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Rewiring Gram-Negative Bacteria Cell Surfaces with Bio-Orthogonal Chemistry via Liposome Fusion

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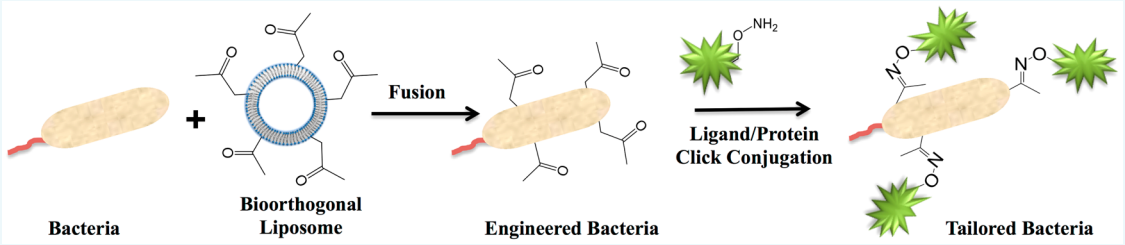

ABSTRACT: The ability to tailor bacteria cell surfaces with non-native molecules is critical to advance the study of bacteria communication, cell behavior, and for next-generation therapeutics to improve livestock and human health. Such modifications would allow for novel control over cell behavior, cell−cell interactions, biofilm formation, adjuvant conjugation, and imaging. Current methods to engineer bacteria surfaces have made major advances but rely on complicated, slow, and often expensive molecular biology and metabolic manipulation methods with limited scope on the type of molecules installed onto the surface. In this report, we introduce a new straightforward method based on liposome fusion to engineer Gram-negative bacteria cells with bio-orthogonal groups that can subsequently be conjugated to a range of molecules (biomolecules, small molecules, probes, proteins, nucleic acids, ligands, and radiolabels) for further studies and programmed behavior of bacteria. This method is fast, efficient, inexpensive, and useful for installing a broad scope of ligands and biomolecules to Gram-negative bacteria surfaces.

INTRODUCTION

The understanding and manipulation of the bacteria cell surface is important for a range of fundamental studies of bacteria behavior and for therapeutic applications to improve human health.[1] The first studies of modifying bacteria surface proteins were performed in the 1980s and led to the critical development of tools to tailor bacteria cells with biological macromolecules and synthetic man-made molecules for applications in microbiology, vaccine development, and biotechnology.[2,3] In particular, Gram-negative bacteria including *Pseudomonas aerugonosa* and *E. coli* have shown increasing importance due to their implications in infection and pathogenesis.[4,5] These bacteria also serve as model single cell organisms to study fundamental cell behavior as well as various bacteria associated diseases. Due to their central importance in livestock, agriculture, and human health, methods to tailor bacteria cell surfaces are essential to generate new bacteria platforms that have the ability to display a wide range of molecules to further explore and manipulate bacteria behavior and to design next generation therapeutics.

Due to the complexity of the bacteria cell surface, few straightforward and robust technologies exist to add biomacromolecules or synthetic small molecules and ligands to the membrane with high efficiency and fidelity. Current methods use three main strategies: 1, a molecular biology approach that uses genetic surface display technologies; 2, a metabolic engineering approach where foreign substrate ligands are fed to bacteria and then processed through the bacteria enzymatic machinery to be displayed on the cell surface; and 3, direct chemical display of molecules to the bacteria cell surface via chemical ligation to membrane proteins or carbohydrates. Through genetic surface display technologies, a range of applications including environmental remediation,[6] biofuel production,[7] biocatalysis,[8] biosensing,[9] protein library screening,[10] cancer therapeutics,[11] vaccine development,[12,13] and intestinal probiotic therapy[14] have been investigated. However, these technologies traditionally cannot be applied to install nongenetically encoded molecules such as lipids, carbohydrates, and a variety of non-native chemical compounds. To address this limitation, chemical surface display technologies, which allow for installation of non-native compounds onto cell surfaces, have become increasingly of interest to researchers in recent years. These technologies are commonly based on metabolic labeling of small peptides, which relies on intercepting the machinery pathways of cell-wall biosynthesis. Thus, they are well suited for manipulating the surface glycans as well as subsequent conjugation of other attachable compounds. These technologies led to many studies including

