Site-Specific Fatty Acid-Conjugation to Prolong Protein Half-Life *In Vivo*

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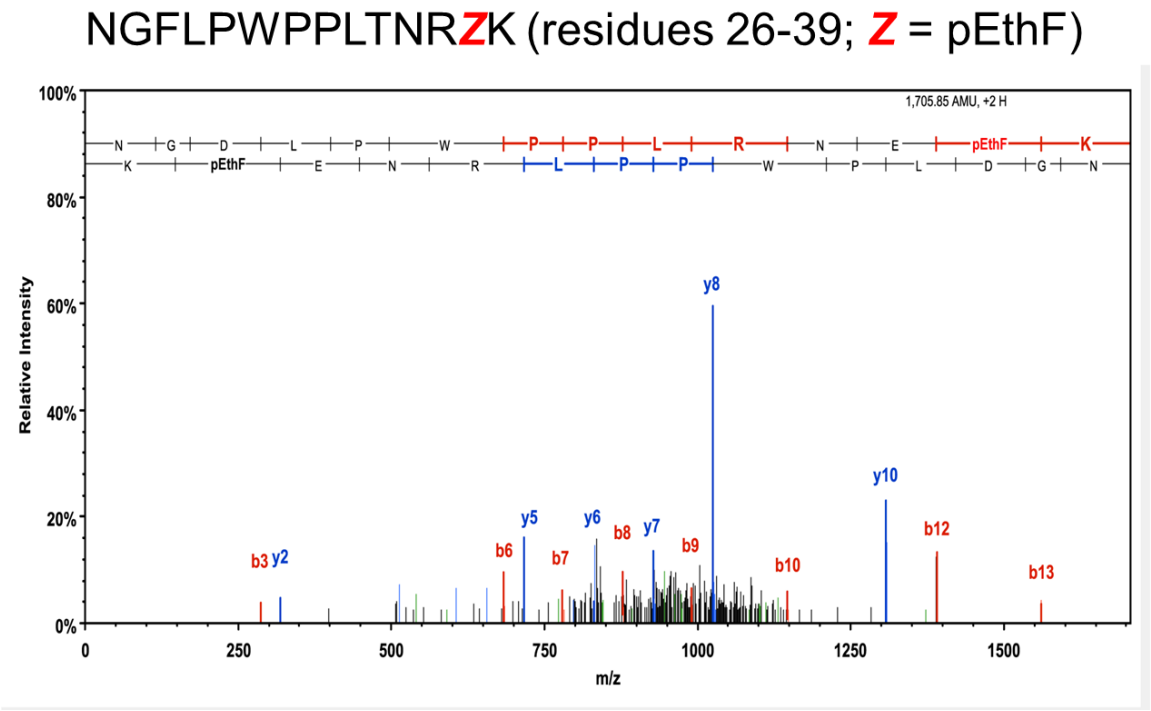
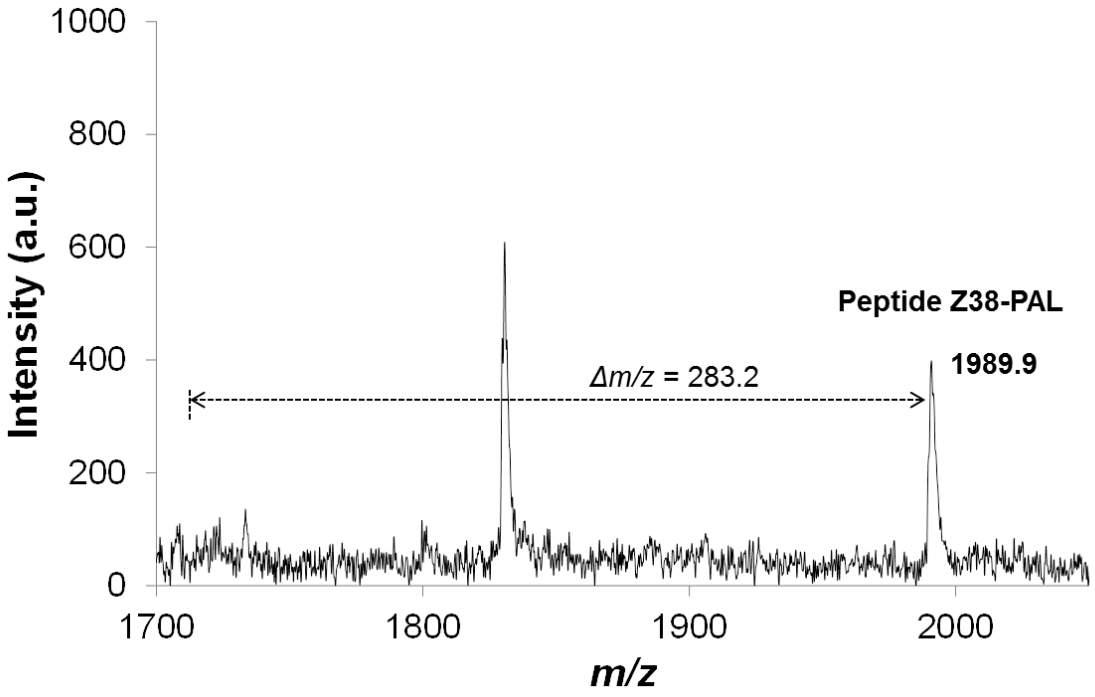
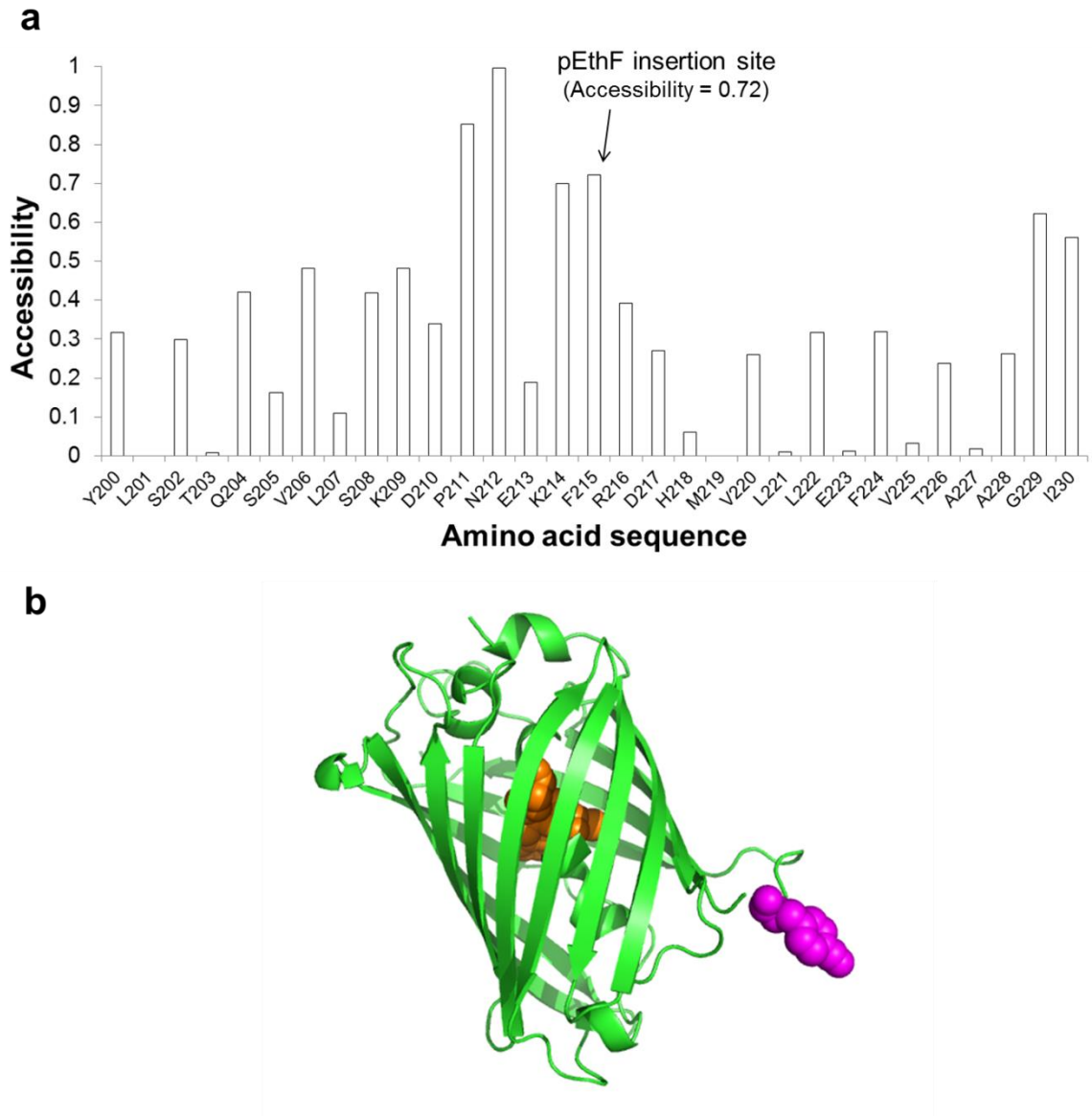
Supplementary Information (SI)



