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Real-time bioelectronic sensing of environmental contaminants

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Supplementary Materials for

Real-time monitoring of contaminants using living electronic sensors

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Supplementary Text

Evaluation of sulfur source on assimilation and H_2S evolution.

E. coli assimilates inorganic sulfur sources (*e.g.,* sulfate, sulfite, thiosulfate) that vary in energetic costs for fixation into L-cysteine (Extended Data Fig. 3A). Sulfur metabolism is tightly regulated in *E. coli* to limit the overproduction of L-cysteine, maintaining a total sulfur-atom concentration inside *E. coli* of ~0.13 M[1]. This regulation is vital as at high concentrations L-cysteine can cause DNA damage through the Fenton reaction[2] and its degradation to sulfide can lead to cytochrome *bo* oxidase inhibition[1]. Sulfate assimilation requires 3 mol of ATP and 4 mol of NADPH/mol of SO_4^{2-} assimilated to yield one mol of L-cysteine. In contrast, thiosulfate assimilation is energetically less expensive yielding 2 mol of L-cysteine/mol of $S_2O_3^{2-}$, while only requiring only 1 mol of ATP and 4 mol of NADPH (Extended Data Fig. 3A). Thiosulfate, sulfate, or sulfite enter the cytosol through the ATP-dependent anion permease consisting of CysAUW and the periplasmic binding protein CysP or Sbp or the proton-dependent symporter CysZ. Sulfate proceeds through the canonical sulfate assimilation pathway becoming activated by ATP sulfurylase (CysDN) and APS kinase (CysC) using 2 ATP and reduced by PAPS reductase (CysH) using 1 NADPH to generate sulfite. Sulfite serves as the substrate for SIR using 3 NADPH to generate sulfide with FNR and Fd delivering these electrons to SIR in our engineered strains. Sulfide is then attached to the carbon skeleton *O*-acetyl-serine (OAS) to generate L-cysteine by cysteine synthase (CysK). In contrast, thiosulfate is initially attached to the OAS skeleton by cysteine synthase B (CysM) to form *S*-sulfocysteine which undergoes reductive cleavage by glutaredoxin using 1 NADPH to form L-cysteine and sulfite[3]. Sulfite can be assimilated into an additional L-cysteine through SIR and CysK. OAS is generated from L-serine by serine acetyltransferase (CysE) and flux is regulated post-translationally through feedback inhibition by L-cysteine. When OAS becomes limiting, sulfide accumulates in the cell and negatively regulates expression of the sulfate assimilation pathway by inhibiting the CysB regulon. If sulfide or thiosulfate becomes limiting, *N*-acetylserine (NAS) forms from OAS and positively regulates expression of the sulfate assimilation pathway by activating the CysB regulon.

To determine how Fd expression influences sulfide production from these different sulfur sources, we monitored growth (Extended Data Fig. 3B) and H_2S evolution (Extended Data Fig. 3C) from $I^+C^-O^-$ cultures grown in medium containing these anions. In sulfate and sulfite media, cells displayed Fd-dependent growth that increased with aTc concentration. In contrast, H_2S evolved from these cultures only at intermediate aTc concentrations. Cells grown with thiosulfate grew at all aTc concentrations. Additionally, thiosulfate cultures evolved similar levels of H_2S at all aTc concentrations. The differences in H_2S evolution at high aTc concentrations was interpreted to be caused by these differences in regulatory topologies with sulfate assimilation structured as an incoherent feedforward loop which can lead to band-pass filter behavior[4,5] while thiosulfate assimilation is regulated by a post-translational negative feedback loop. Because of this difference in regulatory topology we chose to use thiosulfate as a substrate to support EET in our living electronic sensors.

Table S1. List of plasmids. For each plasmid, the protein components expressed are noted.

Plasmid Name	Description	Addgene ID
pSAC01	FNR/SIR expressing plasmid	131826
pSAC01_SQR1	FNR/SIR/rcSQR expressing plasmid	188461
pSAC01_SQR2	FNR/SIR/gsSQR expressing plasmid	188462
p(e-)nzymes_NC	Ccm/FNR/SIR expressing plasmid	188463
p(e-)nzymes_1	Ccm/FNR/SIR/rcSQR expressing plasmid	188464
p(e-)nzymes_2	Ccm/FNR/SIR/gsSQR expressing plasmid	188465
pFd007/lacI	Fd expressing plasmid	131832
pFd007_C42A/lacI	Fd(C42A) expressing plasmid	131831
pBW014	sFd-55-ER expressing plasmid	131817
pFd007/lacI/fnr/pgl	Fd/Fnr/Pgl expressing plasmid	188466
pFd007_C42A/lacI/fnr/pgl	Fd(C42A)/Fnr/Pgl expressing plasmid	188467
pERA007.55/lacI/fnr/pgl	sFd-55-ER/Fnr/Pgl expressing plasmid	188468
pSIM19	lambda red recombinase expressing plasmid	-
pX2-Cas9	Cas9 expressing plasmid	85811

pSS9-RNA	gRNA expressing plasmid	71656
pSS9	ss9 homology arm containing plasmid	71655
pSS9:cymAmtrCAB	ss9 homology arm flanking cymA-mtrCAB operon	188469

