Supplementary Materials

Supplementary Results

Supplementary Figure S1—Cell-free system productivity as a function of temperature and chaperone expression in the extract.

Supplementary Figure S2—Optimization of chaperone-transformed extract.

Supplementary Figure S3—Chaperone effects on cell-free system preservation.

Supplementary Figure S4—Effect of WZY2 on *E. coli* cell growth.

Supplementary Figure S5—Integration result of the chaperone expression curves from Figure 1.

Supplementary Figure S6—Polynomial regression models for the chaperone-augmented cell-free systems, including WZY2, CsLEA11, and PtDRG1.

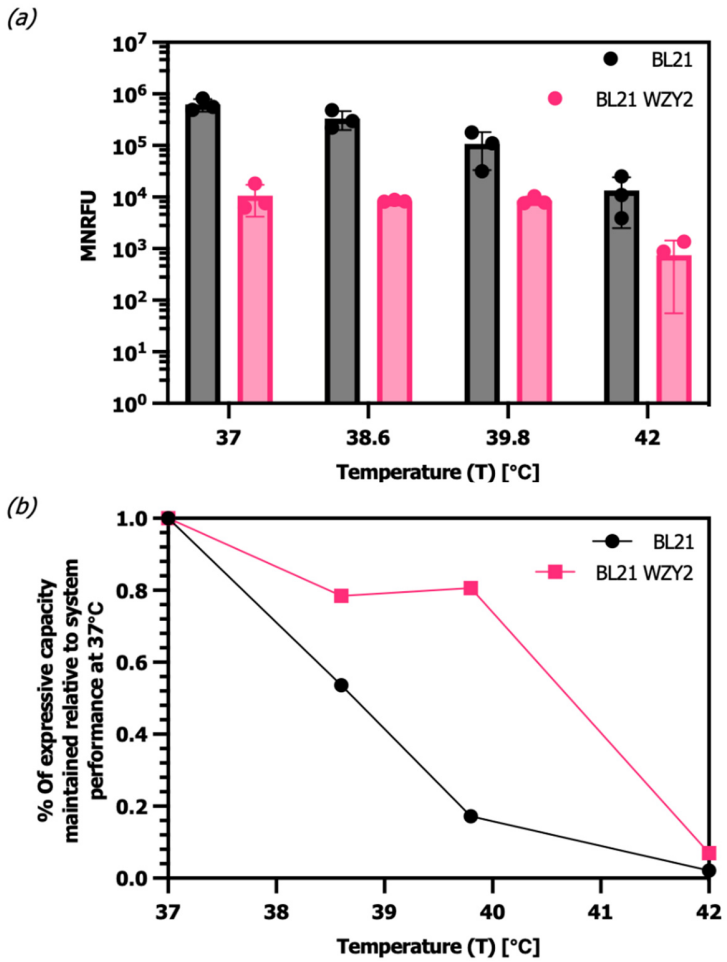

Figure S1. Extract prepared from transformed and non-transformed cells was evaluated in cell-free systems over temperatures ranging from 37 °C to 42 °C. (**a**) Cell-free system efficacy was evaluated by monitoring EGFP fluorescence following system incubation at 37 °C, 38.6 °C, 39.8 °C, and 42 °C. The transformed and non-transformed extract, referred to as 'BL21 WZY2' and 'BL21', respectively, was used. (**b**) This plot represents the system's expressive capacity relative to its highest-performing measure, occurring at 37 °C. Of note, the 'BL21 WZY2' system maintains much of its expressive capacity at temperatures as high as 39.8 °C.

