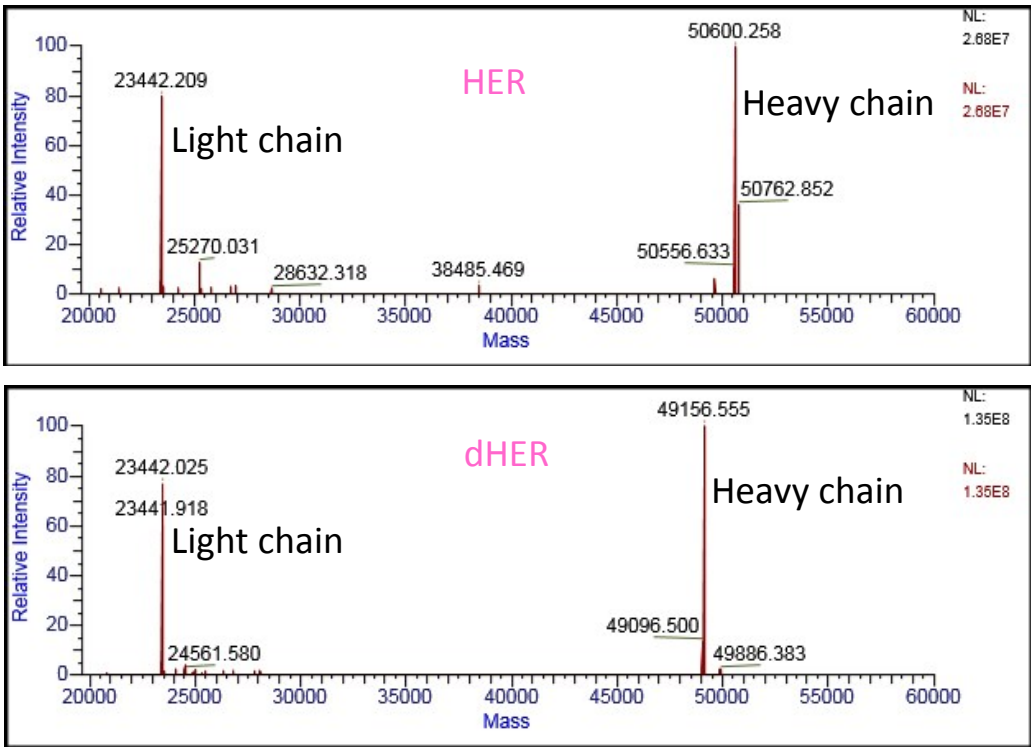
Electronic Supplementary Material (ESI) for Chemical Science.

