CHEMBIOCHEM

Supporting Information

DOI: 10.1002/cbic.200800703

Supporting Information

for

Position-Specific Incorporation of Fluorescent Non-natural Amino Acids into Maltose-Binding Protein for Detection of Ligand Binding by FRET and Fluorescence Quenching

Issei Iijima and Takahiro Hohsaka*

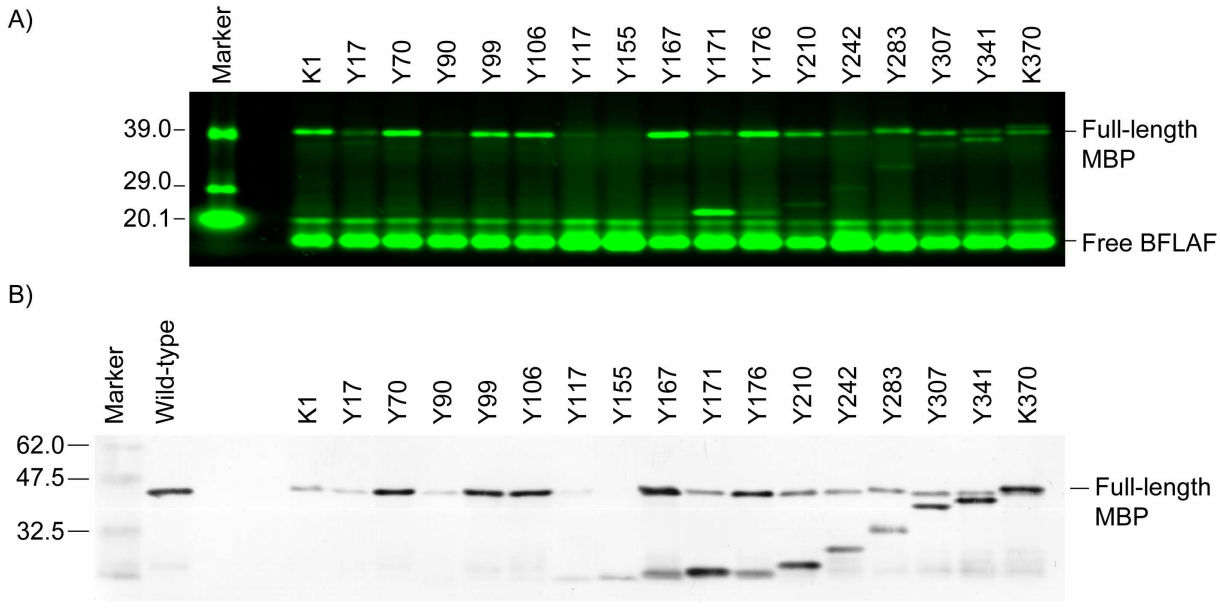

Figure S1. A) Fluorescence image of SDS-PAGE gel and B) Western blotting using anti-T7 tag antibody for unpurified MBPs containing BFLAF at Lys1, 15 tyrosines, and Lys370. BFLAF-containing MBPs were detected with excitation at 488 nm and emission at 520 nm.

