# Comparative studies of the serum half-life extension of a protein via site-specific conjugation to a species-matched or -mismatched albumin

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## Electronic Supplementary Information (ESI)

**Circular dichroism analysis of sfGFP-WT and sfGFP-AzF**

The circular dichroism spectra of sfGFP-WT and sfGFP-AzF were measured by Jasco J-815 circular dichroism spectropolarimeter. The protein sample with a concentration of 50 μM in 10 mM sodium phosphate (pH 7.0) was loaded in a 1-mm path length quartz cuvette. The instrument detection was performed at 50 nm/min of scanning speed, and 30-time accumulations, and the scanning range from 190 to 250 nm. The sample spectra were deconvoluted by Spectra Measurement Data Analysis program (Jasco, Oklahoma City, OK).

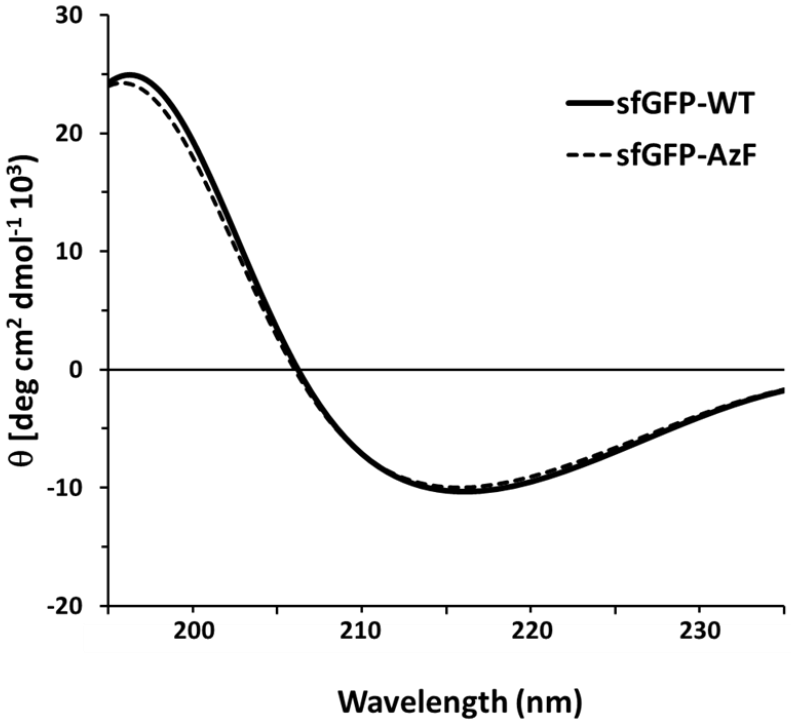
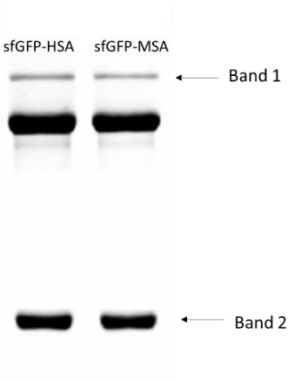



**Figure S1.** CD spectra of sfGFP-WT (solid) and sfGFP-AzF (dashed).

Circular dichroism (CD) spectroscopy of sfGFP-WT and sfGFP-AzF was performed to investigate structural change of the proteins. The spectrum showed no significant difference between sfGFP-WT and sfGFP-AzF (Figure S1). These results supported that site-specific incorporation of AzF into the protein does not affect folded structures.

**Determination of conjugation yield of sfGFP to HSA/MSA**

Lanes 4 and 5 in Figure 4 were shown below for conjugation yield determination. The gel image was analyzed by ImageJ software (NIH) to estimate band intensity.




| Conjugate | Band | Identity | Intensity (a.u.) | MW (Da) | Mole (Intensity / MW, a.u.) | Conjugation yield (%)[a] |
|---|---|---|---|---|---|---|
| sfGFP-HSA | 1 | sfGFP conjugated to HSA | 1,434 | 94,060 | 0.015 | 4.9 |
| | 2 | sfGFP-AzF | 8,079 | 27,560 | 0.29 | |
| sfGFP-MSA | 1 | sfGFP conjugated to MSA | 1,013 | 94,560 | 0.011 | 4.1 |
| | 2 | sfGFP-AzF | 7,173 | 27,560 | 0.26 | |

[a] Percentage of ratio of sfGFP AzF Mole (Intensity / Mw) and sum of sfGFP-AzF Mole and sfGFP conjugated to HSA Mole

**Figure S2.** Determination of conjugation yields of sfGFP to HSA/MSA.

The MW (Molecular weight) of the sfGFP-HSA/MSA conjugates are the sum of molecular weights of sfGFP and HSA/MSA. Conjugation yield of each species was calculated assuming that the band intensity is proportional to the amount of protein. Then, the determination of conjugation yield of sfGFP to MSA was carried out similarly to sfGFP-HSA. As a result, the conjugation yields of sfGFP-HSA and sfGFP-MSA were determined to 4.9 and 4.1 %, respectively.