Supplementary material

A Cost-Effective Polyphosphate-Based Metabolism Fuels an All *E. coli* Cell-Free Expression System

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Abbreviations: TX-TL, transcription-translation; ATP, adenosine triphosphate; iP, inorganic phosphate; eGFP, enhanced green fluorescent protein; PEP, phosphoenolpyruvate; 3-PGA, 3-phosphoglycerate; NAD, nicotinamide; CoA, coenzyme A; cAMP, adenosine 3',5'-cyclic monophosphate; DTT, dithiothreitol; M, maltose; Mx, maltodextrin; HMP, hexametaphosphate; poly(P), polyphosphate; RB, reaction buffer.

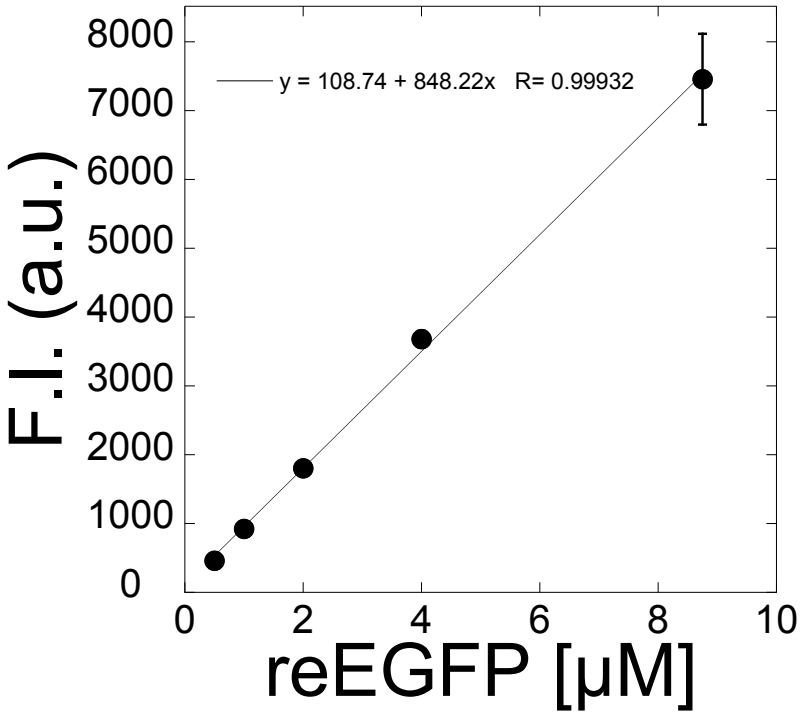

Fig. S1: Calibration line used to retrieve deGFP concentration. Measurements were taken in a 384 well-plate, 10 µL of reGFP diluted in the cell extract (Ex 485 nm – Em 528 nm). Each point is the average of three different measurements.

