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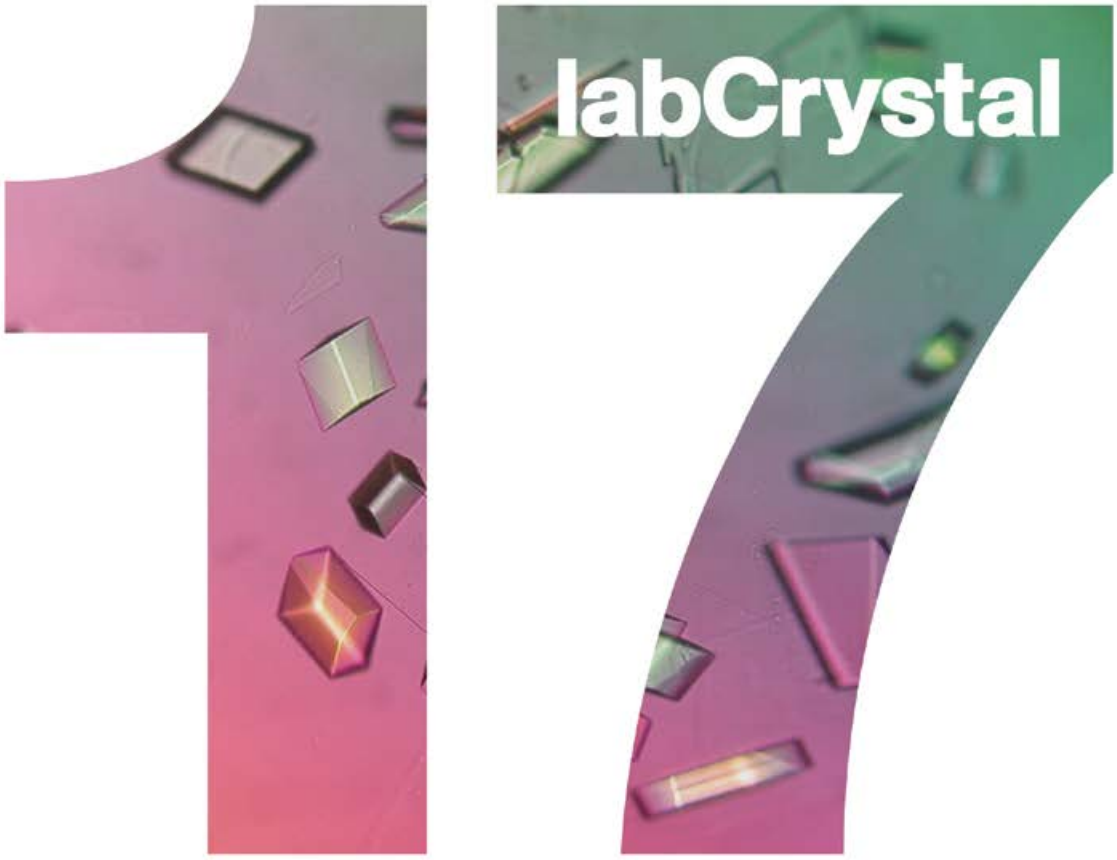
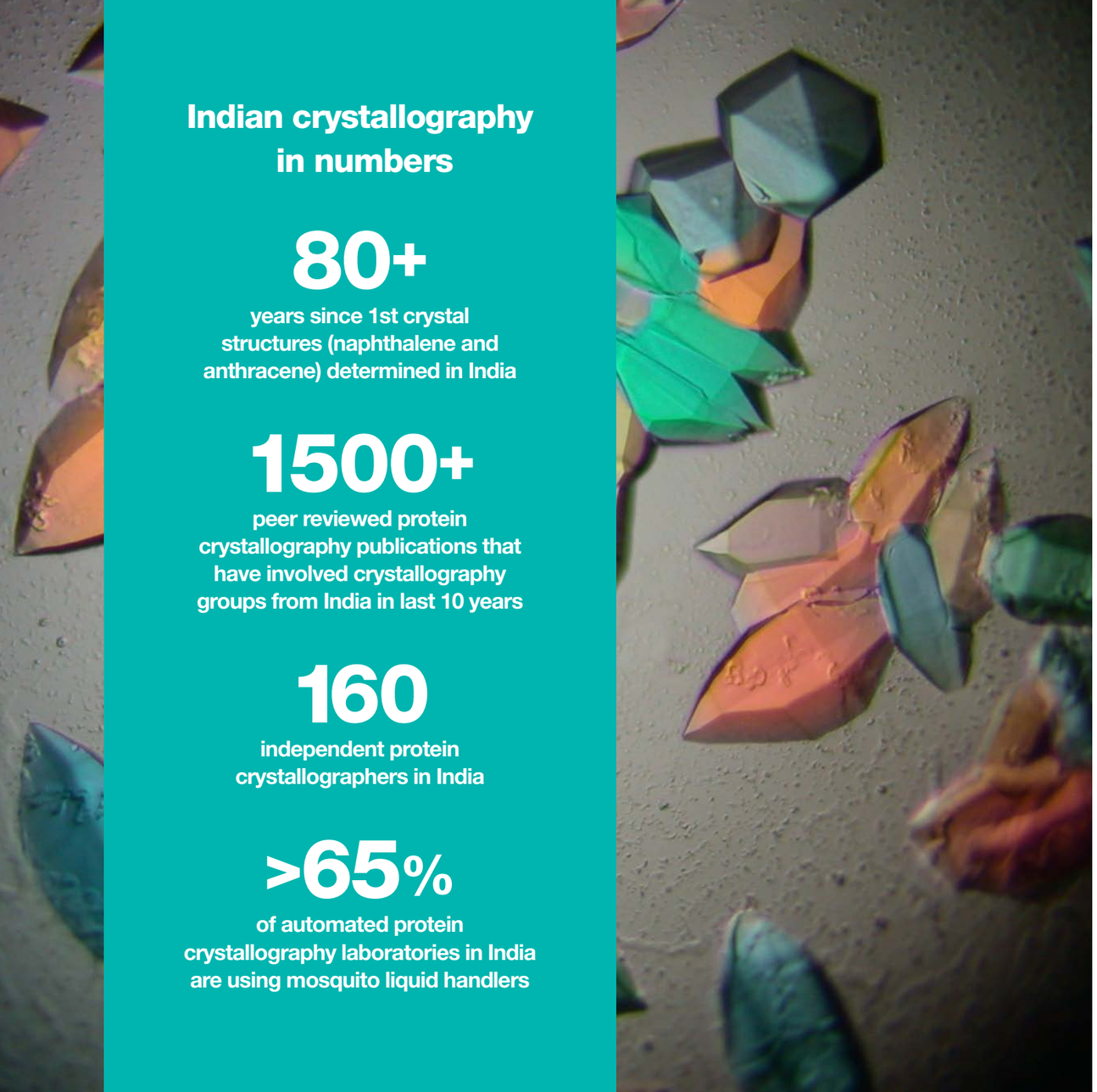


