**Supporting Information**

# Living Bacteria-Nanoparticle Hybrids Mediated through Surface-displayed Peptides

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## EXPERIMENTAL METHODS

### Cloning of E. coli Expression Plasmids

To create the "pDSJR No P2X" plasmid, primers were designed and ordered from IDT for plasmid PCR, excluding the region encoding the P2X peptide by starting the forward primer just after the P2X site (5'-CAAGCTTGGCTGTTTTGGCG-3') and ending the reverse primer just before the P2X site (5'-CTATTATTGGCCACCTTGGCCGC-3'), replacing P2X with stop codons. Using the pDSJR plasmid as template, the no P2X version was amplified by PCR using Q5 DNA Polymerase, phosphorylated using T4 Polynucleotide Kinase (PNK) enzyme for 1 hour at 37°C, heat inactivated for 20 min at 65°C, then ligated using T4 DNA ligase for 1 hour at room temperature. The absence of the P2X coding sequence was confirmed by DNA sequencing (Genewiz).

Cloning of the various gold binding peptides into the pDSJR and pDSJR-No-P2X plasmids was performed by ordering the DNA mini genes encoding the desired peptide sequences (see Table 1) and flanking regions from Bio Basic, digesting inserts with AatII and XhoI (or KpnI-HF and XhoI for an equivalent result) overnight at 37°C, and ligating appropriate fragments into similarly digested pDSJR or pDSJR-No-P2X using T4 DNA ligase for 1 hour at room temperature. Some of the peptide sequences described in literature were first inserted into a similar version of the plasmid that has been previously described[1-2] and were moved to this pDSJR version (which alters N-terminal restriction site and translated peptide flanking sequence to remove an N-terminal cysteine residue as compared with the previously described version) using standard PCR techniques with the following primers: forward: 5'-TTCCGTAGCTGGGACGTCTGGCCAG-3' and reverse: 5'-CACCGCTGCCACCGCT-3',

similarly to previously described methods[1], for an equivalent final product in pDSJR. The insertion of each peptide encoding sequence was confirmed by DNA sequencing (Genewiz).

For all relevant steps, DNA fragments were separated using agarose gels and extracted from the gels using a Zymoclean Gel DNA Recovery Kit (Zymo Research) as needed. All enzymes were from New England Biolabs. Each variant created above was transformed into "Z-competent" MC1061 *E. coli* (ATCC) made competent by following the instructions for the Mix & Go *E. coli* Transformation Kit & Buffer Set (Zymo Research).

**Assessment of eCPX Scaffold Expression**

For each new peptide inserted into the pDSJR plasmid (with P2X) and transformed into MC1061 *E. coli* (see Table 1), peptide expression level was assessed. This could not be performed with the pDSJR-No-P2X plasmid due to lack of binding to YPet Mona. Each sample, including a peptide-free negative control, was grown to $OD_{600}$ of about 0.5 and induced with 0.04% L-arabinose for 1 hr at 37°C. The samples were placed on ice to stop induction and 5 µl cells were incubated with 25 µl PBS alone or PBS containing 150 nM YPet Mona for 45 min on ice. Samples were centrifuged at 6000 x g for 5 min and the supernatant removed. The cell pellet was then diluted in 500 µl FACSFlow solution (BD) and fluorescence intensity measured using a FACSCanto II flow cytometer with FACSDiva software. A scatterplot of FSC-A vs SSC-A was created to gate the *E. coli* cell population using cells in PBS alone. Using this scatterplot as a parent, a scatterplot of FITC-A vs FSC-A was created and cells in PBS alone were again gated, then all samples were run one by one to assess YPet Mona binding to the cells. The percentage of cells falling outside of this gate was recorded and corresponds to the percentage of cells expressing the P2X peptide (and therefore the eCPX scaffold). Additionally, median fluorescence intensity (MFI) was recorded and normalized to the negative control (NC,

expressing the eCPX scaffold with no N-terminal peptide) incubated with YPet Mona such that the normalized median fluorescence intensity (nMFI) of the negative control is equal to 1.0. This method is further described through written protocol and video in Sarkes *et al.* 2017.[3]

