Supplementary material

Synthesis of 2.3 mg/mL of Protein with an all *E. coli* Cell-Free Transcription Translation System

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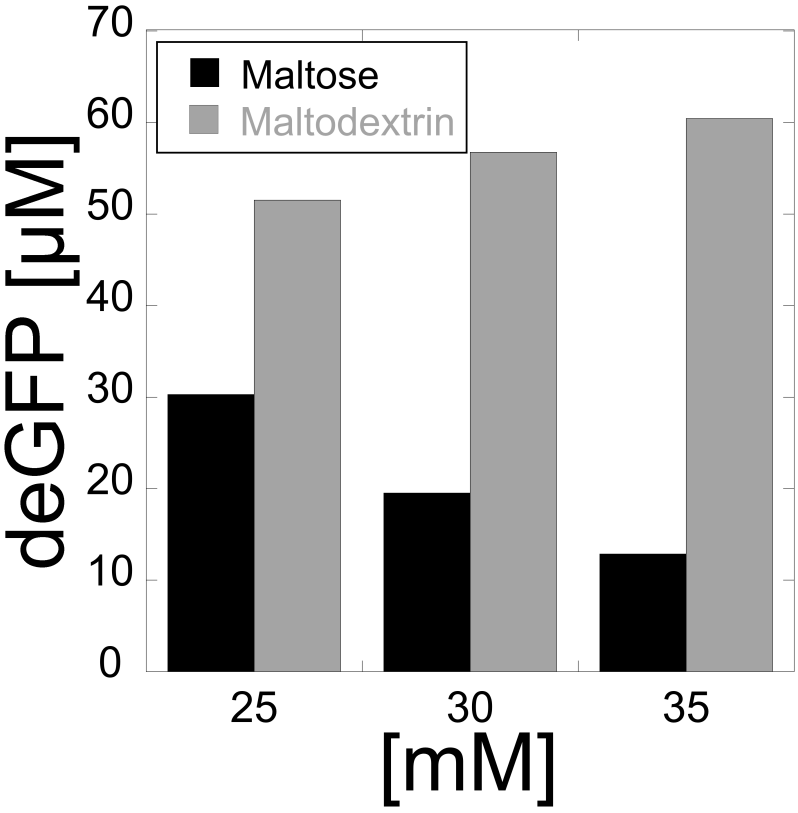


Fig. S1: Concentration ranges in batch mode of maltose and maltodextrin with 5 nM pBEST-OR2-OR1-Pr-UTR1-deGFP-T500 and 3 mM amino acids.

