Supplementary Information

Genetic Control of Radical Crosslinking in a Semi-Synthetic Hydrogel

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Table S1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Description/Genotype	Reference or source
***S. oneidensis* Strains**		
MR-1	MR-1 (ATCC700550), wild-type strain	American-Type Culture Collection
JG749	Lacks outer membrane cytochromes, MtrC and OmcA; Δ*mtrC*Δ*omcA*	[1]
JG596	Lacks out membrane cytochromes MtrC, OmcA, and MtrF; Δ*mtrC*Δ*omcA*Δ*mtrF*	[1]
JG1194	Lacks numerous proteins responsible for EET, including outer membrane cytochromes, β-barrel cytochromes, and periplasmic electron carriers; ΔMtr	[2]
MR-1 + pCD7sfGFP	Wild-type with a LacI repressed *sfGFP* circuit (Fig. S16-17)	This work
JG596 + pCD7sfGFP	JG596 with a LacI repressed *sfGFP* circuit (Fig. S16-17)	This work
MR-1 + pCD8	Wild-type with an empty vector on the LacI repressed circuit	This work
JG596 + pCD8	JG596 with an empty vector on the LacI repressed circuit (Fig. S17)	This work
JG596 + pCD24r1	JG596 with a LacI repressed *mtrC* circuit (Fig. S16-17)	This work
***E. coli* Strains**		
MG1655	Wild-type strain	Lydia Contreras, U. of Texas at Austin
Plasmids		
pCD7sfGFP	See Fig. S16-17	This work
pCD8	See Fig. S17	This work
pCD24r1	See Fig. S16-17	This work

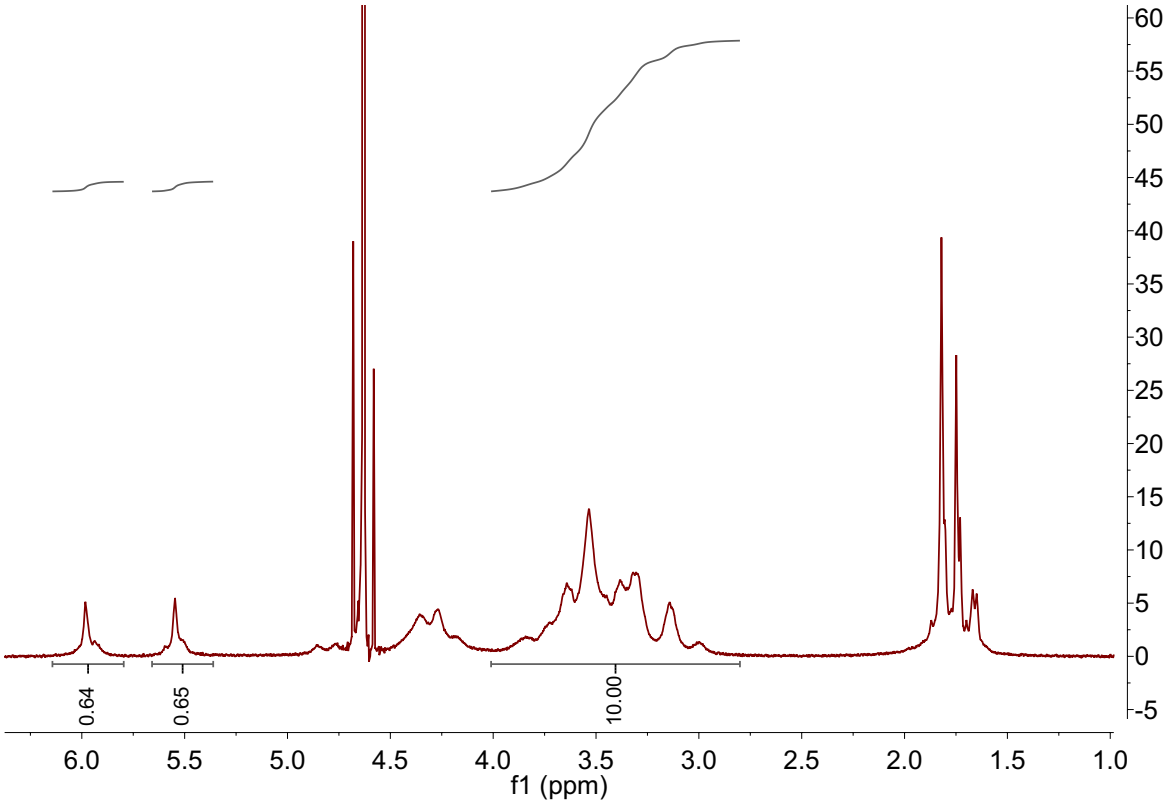

Figure S1. ^{1}H-NMR spectrum of 65% methacrylated hyaluronic acid macromer dissolved in D_2O after synthesis, dialysis, and lyophilization.

Table S2. Ingredients in *Shewanella* Basal Medium. Growth media was supplemented with casamino acids and Wolfe's mineral solution, whereas crosslinking media was only supplemented with casamino acids.

Ingredient	Quantity for 1L of 1x SBM
K_2HPO_4	225 mg
KH_2PO_4	225 mg
NaCl	460 mg
$(NH_4)_2SO_4$	1.703 mL of 1 M stock
$MgSO_4 \cdot 7H_2O$	0.475 mL of 1 M stock
HEPES	100 mL of 1 M stock
Casamino acids	5 mL of 10% stock
Wolfe's mineral solution	5 mL of 200x stock
ddH_2O	Up to 1 L, adjust to pH = 7.2

Table S3. Ingredients in Wolfe's mineral solution, adapted from ATCC recipe.

Reagent	Quantity in 1L of 200X Stock
EDTA	0.5 g (2.69 mL of 0.5 M stock)
$MgSO_4•7H_2O$	3.0 g
$MnSO_4•H_2O$	0.5 g
NaCl	1.0 g
$FeSO_4•7H_2O$	0.1 g
$Co(NO_3)_2•6H_2O$	0.1 g
$CaCl_2$	0.9 mL from 1 M stock
$ZnSO_4•7H_2O$	0.1 g
$CuSO_4•5H_2O$	10 mg
$AlK(SO_4)_2$	10 mg
H_3BO_3	10 mg
$Na_2MoO4•2H_2O$	10 mg
Na_2SeO_3	1 mg
$Na_2WO_4•2H_2O$	10 mg
$NiCl_2•6H_2O$	20 mg
ddH_2O	1 L

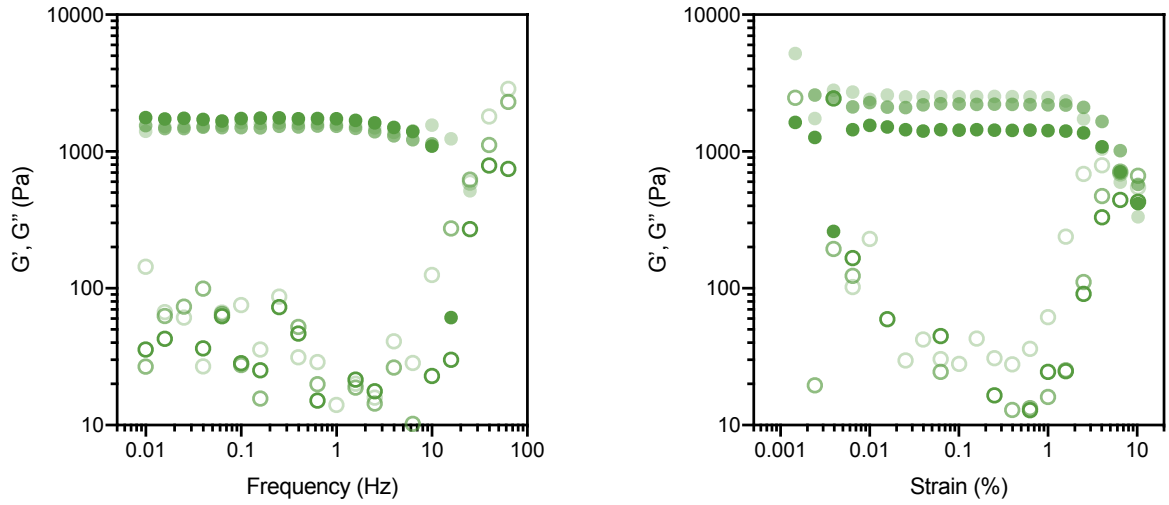

Figure S2. Strain and frequency sweeps of MeHA hydrogels crosslinked by *S. oneidensis* MR-1 at standard conditions (OD_{600} = 0.2 cells, 3 wt.% MeHA65, 10 μM Cu-TPMA, 500 μM HEBIB, 20 mM lactate, 40 mM fumarate) to determine storage modulus (G', closed circles) and loss modulus (G'', open circles) for *n* = 3 biological replicates. Storage modulus calculations for all subsequent gels were made using frequency sweeps at 0.1% strain, which is within the linear viscoelastic regime.

