Supplementary Information for:

# Specific modification at the C-terminal lysine residue of the green fluorescent protein variant, GFPuv, expressed in *Escherichia coli*

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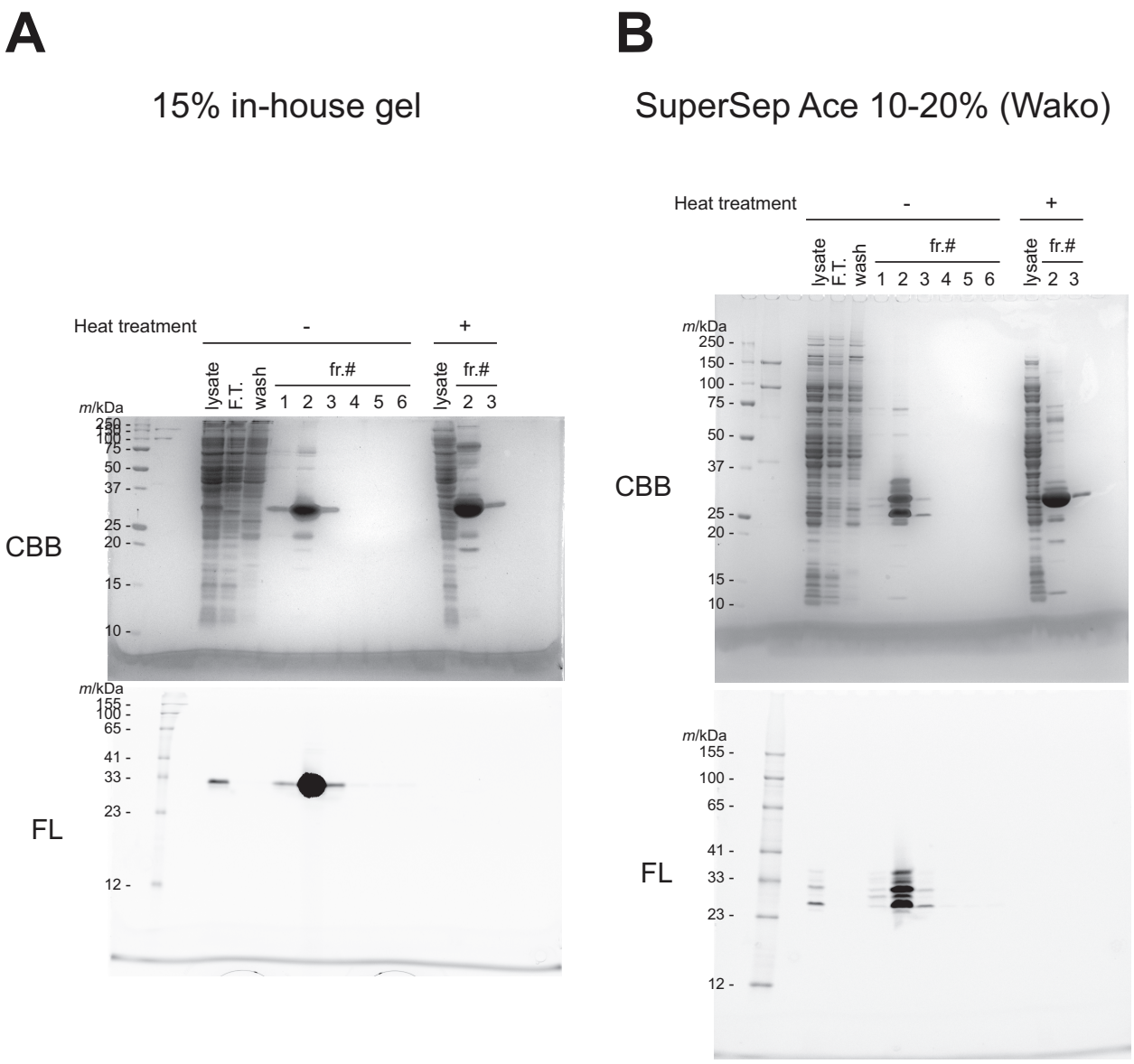
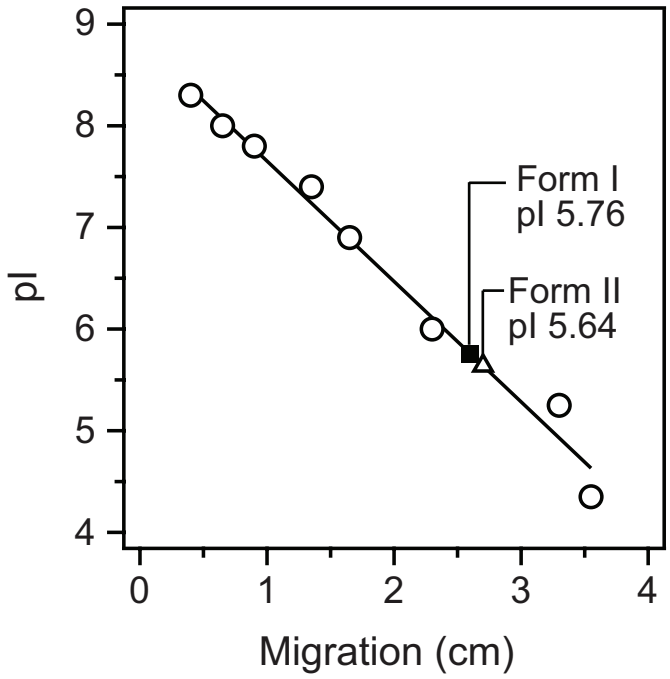
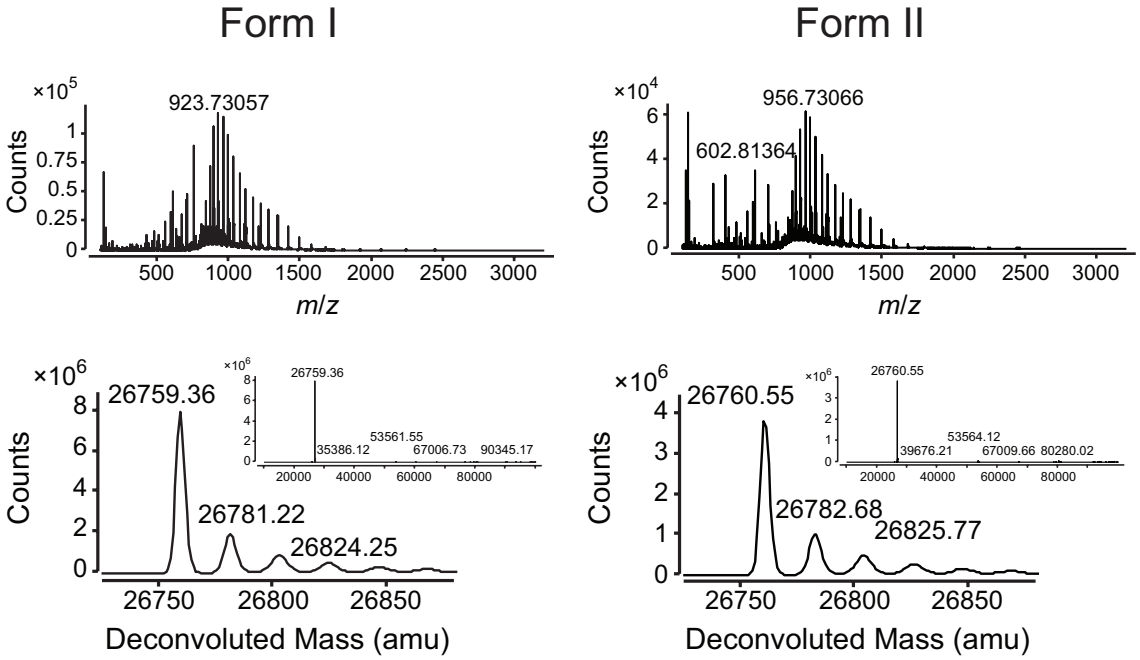


