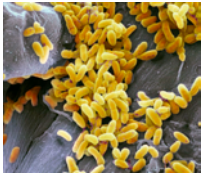


IDENTIFICATION, CHARACTERIZATION AND EXPLOITATION OF NOVEL GRAM-NEGATIVE DRUG TARGETS

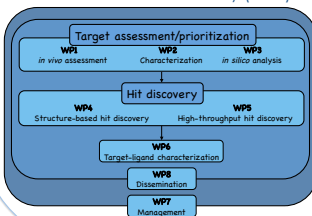
Vincent Rao, Richard Bickerton, Andrew Hopkins, William Hunter and the AEROPATH consortium
Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee, DD1 5E

THE AEROPATH CONSORTIUM



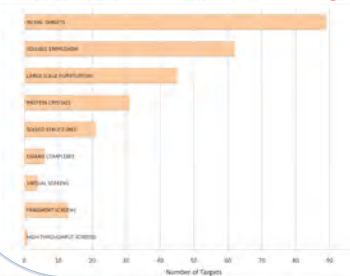
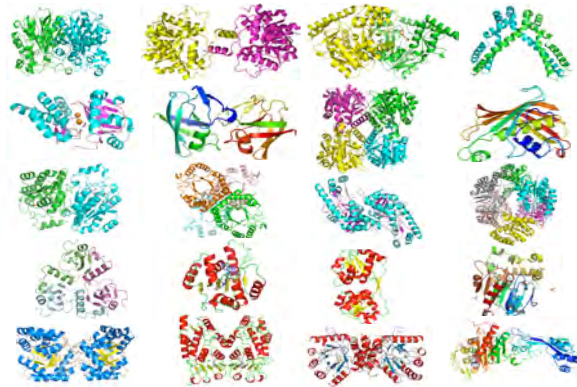
Pseudomonas aeruginosa is an important Gram-negative bacterial pathogen. It is of major clinical significance as a cause of pneumonia, septic shock, urinary tract and gastrointestinal infections that presents particular problems for Cystic Fibrosis patients and burn victims. It is increasingly exhibiting worrying antibiotic resistance.

The barren state of industrial development pipelines for new antibiotics against Gram-negative pathogens¹ prompted the European Union to fund the AEROPATH project €4.6 million over 4 years, more than half of which came to the UK. The AEROPATH consortium is led by the University of Dundee and also includes the University of St. Andrews, The Karolinska Institute in Sweden and two German-based biotech companies (Lionex and mfd Diagnostics). Much of the capacity of consortium members to contribute to AEROPATH is attributable to the BBSRC's previous investment in the Scottish Structural Proteomics Facility (SSPF).



Potential targets are identified by chemogenomic *in silico* analysis. Targets are validated by gene knockout and animal models before further characterization by biochemical and structural studies. High throughput, virtual and fragment screening approaches are used to identify active small molecule hit compounds before being further developed into leads.

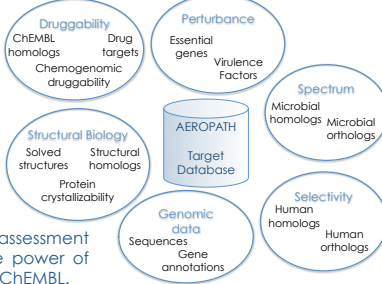
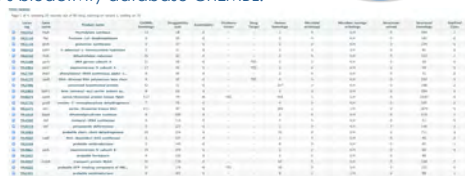
ACHIEVEMENTS TO DATE



In under two years the consortium has succeeded in solving the structures of more than 20 bacterial proteins, performed 13 fragment screens, 4 virtual screens and 1 high throughput screen. Further target and cell-based high throughput screens are already underway.

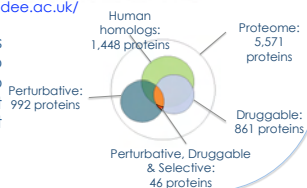
AEROPATH TARGET DATABASE

The AEROPATH Target Database aids the identification of potential drug targets from the genome of *P. aeruginosa*. Conventional biological criteria for target selection, including essentiality, selectivity, likely spectrum of activity and amenability to structural biology, are integrated with a novel chemistry-driven druggability assessment procedure that harnesses the power of the EBI's bioactivity database ChEMBL.

<http://aeropath.lifesci.dundee.ac.uk/>

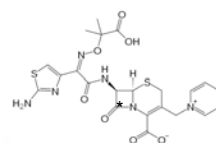
The complete bacterial genome is presented in a publicly accessible web framework that enables researchers to filter and sort genes by the relevant criteria to rapidly identify the most tractable targets in the genome.



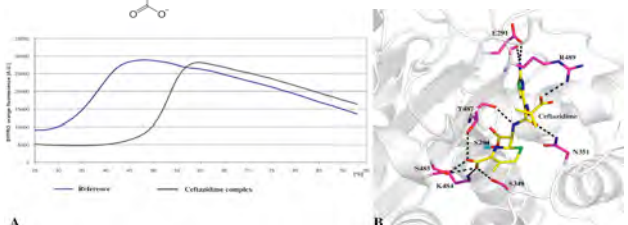
HIT DISCOVERY



In collaboration with the MRC-funded Oxford Protein Production Facility (OPPF), the first Penicillin-Binding Protein (PBP) from *P. aeruginosa*, PBP3, has been structurally characterized³. PBP3 is a high-molecular mass (HMM) protein involved in the cross-linking of muramyl peptides (transpeptidase activity). PBP3 has been identified as the primary target of a number of β -lactams used to treat pseudomonal infections, including the cephalosporin analogue ceftazidime⁴.



Chemical structure of ceftazidime. The * marks the position where the catalytic serine attacks the drug.



Binding of ceftazidime to PBP3 was confirmed using a thermal shift assay (A)^{5,6}. This assay indicated a significant increase in thermostability, with shifts in the midpoint transition temperature (ΔT_m) of 14.5 ± 0.1 °C following incubation with 0.5 mM ceftazidime. Ceftazidime covalently bound to the active site of PBP3 is shown (B). The side chain of the catalytic serine (S294) is highlighted in cyan making contact with the inhibitor.

Team Members

The lead investigators are Prof William Hunter from the University of Dundee, Dr Bernd Lecher and Dr Marko Maringer from mfd Diagnostics, Prof Jim Naismith from the University of St. Andrews, Dr Manavir Singh from Lionex and Prof Gunter Schneider from the Karolinska Institute. Staff in Dundee are Dr Richard Bickerton, Dr Ruth Brenk, Prof Julie Frearson, Dr Mary Gardiner, Dr David Gray, Prof Andrew Hopkins, Dr Daniel Muthas, Dr Stuart McIlroy, Vincent Rao, Dr Aurijit Sarker and Sharon Shepherd.

References

1. Drugs for bad bugs: confronting the challenges of antibacterial discovery. Payne et al. 2007
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3. Crystal Structures of Penicillin-Binding Protein 3 from *P. aeruginosa*: Comparison of Native & Antibiotic-Bound forms. Sainsbury et al. 2010
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6. Evaluation of fluorescence-based thermal shift assays for hit identification in drug discovery. Lo et al. 2004

Acknowledgements

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