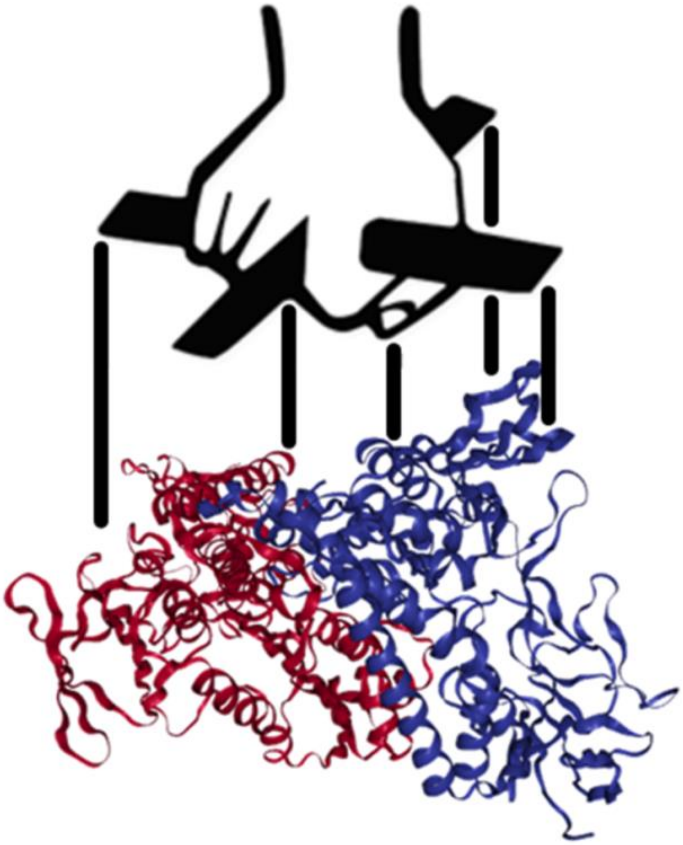
Thursday June 6, 2019
O|2 Auditorium, VU Amsterdam

VU Amsterdam – X-Ray Crystallography &
The Nederlandse Vereniging voor Kristallografie present:

Structural Biology Symposium 2019
Interacting with Proteins :
A structural view



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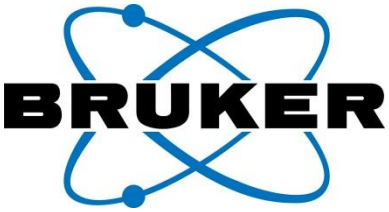

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Program

Start	Speaker or event	Affiliation	Topic
9:30	**Registration & Coffee**		
10:00	Sven Hennig	VU Amsterdam	Welcome
10:10	Joost Uitdehaag	NTRC	Inhibitor complexes of Arginase-1, a target for cancer immunotherapy
10:35	Loes Kroon-Batenburg	Univ. Utrecht	Rigid body motion is the main source of diffuse scattering in protein crystallography
11:00	Kenneth Verstraete	VU Gent	Structural basis for cytosolic acetyl-CoA production by ATP citrate lyase
11:25	Nicholas Pearce	Univ. Utrecht	Intuitive structural-disorder analysis and multi-dataset crystallographic parameterisation
11:50	Max Clabbers	Univ. Stockholm	Macromolecular structure determination by microcrystal electron diffraction
12:15	**Lunch & Posters**		
13:15	Kerstin Wallraven	VU Amsterdam	Crystal Structures of Chemically Modified Peptides in Complex with 14-3-3 zeta
13:30	Jan Vilim	Univ. van Amsterdam	Elucidating the structure of engineered amine dehydrogenase using X-ray crystallography
13:45	Abril Gijsbers Alejandre	M4I	From monomer to oligomer: the different faces of EspB
14:00	Antonella Fioravanti	VU Brussel	Discovering and targeting Bacillus anthracis' Achilles heel: the new way to fight anthrax
14:25	Albert Guskov	Univ. Groningen	Transport of enantiomeric substrates by archaeal homologs of mammalian glutamate transporters
14:50	**Tea & Posters**		
15:10	Meindert Lamers	LUMC	Structural features of high-fidelity DNA replication
16:10	Wine and Cheese Poster Session		
17:40	**Poster Prizes & Closing**		
18:00	Walk to restaurant		
18:30	Speakers Dinner	Kookstudio Nine	Arnold Schönberglaan 9, 1082 MJ Amsterdam