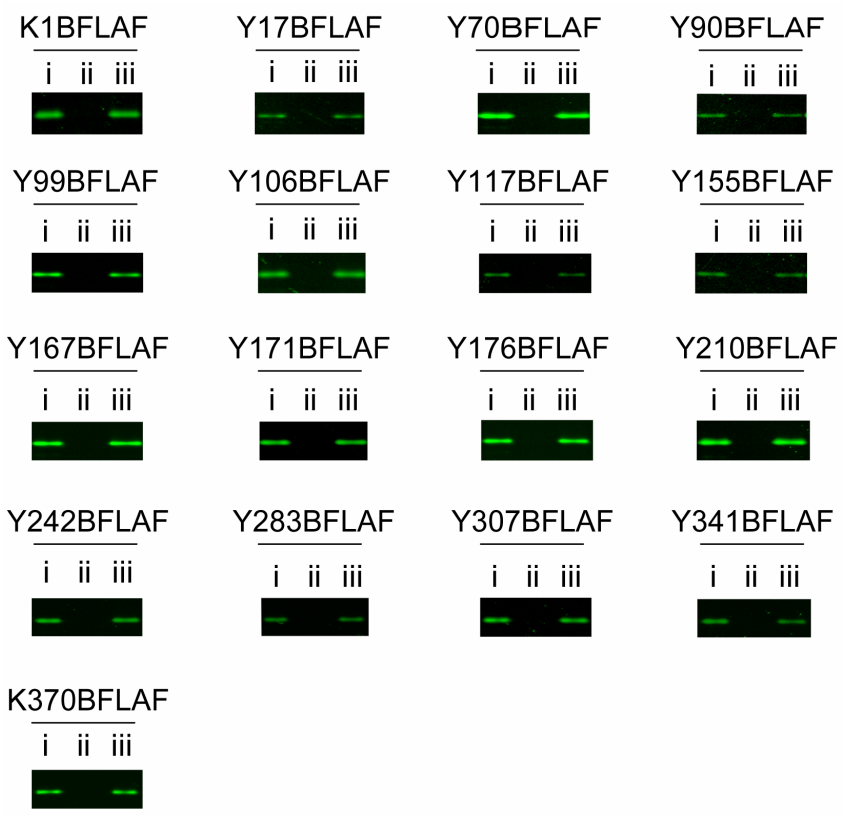

Figure S2. Amylose bead assay for purified BFLAF-containing MBPs. Each lane contained purified MBP (i), and supernatants of the mixture of the purified MBP and amylose beads in the absence of maltose (ii) and in the presence of maltose (iii).

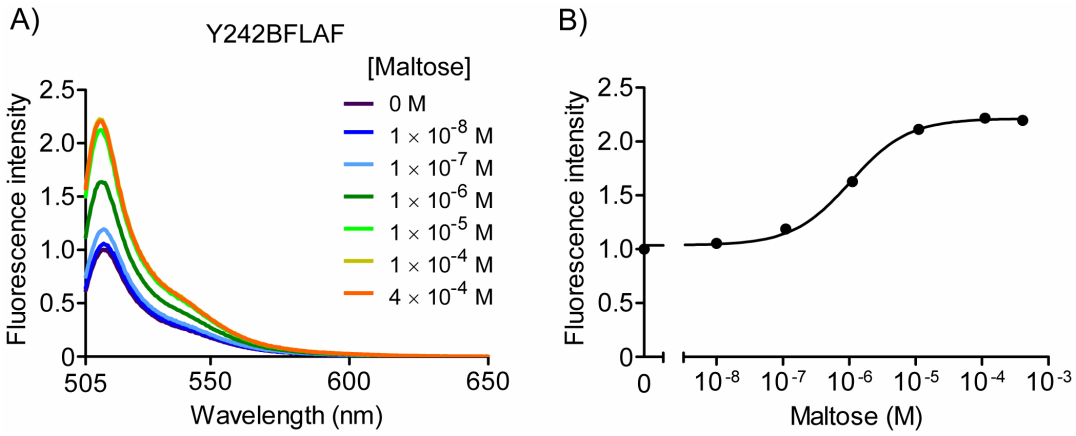

Figure S3. A) Fluorescence spectra and B) titration curve of Y242BFLAF with excitation at 490 nm in the absence and presence of maltose.

