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Supporting Information for
Engineering transmembrane signal transduction in synthetic membranes using two-component systems

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This PDF file includes:

Supplementary Figures

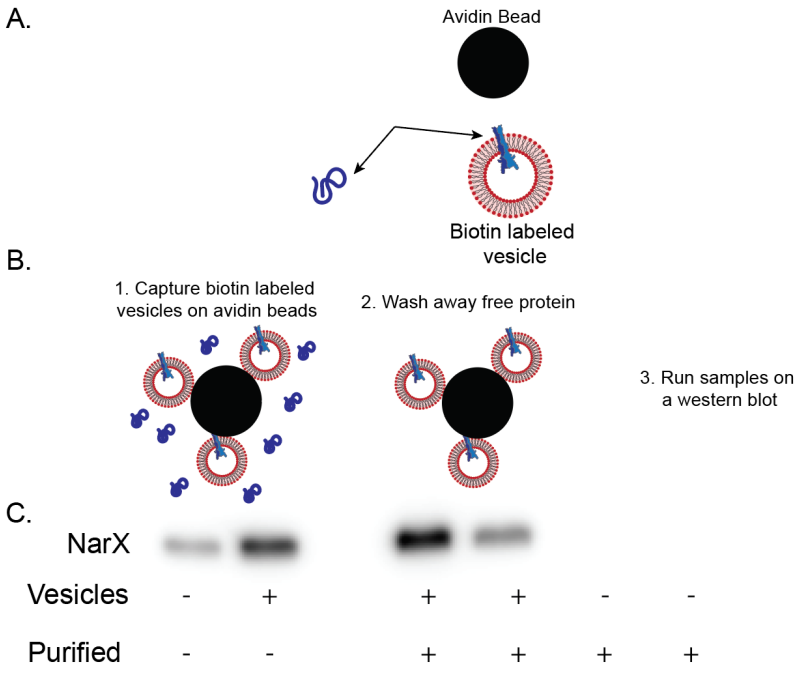

Figure S1. NarX associates with synthetic membranes. (A) NarX was expressed with and without DMPC vesicles labeled with biotin. (B) Vesicles were then pulled down with avidin coated magnetic beads and subsequently washed to remove non-vesicle associated protein. A western blot was then performed on the purified samples, probing for myc-tagged NarX. (C) NarX associates with DMPC vesicles as determined by Western Blot. NarX was expressed as determined by probing samples prior to purification. However, NarX was only retained only in samples with vesicles present in the reaction following purification.

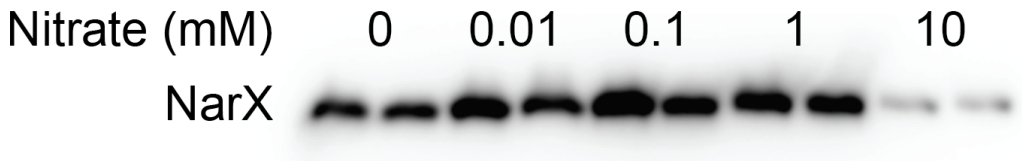

Figure S2. Protein expression is inhibited at high nitrate concentrations. Cell-free reactions were performed with increasing amounts of nitrate and expression of NarX was probed via western blot. At high concentrations of nitrate band intensity decreased, suggesting that nitrate inhibits protein expression. Each lane pair represents a replicate (*n=2*).

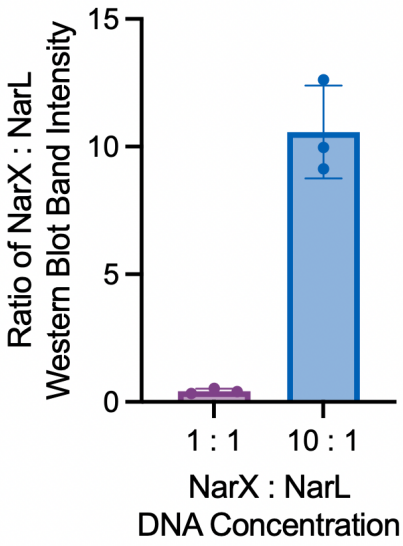

Figure S3. Protein expression can be tuned by altering DNA concentrations in cell-free reactions. Cell-free reactions containing 1 NarX : 1 NarL and 10 NarX : 1 NarL mol DNA (6.6 nM total) were run and protein expression was quantified by western blot. The ratio of band intensities of NarX to NarL increases with increasing ratio of the respective DNA concentrations.

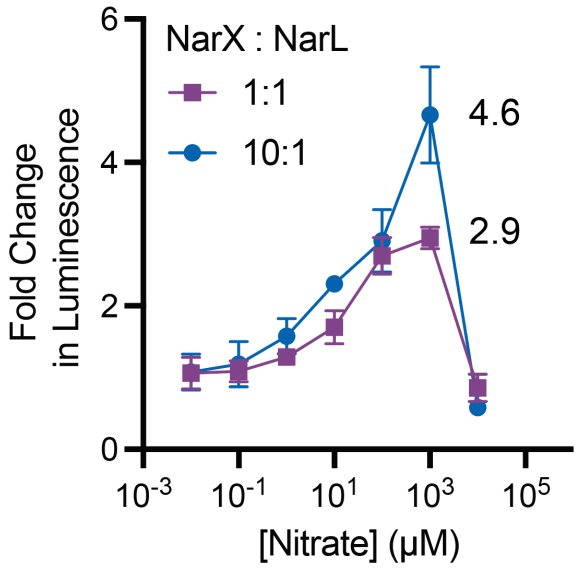

Figure S4. Nitrate titration for cell-free reactions with DMPC vesicles and either 1:1 or 10:1 NarX:NarL plasmid ratio. By altering the DNA ratio of NarX and NarL, the fold increase in luminesce in response to nitrate is increased. The sum of NarX and NarL plasmid concentrations were kept at 6.6 nM. All error bars represent the S. E. M. for *n=3* independent replicates.

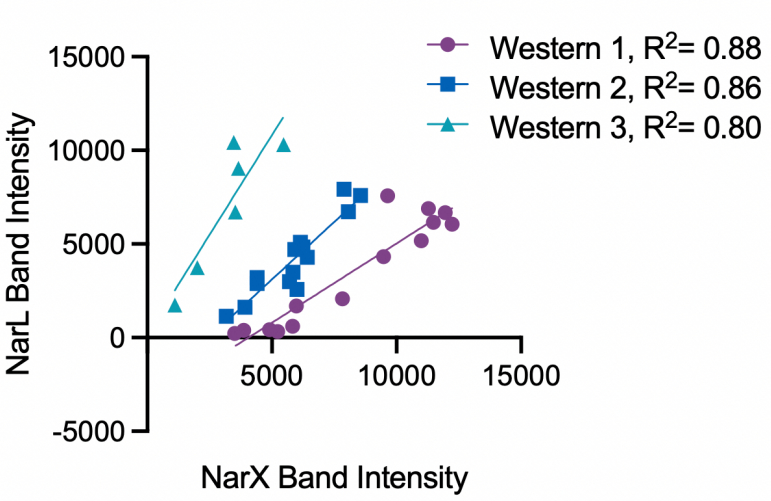

Figure S5. NarL band intensity correlates with NarX band intensity. Protein expression of each component is interrelated, as unfolded or aggregated protein likely aggregates nascent and expressed proteins. Each line represents a different western blot.