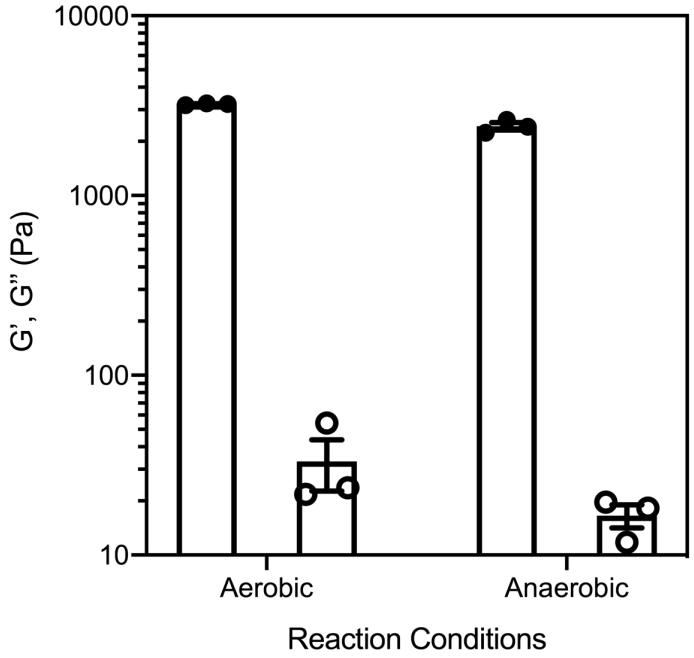

Figure S3. Storage modulus (G', filled circles) and loss modulus (G'', open circles) of hydrogels crosslinked by *S. oneidensis* MR-1 at standard conditions in either aerobic or anaerobic environments. Data shown are mean ± SEM for n = 3 biological replicates. MeHA65 networks are predominately elastic in nature, as demonstrated by significantly larger G' than G''.

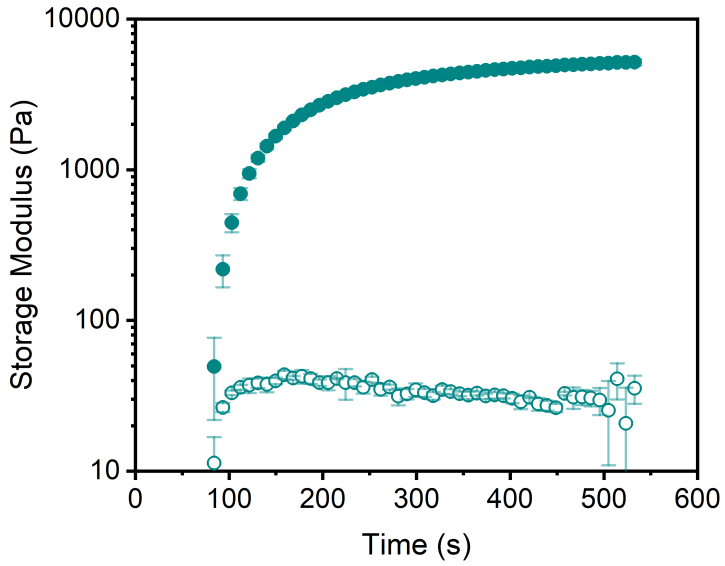

Figure S4. *In situ* rheology showing G' (filled circles) and G'' (open circles) of MeHA65 gels synthesized using 500 μM lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as a photoinitiator with 365 nm light at 10 mW/cm^2 laser intensity. Data are reported as the mean ± SD of n = 3 gels.

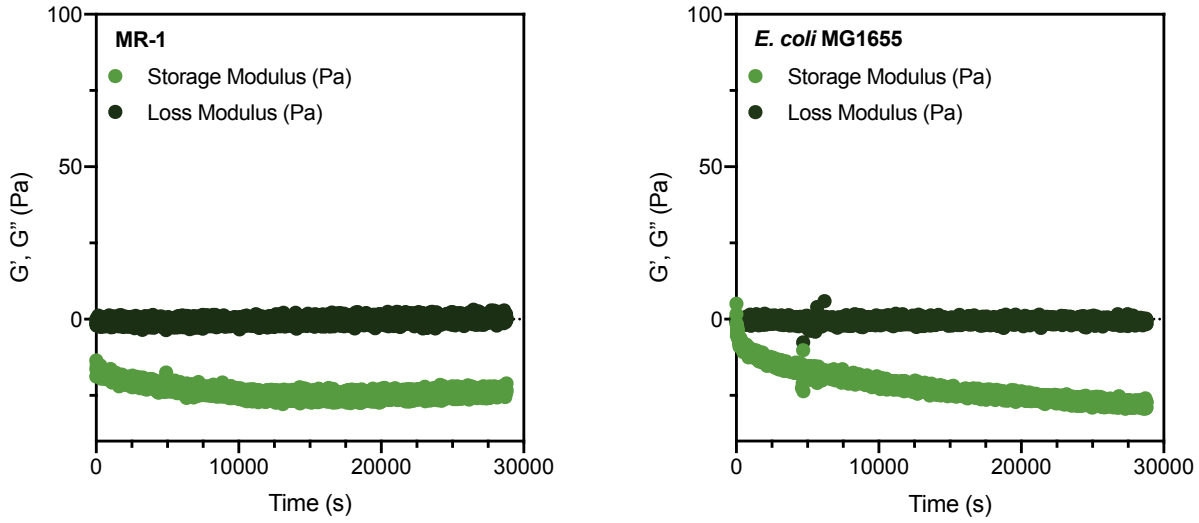

Figure S5. *In situ* rheology showing G' and G'' of non-functionalized HA solutions containing all crosslinking components and either MR-1 (left) or *E. coli* MG1655 (right). Gels did not form; negative G' is attributed to dominant inertial forces and small plate geometry (weak elastic component) (3).

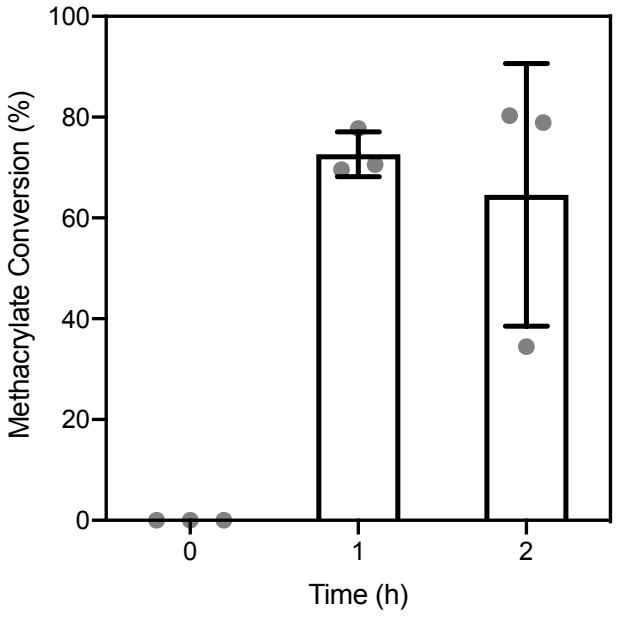

Figure S6. Methacrylate functional group conversion as determined by ^{1}H-NMR spectroscopy after enzymatically degrading MeHA hydrogels with hyaluronidase. Samples were diluted in D_2O and the integration values of the methacrylate peaks were normalized against the average value at t = 0 h to determine conversion. Gels were formed at standard conditions using *S. oneidensis* MR-1. Data reported are the mean ± SD of n = 3 gels.