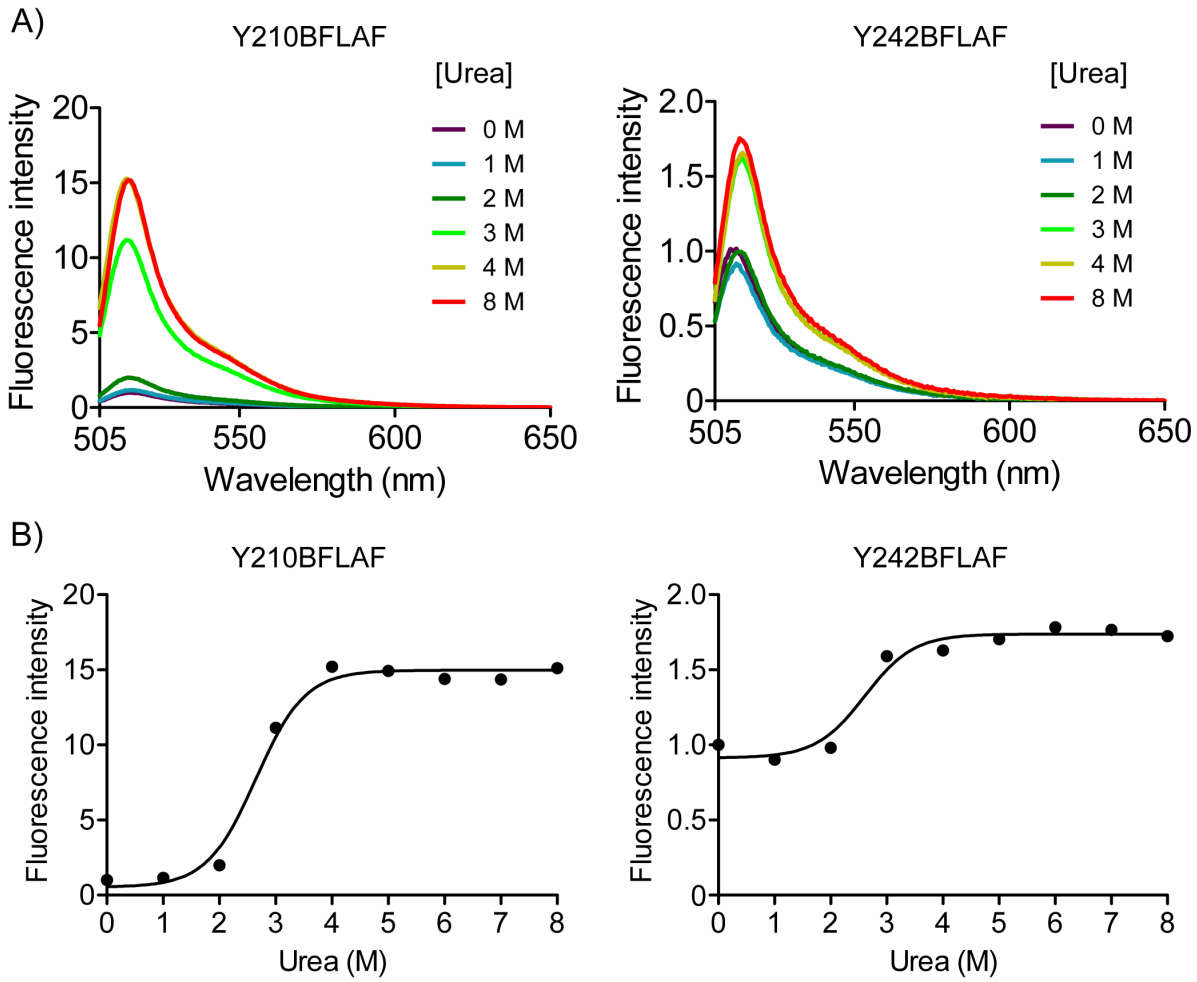

Figure S4. A) Fluorescence spectra and B) titration curves of Y210BFLAF and Y242BFLAF with excitation at 490 nm in the absence and presence of urea. Fluorescence intensities are relative values compared with those in the absence of urea.

