

Quantifying hormone disruptors with an engineered bacterial biosensor

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1. SUPPORTING FIGURES

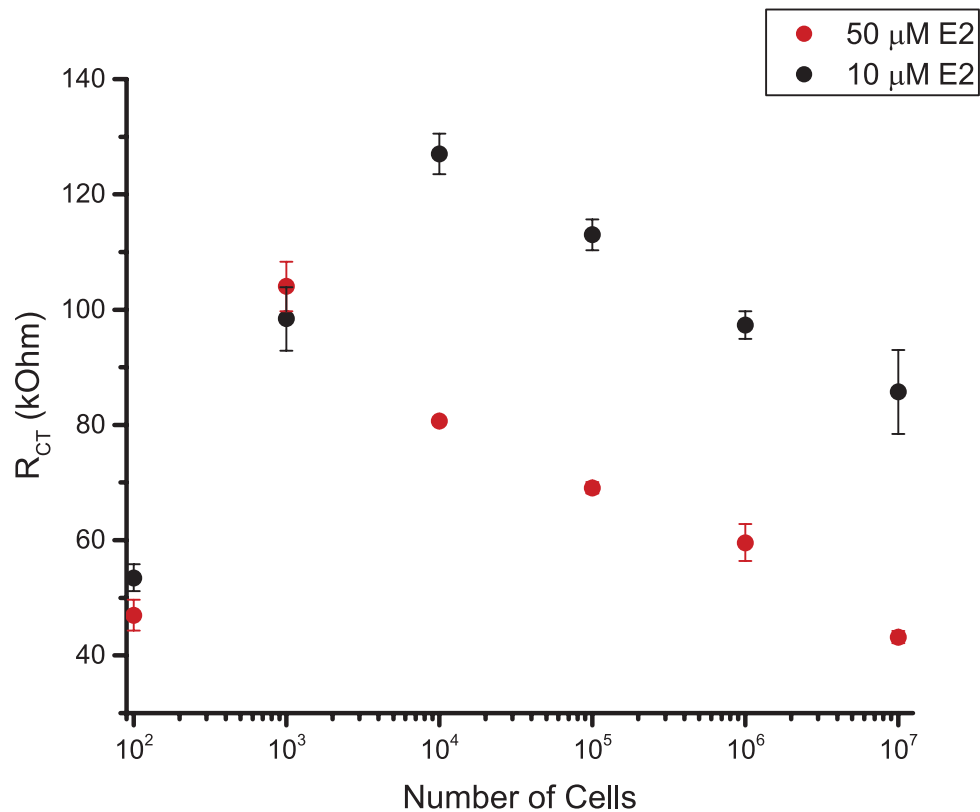


Figure S1. Optimization of number of cells applied to electrodes. The number of lyophilized cells on the electrodes was optimized at saturating estradiol (E2) levels: 50 μ M E2 (red) and 10 μ M E2 (black). R_{CT} values for varying numbers of cells at each concentration are shown. Error bars represent the SD for n=3 replicates.