## TABLES AND FIGURES

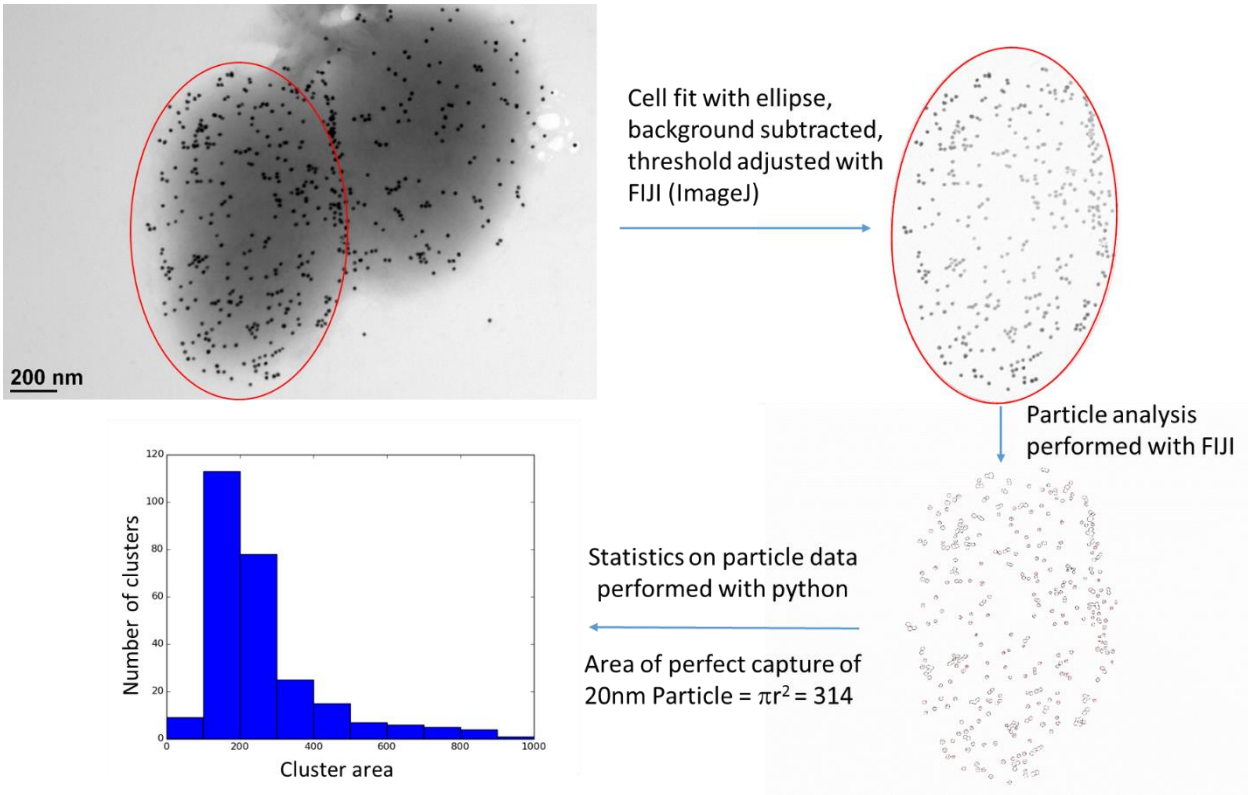



**Figure S1.** An example of particle analysis on bacterial cells. For representative images from hybrids of each cell type and Au NP size tested, captured TEM images were used to count bound Au NPs using Fiji (ImageJ). Cells were first fit to an ellipse, background subtracted, and threshold adjusted with Fiji. Particle analysis was performed for all particles found within the ellipse, and statistical analysis was performed using python. These results are shown in Table S1 and Figure 4.