Speakers & Abstracts

Joost Uitdehaag
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Inhibitor complexes of Arginase-1, a target for cancer immunotherapy

Arginase-1 is a manganese-dependent metalloenzyme that catalyzes the hydrolysis of L-arginine into L-ornithine and urea. Recently, Arginase-1 has gained much attention as a target for cancer immunotherapy, as it is abundantly expressed by tumor-infiltrating myeloid cells that promote tumor immunosuppression. Interestingly the enzyme has a very high pH optimum of 9.0. Here we will discuss the crystal structure of the human Arginase-1 in complex with various inhibitors at various pH values, and discuss the implications for drug discovery.

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Rigid body motion is the main source of diffuse scattering in protein crystallography

The origin of diffuse X-ray scattering from protein crystals has been the subject of debate over the past three decades regarding whether it arises from correlated atomic motions within the molecule or from rigid-body disorder. Here, a supercell approach to modelling diffuse scattering is presented that uses ensembles of molecular models representing rigid-body motions as well as internal motions as obtained from ensemble refinement. This approach allows oversampling of Miller indices and comparison with equally oversampled diffuse data, thus allowing the maximum information to be extracted from experiments. It is found that most of the diffuse scattering comes from correlated motions within the unit cell, with only a minor contribution from longer-range correlated displacements. Rigid-body motions, and in particular rigid-body translations, make by far the most dominant contribution to the diffuse scattering, and internal motions give only a modest addition. This suggests that modeling biologically relevant protein dynamics from diffuse scattering may present an even larger challenge than was thought.

Kenneth Verstraete
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Structure of ATP citrate lyase and the origin of citrate synthase in the Krebs cycle

Across different kingdoms of life, ATP citrate lyase (ACLY) catalyzes the ATP-dependent and coenzyme A (CoA)-dependent conversion of citrate, a metabolic product of the Krebs cycle, to oxaloacetate and the high-energy biosynthetic precursor acetyl-CoA. The latter fuels pivotal biochemical reactions such as the synthesis of fatty acids, cholesterol and acetylcholine, and the acetylation of histones and proteins. In autotrophic prokaryotes, ACLY is a hallmark enzyme of the reverse Krebs cycle (also known as the reductive tricarboxylic acid cycle), which fixates two molecules of carbon dioxide in acetyl-CoA. In humans, ACLY links carbohydrate and lipid metabolism, and is strongly expressed in liver and adipose tissue, and in cholinergic neurons. The structural basis of ACLY's function remained unknown. Here we report high-resolution crystal structures of bacterial, archaeal and human ACLY, and via distinct substrate-bound states we link the conformational plasticity of ACLY to its multistep catalytic itinerary. Such detailed insights will provide the framework for targeting human ACLY in cancer and hyperlipidaemia. Our structural studies also unmask a fundamental evolutionary relationship that links citrate synthase, the first enzyme of the oxidative Krebs cycle, to an ancestral tetrameric citryl-CoA lyase module that operates in the reverse Krebs cycle. This molecular transition marked a key step in the evolution of metabolism on earth.
Reference: Verschueren, K.H.G., Blanchet, C., Felix, J., Dansercoer, A., De Vos, D., Bloch, Y., Van Beeumen, J., Svergun, D., Gutsche, I., Savvides, S.N., and Verstraete, K. Structure of ATP citrate lyase and the origin of citrate synthase in the Krebs cycle. Nature (2019)

Nicholas Pearce
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Re-factoring the B-factor: enabling intuitive structural-disorder analysis and multi-dataset crystallographic parameterisation

Displacement parameters (or temperature factors, or B-factors) describe the positional disorder of an atom in an atomic model. Though they typically constitute at least 20% of atomic model parameters and contain detailed information about atomic motions, they are difficult to interpret and are thus little used in quantitative structural analysis. Furthermore, the permitted complexity of an atomic disorder model is strongly restricted by the resolution of the crystallographic data. We present a new approach for decomposing molecular disorder into a hierarchical series of contributions, which provides an intuitive basis for quantitative structural analysis. Additionally, this formalism allows for the simultaneous parameterisation of closely-related macromolecular structures, decreasing the data-parameter ratio and reducing overfitting, particularly for low resolution models. We demonstrate the visualisation of disorder in several biologically-relevant systems, and show that multi-crystal experiments offer an opportunity to improve our understanding of dynamics within macromolecular crystals.

