**Enabling Cargo-Carrying Bacteria via Surface Attachment and Triggered Release**

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**Supporting Information**

This document contains, details of the methods for: -

1) Fabrication and patterning of the substrates
2) Attachment of beads and bacteria on the gold patterns on the substrate
   - Supplementary Figure 1
3) Chemical release of bacteria–bead conjugates from the gold patterns
   - Supplementary Figure 2
4) Tracking motion of individual bacteria-bead conjugates

Also included are video captions for supplementary videos of mobile bacteria-bead conjugates

a. Video S1
b. Video S2

## 1. Fabrication and patterning of the substrates

a. Preparation of the gold (Au) patterns on the silicon (Si) wafers

Si wafer substrates (Montco Silicon Technologies) were cleaned by rinsing with acetone, 2-propanol, deionized water (DIW) and then dried under a stream of compressed nitrogen gas ($N_2$). Before use, the wafers were subjected to a dehydration bake on a hotplate at 115 $^oC$ for 1 min. In order to create patterns on the wafers, a layer of the positive photoresist SC 1827 (Microposit™) was spin-coated on the surface of the cleaned wafers and the wafers were baked for 1 min at 115 $^oC$. An array of squares 80 μm x 80 μm squares spaced 40 μm apart was then printed on the photoresist using a photomask and UV exposure (45 sec). After developing in 351 Developer (Microposit™), the wafers were transferred to a thermal evaporator where a 20 nm layer of chromium (Cr) was deposited by thermal evaporation followed by a 50 nm layer of Au. After thermal evaporation, the photoresist was lifted-off with acetone leaving behind an array of 80 μm x 80 μm Au squares (over a Cr adhesion layer) spaced 40 μm apart.

b. Silanization of Si and thiolation of Au

The wafers containing the Au patterns were then immersed in hot piranha solution [NOTE: Piranha solution must be handled with extreme caution as is it is extremely energetic and potentially explosive] for 10 minutes (1:4 v/v 30 % hydrogen peroxide/98 % sulfuric acid) to clean and graft hydroxyl groups on the surface of the wafers. The wafers were rinsed thoroughly with DIW, dried under a stream of compressed $N_2$ and then immersed in a solution of polyethylene glycol silane (PEG Silane; Gelest Inc.) in toluene. The silanization reaction mixture contained 2 mL PEG silane (used as supplied) and 0.8 mL concentrated hydrochloric acid per L of toluene mixed by ultrasonication for 10 minutes before addition to the wafers. The silanization reaction was carried out for 2 h at room temperature so as to render the Si surface repellant to protein adsorption.[1] After silanization, the wafers were rinsed thoroughly with toluene, 2-propanol and then dried with compressed $N_2$. The silanized wafers were immersed in a solution containing 0.1 mM nitrilotriacetic acid thiol (NTA thiol; Prochimia) in fresh anhydrous ethanol for 16 hours to thiolate the surface of the Au patterns so as to be able to bind histidine tagged protein (his-tag).[2] After thiolation, the wafers were rinsed with ethanol, 2-propanol and then dried with compressed $N_2$. For long term storage and to prevent

sample surface contamination and scratching, the silanized and thiolated wafers were coated with a layer of SC 1827 by spin-coating.

c. Substrate preparation for cargo attachment

Prior to use, the wafers were diced into rectangular shaped substrates approximately 5 mm x 20 mm in dimensions. The photoresist was removed by immersing in acetone, rinsing with 2-propanol and drying with compressed air. To further block the non-specific adsorption of proteins, the substrates were dipped in 1 % bovine serum albumin (BSA; Sigma) in phosphate buffered saline pH 7.4 (PBS; Sigma) for 16 h at room temperature. After blocking the substrates were rinsed under a stream of DIW and then immersed in a 1 M solution on nickel sulfate in DIW for 1 h at room temperature. This step ensures that $Ni^{2+}$ ions are chelated by the NTA thiol on the Au patterns. The substrates were rinsed with DIW and then placed in a solution of his-tag protein G (Abcam; 0.5 μM protein G + 1 % BSA in PBS) for 1 h at room temperature. The $Ni^{2+}$-NTA binds the his-tagged protein G onto the Au patterns on the substrate. The substrates were then rinsed with PBS and placed in a solution of the cargo capturing antibody; goat anti-rabbit IgG (AbD Serotec; 0.2 μM IgG + 1 % BSA in PBS) for 1 h at room temperature. The protein G immobilized on the Au patterns bind the $F_c$ region of the cargo capturing antibody.[3, 4] The substrates were then rinsed with PBS and stored in fresh PBS prior to addition of the cargo.