news from the world of protein crystallography



Indian crystallography in numbers

80+

years since 1st crystal structures (naphthalene and anthracene) determined in India

1500+

peer reviewed protein crystallography publications that have involved crystallography groups from India in last 10 years

160

independent protein crystallographers in India

>65%

of automated protein crystallography laboratories in India are using mosquito liquid handlers

welcome to labCrystal

This year's edition of labCrystal has a special focus on crystallography in India

India has supported crystallography research from the 1930's, although it was the 1950's when GN Ramachandran was named as a founding father of structural biology.

GN Ramachandran was an Indian physicist best known for his development of the Ramachandran plot for validating protein structures but he was also the first person to propose a triple-helical model for the structure of collagen.

Since then, India has gone through turbulent times but has excelled in bioinformatics. Thanks to the generous support from a few individuals, funding from the Department of Science and Technology (DST) and other agencies; crystallographic research is on par with leading research laboratories in the rest of the world. In emerging areas, India has established itself among the top publishing countries.

This year, the internationally important IUCr Congress is being hosted in India (21st - 28th August). TTP Labtech will honor our 25th Indian liquid handling instrument customer during IUCr and celebrate the relocation of our India office to new facilities.

Our new office, providing sales, application and engineering support, aims to deliver the very best to Indian scientists. By partnering with other highly respected companies in the field, we can offer a complete range of products and services. Including screening plates and detergents, to automation and storage solutions.

Read on to find out more about crystallography in India from Drs. Sureshkumar Ramasamy, NCL and Balaji Prakesh, CSIR-CFTR, and from some of our other customers worldwide.

Sarveshwar Johri
Business Head, TTP Labtech India Pvt Ltd

in this issue

Semi-synthetic penicillins - let's not forget the Gram-negative bacterial enzymes!

Penicillin acylases and bile salt hydrolases are routinely employed as synthetic antibiotics in the pharmaceutical and food industry as an alternative to naturally occurring antibiotics. In this article, Dr. Sureshkumar Ramasmay, CSIR-National Chemical Laboratory, India describes the determination of the three-dimensional structure of a novel penicillin V acylase (PVA) from the Gram-negative bacterium *Pectobacterium atrosepticum* (PaPVA). This enzyme shows many preferential properties compared to other PVAs characterised from Gram-positive bacteria. TTP Labtech's mosquito LCP demonstrated reliable, automated liquid handling. This enabled Dr. Ramasamy to screen much more crystallisation space then he would have done previously using manual drop set-ups.

