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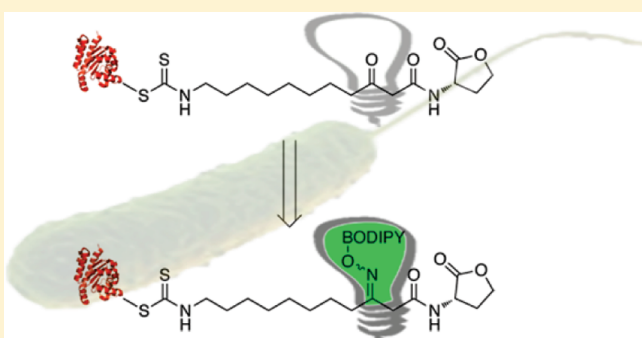
Live Cell Labeling of Native Intracellular Bacterial Receptors Using Aniline-Catalyzed Oxime Ligation

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S Supporting Information

ABSTRACT: Live cell fluorescent labeling of proteins has become a seminal tool in biology and has led to hallmark discoveries in diverse research areas such as protein trafficking, cell-to-cell interactions, and intracellular network dynamics. One of the main challenges, however, remains the ability to label intracellular proteins using fluorescent ligands with high specificity, all the while retaining viability of the targeted cells. Here, we present the first example of live cell labeling and imaging of an intracellular bacterial receptor involved in cell-to-cell communication (i.e., quorum sensing), using a novel two-step approach involving covalent attachment of a reactive mimic of the primary endogenous *Pseudomonas aeruginosa* quorum-sensing signal to its receptor, LasR, followed by aniline-catalyzed oxime formation between the modified receptor and a fluorescent BODIPY derivative. Our results indicate that LasR is not distributed evenly throughout the cytoplasmic membrane but is instead concentrated at the poles of the *P. aeruginosa* cell.



INTRODUCTION

Cell-to-cell communication is employed by single-cell organisms to sense their population density and coordinate gene expression in a manner that benefits their ability to compete and adapt to changing environments. This form of interbacterial chemical communication is known as “quorum sensing” (QS).^{1,2} Examples of QS-controlled group behavior include the formation of biofilms, the expression of virulence factors, antibiotic production and bioluminescence. Each of these processes is beneficial to a bacterial population only when carried out in a synchronized manner, at high cell density. While significant progress has been made in understanding the mechanisms behind QS in recent years, many important molecular details remain to be elucidated. One such point in question addresses the regulation of QS in *Pseudomonas aeruginosa*, an organism often resistant to common antibiotic agents and commonly associated with severe hospital-acquired infections, especially in immuno-compromised individuals.³ The primary QS signal in this bacterium is 3-oxo-dodecanoyl homoserine lactone (3-oxo- C_{12} -HSL, **1**, Figure 1a), which binds to the transcriptional activator, LasR, once thresholds of cell density and concentration have been reached. Correct folding of LasR, which is bound to the cytoplasmic membrane, has been suggested to occur only upon ligand binding, enabling the formation of a homodimer that is able to bind to its target DNA promoter, leading to gene expression.⁴ It is less clear, however, if LasR remains associated with the membrane or whether it is evenly distributed throughout the cell.⁵

It is known that small changes in the structure of the LasR cognate ligand, 3-oxo- C_{12} -HSL, may lead to a significant decrease in ligand affinity for its receptor. However, by covalently labeling LasR with a reactive 3-oxo- C_{12} -HSL analogue containing a keto group, we enabled the exploration of bio-orthogonal ligation reactions on the protein.

Only limited examples for the specific intracellular labeling of proteins with fluorescent ligands have been reported.^{6,7} The reason for this is that the use of a conventional approach, in which a ligand is labeled with a fluorescent tag, often results in a significant decrease in affinity of the modified ligand for its target protein, particularly when the ligand is small.⁸ In the case of larger ligands, such as peptides or proteins, the main obstacle for fluorescent labeling remains penetration of modified ligands into the cell such that membrane structure and polarity are not compromised. Recent progress in the labeling of proteins in live cells by fluorescent probes has been achieved through the development of powerful bio-orthogonal chemistry, such as Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) assisted by a tris(triazolylmethyl)amine-based ligand,⁹ copper-free reactions between azides and strained cyclo-octynes,¹⁰ Staudinger ligation between azides and phosphines,¹¹ and thiol–ene reactions.¹²

One of the few reactions considered to be bio-orthogonal and involving carbonyl moieties is the formation of oximes/hydrazines

