1. a. Use the R-groups to help you start identifying amino acids, then look at the order of N-H, Cα, and carbonyl groups to follow the peptide backbone:

   Cys-Tyr-Phe-Gln-Asn-Pro-Arg-Gly-(NH₂)

b. 3 ionizable groups: N-term (pKa ~8), Tyr –OH (pKa ~10.5), Arg –\(^\text{+}NH₂\) (pKa ~12.5)

c. Buffering ranges for each: 7-9, 9.5-11.5, 11.5-13.5; combined: 7-9, 9.5-13.5

d. To calculate the pI, first find the pH range where the zero charge state predominates:

<table>
<thead>
<tr>
<th>pH range</th>
<th>Predominant charge on N-term</th>
<th>Predominant charge on Tyr</th>
<th>Predominant charge on Arg</th>
<th>Predominant net charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH &lt; 8</td>
<td>+1</td>
<td>0</td>
<td>+1</td>
<td>+2</td>
</tr>
<tr>
<td>8 &lt; pH &lt; 10.5</td>
<td>0</td>
<td>0</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>10.5 &lt; pH &lt; 12.5</td>
<td>0</td>
<td>-1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12.5 &lt; pH</td>
<td>0</td>
<td>-1</td>
<td>0</td>
<td>-1</td>
</tr>
</tbody>
</table>

Since the predominant net charge is zero between pH 10.5 and 12.5, use these pKa’s to calculate pI: 

\[
 pI = \frac{pK_a + pK_a}{2} = \frac{10.5 + 12.5}{2} = 11.5
\]
1. e. To find the average charge at pH 7, find the average charge (= fraction charged * charge) for each charged group, then sum the charges:

**N-term NH$_3^+$:**
\[
\text{ratio: } \frac{[\text{NH}_3]}{[\text{NH}_3^+] + [\text{NH}_2]} = 10^{pH-pK_a} = 10^{7-8} = 10^{-1} = \frac{0.1}{1} = \frac{1}{10}
\]
\[
\text{fraction charged: } \frac{[\text{NH}_3]}{[\text{NH}_3^+] + [\text{NH}_2]} = \frac{10}{10+1} = \frac{10}{11} = 0.91
\]
\[
\text{average charge: } 0.91 \times +1 = +0.91
\]

**Tyr OH:**
\[
\text{ratio: } \frac{[\text{O}^-]}{[\text{OH}]} = 10^{pH-pK_a} = 10^{7-10.5} = 10^{-3.5} = 0.0003 = \frac{0.0003}{1}
\]
\[
\text{fraction charged: } \frac{[\text{O}^-]}{[\text{OH}]+[\text{O}^-]} = \frac{0.0003}{1+0.0003} = 0.0003
\]
\[
\text{average charge: } 0.0003 \times -1 = -0.0003
\]

**Arg NH$_2^+$:**
\[
\text{ratio: } \frac{[\text{NH}_2]}{[\text{NH}_2^+] + [\text{NH}_3]} = 10^{pH-pK_a} = 10^{7-12.5} = 10^{-5.5} = 3.2 \times 10^{-6} = \frac{3.2 \times 10^{-6}}{1}
\]
\[
\text{fraction charged: } \frac{[\text{NH}_2]}{[\text{NH}_2^+] + [\text{NH}_3]} = \frac{1}{1+3.2 \times 10^{-6}} = 1.0
\]
\[
\text{average charge: } 1.0 \times +1 = +1.0
\]

Sum the average charges of each ionizable group in solution at pH 7 to find the average charge of vasopressin at pH 7:
\[
(+0.91) + (-0.0003) + (+1.0) = +1.9
\]

f. Most molecules of human vasopressin have a net charge of +2 at pH 7, and a few have a net charge of +1.