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Macromolecular structure determination by micro-crystal electron diffraction

Micro-crystal electron diffraction (MicroED) has recently shown potential for structural biology. It enables macromolecular structure determination when only small micron-sized three-dimensional crystals are available that are beyond what can be resolved by conventional X-ray crystallography. However, up until now MicroED had only been applied to refine known protein structures that were already solved previously by X-ray diffraction. Here we present the first unknown protein structure of a novel R2lox metalloenzyme solved using MicroED. The structure was phased by molecular replacement using a search model of 35% sequence identity, and the resulting electrostatic scattering potential map at 3.0 Å resolution was of sufficient quality to allow accurate model building and refinement. Our results illustrate that MicroED has the potential to become a widely applicable technique for protein structure determination, complementing X-ray crystallography when crystal volume is the limiting factor, with ongoing developments aimed at optimizing sample preparation and data acquisition, as well as investigating possible strategies for experimental phasing including isomorphous replacement and high-resolution imaging.

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Discovering and targeting Bacillus anthracis' Achilles heel: the new way to fight anthrax

Anthrax is a highly resilient and deadly disease caused by the spore-forming bacterial pathogen *Bacillus anthracis*. Today, anthrax mostly affects wildlife and livestock, but remains a concern for human public health primarily in persons handling contaminated animal products and as a bioterror threat due to the high resilience of spores, the high case-fatality rate even with the aggressive use of antibiotics and the lack of a civilian vaccine program. As part of its immune evasion strategy, the bacterium presents a dynamic cellular surface with a complex composition. In its vegetative form, the cell surface of *B. anthracis* is covered by one of two protective paracrystalline protein arrays known as the Sap or EA1 S-layer (surface layer), present during exponential and stationary growth phase, respectively. The self-assembling characteristic of these S-layer proteins has thus far hampered their detailed structural and biophysical characterization. Here, we applied Nanobodies (Nbs) as a bio-tool to control Sap polymerization and to accomplish its crystallization and structure determination, unveiling a new class of S-layer proteins. The Sap assembly domain consists of six β-sandwich domains that organize into a flat, tile-shaped unit that self-assembles independent of calcium. Amongst the isolated Nbs we identified inhibitory nanobodies that prevented Sap assembly and depolymerized existing Sap S-layers *in vitro*. When applied *in vivo*, nanobody-mediated destruction of the Sap S-layer resulted in severe morphological defects and proved bacteriostatic unlike the genetic knockout of sap. In a mouse model of ongoing *B. anthracis* infection, subcutaneous administration of Sap inhibitory Nanobodies resulted in clearance of infection and a cure of lethal anthrax disease. These findings expose, for the first time, the disruption of S-layer integrity as a mechanism with therapeutic potential in S-layer carrying pathogens.

Ref.: Fioravanti A.* et al., Manuscript accepted, Nat. Microbiol. - 18081671B

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Towards time-resolved crystallography on glutamate transporters

Mammalian glutamate transporters (belonging to Solute Carrier Family 1 of transporters) are crucial players in neuronal communication as they perform neurotransmitter reuptake from the synaptic cleft. Besides L-glutamate and L-aspartate, they also recognize D-aspartate, which might participate in mammalian neurotransmission and/or neuromodulation. We investigated binding and transport of enantiomeric substrates in archaeal homologue of glutamate transporters - GltTk from *Thermococcus kodakarensis*. We observed that GltTk transports D-aspartate with identical Na^+:substrate coupling stoichiometry as L-aspartate, and that the affinities (K_d and K_m) for the two substrates are very similar. Additionally, we solved a crystal structure of GltTk with bound D-aspartate at 2.8 Å resolution and compared it with the L-aspartate bound GltTk structure. The new structure explains how the geometrically different molecules L- and D-aspartate are recognized and transported by the protein in the same way and provides a clue to explain the puzzling observation why mammalian SLC1A transporters readily transport L- but not D-glutamate.