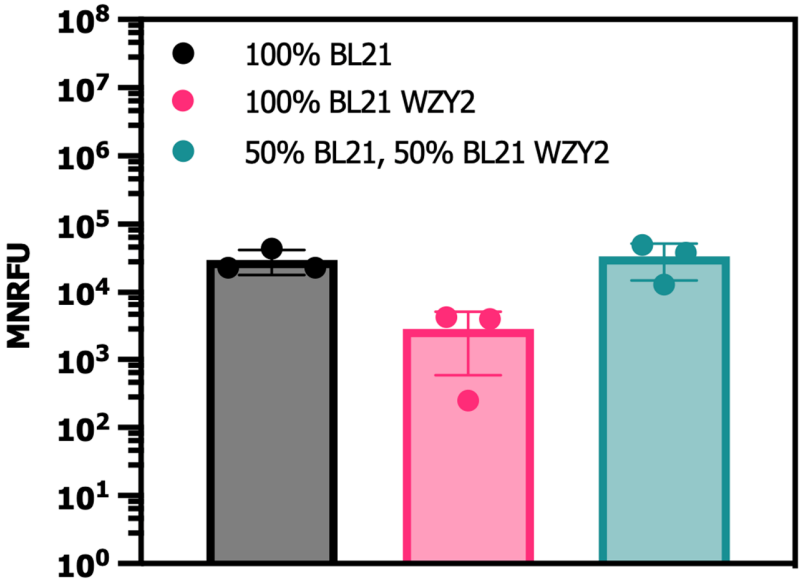

Figure S2. Effects of extract optimization. Considering the growth differences between the transformed and non-transformed cells from which the extract was prepared, we evaluated whether combining the two in equal amounts, given as '50% BL21, 50% BL21 WZY2', would improve system performance. The hypothesis was such that we could supplement the loss of translational efficacy of the transformed extract resulting from chaperone expression while maintaining the benefit of having a chaperone present. System performance was evaluated by measuring EGFP fluorescence at 42 °C.

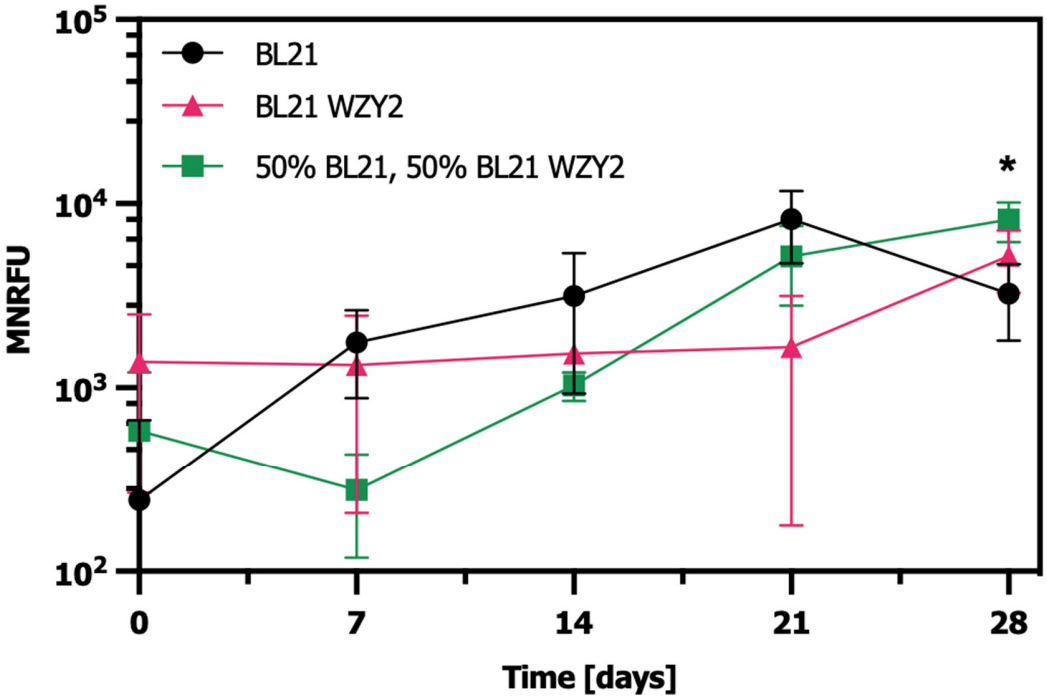

Figure S3. Preservation analysis of cell-free systems with and without the chaperone WZY2. Two chaperone systems were evaluated, including 'BL21 WZY2' and '50% BL21, 50% BL21 WZY2', the latter consisting of an equal mixture of the transformed and non-transformed extract. This assay was carried out by combining reaction buffer and transformed/non-transformed extract, plating these solutions, and air-drying them at 37 °C for 5 h. The samples were reconstituted with water at weeks 0, 1, 2, 3, and 4, the EGFP reporter plasmid was added, and fluorescence was measured after 4 h of incubation at 37 °C. Results showed that the chaperone systems offer unparalleled protection to cell-free system components at 4 weeks of system preservation.

