PoC study shows protein synthesis errors can cause activity losses in recombinant protein

Proof of concept funding from BioProNET has enabled Tobias von der Haar from the University of Kent and his collaborators to develop a new way of determining the accuracy of protein synthesis. In addition, they were able to use this new technique to show that minor inaccuracies in translation – such as amino acid substitutions – can affect the activity of a recombinant protein.

Cells can be reprogrammed to make many types of recombinant proteins, but this creates additional demand on the cellular protein synthesis machinery that could lead to a decrease in the accuracy of translation and mean that resultant proteins contain more errors compared to endogenous proteins in normal cells. This in turn could lead to changes in the efficacy, bioavailability and immunogenicity of therapeutic and diagnostic proteins.

Working with Cobra Biologics and MRC Technology, Tobias and colleagues sought to establish what effects a loss of translation optimization and decreased protein synthesis accuracy had on the resultant protein. First they developed a new computational tool to generate a database of all possible single-amino acid substitutions in a recombinant protein, as well as LC-MS protocols for analysing mis-incorporated amino acids in a peptide sequence. These tools were then used to analyse recombinant proteins produced in yeast and *E. coli* – two popular bioprocessing hosts.

The tools could detect minor variations in the amino acid sequence. "The sequence variations would have escaped detection with standard mass spectrometry approaches, but can be reliably visualised using our novel approach," says Lyne Jossé, who carried out the experimental work.

Many of the observed substitutions were shown to be the result of specific biological mechanisms, such as non-optimal codon usage, that generate specific, predictable translational errors. Interestingly, many other observed errors were universal, occurring in all peptide sequences that were tested from both yeast and *E. coli*. The source of these latter errors is currently not well understood.

A key aspect of this study was the demonstration that errors in protein synthesis can affect the properties of the resultant protein. Surprisingly, a protein translated from a non-codon-optimised DNA sequence had only about 60% of the specific enzymatic activity of the same protein produced from a codon-optimised DNA sequence in *E. coli* (but this was not true for yeast). "To our knowledge, this is the first direct demonstration of DNA sequence-dependent activity differences," highlights Tobias.

"The collaboration has significantly increased our understanding of the potential issues relating to the production of heterologous proteins in *E.coli*," says Steve Williams from Cobra Biologics. The study also seeded opportunities for further work – Tobias intents to apply for further funding to investigate the biological mechanisms that cause the observed amino acid substitutions.

"To our knowledge, this is the first direct demonstration of DNA sequence-dependent activity differences"