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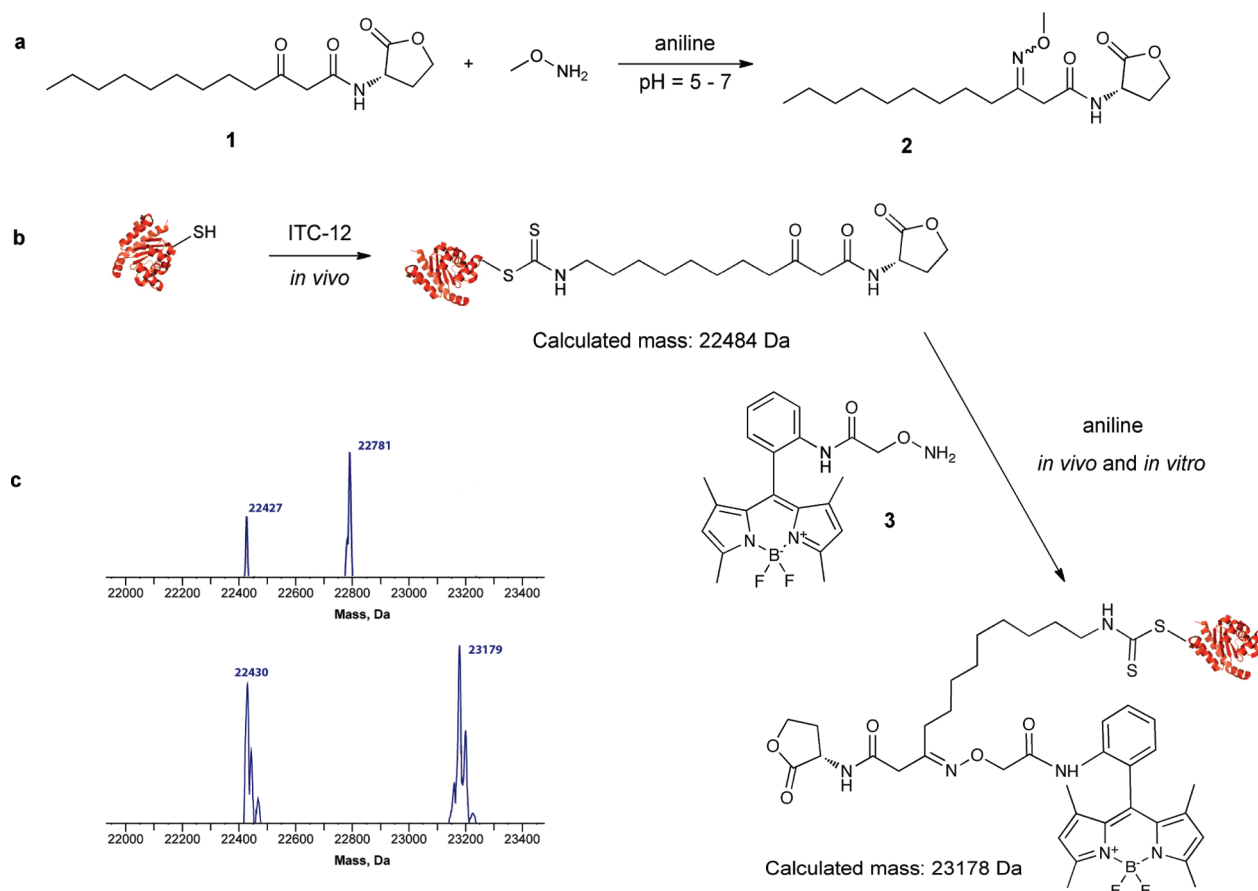


Figure 1. QS receptor labeling using aniline-catalyzed oxime chemistry. (a) Condensation reaction between 3-oxo-C₁₂-HSL **1** and methoxyamine. (b) Selective *in vivo* and *in vitro* labeling of ITC-12 covalently bound to a quorum sensing receptor, LasR-LBD, by aminoxy-BODIPY. (c) Deconvoluted ESI mass spectra of the *in vitro* reaction (pH 5, 1 mM aniline, 100 μ M aminoxy-BODIPY, and 1 μ M LasR-ITC-12) at *t* = 0 h (top) and 12 h (bottom). The 22 430 Da product corresponds to unreacted LasR-LBD.

upon reaction with an appropriate amine. The reaction of aminoxy compounds or hydrazines with carbonyl moieties usually takes several days for completion at neutral pH, while shorter reaction times can be achieved by significantly lowering the pH (pH < 4.5). Such considerations render the reaction not useful for *in vivo* studies. Elegant studies by Dawson and co-workers, however, led to the discovery that aniline catalyzes the reaction between aldehydes and oxyamines at neutral pH, resulting in swift oxime formation with good reaction rates.^{13–15} Accordingly, we have adopted this strategy to label LasR in two steps in live *P. aeruginosa* cells, after covalent modification with a reactive 3-oxo-C₁₂-HSL analogue, isothiocyanate ITC-12 (Figure 1b), followed by reaction with a fluorescent aminoxy-tag (BODIPY-ONH₂). This novel and highly specific live cell dual tagging approach has enabled the first fluorescent labeling of a native intracellular bacterial receptor.

RESULTS AND DISCUSSION

In Vitro Ligation. To explore the suitability of this oxime formation-based methodology for *in vivo* imaging in *P. aeruginosa*, we began our study by characterizing the product resulting from a reaction between a model aminoxy reagent, methoxyamine, and the *P. aeruginosa* cognate ligand, 3-oxo-C₁₂-HSL (**1**). This reaction proceeds smoothly under acidic conditions (pH 5, Supporting Information (SI), Table S1). The single

