Supporting Information

Cell-free protein synthesis and *in situ* immobilization of deGFP-MatB in polymer microgels for malonate-to-malonyl CoA conversion

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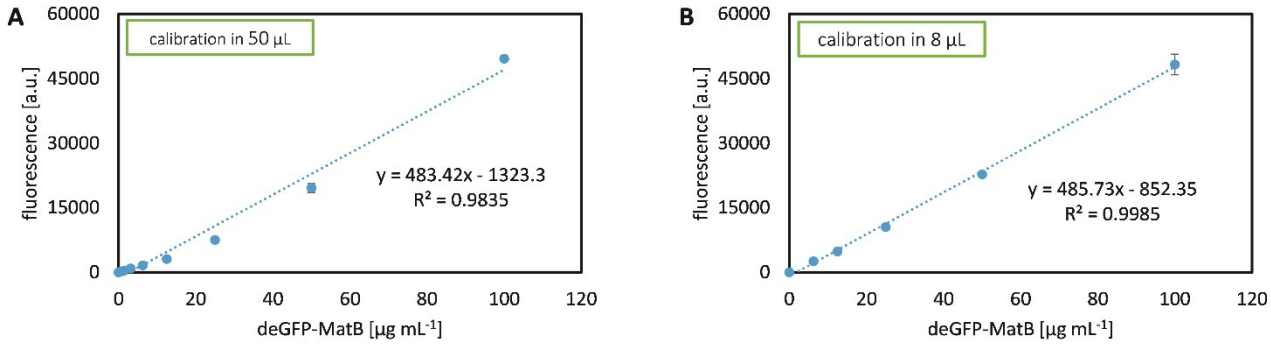

Figure S1: Calibration curve for deGFP-MatB. Calibration was done in different volumes 50 µL (A) and 8 µL (B) in black 96-well or 384-well plates, respectively.

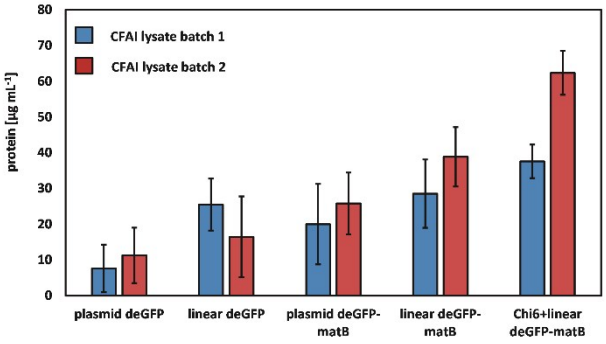

Figure S2: Comparison of lysates regarding their overall protein production. Identifying suitable templates comparing two cell-free auto-induction (CFAI)-based lysate preparations (blue and red). As-prepared lysates were tested with either plasmid or linear DNA, encoding for deGFP and deGFP-MatB, respectively, to investigate the synthesized amount of protein after 24 h. As the plasmid yields comparably low amounts of deGFP and deGFP-MatB for both lysates, linear DNA is identified as more suitable template for cell-free protein synthesis. Additional Chi6 DNA pushes the protein yield for deGFP-MatB to 62.4 µg mL^{-1}.

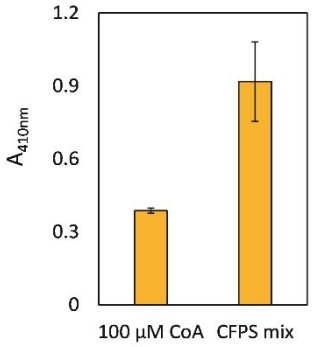

Figure S3: DTNB assay for determining the enzymatic activity. Absorbance of DTNB assay with 100 µM of CoA without enzymatic conversion (left) and a CFPS mixture containing 0.5 µg of non-purified deGFP-MatB (right). Error bars indicate the standard deviation of three measurements.

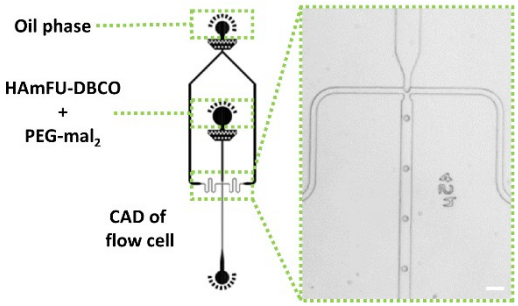

Figure S4: Microfluidic water-in-oil (W/O) emulsion preparation containing HAmFU-DBCO-DNA, pre-functionalized with NTA-mal, and PEG-mal_2 as crosslinker. Microfluidic device design (left) for producing tailor-made emulsion droplets. Scale bar indicates 50 µm.