

## Answer Key - Quantitative Question from Lecture #6

(a) Based on your knowledge of the approximate  $pK_a$  values of the ionizable groups in (free) tyrosine, what is the approximate  $pI$  value (isoelectric point) for this amino acid?

$pK_a$  of phenolic-OH on side chain =  $\sim 10$

$pK_a$  of  $\alpha$ -NH<sub>2</sub> =  $\sim 9$

$pK_a$  of  $\alpha$ -COOH =  $\sim 2$

$pI$  is the value of the pH where Tyr will have a net charge of zero, which will occur at a pH exactly half-way between the  $pK_a$ 's of the  $\alpha$ -NH<sub>2</sub> and  $\alpha$ -COOH groups, namely

$$(9 + 2) / 2 = 11 / 2 = \boxed{5.5}$$

At this pH, the phenolic-OH on the side chain will be completely protonated and, hence, uncharged. At this pH, the  $\alpha$ -COOH will be completely deprotonated and, hence, in the -1 state, and the  $\alpha$ -NH<sub>2</sub> will be completely protonated and, hence, in the +1 state, which will counter-balance each other.

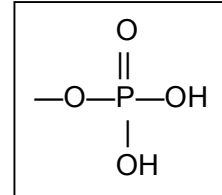
(b) The side chain of tyrosine can be phosphorylated. Based on the fact that the approximate  $pK_a$ 's of the two ionizable groups present on an esterified phosphate (see box) are  $pK_{a1} = 1$  and  $pK_{a2} = 7$ , what is the approximate  $pI$  value for (free) phospho-tyrosine?

$pK_a$  of  $\alpha$ -NH<sub>2</sub> =  $\sim 9$

$pK_a$  of 1st —OH on phosphate =  $\sim 7$

$pK_a$  of  $\alpha$ -COOH =  $\sim 2$

$pK_a$  of 2nd —OH on phosphate =  $\sim 1$



$pI$  is the value of the pH where phospho-Tyr will have a net charge of zero, which will occur at a pH exactly half-way between the  $pK_a$ 's of the two most acidic groups, namely

$$(2 + 1) / 2 = 3 / 2 = \boxed{1.5}$$

At this pH, the  $\alpha$ -NH<sub>2</sub> will be completely protonated and, hence, in the +1 state, and the —OH on the phosphate with the  $pK_a$  of 7 will be completely protonated and, hence, uncharged. At this pH, the majority of the —OH on the phosphate with the  $pK_a$  of 1 will be deprotonated and, hence, negatively charged. The percentage this hydroxyl that is not deprotonated will just equal (will exactly match) the percentage of the  $\alpha$ -COOH that is not protonated and, hence, is still negatively charged. Thus, at this pH value, the fraction of the phosphate hydroxyl (—O<sup>-</sup>) and the fraction of the  $\alpha$ -carboxyl (—COO<sup>-</sup>) that are charged will sum up to a total of -1 charge, which will counter-balance the +1 charge contributed by the protonated  $\alpha$ -NH<sub>2</sub> group. In other words, addition of a phosphate group has made phospho-Tyr a much more acidic molecule than Tyr itself.