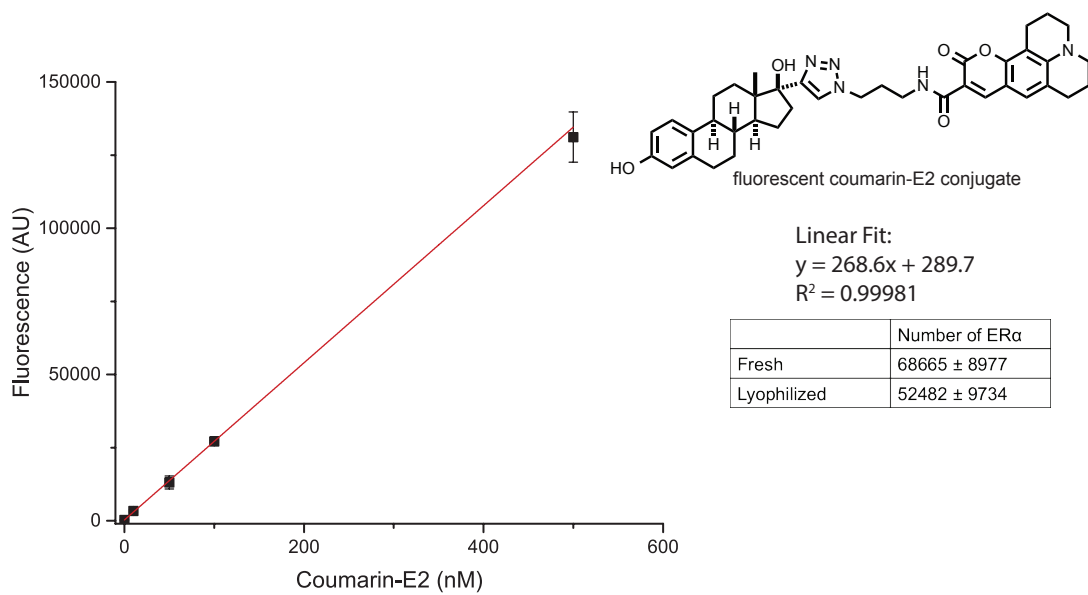


Figure S2. Standard curve of E2-coumarin fluorescence in the linear concentration range. The linear fit for the fluorescence at the peak wavelength (497 nm) was used to determine the number of surface-expressed ER α proteins.

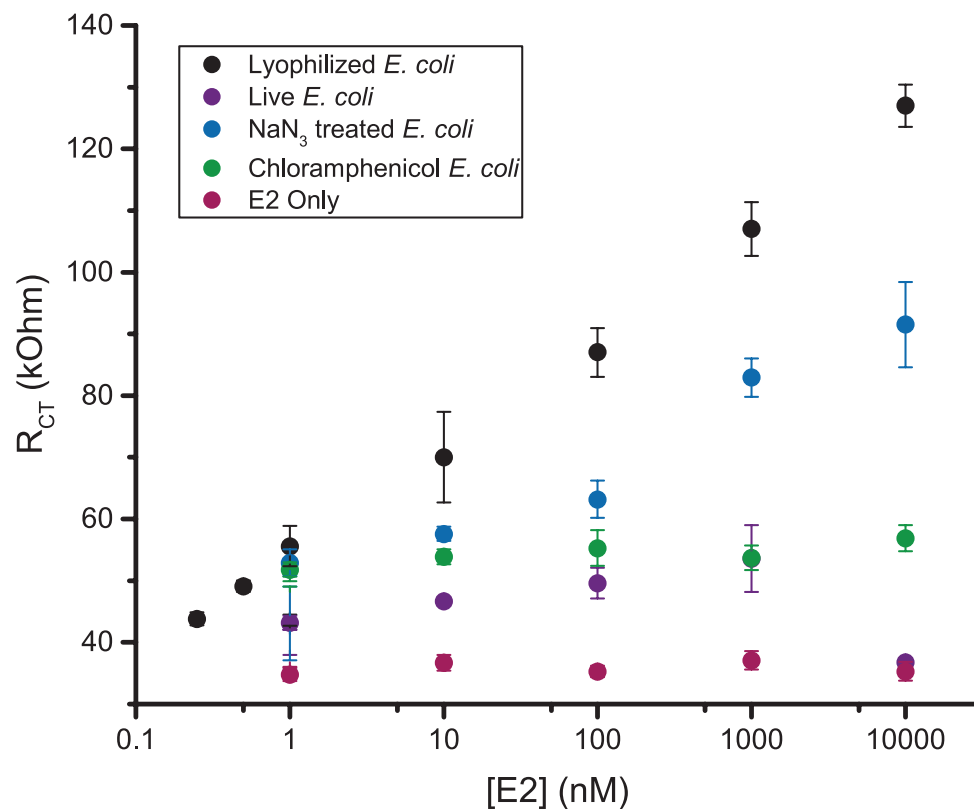


Figure S3. Estradiol concentration dependent R_{CT} for ER α on lyophilized *E. coli* (black), ER α on live *E. coli* (purple), ER α on NaN₃ killed *E. coli* (blue), and ER α on chloramphenicol-treated non-viable live *E. coli* (green). Monobody surfaces treated with only E2 are also shown (pink). Error bars represent the SD for n=3 replicates.

