Supporting Information

Fe(III)-complex mediated bacterial cell surface immobilization of eGFP and enzymes

Lilin Feng, [†a] Liang Gao, [†a] Daniel F. Sauer, [a] Yu Ji, [a] Haiyang Cui, [a] and Ulrich Schwaneberg* [a,b]

[a] Lehrstuhl für Biotechnologie, RWTH Aachen University, Worringerweg 3, 52074 Aachen, Germany

[b] DWI – Leibniz Institut für Interaktive Materialien, Forckenbeckstraße 50, 52074, Aachen, Germany

[†] These authors contributed equally to this work.

*Corresponding author: Prof. Dr. Ulrich Schwaneberg

E-mail: u.schwaneberg@biotec.rwth-aachen.de

Experimental Procedures

Chemicals

All chemicals are of analytical grade or higher purity and were purchased from Merck (Darmstadt, Germany), AppliChem (Darmstadt, Germany), and Carl Roth (Karlsruhe, Germany) if not otherwise specified. The *E. coli* strains DH5α, and BL21 Gold (DE3) were obtained from Agilent Technologies (Santa Clara, USA). The expression strain *Pichia pastoris* (*Komagataella phaffii*) BSYBG11 and plasmid pBSYA1S1Z were purchased from Bisy GmbH (Hofstätten/Raab, Austria).

Cell culture

E. coli BL21 Gold (DE3), *E. Coli* K12, and *Bacillus subtilis* were cultured at 37 ℃, 250 rpm in Lysogeny Broth medium (5 g/L yeast extract, 10 g/L peptone, 10 g/L NaCl) overnight. *Corynebacterium glutamicum* was cultured at 30 ℃, 250 rpm in BHI medium (37 g/L BHI powder, 10 g/L D-glucose) overnight. The cells were harvested and washed two times with 0.9% (w/v) NaCl aqueous solution and stored in 0.9% (w/v) NaCl aqueous solution on ice prior to be used.

Immobilization of His_6-tagged eGFP on *E. coli* cell surface mediated by metal ions

The immobilization test of His_6-tagged eGFP ($eGFP^{His}$) on the *E. coli* cell surface was performed by addition of 100 μM metal-chloride solution (*e.g.,* Fe^{3+}, Ni^{2+}, $Co^{2+/3+}$, Cu^{2+}, and Zn^{2+}) and 5 μM $eGFP^{His}$ to the suspension of cells (0.9% (w/v) NaCl; OD_{600} = 4) for 10 min at room temperature (total volume = 100 μL). Then, the *E. coli* cells were collected by centrifugation at 11000 rpm for 1 min, washed with 100 μL 0.9% (w/v) NaCl two times at room temperature resuspended in 100 μL 0.9% (w/v) NaCl. A suspension of *E. coli* cells mixed with Fe^{3+}/eGFP (no His_6-Tag) or only $eGFP^{His}$ were used as controls, respectively. The fluorescence was detected *via* fluorescence microscope (Olympus Corporation, Japan) at the exposure time of 109.7 ms and the gain of 2.9 dB.

Immobilization of $eGFP^{His}$ on other bacterial cell surface mediated by Fe^{3+}

For *E. coli* K12 and *Corynebacterium glutamicum*, the immobilization was performed according to the method mentioned above. For *Bacillus subtilis* cells, the immobilization was performed by addition of 300 μM $FeCl_3$ (final concentration) into the cell suspension (0.9% (w/v) NaCl;

OD_{600} = 4; total volume = 100 µL) for 10 min at room temperature followed by washing the cells one time with 0.9% (w/v) NaCl to remove unbound Fe^{3+}. Afterwards, the cells were resuspended in 100 µL 0.9% (w/v) NaCl and mixed with 2 µL 250 µM $eGFP^{His}$ (final concentration 5 µM) for 5 min at room temperature. Finally, the *Bacillus* cells were harvested by centrifugation at 8000 rpm for 2 min, washed with 0.9% (w/v) NaCl two times at room temperature, and resuspended in 0.9% (w/v) NaCl. The suspensions of these bacteria cells mixed with Fe^{3+}/eGFP (no His_6-Tag) or only $eGFP^{His}$ were used as controls. The fluorescence was analyzed via fluorescence microscope (Olympus Corporation, Japan) at the exposure time of 109.7 ms and the gain of 2.9 dB.

Flow Cytometry Analysis

Single cell immobilization efficiency was detected by transferring the resulting cell suspensions (in 0.9% (w/v) NaCl) to polystyrene tubes for flow cytometry analysis (BD Influx™, USA). The population of cells was gated based on forward and side scatter emission, and the eGFP signal was determined by fluorescence intensity on the 488/30 BP Filter emission channel.

Metal absorption capacities of *E. coli* cells

5 mL of *E. coli* cells (OD_{600} = 4) were resuspended in 0.9% (w/v) NaCl and then supplemented with 100 µM of $FeCl_3$, $ZnCl_2$, $CuCl_2$, $NiCl_2$, and $CoCl_2$. After 10 min, the cells were washed two times with 5 mL 0.9% (w/v) NaCl, and then the pellets were collected. The resulting pellets were incubated overnight in 5 mL 70% nitric acid (22.22 M) at 50 ℃. Metal contents of these samples was determined by atomic emission spectroscopy (ICP-OAS) (PlasmaQuant PQ9000 Elite, Analytik Jena) [1].

Outer membrane fractionation

E. coli cells grown overnight at 37 ℃ were collected and suspended in 0.9 % (w/v) NaCl (OD_{600} of 4.0; total volume = 200 mL). The suspensions, containing 50 µg/mL of deoxyribonuclease and 100 µg/mL of ribonuclease, were then passed through French press to lyse the cells. The cell lysates were centrifuged at 5,000 g (4 ℃, 1 h). The resulting pellets were resuspended in 0.9 % (w/v) NaCl supplemented with 2% (v/v) Triton X-100 and incubated at room temperature for 30 min. The suspension was subsequently centrifuged at 100,000 g (4 ℃, 30 min). The

resulting pellet contained the outer membrane fraction was dried by lyophilization for two days prior to FTIR analysis. This method is based on a protocol developed by Beveridge et al. [2]

FITR analysis of *E. coli* BL21 whole cells and outer membrane fraction

The FTIR was performed with a FTIR spectrometer (Avatar Nicolet 360 FT-IR, ThermoFisher, USA). 5 mg of dried outer membrane fraction was mixed with 150 mg of KBr (Spectral) in an agate mortar. The translucent discs were prepared by pressing the KBr mixture with the aid of 10 t pressure bench press. The disc was immediately analyzed using a spectrophotometer in the range of 4000-400 cm^{-1}. Atmospheric water and CO_2 were subtracted.

Detachment test

Detachment test of $eGFP^{His}$ on the cell surface was carried out by using L-ascorbic acid to reduce Fe^{3+} to Fe^{2+}. 100 µL cell suspension with immobilized $eGFP^{His}$ (0.9% (w/v) NaCl; OD_{600} = 4) was incubated with L-ascorbic acid (250 µM) for 30 min at room temperature. Afterwards the cells were washed twice with 100µL 0.9% (w/v) NaCl and resuspended in 100µL 0.9% (w/v) NaCl. Cell-surface immobilized $eGFP^{His}$ without addition of L-ascorbic acid served as control. The influence of L-ascorbic acid on the fluorescence of eGFP was investigated by supplementing 250 µM L-ascorbic acid to 100 µL solution containing 5 µM $eGFP^{His}$ for 100 min at room temperature. $eGFP^{His}$ without L-ascorbic acid was used as control. Fluorescence was detected *via* Tecan Infinite M1000 Pro plate reader (Tecan Group, Mannedorf, Switzerland; λ_{ex} = 488 nm and λ_{em} = 507 nm).

