Supplementary Information for
Cell-free biosensors for rapid detection of water contaminants

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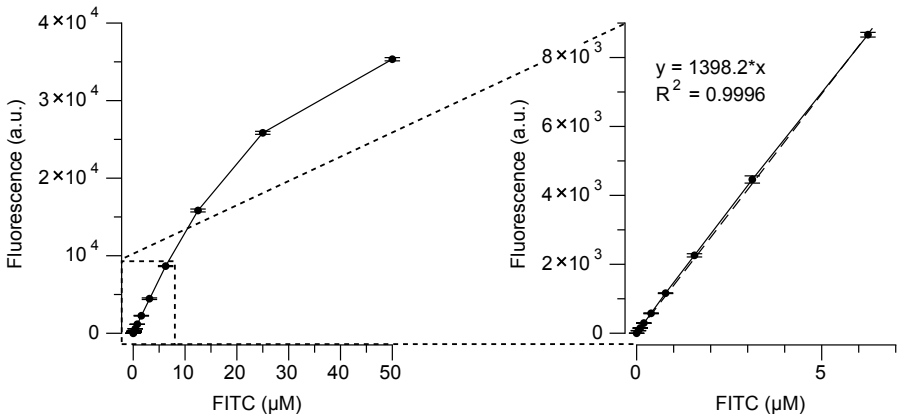
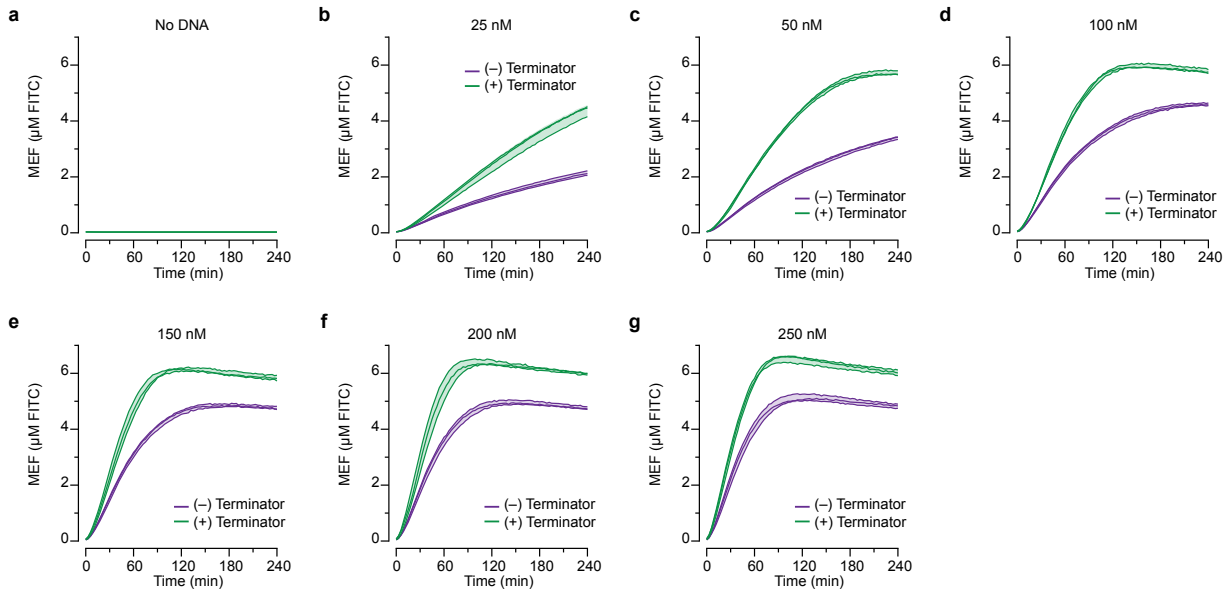
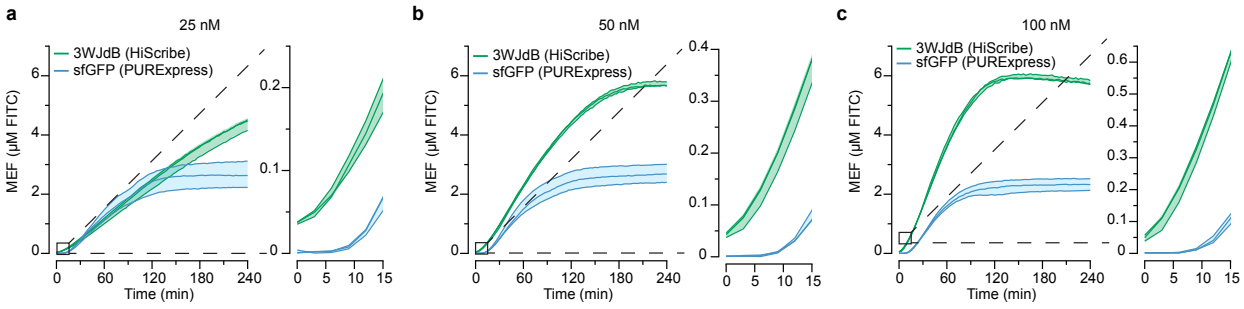