**Table S1.** Quantification of nanoparticles on hybrid bacterial cells.

| | Surface-displayed Peptides | | Cluster Area/Cell Area | Average Cluster Size $nm^2$ | Median | Skewness | Max Cluster Size | N (sample size) |
|---|---|---|---|---|---|---|---|---|
| **Bacterial Cells with 20 nm Au NPs** | **Un-induced M6G9** | **P2X** | 1.643E-03 | 401.32 | 342.02 | 0.928 | 659.81 | 3 |
| | **M6G9** | **P2X** | 4.949E-02 | 416.49 | 285.22 | 5.391 | 6140.80 | 6 |
| | **NC** | **P2X** | 1.687E-02 | 342.72 | 222.70 | 3.445 | 2325.23 | 4 |
| | **GBP1** | **P2X** | 4.451E-02 | 262.90 | 203.35 | 2.817 | 1312.06 | 4 |
| | **GBP2** | **P2X** | 3.635E-02 | 284.00 | 191.15 | 3.206 | 1835.58 | 4 |
| | **H6G9** | **P2X** | 5.591E-02 | 578.33 | 289.61 | 3.882 | 5326.47 | 6 |
| | | | | | | | | |
| | **NC** | **No P2X** | 5.668E-04 | 479.46 | 479.49 | NA | 537.93 | 2 |
| | **M6G9** | **No P2X** | 5.211E-02 | 342.75 | 227.76 | 4.426 | 3020.42 | 3 |
| | **GBP1** | **No P2X** | 2.165E-02 | 492.67 | 245.17 | 3.938 | 4415.78 | 3 |
| | **GBP2** | **No P2X** | 7.631E-03 | 601.16 | 324.62 | 2.700 | 3376.62 | 3 |
| | **H6G9** | **No P2X** | 4.333E-02 | 396.09 | 268.73 | 5.137 | 4155.55 | 3 |
| | | | | | | | | |
| **Bacterial Cells with 10 nm Au NPs** | **NC** | **No P2X** | 4.201E-03 | 192.16 | 82.17 | 2.740 | 1187.15 | 3 |
| | **NC** | **P2X** | 9.481E-02 | 178.98 | 69.06 | 5.243 | 3733.76 | 3 |
| | **M6G9** | **No P2X** | 1.048E-01 | 137.69 | 66.33 | 5.180 | 2678.67 | 4 |
| | **M6G9** | **P2X** | 9.788E-02 | 132.47 | 67.95 | 5.622 | 2610.60 | 5 |
| | **GBP1** | **No P2X** | 1.089E-01 | 307.60 | 108.81 | 6.543 | 8618.74 | 3 |
| | **GBP1** | **P2X** | 1.029E-01 | 124.55 | 62.92 | 5.502 | 2386.79 | 5 |