Naturally occurring antibiotics often have low acid stability and increasing resistance problems which have initiated the development of semi-synthetic antibiotics (SSA's). However, chemical synthesis of these synthetic antibiotics often requires harsh conditions, environmentally harmful processes and high downstream processing costs.

why study Penicillin V acylase (PVA) enzymes from Gram-negative bacteria?

Dr. Ramasamy's previous work contributed to the cloning and characterisation of a Penicillin V acylase (PVA) enzyme from the Gram-negative plant pathogen, *Pectobacterium atrosepticum* (PaPVA) [1]. PVAs belong to the Ntn hydrolase super family of enzymes that catalyse the deacylation of the side chain from phenoxymethyl penicillin (penicillin V). This work demonstrated that PaPVA possessed better enzyme properties and enhanced activity compared to other PVAs from Gram-positive bacteria. Sequence analysis and characterisation revealed the distinctive nature of these enzymes and underscored the need to study PVAs from Gram-negative bacteria.

completing the structure of PaPVA for the first time

More recently Dr. Ramasamy's group have published work that describes the structural analysis of PaPVA [2]. This was the first complete structure of a functionally characterised penicillin V acylase from Gram-negative bacteria.

Fig 1. Crystals of Penicillin V Acylase from *Pectobacterium atrosepticum* (PaPVA) obtained by fine screening with mosquito LCP

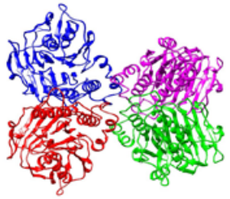


Fig 2. Quaternary structure of PaPVA after refinement (front view and rotated 90°). The four subunits are shown in different colours.

The initial process of finding suitable conditions for crystallisation requires the use of a high-throughput low-volume screening approach. In the case of PaPVA, crystal drops were set up using mosquito LCP (TTP Labtech) in 96-well plates for different commercially available crystal screens using the sitting drop vapour diffusion technique; 200 nL of protein was mixed with 200 nL of each condition. Crystal hits occurred after 3 days, observed as needles and plates in 37 drops from 278 conditions (Fig. 1). From here, it was easily possible to find the perfect condition for a good-quality crystal. Further optimisation resulted in cuboid crystals that diffracted at 2.5Å resolution. The overall structure displayed the characteristic αββα core catalytic fold of an Ntn hydrolase, comprising two anti-parallel β-sheets packed against each other and sandwiched between two α-helices (Fig. 2).

screening more conditions in less time

mosquito LCP dispensed all the reagents required for these conditions, rapidly and accurately. This shortened the time and effort taken for narrowing the range of optimum conditions and protein concentrations.

"The rapid and robust nature of mosquito LCP has been of tremendous support for our research activities. We are now able to screen a wide variety of crystallisation conditions with utmost precision in a minimum time frame, thus making it possible to explore structure-function relationships for a large number of proteins from our group, as well as our collaborators," remarked Dr. Ramasamy.

Dr. Sureshkumar Ramasamy

Dr. Sureshkumar Ramasamy (shown third from the left with team) is an Assistant Professor at CSIR - National Chemical Laboratory, Pune, India. He is interested in studying protein-protein interaction networks of translocation pathways and lipid signalling. Specifically, he investigates the structure and dynamics of protein complexes with respect to protein translocation and cell development. The basic research question to be answered is 'what could be the differences in the modulation and dynamics of protein complexes/ lipids between normal cells and under stress conditions?'

> The rapid and robust nature of mosquito LCP has been of tremendous support for our research activities

references

[1] Avinash, VS *et al*. Penicillin V acylase from *Pectobacterium atrosepticum* exhibits high specific activity and unique kinetics. (2015) *Int. J. Biol. Macromol.* 79:1-7

[2] Avinash, VS *et al*. Structural analysis of a penicillin V acylase from *Pectobacterium atrosepticum* confirms the importance of two Trp residues for activity and specificity. (2016) *J. Struct. Biol.* 193: 85-94

Breaking down the cell walls of *mycobacterium tuberculosis*

N-Acetylglucosamine-1-phosphate uridyltransferase (GlmU) is a bifunctional enzyme exclusive to prokaryotes and belongs to the family of sugar nucleotidyltransferases (SNTs). GlmU utilises UTP and GlcNAc-1-P as its natural substrates and synthesises UDP-GlcNAc, which is a crucial element of the cell wall of several bacteria. Given this significant role, GlmU is an established target for antimicrobial drugs. In this article, Ms. Neha Vithani and Dr. Balaji Prakash, CSIR-CFTR India describe how they characterised the substrate specificity of the uridyltransferase reaction in *Mycobacterium tuberculosis* (*M.tb*) using biochemical and crystallographic studies. TTP Labtech's mosquito® Crystal enabled the crystallisation of GlmU to be performed in low volumes and in high-throughput.

