Cell-Free Protein Synthesis: Applications Come of Age

Erik D. Carlson[1,2,*], Rui Gan[1,2,*], C. Eric Hodgman[1,2], and Michael C. Jewett[1,2,3,†]

[1]Department of Chemical and Biological Engineering
[2]Chemistry of Life Processes Institute
[3]Member, Robert H. Lurie Comprehensive Cancer Center
Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, USA

*These authors contributed equally
†Correspondence to m-jewett@northwestern.edu

Supplementary Information

1. Supplementary Tables

Supplementary Table 1 – Historical Trends of Cell-Free Protein Synthesis Systems

Figure 3a – Protein Yield (Batch)

Year	*System*	*Yields (mg/mL)*	*Protein Expressed*	*Citation*
1996	ECE	0.1	chloramphenicol acetyltransferase	(Kim *et al.*, 1996)
1999	ECE	0.15	chloramphenicol acetyltransferase	(Kim and Swartz, 1999)
2000	WGE	0.6	tobacco mosaic virus	(Madin *et al.*, 2000)
2001	ECE	0.52	chloramphenicol acetyltransferase	(Kim and Swartz, 2001)
2004	ECE	0.7	chloramphenicol acetyltransferase	(Jewett and Swartz, 2004a)
2004	ECE	0.92	chloramphenicol acetyltransferase	(Chumpolkulwong *et al.*, 2004)
2005	ECE	0.56	chloramphenicol acetyltransferase	(Voloshin and Swartz, 2005)
2005	ECE	0.7	chloramphenicol acetyltransferase	(Calhoun and Swartz, 2005a)
2008	ECE	1.2	chloramphenicol acetyltransferase	(Jewett and Swartz, 2004a)
2010	ICE	0.05	β-galactosidase	(Ezure *et al.*, 2010)
2010	RRL	0.006	firefly luciferase	(Promega_http://www.promega.com/resources/articles/pubhub/enotes/optimize-your-tnt-reticulocyte-lysate-systems-reaction/)
2011	ECE	1.7	chloramphenicol acetyltransferase	(Kim *et al.*, 2011)

Figure 3b – Protein Yield (Fed-Batch/CECF)

Year	*System*	*Reaction Format*	*Effective Yield (mg/mL)**	*Protein Expressed*	*References*
1988	ECE	CECF	0.002	calcitonin polypeptide	(Spirin *et al.*, 1988)
1999	ECE	Semi-continuous	0.55	chloramphenicol acetyltransferase	(Kigawa *et al.*, 1999)
2004	ECE	Semi-continuous	0.08	enhanced green fluorescent protein	(Noireaux and Libchaber, 2004)
2004	ECE	Fed-batch	1.4	chloramphenicol acetyltransferase	(Jewett and Swartz, 2004b)
2005	ECE	Semi-continuous	0.4	G-protein coupled receptor	(Klammt *et al.*, 2005)
2000	WGE	Semi-continuous	0.36	dihydrofolate reductase	(Madin *et al.*, 2000)
2002	WGE	Semi-continuous	2.19	ζ-crystallin quinone reductase	(Sawasaki *et al.*, 2002b)
2002	WGE	Semi-continuous (bilayer)	1.62	green fluorescent protein	(Sawasaki *et al.*, 2002a), (Endo and Sawasaki, 2006)
2004	WGE	Semi-continuous (bilayer)	0.03	protein kinase library from *Arabidopsis thaliana*	(Sawasaki *et al.*, 2004)
2008	WGE	Semi-continuous (bilayer)	0.002	average of 13,364 human proteins	(Goshima *et al.*, 2008)
2009	WGE	Semi-continuous	0.09	renilla luciferase	(Park *et al.*, 2009)

**Volume taken as final volume for fed-batch reactions, or total volume (reaction + feed solution) for CECF reactions*

Figure 3c – Active Reaction Duration (Batch)

Year	*System*	*Active Reaction Time (hr)*	*Protein Expressed*	*Citation*
1996	ECE	1	chloramphenicol acetyltransferase	(Kim *et al.*, 1996)
1999	ECE	2	chloramphenicol acetyltransferase	(Kim and Swartz, 1999)
2000	WGE	4	dihydrofolate reductase	(Madin *et al.*, 2000)
2001	ECE	2	chloramphenicol acetyltransferase	(Kim and Swartz, 2001)
2004	ECE	6	chloramphenicol acetyltransferase	(Jewett and Swartz, 2004a)
2005	ECE	4	chloramphenicol acetyltransferase	(Voloshin and Swartz, 2005)
2005	ECE	3	chloramphenicol acetyltransferase	(Calhoun and Swartz, 2005b)
2008	ECE	8	chloramphenicol acetyltransferase	(Jewett *et al.*, 2008)
2011	ECE	12	chloramphenicol acetyltransferase	(Kim *et al.*, 2011)

