

## Application Note

# How PURE is your cell free protein synthesis: We call it cell free, but exceptional quality protein extraction is required at the outset to properly kick start the process in vitro!

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PURE systems demand purified components for translation such as tRNA synthetases, initiation / elongation / termination factors, energy regeneration components, and even if a few key enzymes are damaged, translation stops! The construction of a self-regenerating synthetic cell represents a central goal in synthetic biology. A landmark study by Matthaeus Schwarz-Schilling et al in Nature Communication, "*Autonomous biogenesis of all thirty proteins of the Escherichia coli translation machinery*" demonstrates the simultaneous synthesis and functional regeneration of all **30 translation proteins** in a reconstituted transcription-translation system. This autonomous biogenesis required exceptionally high-quality, functional translation components and extract preparation – made possible through robust upstream processing enabled by the Constant Systems platform.

Reconstituting a self-regenerating translation system demands:

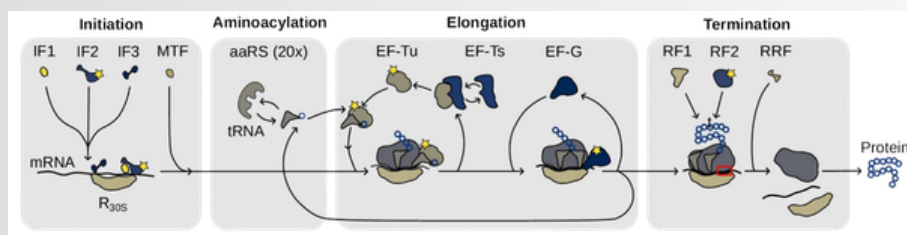
- Fully functional ribosomal and translation-associated proteins
- Preserved enzymatic activity
- Accurate threshold concentration control
- Reproducible transcription-translation extracts
- Stability for surface-based compartmentalized reactions



The authors first demonstrated that each translation protein could regenerate itself:

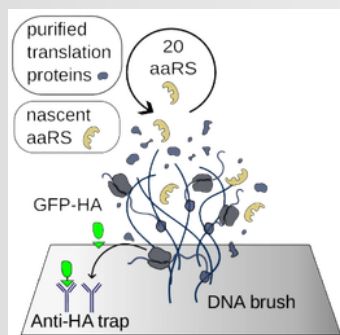
**"We first establish self-regeneration of every translation protein by determining the threshold concentration required for its own synthesis... thereby demonstrating the functionality of all nascent proteins."**

Such precision requires intact translational machinery from the earliest preparation steps.



### Ribosome purification

Ribosomes were purified from the E. coli strain A19 (no antibiotics). The pelleted cells were lysed using a French press Constant Systems Cell Disruptor, 12,000 PSI 4°C



### Scheme:

DNA brush immobilized on a surface localizing the purified (dark blue) translation proteins and nascent (yellow) aaRS (aminoacyl-tRNA synthetases)

### How the Constant Systems Platform Enabled Progress

The Constant Systems platform supported the preparation of high-quality Escherichia coli-derived components, critical for cell-free transcription-translation systems, efficiently and without damaging the proteins. The platform employs fixed orifice cell disruption and critically important, instant and active cooling of these very valuable biomolecules

## About The Continuous Flow Cell Disruptor

The CF Cell Disruptor offers Continuous Flow Processing with two models, the CF1 and CF2. Both models offer the same process and differ only by process speed.

The CF1 offers up to 6L per hour and the CF2 offers up to 24L per hour processing rates.

Both models benefit from a HMI control, a maximum process pressure of 40kpsi (2700 bar), integrated sample cooling jacket, 200mL inlet reservoir (hopper), auto shut down feature for when the process is complete and both models take advantage of Constant Systems precise and consistent hydraulic control which is fully scaleable through the whole product range from 0.5mL single preparation processing to large volume processing at 150L per hour. This precise control ensures that the entire sample is processed at the operators set pressure to ensure accurate and consistent results and in most cases a single process or pass is adequate meaning multiple passes are not required. Both models are capable of processing fluid or re-suspended sample types and are utilised for many sample types such as bacteria, yeast, algae and mammalian / Insect cell types.

### CF1 Model

At approximately 700mm<sup>2</sup> the CF1 is small enough to bench mount if required but is best suited when used on its tailor made trolley. The CF1 trolley ensures that the equipment is situated at its optimum working height and is mounted on full swivel and lockable casters that enables safe and free movement of the equipment when needed. The CF1 is recommended for process volumes in the range of 15mL through to 10L. For those processing larger volumes in this range the CF1 can be offered with an integrated peristaltic pump fully controlled through the HMI which will ensure that the inlet reservoir is continually fed whilst recirculating the sample to help avoid any settling until the entire sample is processed.

### CF2 Model

With a footprint of approx. 700mm<sup>2</sup> the CF2 is a floor standing model that is recommended for process volumes from 15mL through to 100L. The CF2 is offered with an integrated peristaltic pump as standard. The CF2 is mounted on full swivel and lockable castors that enables safe and free movement of the equipment when needed.



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