**BPTI**

| Site | H++ p$K_{1/2}$ | Experimental p$K$ |
|---|---|---|
| **Nter1** | **6.30** | 8.1 |
| **Asp3** | **3.45** | 3.4 |
| **Glu7** | **5.67** | 3.7 |
| **Lys15** | **10.38** | 10.6 |
| **Lys26** | **10.42** | 10.6 |
| **Lys41** | **10.18** | 10.8 |
| **Lys46** | **9.77** | 10.6 |
| **Glu49** | **4.16** | 3.8 |
| **Asp50** | **2.40** | 3.0 |
| **Cter58** | **3.79** | 2.9 |

**HEWL**

| Site | H++ p$K_{1/2}$ | Experimental p$K$ |
|---|---|---|
| **Nter1** | **6.77** | 7.90 |
| **Lys1** | **9.65** | 10.80 |
| **Glu7** | **3.34** | 2.85 |
| **Lys13** | **10.44** | 10.50 |
| **His15** | **5.61** | 5.36 |
| **Asp18** | **1.65** | 2.66 |
| **Tyr20** | **15.72** | 10.30 |
| **Tyr23** | **10.67** | 9.80 |
| **Lys33** | **11.00** | 10.60 |
| **Glu35** | **5.12** | 6.20 |
| **Asp48** | **-0.63** | 1.60 |
| **Asp52** | **2.10** | 3.68 |
| **Tyr53** | **26.16** | 12.10 |
| **Asp66** | **-2.22** | 0.90 |
| **Asp87** | **0.97** | 2.07 |
| **Lys96** | **11.09** | 10.80 |
| **Lys97** | **10.82** | 10.30 |
| **Asp101** | **4.60** | 4.09 |
| **Lys116** | **9.15** | 10.40 |
| **Asp119** | **3.32** | 3.20 |
| **Cter129** | **3.64** | 2.75 |

Table 1: p$K_{1/2}$ values generated by H++ for two common benchmark proteins are compared to experimental p$K$ , where avaialbe. The following physical conditions $\epsilon_{solute} = 4$, $\epsilon_{solvent} = 80$, $salt = 0.15$, and the Poisson-Boltzmann method are used. These PDB structures serve as input: 2LZT (lysozyme) and 4PTI (BPTI). The large computed p$K_{1/2}$ for Tyr 53 and Tyr 20 of 2LZT likely indicate that these groups remain protonated for all reasonable pH values. Likewise, the negative p$K_{1/2}$ for Asp66 and Asp48 should be interpreted in terms of their protonation states – most likey deprotonated for any reasonable pH. Extreme low/high p$K_{1/2}$ often result form protonation curves that deviate from the classic Henderson–Hasselbalch shape and for which p$K_{1/2}$ lose their text book meaning (p$K_{1/2} \neq pK_a$). More on this problem can be found in Alexey Onufriev, D.A. Case and G. M. Ullmann, " A Novel View of pH Titration in Biomolecules", *Biochemistry*, 40, 3413 (2001).