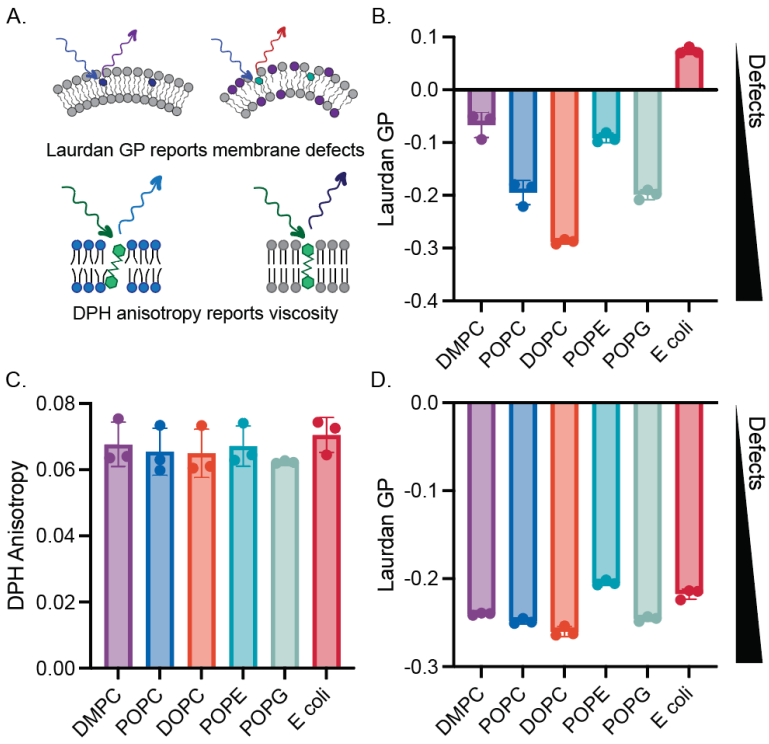

Figure S6. Measurement of membrane defects and viscosity for membranes used in this study. (A) Laurdan GP was used to measure membrane defects, while DPH anisotropy was used to measure membrane viscosity. (B) Laurdan GP measurements of membranes in Figure 2. (C) DPH Anisotropy and (D) Laurdan GP for membranes in Figures 3. Differences in DPH Anisotropy were not observed as POPC made up of 90 mol% in each sample, while only 10 mol% was altered. All error bars represent the S. E. M. for *n=3* independent replicates.

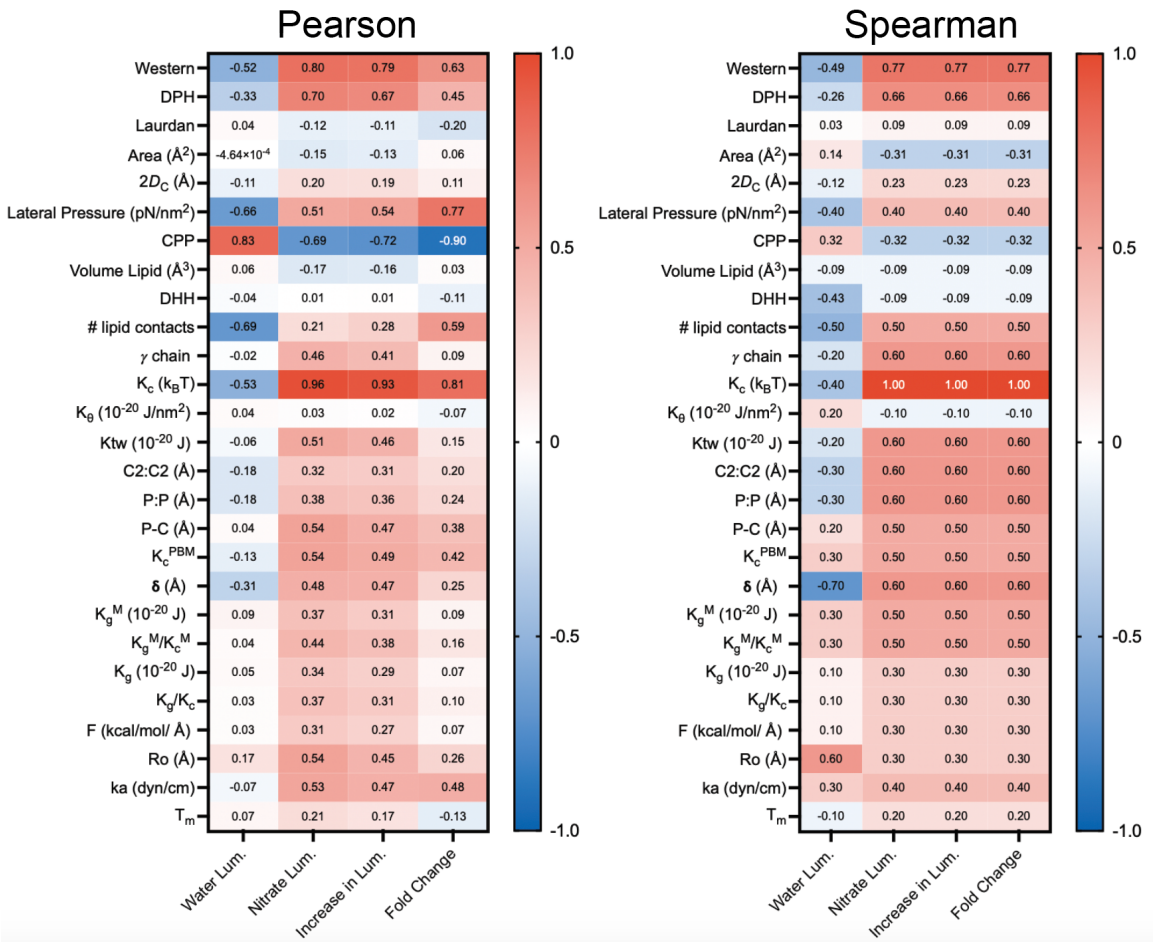

Figure S7. Pearson and Spearman correlations for data in Figure 2.

