26th Swedish Conference on

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PROGRAM

Friday June 16t

16:30 – 18:00 Conference desk open

18:00 – 19:30 Dinner

Session Ia: Opening and Awards

Chair: Dr. Burmann & Dr. Knecht

19:45 – 20:15 Welcome, presentation of Keynote Lecturers and the The Svedberg Awardee

20:15 – 21:00 The Svedberg Award Lecture: **Simon Elsässer** (Karolinska Institutet)

21:00 – 22:00 PI meeting

Saturday June 17th

07:00 – 08:45 Breakfast

Session II: Beyond classical structural biology

Chair: Dr. Neutze

09:00 – 09:45 Keynote 1: **Serena DeBeer** (MPI Mühlheim, Germany)

Spectroscopic and computational studies of biological nitrogen reduction

09:45 – 10:05 **Katja Bekcic** (Karolinska Institutet)

Optimizing large-scale production of circular RNA for in-cell NMR of nucleic acids

10:05 – 10:25 **Nicolas Pearce** (Linköping University)

Refactoring the B-factor: intuitively extracting structural dynamics from macromolecular disorder

10:25 – 10:50 Coffee

10:50 – 11:35 Keynote 2: **Alan Brown** (Harvard Medical School, USA)

Using cryo-EM to build atomic models of ciliary axonemes

11:35 – 11:55 **Johanna Höög** (University of Gothenburg)

3D TEM Studies of Mammalian Sperm Extra-Axonemal Structures.

11:55 – 12:15 **Juliane John** (Stockholm University)

XFEL investigation of redox-dependant flavin strain in the ribonucleotide reductase R2b-NrdI complex

12:20 – 13:40 Lunch

Session III: Membrane proteins in health and disease I

Chair: Dr. Esbjörner

13:40 – 14:25 Keynote 3: **Layara Akemi Abiko** (Biozentrum Basel, Switzerland)

Influencing the β1-adrenergic receptor conformational equilibrium by targeting its empty cavities

14:25 – 14:45 **Yue Chen** (KTH Stockholm)

Mechanism of ligand-dependent D2 dopamine receptor activation revealed by free-energy landscapes

14:45 – 15:05 **Jessica Glas** (University of Gothenburg)

High-resolution structure of a fish aquaporin reveals a novel extracellular fold

15:05 – 15:25 **Lightning talks**

15:25 – 16:00 Coffee

Session IV: Membrane proteins in health and disease II

Chair: Dr. Hedfalk

16:00 – 16:45 Keynote 4: **Patrick Sexton** (Monash University, Australia)

Advancing GPCR drug discovery using cryo-EM

16:45 – 17:05 **Nour Aldin Kahlous** (Uppsala University)

Design of drug efficacy guided by free energy simulations of G protein-coupled receptors

17:05 – 17:25 **Alexandra Berg** (Umeå University)

Nematocida displodere mechanosensitive ion channel of small conductance 2 assembles into a unique six-channel super-structure *in vitro*

17:25 – 17:45 **Johannes Thoma** (University of Gothenburg)

Exploiting OMVs for the *in-situ* characterization of bacterial envelope proteins

18:00 – 19:30 Dinner

20:00 – 21:30 **Poster Session II**

Sunday June 18th

07:00 – 08:45 Breakfast

Session Ib: Awards

Chair: **Dr. Morud Lekholm**

09:00 – 09:15 Presentation of The Theorell Awardee.

09:15 – 10:00 The Theorell Award Lecture: **Marta Carrroni** (SciLifeLab Stockholm)

10:00 – 10:30 Coffee

Session V: Integrated structural biology

Chair: **Dr. Burmann**

10:30 – 11:15 Keynote 5: **Sebastian Hiller** (Biozentrum Basel, Switzerland)

Integrative structural biology to resolve molecular machines and membrane megaprores

11:15 – 11:35 **Annika Breidenstein** (Umeå University)

Structural and functional characterization of the relaxase TraI – Implications for Gram-negative Type 4 secretion systems

11:35 – 11:55 **Vamsi Krishna Moparthi** (Linköping University)

Regulation of MYC-MAX DNA binding in cancer

11:55 – 12:15 **Oksana Koshla** (Uppsala University)

The iron sensing transcriptional factor IdeR and its function in the metabolism of *Saccharopolyspora erythraea*

12:20 – 13:40 Lunch

Session VI: Success stories using research infrastructures in Sweden

Chair: **Dr. Göran Karlsson**

13:40 – 14:00 **Bärbel Lorenz** (Lumicks)

Molecular tractor beams? Unravelling biological mechanisms by looking at single molecules in real-time

14:00 – 14:20 **Simon Ekström** (Lund University)

Cross-linking and Hydrogen-Deuterium Exchange Mass Spectrometry as a National Infrastructure Service

14:20 – 14:40 **Huanbing Wang** (Stockholm University/China)

The structural biology in protein folding and aggregation.

14:40 – 15:00 **Atsarina Larasati Anindiya** (University of Gothenburg)

Prediction of peptide binding to survivin based on experimental microarray fluorescence intensities.

15:20 – 15:40 **Tristan Kenney** (Linköping University/University of Toronto)

The dynamic interactions between Myc and RNA Polymerase II component TFIIF component

15:40 – 16:10 Coffee

16:20 – 18:00 Annual football match

18:00 – 19:30 Dinner

20:00 – 21:30 **Poster Session II**

Monday 19th June

07:00 – 08:45 Breakfast

Session VII: Drug discovery

Chair: **Dr. Käck**

09:00 – 09:45 Keynote 6: **Alessio Ciulli** (University of Dundee, UK)

How PROTAC degraders work: Molecular recognition and design principles

09:45 – 10:05 **Wolfgang Knecht** (Lund University)

Structural basis for small molecule binding to SARS-COV-2 nonstructural protein 10

10:10 – 10:30 Coffee

10:30 – 10:50 **Andreas Luttens** (Uppsala University)

Virtual Fragment Screening for DNA Repair Inhibitors in Vast Chemical Space

10:50 – 11:10 **Adrian Gonzales Lopez** (Uppsala University)

Cryo-EM structures of Staphylococcus aureus 70S ribosomes in complex with elongation factor G and fusidic acid

11:10 – 11:30 **Sayyed Jalil Mahdizadeh** (University of Gothenburg)

I-TripleD, an AI tool for *de novo* drug discovery

11:35 – 11:50 Closing of meeting and award of prizes for best presentation and poster

12:00 – 13:00 Lunch + Departure

Spectroscopic and computational studies of biological nitrogen reduction

Serena DeBeer[1]

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The biological conversion of N_2 to NH_3 is accomplished by the nitrogenase family, which is collectively comprised of three closely related but unique metalloenzymes. In this talk, I will present recent comparative studies using a combination of the synchrotron-based technique of ^{57}Fe nuclear resonance vibrational spectroscopy together with DFT-based quantum mechanics/molecular mechanics (QM/MM) calculations to probe the electronic structure and dynamics of the catalytic components of each of the three unique M N_2ase enzymes (M = Mo, V, Fe) in both the presence (holo-) and absence (apo-) of the catalytic FeMco clusters (FeMoco, FeVco and FeFeco). In addition, recent studies on the interaction of CO with the FeMoco site of nitrogenase will be presented for both the native enzyme, as well as biohybrids immobilized on electrodes.

