
The Saturation Game: Teaching Protein-Ligand Binding with a Playing Card Analogy

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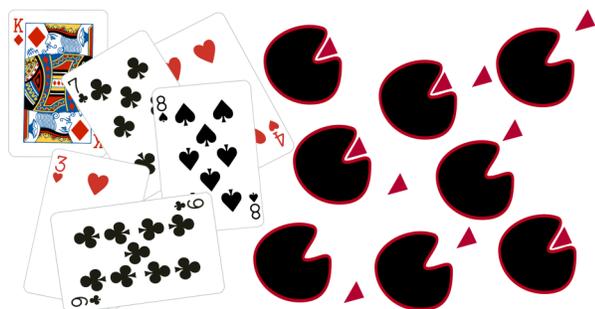
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ABSTRACT

The dissociation constant of a protein-ligand complex is commonly measured by a saturation binding experiment, in which a protein is progressively saturated by repeated additions of a ligand. Students often find it hard to understand how the hyperbolic binding curve, described by the Hill-Langmuir equation, arises from an unchanging dissociation constant. In this activity, students played a pre-lab card game which taught them the stochastic origin of the binding curve's shape. The saturation game almost doubled the number of students who chose the correct explanation of the curve's shape and decreased the popularity of the most common misconception by a factor of 2.5.

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GRAPHICAL ABSTRACT



KEYWORDS

First-Year Undergraduate / General; Biochemistry; Analogies / Transfer; Hands-On Learning / Manipulatives; Humor/Puzzles/Games; Inquiry-Based / Discovery Learning; Bioanalytical Chemistry; Biophysical Chemistry; Equilibrium; Proteins/Peptides

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INTRODUCTION

Much enzymology and pharmacology rests on the reversible binding of a single, small-molecule “ligand” (L) to a protein “receptor” (R), making the process shown in eqn. 1 an important application of thermodynamics in the undergraduate biochemical curriculum.



Protein-ligand binding strength is typically measured as the equilibrium dissociation constant (K_d) of the complex LR . The value of this constant is found by a saturation binding experiment and the analysis of the Hill-Langmuir equation.¹ The simplest version of this equation (eqn. 2) describes how the concentration of the ligand-receptor complex changes when a protein at low and constant concentration is progressively saturated by a ligand which binds independently to each protein.¹

$$[LR] = [R]_0 \cdot \frac{[L]}{[L] + K_d} \quad (2)$$

where $[R]_0$ is the total receptor protein concentration.

The shape of the saturation binding curve described by eqn. 2 is shown in Figure 1.¹ That an unchanging value of K_d should produce a hyperbolic curve rather than a straight line is hard for many students to understand, and our activity was designed to overcome that conceptual difficulty.

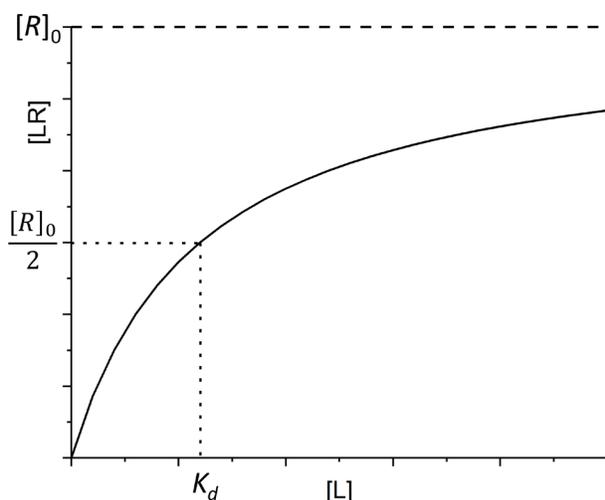


Figure 1. An ideal saturation binding curve, showing the hyperbolic dependence of $[LR]$ on $[L]$ according to eqn. 2. The asymptote of the line, corresponding to the total protein receptor concentration $[R]_0$, is shown as a dashed line. The dissociation constant K_d is the concentration of L at which $[LR]$ is equal to $[R]_0/2$ (shown by dotted line).