1, 10-phenanthroline assay

The cell surface reduced Fe^{2+} was measured through 1,10-phenanthroline assay (Fe^{2+} form a deep red solution with 1,10-phenanthroline) [2]. After the $eGFP^{His}$ detachment procedure, 400 µL resulting cells suspension was added to 150 µL 1,10-phenanthroline solutions (5 mM). Subsequently, 450 µL sodium acetate buffer (200 mM, pH 4.5) was added, and the mixture was incubated for 10 min at room temperature. Fe^{3+} decorated cells without L-ascorbic acid were used as control.

Cell viability test of the Fe^{3+} mediated immobilization method

After immobilization, the cell viability was determined by adding 5 µL cell suspension into 195 µL LB_{Kan} medium in the microtiter plate. The cell OD_{600} was measured by Tecan Sunrise™ plate reader (Tecan Group, Mannedorf, Switzerland; 600 nm). After 30 min and 60 min of culture,

5 µL of the cultures were taken out for the fluorescence microscope detection, respectively. The cells without immobilization were used as control.

Expression and purification of His_6-tagged enzymes

Cloning and expression of His_6-tagged *Bacillus licheniformis* laccase ($BlcotA^{His}$)[3], His_6-tagged *Bacillus subtilis* lipase A ($BSLA^{His}$)[4], and His_6-tagged *Candida tropicalis* fatty alcohol oxidase ($CtFAO^{His}$)[5] were performed according to the published procedures. After expression, cells were harvested by centrifugation at 4,000 rpm for 15 minutes then resuspended in 20 mL optimal buffer (according to the enzymes) containing 150 mM NaCl, 10 mM imidazole. Cells were disrupted by sonication and debris removed by centrifugation at 15,000 × g for 1 hour (Sorvall, ThermoFischer Scientific, Germany). The supernatant was then applied to the Protino1 Ni-TED 2,000 packed columns (Macherey-Nagel). Afterwards, PD-10 desalting column (GE Healthcare, Germany) was used to remove salts. The purified lipases were stored at glycine buffer (10 mM, pH 10.5) in small aliquots at -80 ℃, and each aliquot was used only once after thawing. Purity was determined using the ExperionTM system from Bio-Rad (München, Germany).

Immobilization of His_6-tagged enzymes on the cell surface mediated by Fe^{3+}

Immobilization of His_6-tagged enzymes on the cell surface was performed by adding $FeCl_3$ (0; 50; 100 µM, respectively) and 1 µM His_6-Tag enzymes to *E. coli* cell suspensions (0.9% (w/v) NaCl; OD600 = 4; total volume = 100 µL). The solution was incubated for 10 min at room temperature, and then the cells were washed two times and resuspended with 100 µl 0.9% (w/v) NaCl. The activity of $BlcotA^{His}$ and $CtFAO^{His}$ on the cell surface were detected via ABTS assay, 30 µL cell suspension was added into 170 µL ABTS mix solution (3 mM in 100 mM HAc-NaAc buffer, pH 4.5 for $BlcotA^{His}$; 50 mM KPi buffer, pH 7.4 for $CtFAO^{His}$). The increase in absorbance was measured at 420 nm for 20 min via Tecan Sunrise™ plate reader. The activity of $BSLA^{His}$ on the cell surface was detected via *p*-nitrophenyl butyrate (*p*NBP) assay. *p*NBP stock solution (10 mM in acetonitrile) was prepared in advance, and then the master mix solution was prepared by adding 9 mL TEA buffer (Triethanolamin, pH 7.4) to 1 mL *p*NBP stock solution. The measurement was initiated by adding 50 µL cells suspension to 150 µL master mix solution, and then the absorbance was measured at 410 nm for 20 min via Tecan Sunrise™ plate reader.

Construction and expression of Strep-tag II_enzymes_His_6-tag

Strep-tag II (eight amino acids: **WSHPQFEK**) was introduced into the *N*-terminus of each protein as **5'-ASA-strep-tag II-SG-BlcotA_His_6-tag-3' (StrepII_$BlcotA^{His}$)**, **5'-ASA-strep-tag II-SG-BSLA_His_6-tag-3' (StrepII_$BSLA^{His}$)** and **5'-ASA-strep-tag II-SG-CtFAO_His_6-tag-3' (StrepII_$CtFAO^{His}$)**. The protein expression and purification were performed according to the protocol mentioned above.

Staining Strep-tag II with Strep-Tactin Chromeo™ 546 conjugate

After immobilization (according to the method mentioned above), 2 μL Strep-Tactin Chromeo™ 546 conjugate (0.5 mg/ml solution in PBS, 10 × dilute before usage, IBA Lifesciences, Göttingen, DE) was added into 50 μL of cell suspension immobilized with StrepII_enzymes_His_6-tag (0.9% (w/v) NaCl; OD_{600} = 4). The mixture was incubated at room temperature with 900 rpm shaking for 15 min, and then cells were washed twice and resuspended in 50 μL 0.9% (w/v) NaCl. Afterwards, cells were analyzed by fluorescence microscopy (λ_{ex} = 555 nm; λ_{em} = 640 nm). To analyze the conjugation of the immobilized enzymes in different buffers, 30 μL of cell suspension immobilized with StrepII_$BlcotA^{His}$ or StrepII_$CtFAO^{His}$ were transferred into 170 μL ABTS mix solution (100 mM HAc-NaAc buffer, pH 4.5 and 50 mM KPi buffer, pH 7.4; respectively) for 30 min at room temperature; 50 μL of cell suspensions immobilized with StrepII_$BSLA^{His}$ were transferred into 150 μL *p*NBP master mix solution (50 mM TEA buffer, pH 7.4)) for 30 min at room temperature. After incubation, the cells were washed twice and resuspended in 50 μL 0.9% (w/v) NaCl. The following staining step was performed as described above. The resulting cells were analyzed by fluorescence microscopy (λ_{ex} = 555 nm; λ_{em} = 640 nm).

Immobilization of multiple proteins on the cell surface through Fe^{3+}

StrepII_$BlcotA^{His}$ and $eGFP^{His}$ were used to perform the cell surface immobilization of different proteins. To this end, 40 μM StrepII_$BlcotA^{His}$ and 40 μM $eGFP^{His}$ were mixed. 10 μL mixture was then add to 100 μL cell suspension supplied with 100 μM $FeCl_3$ (0.9% (w/v) NaCl; OD_{600} = 4). After washing the cells twice, the cells were resuspended in 0.9% (w/v) NaCl. The immobilization of StrepII_$BlcotA^{His}$ was visualized by staining the StrepII tag with Strep-Tactin conjugated Chromeo™ 546 (the details were shown above). The fluorescence was detected via fluorescence microscope (Olympus Corporation, Japan) at the exposure time of 109.7 ms and the gain of 2.9 dB.

Construction of HRP-mCherry-His_6 tag

Horseradish peroxidase (HRP, EC 1.11.1.7, Genebank accession: AIV09214.1) from *Armoracia rusticana* [6] was synthesized by GeneScript with codon optimization for *Pichia pastoris* BSYBG11. HRP gene was then cloned and fused to the *N*-terminus of mCherry (red fluorescence protein) with a flexible linker GGGS as shown as 5'-HRP-GGGS-mCherry-His_6 tag-3'. The whole construct was inserted into pBSYA1S1Z plasmid and then transformed into chemical-competent *E. coli* BL21 DH5α. The resulting plasmid, pBSYA1S1Z-HRP-mCherry-His_6 tag, was isolated and transformed into *Pichia pastoris* BSYBG11 via electroporation.