Optimizing large-scale production of circular RNA for in-cell NMR of nucleic acids

Katja Bekcic[1], Henry Annecke[1#], Rubin Dasgupta[#] and Katja Petzold[#,*]

[1]Equal contribution [#]Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden, *Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

To perform its cellular functions, RNA undergoes changes in structure, also referred to as dynamics. These dynamics are essential for cellular biochemistry. Therefore, studying them allows us to reveal the mechanism of biological processes involving RNA such as microRNA targeting or ribosome function and assists in designing drugs to target RNAs. Currently, these dynamics are predominantly studied *in vitro* using NMR spectroscopy, which differs from the crowded and variable environment of a cell. In recent years, in-cell NMR experiments have shown that RNA structure and dynamics can be probed within the cellular environment.[1] One main limitation of in-cell NMR compared to *in vitro* NMR is the presence of RNases, which rapidly degrade RNA and consequently limit NMR measurement time. However, RNA lacking a free 3′-hydroxyl & 5′-phosphate, such as circular RNA, is resistant to degradation by exonucleases.[2] To investigate the ability of circular RNA to improve in-cell NMR we created a pipeline for the large-scale production of circular RNA. The circularization of GUG, an artificial RNA with dynamics in a single uridine bulge was accomplished with the addition of a UUCG tetraloop to the end of the hairpin construct. Ligation was performed with T4 RNA ligase 2. We observed that RNA synthesized by *in vitro* transcription and purified by preparative gel had greatly reduced ligation efficiency. Dynamics studies using ^{1}H $R_{1\rho}$ relaxation dispersion[3] of the circular-GUG and comparison with its linear counterpart revealed the addition of a UUCG loop reduced R_1 and R_2 rates. This is likely due to coaxial stacking of the linear construct, which is prevented by the UUCG loop in the circular construct thereby reducing the overall rotational correlation time. Additionally, both constructs favor a three-state exchange model. It is anticipated that circularization of RNA, which confers protection towards nucleases, can be a strategy to study biologically relevant RNA dynamics in the cellular context using NMR spectroscopy.

1. Schlagnitweit J, Friebe Sandoz S, Jaworski A, et al. Observing an Antisense Drug Complex in Intact Human Cells by in-Cell NMR Spectroscopy. *ChemBioChem.* 2019;20(19):2474-2478.
2. Abe N, Abe H, Ito Y. Dumbbell-shaped nanocircular RNAs for RNA interference. *J Am Chem Soc*. 2007;129(49):15108-15109.
3. Marušič M, Schlagnitweit J, Petzold K. RNA Dynamics by NMR Spectroscopy. *ChemBioChem*. 2019;20(21):2685-2710.

Refactoring the B-factor: intuitively extracting structural dynamics from macromolecular disorder

Nicholas M Pearce* and Piet Gros‡

* Linköping University, Sweden, ‡ Utrecht University, The Netherlands

Displacement parameters (B-factors) play a crucial role in macromolecular structure determination yet are rarely used for biological interpretation. This is somewhat egregious, since these are the experimental parameters that account for the local flexibility of conformational states. The absence of approaches for extracting flexibility from experimental structures has meant this is largely left to computational approaches such as molecular dynamics. We have recently developed a new approach for extracting molecular, domain, secondary structure, and atomic motions from the disorder in macromolecular structures, which is available as an open-source tool. This method makes both large-scale molecular and small-scale atomic disorder intuitively understandable and allows a widespread reinterpretation of experimental macromolecular structures. We demonstrate the method by applying it to SARS-CoV-2 structures, where we characterize the flexibility of the binding site of the main protease, and clearly reveal the collective motions of the receptor binding domains of the spike glycoprotein that correspond to transitions from closed to open states. Lastly, we demonstrate how flexibility can link structure to function, through an analysis of the cryo-EM structure of STEAP4, an iron reductase. We find that analysis of experimental disorder points the way to closing the gap between the static structures of crystallography and cryo-electron microscopy, and the dynamic ensembles obtained from molecular dynamics simulations.

1. Pearce, N. M. & Gros, P. A method for intuitively extracting macromolecular dynamics from structural disorder . *bioRxiv* 2021.03.22.436387 (2021) doi:10.1101/2021.03.22.436387.

Using cryo-EM to build atomic models of ciliary axonemes

Alan Brown*

*Harvard Medical Scholl, Boston, USA

Motile cilia (or flagella, as they are sometimes known) are responsible for the movement of many unicellular organisms including algae and protozoan parasites, and in the human body, for the swimming of sperm cells and the movement of liquids, such as mucus and cerebrospinal fluid. Defective ciliary motility can lead to male infertility and a rare genetic disorder called primary ciliary dyskinesia (PCD), where impaired mucociliary clearance leads to progressive respiratory failure. Ciliary motility is generated by the axoneme, a molecular machine so large it occupies almost the entire volume of each cilium. Each axoneme consists of geometrically complex arrangement of microtubules, ATP-powered dynein motors, and their mechanoregulatory complexes. The size and complexity of the axoneme has long impeded an atomic model, hindering efforts to understand how all the components are integrated together to orchestrate the beating of a cilium. In this study, we have utilized recent advances in cryo-electron microscopy (cryo-EM) and artificial intelligence (AI)-enabled structure prediction to determine the 96-nm modular repeats of axonemes from *Chlamydomonas reinhardtii* flagella and human respiratory cilia. Our atomic models provide insights into the conservation and specialization of axonemes, the interconnectivity between dyneins and their regulators, and the mechanisms that maintain axonemal periodicity and regulate motility. Cryo-EM structures of respiratory cilia doublet microtubules from organoids derived from individuals with PCD reveal how the loss of individual docking factors can selectively eliminate periodically repeating structures, and provide a roadmap for the future of personalized structural biology.

