

# What's been happening on the AEROPATH Project?

## January 2012 Update



Around one tenth of infections acquired during a hospital stay are caused by *Pseudomonas aeruginosa*.

This free-living bacterium is most commonly found in soil and water. However, it also occurs regularly on the surfaces of plants and occasionally on animals.

For most healthy people, this Gram-negative bacterium seldom poses a problem. Rather, *P. aeruginosa* is an opportunistic pathogen that infects humans with compromised natural defences. As a result, many *P. aeruginosa* infections occur after patients have been hospitalized. The bacterium causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bone and joint infections, gastrointestinal infections and a variety of systemic infections. *P. aeruginosa* infection is a serious problem for people whose immune system is compromised either due to transplant surgery, chemotherapy, people who have HIV/AIDS, who are recovering from burns and also young people with cystic fibrosis whose lungs struggle to cope with dangerous Gram-negative bacteria.

Infections caused by Gram-negative bacteria, often called 'superbugs', present a serious problem because these bacteria are tolerant to a wide variety of physical conditions, including temperature, and are resistant to high concentrations of salts and dyes, weak antiseptics and many commonly used antibiotics. They are also capable of acquiring resistance to many others, making treatment difficult.

'The identification, characterisation and exploitation of novel Gram-negative drug targets' (**AEROPATH**), a project funded under the EU's 7th Framework Programme (FP7), is taking a multidisciplinary approach aimed at promoting the development of relevant antimicrobial drugs. Researchers from the universities of Dundee and St Andrews in Scotland, the Karolinska Institutet in Sweden and two German-based SMEs (LIONEX and mfd Diagnostics) aim to better understand the biology of Gram-negative bacteria at a molecular level, using a *P. aeruginosa* model, and to characterise potential new drug targets. The overarching goal is to develop new compounds ('hits') that can bind to the target proteins and weaken or interfere with the bacterium's ability to cause infection. Information derived from these hits will inform on the potential of using the targets for drug discovery and indeed could provide useful starting points.

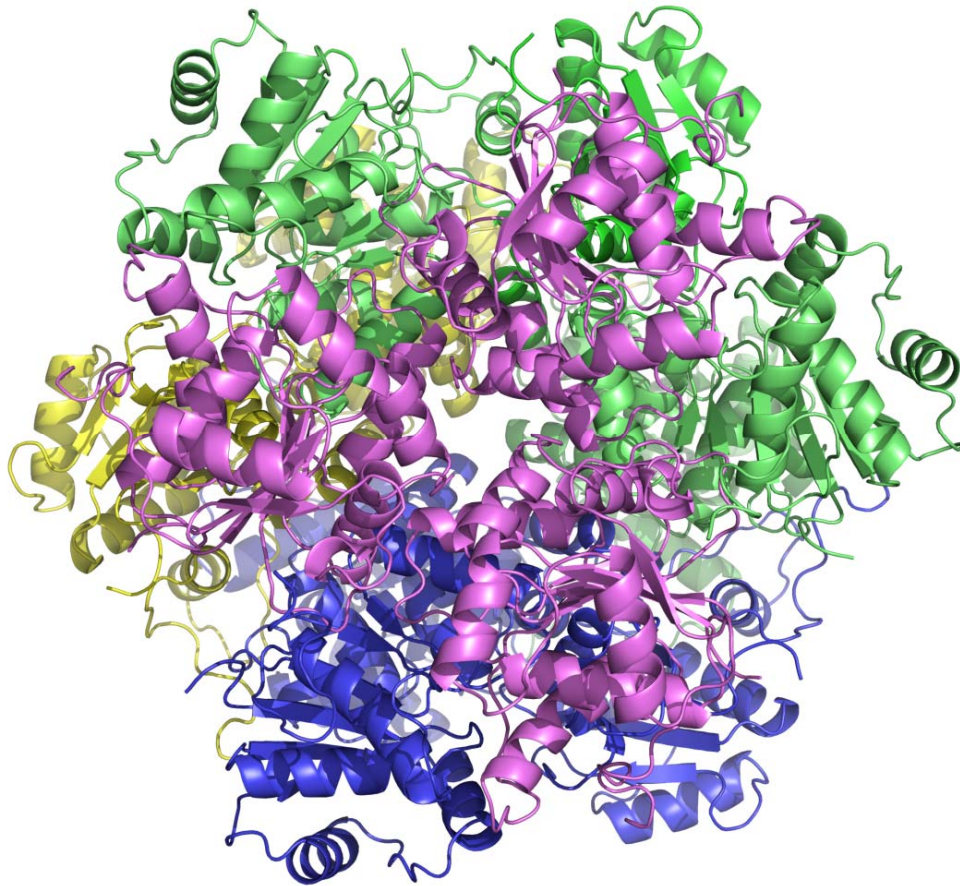


The AEROPATH research activity covers experimental validation and identification of novel targets, cloning, characterisation, biochemical and structural biology of these novel targets. Identification of hits is achieved by virtual screening (VS) and high-throughput screening (HTS), characterisation of interactions between the drug target and the inhibitor, and initial biological testing for efficacy against Gram-negative bacteria.

AEROPATH has used chemo and bioinformatics analyses to prioritise potential drug targets, and researchers are using initial results from work in the above areas to understand how essential proteins can be targeted. This information is crucial for future activities aimed at developing chemicals, the hits, which can disrupt a biological reaction and kill off bacteria.

Achievements in the first 3 years of the AEROPATH project include computer-based druggability assessment of over 5000 targets resulting in the release of the AEROPATH Target database, validation of 28 targets using gene knockout, cloning of more than 100 genes of prioritised targets and crystallisation trials for over 60 protein samples. Project partners have determined 30 crystal structures and assessed 20 targets in fragment screening assays, 8 in VS and 3 in HTS.

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*The structure of an aromatic acid decarboxylase from Pseudomonas. The structure of this dodecameric assembly was determined by partners at the Karolinska Institutet in Stockholm.*

A particular highlight was the derivation of the 3D molecular image of the penicillin binding protein PBP3 from *Pseudomonas aeruginosa*. Having this accurate 3D picture of the enzyme and knowing where molecules called inhibitors bind gives a clear understanding of the interactions that are involved in inhibiting this drug target. The structures identified suggest that there could be scope to develop new drugs that work in combination with existing PBP inhibitors to make them more effective and able to overcome resistance. The research, carried out in collaboration with Oxford University and the Oxford Protein Production Facility (OPPF), was published in the *Journal of Molecular Biology*<sup>1</sup>.

As the research continues, more detailed analyses will be performed on the PBP structure and on the others that have been determined. The details and binding properties of the screening campaigns' most promising hits will be further investigated.

A number of new targets have been proven essential and so become priorities for the screening methods in the final year of the project. The AEROPATH researchers are confident of their potential to underpin early-stage drug discovery for development of antibiotic drugs.

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<sup>1</sup>Sainsbury S, Bird L, Rao V, Shepherd SM, Stuart DI, Hunter WN, Owens RJ, Ren J, Crystal Structures of Penicillin-Binding Protein 3 from *Pseudomonas aeruginosa*: Comparison of Native and Antibiotic-Bound Forms, *J. Mol. Biol.* (2011) 405, 173-184