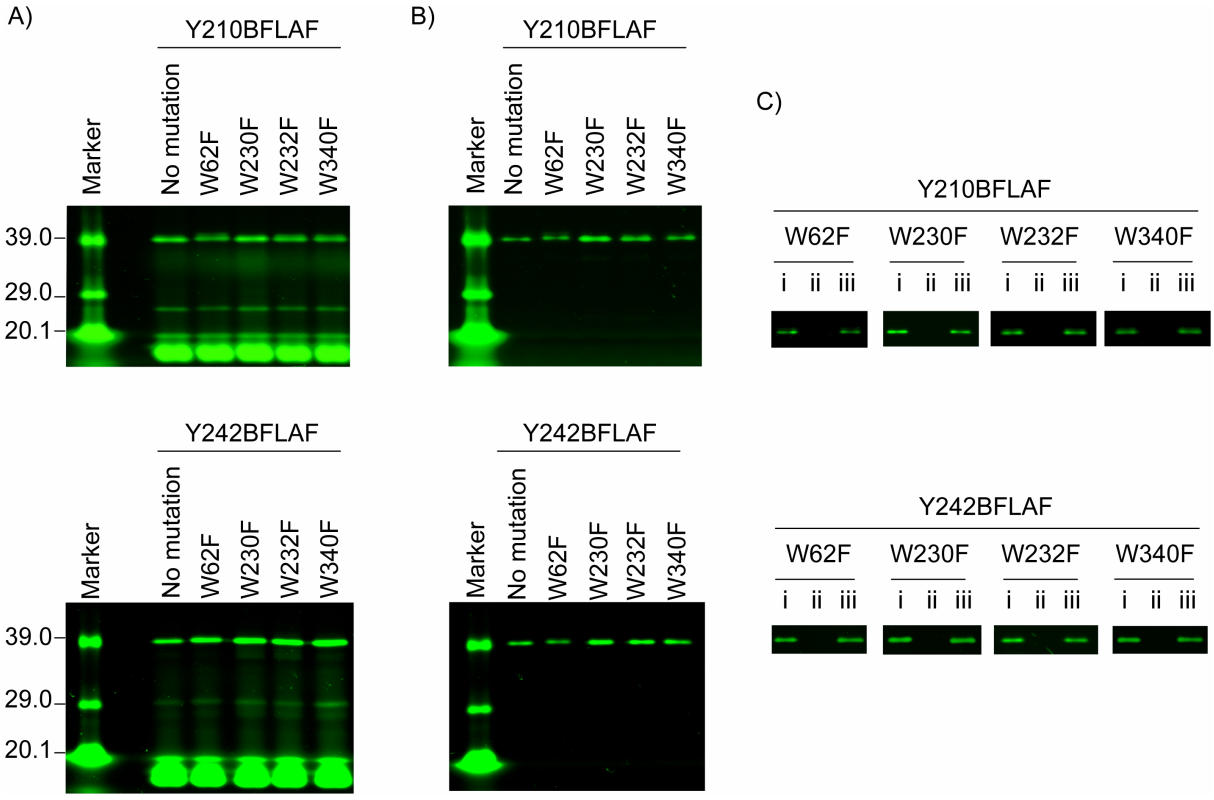

Figure S5. A, B) Fluorescence images of SDS-PAGE gel for A) unpurified and B) purified BFLAF-containing MBPs with tryptophan to phenylalanine substitutions. C) Amylose bead assay for the purified BFLAF-containing MBPs. Each lane contained purified MBP (i), and supernatants of the mixture of the purified MBP and amylose beads in the absence of maltose (ii) and in the presence of maltose (iii).