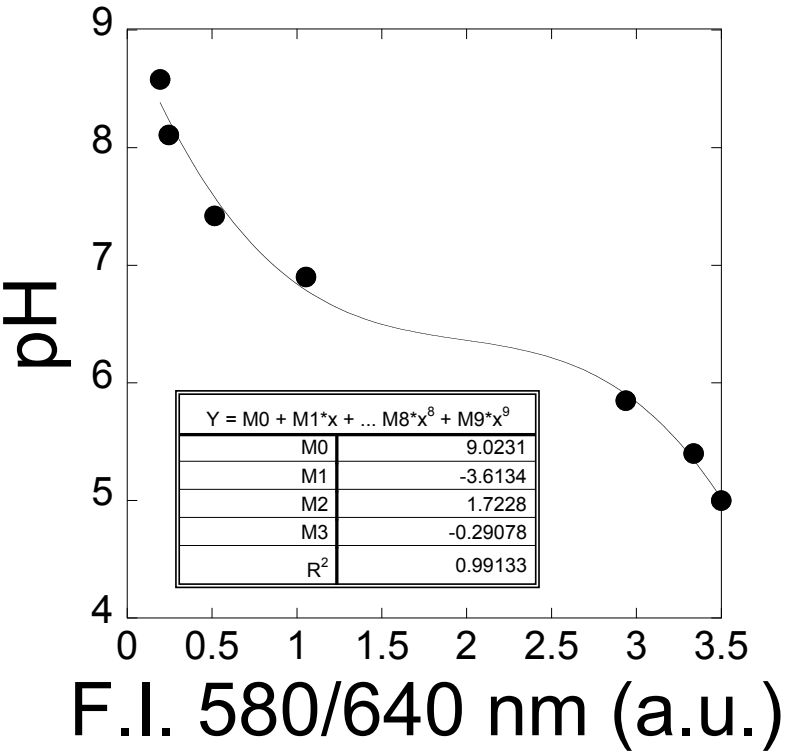

Fig. S2: Calibration curve of 100 µM SNARF-5F dye in aqueous solution buffered with 100 mM Tris, Ex at 514 nm. The curve was fit with a polynomial up to x^3. 5 µL were measured in a 96-well plate with conical bottom.

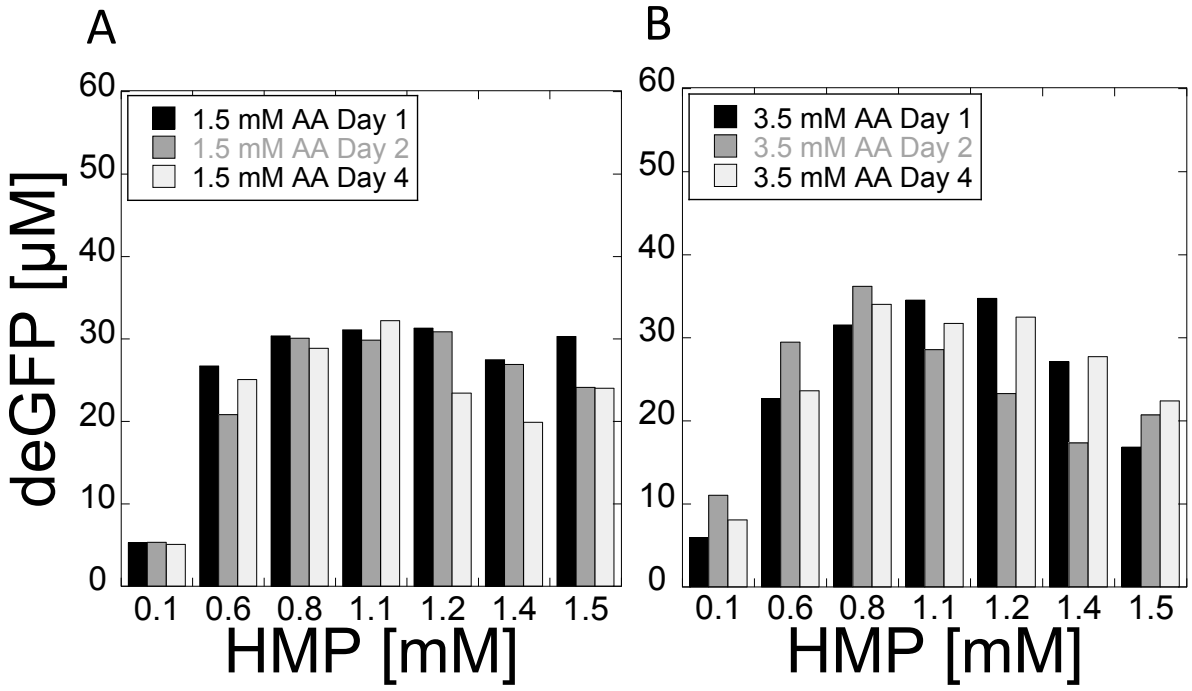

Fig. S3: Concentration range of hexametaphosphate (HMP) prepared at room temperature (RT). Cell-free reactions performed with 15 mM maltose and (A) 1.5 mM amino acids (B) 3.5 mM amino acids (6 nM pBEST-OR2-OR1-Pr-UTR1-deGFP-T500). Measurements taken with HMP stored at room temperature up to 4 days. Cell-free reactions (12 µL) were incubated at 29°C for 18 hours before measurement in a 384-well plate.

