Supporting Information for:

A Simplified Protein Purification Method through Nickel Cleavage of Recombinant Protein from *Escherichia coli* Cell Surface

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Experimental procedures

Materials. Unless otherwise noted, all the reagents used in this study were of analytical grade and purchased from commercial sources. Milli-Q water was used to prepare all the buffer solutions.

Generation and expression of LOS-GB1, LOS-sfGFP, 6His-GB1 and 6His-sfGFP constructs. The optimized genes encoding *LOS-GB1* and *LOS-sfGFP* were synthesized by Sangon Biotechnology (Shanghai), and were cloned into the pet30a plasmid between *NdeI* and *XhoI* restriction sites, respectively. The plasmids were then transformed into the expression strain of *E. coli* (DE3). The cells carrying *LOS-GB1* or *LOS-sfGFP* genes were grown in Luria broth (LB) with 30 μg/mL kanamycin at 37 ℃ to obtain a cell culture with an OD_{600} of 0.5–0.6. These genes were overexpressed upon induction with 0.5 mM IPTG for 5 h at 30 ℃. The overexpressed cells were harvested by centrifugation, flash frozen by liquid N_2 and stored at -80 ℃ until further use.

The optimized genes encoding *GB1* and *sfGFP* were also synthesized by Sangon Biotechnology (Shanghai), and were cloned into pet28a plasmid between *NdeI* and *XhoI* restriction sites, respectively. Because these genes were cloned in-frame with the C-terminus of the His-tag of the pet28a plasmid, the expression products carried a His-tag fusion. The cells carrying the 6His-GB1 or 6His-sfGFP genes were expressed and harvested in a similar way as described above.

Sodium dodecyl sulfide-polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE experiments were conducted using the Invitrogen NuPAGE® Bis-Tris Mini Gels system. The protein samples were run through a 4-12% Bis-Tris 1.5 mm gel with MES SDS running buffer at 200 V.

Characterization of cytoplasmic and membrane extracts by SDS-PAGE. The harvested *E. coli* cells expressing the respective fusion proteins were resuspended in 10 mM PBS (pH 7.4) and lysed by sonication. After centrifugation (15000 rpm,15 min),

the membrane extracts (precipitate) were washed twice with TDSET buffer (1% Triton X-100, 0.2% sodium deoxycholate, 0.1% SDS, 10 mM tetrasodium EDTA, and 10 mM Tris/HCl) and then resuspended in 10 mM PBS (pH 7.4). The supernatant and resuspended precipitate were heated at 95 ℃ for 10 min, and then were subjected to SDS-PAGE analysis.

Protein purification. A total of 100 mL of bacterial culture expressing the LOS-GB1 fusion protein was washed thrice with 15 mL of washing buffer (0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl). The washed cells were resuspended in 50 mL of standard cleavage buffer (0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl, 1 mM $NiCl_2$) (OD_{600} = 2.0) and incubated on a rotator for 18 h at room temperature (25 ℃). The solution was then centrifuged at 6000 rpm for 5 min. The purified LOS-GB1 protein was obtained from the supernatant upon passing it through a 0.45 μm filter. The purification of LOS-sfGFP was carried out in a similar way as described for LOS-GB1.

Both 6His-GB1 and 6His-sfGFP proteins were purified by affinity chromatography following the manufacturer's instructions. Briefly, 1 L of bacterial culture was resuspended in 30 mL of lysis buffer (10 mM Tris-HCl, pH 7.4, 0.1 M NaCl, 1 mM PMSF, and 10% glycerol), and was lysed by sonication. After centrifugation (15000 rpm, 20 min), the supernatant was passed through a 0.45 μm filter and applied to a HisTrap HP column (5 mL, GE Healthcare). The column was equilibrated with 10% buffer B and eluted with 10% to 100% buffer B at five different column volumes (buffer A: 10 mM Tris-HCl, pH 7.4, 0.1 M NaCl, 10 mM β-ME; buffer B: buffer A with 500 mM imidazole).

All the purified proteins were characterized by MALDI-TOF MS (Bruker) and SDS-PAGE. The concentration of the purified protein was determined by performing the Bradford assay.[1] The purity and cleavage efficiency was quantified using the Imagelab software on the Bio-Rad ChemiDoc MP imagine system.[2]

Flow cytometric analysis of fluorescence intensity before and after Ni^{2+}-mediated

cleavage. The change in fluorescence intensity of *E. coli* cells was tested through the BD LSRFortessa cell analyzer. The washed cells were resuspended either in cleavage buffer (0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl, 1 mM $NiCl_2$) or washing buffer (0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl). After incubation on a rotor shaker for 18 h at 25 ℃, the cells were centrifuged and diluted to an OD_{600} of 0.5 in washing buffer before testing.

Characterization of recombinant bacteria through confocal microscopy. The confocal microscope images of *E. coli* cells were obtained using the Leica TCS SP8. The harvested *E. coli* cells expressing the LOS-sfGFP protein were resuspended in standard cleavage buffer (0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl, 1 mM $NiCl_2$) to obtain an OD_{600} of 1.0, and then were incubated on a rotor shaker at 25 ℃ for 18 h. The cells were then transferred onto a glass slide to visualize under the confocal microscope and the images were captured. *E. coli* cells resuspended in the buffer without Ni^{2+} (0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl) were prepared and tested subsequently. The wavelength of stimulating laser was set at 488 nm, and the emission was collected at a range of 500 to 600 nm of wavelength.

