Supplementary Information

Gene expression dynamics in input-responsive engineered living materials programmed for bioproduction

Widianti Sugianto[1,2,3], Gokce Altin-Yavuzarslan[2,4], Benjamin I. Tickman[1,2,3], Cholpisit Kiattisewee[2,3], Shuo-Fu Yuan[5] ,Sierra M. Brooks[6], Jitkanya Wong[4], Hal S. Alper[5,6], Alshakim Nelson[2,4,*], James M. Carothers[1,2,3,*]

1: Department of Chemical Engineering
University of Washington
Seattle, WA 98195
United States

2: Molecular Engineering & Sciences Institute
University of Washington
Seattle, WA 98195
United States

3: Center for Synthetic Biology
University of Washington
Seattle, WA 98195
United States

4: Department of Chemistry
University of Washington
Seattle, WA 98195
United States

5: Institute for Cellular and Molecular Biology
University of Texas at Austin
Austin, TX 78712
United States

6: McKetta Department of Chemical Engineering
University of Texas at Austin
Austin, TX 78712
United States

*Corresponding authors.
alshakim@uw.edu

jcaroth@uw.edu

Supplemental information:

Supplementary Tables

Supplementary Table S1. Plasmids used in this study

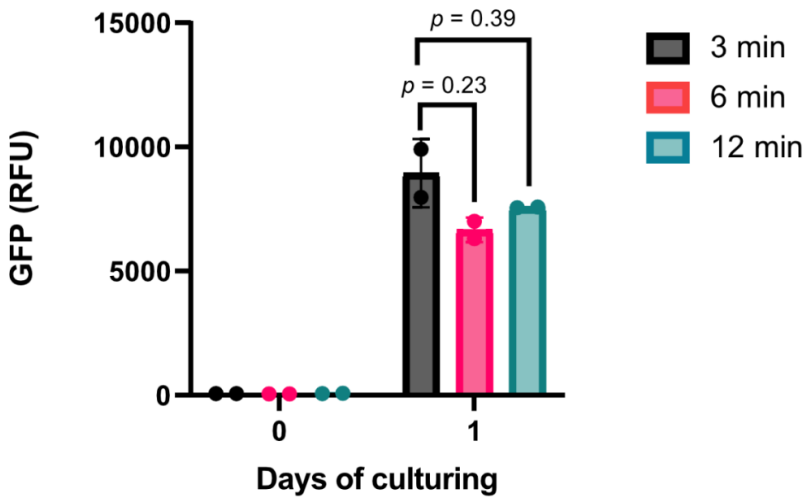
Plasmid or Integrative Cassette	Promoter	Minimal Promoter	CDS	gRNA	Terminator	Marker	Ori	Description
pJF143.J3.J23106.mRFP	J3	J23106	mRFP1	X	dbl Term	Carb	pSC101**	mRFP1 reporter plasmid (Supplementary Figs. S2 and S3)
pWS028.J3.J23106.sfGFP	J3	J23106	sfGFP	X	dbl Term	Carb	pSC101**	sfGFP reporter plasmid (Fig. 1c, Supplementary Fig. S1)
pCK430	X	X	Flp	X	X	Carb/ Kan	p15A	No reporter plasmid, expressing non-fluorescent protein (Fig. 1c)
pBT001-J2:RR2.CM.J23118	X	J23118	sfGFP	X	ECK120010818 Term	Carb	pSC101**	sfGFP reporter plasmid (Fig. 1b)
	J2	J23117	RR2		TrrnB			
	J3		mRFP1		dbl Term			
pCK389.306	X	J23119	X	J306	TrrnB	Chlor	p15A	Plasmid containing Inducible CRISPRa components, where pTet controls expression of TetR and MCP-SoxS (Figs. 2,3, and 4)
		Sp. Cas9	dCas9	X	dbl Term			
		pTet	MCP-SoxS	X	BBa_B102			
pPC003.J3.J23117.GFP	J3	J23117	sfGFP	X	dbl Term	Carb	pSC101**	sfGFP reporter plasmid (Figs. 2 and 3)
pCK014	J3	J23117	GTPCH	X	dbl Term	Carb	pSC101**	Multi-gene pathway of Pteridines (Fig. 4)
			PTPS	X	dbl Term			