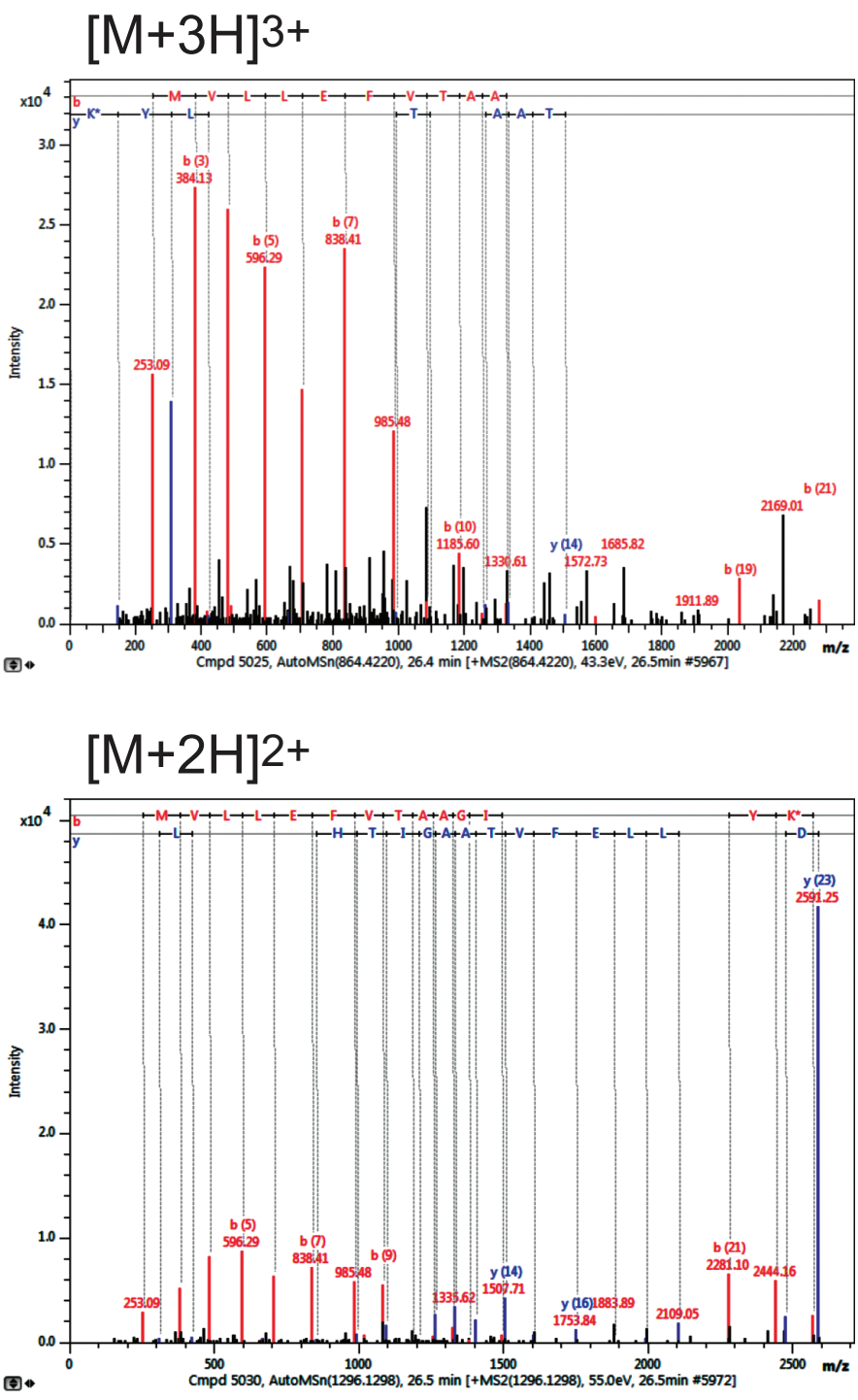

**Supplementary Figure S1.** Gel dependency of the separation of GFPuv form I and form II by SDS-PAGE. SDS-PAGE analysis of the fractions from a Ni-NTA column during the purification of HFT-GFPuv on a 15% in-house gel (A) and a precast 10–20% gradient gel (B).




**Supplementary Figure S2.** Estimation of the isoelectric point (pI) of GFPuv form I and form II using standard proteins. pIs of the standard proteins are plotted against their migration distance from the cathode on the gel in Fig. 3C. Cytochrome c from pig heart (pI 10.7), ribonuclease A from bovine pancreas (pI 9.5), a lectin from *Lens culinaris* (pI 8.3, 8.0, 7.8), myoglobin from horse muscle (pI 7.4, 6.9), carbonic anhydrase from bovine erythrocytes (pI 6.0), β-lactoglobulin from bovine milk (pI 5.3, 5.2), trypsin inhibitor from soybean (pI 4.5), glucose oxidase from *Aspergillus niger* (pI 4.2), amyloglucosidase from *Aspergillus niger* (pI 3.5). The closed square and open triangle indicates the position of GFPuv form I and form II, respectively.

