Supplemental Information: pKa Determination of a Histidine Residue in a Peptide Using the Raman C-D Stretch

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Supplementary Equations

The difference spectra in Figure 4 were overlaid, and the maximum peak intensity at 2362 cm^{-1} of each difference spectrum was plotted versus pH. This data was then fit to a sigmoidal dose-response curve (Equation 1). The pKa was extracted from the data by finding the midpoint ($Logx_0$) of this sigmoidal curve from its equation. In the case of Figure 5, the resulting sigmoid fit is shown by Equation S1.

$$y = 18282.4841 + \frac{235463.21863}{1+10^{(6.82427-x)*1.12812}} \qquad \text{(S1)}$$

The same equation was used for subsequent fits to the NMR data in Figure 6 and alternate-region Raman spectrum (Figure S2, below).

Supplementary Figures

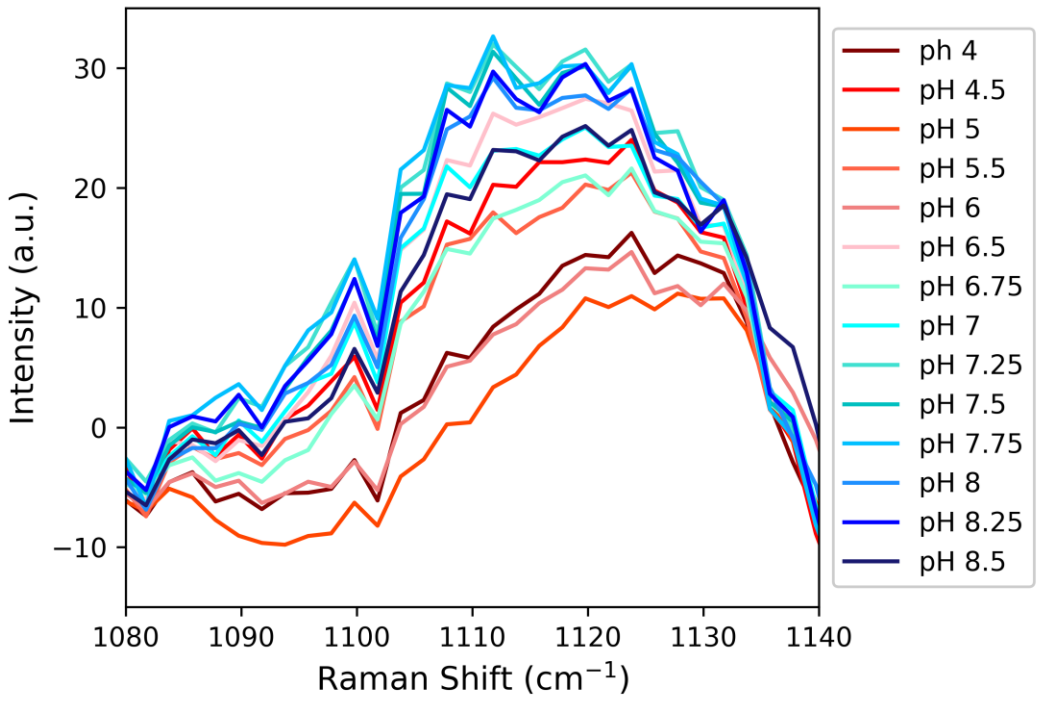

Figure S1. pH-dependent Raman spectra of H(C2-D)VD in the region of the C4-N3 stretch near 1115 cm^{-1}. The C4-N3 stretch does appear to change with pH, but not in a titratable manner. This signal was therefore found not to be useful for determining the pKa of His in the tripeptide HVD.