pWS027.J3.dbl Term	J3	X	X	X	dbl Term	Carb	pSC101**	No reporter (no pathway) plasmid (Supplementary Fig. S7)
PITy3-GAL1-ScPEP4 (integrative cassette)	pGal1	X	ScPEP4	X	tPRM9	G418 (*S. cerevisiae*), Kan (*E. coli*)	ColE1	pITy3 backbone (Parekh, R.N., Shaw, M.R. and Wittrup, K.D. Biotechnol Progress, 1996) protease-production plasmid for integration into *S. cerevisiae* (Fig. 5)
P415-UraInt-pCup1-MjDOD-CYP76AD5 (integrative cassette)	pCUP1 (gene 1) pTDH3 (gene 2)	X	Gene 1: MjDOD (T261N) Gene 2: CYP76AD1	X	tTDH1	Leu (*S. cerevisiae*), Amp (*E. coli*)	ColE1	P415 Mumberg Backbone (Mumberg D, et al. Gene, 1995) betaxanthins-production plasmid for integration into *S. cerevisiae* (Fig. 5)

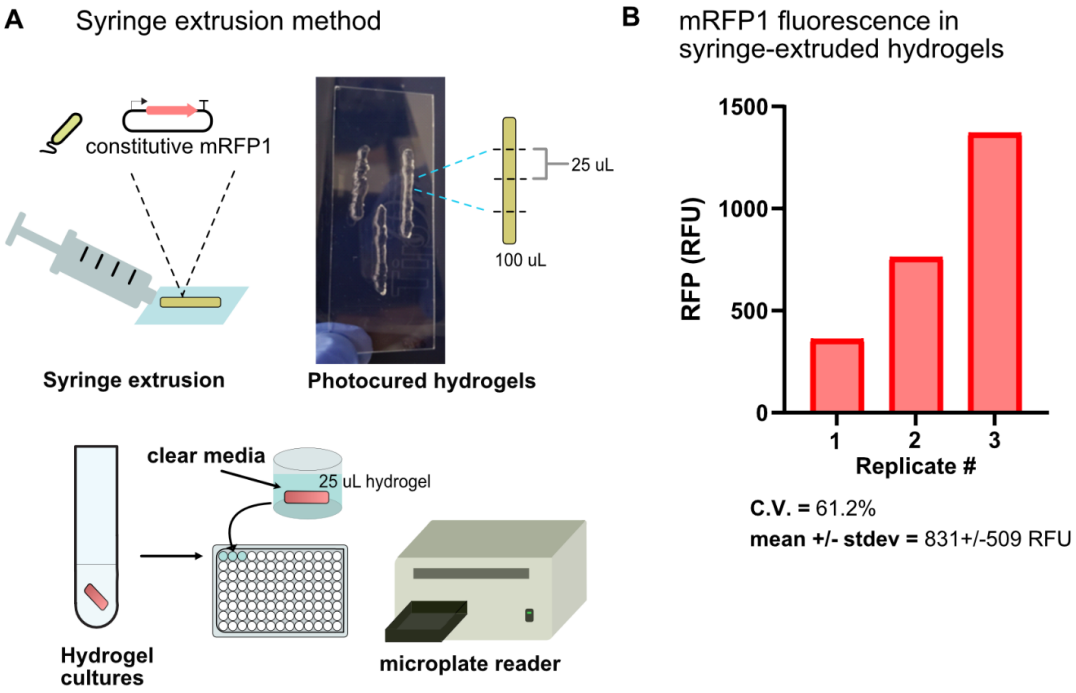
Supplementary Table S2. Direct-gel measurement parameters (mean expression, standard deviation, and coefficient of variation) for 3mm- and 4mm-diameter hydrogels expressing mRFP1 and sfGFP reporters.

