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Engineering Bacterial Two-Component System PmrA/PmrB to Sense Lanthanide Ions

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Supporting Information

ABSTRACT: The *Salmonella* PmrA/PmrB two-component system uses an iron(III)-binding motif on the cell surface to sense the environmental or host ferric level and regulate PmrA-controlled gene expression. We replaced the iron(III)-binding motif with a lanthanide-binding peptide sequence that is known to selectively recognize trivalent lanthanide ions. The newly engineered two-component system (PmrA/PmrB) can effectively sense lanthanide ion and regulate gene expression in *E. coli*. This work not only provides the first known lanthanide-based sensing and response in live cells but also demonstrates that the PmrA/PmrB system is a suitable template for future synthetic biology efforts to construct bacteria that can sense and respond to other metal ions in remediation or sequestration.

The ability to detect, remediate, and sequester heavy metals and rare earth elements in the environment is highly desirable.[1−3] In the past genetically encoded sensors that can detect metal ions or other small molecules have been successfully constructed.[4−7] However, systems that can sense and respond to lanthanide and actinide ions with live cells are still lacking. The lanthanide series is comprised of 15 elements from lanthanum to lutetium. The most successful lanthanide binders are lanthanide-binding tags (LBTs) developed by Imperiali et al. that can selectively bind lanthanide ions with high affinities.[8] LBTs have shown a wide range of applications in biochemistry including protein structure determination and the investigation of protein trafficking and metal/protein interactions.[9,10] However, these peptides have yet to be implanted as genetically encoded lanthanide probes that could detect lanthanide ions with live cells. We envisioned constructing a lanthanide-sensing system by fusing LBTs with bacterial two-component systems that are widely used by microbes to sense and respond to nutrients and environmental stresses.

Bacteria rely primarily on two-component signal transduction systems to sense and respond to environmental changes.[11] These systems typically consist of a sensor histidine and a response regulator. Upon sensing a specific signal, the sensor kinase autophosphorylates a highly conserved histidine residue and subsequently transfers the phosphoryl group to a conserved aspartate residue in its cognate response regulator, which in turn activates or represses the transcription of its target genes.[12,13] The PmrA/PmrB two-component system in *Salmonella* is such a system necessary for resistance to both polymyxin B and neutrophil antimicrobial proteins.[14,15] It binds and senses extracytoplasmic ferric ion via its periplasmic domain that harbors two copies of an ExxE motif shown to bind iron(III).[16] This extramembrane iron(III)-binding loop contains ∼30 amino acids (Figure 1). It is most likely unstructured in the absence of iron(III). Upon iron(III) binding the folding of the loop induces a stretch of the transmembrane domain, which delivers the signal for the auto His phosphorylation.

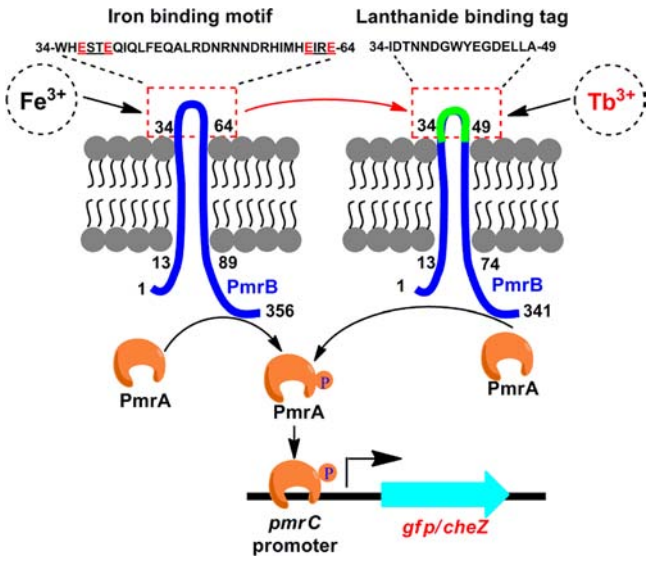

Figure 1. Design and construction of a lanthanide-responsive system based on the PmrA/PmrB two-component system that consists of the histidine kinase PmrB and the response regulator PmrA. Binding of the ferric ion by the exposed iron(III)-binding motif in PmrB activates the autophosphorylation of PmrB, which subsequently phosphorylates PmrA. The phosphorylated PmrA activates its own operon (*pmrCAB*). We envision replacing the iron-binding motif (amino acids 34−64) of PmrB with a lanthanide-binding tag (amino acids 34−49 are highlighted in green). The engineered PmrB is expected to sense and respond to the external lanthanide ions.

Two-component systems have been employed as templates for engineering artificial sensors; Voigt et al. have successfully used the EnvZ-OmpR two-component system by fusing a

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cyanobacterial photoreceptor to an EnvZ histidine kinase domain to engineer *E. coli* to sense light.[17] We here present the design and engineering of the PmrA/PmrB two-component system that specifically senses and responds to lanthanide ions in *E. coli* (Figure 1).

