pH Ruler Java Applet for Developing Interactive Learning Exercises on Protonation States and Titration Curves

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Abstract

Students in introductory biochemistry courses often struggle to understand the basic concepts underlying the titration of weak acids, especially for important biological molecules like amino acids with more than one titratable group. In the past, we have only had limited success in trying to develop interactive laboratory and tutorial exercises to help students learn these extremely important concepts. To provide students with an intuitive and flexible graphical interface for learning about protonation states and titration curves, we have written a Java applet that allows students to explore, in a self-directed and interactive manner, how variations in pH affect the protonation states of carboxylate, amino, imidazole and thiol groups in amino acids and peptides. This simple software tool provides an effective means for developing inquiry-based computer laboratory exercises to help students improve their understanding of basic concepts involving ionization states. We also envision that once these basic concepts have been mastered, students can use the same applet to develop a deeper understanding of more advanced concepts in biochemistry, including protein purification procedures like ion exchange chromatography, protein characterization techniques like isoelectric focusing, protein-ligand interactions and enzyme catalysis.

Introduction

In introductory biochemistry courses, it is often a struggle to teach the basic concepts of pH and titration and to demonstrate how these concepts apply to biological systems. The task becomes even more difficult when students in the class have very diverse backgrounds and interests.

This problem often arises in BCEM 341 (Biochemistry of Life Processes), an introductory biochemistry course taken by approximately sixty non-biological sciences majors at the University of Calgary. In this course, students from kinesiology or physical education are often interested in how the pH changes that arise during exercise affect the physiology of tissues and cells. However, many of these students lose interest as the dry and abstract details of the Henderson-Hasselbach equation are presented in the traditional manner prescribed by most textbooks. Chemistry majors on the other hand are more familiar with the theory of pH and titrations, but struggle instead with how changes in pH relate to biochemical and physiological processes.

To more effectively engage and challenge students, we have developed a new strategy that introduces a simplification of the terminology and provides several inquiry-based, self-directed learning exercises.

Problems with the use of conjugate acid (HA) and conjugate base (A⁻) terminology

Most textbooks present the dissociation of a conjugate acid (HA) into a conjugate base (A^-) and a proton (H^+). Often, this leads students to believe that HA does not carry a charge, whereas in reality, many biological compounds are positively charged in the protonated state. Similarly, A^- implies that conjugate bases contain a negative charge, whereas many biological compounds are neutral, when deprotonated.

Key concept: Students often mistakenly think HA is acidic and neutral, and that A^- is negatively charged and alkaline.

Introducing a simplified terminology for conjugate acids and bases

To avoid the confusion introduced by traditional HA & A^- terminology, we suggest the following abbreviations:

conjugate acid: CA
conjugate base: CB

Thus strong acids can dissociate in water as follows:
$$H_2O + CA \longrightarrow H_3O^+ + CB$$

Weak acids can form the following equilibrium:
$$H_2O + CA \rightleftharpoons H_3O^+ + CB$$

The equilibrium formed between a weak acid and its conjugate base forms the basis for how buffers work. When the CA and CB terminology is used, there is no implication that CA must be neutral as was conveyed in the old HA terminology. Similarly, use of CB to represent the conjugate base does not imply a negatively charged species, as was often mistakenly implied by the A^- terminology.
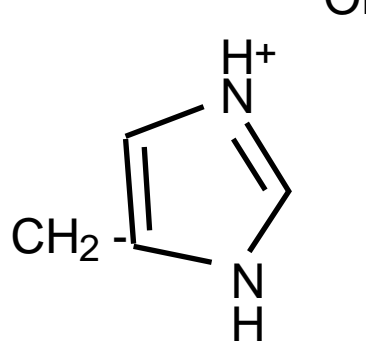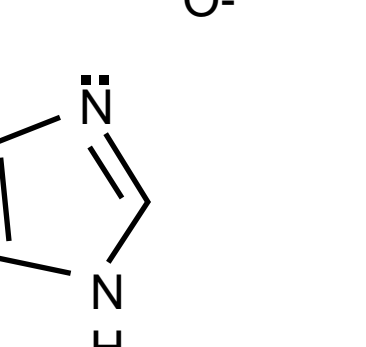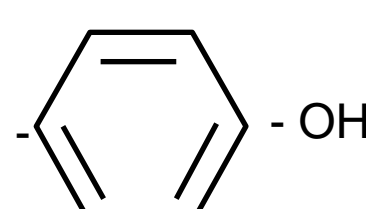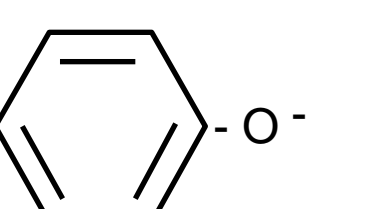
Applying the simplified terminology to biological molecules