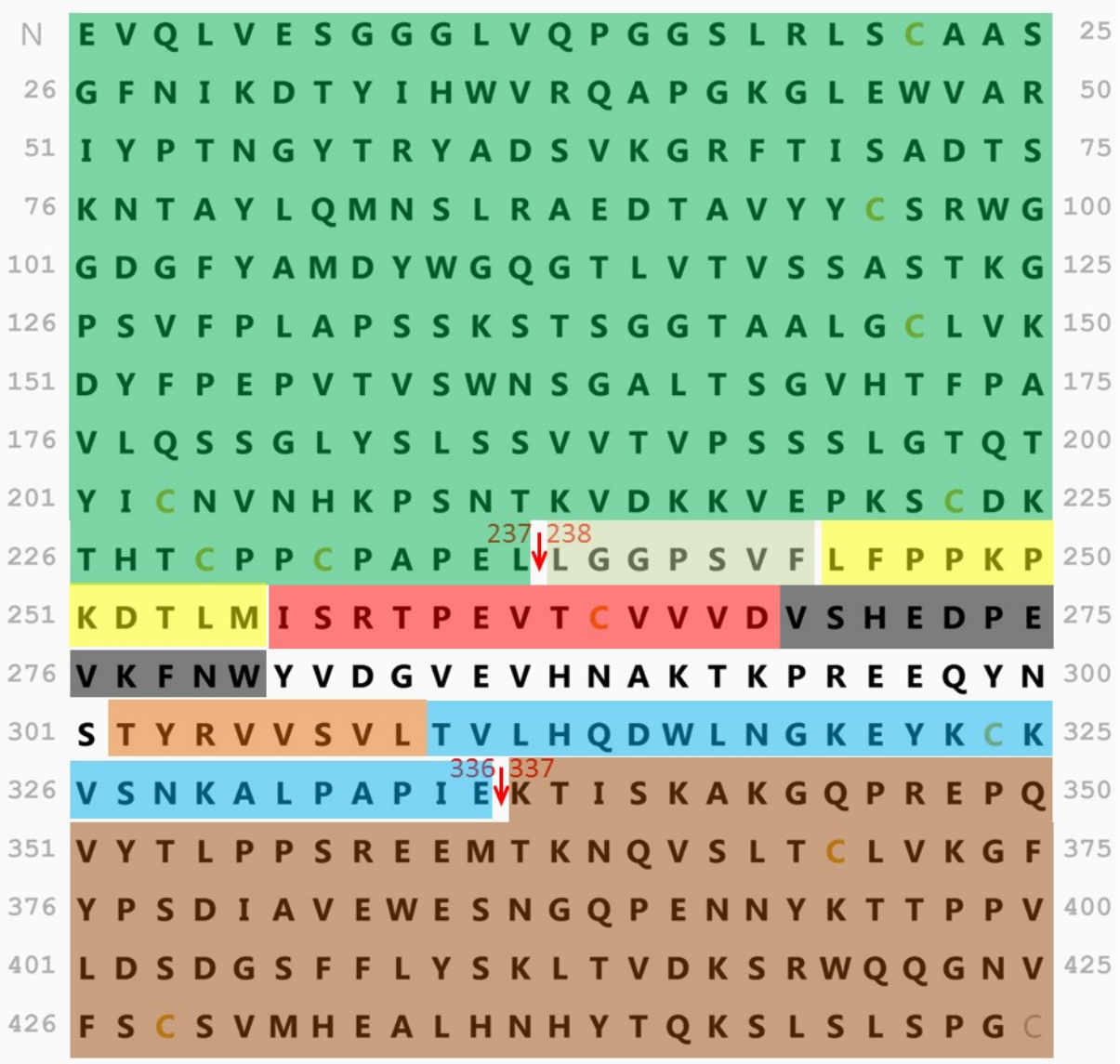

# Supplementary Information

**Figure S1:** Intact mass measurements of reduced HER and dHER.



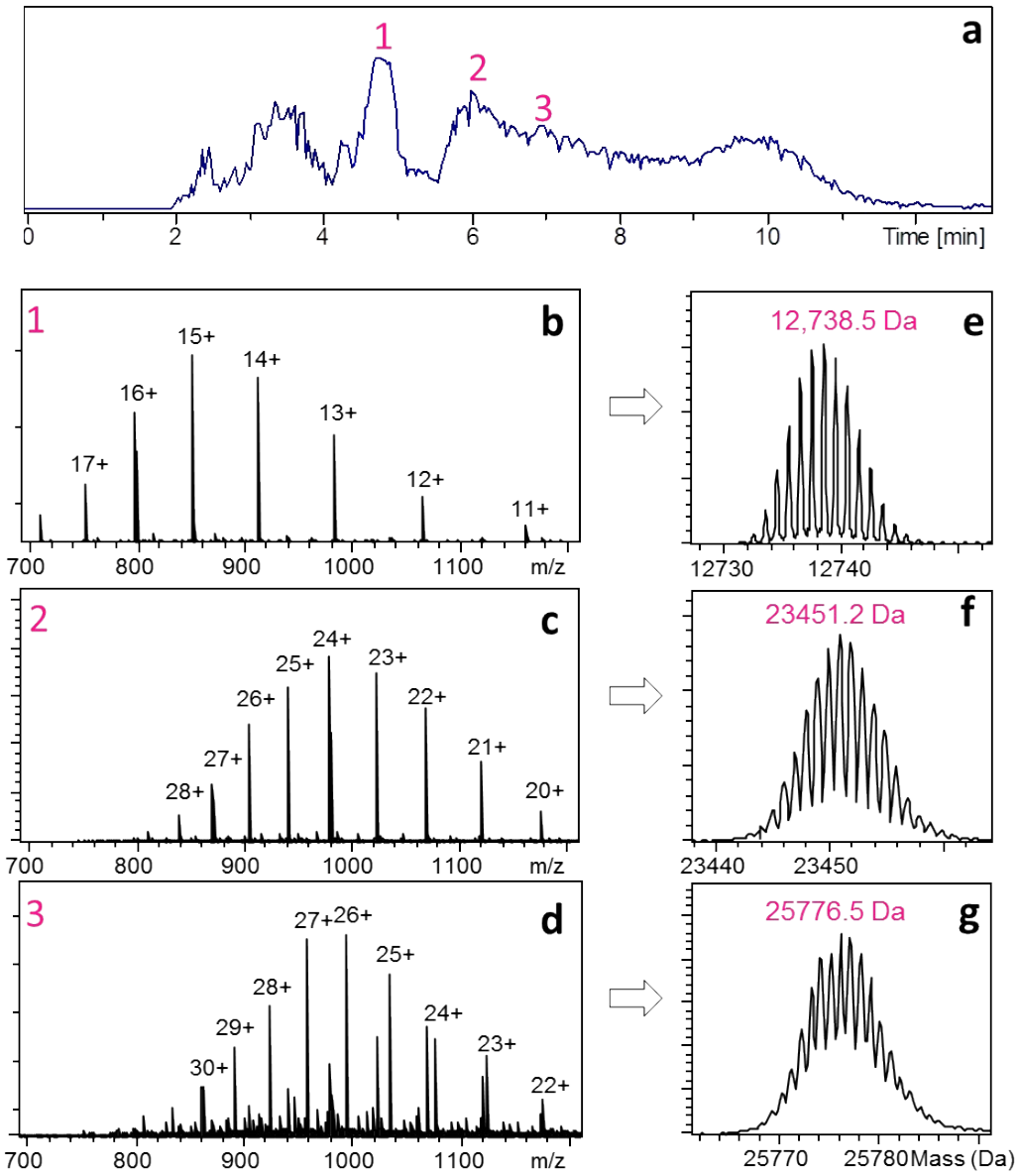

Deconvoluted masses of the light chain and heavy chain. (Top) reduced HER, (bottom) reduced dHER. A difference between before and after deglycosylation was only observed for the heavy chain because there is no glycosylation site on the light chain.

