Supporting information

for

Engineering Biorthogonal Phage-based Nanobots for Ultrasensitive, *in situ* Bacteria Detection

Hannah S. Zurier, Michelle M. Duong, Julie M. Goddard, and Sam R. Nugen*

*srn6@cornell.edu

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Table S1: Bacteria and Phages used in this study

Strain	Strain source
Escherichia coli strain DH5α	Invitrogen
Escherichia coli strain BL21 (DE3)	Invitrogen
Escherichia coli strain 10G	Lucigen
Escherichia coli strain ECOR#13	University of Texas, Austin, TX, USA (human isolate)
Listeria monocytogenes	FSL C7-0239 (environmental water isolate)
Pseudomonas aeruginosa	ATCC 27853
Spingopyxis alaskiensis	NRG-0228 (tap water isolate)
E. coli phage T4	ATCC 11303-B4
NRGp17	*E. coli* phage T4 Nluc-CBM (Duong *et al.* 2020)[32]
E. coli phage RB69	Paul Sabatier University, Toulouse, FR
E. coli phage T7	ATCC BAA-1025-B2

Table S2: Primers used in this study

Template	Purpose	Direction	Sequence (5′ to 3′)
pET 11a	Adds SOC homology and 6X His tag	Forward	agtaagtggtcatcaccatcaccatcactaag atccggctgctaacaaag
pET 11a	Adds SOC homology and amber codon	Reverse	cataaccaccctacatatgtatatctccttcttaa agttaaacaaaattatttc
RB69	Amplifies SOC gene, adds pET 11a homology and amber codon	Forward	acatatgtagggtggttatgtaaacatcaaaac
RB69	Amplifies SOC gene, adds pET 11a homology and 6X His tag	Reverse	ttagtgatggtgatggtgatgaccacttactggt gtagg
pET 11a	Adds SOC homology	Reverse	taaccacccatatgtatatctccttcttaaagtta aacaaaattatttc
RB69	Amplifies SOC gene, adds pET 11a homology	Forward	gatatacatatgggtggttatgtaaacatc

Table S3: Raw fluorescent cycloaddition data. Data are displayed in Figure 3a.

	Fluorescence (RFU) by sample type			
Replicate	**Click SOC**	**WT SOC**	**Free Prp**	**Buffer only**
1	602	328	867	326
2	642	359	481	341
3	723	281	828	298
4	628	348	781	255

Table S4: Raw bead infectivity data. Data are displayed in Figures 4a and 4b.

		Plate count (pfu/ml) by SOC protein used		
Wash	**Replicate**	**Click SOC**	**No SOC**	**WT SOC**
supernatant	1	2.80E+07	1.60E+07	1.00E+08
supernatant	2	2.00E+07	1.50E+07	8.00E+07

supernatant	3	3.50E+07	8.00E+06	9.00E+07
wash 1	1	5.00E+05	2.00E+05	1.00E+06
wash 1	2	2.00E+05	2.00E+05	4.00E+06
wash 1	3	7.00E+05	3.00E+05	3.00E+06
wash 2	1	9.00E+04	7.00E+04	8.00E+05
wash 2	2	4.00E+04	5.00E+04	5.00E+05
wash 2	3	5.00E+04	5.00E+04	7.00E+05
wash 3	1	3.00E+03	3.00E+03	2.00E+04
wash 3	2	2.00E+03	5.00E+03	2.00E+04
wash 3	3	3.00E+03	4.00E+03	2.00E+04
Resuspended beads	1	2.00E+05	1.00E+03	9.00E+03
Resuspended beads	2	1.00E+05	1.00E+03	1.40E+04
Resuspended beads	3	1.00E+05	1.00E+03	8.00E+03

Table S5: Raw reagent sensitivity luminescent data. Data are displayed in Figure 5a.

		Luminescence (RLU) by detection reagent			
***E. coli* concentration**	**Replicate**	**MNPs**	**Free NRGp17**	**WT SOC NRGp17 MNPs**	**Click SOC NRGp17 MNPs**
3.64 ± 0.32 log10 cfu/ml	1	6	40	34	304
3.64 ± 0.32 log10 cfu/ml	2	1	152	70	198
3.64 ± 0.32 log10 cfu/ml	3	5	86	89	132
3.64 ± 0.32 log10 cfu/ml	4	2	19	106	58
0 cfu/ml	1	11	21	25	18
0 cfu/ml	2	2	21	25	24
0 cfu/ml	3	1	16	25	13
0 cfu/ml	4	0	20	21	15

Table S6: Raw reagent specificity data. Data are displayed in Figure 5b.

	Luminescence (RLU) by organism detected			
Replicate	**ECOR#13 with non-coliforms**	**Non-coliforms alone**	**ECOR#13 alone**	**Buffer control**
1	837	24	765	17
2	733	20	1222	17
3	550	13	1342	20
4	777	19	1270	23

Table S7: Raw large-scale detection data. Bacteria concentration is reported as the mean ± standard deviation of 12 plate counts. Data are displayed in Figures 6a and 6b.

Bacteria concentration (cfu/100 mL)	**Replicate**	**Luminescence (RLU)**
132 ± 33.6	1	54

132 ± 33.6	2	50
132 ± 33.6	3	69
6.58 ± 1.68	3	29
6.58 ± 1.68	1	26
6.58 ± 1.68	2	32
0	1	11
0	2	5
0	3	5

Table S8: Raw large-scale biological replicated data. Bacteria concentration is reported as the mean ± standard deviation of 12 plate counts.

Biological replicate	Bacteria concentration (cfu/100 mL)	Luminescence (RLU)
1	79.4 ± 9.8	38
1	3.97 ± 0.49	35
1	0	27
2	81.6 ± 31	130
2	4.08 ± 1.55	25
2	0	8
3	71.7 ± 27.6	54
3	3.58 ± 1.38	32
3	0	11

Table S9:.MNP size distribution. Data were obtained by dynamic light scattering and sizes are reported as mode ± standard deviation

Peak	% mass in peak	Hydrodynamic radius (nm)	Molecular weight (kDa)	Polydispersity (%)
1	99.8	8.721 ±1.472	105.6 ± 22.9	16.7
2	0.1	105.7 ± 16.72	3.62E+04 ± 5.43E+03	16.6
3	0.2	396.1 ± 87.76	7.96E+05 ± 1.46E+05	21.2

Figure S1. Click SOC construct design
a Schematic of dual plasmid system used for amber suppression. Arrows indicate promoters, star indicates amber codon. RB69 SOC gene is shown in pink, Prp tRNA synthetase gene is shown in green.
b Structure of propargylglyoxy phenylalanine (Prp)

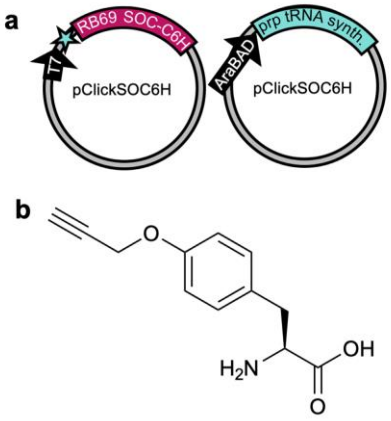

Figure S2. Click SOC and WT SOC purification
Total indicates whole cell lysate. Load indicates clarified lysate. Resin was washed with 25 mM imidazole and protein was eluted with 150 mM imidazole. Numbers indicate approximate molecular weight of bands on ladder.
a SDS-PAGE of Click SOC purification.
b Western blot of Click SOC purification
c SDS-PAGE of WT SOC purification