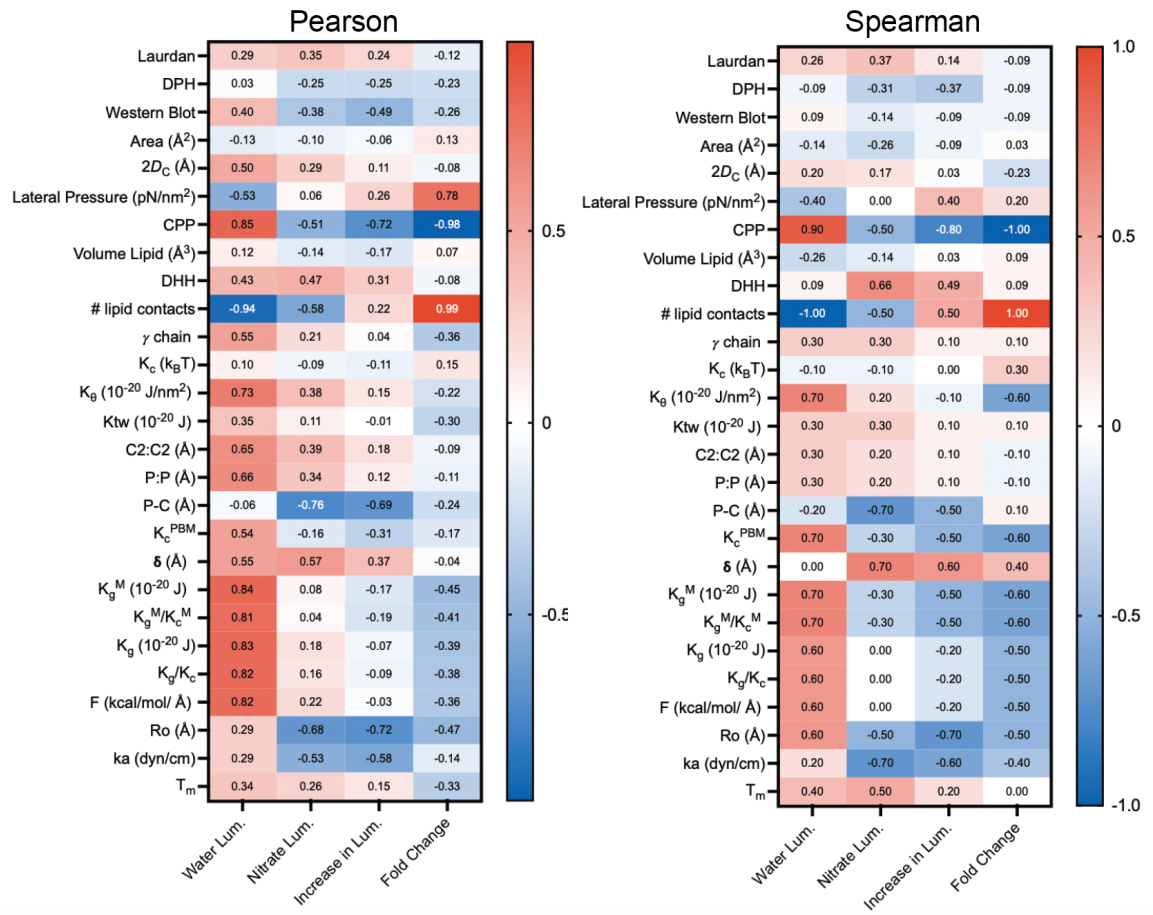

Figure S8. Pearson and Spearman correlations for data in Figure 3.

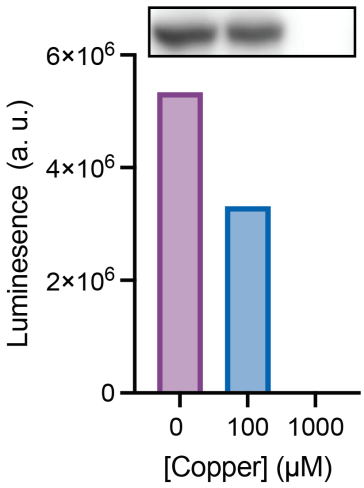

Figure S9. Copper inhibits cell-free protein synthesis at relevant concentrations. Protein expression as determined by NarX Western Blot band intensity and luminescence decreased with increasing copper concentration.

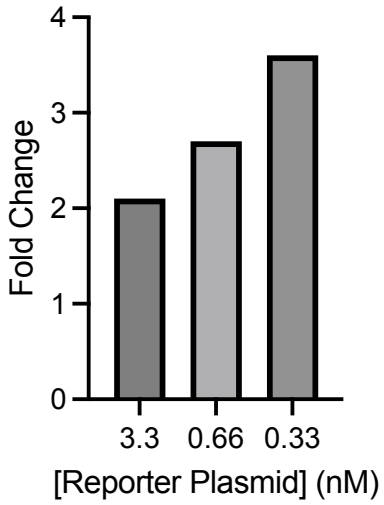

Figure S10. Tuning the concentration of reporter plasmid enhances chimera sensor fold change. Cell-free reactions were assembled with POPC liposomes and the NrsS chimeric protein, varying the concentration of the reporter plasmid from 3.3 to 0.33 nM. Fold change of luciferase expression in the presence and absence of nickel was calculated. As the concentration of reporter plasmid was decreased, the fold change in response to nickel increased (*n=1*).

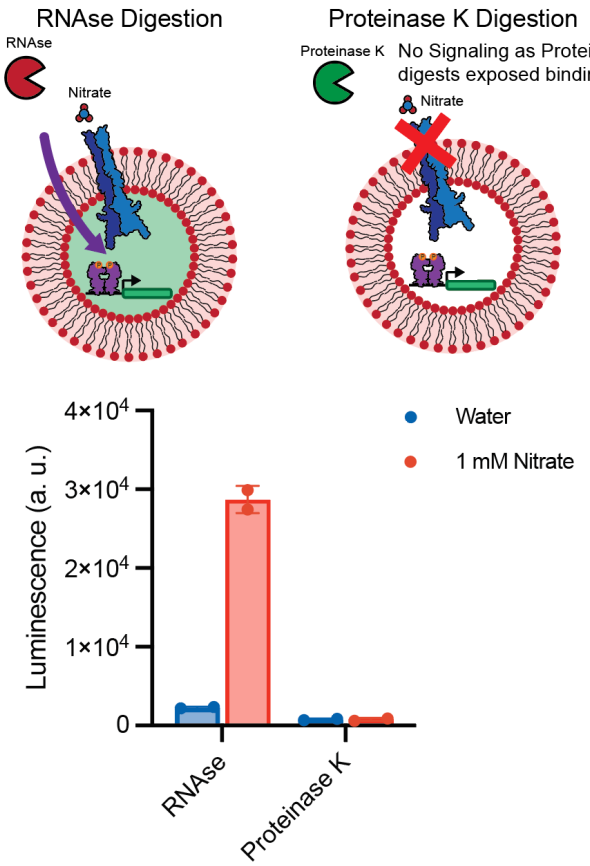

Figure S11. Encapsulated TCS signaling is inhibited upon incubation of Proteinase K but not RNAse. External Proteinase K likely digests externally displayed nitrate binding domains, inhibiting downstream, nitrate dependent signaling. *n=2* , error bars represent S. E. M.