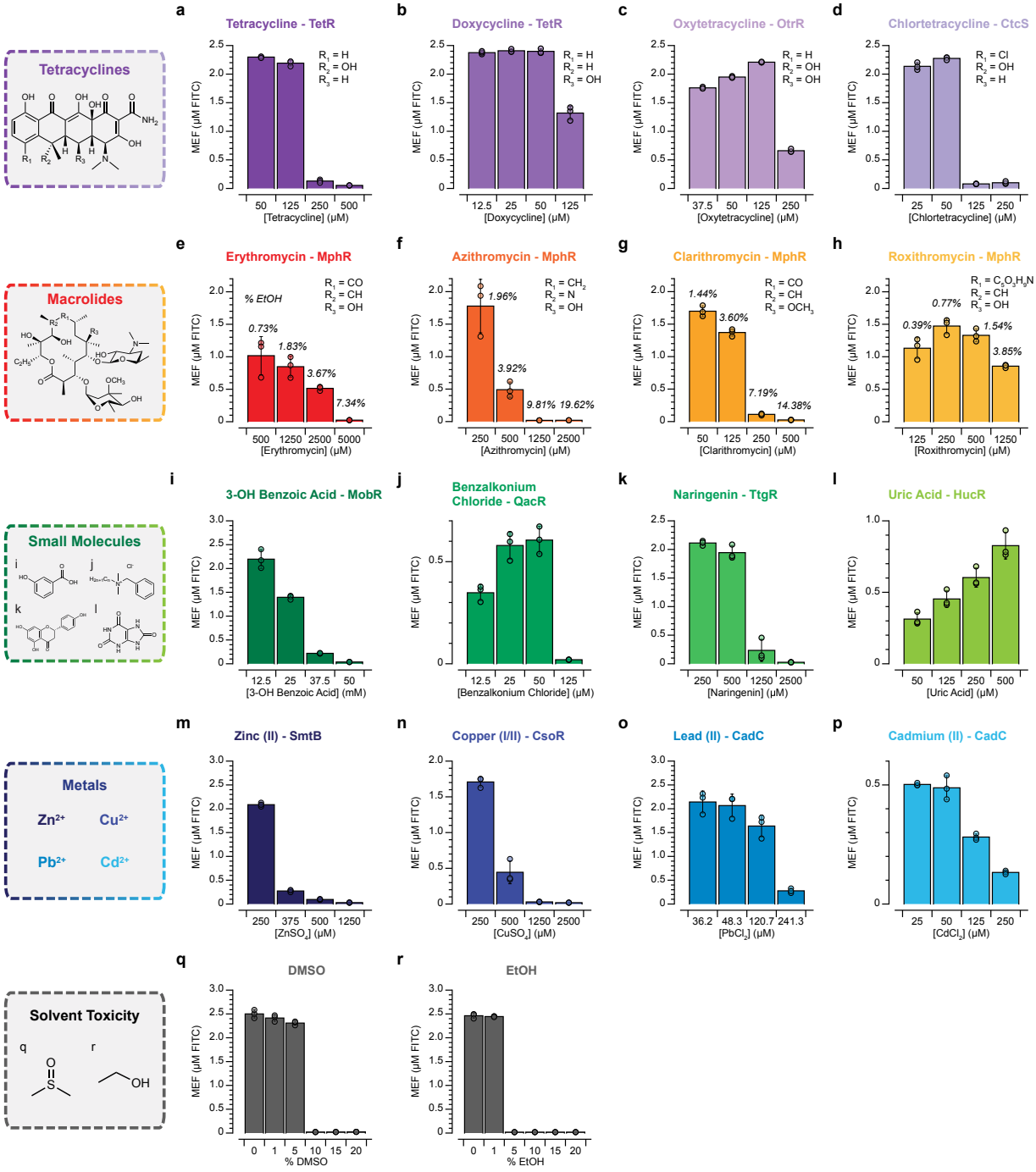
Supplementary Fig. 1 | Micromolar Equivalent Fluorescein (MEF) standardization. Arbitrary units of fluorescence were standardized to μM concentrations of fluorescein (FITC) using a NIST traceable standard (see Methods). In the representative example shown here, a dilution series of FITC was prepared in buffer (100 mM sodium borate, pH 9.5) and measured on a plate reader using the same settings for measuring 3WJdB signal (472 nm excitation, 507 nm emission). The resulting curve, calculated over the linear range 0–6.25 μM, was then used to standardize fluorescence measured from ROSALIND reactions. The standard curve was generated at regular intervals for each plate reader and each measurement setting. Data shown are for n=9 replicates (3 experimentally independent replicates each with 3 technical replicates). Error bars indicate standard deviation computed over n=9 replicates.