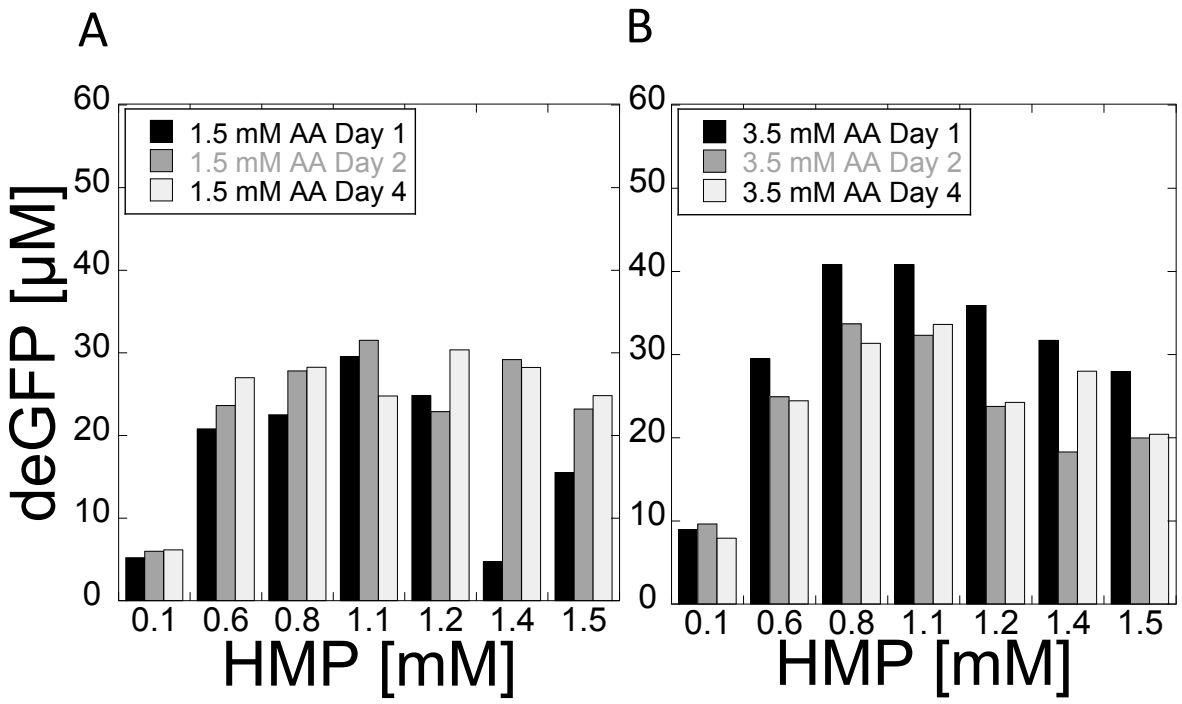

Fig. S4: Concentration range of hexametaphosphate (HMP) prepared at room temperature and incubated at 100C for 5 minutes before each experiment. Cell-free reactions performed with 15 mM maltose and (A) 1.5 mM amino acids (B) 3.5 mM amino acids (6 nM pBEST-OR2-OR1-Pr-UTR1-deGFP-T500). Measurements taken with a HMP solution stored at room temperature up to 4 days. Cell-free reactions (12 μL) were incubated at 29°C for 18 hours before measurement in a 384-well plate.