**Figure S2:** Sequence of the HER heavy chain.



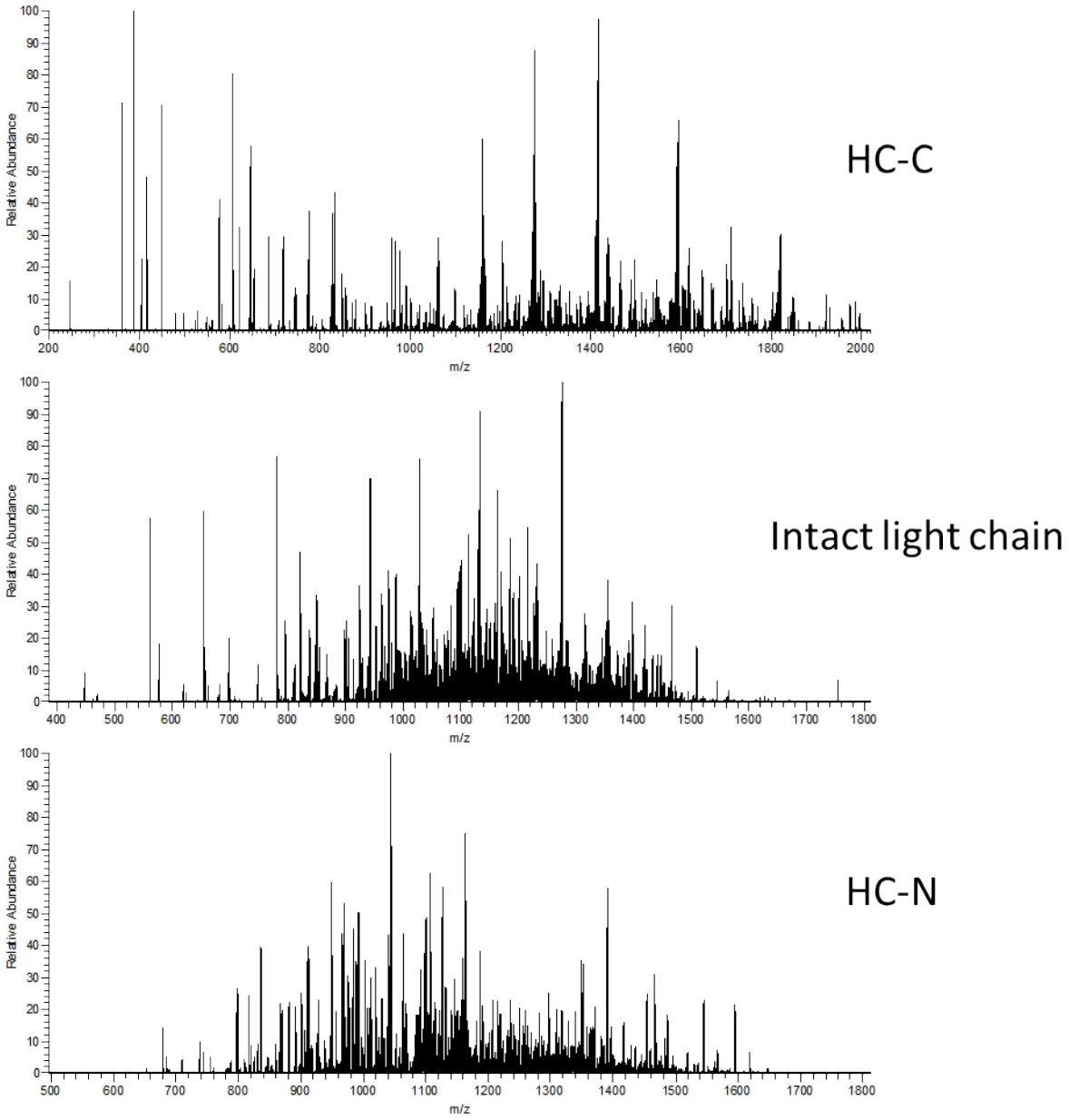

The specific peptic fragments from HER heavy chain are shown in different colors (one color represents one fragment). The two main cleavage sites of pepsin are indicated by red arrows.