Supplementary Fig. 2 | Fluorescence-activation of 3WJdB during run-off *in vitro* transcription reactions is improved by additionally encoding a T7 terminator. *In vitro* transcription reactions using a commercially available kit (NEB HiScribe™ T7 Quick High Yield RNA Synthesis Kit) were performed across a range of DNA template concentrations, with and without an additional T7 terminator sequence. **a,** Reactions containing no DNA failed to generate signal, while all other DNA containing reactions (**b**-**g**) generated measurable fluorescence that improved with the addition of a template-encoded T7 terminator. We hypothesize that this terminator-dependent increase in fluorescence could be due to the terminator structure encouraging faster recycling of T7 RNAP from the elongation phase, to the termination phase, and then to initiation phase. Although less likely due to the highly structured design of 3WJdB, the terminator-dependent increase could be due to the terminator structure stabilizing the productive, fluorescence-activating fold of 3WJdB. All data shown for n=3 independent biological replicates as lines with raw fluorescence values standardized to μM FITC. Shading indicates the average value of 3 independent biological replicates ± standard deviation.

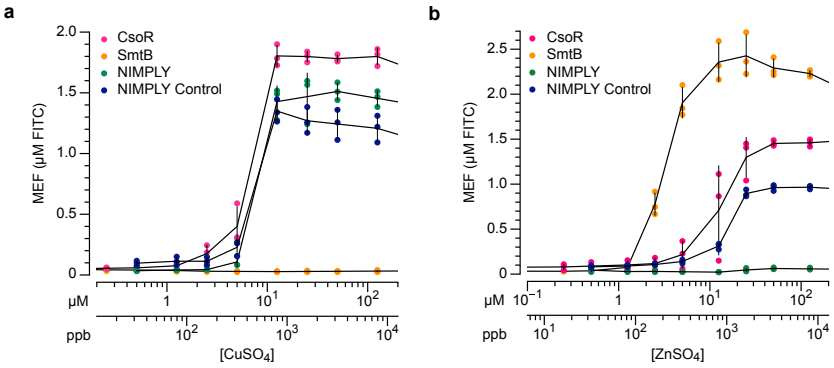


Supplementary Fig. 3 | Fluorescence-activation from 3WJdB transcription reactions is faster and brighter than fluorescence from transcription-translation (PURE) reactions of sfGFP. All data shown for n=3 independent biological replicates as lines with raw fluorescence values standardized to μM FITC. Shading indicates the average value of 3 independent biological replicates ± standard deviation.