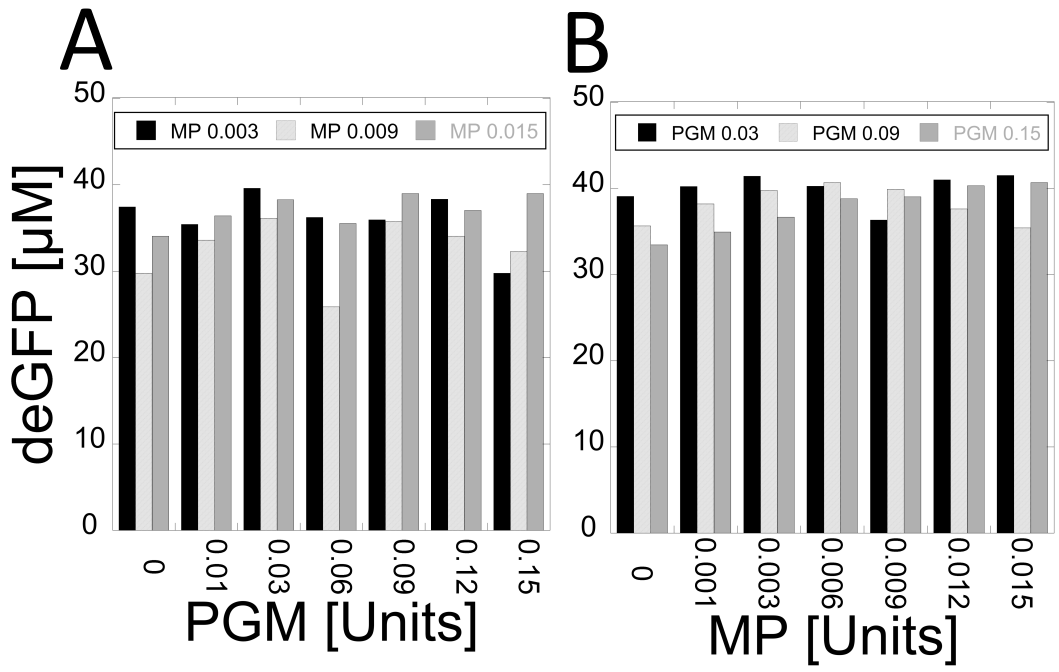
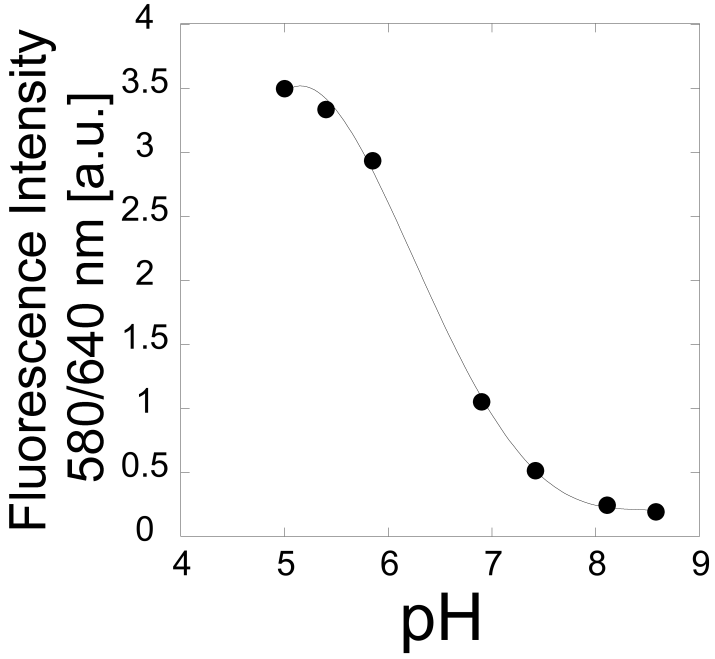


Fig. S2: Concentration ranges of maltose phosphorylase (MP) and phosphoglucomutase (PMG) with 12 mM maltose, 5 nM pBEST-OR2-OR1-Pr-UTR1-deGFP-T500 and 1.5 mM amino acids. The enzymes were diluted at desired concentrations in water before use. (A) The MP was fixed at three concentrations whereas PGM was varied along a concentration range. (B) The PGM was fixed at three concentrations whereas MP was varied along a concentration range.



Y = M0 + M1*x + ... M8*x^8 + M9*x^9	
M0	-213.84
M1	129
M2	-27.868
M3	2.5956
M4	-0.088697
R	0.99943

Fig. S3: SNARF-5F calibration at 100 μM in 100 mM Tris buffered with concentrated acetic acid and polynomial fitting for computing the emission ratio at 580/640 nm of the fluorescence dye during pH variation.