Time (hr)	**sfGFP (RFU)**						**mRFP1 (RFU)**					
	d_{gel} = 3 mm			**d_{gel} = 4 mm**			**d_{gel} = 3 mm**			**d_{gel} = 4 mm**		
	Avg	**stdev**	**C.V. (%)**	**Avg**	**stdev**	**C.V. (%)**	**Avg**	**stdev**	**C.V. (%)**	**Avg**	**stdev**	**C.V. (%)**
0	29.2	1.1	3.8	37.8	3.3	8.7	57.6	8.4	14.7	106.2	16.6	15.6
12	40.8	8.2	20.0	56.4	5.1	9.1	102.0	20.5	20.1	144.2	10.5	7.3
24	**76.0**	**14.8**	**19.4**	**130.2**	**10.9**	**8.4**	**2096.6**	**1770.2**	**84.4**	**1568.0**	**1081.6**	**69.0**
36	88.0	11.7	13.3	191.2	17.9	9.4	2313.8	987.5	42.7	1732.4	835.8	48.2
Mean± stdev of C.V.	14.1 ± 7.6%			8.9 ± 0.4%			40.5 ± 31.7%			35.0 ± 28.7%		

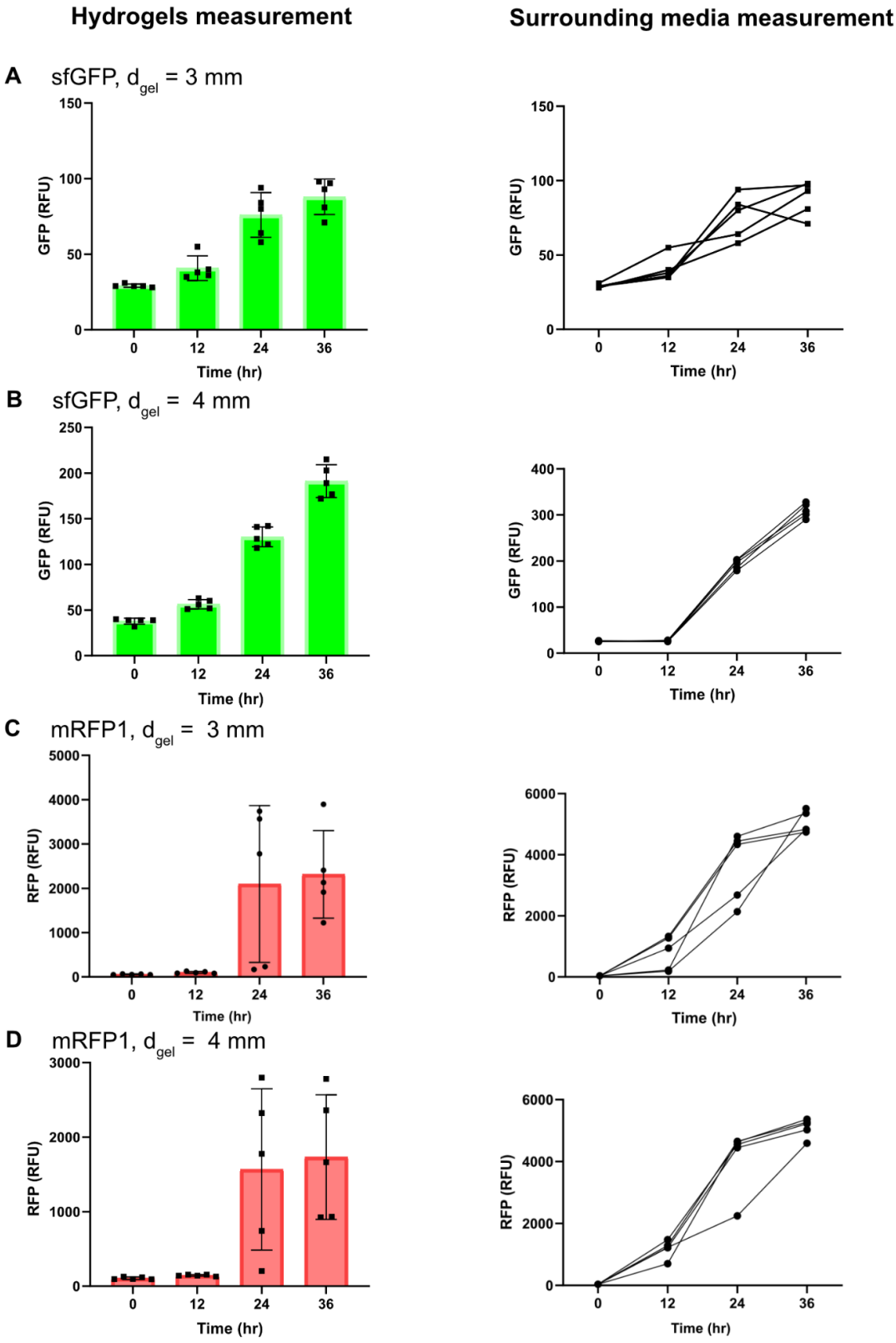
Supplementary Figures



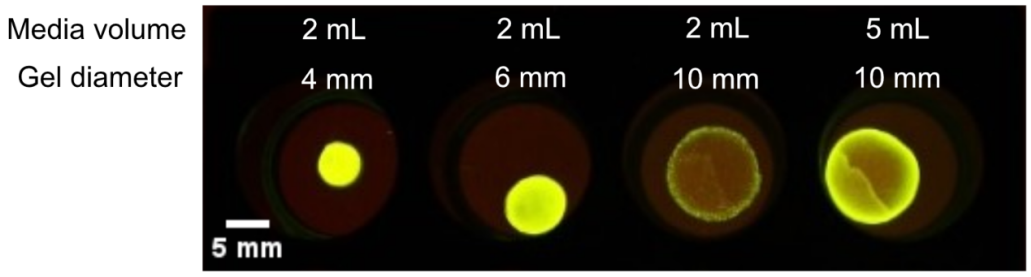
Supplementary Figure S1. Effect of UV curing duration on gene expression in ELMs. *E. coli* ELMs (diameter = 4 mm) were fabricated as described in the methods section (Section 2.1.3). ELMs were photocured under a UV 365 nm lamp for a total duration (curing both sides of the mold assembly) of 