Supporting Information

Sensitive and Unequivocal Determination of pK_a Values of Individual Histidine Residues in Proteins Using Mass Spectrometry

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Supplemental Figures:

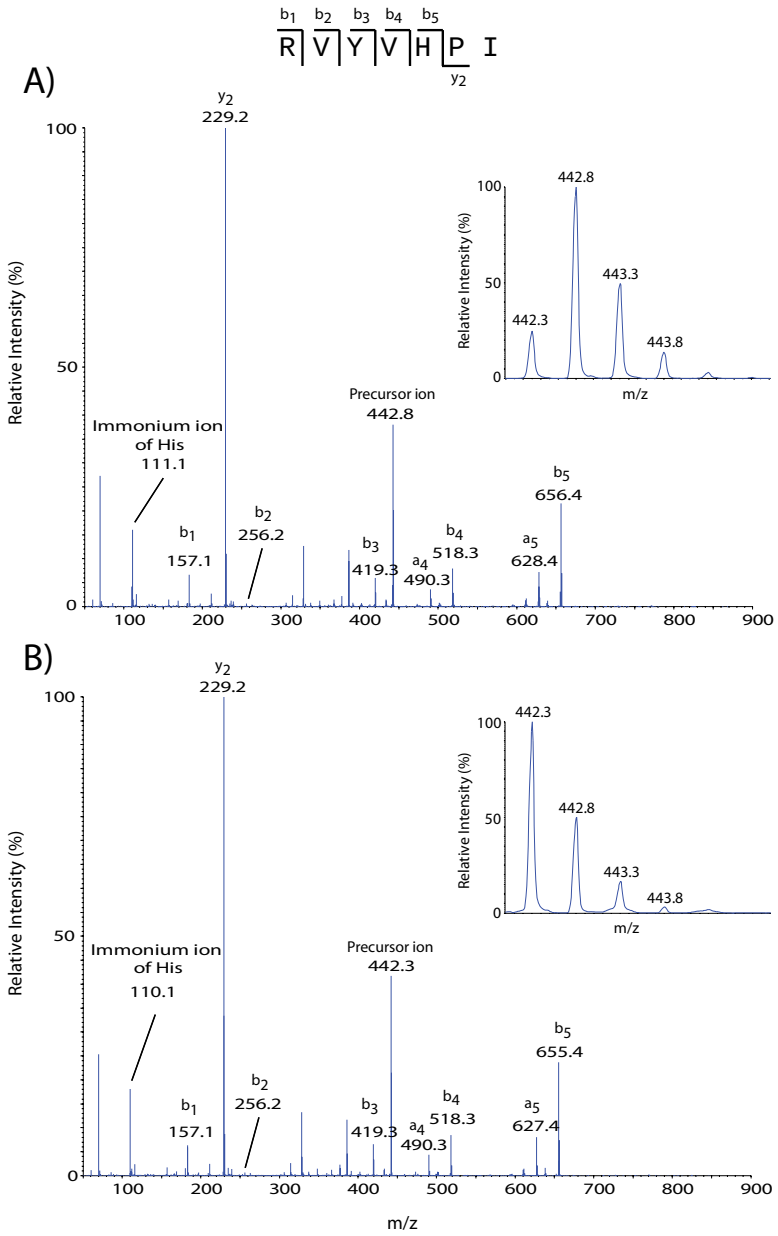

Figure S-1. Tandem mass spectrum of deuterated angiotensin III. Angiotensin III was incubated in D_2O buffered with MES (pH 7.0) at 37 °C for 7 days, and the resulting peptide were analyzed by tandem mass spectrometry. Tandem mass spectra of A) deuterated- (precursor ion: m/z 442.8) and B) nondeuterated (precursor ion: m/z 442.3) angiotensin III are shown with their precursor ion spectra (inserted spectra). Deuterated angiotensin III gave 1 Da higher m/z values for the product ions that contain the histidine residue (a_5, b_5 and immonium ion of histidine residue) than those ions produced from nondeuterated angiotensin III, while the same m/z values for the product ions that do not contain the histidine residue (b1, b2, b3, a4, b4 and y2) were given from deuterated and nondeuterated angiotensin III. The result strongly suggest that the histidine residue in angiotensin III is the site of deuterium incorporation.