Expression and purification of HRP-mCherry-His_6 tag

HRP-mCherry-His_6 tag (HRP-$mCherry^{His}$) was overexpressed in *Pichia pastoris* BSYBG11. The pre-cultures were grown in 10 mL YPD medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L D-glucose, 100μg/mL zeocin) using a 100 mL flask (30 ℃, 200 rpm). Main cultures were inoculated using the pre-cultures as inoculum to a start OD_{600} = 0.5 in 200 mL YPD medium. After cultivation (30 ℃, 200 rpm), the culture supernatant was separated from the cell broth by centrifuging (Sorvall, ThermoFischer Scientific, Germany, 4 ℃, 4000 × g, 30 min). Culture supernatant was concentrated 10 folds using an Amicon Ultra-15 Centrifugal Filter Unit (Merck Millipore Ltd. Tullagreen, Ireland; Cut off: 10 kDa). HRP-$mCherry^{His}$ was purified by using a Ni-IDA 2000 column. Samples eluted with 50 mM imidazole are dialyzed and stored in 0.9% (w/v) NaCl solution at -80 ℃ for further application.

Immobilization of HRP-$mCherry^{His}$ on the cell surface mediated by Fe^{3+}

HRP-$mCherry^{His}$ (0.06 mg/mL) and different concentration of $FeCl_3$ (0; 30; 50 μM) were added into 100μl fresh suspension of *E. coli* cells (0.9% (w/v) NaCl; OD_{600} = 4) for 10 min at room temperature. After two times washing with 100μl 0.9% (w/v) NaCl, the resulting cells were examined by fluorescence microscope (λ_{ex} = 555 nm; λ_{em} = 640 nm) and ABTS colorimetric assay (details were shown above).

Modification of alginate with fluorescein and phenol group

The alginate with fluorescein and phenol group (Fluor-Alg-Ph) was prepared based on previously reported [7, 8]. Briefly, sodium alginate was dissolved in the 50 mM MES buffer (pH 6) with a concentration of 10 g/L. Then, 40 mM Tyramine hydrochloride, 10 mM *N*-hydroxysuccinimide (NHS), and 20 mM 1-ethyl-3-(3 dimethyl aminopropyl) carbodiimide-

hydrochloride (EDC) were added, respectively. The mixture was under continuous stirring by adding 2 mM 5-aminofluorescein (144 mM stock in DMSO) at room temperature for overnight. The modified polymer was precipitated with 80% cooled ethanol solution and collected by centrifugation (4000 × g, 4 ℃ for 10 min). The pellet was washed twice with 80% ethanol and then dissolved in water for lyophilization. The modified alginate was analyzed by ^{1}H-NMR (nuclear magnetic resonance, 300 MHz, 23 °C) in D_2O.

Cell encapsulation in fluorescent alginate

In case of single cell encapsulation based on Fluor-Alg-Ph polymer, HRP-mCherry^{His} immobilized *E. coli* cells (100 µL, OD_{600} = 4) are harvested and resuspended in 1% (w/v) Fluor-Alg-Ph solution (100 µL, 0.9% (w/v) NaCl) containing 1 mM H_2O_2. The whole mixture was incubated at room temperature for 10 min and then harvested (11000 rpm, 2 min) and washed with 100 µL 0.9% (w/v) NaCl aqueous solution four times. The resuspended samples were transferred to fluorescence microscope for checking hydrogel formation (λ_{ex}=470 nm, λ_{em}=535 nm).

Supplementary results

Supplementary Table 1: Metal ions adsorption after exogenous supplementation of different metal ions

E. coli cells retained 3.05 mg/g_{cells} of Fe^{3+} compared to the other probed metals. The metal absorption analysis explains to some extent the reason why other metal ions (e.g., Ni^{2+}, Co^{2+}, and Zn^{2+}) with high His_6-tagged affinity show no detectable immobilization ability of $eGFP^{His}$. Notably, *E. coli* BL21 cells also exhibited high adsorption capacity towards Cu^{2+}. However, in contrast to the fluorescence results obtained with Fe^{3+}, the low labeling ability can be explained by non-optimal binding conditions of Cu^{2+} towards the His_6-tagged [9].

Table S1: Metal ions adsorption by *E. coli* cells

	Metal ions absorbed (mg/g_{cells})				
	Fe^{3+}	Zn^{2+}	Cu^{2+}	Ni^{2+}	Co^{2+}
E. coli BL21	0.09	0.06	< 0.02	<< 0.01	0.0
E. coli BL21 + Metal ions	3.05	0.84	2.79	0.31	0.11

5 mL of Cells (OD_{600} = 4) were resuspended in 0.9% (w/v) NaCl and then supplemented with 100 µM of $FeCl_3$, $ZnCl_2$, $CuCl_2$, $NiCl_2$, and $CoCl_2$, respectively. After 10 min, cells were washed twice with 0.9% (w/v) NaCl and then the pellets were collected. The resulting pellets were incubated overnight in 70% nitric acid (22.22 M) at 50°C. Metal content of these samples was determined by ICP-OAS.

Supplementary Figure 1: The effects of metal ions on the fluorescence of eGFP^{His}

The effect of metal ions on the fluorescence of eGFP^{His} was examined. For Fe^{3+}, metal concentrations below 100 µM showed a slight quenching effect on eGFP, but when the concentration was increased above 150 µM, the fluorescence of eGFP was strongly suppressed. For Cu^{2+}, low concentrations of metal ions already have caused about 40% loss of eGFP fluorescence. The other tested metal ions (Ni^{2+}; Co^{2+}; Zn^{2+}) have only a negligible effect on the fluorescence of eGFP.

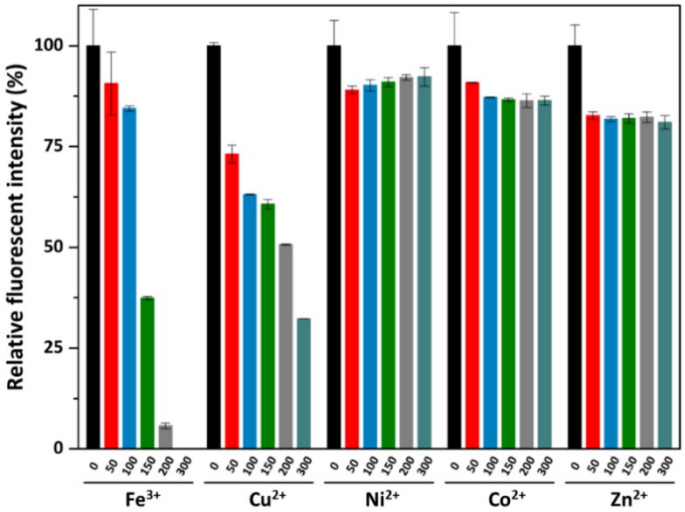

Figure S1: The metal ions were incubated with 5 µM eGFP^{His} for 10 min, and then the residual fluorescence intensity was measured at λ_{ex} = 488 nm; λ_{em} = 507 nm. For each metal ions, six different concentrations were tested (0; 50; 100; 150; 200; 300 mM). Error bars indicate standard deviations of two technical replicates.

Supplementary Figure 2: Optimization and quantification of the immobilization method

The immobilization conditions were optimized. Firstly, we tested the saturation concentration of $eGFP^{His}$ for 100 µM Fe^{3+}. The highest fluorescence intensity was reached at 5 µM $eGFP^{His}$, despite testing the number of cells (Figure S2A). Then under this concentration of $eGFP^{His}$, the optimal cell concentration was measured utilizing 100 µM Fe^{3+}. It was shown that when cell OD_{600} is 4, the fluorescence intensity reached highest and no obvious fluorescence increased by adding more cells. It indicated 100 µM Fe^{3+} realized saturation at cell OD_{600} = 4 (Figure S2B). Additionally, the buffer system was optimized (Figure S2C). It was shown that the best working solution for immobilization is 0.9 % (w/v) NaCl. Hence, the optimal condition was determined that 100 µM Fe^{3+} and 5 µM $eGFP^{His}$ are add to the *E. coli* cells (suspend in 0.9% (w/v) NaCl; OD_{600} = 4). Under this optimal condition, it can be calculated that 100 µM Fe^{3+} could mediate around 3.82 µM $eGFP^{His}$ immobilized on the cell surface when the cell OD_{600} is 4, comparing with the calibration curve ($y = exp(-4.8003 + 4.98x - 0.413x2)$; R^2 = 0.997) (Figure S2D).