Meindert Lamers – *Keynote Speaker*
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Structural features of high-fidelity DNA replication

During DNA replication, the DNA polymerase rapidly responds to different challenges in the DNA. Incorporation of a wrong nucleotide induces transfer of the DNA to the exonuclease where the mis-incorporated nucleotide is excised. Encounter with a damaged base in the template strand results in exchange with specialized repair DNA polymerases that can bypass the damaged base. Finally, on the lagging strand where DNA synthesis is discontinuous, the polymerase needs to repeatedly dissociate from the DNA, more than 10,000 times per replication cycle. How a single DNA polymerase is able to respond to all these different signals is not understood. Using cryo-electron microscopy and single molecule light microscopy, we reveal how the DNA polymerase responds to the different signals. Multiple cryo-EM structures show the molecular motions within the polymerase that enable it to adapt to the different signals, while the single molecule studies reveal a carefully orchestrated cooperation between different proteins that load and release the polymerase from the DNA.

Poster Abstracts

P01

Crystal Structures of Chemically Modified Peptides in Complex with 14-3-3zeta

Kerstin Wallraven, Tom N. Grossmann

Stabilization of peptide secondary structures is an established strategy to constrain peptides with a biological relevance in their bioactive conformation and thereby improving their affinty towards a target. Peptide stapling is commonly used method to achieve such a stabilization. Here, two alpha-methyl, alpha-alkenyl amino acid building blocks are introduced into a peptide sequence during peptide synthesis and subsequently crosslinked through a ring-closing metathesis (RCM) reaction. Initially, this strategy has been developed to stabilize alpha-helical peptide sequences. However, Grossmann and coworkers adopted this method to constrain the irregular conformation of binding epitope-derived peptides to interfere the pathogenic 14-3-3/ExoS protein-protein-interaction. Interestingly, the crosslinks of those peptides are involved in binding to 14-3-3 and thereby show potential for chemical modifications to modulate their affinities. Based on those peptides, peptide EtMe bearing an alpha-ethyl, alpha-alkenyl amino acid and peptide H containing an alkyne moiety have been developed. Their binding modes to 14-3-3zeta have been elucidated via X-ray crystallography.

P02

Structure-based Design of Macrocyclic Peptides binding Transcription Factor NF-Y

Mathias Wendt, Sadasivam Jeganathan, Sebastian Kiehstaller, Diego Brancaccio, Arne Kuepper, Nicole Pospiech, Alfonso Carotenuto, Ettore Novellino, Sven Hennig and Tom N. Grossmann

Peptide-based modulators of Protein–Protein Interactions (PPIs) have increased attention in recent years, due to developments in chemical modification strategies, that can enhance the bioactivity of natural peptides. In particular, hydrocarbon peptide stapling has been succesfully used for the modulation of PPIs.[1] The approach combines two features for constaining a peptide in its helical conformation: i) α-methylation and ii) macrocyclization via side chains using ring closing metathesis. Here, we show the structure-based design of macrocylic peptides targeting the Nuclear Transcription Factor Y (NF-Y).[2] NF-Y consists out of three subunits. Subunits B and C form a histone-fold domain, which can interacte with helix A1 of subunit A, while helix A2 of subunit A is interacting with the minor groove of the DNA target sequence. Having the X-ray crystal structure of subunits B/C in complex with helix A1 allowed us to design macrocyclic peptides using hydrocarbon peptide stapling. Characterization and structural analysis of these peptides encouraged a reconsideration of the peptides' design. The alteration of the α-methylation pattern reveald the influence of α-methylation on the binding affinity. Removing a single methyl group lead to an 15-fold increase. Finally, the optimized peptides were not only used for the inhibition of the PPI itself, but also for NF-Y's DNA binding.
References: [1] Cromm, P. M. et al. ACS Chem. Biol. 10, 1362-1375, 2015. [2] Nardini, M. et al. Cell 152, 132-143, 2013.