reaction product, 3-methoxyimino-C₁₂-HSL (**2**), was isolated and characterized by electrospray ionization mass spectrometry (ESI-MS) and NMR spectroscopy (SI, Figure S1). Full conversion was achieved within 10 h at room temperature, which is faster than a similar reaction reported by Fleissner et al.¹⁶ but slower than reported reactions with aldehydes.^{13,15} At neutral pH, a moderate conversion (60%, 8 h) was obtained using a 100-fold excess of the aminoxy reagent (SI, Table S1). Once the optimal conditions for this model reaction were determined, we next assessed whether the aminoxy reagent could also specifically react with the keto moiety of a 3-oxo-C₁₂-HSL analogue, ITC-12,¹⁷ covalently bound to the ligand-binding domain (LBD) of a bacterial quorum-sensing regulator, LasR. We previously established that this covalent binder reacts specifically with Cys79 in the LasR-LBD binding pocket (Figure 1b).¹⁷ Indeed, upon applying the procedure used in the reaction between methoxyamine and 3-oxo-C₁₂-HSL, we were able to observe efficient labeling of LasR-LBD-ITC-12 with BODIPY-ONH₂ (SI, Figure S2). Briefly, the reaction performed at pH 5 in the presence of 100 μ M of BODIPY-ONH₂ and 1 mM aniline yielded a single product with a mass of 23 179 Da after 12 h, as revealed by ESI-MS. This value corresponds to the expected mass of the conjugated product (Figure 1c).

Next, we set out to examine whether this methodology could be applied to label the ITC-12-LasR complex *in vitro*, in the

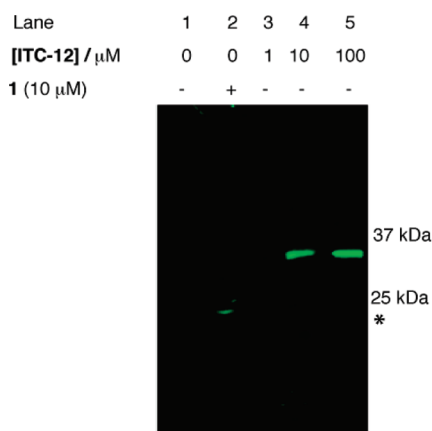


Figure 2. *In vitro* labeling of LasR in a whole cell lysate of *Pseudomonas aeruginosa*. Ligation reactions were carried out after incubation of *P. aeruginosa lasI-rhlI* double mutant (PAO-JP2) with different concentrations of ITC-12. Cell lysates were treated with 100 μM of aminoxy-BODIPY and 1 mM aniline for 12 h at pH 5.1. The labeled proteome was resolved by SDS–PAGE (12%) and analyzed by in-gel fluorescence scanning. See SI, Figure S3 for the corresponding Coomassie blue-stained gel. *Nonspecific labeling. The positions of molecular weight markers are indicated.

context of the complete *P. aeruginosa* proteome. This is not a trivial matter when one considers the relatively low solubility, stability, and expression levels of LasR.¹⁸ However, if both reactions (i.e., the initial reaction of the affinity-based covalent probe (ITC-12) with LasR followed by reacting bound ITC-12 with BODIPY-ONH₂) are efficient and highly specific, we would expect to observe a single band in the fluorescent readout of the proteome following separation by SDS–PAGE. To determine whether this was the case, bacteria were grown in the presence or absence of ITC-12, after which the cells were washed and lysed, and the supernatant was incubated at pH 5.1 in the presence of 100 μM BODIPY-ONH₂ (**3**) and 1 mM aniline for 12 h. When the samples were analyzed by SDS–PAGE (as shown in Figure 2), robust labeling of a protein with an apparent molecular weight of approximately 30 kDa (calculated molecular weight for ligated full length LasR is 28.5 kDa) was observed when the bacteria were incubated in the presence of 10 and 100 μM ITC-12 (lanes 4 and 5, respectively). In contrast, when the concentration of ITC-12 was reduced to 1 μM (lane 3), no labeling was observed. Furthermore, no labeling of this protein was observed when the bacteria were grown in the presence of **1** (lane 2), although a small degree of nonspecific labeling of an unknown protein with smaller molecular weight was observed, reflecting the nearly complete chemoselectivity of the ligation.

In Vivo Labeling of LasR in *E. coli* and *P. aeruginosa*. Although oxime/hydrazine ligation has already been employed for labeling aldehydes on the cell surface,^{13,19,20} in which one study used aniline mediated conjugation,¹³ it has been previously suggested that this mode of ligation is not suitable for *in vivo* intracellular imaging due to competition with endogenous aldehydes and ketones.²¹ Zhang et al., however, reported *in vivo* intracellular labeling of an unnatural amino acid, m-acetylphenylalanine, with fluorescein hydrazide,²² suggesting a method we could adopt to label LasR intracellularly with a fluorescent aminoxy tag. To validate this approach, we first performed the conjugation in *E. coli* overexpressing LasR-LBD. Cells were incubated in the presence or absence of ITC-12 for 12 h, and then