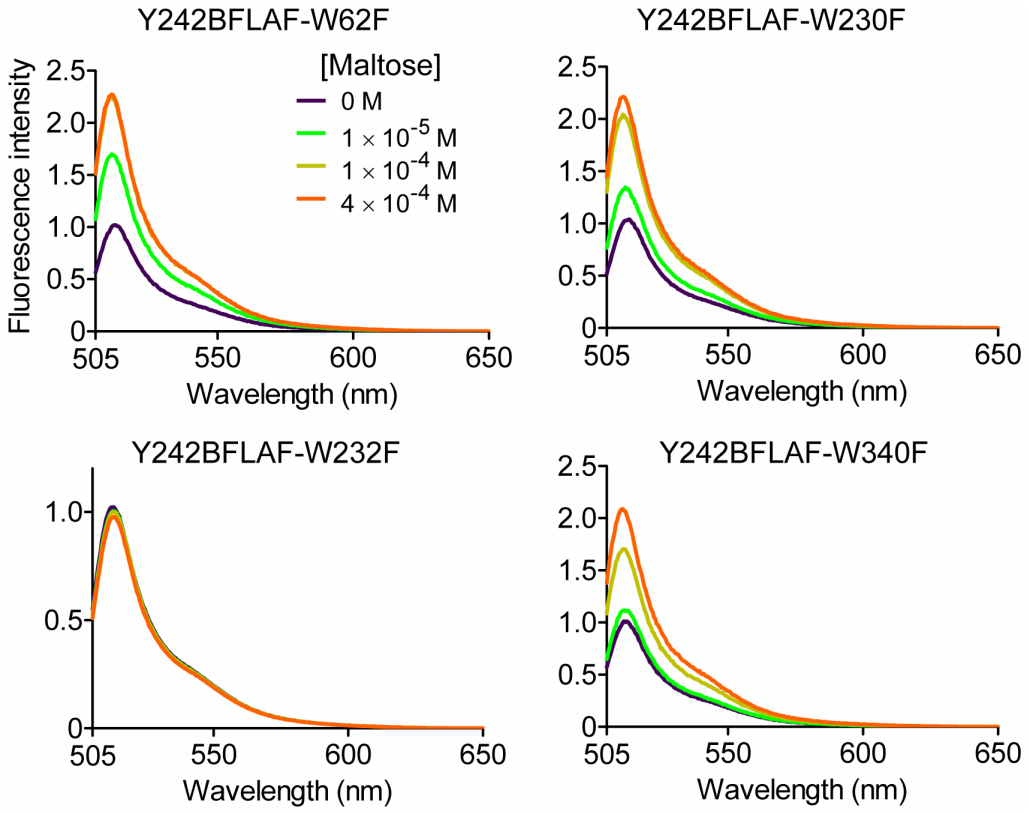

Figure S6. Fluorescence spectra of Y242BFLAF containing the W62F, W230F, W232F, or W340F mutation with excitation at 490 nm in the absence and presence of maltose.

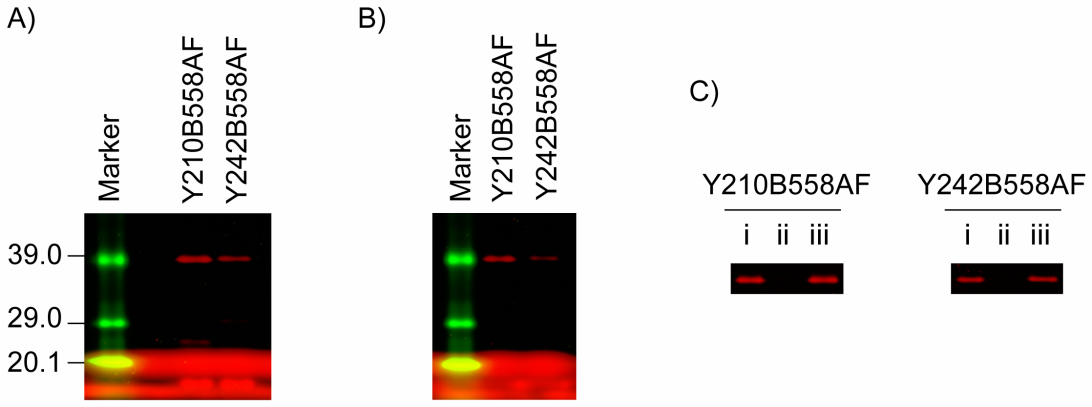

Figure S7. A, B) Fluorescence images of SDS-PAGE gel for A) unpurified and B) purified B558AF-containing MBPs at Tyr210 or Tyr242. C) Amylose bead assay for the purified B558AF-containing MBPs. MBPs were detected with excitation at 532 nm and emission at 605 nm. Each lane contained purified MBP (i), and supernatants of the mixture of the purified MBP and amylose beads in the absence of maltose (ii) and in the presence of maltose (iii).