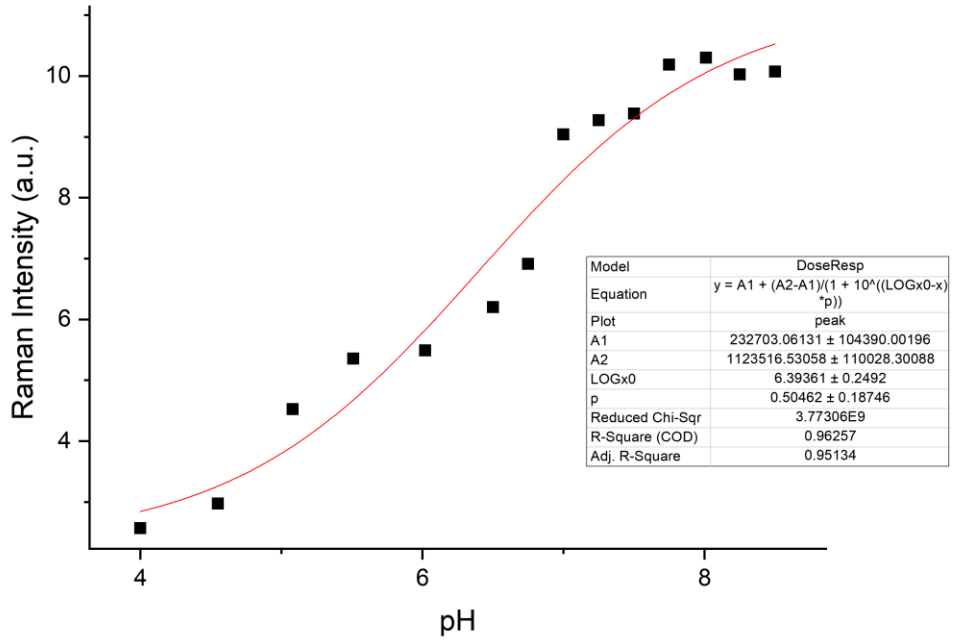

Figure S2. The maximum Raman intensity from the C4-C5 stretch at 1580 cm^{-1} (Figure 7) vs pH. A dose-response curve was then attempted to fit the data; however, the curve fits the data poorly and the pKa (6.39 ± 0.25) is reported with error margins of error and does not match the value reliably determined by ^{1}H NMR spectroscopy.

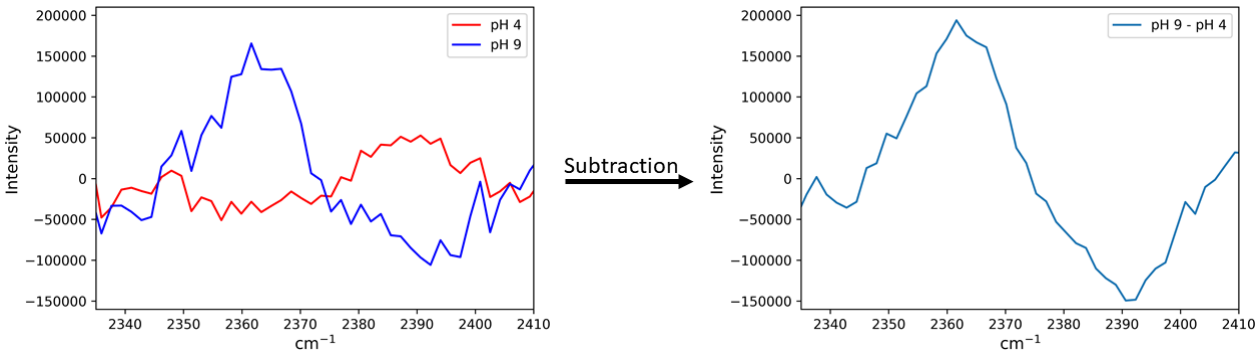

Figure S3. Difference spectra were produced by subtracting the Raman trace of a titration point (blue curve, left) from the curve from an acidic standard pH 4 sample (red curve, left). This resulted in a wave like pattern difference spectrum that displayed the disparity in peak heights between the two samples (right).