Both iron and zinc are known to induce the *E. coli* BasS/BasR system, which is homologous to PmrA/PmrB.[18] We employed *Salmonella* PmrA/PmrB known to be selective to iron(III) and an *E. coli* host with deletion of *basS*. A PmrA/PmrB-responsive reporter plasmid pJBA25-*pmrC* was constructed, in which the *pmrC* promoter was fused to the *gfp* gene encoding a green fluorescent protein. The plasmids of pBAD33-*pmrA*/*pmrB* (to express both PmrA and PmrB) and pJBA25-*pmrC* were transformed into the *E. coli*(Δ*basS*) mutant (the system was designated PmrA/PmrB/*pmrC-gfp*), and the level of *pmrC-gfp* was measured in the presence of 50 or 100 μM Fe^{3+}. The result showed that the expression of *pmrC-gfp* was induced by high concentrations of Fe^{3+} (Figure S1B), which was further confirmed by Western blot and imaging assays (Figure S1C,D). This result is consistent with the previous study showing that the PmrA-controlled genes are responsive to high extracytoplasmic ferric ion through binding to PmrB.[12]

Further experiments were conducted to rule out the possibility that Fe^{3+} can induce the expression of GFP. We introduced pBAD33-*pmrA*/*pmrB* and a pJBA25 plasmid without the promoter sequence that can be recognized by PmrA into the *E. coli* (Δ*basS*) strain and tested the fluorescent response to Fe^{3+}. The results showed that the pJBA25 alone exhibited no fluorescence change in the presence of Fe^{3+} or other ions (Figure S2).

Extracellular iron is known to be recognized by an outer membrane loop containing two ExxE sequences in PmrB, which transduces the signal to PmrA, which in turn regulates its target genes in *Salmonella*.[16] To create the lanthanide-sensing system, we replaced the iron-sensory loop with a lanthanide-binding tag sequence (Figure 1). We transformed pBAD33-*pmrA*/*pmrB*′ (engineered) that contained the lanthanide-binding tag and pJBA25-*pmrC* into the *E. coli*(Δ*basS*) mutant. Spectroscopic experiments were performed with different concentrations of Tb^{3+} (0.2, 0.5, 1.0, or 2.0 μM). The fluorescence emission at 515 nM of the induced GFP showed an almost 3-fold increase upon excitation at 485 nM in the presence of 1.0 or 2.0 μM Tb^{3+} added to the medium (Figure 2A). This result was also confirmed by Western blot and imaging assays (Figure 2B,C). Other metal ions were tested, and as expected, the PmrB′-based system showed high selectivity toward Tb^{3+} (Figure 2D). Ca^{2+}, at 50−100 μM, could induce a 2-fold increase of the fluorescence (Figure S2C).

With a two-component system selectively sensing and responding to extracellular lanthanide ions, we further engineered the system to regulate *E. coli* chemotaxis in response to lanthanide ions by employing previous strategies.[7,19,20] We used *cheZ* as a reporter gene, which plays a critical role in *E. coli* chemotaxis by dephosphorylating the CheY-P protein that binds to the flagellar motor and causes cells to tumble.[21] CheY controls the rotational direction of the flagellar motor. When phosphorylated, CheY binds to the flagellar switch protein FilM and causes *E. coli* cells to migrate on semisolid media.[22,23] However, *E. coli* lacking CheZ cannot desphosphorylate CheY-P and thus becomes nonmotile. Induction of CheZ can restore motility in a *cheZ* knockout strain by reactivating wild-type chemotaxis.[20,24−26] Accordingly,