The glmU gene is essential to *M.tb*, being required for optimal bacterial growth, and has been selected as a possible drug target for structural and functional investigation. The GlmU enzyme binds GlcNAc-1-P and UTP and catalyses a uridyltransfer reaction to synthesize UDP-GlcNAc, an important precursor for cell-wall biosynthesis.

Previously, Dr. Prakesh characterised GlmU from *M.tb* (GlmUMtb) and attempted to understand the structure–function relationships that explain its catalytic mechanisms and regulation. As several SNTs possess wide substrate utilisation, Dr. Prakash's PhD student, Neha Vithani set out to characterise the substrate specificity of the uridyltransferase reaction in *M.tb* using biochemical and crystallographic studies [1].

low volume crystallisation of protein-DNA complexes

Crystals of GlmU in apo (nucleotide-free state) were obtained from *M.tb* by sitting-drop vapor-diffusion method at 4°C using a reservoir of 100 mM Hepes (pH 7.5), 1 mM DTT, and 8% polyethylene glycol (PEG) 8000, in a Greiner XTL 96-well plate. The reservoir solution in each well was prepared by mixing 400 nL of protein solution with 400 nL of reservoir solution using TTP Labtech's mosquito Crystal. 4% t-butanol (Hampton Research) was used as an additive in the mother liquor. Multiple variations of conditions using low volumes required consistent repeatability which was achieved with mosquito Crystal. Crystals suitable for X-ray analysis were obtained in 15–18 days.

Crystals of GlmU in complex with ligands (ATP and GlcNAc-1-P) and three Mg^{2+} ions were obtained by soaking apo GlmUMtb crystals in soaking solution for 4 hours.

de novo GlmUMtb inhibition

Although GlmUMtb appears to be highly specific for its natural substrates UTP and GlcNAc-1-P, it can also bind to ATP. In the new structure, termed GlmUMtb[GlcNAc-1- P:ATP] an unusual conformation was observed that resulted in an inactive enzyme–nucleotide complex (Fig 1). This may provide a platform for the *de novo* design of an inhibitor against GlmU and a novel antimicrobial drug for *M.tb* infection.

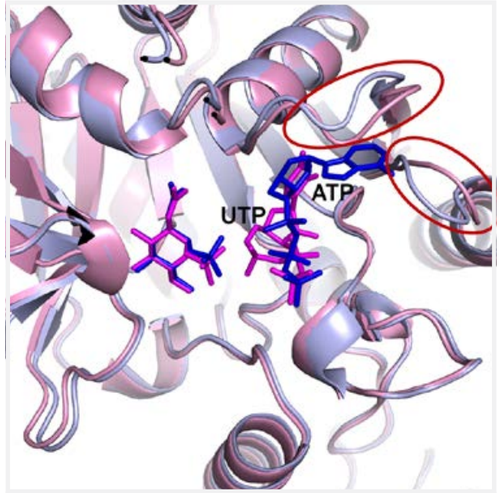


Fig 1. A structural comparison of GlmUMtb[GlcNAc-1-P:ATP] with GlmUMtb[GlcNAc-1-P:UTP] is shown as ribbons coloured purple and pink, respectively. The nucleotides ATP and UTP bound at the active site are shown as sticks coloured blue and magenta, respectively. The loop regions that show a conformational change are indicated.

Dr. Prakash commented, "The crystallisation of GlmU required handling protein and reservoir solutions in very small volumes, down to 400 nL but the assay was very sensitive to variations in these volumes within the droplets. mosquito Crystal enabled us to successfully set up the crystallisation of GlmU with consistent conditions, in low volumes and in a high-throughput manner."

Dr. Balaji Prakash

Dr. Prakash is a Senior Principal Scientist and Head of the Department of Molecular Nutrition at CSIR-CFTR, Mysore, India. Ms Neha Vithani is a student at Indian Institute of Technology (IIT), Kanpur. Using a combination of biochemistry, structural Biology and bioinformatics, Dr. Prakash's work addresses catalytic mechanisms of important families of enzymes, such as GTPases, kinases and sugar nucleotidylyltransferases. By targeting these, he develops novel peptides/molecules with potential applications and relevance to food science and drug discovery.