E. coli cells expressing the 6His-sfGFP were used as the control. The cells were prepared, and analyzed through the confocal microscope in a similar way as that of LOS-sfGFP cells.

Total luminescence spectra, the absolute quantum efficiency and fluorescence lifetime. The fluorescence properties of sfGFP and 6His-sfGFP were measured using the HORIBA FL-3 fluorescence spectrometer at 25 ℃. The buffer of the purified sfGFP and 6His-sfGFP proteins were exchanged with a new buffer (10 mM Tris-HCl, pH 7.4, 0.1 M NaCl) by passing through a desalting column (GE). Samples were analyzed at a concentration of 10 μM using the quartz cuvettes at 1 cm pathlength. For the total luminescence spectra and absolute quantum efficiency, xenon lamp was used as the source of excitation. However, for the fluorescence lifetime analysis, the LED with 366 nm spectra was used as the source of excitation.

Purification efficiency of GB1 under different conditions. We investigated the effect of pH, salt concentration, Ni^{2+} concentration, temperature, different types of buffer, and cell density on the purification efficiency of GB1 from the *E. coli* cell surface. Unless otherwise noted, the cell density (OD_{600}) during the cleavage incubation was ~2.0.

To analyze the effect of pH, 20 mL bacterial culture expressing LOS-GB1 fusion protein was washed thrice with 10 mL of buffer (0.1 M CHES, 0.1 M acetone oxime, 0.1 M NaCl) at different pH (6.0, 7.0, 8.0, 9.0, and 10.0). The washed cells were resuspended in 10 mL of buffer (0.1 M CHES, 0.1 M acetone oxime, 0.1 M NaCl, 1 mM $NiCl_2$) with the corresponding pH.

To analyze the effect of salt concentration, *E.coli* cells were washed thrice with 10 mL of buffer with the following composition: 0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl. Thereafter, the washed cells were resuspended in 10 mL of buffer with the following composition: 0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 1 mM $NiCl_2$, and with a gradient concentration of NaCl (0, 0.3, and 0.5 M), respectively. Purification with two different concentration of Ni^{2+} (0.5 and 2 mM) was conducted by resuspending the washed cells in 10 mL of buffer with the following composition: 0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl. We also examined the effect of reducing reagent on the purification efficiency by resuspending the washed cells in 10 mL of buffer with the following composition: 0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl, 0.5 mM TCEP.

To analyze the effect of different types of buffer, *E. coli* cells were washed thrice using 10 mL of solution (0.1 M acetone oxime, 0.1 M NaCl) with different buffers (0.1 M PBS, pH 8.0; 0.1 M HEPES, pH 8.2 or 0.1 M Tris-HCl, pH 8.6). The washed cells were resuspended in 10 mL of cleavage solution (0.1 M acetone oxime, 0.1 M NaCl, 1 mM $NiCl_2$) comprising corresponding types of buffer.

To analyze the effect of cell density (OD_{600}) on the cleavage reaction, we tested the cleavage efficiency of GB1 under different cell densities (2.0, 4.0, 8.0, and 16.0). *E. coli* cells were washed thrice with 10 mL of washing buffer (0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl). The washed cells were resuspended in standard

cleavage buffer (0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl, 1 mM $NiCl_2$) at various cell densities ranging from 2.0 to 16.0. After cleavage, all of the incubated samples were diluted to a final OD_{600} of 2.0 and then analyzed using SDS-PAGE.

All the resuspended samples were incubated on a rotor shaker for 18 h at 25 ℃. After centrifugation, the supernatants and precipitates were analyzed by SDS-PAGE.

Additionally, the purification of GB1 at 4 ℃ using the standard cleavage buffer (0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl, 1 mM $NiCl_2$) was also analyzed.

SI Figures

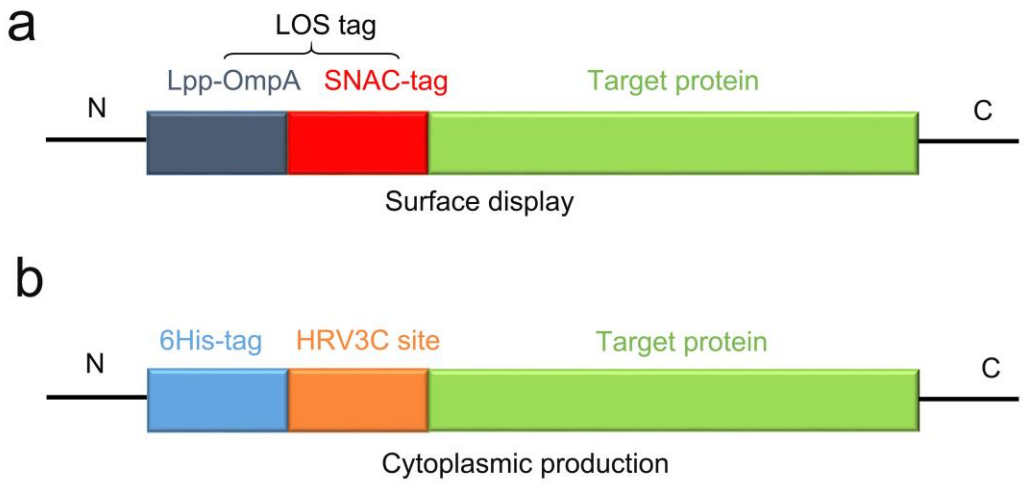

Figure S1. Schematic representation of the genetic organization of *Escherichia coli* (a) surface display, and (b) cytoplasmic production of target protein.