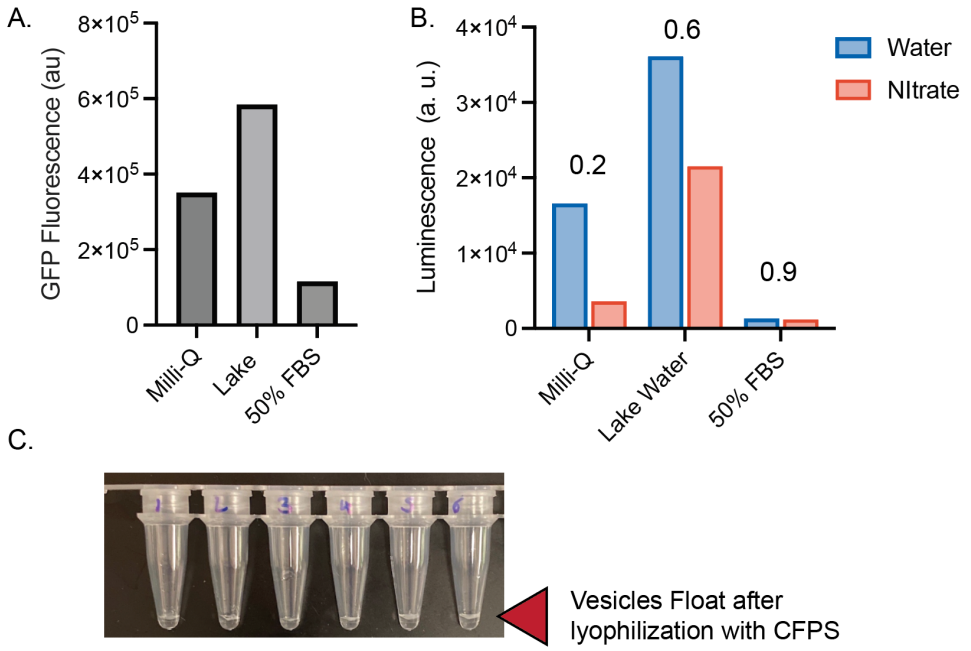

Figure S12. Characterizing the performance of lyophilized NarX-L sensors. (A) Background subtracted eGFP fluorescence following lyophilization and rehydration with different media. (B) Lyophilized NarX-L activity when vesicles are lyophilized with cell-free components. (C) Vesicles appear to float when lyophilized with cell-free reactions, likely leading to poor fold increase in response to nitrate. Experiments were repeated with vesicles in reactions prior to lyophilization multiple times and always produced fold increases opposite to what was observed in non-lyophilized experiments; representative data is shown (*n=1*).

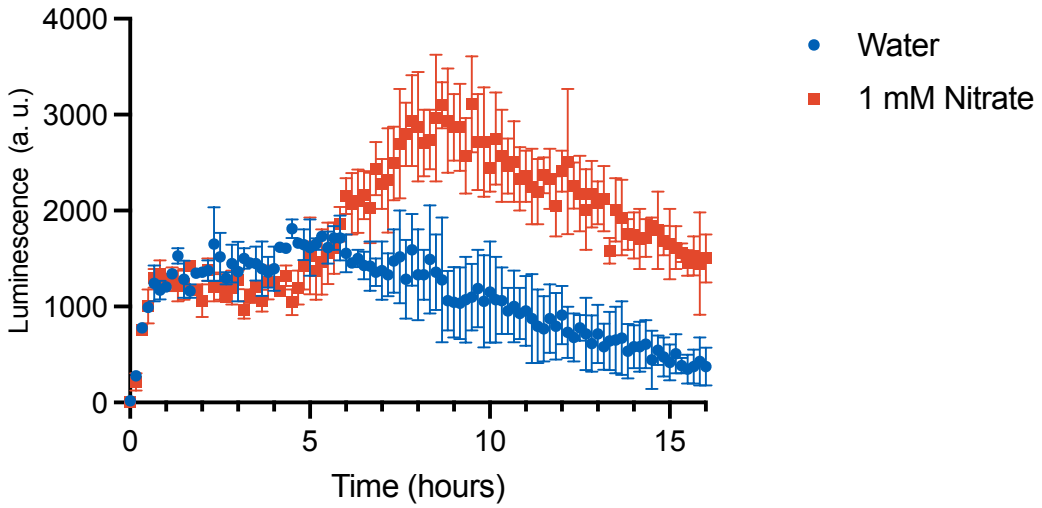

Figure S13. Kinetics of the NarX sensor. Cell-free reactions were set up and luminescence was read on the plate reader over time. The nitrate sample was significantly higher than the water sample after 6.5 hours ($p<0.03$). Luminescence decreases over time likely due to the reduction in substrate as it is used. Error bars represent the standard deviation, $n=2$.

Table S1. Dynamic light scattering of vesicles compositions used in Figure 2. Presented is an average and standard deviation of 2 independently prepared samples.

	Diameter (nm)	***SD (nm)***	***P.D.I.***
100% DMPC	127.44	43.22	0.115
100% POPC	128.14	66.41	0.269
100% DOPC	124.71	62.77	0.253
30% POPE, 70% POPC	118.24	47.02	0.158
30% POPG, 70% POPC	112.91	61.28	0.295
100% E. coli Polar Lipid Extract	127.19	50.47	0.157

Table S2. Lipid physical properties found in literature.

	POPC	DOPC	DMPC	POPE	POPG	E. coli
Area ($Å^2$) (1)	64.3	67.4	60.6	56.6	66	56.8
$2D_c$ (Å) (2)	28.8	28.8	25.4	32.6	27.9	30
Lateral Pressure (pN/nm^2) (3)	517	480	442	456		
CPP (4)	0.61	1.09	1	1.08	0.7	
Lipid Volume (A^3) (3)	1231.2	1304.5	1100	1176.8	1226.6	1186.6(1)
D_{HH} (Å) (2)	37	36.7	35.3	43.4	38.5	40.8(1)
# of lipid contacts (5)	2.75			1.5		2.25
γ chain (6)	0.198	0.18(5).221	0.247	0.188		
K_c (k_BT) (6)	31.7	28.8	29.3	31.2	26.9	
K_θ (10^{-20} J/nm^2) (6)	5.52	6.4	4.02	8.04	6.15	
K_{tw} (10^{-20} J) (6)	1.45	0.99	2.18	2.36	1.05	
C2:C2 (Å) (6)	27.52	27.37	24.54	30.67	26.88	
P:P (Å) (6)	38.54	38.35	35.65	41.55	37.49	
P-C (Å) Head group thickness (6)	11.02	10.98	11.11	10.88	10.61	
K_c^{PBM} (6)	9.5	9.71	5.75	10.78	5.67	
δ (Å) (6)	9.64	9.41	9.48	10.12	9.57	
K_g^M (10^{-20} J) (6)	-1.09	-0.84	-1.32	0.06	-1.43	
K_g^M/K_c^M (6)	-0.17	-0.14	-0.22	0.01	-0.25	
Kg (10^{-20} J) (6)	-1.38	-0.08	-3.23	5.85	-2.76	
K_g/K_c (6)	-0.1	-0.01	-0.26	0.44	-0.24	
F (kcal/mol/ Å) (6)	0.0303	0.061	-0.0225	0.2041	0.0036	
R_o (Å) (6)	-315	-1(5)392	-47	-2220		
K_a (dyn/cm) (6)	280	290	210	260	180	
Tm (7)	-9	-17	24	25	-2	