3, 6, and 12 minutes. The ELMs were cultured in EZ-RDM media as described in the methods section (Section 2.1.3) for 1 day. No significant differences (Student's *t*-test $p > 0.05$) in ELM sfGFP expression were observed among those three UV curing durations. Put another way, neither halving nor doubling the UV curing duration appears to impact ELM gene expression after one day of culturing compared to our standard 6 minute curing condition. Values represent the mean ± standard deviation from $n = 2$ technical replicates.



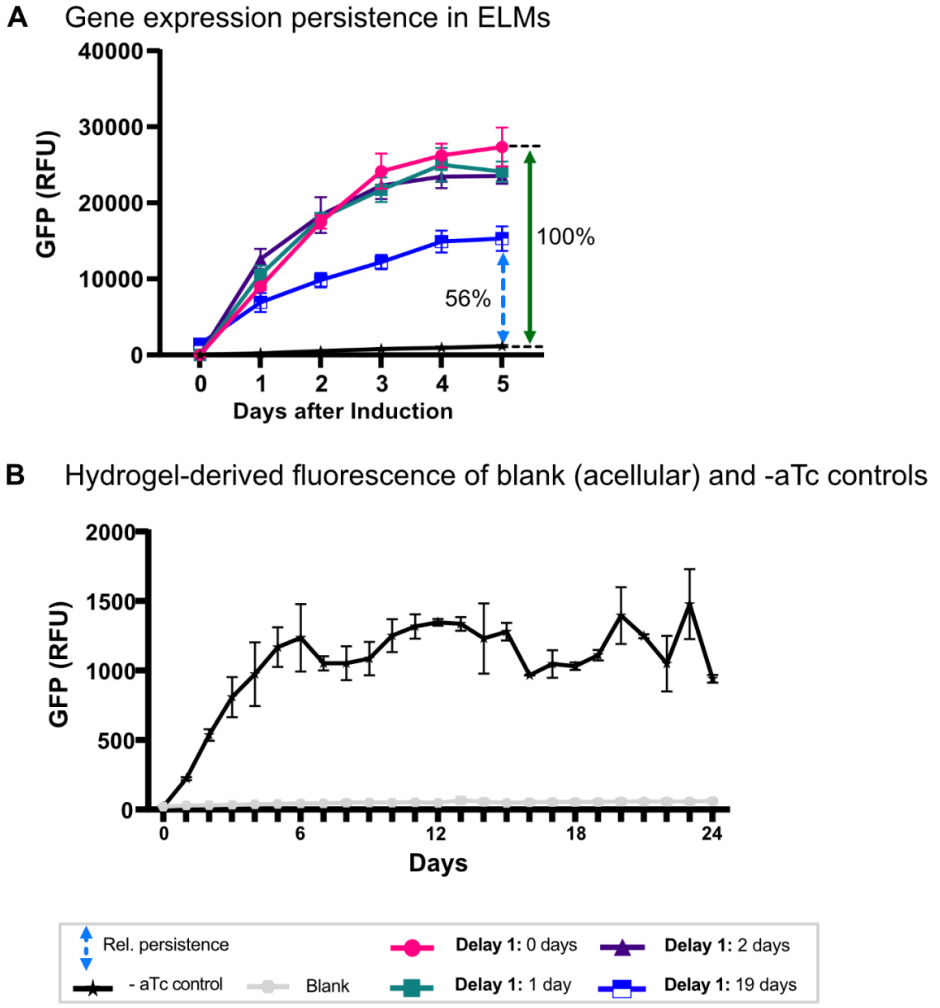
Supplementary Figure S2. Direct-gel measurement method for uncast (syringe-extruded) *E. coli*-laden hydrogels. **a)** 100 µL syringe-extruded hydrogel was seeded with mRFP1-expressing *E. coli* cells and divided into four equal parts (~25 µL) before culturing in EZ-RDM overnight. Plate reader measurement was conducted after culturing for 24 hours. **b)** Measured hydrogel-derived RFP fluorescence per hydrogel replicate (n = 3 technical replicates).


