Supporting Information

Smart Microbial Cells Couple Catalysis and Sensing to Provide High Throughput Selection of an Organophosphate Hydrolase

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Supplementary Tables

Table S1. Raw whole cell fluorescence intensity[a] expressing sfGFP-PON1 fusion under positive feedback regulation of PNP sensor and measured using a flow cytometer.

PON1 variant	UI[b]	PNP (200 μM)	PXN (1.6 mM)
G3C9	168 (164, 171)	7052 (7140, 6964)	1761 (1873, 1649)
H115W	301 (271, 330)	13934 (13367, 14501)	10276 (10003, 10549)
V346A	174 (153, 194)	6263 (6222, 6303)	895 (922, 867)
H115W/V346A	248 (273, 222)	11484 (12920, 10047)	2410 (2883, 1937)

[a]: Mean cell fluorescence intensity from biological duplicates. The individual fluorescence intensity readout of the replicates is shown in parentheses.
[b]: Uninduced

Table S2. Mutational library 1 (Lib1)[a]

	L69	K70	Y71	P72	G73	I74
Diversified codon	DTA	NWW[b]	NDT	NCN	RST	AHT
Amino acids	LVI	YHNDQKEFL(3)I(2)V(2)[c]	RNDCGHILFSYV	S(4)P(4)T(4)A(4)	TSAG	ITN
Unique variants	3	12	12	4	4	3

[a]: Theoretical library diversity = 3×12×12×4×4×3 = 20736
[b]: one-sixteenth of the variants have a premature STOP codon
[c]: Number in parenthesis denotes number of degenerate codons for a particular amino acid

Table S3. Mutational library 2 (Lib2)[a]

	G116	N168	F222	N224	L267	N270	I291	F292
Diversified codon	RST	VAT	WWT	VAC	VTT	VAC	NTC	NTC
Amino acids	TSAG	HND	INYF	HND	LIV	HND	FLIV	FLIV
Unique variants	4	3	4	3	3	3	4	4

[a]: Theoretical library diversity = 4×3×4×3×3×3×4×4 = 20736

Table S4. Fluorescence measurement of lysate from cells expressing PON1 variants in fusion with sfGFP

Variant	**H115W**	**A3**	**A9**	**A12**	**B7**	**B12**	**D10**	**D12 (Lib2)**	**C2.2**	**C3.3**	**C4.1**
Cell lysate fluorescence (au)	5003	3619	4149	2732	3167	4177	4659	2750	3854	4745	2892
Relative PON1 concentration	1	0.72	0.83	0.55	0.63	0.84	0.93	0.55	0.77	0.95	0.58

Supplementary Figures

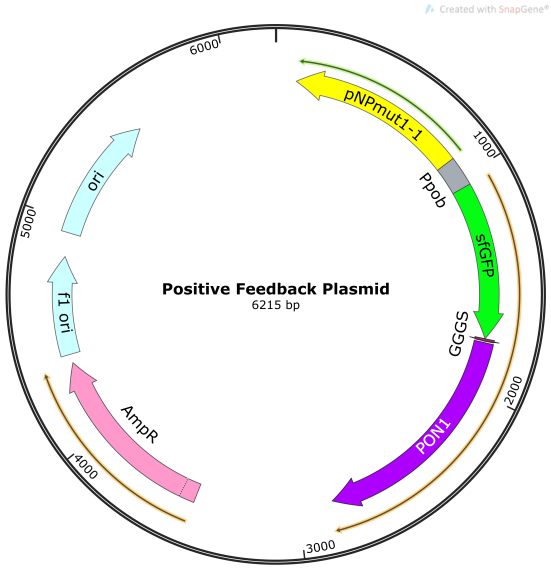

Figure S1. Plasmid map showing an arrangement of genes to establish a positive feedback regulation of the PON1 activity. The genetic sequences for the reporter (sfGFP) and enzyme (PON1) linked with codons for glycine-serine rich linker (GGGS) gives sfGFP-GGGS-PON1 fusion protein when the sensor is activated. The PON1 enzymatic product, PNP, regulates the sensor activity (pNPmut1-1, an engineered transcription factor)[1] resulting in expression of sfGFP-GGGS-PON1 from P_{pob} promoter.

