Joined Workshop between the Institute of Crystallography-CNR (IC-CNR) and the Maastricht MultiModal Molecular Imaging Institute (M4i), Maastricht University, The Netherlands

Report of Contributions

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

Discussion and Conclusions

Tuesday, 7 March 2023 16:30 (10 minutes)

Speakers: Dr. Cinzia Giannini (IC-CNR) and Prof Ron Heeren (M4I)

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

Welcome and intro IC-CNR & M4I

Tuesday, 7 March 2023 13:00 (15 minutes)

Presenters: Dr GIANNINI, Cinzia (IC-CNR);   Prof. HEEREN, Ron (M4I)

Session Classification: Session

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

Machine learning-based prediction of hERG-mediated cardiotoxicity: a structure-based investigation

Tuesday, 7 March 2023 13:15 (30 minutes)

Giuseppe Felice Mangiatordi(1) Pietro Delre(1,2) Teresa Maria Creanza(3), Nicola Ancona,(3) Giovanni Lentini,(4) Michele Saviano(1).

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Prioritizing drug candidates based on their human ether-à-go-go-related gene potassium channel (hERG) blocking potential is a mandatory step in the early preclinical stage of a drug discovery program. The hERG blockade is, in fact, considered the main cause of cardiotoxicity in post-marketing surveillance. Several ligand-based approaches were therefore developed in the last years and are currently employed in a drug discovery process for in silico cardiac safety assessment of drug candidates. Herein, the first structure-based classifiers able to discern hERG binders from non-binders will be presented.(1) LASSO regularized Support Vector Machines were applied to integrate docking scores and protein-ligand interaction fingerprints. 396 models were trained and validated based on: i) high-quality experimental bioactivity information returned by 8,337 curated compounds extracted from ChEMBL (version 25(2)) and ii) structural predictor data. Molecular docking simulations were performed by using GLIDE and GOLD software programs and different hERG structural models, namely the recently published structures obtained by cryo-electron microscopy (PDB codes: 5VA1(3)and 7CN1(4)) and two published homology models selected for comparison. Remarkably, some models return performances comparable to ligand-based classifiers in terms of accuracy (AUCMAX = 0.86±0.01) and negative predictive values (NPVMAX = 0.81±0.01) thus putting forward the herein developed computational workflow as a valuable tool for predicting hERG mediated cardiotoxicity without the limitations of ligand-based models, typically affected by low interpretability and a limited applicability domain. The study highlights the importance of using hERG structural models accounting for ligand-induced fit effects and allowed us to select the best performing protein conformation to be employed for a reliable structure-based prediction of hERG-related cardiotoxicity.

References
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R.; Yonemori, K.; Igaki, S.; Yasuda, S.; Ogasawara, S.; Senda, T.; Murata, T. Cryo-EM Structure of K+-Bound HERG Channel Complexed with the Blocker Astemizole. Structure 2021, 29 (3), 203-212.

Presenter: Dr MANGIATORDI, Giuseppe Felice (IC-CNR (Bari))

Session Classification: Session

Contribution ID: 7
Type: **not specified**

Insights on the molecular and structural basis of human dystroglycanopathies

Tuesday, 7 March 2023 15:30 (30 minutes)

Cassetta A(1), Longo F(1), Sciandra F(2), Bozzi M(2,3), Bigotti MG,(4) Hübner W(5), Brancaccio A(2), Covaceuszach S(1)

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Dystroglycanopathies are neuromuscular disorders associated with abnormal neuronal migration and muscular dystrophy. Clinical manifestations are extremely variable, and include a wide spectrum of phenotypic severity. Dystroglycanopathies are mainly due to abnormal glycosylation of dystroglycan (DG) which is a cell-surface glycoprotein that links the cytoskeleton with the extracellular matrix acting as a receptor for extracellular matrix proteins containing laminin-G domains. Dystroglycan is composed of two subunits: the extracellular highly glycosylated α-DG and the transmembrane β-DG. A multistep glycosylation process is necessary to decorate the α-DG subunit with complex glycans that are crucial for its interaction with the extracellular matrix proteins, such as laminins. Most of the dystroglycanopathies are due to an impaired functional state of the enzymes involved in α-DG maturation. Nevertheless, a set of missense mutations has been recently identified on the N-terminal region of α-DG (a.a. 50-313 in mouse) that determine the hypoglycosylation of the DG complex, due to the impairment of a key step in α-DG glycosylation operated by the bifunctional glycosyltransferase LARGE1.

With the aim of elucidating the molecular and structural implications of the pathological mutations leading to dystroglycanopathies, we have undertaken a multi-technique study, including synchrotron radiation approaches to gain insight on the molecular structure of a recently discovered α-DG point pathological mutant (L84F) in both crystals and solution. Indeed, we determined the high-resolution molecular structure of L84F mutant by X-ray crystallography. Moreover, Small Angle X-ray Scattering (SAXS) has been employed as a complementary approach to get low resolution information on the flexibility, conformation and the structural organization of this α DG mutant at near-physiological conditions. The results of the synchrotron radiation-based experiments, combined with biochemical, cellular and microscopic data, allowed us to shed light on the molecular and structural basis of dystroglycanopathies.