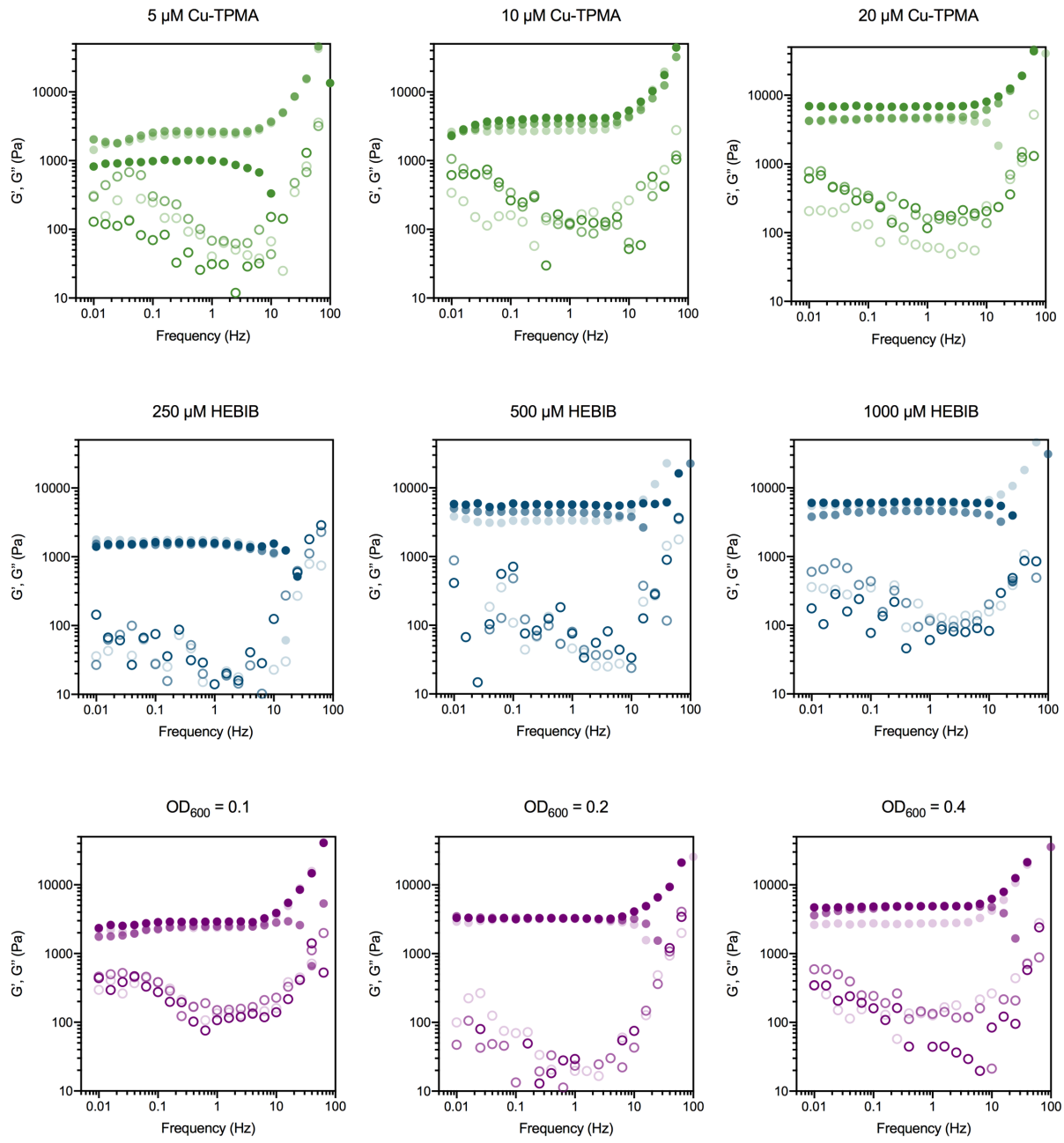

Figure S7. Rheological frequency sweeps for end-point measurements in Fig. 2. G' (filled circles) and G'' (open circles) for *n* = 3 biological replicates.

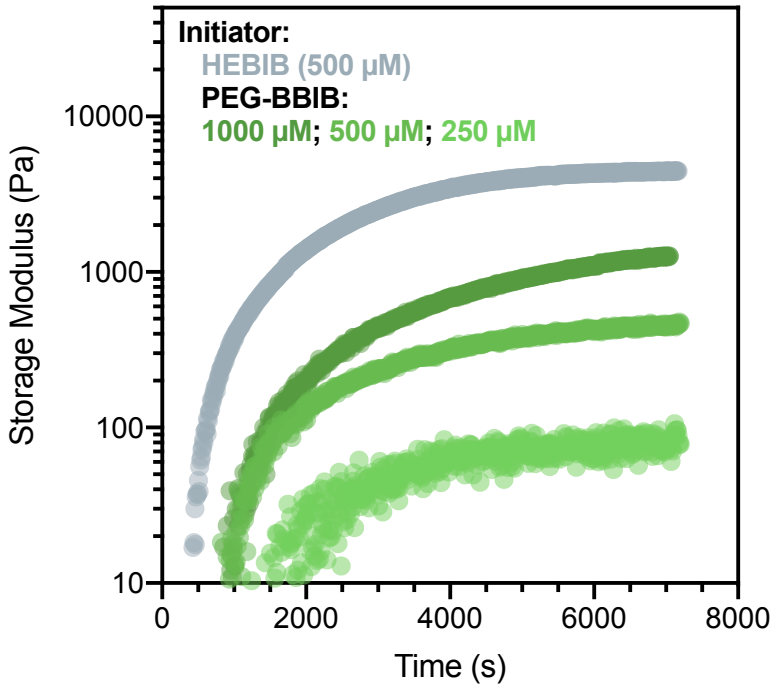

Figure S8. *In situ* G' measurement of MeHA65 gels synthesized using varying concentrations of poly(ethylene glycol) bis(2-bromoisobutyrate) ($M_{n,avg}$ = 700 g/mol, PEGBBiB). HEBiB, the initiator for standard gelation conditions, is included for comparison.

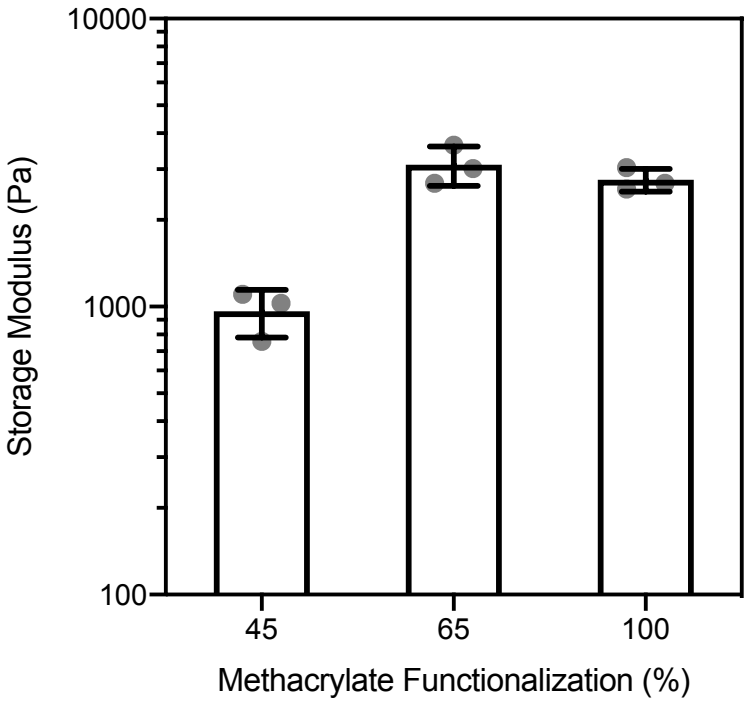

Figure S9. Storage modulus for MeHA hydrogels crosslinked at standard conditions for 2 hours with varying degrees of methacrylate functionalization of the HA backbone.

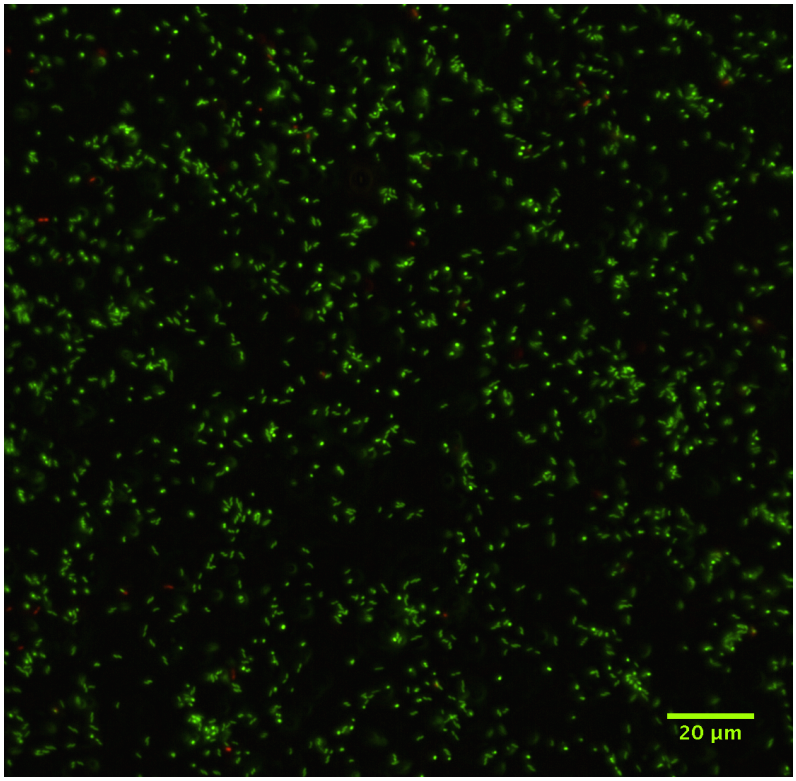

Figure S10. Viability analysis 5 days after crosslinking as determined by the BacLight Live/Dead stain. Cells are almost completely viable (green) as opposed to non-viable (red).

