Supporting Information:

In-vitro Protein Synthesis in Semi-Permeable Artificial cells

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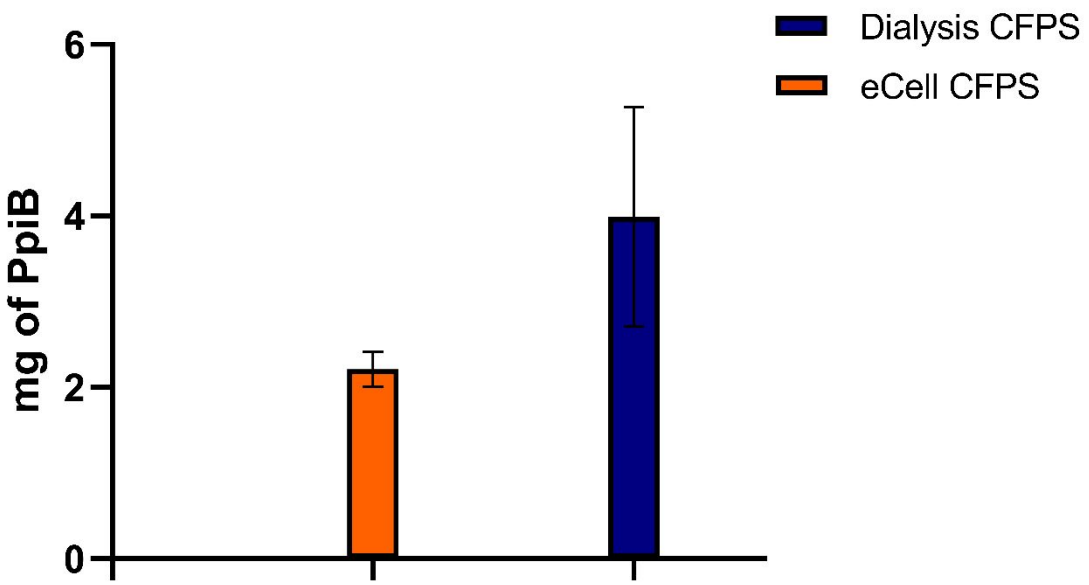
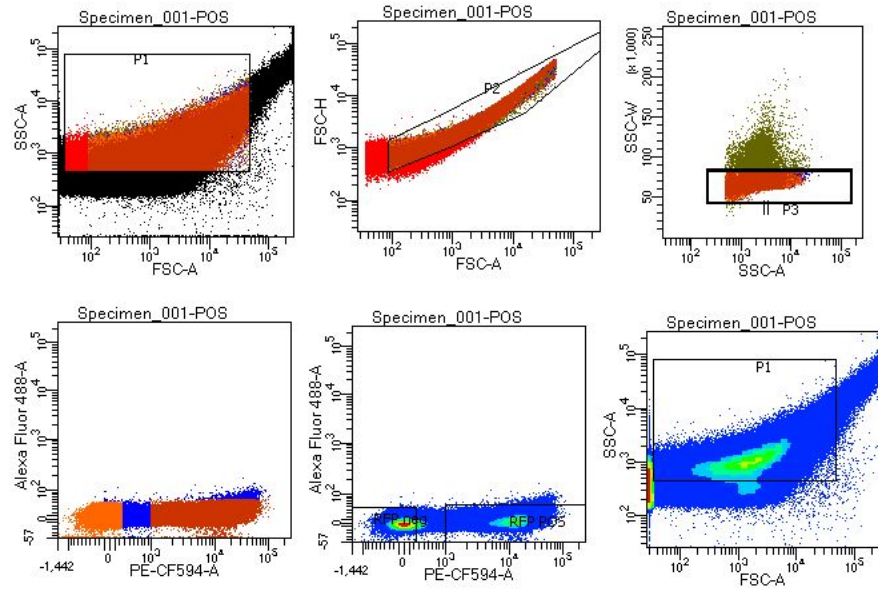
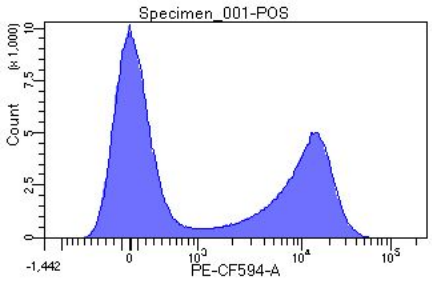


SI Figure 1.) A comparison of WT PpiB production between dialysis and eCell CFPS. eCell based CFPS is 55% of the yield between standard dialysis CFPS. This is quite good expression for eCell based CFPS and is competitive with standard dialysis CFPS, especially when cost is taken into consideration. See comparison methods in methods section.

