populated, high-energy conformational states of proteins with population fractions close to 1% and lifetimes from μs to ms. The method is non-invasive and non-destructive and can study biomolecules under near physiological conditions even in living cells. In addition, new hardware, software, isotope labelling strategies, and pulse sequences have helped to move the main limitation of solution NMR spectroscopy, namely the molecular weight, to the order of 1 MDa. Solution NMR spectroscopy can detect ligand binding over a wide range of binding affinities and is uniquely suited for characterizing protein weak binders. Regardless of the thermodynamic nature of the interactions, the heteronuclear single quantum coherence NMR method is capable of quantitatively determining the residue-specific parameters for very weak binding events (KD > 10 mM) for which other traditional biophysical methods are not reliable.
The potential of ligand-based and protein-based NMR methods for the identification and characterization of ligand-protein interactions will be presented using the results of our studies of ligand binding to the protein targets for the development of antimicrobial agents such as muramyl ligase D and sterol 14-alpha; demethylase [1], including studies of transient interactions of endogenous modulators with nerve growth factor [2].

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Presenter: Prof. GRDADOLNIK, Simona Golič (KEMIJSKI INSTITUT)

Session Classification: Session

Contribution ID: **8** Type: **not specified**

New therapeutic targets against Non-Tubercolulous Mycobacteria: Salycilate Synthase from M. abscessus

Wednesday, 21 June 2023 15:00 (15 minutes)

Non-tubercolous mycobacteria (NTM) encompass a diverse group of 172 species with distinct virulence features, that differentiate from the M. tuberculosis complex. NTM are widely distributed in the environment and affect individuals with chronic pulmonary diseases like cystic fibrosis (CF), leading to severe infections.
Among NTM, M. abscessus is emerging as one of the most virulent pathogens, contributing to increased morbidity and mortality in CF patients. Complex M. abscessus infections are extremely difficult to treat due to their high drug and disinfectant resistance. Novel therapeutic strategies are essential to enhance clinical outcomes in CF patients with M. abscessus infections, and targeting iron intake, essential for many virulence factors, appears promising for inhibiting M. abscessus proliferation pathogenicity.
In this study, we employed a Structure-Based Drug Discovery approach to identify potential inhibitors with a furan-based scaffold against M. abscessus Salycilate Synthase (Mab-SAS), an enzyme involved in siderophore biosynthesis and iron intake. We conducted Grating Coupled Interferometry measurements on repurposed Mab SAS inhibitors and newly developed compounds. Additionally, the crystal structure of Mab-SAS was determined to serve as a structural foundation to virtual screening.
These findings hold implications for the discovery of novel therapeutic candidates against M. abscessus infections in CF patients. Targeting Mab-SAS to inhibit iron intake could provide an effective approach to limit the proliferation and pathogenicity of M. abscessus.

Faria S., et al. J. Pathog. 2015, 2015,809014
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Mori M. et al. Pharmaceutics, 2023, 15, 502

Presenter: Dr CASSETTA, Alberto (IC-CNR)

Session Classification: Session

Contribution ID: **16** Type: **not specified**

Small Angle X-ray Scattering (SAXS) in combination with other complementary techniques to tackle challenging protein structures

Wednesday, 21 June 2023 14:30 (15 minutes)

Small Angle X-ray Scattering (SAXS) is widely used in the study of biomolecules, providing valuable insights into their structural properties and behavior in solution. SAXS is particularly suitable for investigating biomolecules' overall shape, size, conformation, and flexibility, including proteins, nucleic acids, and complexes. In the case of proteins, SAXS can provide information about their tertiary and quaternary structure, domain organization, and overall shape. SAXS is also useful for studying protein-protein interactions and the assembly of biomolecular complexes. One of the advantages of SAXS in studying biomolecules is its ability to analyze molecules in solution under near-physiological conditions. This allows for the investigation of biomolecular behavior in their native state, providing insights into their dynamics and conformational changes. Small Angle X-ray Scattering (SAXS) is often combined with other complementary techniques, such as X-ray Crystallography, Nuclear Magnetic Resonance (NMR) Spectroscopy, Electron Microscopy (EM), Mass Spectroscopy, Molecular Dynamic simulations (DM) and protein-protein docking, to tackle challenging protein structures and obtain a more comprehensive understanding of their properties. Results obtained from the study of proteins and their complexes involved in the mechanism of diseases such as Shwachman Diamond [1-2] and Jalili [3] syndromes, and tuberculosis [4], will be shown.

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Presenter: Dr SILIQI, Dritan (IC-CNR)

Session Classification: Session

Contribution ID: **18** Type: **not specified**

Simulations of water transport in membrane proteins

Wednesday, 21 June 2023 14:45 (15 minutes)

Computer simulations are becoming an inevitable tool for describing the dynamics and function of biological macromolecules at various levels of resolution, in particular at the atomistic level. Among motions particularly important are those related to the transport processes. The complex topology of macromolecular channels and the transient nature of the penetrant passage pose difficulties in the modeling of the penetrant entry/escape pathways. We elucidate the basic physical factors influencing the water permeation in sodium glucose cotransporter SGLT1 including the channel opening as well as dynamic flexibility and its relationship with the domain motion.

