

Electronic Supplementary Information

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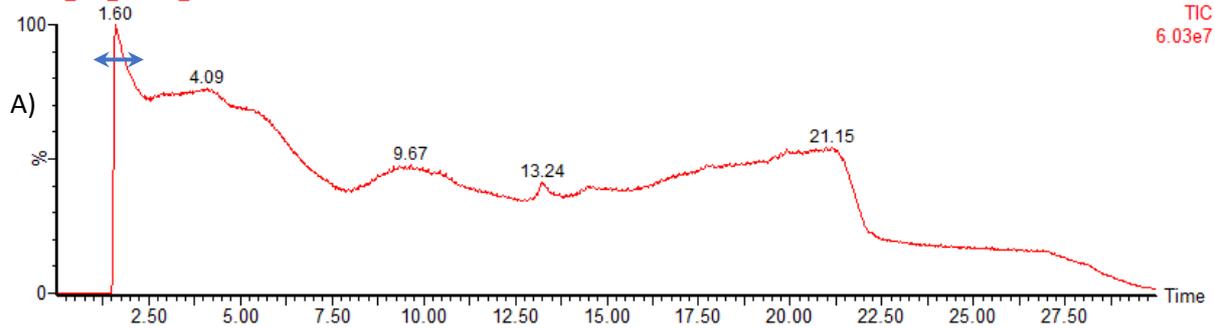
Liquid chromatography–mass spectrometry (LC–MS).

LC–MS was performed on a Xevo G2-S TOF mass spectrometer connected to an Acquity UPLC system using an Acquity UPLC BEH300 C4 column (1.7 μm , 2.1 % 50 mm). The eluent was solvent A, water with 0.1% formic acid and B, 71% acetonitrile, 29% water with 0.075% formic acid, at a flow rate of 0.2 mL/min. The gradient was programmed from solvent A/B (72:28) to 100% B over 25 min, followed by solvent B for 2 min and then up to solvent A/B (72:28) over 18 min. The electrospray source was operated with a capillary voltage of 2.0 kV and a cone voltage of 40 V. Nitrogen was used as the desolvation gas at a total flow of 850 L/h. Total mass spectra were reconstructed from the ion series using the MaxEnt algorithm preinstalled on MassLynx software (Waters). To obtain the ion series described, the major peak(s) of the chromatogram were selected for integration and further analysis.

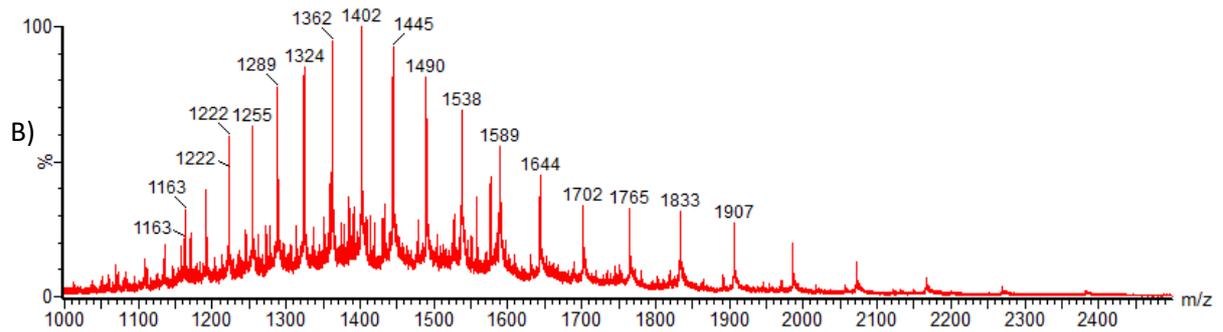
Native_Fab_240816_1_PBS

Native_Fab_240816_1

1: TOF MS ES+
TIC
6.03e7



Native_Fab_240816_1_PBS



Native_Fab_240816_1_PBS

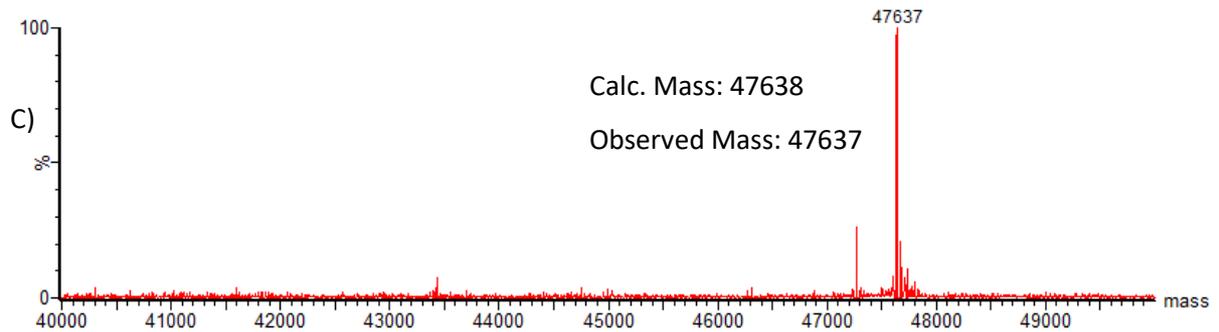


Figure S1. LC–MS data and processing of native Fab. (A) Total ion count trace of liquid chromatography step. (B) The peak corresponding to protein ions (time range indicated with blue arrow on LC chromatography) is combined and reported as m/z. (C) Deconvolution of the m/z charge spectrum identifies the observed mass.