washed with PBS containing 0.01% pluronic F-127 to remove unreacted ITC-12. In each experiment, cell density was normalized to an OD₆₀₀ of 2, and ligations were carried out at pH 6.6. After incubation with the membrane-permeable aminoxy probe for 12 h at 8 °C, the cells were pelleted, washed with PBS containing 0.01% pluronic F-127, resuspended in SDS sample buffer, sonicated, and the proteome was resolved on 12% SDS–PAGE. In agreement with Zhang et al.,²² strong labeling of the protein target was only observed upon introduction of a reactive ketone into the protein (Figure 3a, lane 5). To further explore the specificity of the ligation reaction, we performed a competition experiment with **1**. As expected, due to the higher affinity of **1** for the receptor, the fluorescent band corresponding to the labeled LasR-ITC-12 complex disappeared upon preincubation with an equimolar amount (10 μM) of the cognate LasR ligand (Figure 3a, lane 4). As mentioned, we ultimately aimed to label the bacterial virulence regulator, LasR, in its endogenous environment. Toward this end, we applied the same conditions used in the *E. coli* ligations in repeating the approach with live *P. aeruginosa* cultures, taking into account the increased challenge to success due to the presence of lower levels of LasR expression. Encouragingly, as seen in the *in vitro* studies, incubation of intact cells with **3** showed specific labeling of a protein with an apparent molecular weight of approximately 30 kDa (Figure 3b; full length BODIPY-labeled LasR-ITC-12 has a mass of 28.5 kDa). Lowering the concentration of **3** from 100 to 10 μM decreased the intensity of this band. No labeling was detected in the absence of the probe. Finally, the fluorescent band was excised, digested with trypsin, and its identity was assessed by mass spectrometry using a linear trap quadrupole (LTQ) Orbitrap apparatus (Figure 3c). SEQUEST searches of the resulting LC-MS/MS data set identified three LasR-derived peptides, corresponding to a total sequence coverage of 32%.

To further corroborate these results, the ligation reaction was analyzed by flow cytometry. *Pseudomonas* cells were treated with ITC-12 and incubated with the BODIPY-aminoxy probe using the same methodology as employed for *in vivo* labeling. In preliminary experiments in which the cells were washed extensively with PBS buffer, a relatively high background signal was observed for cells that were not labeled with ITC-12 (SI, Figure S4). However, through use of a modified procedure described by Aharoni et al.,²³ remaining unreacted probe was easily removed. Briefly, cells were subjected to two 5 min cycles of incubation with fresh LB media. With this washing procedure, it is likely that products of the reaction of compound **3** with small metabolites are released from the cells. As shown in Figure 4, the negative control displays only a slight increase in mean fluorescence over the background. Significantly, when cells were pretreated with ITC-12, a concentration-dependent increase in the fluorescent readout was detected (Figure 4a). Furthermore, these results are in good agreement with those obtained in the gel fluorescent readout (Figure 3b). Additionally, when cells were preincubated with increasing concentrations of 3-oxo-C₁₂-HSL, a marked decrease in fluorescence was observed (SI, Figure S5). Finally, we set out to determine the effect of cell density on the extent of LasR labeling. As several studies have shown a clear relationship between the expression of LasR and cell density,^{18,24} we expected an increase in overall fluorescence with increasing cell density at the time of labeling. To determine whether this was indeed the case, bacteria were grown to different cell densities (OD₆₀₀ = 0.4–6) in the presence of ITC-12 (10 and 100 μM), followed by incubation with the aminoxy probe (cell densities