Presenter: Dr MERZEL, Franci (KEMIJSKI INSTITUT)

Session Classification: Session

Contribution ID: **19** Type: **not specified**

Structural and functional insights into plant pathogens

Wednesday, 21 June 2023 15:15 (15 minutes)

The interplay between pathogens and their targets is the focus of research in our department. We are particularly interested in the molecular interactions and molecular mechanisms of action that lead to various pathological conditions in humans, animals or plants. Plants are vital and an essential resource for food, water, medicine, oxygen, habitat, climate and more. But plants also get sick. We study the molecular background of plant diseases caused by microorganisms such as bacteria, fungi, oomycetes and viruses that cause major agricultural losses worldwide. In my talk, I will present a selection of structural and functional studies of plant pests studied in our research group.

Presenter: Prof. PRESENTED BY DR. MATIC KISOVEC, Marjetka Podobnik (KEMIJSKI INSTITUT)

Session Classification: Session

Contribution ID: **20** Type: **not specified**

Understanding the interaction between Aβ42 and β-amyloid aggregation inhibitors: a mass spectrometry based-approach

Wednesday, 21 June 2023 16:40 (15 minutes)

Neurodegenerative disorders (NDs) such as Alzheimer's disease (AD), Parkinson's disease (PD) and prion diseases are some of the most common forms of age-related diseases. Even if pathogenesis of these neurodegenerative diseases remains unclear, increasing evidence point out a common critical molecular process involving the assembly of various aggregated proteins with a β-sheet conformation, referred to as amyloids.[1] The inhibition of this process could be a viable therapeutic strategy for the treatment of neurodegenerative diseases. Peptide based inhibitors of β-amyloid fibrillation can prevent Aβ aggregation into fibrils by binding the protein and are emerging as safe drug candidates as well as interesting compounds for early diagnosis of AD.[2] The identification of adducts formation by means of mass spectrometry techniques, can be used to obtain a direct evidence of the interaction between Aβ42 and its aggregation inhibitors. These interactions can alter peptide-chain flexibility affecting the cleavage of the peptide bonds by a protease. Moreover, interactions can also occur at the peptide bonds involved in the proteolytic cleavage in turn affecting enzyme's accessibility to the cleavage sites. Therefore, the identification of proteolysis resistant peptides fragments, by mass spectrometry, may reveal the amino acid residues involved in the interaction of Aβ42 with specific molecules. Here we report studies using mass spectrometry techniques to investigate the interaction of Aβ42 monomer with conjugated peptides that we recently proposed as aggregation inhibitors.[3-5] All the results observed indicate a different behaviour of the proposed molecules on the proteolytic pattern of Aβ. Compelling evidences were observed when interactions concerned the N-terminal domain of the protein. Understanding the effect of these interactions on the aggregation processes, at the molecular level, will enable to outline the features of new and more effective aggregation inhibitors.

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Presenter: Dr DI NATALE, Giuseppe (IC-CNR)

Session Classification: Session

Contribution ID: **21** Type: **not specified**

The cryo-electron microscopy facility at the National Institute of Chemistry

Wednesday, 21 June 2023 15:55 (15 minutes)

The field of cryo-electron microscopy (cryo-EM) has seen rapid development and significant technical improvements in the last decade. Perhaps most important have been the advances in sample preparation, direct electron detectors and data analysis for which the Nobel Prize in Chemistry was awarded in 2017. Cryo-EM has become an important tool for gaining structural insights into cells, organelles, biomolecular complexes, proteins, peptides and small crystalline molecules. In addition to biological samples, cryo-EM can also be a successful tool for structural analysis of some radiation-sensitive organic and inorganic samples, as well as various industrial samples, especially from the pharmaceutical and food industries. At atomic or near-atomic resolution, cryo-EM offers a unique combination of features.
The cryo-EM facility at the National Institute of Chemistry was established in 2019 and remains the only one in the wider south-eastern EU region. It houses the 200 kV Glacios cryo-transmission electron microscope, which can perform single particle analysis (SPA), tomography (cryo- ET) and microcrystal electron diffraction (MicroED) experiments.
Researchers can test and screen various samples (isolated proteins, (synthetic) protein complexes, biological and synthetic membranes, viruses and virus-like particles, nanoparticles, etc.) and perform sample optimization for final data collection, which can be performed either at this microscope or at other cryo-EM facilities.
Samples are vitrified by plunge-freezing (Vitrobot), and the Falcon 3 detector enables acquisition of high-quality data. High performance computing (HPC) infrastructure is available for data storage and analysis.
The cryo-EM facility is part of the Centre for Molecular Interactions and Structural Biology (CMISB) within the Department of Molecular Biology and Nanobiotechnology and is open to internal and external users from academia and industry. The facility can be contacted via email cryoem@ki.si.