> mosquito Crystal enabled us to successfully set up the crystallisation of GlmU with consistent conditions, in low volumes and in a high-throughput manner

references

[1] Vithani N, Bais V and Prakash B. GlmU (N-acetylglucosamine-1-phosphate uridyltransferase) bound to three magnesium ions and ATP at the active site. (2014) *Acta Cryst* F70: 703-708

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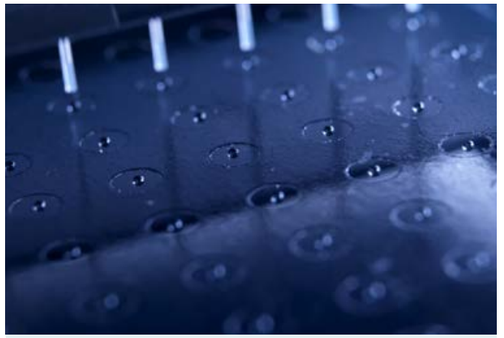

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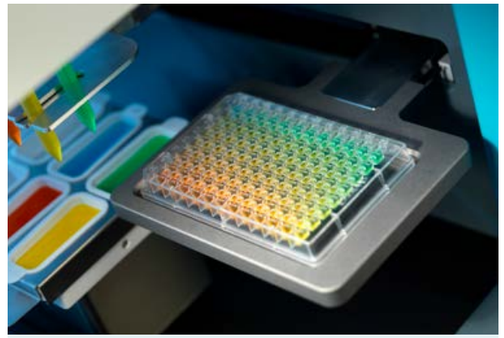
- the 'breeder facility' produces > 1 million tips per day, equivalent to more than 10,000 standard tip boxes
- tips undergo 100% QC whereby the physical parameters of each tip are checked via imaging, and volumetric testing of every spool is also conducted
- performance of tips is guaranteed when combined with TTP Labtech instruments



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Toppling across a membrane to take up nutrients

Energy-coupling factor (ECF) transporters mediate the uptake of micronutrients in prokaryotes. The mechanism of transport is poorly understood but involves an unusual step in which a membrane-embedded component topples over to carry the substrate across the membrane. In this article, Dr. Albert Guskov and Prof. Dirk Slotboom, University of Groningen, The Netherlands present details of a study that led to the proposal of a novel model for transportation of micronutrients in prokaryotes.

crystal structures define micronutrient transportation

In their recent work, Dr. Guskov and Prof. Slotboom describe the crystal structure of a folate-specific transporter (ECF–FolT) from *Lactobacillus delbrueckii* [1].

Energy-coupling factor (ECF) transporters consist of an S-component that binds the transported substrate, and an ECF module that binds and hydrolyses. The membrane-embedded S-component serves as both the substrate-binding and the substrate-translocation domain.

The crystal structures of a folate-specific transporter (ECF–FolT) from *L. delbrueckii* were determined in three different states:

1. apo-form (unbound state, but still connected to ECF)

Initial crystals of the apo ECF– FolT2 complex were identified in the MemGold2 HT-96 screen (Molecular Dimensions) and diffracted up to 8Å resolution. Optimisation conditions using several additives and detergents, were assessed. Crystals were yielded that diffracted up to 3.7Å resolution. Multiple rounds of seeding resulted in diamond-shaped crystals, which grew in clumps and diffracted up to 2.7Å resolution.

2. in complex with one of its substrates (ATP analogue AMP–PNP)

Using the apo ECF–FolT2 crystals (Fig 1) for seeding ECF–FolT2 crystallised in single diamond-shaped crystals diffracting up to 3.0Å resolution.

Fig 1. The optimised crystals of apo ECF complex before a seeding procedure

3. in isolation (lone S-component with a bound substrate but not connected to ECF

Crystals of FolT1 were obtained using the hanging-drop vapour-diffusion technique. These crystals were rectangular in shape and diffracted up to 2.8Å resolution.

All these crystallisation and optimisation studies utilised mosquito® Crystal (TTP Labtech) to pipette low volumes of protein (down to 75 nL) and reagents for each screen (total reservoir solution 100 nL). As the transporter was crystallised in several different states, it was necessary to re-screen a lot of conditions.