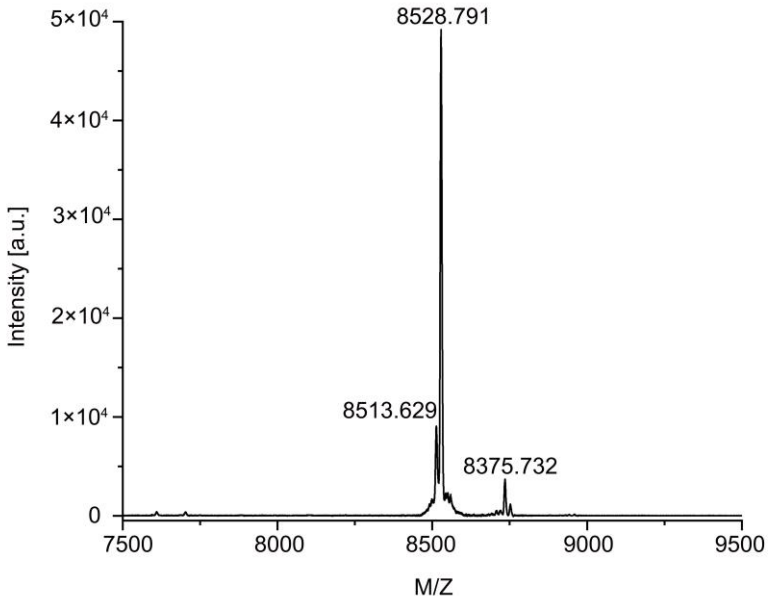

Figure S2. MALDI-TOF mass spectra of 6His-GB1 (calc. 8529.263 Da, obsd. 8528.791 Da) that was purified using metal affinity chromatography. 2, 5-Dihydroxybenzoic acid was used as the matrix and the spectra were acquired in a positive mode.

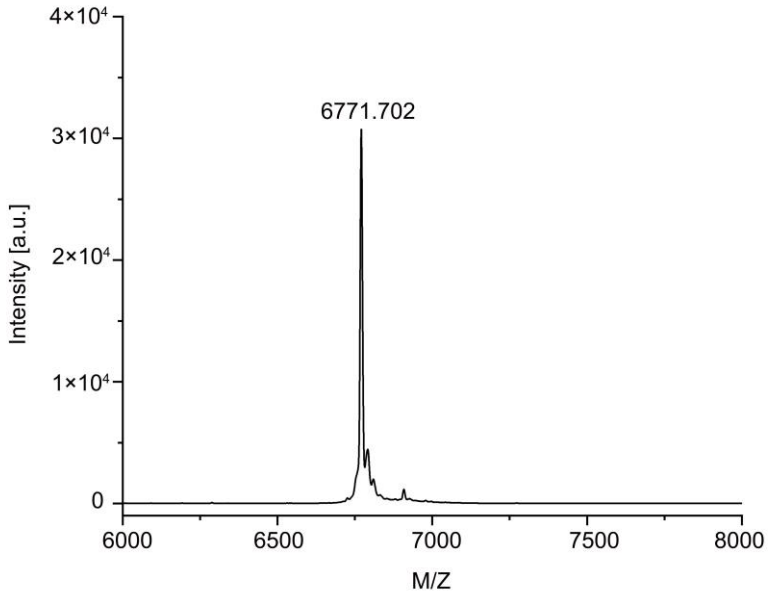

Figure S3. MALDI-TOF mass spectra of GB1 (calc. 6770.394 Da, obsd. 6771.702 Da) that was purified using Ni^{2+} cleavage from the *Escherichia coli* cell surface. 2, 5-Dihydroxybenzoic acid was used as the matrix and the spectra were acquired in a positive mode.

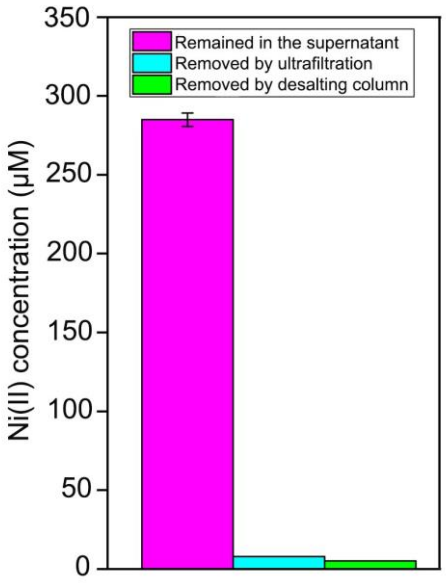

Figure S4. Concentration of Ni^{2+} in purified GB1 solution. Approximately 98% of excess Ni^{2+} could be removed by conducting ultrafiltration (MW: 3 K, Millipore) and using desalting column (GE Healthcare).