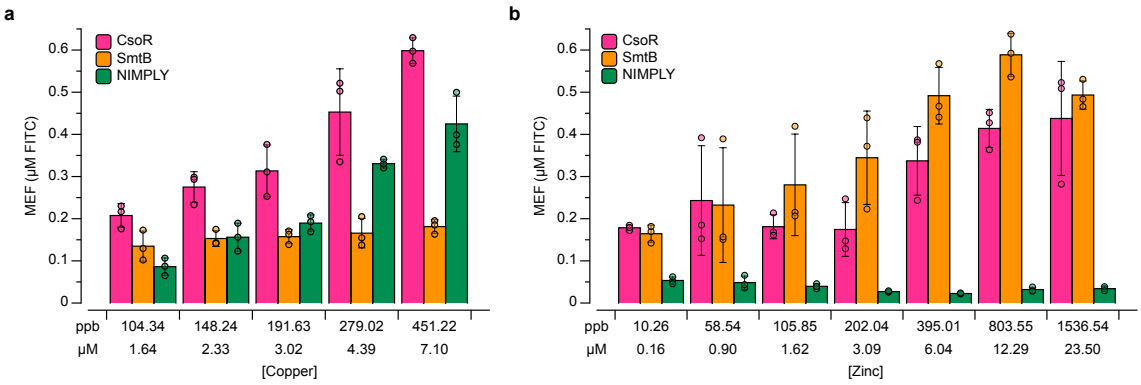


Supplementary Fig. 4 | Ligand and solvent inhibitory effects in ROSALIND. a – d, Tetracycline sensors can tolerate up to hundreds of micromolar of tetracyclines. **e – h**, Varying amounts of different macrolides are tolerated by ROSALIND, and the inhibitory effects of macrolides appears to be caused by the solvent in which the ligand is dissolved. %EtOH indicated above each bar. ROSALIND reactions are poisoned at the tens of millimolar range of 3-hydroxy benzoic acid **i**, at the hundreds of micromolar range of benzalkonium chloride **j**, and at a low millimolar range of naringenin **k**. **l**, No inhibitory effect of uric acid is observed at its maximum solubility limit. **m – p**, Inhibitory effects of metals vary. **q, r**, There are appreciable inhibitory effects due to the solvents DMSO and EtOH used to dissolve several of the ligands. All data shown for

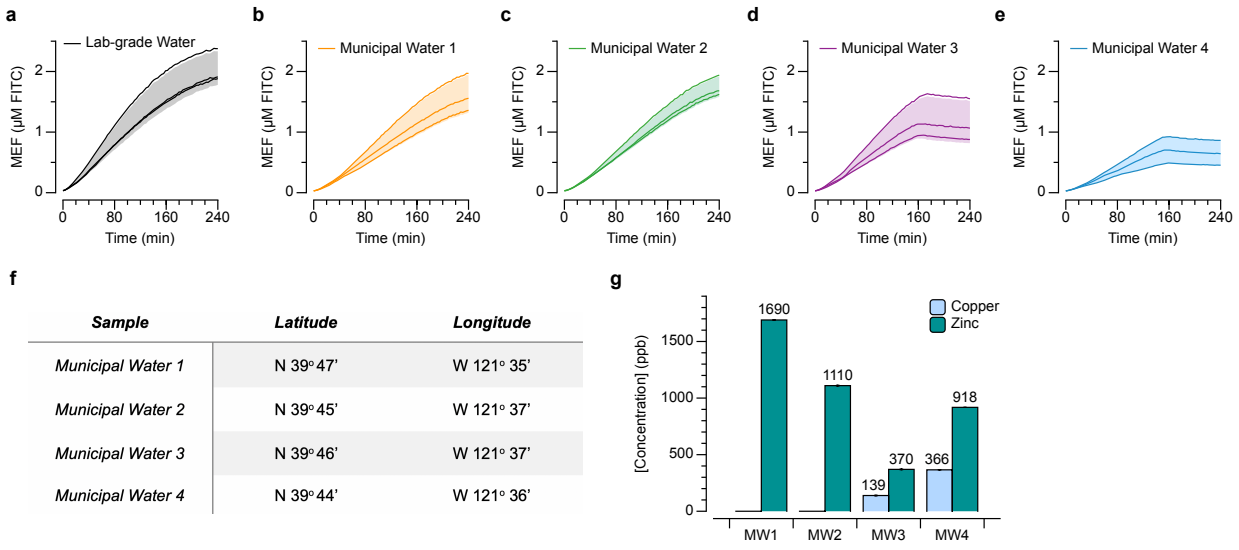
n=3 independent biological replicates as points with raw fluorescence values standardized to μM FITC, and bars representing averages of the replicates. Error bars indicate the average value of 3 independent biological replicates ± standard deviation.