P03

MicroED: Fast and Furious

Bart Buijsse, Michael Janus, Abhay Kotecha, Lingbo Yu, Hans Raaijmakers

3D electron diffraction allows crystallographic analysis at the atomic resolution from simple micro-crystals. With our Talos/Glacios TEM systems, a specialized camera, and dedicated acquisition software we solved a protein structure up to high resolution. The movie mode acquisition of a continuous tilt series yields fast and reliable results. Electron doses are about an order-of-magnitude smaller than what is typically used in Single Particle Analysis: Radiation damage ought to be less severe. Last October, two publications appeared on analysis of small molecules. The input material are simple powders composed of microcrystals. Data collection can be done in minutes and is sufficient to obtain a sub-Angstrom resolution structure. Especially the speed of the complete workflow triggered the interest of the pharma and chemical industry. In order to demonstrate the small-molecule application to our customers we have tested our (prototype) product on paracetamol.

P04

In-silico *investigations of structure-kinetic relationships of phosphodiesterases (PDEs) inhibitors during fragment growing*

Lorena Zara, Antoni R. Blaazer, Abhimanyu K. Singh, Albert Kooistra, Marta Arimont, Maciej Majewski, Xavier Barril, David Brown, Jacqueline van Muijlwijk-Koezen, Iwan J. P. de Esch

Detailed understanding of the molecular determinants of ligand-protein binding enables efficient Fragment-Based Drug Design (FBDD). We use binding kinetics in the design process by developing a stepwise computational protocol that explores the ligand-protein unbinding events. First, we applied the dynamic undocking (DUck) approach to calculate the work necessary to break the key H-bonding of the ligand with the receptor. Next, the complete dissociation trajectory is studied using Random Acceleration Molecular Dynamics (RAMD). The MD trajectories generated were further investigated using Interaction FingerPrints2 (IFPs) to map various features of ligand-protein and ligand-water-protein interactions. This study explores *Trypanosoma brucei* phosphodiesterase B1 (TbrPDEB1s), a drug target for the treatment of African sleeping sickness4. This is a challenging protein because most of TbrPDEB1 inhibitors show similar activity for the (off-target) human PDE4 enzymes. In-house SPR measurements showed these inhibitors to have different binding kinetics in the two species. The stepwise computational protocol can predict the different kinetic profiles and pinpoint important features that explain the differences among them. A well-defined water network in TbrPDEB1 that is connected to a parasite specific pocket is shown to play a key role. The protocol and insights gained can be applied in order to develop potent and more selective TbrPDEB1 inhibitors.

P05

Identification of a 14-3-3 binding epitope in aminopeptidase N

Sebastian Kiehstaller, Sven Hennig

Aminopeptidase N (APN, CD13) is a zinc-dependent type II trans¬membrane ectopeptidase which is located on the surface of many cell types such as fibroblasts, epithelial and myeloid cells. It plays an important role in different cellular mechanisms including tumour cell invasion and angiogenesis. Elevated levels of APN were also found in several kinds of malignancies. One interaction partner of APN is the adapter protein 14-3-3. Previous research has shown, that extracellular binding of 14-3-3 to APN leads to an upregulation of MMP1 transcription within fibroblasts. Additionally, it has been observed that 14-3-3ε binding to the surface of chondrocytes seems to be dependent on APN expression. The family of 14-3-3 proteins are highly conserved eukaryotic proteins and 7 different homologues have been detected in mammals. 14-3-3 forms homo- and heterodimers with other homologues and serves as an adapter protein for more than 200 binding partners. The monomer consists of 9 α-helices that form an amphiphatic binding groove. Although the cellular effects of the interaction have been shown, the binding mechanism and the activation of APN by 14-3-3 are still unknown. We therefore used an in silico approach to identify potential binding motifs in APN and analysed most promising epitopes in vitro. We could identify and characterize a best binding protein sequence and analysed its binding mode by X-ray crystallography.