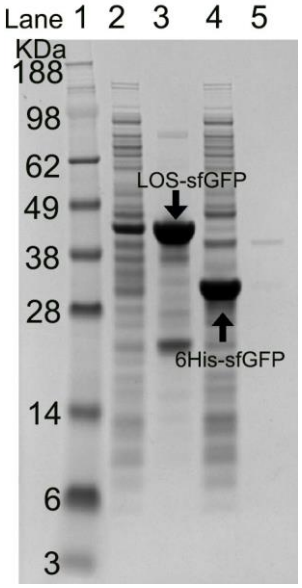

Figure S5. The cytoplasmic and membrane extracts of *Escherichia coli* cells expressing LOS-sfGFP (lane 2 and 3) and 6His-sfGFP (lane 4 and 5). Lane 1: MW marker; lane 2 and 4: cytoplasmic extracts; lane 3 and 5: membrane extracts.

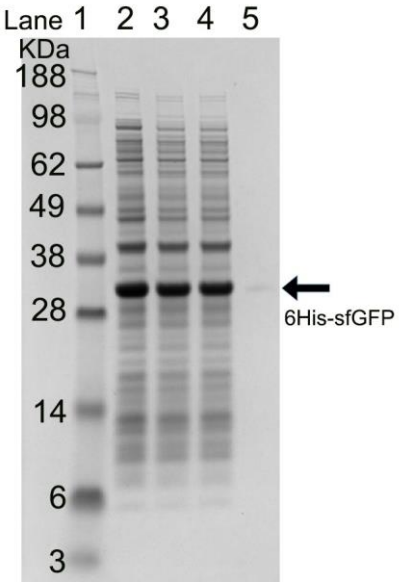

Figure S6. Control purification by Ni^{2+} cleavage from the surface of *Escherichia coli* cells expressing 6His-sfGFP. Lane 1: MW marker; lane 2 and 3: cell extract before and after purification, respectively; lane 4 and 5: precipitate and supernatant after Ni^{2+} cleavage from the cell surface, respectively.

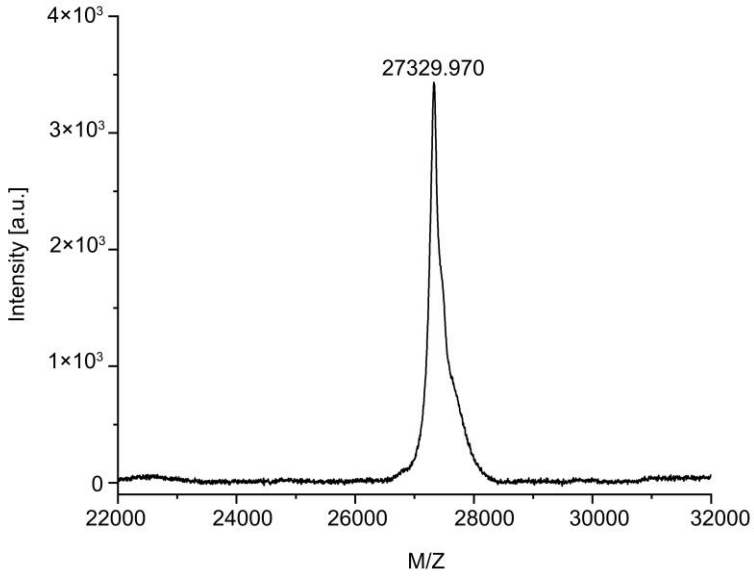

Figure S7. MALDI-TOF mass spectra of sfGFP (calc. 27328.710 Da, obsd. 27329.970 Da) that was purified from the *Escherichia coli* cell surface using Ni^{2+} cleavage. 2, 5-Dihydroxybenzoic acid was used as the matrix and the spectra were acquired in a positive mode.

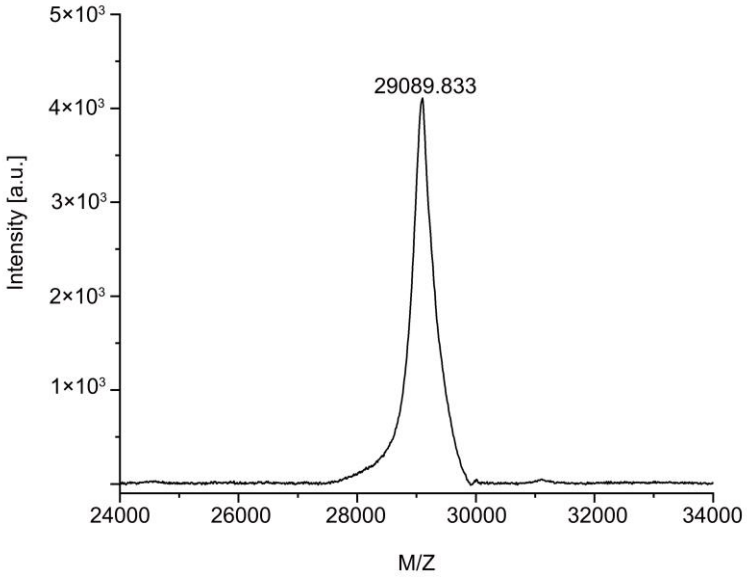

Figure S8. MALDI-TOF mass spectra of 6His-sfGFP (calc. 29087.580 Da, obsd. 29089.833 Da) that was purified by metal affinity chromatography. 2, 5-Dihydroxybenzoic acid was used as the matrix and the spectra were acquired in a positive mode.