Supplementary Fig. 5 | Copper and Zinc dose response curves of NIMPLY circuits. The Cu NIMPLY Zn circuit, a control NIMPLY circuit where the KB2-encoding sequence was replaced by the reverse sequence and CsoR- or SmtB-regulated reactions were each tested and found to function as expected for a range of **a**, $CuSO_4$ and **b**, $ZnSO_4$ concentrations. All data shown for n=3 independent biological replicates as points with raw fluorescence values standardized to MEF (μM FITC). Error bars indicate the average value of 3 independent biological replicates ± standard deviation. Reactions are configured as described in **Fig. 4c**.



Supplementary Fig. 6 | ROSALIND reactions can be freeze-dried and rehydrated with a drinking water source. The ROSALIND reactions described in **Fig. 6** were constructed, freeze-dried and rehydrated with a municipal water sample from Evanston, Illinois that was filtered and spiked with a range of concentrations of **a,** $CuSO_4$ and **b**, $ZnSO_4$. The reactions were then incubated and characterized for fluorescence by plate reader. X-axis concentrations were obtained from FAAS analysis of these same samples. In each case, the reactions behaved as expected. All data shown for n=3 independent biological replicates as points with raw fluorescence values standardized to MEF (μM FITC), and bars representing averages of the replicates. Error bars indicate the average value of 3 independent biological replicates ± standard deviation. Reactions are configured as described in **Fig. 4c**.



f

Sample	*Latitude*	*Longitude*
Municipal Water 1	N 39° 47’	W 121° 35’
Municipal Water 2	N 39° 45’	W 121° 37’
Municipal Water 3	N 39° 46’	W 121° 37’
Municipal Water 4	N 39° 44’	W 121° 36’

Supplementary Fig. 7 | Paradise Municipal Water Sample Analysis. a – e, Unregulated ROSALIND reactions with 25 nM of 3WJdB template were freeze-dried and rehydrated with Paradise municipal water samples used in **Fig. 6c-f** to investigate matrix effects. While little toxicity effects are observed from the samples with zinc only (**b**, **c**), the samples with copper and zinc (**d**, **e**) seem to inhibit signal from 3WJdB, and the sample with higher copper concentration (municipal water 4) is more heavily affected. All data shown for n=3 independent biological replicates as lines with raw fluorescence values standardized to MEF (μM FITC). Shading indicates the average value of 3 independent biological replicates ± standard deviation. **f**, GPS coordinates of the water samples. GPS coordinates are reported to the nearest minute resolution and thus represent regions rather than exact locations. **g**, FAAS measurements of the undiluted filtered water samples used in **Fig. 6c-f**. Bars represent the average values of n=3 independent replicates with ± standard deviation.

Supplementary Table 1 | US EPA, EU Drinking Water Directive and WHO Guidelines for ROSALIND Ligands in Drinking Water. A dash indicates no regulatory guidelines are currently available.

Contaminant	Environmental Protection Agency (US)	Drinking Water Directive (EU)	WHO Guideline
Tetracyclines	–	–	–
Macrolides (i.e. Erythromycin)	Candidate Contaminant List 4[a]	–	–
3-OH Benzoic Acid	–	–	–
Benzalkonium chloride	–	–	–
Naringenin	–	–	–
Uric Acid	–	–	–
Zinc	5 mg/L[b]	3 mg/L	–
Copper	1.3 mg/L (1 mg/L[b])	2 mg/L	2 mg/L
Lead	15 µg/L	10 µg/L	10 µg/L
Cadmium	5 µg/L	5 µg/L	3 µg/L

[a]Candidate contaminants are under consideration for regulation.
[b]National secondary drinking water regulation (unenforced)

Supplementary Table 2 | Concentrations of ROSALIND components in each sensor.