Figure 3d – Protein Synthesis Rate

Year	*System*	*Reaction Format*	*Yield (mg/mL-hr)*	*Protein Expressed*	*Citation*
1988	ECE	Batch	0.0043	calcitonin polypeptide	(Spirin *et al.*, 1988)
2000	WGE	Semi-continuous	0.008*	dihydrofolate reductase	(Madin *et al.*, 2000)
2001	ECE	Batch	0.3	chloramphenicol acetyltransferase	(Kim and Swartz, 2001)
2004	ECE	Batch	0.37	chloramphenicol acetyltransferase	(Jewett and Swartz, 2004a)
2008	ECE	Batch	0.6	chloramphenicol acetyltransferase	(Jewett *et al.*, 2008)
2011	ECE	Batch	0.7	chloramphenicol acetyltransferase	(Kim *et al.*, 2011)

**Volume taken as final volume for fed-batch reactions, or total volume (reaction + feed solution) for CECF reactions*

Figure 3e – Yield (mg/$)†

Year	*System*	*Reaction Format*	*Protein Expressed*	*Yield (mg /$ substrate)*	*Yields (mg/mL)**	*Citation*
1988	ECE WGE	Continuous Exchange	calcetonin polypeptide	0.005	0.002	(Spirin *et al.*, 1988)
2000	ECE	Fed-batch	chloramphenicol acetyltransferase	0.002	0.42	(Kim and Swartz, 2000)
2000	WGE	Batch	dihydrofolate reductase	0.543	0.36	(Madin *et al.*, 2000)
2002	WGE	Semi-continuous (Bilayer)	green fluorescent protein	1.835	1.6	(Sawasaki *et al.*, 2002a), (Endo and Sawasaki, 2006)
2004	ECE	Batch	chloramphenicol acetyltransferase	1.200	0.7	(Jewett and Swartz, 2004a)
2005	ECE	Batch	chloramphenicol acetyltransferase	0.237	0.56	(Voloshin and Swartz, 2005)
2005	ECE	Batch	chloramphenicol acetyltransferase	18.287	0.68	(Calhoun and Swartz, 2005a)
2008	ECE	Batch CSTR (Cytomim)	chloramphenicol acetyltransferase	9.554	0.62	(Jewett *et al.*, 2008)
2008	WGE	Semi-continuous (Bilayer)	average of 13,364 human proteins	0.002	0.002	(Goshima *et al.*, 2008)
2009	ECE	Batch	esterase 2	0.059	0.28	(Wang and Zhang, 2009)
2011	ECE	Batch CSTR	human granulocyte macrophage colony-stimulating factor	10.439	0.7	(Zawada *et al.*, 2011)
2011	ECE	Batch	chloramphenicol acetyltransferase	3.056	1.7	(Kim *et al.*, 2011)

**Volume taken as final volume for fed-batch reactions, or total volume (reaction + feed solution) for CECF reactions*

†*Yield based on mg of protein synthesized per $ nucleotides (i.e., ATP, ADP, AMP, GTP, etc.), secondary energy source (i.e., phopshoenolpyruvate, creatine phosphate, sodium pyruvate, glucose, etc.), and enzymes necessary for energy regeneration (i.e., pyruvate kinase, creatine phosphokinase, etc.). Prices for products were all determine from http://www.sigmaaldrich.com/united-states.html last accessed August 5th, 2011.*

Figure 3f – Reaction Volume (Batch)

Year	*System*	*Protein Expressed*	*Scale (L)*	*Yields (mg/mL)*	*Citation*
1996	ECE	chloramphenicol acetyltransferase	2E-5	0.1	(Kim *et al.*, 1996)
1999	ECE	chloramphenicol acetyltransferase	6E-5	0.15	(Kim and Swartz, 1999)
2001	ECE	chloramphenicol acetyltransferase	1E-4	0.23	(Kim and Swartz, 2001)
2005	ECE	chloramphenicol acetyltransferase	5E-4	0.56	(Voloshin and Swartz, 2005)
2008	ECE	chloramphenicol acetyltransferase	2E-3	1.2	(Jewett *et al.*, 2008)
2011	ECE	human Granulocyte macrophage colony-stimulating factor	1E+2	0.7	(Zawada *et al.*, 2011)