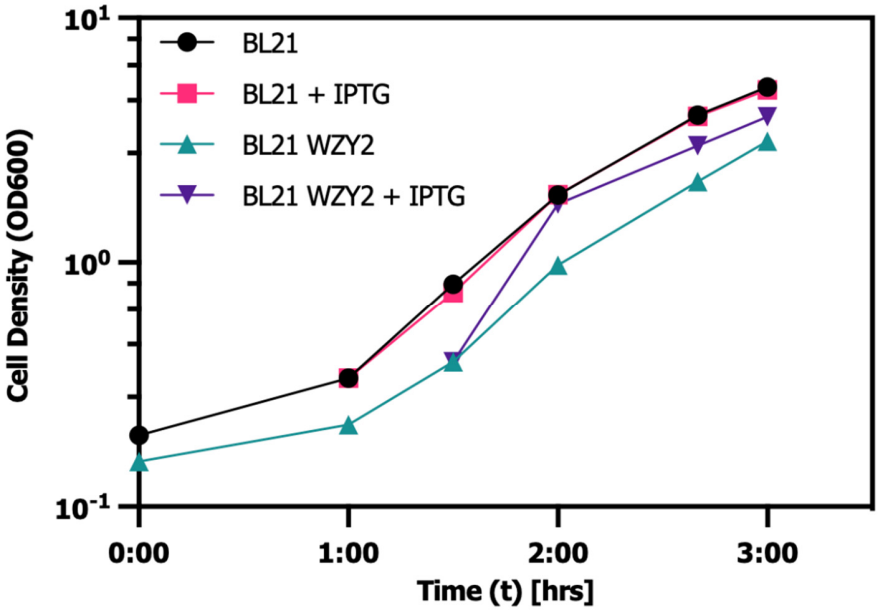

Figure S4. Growth analyses of BL21 Star (DE3) cells transformed with and without the WZY2 chaperone component at 37 °C. In anticipation of future preservation and other cell-free studies, we examined the effects of WZY2 on *E. coli* cell growth. BL21 Star (DE3) cells transformed with and without WZY2 were grown for 3 h at 37 °C and 225 revolutions per minute (rpm). IPTG was added at 1 h for the non-transformed cells and 1.5 h for the transformed cells. Growth slowed considerably for the transformed cells, and the outright dry cell weight (dcw) was lower than the non-transformed sample following centrifugation.