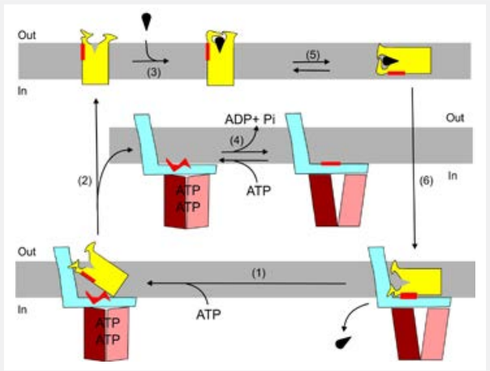

Fig 2. Working model for the transport mechanism of group II ECF transporters. Binding of ATP releases the empty S-component by disruption of the hydrophobic interface (red) (step (1)). The S-component will reorient to the outward-facing state (2) and can bind substrate on the extracellular side of the membrane (3). ATP hydrolysis in the ECF module regenerates the binding platform for the S-component (4). Possibly futile ATP hydrolysis takes place in this stage. The substrate-bound S-component can topple over in the membrane possibly aided by the vicinity of the ECF module (5). The toppled S-component binds to the ECF module via the complementary hydrophobic surfaces, coloured in red (6). Binding of the S-component to the ECF module forces the disruption of the substrate-binding site and release of the substrate into the cytoplasm

challenging previous models of membrane transport

After re-evaluation of the existing data, a new working model was proposed which features the S-component toppling between the inside and outside of the membrane dissociating from the ECF module and driven by binding of ATP or substrate (Fig 2).

Dr. Albert Guskov

Dr. Albert Guskov (left) is a group leader for protein crystallography in the membrane enzymology lab (headed by Prof Dirk Jan Slotboom, pictured right) at University of Groningen, The Netherlands. The enzymology group focuses on the molecular mechanisms of transport across biological and synthetic membranes. Biochemical and X-ray crystallography studies are combined with (single-molecule) microscopy to yield mechanistic insight into membrane transport.

“”

mosquito Crystal is the best investment in lab equipment we have ever made!

references

[1] Swier, LJYM, Guskov, A and Slotboom, DJ (2016) Structural insight in the toppling mechanism of an energy-coupling factor transporter. *Nature Communications* 7:11072

Regulating bacterial transcription in a disorderly fashion

Toxin-Antitoxin (TA) systems are widespread among mobile genetic elements and bacterial genomes. Their function is still far from well understood. They seem to play a major role in bacterial stress physiology by temporarily halting cell division when nutrients are scarce. In this article, Dr. Abel Garcia-Pino, Brussels University (ULB), Belgium presents structural data describing the role of an intrinsically disordered region (IDR) of the Phd antitoxin in the modulation of the toxin regulatory process.

Most antitoxins contain an intrinsically disordered region (IDR) that typically is involved in toxin neutralisation and repressor complex formation. In a recent publication, Dr Garcia Pino addresses how the antitoxin IDR is involved in transcription regulation by studying the *phd-doc* operon from bacteriophage P1 [1]. It was found that the IDR of Phd provides an entropic barrier precluding full operon repression in the absence of Doc. Binding of Doc then results in a cooperativity switch and consequent strong operon repression, enabling context-specific modulation of the regulatory process.

structural challenges of protein-nucleic acid complexes

This work involved the integration of a myriad of biophysical measurements, all hinged on the crystal structure of Phd bound to its operator. This structure was the first of its type and remained, for years, one of the few bacterial transcription factors for which structural data bound to nucleic acids was ever obtained. It was fundamental to analyse additional thermodynamic and structural data to model the allosteric nature of the system, however crystallising the complex was not a minor undertaking.

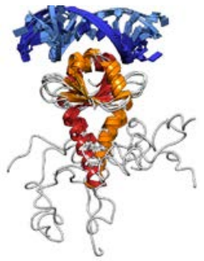

Fig 1. SAXS-based model of the Phd:DNA solution ensemble including the disordered region illustrating the challenges of crystallising such a molecule

One-third of Phd is intrinsically disordered which adds to the known difficulties of crystallising protein-nucleic acid complexes (Fig 1). In addition, this makes the protein very unstable and aggregation-prone at high concentrations.

finding the right equipment to overcome the hurdles

The use of TTP Labtech's mosquito® Crystal liquid handler was crucial to the determination of the protein-nucleic acid complex. In this setup, dozens of different duplex DNA fragments were screened at various temperatures and concentrations to find a "crystallisable" complex (Fig 2). Moreover, the speed of the instrument, completing a full plate in a few minutes, was also an advantage to prevent unwanted precipitation of the complex. The versatility of the system for screening using sitting and hanging drop configuration allowed an additional screening parameter. In the end, this fast and reliable set-up helped identify two hits (within several thousand screens) that were harvested directly, without further optimisation, and led to determination of the complex structure at 3.1Å and 3.8Å resolution (Fig 1 and 3).

