

## Estimate the pI value of the following hexapeptide:

### Phe-Lys-Asp-Cys-Thr-Tyr

- Step 1: Determine the total positive charge on the peptide when all acidic and basic groups are fully protonated (at low pH).
- Step 2: Determine the total negative charge on the peptide when all the groups are titrated (at high pH).
- Step 3: List the  $pK_a$  values of all acidic and basic groups in order from lowest ( $pK_{a1}$ ) to highest.
- Step 4: Calculate the pI as the average of the values for  $pK_a$  value of the proton dissociation forming a neutral species from a +1 species, and  $pK_a$  value of the proton dissociation forming a -1 species from the neutral species.

#### So for this peptide

Step 1: charge when fully protonated +2

Step 2: charge when fully de-protonated -4

Step 3:  $pK_a$  values are:

9.0(N-term), 10.5(Lys), 3.9(Asp), 8.4 (Cys), 10.5(Tyr), 3.5(C-term)

List from lowest to highest

pKa		3.5	3.9	8.4	9.0	10.5	10.5	
Charges	+2	↔ +1	↔ 0	↔ -1	↔ -2	↔ -3	↔ -4	

Step 4: The pI is  $(3.9 + 8.4)/2 = 6.2$