3D TEM Studies of Mammalian Sperm Extra-Axonemal Structures.

Vajradhar Acharya[1], Davide Zabeo[1,2], Jacob Croft[1,3] and Johanna Höög[1]

[1]Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg 41390, Sweden,
[2]Electron Bio-imaging Centre (eBIC), Harwell Science and Innovation Campus, Didcot OX11 0DE, UK,
[3]Department of Biochemistry, University of Washington, Seattle, WA 98195.

Mammalian spermatozoa are highly specialized cells, responsible for fertilization and possess a motile flagellum. Inside of the sperm tail, there is the microtubule based motile machinery (the axoneme) as well as extensive, sperm specific, associated structures such as the annulus, outer dense fibers and the fibrous sheath. Although defects in proteins within these extra-axonemal structures cause male infertility their structure, organization and interactions with other flagellar components remain poorly understood.

Here, cryo-electron tomography is combined with serial-section room temperature electron tomography to study bovine and human sperm flagella. Artificial intelligence segmentation (Dragonfly) of obtained tomograms was performed to semi-automatically annotate all flagellar components and generate 3D models of the cells. Our results revealed details about the spatial relationships of the annulus, ODFs and the FS with each other along with their connections to the axoneme. The structural changes of the accessory structures along the length of the flagellum is also described. We are currently investigating if sub-tomogram averaging can be applied to our cryo-electron tomograms of intact sperm tails to unveil the molecular architecture of the fibrous sheath.

Our study provides a 3D map of the sperm tail into which future high-resolution 3D structural information can be docked. An improved understanding of the structural arrangements inside the sperm tail might reveal how the accessory structures contribute to the sperm motility and thus, male fertility. Our results may also have implications for the development of new diagnostic and therapeutic strategies for male infertility, or the development of a non-hormonal male contraceptive.

XFEL investigation of redox-dependant flavin strain in the ribonucleotide reductase R2b-Nrdl complex

Juliane John*

*Stockholm University

In the class Ib ribonucleotide reductase (RNR) system the flavoprotein Nrdl produces superoxide, which is shuttled to the R2b active site and used to generate a tyrosine radical. This radical is essential for a functional RNR and hence the reduction of ribonucleotides.
Here, we show two different oxidation states of the R2b-Nrdl complex and describe redox-dependant crosstalk within the complex. High-resolution structures obtained by femtosecond crystallography ascertain defined redox-states of the Nrdl and show structural rearrangements around the flavin binding pocket. We discuss mechanistic implications of redox control for the R2b radical generation.

Influencing the β1-adrenergic receptor conformational equilibrium by targeting its empty cavities

Layara Akemi Abiko, Raphael Dias Teixeira, and Stephan Grzesiek
Focal Area Structural Biology and Biophysics, Biozentrum, University of Basel, CH-4056 Basel, Switzerland

G protein-coupled receptors (GPCRs) are important drug targets, which mediate the majority of cellular responses to a wide variety of extracellular stimuli across the plasma membrane.[1] The functional states of GPCRs involve highly dynamic equilibria between multiple conformations.[2] The conformational equilibria of GPCRs are sensitive to many factors, for example point mutations, pressure and the lipid environment. We have recently shown that pressure also modulates the conformational equilibria of the β1-adrenergic receptor (β1AR).3 When subjected to pressure, β1AR is shifted from a mixture of inactive and active conformations to a fully populated active conformation even in the absence of a G protein or G protein-mimicking nanobody. This pressure dependence shows that the active conformation has an about 100.[3] smaller volume than the preactive conformation, which must be due to the collapse of empty (not water-filled) voids within the receptor-detergent micelle.

Here we explore how such empty cavities within the receptor modulates its conformational equilibrium by using an integrative structural biology approach. We were able to localize the dry pockets using X-ray crystallography, and to measure the population of the well-defined functional states of β1AR by solution state NMR.[4] We found out that by partially filling the empty cavities with extrinsic molecules, the β1AR conformational equilibrium is shifted towards the inactive state, blocking the activating movements of the conserved GPCR microswitches, and revealing hot spots for allosteric regulation. This may provide new routes for the development of new, highly selective allosteric drugs.

References:

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[2] Latorraca, N. R.; Venkatakrishnan, A. J.; Dror, R. O. Chem. Rev. 117 (1), 139–155 (2017).

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[4] Abiko, L. A.*;Teixeira, R. D.; Engilberge, S.; Grahl, A.; Grzesiek, S.* Nat. Chem. 14, 1133–1141 (2022)

Mechanism of Ligand-dependent D2 Dopamine Receptor Activation Reveled by Free-energy Landscapes

Yue Chen[1], Jens Carlsson[2], Lucie Delemotte[1]

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G-protein-coupled receptors (GPCRs) respond to a variety of extracellular stimuli to mediate diverse cellular signals. Despite the high similarity of their orthosteric binding sites and endogenous ligand chemistry, GPCRs are selectively activated by specific ligands/drugs. Thus, understanding and predicting the underlying mechanisms for ligand-dependent signaling responses is critical for the development of efficient drugs, which remains a major challenge. Here we used enhanced sampling molecular dynamic (MD) simulations[1] to disclose the activation mechanism for D2 dopamine receptor (D2R) in the presence of agonists with similar chemical structures but various efficacies. Free energy landscapes projected along functional microswitches located from ligand-binding to G-protein binding sites revealed agonist-induced conformational transitions between the inactive and the active states. Further analyses of the correlation between ligand efficacy and the outward movement of transmembrane helix 6 (TM6) identify that D2R exists in a ligand efficacy-dependent equilibrium between an inactive, partially active, and fully active conformation. Together, these findings provide mechanistic insights into the dynamic activation of GPCRs and useful information for designing specific GPCR ligands with desired activity profile.

References

1. Fleetwood, Oliver, et al. "Energy landscapes reveal agonist control of G protein-coupled receptor activation via microswitches." Biochemistry 59.7 (2020): 880-891

Acknowledgments

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High-resolution structure of a fish aquaporin reveals a novel extracellular fold

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Aquaporins are protein channels embedded in the lipid bilayer in cells from all organisms on earth that are crucial for water homeostasis. In fish, aquaporins are believed to be important for osmoregulation; however, the molecular mechanism behind this is poorly understood. Here, we present the first structural and functional characterization of a fish aquaporin; cpAQP1aa from the fresh water fish climbing perch (*Anabas testudineus*), a species that is of high osmoregulatory interest because of its ability to spend time in seawater and on land. These studies show that cpAQP1aa is a water-specific aquaporin with a unique fold on the extracellular side that results in a constriction region. Functional analysis combined with molecular dynamic simulations suggests that phosphorylation at two sites causes structural perturbations in this region that may have implications for channel gating from the extracellular side.