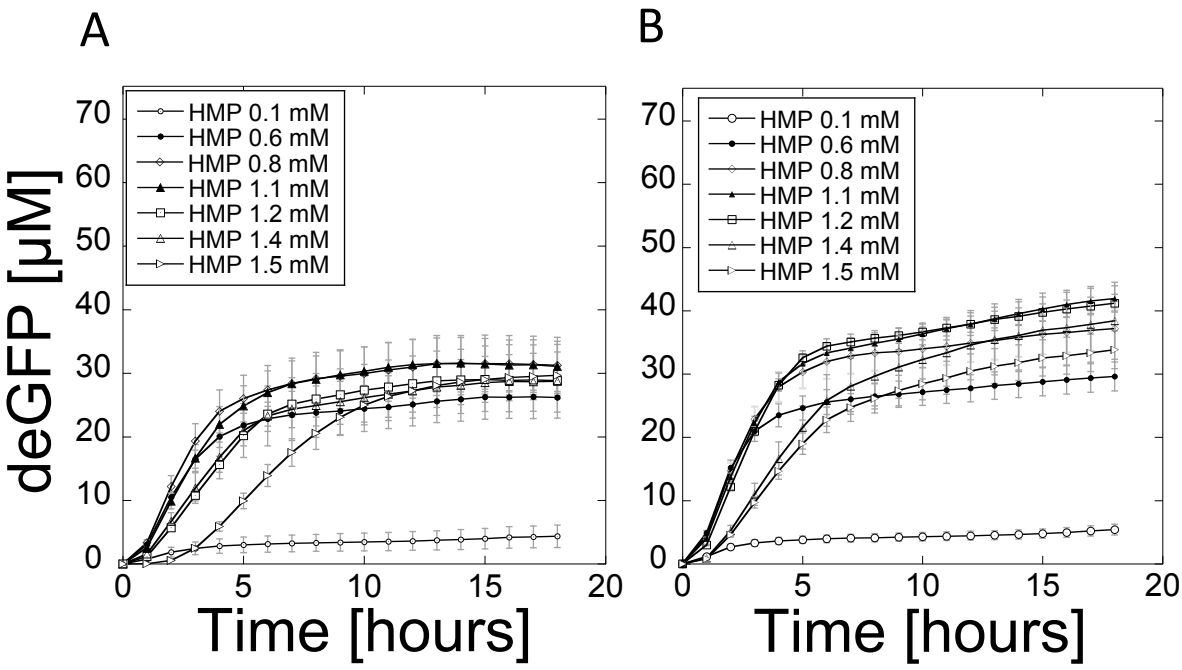

Fig. S5: Kinetics of *in vitro* deGFP synthesis with 15 mM maltose (A) or 35 mM maltodextrin (B) and different concentrations of HMP (1.5 mM amino acids, 6 nM plasmid pBEST-OR2-OR1-Pr-UTR1-deGFP-T500). Kinetics of cell-free reactions (5 µL) were performed at 29°C in a sealed 96-well plate with conical bottom for 18 hours. Error bars represent the standard deviation of three experiments.

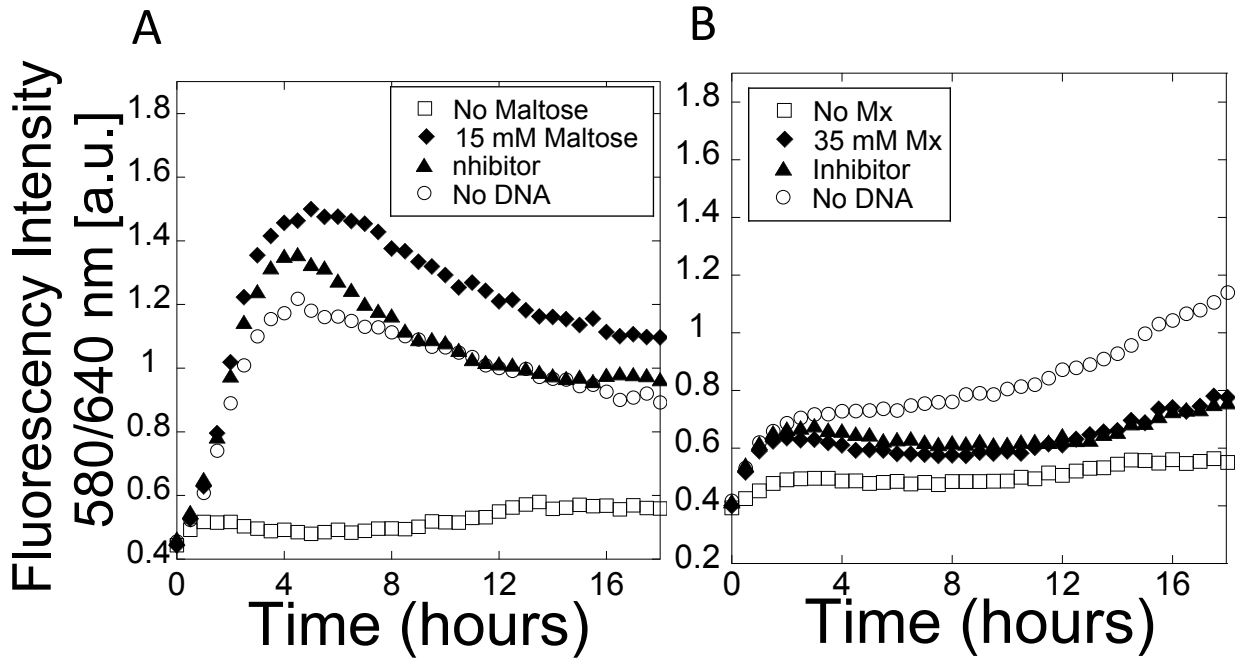

Fig. S6: Fluorescence kinetics of pH change (100 μM SNARF-5F, Ex at 514 nm) during *in vitro* protein synthesis (6 nM pBEST-OR2-OR1-Pr-UTR1-deGFP-T500, 0.8 mM HMP and 3.5 mM amino acids). Raw data used for graphs in Fig. 4. Cell-free reactions (5 μL) were incubated at 29°C in a sealed 96-well plate with conical bottom for 18 hours.. Error bars represent the standard deviation of three experiments.