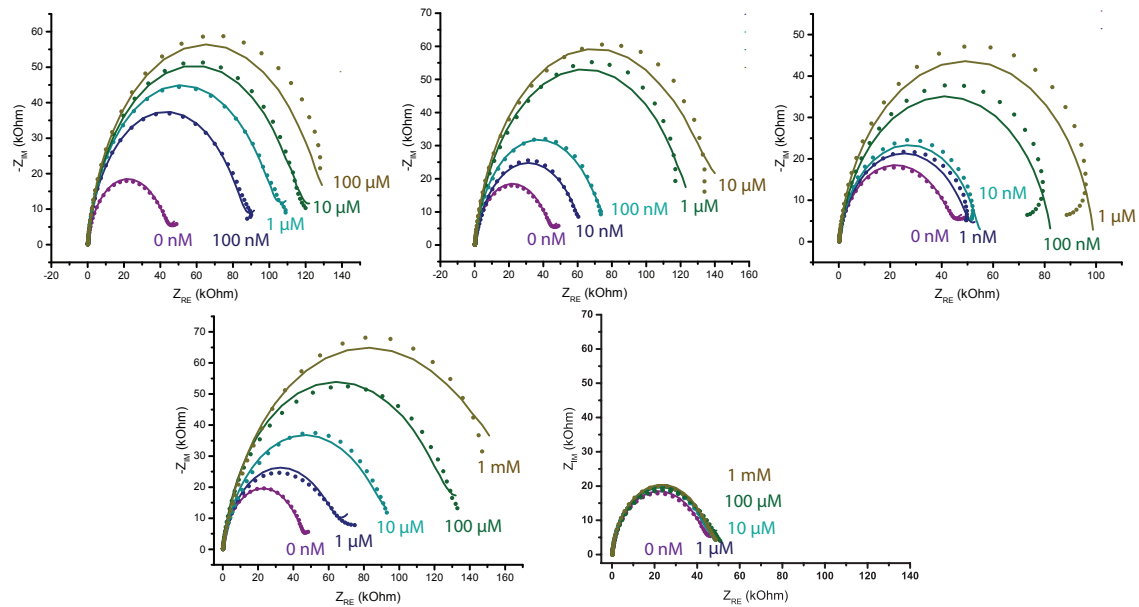


Figure S4. Nyquist plots with CPE fits for the endocrine disrupting compounds 4-nonylphenol (top left), genistein (top center), diethylstilbestrol (top right), bisphenol A (bottom left), and the non-binding hormone progesterone (bottom right).

