CONCEPT: ISOELECTRIC POINT OF AMINO ACIDS WITH IONIZABLE R-GROUPS

●Amino acids with ionizable R-groups have _____ pK_a values, but pI is still an *average* of *only* _____ pK_a values.

□ *Question*: So how do you know which pK_a values to average?

1) Know the net charge of the protonated & deprotonated forms of _____ R-groups.

2) Order the 3 pK_a values from _____ to largest & use the middle pK_a as a guide.

3) Determine *net* _____ of predominate amino acid structures at any pH between adjacent pK_a values.

4) Average _____ pK_as: the middle pK_a with the pK_a closest to the pH that gives a neutral net charge.

EXAMPLE: Calculate the isoelectric point of Tyr. pK_{a1} = 9.1. pK_{a2} = 2.2. pK_R = 10.1.

Step #1: _____ how Amino Acid R-groups ionize.

Step #2: _____ the 3 pK_a values from smallest to greatest.

$pK_{a1} < pK_{a2} < pK_{a3}$

Step #3: Determine net _____ of predominate structures at any pH between each pair of adjacent pK_a's (by comparing pH to all pK_a values).

pH: pK_{a1} ····· pK_{a2} ····· pK_{a3}

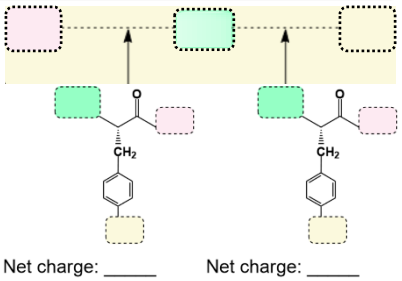
Structure #1 Net Charge

Structure #2 Net Charge

Imagine/draw predominate structures to determine their net charges.

Step #4: Calculate pI by _____ middle pK_{a2} & the pK_a "sandwiching" the pH where the predominate structure has a *neutral* net charge.

$$pI = \frac{pK_{a2} + (\text{either } pK_{a1} \text{ or } pK_{a3})}{2}$$



Net charge: _____ Net charge: _____

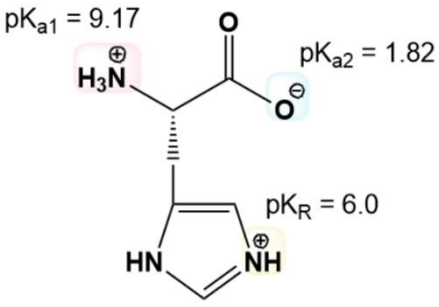
a) 7.48

b) 3.22

c) 6.96

d) 5.65

PRACTICE: What is the pI of His?



a) 7.59

b) 6.58

c) 3.91

d) 5.5

PRACTICE: Electrophoresis separates molecules by migration through a gel only if they have a net charge. How do you expect an amino acid to migrate during electrophoresis when the pH = pI?

a) Migrate from the (-) end to the (+) end. b) Migrate from the (+) end to the (-) end. c) No migration.

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PRACTICE: Draw Glu and calculate its isoelectric point. pK_{a1} = 9.67. pK_{a2} = 2.19. pK_R = 4.25.

a) 7.48

b) 3.22

c) 6.96

d) 5.93

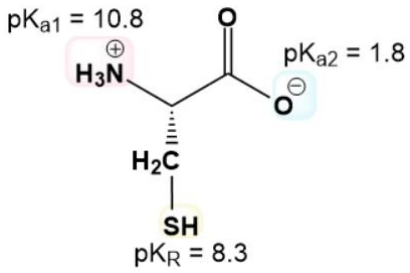
PRACTICE: Calculate Cys's pI.

a) 2.3

b) 3.14

c) 5.05

d) 6.05



PRACTICE: Calculate the pI of Arg. pK_{a1} = 9. pK_{a2} = 2.2. pK_R = 12.5.

a) 1.9

b) 10.75

c) 3.6

d) 12.5

PRACTICE: Calculate the pI of Asp.

a) 1.4

b) 3

c) 5.95

d) 6.85