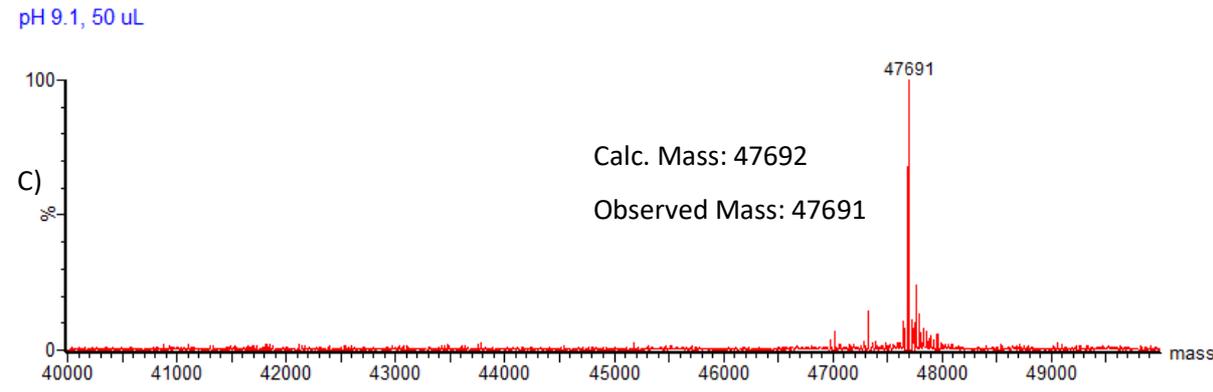
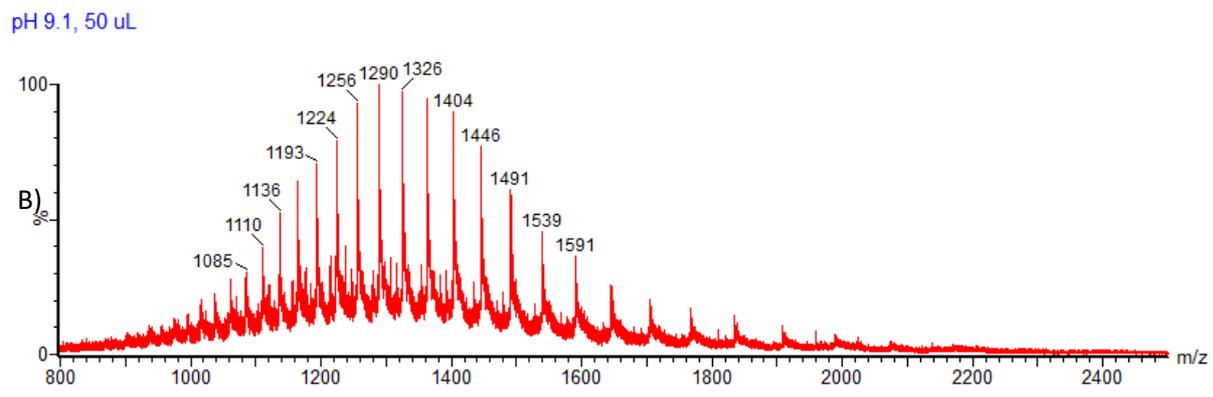
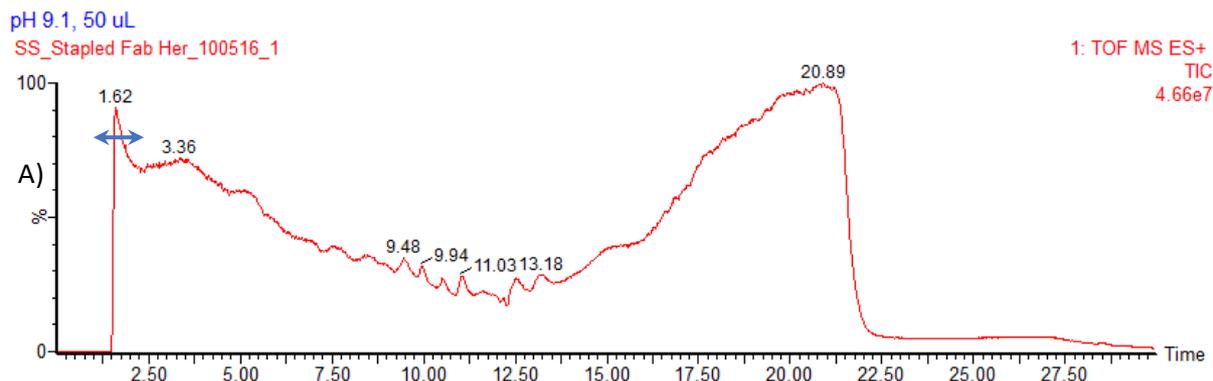


Figure S2. LC–MS data and processing of stapling of Fab in 50 mM NaPi buffer pH 9.1. (A) Total ion count trace of liquid chromatography step. (B) The peak corresponding to protein ions (time range indicated with blue arrow on LC chromatography) is combined and reported as m/z. (C) Deconvolution of the m/z charge spectrum identifies the observed mass.

