MX2082 biennial report

The **Southampton, UCL Royal Free, Exeter, Bristol, Essex BAG** reports 16 papers (1 in press) and 26 structures deposited in the PDB. Their new application is strengthened by further engagement from Bristol (two groups) and Portsmouth joining the bag (two groups); Essex do not report here and leave this BAG. The BAG submits three highlight reports.

The Tews group reports studies of monoclonal antibodies directed against key cellular receptors. The antibodies were developed at Southampton for use in cancer therapy, and several are in clinical testing. Work is undertaken with the vision to understand structure function relationships to use this information for improving clinical properties.

- The mechanism of how CD40 antibodies can vary in their activity to agonise the receptor was investigated. A structure of an antibody-receptor complex was published in 2017 in Cancer Cell and selected as a report for the ESRF Highlights 2018.
- Novel methodology has been developed to relate SAXS data to Molecular Dynamics Simulations. Starting with a structure of a CD32b receptor – antibody complex structural changes to the F(ab) fragment recognizing CD32b were investigated. The method allows not only the detection of global changes but also allows identification of localized motions. Published in Biophys J, it is submitted here as a highlight report.

The Tews group have a long history in carrying out work on Vitamin B6 biosynthesis and use advanced spectroscopy for detection of enzyme intermediate complexes, both online and offline. The work has used beamtimes over a timeframe of nine years and was published in Nature Chemical Biology. A highlight report is included.

The Tews group determined structures of ABC transporter substrate binding domains, studying ecologically important marine cyanobacteria. The work was recently published in J. Biol Chem. A recent success is work on a vaccine target from *Neisseria gonorrhoeae*, an organism that has become a public health threat with the emergence of multiple antibiotic-resistance. The study is published in mSphere.

The Doyle group studies interactions with the NMDAR glutamate receptors that are central in cognitive processes and long-term memory. NMDAR activity loss in synapses is associated with neurological diseases such as Alzheimer's. A number of structures of PDZ domains derived from the protein PSD-95 were determined with a series of C-terminal peptide fragments of the NMDA receptors NR2A and NR2B (in preparation).

The Berger group studies the regulation of transcription, in particular human general transcription factors and their structure, interaction, mechanism and cellular assembly. TFIID is the largest of the major general transcription factor (GTF) and composed of a TATA-box binding protein (TBP) and 13 TBP associated factors (TAFs). The Berger group a track record of producing high quality protein complexes recombinantly and studied or discovered various modes of regulation via these TAFs, using an integrative structural biology approach.

Structures and interactions of TAF5/TAF6/TAF9 and TAF11/TAF13/TBP complexes were studied. A highlight report is attached.

Isupov is involved in work with Littlechild studying thermophilic enzymes with potential for application in industrial biotechnology. Here, collaborative work is reported covering involvement at the phasing/refinement that reflects a substantial contribution by Isupov (NB this work may therefore be covered in other BAG reports).

The Spencer group studies mechanisms of antibiotic resistance and publishes a study on novel beta-lactamase inhibitors.

The Coker/Cooper groups work on a range of medically important proteins focusing on both understanding their mechanism of action and rational drug design.

MX2082 beamline performance

Excellent. Thank you for support on beam lines.

We note that ID30B and ID23-1 have been particularly useful for studying metal binding and collect S/MAD data.
We note that use of spectroscopy facilities, both on and offline (ID29S) has been instrumental.
We are mindful to further incentivize uptake of automated data collection by users in this bag (MASSIF).

In the following, we report some use of beamlines, and note that these still contain structures / papers arising from data collection on ID14 beamlines.

In the study of Vitamin B6 biosynthesis, the Tews group have made use of both online and offline spectroscopy using beamlines BM30 and ID29, as well as ID29S. Crystallographic work used several ESRF beamlines, namely ID14-1, ID14-4, ID23-1 and ID29 over a timeframe of nine years and was published in Nature Chemical Biology. Work like this requires an integrated approach and the mixed portfolio of beamlines that ESRF offers.

The large complexes of the general transcription factor TFIID studied by Berger make particular use of an integrative structural biology approach, using SAXS data to complement crystallographic structure determination, and also EM.

In immunology work Tews compares crystallographic structure with in-solution behavior determined by SAXS and brings in molecular dynamics simulation for interpretation of the biology. This is a further example of an integrated approach and has benefitted from the availability of a high quality SAXS station and support at BM29, essential to this work.

PDB codes published by the bag:
5MHB, 5HMS, 5I6J, 5K4Q, 5LC1, 5LNR, 5LNS, 5LNT, 5LNU, 5LNW, 5L9A, 5M4S, 5OCC, 5O44, 6FAX, 6F3T, 6GQ4, 6G7N, 6G7P, 6G7Q, 6H77, 6H78, 6IAA, 6I7S, 6QWV, 6TD0.