**2. Attachment of beads and bacteria on the gold patterns on the substrate**

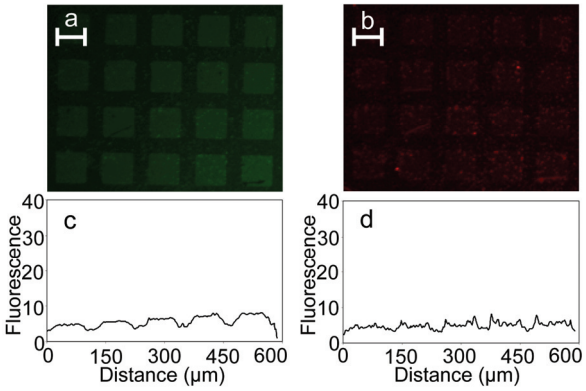
a. Attachment of the beads on the gold patterns

The cargo used in this study comprised of polystyrene beads (diameter 0.4 – 0.6 μm; Spherotech) coated with fluorescein isothiocyanate FITC labeled bacteria capturing antibody (rabbit anti-*E.coli*; AbD Serotec). The beads were coated with the antibody by contacting a 5 mg/mL suspension of beads with 2 μM labeled antibody in PBS at room temperature for at least 16 h. Subsequently, the beads were separated from the unbound antibody by collecting the beads by centrifugation (8000 xg for 5 minutes) and re-suspending in PBS multiple times (> 8 times to ensure that the concentration of the free unbound antibody in suspension is very low). To spatially attach the antibody labeled beads, the substrate was immersed in a solution containing the antibody coated beads (0.1 mg/mL + 1 % BSA in PBS) for 1 h at 30 $^{o}$C and 150 rpm (to gently mix the beads in suspension). The suspension was protected from light by covering using aluminum foil. After contact, the substrate was rinsed twice by dipping in PBS for 5 min at 30 $^{o}$C and 150 rpm. The experimental sample comprised of the substrate prepared and treated as described above. The control sample was subjected to all the above treatment steps except the addition of protein G and cargo capturing antibody (thus could not specifically capture the antibody coated beads). The spatial attachment of the beads in both the experimental and control substrates was separately imaged by measuring the green fluorescence produced by the beads using a Nikon AZ100 multizoom microscope (B-2E/C filter set; Nikon Microscopy). To generate the fluorescence intensity profiles, line plots were generated for a given sample using ImageJ (NIH) and the mean of four separate line plots was taken.

b. Attachment of the bacteria to the beads attached to the gold patterns

The bacterial strain used in this study is *Escherichia coli* W3110 (ATCC; # 39936). The bacteria were inoculated from freezer stock into tryptone broth (TB; 10 g/L tryptone (Difco) and 5 g/L sodium chloride (Sigma)) for 12 h at 30 $^{o}$C and 150 rpm. The 12 h cultures were diluted 1:20 in fresh TB and grown for a further 5 h at 30 $^{o}$C and 150 rpm ($OD_{600}$ ~ 1). The culture conditions were chosen to facilitate growth of motile bacteria.[5, 6] After the said growth period, the bacteria were collected by centrifuging (2000 xg for 5 minutes) and