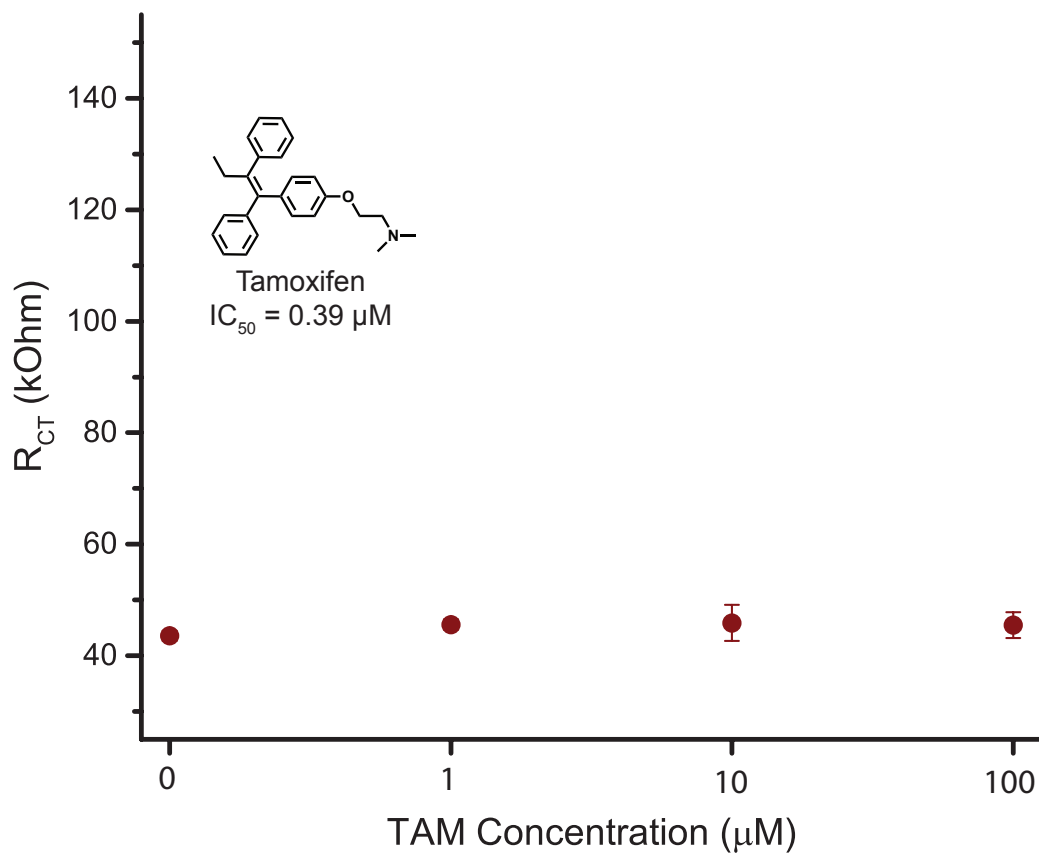


Figure S5. Chemotherapeutic and ER α antagonist Tamoxifen concentration dependent R_{CT} for ER α on lyophilized *E. coli*; no R_{CT} response is observed independent of concentration, indicating that the antagonist-ER α complex does not bind to the monobody. Error bars represent the SD for n=3 replicates.

Table S1. Concentrations of analytes used in Figure 4c of the main text.^a

Sample	Compound	%	0 Kd	1 Kd	10 Kd	100 Kd	1000 Kd
Control	E2	100	0	1.00E+00	1.00E+01	1.00E+02	1.00E+03
1	BPA	50	0	5.00E+03	5.00E+04	5.00E+05	5.00E+06
	4-NP	16.6	0	3.30E+02	3.30E+03	3.30E+04	3.30E+05
	DES	16.6	0	3.30E-02	3.30E-01	3.32E+00	3.32E+01
	GEN	16.6	0	8.00E+01	8.00E+02	8.00E+03	8.00E+04
2	BPA	16.6	0	1.66E+03	1.66E+04	1.66E+05	1.66E+06
	4-NP	50	0	1.00E+03	1.00E+04	1.00E+05	1.00E+06
	DES	16.6	0	3.30E-02	3.30E-01	3.32E+00	3.32E+01
	GEN	16.6	0	8.00E+01	8.00E+02	8.00E+03	8.00E+04
3	BPA	16.6	0	1.66E+03	1.66E+04	1.66E+05	1.66E+06
	4-NP	16.6	0	3.30E+02	3.30E+03	3.30E+04	3.30E+05
	DES	50	0	1.20E-01	1.20E+00	1.20E+01	1.20E+02
	GEN	16.6	0	8.00E+01	8.00E+02	8.00E+03	8.00E+04

^aAll concentrations in nM.

2. PLASMID SEQUENCES

2.1. *Monobody encoding pSKB3 vector.*

CCATGGGCTGCAGCCATCATCATCATCACGATTACGATATCCCAACGACCGAAAACCTTTA
CTTCCAGGGATCCGTCTCTGACGTACCTCGGACTTAGAGGTGGTCGCGCCACGCCACGAGC
CTGTTGATTAGCTGGGATGCTCCGGCAGTTACCGTGCCTATTATCGCATCACATATGGTGAGA
CGGGCGGCAACTCCCCAGTACAGGAATTCACCGTGCCGGGTAGTAAATCAACAGCCACCATTTC
AGGTTTGAAGCCGGGAGTGGATTATACTATCACCGTTTATGCAGTTACGGGCCTTCGCCTGATG
CTGGCTGGCAGCAAACCGATCTCAATCAACTATCGCACGTAAGCGGCCGCACTCGAG