BRISTOL

BERGER PDBs

1. 6F3T
2. 5M4S

BERGER PAPERs

1. Antonova, S. V., Haffke, M., Corradini, E., Mikuciunas, M., Low, T. Y., Signor, L., Van-Es, R. M., Gupta, K., Scheer, E., Vos, H. R., Tora, L., Heck, A. J. R., Timmers, H. T. M. & Berger, I., Dec 2018, Chaperonin CCT Checkpoint Function in Basal Transcription Factor TFIID Assembly. Nature Structural and Molecular Biology. 25, 12, p.1119-1127 **BEAMLINE: ESRF ID14-4, plus SOLEIL Proximal**

2. Gupta, K., Watson, A. A., Baptista, T., Scheer, E., Chambers, A. L., Koehler, C., Zou, J., Obong-Ebong, I., Kandiah, E., Temblador, A., Round, A., Forest, E., Man, P., Bieniossek, C., Laue, E. D., Lemke, E. A., Rappsilber, J., Robinson, C. V., Devys, D., Tora, L. & Berger, I., Architecture of TAF11/TAF13/TBP complex suggests novel regulation properties of general transcription factor TFIID. eLife. 6, 2017, e30395. **BEAMLINE: ESRF only, ID29 and BM29**

SPENCER PDBs

1. 6TD0

SPENCER PAPERs

1. Catherine L. Tooke, Philip Hinchliffe, Alen Krajnc, Adrian J. Mulholland, Jürgen Brem, Christopher J. Schofield and James Spencer. Cyclic boronates as versatile scaffolds for KPC-2 β-lactamase inhibition. RSC Med Chem (in press) **BEAMLINE: ESRF ID23-1, ALBA BL13-XALOC.**

EXETER

LITLECHILD/ISUPOV PDBs

1. 6i7s
2. 6qwv
3. 6h77, 6h78
4. 6iaa
5. 5o44
6. 5i6j

LITLECHILD/ISUPOV PAPERs

1. Biterova EI, Isupov MN, Keegan RM, Lebedev AA, Sohail AA, Liaqat I, Alanen HI, Ruddock LW. The crystal structure of human microsomal triglyceride transfer protein. Proc Natl Acad Sci U S A. 2019 Aug 27;116(35):17251-17260. **BEAMLINE: ESRF only, Massif1**
2. Sporny M, Guez-Haddad J, Lebendiker M, Ulisse V, Volf A, Mim C, Isupov MN, Opatowsky Y. Structural Evidence for an Octameric Ring Arrangement of SARM1. J Mol Biol. 2019 Sep 6;431(19):3591-3605. **BEAMLINE: ESRF ID23-1**
3. Soudah N , Padala P., Hassouna F, Kumar M, Mashahreh B, Lebedev AA, Isupov MN, Cohen-Kfir E, and Wiener R. (2019) An N-terminal extension to UBA5 adenylation domain boosts UFM1 activation: Isoform-specific differences in ubiquitin like protein activation. J Mol Biol 431, 463-478. **BEAMLINE: ESRF ID29, Massif1**
4. Barak R, Yom-Tov G, Guez-Haddad J, Gasri-Plotnitsky L, Maimon R, Cohen-Berkman M, McCarthy AA, Perlson E, Henis-Korenblit S, Isupov MN and Opatowsky Y (2019). Structural Principles in Robo Activation and Auto-Inhibition. Cell, 177, 272-285. **BEAMLINE: ESRF ID29, BESSY14-2**
5. Padala P, Soudah N, Giladi M, Haitin Y, Isupov MN, Wiener R. (2017) The Crystal Structure and Conformations of an Unbranched Mixed Tri-Ubiquitin Chain Containing K48 and K63 Linkages. J Mol Biol. 429, 3801-3813. **BEAMLINE: ESRF only: ID30B and BM29**
6. Sporny M, Guez-Haddad J, Kreusch A, Shakartzi S, Neznansky A, Cross A, Isupov MN, Qualmann B, Kessels MM, Opatowsky Y. (2017). Structural History of Human SRGAP2 Proteins. Mol Biol Evol. 34, 1463-1478, **BEAMLINE: ESRF only: ID29**

SOUTHAMPTON

TEWS PDBs

1. 6GQ4
2. 6G7N, 6G7P, and 6G7Q
3. 5OCC
4. 6FAX
5. 5LNR, 5LNS, 5LNT, 5LNU, 5LNW

TEWS PAPERs

1. Almonacid-Mendoza HL, Humbert MV, Dijokaite A, Cleary DW, Soo Y, Hung MC, Orr CM, Machelett MM, Tews I, Christodoulides M.Structure of the Recombinant Neisseria gonorrhoeae Adhesin Complex Protein (rNg-ACP) and Generation of Murine Antibodies with Bactericidal Activity against Gonococci. mSphere. 2018 Oct 10;3(5). pii: e00331-18. **BEAMLINE: ESRF only, ID23-1**
2. Polyviou D, Machelett MM, Hitchcock A, Baylay AJ, MacMillan F, Moore CM, Bibby TS, Tews I. Structural and functional characterization of IdiA/FutA (Tery_3377), an iron-

binding protein from the ocean diazotroph Trichodesmium erythraeum. J Biol Chem. 2018 Nov 23;293(47):18099-18109. **BEAMLINE: ESRF ID30B, DLS I02**