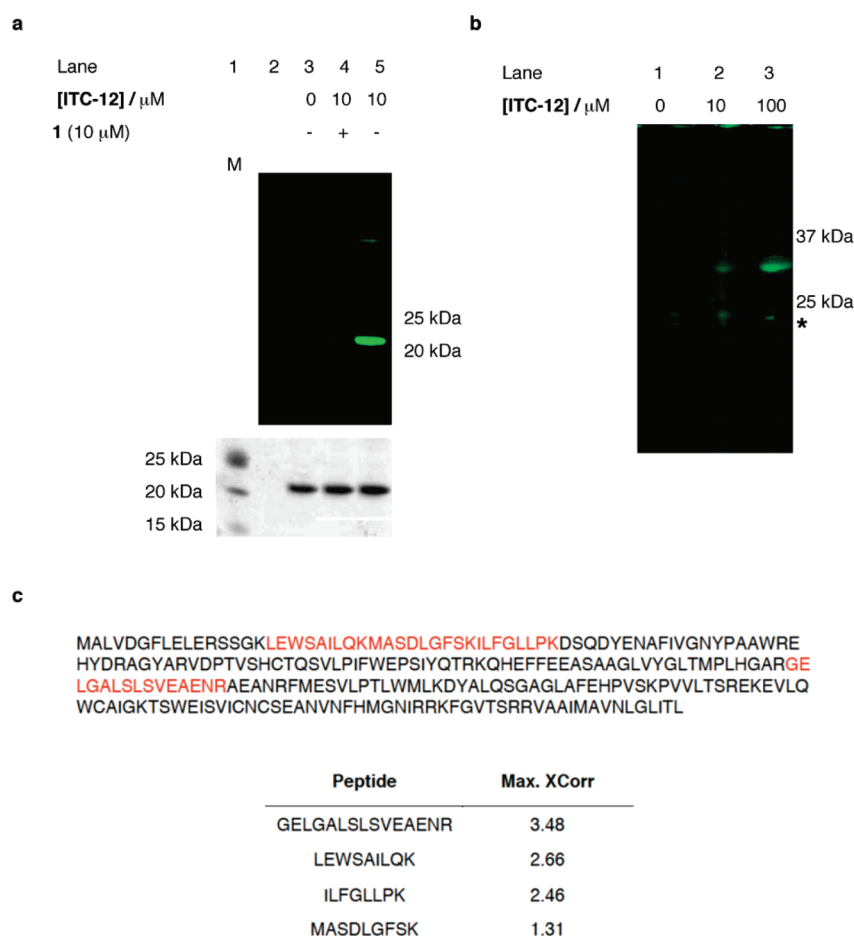


Figure 3. *In vivo* labeling of LasR by aminooxy-BODIPY. Ligation reactions were carried out at pH 6.6 with 100 μ M of aminooxy-BODIPY and 1 mM aniline for 12 h at 8 $^{\circ}$ C. A total cell protein (TCP) extract was separated by SDS–PAGE (12%). (a) TCP of *E. coli* BL21 (DE3) overexpressing LasR-LBD, in the presence (lanes 4 and 5) or the absence (lane 3) of ITC-12. The fluorescent readout (top) and Coomassie-stained SDS–PAGE (bottom) gels are shown. Pretreatment of cells with 3-oxo- C_{12} -HSL (lane 4) diminished the labeling. Lane 1, molecular weight markers; lane 2, empty. (b) Specific labeling of LasR in *P. aeruginosa* in the presence (10 and 100 μ M) or absence (negative control) of ITC-12. See SI, Figure S3 for the corresponding Coomassie blue-stained gel. *Nonspecific labeling. (c) High-resolution mass spectrometry analysis of the 30 kDa band after digestion with trypsin (see Supporting Information for details). The primary sequence LasR (top) and peptides identified by LC-MS/MS (ESI) and the SEQUEST search algorithm (bottom) are shown. The sequences covered by the identified peptides are marked in red.

were normalized to 2 in all labeling experiments). As shown in Figure 4b, an increase in fluorescence correlates with increased cell density, and thus with an increase in LasR concentration. However, at high cell densities ($OD_{600} = 6$) the fluorescence is diminished. A possible explanation for this phenomenon is that upon reaching the threshold cell density, i.e. when the quorum has been reached and sensed, any further increase in cell density is accompanied by degradation of both quorum signals and receptors, as prolonged sensing of the population size may not be needed and would likely even be wasteful. Collectively, these two experiments performed in the presence or absence of ITC-12 and at different LasR expression levels proves that ligation involves covalently bound ITC-12 and not any free affinity probe that might remain in the cell.

In Vivo Imaging of LasR. The current model describing LasR activity in *P. aeruginosa* QS is based on the assumption that the N-terminal region of LasR is inserted in the cytoplasmic membrane and that binding of its cognate ligand, 3-oxo- C_{12} -HSL, results in structural changes that lead to a conformation that allows LasR to bind its target DNA. It is not clear whether the interaction of LasR with the membrane is retained upon such

structural change. A second question regarding the localization of LasR asks whether this QS receptor is distributed evenly throughout the cell, or instead, whether a certain degree of compartmentalization occurs. To address these points, *P. aeruginosa* cells were treated with 3 using the same methodology as described *vide supra* for the *in vivo* labeling and flow cytometry experiments above (Figure 4). Corresponding with the observations made upon analysis of the SDS–PAGE fluorescent readout and flow cytometry measurements, a concentration-dependent increase in LasR labeling was observed, proving the suitability of the aniline-catalyzed oxime chemistry methodology for *in vivo* labeling of this bacterial virulence regulator. When cells were pretreated with ITC-12 (Figure 5a), the resulting fluorescent staining was not consistent over the entire bacterial surface; instead, it appeared to be localized at the poles of the bacteria. This effect was enhanced when a higher concentration (100 μ M) of ITC-12 was used. It is worth mentioning that a similar labeling profile was obtained by Ventre et al.²⁵ by expressing RhlR (another LuxR family type protein in *P. aeruginosa*) as an EGFP fusion protein.

Figure 5b shows a clear association of the labeled protein with the cell membrane, while in cells that were treated with a higher

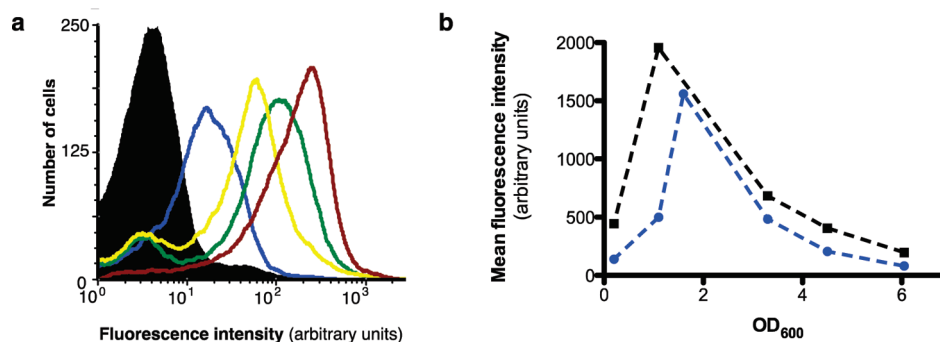


Figure 4. Analysis of *in vivo* QS receptor labeling by flow cytometry. (a) *Pseudomonas aeruginosa* cells were incubated in the absence (blue) or presence of 10 (yellow), 100 (green), or 250 μ M ITC-12. After removal of most of the unreacted ITC-12, the cell density in all samples was normalized to an OD_{600} of 2 and treated at 8 $^{\circ}$ C and pH 6.6 with 100 μ M aminooxy-BODIPY (3) and 1 mM aniline for 12 h. As negative control (black), absence of 3. (b) Mean fluorescence as function of cell density after incubation with ITC-12 ((\bullet) 10 μ M, (\blacksquare) 100 μ M) and reaction with probe 3. Before the ligation reaction with 3, cell densities were normalized to an OD_{600} of 2.