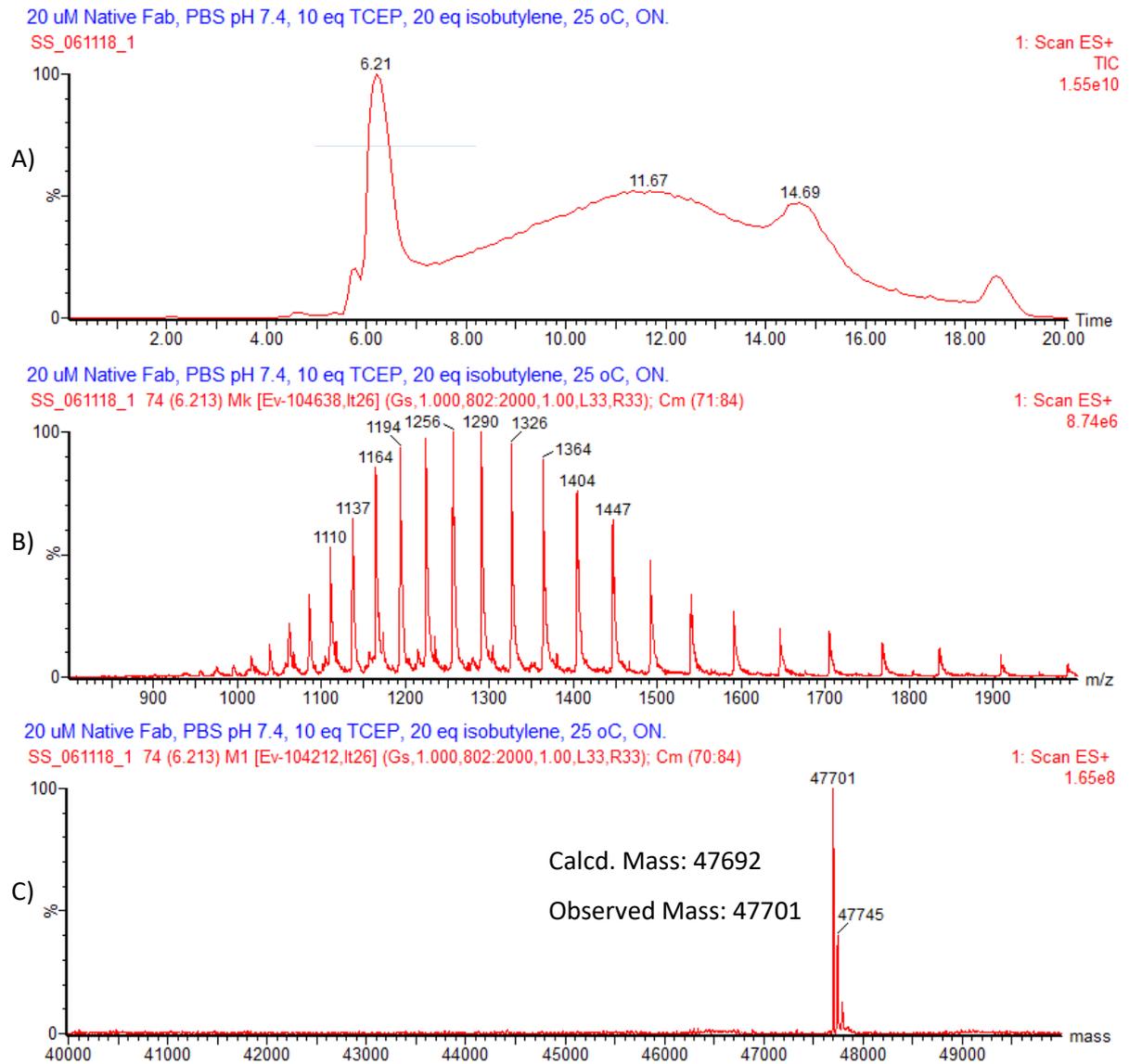


Figure S3. LC–MS data and processing of stapled Fab. (A) Total ion count trace of liquid chromatography step. (B) The peak corresponding to protein ions (time range indicated with blue arrow on LC chromatography) is combined and reported as m/z. (C) Deconvolution of the m/z charge spectrum identifies the observed mass.