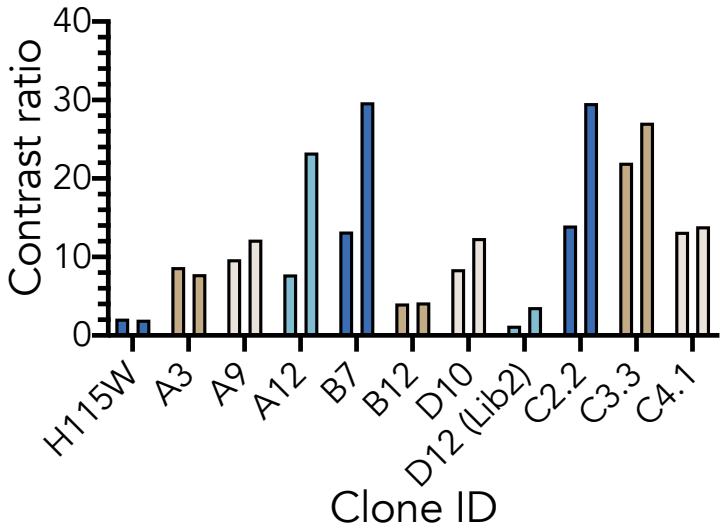

Figure S2. Whole cell catalysis and sensing using the visually picked clones from DH5α cells expressing PON1 libraries Lib1 and Lib2. The clones were grown as liquid culture and supplemented with 400 μM PXN. The contrast ratio is defined as total fluorescence of the cells in the presence of PXN over the fluorescence of the cells in the absence of PXN (background fluorescence). H115W is the starting PON1 scaffold on which libraries were constructed. The data represent results from biological replicates performed on different days to compare variability in the fluorescence response.

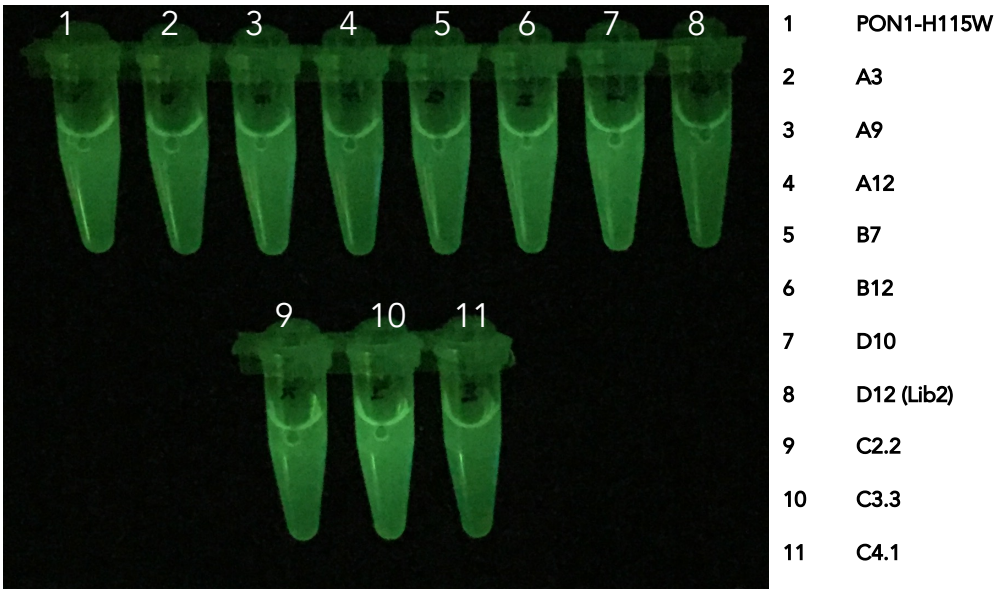

Figure S3. Clarified cell lysates from DH5α cells expressing sfGFP-PON1 fusion. The lysates were illuminated under IllumaTool (LightTools Research) at 488 nm excitation wavelength and observed through a 515 nm filter.