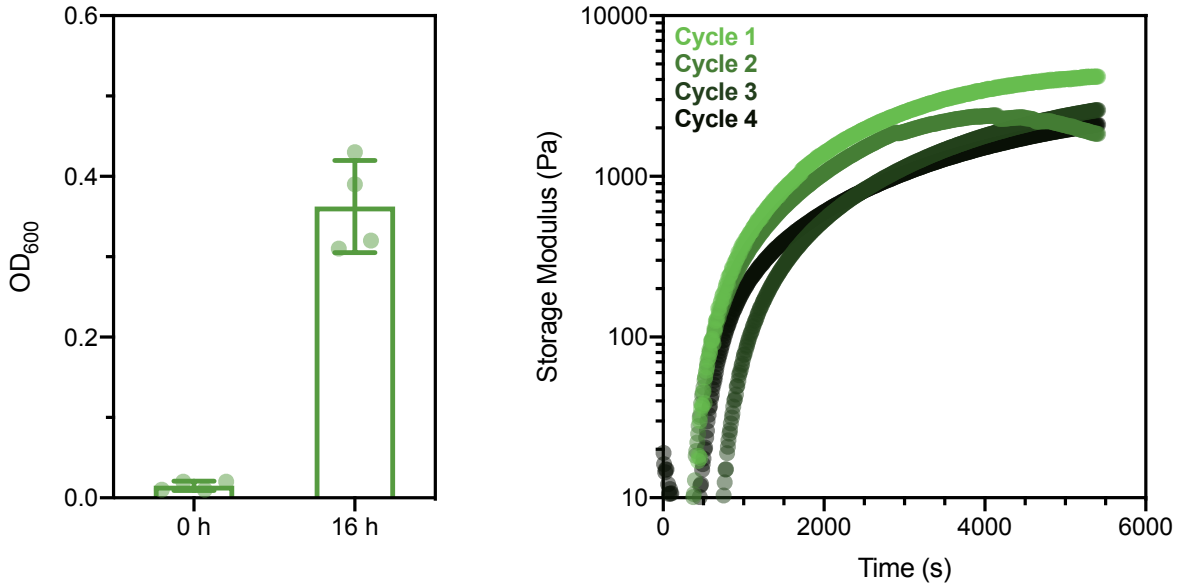

Figure S11. Cells can be recovered after crosslinking to synthesize new gels. Left, OD_{600} measurements of cultures after inoculating from swelling mixture into growth media, data are shown as mean ± SEM of *n* = 4 measurements. Right, *in situ* storage modulus measurements (right) of gels crosslinked by recovered bacteria cultures (inoculated from hydrogel swelling media and grown overnight).

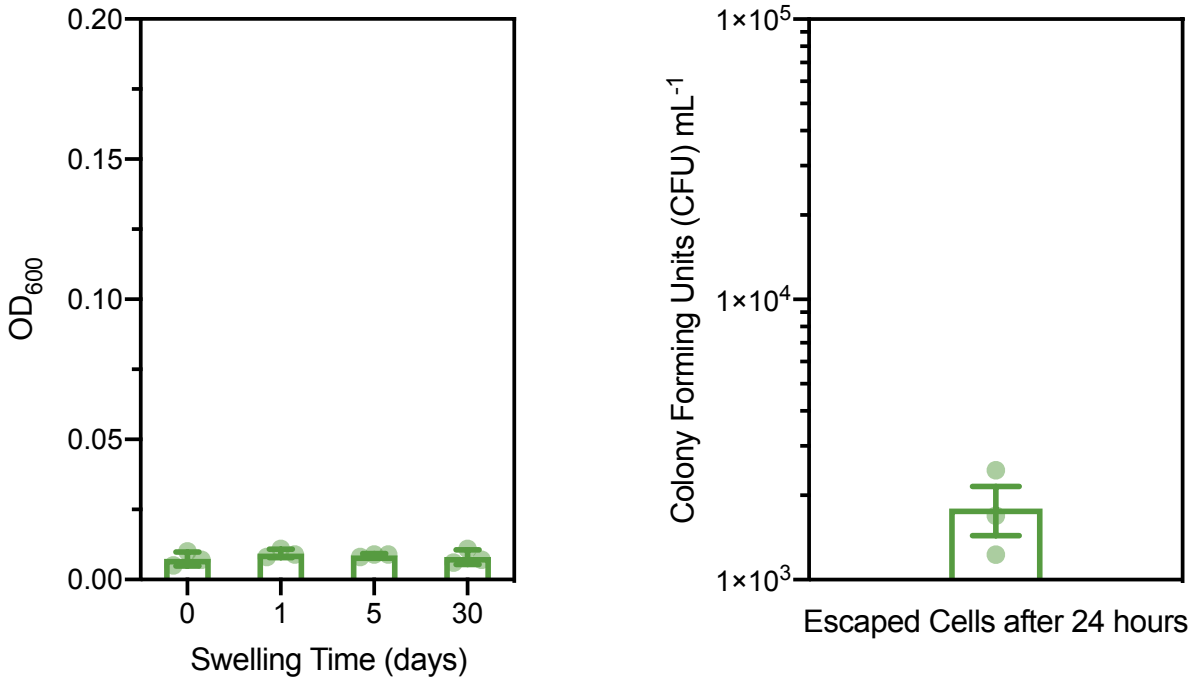

Figure S12. Bacteria do not appreciably escape the gels after synthesis. OD_{600} measurements of swelling media (left) after washing gels 3x in PBS and allowing to swell in 1 mL PBS for varying lengths of time. Colony counting (right) confirms that escaped cells account for < 0.005% of inoculating density (~4 x 10^7 CFU/mL or OD_{600} = 0.2) after 3x washing in PBS. Data are shown as mean ± SEM for *n* = 3 biological replicates.

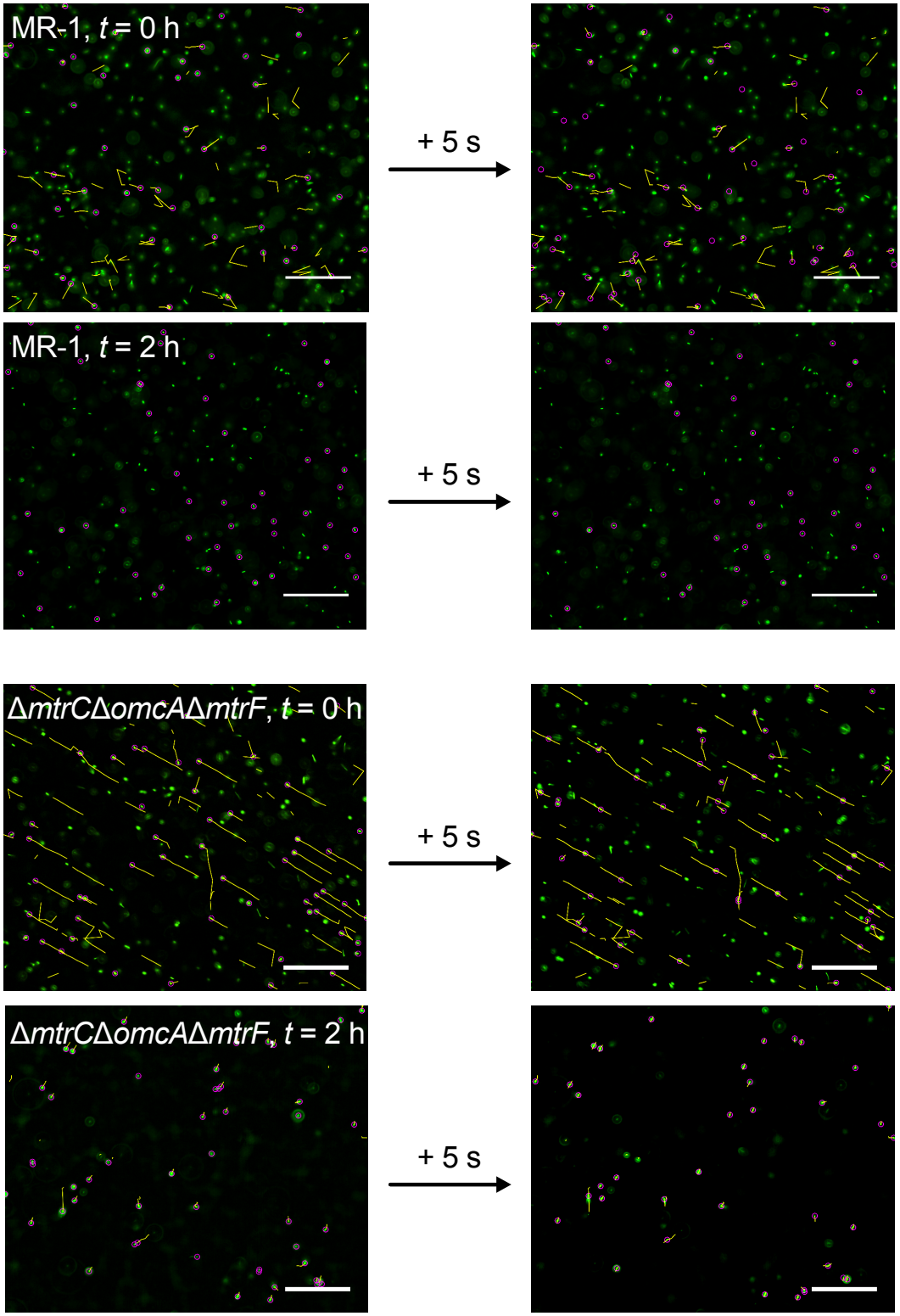

Figure S13. Fluorescence microscopy images of 1000 μM IPTG induced MR-1 and Δ*mtrC*Δ*omcA*Δ*mtrF* strains show swimming and convective movement over 5 seconds. Cells (purple circles) were tracked over 5 s (yellow lines) using TrackMate in Fiji 1.0. The average displacement of highest quality tracks (as determined by the software, n = 33) was then calculated for each strain at 0 and 2 hours. Scale bars are 40 μm.