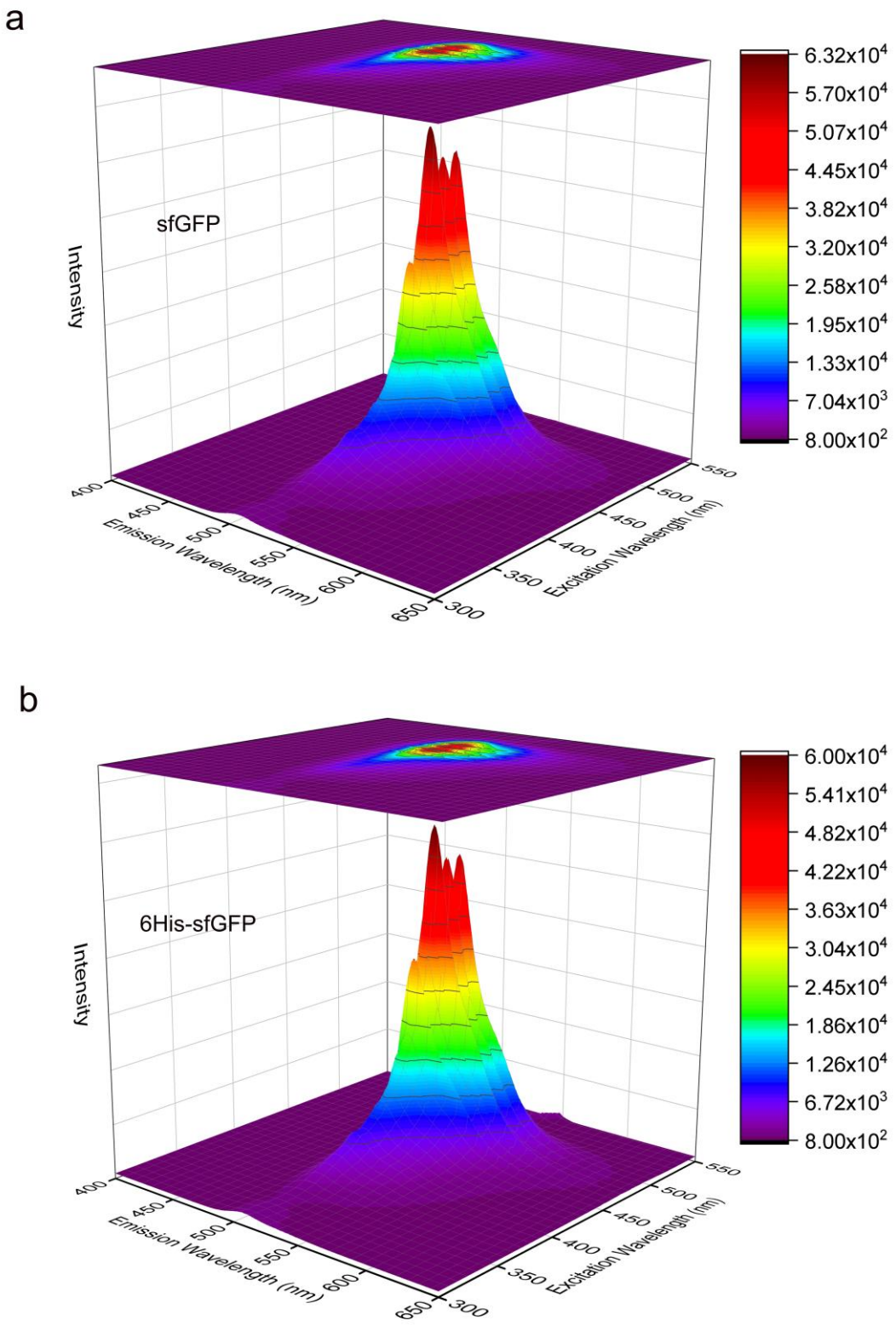

Figure S9. Comparison of 3D total luminescence spectra between (a) sfGFP purified by Ni^{2+} cleavage from *E. coli* cell surface and (b) 6His-sfGFP purified by metal affinity chromatography. Proteins were analyzed at a concentration of 10 μM in the buffer containing 10 mM Tris-HCl (pH 7.4) and 0.1 M NaCl.