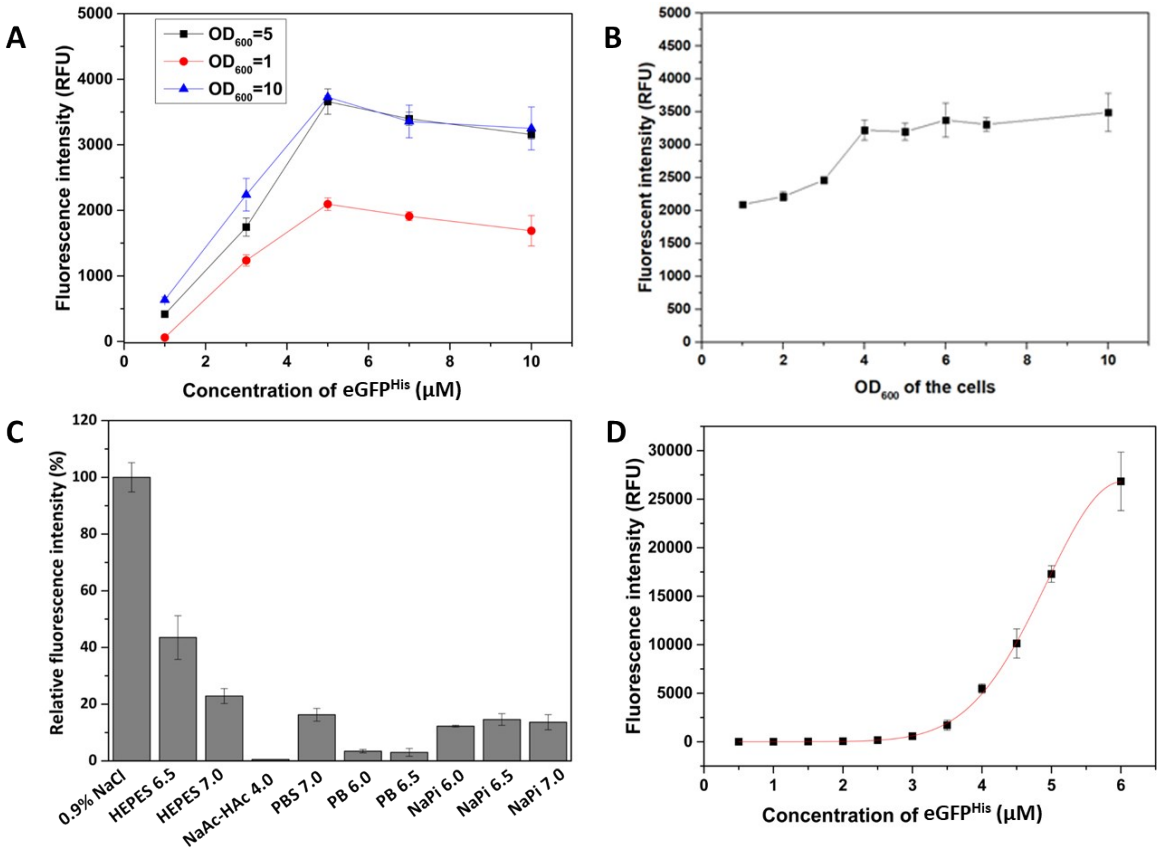

Figure S2: (A) Saturation line of 100 µM Fe^{3+} for different concentration $eGFP^{His}$. 100 µM Fe^{3+} and different concentration of $eGFP^{His}$ were added to the suspension of *E. coli* cells (0.9% (w/v) NaCl). Red line: cell suspension

at OD_{600} = 1; black line: cell suspension at OD_{600} = 5; blue line: cell suspension at OD_{600} = 10. (B) Saturation line of 100 µM Fe^{3+} for different cell density (OD_{600}). 100 µM Fe^{3+} and 5 µM $eGFP^{His}$ were added to the suspension of *E. coli* cells (0.9% (w/v) NaCl), whereas the concentration of cells was varied. (C) Cell binding of $eGFP^{His}$ in different solutions. The fluorescence intensity of immobilized $eGFP^{His}$ was measured at λ_{ex} = 470 nm; λ_{em} = 535 nm. Error bars indicate standard deviations of two technical replicates. (D) Calibration curves of $eGFP^{His}$ under 100 µM Fe^{3+}. Different concentration of $eGFP^{His}$ was incubate with 100 µM $eGFP^{His}$ for 10 min at room temperature.

Supplementary Figure 3: Cell-surface immobilization of eGFP^{His} for *E. coli* K12, *Bacillus subtilis* and *Corynebacterium glutamicum*

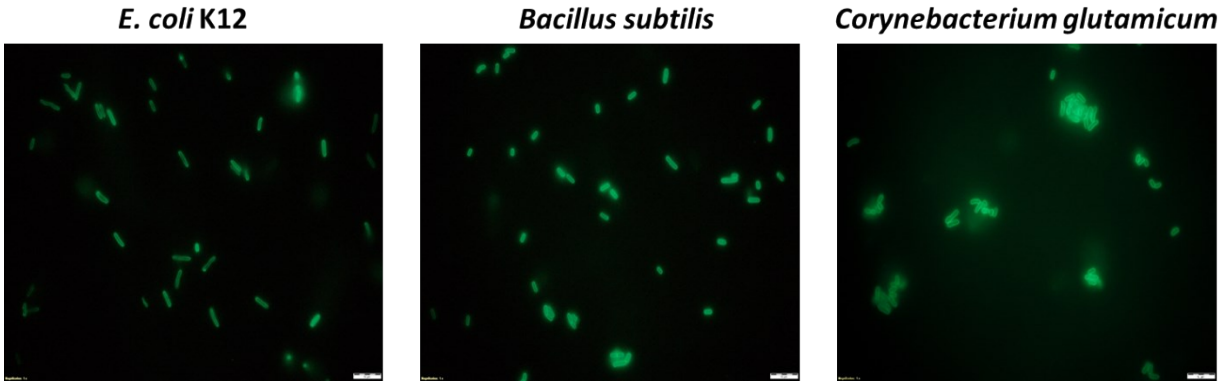

Figure S3: Fluorescence image of the cell surface of different strains immobilized with eGFP^{His}.

Supplementary Figure 4: FTIR analysis of *E. coli* BL21 and the isolated outer membrane fraction

FTIR spectra of *E. coli* BL21 and its isolated outer membrane fraction, in the range of 4000 - 500 cm^{-1}, were taken to confirm the presence of functional groups (Fig. S4). FTIR suggests that carbonyl groups belonging to carboxylic acids or amides as well as free amines are present for metal coordination.