Dr. Garcia Pino stated, "Our experience with the mosquito Crystal has been a sound success and trouble-free. Its throughput, simplicity and user-friendly interface make the system accessible to all the members of our group from the start; even for those just introduced to the method".

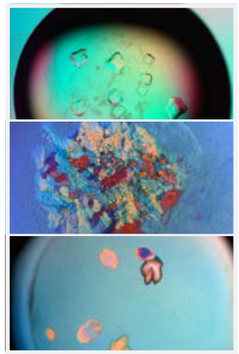

Fig 2. Typical crystals of the Phd:DNA complex obtained using the mosquito Crystal system

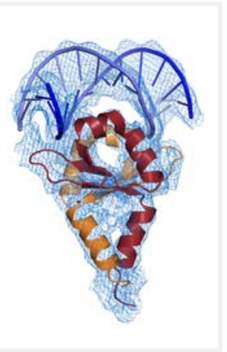

Fig 3. Electron density map representation of the 2mFo-DFc Phd:DNA map superimposed with the model of the complex

references

[1] Garcia-Pino A *et al*. An intrinsically disordered entropic switch determines allostery in Phd–Doc regulation. (2016) *Nat. Chem. Biol.* 12(7): 490-6

Dr. Abel Garcia-Pino

Abel Garcia-Pino is an Assistant Professor in the Institut de biologie et de médecine moléculaires (IBMM), Université Libre de Bruxelles (ULB), Belgium. His group are studying the mechanistic bases of regulatory processes using structural biology and biophysical techniques. This involves using Toxin-Antitoxin (TA) modules as probes to understand how basic biochemical mechanisms integrate in the myriad of processes leading to cell growth arrest. In addition, the group is also interested in basic protein chemistry processes involving intrinsically disordered proteins (IDP) and the study of signal transduction by membrane receptors.

> The throughput, simplicity and user-friendly interface of the mosquito Crystal liquid handling system makes it accessible to all the members of our group from the start; even for those just introduced to the method

Targeting the BAM complex against multidrug resistance in pathogenic bacteria

The β-barrel assembly machinery (BAM) is important in the integrity of the membrane and the assembly of surface exposed virulence factors. This makes it an attractive therapeutic target against pathogenic bacteria. The mechanism by which BAM functions remains elusive. To address this, Dr. Nicholas Noinaj and his team at Purdue University, West Lafayette, USA, reported on the structure of the BAM complex from *E. coli* [1]. This article also describes the essential use of TTP Labtech's mosquito® LCP and dragonfly® in setting up fast and reliable crystallisation screens.

While α-helical membrane proteins can be found in nearly all membranes, β-barrel membrane proteins can only be found in the outer membranes (OM) of Gram-negative bacteria, mitochondria, and plastids, such as chloroplasts.

The β-barrel assembly machinery (BAM) is a multicomponent complex responsible for the biogenesis of β-barrel OMPs in Gram-negative bacteria. Potential antimicrobial compounds could directly interact with BAM at the surface of the bacteria rather than having to cross one or two membranes to reach the intended target.

Structural studies of individual components of BAM have offered clues to how each component may function within the complex, however, the lack of structural information regarding the fully assembled complex has hindered progress towards exploring the mechanism further.

facilitating the screening of thousands of conditions!

Dr. Noinaj set up numerous crystallisation trials of BAM in commonly available screens using the hanging drop method.

Initial screening of more than 2,000 conditions was set up using 200 nL of protein and 200 nL of well solution per drop. Additionally, LCP crystallisation screening of more than 1,000 conditions was also performed using 75 nL of LCP mix and 1 mL of well solution in sandwich plates. An initial hit was improved by additive screening using the AdditiveHT screen (Hampton Research) with final crystals grown at 21°C (Fig 1).

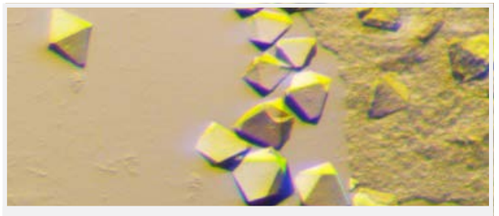

Fig 1. An example of a BAM crystal grown using the crystallisation pipeline that includes mosquito LCP and dragonfly. This crystal produced led to a resolution of 3.4Å.

Dr. Noinaj commented, "The mosquito LCP truly is an ESSENTIAL piece of equipment in modern membrane protein crystallography and with the humidification chamber (a must have!), you never have to worry about your drops drying out! The main advantages of mosquito LCP are that of precision, speed and accuracy enabling a 96-well hanging drop plate to be setup in just over 3 minutes."

membrane protein optimisation screening in minutes!