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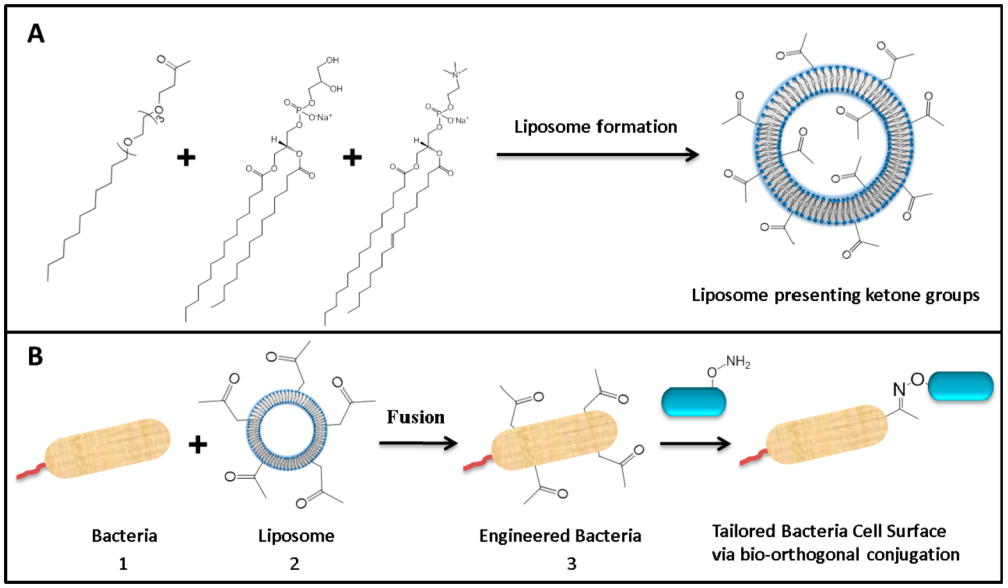

Figure 1. Schematic describing the generation of bio-orthogonal liposomes for bacteria surface engineering. (A) Ketone-terminated lipid-like molecule is mixed with 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (DMPG) to form ketone-functionalized liposomes. (B) Bacteria cells (**1**) are incubated with the ketone-functionalized liposomes (**2**) at pH 4.5 buffer at 37 °C for 1 h. Through liposome fusion, the bacteria surface can be engineered to present ketone groups (**3**), which can subsequently react with an oxyamine tailored FITC molecule via bio-orthogonal oxime conjugation. Based on this bacteria surface engineering system, a range of ligands, proteins, small molecules, and probes containing the oxyamine group can be installed onto the bacteria surface through oxime ligation.

directed evolution,[15] proteomic analysis,[16] immunotherapy,[17,18] molecular imaging,[19] and host−pathogen interactions.[20]

Herein, we introduce a rapid and straightforward Gram-negative bacteria surface engineering method that bypasses traditional genetic and metabolic manipulations. This approach relies on integrating a robust delivery system (liposome fusion) with bio-orthogonal lipids to tailor bacteria surfaces. The liposome fusion method combined with bio-orthogonal ligation strategies allows for the presentation of stealthlike molecules on the bacteria surface for subsequent decoration with a wide variety of ligands to simultaneously track bacteria, augment bacteria behavior, and to provide theranostic type studies and applications. In this report, we use for the first time an oxime reaction to tailor *Pseudomonas aeruginosa* and *E. coli* bacteria cell surfaces with a range of ligands. Based on this powerful, fast, and straightforward surface engineering technique, a wide range of functional molecules can be installed onto the bacteria surface without disturbing regular cellular activities. In this work, we fully characterize and demonstrate this new strategy by employing flow cytometry, phase contrast microscopy, membrane isolation, mass spectrometry, and surface chemistry methods to study the tailoring of bacteria cell surfaces.

Liposome fusion has been used as a technique for the delivery of chemical and biological cargoes into mammalian cells for many years.[21,22] In this study, we used the liposome strategy to deliver novel lipid-like functional molecules efficiently to a bacteria surface. The liposome fusion method takes advantage of hydrophobic interactions of micelles and lipid bilayers to insert into the outer membrane structure. Although such interactions are electrostatic in nature and thermodynamically favored, the composition of the liposome nanoparticle plays a significant role to induce adhesion and fusion.

In order to tailor bacteria surfaces with a range of molecules, a general class of reactions that can be performed at physiological conditions without side reactions in a biological environment is required. Tremendous research has been performed to investigate and expand the scope of these special chemical reactions termed bio-orthogonal chemistry.[23−25] Several new types of bio-orthogonal reactions have been discovered including click, hydrazone, thiol−ene, diels-alder, and so forth. However, the most popular click reaction is the copper catalyzed Huisgen 3 + 2 alkyne and azide reaction, hydrazone and oxime chemistry. Pioneering efforts by several researchers have shown the utility of these reactions for many biological applications ranging from in vivo targeting, drug delivery, antibody drug conjugates, protein engineering, proteomics, imaging, and biosensing.[26−28]