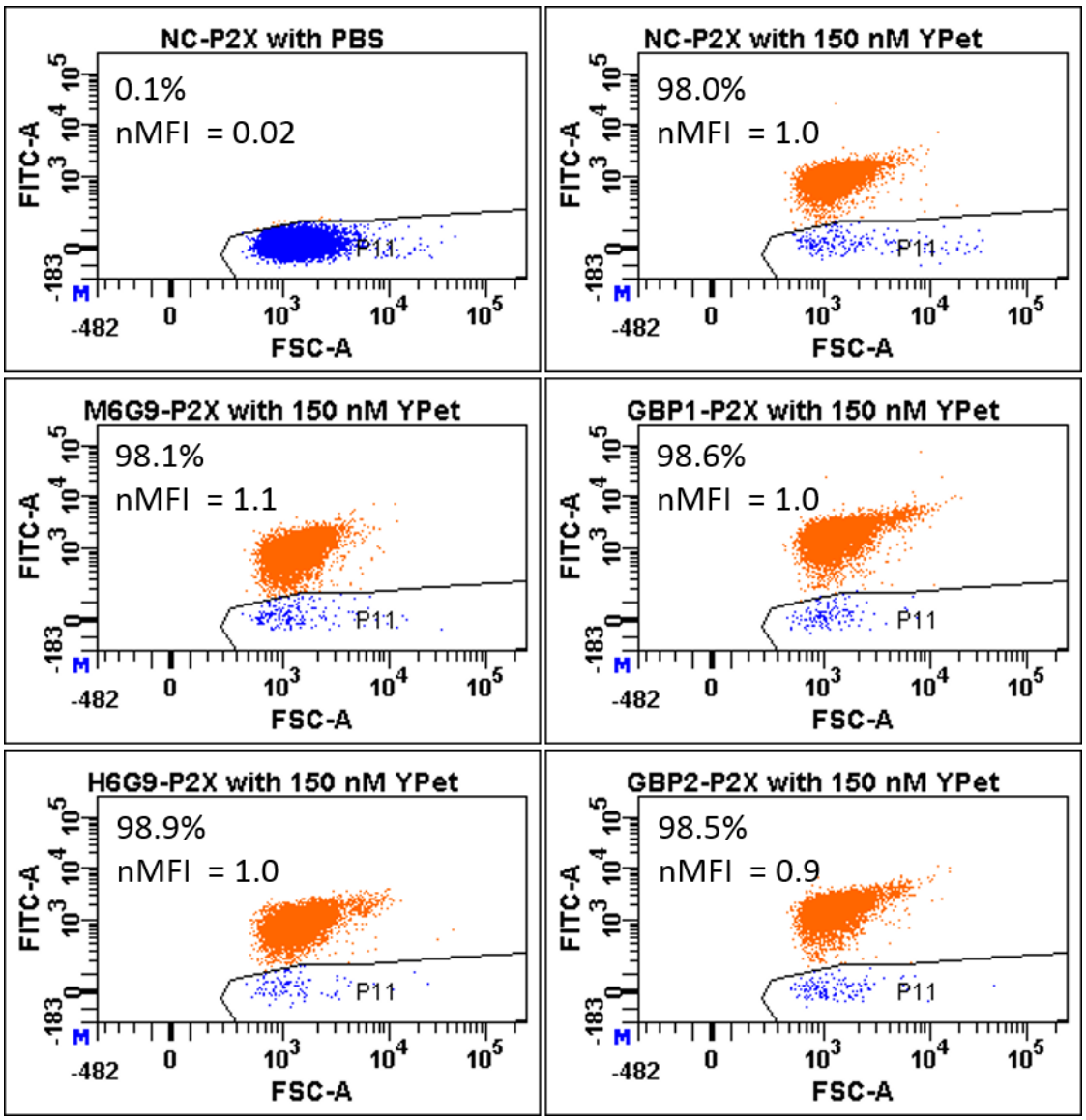



**Figure S2.** Analysis of peptide expression levels using FACS. Each strain expressing both the N-terminal peptide shown in the figure and the C-terminal P2X peptide, which binds to the fluorescent protein YPet Mona, were incubated with PBS (as negative control for gating) or 150 nM YPet Mona (for confirmation of expression) for 45 min after a 1 hour induction. The percentage of cells falling outside the gate drawn around the population of unbound cells (P11) is shown and indicates the percentage of cells that express P2X and therefore the N-terminal peptide as well. The normalized median fluorescence intensity (nMFI), normalizing against NC-P2X cells incubated with 150 nM YPet Mona, is also shown and should be close to 1.0 after incubation with YPet Mona if the peptide expression level for each strain is similar to the expression level achieved by negative control cells. Only NC-P2X incubated with PBS alone is shown here, but is representative of binding level in PBS alone for all strains.