Table S3. Dynamic light scatting of vesicle compositions used in Figure 3. All vesicles are composed of 90% POPC and 10% of lipid listed in the table. Presented is an average and standard deviation of 2 independently prepared samples.

	Diameter (nm)	***SD (nm)***	***P.D.I.***
DMPC	129.61	60.54	0.218
POPC	128.14	66.41	0.269
DOPC	129.86	72.76	0.271
POPE	125.23	61.91	0.244
POPG	121.16	51.83	0.183
E. coli	128.33	47.69	0.209

Table S4. Dynamic light scatting of 100% POPC vesicles in the presence of ligands used in this study. Presented is an average and standard deviation of 2 independently prepared samples.

	Diameter (nm)	***SD (nm)***	***P.D.I.***
1 mM Nitrate	123.26	64.93	0.278
1 mM Nickel	123.27	54.07	0.192
100 µM Iron	118.62	43.63	0.135
1 mM Vancomycin	124.88	54.32	0.189

Table S5. DNA sequences used in this study. All genes were placed under the control of a T7 promoter, except nanoluciferase and GFP. These constructs were under the control of the PydfJ115 promoter. The promoter sequence is listed in with the gene.

Plasmid/Gene	Sequence
NarX	ATGCTTAAACGTTGTCTCTCTCCGCTCACCCTGGTTAATCAGGTTG CGCTTATTGTGTTGCTTTCTACTGCTATTGGACTGGCAGGGATGGC GGTTTCTGGCTGGCTGGTGCAAGGCGTTCAGGGCAGCGCCCATGC GATCAACAAAGCGGGATCGCTGCGCATGCAAAGTTACCGTCTGTTG GCGGCAGTGCCATTAAGCGAGAAAGACAAGCCCTTAATTAAAGAGA TGGAACAAACGGCATTTAGCGCCGAGTTGACTCGAGCAGCAGAAC GAGACGGACAACTGGCGCAATTACAGGGTTTACAAGATTACTGGC GTAATGAACTGATCCCTGCGCTGATGCGTGCACAAAACCGAGAAAC GGTGTCAGCGGATGTCAGCCAGTTTGTTGCCGGGCTTGATCAACT GGTATCTGGTTTTGACCGCACCACGGAAATGCGCATCGAGACAGT GGTACTGGTCCATCGGGTAATGGCGGTATTTATGGCACTTTTACTG GTGTTCACTATTATCTGGTTGCGGGCGCGACTGCTACAACCGTGGC GGCAACTGCTGGCAATGGCGAGTGCCGTCAGTCATCGCGATTTTA CCCAACGCGCAAACATCAGCGGGCGCAACGAAATGGCGATGCTTG GAACTGCGTTGAACAATATGTCTGCAGAACTGGCCGAAAGTTATGC CGTACTTGAGCAGCGGGTTCAGGAGAAAACCGCCGGGCTGGAGCA TAAAAATCAGATCCTCTCTTTTTTATGGCAGGCTAACCGCCGTTTGC ATTCCCGCGCCCCGCTGTGTGAACGCCTGTCACCTGTACTCAACG GCTTACAGAATTTAACCCTGCTACGTGATATCGAATTGCGGGTGTA TGACACTGATGATGAAGAGAATCATCAGGAGTTTACCTGCCAGCCA GATATGACTTGTGATGATAAAGGCTGCCAGCTCTGCCCGCGCGGC GTATTACCCGTTGGTGATCGCGGCACGACCCTGAAGTGGCGGCTG GCTGACTCTCATACGCAGTACGGTATTTTGCTGGCGACCCTGCCAC AGGGGCGTCATCTTAGCCATGATCAACAACAACTGGTGGATACCCT GGCTGAACAACTCACCGCCACGCTGGCGCTGGATCGCCATCAGGA ACGTCAGCAACAGTTGATCGTGATGGAAGAGCGTGCCACCATTGC GCGCGAACTGCATGATTCTATTGCCCAATCTCTCTCTTGCATGAAG ATGCAGGTGAGTTGTTTACAGATGCAGGGCGATGCGCTGCCAGAA AGCAGCCGCGAACTGTTAAGTCAGATCCGTAACGAACTGAATGCAT CCTGGGCGCAGTTGCGTGAATTGCTCATCACATTCCGCTTGCAGCT CACCGAGCCTGGATTACGTCCGGCGCTGGAGGCGAGTTGCGAAGA GTACAGCGCCAAATTTGGCTTCCCGGTGAAGCTGGATTATCAATTG CCGCCTCGCCTGGTGCCTTCGCATCAGGCAATCCACTTGTTGCAAA TTGCCCGTGAGGCATTAAGTAACGCCCTCAAACATTCGCAAGCGAG TGAAGTCGTGGTGACGGTGGCGCAAAACGATAATCAGGTCAAACT GACCGTCCAGGATAACGGCTGCGGCGTGCCTGAAAATGCCATCCG CAGCAATCACTACGGCATGATAATAATGCGCGATCGTGCGCAAAGT TTACGAGGCGATTGCCGCGTCCGCCGTCGTGAATCAGGTGGCACC GAAGTGGTGGTCACCTTTATTCCCGAAAAAACTTTCACAGACGTCC AAGGAGATACCCATGAGGGAGGAGGAAGCGAGCAGAAACTCATCT CTGAAGAGGATCTGTAA
NarL	ATGAGTAATCAGGAACCGGCTACTATCCTGCTGATTGACGATCACC CGATGCTGCGAACTGGCGTAAAACAGCTTATCAGTATGGCACCAGA TATCACCGTGGTTGGCGAAGCGAGTAATGGCGAACAGGGTATTGA ACTGGCGGAGTCTCTTGATCCCGATCTGATCCTGTTAGATCTCAAT ATGCCCGGCATGAACGGTCTGGAAACGCTGGATAAACTGCGCGAA AAGTCCCTCTCAGGGCGCATTGTGGTATTCAGCGTCTCTAACCATG AAGAAGATGTGGTCACCGCACTGAAACGCGGCGCGGATGGCTATC TGTTAAAAGATATGGAACCGGAAGATCTGCTGAAAGCATTGCATCA GGCAGCTGCTGGCGAAATGGTATTAAGCCCTGATATCCTTAAACGT CTGCAAGAAATCCAATTTGAGCGGATGAAAAAGCAGCGCAATGAGA