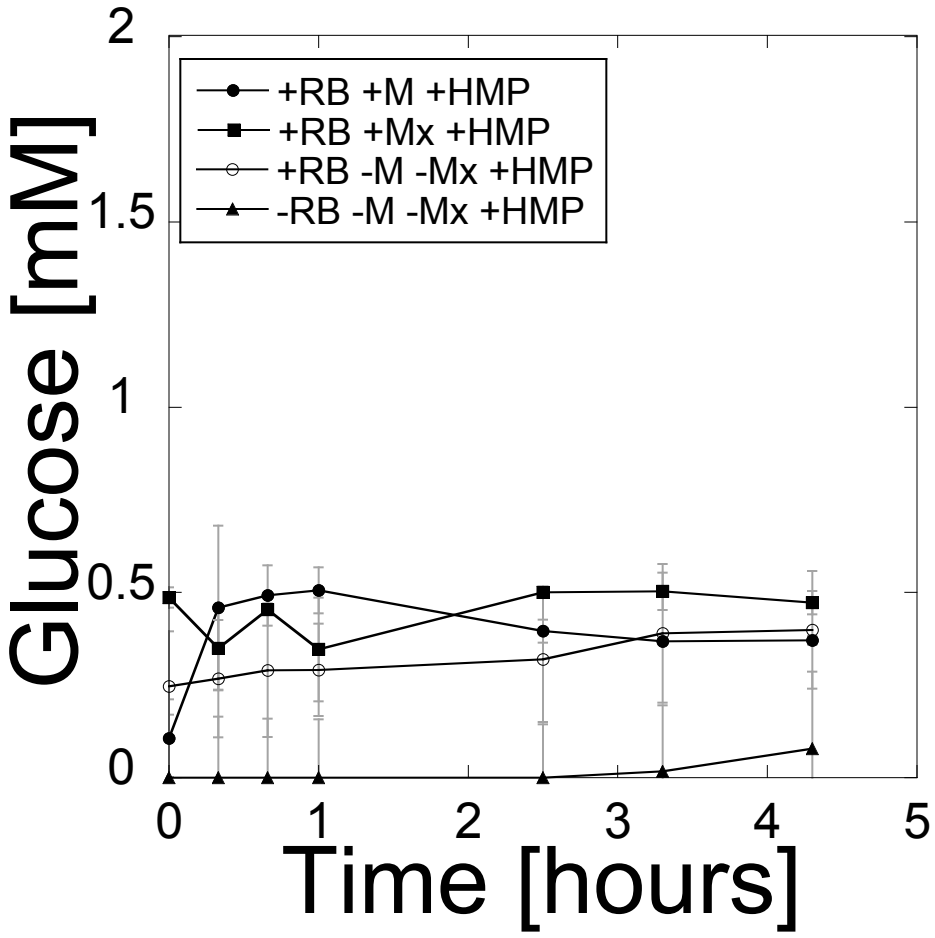

Fig. S7: Time course of glucose concentration during cell-free protein synthesis. Cell-free reactions (12 µL) were incubated at 29°C and snap frozen in liquid nitrogen before determination of glucose concentrations by enzymatic colorimetric assays. 15 mM maltose, 35 mM maltodextrin, 3.5 mM amino acids, 1.1 mM HMP and 6 nM of plasmid pBEST-OR2-OR1-Pr-UTR1-Luc-T500 were used. Error bars represent the standard deviation of two experiments.