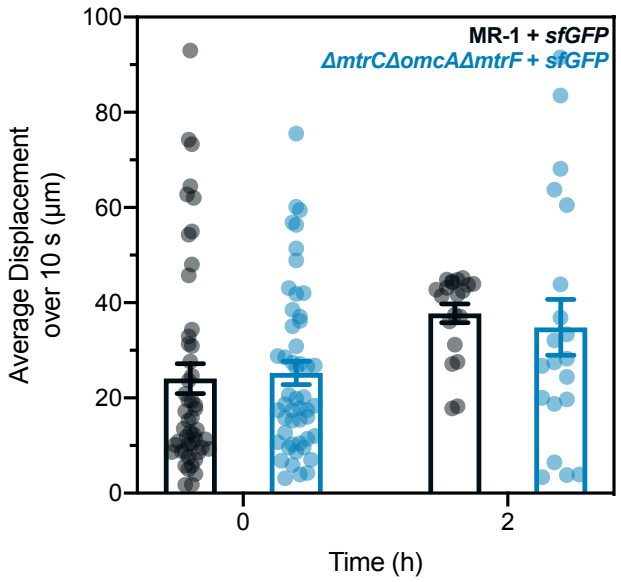

Figure S14. Average cell displacement within a non-functionalized 3 wt.% HA solution as measured by microscopy over 10 s time-lapses at 0 and 2 hours. Displacement was quantified using TrackMate in Fiji 1.0. Student t-test shows no statistical difference (p = 0.95 at 0 hours, n = 50; p = 0.76 at 2 hours, n = 20), indicating that differences in the functionalized MeHA is due to crosslinking. Data are shown as mean ± SEM.

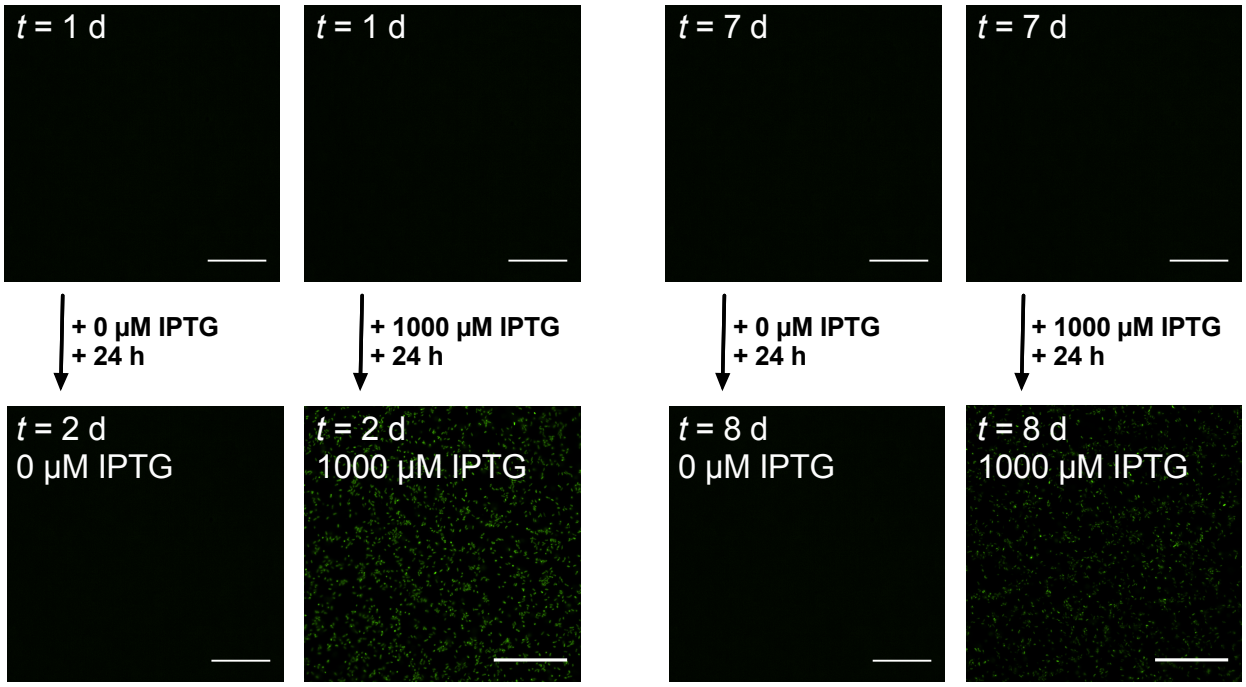

Figure S15. sfGFP fluorescence demonstrates metabolic activity for extended time periods after crosslinking. Crosslinked gels were swollen in 1x PBS for 1 and 7 days after crosslinking, then moved into 0 or 1000 μM IPTG-containing PBS for 24 h and imaged by microscopy. Gels imaged before overnight induction with IPTG did not fluoresce. All images taken with equivalent exposure times to allow comparison of protein expression and circuit maintenance. Scale bars are 40 μm.

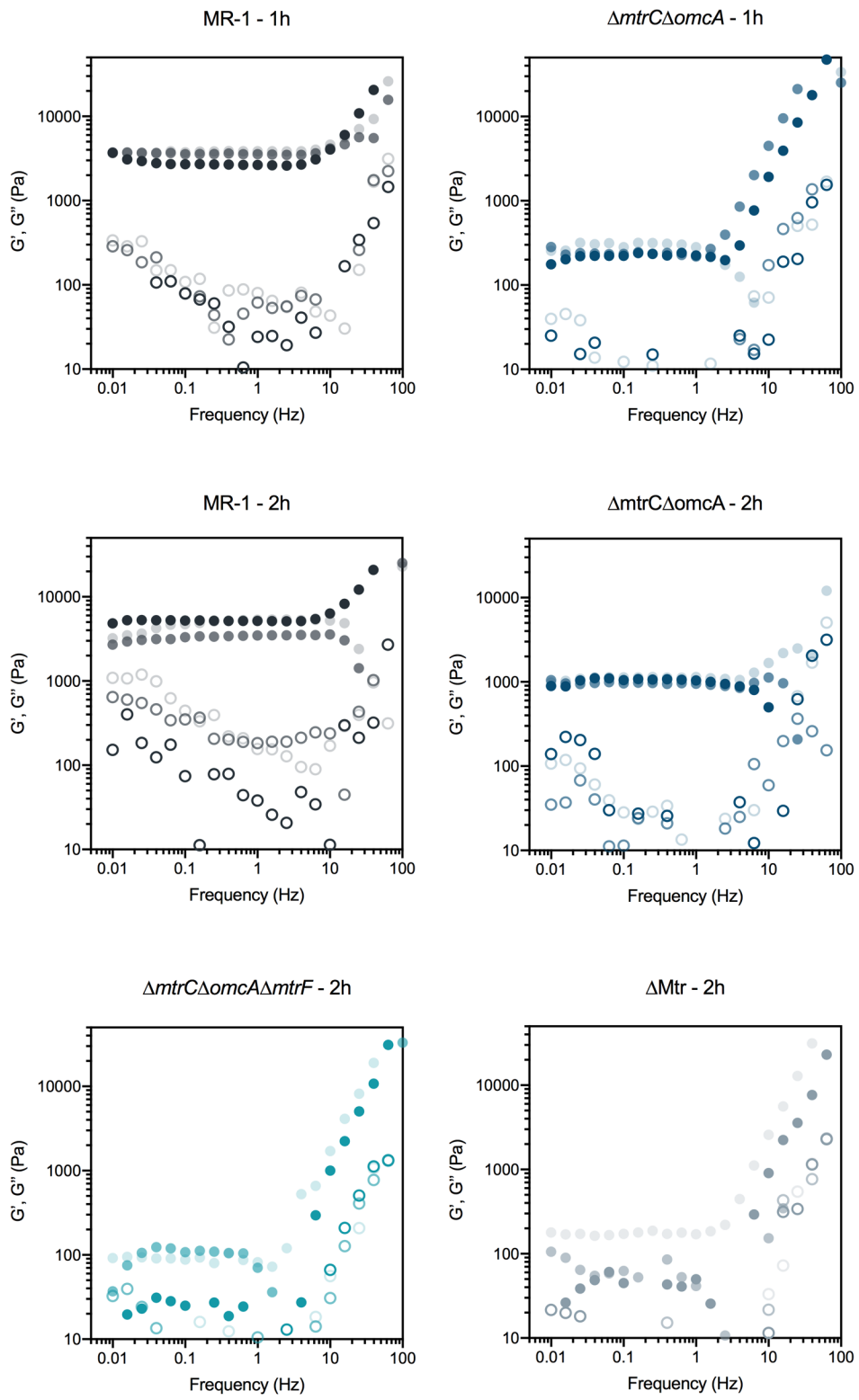

Figure S16. Rheological frequency sweeps for end-point measurements in Fig. 3. G' (filled circles) and G'' (open circles) for individual replicates.