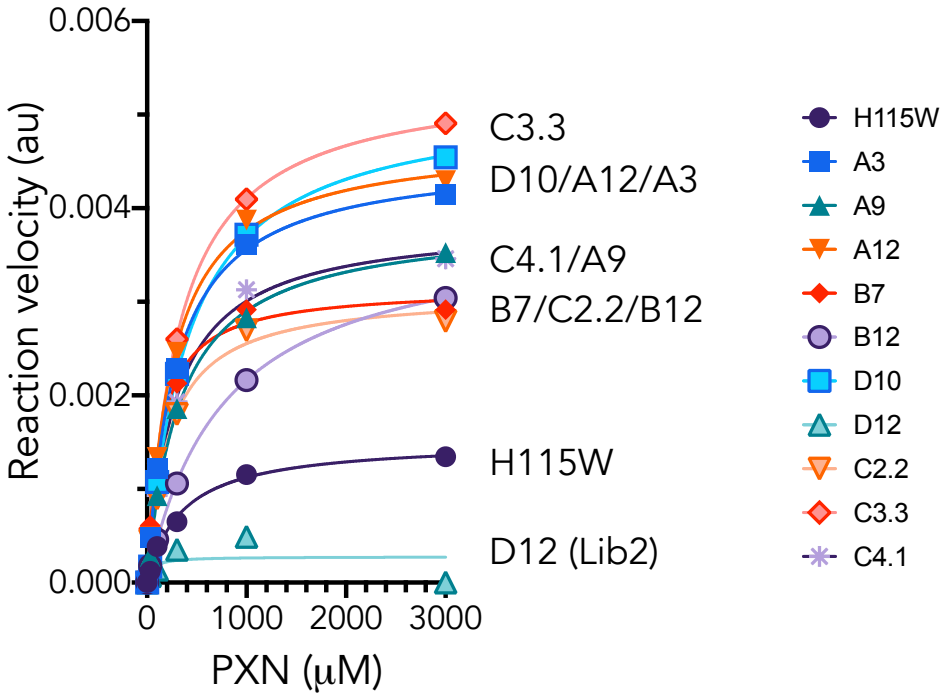

Figure S4. Protein stability and Specific Activity dose-response: *In vitro* spectrophotometric assay using clarified cell lysate normalized for GFP fluorescence. The cell lysates were stored in a refrigerator for 3 days and tested in the same format as described in Figure 4B. Overall there was a depletion in activity in all proteins while the relative ranking of the variants was comparable with the ranking in Figure 4B. Clone D12 (Lib2) showed significant loss of activity compared to other variants.

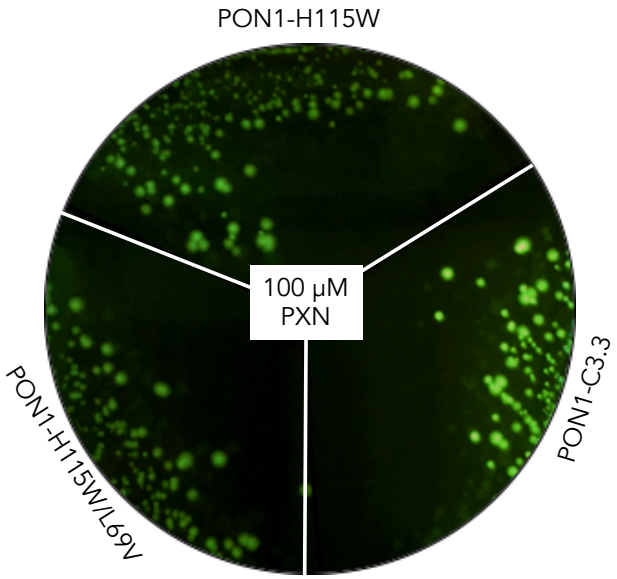

Figure S5. Comparison of DH5α cells expressing PON1 variants and grown on LB agar plates supplemented with 100 µM PXN. PON1-C3.3, compared with PON1-H115W (starting scaffold) and PON1-H115W/L69V. The plates were visualized under IllumaTool (LightTools Research) with 488 nm excitation and observed through a 515 nm filter.

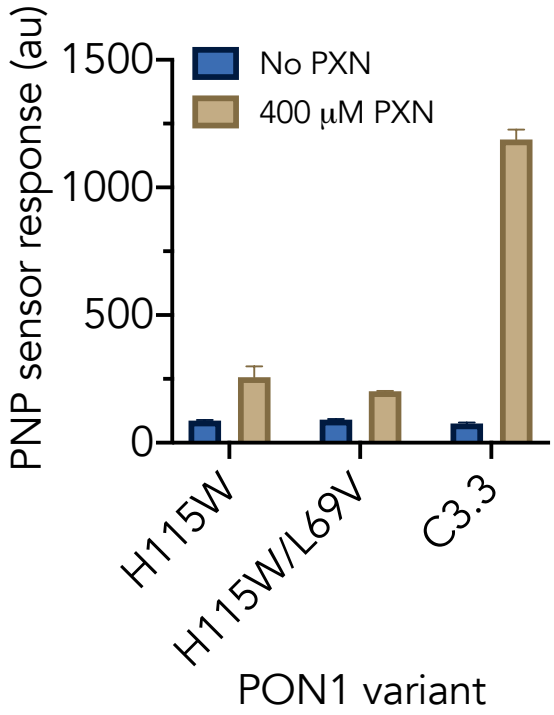

Figure S6. Comparison of cellular fluorescence of DH5α cells expressing sfGFP-PON1 fusion and grown in the presence of PXN. Cultures with no PXN were used as a control for comparison of the background signal. The cells were analyzed using Accuri C6 plus (BD Biosciences) flow cytometer at 488 nm excitation and 533/30 nm emission setting. The data represent mean and standard deviation of two biological replicates.

Reference

(1) Jha, R. K.; Kern, T. L.; Kim, Y.; Tesar, C.; Jedrzejczak, R.; Joachimiak, A.; Strauss, C. E. M. A Microbial Sensor for Organophosphate Hydrolysis Exploiting an Engineered Specificity Switch in a Transcription Factor. *Nucleic Acids Res* **2016**, *44* (17), 8490–8500.