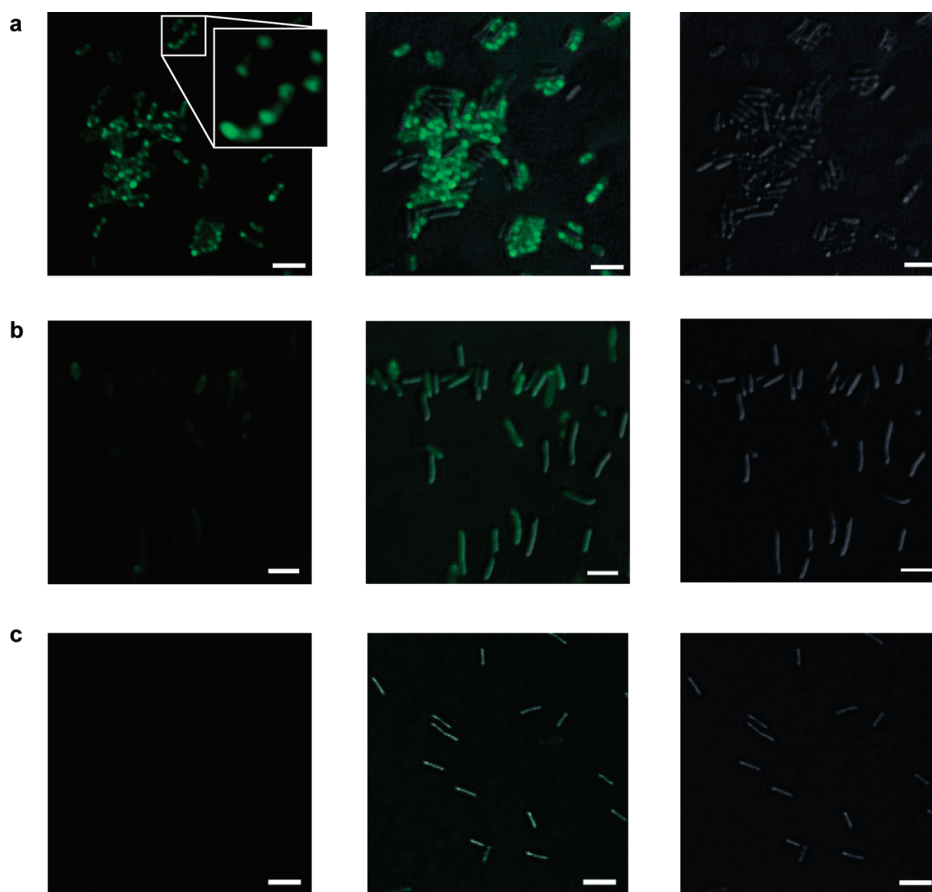


Figure 5. Imaging of aniline-catalyzed aminooxy ligation of QS receptors on live cells. *Pseudomonas aeruginosa* cells were incubated in the presence of 100 μ M (a), in the presence of 10 μ M (b), or in the absence (c) of ITC-12. Cells were washed and subsequently labeled at pH 6.6 with 100 μ M of aminooxy-BODIPY and 1 mM aniline for 12 h. Left, fluorescence channel. Right, bright field. Middle, overlay of BODIPY fluorescence over bright field. The inset is an expanded section of the fluorescence image. The scale bar in each panel corresponds to 3 μ m.

concentration of ITC-12 (Figure 5a), this association did not appear as strong. This may indicate that LasR indeed dissociates from the membrane upon activation and dimerization. Additional studies including higher resolution fluorescence microscopy, however, will be required to further examine this hypothesis.

LasR Localization. The observation that LasR localizes at the poles of the cell is significant. This finding may have important implications for our understanding of the overall mechanism of quorum sensing signal generation and collection. We propose the following model to explain the observed localization of LasR: Several studies have shown that bacteria distribute proteins to

distinct locations in the cell.²⁶ Here, the localization of LasR observed by fluorescence microscopy (Figure 5) can be explained by considering reports that newly synthesized autoinducers undergo active secretion from *P. aeruginosa* to the environment via the MexAB-OprM efflux pump.²⁷ The precise location of this efflux pump in bacteria is still unknown, although recent studies indicate that a similar efflux pump, Opr86, is mainly localized at the central part of the cell.²⁸ LuxR-type family proteins, such as LasR, are membrane-associated monomers, which in the absence of their cognate ligand misfold and are rapidly degraded by endogenous proteases.¹⁸ Is it likely that through their association with the membrane, these proteins allow bacteria to specifically detect extracellular autoinducers that diffuse passively into the cell, especially if the autoinducers are secreted at a location in the cell that is far removed from the poles, where the receptors appear to be located. In a previous study that strengthens this hypothesis, Zhu and Winans determined that Clp proteases are the primary proteins responsible for TraR (a LuxR-type protein in *Agrobacterium tumefaciens*) turnover.²⁹ In addition, it has been proposed that Clp proteases are deployed mainly at the bacterial poles, and that LasR undergoes degradation at low cell densities.^{30,31} These findings, in combination with the potential benefit conferred to bacteria in terms of noise reduction, by the physical separation of the cellular machineries responsible for autoinducer secretion and sensing functions, suggest that LasR is most abundant at the cell poles (see SI, Figure S6 for a schematic description of this model). The benefit for bacteria to separate localization of autoinducer secretion and sensing would be in reducing the chance that secreted autoinducers bind receptors on the cell that secretes them, thereby increasing sensitivity to gauge the presence and amount of other cells.