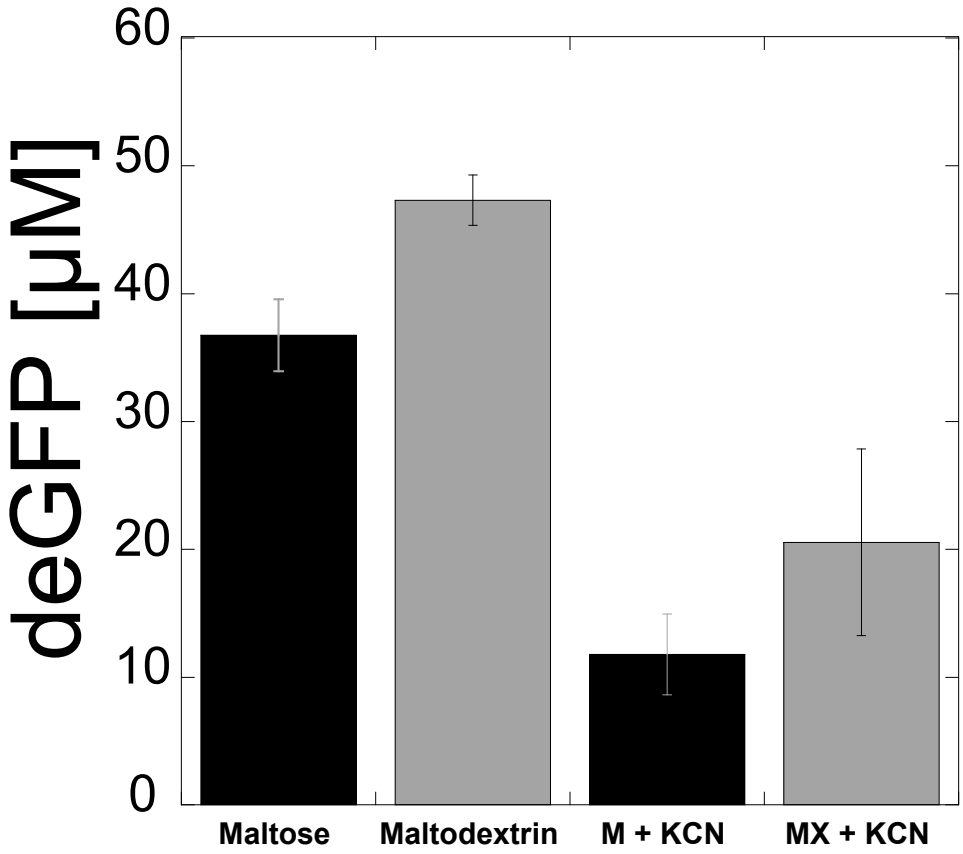

Fig. S8: Histograms of deGFP synthesis yield before and after incubation of the extract with 1 mM KCN for 15 minutes at RT (25°C). Afterward, cell-free reactions (12 µL) were incubated at 29°C for 18 hours before measurements in a 384-well plate; 3.5 mM amino acids, 1.1 mM HMP and 6 nM of plasmid pBEST-OR2-OR1-Pr-UTR1-deGFP-T500 were used. Error bars represent the standard deviation of three experiments.