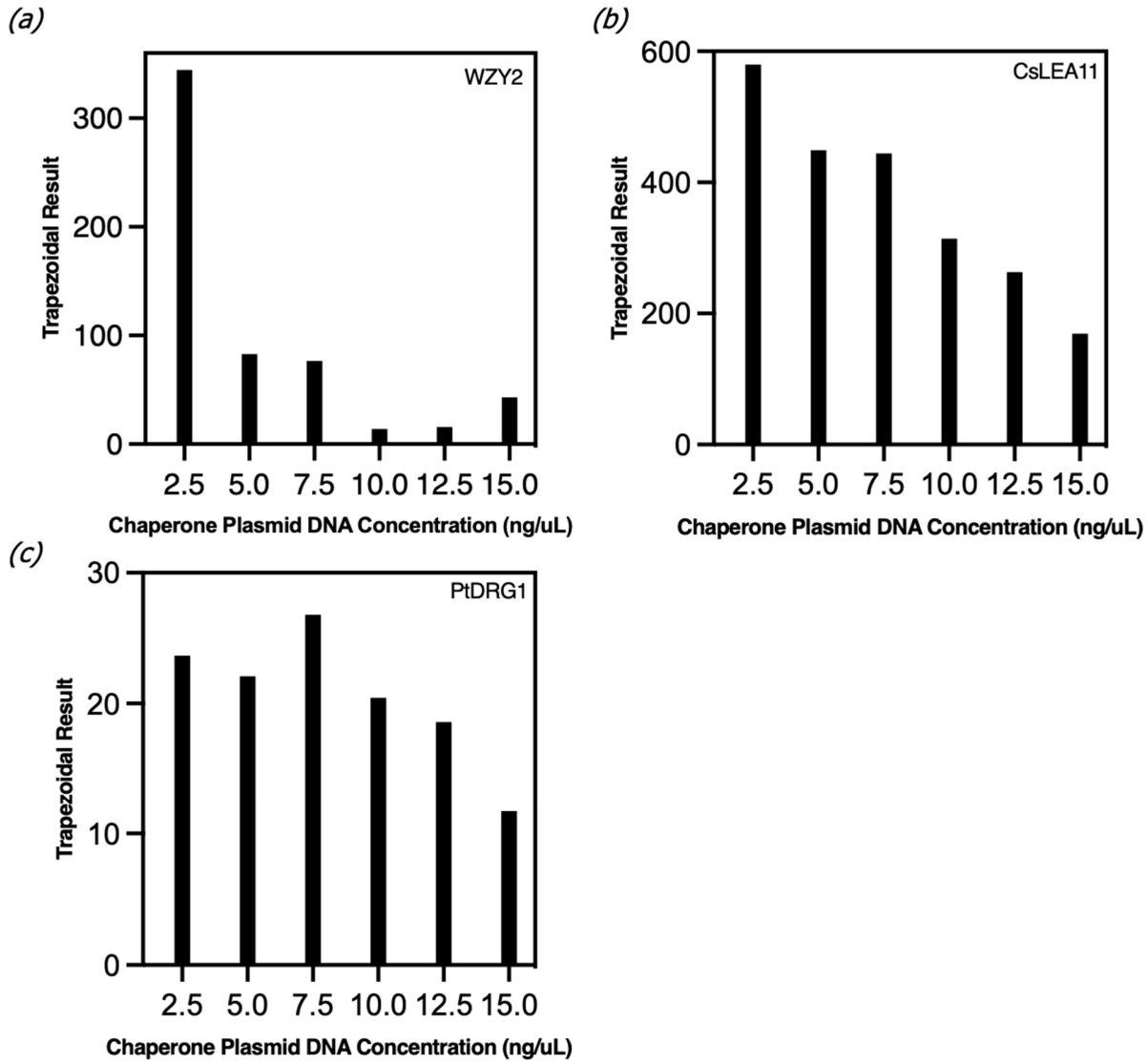

Figure S5. The integration result of the chaperone expression curves from Figure 1. All expression results were normalized to their EGFP expression curve, and the integral was found across each chaperone concentration for the chaperones (a) WZY2, (b) CsLEA11, and (c) PtDRG1. This was done to identify which chaperone DNA concentration was best across the temperatures and conditions tested. For WZY2 and CsLEA11, 2.5 ng/μL gave the largest result. For PtDRG1, 7.5 ng/μL gave the largest result.

(a)

Coefficients	Intercept	Linear term	Quadratic term	Cubic term	Quartic term	R-squared
WZY2	74,917	-494,363	216,369	-27,444	-9,645	0.97
CsLEA11	57,909	-390,690	183,884	-30,009	-20,186	0.93
PtDRG1	69,682	-467,276	216,430	-37,399	-10,088	0.95

(b)

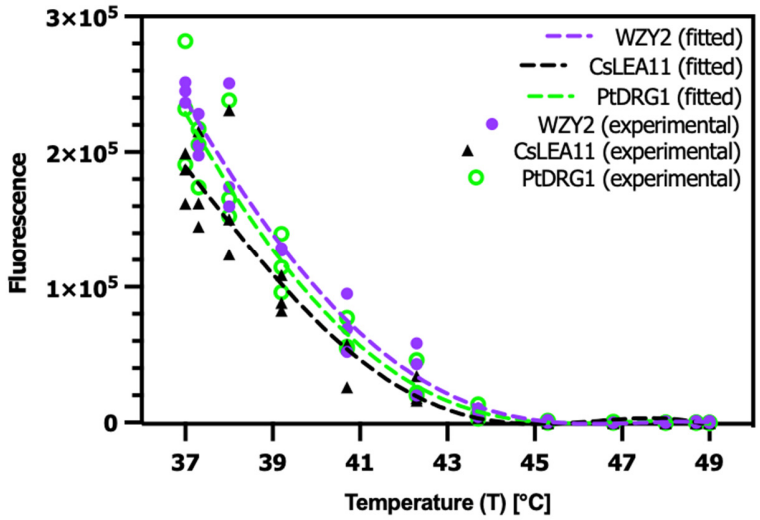

Figure S6. Experimental data for all three chaperone systems were fitted to polynomial regression functions. (**a**) Coefficients for each polynomial equation term are given in addition to the R-squared values. (**b**) Experimental and fitted data were graphed using R. These data are the same as those presented in Figure 3.