Amino acids are biological molecules containing at least two titratable groups:
· the α-amino group (red);
· the α-carboxyl group (blue);
· in some cases (7 out of 20) a titratable side chain (green).
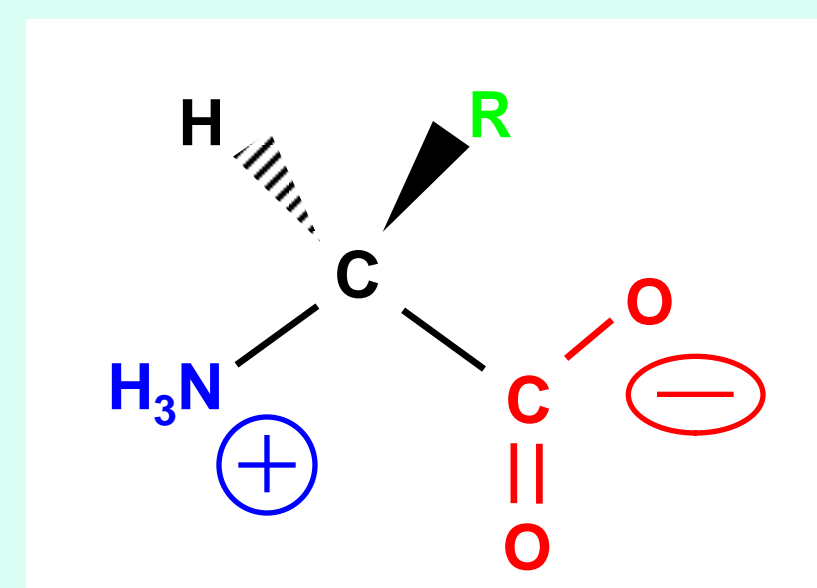
Use of the old HA / A^- terminology led many students to mistakenly assign a neutral charge to all conjugate acids and a negative charge to all conjugate bases. This became particularly problematic when looking at amino acid side chains, where some are positively charged in the protonated (conjugate acid) state and neutral in the deprotonated (conjugate base) state, as shown below. Using the new terminology, students do not automatically assume that the conjugate acid is always neutral and the conjugate base is always negatively charged.
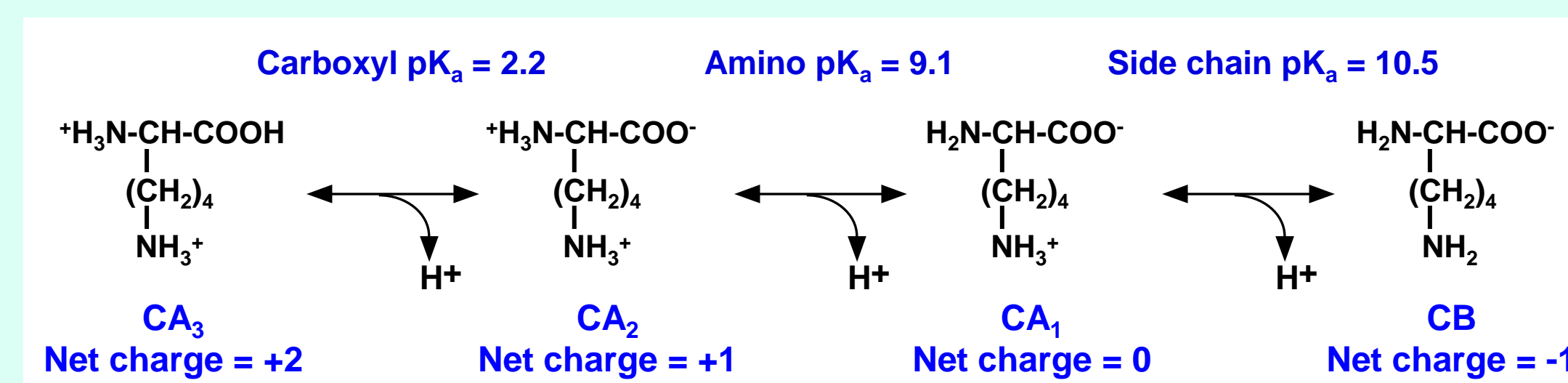
	CA form of R group	CB form of R group	pK_a
Asp	$- CH_2 - C{<}^O_{OH}$	$- CH_2 - C{<}^O_{O^-}$	3.90
Glu	$- CH_2 - CH_2 - C{<}^O_{OH}$	$- CH_2 - CH_2 - C{<}^O_{O^-}$	4.07
His	$- CH_2 -$ (imidazole, protonated)	$- CH_2 -$ (imidazole)	6.04
Cys	$- CH_2 - SH$	$- CH_2 - S^-$	8.37
Tyr	$- CH_2 - $ (phenol) $- OH$	$- CH_2 - $ (phenol) $- O^-$	10.54
Lys	$- (CH_2)_4 - NH_3^+$	$- (CH_2)_4 - NH_2$	10.50
Arg	$- (CH_2)_3 - NH - C{<}^{NH_2^+}_{NH_2}$	$- (CH_2)_3 - NH - C{<}^{NH}_{NH_2}$	12.48

