**MX2373 biennial report**

The **Southampton, Bristol, Exeter, KCL, Portsmouth, UCL Royal Free Block Allocation Group (BAG)** reports 7 papers and 10 structures deposited in the PDB. We note that output has been affected negatively in three ways: (1) COVID, leading to reduced productivity; travel and customs problems, (2) loosing samples through delay in clearing (see remarks in performance); (3) and members retiring from or moving post. While Portsmouth decided to leave the bag, primarily on sample delivery modalities, the resubmitted proposal now includes new members and, significantly, a new institution (Cardiff University). The BAG submits three highlight reports and one ESRF spotlight.

***The Tews group*** reports studies on the study of vitamin biosynthesis, with discovery of a novel intermediate identified by spectroscopy. The work highlights the unique profile of ESRF and is truly a reflection on ESRF's excellence in macromolecular crystallography. Using *in crystallo* spectroscopy at icOS and at the beamline was essential in these discoveries. The study is ESRF data only (ID23-1), the work is described in a highlight report and was selected as spotlight, to be published 1-3-2022. RSC Chem Biol 3 (2022), 227-230.

***The Werner group*** reports new structures collagen VI von Willebrand factor type A domains for the study of congenital myopathies. The study combines crystal structure determination at ID30B and SAXS analysis. Journal of Biological Chemistry, 295 (2020), 12755-12771.

***The Berger group*** reports structures of the Human Karyopherin RanBP5, an Essential Factor for Influenza Polymerase Nuclear Trafficking. J Mol Biol. 432 (2020), 3353-3359.

**Isupov** reports collaborative work on protein ubiquitination with a highlight report, Nat Commun. 12 (2021), 5708.

**The Spencer group** reports new mechanisms of antibiotic resistance and publishes a study on beta-lactamase inhibitors. RSC Med Chem 10 (2020), 491-496.

**The Coker groups** has done seminal COVID work for rational drug design (to be published).

***The Steiner group*** reports a combined neutron/x-ray study on the tetrameric cofactor-independent urate oxidase, an essential enzyme for uric acid catabolism in many organisms. The study reveals a proton-relay system, with room-temperature crystallography revealing functional conformational heterogeneity required to modulates the peroxo hole. IUCrJ. 8 (2021), 46-59.

***The Bax group.*** We include here one further highlight from Cardiff who have just joined the bag, ACS Infect Dis 5 (2019), 570-581.

**MX2373 beamline performance**

Excellent. Thank you for support at beam lines.
Particular thanks to the MAD beamlines ID23-1 and ID30B which have provided essential phase information, e.g. in studying metal sites and disulfide structures (publications to follow).

Particular thanks to the *ic*OS and online spectroscopy which has been instrumental (see ESRF spotlight and highlight report).

**PDB codes (beamline) published by the bag:**
6qtk (ID23-2), 6qtp (ID23-1), 6SNK (ID30B), 6TD0 (ID23-1), 6XTE (ID29), 6XU2 (ID29), 7A0L (BM30A), 7NHE (ID23-1), 7NHF (ID23-1), 7nw1 (ID23-1)

**Note on travel and sending samples:**
Sadly, we have had a number of shipments from the UK to France (at least three) where samples were left in customs. It turned out that customs regulations had tightened, probably as a result of Brexit and COVID. We have learnt that we must use terms like “serum free”, “affinity purified” and “free of microorganisms” when sending antibody or protein samples. This has led to the decision of some PIs in this bag to discontinue using ESRF. Indeed, this must not be taken as dissatisfaction; indeed, everyone commented on the help we have received from ESRF stores and also from beamline scientists to accommodate late samples. Particular thanks to all at the User office and to Deborah Davison who was a real help in getting the right people on the case, many thanks Debbie!

# Combining UV-vis absorption spectroscopy, mass spectrometry and X-ray crystallography to study the biosynthesis of vitamin B6

Matthew J Rodrigues[1,2], Nitan Giri[3], Antoine Royant[4,5], Yang Zhang[6], Rachel Bolton[1,2], Gwyndaf Evans[2], Steve E. Ealick[6], Tadhg Begley[3], Ivo Tews[1].