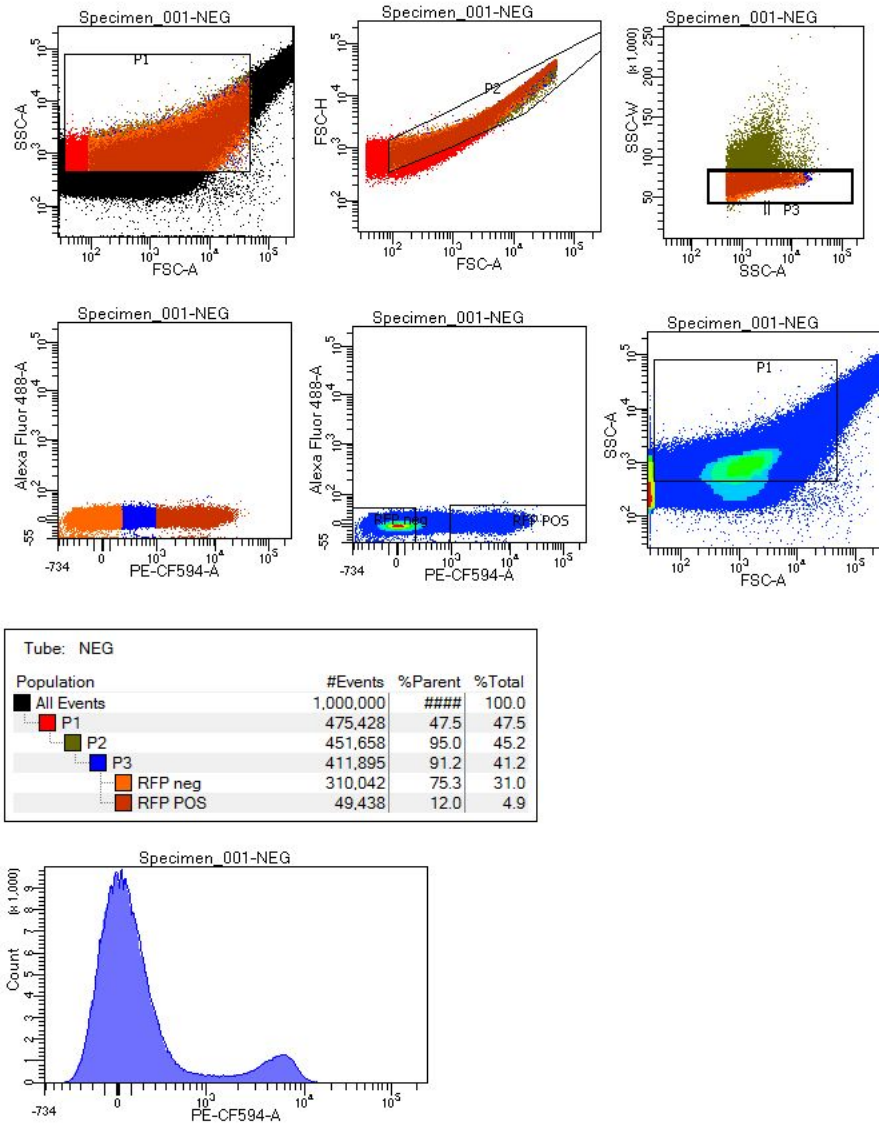


Tube: POS

Population	#Events	%Parent	%Total
All Events	1,000,000	####	100.0
P1	554,551	55.5	55.5
P2	530,087	95.6	53.0
P3	497,470	93.8	49.7
RFP neg	237,623	47.8	23.8
RFP POS	211,454	42.5	21.1