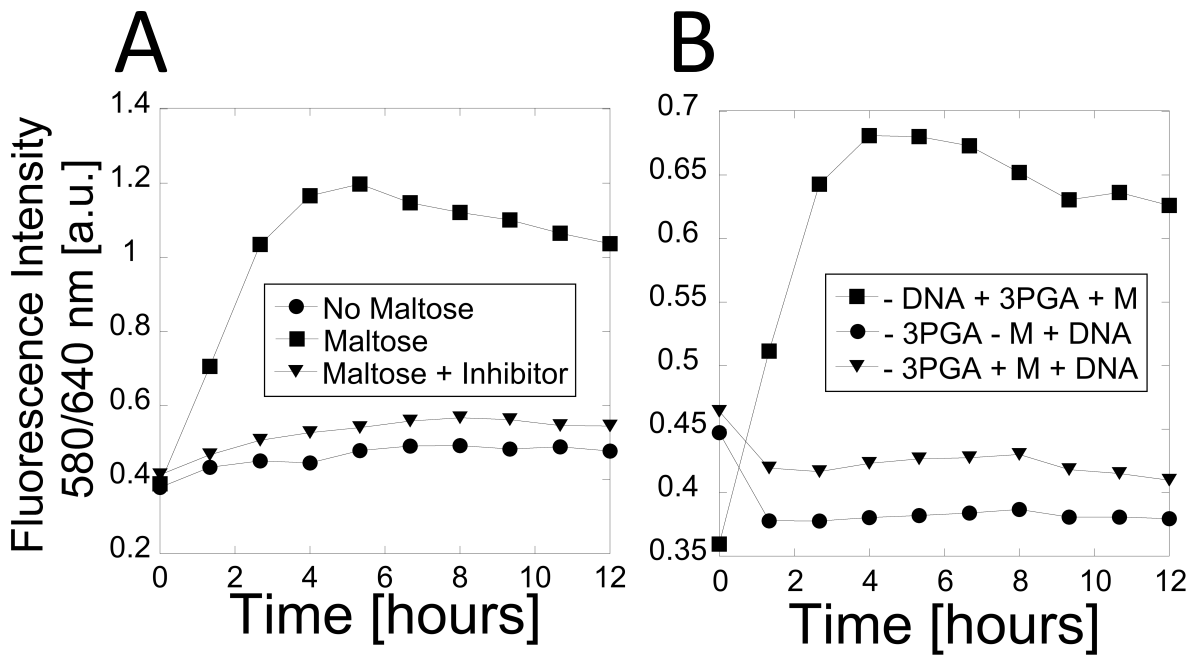


Fig. S4: Raw data of the kinetics of SNARF-5F fluorescence during cell-free reaction with 5 nM pBEST-OR2-OR1-Pr-UTR1-Luc-T500 and 3 mM amino acids. Maltose at 12 mM. These data were used with the calibration in Fig. S3 to produce Fig. 4.

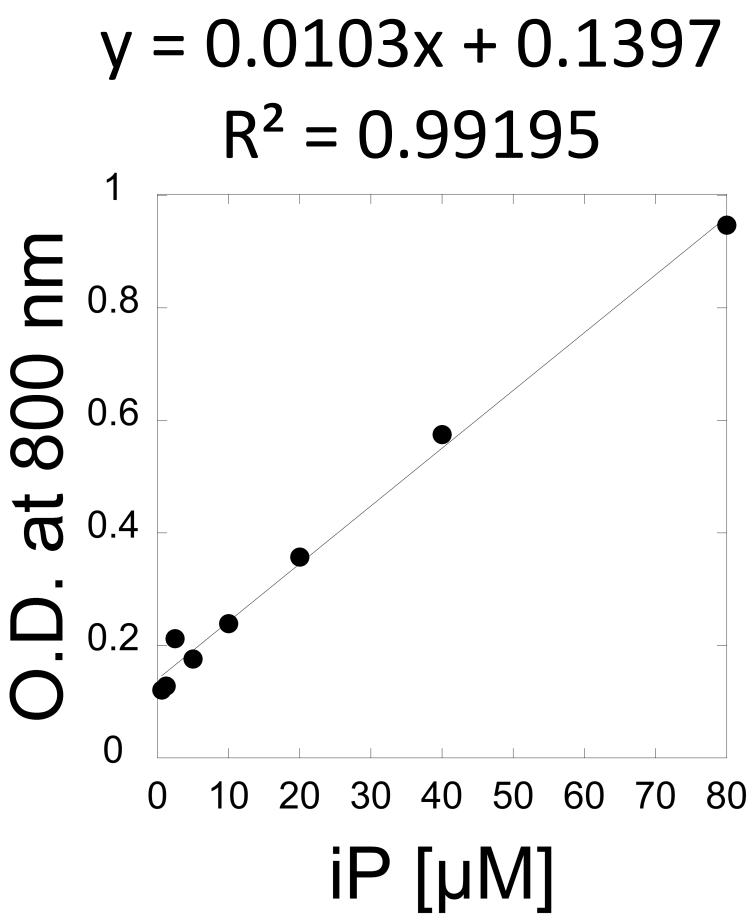


Fig. S5: Calibration line used for the inorganic phosphate assay. The calibration was made with known concentration of potassium phosphate monobasic.