Hydrogels measurement
Surrounding media measurement
A
sfGFP, d gel = 3 mm
B
sfGFP, d gel = 4 mm
C
mRFP1, d gel = 3 mm
D
mRFP1, d gel = 4 mm
GFP (RFU)
RFP (RFU)
Time (hr)
0
12
24
36

Supplementary Figure S3. Direct-gel measurement for cast *E. coli* hydrogels of various diameter and fluorescent reporter combinations. Hydrogel-based fluorescence was measured using workflow depicted in Fig. 1 and reported as relative fluorescence units (RFU). Fluorescence of planktonic cells in surrounding media were also measured using a microplate reader. Values represent the mean ± standard deviation from n = 5 technical replicates. **a)** sfGFP, 3-mm diameter gels **b)** sfGFP, 4-mm diameter gels **c)** mRFP1, 3-mm diameter gels **d)** mRFP1, 4-mm diameter gels.



Supplementary Figure S4. GFP expression as a function of cast-gel diameter and culture volume. Cylindrical hydrogels (constant height = 2 mm) containing sfGFP-expressing *E. coli* were cast into molds of varying diameters (D = 4, 6, and 10 mm). Surface area-to-volume ratio (SA/V) of the hydrogels was decreased from 2.0 (D = 4 mm), to 1.7 (D = 6 mm), to 1.4 (D = 10 mm). Qualitatively, fluorescence is saturated in hydrogels with larger SA/V (D = 4 and 6 mm) cultured for 24 hours in 2 mL of EZ media. Relatively low fluorescence was observed in hydrogels with the smallest SA/V (D = 10 mm) cultured in 2 mL of media. Compared to this condition, increasing the culture volume to 5 mL for the largest (D = 10 mm) hydrogel increased the fluorescence.



Supplementary Figure S5. Dynamic sfGFP expression in *E. coli* ELMs. All values represent mean ± standard deviation from *n* = 5 technical replicates, except for 19-day delay* and uninduced** samples (**n* = 3, ***n*= 2 technical replicates). **a)** Gene expression persistence throughout 5 days of induction in ELMs, overlaid for all delay periods. **b)** Zoomed-in plot of blank (acellular) and uninduced (no aTc) gels controls for dynamic gene expression experiment (as depicted in Fig. 3b).