RESULTS AND DISCUSSION

In previous studies from our laboratory, nanoparticle liposomes made from POPC/DOTAP and bio-orthogonal lipids have shown broad scope results in decorating the surface of mammalian cells.[29−34] However, this particular liposome composition may not be necessarily compatible with the majority of Gram-negative bacterial cell membranes. Recent reports have shown successful delivery of antimicrobial cargo into the cytoplasm of bacterial cells using DPPC/DMPG lipids. Therefore, we designed our bio-orthogonal/liposome delivery system based on a combination of 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (DMPG) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and a bio-orthogonal lipid like molecule. As shown in Figure 1A, 2-dodecanone, DMPG, and DPPC were mixed together at various ratios to prepare ketone-presenting liposomes to be delivered to bacteria for display on their cell surfaces. After fusion the bacteria surface presents ketone molecules, which can subsequently be reacted with oxyamine ($RONH_2$) containing ligands, proteins, or small molecules via oxime ligation. The addition of the ketone-lipid-like molecule directly (not in liposome format) to bacteria did not result in incorporation into the bacteria cell membrane.

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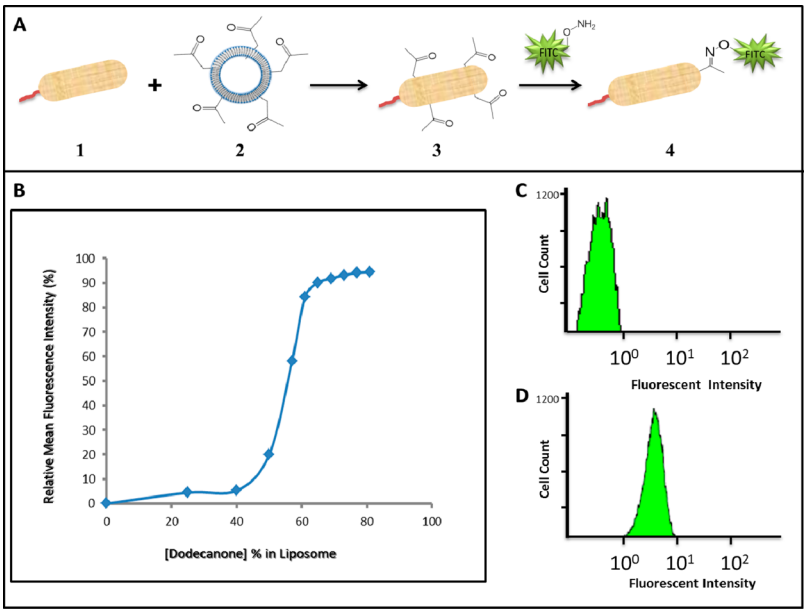

Figure 2. Quantitative characterization of bacteria surface engineering via bio-orthogonal chemistry and liposome fusion by flow cytometry. (A) Schematic of the process of bacteria surface engineering. Through liposome fusion, bacteria cells were surface-engineered to present ketone groups (3), which subsequently reacted with a oxyamine tailored fluorescent reporter (FITC-ONH_2) to generate FITC-conjugated bacteria (4). (B) Flow cytometry characterization shows that control bacteria cells treated with blank liposomes containing no ketone and then FITC-ONH_2 expressed no fluorescent signal. (C) Bacteria cells treated with ketone tailored liposomes and then FITC-ONH_2 resulted in fluorescent bacteria due to oxime ligation. (D) Flow cytometry analysis of the relationship between ketone concentration in the liposome and ketone installed onto the bacteria cell surface. The amount of ketone on the cell surface correlates with the amount of conjugated FITC-ONH_2 and can be analyzed by fluorescence intensity. It is observed that controlling ketone concentration in the liposome results in tuning the amount of ketone molecules installed onto the cell surface.