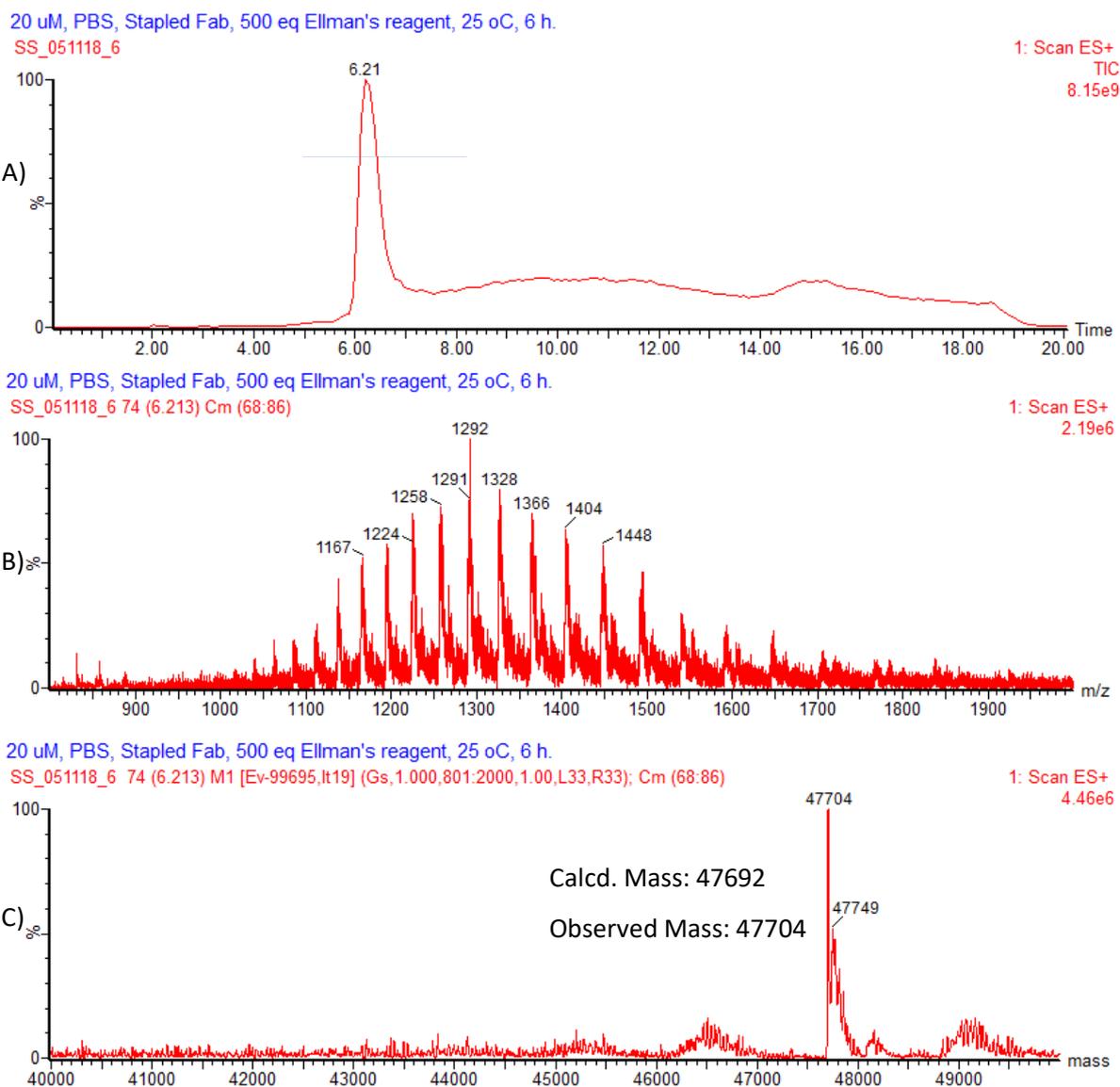
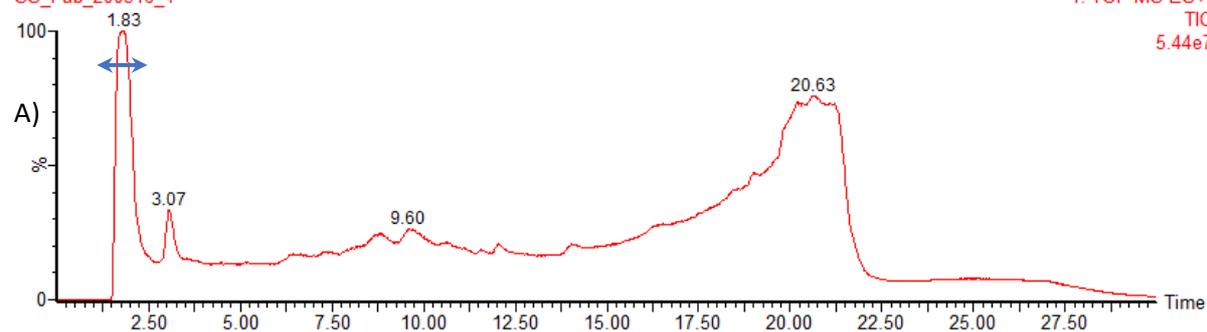


Figure S4. LC–MS data and processing of the Ellman’s test reaction with stapled Fab. (A) Total ion count trace of liquid chromatography step. (B) The peak corresponding to protein ions (time range indicated with blue arrow on LC chromatography) is combined and reported as m/z. (C) Deconvolution of the m/z charge spectrum identifies the observed mass.

