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SUPPORTING INFORMATION

A versatile and economical method for the release of recombinant proteins from *Escherichia coli* by 1-propanol cell disruption

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Transformation of EGFP construct into *E. coli* Rosetta-gami 2 (DE3) host strain

The EGFP gene amplified from pEGFP-N2 vector (Clontech) was cloned into pRSET-B vector (Invitrogen) at *Hind*III and *Bam*HI cutting sites. The pRSET-B vector containing EGFP gene was subsequently amplified and purified. Next, 4 µL of the purified pRSETB-EGFP construct was mixed well with *E. coli* strain Rosetta-gami 2 (DE3) competent cells using a vortexer. The transformation mixture was incubated on ice for 30 min followed by an incubation at 42 °C in static water bath for 1 min. Later, the mixture was immediately chilled on ice for 2 min. The transformation mixture was then transferred to 900-µL pre-warmed Super Optimal broth with Catabolite repression (SOC) medium [2% (w/v) tryptone; 0.5% (w/v) yeast extract; 10 mM NaCl; 2.5 mM KCl; 10 mM $MgCl_2$; 20 mM glucose; pH 7] and was shaken for 1 hr at 250 rpm and 37 °C. The cell culture was then spread on a plate of LB agar containing 100 µg mL^{-1} ampicillin. The *E. coli* strain Rosetta-gami 2 (DE3) tranformants were selected from the LB agar plate using blue/white method.

Preparation of buffered 1-PrOH solution at different pH

Firstly, the buffer at a given pH was prepared according to the methods described below. The molarity of the buffer is between 0.05 M and 0.1 M. The buffered 1-PrOH solution was then prepared at a desired concentration (%, v/v) by mixing the known volumes of 99.5% 1-PrOH and the specific buffer solution. Lastly, the pH of the mixture was adjusted with an acid solution (acetic acid for sodium acetate buffer; phosphoric acid for Sørensen's phosphate buffer) or NaOH solution.

1-PrOH + sodium acetate buffer (pH 5)

Sodium acetate buffer (pH5) was prepared using sodium acetate and acetic acid. [1]

1-PrOH + Sørensen's phosphate buffer (pH 6.2 and pH 8)

Sørensen's phosphate buffer (pH 6.2 and pH 8) was prepared using sodium phosphate monobasic and sodium phosphate dibasic. [2]

1-PrOH + glycine–NaOH buffer (pH 8.8 and pH 9.8)

Glycine–NaOH buffer (pH 8.8 and pH 9.8) was prepared using glycine and NaOH. [3]

1-PrOH + sodium phosphate –NaOH buffer (pH 11)

Sodium phosphate–NaOH buffer (pH 11) was prepared using sodium phosphate dibasic and NaOH solution. [4]

Preparation of EGFP standard

The EGFP expressed by the recombinant *E. coli* strain contains a polyhistidine tag that acts as the metal binding domain used in the purification via immobilized metal affinity chromatography (IMAC). In brief, the harvested cell culture was first homogenized by ultrasonication[5] followed by centrifugation at 5000 rpm and 4 °C for 30 min. The supernatant was subsequently loaded to a HiTrap™ chelating HP column (Amersham Bioscience, USA) connected to a fast performance liquid chromatography (FPLC) system (ÄKTA purifier 10; GE Healthcare Life Sciences, Sweden). The column was pre-equilibrated with binding buffer (20 mM sodium phosphate buffer; 0.5 M NaCl; 20 mM imidazole; pH 7.4). After the purification step, the elution of EGFP was carried out by flowing the elution buffer (20 mM sodium phosphate; 0.5 M NaCl; 500 mM imidazole; pH 7.4) through the column at 1 mL min^{-1}. The EGFP-containing fractions were subsequently pooled and concentrated using centrifugal concentrator [Vivaspin 500 (molecular weight cut-off = 3000 Da), Sartorius, Germany]. The purified EGFP sample was applied as a standard protein in SDS-PAGE analysis.