3. Sutton EJ, Bradshaw RT, Orr CM, Frendéus B, Larsson G, Teige I, Cragg MS, Tews I, Essex JW. Evaluating Anti-CD32b F(ab) Conformation Using Molecular Dynamics and Small-Angle X-Ray Scattering. Biophys J. 2018 Jul 17;115(2):289-299. **BEAMLINE: ESRF only, ID23-2 and BM29**
4. Yu X, Chan HTC, Orr CM, Dadas O, Booth SG, Dahal LN, Penfold CA, O'Brien L, Mockridge CI, French RR, Duriez P, Douglas LR, Pearson AR, Cragg MS, Tews I, Glennie MJ, White AL. Complex Interplay between Epitope Specificity and Isotype Dictates the Biological Activity of Anti-human CD40 Antibodies. Cancer Cell. 2018 Apr 9;33(4):664-675.e4. **BEAMLINE: ESRF only, ID23-1. MX1931 Highlight. ESRF HIGHLIGHT 2018**
5. Rodrigues MJ, Windeisen V, Zhang Y, Guédez G, Weber S, Strohmeier M, Hanes JW, Royant A, Evans G, Sinning I, Ealick SE, Begley TP, Tews I. Lysine relay mechanism coordinates intermediate transfer in vitamin B6 biosynthesis. Nat Chem Biol. 2017 Mar;13(3):290-294. **BEAMLINE: ESRF ID14-1, ID14-4, ID23-1 and ID29, and also at DLS I04-1. ESRF Spotlight 2017.**

UCL

COKER/COOPER PDBs

1. 5K4Q, 5L9A, 5LC1
2. 5HMS, 5MHB

COKER/COOPER PAPERs

1. Structure and function of L-threonine-3-dehydrogenase from the parasitic protozoan Trypanosoma brucei revealed by X-ray crystallography and geometric simulations. Adjogatse, E., Erskine, P., Wells, S. A., Kelly, J. M., Wilden, J. D., Chan, A. W. E., Selwood, D. Coker, A., Wood, S. and Cooper, J. B. (2018). Acta Crystallogr. D74, 861-876. **BEAMLINE: ESRF ID14-4, ID23-2, ID29 and DLS I02, I04-1**
2. Mills-Davies, N., Butler, D., Norton, E., Thompson, D., Sarwar, M., Guo, J., … Shoolingin-Jordan, P. M. (2017). Structural studies of substrate and product complexes of 5-aminolaevulinic acid dehydratase from humans, Escherichia coli and the hyperthermophile Pyrobaculum calidifontis. *Acta Crystallographica Section D: Structural Biology*, *73*(1). **BEAMLINE: ESRF BM14, ID14-2, DLS I02, DESY/EMBL X11 and SRS PX9.5**

Evaluating Anti-CD32b F(ab) Conformation Using Molecular Dynamics and Small-Angle X-Ray Scattering

Emma J. Sutton[1,2,4], Richard T. Bradshaw[2], Christian M. Orr[1,4], Bjorn Frendeus,[3] Gunilla Larsson,[3] Ingrid Teige,[3] Mark S. Cragg,[1] Ivo Tews,[4] and Jonathan W. Essex[2,*]

[1]Antibody & Vaccine Group, Cancer Sciences Unit, Centre for Cancer Immunology, Faculty of Medicine, University of Southampton, Southampton General Hospital, Southampton, United Kingdom; [2]Department of Chemistry, University of Southampton, Highfield Campus, Southampton, United Kingdom; [3]BioInvent International AB, Lund, Sweden; and [4]Department of Biological Sciences, Institute for Life Sciences, University of Southampton, Highfield Campus, Southampton, United Kingdom