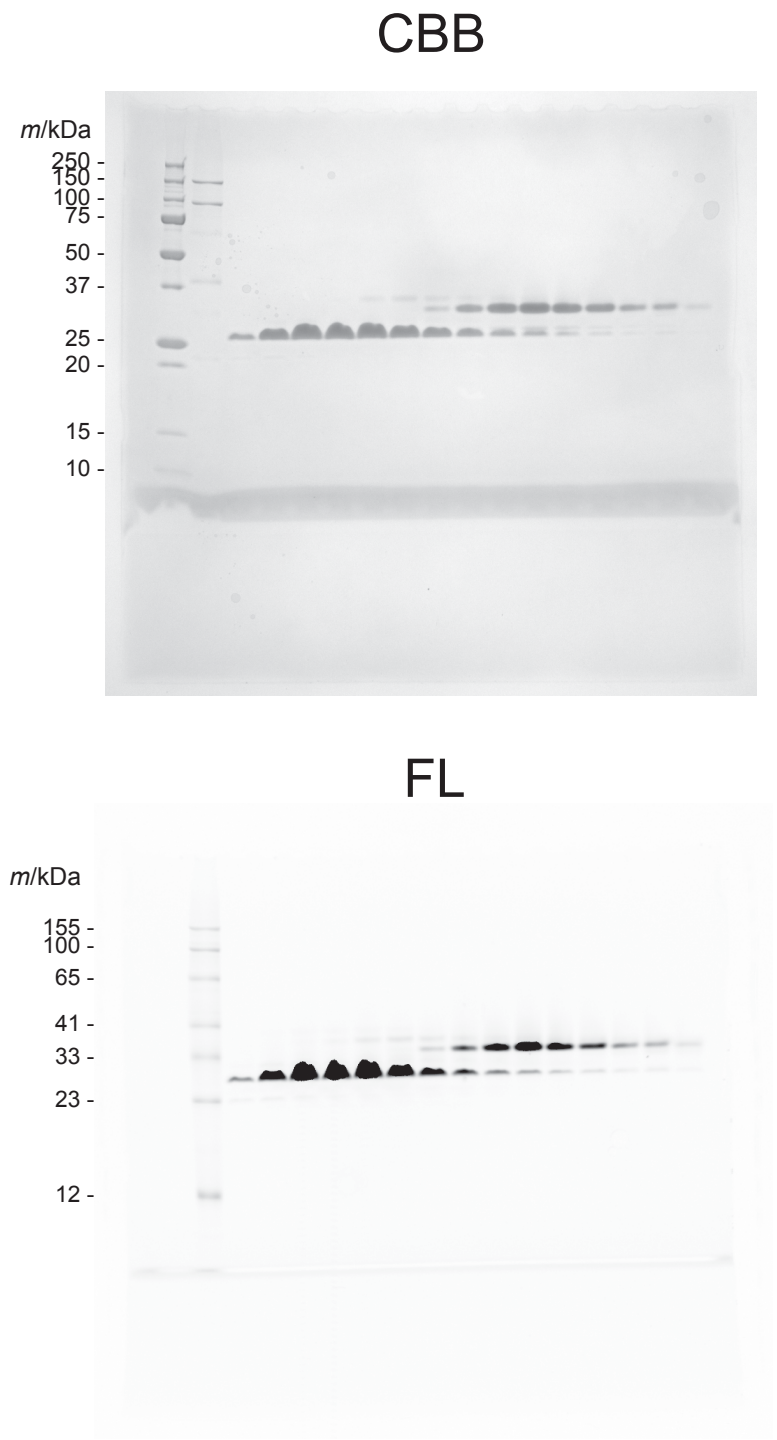



**Supplementary Figure S3.** ESI-MS spectra of GFPuv form I and form II. Mass spectra (*top*) and deconvoluted mass spectra (*bottom*) of the intact GFPuv form I and form II are shown. Estimated mass values of form I and form II are 26759.36 and 26760.55, respectively.




**Supplementary Figure S4.** Top-down sequencing of the $H_{231}$GMDELYK$_{238}$ fragment of GFPuv form II using MALDI In-Source-Decay. Mass spectra for $[M+3H]^{3+}$ (top) and $[M+2H]^{2+}$ (bottom) ions are shown. Identified b-ions (red) and y-ions (blue), as well as unidentified signals (black), are indicated in the spectra.


**Supplementary Figure S5.** Structure-based amino acid sequence alignment of GFP and its related proteins. AvGFP and AcGFP are derived from *Aequorea victoria* and *Aequorea coerulescens*, respectively. UniProtKB accession numbers are as follows: AvGFP (P42212), EGFP (C5MKY7), and DsRed (Q9U6Y8). The GenBank accession number of GFPuv is AAC53663.1. The sequence of AcGFP was obtained from Clontech Laboratories Inc. (http://www.clontech.com). The sequences were aligned with the PROMALS3D multiple sequence and structure alignment server [1] based on the structures of GFPuv (PDB ID: 1B9C) and DsRed (PDB ID: 1GGX).




**Supplementary Figure S6.** The original gel images displayed on Figure 3A. Fluorescent bands (*bottom*) were visualized under ultraviolet light without any staining processes. After the detection of the fluorescent signal, the same gel was stained with CBB (*top*).

## Reference for Supplementary Information

1 Pei, J., Kim, B. H. & Grishin, N. V. PROMALS3D: a tool for multiple protein sequence and structure alignments. *Nucleic acids research* **36**, 2295-2300, doi:10.1093/nar/gkn072 (2008).