	CGCAGCTGACAGAAAAGGAAGTCATTGTTCTAAAAGCAATTGCTAA AGGTCTTAAAAGCAAAGCGATTGCCTTTGATTTGGGCGTCTCTGAG CGAACAGTAAAGTCCAGATTAACGTCCATTTACAATAAATTAGGCGC GAATTCAAGAACTGAGGCAGTAACGATTGCCATGCAAAAAGGTATT CTGACAATAGACAACggAGGAGGAAGCGATTACAAGGATGACGACG ATAAGTAA
PYdfJ115_nanoluc	ACTGCATATTTGAAAATTGCCCAAACGTACATGCCCGAATGTACGTT TTTTTCATTTCATTGTCAACTACAATGAGAAAGAATGTGATCAAGCA ATGTGTTGAAAGGAGATTATCACGTCGACTCTCGAGTGAGATTGTT GACGGTACCGTATTTTGGATCTAGGAGGAAGGATCTATGGTGTTTA CGCTGGAGGATTTCGTCGGTGACTGGCGTCAGACAGCTGGGTATA ACCTTGACCAGGTACTTGAACAAGGCGGCGTTTCCAGCTTATTTCA AAATCTGGGGGTGTCTGTCACACCAATTCAGCGCATTGTCTTGTCT GGGGAGAATGGTCTTAAAATTGATATTCATGTTATCATCCCTTACGA AGGTTTGTCCGGTGACCAAATGGGACAAATCGAGAAAATTTTCAAA GTGGTTTATCCAGTGGATGATCATCACTTTAAAGTCATTTTACACTA TGGTACGCTGGTAATCGACGGTGTGACACCGAACATGATTGATTAT TTCGGGCGTCCGTATGAAGGAATCGCCGTTTTTGATGGGAAGAAAA TCACCGTAACAGGTACTCTGTGGAACGGAAACAAAATCATCGACGA ACGCTTGATTAACCCCGATGGTAGTTTGTTGTTCCGTGTTACGATTA ACGGAGTTACGGGTTGGCGCCTTTGTGAGCGTATCTTGGCCGGAA GCGGAAGCGAGCAGAAACTCATCTCTGAAGAGGATCTGTAA
PYdfJ115_GFP	ACTGCATATTTGAAAATTGCCCAAACGTACATGCCCGAATGTACGTT TTTTTCATTTCATTGTCAACTACAATGAGAAAGAATGTGATCAAGCA ATGTGTTGAAAGGAGATTATCACGTCGACTCTCGAGTGAGATTGTT GACGGTACCGTATTTTGGATCTAGGAGGAAGGATCTATGAGCAAAG GAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGAT GGTGATGTTAATGGGCACAAATTTTCTGTCCGTGGAGAGGGTGAAG GTGATGCTACAAACGGAAAACTCACCCTTAAATTTATTTGCACTACT GGAAAACTACCTGTTCCGTGGCCAACACTTGTCACTACTCTGACCT ATGGTGTTCAATGCTTTTCCCGTTATCCGGATCACATGAAACGGCA TGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGC ACTATATCTTTCAAAGATGACGGGACCTACAAGACGCGTGCTGAAG TCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAGGGT ATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAACTCGAGTA CAACTTTAACTCACACAATGTATACATCACGGCAGACAAACAAAAGA ATGGAATCAAAGCTAACTTCAAAATTCGCCACAACGTTGAAGATGG TTCCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCG ATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGACACAATCT GTCCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTTC TTGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGATGAGCT CTACAAATAA
NarX-CusS Chimera	ATGGTGAGCAAACCGTTTCAGCGTCCGTTTAGCCTGGCAACCCGTC TGACCTTTTTCATTTCACTGGCAACCATTGCAGCATTTTTCGCATTT GCATGGATTATGATCCATAGCGTGAAAGTGCATTTTGCCGAACAGG ATATTAACGACCTGAAAGAAATTAGCGCAACCCTGGAACGTGTTCT GAATCATCCTGATGAAACCCAGGCACGTCGTCTGATGACCCTGGAA GATATTGTTAGCGGTTATAGCAATGTTCTGATTAGTCTGGCAGATAG CCAGGGTAAAACCGTTTATCATAGTCCGGGTGCACCGGATATTCGT GAATTTACCCGTGATGCAATTCCGGATAAAGATGCACAAGGTGGTG AAGTTTATCTGCTGAGCGGTCCGACCATGATGATGCCTGGTCATGG TCATGGCCACATGGAACATAGCAATTGGCGTATGATTAATCTGCCG GTTGGTCCGCTGGTTGATGGTAAACCGATTTATACCCTGTATATTG CCCTGAGCATTGATTTTCATCTGCACTATATCAACGATCTGATGAAC AAACTGATTATGACCGCAAGCGTTATTAGCATCCTGATTGTTTTTAT TGTTCTGCTGGCAGTTCATAAAGGTCATGCACCGATTCGTAGCGTT