Ligand – aTF Pair	[DNA] (nM)	[aTF] (μM)	[Ligand] (μM)	Ligand Solvent	[Ligand Stock] (μM)	Ligand Purchased From
Anhydrotetracycline – TetR	25	1.25	0.1 – 50	Ethanol	5 – 500	Sigma-Aldrich (Cat#: 37919–100MG–R)
Tetracycline – TetR	25	1.25	0.05 – 500	Lab-grade H_2O	24 – 14,383	Gold Biotechnology (Cat#: T–101–25)
Doxycycline – TetR	25	1.25	0.05 – 125	Lab-grade H_2O	8 – 800	Gold Biotechnology (Cat#: D–500–1)
Oxytetracycline – OtrR	25	2.5	0.25 – 250	Lab-grade H_2O	14.5 – 1,450	Gold Biotechnology (Cat#: O–410–10)
Chlortetracycline – CtcS	25	1.25	0.125 – 125	Lab-grade H_2O	5.4 – 5,400	Gold Biotechnology (Cat#: C–840–5)
Erythromycin – MphR	25	0.625	0.05 – 5,000	Ethanol	6.8 – 6,800	Sigma-Aldrich (Cat#: E5389–1G)
Azithromycin – MphR	25	0.625	0.125 – 2,500	Ethanol	12.7 – 12,700	Sigma-Aldrich (Cat#: PHR1088–1G)
Clarithromycin – MphR	25	0.625	0.25 – 500	Ethanol	3.5 – 3,500	Sigma-Aldrich (Cat#: PHR1038–500MG)
Roxithromycin – MphR	25	0.625	0.5 – 1,250	Ethanol	32.4 – 32,400	Sigma-Aldrich (Cat#: R4393–1G)
3-OH Benzoic Acid – MobR	10	100	5 – 50,000	1M Tris-base Buffer, pH 8	109.6 – 225,410	Sigma-Aldrich (Cat#: H20008–5G)
Benzalkonium Chloride – QacR	25	2.5	0.5 – 125	Lab-grade H_2O	41.33 – 4,133	Sigma-Aldrich (Cat#: 12060–100G)
Naringenin – TtgR	10	12.5	0.5 – 2,500	DMSO	73.5 – 36,731	Sigma-Aldrich (Cat#: N5893 – 1G)
Uric Acid – HucR	17.5	2.15	2.5 – 500	1mM NaOH	77 – 770	Sigma-Aldrich (Cat#: U0881–10G)
$ZnSO_4$ – AdcR	7.5	1.5	30	Lab-grade H_2O	200	Sigma-Aldrich (Cat#: 83265–250mL–F)
$ZnSO_4$ – SmtB	25	5	0.05 – 1,250	Lab-grade H_2O	2 – 2,000	Sigma-Aldrich (Cat#: 83265–250mL–F)
$CuSO_4$ – CsoR	25	2.5*	0.05 – 2,500	Lab-grade H_2O	2.7 – 24,430	Sigma-Aldrich (Cat#: 209198–5G)
$PbCl_2$ – CadC	25	1.5	0.005 – 241.3	Lab-grade H_2O	0.2 – 200	Sigma-Aldrich (Cat#: 203572–10G)
$CdCl_2$ – CadC	25	1.5	0.05 – 250	Lab-grade H_2O	5.2 - 5,200	Sigma-Aldrich (Cat#: 202908–10G)

*All aTF concentrations listed are dimer concentrations except for CsoR, which is a tetramer.

Supplementary Table 3 | Estimated cost per ROSALIND reaction*

Component	Supplier	Catalog Number	Quantity	Price (USD)	[Stock]	Stock Volume (mL)	Amount / Reaction (mL)	# Reactions	Cost / 20 µL Reaction (USD)
DFHBI-1T	Tocris	5610	10 mg	$240.59	40 mM	0.781	0.00113	691	$0.35
ATP	Sigma	A2383-25G	25 g	$466.48	100 mM	453.605	0.00057	795798	< $0.01
CTP	Sigma	C1506-1G	1 g	$448.84	100 mM	18.971	0.00057	33282	$0.01
GTP	Sigma	G8877-1G	1 g	$600.74	100 mM	19.114	0.00057	33533	$0.02
UTP	Sigma	U6625-1G	1 g	$495.88	100 mM	18.179	0.00057	31893	$0.01
Tris	Sigma	RDD009-2.5KG	2.5 kg	$166.56	2 M	7931.5	0.00020	39657500	<< $0.01
$MgCl_2$	Sigma	M2670-1KG	1 kg	$109.81	800 mM	6148.5	0.00020	30742500	<< $0.01
Spermidine	Sigma	S2626-25G	25 g	$498.82	200 mM	860.585	0.00020	4302926	<< $0.01
NaCl	Sigma	746398-25KG	25 kg	$198.50	2 M	213894.6	0.00020	1069473000	<< $0.01
DTT	Gold Bio	DTT500	500 g	$1,425	1 M	3241.5	0.00020	16207500	<< $0.01
Sucrose	Sigma	S0389-5KG	5 kg	$61.53	5 M	2921.4	0.00020	14607000	<< $0.01
Mannitol	Sigma	M4125-5KG	5 kg	$173.01	1 M	27446.9	0.00500	5489380	<< $0.01
TIPP	NEB	M0296L	1250 U	$244.79	2 U/µL	0.625	0.00015	4167	$0.06
T7 RNAP[a]	n/a	n/a	50 mg	$400.00	1 mg/mL	50	0.0002	250000	< $0.01
aTF[a]	n/a	n/a	10 mg	$400.00	1 mg/mL	10	0.0002	50000	< $0.01
DNA Template[b]	n/a	n/a	20 µg	$42.81	0.1 µg/µL	0.2	0.001	200	$0.21
TOTAL									**~ $0.67 / reaction**