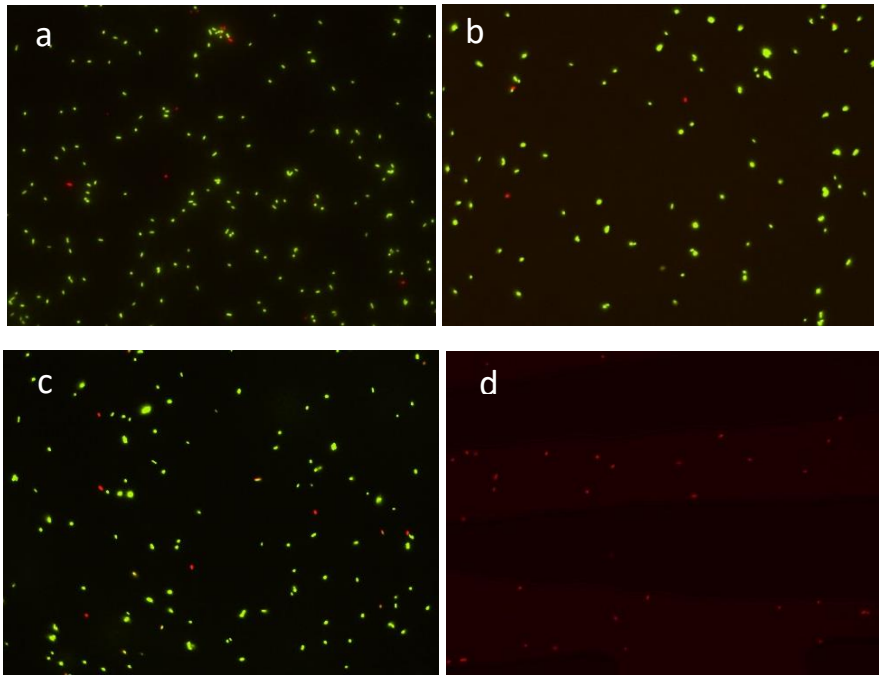
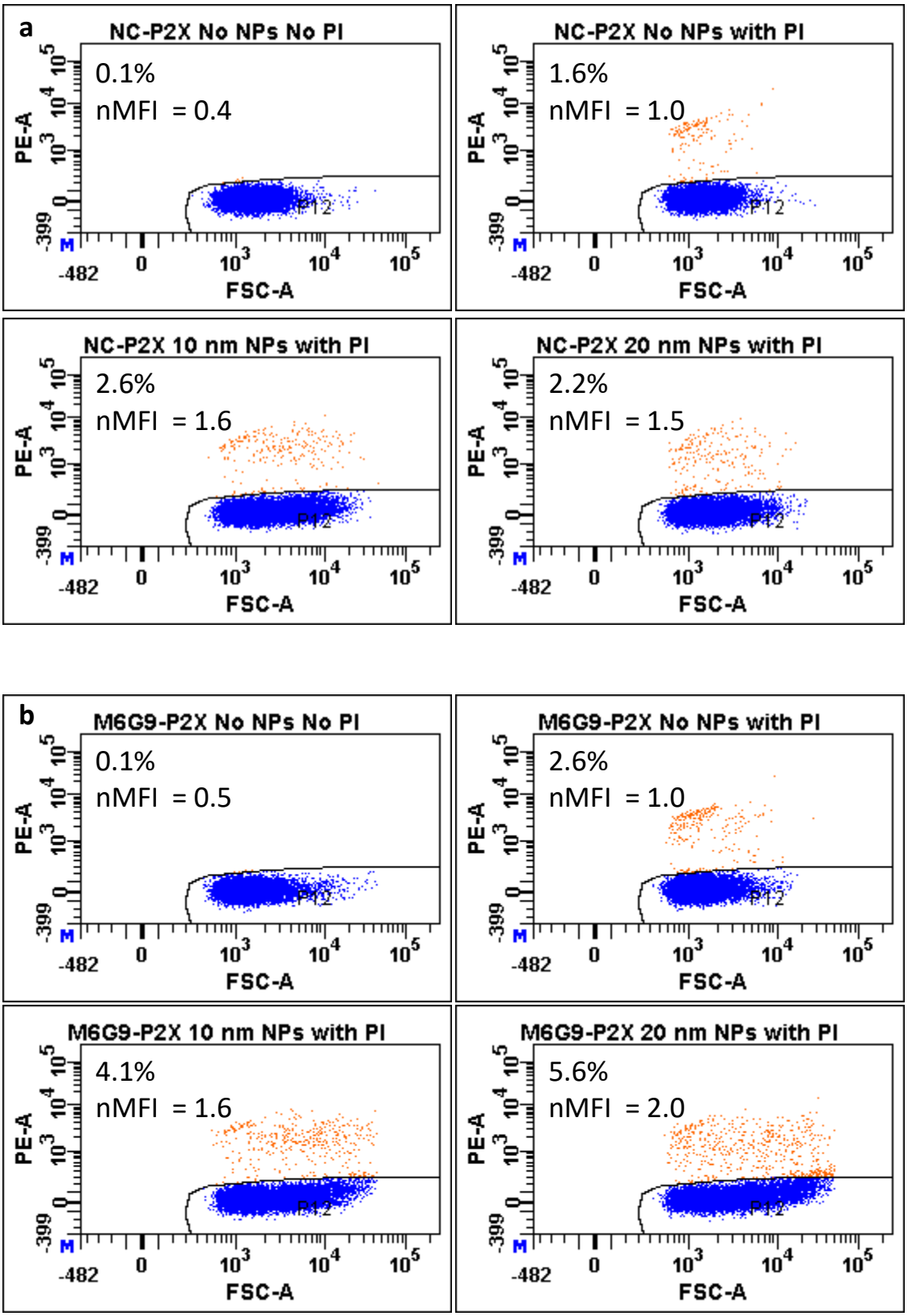



**Figure S3.** Fluorescent microscopy images of live/dead bacterial cells (with P2X). (a) NC-P2X with 20 nm Au NPs, (b) H6G9-P2X with 20 nm Au NPs, (c) GBP1-P2X with 10 nm Au NPs. (d) M6G9-P2X cells after mixing with 1 mM $HAuCl_4$ in PBS solution for 1 hour. Cells with green fluorescence are representative of live cells, while red fluorescing cells are representative of dead or compromised cells.


a
NC-P2X No NPs No PI
0.1%
nMFI = 0.4
NC-P2X No NPs with PI
1.6%
nMFI = 1.0
NC-P2X 10 nm NPs with PI
2.6%
nMFI = 1.6
NC-P2X 20 nm NPs with PI
2.2%
nMFI = 1.5
b
M6G9-P2X No NPs No PI
0.1%
nMFI = 0.5
M6G9-P2X No NPs with PI
2.6%
nMFI = 1.0
M6G9-P2X 10 nm NPs with PI
4.1%
nMFI = 1.6
M6G9-P2X 20 nm NPs with PI
5.6%
nMFI = 2.0
PE-A
FSC-A
P12