**Figure S3:** LC-MS of the middle-down fragments from Bevacizumab



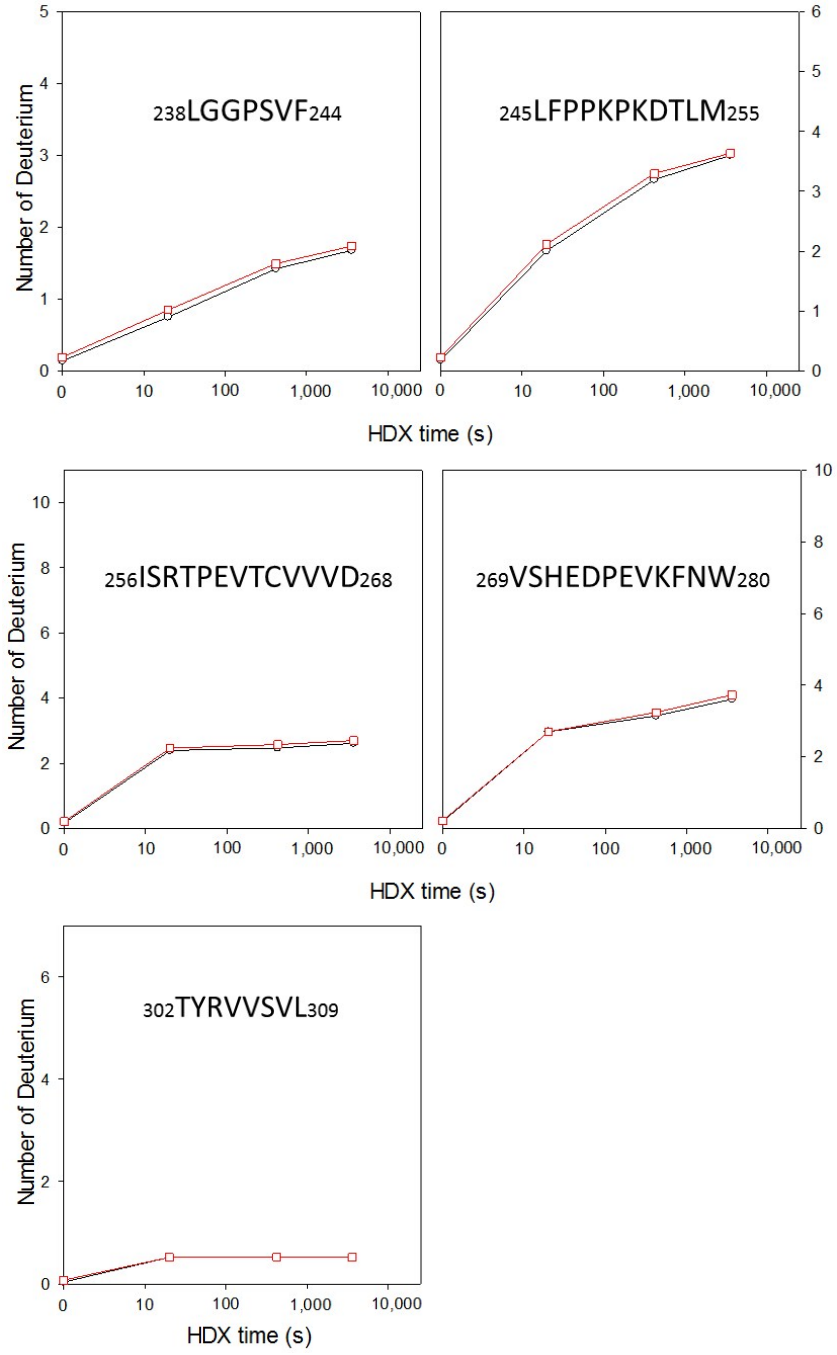

HPLC elution profile and intact mass measurements of Bevacizumab fragments obtained after 1-min pepsin digestion and 3-min reduction at pH 2.5. The ESI-MS spectra of the three main HPLC peaks (labeled as 1, 2, and 3) are shown in (**b**), (**c**), and (**d**), respectively. The corresponding masses after deconvolution are given in (**e**), (**f**), and (**g**).

