Supplementary Information

A label-free optical whole-cell *Escherichia coli* biosensor for the detection of pyrethroid insecticide exposure

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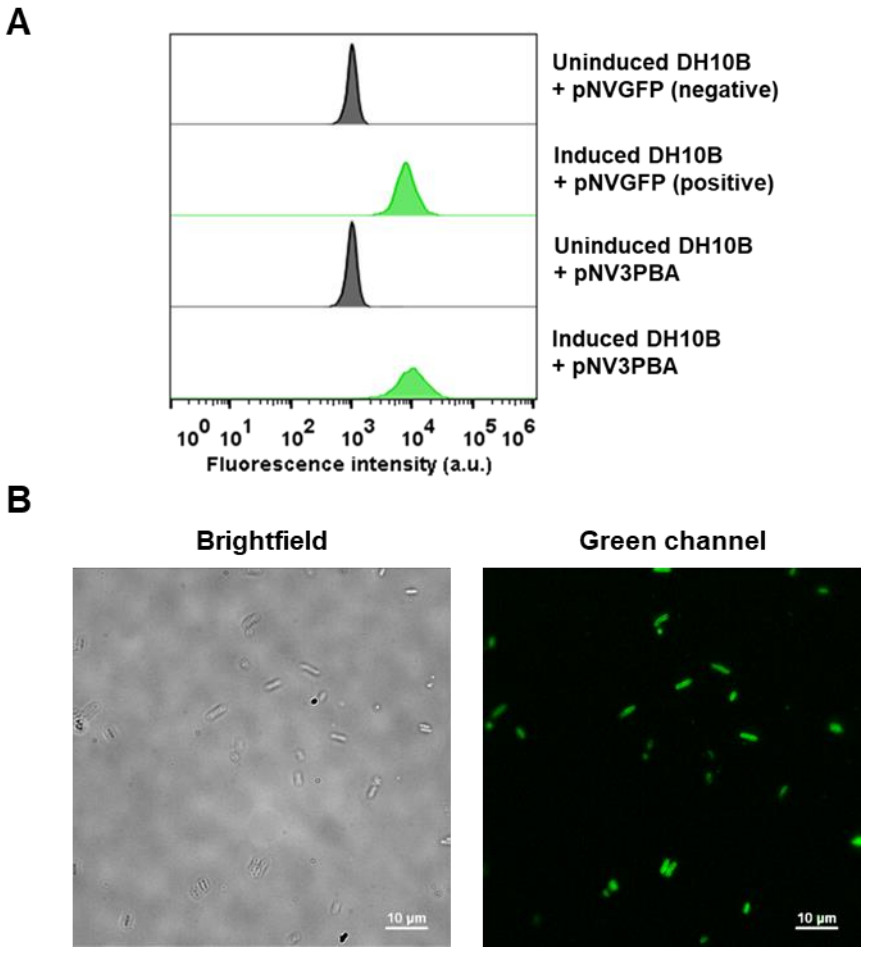

Figure SI1. The cell surface expression of VHH3-PBA. (A). Flow cytometry analysis of *E. coli* DH10B cells displaying VHH3-PBA on the surface. Cells labelled with anti-Myc-Tag (9B11) Mouse mAb (Alexa Fluor® 488 conjugate) were excited by a 488-nm blue laser and detecting through a 530/30 band-pass filter (BL1-H). Data were analyzed using the FlowJo software tool. (B). The brightfield and green fluorescent images of the antibody-bound cells were taken using a 60x objective at 100 ms exposure time using a laser with 488 nm excitation.