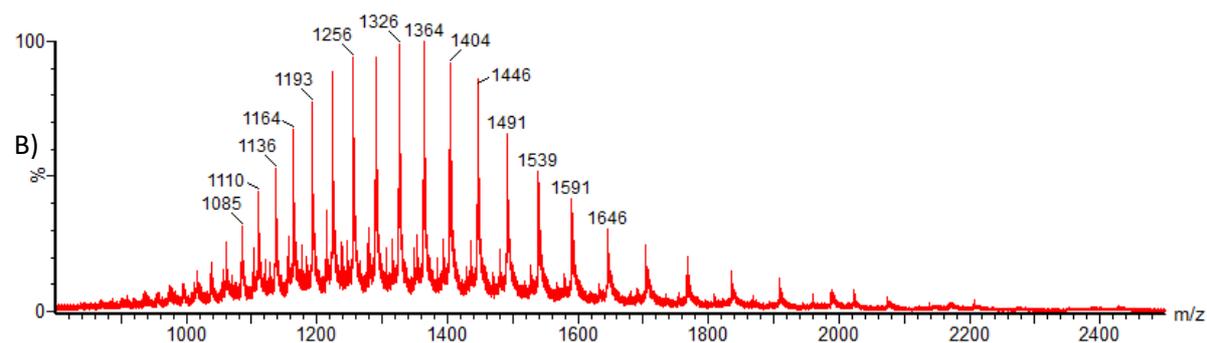
GSH, PBS, 24 h, Stapled Fab Stability GSH_200516

SS_Fab_200516_1

1: TOF MS ES+
TIC
5.44e7



GSH, PBS, 24 h, Stapled Fab Stability GSH_200516



GSH, PBS, 24 h, Stapled Fab Stability GSH_200516

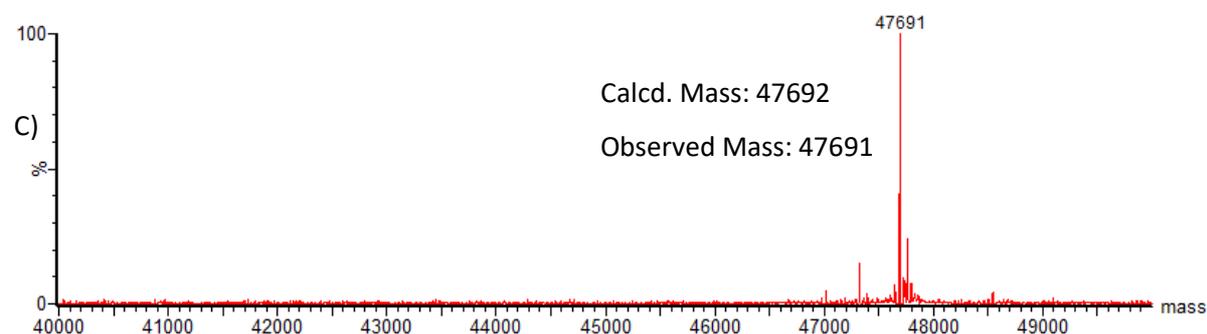
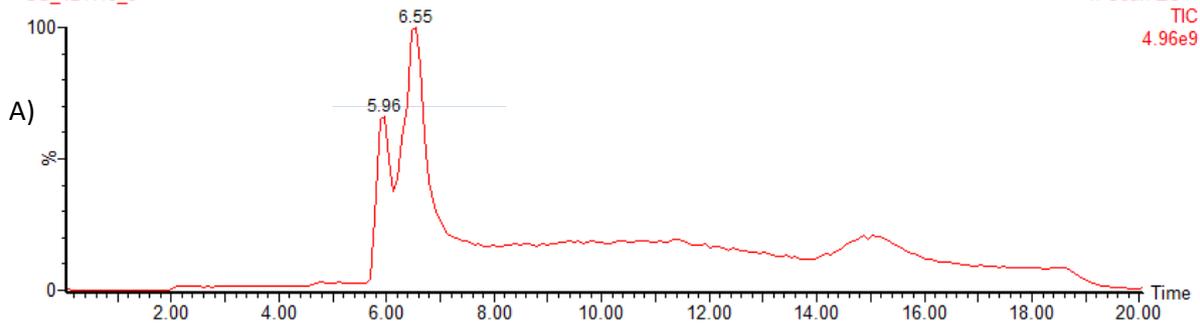
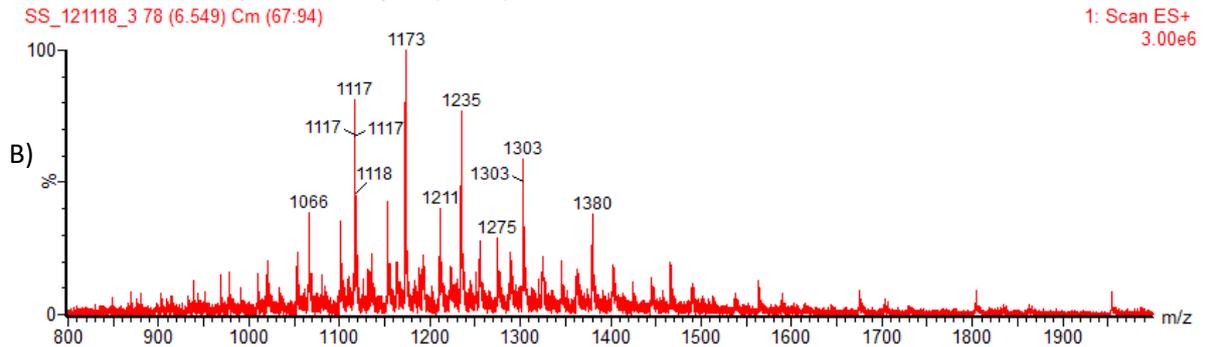