Supplementary Table 2 – CFPS advancement timeline

Figure 4 – CFPS advancement timeline

Year	*System*	*Protein Expressed*	*Yield (mg/mL)*	*Disulfide Bonds*	*Citation*
2002	WGE	ζ-crystalline quinone reductase	2.2		(Sawasaki *et al.*, 2002b)
2003	WGE	single-chain antibody variable fragment against Salmonella O-antigen	0.013	2	(Kawasaki *et al.*, 2003)
2004	ECE	urokinase protease	0.04	6	(Kim and Swartz, 2004)
2004	ECE	variant of human tissue-type plasminogen activator	0.06	9	(Yin and Swartz, 2004)
2005	ECE	granulocyte macrophage colony-stimulating factor	0.043	2	(Yang *et al.*, 2005)
2006	ECE	insulin-like growth factor I	0.4	3	(Swartz, 2006)
2007	ECE	single chain Fv antibody fragment / granulocyte macrophage colony-stimulating factor fusion protein	0.03	2	(Kanter *et al.*, 2007)
2008	ECE	[FeFe] hydrogenase	0.022		(Boyer *et al.*, 2008)
2008	WGE	average of 13,364 human proteins	0.002	variable	(Goshima *et al.*, 2008)
2011	ECE	human Granulocyte macrophage colony-stimulating factor	0.7	2	(Zawada *et al.*, 2011)
2011	ECE	human consensus interferon-alpha	0.4	2	(El-Baky *et al.*, 2011)

2. Supplementary References

Boyer ME, Stapleton JA, Kuchenreuther JM, Wang C-w, Swartz JR. Cell-free synthesis and maturation of [FeFe] hydrogenases. Biotechnol Bioeng. 2008;99:59-67.

Calhoun KA, Swartz JR. An economical method for cell-free protein synthesis using glucose and nucleoside monophosphates. Biotechnol Prog. 2005a;21:1146-53.

Calhoun KA, Swartz JR. Energizing cell-free protein synthesis with glucose metabolism. Biotechnol Bioeng. 2005b;90:606-13.

Chumpolkulwong N, Hori-Takemoto C, Hosaka T, Inaoka T, Kigawa T, Shirouzu M, et al. Effects of *Escherichia coli* ribosomal protein S12 mutations on cell-free protein synthesis. Eur J Biochem. 2004;271:1127-34.

Endo Y, Sawasaki T. Cell-free expression systems for eukaryotic protein production. Curr Opin Biotechnol. 2006;17:4:373-380.

El-Baky NA, Omar SH, Redwan EM. The anti-cancer activity of human consensus interferon-alpha synthesized in cell-free system. Protein Expr Purif. 2011;In Press, Uncorrected Proof.

Ezure T, Suzuki T, Shikata M, Ito M, Ando E. A cell-free protein synthesis system from insect cells. Methods Mol Biol. 2010;607:31-42.

Goshima N, Kawamura Y, Fukumoto A, Miura A, Honma R, Satoh R, et al. Human protein factory for converting the transcriptome into an *in vitro*-expressed proteome. Nat Methods. 2008;5:1011-7.

Jewett MC, Calhoun KA, Voloshin A, Wuu JJ, Swartz JR. An integrated cell-free metabolic platform for protein production and synthetic biology. Mol Syst Biol. 2008;4.

Jewett MC, Swartz JR. Mimicking the *Escherichia coli* cytoplasmic environment activates long-lived and efficient cell-free protein synthesis. Biotechnol Bioeng. 2004a;86:19-26.

Jewett MC, Swartz JR. Substrate replenishment extends protein synthesis with an *in vitro* translation system designed to mimic the cytoplasm. Biotechnol Bioeng. 2004b;87:465-71.

Kanter G, Yang J, Voloshin A, Levy S, Swartz JR, Levy R. Cell-free production of scFv fusion proteins: an efficient approach for personalized lymphoma vaccines. Blood. 2007;109:3393-9.

Kawasaki T, Gouda MD, Sawasaki T, Takai K, Endo Y. Efficient synthesis of a disulfide-containing protein through a batch cell-free system from wheat germ. Eur J Biochem. 2003;270:4780-6.