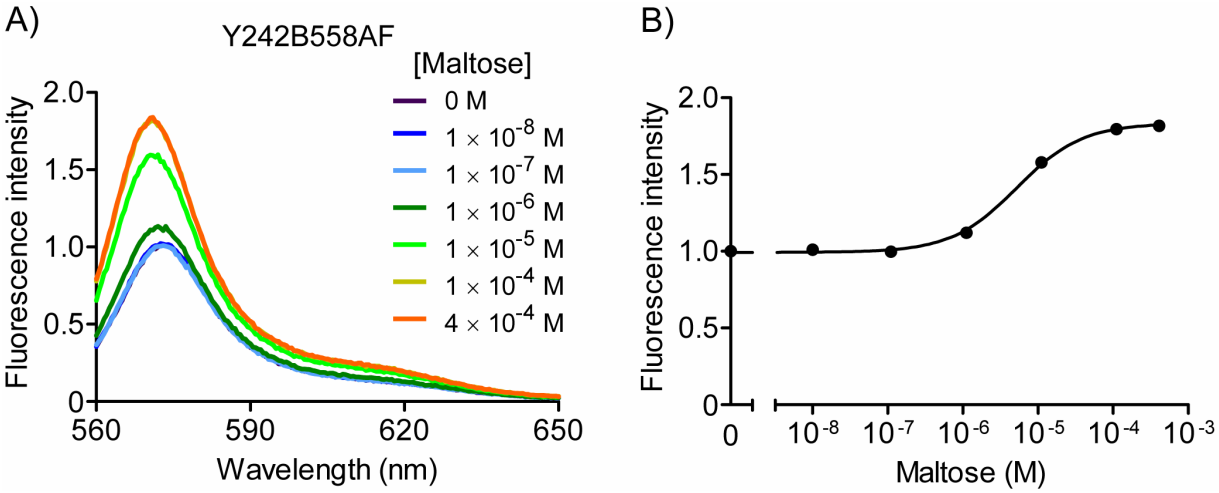

Figure S8. A) Fluorescence spectra and B) titration curve of Y242B558AF with excitation at 545 nm in the absence and presence of maltose.