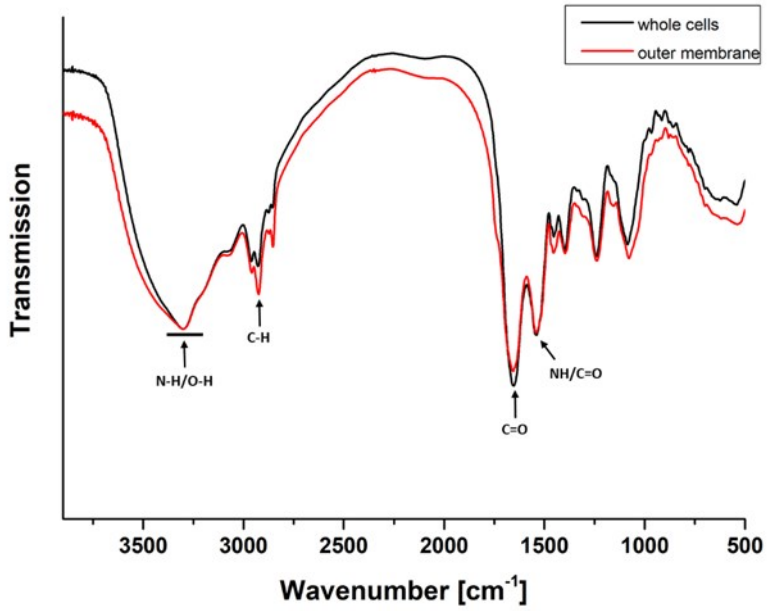

Figure S4: FTIR spectra of *E. coli BL21* whole cells (black line) and outer membrane fraction of *E. coli* BL21 (red line).

Supplementary Figure 5: The influence of L-ascorbic acid on eGFP^{His}

The time-dependent fluorescence curve shows that L-ascorbic acid has no effect on the fluorescence of eGFP^{His} (Figure S5).

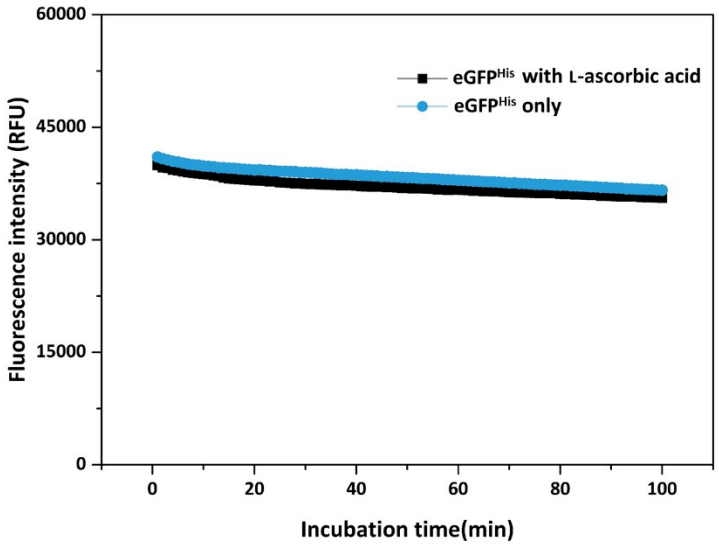

Figure S5: Time-dependent fluorescence curve of the influence of L-ascorbic acid on the fluorescence of eGFP^{His}. Black line: 250 µM L-ascorbic acid was added to 5 µM eGFP^{His} for 100 min; blue line: only 5 µM eGFP^{His} was incubated for 100 min. The fluorescence intensity was measured by Tecan Infinite M1000 Pro plate reader (λ_{ex} = 488 nm; λ_{em} = 507 nm).

Supplementary Figure 6: the immobilization of $eGFP^{His}$ on the cell surface while cell growth

During cell growth, the immobilization of $eGFP^{His}$ on the cell surface was inspected by fluorescence microscopy. As shown in Figure S6, after 30 min and 60 min culture, $eGFP^{His}$ remained on the cell. During cell growth, the cell-mass increases, which leads to reduced fluorescence intensity, since the total amount of eGFP is constant. For applications, either the cells need to be kept in the static phase or new Fe^{3+}/His_6-tagged protein need to be supplemented to ensure homogeneous distribution of the protein on the cell surface.

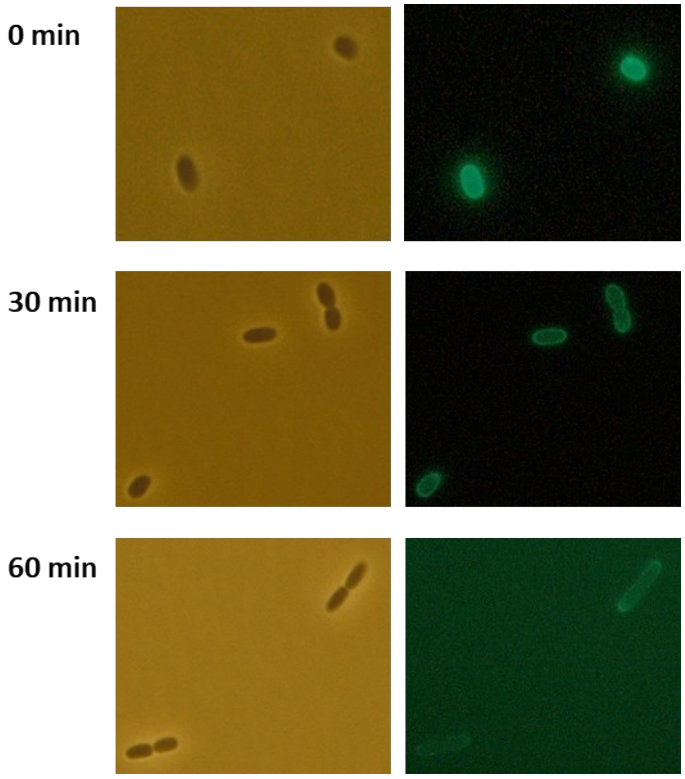

Figure S6: Fluorescence images of $eGFP^{His}$ immobilized on the cell surface during cell growth. The image was inspected by fluorescence microscope in bright and green fields (λ_{ex} = 470 nm, λ_{em} = 535 nm) at the exposure time of 109.7 ms and the gain of 2.9 dB.

Supplementary Figure 7: SDS-PAGE for the purified His_6-tagged enzymes

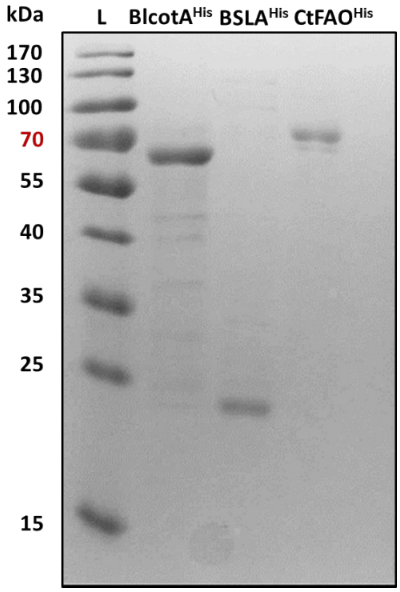

Figure S7: SDS-PAGE of purified BlcotA^{His}, BSLA^{His}, and CtFAO^{His}. The molecular weight of each protein is 60.4, 19.3, 78 kDa, respectively. L: Ladder (PageRulerTM Prestained Protein Ladder (Thermo Scientific).

Supplementary Figure 8: visualization of the His_6-tagged enzymes on the cell surface

Direct visualization of the His_6-tagged enzymes on the cell surface was performed by Strep-tag II, which can combine with Strep-Tactin conjugated fluorophore (Chromeo™ 546). Fluorescence microscopy revealed that the His_6-tagged enzymes successfully remained on the cell surface after immobilization and their corresponding activity assay, which was performed in a different buffer.