Kigawa T, Yabuki T, Yoshida Y, Tsutsui M, Ito Y, Shibata T, et al. Cell-free production and stable-isotope labeling of milligram quantities of proteins. FEBS Lett. 1999;442:15-9.

Kim D-M, Kigawa T, Cha-Yong C, Yokoyama S. A highly efficient cell-free protein synthesis system from *Escherichia coli*. Eur J Biochem. 1996;239:881-6.

Kim D-M, Swartz JR. Prolonging cell-free protein synthesis with a novel ATP regeneration system. Biotechnol Bioeng. 1999;66:180-8.

Kim D-M, Swartz JR. Prolonging cell-free protein synthesis by selective reagent additions. Biotechnol Prog. 2000;16:385-90.

Kim D-M, Swartz JR. Regeneration of adenosine triphosphate from glycolytic intermediates for cell-free protein synthesis. Biotechnol Bioeng. 2001;74:309-16.

Kim D-M, Swartz JR. Efficient production of a bioactive, multiple disulfide-bonded protein using modified extracts of *Escherichia coli*. Biotechnol Bioeng. 2004;85:122-9.

Kim H-C, Kim T-W, Kim D-M. Prolonged production of proteins in a cell-free protein synthesis system using polymeric carbohydrates as an energy source. Process Biochemistry. 2011;46:1366-9.

Klammt C, Schwarz D, Fendler K, Haase W, Dötsch V, Bernhard F. Evaluation of detergents for the soluble expression of α-helical and β-barrel-type integral membrane proteins by a preparative scale individual cell-free expression system. FEBS J. 2005;272:6024-38.

Madin K, Sawasaki T, Ogasawara T, Endo Y. A highly efficient and robust cell-free protein synthesis system prepared from wheat embryos: Plants apparently contain a suicide system directed at ribosomes. Proc Natl Acad Sci U S A. 2000;97:559-64.

Noireaux V, Libchaber A. A vesicle bioreactor as a step toward an artificial cell assembly. Proc Natl Acad Sci U S A. 2004;101:17669-74.

Park N, Um SH, Funabashi H, Xu J, Luo D. A cell-free protein-producing gel. Nat Mater. 2009;8:432-7.

Promega. Optimize your TnT reticulocyte lysate systems reaction. 2010. http://www.promega.com/resources/articles/pubhub/enotes/optimize-your-tnt-reticulocyte-lysate-systems-reaction/) Accessed: August 8th, 2011

Sawasaki T, Hasegawa Y, Morishita R, Seki M, Shinozaki K, Endo Y. Genome-scale, biochemical annotation method based on the wheat germ cell-free protein synthesis system. Phytochemistry. 2004;65:1549-55.

Sawasaki T, Hasegawa Y, Tsuchimochi M, Kamura N, Ogasawara T, Kuroita T, et al. A bilayer cell-free protein synthesis system for high-throughput screening of gene products. FEBS Lett. 2002a;514:102-5.

Sawasaki T, Ogasawara T, Morishita R, Endo Y. A cell-free protein synthesis system for high-throughput proteomics. Proc Natl Acad Sci U S A. 2002b;99:14652-7.

Spirin A, Baranov V, Ryabova L, Ovodov S, Alakhov Y. A continuous cell-free translation system capable of producing polypeptides in high yield. Science. 1988;242:1162-4.

Swartz J. Developing cell-free biology for industrial applications. J Ind Microbiol Biotechnol. 2006;33:476-85.

Voloshin AM, Swartz JR. Efficient and scalable method for scaling up cell free protein synthesis in batch mode. Biotechnol Bioeng. 2005;91:516-21.

Wang Y, Zhang Y-HP. Cell-free protein synthesis energized by slowly-metabolized maltodextrin. BMC Biotechnol. 2009;9:58.

Yang J, Kanter G, Voloshin A, Michel-Reydellet N, Velkeen H, Levy R, et al. Rapid expression of vaccine proteins for B-cell lymphoma in a cell-free system. Biotechnol Bioeng. 2005;89:503-11.

Yin G, Swartz JR. Enhancing multiple disulfide bonded protein folding in a cell-free system. Biotechnol Bioeng. 2004;86:188-95.

Zawada JF, Yin G, Steiner AR, Yang J, Naresh A, Roy SM, et al. Microscale to manufacturing scale-up of cell-free cytokine production—a new approach for shortening protein production development timelines. Biotechnol Bioeng. 2011;108:1570-8.