Advancing GPCR drug discovery using cryo-EM

Patrick M. Sexton

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G protein-coupled receptors (GPCRs) are the largest family of cell surface receptor proteins and critical targets for current and future therapeutics. Technological evolution in cryo-EM combined with continuing advances in biochemical approaches for the stabilisation of active-state complexes of GPCRs with different transducer proteins is now enabling structural interrogation of agonist binding, receptor activation and transducer engagement. Indeed, cryo-EM has become the predominant method for GPCR structure determination. Such cryo-EM enabled structures provide key insight into both peptide and small molecule drug action, while the breadth of application of cryo-EM for study of GPCRs has greatly expanded the number of independent receptors where structures have been determined. Moreover, cryo-EM can access conformational ensembles of GPCR complexes that are present during vitrification, which can provide a window into the dynamics of these complexes. Using exemplar receptors, I will discuss how we are using cryo-EM to provide novel insight into the structure and dynamics of drug-receptor complexes, including enhanced understanding of the molecular basis for distinct ligand pharmacology, how small molecules can potentially mimic peptide pharmacology and how small molecules and interacting proteins can modulate receptor engagement with endogenous ligands.

PMS is a scientific co-founder of Septerna Inc. and DACRA Therapeutics.

Design of drug efficacy guided by free energy simulations of G protein-coupled receptors

Nicolas Panel[1], Duc Duy Vo,[1,&], Nour Aldin Kahlous[1,&], Harald Hübner[2], Stephanie Tiedt[2], Pierre Matricon[1], Jody Pacalon[1], Oliver Fleetwood[3], Stefanie Kampen[1], Andreas Luttens[1], Lucie Delemotte[3], Jan Kihlberg[4], Peter Gmeiner[2,*], Jens Carlsson[1, *]

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G protein-coupled receptors (GPCR) play important roles in physiological processes and are modulated by drugs that either activate or block signaling. Rational design of the pharmacological efficacy profiles of GPCR ligands could enable the development of more efficient drugs, but is challenging even if high-resolution receptor structures are available. We performed molecular dynamics simulations of the ß2 adrenergic receptor (ß2R) in active and inactive conformations to assess if binding free energy calculations can predict differences in ligand efficacy for closely related compounds. Previously identified ligands were successfully classified into groups with comparable efficacy profiles based on the calculated shift in ligand affinity upon activation. A series of ligands were then predicted and synthesized, leading to the discovery of partial agonists with nanomolar potencies and novel scaffolds. Our results demonstrate that free energy simulations enable the design of ligand efficacy and the same approach can be applied to other GPCR drug targets.

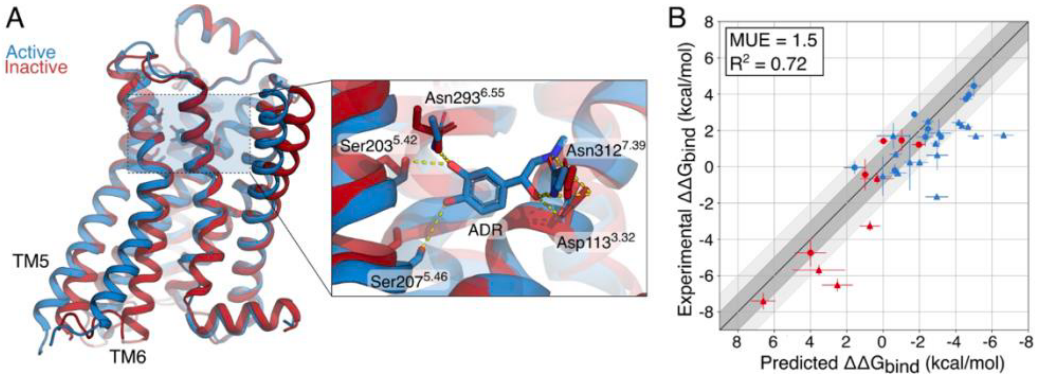

Figure1. Molecular basis of β2R activation and relative binding free energy calculations in active and inactive receptor conformations. **A)** Structures of the active and inactive conformations of the β2R. Adrenaline and key binding site residues. **B)** Correlation between calculated and experimental relative binding-free energies of β2R ligands.

References Panel N, Vo D, Kahlous N, Hübner H, Tiedt S, Matricon P, Pacalon J, Fleetwood O, Kampen S, Luttens A, Delemotte L, Kihlberg J, Gmeiner P, Carlsson J (2023) Design of Drug Efficacy Guided by Free Energy Simulaitons of the β2-Adrenoceptor. *Angew. Chem. Int. Ed.* e202218959. DOI: 10.1002/anie.202218959

Nematocida displodere mechanosensitive ion channel of small conductance 2 assembles into a unique six-channel super-structure *in vitro*

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Mechanosensitive ion channels play an essential role in reacting to environmental signals and sustaining cell integrity by facilitating ion flux across membranes. For obligate intracellular pathogens like microsporidia, adapting to changes in host environment is crucial for survival and propagation. Despite representing a eukaryote of extreme genome reduction, microsporidia have expanded the gene family of mechanosensitive ion channels of small conductance (MscS) through repeated gene duplication and horizontal gene transfer. At least five copies of MscS are present in each to-date characterized microsporidian genome: One subfamily related to eukaryotic MscS and the other of bacterial origin. A single copy of the bacterially derived mechanosensitive ion channel of small conductance 2 (MscS2) is highly conserved amongst all microsporidian species sequenced to date. However, compared to its bacterial counterpart it is extremely reduced and it is unclear if MscS2 forms a channel protein and if so, what role it plays in microsporidia.

Here, we investigated the cryo-electron microscopy structure of MscS2 from *Nematocida displodere*, an intracellular pathogen of *Caenorhabditis elegans*. We purified MscS2 and used size exclusion chromatography, negative-stain transmission electron microscopy, cryo-electron microscopy, and mass photometry to analyze size and structure.

Nematocida displodere MscS2 assembles into a unique superstructure *in vitro* with six heptameric MscS2 complexes interacting through their transmembrane domains. Individual MscS2 channels are oriented in a heterogeneous manner to one another, resembling an asymmetric, flexible sixway cross joint. This highly unusual assembly provides a novel basis to design oligomers that interact through hydrophobic interfaces.