P06

Towards automated serial electron diffraction for macromolecular crystallography

Stef Smeets, Arjen Jakobi

Electron diffraction (ED) techniques have reached a level where high-quality diffraction data can be collected from crystals that are orders of magnitude smaller than those needed for conventional X-ray diffraction experiments. However, data collection is still mainly following ad-hoc protocols that are very time-consuming, laborious and often poorly reproducible. Therefore, we are developing strategies to standardize and automate data collection for electron diffraction experiments, which should significantly increase the efficiency of this method. These procedures automatically screen for crystals and collect ED data for macromolecular crystallography. In a serial electron diffraction (SerialED) experiment, micron-sized crystals are detected at a low magnification using low-dose illumination with image processing algorithms. For each crystal, diffraction data are collected while continuously rotating the crystal in the beam, making use of automated tracking algorithms to predict crystal movement during rotation. There are several aspects of transmission electron microscopes (TEM) that make them well suited for serial crystallography: (1) crystals can be observed directly in imaging mode, (2) there is a TEM available in many laboratories, and (3) modern TEMs and cameras are computer controlled, such that the entire data collection process can be automated through software. We have recently shown in examples from materials science that our automated data acquisition can run unsupervised for hours. Current developments include the extension of our approach for the application to macromolecular crystallography, such as novel strategies to minimize radiation damage to protein crystals and other beam-sensitive materials.

P07

Elucidating the structure of engineered amine dehydrogenase using X-ray crystallography

Jan Vilim, Marcelo F. Masman, Tom N. Grossmann, Sven Hennig, Francesco G. Mutti

We have engineered amino acid dehydrogenase from *Geobacillus stearothermophillus* into amine dehydrogenase (LE-AmDH_v1) using the three-template composite homology model. To validate the structural implications derived from the homology model, we have crystallized LE-AmDH_v1 and determined the 3D structure using molecular replacement. We have obtained crystal structures of apo-LE-AmDH_v1 and LE-AmDH_v1 with bound NAD^+. Interestingly, use of composite homology model as a search template allowed us to elucidate the structure by molecular replacement, since other possible search templates were sharing to low sequence homology too allow solving the structure. Moreover, we offer some insights into binding modes of LE-AmDH_v1, since homologous proteins often exhibit open and closed conformation upon binding of the substrate in the active site.

P08

Crystal structure of a Pseudomonas aminotransferase involved in caprolactam metabolism

Henriëtte J. Rozeboom, Cyntia M. Palacio, Elisa Lanfranchi, Qinglong Meng, Marleen Otzen, Dick B. Janssen

The biodegradation of the nylon-6 precursor caprolactam by a strain of Pseudomonas jessenii proceeds via ATP-dependent hydrolytic ring-opening to 6-aminohexanoate. This non-natural ω amino acid is converted to 6-oxohexanoic acid by an aminotransferase (PjAT) belonging to the fold type I PLP enzymes. To understand the structural basis of 6-aminohexanoatate conversion, we solved five distinct different crystal structures and determined the substrate scope with a range of aliphatic and aromatic amines. The structure of the aldimine intermediate formed from 6-aminohexanoate and the PLP cofactor explains the high activity and selectivity of the PjAT with 6-aminohexanoate.