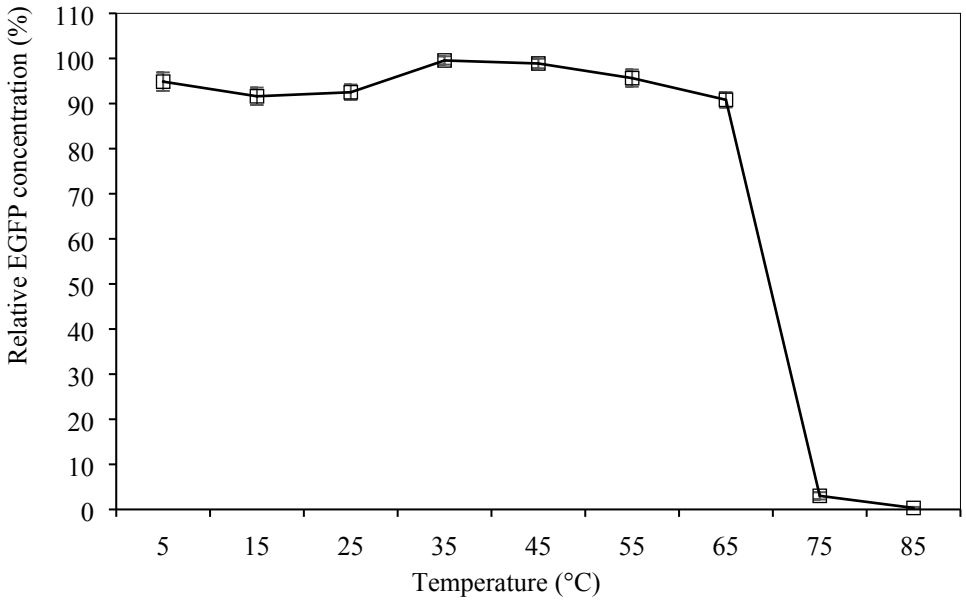

Figure S1: Percentage of relative EGFP stability (□) at different temperature. Experimental condition: pH 7.4 and 2 hr of incubation. The relative EGFP concentration (%) was calculated as the ratio of relative fluorescent unit (RFU) in the treated sample to that of the control sample. The EGFP suspended in phosphate buffer (pH 7.4) at room temperature was served as a control.

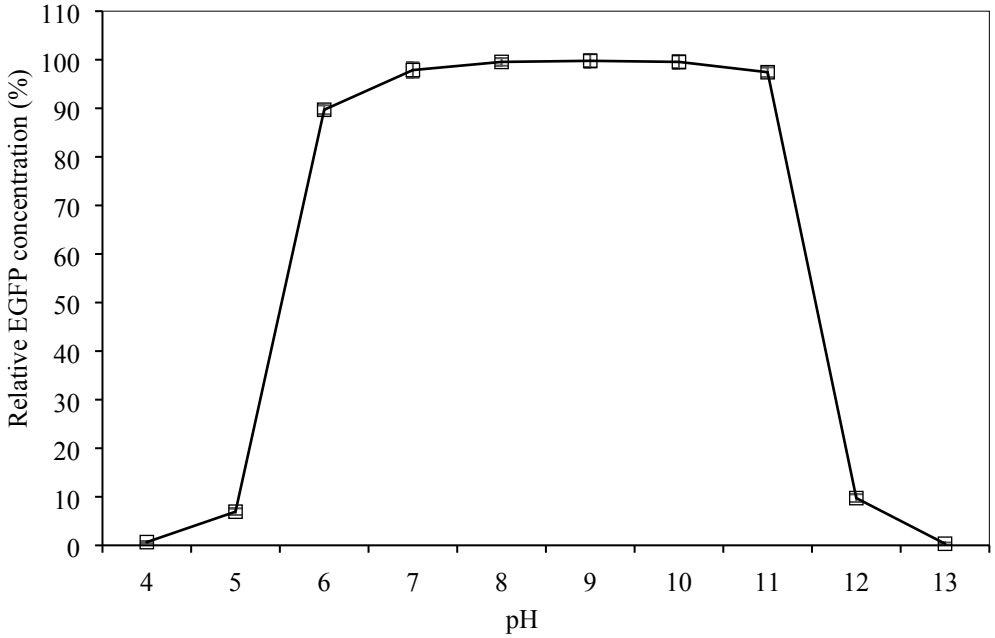

Figure S2: Percentage of relative EGFP concentration (□) at different pH. Experimental condition: room temperature and 2 hr of incubation. The relative EGFP concentration (%) was calculated as the ratio of relative fluorescent unit (RFU) in the treated sample to that of the control sample. The EGFP suspended in phosphate buffer (pH 7.4) at room temperature was served as a control.