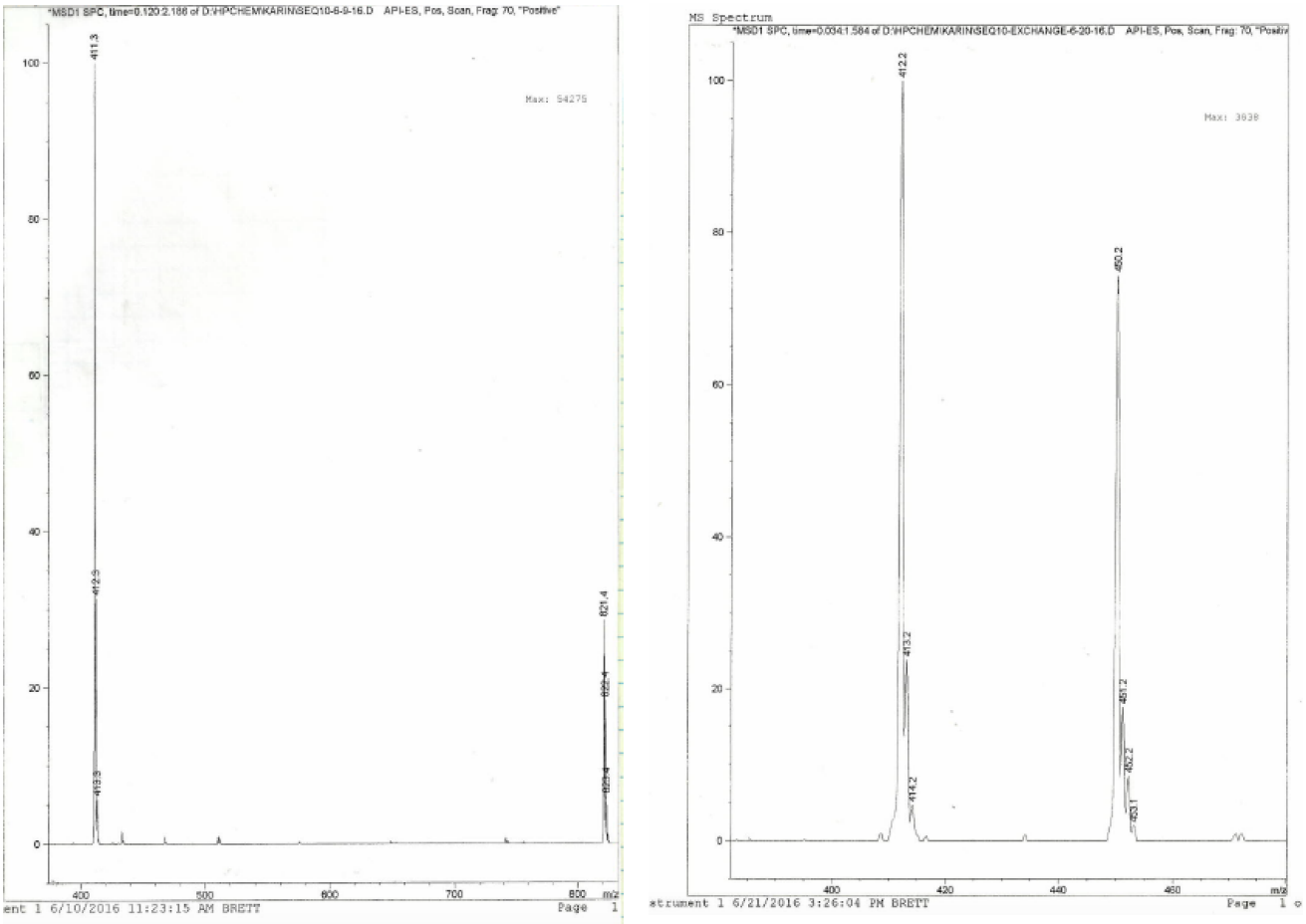

Figure S4. ES MS spectra of HVD (left) $[M+H]^+$ *m/z* = 411.3 (found), $[M+H]^+$ *m/z* = 411.2; $[2M+H]^+$ *m/z* = 821.4 (found), $[2M+H]^+$ *m/z* = 821.4 (expected); and H(C2-D)VD (right) $[M+H]^+$ *m/z* = 412.2 (found), $[M+H]^+$ *m/z* = 412.2; $[M+K]^+$ *m/z* = 450.2 (found), $[2M+H]^+$ *m/z* = 450.2.

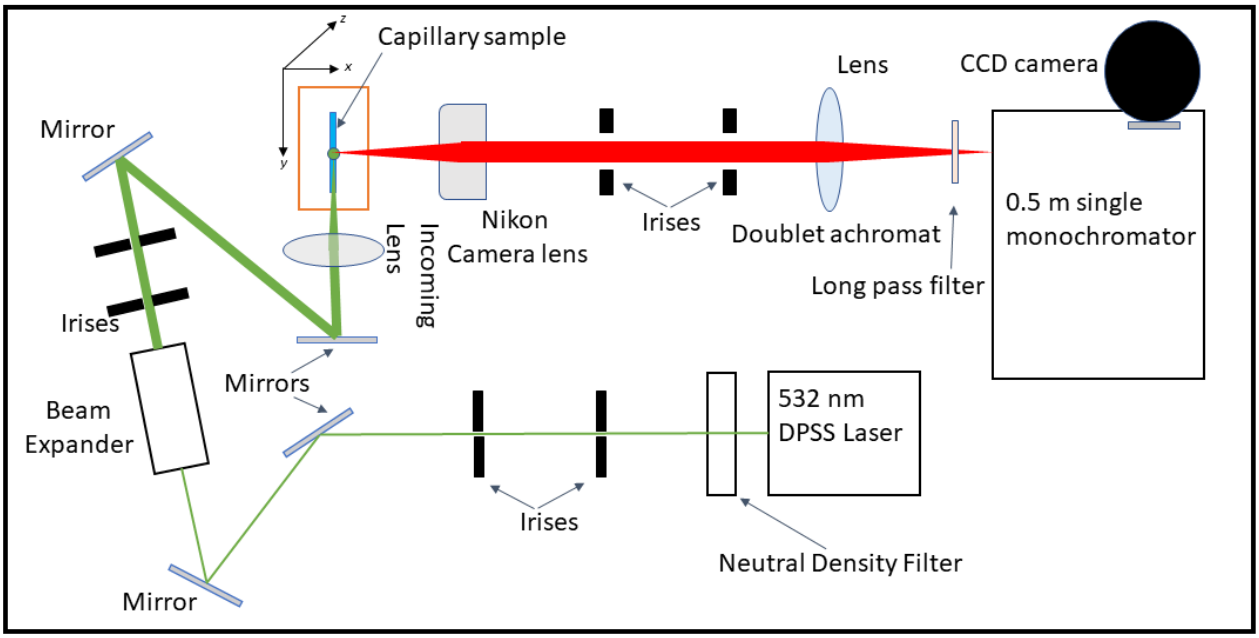

Figure S5. Box diagram of the continuous wave Raman spectrometer set-up in the Londergan lab at Haverford College.