## Applying the simplified terminology to the titration of a triprotic amino acid

If an amino acid has a titratable side chain, it is an example of a triprotic acid, designated as CA_3 with our simplified terminology. To demonstrate how changes in pH affect the ionization states of the three titratable groups, students are asked to draw the amino acid in its most protonated state and then titrate it sequentially from pH zero to 14.

Example: titration of lysine

Starting at the left, at pH zero, the CA_3 form of lysine has a net charge of +2. The group with the lowest pK_a is the α-carboxylate group, which is the first group to lose its proton as base is added. Once this first proton is removed, the CA_2 form of lysine is produced, which has a net charge of +1. The α-amino group has the next lowest pK_a and loses its proton next to produce CA_1, a zwitterionic form with a net charge of 0. The side chain amino group has the highest pK_a and releases the final proton to produce the CB form with a net charge = –1. charge.

Carboxyl pK_a = 2.2   Amino pK_a = 9.1   Side chain pK_a = 10.5

^+H_3N-CH-COOH ^+H_3N-CH-COO$^-$ H_2N-CH-COO$^-$ H_2N-CH-COO$^-$
$(CH_2)_4$ $(CH_2)_4$ $(CH_2)_4$ $(CH_2)_4$
NH_3^+ NH_3^+ NH_3^+ NH_2

CA_3 — Net charge = +2 CA_2 — Net charge = +1 CA_1 — Net charge = 0   CB — Net charge = -1