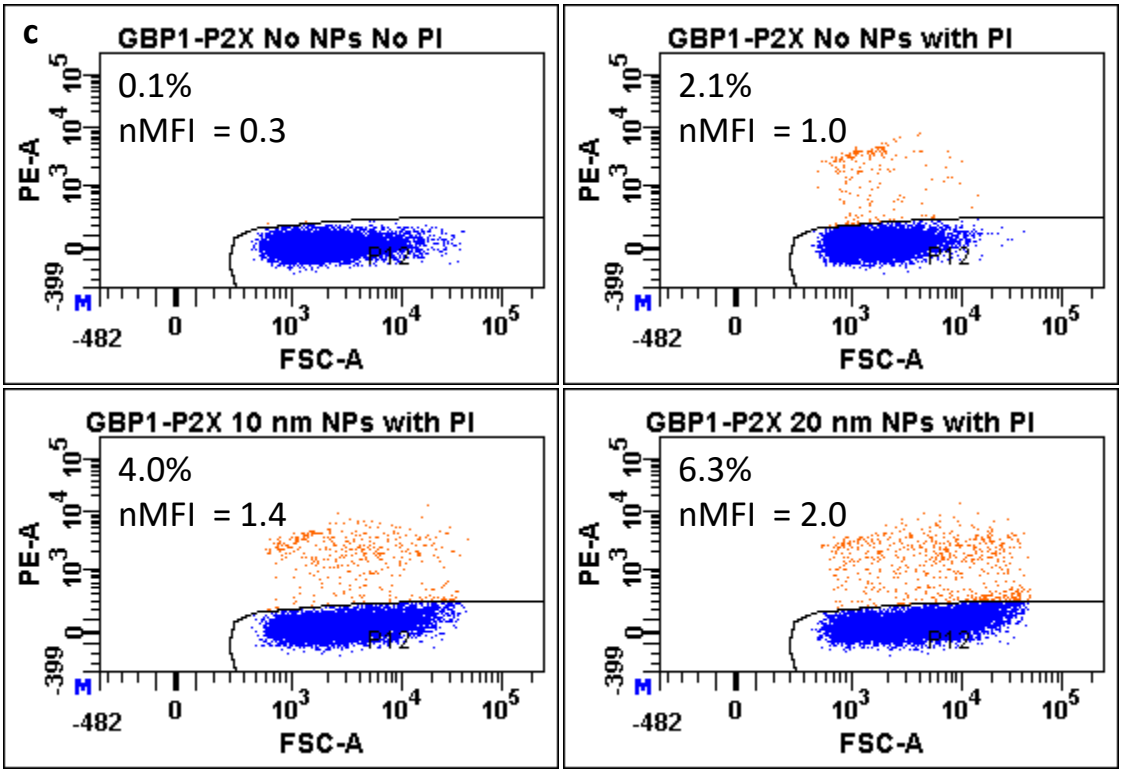



**Figure S4**. Viability evaluation of (a) NC-P2X, (b) M6G9-P2X and (c) GBP1-P2X cells with or without 10 nm or 20 nm Au NPs, using FACS. Unstained cells were used for gating the autofluorescence of the cell population (indicated in panels labeled "No NPs No PI"). Remaining panels display cells that were stained for 15 minutes with propidium iodide, with or without bound 10 or 20 nm Au NPs. The percentage of cells falling outside a gate (orange) drawn around the population of unlabeled cells (P12) is shown and indicates the percentage of cells that have taken up the propidium iodide, indicating that their membrane has been compromised. The percentage of cells remaining in the P12 gate (blue) are considered viable and are reported in Figure 8e, after normalization to percent viability without Au NPs. The normalized median fluorescence intensity (nMFI) is also shown here. For each condition listed, the MFI has been normalized to the MFI of the same strain labeled with propidium iodide but not incubated with Au NPs (designated "No NPs with PI"). Higher nMFI therefore indicates an increase in cell death after the addition of Au NPs, while cells with no Au NPs have an nMFI of 1.0 by definition. The MFI can increase as a result of individual cells having higher uptake of PI and therefore nMFI is not a direct measure of % viability.

