Warwick and JEOL Strike Gold in Electron Microscopy Collaboration

Escherichia coli is a popular system for the production of recombinant proteins, but little is known about the distribution and shape of structural elements of *E. coli* that drive protein expression and export to the periplasm. To investigate this, Corinne Smith from University of Warwick and colleagues used business interaction voucher funding from BioProNET to collaborate with electron microscope specialist JEOL UK.

The collaboration drew on JEOL's expertise in zero-loss cryo-electron tomography and direct electron detection to investigate the export of human growth hormone by the twin-arginine translocation (TAT) system in *E. coli*. This system is responsible for the export of fully folded proteins — endogenous and recombinant — from the cytoplasm, across the inner membrane and into the periplasm.

"A better understanding of protein export by the TAT system will facilitate better bioprocessing technologies," says Corinne.

After first using biochemical studies to show that human growth hormone was exported to the periplasm by the TAT machinery, the collaborators then optimised an immunogold labelling procedure to unambiguously identify human growth hormone in *E. coli*.

Electron microscopy data of immunogold-labelled growth hormone showed that a proportion of the protein forms inclusion bodies in the cytoplasm, meaning that it cannot be exported and so would affect the yield of protein. The growth hormone that was available for export at the cytoplasmic membrane was randomly distributed throughout membrane, and did not appear to effect the membrane structure.

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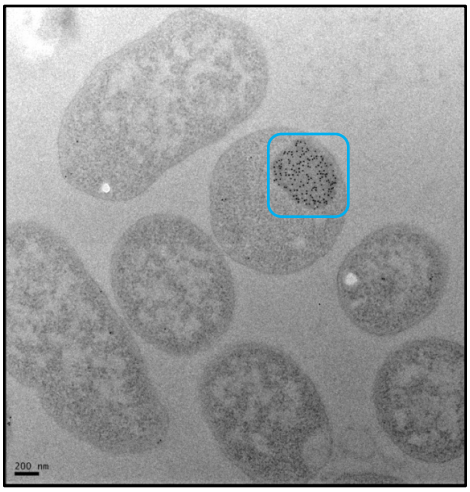
Sarah Smith, who undertook the experimental work, gained valuable new skills. "This project gave me training in difficult electron microscopy techniques such as imaging of resin-embedded *E. coli* and electron tomography sections, as well experience of automated image acquisition software, which together which enabled us to gain high resolution data."

Sarah also showed that a mutant form of growth hormone that cannot be processed for export was randomly distributed in the inner membrane without affecting membrane structure. "In principle this represents a novel way of displaying a protein on the periplasmic face of the *E. coli* inner membrane, which could have applicability in library screening, protein engineering or whole cell biocatalysts", she notes.

Moving forward, Corinne and Sarah are collaborating with colleagues at University College London to quantify how much human growth hormone can be made by the system, and hope to combine data with results from this study to publish as a paper on a new method of producing proteins in *E. coli*.

"This successful project established a working relationship between JEOL and scientists from the University of Warwick, which will be a catalyst for future electron microscopy-based research projects," concludes Andrew Yarwood from JEOL.



Immunolabelling experiments confirmed the formation of inclusion bodies in *E. coli* upon overexpression of recombinant human growth hormone. Scale bar = 200 nm.