2. a. False; although there is no rotation about the peptide bond, the N-C$_\alpha$ and C$_\alpha$-C bonds (about which $\phi$ and $\psi$ are measured) do sit in the peptide plane. (There is also rotation about the N-H bond.)

b. True, although a more specific term than homologs would be ‘orthologs.’ (Written as it is, the statement is not false, but written in reverse, that is, “Homologs are related proteins that have the same function but are found in different species” would be false.)

c. True

3. a. - Given the direction of the curve, the x-axis label should be “OH’ equivalents.”
- There are 3 ionizable groups (N-term amino, Glu carboxyl, Tyr hydroxyl), there should be only 3 buffering regions (not the 4 shown)
- Each ionizable group requires only one equivalent to titrate, so the x-axis numbers should be 1, 2, 3 instead of 2, 4, 6
- The pKa’s of the ionizable groups are 4, 8, and 10.5 (none near the 2 and 13 shown)
- The buffering regions are too flat; the pH should increase somewhat over these regions
3. b. Average charge at pH 7: calculate the average charge on each ionizable group at pH 7 (by multiplying the fraction charged by the value of the charge) then sum these average charges:

N-term NH$_3^+$:

- ratio: $\frac{[NH_3]}{[NH_3^+]} = 10^{pH-pKa} = 10^{7-8} = 10^{-1} = 0.1 \equiv \frac{1}{10}$
- fraction charged: $\frac{[NH_3^+]}{[NH_3] + [NH_3^+]} = \frac{10}{10+1} = \frac{10}{11} = 0.91$
- average charge: $0.91 \times +1 = +0.91$

Glu COOH:

- ratio: $\frac{[COO]}{[COOH]} = 10^{pH-pKa} = 10^{7-4} = 10^3 = 1000 \equiv \frac{1000}{1}$
- fraction charged: $\frac{[COO]}{[COOH] + [COO]} = \frac{1000}{1+1000} = 1.0$
- average charge: $1.0 \times (-1) = -1.0$

Tyr OH:

- ratio: $\frac{[O]}{[OH]} = 10^{pH-pKa} = 10^{7-10.5} = 10^{-3.5} = 0.0003 \equiv \frac{0.0003}{1}$
- fraction charged: $\frac{[O]}{[OH] + [O]} = \frac{0.0003}{1+0.0003} = 0.0003$
- average charge: $0.0003 \times (-1) = -0.0003$

Sum the average charges of each ionizable group in solution at pH 7 to find the average charge of the peptide at pH 7:

\[(+0.91) + (-1.0) + (-0.0003) = -0.1\]

4. D. There are 20 amino acids possible in each of the four positions: $20 \times 20 \times 20 \times 20 = 20^4$

5.

a. Serotonin lacks the carboxyl group of tryptophan. (It also contains a hydroxyl group, but this should have minimal effect on the amino group’s pKa because it’s so far away.)

b. B (inductive effect) of the carboxylate oxygens in tryptophan lowers the pKa of the amino group relative to serotonin’s amino group. E (electrostatic effect) of the negative charge on tryptophan’s carboxylate raises the pKa of the amino group relative to serotonin’s.
6. Two peptides having the same titration curve will have the same number of ionizable groups. Counting the ionizable groups provides a quick first check to see which peptides could have the same titration curve (aa’s with ionizable R-groups are underlined; all of the listed peptides will also have ionizable N- and C-termini):
   a. Asp-Gly-Ser-Ser-Gln-Glu-Tyr-Cys-Arg-Val → 9 ionizable groups
   b. Ala-Glu-Tyr-Arg-Cys-Val-Ser → 7 ionizable groups
   c. Asp-Gly-Ser-Ser-Gln-Thr-Glu-Tyr-Cys-Arg-Val → 10 ionizable groups
   d. Trp-Tyr-His-Glu-Glu-Arg-Thr-Cys-Asn-Asn-Lys-Ser → 11 ionizable groups
   e. DDEMRTCSP → 9 ionizable groups
   f. ACDEFGILMNPOSTVWX → X is not an amino acid! This is not a real peptide.
   g. DDLQNRSASYNCW → 9 ionizable groups
   h. KKGEFMNRTSSCYQ → 11 ionizable groups

So, a, e, and g all have 9 ionizable groups, and d and h have 11.