	AGCCGTCAGATTCAGAATATTACCAGCAAAGATCTGGATGTTCGTC TGGATCCGCAGACCGTTCCGATTGAACTGGAACAGCTGGTTCTGA GCTTTAATCATATGAGCGCAGAACTGGCAGAAAGCTATGCAGTTCT GGAACAGCGTGTTCAAGAGAAAACCGCAGGCCTGGAACATAAAAAT CAGATTCTGAGCTTTCTGTGGCAGGCAAATCGTCGTCTGCATAGCC GTGCACCGCTGTGTGAACGTCTGAGTCCGGTTCTGAATGGTCTGC AGAACCTGACACTGCTGCGTGATATTGAACTGCGTGTTTATGATAC CGATGATGAAGAAAACCACCAAGAATTTACCTGTCAGCCGGATATG ACCTGTGATGATAAAGGTTGTCAGCTGTGTCCGCGTGGTGTTCTGC CGGTGGGTGATCGTGGTACAACCCTGAAATGGCGTCTGGCCGATA GTCATACCCAGTATGGTATCCTGCTGGCGACACTGCCGCAGGGTC GTCATCTGAGCCATGATCAGCAGCAGCTGGTAGATACCCTGGCCG AACAGCTGACCGCAACACTGGCACTGGATCGTCATCAAGAACGTCA GCAGCAACTGATTGTTATGGAAGAACGTGCCACCATTGCGCGTGAA CTGCATGATAGCATTGCACAGAGCCTGAGCTGTATGAAAATGCAGG TTAGCTGTCTGCAGATGCAGGGTGATGCACTGCCGGAAAGCTCAC GCGAACTGCTGAGCCAGATTCGCAATGAACTGAATGCCAGCTGGG CACAGCTGCGTGAGCTGCTGATTACCTTTCGTCTGCAGCTGACAGA ACCGGGTCTGCGTCCGGCACTGGAAGCAAGCTGTGAAGAATATTC AGCGAAATTTGGTTTTCCGGTGAAACTGGATTATCAGCTGCCTCCG CGTCTGGTTCCGAGCCATCAGGCAATTCATCTGCTGCAAATTGCAC GTGAAGCACTGAGCAATGCACTGAAACATTCACAGGCAAGCGAAGT TGTTGTTACCGTTGCACAGAATGATAACCAGGTTAAACTGACCGTT CAGGATAATGGTTGTGGTGTTCCGGAAAATGCAATTCGTAGCAATC ATTATGGCATGATCATTATGCGTGATCGTGCCCAGAGCCTGCGTGG TGATTGTCGTGTTCGTCGTCGTGAAAGCGGTGGTACAGAAGTGGT GGTTACCTTTATTCCTGAGAAAACCTTTACCGATGTTCAGGGTGATA CCCATGAAGGTGGTGGTAGCGAACAGAAACTGATTTCAGAAGAAGA TCTGTAA
NarX-NrsS Chimera	ATGAATACCCGTCGTCTGTTTGCACGTAGCCGTCTGCAGCTGGCAT TTTGGTATGCACTGGTTATGGGTGGTATTCTGACCCTGTTAGGTCT GGGTGTTTATCGTGCAATTGTTCAGGCAAATTGGATGGCACTGGAA CGTGAAGTTGAAAGCATTGCAGGCACCCTGCATGATAGCCTGGAA CCGATGCTGCCGAGCAATGCAAGCCCGACCGGTGTGCTGCAGAAA ATGCTGCCGGATCTGTGTCTGGTTAATCAGCCGTGCCAGGTTAATC CGACACTGATTGAACGTCATACCCTGGGTATTAGCGATCGTAGCCT GTATTATATCCGCCTGTTTGATTATCAGGGTAATCTGCTGGCGTTTA GCCCGAATCAGCCTGCAAGCCTGAGCAGCATCTTTAATCAAGAAAC CTGGCAGACCATTCATCCGCCTACCGGTGATCGTTATCGTCAGTTT ACCACCATTCTGCATAGCGCAGGTAATACCGATAAAAGCAGCTGGG GTTATCTGCAAATTGGTCGTAGTCTGGCAGCCTTTGATGCCGAAAA TAAACGTATTCTGTGGATTCTGGGTCTGAGCTTTCCGATTGCACTG GGTTTAGTTGCATTTAGCAGTTGGGGTTTAGCAGGTCTGGCAATGC GTCCGATTTATCAGAGCTATCAGCAGCAGCAACAGTTTACCGCAAA TGCAGCACATGAACTGCGTAGTCCGCTGGCAAGCCTGCTGGCAAC CGTTGAAGCAGTTCTGCGTATTGATAGCAGCCATAGTCCGGAAATT AACACCATGTCTGCAGAACTGGCCGAAAGTTATGCCGTACTTGAGC AGCGGGTTCAGGAGAAAACCGCCGGGCTGGAGCATAAAAATCAGA TCCTCTCTTTTTTATGGCAGGCTAACCGCCGTTTGCATTCCCGCGC CCCGCTGTGTGAACGCCTGTCACCTGTACTCAACGGCTTACAGAAT TTAACCCTGCTACGTGATATCGAATTGCGGGTGTATGACACTGATG ATGAAGAGAATCATCAGGAGTTTACCTGCCAGCCAGATATGACTTG TGATGATAAAGGCTGCCAGCTCTGCCCGCGCGGCGTATTACCCGT TGGTGATCGCGGCACGACCCTGAAGTGGCGGCTGGCTGACTCTCA TACGCAGTACGGTATTTTGCTGGCGACCCTGCCACAGGGGCGTCA TCTTAGCCATGATCAACAACAACTGGTGGATACCCTGGCTGAACAA

