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The function of the aromatic acid transporter VanK

The Challenge

The aromatic acid:H+ symporters (AAHS) are a diverse and widespread transporter family that are responsible for the influx of aromatic acids into bacterial cells.

Aromatic acid transport could be exploited in bioremediation, microbial cell factories and in developing novel orthogonal components for synthetic biology. However, we currently lack the fundamental understanding of protein structure and function that will be required to support such applications.

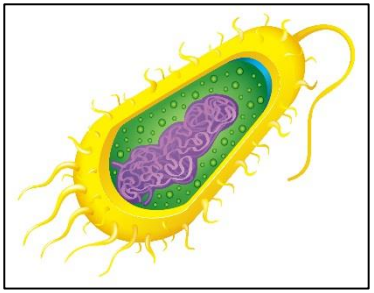
Can we develop in vitro methods to study the transport kinetics and substrate specificity of the AAHS proteins?

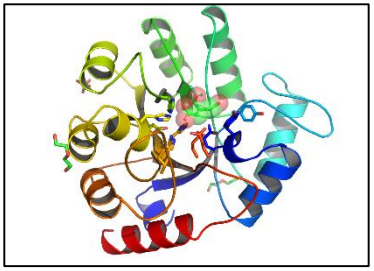
The Research

Dr Paul Curnow is a Senior Research Fellow at the University of Bristol. He researches mainly in to membrane proteins, biochemistry and 'environmentally friendly' cell-factories.

He applied for a CBMNet Vacation Scholarship which allowed a undergraduate student to be trained in practical techniques common across protein chemistry (microbial culture, IPTG induction, affinity purification, SDS-PAGE, Western blotting, gel filtration) as well as those specific to membrane transport proteins (detergent solubilization, reconstitution, substrate transport assays).

They purified the AAHS transporter VanK after recombinant expression in *E. coli*. The purified VanK was then reconstituted into synthetic proteoliposomes and fluorescence methods were used to assess substrate transport.





CBMNet Vacation Scholarship

The Result

The CBMNet Vacation Scholar showed that VanK could be expressed into *E. coli* membranes, solubilized in a surfactant and purified to homogeneity.

They showed that VanK was an alpha-helical membrane protein that formed a relatively stable homotrimer in surfactant micelles. For functional assays, the Scholar adopted a popular method that used the pH responsive dye pyranine to track the cotransport of H^+.

The student did observe differences in transport data between the full treatment and control samples, although the effect was subtle.

The Future

This was a basic research project that provided excellent training for the CBMNet Vacation Scholar in studying membrane transport proteins *in vitro*.

This included generating publication-quality data that the host lab can build upon in the future.

For example, we are now targeting a specific industrial application and will bid for CBMNet proof-of-concept funding to enable this.

The student is aiming to undertake a PhD studying membrane transport proteins.

"The CBMNet funding was a great way for my lab to explore a new area of research. The success of the project gives us convincing data that we can now use to engage industrial partners within the network."

Dr Paul Curnow, University of Bristol

"Working independently in this exiting area of biochemistry was an incredibly rewarding experience. This project confirmed my ambition for a career in science, starting with a PhD in membrane protein biochemistry after graduation."

Grant Pellowe, CBMNet vacation scholar

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