[a]Homemade, estimated for 2 L expression culture using core facility pricing (NU rPPC, March 2019).
[b]Calculated for the cost of a 2 mL PCR and purification using NEB #M3050 ($34.96/prep), NEB #N0447L ($2.45/prep), Qiagen #28106 ($4.40/prep), and PCR primers (< $1/prep).
*Calculated using institutional pricing (March 2019). Not included in calculation: labor, overhead, equipment, consumables (*e.g.* pipette tips) and additional materials (*e.g.* for pH adjustments), laboratory grade water, storage, shipping.

Supplementary Table 4 | Comparison between traditional laboratory-based methods for assessing water contaminants and synthetic biology approaches. Techniques are assessed by documented sensitivity, specificity, portability for the field, the types of protocol steps needed to perform the tests and sample volume/equipment needs. Sections are divided based on metal detection and antibiotic detection.

	Sensitivity (Detection Limit / Range)				***Specificity***	***Port-ability***	***Protocol Steps***	***Sample Volume – Equipment Needs***
Metals: Zn, Cu, Pb, Cd								
Atomic Spectroscopy	**Zn**	**Cu**	**Pb**	**Cd**				
Flame Atomic Absorption (FAAS)[1]	15 nM	50 nM	50 nM	14 nM	Total dissolved element	No	Sample Filtration (SF), Acidification, Calibration	Chemical Laboratory Infrastructure and Instrumentation
Inductively Coupled Plasma – Optical Emission[1]	1 nM	5 nM	5 nM	1 nM	Total dissolved element	No	SF, Acidification, Calibration	Chemical Laboratory Infrastructure and Instrumentation
Graphite Furnace AAS (GFAAS)[1]	115 pM	1 nM	0.2 nM	0.2 nM	Total dissolved element	No	SF, Acidification, Calibration	Chemical Laboratory Infrastructure and Instrumentation
Inductively Coupled Plasma Mass Spectrometry (ICP-MS)[1]	15 pM	8 pM	0.5 pM	1 pM	Total dissolved element	No	SF, Acidification, Calibration	Chemical Laboratory Infrastructure and Instrumentation
Electrochemistry								
Potentiometry[2]	–	10 nM – 0.1 M	1 µM – 0.1 M	100 nM – 0.1 M	Free metal ion activity	Yes	SF, Ionic Strength Buffer	High impedance entry voltmeter / pH meter
Voltammetry[3, 4]	1 nM	1 nM	1 nM	1 nM	Electro-labile concentration	No	SF, Background electrolyte, Buffer	Potentiostat, Electrochemical Cell, N_2 for purging O_2
Voltammetry[3-5]	150 nM – 1.5 µM	160 nM – 1.6 µM	10 nM – 0.5 µM	10 nM – 1 µM	Electro-labile concentration	Yes	SF, Background electrolyte, Buffer	Portable Potentiostat, Screen printed electrodes
Molecular Spectroscopy								
Colorimetry[3]	160 nM – 1.6 µM	1.6 µM – 13 µM	0.5 µM – 10 µM	200 nM – 3 µM	Interferences	Yes	SF, adding colorimetric reagent	Portable Spectrophotometer
Synthetic Biology								

