A robust, rapid, and scalable cell free expression system for the production and engineering of Fab antibodies

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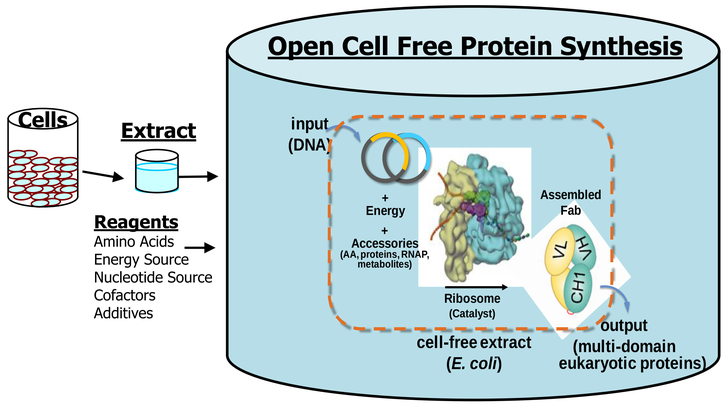
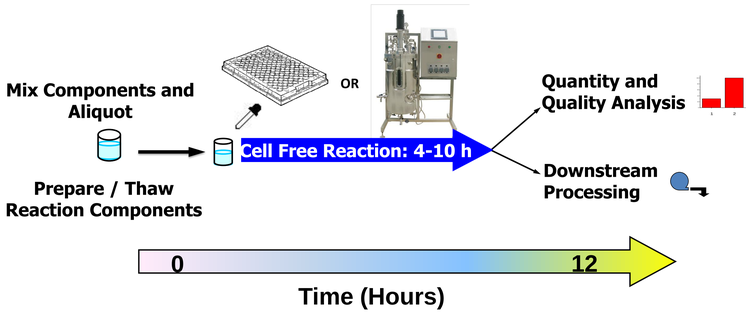
Abstract: Open Cell Free Synthesis (OCFS) is a rapid and scalable protein engineering and expression platform that alleviates challenges generally faced in traditional cell-based expression systems. Here we present an *Escherichia coli*-based OCFS to rapidly produce functionally assembled Fab antibodies to human receptor IL-13-α1 from microtiter plates to 5L bioreactors at approximately 300 mg/L in 8 hours. A high throughput label-free ForteBio antigen binding assay was developed to quantitate functionally assembled Fab directly from OCFS crude reactions. We combinatorially optimized heterodimeric Fab assembly based on the ratio, timing of addition, and absolute amounts of HC and LC genes added in the OCFS. Sutro is actively working on extending OCFS processes to cGMP manufacturing of proteins with the ability to site-specifically incorporate novel non-natural amino acids.

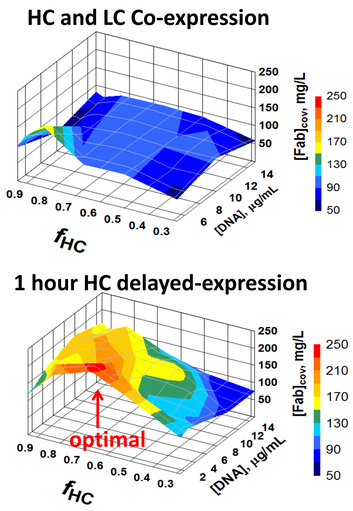


Rapid product and process development



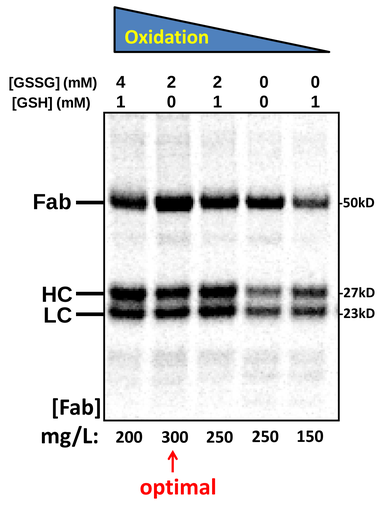
Delaying expression of HC significantly improves Fab production. Optimizing cell-free redox conditions via glutathione addition facilitates better Fab assembly.