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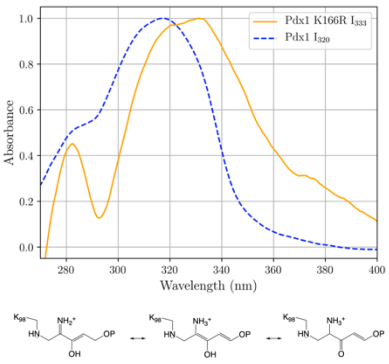
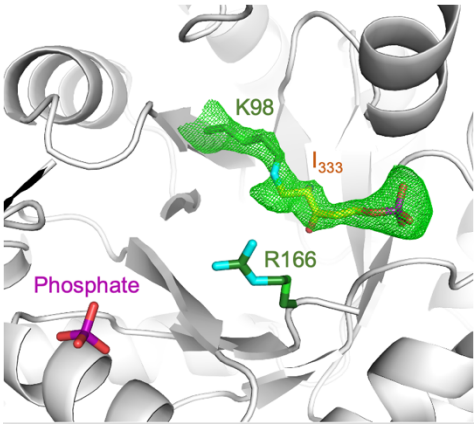
The enzyme PLP synthase uses two simple sugars and an amino acid as building blocks for vitamin B6 (pyridoxal-phosphate, PLP). Arguably the most intriguing step is incorporation of ammonia into the growing vitamin. The result of this reaction is an intermediate that is held between two protein amino acids by covalent bonds. Characterisation of a novel reaction intermediate by optical spectroscopic methods *in cristallo*, structure determination of the enzyme-intermediate complex, and mass spectrometry can explain the stereoselectivity of catalytic steps leading to the vitamin's biosynthesis.




The enzyme PLP synthase first binds the carbohydrate ribose 5-phosphate in a covalent bond with a protein lysine residue. When ammonia adds, a second covalent linkage to another lysine results in formation of the double imine chromophore I320, named after its characteristic absorbance at 315 nm, shown as blue dashed line. Modification of the enzyme by exchange of this second lysine allowed characterisation of the novel intermediate that immediately precedes I320 formation. This new species has a characteristic absorbance at 333 nm, shown as orange line.

The ability to perform these experiments on protein crystals at icOS meant that specific crystals could be selected for diffraction data collection. Equally, the spectroscopic signature proves the integrity of the specific intermediate for protein crystals from which the enzyme-intermediate structure was determined using a crystallographic experiment was carried out at ESRF beamline ID23-1. The electron density map shows the simulated annealing electron density contoured at 2.5σ (calculated with Lys98–I333 residues removed). A phosphate ion is seen in the site where PLP would form. Interpretation of this electron density is not unambiguous, and various tautomeric structures were tested to give the final structure. The sum formula of I333 had been determined by mass spectrometry. Knowing the detailed structure if I333 now allows to propose how I320 is formed, explaining this central and rate limiting step in PLP biosynthesis.

**Published in RSC Chem. Biol., 3 (2022), 227–230.**
**PDB depositions 2: 7NHE, 7NHF.**
**Crystallographic data were collected at ID23-1.**
**Spectroscopic data were collected on *ic*OS. ESRF Spotlight 2022.**

# Structural basis for UFM1 transfer from UBA5 to UFC1

Kumar M[1], Padala P[1,2], Fahoum J[1], Hassouna F[1], Tsaban T[3], Zoltsman G[4], Banerjee S[1], Cohen-Kfir E[1], Dessau M[5], Rosenzweig R[4], Isupov MN[6], Schueler-Furman O[3], Wiener R[7]