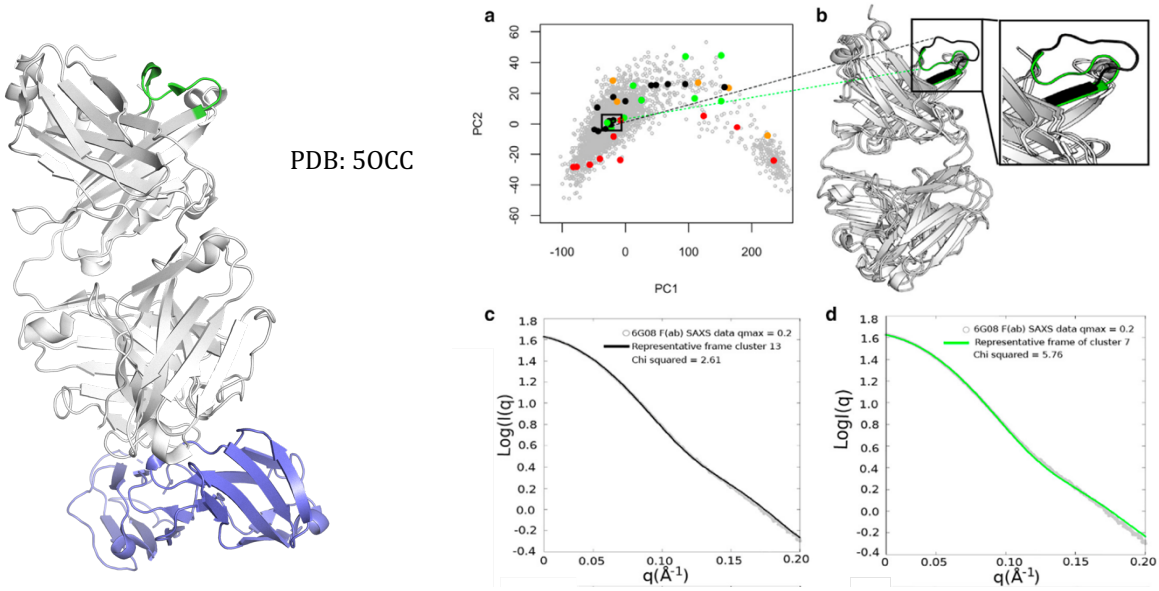


Fig 1. Left, Crystal structure of CD32b extracellular domain (blue), in complex with 6G08 F(ab) (white), with the loop of interest highlighted in green. **Right, MD/SAXS analysis** (a) Representative structures from clustering of MD trajectories, where red/green colors indicate agreement to the SAXS data. (b) Overlay of two representative structures with good (green) and less good (black) agreement, highlighting difference in a loop furthest away from the CD32b binding site. (c, d) Theoretical SAXS scattering profiles, fit to SAXS data; the bottom panels show the residual plots for the respective fits. χ^2 scores are calculated in CRYSOL.

We determined the X-ray crystallographic structure of a F(ab) fragment in complex with CD32b, the only inhibitory Fc-gamma receptor in humans, and compared the structure of the F(ab) from the crystal complex with SAXS data for the F(ab) alone in solution. We investigated changes in F(ab) structure by predicting theoretical scattering profiles for atomistic structures extracted from molecular dynamics (MD) simulations and assessed their agreement with experimental SAXS data. Principal component analysis from the MD trajectory allows the evaluation of the influence of structural motions on the fit. Changes in the F(ab) elbow angle were found to be important to reach agreement between the crystal structure and the experimental SAXS data; however, further discrepancies were apparent. By analysing multiple MD structures observed in similar regions of the principal component analysis, we were able to pinpoint discrepancies to a specific loop region in the F(ab) heavy chain. This method, therefore, not only allows determination of global changes but also allows identification of localized motions important for determining the agreement between atomistic structures and SAXS data. In this particular case, the findings allowed us to discount the hypothesis that structural changes were induced upon complex formation, a significant finding informing the drug development process.

Published in: Biophys J. 2018 Jul 17;115(2):289-299.
Crystallographic data were collected at ID23-2. SAXS data were collected at BM29.

Architecture of TAF11/TAF13/TBP complex suggests novel regulation properties of general transcription factor TFIID

[1]The School of Biochemistry, Biomedical Sciences and BrisSynBio Centre, University of Bristol, Tankard's Close, Bristol BS8 1TD, United Kingdom; [2]European Molecular Biology Laboratory, 71 Avenue des Martyrs, 38000 Grenoble, France; [3]Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA, United Kingdom; [4]Institut de Génétique et de Biologie Moléculaire et Cellulaire IGBMC, Illkirch, France; [5]Centre National de la Recherche Scientifique, UMR7104, Illkirch, France; [6]Institut National de la Santé et de la Recherche Médicale, U964, Illkirch, France; [7]Université de Strasbourg, Illkirch, France; [8]European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany; [9]Wellcome Trust Centre for Cell Biology, University of Edinburgh, Max Born Crescent, Edinburgh, EH9 3BF, United Kingdom and Chair of Bioanalytics, Institute of Biotechnology, Technische Universität Berlin, 13355 Berlin, Germany; [10]Physical and Theoretical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ, United Kingdom; [11]Institut de Biologie Structurale IBS, 71 Avenue des Martyrs, 38042 Grenoble, France; [12]BioCeV - Institute of Microbiology, The Czech Academy of Sciences, Prumyslova 595, 252 50 Vestec and Faculty of Science, Charles University, Hlavova 8, 128 43 Prague, Czech Republic