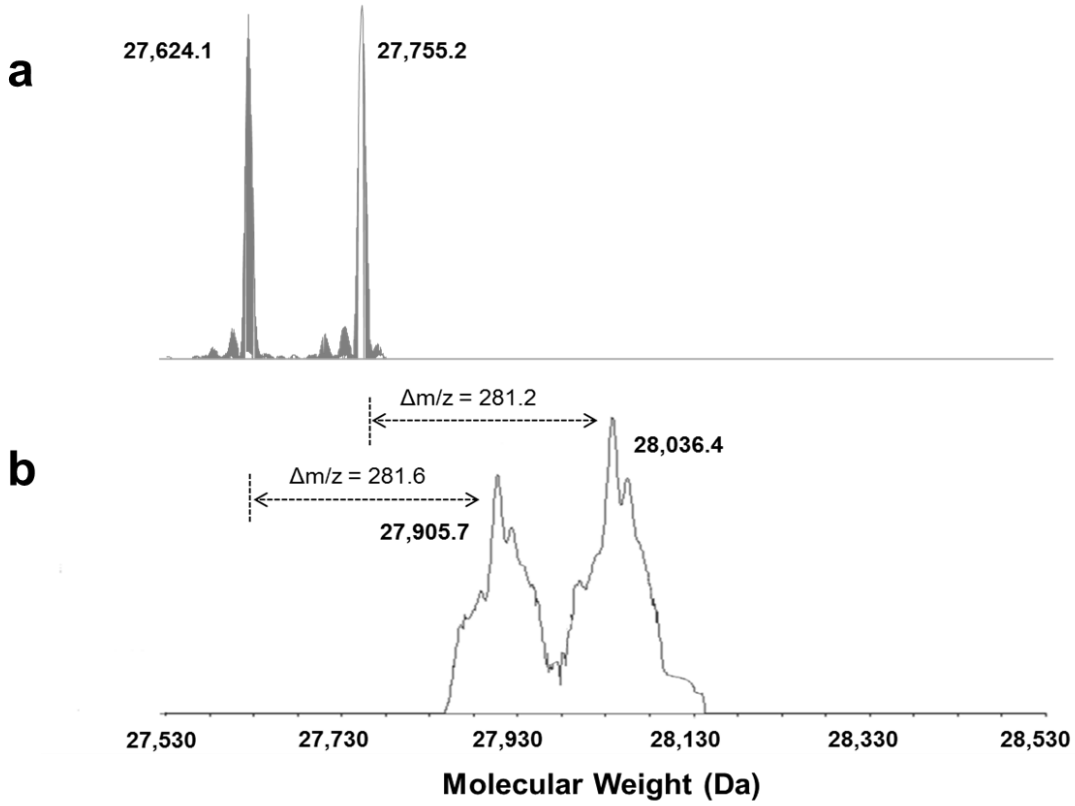
Supplementary Fig. S1. Liquid chromatography tandem MS of Peptide Z38 (m/z = 1706.8, NGDLPWPPLRNEAZK) of the mDHFR-pEthF (Fig. 2). The system consisted of a Thermo Electron LTQ Orbitrap XL mass spectrometer interfaced to a Phenomenex Jupiter C18. The analysis was performed by acquiring a mass spectrum using Fourier transform ion cyclotron resonance.