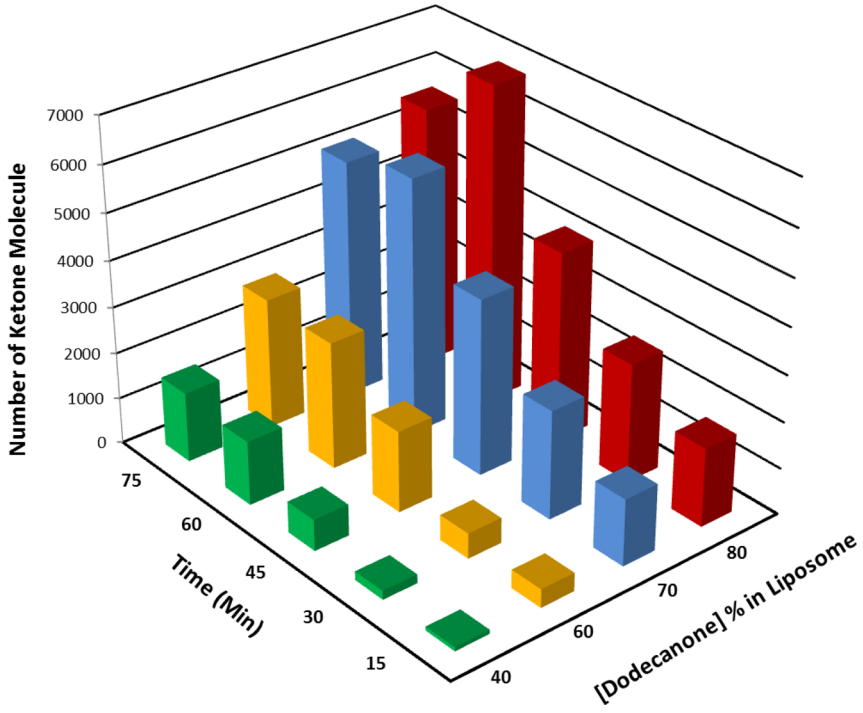

Figure 3. Three-dimensional (3D) plot describing the interplay between liposome composition, liposome fusion/incubation time, and the amount of ketone molecules on the bacteria cell surface. The ketone groups on the cell surface were conjugated with a fluorescent probe FITC-ONH_2 and then quantified per bacteria cell using flow cytometry. From the data the number of ketone molecules on the bacteria surface is directly related to the ketone content in the liposome as well as the liposome fusion/incubation duration. By tuning the ketone content in the liposome and incubation time (liposome fusion time), the amount of ketone on the cell surface can be directly modulated.

As a representative application, a synthetically modified fluorescent probe FITC-ONH_2 was synthesized to click with the ketone group on the bacteria surface and thus label the bacteria with a fluorescent probe (Figure 1B). Through the bio-

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orthogonal oxime conjugation between oxyamine and ketone, the bacteria surface can be fluorescently labeled with the FITC moiety. It was observed that cells treated with these liposomes were indistinguishable in behavior and morphological integrity from untreated bacteria. The oxime reaction conjugate offers a very powerful ligation method that is well tolerated by living cells. This reaction is also very efficient with no side reactions or byproducts under physiological conditions.

To characterize the bio-orthogonal liposome fusion and conjugation method to tailor cell surfaces we used oxyamine probes and flow cytometry (Figure 2). Bacteria cells were treated with liposomes for 1 h containing different amounts of ketone groups and then exposed to FITC-ONH_2 (a fluorescent-oxyamine). Only cells presenting ketone groups reacted via a bio-orthogonal oxime ligation and became fluorescent. These cells were then quantified for the amount of fluorescence, which directly corresponds to the successful liposome fusion and therefore the amount of ketone presentation on bacteria surfaces with flow cytometry. The data shows that the FITC-ONH_2 efficiently clicked to the ketone-presenting bacteria and reported positive fluorescent signal after thoroughly washing and removing excess FITC-ONH_2. In contrast, the control bacteria (liposome exposure but without ketone-lipid) reported no fluorescent signal under the same conditions. We also conducted a further study to determine the role of the amount of bio-orthogonal lipid group (ketone) concentration within the liposome on the oxime conjugation of the fluorescent probe (FITC-ONH_2) (Figure 2D). It was clear that increasing the ketone concentration of the liposome resulted in increased ketone group content on the cell surface through a standard titration curve.

To further explore the parameters that affect bacteria cell surface engineering, we investigated how the interplay of liposome composition and liposome fusion/incubation time impacted the amount of bio-orthogonal group displayed on the bacteria surface (Figure 3). Moreover, the ability to precisely determine and therefore tune the number of bio-orthogonal groups (ketones) on the bacteria surface may be relevant for a number of applications that require precise amounts of ligands or proteins to be conjugated to the cell surface. Furthermore, interfacial kinetic information for conjugation may be obtained for further biophysical studies of membrane dynamics. To quantitatively analyze the number of ketone groups on the bacteria surface, a standard fluorescence quantification kit (Quantum FITC-5 MESF from Bangs Laboratories, Inc.) was used for measuring the amount of fluorescence from the FITC-ONH_2 treated ketone-presenting bacteria. The 3D plot shows that the number of ketone groups on the bacteria surface is directly related to the concentration of ketones within the liposome and the duration of liposome fusion. Therefore, the amount of bio-orthogonal group on the cell surface can be quantitatively determined by controlling parameters that directly influence the liposome fusion conditions. It was also determined that only a small amount (several thousand) of bio-orthogonal groups installed onto the cell surface was required for ligand immobilization detection. An important feature of the liposome fusion cell surface engineering method described is the ability to rapidly alter cell surfaces and to precisely tune the amount of bio-orthogonal lipid and therefore ligand conjugation without the use of cumbersome molecular biology or metabolic engineering strategies. Furthermore, due to the mild liposome fusion conditions and the fast bio-orthogonal surface conjugation method, no toxicity or disruption of regular cellular activity was observed. The data in the 3D plot provides a matrix of conditions to tailor a cell's surface with precise amounts of ligands and/or proteins. This could prove valuable in clinical applications where exact amounts of ligand bioconjugation are required for cell surface display. For example, various cancer biomarkers, viral infections, adjuvants, and other immunophenotyping analyses are all diagnosed via their relative abundance on the cell surface.[35]