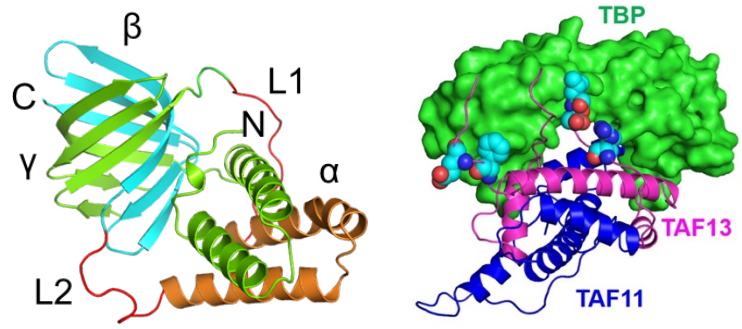


Fig 1. Left, Crystal structure of single chain version of general transcription factor TFIIA. **Right,** Model of TAF11/TAF13/TBP complex generated using SAXS data, available crystal structures and crosslinking data in an integrative biology approach. Amino acid residues crucial for complex formation are highlighted (light blue, space filling).

General transcription factor TFIID is a key component of RNA polymerase II transcription initiation. Human TFIID is a megadalton-sized complex comprising TATA-binding protein (TBP) and 13 TBP-associated factors (TAFs). TBP binds to core promoter DNA, recognizing the TATA-box. We identified a ternary complex formed by TBP and the histone fold (HF) domain-containing TFIID subunits TAF11 and TAF13. We demonstrate that TAF11/TAF13 competes for TBP binding with TATA-box DNA, and also with the N-terminal domain of TAF1 previously implicated in TATA-box mimicry. In an integrative approach combining crystal coordinates, biochemical analyses and data from cross-linking mass-spectrometry (CLMS), we determine the architecture of the TAF11/TAF13/TBP complex, revealing TAF11/TAF13 interaction with the DNA binding surface of TBP. We identify a highly conserved C-terminal TBP-interaction domain (CTID) in TAF13, which is essential for supporting cell growth. Our results thus have implications for cellular TFIID assembly and suggest a novel regulatory state for TFIID function.

ESRF played a significant role in this study by providing access to x-ray and SAXS beamlines. ID29 was used to obtain the crystal structure of $TFIIA^{S\text{-}C}$. BM29 was instrumental in obtaining high quality of SAXS data used in this study to build ab-initio models of various TAF complexes. This SAXS data was then used to generate the model of the whole TAF11/TAF13/TBP complex which enabled us to do mutational analysis in order to discover the novel mechanism of transcription regulation.

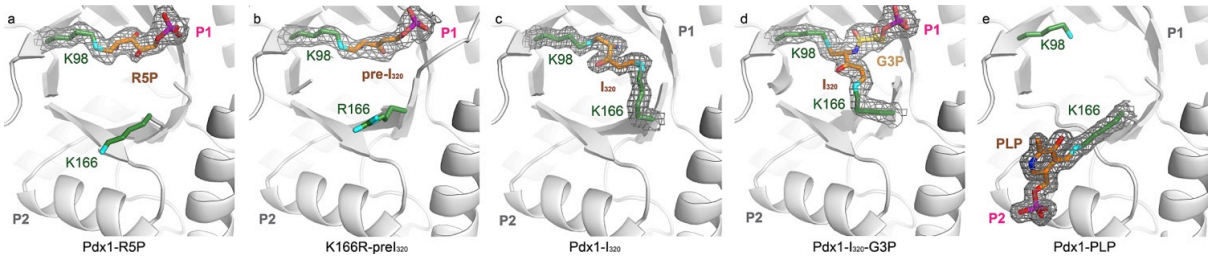
Published in: eLife (2017), 6:e30395 DOI: 10.7554/eLife.30395.
Crystallographic data was collected at ID29 and SAXS data was collected at BM29.

Lysine relay mechanism coordinates intermediate transfer in vitamin B6 biosynthesis

Matthew J Rodrigues[1,2], Volker Windeisen[1,3], Yang Zhang[4], Gabriela Guédez[3], Stefan Weber[3], Marco Strohmeier[3], Jeremiah W Hanes[4,5], Antoine Royant[6,7], Gwyndaf Evans[2], Irmgard Sinning[3], Steve E Ealick[4], Tadhg P Begley[8] and Ivo Tews[1,3].

[1]Biological Sciences, University of Southampton, Southampton, UK. [2]Diamond Light Source, Harwell Science and Innovation Campus, Didcot, UK. [3]Heidelberg University Biochemistry Center (BZH), Heidelberg, Germany. [4]Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York, USA. [5]Pacific Biosciences, Menlo Park, California, USA. [6]Institut de Biologie Structurale, Université Grenoble Alpes, CNRS, CEA, Grenoble, France. [7]European Synchrotron Radiation Facility, Grenoble, France. 8Department of Chemistry, Texas A&M University, College Station, Texas, USA.