resuspended in PBS containing 0.5 % BSA. The substrates containing the spatially attached beads were immersed in bacterial suspension in BSA/PBS for 30 min at 37 $^{o}$C and 150 rpm. Once again the conditions were selected to facilitate bacteria settling onto the substrate and contacting the spatially attached beads. The substrates were then removed from the bacterial suspension and rinsed with motility buffer (10 mM phosphate buffer, 0.1 mM EDTA, 1 µM methionine and 10 mM lactic acid; pH 7.4) twice for 5 min at 30 $^{o}$C. To better observe the bacteria attached to the beads on the substrate, the bacteria were stained by dipping the substrate in 1 µM Syto 61 (red fluorescent nucleic acid stain; Invitrogen) in 0.1 M Tris-Cl buffer pH 8 (Sigma) for 20 minutes at 30 $^{o}$C and 150 rpm. After staining, the substrates were once again rinsed with motility buffer and imaged by measuring the red fluorescence produced by the bacteria using a Nikon AZ100 multizoom microscope (G-2E/C filter set; Nikon Microscopy). The fluorescence intensity profiles were generated as described above using ImageJ. Images of the attached beads and bacteria for the experimental sample show specific attachment of bacteria and beads (Fig. 2 of manuscript) while fluorescence images of a negative control sample (subjected to all the treatment steps for the experimental sample except for the addition of protein G and cargo capturing antibody) show significantly less specific attachment of beads or bacteria to the Au patterns (Supplementary Fig. 1) indicating specificity of our molecular attachment methodology.



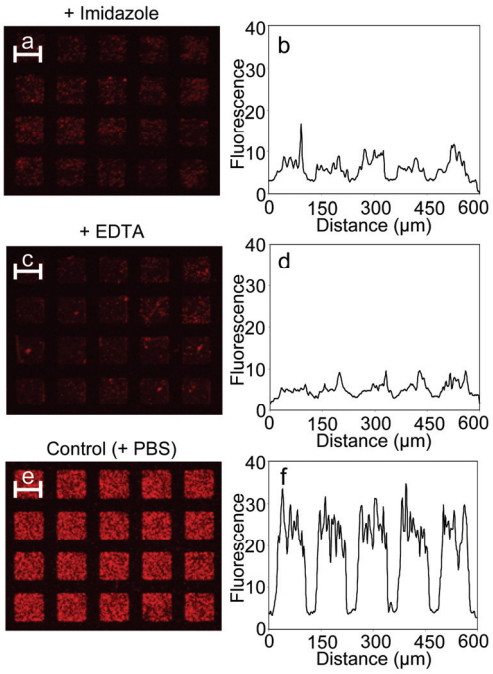

**Supplementary Figure 1**. **Verification of the relative non-specificity of attachment in the negative control sample (compared to Fig. 2 of manuscript).** (a-b) Fluorescence images and (c-d) line intensity plots of the control sample after treatment with beads (green; a and c) and *E. coli* (red; b and d) to Au patterns on Si substrates. Scale bars in panels a, b are 80 µm long.

To image the bacteria-bead conjugates assembled on the substrate via scanning electron microscopy (SEM), the samples containing the bacteria bound to the beads were first fixed at room temperature for 1 h with a fixative containing 3.0 % formaldehyde, 1.5 % glutaraldehyde, 5 mM $Ca^{2+}$ and 2.5 % Sucrose in 0.1 M sodium cacodylate, pH 7.4. The substrates were rinsed thrice (15 min each) in 0.1 M sodium cacodylate containing 2.5% sucrose. The substrates were then post fixed with 1% osmium tetroxide for 5 minutes protected from light and rinsed thrice with 0.1 M sodium cacodylate. The substrates were quickly rinsed in DIW and then with 50 % cold ethanol. Subsequently, the samples were dehydrated with a graded series of cold ethanol (70 → 90 → 100 %) and then washed thrice for 15 minutes each in freshly opened 100 % ethanol at room temperature. The samples were then placed in 50 % ethanol/ 50 % hexamethyldisilazane (HMDS) for 5 minutes (rocking gently) and then in 100 % HMDS for 10 minutes with gentle rocking. Finally, the substrates were placed in fresh 100 % HMDS and the excess HMDS was removed by pipetting and the substrates were allowed to dry uncapped in the hood. The air dried substrates were mounted

on SEM mounts using carbon tape, sputter coated with 4 nm platinum (Anatech Hummer) and imaged using SEM.