Figure S5. LC–MS data and processing of stapled Fab, being incubated with glutathione under 37 °C for 24 h. (A) Total ion count trace of liquid chromatography step. (B) The peak corresponding to protein ions (time range indicated with blue arrow on LC chromatography) is combined and reported as m/z. (C) Deconvolution of the m/z charge spectrum identifies the observed mass.

20 μ M Native Fab, PBS pH 7.4, 1000 eq GSH, 37 °C, 24 h.
SS_121118_3



20 μ M Native Fab, PBS pH 7.4, 1000 eq GSH, 37 °C, 24 h.
SS_121118_3 78 (6.549) Cm (67:94)



20 μ M Native Fab, PBS pH 7.4, 1000 eq GSH, 37 °C, 24 h.

SS_121118_3 78 (6.549) M1 [Ev-98650,It24] (Gs.2.000,798:2000,1.00,L33,R33); Cm (66:86)

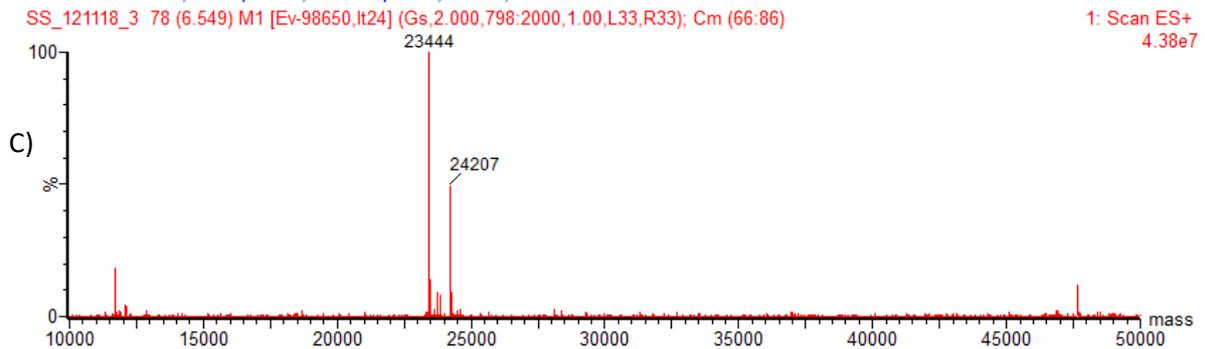
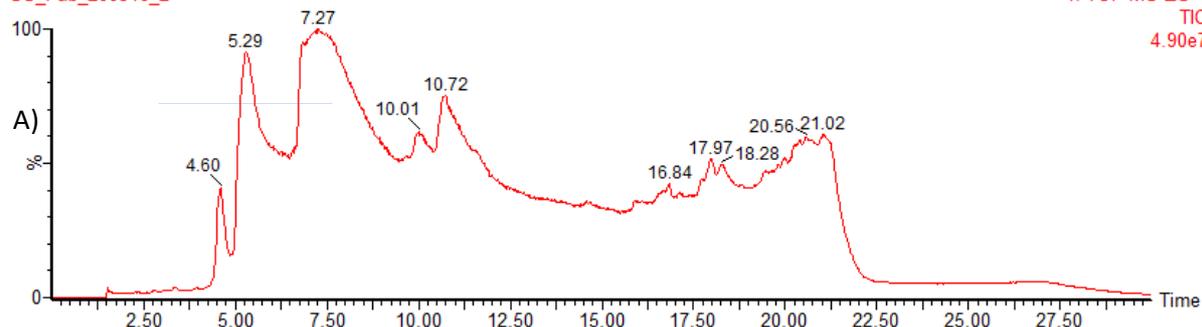