**Figure S4:** ETD spectra of representative HER fragments.



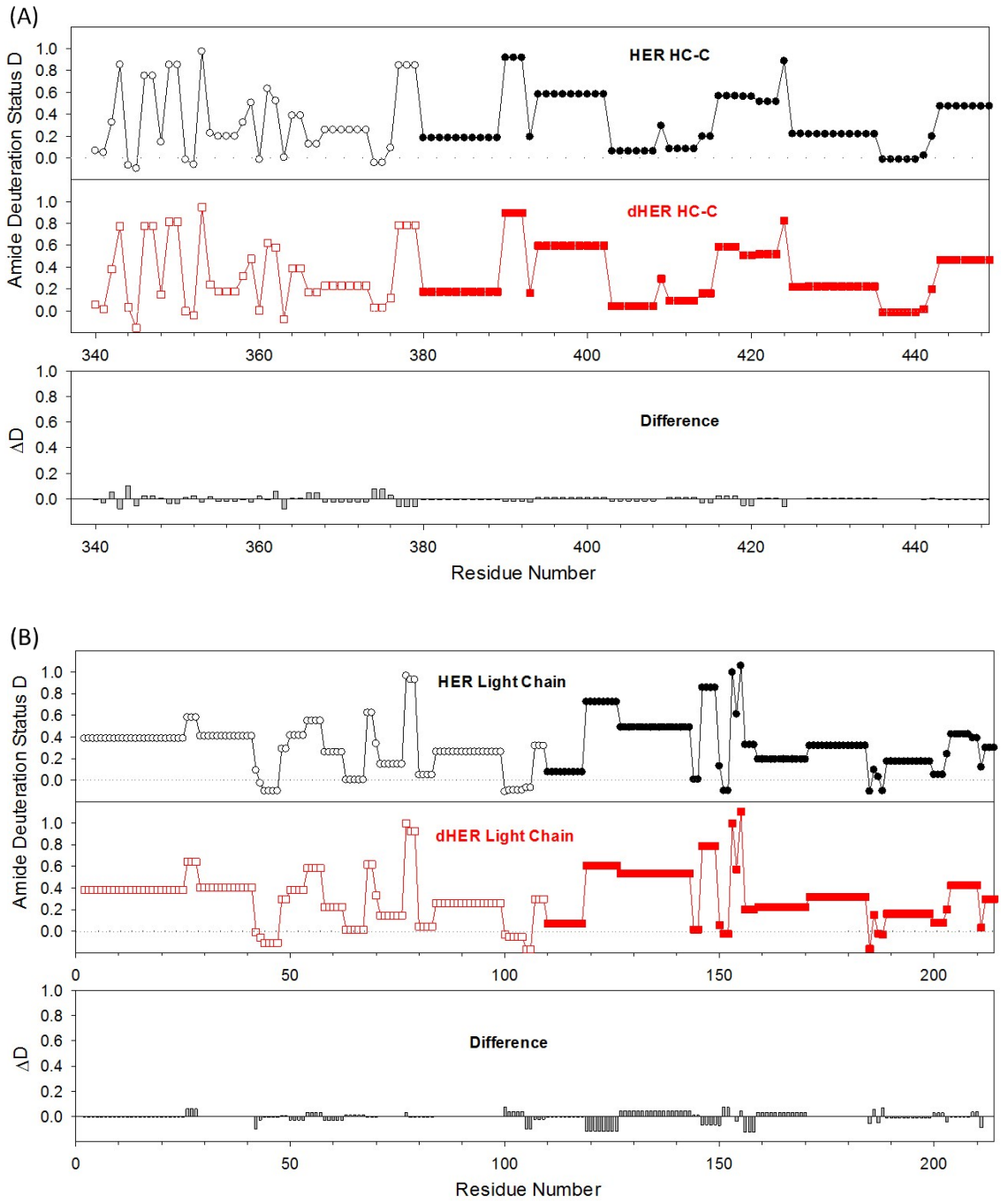

Orbitrap MS spectra obtained after online ETD of the intact peptic fragments produced after a 1-min digestion: (top) HC-C, (middle) light chain, (bottom) HC-N.