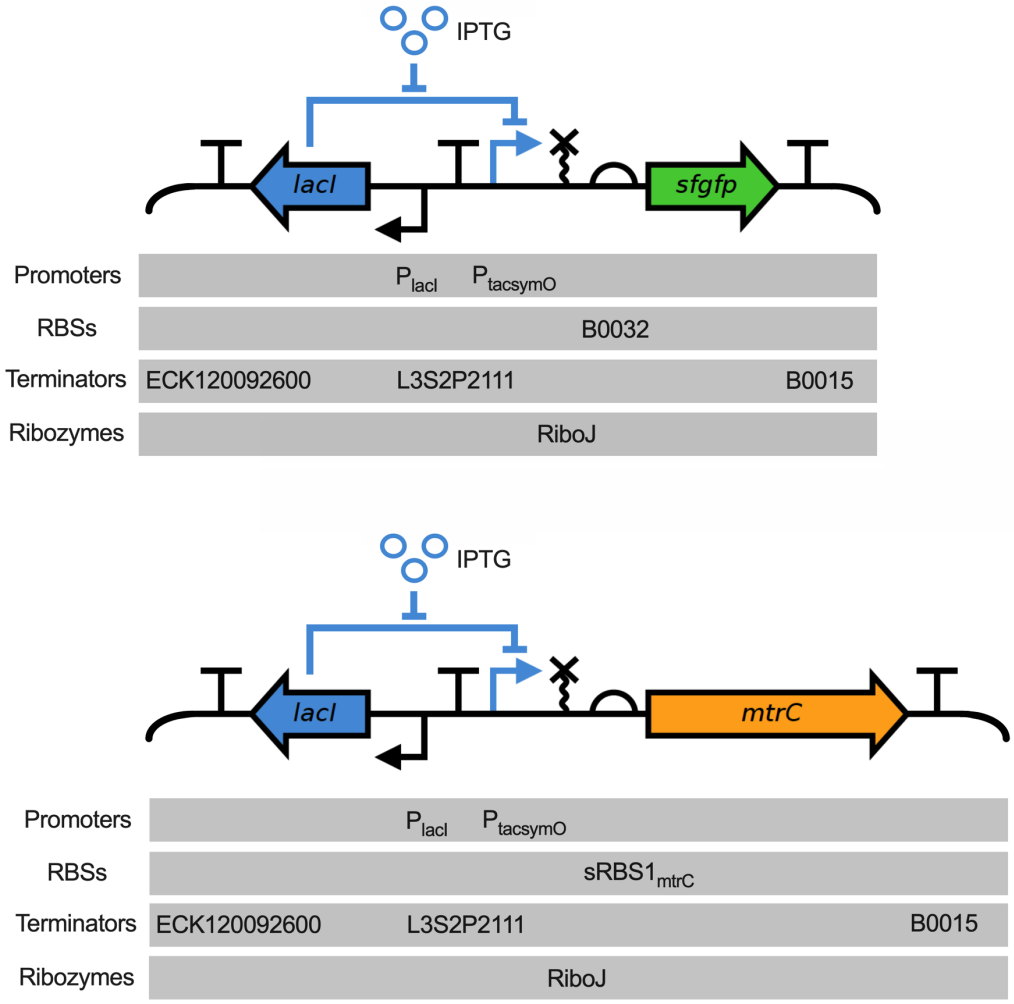

Figure S17. Gene circuit maps for both the *sfgfp* (top, pCD7sfGFP) and *mtrC* (bottom, pCD24r1) expression vectors. Outside of the flanking terminators, both vectors are identical and contain the ColE1 origin of replication and a kanamycin resistance marker. The empty vector (pCD8) is identical to pCD7sfGFP, but lacks *sfgfp*.

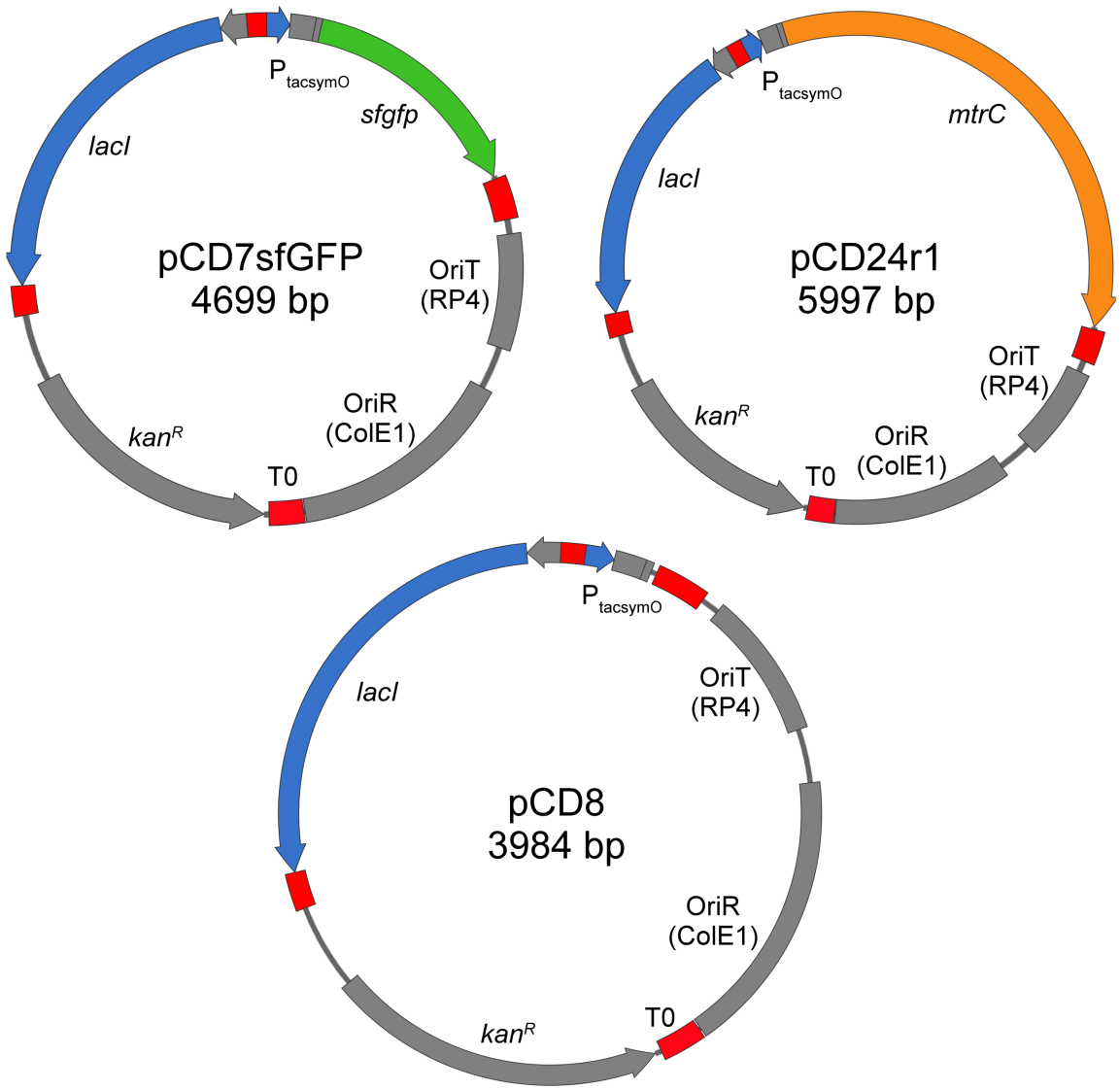

Figure S18. Plasmid maps for all plasmids used in this study. Terminator sequences are depicted as red, *lacI* and $P_{tacsymO}$ as blue, *sfgfp* as green, and *mtrC* as orange.

Table S4. Genetic parts/sequences used to construct the plasmids in this study.