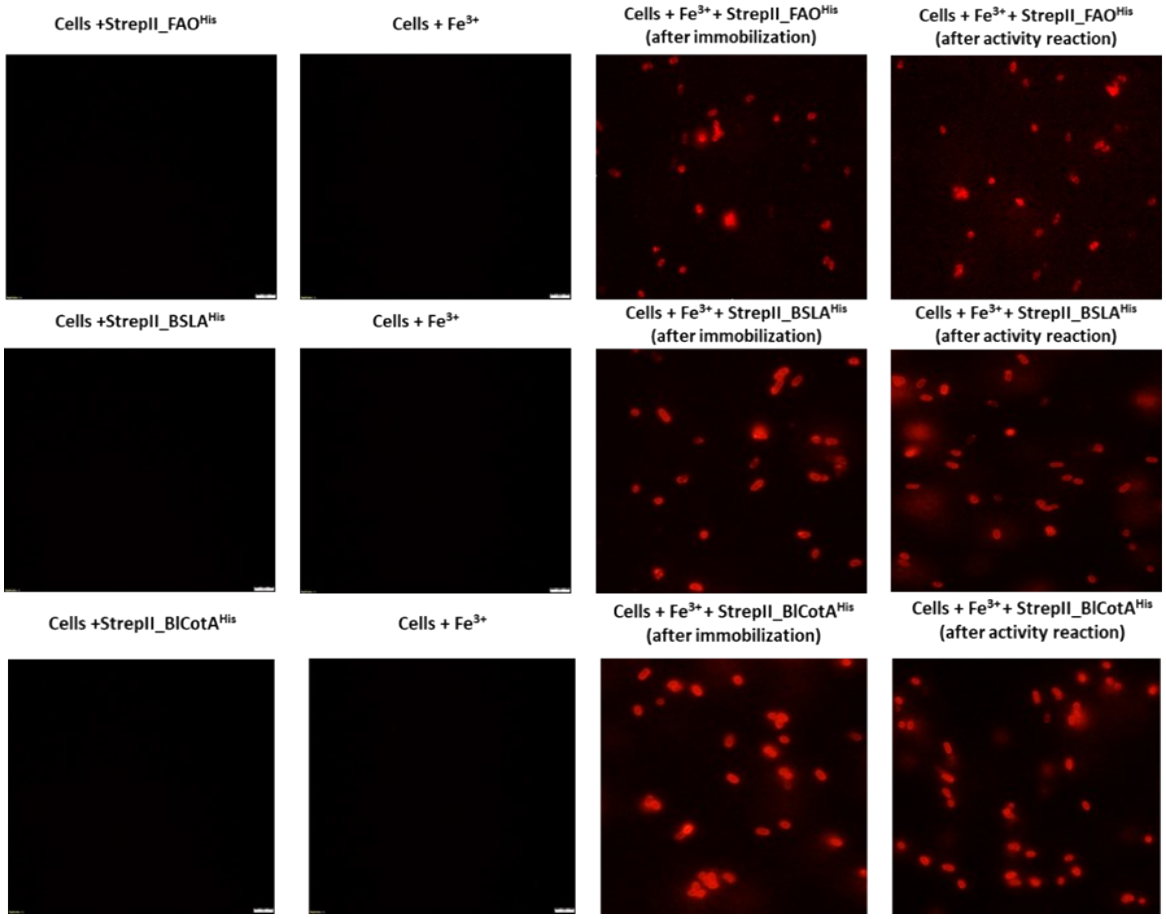

Figure S8: Fluorescence images of cells immobilized with Strep-tag II_enzymes_His_6-tag after staining with Strep-Tactin Chromeo™ 546 conjugate. The red fluorescence signal (Chromeo™ 546) directly indicated the His_6-tagged enzymes are successfully immobilized on the cell surface before and after activity assay reaction. The image was inspected by fluorescence microscope in red fields (λ_{ex} = 555 nm; λ_{em} = 640 nm) at the exposure time of 109.7 ms and the gain of 2.9 dB.

Supplementary Figure 9: Two proteins immobilized in the cell surface

The images of fluorescence microscopy confirms that both proteins, StrepII_BlCotA^{His} and eGFP^{His}, are presented on *E. coli* cells.

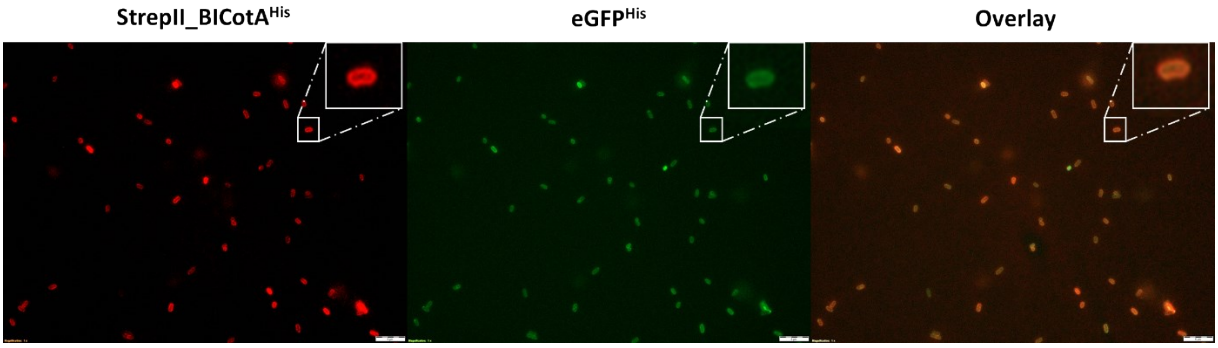

Figure S9: Fluorescence images of cells immobilized with StrepII_BlCotA^{His} and eGFP^{His}. The immobilization of StrepII_BlcotA^{His} was visualized by staining the Strep tag II with Strep-Tactin conjugated Chromeo^{TM} 546. The cells were inspected by fluorescence microscopy in green field (λ_{ex} = 470 nm, λ_{em} = 535 nm) and red field (λ_{ex} = 555 nm; λ_{em} = 640 nm) at the exposure time of 109.7 ms and the gain of 2.9 dB.

Supplementary Figure 10: Modification of alginate with phenols and fluorophores (Fluor-Alg-Ph)

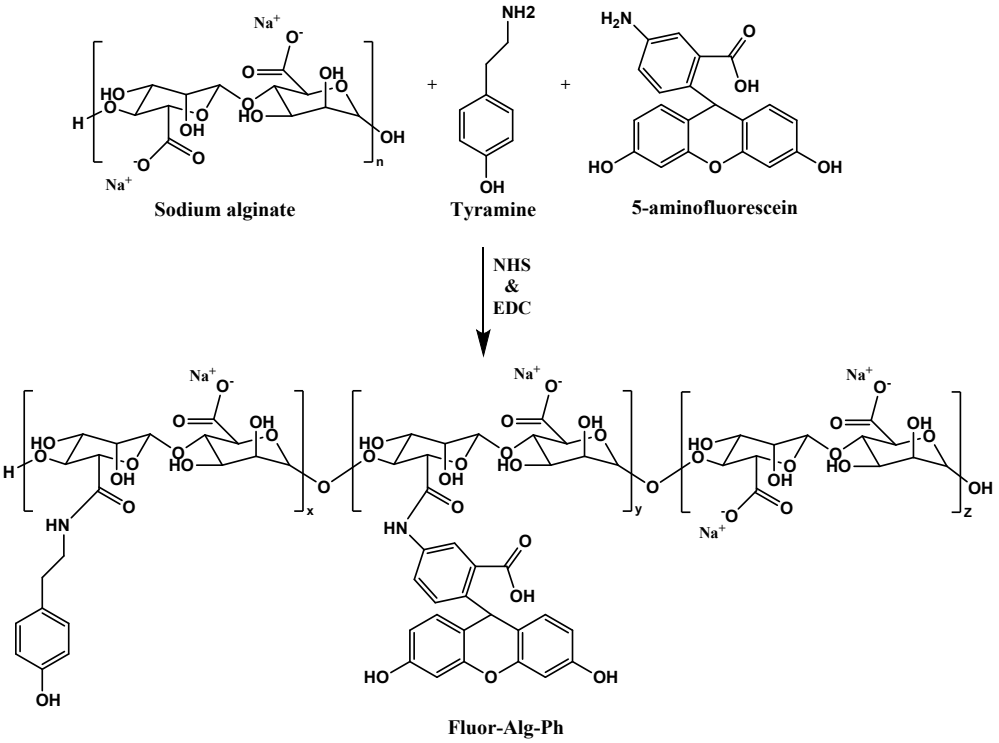

Figure S10: Reaction scheme for the production of alginate macromonomers (Fluor-Alg-Ph).