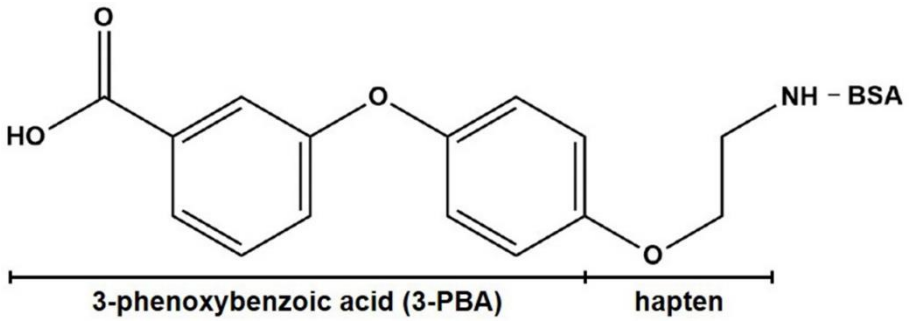

Figure SI2. Structure of 3-PBA hapten-BSA (cAg01). Hapten linker was attached to the 4-position of the distal phenyl group of 3-PBA and primary amine of lysine residue in BSA molecule.[1]

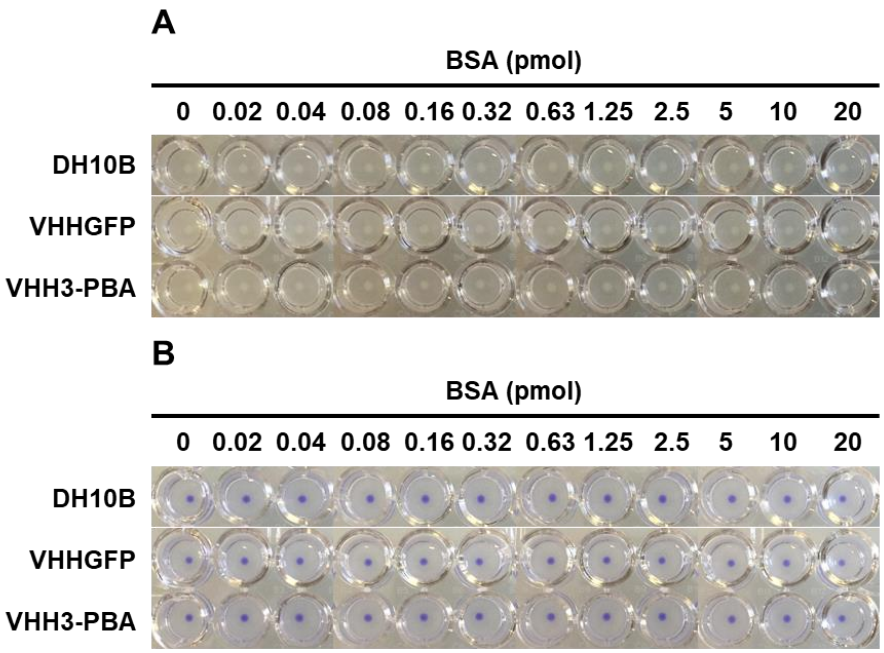

Figure SI3. BSA has no effect on the cell agglutination reaction. *E. coli* DH10B cells displaying VHH3-PBA in the absence (A) and presence (B) of amilCP were mixed with BSA at concentrations equivalent to those of 3-PBA hapten-BSA in the experiments. The reactions were statically incubated at room temperature for 16 h before observation. Pictures are a composite of photos that were taken every three or six wells per frame using a phone camera. This experiment was carried out in three technical replicates.