Genetic Part	DNA Sequence (5' to 3')
Promoters	
$P_{tacsymO}$	TGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGCTCACA ATTCTATGGACTATGTTT
P_{lacI}	GCGGCGCGCCATCGAATGGCGCAAAACCTTTCGCGGTATGGCATGATAGCG CCCGGAAGAGAGTCAATTCAGGGTGGTGAAT
Ribosome Binding Sites	
B0032	TCACACAGGAAAGTACTAG
$sRBS1_{mtrC}$	GGGGAAAAACAGCAGTGCGAT
Terminators	
ECK120029600	TTCAGCCAAAAAACTTAAGACCGCCGGTCTTGTCCACTACCTTGCAGTAATGC GGTGGACAGGATCGGCGGTTTTCTTTTCTCTTCTCAA
L3S2P2111	TCGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGT TTTGGTCC
B0015	TAATCTAGACCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGC CTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTG GCTCACCTTCGGGTGGGCCTTTCTGCGTTTATA
Ribozymes	
RiboJ	AGCTGTCACCGGATGTGCTTTCCGGTCTGATGAGTCCGTGAGGACGAAACAG CCTCTACAAATAATTTTGTTTAA
Genes	
lacI	GTGAAACCAGTAACGTTATACGATGTCGCAGAGTATGCCGGTGTCTCTTATCA GACCGTTTCCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCG GGAAAAAGTGGAAGCGGCGATGGCGGAGCTGAATTACATTCCCAACCGCGT GGCACAACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCC AGTCTGGCCCTGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCG CCGATCAACTGGGTGCCAGCGTGGTGGTGTCGATGGTAGAACGAAGCGGCG TCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCGTCAGTG GGCTGATCATTAACTATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGC TGCCTGCACTAATGTTCCGGCGTTATTTCTTGATGTCTCTGACCAGACACCCA TCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGACTGGGCGTGGAGCA TCTGGTCGCATTGGGTCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAAGT TCTGTCTCGGCGCGTCTGCGTCTGGCTGGCTGGCATAAATATCTCACTCGCA ATCAAATTCAGCCGATAGCGGAACGGGAAGGCGACTGGAGTGCCATGTCCG GTTTTCAACAAACCATGCAAATGCTGAATGAGGGTATCGTTCCCACTGCGATG CTGGTTGCCAACGATCAGATGGCGCTGGGCGCAATGCGCGCCATTACCGAG TCCGGGCTGCGCGTTGGTGCGGATATCTCGGTAGTGGGATACGACGATACC GAAGACAGCTCATGTTATATCCCGCCGTTAACCACCATCAAACAGGATTTTCG CCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCA GGCGGTGAAGGGCAATCAGCTGTTGCCCGTGTCACTGGTGAAAAGAAAAACC ACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCAT TAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGA
sfgfp	ATGCGTAAAGGCGAAGAGCTGTTCACTGGTGTCGTCCCTATTCTGGTGGAAC TGGATGGTGATGTCAACGGTCATAAGTTTTCCGTGCGTGGCGAGGGTGAAGG TGACGCAACTAATGGTAAACTGACGCTGAAGTTCATCTGTACTACTGGTAAAC TGCCGGTACCTTGGCCGACTCTGGTAACGACGCTGACTTATGGTGTTCAGTG CTTTGCTCGTTATCCGGACCATATGAAGCAGCATGACTTCTTCAAGTCCGCCA TGCCGGAAGGCTATGTGCAGGAACGCACGATTTCCTTTAAGGATGACGGCAC GTACAAAACGCGTGCGGAAGTGAAATTTGAAGGCGATACCCTGGTAAACCGC ATTGAGCTGAAAGGCATTGACTTTAAAGAAGACGGCAATATCCTGGGCCATAA GCTGGAATACAATTTTAACAGCCACAATGTTTACATCACCGCCGATAAACAAA AAAATGGCATTAAAGCGAATTTTAAAATTCGCCACAACGTGGAGGATGGCAGC GTGCAGCTGGCTGATCACTACCAGCAAAACACTCCAATCGGTGATGGTCCTG TTCTGCTGCCAGACAATCACTATCTGAGCACGCAAAGCGTTCTGTCTAAAGAT

	CCGAACGAGAAACGCGATCATATGGTTCTGCTGGAGTTCGTAACCGCAGCGG GCATCACGCATGGTATGGATGAACTGTACAAATGATGA
mtrC	ATGATGAACGCACAAAAATCAAAAATCGCACTGCTGCTCGCAGCAAGTGCCG TCACAATGGCCTTAACCGGCTGTGGTGGAAGCGATGGTAATAACGGCAATGA TGGTAGTGATGGTGGTGAGCCAGCAGGTAGCATCCAGACGTTAAACCTAGAT ATCACTAAAGTAAGCTATGAAAATGGTGCACCTATGGTCACTGTTTTCGCCAC TAACGAAGCCGACATGCCAGTGATTGGTCTCGCAAATTTAGAAATCAAAAAAG CACTGCAATTAATACCGGAAGGGGCGACAGGCCCAGGTAATAGCGCTAACTG GCAAGGCTTAGGCTCATCAAAGAGCTATGTCGATAATAAAAACGGTAGCTATA CCTTTAAATTCGACGCCTTCGATAGTAATAAGGTCTTTAATGCTCAATTAACGC AACGCTTTAACGTTGTTTCTGCTGCGGGTAAATTAGCAGACGGAACGACCGTT CCCGTTGCCGAAATGGTTGAAGATTTCGACGGCCAAGGTAATGCGCCGCAAT ATACAAAAAATATCGTTAGCCACGAAGTATGTGCTTCTTGCCACGTAGAAGGT GAAAAGATTTATCACCAAGCTACTGAAGTCGAAACTTGTATTTCTTGCCACACT CAAGAGTTTGCGGATGGTCGCGGCAAACCCCATGTCGCCTTTAGTCACTTAA TTCACAATGTGCATAATGCCAACAAAGCTTGGGGCAAAGACAATAAAATCCCT ACAGTTGCACAAAATATTGTCCAAGATAATTGCCAAGTTTGTCACGTTGAATC CGACATGCTCACCGAGGCAAAAAACTGGTCACGTATTCCAACAATGGAAGTC TGTTCTAGCTGTCACGTAGACATCGATTTTGCTGCGGGTAAAGGCCACTCTCA ACAACTCGATAACTCCAACTGTATCGCCTGCCATAACAGCGACTGGACTGCT GAGTTACACACAGCCAAAACCACCGCAACTAAGAACTTGATTAATCAATACGG TATCGAGACTACCTCGACAATTAATACCGAAACTAAAGCAGCCACAATTAGTG TTCAAGTTGTAGATGCGAACGGTACTGCTGTTGATCTCAAGACCATCCTGCCT AAAGTGCAACGCTTAGAGATCATCACCAACGTTGGTCCTAATAATGCAACCTT AGGTTATAGTGGCAAAGATTCAATATTTGCAATCAAAAATGGAGCTCTTGATC CAAAAGCTACTATCAATGATGCTGGCAAACTGGTTTATACCACTACTAAAGAC CTCAAACTTGGCCAAAACGGCGCAGACAGCGACACAGCATTTAGCTTTGTAG GTTGGTCAATGTGTTCTAGCGAAGGTAAGTTTGTAGACTGTGCAGACCCTGC ATTTGATGGTGTTGATGTAACTAAGTATACCGGCATGAAAGCGGATTTAGCCT TTGCTACTTTGTCAGGTAAAGCACCAAGTACTCGCCACGTTGATTCTGTTAAC ATGACAGCCTGTGCCAATTGCCACACTGCTGAGTTCGAAATTCACAAAGGCA AACAACATGCAGGCTTTGTGATGACAGAGCAACTATCACACACCCAAGATGCT AACGGTAAAGCGATTGTAGGCCTTGACGCATGTGTGACTTGTCATACTCCTGA TGGCACCTATAGCTTTGCCAACCGTGGTGCGCTAGAGCTAAAACTACACAAA AAACACGTTGAAGATGCCTACGGCCTCATTGGTGGCAATTGTGCCTCTTGTCA CTCAGACTTCAACCTTGAGTCTTTCAAGAAGAAAGGCGCATTGAATACTGCCG CTGCAGCAGATAAAACAGGTCTATATTCTACGCCGATCACTGCAACTTGTACT ACCTGTCACACAGTTGGCAGCCAGTACATGGTCCATACGAAAGAAACCCTGG AGTCTTTCGGTGCAGTTGTTGATGGCACAAAAGATGATGCTACCAGTGCGGC ACAGTCAGAAACCTGTTTCTACTGCCATACCCCAACAGTTGCAGATCACACTA AAGTGAAAATGTAA

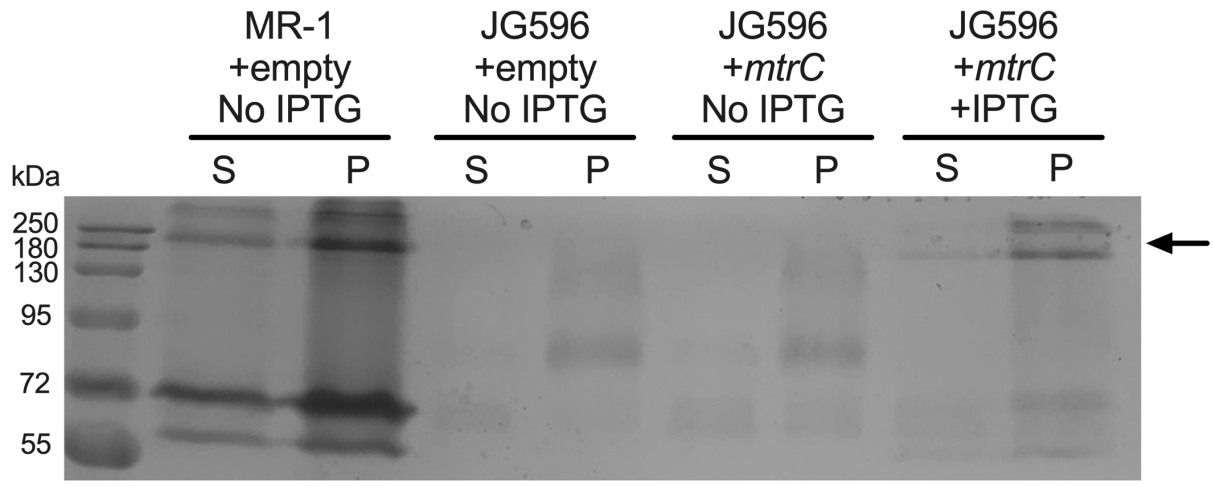

Figure S19. Heme-stained SDS-PAGE gel of uninduced (0 μM IPTG) and induced (1000 μM IPTG) *S. oneidensis* strains. 10 μg of protein (as determined by Bradford assay) was loaded into each lane. +empty indicates strains harboring the empty expression vector (pCD8) and +*mtrC* indicates strains harboring the IPTG-inducible *mtrC* vector (pCD24r1). After sonication, cell lysate was pelleted at 10,000 rcf for 5 minutes and the soluble fraction (S) and pellet (P) were separated for analysis. The arrow indicates the bands that appear in the JG596 +*mtrC* +IPTG lanes, which correspond to the apparent size of the MtrCAB complex (~210 kDa) (4).

