

From DNA to Protein in under 4 hours using Automated Cell-Free Protein Synthesis

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Understanding biomolecular structures, physicochemical properties, and their functional implications requires the production of high-quality biomolecules. Cell-free protein synthesis (CFPS) has emerged as a powerful technique for the on-demand production of challenging biomolecules by harnessing cellular transcriptional and translational machinery *in vitro*. CFPS is a robust and flexible platform with strong potential to overcome limitations associated with existing *in vivo* biotherapeutic production methods.

The CFPS platform at the Swedish NMR Centre plays an integral role in Protein Production Sweden (PPS), a national research infrastructure dedicated to generating high-quality protein samples. The platform provides a versatile toolbox for rapid and cost-effective *in vitro* protein synthesis, enabling the production of diverse protein samples, including soluble, membrane-bound, and toxic proteins, from plasmid templates using isolated bacterial (*E. coli*) translation machinery. NMR-optimized isotopic labeling of proteins through the CFPS protocol, followed by NMR data acquisition and analysis, is also available.

This poster describes the cell-free protein synthesis capabilities available at the Swedish NMR Centre, University of Gothenburg. Our fully automated workflow enables rapid, flexible, and scalable CFPS, accelerating the path from DNA to purified protein. Using Obi-Wan, an *Opentrons Labworks Inc. OT-2* liquid-handling robot, CFPS reactions are assembled in a plate-based format with high precision and reproducibility. This setup supports anything from a single construct to 96 or 384 unique reactions in parallel, making it ideal for expression screening and small-scale protein production. Following expression, Boba, an *Agilent Technologies Bravo* liquid-handling platform, performs automated affinity tag-based protein purification using Biotage PhyTips®. This seamless integration of expression and purification ensures consistent handling, minimal hands-on time, and rapid turnaround. The entire workflow, from plasmid DNA to purified protein, is completed in less than four hours, enabling same-day decision-making and iterative experimental design.

This methodology is available to local and external researchers upon request through PPS projects.