Next, check the pKa’s of the ionizable groups:
   a. (N→C): 8, 4, 13, 13, 4, 10.5, 8.5, 12.5, 3; (in number order): 3, 4, 4, 8, 8.5, 10.5, 12.5, 13, 13
   b. (N→C): 8, 4, 4, 4, 12.5, 8.5, 10.5, 13, 3; (in number order): 3, 4, 4, 4, 8, 8.5, 10.5, 12.5, 13
   c. (N→C): 8, 4, 4, 12.5, 13, 13, 10.5, 8.5, 3; (in number order): 3, 4, 4, 8, 8.5, 10.5, 12.5, 13, 13
   d. (N→C): 8, 10.5, 6, 4, 4, 12.5, 13, 8.5, 10.5, 13, 3;
      (in number order): 3, 4, 4, 6, 8, 8.5, 10.5, 10.5, 12.5, 13, 13
   e. (N→C): 8, 10.5, 10.5, 4, 12.5, 13, 13, 13, 10.5, 8.5, 3;
      (in number order): 3, 4, 8, 8.5, 10.5, 10.5, 10.5, 12.5, 13, 13, 13

Peptides a and g have the same set of pKa’s, so they’ll give rise to the same titration curve:

7. a. D. not able to be determined (since you don’t know which ionizable groups it has)
   b. B. positive (since a few more groups will be protonated than when the protein has zero average charge)
   c. A. zero
   d. A. zero
   e. C. fewer acidic than basic residues – since the pKa is above 7, this means the pH has to be raised to neutralize positive charge, which would come from basic residues.
8. a. A. the charged side chains: asp, glu, his, lys, arg  
   B. cys  
   C. asp, glu, his, lys, asn, gln, arg, ser, thr, trp, tyr  
   D. all of them!  
   E. cys, asp, glu, his, lys, arg, ser, thr, tyr  

   b. Since each buffering range spans less than one equivalent, each corresponds to one ionizable group. The buffering ranges are centered around pH 3, 8, and 13, so these are the pKa’s of the ionizable groups. pKa’s 3 and 8 correspond to the C- and N-termini, respectively, so 13 corresponds to an R-group: Ser or Thr. This means that one residue of the dipeptide must be Ser or Thr. And the other residue must not have an ionizable R-group, because there is no additional group titrated in the curve. Part E above lists the ionizable R-groups; there are 9. So there are 11 amino acids (20 – 9 = 11) that have non-ionizable (NI) R-groups (ala, phe, gly, ile, leu, met, asn, pro, gln, val, trp). So the different possible dipeptides are:

   (Ser/Thr)–(NI) = 2 x 11 = 22 possible sequences  
   or (NI)–(Ser/Thr) = 11 x 2 = 22 possible sequences  
   22 + 22 = 44 possible sequences

   c.

<table>
<thead>
<tr>
<th>pH range</th>
<th>Predominant charge on C-term</th>
<th>Predominant charge on N-term</th>
<th>Predominant charge on Ser/Thr</th>
<th>Predominant net charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH &lt; 3</td>
<td>0</td>
<td>+1</td>
<td>0</td>
<td>+1</td>
</tr>
<tr>
<td>3 &lt; pH &lt; 8</td>
<td>-1</td>
<td>+1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8 &lt; pH &lt; 13</td>
<td>-1</td>
<td>0</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>13 &lt; pH</td>
<td>-1</td>
<td></td>
<td>-1</td>
<td>-2</td>
</tr>
</tbody>
</table>

   \[ pI = \frac{pK_{a_i} + pK_{a_j}}{2} = \frac{3 + 8}{2} = 5.5 \]

9. c

10.

\( \alpha \)-D-altropyranose   \( \beta \)-D-altropyranose

11. The angle between two planes.

12. a. Ramachandran plot  
   b. Darkest are favored dihedral angles, medium are allowed, and white are disallowed angles.  
   c. It corresponds to a glycine in the structure, which can adopt angles that are disallowed for other amino acids.  
   d.  
   e. The N-terminal amino group has no peptide bond, so there’s no reference for measuring \( \phi \).