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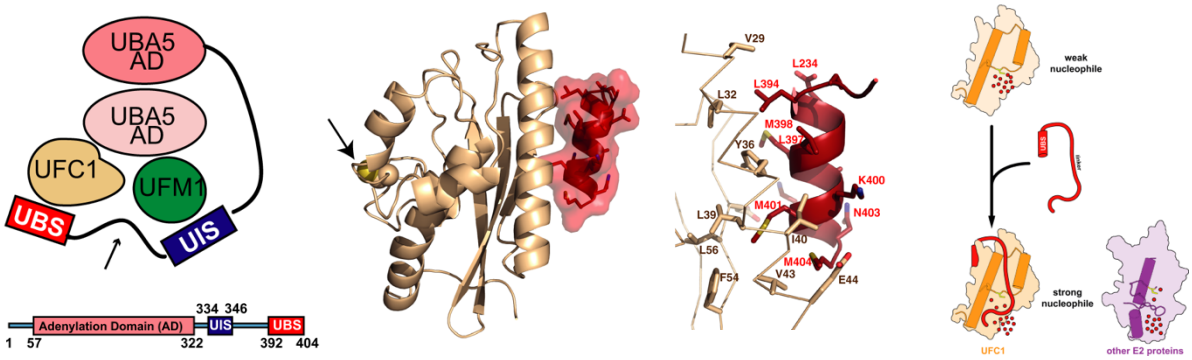
Ufmylation is a post-translational modification essential for regulating key cellular processes. A three-enzyme cascade involving E1, E2 and E3 is required for UFM1 attachment to target proteins. How UBA5 (E1) and UFC1 (E2) cooperatively activate and transfer UFM1 is still unclear. Here, we present the crystal structure of UFC1 bound to the C-terminus of UBA5, revealing how UBA5 interacts with UFC1 via a short linear sequence, not observed in other E1-E2 complexes. We find that UBA5 has a region outside the adenylation domain that is dispensable for UFC1 binding but critical for UFM1 transfer. This region moves next to UFC 1's active site Cys and compensates for a missing loop in UFC1, which exists in other E2s and is needed for the transfer.




We found the structural basis for how UBA5 gains its binding specificity to UFC1 via a short linear sequence located at the C-terminus. In parallel, UFC1 uses a previously unknown mechanism to regulate its nucleophilic activity. Specifically, UFC1 lacks a key structural element that is needed for its activity and is contributed by UBA5. Overall, our findings advance the understanding of UFM1's conjugation machinery and may serve as a basis for the development of ufmylation inhibitors.

**Published in Nat Commun. 12(2021), 5708.**
**PDB depositions 7nw1.**
**Crystallographic data were collected at ID23-1.**

# Will gepotidacin become the first novel antibiotic approved for use against gram-negative bacteria in the 21st century?

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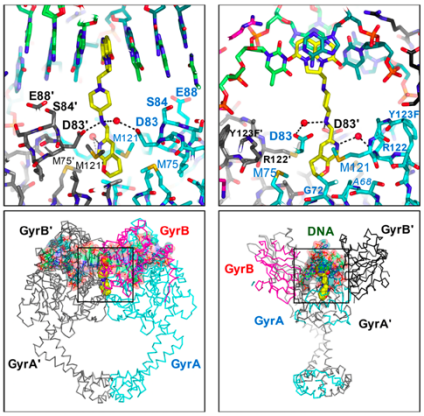
Antimicrobial resistance (AMR) remains a major risk and, according to a world health organization report in 2017, the top eleven most serious bacterial threats were all Gram-negative (G-ve). However, bringing new antibiotics to market is difficult and, between 2000 and 2019, only five new classes of antibacterial drugs successfully progressed through clinical trials to come to market, and these five were all approved for Gram-positive (G+ve) bacteria only[1,2]. Gepotidacin is a first in class novel bacterial topoisomerase inhibitor (NBTI), developed by GlaxoSmithKline, currently in two phase III clinical trials (the last phase before a drug comes to market), for two different types of Gram-negative bacterial infections[3,4].

The development of NBTIs within GlaxoSmithKline was supported by structural biology[5]. In a paper published in 2019[6] crystal structures of gepotidacin in complex with DNA and DNA gyrase were described (see figure). Gepotidacin was shown, like other NBTIs, to bind midway between the two, four base-pair staggered, DNA-cleavage sites in DNA gyrase and stabilizes single-stranded DNA-cleavage complexes.




**PDB depositions: 6qtk, 6qtp.**
**Publication: ACS Infect Dis 5 (2019), 570-58.**
**Crystallographic data collected by GlaxoSmithKline on ID23-1 and ID23-2.**

References

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