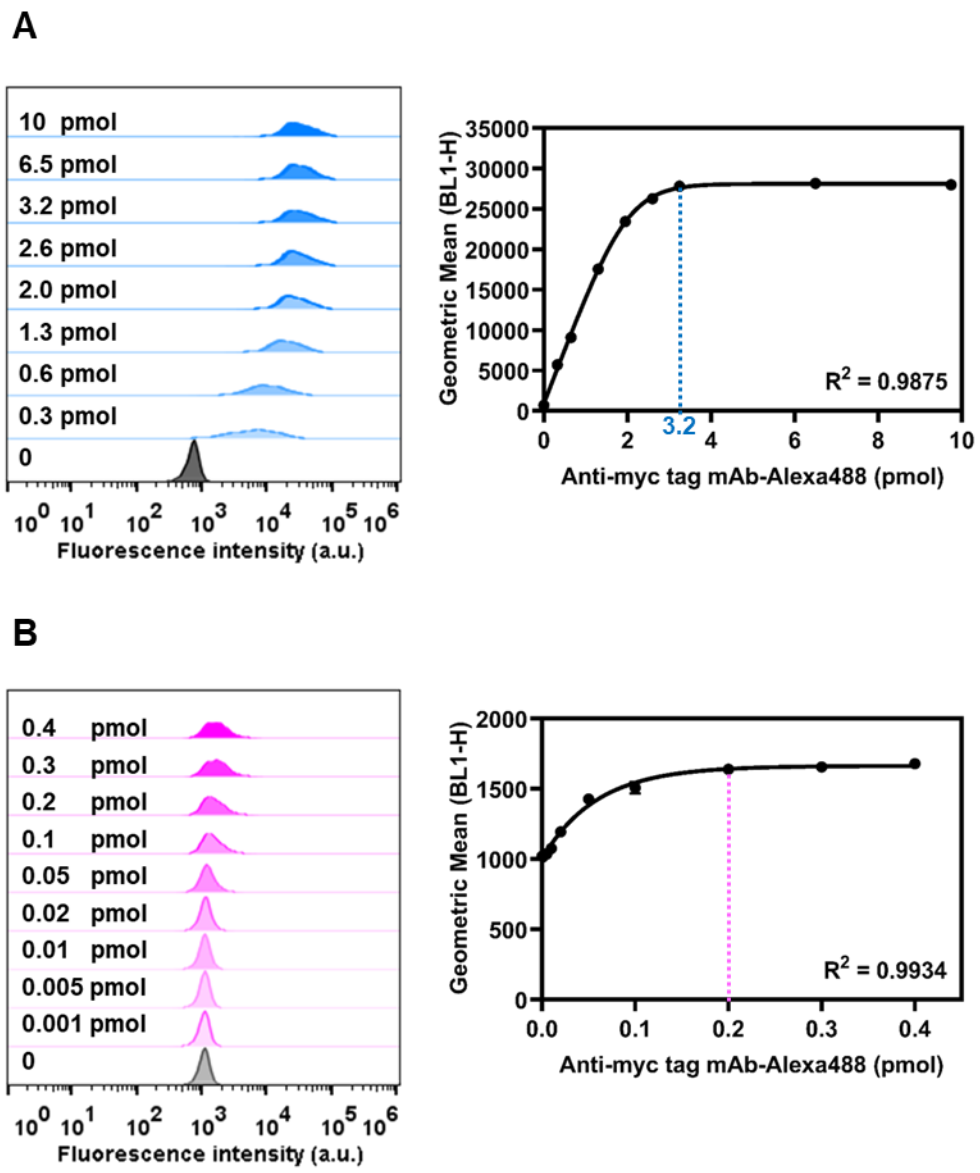

Figure SI4. Quantification of VHH3-PBA displayed on the surface of *E. coli* DH10B carrying pNV3PBA original (A) and V2 constructs (B) in the presence of amilCP. The induced cells were labelled with various amounts of the anti-Myc-Tag (9B11) Mouse mAb (Alexa Fluor® 488 conjugate) and then analyzed by flow cytometry. From left to right**,** histograms of the cell fluorescence intensity and the plotted curve of geometric means against the labelling antibody amounts was fitted using a non-linear least squares regression model in Prism 7 software (GraphPad). Dotted lines represent the initial point of the plateau assuming full occupancy of the labelled VHH. Mean and standard deviation were analyzed from three biological triplicates.