Substrate channeling has emerged as a common mechanism for enzymatic intermediate transfer. A conspicuous gap in knowledge concerns the use of covalent lysine imines in the transfer of carbonyl-group-containing intermediates, despite their wide use in enzymatic catalysis. Here we show how imine chemistry operates in the transfer of covalent intermediates in pyridoxal-phosphate biosynthesis by the Arabidopsis thaliana enzyme Pdx1. An initial ribose-5-phosphate lysine imine is converted to the chromophoric I_{320} intermediate, simultaneously bound to two lysine residues and partially vacating the active site, which creates space for glyceraldehyde 3-phosphate to bind. Crystal structures show how substrate binding, catalysis and shuttling are coupled to conformational changes around strand 6 of the Pdx1 ()$_8$-barrel. The dual-specificity active site and imine relay mechanism for migration of carbonyl intermediates provide elegant solutions to the challenge of coordinating a complex sequence of reactions that follow a path of over 20 Å between substrate- and product-binding sites.



X-ray and spectroscopy data collected on ID29 and ID29S have been instrumental in the study of the catalytic mechanism of PLP synthase. To characterise the PLP biosynthetic activity in protein crystals, we delivered structural snapshots of reaction intermediates, adding ribose-5-phosphate, ammonia and glyceraldehyde 3-phosphate. Reactions leading to the key intermediate I320, named after its characteristic absorbance, were investigated at ID29. The structures revealed how substrate binding, catalysis and intermediate shuttling are coupled to conformational changes. For PLP synthase, we used conventional methods based on data collected from single or few crystals. We recently investigated specific radiation damage using serial multi-crystal approaches and online UV/Vis spectroscopy.

Published in: Nat Chem Biol. 2017 Mar;13(3):290-294.
Crystallographic data were collected at ID14-1, ID14-4, ID23-1 and ID29. Spectroscopic data were collected on ID29S. ESRF Spotlight 2017.

The remarkable complexity of vitamin B6 biosynthesis

News

SPOTLIGHT ON SCIENCE

13-04-2017

Vitamin B6 biosynthesis has been studied with X-ray crystallography and in crystallo spectroscopy. An unprecedented use of imine chemistry creates covalently-linked intermediates that allow migration of the reaction across a path of over 20 Å between substrate- and product-binding sites.

The mechanism of vitamin B6 biosynthesis has long been enigmatic [1]. The main biosynthetic route uses an enzyme complex known as pyridoxal 5-phosphate (PLP) synthase. The full PLP synthase complex consists of twelve copies each of the Pdx1 and Pdx2 proteins (**Figure 1**) [2]. While Pdx2 simply catalyses hydrolysis of glutamine to generate the ammonia required for PLP biosynthesis, Pdx1 catalyses the condensation of two carbohydrate substrates and ammonia in a complex reaction involving more than ten catalytic steps.

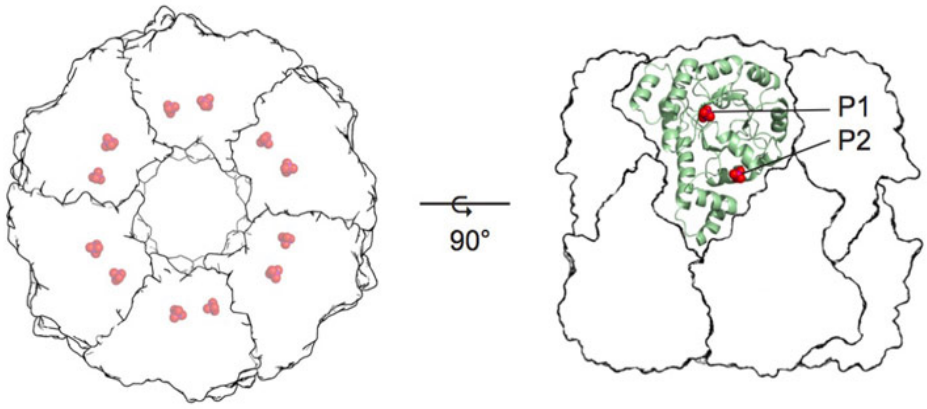


Figure 1. Crystal structure of pyridoxal 5-phosphate synthase. P1 and P2 indicate phosphate-binding sites.

The Pdx1 enzyme has a $(\beta\alpha)_8$-barrel fold and forms a dodecamer. Each Pdx1 enzyme contains two phosphate-binding sites, named P1 and P2 that are separated by 21 Å (phosphorus to phosphorus) [1]. The first Pdx1 substrate is ribose 5-phosphate (R5P), which binds with its phosphate group in the P1 site [3,4]. Addition of the second Pdx1 substrate, ammonia, leads to the formation of the chromophoric I_{320} intermediate with an absorbance maximum λ = 320 nm [5]. The formation of I_{320} partially vacates the P1 active site and creates space for the third substrate, glyceraldehyde 3-phosphate (G3P), making the P1 site a dual specificity active site. As the product, PLP, is observed with its phosphate group bound in the P2 site, the bridging position of the I_{320} intermediate between P1 and P2 sites explains intermediate transfer, a novel example of channelling.