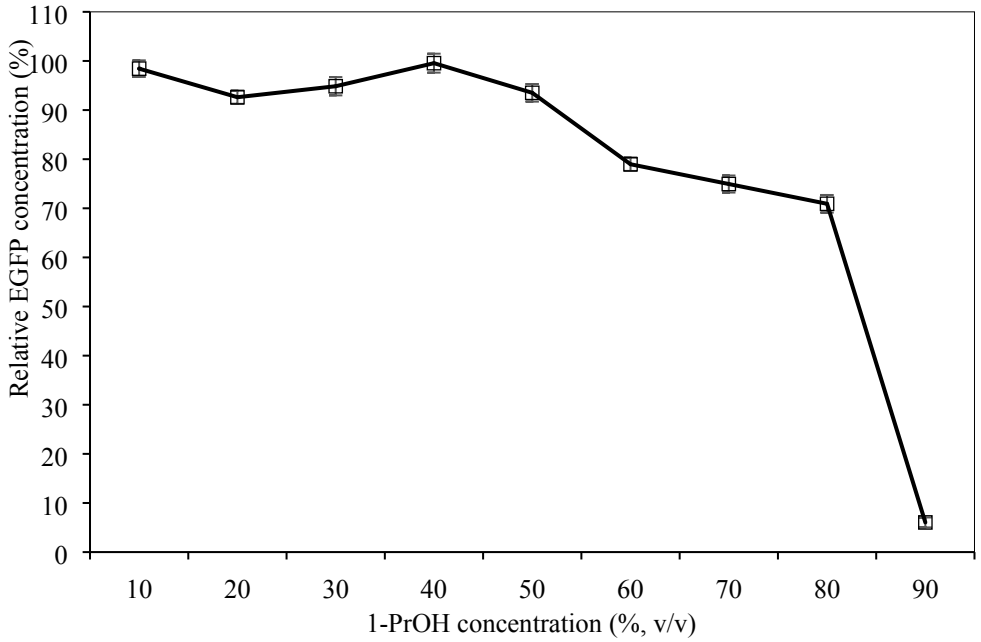

Figure S3: Percentage of relative EGFP concentration (□) at different concentrations of 1-PrOH. Experimental condition: room temperature and 2 hr of incubation. The relative EGFP concentration (%) was calculated as the ratio of relative fluorescent unit (RFU) in the treated sample to that of the control sample. The EGFP suspended in phosphate buffer (pH 7.4) at room temperature was served as a control.

Table S1. ANOVA for active EGFP release and total protein release in CCD.

a) active EGFP release

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	4.82	9	0.54	428.53	< 0.0001
A	0.18	1	0.18	145.72	< 0.0001
B	0.25	1	0.25	196.8	< 0.0001
C	0.68	1	0.68	545.21	< 0.0001
A2	1.51	1	1.51	1211.2	< 0.0001
B2	1.21	1	1.21	966.78	< 0.0001
C2	0.78	1	0.78	625.11	< 0.0001
AB	7.64×10^{-3}	1	7.64×10^{-3}	6.11	0.0386
AC	0.024	1	0.024	18.84	0.0025
BC	0.091	1	0.091	72.9	< 0.0001
Residual	0.01	8	1.25×10^{-3}		
Lack of Fit	4.67×10^{-3}	3	1.56×10^{-3}	1.46	0.3313
Pure Error	5.333×10^{-3}	5	1.067×10^{-3}		
Std. Dev.	0.035	R-Squared	0.9979		
Mean	0.7	Adj R-Squared	0.9956		
C.V.	5.03	Pred R-Squared	0.9845		
PRESS	0.075	Adeq Precision	49.24		

b) total protein release

Source	Sum of squares	DF	Mean square	F value	Prob > F
Model	16.99	9	1.89	106.19	< 0.0001
A	0.13	1	0.13	7.44	0.0259
B	1.11	1	1.11	62.46	< 0.0001
C	7.12	1	7.12	400.41	< 0.0001
A2	0.41	1	0.41	23.02	0.0014
B2	2.18	1	2.18	122.32	< 0.0001
C2	0.1	1	0.1	5.72	0.0437
AB	0.69	1	0.69	38.81	0.0003
AC	1.8	1	1.8	101.11	< 0.0001
BC	0.13	1	0.13	7.21	0.0277
Residual	0.14	8	0.018		
Lack of Fit	0.059	3	0.02	1.16	0.4099
Pure Error	0.084	5	0.017		
Std. Dev.	0.13	R-Squared	0.9917		
Mean	2.9	Adj R-Squared	0.9824		
C.V.	4.59	Pred R-Squared	0.9195		
PRESS	1.38	Adeq Precision	33.927		

Abbreviations: DF, degree of freedom; Prob>F, proportion of time or probability expect to get the stated F value; Std. Dev., standard deviation; C.V., coefficient of variance; PRESS, prediction residual sum of squares; R-squared, coefficient of determination; Adj R-squared; Adjusted R-squared; Pred R-squared, predicted R-squared; Adeq precision, adequate precision.

Table S2: Predicted and experimental values of active EGFP release obtained from the optimized condition of 1-PrOH cell disruption.

Optimized condition	Predicted value (mg mL^{-1})	Experimental value (mg mL^{-1})	Difference (%)
32.2% (v/v) 1-PrOH, 25 °C and pH 8.8	1.39	1.27	8.63

References

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