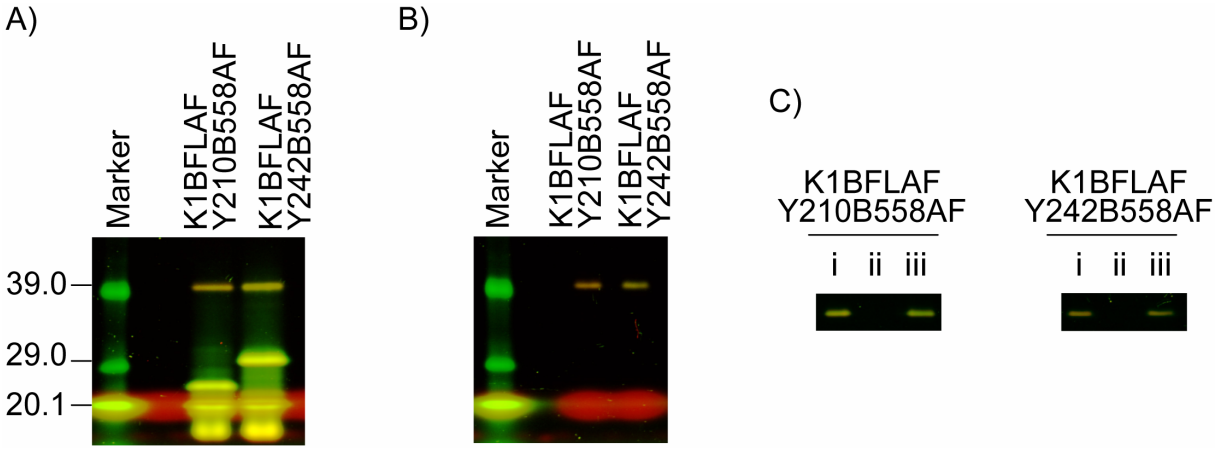

Figure S9. A, B) Fluorescence images of SDS-PAGE gel for A) unpurified and B) purified MBPs containing both BFLAF and B558AF. C) Amylose bead assay for purified MBPs containing both BFLAF and B558AF. MBPs were detected with excitation and emission at 488 nm and 520 nm (green) and at 532 nm and 605 nm (red). Each lane contained purified MBP (i), and supernatants of the mixture of the purified MBP and amylose beads in the absence of maltose (ii) and in the presence of maltose (iii).

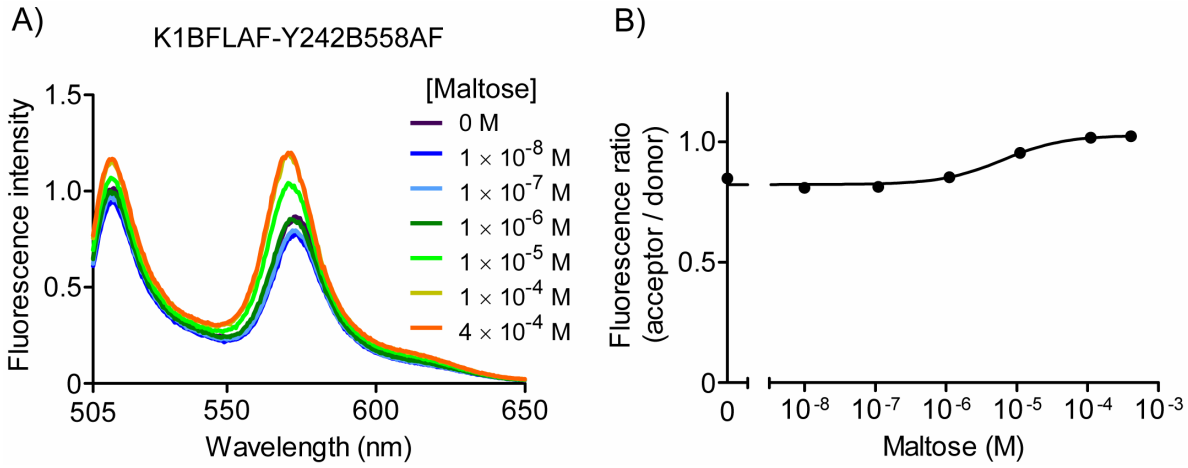

Figure S10. A) Fluorescence spectra of K1BFLAF-Y242B558AF with excitation at 490 nm in the absence and presence of maltose. B) Titration curve of fluorescence intensity ratio at 511 nm and 572 nm.

Table S1. Relative fluorescence intensities for BFLAF-containing MBPs in the absence and presence of maltose (4×10^{-4} M).[a]

Position	no maltose	+ maltose
K1	1.00	1.03
Y17	2.75	2.74
Y70	0.44	0.44
Y90	1.27	0.91
Y99	1.05	0.92
Y106	3.20	2.92
Y117	1.98	1.72
Y155	0.78	0.73
Y167	1.64	1.52
Y171	2.00	1.80
Y176	0.63	0.65
Y210	0.10	0.92
Y242	0.59	1.29
Y283	3.29	2.88
Y307	1.86	1.56
Y341	0.71	0.81
K370	0.52	0.56

[a] Relative fluorescence intensities were estimated by correcting the fluorescence intensities at maximum emission wavelength with relative concentrations of the purified MBPs that were determined from the fluorescence band intensities on the SDS-PAGE.