Table S2. List of strains. In total, three different strains were used, including XL1-Blue, EW11, and EW11-JA01. To create strains that express different combinations of the I, C, and O module components, strains were transformed with one or two plasmids.

Strain	Designation	Plasmids	Input	Coupling	Output
Cloning Strain	XL1-Blue	-	-	-	-
$I^{-}C^{-}O^{-}$	EW11	-	-	-	-
$I^{-}C^{-}O^{+}$	EW11-JA01	pSS9-RNA	-	-	CymA-MtrCAB
$I^{C42A}C^{-}O^{-}$	EW11	pFd007_C42A/lacI/fnr/pgl + p(e-)nzymes_NC	Fd(C42A)	-	-
$I^{C42A}C^{-}O^{+}$	EW11-JA01	pFd007_C42A/lacI/fnr/pgl + p(e-)nzymes_NC	Fd(C42A)	-	CymA-MtrCAB
SQR Analysis	EW11	pFd007_C42A/lacI/fnr/pgl + p(e-)nzymes_NC	Fd(C42A)	-	-
SQR Analysis	EW11	pFd007_C42A/lacI/fnr/pgl + p(e-)nzymes_1	Fd(C42A)	rcSQR	-
SQR Analysis	EW11	pFd007_C42A/lacI/fnr/pgl + p(e-)nzymes_2	Fd(C42A)	gsSQR	-
$I^{C42A}C^{+}O^{+}$	EW11-JA01	pFd007_C42A/lacI/fnr/pgl + p(e-)nzymes_1	Fd(C42A)	rcSQR	CymA-MtrCAB
$I^{+}C^{+}O^{+}$	EW11-JA01	pFd007/lacI/fnr/pgl + p(e-)nzymes_1	Fd	rcSQR	CymA-MtrCAB
$I^{S}C^{+}O^{+}$	EW11-JA01	pERA007.55/lacI/fnr/pgl + p(e-)nzymes_1	sFd-55-ER	rcSQR	CymA-MtrCAB

Table S3. The estimated detection limit (DL) of thiosulfate sensing. DL was estimated as *3.3 * σ / S*, *w*here *σ* is estimated based on the standard deviation of the current response, and *S* is the slope of the linear fit. The data range for this calculation is between 0.1 mM to 10 mM.

	Sensor 1		**Sensor 2**		**Sensor 3**		**DL (mM)**
	σ1	**S1**	**σ2**	**S2**	**σ3**	**S3**	
+gel, 30min (R^2=0.998)	0.00745	0.32679	0.01181	0.32976	0.05652	0.28213	0.28

Table S4. A comparison of state-of-art sensing techniques for thiosulfate and estrogenic compounds. The bioelectronic sensors described herein specifically detect analytes with faster response times, no need for sample preparation, and require instrumentation with lower costs.

	Sample preparation required	Figures of Merit for thiosulfate				Figures of Merit for 4-hydroxytamoxifen				instrumentation required (cost)	Reference
		response time	limit of detection	linear range	sensitivity	response time	limit of detection	linear range	sensitivity		
This work	No	<10 min	~0.4 mM	0.1-20 mM	0.06 ± 0.004 μA/mM[a]	3 min	N.D.	N.D.	0.93 ± 0.33%[b]	potentiostat ($60 to $6000)	(This work)
Liquid chromatography	Yes	60 min (sample prep <5 min)	low μM range	3.3 nM to 6.67 mM	1333:1[c]	-	-	-	-	UPLC ($30-60,000)	[6]
Two-component fluorescence sensor	Yes	360 min	~0.1 mM	N.D.	N.D.	-	-	-	-	flow cytometer ($25,000)	[7]
Liquid chromatography	Yes	-	-	-	-	20 min (sample prep <5 min)	258 pM	258 pM to 130 nM	N.D.	UPLC ($30-60,000)	[8]
Split fluorescence based sensor	Yes	-	-	-	-	240 min	EC_{50} = 2.5 nM	N.D.	N.D.	flow cytometer ($25,000)	[9]

[a] Quantified at 5 min sensing time.

[b] Quantified at 12.5 μM.

[c] Quantified at 33.35 μM.

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