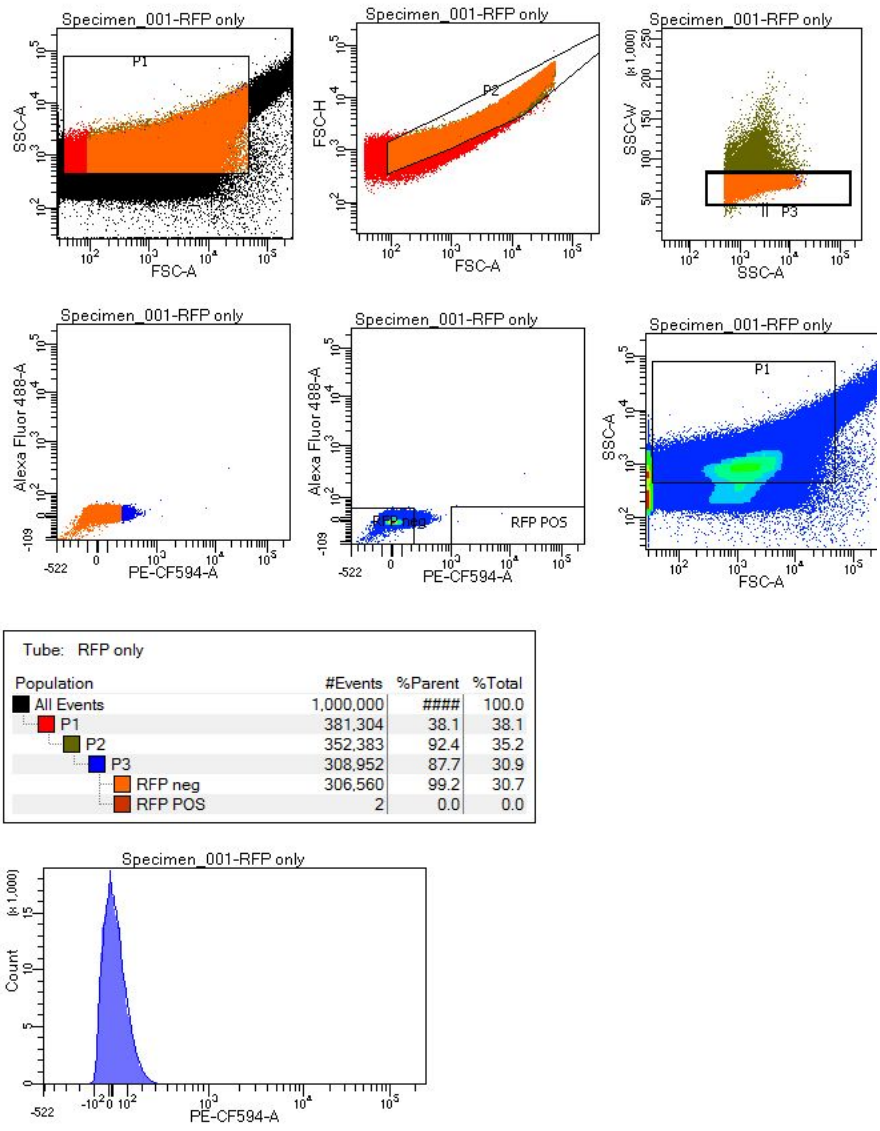
SI Figure 2.) FACS graphs detailing FSC, SSC and fluorescence of the +aaRS +ncAA sample. Events were set to 10^6 consistently between samples and gating was set for only single capsules. Gating to record fluorescence (RFP pos) was set at 10^3 and higher, so that noise and debris were excluded. 42.5% of capsules held significant RFP fluorescence, while 47.8% were inactive.



Tube: NEG

Population	#Events	%Parent	%Total
All Events	1,000,000	####	100.0
P1	475,428	47.5	47.5
P2	451,658	95.0	45.2
P3	411,895	91.2	41.2
RFP neg	310,042	75.3	31.0
RFP POS	49,438	12.0	4.9

SI Figure 3.) FACS graphs detailing FSC, SSC and fluorescence of the +aaRS +ncAA sample. Events were set to 10^6 consistently between samples and gating was set for only single capsules. Gating to record fluorescence (RFP pos) was set at 10^3 and higher, so that noise and debris were excluded. 12.5% of capsules held significant RFP fluorescence, while 75.3% were inactive.