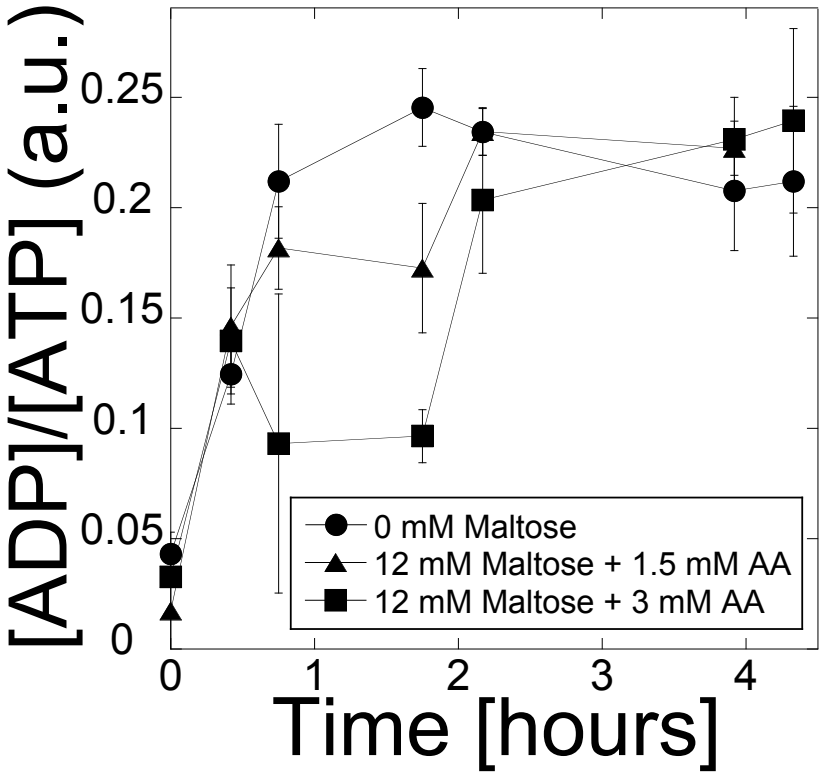
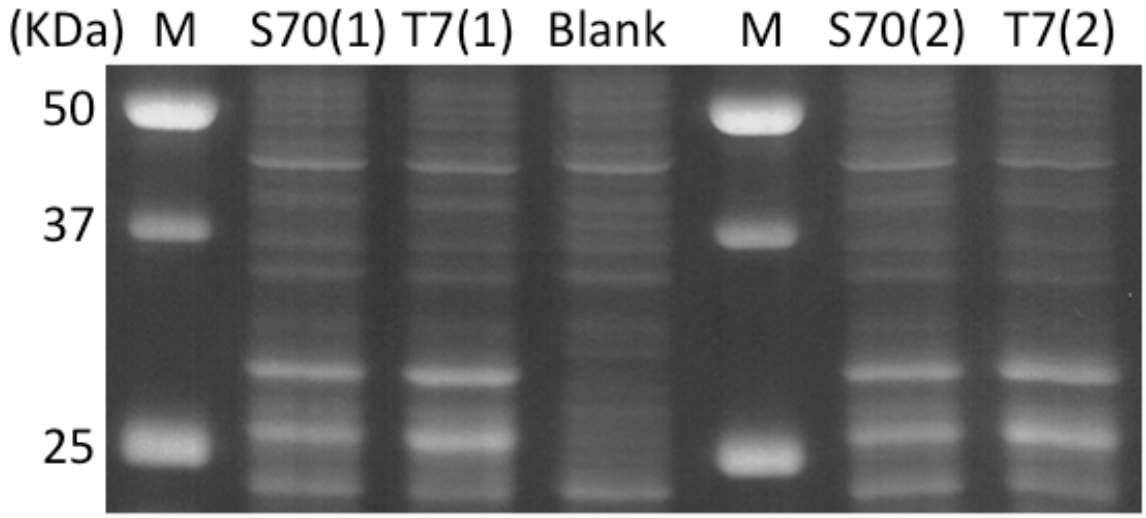


Fig. S6: Kinetics of ADP/ATP ratio in the presence of 12 mM maltose with 5 nM plasmid pBEST-OR2-OR1-Pr-UTR1-deGFP-T500. Error bars represent the standard deviation of three different samples.



Sample	Lane	Area	Mean
Blank rEGFP	Blank	0.034	43.504
2 mg/mL rEGFP	T7 (1)	0.034	60.243
1.5 mg/mL rEGFP	S70 (1)	0.034	59.183
Blank deGFP	Blank	0.034	48.377
T7	T7 (1)	0.034	68.297
S70	S70 (1)	0.034	62.102
S70	S70 (2)	0.034	63.307
T7	T7 (2)	0.034	70.117
2 mg/mL rEGFP	T7 (2)	0.034	62.938
1.5 mg/mL rEGFP	S70 (2)	0.034	60.141

Fig. S7: Black and white inverted 16-bit image obtained with ImageJ software. The image was used as displayed to quantify the amount of deGFP expressed. Table: the average value of the intensity pixel for a selected region on the gel are reported and used for quantification with respect to known concentration of rEGFP.