Whole Cell Biosensors (WCBs)[6-9]	0.5 µM - 4.5 mM	1.5 µM – 2 mM	15 nM – 1.5 mM	5 nM – 27 µM	Known Crosstalk	No	SF, C source / O_2 / Metabolic Activity	~ 1 mL, cell growth, for detection: bioluminescence, fluorescence
Whole Cell Biosensors (WCBs)[10]	–	–	–	–	Known crosstalk	Yes*	SF, C source	Portable luminometer
ROSALIND	2.5 µM – 375 µM	5 µM – 500 µM	1.25 µM – 250 µM	1.25 µM – 250 µM	Circuits can fix crosstalk	Yes	SF	20 µL, Illuminator
Antibiotics: Tetracyclines, Macrolides								
Separation Methods	**Tetracyclines**		**Macrolides**					
LC/MS/MS – QTRAP – MS/MS[11-13]	0.2 nM – 2.0 nM		0.3 nM – 1.5 nM		–	No	Solid Phase Extraction (SPE)	Chemical Laboratory Infrastructure and Instrumentation
Electrochemistry								
Voltammetry[14]	~ 1 nM		–		Unknown	No	Adsorptive stripping	Potentiostat, Electrochemical Cell, N_2 for purging O_2
Voltammetry[15]	–		~ 2 µM		Unknown	No	Differential Pulse Polarography	Potentiostat, Electrochemical Cell, N_2 for purging O_2
Synthetic Biology								
Whole Cell Biosensors (WCBs)[9, 16]	~45 nM		~10 µM		Known Crosstalk	Yes	SF, Optional Pre-concentration	Varies: Cell encapsulation, growth, biocontainment, detection
ROSALIND	~125 nM (Circuits can enhance sensitivity)		~2.5 µM (Circuits can enhance sensitivity)		Known Crosstalk	Yes	SF, Optional Pre-concentration	20 µL, Illuminator

*Field deployment shown for As only.

Supplementary Table 5 | Plasmid constructs and purification methods of aTFs.

aTF	Type of Purification	Tag Location	TEV Cleavage	Columns Used
TetR	His-tag affinity followed by size exclusion	C-terminus 6XHis-tag	N/A	HisTrap FF 5mL column for affinity, and Superdex HiLoad 26/600 200 pg column for size exclusion
OtrR	His-tag affinity followed by size exclusion	C-terminus TEV followed by 6XHis-tag	No	HisTrap FF 5mL column for affinity, and Superdex HiLoad 26/600 200 pg column for size exclusion
CtcR	His-tag affinity followed by size exclusion	C-terminus 6XHis-tag	N/A	HisTrap FF 5mL column for affinity, and Superdex HiLoad 26/600 200 pg column for size exclusion
MphR	His-tag affinity followed by size exclusion	C-terminus 6XHis-tag	N/A	HisTrap FF 5mL column for affinity, and Superdex HiLoad 26/600 200 pg column for size exclusion
MobR	His-tag affinity followed by size exclusion	C-terminus TEV followed by 6XHis-tag	No	HisTrap FF 5mL column for affinity, and Superdex HiLoad 26/600 200 pg column for size exclusion
QacR	His-tag affinity	C-terminus 6XHis-tag	N/A	Gravity flow column packed with Qiagen Ni-NTA Agarose
TtgR	His-tag affinity followed by size exclusion	C-terminus 6XHis-tag	No	HisTrap FF 5mL column for affinity, and Superdex HiLoad 26/600 200 pg column for size exclusion
HucR	His-tag affinity	N-terminus 6XHis-tag	No	Gravity flow column packed with Qiagen Ni-NTA Agarose
AdcR	PEI precipitation, $(NH_4)_2SO_4$ precipitation, ion exchange followed by size exclusion	N/A	N/A	SP-Sepharose fast flow column for ion exchange, and Superdex 75 preparative-grade column for size exclusion
SmtB	His-tag affinity	C-terminus TEV followed by 6XHis-tag	No	Gravity flow column packed with Qiagen Ni-NTA Agarose
CsoR	PEI precipitation, $(NH_4)_2SO_4$ precipitation, ion exchange followed by size exclusion	N/A	N/A	SP-Sepharose fast flow column for ion exchange, and Superdex 75 preparative-grade column for size exclusion

CsoR (Extended Data Fig. 7a)	His-tag affinity	N-terminus TEV followed by 6XHis-tag	Yes	Gravity flow column packed with Qiagen Ni-NTA Agarose
CadC	PEI precipitation, $(NH_4)_2SO_4$ precipitation, ion exchange followed by size exclusion	N/A	N/A	SP-Sepharose fast flow column for ion exchange, and Superdex 75 preparative-grade column for size exclusion

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