Collaboration creates a recipe for success in cell-free protein synthesis

Proof of concept funding from BioProNET has allowed Karen Polizzi and Rochelle Aw from Imperial College London to work with Fufifilm Diosynth Biotechnologies on a project that tested if cellular extracts from the yeast *Pichia pastoris* could be used to synthesise proteins.

Protein-based drugs are often synthesised in whole cells. However, the use of cell-free protein synthesis systems — that is, the cell's internal machinery in the absence of the cell wall — has several potential advantages. Compared to whole cell synthesis, this method allows for quicker synthesis, enables the production of proteins that are toxic to living cells and can can be scaled to large volumes more easily.

Currently, cell-free protein synthesis extracts from yeast are not commercially available. "This project has proved the concept that *P. pastoris* can be used for cell-free protein expression", says Ian Hodgson from Fujifilm. "To our knowledge is the first time this has been done."

As a test system, the scientists investigated the synthesis of green fluorescent protein (GFP) and luciferase. The initial phases of the project determined the best way to lyse yeast cells to release the optimum amount of cellular machinery, and developed a recipe to stabilise RNA transcripts and increase the yield of RNA encoding for the reporter proteins.

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The main phase of the project showed evidence of combined transcription and translation in the extract from the yeast cells. "The most important outcome of the work was that we were able to generate a working cell-free protein synthesis extract from *P. pastoris*", says Karen.

The final titres of GFP and luciferase observed were similar to that observed with a cell extract from another strain of yeast, *Saccharomyces cerevisiæ,* using the same protocol. However, the protein synthesis reaction had a much longer lag phase, and despite initial evidence that the cell-free system was functional, yields of protein were low.

"The project has given us a strong basis to further build upon the results," highlights Karen. "Optimisation will be key to maximising the productivity of the system."

In addition, the project has benefited the industrial partner. "The project has also allowed Fujifilm to understand some of the factors that would be important in utilising cell-free extracts for commercial use."

As a next step, Imperial and Fujifilm hope to continue their collaboration by focusing on the production of a more complex, industrially relevant proteins with the *P. pastoris* system.