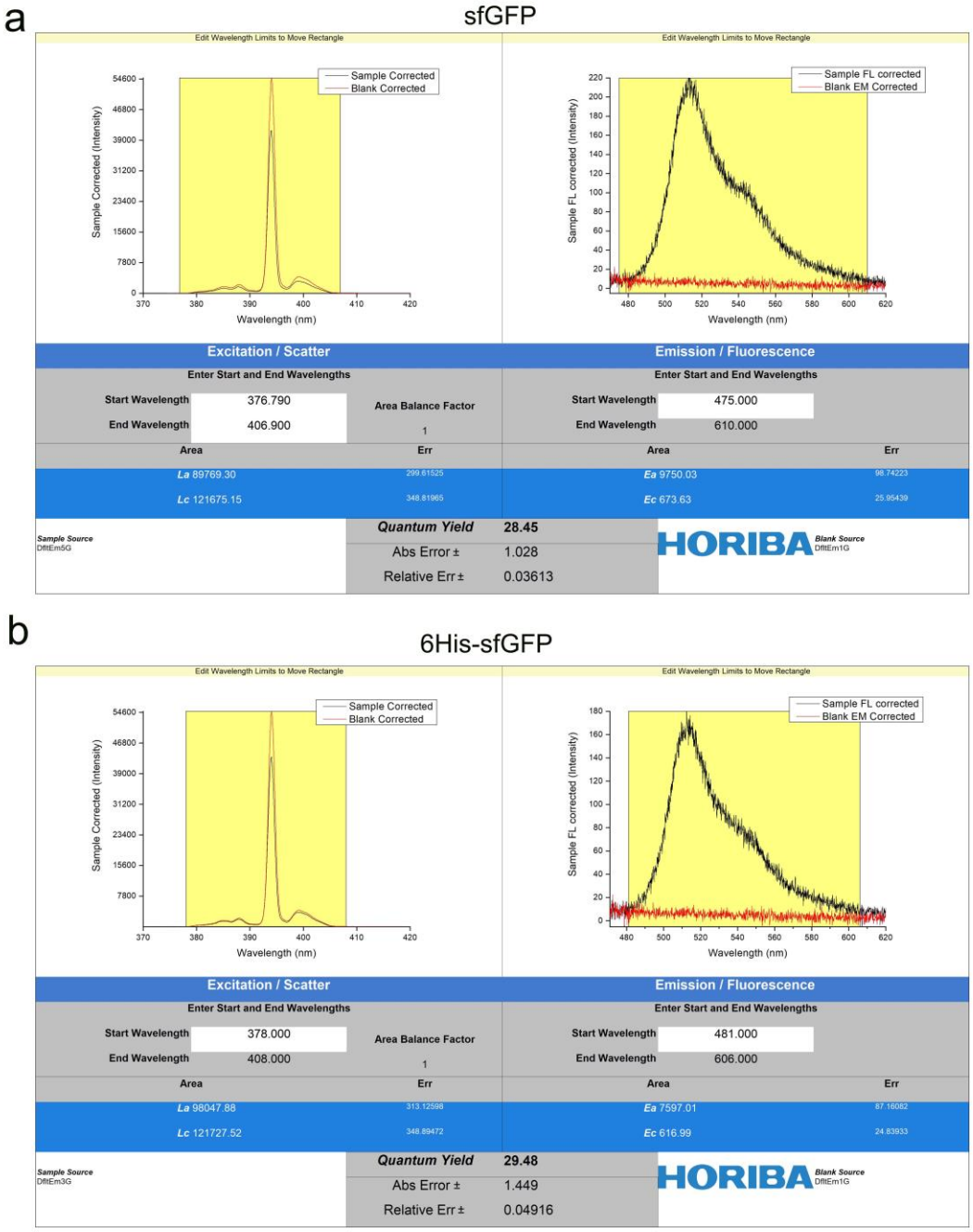

Figure S10. Absolute quantum efficiency of (a) sfGFP purified by Ni^{2+} cleavage from the *Escherichia coli* cell surface and (b) 6His-sfGFP purified by metal affinity chromatography.

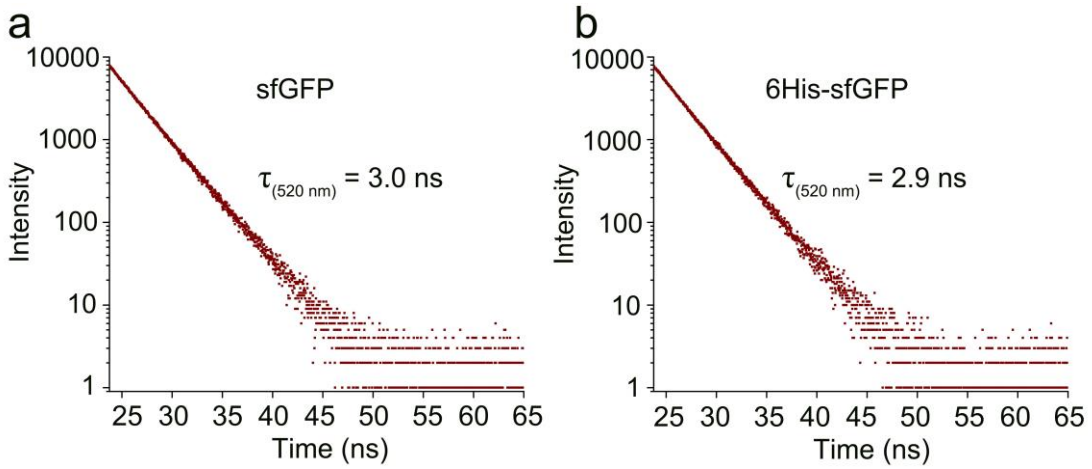

Figure S11. Transient fluorescence decays of (a) sfGFP purified by Ni^{2+} cleavage from the *Escherichia coli* cell surface and (b) 6His-sfGFP purified by metal affinity chromatography.