Underlined restriction sites in order of sequence: NcoI/BamHI/NotI/XhoI

Green: 6His affinity tag encoding sequence

Orange: TEV encoding sequence

Purple: Monobody encoding sequence

2.2. *MBP-ERa encoding pSKB3 vector.*

CCATGGCCAGCAGCCATCATCATCATCACGATTACGATATCCCAACGACCGAAAACCTTTA
CTTCCAGGGATCCGAAAACCTTTACTTCCAGGGATCCAAAATCGAAGAAGGTAAACTGGTAATC
TGGATTAACGGCGATAAAGGCTATAACGGTCTCGCTGAAGTCGGTAAGAAATTCGAGAAAGATA
CCGGAATTAAGTCACCGTTGAGCATCCGGATAAACTGGAAGAGAAAATCCCACAGGTTGCGGC
AACTGGCGATGGCCCTGACATTATCTTCTGGGCACACGACCGCTTTGGTGGCTACGCTCAATCT
GGCCTGTTGGCTGAAATCACCCGGACAAAGCGTTCAGGACAAGCTGTATCCGTTTACCTGGG
ATGCCGTACGTTACAACGGCAAGCTGATTGCTTACCCGATCGCTGTTGAAGCGTTATCGCTGAT
TTATAACAAAGATCTGCTGCCGAACCCGCCAAAACCTGGGAAGAGATCCCGGCGCTGGATAAA
GAACTGAAAGCGAAAGGTAAGAGCGCGCTGATGTTCAACCTGCAAGAACCGTACTTCACCTGGC
CGCTGATTGCTGCTGACGGGGGTTATGCGTTCAAGTATGAAAACGGCAAGTACGACATTAAGA
CGTGGGCGTGGATAACGCTGGCGCGAAAGCGGGTCTGACCTTCCTGGTTGACCTGATTAAAAAC
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CGATGACCATCAACGGCCCCTGGGCATGGTCCAACATCGACACCAGCAAAGTGAATTATGGTGT
AACGGTACTGCCGACCTTCAAGGGTCAACCATCCAAACCGTTCGTTGGCGTGCTGAGCGCAGGT
ATTAACGCCCGCCAGTCCGAACAAAGAGCTGGCAAAGAGTTCCTCGAAAACCTATCTGCTGACTG
ATGAAGGTCTGGAAGCGGTTAATAAAGACAAACCGCTGGGTGCCGTAGCGCTGAAGTCTTACGA
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TTTCGTATGATGAACCTTCAAGGGGAAGAATTTGTTTGTCTGAAGTCAATCATCTGCTGAACT
CTGGAGTGTATACGTTCTTGAGTTCTACGCTGAAATCATTAGAAGAGAAAGATCACATTCATCG

TG TACTGGACAAAATCACAGATACGTTAATCCACCTTATGGCAAAGCAGGCTTGACTCTGCAG
CAGCAACATCAACGCCTGGCGCAGTTGCTGTTGATTCTGAGTCACATCCGCCATATGAGCAATA
AAGGCATGGAACATCTGTACTCCATGAAATGCAAAAATGTTGTCCCTTTATATGATCTGCTGTT
AGAAATGCTGGATGCGCACCGTTTGACGCGCCGTAAGCGGCCGCACTCGAG

Underlined restriction sites in order of sequence: NcoI/BamHI/NheI/NotI/XhoI
Green: 6His affinity tag encoding sequence
Orange: TEV encoding sequence
Blue: Maltose Binding Protein encoding sequence
Purple: N₁₀ linker encoding sequence
Red: ER α encoding sequence

2.3. INPNC-ER α encoding pSKB3 vector.

CCATGGCTCTCGACAAGGCGTTGGTGCTGCGTACCTGTGCAAATAACATGGCCGATCACTGCGG
CCTTATATGGCCCGCGTCCGGCACGGTGGAAATCCAGATACTGGCAGTCAACCAGGCGGCATGAG
AATGGTCTGGTTCGGTTTACTGTGGGGCGCTGGAACCAGCGCTTTTCTAAGCGTGCATGCCGATG
CTCGATGGATTGTCTGTGAAGTTGCCGTTGCAGACATCATCAGTCTGGAAGAGCCGGGAATGGT
CAAGTTTCCGCGGGCCGAGGTGGTTCATGTCCGGCAGCAGGATCAGCGCGTCACACTTCATTTCCG
GCACGTCAGGCCGACCCTGCGTCAACGTCAACGTCAACGTCAACGTCAACGTTAACGCCAATGC
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CGTCATGAAGTGTTCGATGTCCGCTCGGTTCAGCGCGGCTGCCGCCCCAGTAAACACCCTGCCGG
TGACGACGCCGAGAATTTGCAGACCAGATCCAGACTCTGGGACGGGAAGAGGTACAGGCAACT
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TGGACAAAATCACAGATACGTTAATCCACCTTATGGCAAAGCAGGCTTGACTCTGCAGCAGCA
ACATCAACGCCTGGCGCAGTTGCTGTTGATTCTGAGTCACATCCGCCATATGAGCAATAAAGGC
ATGGAACATCTGTACTCCATGAAATGCAAAAATGTTGTCCCTTTATATGATCTGCTGTTAGAAA
TGCTGGATGCGCACCGTTTGACGCGCCGTAAGCGGCCGCACTCGAG

Underlined restriction sites in order of sequence: NcoI/NheI/NotI/XhoI
Orange: INP_N encoding sequence
Blue: INP_C encoding sequence
Red: ER α encoding sequence