Supplementary Figure 11: ^{1}H NMR spectroscopy of Fluor-Alg-Ph

^{1}H NMR spectra were recorded on a Bruker Avance III 300 MHz NMR spectrometer (Bruker, Massachusetts). Deuterium oxide (D_2O) was used as NMR solvent and the NMR signals are reported relative to the remaining proteo solvent at 4.75 ppm [10]. The signals above δ = 6.8 ppm indicate the aromatic moieties attached upon the EDC/NHS treatment as shown in Figure S11.

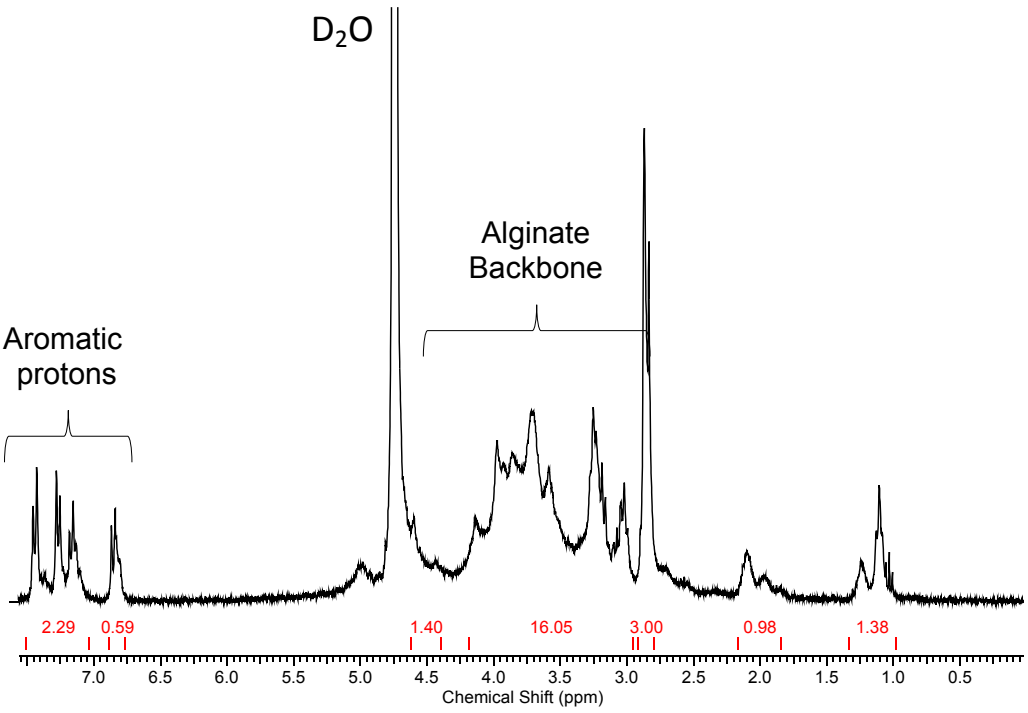

Figure S11: ^{1}H NMR spectrum (300 MHz, 23 °C, D_2O) of Fluor-Alg-Ph.

Supplementary Figure 12: Immobilization of HRP-mCherry^{His} on *E. coli* surface mediated by Fe^{3+}

The immobilization of HRP-mCherry^{His} on the cell surface was inspected by fluorescence microscope. As shown in Figure S12, the cells were decorated with red fluorescence upon treatment with Fe^{3+} and HRP-mCherry^{His}, which indicates that HRP-mCherry^{His} was successfully immobilized on the cell surface.

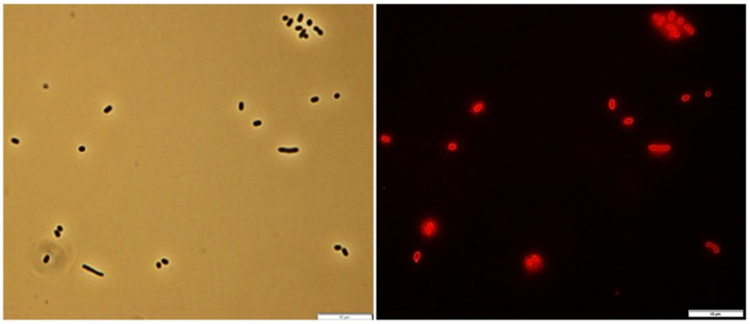

Figure S12: Fluorescence images of HRP-mCherry^{His} immobilized on the cell surface. The red fluorescence signal (mCherry) indicated the HRP-mCherry^{His} was successfully immobilized on *E. coli* cell surface mediated by Fe^{3+}. The image was inspected by fluorescence microscope in bright and red fields (λ_{ex} = 555 nm; λ_{em} = 640 nm) at the exposure time of 109.7 ms and the gain of 2.9 dB.

Supplementary Figure 13: Reaction scheme for hydrogel polymerization reaction initiated by HRP

The biocompatible hydrogel polymerization was initiated by a peroxidase-catalyzed oxidation reaction [11]. The Fluor-Alg-Ph hydrogel was formed through the controlled radical polymerization using H_2O_2 as an oxidant of horseradish peroxidase (HRP) to generate hydroxyl radical to initial the coupling of the phenol moiety.

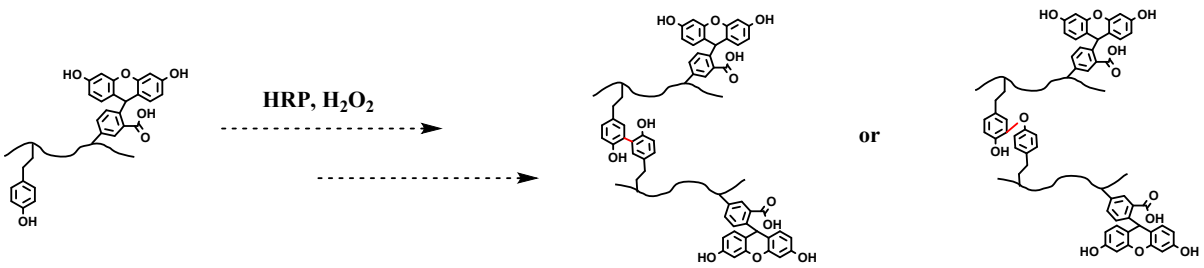

Figure S13: Illustration for Fluor-Alg-Ph hydrogel formation initiated through H_2O_2 and HRP.

Supplementary Figure 14: Fluorescence image of *E. coli* cells encapsulated in a conformal alginate shell

E. coli cell encapsulation with alginate shell was inspected by fluorescence microscope. As shown in Figure S14, after soaking in the solution containing Fluor-Alg-Ph and H_2O_2, the hydrogel sheath was formed on the cells immobilized with HRP-mCherry^{His} (Fig S14A). In contrast, the cells whitout Fe^{3+} (treated with free HRP-mCherry^{His} or commercial HRP (no His_6-Tag) as shown in Fig S14 B and C) cannot form any hydrogel sheath around the cell surface. These results confirmed hydrogel formation occurred only on the cell surface carrying HRP-mCherry^{His}.