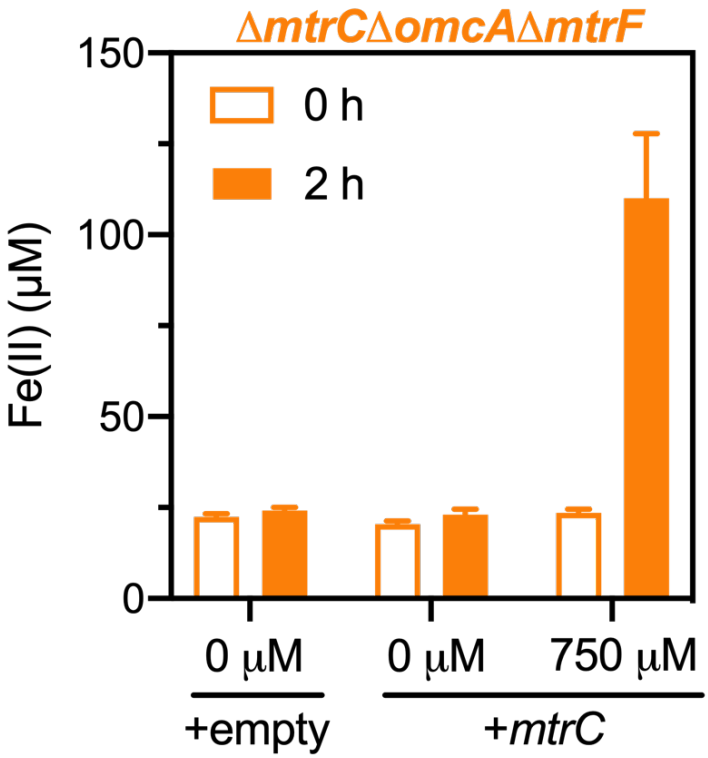

Figure S20. Reduction of Fe(III)-citrate over 2 hours by *S. oneidensis* Δ*mtrC*Δ*omcA*Δ*mtrF* carrying either pCD24r1 (+*mtrC*) or pCD8 (+empty). Cells were either uninduced (0 μM) or induced (750 μM) with IPTG at 0 h. Data shown are mean ± SD for *n* = 3 biological replicates.

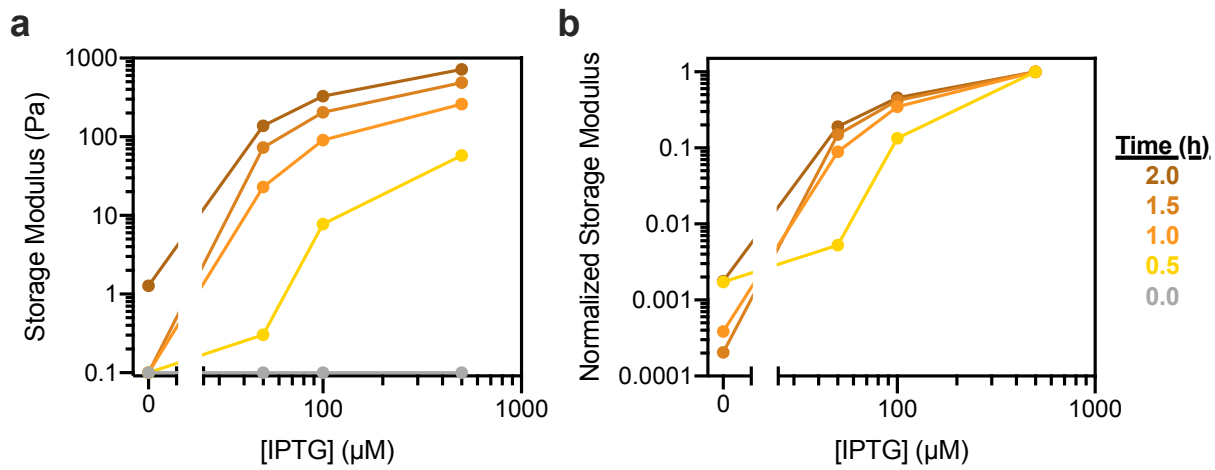

Figure S21. Dynamic range of storage modulus response function changes with time. (a) Storage moduli of hydrogels crosslinked by *S. oneidensis* Δ*mtrC*Δ*omcA*Δ*mtrF* carrying pCD24r1 (+*mtrC*) and (un)induced at various IPTG levels. Storage modulus was arbitrarily set at 0.1 Pa when stiffness was below the limit of detection. (b) Storage moduli from each timepoint when normalized to the highest IPTG concentration (500 μM). Past 1.0 h, all response curves collapse to similar functions. Each curve represents data from a single timepoint. Data shown are values from individual *in situ* rheology experiments (n = 1 biological replicate) that are also depicted in Figure 4b.

Note S1. Models of inducible gene expression and storage moduli.

Hill Function Model

The activating Hill function has been extensively used to describe inducible gene expression (5). Its general form is depicted below:

$$y = Min + (Max - Min)\frac{[I]^n}{EC_{50}{}^n + [I]^n} \qquad [1]$$

where y corresponds to storage modulus (G') or relative expression units (REU), Min corresponds to the lower-bound plateau value, Max corresponds to the upper-bound plateau value, $[I]$ corresponds to IPTG concentration, n corresponds to the hillslope, and EC_{50} corresponds to the half-maximal effective concentration.

Derivation of Linear Relationship Between REU and G'

We observed that inducible transcriptional rate/sfGFP fluorescence (REU, Figure 4c) and hydrogel storage modulus (G', Figure 4d) fit well to [1] and that similar n and EC_{50} values were obtained from fitting the model to each data set. Assuming the fitted n and EC_{50} values are identical for REU and G', the $[I]$, n and EC_{50} terms can be rewritten as an expression that relates G' as a function of REU. This expression dependent on experimental REU values and fitted Hill function parameters is shown below:

$$\frac{[I]^n}{EC_{50}{}^n + [I]^n} = \frac{REU - Min_{REU}}{(Max_{REU} - Min_{REU})} \qquad [2]$$

By inserting [2] into [1], where G' is the dependent variable, a linear relationship of the form $y = mx + b$ is obtained. The final relationship is depicted below:

$$G' = \frac{(Max_{G'} - Min_{G'})}{(Max_{REU} - Min_{REU})} REU + \left(Min_{G'} - \frac{(Max_{G'} - Min_{G'})}{(Max_{REU} - Min_{REU})} Min_{REU}\right) \qquad [3]$$

Using the normalized Hill function-derived values for each Max and Min value, [3] is predicted:

$$G' = 1.25REU - 0.21 \quad [4]$$

which is similar to the fitted values obtained from linear regression (Figure 4e). Assuming $[MtrC]$ is proportional to transcriptional rate (REU) (6), this generates a linear relationship between G' and $[MtrC]$.

Table S5. Nonlinear fit parameters for products of *sfgfp* expression (fluorescence) and *mtrC* expression (storage modulus)

Parameter	*sfgfp* Nonlinear Fit Value (units)	*mtrC* Nonlinear Fit Value (units)
Best-fit Values		
Hillslope	1.569	1.402
EC_{50}	98.58 (μM)	96.38 (μM)
Max	1.032 (REU)	3360 (Pa); 1.08 (Relative Hydrogel Modulus)
Min	0.2086 (REU)	158.4 (Pa); 0.05 (Relative Hydrogel Modulus)
95% Confidence Interval		
Hillslope	1.028 to 2.505	0.3979 to 4.671
EC_{50}	78.01 to 132.5 (μM)	50.92 to 3650 (μM)
Max	0.9654 to 1.137 (REU)	2730 to 9795 (Pa)
Min	0.1475 to 0.2644 (REU)	-401.7 to 568.6 (Pa)
Goodness of Fit		
R^2	0.9314	0.8632

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