To investigate the retention of (bio-orthogonal groups) ketone groups on the cell surface over time we analyzed the amount of ketone via flow cytometry (Figure 4). This transient

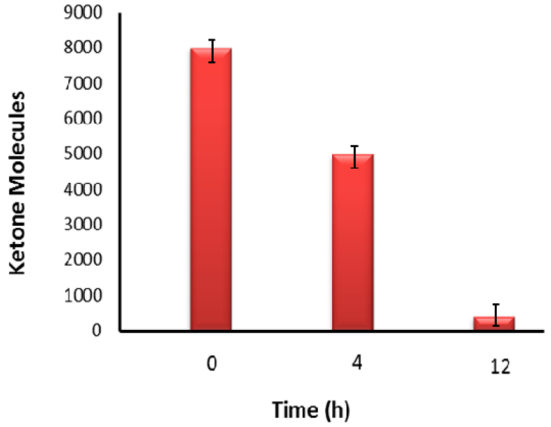

Figure 4. Transient transfection of bio-orthogonal groups on a bacteria surface. Time course of ketone content and viability of functionalized cells studied over 12 h, with incubating in fresh LB media at 37 °C. After 12 h incubation in fresh media, cells completely dilute the ketone content and are no longer surface tailored with bio-orthogonal groups and are indistinguishable from nontailored bacteria.

transfection may be valuable in modifying bacteria for specific time-sensitive applications. To determine the quantity of available ketone molecules on the bacteria cell surface, a fluorescent molecule FITC-ONH_2 was used to conjugate with the ketone on the bacteria surface. An aliquot withdrawn from the culture incubated in fresh LB media showed significant reduction in the number of ketones reported after 4 h growth and showed negligible amounts after 12 h of growth. The fluorescently labeled bacteria was characterized using flow cytometry and the quantity of fluorescent molecule was calibrated based on a standard fluorescence quantification kit (Quantum FITC-5 MESF from Bangs Laboratories, Inc.). The quantification kit contains a group of fluorescent beads labeled with varying amounts of FITC. A QuickCal analysis template is provided with the kit. After using flow cytometry to measure the FITC intensity of the group of beads, a standardized calibration system can be established based on the analysis template. Therefore, the FITC intensity can be directly translated into the amount of surface conjugation. We found no difference in toxicity by the liposome fusion method. We observed no difference in cell growth kinetics or cell numbers when the cells were treated with blank liposomes, bio-orthogonal liposomes, or no liposomes. This transient feature may be useful for applications that require the bacteria to only present probes or ligands for short periods for time sensitive assays and tracking.

To demonstrate the scope of the bio-orthogonal bacteria surface method for protein display we used the classic

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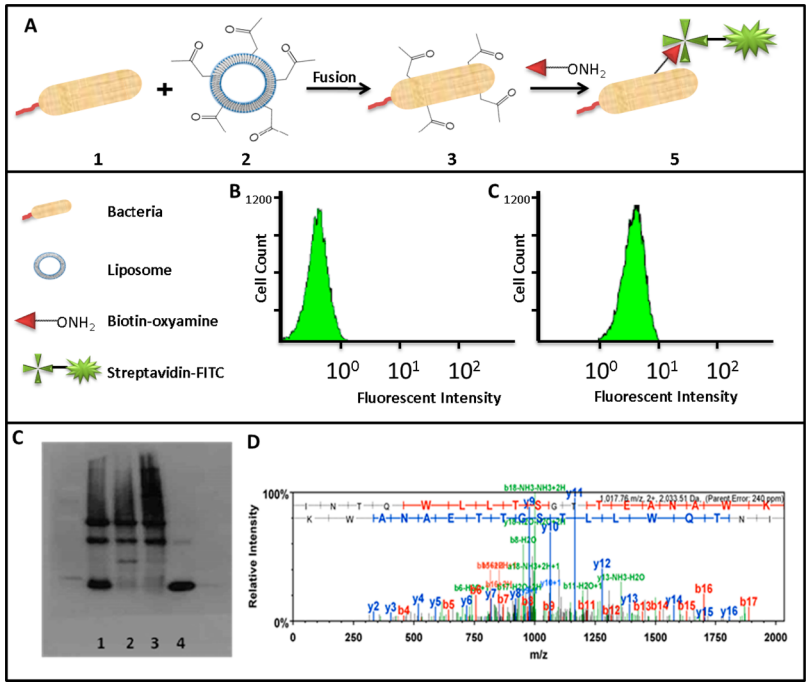