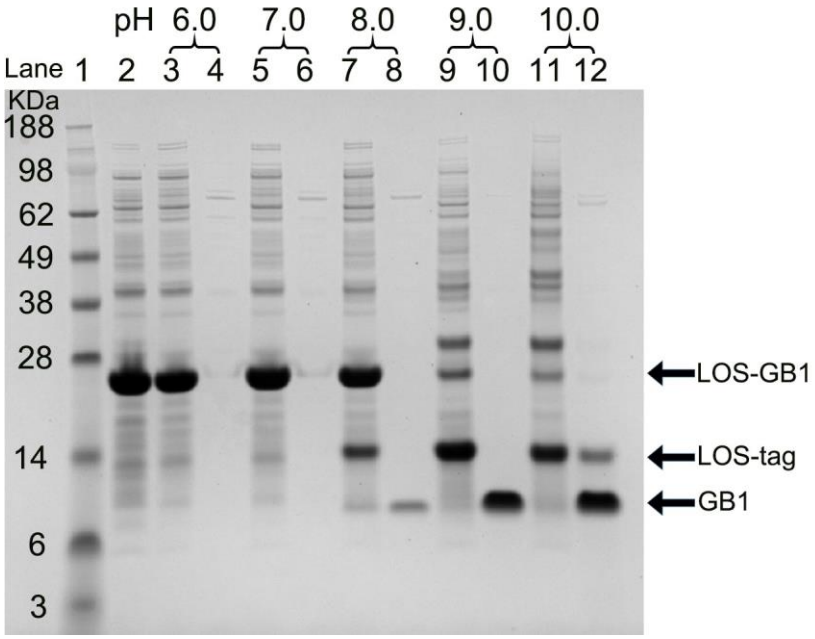

Figure S12. Purification of GB1 from the *Escherichia coli* cell surface at different pH values. Purification conditions: 0.1 M CHES (6.0–10.0 pH range), 0.1 M acetone oxime, 0.1 M NaCl, 1 mM Ni^{2+}, 25 ℃, 18 h. Lane 1: MW marker; lane 2: control sample of *E. coli* cell extract before purification; lanes 3, 5, 7, 9, and 11: precipitates after Ni^{2+} cleavage from the cell surface at different pH; lanes 4, 6, 8, 10, and 12: supernatant after Ni^{2+} cleavage from the cell surface at different pH.

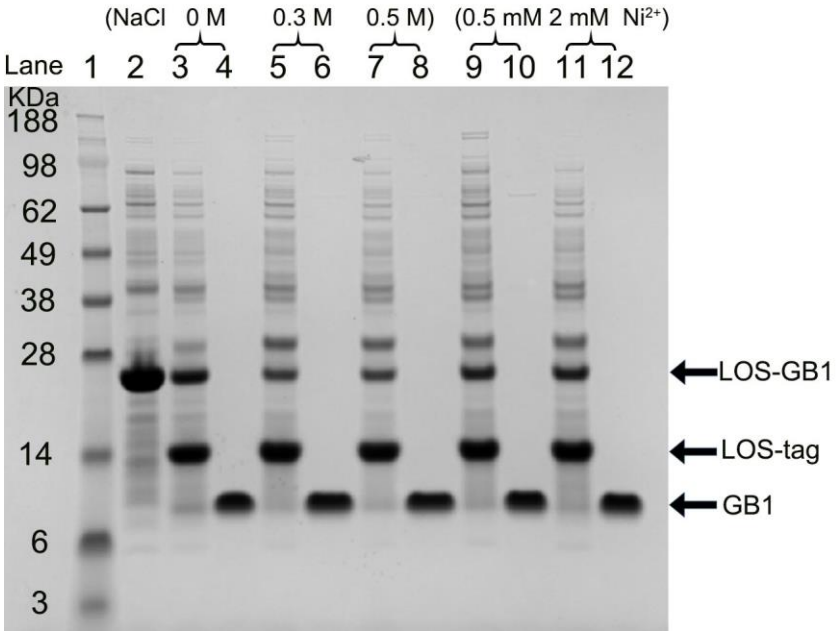

Figure S13. Purification of GB1 from the *Escherichia coli* cell surface at different concentrations of NaCl or Ni^{2+}. Lane 1: MW marker; lane 2: control sample of *E. coli* cell extract before purification. Lanes 3–8 represents the extracts purified under different NaCl concentrations. Purification conditions: 0.1 M CHES, pH 8.6, 0.1 M acetone oxime, NaCl (0–0.5 M), 1 mM Ni^{2+}, 25 ℃, 18 h. Lanes 3, 5, and 7: the precipitate after Ni^{2+} cleavage from the cell surface; lanes 4, 6, and 8: the supernatant after Ni^{2+} cleavage from the cell surface. Lanes 9–12 represents the extracts purified under different Ni^{2+} concentrations. Purification conditions: 0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl, Ni^{2+} (0.5 and 2 mM), 25 ℃, 18 h. Lanes 9 and 11: precipitates after Ni^{2+} cleavage from the cell surface at 0.5 and 2 mM concentrations of Ni^{2+}, respectively; lanes 10 and 12: supernatants after Ni^{2+} cleavage from the cell surface at 0.5 and 2 mM concentrations of Ni^{2+}, respectively.

