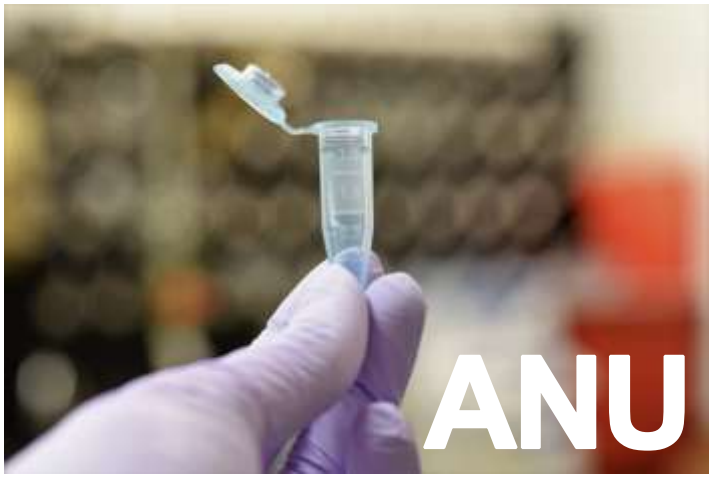
Australian National University

TECHNICAL SUMMARY

Efficient and cost-effective method for cell-free protein synthesis



Recombinant protein production is prevalent in two systems; an in-vivo (living) or in-vitro (non-living) system. Oftentimes, Escherichia coli (E. coli) is used as a model organism for large scale production of protein using both systems. In-vitro protein synthesis or Cell-Free Protein Synthesis (CFPS) is the functional use of biological transcription/translation processes without the use of living cells. It includes the use of a lysate that holds all the necessary macromolecules that facilitate energy production, transcription, translation and protein folding. Unlike in-vivo systems, in-vitro protein synthesis is not constrained by a cell wall or homeostatic conditions. This enables direct access and chemical control of the expression environment. Toxins, biopharmaceutical proteins and membrane proteins can easily be expressed in CFPS with high yields, all without the energy consuming ancillary processes of the cell system. The relative speed and ease that CFPS imparts is one of the major advantages that is associated.As appealing as CFPS sounds, there are problems surrounding lysate preparation for CFPS as cell extracts have issues with consistent activity between batches. In addition, preparation of cell lysate is time-consuming and expensive. As a result, there exists a need for improved methods of CFPS.

Technology (TT2018-011)

Researchers from The Australian National University, have overcome these limitations by developing a novel method for efficiently producing capsules based on the lysate of E. coli. This facile method for CFPS utilizes encapsulated cell-like structures (eCells) which maintain many of the advantages of living cells while at the same time allow full control over the environment, like in CFPS. Established studies utilising eCells have shown that they are competitive (to traditional CFPS) and large amounts can be produced faster. The nano-size of the technology further allows experimenters to demarcate unique protein features in single capsules and sort them from the existing populations in large libraries DOI: 10.1021/acssynbio.1c00044

Potential benefits

> **Pioneering:** The first cost effective and easy to produce capsule based in-vitro expression system.
> **Flexible:** significant data exists on the use of eCells in a variety of disciplines ranging from NMR studies, to biocatalysis of precursor drug compounds.
> **Environmentally impactful:** substantial data on the decontamination of herbicides/ sensing of heavy metals with no secondary biological contamination.
> **Convenient:** eCells are easily customized for target protein expression, enzyme encapsulation and marker protein expression
> **Stable:** eCells can hold and protect proteins that are prone to denaturation and are considered non-living entities which pose no risk of secondary contamination.
> **Inexpensive:** standard CFPS performed using expensive reagents and cell lysate preparation. Using eCells reduces the cost of in-vitro protein production dramatically.
> **Efficient:** eCell production is efficient (i.e. there is no need to express and then purify supplementary proteins and eCell production requires limited use of reagents)
> **Scalable:** rapid, easy generation of capsules, that can be scaled up to industrial scale and reduced to save reagent cost.
> **User friendly:** simple, cost-effective capsule preparation requiring minimal lab equipment/training.
> **Portable:** eCell production is efficient (i.e. there is no need to express and then purify supplementary proteins and eCell production requires limited use of reagents)

Potential applications

> Protein synthesis
> Protein expression
> Recombinant protein therapeutics
> Recombinant protein-based vaccines
> Antigen specific therapeutic peptides
> Veterinary and biochemical research
> Genomic research (recombinant and native enzymes development)
> Environmental and catalytic biotechnology
> Bioassays/Bioprocessing/Biosensors
> Biomolecule analysis
> Environmental sensing (Bioremediation and Biosensing)
> Nanotechnology
> Cell therapy
> Isotope labelling
> Enzymatic biosynthesis
> Synthetic biology

Opportunity

ANU is seeking an option/license arrangement with a commercial R&D collaboration, wherein the technology is further developed via a collaboration with an industry partner, in return for an end option/license to the technology. ANU is able to facilitate the leverage of commercial research grant schemes in Australia with commercial partners

IP status

The principles behind the eCells technology are owned by ANU and can be accessed via the publication number WO/2020/150789 (International application number PCT/AU2020/050050).

https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2020150789

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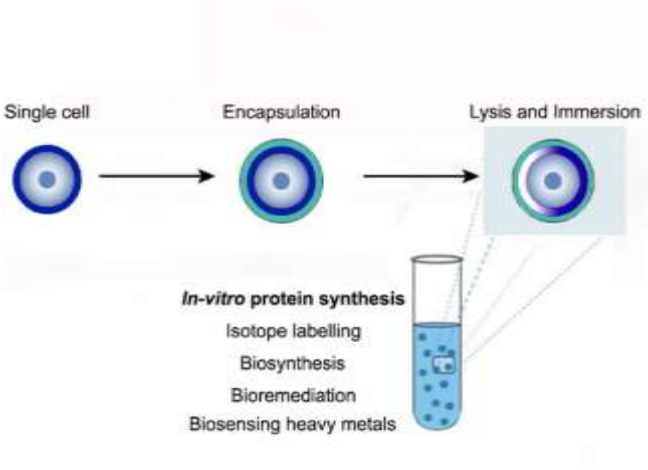

Figure 1: Pictorial representation of how the eCell technology works