	CTCACCGCCACGCTGGCGCTGGATCGCCATCAGGAACGTCAGCAA CAGTTGATCGTGATGGAAGAGCGTGCCACCATTGCGCGCGAACTG CATGATTCTATTGCCCAATCTCTCTCTTGCATGAAGATGCAGGTGA GTTGTTTACAGATGCAGGGCGATGCGCTGCCAGAAAGCAGCCGCG AACTGTTAAGTCAGATCCGTAACGAACTGAATGCATCCTGGGCGCA GTTGCGTGAATTGCTCATCACATTCCGCTTGCAGCTCACCGAGCCT GGATTACGTCCGGCGCTGGAGGCGAGTTGCGAAGAGTACAGCGC CAAATTTGGCTTCCCGGTGAAGCTGGATTATCAATTGCCGCCTCGC CTGGTGCCTTCGCATCAGGCAATCCACTTGTTGCAAATTGCCCGTG AGGCATTAAGTAACGCCCTCAAACATTCGCAAGCGAGTGAAGTCGT GGTGACGGTGGCGCAAAACGATAATCAGGTCAAACTGACCGTCCA GGATAACGGCTGCGGCGTGCCTGAAAATGCCATCCGCAGCAATCA CTACGGCATGATAATAATGCGCGATCGTGCGCAAAGTTTACGAGGC GATTGCCGCGTCCGCCGTCGTGAATCAGGTGGCACCGAAGTGGTG GTCACCTTTATTCCCGAAAAAACTTTCACAGACGTCCAAGGAGATA CCCATGAGGGAGGAGGAAGCGAGCAGAAACTCATCTCTGAAGAGG ATCTGTAA
NarX-VanS Chimera	ATGGTGATCAAGCTGAAGAACAAGAAAAACGACTACAGCAAACTGG AACGCAAGCTGTATATGTATATTGTTGCCATTGTTGTGGTGGCCATT GTGTTTGTTCTGTATATTCGTAGCATGATCCGTGGCAAATTAGGTGA TTGGATTCTGAGCATTCTGGAAAACAAATATGATCTGAATCACCTGG ATGCCATGAAACTGTATCAGTATAGCATTCGCAACAACATCGACATC TTTATCTATGTGGCGATTGTGATTAGCATTCTGATTCTGTGTCGTGT GATGCTGAGTAAATTCGCCAAATATTTCGATGAAATCAACACCGGC ATTGATGTGCTGATTCAGAATGAAGATAAGCAGATTGAACTGAGCG CAGAAATGGATGTTATGGAACAGAAACTGAACACCATGTCTGCAGA ACTGGCCGAAAGTTATGCCGTACTTGAGCAGCGGGTTCAGGAGAA AACCGCCGGGCTGGAGCATAAAAATCAGATCCTCTCTTTTTTATGG CAGGCTAACCGCCGTTTGCATTCCCGCGCCCCGCTGTGTGAACGC CTGTCACCTGTACTCAACGGCTTACAGAATTTAACCCTGCTACGTG ATATCGAATTGCGGGTGTATGACACTGATGATGAAGAGAATCATCA GGAGTTTACCTGCCAGCCAGATATGACTTGTGATGATAAAGGCTGC CAGCTCTGCCCGCGCGGCGTATTACCCGTTGGTGATCGCGGCACG ACCCTGAAGTGGCGGCTGGCTGACTCTCATACGCAGTACGGTATTT TGCTGGCGACCCTGCCACAGGGGCGTCATCTTAGCCATGATCAAC AACAACTGGTGGATACCCTGGCTGAACAACTCACCGCCACGCTGG CGCTGGATCGCCATCAGGAACGTCAGCAACAGTTGATCGTGATGG AAGAGCGTGCCACCATTGCGCGCGAACTGCATGATTCTATTGCCCA ATCTCTCTCTTGCATGAAGATGCAGGTGAGTTGTTTACAGATGCAG GGCGATGCGCTGCCAGAAAGCAGCCGCGAACTGTTAAGTCAGATC CGTAACGAACTGAATGCATCCTGGGCGCAGTTGCGTGAATTGCTCA TCACATTCCGCTTGCAGCTCACCGAGCCTGGATTACGTCCGGCGC TGGAGGCGAGTTGCGAAGAGTACAGCGCCAAATTTGGCTTCCCGG TGAAGCTGGATTATCAATTGCCGCCTCGCCTGGTGCCTTCGCATCA GGCAATCCACTTGTTGCAAATTGCCCGTGAGGCATTAAGTAACGCC CTCAAACATTCGCAAGCGAGTGAAGTCGTGGTGACGGTGGCGCAA AACGATAATCAGGTCAAACTGACCGTCCAGGATAACGGCTGCGGC GTGCCTGAAAATGCCATCCGCAGCAATCACTACGGCATGATAATAA TGCGCGATCGTGCGCAAAGTTTACGAGGCGATTGCCGCGTCCGCC GTCGTGAATCAGGTGGCACCGAAGTGGTGGTCACCTTTATTCCCGA AAAAACTTTCACAGACGTCCAAGGAGATACCCATGAGGGAGGAGG AAGCGAGCAGAAACTCATCTCTGAAGAGGATCTGTAA
NarX-RssA	ATGATCGGCTTCAAAAGCTTCTTTATGCGCACCATTATCTTTCAGGT TCTGGCAATTCTGCTGCTGTGGGGTCTGCTGGTTGCATGGGTGAAA TATTGGTATTATCCGGACATGGAAAAATACTTTGATAACCAGCAGCG TATTGTTGCAGCAGGTATTGCAAATATTCTGGATGAAACCGGCACC

	GACAATATTGATTATCGCGGTATTATCAAAACCATCGAGGGCATGTA TATCGACAGCATTAATAACGGTATGCAGGATGAGATCGATTATCATC CGCTGTTTGTTGTGTATGATCGTGATAATCGTGTGCTGTATAGCAGT CAGACCCAGGGTGAACCGCTGCGTCTGCCTCCGAGCGTTCTGAGC GGTAGCGTTAATTATGCCGGTGCAAATTGGCATCTGGCAGGTAGCT GGAAAGAAAAACGTCAGTATCGTGTTATTGTGGGCGAAAGCTTTAA TGATCGTACCACACTGTTTGGTAATCCGGCAGATGTTCCGCTGCTG GGTATTCTGGCAGCAATTATTGTTACCCTGCTGTTTACCGCATATTT CAGCCTGCGTCCGCTGCGCCAGATTGCACGTACCATTAGCGATCG TCAGCCTGGTAATCTGAGCCCGATTAATGTTAGCGAACAGTATCAA GAAATTCGTCCGGTTGTTATGGAAGTGAACAAAATGTCTGCAGAAC TGGCCGAAAGTTATGCCGTACTTGAGCAGCGGGTTCAGGAGAAAA CCGCCGGGCTGGAGCATAAAAATCAGATCCTCTCTTTTTTATGGCA GGCTAACCGCCGTTTGCATTCCCGCGCCCCGCTGTGTGAACGCCT GTCACCTGTACTCAACGGCTTACAGAATTTAACCCTGCTACGTGAT ATCGAATTGCGGGTGTATGACACTGATGATGAAGAGAATCATCAGG AGTTTACCTGCCAGCCAGATATGACTTGTGATGATAAAGGCTGCCA GCTCTGCCCGCGCGGCGTATTACCCGTTGGTGATCGCGGCACGAC CCTGAAGTGGCGGCTGGCTGACTCTCATACGCAGTACGGTATTTTG CTGGCGACCCTGCCACAGGGGCGTCATCTTAGCCATGATCAACAA CAACTGGTGGATACCCTGGCTGAACAACTCACCGCCACGCTGGCG CTGGATCGCCATCAGGAACGTCAGCAACAGTTGATCGTGATGGAA GAGCGTGCCACCATTGCGCGCGAACTGCATGATTCTATTGCCCAAT CTCTCTCTTGCATGAAGATGCAGGTGAGTTGTTTACAGATGCAGGG CGATGCGCTGCCAGAAAGCAGCCGCGAACTGTTAAGTCAGATCCG TAACGAACTGAATGCATCCTGGGCGCAGTTGCGTGAATTGCTCATC ACATTCCGCTTGCAGCTCACCGAGCCTGGATTACGTCCGGCGCTG GAGGCGAGTTGCGAAGAGTACAGCGCCAAATTTGGCTTCCCGGTG AAGCTGGATTATCAATTGCCGCCTCGCCTGGTGCCTTCGCATCAGG CAATCCACTTGTTGCAAATTGCCCGTGAGGCATTAAGTAACGCCCT CAAACATTCGCAAGCGAGTGAAGTCGTGGTGACGGTGGCGCAAAA CGATAATCAGGTCAAACTGACCGTCCAGGATAACGGCTGCGGCGT GCCTGAAAATGCCATCCGCAGCAATCACTACGGCATGATAATAATG CGCGATCGTGCGCAAAGTTTACGAGGCGATTGCCGCGTCCGCCGT CGTGAATCAGGTGGCACCGAAGTGGTGGTCACCTTTATTCCCGAAA AAACTTTCACAGACGTCCAAGGAGATACCCATGAGGGAGGAGGAA GCGAGCAGAAACTCATCTCTGAAGAGGATCTGTAA

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