Initial lead conditions were expanded and optimised using TTP Labtech's dragonfly screen optimisation robot in a 96-well format. These optimisation plates were then rescreened using the mosquito LCP crystallisation robot again, and the process was repeated with finer and finer screen gradients until crystals were grown to a suitable size for screening and data collection.

"The 10-heads of the dragonfly are more than sufficient to create screens ranging in complexity from simple x,y gradients to multi-zone optimisation. Screens that used to take hours to prepare can now be made in under 10 minutes using the user-friendly software and intuitive design of the dragonfly," continued Dr. Noinaj.

The crystal structure of the BAM complex was solved to 3.4Å resolution by molecular replacement and together with the previously reported partial structure of BamAB, a model of the fully assembled BAM complex was reported (Fig 2).

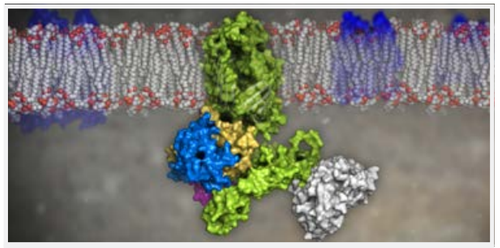

Fig 2. The structure of BAM modelled into a lipid bilayer. The barrel domain of BamA (green) undergoes an unprecedented twisting conformational change thought to be mediated by the interaction with other components of the complex.

Combating multidrug-resistance

The structure revealed that upon binding BamCDE, the β-barrel domain of BamA undergoes an unprecedented conformational twist that dramatically changes the angle of the strands (shear) in the membrane, leading to opening of the exit pore and rearrangement at the lateral gate.

Ultimately, these findings could help to combat multidrug-resistant strains of pathogenic bacteria, by targeting these large conformational changes and preventing BAM function, leading to cell death.

Dr. Nicholas Noinaj

Dr. Nicholas Noinaj (right with Dr. Jeremy Bakelar) is an Assistant Professor in the Structural and Computational Biology and Biophysics group at Purdue University, USA. His research interests are focused on understanding how pathogenic Gram-negative bacteria can use virulence factors found on their outer membrane to mediate infection. Using a combination of techniques including X-ray crystallography, his lab investigates the structural and functional characteristics of outer membrane proteins (OMPs) which serve as the foundation for successful antibiotic discovery and development.

> "mosquito LCP and dragonfly liquid handers are essential tools to streamline structure determination

references

Bakelar, J., Buchanan, S.K. and Noinaj, N. (2016) The structure of the β-barrel assembly machinery complex. *Science* 351(6269): 180-186

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Applications include: hanging drop, sitting drop, bicelle, seeding, additive screening, soaking, microbatch, LCP

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25 nL–1200 nL	pipetting range	25 nL–1200 nL
10 nL	minimal accessible volume	10 nL
48-, 96-, 384-	primary SBS plate format	48-, 96-, 384-
< 2 mins/ 96-well plate 4 mins/ 288 drops	throughput	2 mins/ 96-drop plate for vapour diffusion 4 mins/ 96-drop LCP plate
active humidity chamber	optional extras/ accessories	active humidity chamber, LCP mixer, LCP syringe coupling and needles

dragonfly®

optimisation optimised

TTP Labtech's dragonfly enhances protein crystal screen optimisation. Once the initial crystal 'hits' are identified, dragonfly easily automates the set-up of optimised conditions to grow better diffracting crystals. It is the ideal system to complement TTP Labtech's mosquito in the protein crystallisation workflow.

- **focused** specifically designed to deliver highly accurate and reproducible optimisation screens
- **easy to use** simple set up enables rapid turn-around and robustness
- **unique** positive displacement ensures unrivalled accuracy across a vast range of viscosities - no liquid classification
- **free, unlimited** software licenses that can be used offline

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pipetting range	0.5 µL–4 mL
primary SBS plate format	15-, 24-, 48-, 96-well plate
reservoir capacity	10 mL
throughput	4–6 ingredient, 96-well plate in 4–8 mins, irrespective of viscosity
optional extras/ accessories	MXone automated in-well mixer, 24-well plate adaptor

MXone

perfect plate mixing

Ensure your optimization gradients are uniform solutions with MXone, a perfect complementary mixer to dragonfly.

- **Fast, efficient** mixing even in small wells
- Mix a **wide range of viscosities** in the same plate
- **Zero spillage** or cross contamination
- ensures uniform solution ready for drop setting

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> A breath of fresh air in the world of crystallisation hits optimisation!
>
> Dr. Dimitri Chirgadze, University of Cambridge, UK speaking about dragonfly

get in touch

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