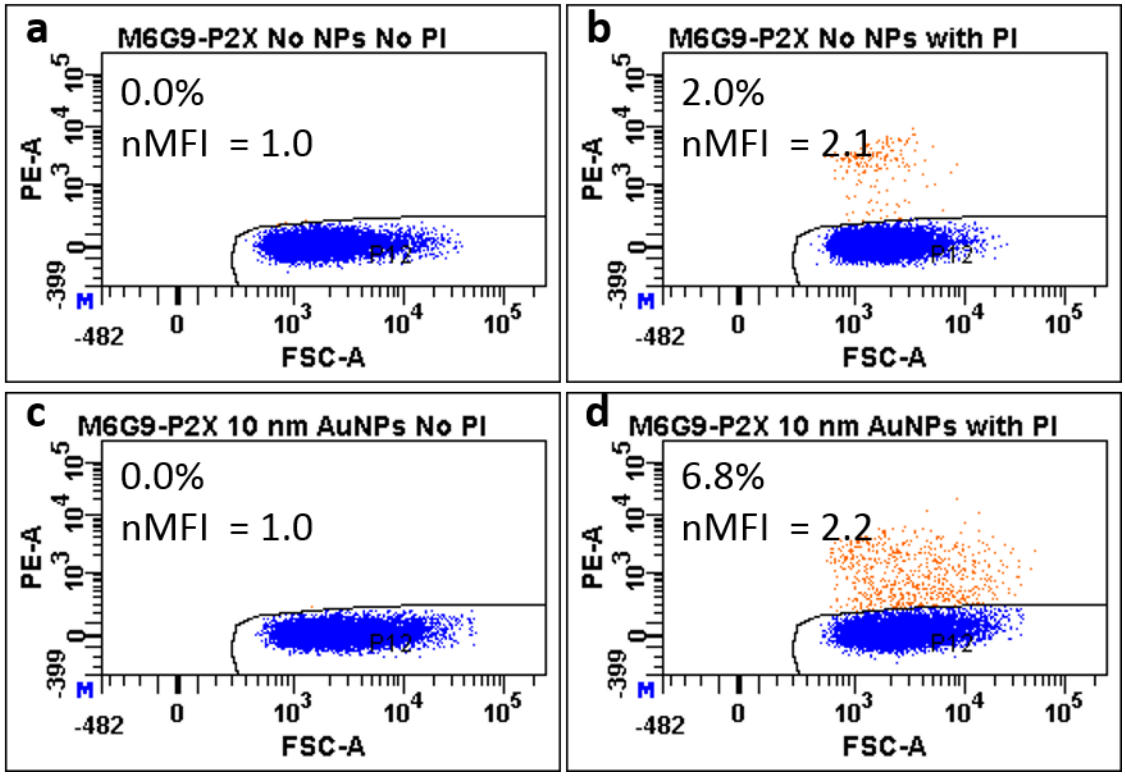



**Figure S5**. Viability evaluation of M6G9-P2X with or without 10 nm Au NPs for 24 hours, using FACS. Unstained cells (a, c) were used for gating the autofluorescence of the cell population (indicated in left panels labeled "No NPs No PI") for (a) bare cells or (b) cells bound to 10 nm Au NPs. Remaining panels at left display cells that were stained for 15 minutes with propidium iodide, with (d) or without (b) bound 10 nm Au NPs. Since the percentage of cells falling outside of the gate shown here indicates dead or compromised cells, the percent viability (100% - % dead cells) for bare cells after 24 hours is about 98% (b) while the percent viability for cells bound to 10 nm Au NPs for 24 hours is 93.2% (d). Normalizing bound to unbound cells (93.2%/98.0% = 95.1%), normalized percent viability for M6G9-P2X cells bound to 10 nm Au NPs for 24 hours at least 95%. See Figure S4 for further explanation of gating and nMFI.