Production as a function of DNA ratio and concentration



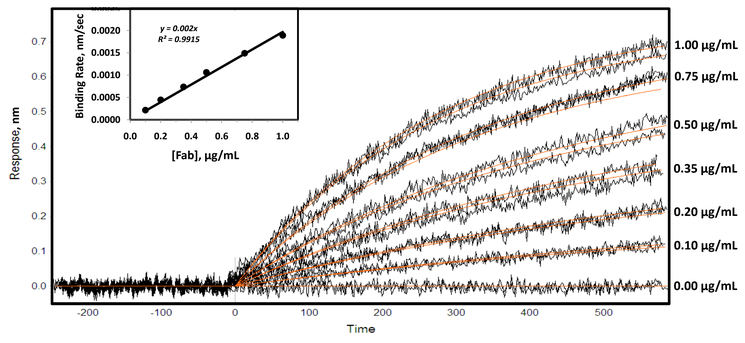
^{14}C-Leu Scintillation Assay

Assembly under various glutathione ratios



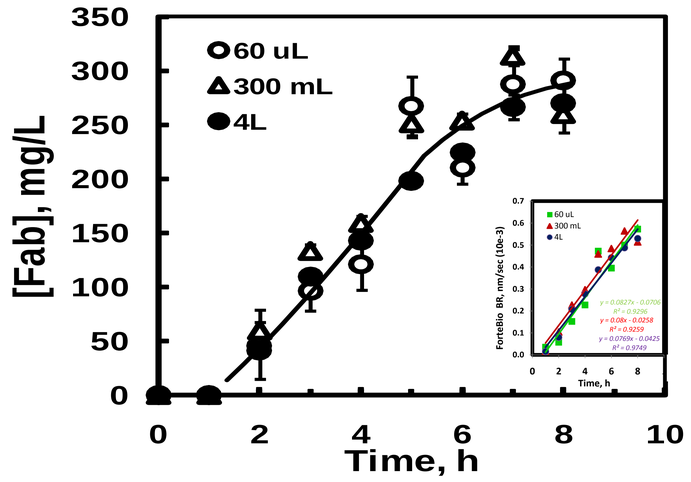
^{14}C-Leu Autoradiogram

Label-free ForteBio assay quantitates functionally assembled Fab direct from crude cell free reaction using biosensor-captured human IL-13Rα1.

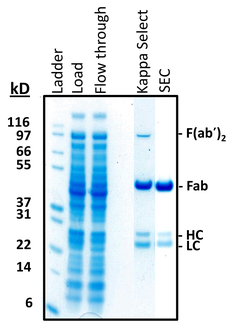


Fab standards in 1/100 cell free matrix

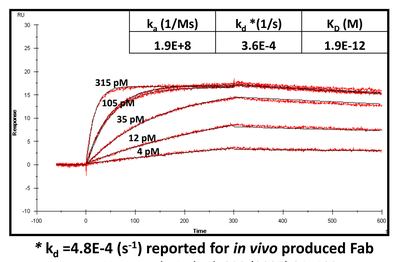
Fab-optimized reaction produced ~300 mg/L of functionally active Fab antibodies in 8 hours as measured by ForteBio anti-hu-IL-13Rα1 binding. Expression is linearly scalable.



Fab was subsequently purified by two column steps and exhibited tight binding kinetics.



Fab Purification



* k_d =4.8E-4 (s^{-1}) reported for *in vivo* produced Fab
J Immunol *Methods* 322 (2007) 94–103

Biacore analysis of purified Fab

Summary

- Fab antibody production in **OCFS** expression system is rapid and linearly scalable.
- The flexibility of **OCFS** system allows engineering of Fab assembly by manipulating: ratios of heavy and light chain plasmid, timing of plasmid addition, and cell free redox conditions via glutathione.
- **OCFS** is a robust and rapid expression system for the production and assembly of functional Fab antibodies.