Analogies, some involving playing cards, have been found to be powerful tools to teach concepts in thermodynamics and equilibria.²⁻⁵ In this work we describe a card game that has helped first-year bioscience undergraduates understand why the graph shown in Figure 1 has the shape it does. In particular, it showed students that the line changes its gradient not because the protein changes its affinity for the ligand, but because of probabilistic effects. Having understood why the line is curved, the students were better placed to analyze an experimental saturation binding curve and extract the value of the dissociation constant K_d .

CONTEXT

The activity was carried out by 114 first-year university students in an introductory biochemistry course, most of whom were studying for a degree in Biomedical Sciences. The laboratory class was taught in two sections of 60 and 54 students, respectively. They played the saturation game in the teaching laboratory before titrating avidin with 2-[(4-hydroxyphenyl)azo]benzoic acid (HABA) to find the dissociation constant of the avidin-HABA complex through its absorbance at 500 nm.^{6,7} Although avidin is homo-tetrameric, recent measurements have confirmed that this system exhibits no cooperativity between the four binding sites, so that the binding of one HABA to each avidin subunit can be described by eqn. 2.⁸ The students had recently attended lectures and solved practice problems in elementary biological thermodynamics, and were familiar with the basic concepts of chemical equilibria. They had previously studied oxygen binding to hemoglobin and myoglobin, but had not quantified these proteins' affinity for oxygen by measuring their p_{50} values in the laboratory. Before they came to the lab, the students had read some introductory material on the avidin titration and all of them had answered three online multiple-choice questions (MCQs) to reinforce their reading (see Supporting Information). The saturation game further prepared students to measure a biochemical equilibrium constant by showing them the physical origin of the binding curve's hyperbolic shape.

A pretest posttest design was used to measure the effect that the activity had on the students' understanding. Before and after they played the game, students were asked the following in-class MCQ through TurningPoint (Turning Technologies): "Adding HABA at the start of the avidin titration will produce a large increase in absorbance at 500 nm. Why will adding the same amount of HABA at the end of the titration produce a smaller increase in absorbance?" The answer options were:

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- A. The added HABA is less likely to bind to avidin than it was at the start.
 - B. The added HABA binds to avidin with a lower affinity than it did at the start.
 - C. The added HABA binds to a different site on avidin than it did at the start.
 - D. The added HABA produces a smaller increase in [HABA] than it did at the start.

75 Option A was the correct answer and option B was intended to be the principal distractor, representing a major misconception that had emerged in the students' previous work. Options C and D were speculative distractors and most students, having completed their pre-lab tasks, were expected to reject them: C is simply incorrect, and the insignificant dilution of the assay solution by the volumes of HABA used cannot explain the shape of the saturation binding curve. Students were not
80 shown whether their answer to the pretest was right or wrong, so that they could not use this information in the posttest. The students' pretest and posttest answers to this question are described in the Results section.

ACTIVITY

Each pair of students was given a shuffled, standard deck of 52 playing cards (without jokers) and
85 a rule sheet (see Supporting Information), which the instructor ran through verbally before they began. Students were told that each card represented a molecule of the ligand, HABA, and that the bench represented the protein, avidin. One student in each pair (the Player) was asked to play the cards onto the bench according to the rules, and the other (the Recorder) to record in a Google Forms questionnaire how many cards had successfully "bound" to the bench each round. The Google Form
90 was connected to a Google Sheets spreadsheet, so that each pairs' results were copied there after they finished playing.