Exploiting OMVs for the *in-situ* characterization of bacterial envelope proteins

Johannes Thoma*, Tanguy LeMarchand**, Tobias Sparrman***, Guido Pintacuda**, and Björn M. Burmann*

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The cell envelope of Gram-negative bacteria is distinguished by a highly asymmetric outer membrane, the outer leaflet of which is formed exclusively by lipopolysaccharides, as well as the gel-like periplasm, which contains proteins in an extraordinarily high concentration. Its complexity makes it virtually impossible to mimic this environment under laboratory conditions, while its low volume ratio contributing only 5-10% to the total bacterial volume marks a challenge for methods such as in-cell NMR spectroscopy.

To overcome this impasse, we recently established a method to modify the protein composition of outer membrane vesicles (OMVs) released from *Escherichia coli* as a unique platform to facilitate the biophysical characterization of envelope proteins within their native cellular environment. OMVs are produced by Gram-negative bacteria in a natural process, have the same asymmetric membrane composition as the parental outer membrane and contain highly dense periplasmic fluid in their lumen, this way providing an isolated cell-envelope microenvironment. These vesicles provide an ideal platform to characterize proteins in a cellular context, which can neither be reconstituted nor mimicked under laboratory conditions

We demonstrate how protein-enriched OMVs can be applied to characterize the structure and dynamics of periplasmic as well as outer membrane proteins *in situ.* Using solution NMR spectroscopy, we obtain high-resolution multidimensional spectra of periplasmic proteins encased in the native lumen of OMVs and utilizing methyl TROSY methods we unravel their dynamics within this crowded cellular environment. Likewise, using solid state NMR spectroscopy allows us to characterize transmembrane domains of outer membrane proteins embedded in the native asymmetric membrane of OMVs. While our work shows that for some proteins the overall fold is similar to what has previously been determined using purified proteins, we observe pronounced structural and dynamic changes imposed by the native environment, which can serve as a proxy for the influence proteins underly in cellular environments in general. Moreover, our work provides a methodological foundation applicable to prokaryotic and eukaryotic extracellular vesicles on a much broader level in the future.

Integrative structural biology to resolve molecular machines and membrane megapores

Sebastian Hiller

Biozentrum, University of Basel, Switzerland

Modern structural biology combines multiple techniques to resolve structure, function, and dynamics of biomolecular systems. Its cornerstones are the three atomic-resolution techniques cryo-electron microscopy, X-ray crystallography and solution NMR spectroscopy, as well as the recent theoretical advance Alphafold.

I will present three case studies, where key functional aspects of challenging biomolecular systems were resolved by a versatile integrative structural biology approach. (i) The molecular Hsp70 chaperone BiP, which features an unexpected 5-step functional cycle at the heart of eukaryotic protein homeostasis. (ii) The disulfide isomerase PDIA6, which condensates to form a phase-separated sub-compartment within the endoplasmic reticulum. (iii) The protein ninjurin-1, which forms gigantic pores to lyse the eukaryotic cell at the endpoint of multiple cell death pathways. The three studies showcase beautiful biology and give insights, how our method set is arranged to handle expected and unexpected challenges.

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- And a lot of unpublished data.

Structural and functional characterization of the relaxase TraI – Implications for Gram-negative Type 4 Secretion Systems

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Rising levels of antibiotic resistance and nosocomial infections represent a great threat to human health. Type 4 Secretion Systems (T4SS) allow bacteria to transfer DNA between each other and spread resistance genes via conjugative plasmids. T4SSs are a highly variable superfamily of secretion systems, which can be found in many bacterial species including Gram-negative and Gram-positive ones. In order for plasmid transfer to occur, the DNA needs to be linearized and unwound through the formation of the relaxosome. I study the T4SS from the conjugative plasmid pKM101. In this system the relaxosome is made up of an accessory factor (TraK), the relaxase (TraI) and the plasmid DNA. TraI consists of a trans-esterase domain, which is responsible for nicking the DNA, a helicase domain for unwinding the strands and a disordered C-terminal region. Although structures of a few relaxases are known, their interactions with the remaining components of the relaxosome are poorly characterized. In this project, we used X-ray crystallography and biochemical assays to study DNA binding of TraI, revealing a conformational change upon substrate binding within the trans-esterase domain. Additionally, we present ongoing work on the interactions between TraI and the accessory factor TraK, where we are using AlphaFold2 to predict protein interactions that inform experimental design to validate and structurally characterize the proposed binding sites, using *in vitro* and *in vivo* methods.

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Breidenstein A, ter Beek J & Berntsson RP-A. Life Science Alliance 2023, 6 (4) e202201775 DOI: 10.26508/lsa.202201775

Regulation of MYC-MAX DNA binding in cancer

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MYC proteins are multifunctional and crucial for various cellular process. MYC proteins act as a universal upregulator of gene expression, except early genes in cells. Deregulated MYC proteins lead to an unregulated expression of many genes that results for transforming normal cell to cancer cell (Meyer and Penn 2008). Thus, MYC proteins are strongly considered as a promising target for anti-cancer drugs. These proteins belong to the MYC family of transcription factors and comprises a large intrinsically disordered region, comprising conserved so called MYC Box (MB0 – IV) regions and a bHLHzip DNA-binding motif (basic helixloop-helix and leucine zipper motif). These regions play an important role in MYC interactions with DNA and other transcription factors. Our research mainly focuses on the understanding of the interactions between MYC and its interaction partner proteins like MAX. MYC-MAX heterodimer assembly is known to play a central hub in cellular growth control. Uncontrolled MYC expression turns the MYC-MAX heterodimer into an oncoprotein multimodular platform and acts a key contributor to the development of numerous cancers.

In this work, we present the structure envelope of extended MYC(MBIV)-MAX and MAX-MAX dimers in complex with DNA, as described by small angle scattering methods, SAXS and SANS. Existing crystal structures of MYC-MAX and MAX-MAX dimers only included the bHLHzip DNAbinding motif without flanking regulatory regions. Our group has previously shown by CD that flanking regions of the MAX bHLHZip core significantly add helical propensity to the dimer fold (Pursglove 2004). We were interested to see whether including the MYC-MBIV region cterminal to the bHLHzip motif would affect DNA affinity and the structural envelope of the MYC-MAX complex. To achieve our aims, we have established the expression and the purification platforms for the MYC and MAX proteins. Further we have developed the biochemical and biophysical (SEC-MALS, ITC, MST, DSF, and DLS) characterization of the tertiary complexes, which is a prerequisite for achieving our aims. Jointly, this work has now led to the first description of the extended MYC(MBIV)-MAX-DNA complex using SAS experimental data and computational modeling, which we will present here. We show that including the MBIV region of MYC significantly stabilizes the MYC-MAX-DNA complex. Characterization of this extended MYC-MAX-DNA complex contributes an extended platform for therapeutic targeting of the MYC oncoprotein.