**Figure S5**: Deuterium uptake at the intact-fragment level.



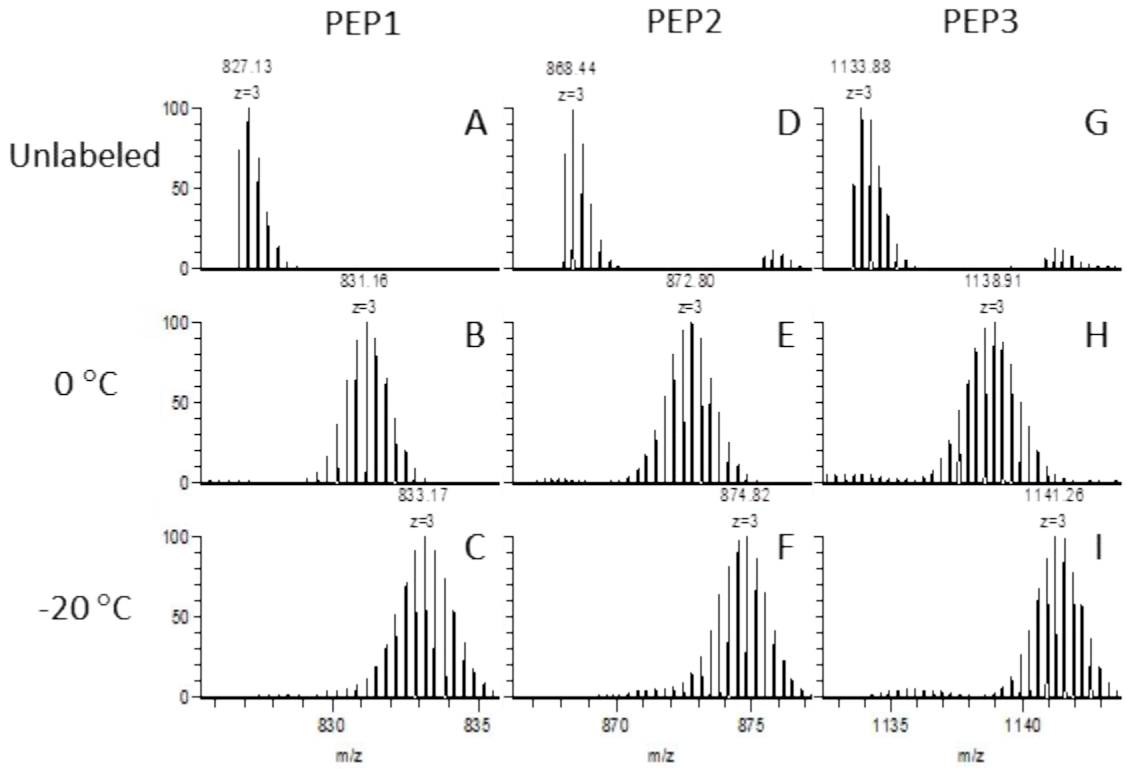

Deuterium uptake plots of the intact peptic fragments from HER (black circle) and dHER (red square). The peptides were all from the middle portion of the heavy chain, as labeled in Figure S2.

**Figure S6**. Amide deuteration levels of the middle-down fragments




Amide deuteration level after HDX for 1 h, as obtained from c ions (open symbol) and z ions (filled symbol). (A) HC-C; (B) Light chain. The data represent an average of triplicate measurements, and the error on the deuterium incorporation of each amide was within ±0.1.

**Figure S7**: Back exchange of peptides.




Mass spectra of three model peptides obtained by LC-MS. First row (A, D, G): unlabeled peptides; second row (B, E, H): deuterated peptides (90% D) after HPLC at 0 °C; Third row: deuterated peptides (90% D) after HPLC at -20 °C.

**Table S1**. Summary of the back-exchange calculations for the three peptides.

| Peptides | Temperature (°C) | Retention time (min) | Charge state | m/z unlabeled | m/z HDX | Total D | Total amides | %D | Theoretical %D | % Back exchange |
|---|---|---|---|---|---|---|---|---|---|---|
| PEP1 | 0 | 2.52 | 3 | 827.30 | 831.21 | 11.73 | 20 | 58.7 | 90 | 31.3 |
| PEP1 | -20 | 2.54 | 3 | 827.30 | 833.17 | 17.61 | 20 | 88.1 | 90 | 1.9 |
| PEP2 | 0 | 2.47 | 3 | 868.62 | 872.8 | 12.54 | 20 | 62.7 | 90 | 27.3 |
| PEP2 | -20 | 2.60 | 3 | 868.62 | 874.48 | 17.58 | 20 | 87.9 | 90 | 2.1 |
| PEP3 | 0 | 2.85 | 3 | 1134.237 | 1138.81 | 13.719 | 26 | 52.8 | 90 | 37.2 |
| PEP3 | -20 | 2.89 | 3 | 1134.237 | 1141.45 | 21.639 | 26 | 83.2 | 90 | 6.8 |