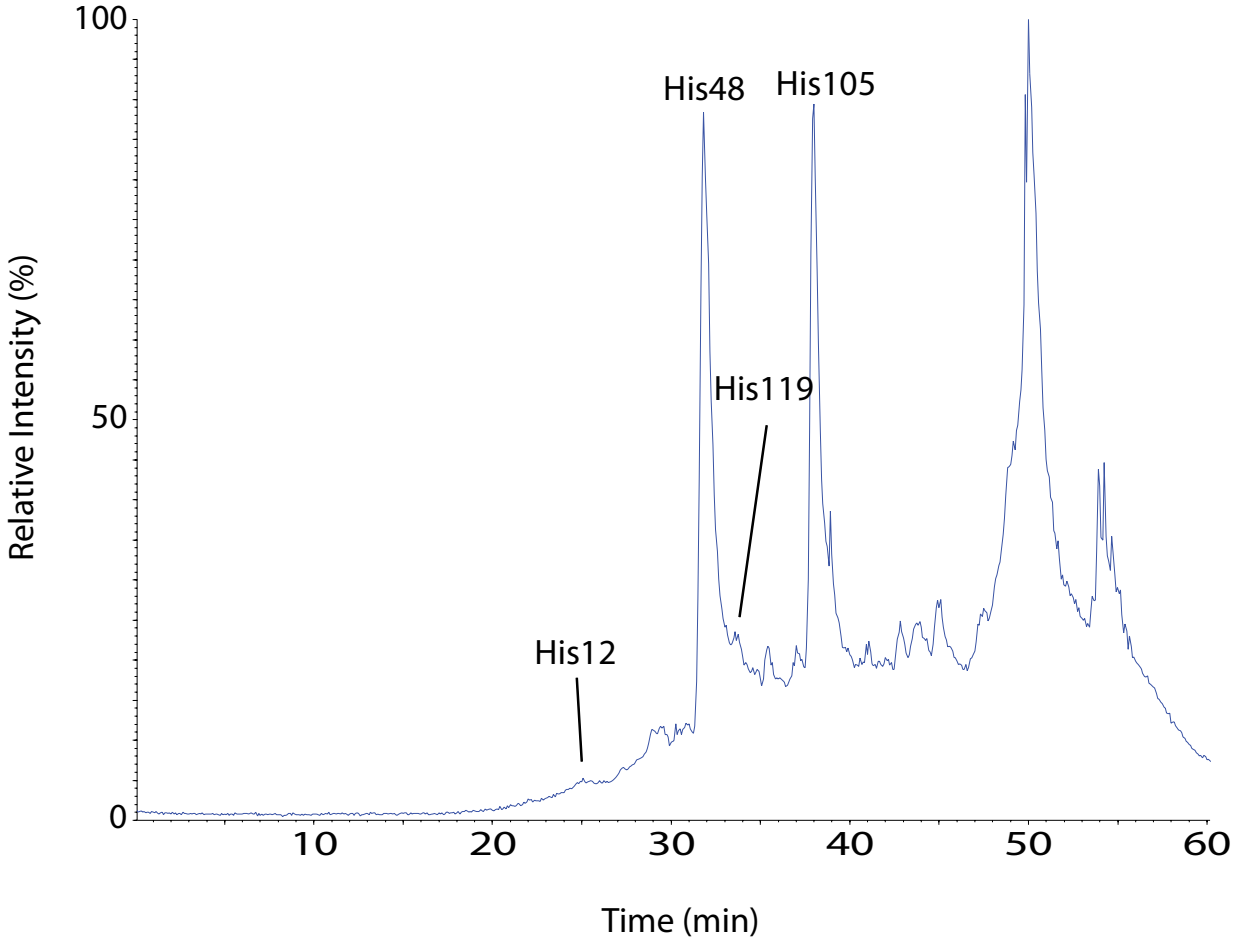

Figure S-2. LC/MS analysis of deuterated RNase A. A typical total ion current chromatogram is shown. the peptide mixture (5 mL, ca. 12.5 pmol) was injected directly into a reverse-phase analytical column (75 mm x 15 cm, Dionex, Sunnyvale, CA) washed with 2% acetonitrile/0.1% formic acid (v/v) in water for 15 min at a flow rate of 350 nL/min. The peptides were then eluted with a 40 min linear gradient of 2% acetonitrile/0.1% formic acid (v/v) in water to 22% acetonitrile/0.1% formic acid (v/v) in water at a flow rate of 350 nL/min. The column effluent was directed on-line to the nano-electrospray ion source. The total ion current was obtained in the mass range of m/z 400-2000 in the positive ion mode with the acquisition time of 5 s for each scan.