Equation S6.A Logistic fit for estimating expression level as a function of time

$$E(t) = \frac{E_{max} \times E_0}{E_0 + (E_{max} - E_0) \times e^{-r_1 t}}$$

Equation S6.B Rate of gene expression derived from logistic fit

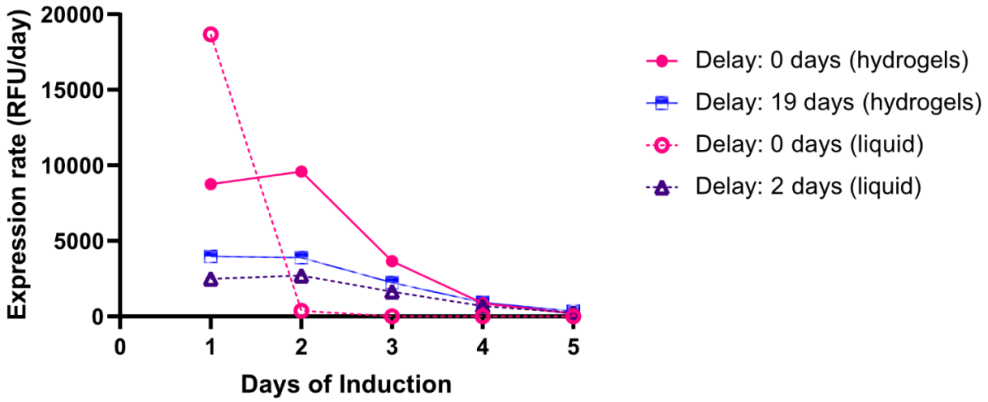
$$R(t) = \frac{A\, e^{r_2 t}}{(B + e^{r_2 t})^2}$$

Symbol	Description	Units
$E(t)$	Expression level as a function of time	RFU
E_{max}	Maximum level	RFU
E_0	Baseline level	RFU
r_1, r_2	Rate of growth	day^{-1}
t	Time	day
$R(t)$	Rate of gene expression	RFU/day
A	Lumped coefficients = $\frac{E_{max} E_0 r (E_{max} - E_0)}{E_0^2}$	-
B	Lumped coefficients = $\frac{(E_{max} - E_0)}{E_0}$	-

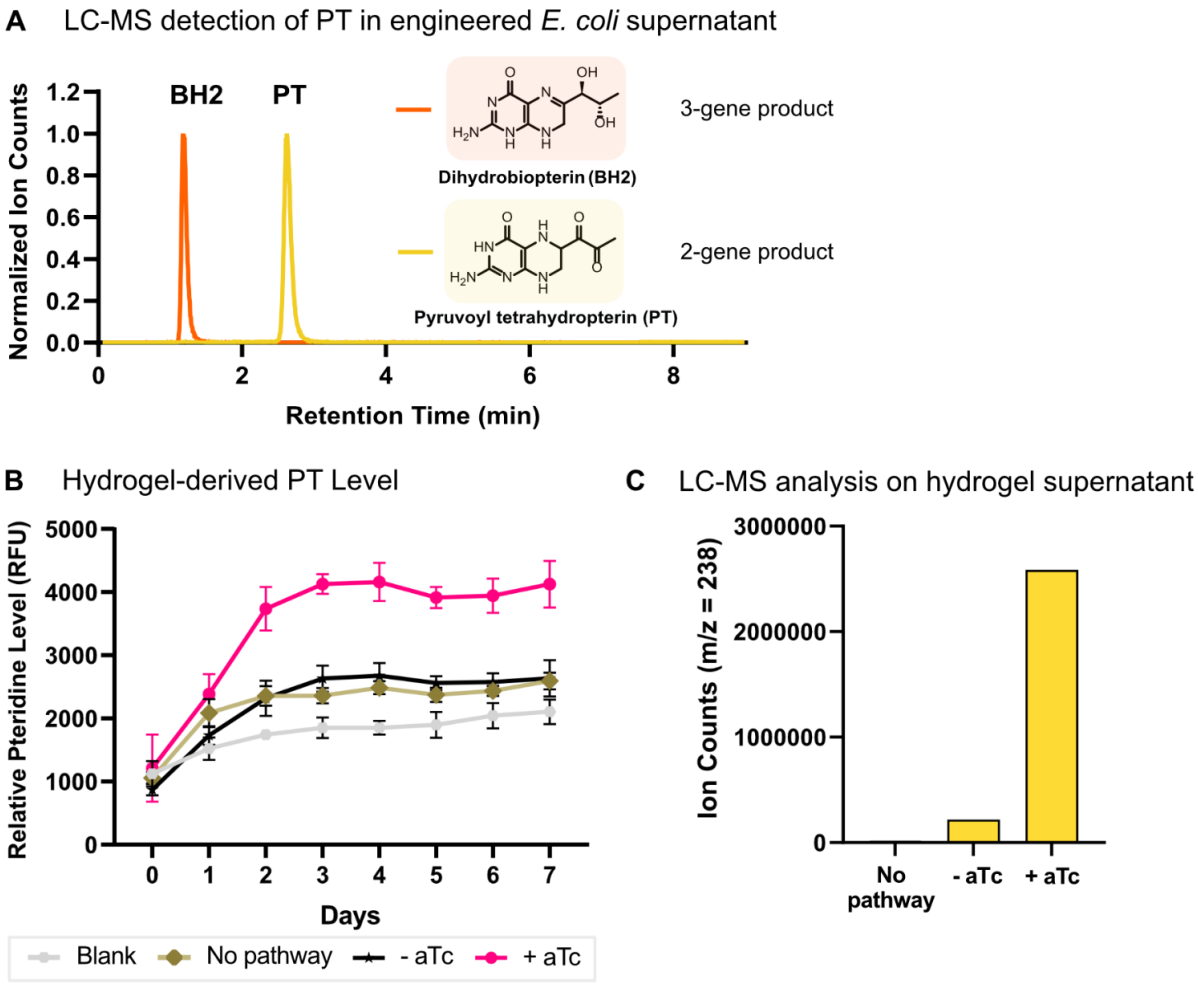
Table S6.1. Gene expression level and rate parameters