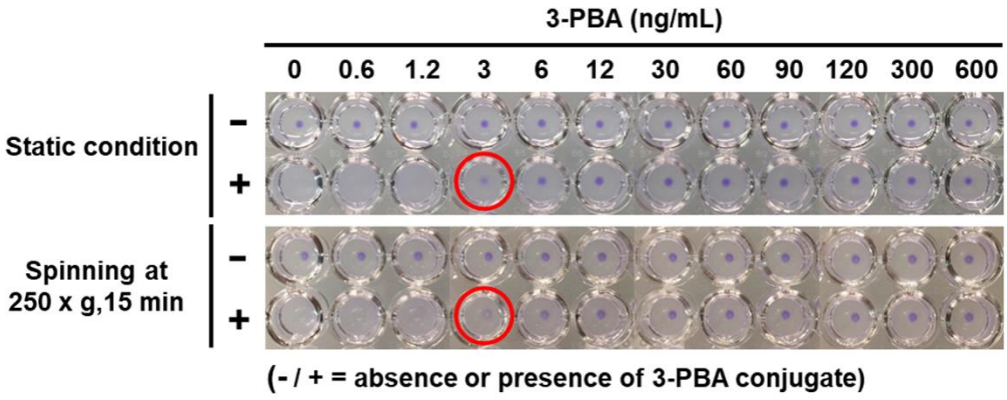

Figure SI5. Minimizing the analysis time by centrifugation. *E. coli* DH10B cells expressing VHH3-PBA (V2 construct) and amilCP were mixed without (-) or with (+) 0.04 pmol of 3-PBA hapten-BSA and various concentrations of 3-PBA diluted in PBS and statically incubated at room temperature for 10 min prior to spinning at 250 x g for 15 min. The standard analysis procedure with 16 h static incubation at room temperature was included as a control (top rows). Red circles represent minimum concentrations causing cell pellet. Pictures are a composite of photos that were taken six wells per frame using a phone camera.

Sequences of the plasmids used in this study

pNV3PBA

This plasmid was constructed using pNVgfp as a template[2] with the replacement of VHHgfp with VHH3PBA, amplified from pComb3xSS.[3]

lacI-plac promoter-NV fusion (IntiminEHEC-**E-tag**-**VHH3PBA**-**myc-tag**)-CmR-pBR322 ori

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pNV3PBA V2 and V4

The original promoter and RBS in the pNV3PBA were replaced with promoters from Anderson promoter collection[4] and RBS using RBS calculator developed by Salis [5] for constitutive expression of VHH3PBA. These BioBrick sequences can be found in Table S1.

Table S1. Sequences of synthetic promoters and RBSs.

Construct	Sequence
V2 (J23105 promoter-RBS(12k))	TTTACGGCTAGCTCAGTCCTAGGTACTATGCTAGCAAAGGAA ATCTA
V4 (J23101 promoter-RBS(12k))	TTTACAGCTAGCTCAGTCCTAGGTATTATGCTAGCAAAGGAA ATCTA

pAmilCP

J23104 promoter-RBS(B0034)T-amilCP-B0010-rrnB T2 terminator-AmpR-p15A ori

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Supplementary references

1 Shan, G. *et al.* A sensitive class specific immunoassay for the detection of pyrethroid metabolites in human urine. *Chemical research in toxicology* **17**, 218-225, doi:10.1021/tx034220c (2004).

2 Salema, V. *et al.* Selection of single domain antibodies from immune libraries displayed on the surface of E. coli cells with two beta-domains of opposite topologies. *PloS one* **8**, e75126, doi:10.1371/journal.pone.0075126 (2013).

3 Kim, H.-J. *et al.* Isolation of Alpaca Anti-Hapten Heavy Chain Single Domain Antibodies for Development of Sensitive Immunoassay. *Analytical chemistry* **84**, 1165-1171, doi:10.1021/ac2030255 (2012).

4 Anderson, J. C. Registry of Standard Biological Parts [parts.igem.org]. (2009).

5 Salis, H. M. The ribosome binding site calculator. *Methods in enzymology* **498**, 19-42, doi:10.1016/B978-0-12-385120-8.00002-4 (2011).