P09

From monomer to oligomer: the different faces of EspB

A. Gijsbers, A. Mathew, A. Siroy, G. Tria, D. Siliqi, S.R.Ellis, R.M.A Heeren, P.J. Peters and R.B.G. Ravelli

Mycobacterium tuberculosis (Mtb) is ingested by macrophages and dendritic cells through a process called phagocytosis. Unlike non-pathogenic strains, virulent Mtb avoids being neutralized by translocating from the phagosome into the cytosol of the immune cell, causing its death (van der Wel et al, 2007). This evasive mechanism depends on a functional type VII secretion system, called ESX-1 (Houben et al, 2012). This machinery is comprised by six proteins and almost 20 substrates and/or regulatory elements (Houben et al, 2014). Among these substrates, the protein EspB is of great interest because knockout mutants significantly attenuate the virulence of the bacteria. Little is known about this protein, except that EspB is cleaved during secretion by a protease called MycP1. The role of this cleavage in virulence has so far remained elusive.
The crystal structure of monomeric EspB at neutral pH is known (Korotkova et al, 2015). During secretion into the phagosome, proteins are exposed to changes in pH. By size exclusion chromatography (SEC), we showed that oligomerization of EspB is favoured in the mature form at acidic pH. We characterized the oligomer composition of EspB using an ultra-high mass range (UHMR) Orbitrap instrument, which resulted in the identification of several oligomeric states of EspB ranging from monomers to nonamers under native conditions. With the help of MS2 fragmentation, we further isolated individual sub-units to characterize these oligomers. We solved the structure of the most abundant oligomer by cryo-Electron Microscopy. Due to a preferential orientation of the protein on EM-grids, we had to use a tilted data collection scheme and reached 3.41Å resolution. Results showed that EspB oligomerizes preferentially in a heptameric form through its N-terminal domain, by a Gln-Gln interaction. It has been suggested that EspK is the chaperon of EspB4. Interestingly, SEC and small angle X-ray scattering studies showed that mutation in the residue Gln48, involved in the oligomerization of EspB, disrupts the binding with EspK as well. This result could propose EspK as an inhibitor of the oligomerization by competing for the same binding interface. Structural studies of the EspB-EspK are ongoing. By a multimodal study involving SAXS, cryo-EM, and native MS, we can have shown that oligomerization of EspB depends on cleavage of its C-terminal domain and this process is promoted by an acidic pH. In vivo, these observations suggest that oligomerization of EspB goes through a checkpoint prior to its secretion into the phagosome.

P10

Obtaining better protein structure models by systematically using homology

Bart van Beusekom, Wouter Touw, Maarten Hekkelman, Anastassis Perrakis & Robbie Joosten

Building, refining, and analysing crystallographic protein structure models can greatly benefit from additional information beyond the primary diffraction data. Rotamer libraries, Engh and Huber restraints, and fold classifications are just some examples of the additional information that is commonly used. Homology is also used as an information source in crystallography: most notably in molecular replacement, but also as reference structures for model refinement. Over 130,000 protein structure models have now been determined experimentally, among which are many homologous proteins. The average protein chain in the PDB has around 30 homologs these days. This recently acquired wealth of data has hardly been used systematically yet, while doing so can improve structure models in many ways. In the PDB-REDO project, we strive to generate ever better structure models. Therefore, we have developed methodology to systematically make use of all available homologous data. With information obtained from homologous structures, we generated hydrogen bond restraints for low-resolution structure models and added missing backbone fragments or loops. By implementing this in PDB-REDO, over 10,000 low-resolution structure models were improved and around 25,000 loops were built in existing PDB structure models. This methodology is available through the PDB-REDO webserver (www.pdb-redo.eu). Now, we aim to extensively compare all homologous structure data on a per-residue basis. By comparing metrics such as backbone conformation, rotamer angles and density fit, but also post-translational modifications and sequence conservation, we uncover errors as well as potentially interesting features in protein structures that will facilitate structure interpretation.

P11

New tools for cryo-EM density interpretation

Arjen Jakobi

Improvements in detector technology and image processing algorithms have rendered single-particle cryo-EM capable of routinely delivering structures of macromolecular assemblies at near-atomic resolution, permitting building and refinement of atomic models. Yet, the interpretation of cryo-EM density maps often remains challenging due to specific properties of these maps such as inherent loss of contrast and spatial variation in map resolution. Variations in map resolution, if combined with global contrast restoration procedures, may result in density maps with locally inappropriate sharpening levels that are prone to hamper or even misguide atomic model building, and hence the chemical interpretation of the structural data. New tools that facilitate more faithful restoration of density contrast along with procedures that permit the assignment of significance to structural features in cryo-EM density maps help to avoid pitfalls in atomic model building resulting from map sharpening artifacts. The presented procedures should help overcome common difficulties in map interpretation using high-resolution cryo-EM density maps, and I will illustrate the relevance of these technical issues on the biological interpretation of real-life examples.

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