Continuous culture type	Delay (days)	Logistic fit parameters				Expression rate parameters		
		E_{max}	E_0	r_1	R^2	A	B	r_2
Liquid	0	17367	280	5	0.96	1349	0.02	-4.7
	2	9997	1364	1	0.97	1782	0.2	-1.1
Hydrogels	0	26976	2036	2	0.99	3503	0.08	-1.6
	19	15449	2573	1	0.97	3389	0.2	-1.1

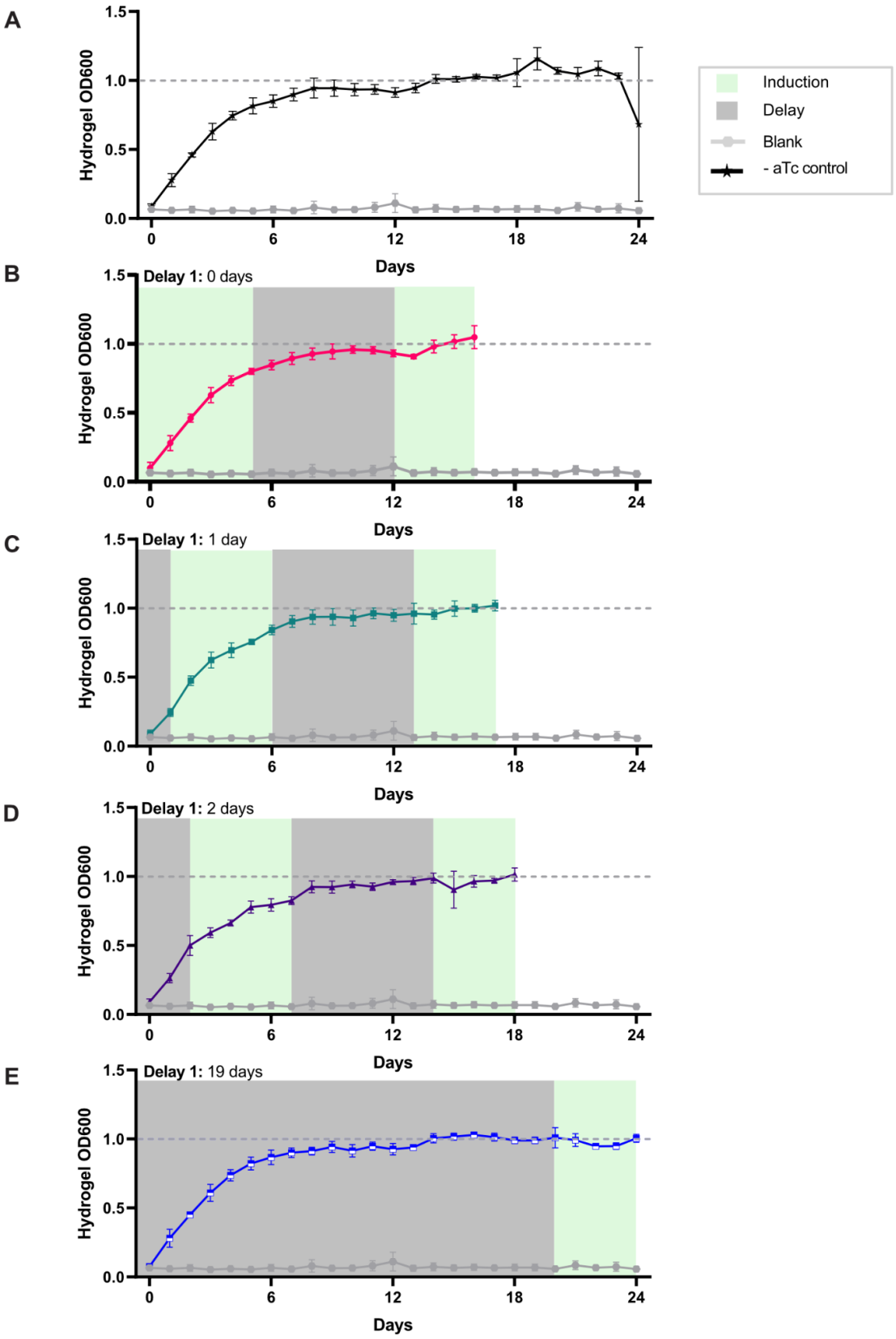
C Instantaneous rate of gene expression



Supplementary Figure S6. Curve fitting for levels and rates of gene expression calculation of *E. coli* in liquid vs. hydrogels continuous cultures. **a)** Logistic equation (**Equation S6.A**) fitted to expression level data for various delays in Fig. 2, resulted in R-squared values between 0.96-0.99. **b)** Rate of gene expression (**Equation S6.B**) obtained from deriving Equation S4.A. **c)** Instantaneous rates of gene expression from Equation S4.B with parameters tabulated in **Table S6.1**.



Supplementary Figure S7. Detection and measurement of PT fluorescence. **a)** Validation of PT production from engineered *E. coli* by LC-MS with m/z = 238.0935 (see Methods). Despite having the same exact mass as dihydrobiopterin (BH_2), PT has a distinct retention time (RT = 2.6 min) compared to that of BH_2 (RT = 1.2 min) produced in a previous work by Kiattisewee *et al.* **b)** Relative PT level measured from hydrogels using direct-gel measurement workflow. No-pathway hydrogels contained *E. coli* with an identical plasmid backbone to the pathway plasmid, but harborning no pathway genes. Values represent the mean ± standard deviation from n = 4 technical replicates. **c)** LC-MS analysis for PT from hydrogel supernatant.