CONCLUSIONS

In summary, our data demonstrate that a combination of isothiocyanate and oxime chemistry can be successfully employed for the visualization of specific native proteins in live cells through a two-step labeling strategy. We have shown, using several different analytical methods, that we can bio-orthogonally label QS receptors. In spite of the propensity of an aminoxy probe to react with endogenous carbonyl moieties (i.e., pyruvic acid, glucose, etc.), we have shown that this problem can be overcome through optimization of the aniline-catalyzed oxime reaction followed by appropriate washing of the cells. Relying on this strategy, we report the first demonstration of activity-based fluorescent labeling and visualization of a quorum sensing receptor in living bacterial cells. This methodology could, moreover, be applied for the intracellular ligation of other types of proteins in bacterial and/or eukaryotic cells in their natural settings, offering the possibility of imaging, mapping and quantifying specific proteins *in vivo*.

EXPERIMENTAL SECTION

In Vitro Proteome Ligation. *P. aeruginosa* strain JP2 (lasI/rhlI-deleted), harboring plasmid pKD201 carrying a LasI reporter coupled to the luxCDABE luminescence system,³² was inoculated into 10 mL of LB media (with 2.5 g/L of NaCl) containing 300 $\mu\text{g/mL}$ of trimethoprim and 3-oxo-C₁₂-HSL (10 μM) or ITC-12 (0, 1, 10, 100 μM). Cells were grown at 37 °C to an optical density (OD₆₀₀) of 2, after which the cells were harvested, washed twice with cold PBS containing 0.01% of pluronic F-127, frozen at -20 °C, and lysed in 50 mM phosphate

buffer, pH 7, 5% glycerol, 250 mM NaCl, 0.3 mg/mL lysozyme, and protease inhibitor cocktail (Merck). The lysate was centrifuged and subjected to buffer exchange with 100 mM sodium acetate (pH 5.1) by repeated concentration and dilution on a centricon YM-10 (Millipore). Ligations (200 μL total reaction volume) were performed with 0.1 mg $\cdot\text{mL}^{-1}$ of proteome in the presence of aniline (1 mM) and 3 (100 μM). After 12 h at room temperature reactions were diluted with 2X SDS sample buffer (without bromophenol blue), heated for 10 min at 70 °C, and subjected to 12% SDS-PAGE. Fluorescence readouts were obtained using a Fuji LAS-3000 scanner.

rLasR-LBD in Vivo Ligation. *E. coli* BL21-DE3 strain, harboring a pETM-11 vector encoding for a shortened, His6-tagged LasR construct, LasR-LBD (ligand-binding domain), was inoculated into 10 mL of LB media containing kanamycin (50 $\mu\text{g/mL}$) and 3-oxo-C₁₂-HSL (10 μM) and/or ITC-12 (0, 10 μM). Cells were grown at 21 °C for 12 h, after which the cells were harvested, washed twice with cold PBS containing 0.01% of pluronic F-127. The cells were then resuspended to an OD₆₀₀ of 2 in 1 mL phosphate buffer (100 mM) containing aniline (1 mM) and 3 (100 μM). After 12 h at 8 °C, cells were harvested and washed twice with cold LB media containing 0.01% of pluronic F-127. Finally, reactions were diluted with 2X SDS sample buffer (without Bromophenol blue), heated for 10 min at 70 °C, and subjected to a 12% SDS-PAGE, and the fluorescence readout was obtained using a Fuji LAS-3000 scanner.

Native LasR in Vivo Ligation. *P. aeruginosa* strain JP2 was grown in the presence and absence of ITC-12 as described above and washed with PBS containing 0.01% of pluronic F-127. Cells were then resuspended to an OD₆₀₀ of 2 in 1 mL of phosphate buffer (100 mM) containing aniline (1 mM) and 3 (100 μM). After 12 h at 8 °C, cells were harvested and washed twice with cold LB media containing 0.01% of pluronic F-127.

Fluorography. Treated bacteria (*P. aeruginosa* JP2 or *E. coli*) were resuspended in 200 μL of 1X SDS sample buffer. Samples were sonicated, heated for 10 min at 70 °C, and subjected to 12% SDS-PAGE. Labeled proteins were visualized by in-gel scanning using a Fuji LAS-3000 scanner and excised from the gel for trypsin digestion. The resulting peptides were analyzed on a LTQ-Orbitrap ion trap mass spectrometer (Thermo-Fisher Scientific). Samples were loaded on custom-made C-18 reverse phase columns (Jupiter 5 μm , 300 Å) and eluted at a flow rate of 300 nL/min (linear gradient of CH₃CN/H₂O). SEQUEST analyses from two independent experiments identified peptides from a *P. aeruginosa* PAO1 genomic V2 database (www.pseudomonas.com).

Microscopy. Labeled (or control) *P. aeruginosa* (strain JP2) bacteria (2 μL of culture with an OD₆₀₀ of 0.1 in PBS) were immobilized on agarose-coated (2%) microscope slides. Images were obtained using an Olympus FV1000 laser-scanning confocal microscope, with a 100 \times oil-immersion lens. Ligated BODIPY was excited by a 488 nm argon laser line, and fluorescence was detected at 520 nm. Images were processed using ImageJ software (National Institutes of Health).