Figure 5. Bacteria surface engineering with bio-orthogonal groups for further ligand and protein conjugation. (A) Bacteria surface-engineered with ketone groups via liposome fusion can be further modified by the conjugation of oxyamine tailored ligands such as Biotin-ONH_2. The biotin ligand present on bacteria surface can then be recognized with Streptavidin labeled with a FITC reporter (Streptavidin-FITC), and characterized by flow cytometry. Cells treated with blank liposomes containing no ketone molecules do not show any fluorescent signal (B), while cells treated with ketone liposomes showed a fluorescent signal (C). (D) Cells with the biotin−streptavidin complex were lysed and the plasma membrane content isolated. The total plasma protein content was analyzed on 0.1% SDS-12% PAGE and the Western blot was stained with anti-streptavidin antibody and then detected with luminol. Lane 1 is the positive analyte of ketone-engineered cells treated with biotin-ONH_2 and then streptavidin (ketone-engineered cells + Biotin-ONH_2 + Streptavidin). Lane 2 is a negative control sample of ketone-engineered cells without biotin-ONH_2 and treated only with streptavidin (ketone-engineered cells + Streptavidin). Lane 3 is a negative control of cells treated with blank liposome with no ketone (blank cells + Biotin-ONH_2 + Streptavidin). Lane 4 is a positive standard of pure streptavidin. (E) Mass spectrometry analysis of digested Lane 1 sample.

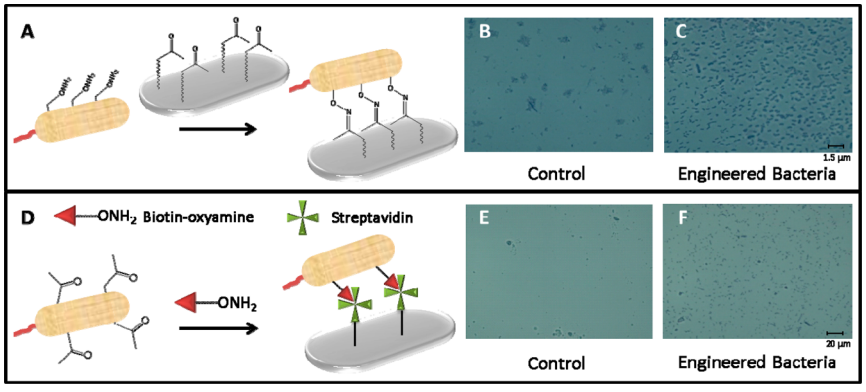

Figure 6. Bio-orthogonal bacteria adhere specifically to tailored materials. (A) Schematic showing surface-engineered bacteria presenting oxyamine adhered to aldehyde-tailored indium tin oxide (ITO) material via oxime immobilization. (B) Images showing that control bacteria with no oxyamine present on the surface do not adhere well to aldehyde-tailored indium tin oxide (ITO). (C) Images showing significant adherence of surface-engineered bacteria, which present oxyamine and bound to the aldehyde-tailored ITO via oxime bonding. (D) Schematic describing further ligand immobilization (Biotin-ONH_2) on ketone-presenting bacteria and subsequent ligand−protein (Biotin−Streptavidin) interaction at the interface of bacteria and material. (E) Microscopy showing no adherence of control bacteria onto Streptavidin-coated glass substrate. (F) Microscopy showing that Biotin-tailored bacteria adhered to Streptavidin-coated glass substrate via Biotin−Streptavidin recognition.

association of biotin and streptavidin. After introduction of ketone groups to the bacteria surface, streptavidin was added and the ligand−protein association characterized by flow cytometry, mass spectrometry, and Western blotting (Figure 5). First, biotin-ONH_2 (Quanta Biodesign) was conjugated to the ketone presenting bacteria surface. The presence of biotin on the surface was then confirmed with the biospecific association of FITC-conjugated streptavidin and quantified by flow cytometry. A Western blot analysis showed the bacteria cell wall contained streptavidin only when bacteria presented biotin groups via bio-orthogonal oxime conjugation. Briefly, after biotin display and streptavidin addition, bacteria cells were lysed and the membrane protein content was isolated according to a straightforward protocol. Samples were then run on SDS gel, blotted, and stained with anti-streptavidin antibody HRP and detected using luminol. Furthermore, the bands in the gel

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lanes were excised, trypsinized, and analyzed using mass spectrometry. Figure 5D clearly shows tandem MS/MS analysis of the bacteria surface containing streptavidin. Several control experiments were performed: 1. Bacteria that did not present ketones on the surface showed no streptavidin binding. 2. Bacteria exposed to liposomes without ketones (blank liposomes) showed no streptavidin binding. 3. Bacteria presenting ketones were reacted with a sacrificial oxyamine and then exposed to biotin-ONH_2 and then to streptavidin showed no streptavidin binding. These experiments showed that only upon liposome fusion of ketone groups followed by biotin-ONH_2 oxime conjugation followed by streptavidin addition resulted in protein tailoring of the bacteria surface.