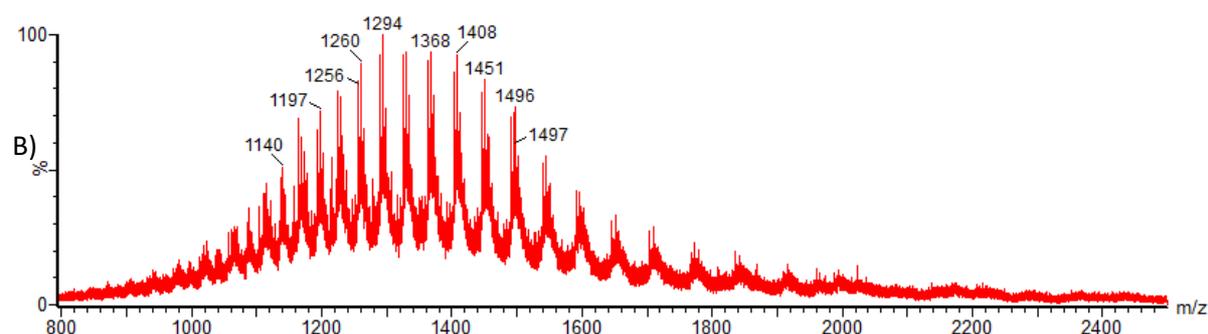
Figure S6. LC–MS data and processing of native Fab, being incubated with glutathione under 37 °C for 24 h. (A) Total ion count trace of liquid chromatography step. (B) The peak corresponding to protein ions (time range indicated with blue arrow on LC chromatography) is combined and reported as m/z. (C) Deconvolution of the m/z charge spectrum identifies the observed mass.

Plasma, PBS, 24 h, Stapled Fab Stability Plasma_200516
SS_Fab_200516_2

1: TOF MS ES+
TIC
4.90e7



Plasma, PBS, 24 h, Stapled Fab Stability Plasma_200516



Plasma, PBS, 24 h, Stapled Fab Stability Plasma_200516

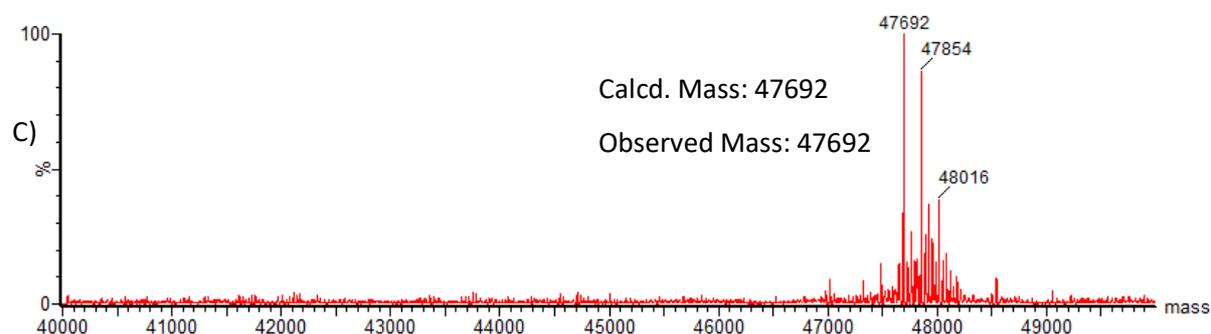


Figure S7. LC-MS data and processing of stapled Fab, being incubated with human plasma under 37 °C for 24 h. (A) Total ion count trace of liquid chromatography step. (B) The peak corresponding to protein ions (time range indicated with blue arrow on LC chromatography) is combined and reported as m/z. (C) Deconvolution of the m/z charge spectrum identifies the observed mass.

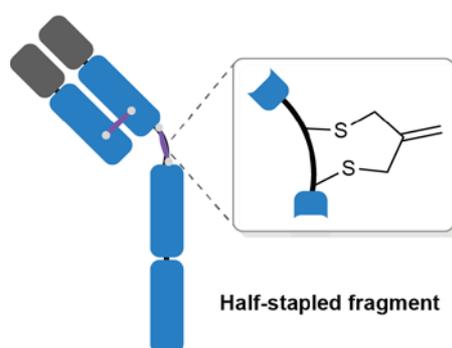


Figure S8. Proposed structure of the half-stapled fragment.