The structures reported are enzyme-intermediate complexes that form the basis for mechanistic proposals of PLP biosynthesis. The data were collected over nearly a decade, testing several Pdx1 enzymes from different organisms. More than 1,000 crystals were tested to optimise soaking protocols used to provide both high-resolution diffraction and homogeneous accumulation of Pdx1 intermediates.

Binding of R5P occurs by covalent attachment through Schiff base formation with Lys98

(**Figure 2a**, collected at ESRF ID14-1, 1.9 Å resolution). Addition of ammonia to Pdx1-R5P complexes leads to formation of I_{320} through a second Schiff base with Lys166 (**Figure 2b**, collected at Diamond I04-1, 1.7 Å resolution). Adding G3P to Pdx1-I_{320} leads to a covalent complex with the G3P phosphate bound in the P1 site (**Figure 2c**, collected at ESRF ID23-1, 1.9 Å resolution). In the product complex, PLP is covalently bound through Schiff base formation with Lys166, with its phosphate bound in the P2 site (**Figure 2d**, collected at ESRF ID14-1, 1.6 Å resolution).

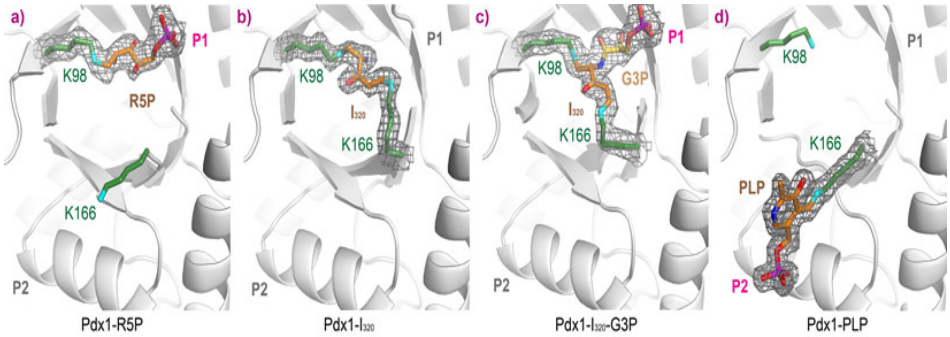


Figure 2. Complexes of Pdx1 after sequential addition of a) ribose 5-phosphate R5P, b) ammonia and c) glyceraldehyde 3-phosphate G3P, leading to the formation of d) pyridoxal 5-phosphate.

The use of micro-spectrophotometry at the ESRF Cryobench was essential to show that crystals used for data collection contained the desired chromophoric intermediates. We have used online spectroscopy to provide proof of the chemical nature of certain intermediates, such as PLP (**Figure 3**); the spectrum of a crystal with PLP bound shows an absorption maximum at λ = 414 nm characteristic for covalently bound PLP, light green, while free PLP in the surrounding buffer has an absorption maximum of λ = 388 nm, dark green (**Figure 3b**, collected at ESRF ID14-1 using on-line micro-spectrophotometry).

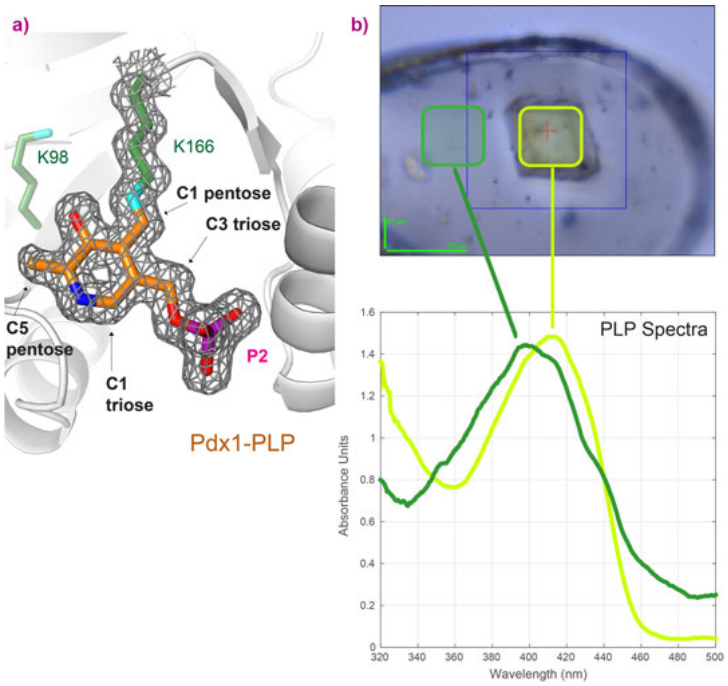


Figure 3. Confirmation of the covalent PLP-enzyme complex by a) structure determination and b) optical spectroscopy at the Cryobench.