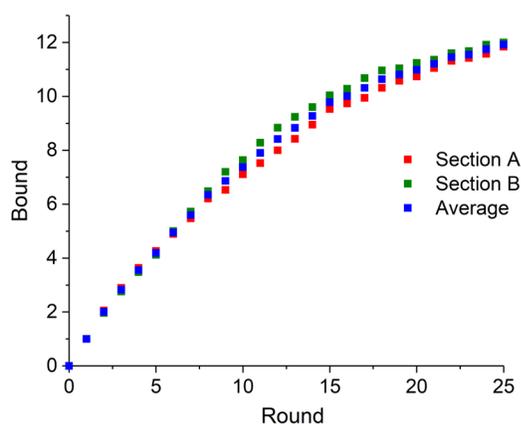
The students were told that each round of the game would represent an increase in the concentration of HABA. Before the game began, in Round 0, no cards had been played, no HABA had been added and thus no ligand had bound to avidin. All the Recorders, therefore, recorded 0 bound
95 cards. The Players then played the first card from the deck, laying it face-up on the bench. This molecule of HABA had bound to an avidin molecule, and the Recorders all noted that 1 card had bound in Round 1.

Up to this point the game had yielded the same results for every pair, but it now became more interesting. For the second and each subsequent round, each Player was told to examine the next card in their deck. If the value of the card (2-10, Jack, Queen, King, Ace) matched one of the cards currently face-up on the bench, the Player discarded it, face down – it did not bind. If the value did *not* match, however, the Player laid it face-up on the bench – it bound. In either case, the Recorder recorded the total number of face-up cards, keeping a cumulative tally up to the maximum number of 13. The game ended after Round 25 had been played or when 13 cards had bound to the bench, whichever came first. If the game ended before Round 25 then the Recorder was asked to record 13 bound cards for all the remaining, unplayed rounds, reflecting the continuing saturation of avidin at higher ligand concentrations.

After about 10 minutes, when all the pairs had finished playing the saturation game and had reported their results, the instructor averaged the class results for each round on Google Sheets and presented them to the students as a scatterplot, with “Round Number” on the x-axis and “Number of cards bound” on the y-axis.

RESULTS

The averaged results of the saturation game obtained from both class sections are shown in Figure 2 as a “Bound *vs* Round” scatterplot.

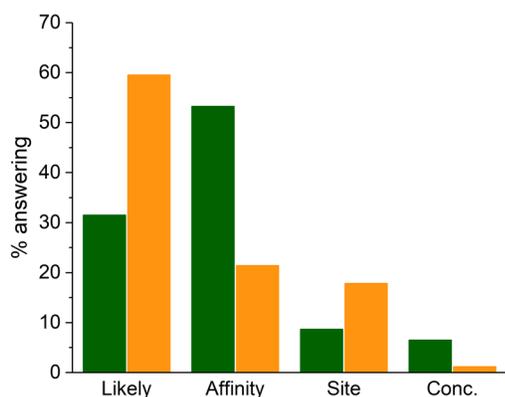


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Figure 2. Number of cards “bound” in each round of the saturation game. The averaged results of Section A (27 student pairs, red squares) and Section B (25 student pairs, green squares) are presented along with the averaged results of the whole class (52 student pairs, blue squares).

120 Students were able to see that their averaged results formed a curve closely resembling a protein saturation binding curve. Having constructed the curve themselves, they could appreciate why the gradient of the line decreased as the game went on: as cards progressively occupied the 13 avidin “binding sites” on the lab bench, it became less and less likely that the next card to be played would bind.

125 With no further comment from the instructor, the students were asked the same question as they had been before they played the game: when you perform the avidin titration, why will adding the same amount of HABA at the end of the titration produce a smaller increase in absorbance than it did at the start? Their pretest and posttest answers to this question are shown in Figure 3. Before they played the saturation game the most popular explanation (given by 53.3% of the students) was that
130 the added ligand would bind to the protein with a *lower affinity* at the end of their titration than it did at the start, and only 31.5% of the students chose the correct answer. After the game, however, the most popular explanation (given by 59.5% of students) was the correct one: the added ligand would be *less likely* to bind to the protein at the end of the titration than it was at the start. The proportion of students who thought that the binding affinity would change fell to 21.4% following the saturation
135 game, and the popularity of the “concentration” distractor fell from 6.5% to 1.2%. The popularity of the “wrong site” distractor, however, was increased from 8.7% to 17.9%.