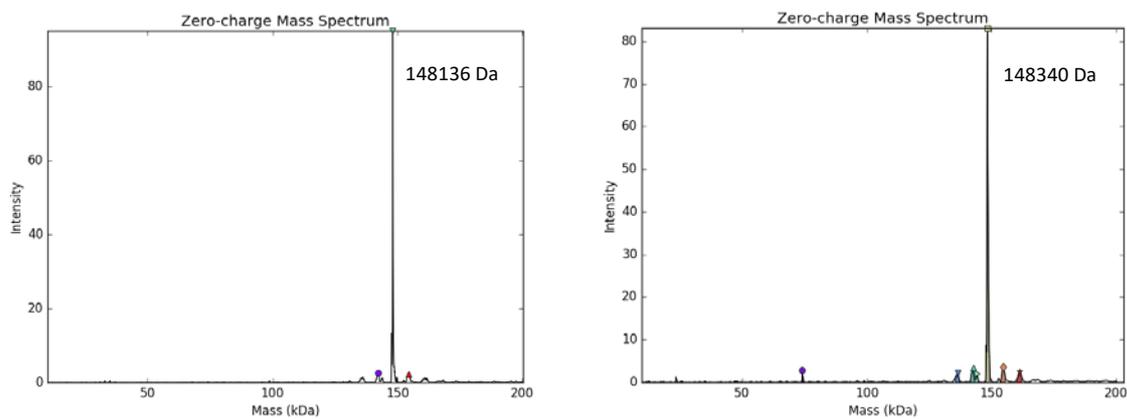
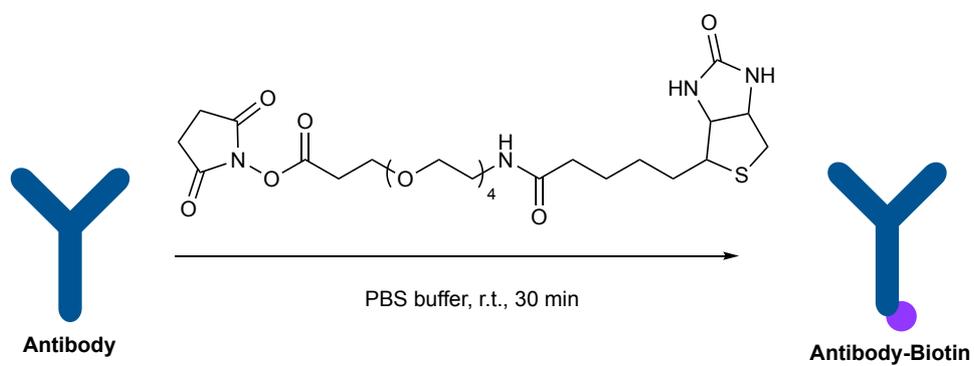
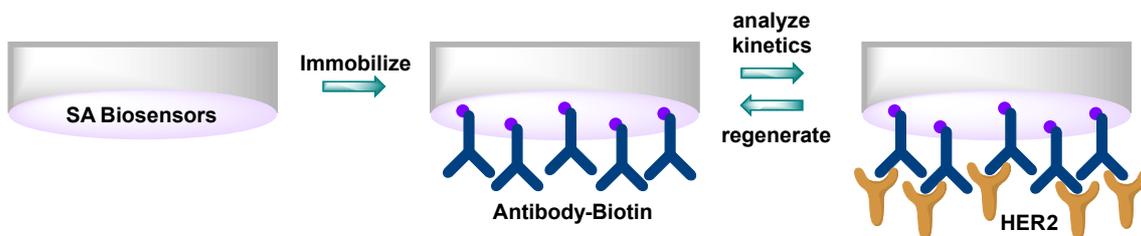


Figure S9. Native mass spectrometry of Native Trastuzumab (left) and Stapled Trastuzumab (right).



Scheme S1. Biotinylation of antibodies.



Scheme S2. Kinetic studies with Bio-layer Interferometry (BLI).

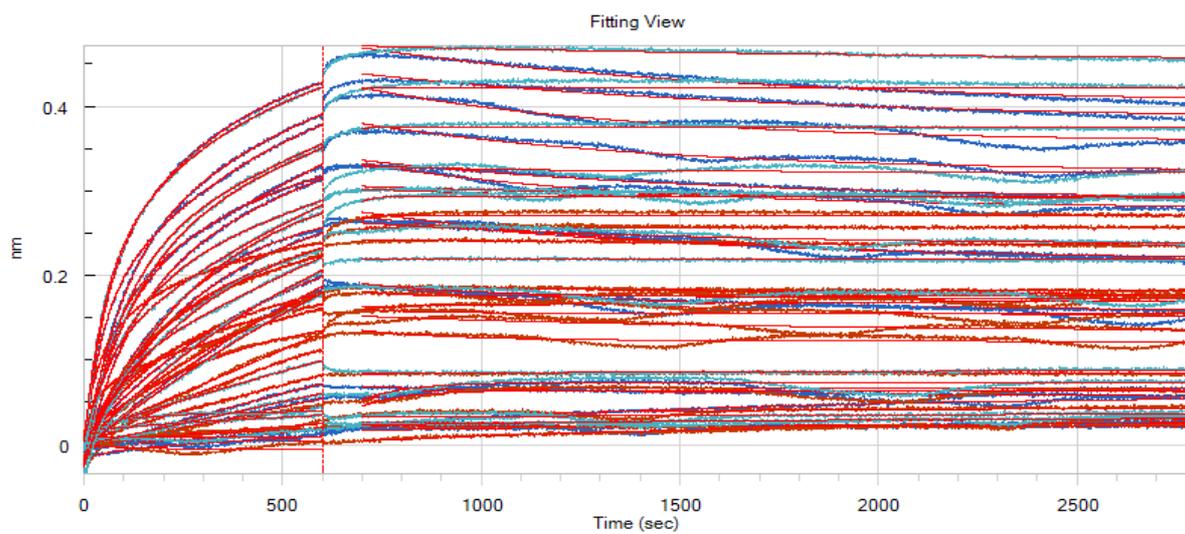


Figure S10. Stacked raw data and fitting curves of BLI.