

Supplementary Information

A relationship between NTP and cell extract concentration for cell-free protein expression

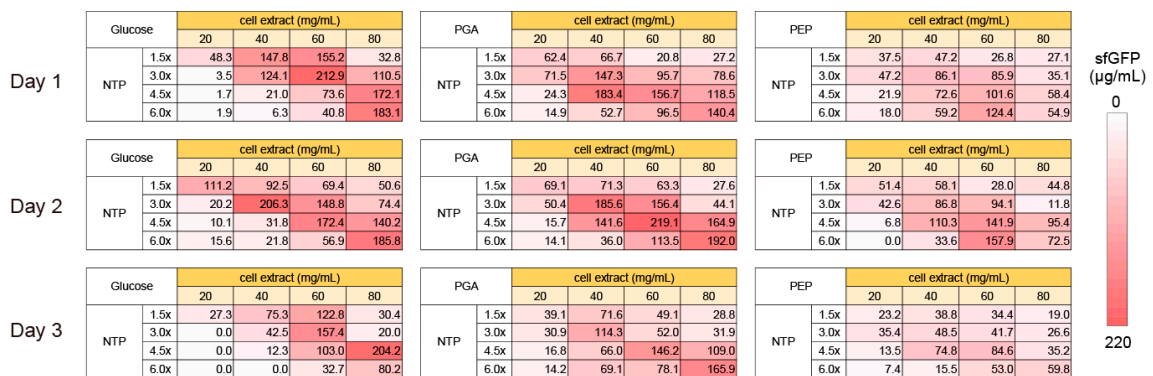
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Supplementary Figures S1-S4

GFP synthesis (μg/mL)		cell extract (mg/mL)			
		20	30	40	50
Mg conc (mM)	5	20±3	102±15	144±9	154±17
	10	205±37	298±27	149±11	118±6
	15	325±38	329±44	221±18	142±31
	20	198±26	332±39	191±9	103±43
	25	71±35	328±41	93±8	28±17

Supplementary Figure S1. Mg dependence of high concentration cell extract on CFPS activity.

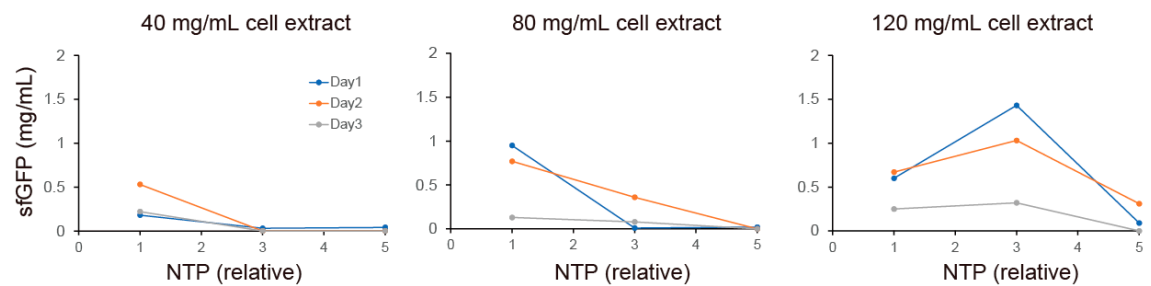
Averages and standard error of 3 times experiments performed in different days were shown.



Supplementary Figure S2. Energy source dependence of high concentration cell extract on CFPS activity. Raw data of Figure 4 are shown. The values indicate the levels of sfGFP synthesis (μg/mL).