Supplementary Fig. S2. Confirmation of the palmitic acid-conjugation to the mDHFR-pEthF by MALDI-TOF MS. Peptide Z38-PAL, one of tryptic digests of the mDHFR-Pal, was detected at a mass of 1989.9.



Supplementary Fig. S3. Determination of an optimal site for mutagenesis. (a) Residue-based solvent accessibility of the sfGFP-pEthF. ASAView [1], an online tool for a graphical representation of solvent accessibility, was used to calculate the relative solvent accessibility of all residues including pEthF at 215th position, based on the PDB file (ID: 2B3P) of sfGFP and a fully automated protein structure homology-modeling server [2] for mutation. (b) Three-dimensional structure of the sfGFP-pEthF generated by Pymol [3]. The chromophore (orange) and pEthF incorporated at the 215th position (magenta) are represented by spheres.



Supplementary Fig. S4. Confirmation of the palmitic acid-conjugation to the sfGFP-pEthF by ESI-MS. ESI-MS spectrum of the sfGFP-pEthF (a) and the sfGFP-Pal (b). Following the reversed-phase high performance chromatography using BEH C4 column (2.1 × 100 mm, 1.7 μm), the molecular weight of a full length protein was analyzed on an LTQ-Orbitrap XL mass spectrometer.

References

[1] S. Ahmad, M. Gromiha, H. Fawareh, A. Sarai, ASAView: database and tool for solvent accessibility representation in proteins. BMC Bioinformatics 5 (2004) 51.

[2] K. Arnold, L. Bordoli, J. Kopp, T. Schwede, The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics 22(2) (2006) 195-201.

[3] The PyMOL Molecular Graphics System, Version 1.2r1 Schrödinger, LLC.