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The iron sensing transcriptional factor IdeR and its function in the metabolism of Saccharopolyspora erythraea

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Iron, as a cofactor for various enzymes, is an essential element for a bacterial cell. Since high intercellular iron levels lead to formation of toxic reactive oxygen species, maintaining specific metal homeostasis is vital. Regulation of iron uptake is performed by iron-sensing transcriptional regulators. In the presence of iron these proteins act as repressors and bind to the regulatory sequences of targeted genes, responsible for iron uptake and/or storage[1]. In GC-rich *Actinobacteria* this function is performed by IdeR-like proteins. IdeR belongs to the DtxR (Diphtheria toxin repressor) family and contains three domains: an N-terminal winged helix-turn-helix DNA-binding domain, a dimerization domain which contains most of metalbinding residues, and a C-terminal SH3-like domain, the function of which is unclear. The function of IdeR/DtxR is studied in pathogens, for example of the genera *Mycobacterium* and *Corynebacterium*, where it was shown to be important not only to control iron homeostasis, but also in some cases to regulate virulence[2,3]. Much less is known about iron metabolism regulation in free-living *Actinobacteria*, many of which are important antibiotic producers, such as the erythromycin producer *Saccharopolyspora erythraea*. The structural studies of *Se*IdeR, performed in our laboratory, revealed some interesting features of the protein. We showed that IdeR performs DNA readout in vitro by its shape rather than the base content, which is a common recognition mechanism for eukaryotic regulators, but has been underestimated for prokaryotic factors. Moreover, our work is the first to describe binding of only one IdeR dimer to the promoter of the *nuo* gene cluster in *S. erythraea* (previously IdeR was believed to always act as a double dimer)[4].

To further expand our knowledge of IdeR regulation, *in vivo* work is undergoing. We are constructing an *ideR*-deficient *S. erythraea* strain to determine the network of genes and regulatory elements controlled and recognized by IdeR. Further experiments involve development of a reporter system to test DNA recognition of IdeR *in vivo* with various promoters, as well as the ability of mutated versions of IdeR to recognize target sequences. Concomitantly we are testing and optimizing the methods for genetic manipulation and engineering of *S. erythraea* strains. This study will provide deeper knowledge of iron-sensing mechanisms among antibiotic-producing microbes. The acquired information can be used for future manipulations of IdeR-like proteins in biotechnology and strains engineering.

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“Molecular tractor beams”?? Unravelling biological mechanisms by looking at single molecules in real-time

Bärbel Lorenz

LUMICKS

Imagine you could directly see the location and dynamics of individual proteins binding to a piece of single DNA. What if you could assemble your biological complex step by step and see it in action in real-time? What if you could manipulate the structure of your biomolecule and quickly change buffer conditions to test your experimental hypotheses? By using “molecular tractor beams”, the C-trap makes that a reality.

Essential biological processes performed by proteins interacting with DNA or cytoskeletal protofilaments are key to cell metabolism and life. In order to understand the molecular basis of life, as well as the pathological conditions that develop when processes go wrong, it is critical to get detailed insights into these processes at the molecular level. Not only at the highest resolution, but also in real time.

Here, we will take you through an exciting journey of different research topics in which dynamic single molecule studies revealed remarkable insights. In each study, the C-Trap system was used; our easy-to-use platform that combines optical tweezers with fluorescence microscopy. We will explain how the ability to control, visualize and manipulate single molecules in real time, changes the way we answer tough scientific questions in the field of DNA processing, single-protein dynamics, liquid-liquid phase separation (LLPS) and beyond.

Cross-linking and Hydrogen-Deuterium Exchange Mass Spectrometry as a National Infrastructure Service

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Cross-linking (XL-MS) and hydrogen-deuterium exchange mass spectrometry (HDX-MS) are powerful techniques in structural proteomics. XL-MS provides evidence of proteins interacting with each other as well as structural information of individual proteins or protein complexes by specifying (alt - identifying or providing?) distance constraints between two cross-linked peptides. The distance constraints arise from the defined length of the cross-linker used. HDX-MS analysis can be used to obtain information on structure, protein-protein interaction sites, allosteric effects, intrinsic disorder, and conformational changes. HDX-MS takes advantage of the labile nature of the exchangeable protons present on protein backbone amides. When dissolved in solution, proteins exchange these protons with hydrogen groups present in a deuterated buffer The rate of hydrogen to deuterium exchange can be measured by MS, and provides a measure of solvent accessibility and used to infer structural information. Examples from successful user projects will be shown, e.g large scale epitope mapping and XL-MS used in combination with X-ray.

The structural biology in protein folding and aggregation

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The folding of proteins into their compact three-dimensional structures is the most fundamental step to generating biological activity, and the mechanism of protein folding could be described by the concept of an energy landscape, and experimentally explored by the phi values analysis. However, direct structure information is rare known during the folding process that involves large conformation changes, and sometimes obtains unexplainable phi values. We combined protein design and X-ray crystallography (collected X-ray diffraction data at MAX IV Laboratory, Lund）, solution NMR (collected data at NMR facility, Stockholm University) etc. structural biology technique, are able to structurally identify the folding intermediated state, multi-conformations of α-helix and a switching in/out hydrophobic residue on folding pathway. In addition, we crystallized a designed infinite protein aggregate. Our trial of structural biology in protein folding studies provide a good example for combined protein design and structural biology strategy that can further helps to solve the protein folding problem.

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Prediction of peptide binding to survivin based on experimentalmicroarray fluorescence intensities

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High throughput techniques such as protein or peptide microarray are mainly seen as useful in the initial phase of discovering novel interactions. While they are useful for screening purposes, potential information on structural patterns influencing interactions that we can mine from these techniques are sometimes overlooked.

In this project, we build prediction models on protein-peptide interactions using survivin as amodel protein. Survivin is a small, 16.5 kDa protein involved in apoptosis and gene transcription in its dimeric form and cell division in its monomeric form. The model is build upon atomic compositions of amino acids, where each amino acid in a sequence is represented by the number of atom types it contains as features, i.e. Cα, carbon in carboxyl group. We compared our model with an existing prediction model1 using Fourier-transform of biophysical value representation of amino acid sequence and found that our model statistically performs better, with 80% mean accuracy and 71% F1, compared to 59% mean accuracy and 32% F1 in said existing model. We also used T-SNE visualization to compare peptide distribution based on atomic compositions and Fourier-transform of amino acid sequences. Correlating these distributions to features, we found that the numbers of carbon and oxygen atoms in carboxyl group correspond to a higher chance of binding to survivin, although no single deterministic feature was found in our model and combinations of features are needed to achieve better statistical parameters for binding prediction.

