

Synthesis and Protein Incorporation of Azido-Modified Unnatural Amino Acids

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Supporting Information

Sequence of wt-sfGFP.

Figure S1 shows the sequence of wt-sfGFP.

MVSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLT¹⁵⁰TKFICTTGKL
PVPWPTLVTTLT¹⁵⁰YGVQCFSRYPDHMKR¹⁵⁰HDFFKSAMPEGYVQERTISFKDDGTYK
TRAEVKFE¹⁵⁰GD¹⁵⁰TLVNRIELKGIDFKEDGNILGHKLEYN¹⁵⁰FN¹⁵⁰SH¹⁵⁰VYITADKQKNGIK
ANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRD
H¹⁵⁰MVLL¹⁵⁰EFVTAAGITHGMD¹⁵⁰ELYKGS¹⁵⁰HHHHHH

Figure S1. Amino acid sequence of wt-sfGFP with residue 150 in italics.

ESI-Q-TOF Mass Analysis.

Table S1 shows electrospray ionization quadrupole time-of-flight (ESI-Q-TOF) mass spectral results for the sfGFP constructs containing an UAA. These results show the successful incorporation of **1**, **2**, or **4** into sfGFP at site 150 as shown in Table S1 by the agreement between the expected and observed molecular weights of the constructs relative to wt-sfGFP, within the error of the measurements.

The ESI-Q-TOF mass analysis was performed on the same purified protein samples used for the linear infrared measurements. This analysis was performed at the Mass Spectrometry Facility at the University of Illinois Urbana-Champaign. The protein samples were desalted into a 20 mM ammonium acetate buffer (pH 7) using PD10 gel filtration columns, lyophilized, and resuspended in 1:1 H₂O:CH₃CN with 0.2% formic acid prior to analysis.

Table S1. ESI-Q-TOF mass spectral results for the sfGFP constructs.

Protein Construct	Expected Molecular Weight Difference from wt-sfGFP (g/mol)	Observed Molecular Weight Difference from wt-sfGFP (g/mol)
sfGFP-150- 1	74	74±1
sfGFP-150- 2	88	88±1
sfGFP-150- 3	112	88±1
sfGFP-150- 4	126	125±1