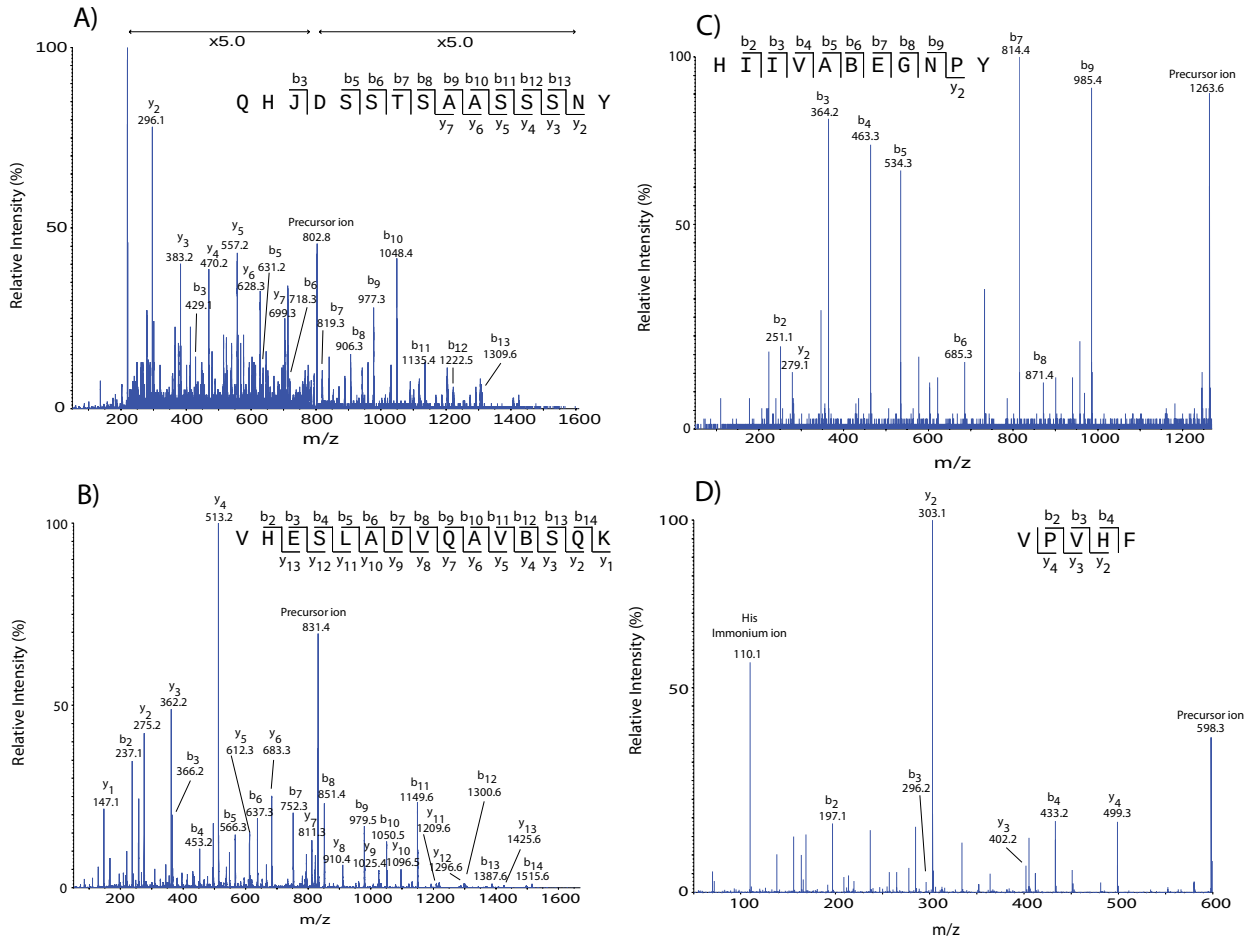

Figure S-3. Tandem mass spectra of His12- (A), His48- (B), His105- (C) and His119-peptide (D). J and B in the amino acid sequences denote methionine sulfone and cysteic acid, respectively. Precursor ions were m/z 802.8 (z = 2), 831.4 (z = 2), 1263.5 (z = 1) and 598.3 (z = 1) for His12-, His48-, His105- and His119-peptide, respectively. Only b and y series product ions are labeled.