Day 1	ATP (mM)	cell extract (mg/mL)				
		sfGFP (μg/mL)	20	40	60	80
		0	69.69	99.04	50.91	32.76
		5	48.81	117.36	98.22	69.72
		10	30.32	75.81	128.49	109.64
Day 2	ATP (mM)	cell extract (mg/mL)				
		sfGFP (μg/mL)	20	40	60	80
		0	28.51	38.93	28.97	12.51
		5	12.43	77.09	59.39	37.98
		10	6.98	35.18	80.59	51.89
Day 3	ATP (mM)	cell extract (mg/mL)				
		sfGFP (μg/mL)	20	40	60	80
		0	52.59	94.47	73.48	19.43
		5	63.66	107.08	111.38	94.32
		10	31.72	52.42	59.88	82.10
Day 1	ADP (mM)	cell extract (mg/mL)				
		sfGFP (μg/mL)	20	40	60	80
		0	69.69	99.04	50.91	32.76
		5	80.58	113.70	100.59	69.80
		10	44.24	111.45	145.83	98.18
Day 2	ADP (mM)	cell extract (mg/mL)				
		sfGFP (μg/mL)	20	40	60	80
		0	28.51	38.93	28.97	12.51
		5	25.01	74.90	62.35	30.25
		10	2.53	87.01	90.19	45.10
Day 3	ADP (mM)	cell extract (mg/mL)				
		sfGFP (μg/mL)	20	40	60	80
		0	52.59	94.47	73.48	19.43
		5	81.83	122.88	105.28	48.53
		10	83.44	85.69	110.24	93.87
Day 1	dATP (mM)	cell extract (mg/mL)				
		sfGFP (μg/mL)	20	40	60	80
		0	69.69	99.04	50.91	32.76
		5	38.62	67.99	111.56	68.24
		10	16.48	44.95	106.27	89.13
Day 2	dATP (mM)	cell extract (mg/mL)				
		sfGFP (μg/mL)	20	40	60	80
		0	28.51	38.93	28.97	12.51
		5	14.53	54.47	54.91	27.94
		10	6.22	21.29	44.28	36.94
Day 3	dATP (mM)	cell extract (mg/mL)				
		sfGFP (μg/mL)	20	40	60	80
		0	52.59	94.47	73.48	19.43
		5	20.01	57.92	74.86	55.12
		10	3.77	16.87	14.72	62.81
Day 1	Adenosine (mM)	cell extract (mg/mL)				
		sfGFP (μg/mL)	20	40	60	80
		0	69.69	99.04	50.91	32.76
		5	77.57	109.32	82.74	58.05
		10	91.00	126.44	97.82	75.38
Day 2	Adenosine (mM)	cell extract (mg/mL)				
		sfGFP (μg/mL)	20	40	60	80
		0	28.51	38.93	28.97	12.51
		5	58.19	87.07	85.09	18.17
		10	44.22	83.05	70.23	27.77
Day 3	Adenosine (mM)	cell extract (mg/mL)				
		sfGFP (μg/mL)	20	40	60	80
		0	52.59	94.47	73.48	19.43
		5	88.37	128.70	108.10	24.10
		10	107.33	145.59	125.45	44.38
Day 1	Cell extract (mg/mL)	sfGFP (μg/mL)				
		20	40	60	80	
		0	69.69	99.04	50.91	32.76
		5	77.57	109.32	82.74	58.05
		10	91.00	126.44	97.82	75.38
Day 2	Cell extract (mg/mL)	sfGFP (μg/mL)				
		20	40	60	80	
		0	28.51	38.93	28.97	12.51
		5	58.19	87.07	85.09	18.17
		10	44.22	83.05	70.23	27.77
Day 3	Cell extract (mg/mL)	sfGFP (μg/mL)				
		20	40	60	80	
		0	52.59	94.47	73.48	19.43
		5	88.37	128.70	108.10	24.10
		10	107.33	145.59	125.45	44.38

Supplementary Figure S3. Effect of ATP and its derivative of high concentration cell extract on CFPS activity. Raw data of Figure 5 are shown. Setting of the heat map is the same as in Figure 5. The values indicate the levels of sfGFP synthesis (μg/mL).



Supplementary Figure S4. NTP requirement of high concentration cell extract for cell-free protein synthesis. Raw data of dialysis-mode results in Figure 6 are shown.