Mapping of survivin binding regions based on microarray fluorescence intensities has been done on crystal structure of polycomb repressor 2 complex (PRC2). Molecular docking simulation of survivin activity with PRC2 reveals survivin binding to SANT1 region of EZH2unit of PRC2 to prevent its methyltransferase activity.2

Finally, we extended our prediction model against human proteome database, the results are currently used to design other peptide microarray experiments. More microarray datasets are also expected to be taken from survivin mutants, as well as crystal structures of survivin-bound peptides.

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Investigating the Interactions Between Myc and the RNAPolymerase II Component TFIIF

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The MYC transcription factor is a potent oncoprotein that is dysregulated in the majority of human cancers. MYC is estimated to bind to 10-15% of the genome, where it regulates several genes necessary for cancer growth. The integral role of MYC in cancer has presented it as an attractive therapeutic target, however, despite numerous efforts to inhibit MYC no small molecule inhibitors have advanced to patient care. This is due, in part, to the intrinsically disordered nature of MYC resulting in a lack of deep pockets and grooves typical of a drug target. Intrinsically disordered proteins tend to adopt secondary structures upon binding to protein interactors, making it possible to specifically disrupt those protein-protein interactions. MYC modulates many of its interactions with other proteins through conserved "MYC box regions" (MBs), each of which is thought to be involved in largely unique cellular processes. We have previously demonstrated that the most recently identified MYC box, MB0, is essential for MYC-driven oncogenesis. One important MB0 interactor is RNA polymerase II pre-initiation complex subunit TFIIF, which is a heterodimer of the proteins GTF2F1 and GTF2F2. The heterodimeric region of TFIIF interacts with low micromolar affinity to MB0, yet the importance of this interaction has yet to be fully understood. Using an assortment of biophysical techniques, including X-ray crystallography and NMR spectroscopy, we aim to describe an atomic-resolution model of the MB0–TFIIF interaction which will facilitate the development of tool compounds to investigate the MYC-TFIIF complex and will deepen our understanding of how MYC functions as part of the transcriptional machinery.

How PROTAC degraders work: Molecular recognition and design principles

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Our laboratory uses molecular information on protein-protein interactions and protein degradation to discover novel therapeutics. Degrader molecules, also known as PROTACs (PROteolysis-Targeting Chimeras) recruit proteins to E3 ligases for targeted protein degradation. Formation of a ternary complex between the PROTAC, the E3 and the target leads to the tagging of the target protein by ubiquitination, and subsequent proteasomal degradation. In cancer, one such drug target is the E3 ubiquitin ligase VHL, which can be hijacked by PROTACs. Our lab solved crystal structures of VHL bound to fragments of its natural substrate and analysed it to design and synthesize novel small molecule ligands of VHL. We tethered the VHL ligand to a small molecule inhibitor targeting BRD4, a protein frequently deregulated in leukemia. The resulting PROTAC MZ1 bridges BRD4 with VHL and removes BRD4 from leukemic cells. Solving the structure of the ternary bridging complex, we unravelled how the PROTAC MZ1 glue BRD4 to VHL, illuminating structural and biophysical insights into PROTAC molecular recognition and mechanism of action. This fundamental understanding has enabled us to develop further small molecules for hard to target proteins and shown how to improve PROTAC activity.

STRUCTURAL BASIS FOR SMALL MOLECULE BINDING TO SARS-COV-2 NONSTRUCTURAL PROTEIN 10

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Coronavirus nonstructural protein 10 (nsp10) has approximately 140 residues and is essential for the stimulation of nsp14 and nsp16, therefore acting primarily as an activator and scaffolding protein. Nsp10 is required for stimulation of both the 3′-5′ exoribonuclease (ExoN) proofreading activity located in nsp14's N-terminal domain and the nsp16 2′-O-MTase activity. This makes nsp10 a central component in both resistance to nucleoside-based drugs and the RNA cap methylation machinery that promotes escape of the virus from innate immunity.

We determined the crystal structure of nsp10 alone using data collected at the BioMAX beamline. This is the first available and only structure of SARS CoV-2 nsp10 in its unbound form [1] and allowed us to use the fragment-screening platform FragMAX at the MAX IV Laboratory [2], to search for fragment hits binding to nsp10. We identified fragments binding to nsp10 and observed ligand-binding sites in conserved interfaces between nsp10 and nsp14/nsp16 [3], a promising start for eventual fragment-based lead generation.

We also solved the crystal structure of nsp10 in complex with the ExoN domain of nsp14 to 1.3 Å resolution and submitted those crystals to a full fragment screen at the FragMAX facility. Surprisingly, 8 of the 14 fragment hits cluster in a region of the nsp10:nsp14 interface. In summary, those and previous hits are interesting starting points for growing, merging and linking fragments supported by structure-guided drug design with the potential to disrupt nsp10:nsp14 interactions and thereby interfering with nsp14 ExoN activity and viral replication.

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Virtual Fragment Screening for DNA Repair Inhibitors in Vast Chemical Space

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Structure-based docking algorithms can rapidly sample and score protein-ligand complexes, enabling evaluations of enormous compound libraries.1,2 The number of commercially accessible compounds is swiftly increasing, and chemical suppliers now offer over 30 billion make-on-demand molecules. But despite the recent expansions of compound catalogs, the coverage of the drug-like chemical space in these libraries remains sparse.

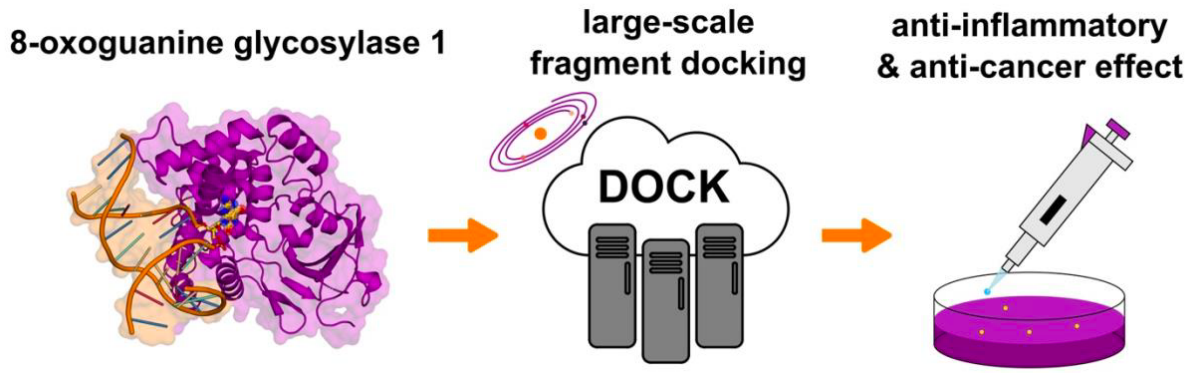

Figure 1: Virtual fragment screening leads to DNA repair inhibitors.