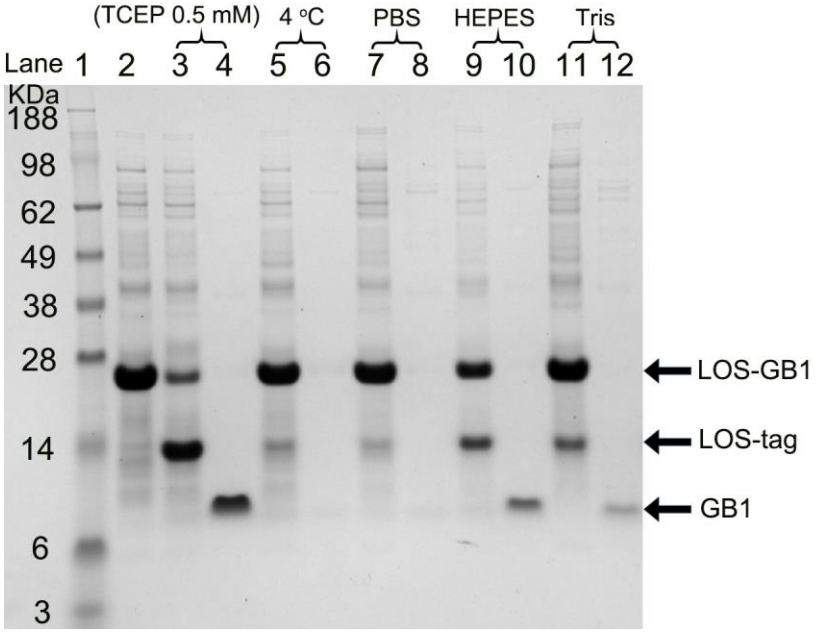

Figure S14. Purification of GB1 from the *Escherichia coli* cell surface in a reducing environment, 4 ℃, or with different types of buffers. Lane 1: MW marker; lane 2: control sample of *E. coli* cell extract before purification. Lanes 3 and 4: the precipitate and supernatant after Ni^{2+} cleavage from cell surface in reducing environment, respectively. Purification conditions: 0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl, 1 mM Ni^{2+}, 0.5 mM TCEP, 25 ℃, 18 h. Lanes 5 and 6: the precipitate and supernatant after Ni^{2+} cleavage from cell surface at 4 ℃, respectively. Purification conditions: 0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl, 1 mM Ni^{2+}, 4 ℃, 18 h. Lanes 7 and 8: the precipitate and supernatant after Ni^{2+} cleavage from cell surface in PBS buffer, respectively. Purification conditions: 0.1 M PBS, pH 8.0, 0.1 M acetone oxime, 0.1 M NaCl, 1 mM Ni^{2+}, 25 ℃, 18 h. Lanes 9 and 10: the precipitate and supernatant after Ni^{2+} cleavage from cell surface in HEPES buffer, respectively. Purification conditions: 0.1 M HEPES, pH 8.2, 0.1 M acetone oxime, 0.1 M NaCl, 1 mM Ni^{2+}, 25 ℃, 18 h. Lanes 11 and 12: the precipitate and supernatant after Ni^{2+} cleavage from the cell surface in Tris buffer, respectively. Purification conditions: 0.1 M Tris, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl, 1 mM Ni^{2+}, 25 ℃, 18 h.

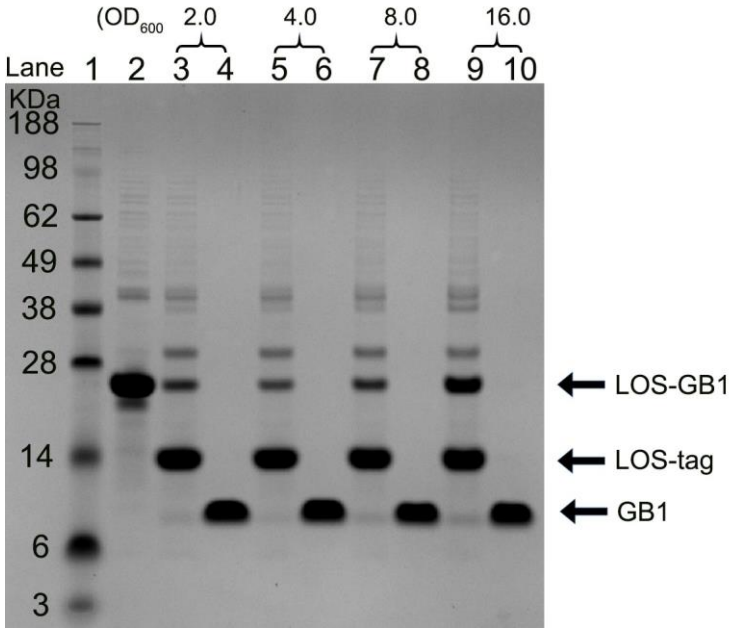

Figure S15. Purification of GB1 from the *Escherichia coli* cell surface at different cell densities. Purification conditions: 0.1 M CHES, pH 8.6, 0.1 M acetone oxime, 0.1 M NaCl, 1 mM Ni^{2+}, 25 ℃, 18 h. Lane 1: MW marker; lane 2: *E. coli* cell extract before purification (control); lanes 3, 5, 7, and 9: precipitates after Ni^{2+} cleavage from the cell surface; lanes 4, 6, 8, and 10: supernatants after Ni^{2+} cleavage from the cell surface. The cleavage efficiency of LOS-GB1 at a cell density (OD_{600}) of 2.0, 4.0, 8.0, and 16.0 was 80%, 85%, 77%, and 60%, respectively. To determine cleavage efficiency, data were quantified using the Imagelab software on the Bio-Rad ChemiDoc MP imaging system.

References

1 Bradford, M. M. *Anal. Biochem.*, 1976, ***72***, 248-254.

2 Dang, B.; Mravic, M.; Hu, H.; Schmidt, N.; Mensa, B.; DeGrado, W. F. *Nature methods*, 2019, ***16***, 319-322.