A
B
C
D
E
Hydrogel OD600
Days
Induction
Delay
Blank
- aTc control
Delay 1: 0 days
Delay 1: 1 day
Delay 1: 2 days
Delay 1: 19 days
0.0
0.5
1.0
1.5
0
6
12
18
24

Supplementary Figure S8. Optical density (OD600) of *E. coli* ELMs as measured using the direct-gel measurement method corresponds to the experimental results plotted in Figures 2 and 3. **a)** Uninduced (no aTc) control **b)** ELM with no induction delay (**Delay 1:** 0 days) **c)** ELM with a 1-day induction delay (**Delay 1:** 1 days) **d)** ELM with a 2-day induction delay (**Delay 1:** 2 days) **e)** ELM with a 19-day induction delay (**Delay 1:** 19 days). The same optical density measurements for blank hydrogel samples without cells are included on all of the subplots for comparison. Values represent the mean ± standard deviation from n = 5 technical replicates, except for 19-day delay samples, where n = 3 technical replicates.

References:

[1] D. Mumberg, R. Müller, M. Funk, Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds, Gene. 156 (1995) 119–122. https://doi.org/10.1016/0378-1119(95)00037-7.

[2] R.N. Parekh, M.R. Shaw, K.D. Wittrup, An Integrating Vector for Tunable, High Copy, Stable Integration into the Dispersed Ty δ Sites of Saccharomyces cerevisiae, Biotechnology Progress. 12 (1996) 16–21. https://doi.org/10.1021/bp9500627.