### 3. Chemical release of bacteria–bead conjugates from the gold patterns

To release the bacteria–bead conjugates from the surface of the substrates, the substrates containing attached bacteria and beads were immersed either in 300 mM imidazole (Sigma) or 50 mM EDTA in motility buffer for 10 minutes at room temperature. After "conditioning" the samples, the substrates were transferred to motility buffer and detachment was completed by pipetting up and down. Alternatively, the substrates could be left in the imidazole or EDTA solutions for longer periods with shaking (i.e. 1 h at 150 rpm) to effect detachment. In our studies, we observed that conjugates remained motile in imidazole (in motility buffer) for around 1 – 2 h after which only Brownian motion of conjugates were observed (data not shown) possibly due to imidazole toxicity or imidazole affecting the flagellar motors. Hence we utilized the conditioning mode of release. Conjugates released in EDTA (in motility buffer) were observed to retain motility even after 24 h. Longer times were not investigated in this study. Control samples were immersed in PBS instead of imidazole or EDTA and treated as described above. The substrates treated with imidazole, EDTA or PBS (controls) were imaged using a Nikon AZ100 multizoom microscope (G-2E/C filter set) and fluorescence intensity profiles were generated as described above using ImageJ. Treatment of the substrates




**Supplementary Figure 2. Release of bacteria-bead conjugates from the substrate in response to imidazole or EDTA.** (a,c,e) Fluorescence images of the substrate surface after treatment with imidazole (a), EDTA (c) and PBS (control; e). (b,d,f) Fluorescence line intensity plots across substrate after treatment with imidazole (b), EDTA (d) and PBS (control; f). Scale bars in panels a, c and e are 80 μm long.

with attached bacteria-beads with imidazole (Supplementary Figs. 2a and b) or EDTA (Supplementary Figs. 2c and d) resulted in significant release of bacteria-bead conjugates from the substrate as compared to controls ((Supplementary Figs. 2e and f) as observed both by fluorescence images and fluorescence intensity profiles.

To image the released bacteria–bead conjugates, the supernatants were fixed with a fixative solution for 1 h at room temperature (described above). After fixing, the samples were imaged using a 3i Marianas microscope (Intelligent Imaging Innovations Inc.) under a GFP/RFP filter set to simultaneously image the red and green fluorescence of the bacteria and beads respectively. Co-localization of the red and green fluorescence in the supernatant (Figs. 3b and d in manuscript) indicate release of intact bacteria-bead conjugates.

**4. Tracking motion of individual bacteria-bead conjugates**

The bacteria-bead conjugates were released from the surface via imidazole or EDTA in motility buffer as described above. The released supernatants were pipetted between two 22 x 22 mm coverslips (Fisher Scientific) separated by a double sticky tape spacer 120μm thick (Secure Seal, Grace Bio-Labs) and individual bacteria-bead conjugates were imaged using a Nikon Eclipse TS100 inverted microscope under a 40 x DIC objective with video imaging (using Nikon's Q Capture Pro software). To track the trajectories of the bacteria-bead conjugates, the video was converted to an image series (TIF series) using ImageJ. The trajectories were plotted and parameters such as the mean speed were calculated using ImageJ's manual tracking plug-in.

**References**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**Captions for videos of mobile bacteria-bead conjugates**

a. Video S1

Video of a single bacterium–bead conjugate imaged after release from the surface that sticks to the coverslip. The conjugate moves in circular trajectories corresponding to that in Fig. 4a. The video runs in real time (file format: avi).

b. Videos S2

Video of a single bacterium–bead conjugate imaged after release from the surface. The conjugates moves in trajectories similar to those associated with motile bacteria. The video corresponds to the Fig. 4b and runs in real time (file format: avi).