Presenter: COVACEUSZACH, Sonia (CNR-IC (Trieste))

Session Classification: Session

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

Towards more dose efficient cryogenic electron microscopy of biological samples

Tuesday, 7 March 2023 15:00 (30 minutes)

Cryo-electron microscopy (cryo-EM) has become an indispensable tool for structural biologists studying the relationship between structure and function of various biomolecules. Recent advancements in transmission electron microscopy (TEM) hardware and data processing software have enabled atomic-level resolution for single particle cryo-EM. However, achieving near-atomic resolution for smaller (<100 kDa), more heterogeneous, and non-symmetric samples remains a significant challenge due to the limited scattering information provided by smaller particles, the high defocus required for image contrast enhancement, and the radiation damage that restricts the number of high-energy electrons per surface area, resulting in low signal-to-noise ratios. To address these challenges, we simulated single particle data sets using realistic parameters for ice layer, dose, detector performance, and beam characteristics for samples that were ideal in terms of homogeneity, distribution, and stability. Our simulations could help expand the size limits of cryo-EM. Meanwhile, we also reviewed alternative TEM techniques such as phase plate and ptychography that hold promise for providing complementary or additional structural information within the limited lifetime of the sample. Finally, we implemented a new event-based electron detector and show experimental data substantiating some of the promises we could simulate.

Presenter: Dr ZHANG, Yue (M4I)

Session Classification: Session

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

Pushing the limits of sample vitrification

Tuesday, 7 March 2023 13:45 (30 minutes)

Cryogenic Electron Microscopy (cryo-EM) is a powerful imaging technique for visualizing molecular structures with unprecedented detail. To prepare samples for cryo-EM a process called vitrification is typically used which involves rapidly freezing a thin layer of sample to create a non-crystalline solid. Plunge freezing is currently the most commonly method used method for preparing samples for Single particle analysis and Cryo Electron Tomography (cryo-ET) workflows. This method works with isolated proteins but is not ideal for thicker samples like whole cells which is necessary for Cryo-ET. To address this challenge, a Vitrojet for cells (VitroJet4Cells) was developed which utilizes ethane jet to vitrify samples. The power of jet has already been proven effective with VitroJet for single particles. A functional prototype of the machine was successfully developed and is currently being used for testing various samples. The cooling potential of the jet is primarily examined using a micro loop set up, which can accommodate a wide range of samples, frozen samples are then examined using an X-ray beam to determine their vitrification quality. The machine is also compatible with standard EM grids. Clipped EM grids with adherent cells are, blotted, vitrified and subsequently utilized for making lamellas to be used in cryo-ET.

Presenter: Dr PREMARAJ, Navya (M4I)

Session Classification: Session

Contribution ID: **17**
Type: **not specified**

Structural insight into mycobacterial protein secretion

Tuesday, 7 March 2023 16:00 (30 minutes)

Mycobacteria tuberculosis employs Type 7 Secretion System (T7SS) to secrete effector proteins to help escape from the host immune system. There are five gene clusters named ESX-1 to ESX-5 belonging to T7SS in M. tuberculosis. ESX-1 is the first identified ESX system and has been shown to be essential for phagosome rapture. Secreted proteins have to cross both membranes of the bacterium. ESX-1 is expected to carry out this process, however, only the inner-membrane core complex components have been identified thus far.

ESX-1 inner core complex consists of five subunits: EccB1, EccCa1, EccCb1, EccD1 and EccE1. It has an estimated molecular mass of ˜2 MDa. All five genes were constructed in multi-cassette vectors with different tags fused to one of the protein subunits. Presence of all five subunits could be detected after purification (by both Western Blot and Mass Spectrometry), however, purified complex from E. coli did not result in useable cryo-EM data thus far. In contrast, good success was obtained for purified ESX-1 components as well as substrates. In here, I will give a short overview about the TB research work done at Maastricht University.

Presenter: Dr GAO, Ye (M4I)

Session Classification: Session

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

Beyond the "Amyloid Hypothesis"? Rescuing proteostasis in Alzheimer's Disease"

Tuesday, 7 March 2023 14:15 (30 minutes)

Danilo Milardi

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Alzheimer's Disease (AD) is the most common form of dementia in the elderly population. The estimated global prevalence of AD in 2015 was 46 million with an incidence rate in 2050 of 131 million. In the absence of medical advancements to prevent, slow down, or stop the disease, there will be dramatic effects on society, global health, and economy. AD is characterized by an abnormal accumulation of Aβ amyloid plaques in the brain. The idea that amyloid Aβ peptide aggregation into amyloid fibrils is an important factor in AD development (Amyloid Hypothesis) is supported by a considerable body of evidence. However, the failure of clinical trials for molecules targeting Aβ misfolding and self-assembly points to the need for a deeper comprehension of the mechanisms behind the impaired proteome maintenance occurring in AD.

Here I propose a brief survey on the intertwined biochemical mechanisms that control Aβ homeostasis (proteostasis) by employing an interdisciplinary approach to screen small molecules (e.g. natural compounds, bioconjugates, and repurposed drugs) for their ability to restore physiological Aβ homeostasis by a multi-target strategy. This research activity spans from fundamental topics related to protein/lipid membrane stability, amyloid aggregation, proteasome activation and ligand protein interactions to applications in medicinal chemistry focusing on the development of bioactive compounds as drug candidates in AD therapy.

Presenter: Dr MILARDI, Danilo (IC-CNR (Catania))

Session Classification: Session