We investigated how large-scale fragment docking could be used as a first step in ligand discovery. Several hundred million molecules were docked against 8-oxoguanine glycosylase 1 (OGG1), an enzyme that is part of the DNA damage and response pathway. Inhibition of OGG1 has recently been demonstrated to be a promising target for the development of drugs against both cancer and inflammation. Our docking screens led to the discovery of starting points for inhibitor development, which were confirmed by protein crystallography. Molecular docking and crystallography guided hit-to-lead generation, leading to novel and potent inhibitors. The most advanced compounds showed anti-cancer and anti-inflammatory effects in cell-based assays. Our results demonstrate that fragment-based docking can identify and elaborate promising hits and this approach can be applied to other challenging drug targets (Figure 1).

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Cryo-EM structures of Staphylococcus aureus 70S ribosomes in complex with elongation factor G and fusidic acid

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Fusidic acid (FA) is an antibiotic effective mainly against gram-positive bacteria and it is commonly used against *Staphylococcus aureus* infections. FA stalls protein synthesis by binding to elongation factor G (EF-G) after translocation and preventing its release from the ribosome. EF-G is a GTPase that is essential in the translocation of the mRNA and tRNA during translation. There are structures of FA-locked ribosomal complexes in *Escherichia coli* (cryo-EM) (1) and *Thermus thermophilus* (X-ray) (2). However, no structures of the clinical target are available.

There are three main types of FA resistance in *S. aureus: fusA*, involving mutations on EF-G (3); fusB, mediated by the expression of a resistance protein (4); and *fusE*, mutations on ribosomal protein L6 (5). To fully understand these mechanisms, a structure of the clinically relevant system is crucial, as it allows for a deeper understanding of the effects of mutations, which might involve specific interactions with the ribosome or the antibiotic. Furthermore, such a structure would allow for target-directed drug design of FA analogues.

We have obtained high-resolution cryo-EM structures of the *S. aureus* FA complex, formed by locking EF-G to the 70S ribosome with FA. Furthermore, we have produced a structure of the same complex with the FA derivative FA cyclopentane (6). We aim to understand why this drug variant is less sensitive to some *fusA*-type resistance mutants. We also provide the highest resolution *S. aureus* 70S structure to date, which allows identification of additional rRNA modifications as well as detailed interactions between EF-G and the antibiotic in its clinical target.

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i-TripleD, an AI tool for *de novo* drug discovery

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Molecular docking is the most employed structure-based technique in traditional computational drug discovery, which is costly, time-consuming, and inefficient, mainly because of the sampling stage. Moreover, the sizes of the chemical libraries available for virtual screening are limited *i.e.*, several billion compounds compared to the size of the druglike chemical space which has been estimated to be as large as 10^{63} unique individual compounds. Thus, only a tiny portion of chemical space can be explored. Therefore, the evaluation of the unseen regions of the druglike chemical space is necessary to discover more potent drugs for current or new upcoming diseases. In addition, traditional scoring functions are not accurate enough to correctly estimate experimental binding affinities of the ligands and accurately rank the compounds. While the more modern scoring functions are more precise in estimating binding affinities, they still require a full picture of the atomic contacts and interactions between the receptor and the ligand molecule, meaning the inefficient sampling stage remains crucial.

Consequently, the necessity of using artificial intelligence (AI) and machine learning (ML) algorithms is apparent. To date, most of these focus on *either* the generation of new chemical entries *or* on the development of scoring functions for binding affinity predictions. Our AI-based drug discovery tool "Intelligent *de novo* Drug Discoverer (*i-TripleD*)" creates a comprehensive pipeline which combines both points mentioned above and does so with superior faster running time and accuracy. *i-TripleD* consists of several interconnected ML-based modules, each trained using the largest datasets to date, yielding results with higher accuracy and several orders of magnitude faster than current drug discovery tools. The state-of-the-art AI-based scoring function *iScore* was extensively benchmarked and compared with most of the available scoring functions and the results show that *i-TripleD*'s scoring function is at the top of modern scoring functions developed so far in terms scoring power, ranking power, and screening power. The generative module *iGen*, is capable of generating new and chemically valid molecules through sampling unseen region of the druglike chemical space with very high level of validity and uniqueness score, at a production rate of ~2000 molecules/s. *iGen* can also be employed to generate libraries of unique molecular derivatives through scaffold decoration techniques. i-*TripleD* hence has the capability to generate entirely novel compounds (*de novo*), screen existing databases, or build on existing scaffolds. Our novel Ultra-Fast Screening (UFS) approach furthermore enables assessments that are several orders of magnitude faster than any available screening tool. As an example, the ZINC15 database (>1 billion compounds) was screened against the SARS-Cov-2 Mpro protein on a single compute node in less than 36 hours.

POSTERS

01 **Sarah Al Hamoud Al Asswad**

Structure Characterization of a thyroid hormone transporter

02 **Ali Ayaan**

CryoEM structure determination of a Zinc transporter

03 **Marta Antonello**

Characterization of a mosquitocidal botulinum toxin-like neurotoxin complex

04 **Dilip Bagujar**

Strucutral understanding of pore formation by *Streptococcus mitis* toxin mitilysin

05 **Krishna Bhattiprolu**

Structure of a Gram-positive type IV secretion system (T4SS)

06 **Szabolcs Bódizs**

Capturing the photoactivation of the *Pseudomonas aeruginosa* phytochrome using cryo-electron microscopy

07 **Charles Burridge**

Structural and dynamic insights into human mitochondrial transcription

08 **Filippo Castegnaro**

Determination of the structural and dynamical properties of the crucial periplasmic chaperone SurA

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Investigation of the new serotonin-gated sodium channel LGC-50

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Adaptation of mixing experiments for time-resolved serial crystallography of proteins at synchrotron and XFEL sources

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