The interaction of Gram-negative bacteria with a range of materials and other cells has important implications in a variety of diverse fields including bioenergy, biofuel, biofilm formation, and the infection of plants and animals.[36,37] In particular, *P. aeruginosa* becomes pathogenic upon cell−cell interactions in a biofilm.[38] To further explore the utility of this bacteria surface engineering system, we showed the specific association between tailored *Pseudomonas aeruginosa* bacteria and tailored materials via bio-orthogonal ligation and ligand−protein interaction (Figure 6).

In our previous work, we established a methodology to rapidly fabricate aldehyde presenting indium tin oxide (ITO) materials for material science, biomaterial, and solar cell applications.[39,40] ITO is a conductive and transparent material and ideal for surface chemistry studies. In the present study we employed the aldehyde-tailored ITO substrates and seeded surface-engineered bacteria presenting oxyamines onto these substrates to evaluate the specific binding of bacteria to ITO substrates via an interfacial oxime ligation. It was observed that control bacteria with no oxyamine present on the surface adhered poorly to the aldehyde-tailored ITO, while the oxyamine-presenting bacteria showed high amounts of binding and significant adherence to the aldehyde-tailored ITO even after washing (approximately 35 cells/mm^2 vs 400 cells/mm^2). In this context, we determined that ligand presenting bacteria should adhere to protein coated slides via a biospecific protein−ligand interaction. Ketone presenting bacteria that were subsequently reacted with biotin-oxyamine showed significant levels of biotin conjugation via oxime ligation as described above. These biotin presenting bacteria were then added to streptavidin coated glass substrates (Arrayit) and adhered efficiently (500 cells/mm^2). Control experiments where ketone, biotin, or streptavidin were not used showed no bacteria adhesion to the materials (20 cells/mm^2). By rewiring the bacteria surface with ketone groups or oxyamine groups it is possible to sort or capture these bacteria in complex mixtures with polymers, beads, or materials presenting complementary bio-orthogonal groups.

CONCLUSION

In summary, a novel and rapid Gram-negative bacteria surface engineering method was developed and demonstrated for *E. coli* and *Pseudomonas aeruginosa*. We introduced a new method based on liposome fusion to engineer Gram-negative bacteria cells with bio-orthogonal groups. These groups can subsequently be conjugated to a range of molecules (biomolecules, small molecules, probes, proteins, nucleic acids, ligands, nanoparticles, radiolabels, etc.). This method can be universal for tailoring a variety of Gram-negative bacteria for various applications in sensing and therapeutics. We showed this strategy is efficient for tailoring *E. coli* and the *Pseudomonas aeruginosa* bacteria strains. This straightforward method is 1, fast; 2, efficient; 3, general for a range of ligand conjugations and bypasses molecular biology and metabolic interference processes to tailor cell surfaces. This method could be of crucial importance to a range of applications including bacteria labeling and probing, interfacial kinetic study, bacteria−material interaction, bacteria−mammalian cell interaction, and vaccine development. Due to the liposome delivery method many different types of bio-orthogonal chemical reactions may be installed onto the cell surface. The reverse strategy where oxyamine liposomes are fused to the bacteria first followed by ketone containing ligands also works to engineer the cell surfaces. Furthermore, a wide range of molecules with lipid-like features may be incorporated into a liposome and subsequently delivered and displayed on a bacteria surface including ligands, probes, and proteins. Serial liposome fusions to the cells may also be performed to install probes at various time points. This method also provided an open platform for easily installing any functional groups, probes, ligands, proteins, and nanoparticles for biological and tissue engineering studies and applications.[41−46]

EXPERIMENTAL SECTION

Liposome Preparation. Liposomes were prepared with DPPC, DMPG, and the corresponding bio-orthogonal pair in various ratios. For example, 160 μL of DPPC 10 mg/mL stock, 10 μL DMPG 10 mg/mL, and 55 μL of 10/mg ketone/oxyamine were mixed in chloroform. After thorough evaporation of the organic solvent, content was resuspended in a HEPES buffer and sonicated for 4 h using tip-sonicator.

Functionalized Fluorescent Assay (Figure 2). Bacteria *E. coli* Bcl2, were grown to OD^{650} = 0.6, corresponding to approximately $4/0^8$ CFU/mL. The cells were harvested by centrifugation at 6000 rpm for 10 min, washed with 100 mM $CaCl_2$. and resuspended in 6 mL of 100 mM $CaCl_2$ for 2 h at 4 °C. Aliquot cells were suspended in 10 mL of 0.5 mM HEPES buffer pH 4.3. Cells were then treated with 6 μL EDTA 50 mM pH 8.4 and incubated at 37 °C for 1 h. Liposome fusion was attempted by addition of various concentrations of Ketone-SUV (10%, 20%, 30%, etc.) to the competent cells at 37 °C for 1 h. Aliquot samples were withdrawn and washed with PBS buffer pH 7.0. 5 μL of 1.6 mMol FITC-PEG-oxyamine was added to the suspension for 5 min at 4 °C. Unbound probe was washed thoroughly three times with PBS buffer to reduce nonspecific binding to cells. The extent of fusion was assessed by measuring fluorescence signal using a Nikon camera fluorescent microscopy at 492 nm excitation and 518 nm emission, along with Beckman Coulter Flow Cytometer at 600 nm excitation for FITC based probe. Control experiment was conducted parallel to other trials, with addition of plain liposomes deficient of bio-orthogonal pairs.