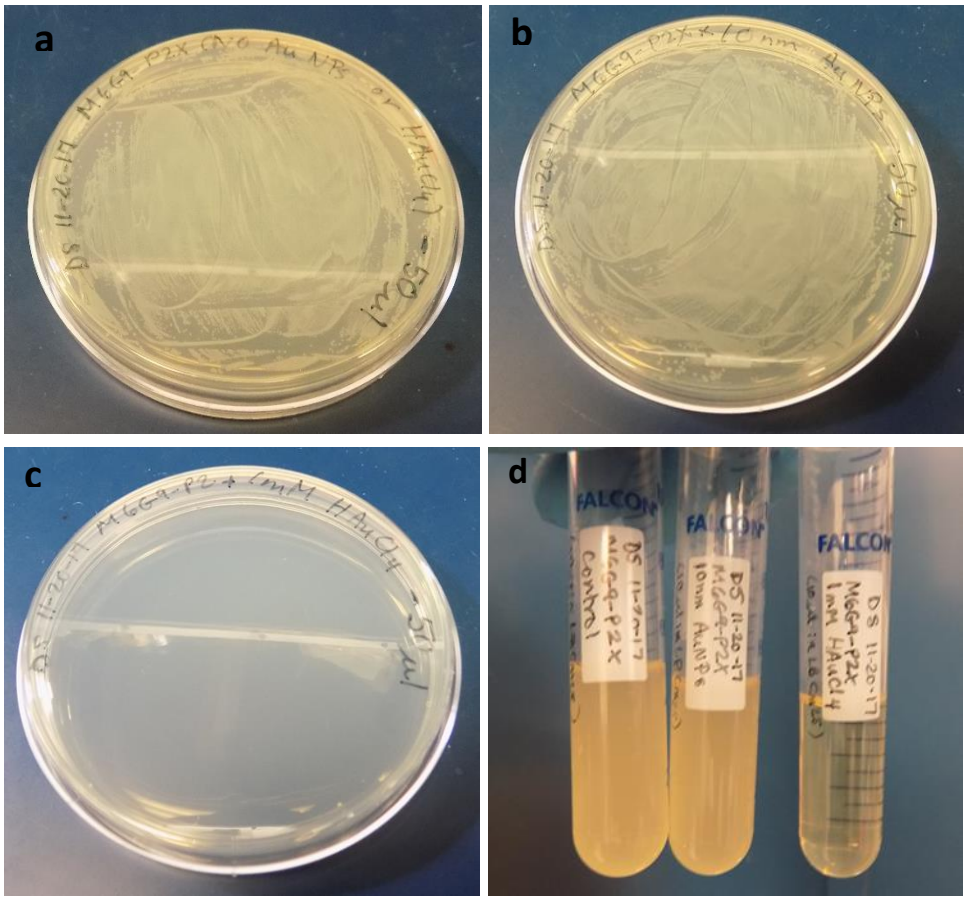



**Figure S6.** (a-d) Cell plating and subculturing for assessment of viability and regrowth after incubation with 10 nm Au NPs or 1 mM $HAuCl_4$. MC1061 *E. coli* containing M6G9-P2X plasmid were incubated or not with 10 nm Au NPs or 1 mM $HAuCl_4$ in PBS pH 7.4 for 1 hour, as described in experimental methods. 50 µl of (a) bare cells with no Au NPs or 1 mM $HAuCl_4$ exposure, (b) cells incubated and therefore decorated with 10 nm Au NPs, or (c) cells incubated with 1 mM $HAuCl_4$ were spread on $LB_{cm25}$ agar plates and grown overnight at 37°C. (d) Similarly, 10 µl of these same cells (from left to right: bare cells, cells with 10 nm Au NPs, and cells with 1 mM $HAuCl_4$) were grown in 5 ml of $LB_{cm25}$ overnight at 37°C, shaking at 225 rpm. No colonies grew on spread plates (c) or in liquid culture (d right) for cells incubated with 1 mM $HAuCl_4$. Meanwhile, overnight cultures were similar for bare cells (a, d left) versus cells decorated with 10 nm Au NPs (b, d middle). The $OD_{600}$'s of the cultures shown in (d) from left to right were 2.9, 2.8, (each using a 1:10 dilution of culture for reading due to high density) and 0.00 (undiluted). Not shown here, spread plates with cells incubated with only 0.1 mM $HAuCl_4$ in PBS pH 7.4 also yielded no colonies.

## References

(1) Adams, B. L.; Finch, A. S.; Hurley, M. M.; Sarkes, D. A.; Stratis-Cullum, D. N., Genetically engineered peptides for inorganics: study of an unconstrained bacterial display technology and bulk aluminum alloy. *Adv. Mater.* **2013,** *25* (33), 4585-4591.
(2) Adams, B. L.; Hurley, M. M.; Jahnke, J. P.; Stratis-Cullum, D. N., Functional and Selective Bacterial Interfaces Using Cross-Scaffold Gold Binding Peptides. *JOM* **2015,** *67* (11), 2483-2493.
(3) Sarkes, D. A.; Jahnke, J. P.; Stratis-Cullum, D. N., Semi-automated Biopanning of Bacterial Display Libraries for Peptide Affinity Reagent Discovery and Analysis of Resulting Isolates. *J. Vis. Exp.* **2017**, e56061.