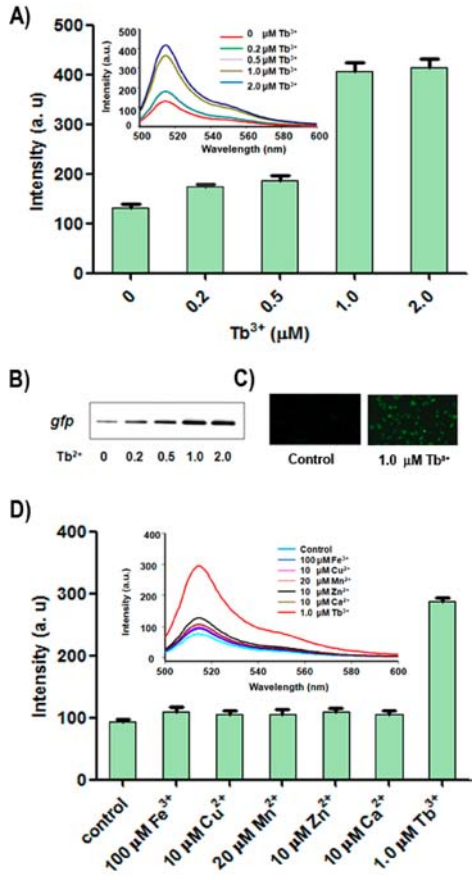

Figure 2. Engineered PmrA/PmrB′/*pmrC-gfp* that specifically senses lanthanide ion. (A) Fluorescence response of *pmrC-gfp* to different concentrations of Tb^{3+}. The inserted graph represents the fluorescence spectra of the probe in the presence of Tb^{3+}. (B) The *E. coli*(Δ*basS*) strain containing plasmids pBAD33-*pmrA*/*pmrB*′ and pJBA25-*pmrC* was grown overnight in the minimal medium with antibiotics, diluted 100-fold in fresh medium with the addition of 0.2, 0.5, 1.0, or 2.0 μM Tb^{3+}. The expression of *gfp* in the stain was tested by Western blot. (C) Imaging of the strains in presence of 1.0 μM Tb^{3+}. (D) Fluorescence response of *pmrC-gfp* to different metal ions. The inserted graph represents the fluorescence spectra of the probe in the presence of metal ions.

we anticipated that the Tb^{3+}-inducible expression system can be used to control the expression of CheZ and thus control cell mobility in the presence of Tb^{3+}. We constructed the *E. coli*(Δ*basS*/*cheZ*) double mutant and the pJBA26-*cheZ* plasmid containing *pmrC-cheZ* which are responsive to PmrA/PmrB (see SI). We introduced the plasmids of pBAD33-*pmrA*/*pmrB* (wild-type) and pJBA26-*cheZ* or pBAD33-*pmrA*/*pmrB*′ (engineered) and pJBA26-*cheZ* into the *E. coli*(Δ*basS*/*cheZ*) strain, respectively. We plated the cells containing wild-type PmrB onto semisolid media containing antibiotics and different concentrations of Fe^{3+} and measured their migration radius after 24 h (Figure S3A). The cells exhibited higher motility with increased Fe^{3+} concentration until reaching a maximum at 100 μM (Figure S3B), although the motility was slightly lower than a control strain using the T7 promoter to drive the expression of *cheZ* under IPTG (Figure S4). By using the same assay, we measured the motility ability of cells containing engineered PmrB′ on the semisolid media with addition of Fe^{3+} and Tb^{3+}. As shown in Figure 3A, these cells could move under the conditions that included the added Tb^{3+} but not Fe^{3+}, again

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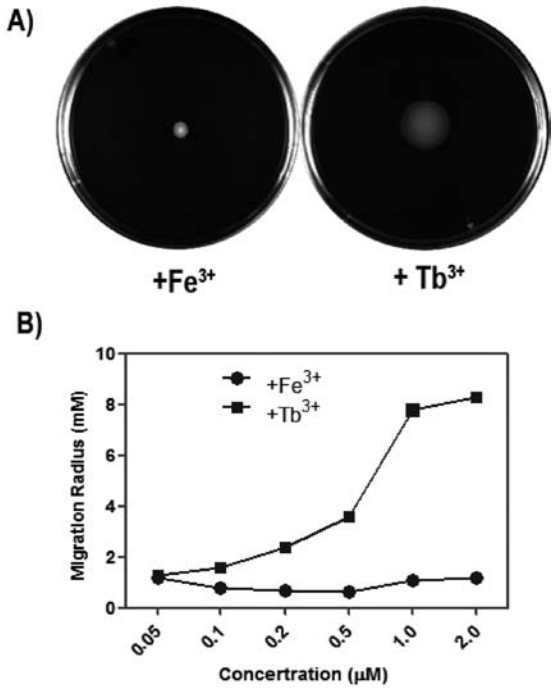

Figure 3. (A) Migration of *E. coli*(Δ*basS*/*cheZ*) which contains pBAD33-*pmrA*/*pmrB*′ and pJBA26-*cheZ* on semisolid media in the presence of Fe^{3+} (100 μM) or Tb^{3+} (1.0 μM). (B) Migration radius of *E. coli*(Δ*basS*/*cheZ*) that contains the engineered PmrB′as a function of the Tb^{3+} concentration. All cells were grown on semisolid media at room temperature for 24 h.

showing selective response to lanthanide ions (Figure 3B). These results clearly show that we can engineer *E. coli* to respond to lanthanide ions and control bacteria motility.

In conclusion, we present the design, construction, and engineering of the two-component system PmrA/PmrB to sense lanthanide ions in *E. coli*. To the best of our knowledge, this represents the first sensory system specifically engineered to detect lanthanide ions and regulate bacterial response. We believe this strategy has great potential in future sequestration and remediation of lanthanide as well as actinide ions.[27] The same template can be applied to engineer sensory systems that recognize other metal ions in various biotechnology applications.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures, supplemental data, and references. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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