Electronic Supplementary Information

Labelling of Endogenous Target Protein via N-S Acyl Transfer-Mediated Activation of *N*-Sulfanylethylanilide

Masaya Denda^{*a*}, Takuya Morisaki^{*a*}, Taiki Kohiki^{*a*}, Jun Yamamoto^{*a*}, Kohei Sato^{*a,b*}, Ikuko Sagawa^{*c*}, Tsubasa Inokuma^{*a*}, Youichi Sato^{*a*}, Aiko Yamauchi^{*a*}, Akira Shigenaga^{**a,d*} and Akira Otaka^{**a*}

E-mail:

shigenaga.akira@tokushima-u.ac.jp (A. Shigenaga)

aotaka@ph.tokushima-u.ac.jp (A. Otaka)

a) Institute of Biomedical Sciences and Graduate School of Pharmaceutical Sciences, Tokushima University, Shomachi, Tokushima 770-8505, Japan

b) Current address: Department of Applied Chemistry and Biochemical Engineering, and Green Energy Research Division, Research Institute of Green Science and Technology, Shizuoka University, 3-5-1 Johoku, Hamamatsu, Shizuoka 432-8561, Japan

c) Institute for Medicinal Research, Graduate School of Pharmaceutical Sciences, Tokushima University, Shomachi, Tokushima 770-8505, Japan

d) JST, PRESTO, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan

Table of Contents

Supplemental Figures S1-S2	.S2
Copies of ¹ H/ ¹³ C NMR spectra	S4

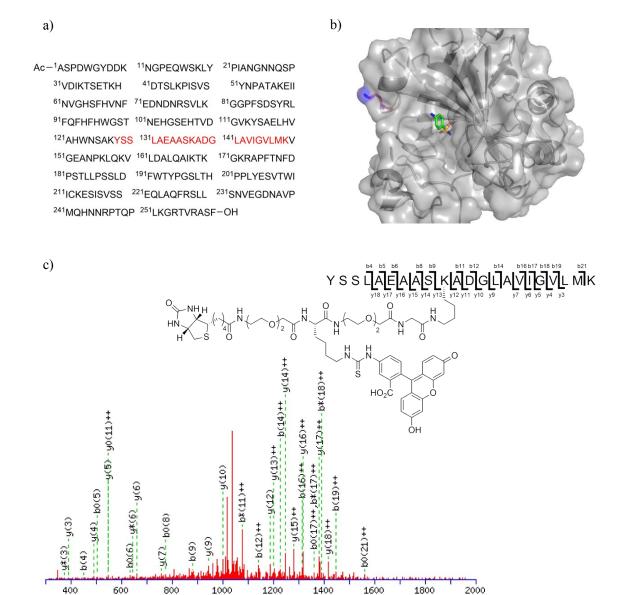
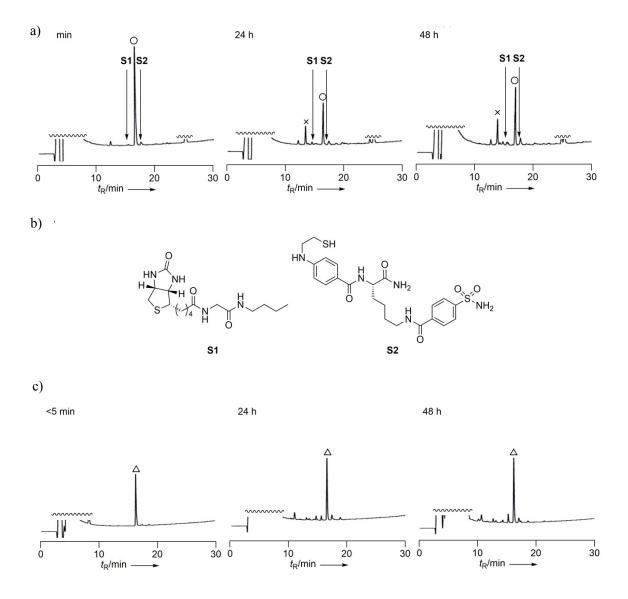
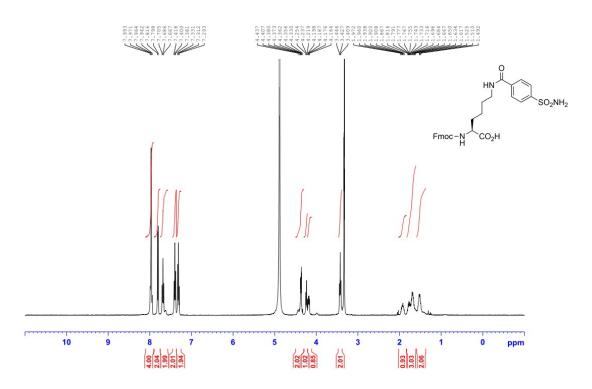


Fig. S1. Mass spectral analysis of the labelling site of hCA1 using SEAL **4**. (a) The primary sequence of hCA1. (b) Crystal structure of hCA1 complexed with 3-Actoxymercuri-4-aminobenzenesulfonamide (PDB ID: 1CZM). The amino acid (Lys 137) modified by **4** is highlighted by a colored stick model. (c) nanoLC-MS/MS analysis of the fragment labelled at Lys137.



S2. (a) HPLC analysis of the SEAL **6** (\bigcirc , 10 µM) with butylamine (1 M) in 50 mM HEPES buffer (pH 7.2) at 37 °C for 5 min > (left), 24 h (middle) or 48 h (right) of incubation. × denotes oxidated SEAL **6**. However, the aminolysis product **S1** and **S2** was not detected. (b) Chemical structures of **S1**, **S2**. (c) HPLC analysis of the SEAL **6** (\triangle , 10 µM) with L-Cys methyl ester (1 mM) in 50 mM HEPES buffer (pH 7.2) at 37 °C for 5 min > (left), 24 h (middle) or 48 h (right) of incubation.

¹H NMR spectrum of Fmoc-L-Lys(4-sulfamoyl-benzoyl)-OH



¹³C NMR spectrum of Fmoc-L-Lys(4-sulfamoyl-benzoyl)-OH