Students can construct a titration curve using a 5-point approach, based on calculations they perform with the Henderson-Hasselbach equation. Each functional group is titrated separately and in sequence starting with the most acidic pK_a and ending with the most alkaline pK_a. The titration curve for the amino acid lysine constructed using the 5-point approach is shown below.
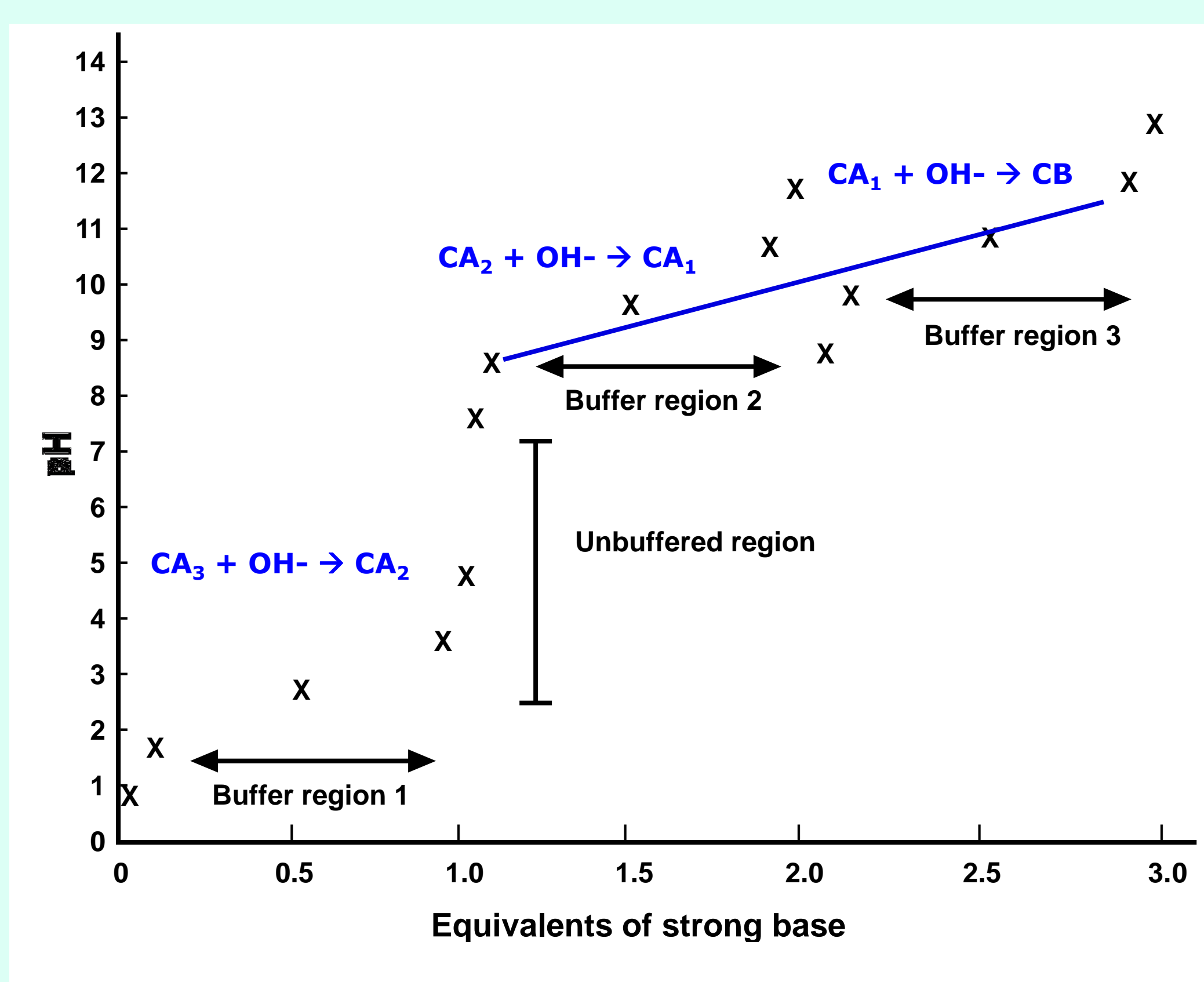
Since all three titratable groups on lysine are weak acids, buffering at or near the pK_a's of these groups can occur in aqueous solutions. Because the pK_a of the α-amino group is so close to that of the ε-amino group of the side chain, these two groups titrate near simultaneously, generating an extended buffer region. In contrast, there is a clearly defined unbuffered region between the CA_3 and CA_2 forms.

Development of an interactive Java Applet for visualizing the distribution of CA and CB forms of amino acids as a function of pH

