

Supplementary information

Labeling yield of PA with F-TrpPA

Mass spectrometry analysis of F-TrpPA. F-TrpPA (500 pmol) was dissolved in 100 μ L 8 M urea and 50 mM ammonium bicarbonate for 5 min to denature the protein. The sample was then diluted to be 2 M urea concentration with 50 mM ammonium bicarbonate, and digested for overnight at 37 °C by 0.75 μ g of Lys-C (Wako Chemicals Richmond, VA). After the digestion, the solution was acidified by adding 10 μ L of formic acid, and the digest was desalted using an Ultra Micro Spin C18 column (Nest Group, Southboro, MA) according to the manufacturer's instructions. The digest was then analyzed by LC-MS/MS using an UltiMate 3000 LC systems (Dionex, San Francisco, Ca) interfaced to a LTQ-Orbitrap XL mass spectrometer (Thermo-Finnigan, Bremen, Germany) as described previously (1).

The ions for F-Trp labeled and unlabeled peptides were extracted from the obtained total ion current chromatogram, and the chromatographic areas between labeled and unlabeled peptide peaks were compared to calculate the labeling yield. An example for peptide containing F-Trp is shown in Figure S1. As can be seen, the area for labeled peptide (Fig, S1B) was 24.6-times larger than that of the unlabeled peptide (Fig, S1C). Table S1 summarizes the labeling yields of F-Trp in PA. Even though we could not confirm the labeling yield of three tryptophan residues (Trp206, 346 and 477), the labeling yields for other tryptophan residues (Trp65, 90, 136 and 226) were >95%. The results indicate that the labeling yield of PA with F-Trp was >95%.

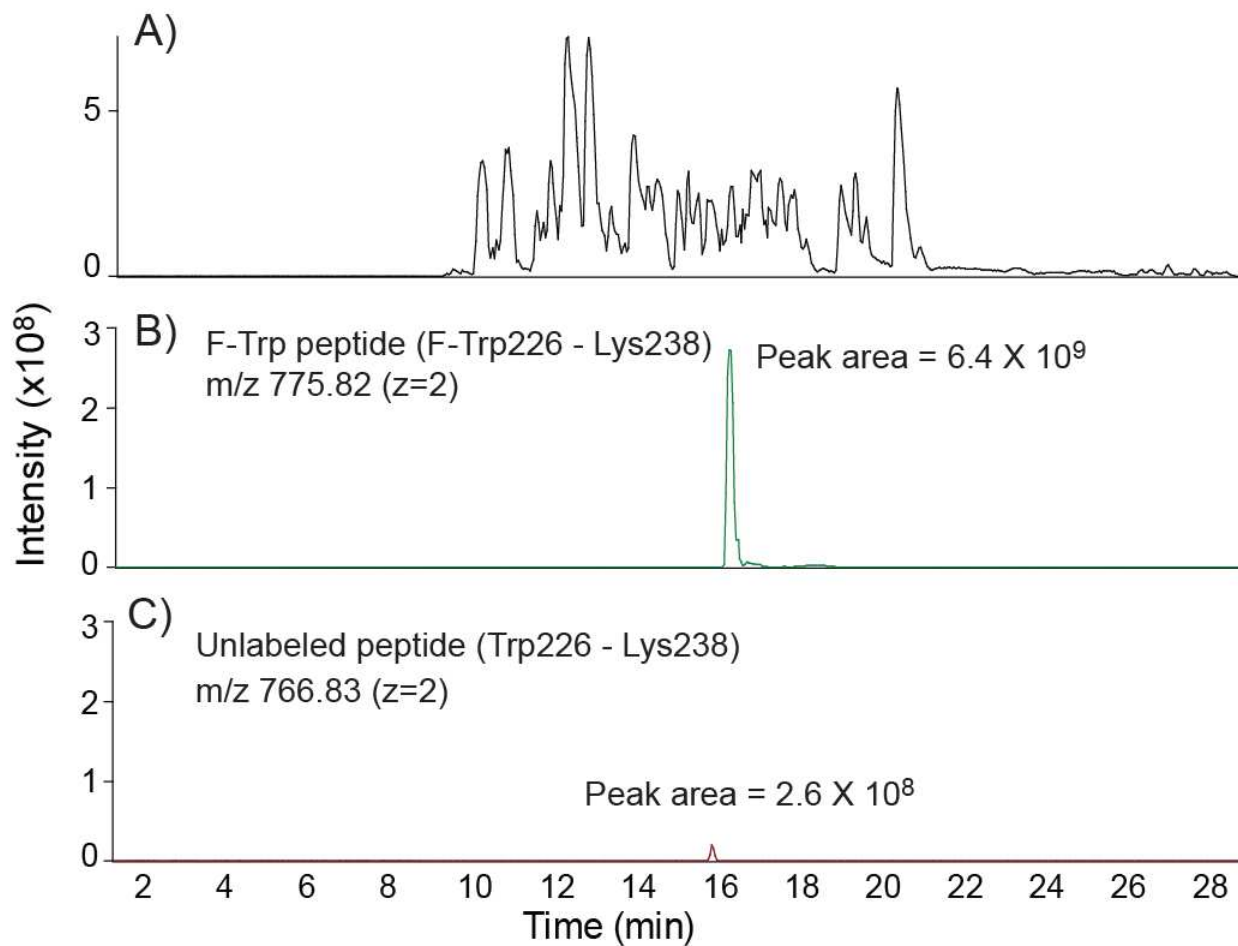


Figure S1. Labeling yield of F-Trp226. A) Total ion current chromatogram, B) Extracted ion (m/z 775.82, z=2) chromatogram of F-Trp labeled peptide, C) Extracted ion (m/z 766.83, z=2) chromatogram of unlabeled peptide.

Table S1. Labeling yields of F-Trp residues in PA

Trp residue	Unlabeled Trp peptide	F-Trp peptide	Labeling yield (%)
Trp65 (Q4-K70) ^a	Not observed	m/z 1494.5 (z=5) R.T. = 25.78 mins Area = 6.8 x 10 ⁷	100%
Trp90 (S74-K99) ^a	Not observed	m/z 1011.77 (z=3) R.T. = 18.35 mins Area = 3.1 x 10 ⁸	100%
Trp136 (L134-K143) ^a	m/z 641.82 (z=2) R.T.= 12.53 mins Area = 6.4 x 10 ⁷	m/z 650.82 (z=2) R.T.= 13.04 mins Area = 2.0 x 10 ⁹	97%
Trp206	Not observed	Not observed	-
Trp226 (W226-K238) ^a	m/z 766.83 (z=2) R.T.= 15.79 mins Area = 2.6 x 10 ⁸	m/z 775.82 (z=2) R.T.= 16.17 mins Area = 6.4 x 10 ⁹	96%
Trp346	Not observed	Not observed	-
Trp477	Not observed	Not observed	-

^a Peptide position

1. Miyagi, M., Wan, Q., Ahmad, M. F., Gokulrangan, G., Tomechko, S. E., Bennett, B., and Dealwis, C. (2011) Histidine hydrogen-deuterium exchange mass spectrometry for probing the microenvironment of histidine residues in dihydrofolate reductase, *PLoS One* 6, e17055.