Spectrophotometry can also be used to monitor specific radiation damage of intermediates, as was done to monitor specific radiation damage of the I_{320} intermediate (**Figure 4**, collected on-line at ESRF ID14-4). Determination of a low-dose structure of the I_{320} complex used about 50 complete data sets for I_{320} complexes (collected at ESRF ID23-1). Data collected before the 245 kGy threshold (corresponding to 80% retained absorbance of the I_{320} species,

not shown) were merged after identifying groups that merged well with the software BLEND, and the final dataset contained 19-wedges from nine crystals to confirm the structure of the l_{320} intermediate as a covalently linked intermediate between two lysine residues.

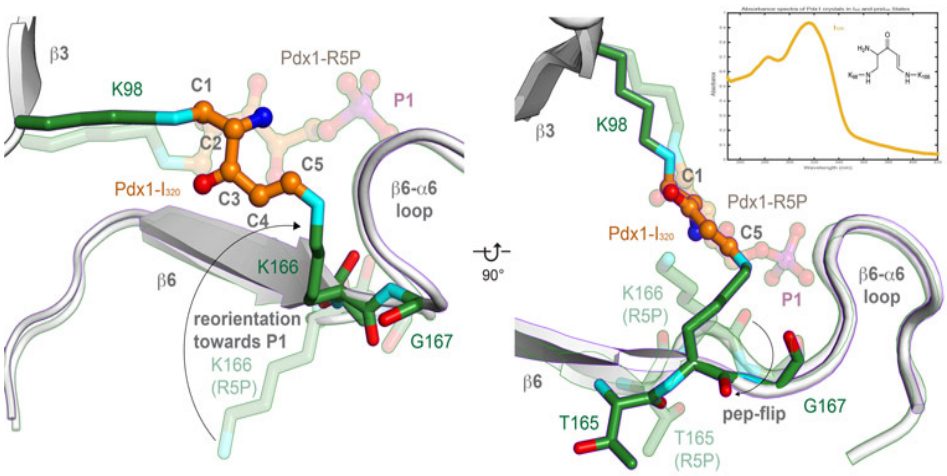


Figure 4. The central intermediate of PLP biosynthesis is the doubly covalently linked l_{320}.

Pdx1 uses covalent tethers in the synthesis of PLP to prevent loss of intermediates to surrounding solvent. This strategy maintains a high local concentration of substrate and protects the reactive l_{320} species. Parallels exist to the phosphopantothenoyl thioesters that are used to transfer intermediates between active sites in the assembly line enzymology of fatty acid synthase, polyketide synthases and non-ribosomal polypeptide synthases. Pdx1 uses a similar transfer strategy to those found in the glycine cleavage system and the classical pyruvate dehydrogenase complex, where lipoic acid is used to chaperone intermediates between active sites. While the use of covalent tethers is common in all these examples, Pdx1 transfers covalent intermediates within a single catalytic domain. The intricate relay mechanism displayed by the Pdx1 subunit of PLP synthase allows the enzyme to maintain precise control of the complex reaction performed across multiple active sites.

Principal publication and authors

Lysine relay mechanism coordinates intermediate transfer in vitamin B6 biosynthesis, M.J. Rodrigues (a,b), V. Windeisen (a,c), Y. Zhang (d), G. Guédez (c), S. Weber (c), M. Strohmeier (c), J.W. Hanes (d,e), A. Royant (f,g), G. Evans (b), I. Sinning (c), S.E. Ealick (d), T.P. Begley (h) and I. Tews (a), *Nat Chem Biol.* **13**, 290-294 (2017); doi: 10.1038/nchembio.2273.
(a) Biological Sciences, University of Southampton, Southampton (UK)
(b) Diamond Light Source, Harwell Science and Innovation Campus, Didcot (UK)
(c) Heidelberg University Biochemistry Center (BZH), Heidelberg (Germany)
(d) Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York (USA)
(e) Pacific Biosciences, Menlo Park, California (USA)
(f) Institut de Biologie Structurale, Université Grenoble Alpes, CNRS, CEA, Grenoble (France)
(g) ESRF
(h) Department of Chemistry, Texas A&M University, College Station, Texas (USA)

References

[1] T.B. Fitzpatrick *et al.*, *Biochem J.* 407, 1-13 (2007).
[2] M. Strohmeier *et al.*, *PNAS* **103**, 19284-9 (2006).
[3] A.M. Smith *et al.*, *J Biol Chem.* **290**, 5226-39 (2015).
[4] G. Guédez *et al.*, *Structure* **20**, 172-84 (2012).
[5] G.C. Robinson *et al.*, *PNAS* **113**, E5821-9 (2016).