Flow Cytometry. Treated bacteria were diluted to an OD₆₀₀ of 0.1 in PBS and analyzed with a FACSCalibur flow cytometer (Becton Dickinson) or alternatively with an Eclipse Analyzer (icyt). The average sorting rate was ~1000 events per second. FACS data were processed using the FCS Express (De Novo Software).

ASSOCIATED CONTENT

S Supporting Information. Synthetic protocols and characterization of compounds reported herein; optimization of conditions for the model reactions; Coomassie blue-stained gels for the *in vivo* and *in vitro* labeling; additional flow cytometry analysis; and proposed model for noise control in *P. aeruginosa*.

This material is available free of charge via the Internet at <http://pubs.acs.org>.

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REFERENCES

- (1) Engebrecht, J.; Neelson, K.; Silverman, M. *Cell* **1983**, *32*, 773–781.
- (2) Fuqua, W. C.; Winans, S. C.; Greenberg, E. P. *J. Bacteriol.* **1994**, *176*, 269–275.
- (3) Arias, C. A.; Murray, B. E. *N. Engl. J. Med.* **2009**, *360*, 439–443.
- (4) Kiratisin, P.; Tucker, K. D.; Passador, L. *J. Bacteriol.* **2002**, *184*, 4912–4919.
- (5) Pappas, K. M.; Weingart, C. L.; Winans, S. C. *Mol. Microbiol.* **2004**, *53*, 755–769.
- (6) Keppler, A.; Pick, H.; Arrivoli, C.; Vogel, H.; Johnsson, K. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 9955–9959.
- (7) Griffin, B. A.; Adams, S. R.; Tsien, R. Y. *Science* **1998**, *281*, 269–272.
- (8) Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686–4687.
- (9) Soriano del Amo, D.; Wang, W.; Jiang, H.; Besanceney, C.; Yan, A. C.; Levy, M.; Liu, Y.; Marlow, F. L.; Wu, P. *J. Am. Chem. Soc.* **2010**, *132*, 16893–16899.
- (10) Jewett, J. C.; Sletten, E. M.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2000**, *132*, 3688–3690.
- (11) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007–2010.
- (12) Dondoni, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 8995–8997.
- (13) Zeng, Y.; Ramya, T. N. C.; Dirksen, A.; Dawson, P. E.; Paulson, J. C. *Nat. Methods* **2009**, *6*, 207–209.
- (14) Dirksen, A.; Dawson, P. E. *Bioconjugate Chem.* **2008**, *19*, 2543–2548.
- (15) Dirksen, A.; Hackeng, T. M.; Dawson, P. E. *Angew. Chem., Int. Ed.* **2006**, *45*, 7581–7584.
- (16) Fleissner, M. R.; Brustad, E. M.; Kálai, T.; Altenbach, C.; Cascio, D.; Peters, F. B.; Hideg, K.; Peuker, S.; Schultz, P. G.; Hubbell, W. L. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 21637–21642.
- (17) Amara, N.; Mashiach, R.; Amar, D.; Krief, P.; Spieser, S. A. H.; Bottomley, M. J.; Aharoni, A.; Meijler, M. M. *J. Am. Chem. Soc.* **2009**, *131*, 10610–10619.
- (18) Siehnell, R.; Traxler, B.; An, D. D.; Parsek, M. R.; Schaefer, A. L.; Singh, P. K. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 7916–7921.
- (19) Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. *Science* **1997**, *276*, 1125–1128.
- (20) Baskin, J. M.; Dehnert, K. W.; Laughlin, S. T.; Amacher, S. L.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 10360–10365.
- (21) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 6974–6998.
- (22) Zhang, Z.; Smith, B. A. C.; Wang, L.; Brock, A.; Cho, C.; Schultz, P. G. *Biochemistry* **2003**, *42*, 6735–6746.
- (23) Aharoni, A.; Thieme, K.; Chiu, C. P. C.; Buchini, S.; Lairson, L. L.; Chen, H.; Strynadka, N. C. J.; Wakarchuk, W. W.; Withers, S. G. *Nat. Methods* **2006**, *3*, 609–614.
- (24) Schuster, M.; Greenberg, E. P. *BMC Genomics* **2007**, *8*, 287.
- (25) Ventre, I.; Ledgham, F.; Prima, V.; Lazdunski, A.; Foglino, M.; Sturgis, J. N. *Mol. Microbiol.* **2003**, *48*, 187–198.
- (26) Shapiro, L.; McAdams, H. H.; Losick, R. *Science* **2009**, *326*, 1225–1228.
- (27) Pearson, J. P.; Van Delden, C.; Iglewski, B. H. *J. Bacteriol.* **1999**, *181*, 1203–1210.
- (28) Tashiro, Y.; Nomura, N.; Nakao, R.; Senpuku, H.; Kariyama, R.; Kumon, H.; Kosono, S.; Watanabe, H.; Nakajima, T.; Uchiyama, H. *J. Bacteriol.* **2008**, *190*, 3969–3978.
- (29) Zhu, J.; Winans, S. C. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 1507–1512.
- (30) Kain, J.; He, G. G.; Losick, R. *J. Bacteriol.* **2008**, *190*, 6749–6757.
- (31) McGrath, P. T.; Iniesta, A. A.; Ryan, K. R.; Shapiro, L.; McAdams, H. H. *Cell* **2006**, *124*, 535–547.
- (32) Duan, K.; Surette, M. G. *J. Bacteriol.* **2007**, *189*, 4827–4836.