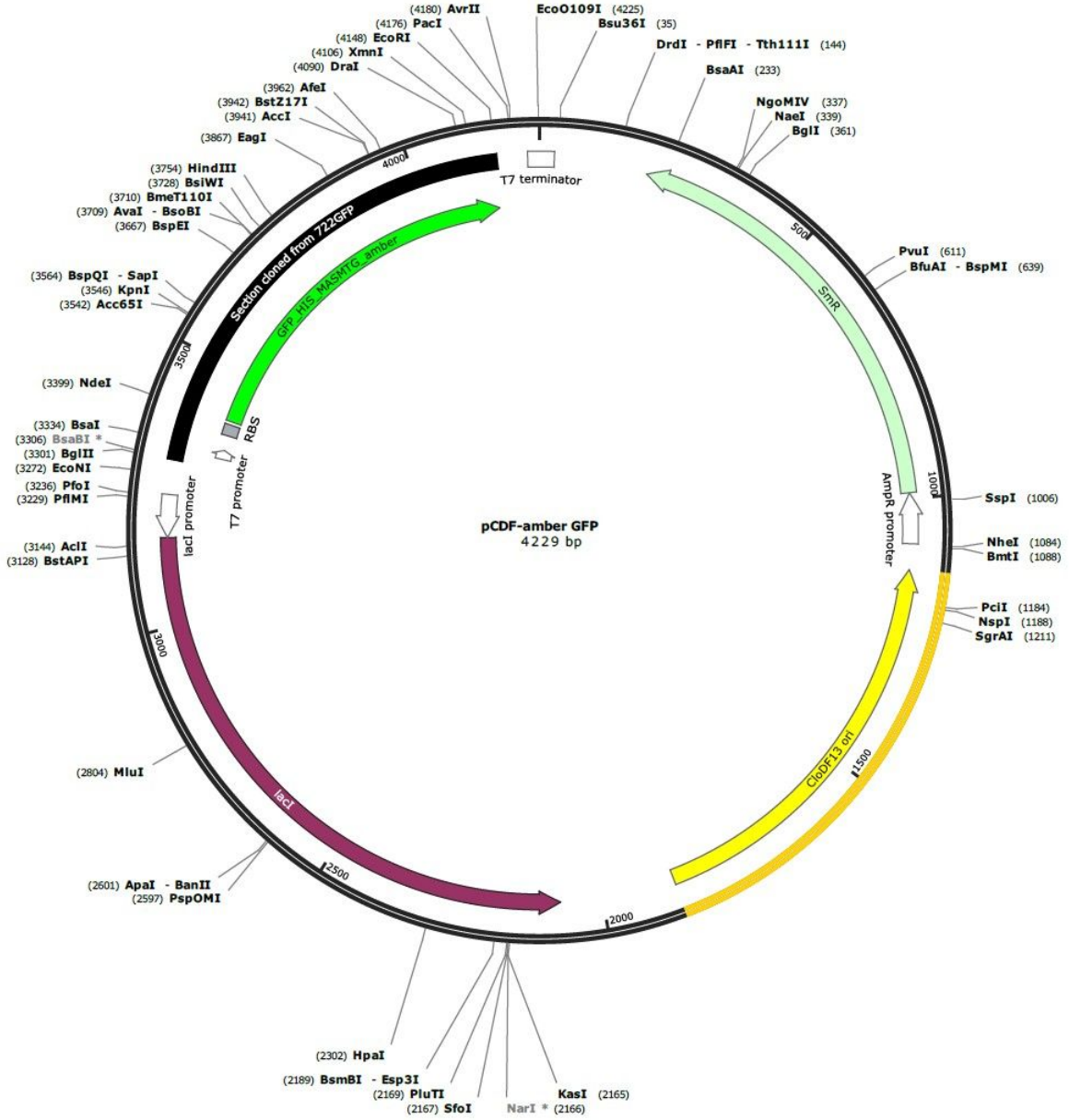


Tube: RFP only

Population	#Events	%Parent	%Total
All Events	1,000,000	####	100.0
P1	381,304	38.1	38.1
P2	352,383	92.4	35.2
P3	308,952	87.7	30.9
RFP neg	306,560	99.2	30.7
RFP POS	2	0.0	0.0

SI Figure 4.) FACS graphs detailing FSC, SSC and fluorescence of the -aaRS -ncAA sample. Events were set to 10^6 consistently between samples and gating was set for only single capsules. Gating to record fluorescence (RFP pos) was set at 10^3 and higher, so that noise and debris were excluded. 0.2% of capsules held RFP fluorescence, while 99% were inactive.