Functionalized Fluorescent Assay (3D plot). Bacteria *E. coli* Bcl2, were grown to OD^{650} = 0.6, corresponding to approximately $4/0^8$ CFU/mL. The cells were harvested by centrifugation at 6000 rpm for 10 min, washed with 100 mM $CaCl_2$, and resuspended in 6 mL of 100 mM $CaCl_2$ for 2 h at 4 °C. Aliquot cells were suspended in 10 mL of 0.5 mM HEPES buffer pH 4.3. Cells were then treated with 6 μL EDTA 50 mM pH 8.4 and incubated at 37 °C for 1 h. Liposome fusion was attempted by addition of various concentrations of Ketone-SUV (40%, 60%, 70%, 80%) to the competent cells at 37 °C for increasing time (15 min intervals). Aliquot samples were

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withdrawn and washed with PBS buffer pH 7.0. 5 μL of 1.6 mMol FITC-PEG-oxyamine was added to the suspension for 5 min at 4 °C. Unbound probe was washed thoroughly three times with PBS buffer to reduce nonspecific binding to cells. The extent of fusion was assessed by measuring fluorescence signal using a Nikon camera fluorescent microscopy at 492 nm excitation and 518 nm emission, along with Beckman Coulter Flow Cytometer at 600 nm excitation for FITC based probe. Control experiment was conducted parallel to other trials, with addition of plain liposomes deficient of bio-orthogonal pairs.

Profile Biotin/Streptavidin Analysis. Bacteria *E. coli* Bcl2, were grown to OD^{650} = 0.6, corresponding to approximately $4l0^8$ CFU/mL. The cells were harvested by centrifugation at 6000 rpm for 10 min, washed with 100 mM $CaCl_2$, and resuspended in 6 mL of 100 mM $CaCl_2$ for 2 h at 4 °C. Aliquot cells were suspended in 10 mL of 0.5 mM HEPES buffer pH 4.3. Cells were then treated with 6 μL EDTA 50 mM pH 8.4 and incubated at 37 °C for 1 h. Liposome fusion was attempted by addition of 70% concentrations of Ketone-SUV to the competent cells at 37 °C for 1 h. Aliquot samples were withdrawn and washed with PBS buffer pH 7.0. Series of different volume 20, 40, 60, 80, 100, and 120 μL of Biotin-PEG-oxyamine 50 mg/mL were added to the suspension and incubated for 3 h min at 4 °C. Unbound biotin was washed thoroughly three times with PBS buffer to reduce nonspecific binding to cells. Conjugated cells were then treated with 50 μL of Streptavidin-FITC for 30 min at 37 °C. The extent of fusion was assessed by measuring fluorescence signal using a Beckman Coulter Flow Cytometer at 600 nm excitation for FITC based probe. Control experiment was conducted parallel to other trials, with the addition of plain liposomes deficient of bio-orthogonal pairs.

Surface Adhesion Analysis. Bacteria *E. coli* Bcl2, were grown to OD^{650} = 0.6, corresponding to approximately $4l0^8$ CFU/mL. The cells were harvested by centrifugation at 6000 rpm for 10 min, washed with 100 mM $CaCl_2$, and resuspended in 6 mL of 100 mM $CaCl_2$ for 2 h at 4 °C. Aliquot cells were suspended in 10 mL of 0.5 mM HEPES buffer pH 4.3. Cells were then treated with 6 μL EDTA 50 mM pH 8.4 and incubated at 37 °C for 1 h. Liposome fusion was attempted by addition of oxyamine-SUV 70% to the competent cells. Aliquot samples were withdrawn and washed with PBS buffer pH 7.0. Indium tin oxide (ITO) plates coated with aldehyde functionality were submerged in 10 mL of cell suspension for 4 h. Plates were removed and washed thoroughly with PBS buffer and Gram-stained for phase contrast microscopic analysis. Surface adhesion experiments were replicated with Arrayit Premium Superaldehyde glass and Streptavidin/Avidin glass substrates (data not shown) and showed similar results to ITO.

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Notes

The authors declare the following competing financial interest(s): Prof. Yousaf has equity in OrganoLinX Inc.

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