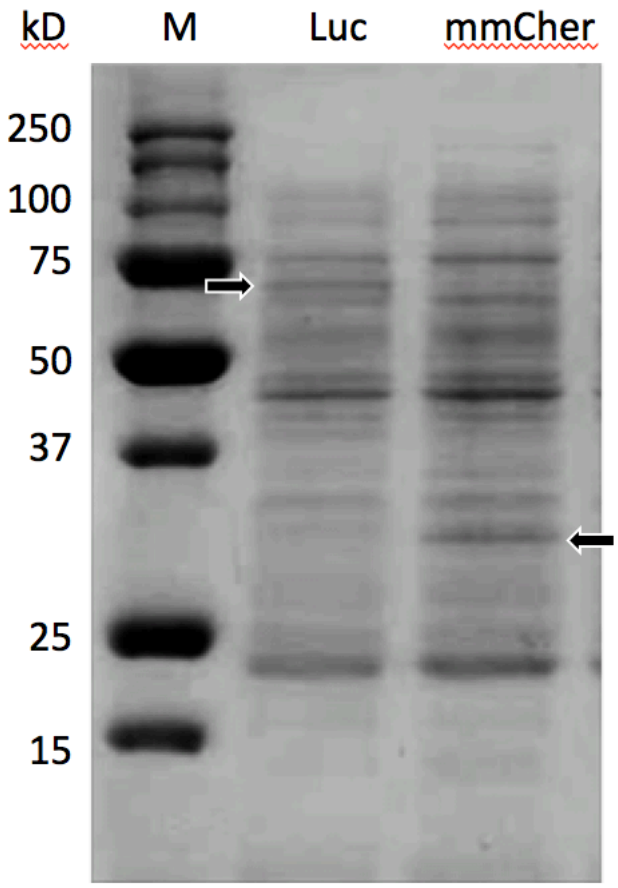

Fig. S9: SDS-PAGE (12% acrylamide). Cell-free reactions were prepared with 35 mM maltodextrin and 1.1 mM HMP. The Luciferase (Luc) protein was synthesized with 6 nM plasmid pBEST-OR2-OR1-Pr-UTR1-Luc-T500 and 60 mM K-Glutamate, 6 mM Mg-Glutamate. The mmCherry (mmCher) gene was synthesized through the T7 cascade with 0.1 nM pBEST-OR2-OR1-Pr-UTR1-T7-T500, 1 nM pIVEX2.3d-mmCherry, 20 mM K-Glutamate and 2 mM Mg-Glutamate. Cell-free reactions were incubated at 29°C for 18 hours.

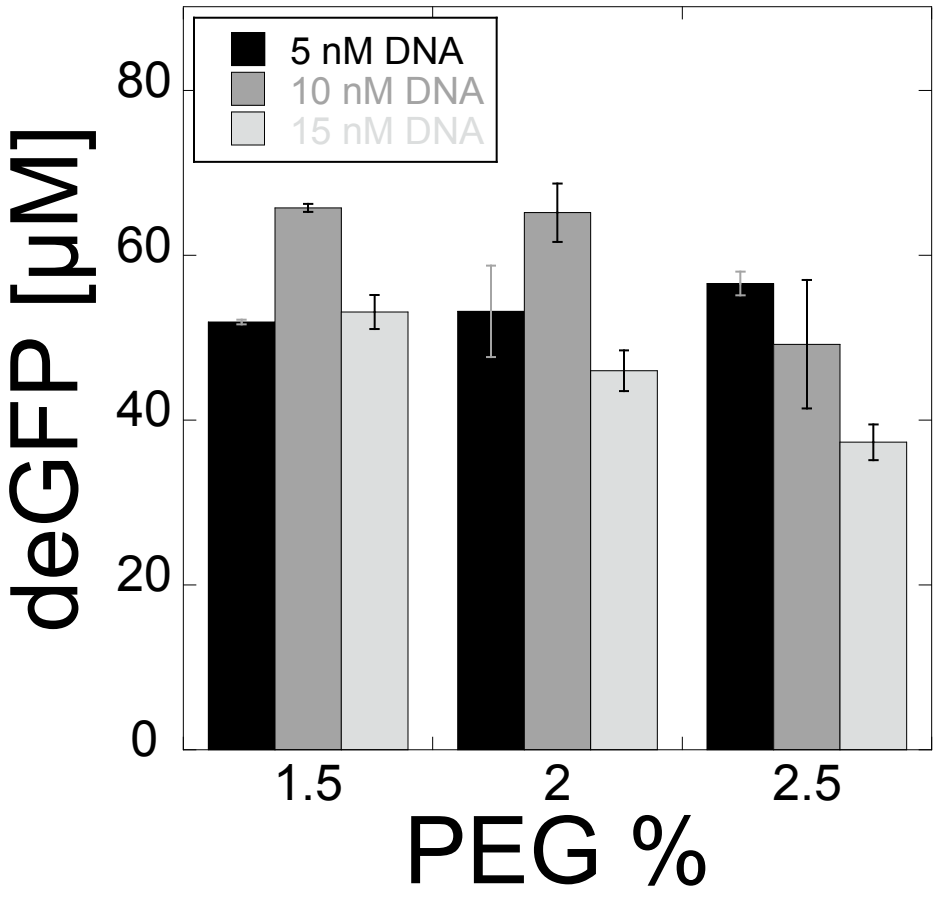

Fig. S10: Cell-free synthesis of deGFP as a function of plasmid (pBEST-OR2-OR1-Pr-UTR1-deGFP-T500) and PEG concentrations (3.5 mM amino acids, 35 mM maltodextrin, 1.1 mM HMP). Cell-free reactions (12 µL) were incubated at 29°C for 18 hours before measurements in a 384-well plate. Error bars represent the standard deviation of three experiments.