We have developed a Java Applet to allow students to visualize the changes in amino acid protonation states as a function of pH. Students have responded extremely positively to the intuitive, graphical "pH ruler" interface. Sliders are used to interactively control pH, as well as the pK_a values of different titratable groups. The Henderson-Hasselbach equation is used to calculate the concentrations of CA and CB forms, and bar graphs provide a visual representation of these concentrations. The three screenshots below illustrate how changes in the pH slider affect the distribution of CA and CB for the simple case of alanine.
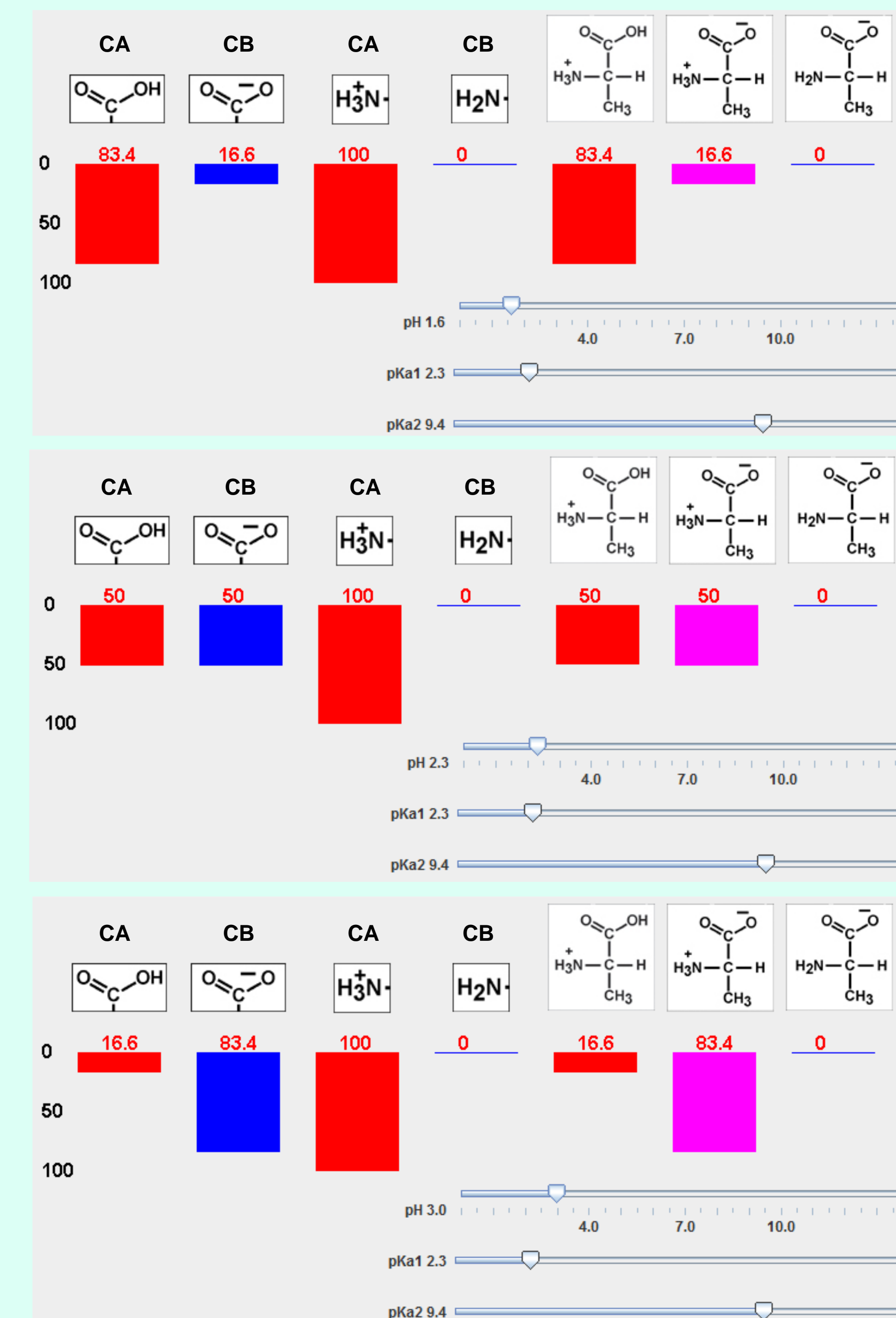
The interactive nature of the applet provides students with a self-directed, inquiry-based learning experience that requires minimal instruction. The applet can be easily modified to accommodate more complex systems with additional titratable groups, including interesting cases like lysine, where the two amino groups can also use similar pK_a values. Instructors can also use the applet to develop exercises to test each student's understanding of pH, buffers and net charge, as well as more advanced concepts central to protein-ligand interactions, enzyme catalysis and protein purification via ion exchange chromatography.

Key concept: The pH ruler Java applet provides students with a simple graphical interface for exploring how changes in pH and variations in the pK_a's of functional groups affect the concentrations of CA and CB forms in solution.