See the attached picture: Three main cryo-EM modalities available at the NIC. Single Particle Analysis - SPA (a), cryo electron tomography - cryo-ET (b), Microcrystal electron diffraction - MicroED (c).

Presenter: Dr KISOVEC, Matic (KEMIJSKI INSTITUT)

Session Classification: Session

Contribution ID: **22** Type: **not specified**

DeLA-Drug: A Deep Learning Algorithm for Automated Design of Drug-like Analogues

Wednesday, 21 June 2023 16:10 (15 minutes)

We present DeLA-Drug,(1) a recurrent neural network (RNN) model composed of two Long Short-Term Memory (LSTM) layers and conceived for data-driven generation of drug-like compounds. DeLA-Drug captures the syntax of SMILES strings of more than 1 million molecules belonging to the ChEMBL28 database and generates analogues starting from a single user-defined query compound by employing a new strategy called Sampling With Substitutions (SWS). The generative model preserves drug-likeness and synthetic accessibility of the known bioactive compounds belonging to the ChEMBL28 repository. The absence of any time-demanding fine-tuning procedure enables DeLA-Drug to perform a fast generation of focused libraries for further high-throughput screening and makes it a suitable tool for performing de-novo design even in low-data regimes. DeLA-Drug, available as a free web platform (http://www.ba.ic.cnr.it/softwareic/deladrugportal/), can help medicinal chemists interested in generating analogues of compounds already available in their laboratories and, for this reason, good candidates for an easy and low-cost synthesis.

See the attached picture: Main steps of the DeLA-Drug workflow.

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Presenter: Dr LAMANNA, Giuseppe (IC-CNR)

Session Classification: Session

Contribution ID: **23** Type: **not specified**

Structural characterization of Coiled-Coil Protein Origami (CCPO) structures

Wednesday, 21 June 2023 16:25 (15 minutes)

Coiled-coil protein origami (CCPO) uses modular coiled-coil building blocks for the de novo design of polyhedral protein nanostructures, using topological design principles distinct from natural globular proteins. While the CCPO strategy has proven successful in designing various protein topologies, obtaining high-resolution structural information has remained challenging due to the small size and high flexibility of these novel protein folds. To overcome these challenge, we employed specific techniques and approaches in our study. Our X-ray crystallography efforts yielded a high-resolution crystal structure of the triangular CCPO by implementing shorter linkers, more stable coiled-coil peptides, and incorporating a natural GCN homodimer. Additionally, we made progress with cryo-electron microscopy by utilizing nanobodies, which allowed us to obtain a high-resolution structure of one vertex of the tetrahedral CCPO.

Presenter: Dr SATLER, Tadej (KEMIJSKI INSTITUT)

Session Classification: Session

Contribution ID: **24** Type: **not specified**

The health seen through the structural hierarchies of collagen: from disease to regeneration

Wednesday, 21 June 2023 15:40 (15 minutes)

In recent decades the extracellular matrix (ECM) has aroused growing interest in biomedical and regenerative fields. It is a complex network made by different macromolecules, in which the main component is the type I collagen, a fibril-forming protein characterized by a tissue-specific morphology. Its hierarchical structure, with specific functional domains, supplies bio-physical support to cells attachment, tissue growth and re-modeling. The multi-level structure possesses a specific arrangement from the molecular order, up to supramolecular scale. In particular it is organized in triple helices assembled in fibrils and fibers, in accordance with a liquid crystalline arrangement at nanoscale, a quasi hexagonal packing observed in corneal tissue.[1] To deeply understand the role of its physiologic features and with the aim to exploit them for biomaterial fabrication useful in regenerative medicine, we investigated type I collagen with both Wide (WAXS) and Small (SAXS) Angle X ray Scattering techniques, of collagen powders and flakes obtained after its extraction from natural tissues and of engineered biomaterial during each step of fabrication process. Both X rays techniques have revealed how manufacturing protocols deeply affect the structural characteristic, both atomic (WAXS) and nanoscale (SAXS), of the biomaterial itself, thus its function, becoming fundamental tools to screen the suitable protocols, according to the tissue to regenerate. [2,3,4,5]
As collagen structure modifications can be related to specific disease prognosis, we also used scanning SAXS and WAXS microscopies for diagnosis, inspecting the structural features in aneurysms biopsies, and in diabetes minimal models.
Precisely, in aneurysms, mapping biopsies allowed to detect and co-localize the nanometric structure of several organic components of the tissues (type I collagen, myofilament and elastin) as well as to identify crystalline phases of pathological micro calcifications.[6] In diabetes, analyses were conducted on decellularized bovine pericardia biotissues, soaked with different sugars (D-glucose, D-galactose, D-ribose) at increasing concentrations (0, 2.5, 5, 10, 20 and 40 mg/ml), and incubated at 37℃ for 3, 14, 30 and 90 days, to identify the sites of glycation and speed of glycation due to glucose/galactose and ribose.[7,8,9]

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2021, 8, 1024-1034

Presenter: Dr TERZI, Alberta (IC-CNR)

Session Classification: Session