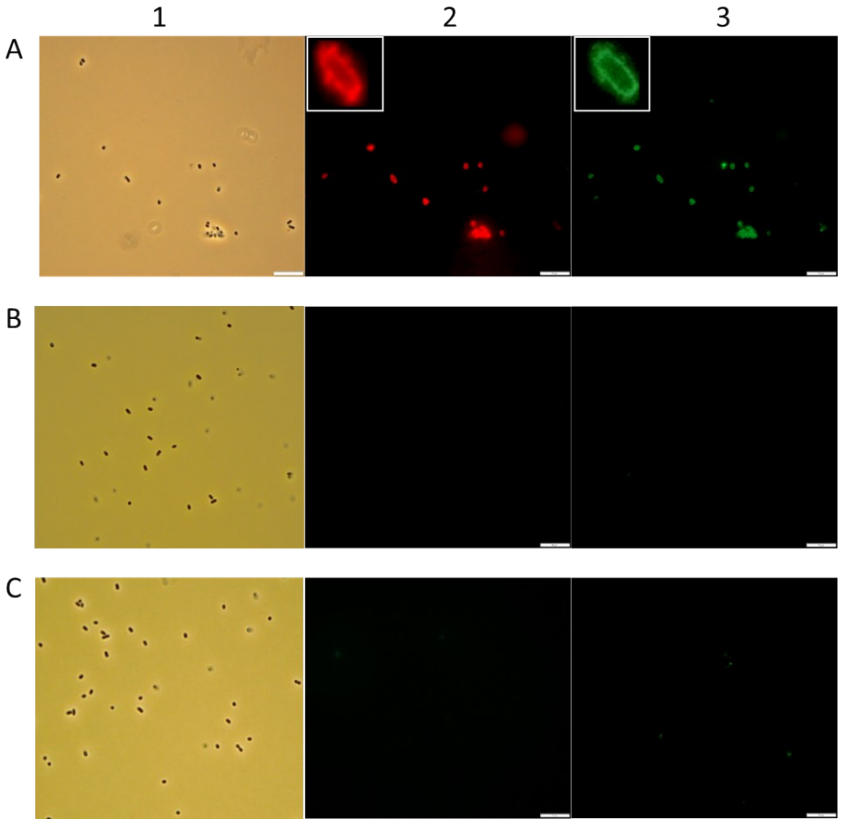

Figure S14: Single cell encapsulation in conformal Fluor-Alg-Ph hydrogel sheath based on controlled radical polymerization using HRP-mCherry^{His}. (A) Cells treated with HRP-mCherry^{His} and Fe^{3+} for hydrogel sheath formation. The red fluorescence from mCherry indicates HRP-mCherry^{His} was still immobilized on *E. coli* cell surface after gel formation process (λ_{ex} = 555 nm, λ_{em} = 640 nm). The green fluorescence from 5-aminofluorescein indicates Fluor-Alg-Ph polymers are surrounded on *E. coli* cell surface (λ_{ex} = 470 nm, λ_{em} = 535 nm). Cells treated with HRP-mCherry^{His} alone (B) or with commercial HRP alone (C) showed no hydrogel sheath around the cell surface. The pictures were inspected by fluorescence microscope in bright field (1), red field (2), and green field (3) at the exposure time of 109.7 ms and the gain of 2.9 dB.

Appendix

Gene of HRP-GGGS-mCherry-His tag

CAGTTGACTCCAACCTTCTACGACAACTCCTGTCCAAACGTTTCCAACATCGTCAGAGACACCATCGT
CAACGAGTTGAGATCTGACCCAAGAATCGCTGCCTCCATCTTGAGATTGCACTTCCACGACTGTTTCG
TGAACGGTTGTGACGCTTCCATCTTGTTGGACAACACCACTTCCTTCAGAACTGAGAAGGACGCTTTC
GGTAACGCTAACTCTGCTAGAGGTTTCCCAGTCATCGACAGAATGAAGGCTGCTGTTGAATCCGCTTG
TCCAAGAACTGTTTCCTGTGCTGACTTGTTGACTATCGCTGCTCAACAGTCCGTTACTTTGGCTGGTG
GTCCATCTTGGAGAGTTCCATTGGGTAGAAGAGATTCCTTGCAGGCCTTCTTGGATTTGGCTAACGCT
AATTTGCCAGCTCCATTCTTCACCTTGCCTCAGTTGAAGGACTCTTTCAGAAACGTCGGTCTGAACAG
ATCCTCCGACTTGGTTGCTTTGTCTGGTGGACACACCTTTGGTAAGAACCAGTGCAGATTCATCATGG
ACAGACTGTACAACTTCTCCAACACCGGTTTGCCAGATCCAACTTTGAACACCACCTACTTGCAGACC
TTGAGAGGTTTGTGTCCACTGAACGGTAACTTGTCCGCTTTGGTTGACTTCGACTTGAGAACCCCAAC
TATCTTCGACAACAAGTACTACGTCAACTTGGAGGAACAGAAGGGTTTGATCCAATCCGACCAAGAGT
TGTTCTCTTCCCCAAACGCTACTGACACTATCCCATTGGTTAGATCCTTCGCCAACTCTACCCAGACT
TTCTTCAACGCTTTCGTTGAGGCTATGGACAGAATGGGTAACATCACTCCATTGACCGGTACTCAGGG
TCAGATTAGATTGAACTGCAGAGTCGTTAACGGAGGCGGTGGTTCCATGGTGAGCAAAGGTGAAGAGG
ATAATATGGCCATCATCAAAGAATTTATGCGCTTTAAAGTGCACATGGAAGGTAGCGTTAATGGCCAT
GAATTTGAAATTGAAGGTGAAGGCGAAGGTCGTCCGTATGAAGGCACCCAGACCGCAAAACTGAAAGT
TACCAAAGGTGGTCCGCTGCCGTTTGCATGGGATATTCTGAGTCCGCAGTTTATGTATGGTAGCAAAG
CCTATGTTAAACATCCGGCAGATATCCCGGATTATCTGAAACTGAGCTTTCCGGAAGGTTTTAAATGG
GAACGTGTGATGAATTTTGAAGATGGTGGTGTTGTTACCGTTACCCAGGATAGCAGCCTGCAGGATGG
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ATTATACCATTGTTGAACAGTATGAACGTGCAGAAGGTCGCCATAGTACCGGTGGTATGGATGAACTG
TATAAACATCATCATCATCATCAC

References

1. N. Cruz, S. L. Borgne, G. Hernandez-Chavez, G. Gosset, F. Valle and F. Bolivar, *Biotechnol. Lett.*, 2000, **22**, 7.
2. P. Komadel, *Clays and Clay Minerals*, 1988, **36**, 379-381.
3. K. Koschorreck, S. M. Richter, A. B. Ene, E. Roduner, R. D. Schmid and V. B. Urlacher, *Appl. Microbiol. Biotechnol.*, 2008, **79**, 217-224.
4. H. Y. Cui, T. H. J. Stadtmuller, Q. J. Jiang, K. E. Jaeger, U. Schwaneberg and M. D. Davari, *Chemcatchem*, 2020, **12**, 4073-4083.
5. S. Q. Meng, J. Guo, K. L. Nie, U. Schwaneberg, T. W. Tan, H. J. Xu and L. Liu, *J Biobased Mater Bio*, 2019, **13**, 79-85.
6. B. Morawski, Z. L. Lin, P. C. Cirino, H. Joo, G. Bandara and F. H. Arnold, *Protein Eng*, 2000, **13**, 377-384.
7. S. Sakai and K. Kawakami, *Acta Biomater*, 2007, **3**, 495-501.
8. Y. Liu, S. Sakai, S. Kawa and M. Taya, *Anal Chem*, 2014, **86**, 11592-11598.
9. J. Watly, E. Simonovsky, R. Wieczorek, N. Barbosa, Y. Miller and H. Kozlowski, *Inorg. Chem.*, 2014, **53**, 6675-6683.
10. G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, *Organometallics*, 2010, **29**, 2176-2179.
11. M. Kurisawa, J. E. Chung, Y. Y. Yang, S. J. Gao and H. Uyama, *Chem Commun* 2005, DOI: 10.1039/b506989k, 4312-4314.