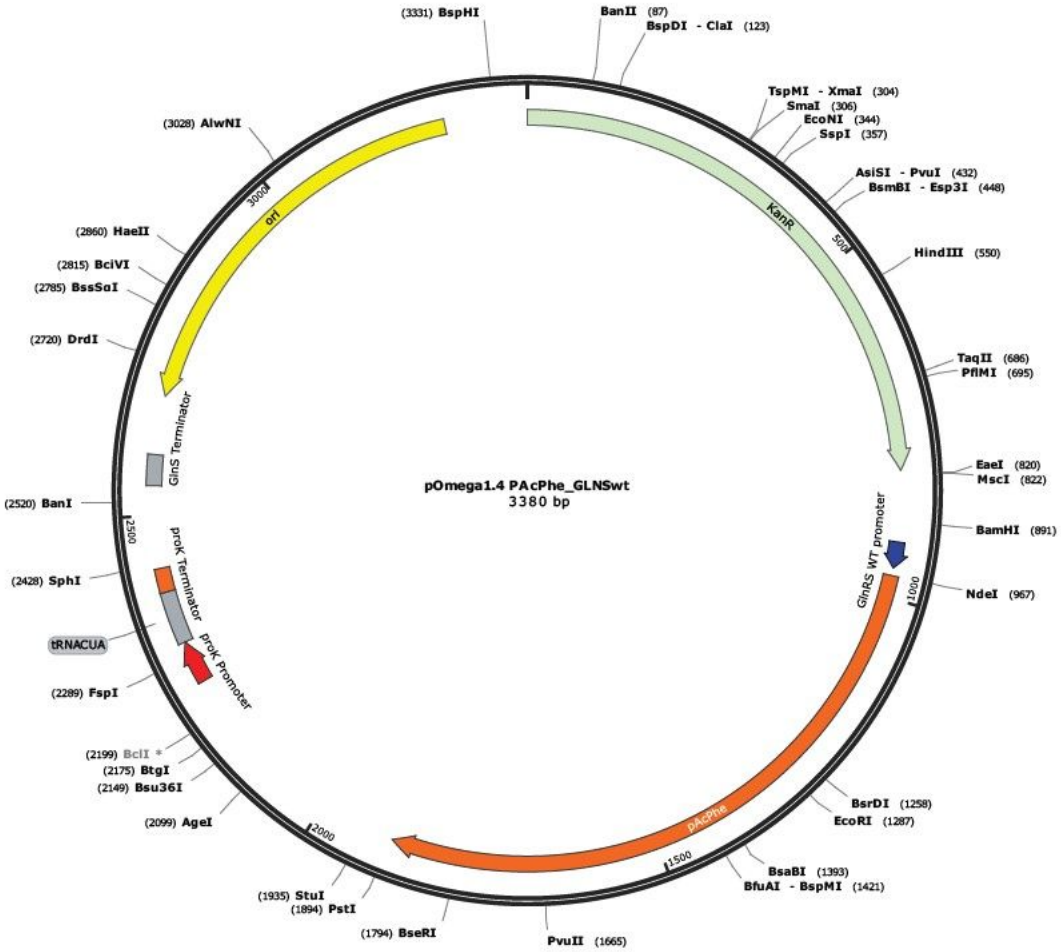
SI Figure 5.) Plasmid with pCloDF13 origin of replication, an mCherry RFP gene with an amber site at position 13 under a T7 promoter with spectinomycin resistance. This plasmid was used as the reporter for FACS.



SI Figure 6.) Plasmid with pCloDF13 origin of replication, a neon GFP gene with an amber site inserted at position 13 (L13) under a T7 promoter with spectinomycin resistance. This plasmid was used as the reporter for FACS. The same gene without the amber site (WT nGFP) in a T7 pET overexpression vector with ampicillin resistance was used for measurements of GFP production in eCells

SI Figure 7.) Plasmid with pCloDF13 origin of replication, a PpiB gene under a T7 promoter with spectinomycin resistance. This plasmid was used to determine yield between dialysis CFPS and eCell CFPS. The same plasmid with an amber site replacing H147 was used to incorporate bocK and PCC. For eCell CFPS with WT PpiB a lac operator was inserted in front of the T7 promoter to eliminate background expression and allow for accurate yield determination.

SI Figure 8.) Plasmid with a pUC origin of replication, kanamycin resistance and the *Methanosarcina mazei* Pyrrolysl tRNA aaRS/$tRNA_{sup}$ that was used to incorporate N^{ε}-(tert-butoxycarbonyl)-L-lysine (boc-lysine) and photocaged-cysteine.



SI Figure 9.) Plasmid with a pUC origin of replication, kanamycin resistance and the *M. jannaschii (Mj)* TyrRS aaRS/tRNA$_{sup}$ with previously described mutations at Y32L, D158G, I159C, L162R and A167A that was used to incorporate p-acetyl-L-phenylalanine in conjunction with A13nGFP.