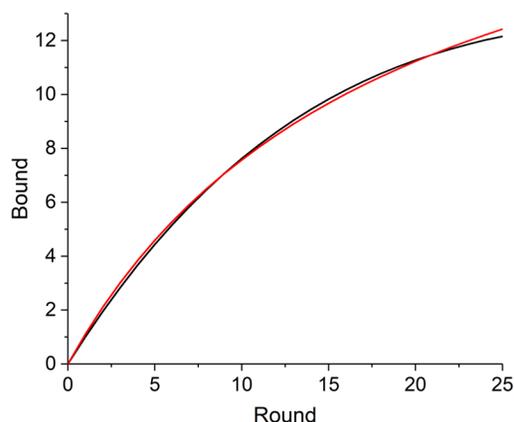
140 Figure 3. Pretest (green) and posttest (orange) answers to the in-class MCQ, asking students why adding the same amount of HABA at the end of the avidin titration produces a smaller increase in absorption than it does at the beginning. “Likely” signifies the correct answer, “The added HABA is less likely to bind to avidin than it was at the start;” “Affinity” signifies the main distractor, “The added HABA binds to avidin with a lower affinity than it did at the start;” “Site” signifies “The added HABA binds to a different site on avidin than it did at the start;” and “Conc.” signifies “The added HABA produces a smaller increase in [HABA] than it did at the start.”

DISCUSSION

145 Playing the saturation game (perhaps discussing it with their partner as they did so) and seeing the averaged class results as a scatterplot (Figure 2) almost doubled a student's chance of selecting the correct, probabilistic explanation for the decreasing gradient of the avidin/HABA saturation binding curve (Figure 3). Figure 3 also shows that the game reduced the popularity of the most common misconception – that the decreasing gradient reflected the decreasing affinity of the ligand for the receptor – by a factor of 2.5. It is likely that these learning gains would be amplified if the
150 instructor explained the analogy after the game had been played. Specifically, the instructor would explain that each round represented the addition of HABA; that a card laid on the table was a HABA molecule bound to avidin; and that as the game progressed, and more binding sites were occupied, it became less and less likely that added HABA would bind to avdin, so that the binding curve had risen
155 less steeply. This was not done in the present study, however, so that the pedagogical power of the game alone could be measured.

 An unwelcome side-effect of the saturation game was to double the popularity of the “different site” distractor, although it remained a minority belief among the class. It may be that the students who chose this answer misinterpreted the analogy, mistaking the different “bound” cards on the bench for
160 different binding sites on the same avidin molecule. Future implementations of the game will have the instructor address this misconception directly.

 The more times the saturation game is played (*i.e.* the bigger the class size) the smoother the resulting “bound *vs.* round” curve will be. This will make its similarity to a real saturation binding curve more impressive, and probably enhance the activity's effectiveness. A numerical approximation
165 of the game's cumulative probability distribution function (see Supporting Information) closely matches a fitted saturation binding curve produced by the Hill-Langmuir equation using suitable parameters (Figure 4).



170 Figure 4. A numerical approximation of the ideal saturation game curve (black line) compared to the closest-fitting Hill-Langmuir equation curve (red line). Details of the numerical approximation can be found in the Supporting Information. The Hill-Langmuir equation parameters were $[R]_0 = 21.68$ and $K_d = 18.62$, obtained using the GRG nonlinear engine in Microsoft Excel's Solver add-in.

The Hill-Langmuir equation parameters used to produce the red line in Figure 4 are artificial; a realistic $[R]_0$ could not exceed 13, which is the maximum number of cards that can be played in the saturation game. This is a reminder that the saturation game is a pedagogical analogy, not a simulation, and like all analogies it should not be pushed too far. If the game is played beyond 25 rounds then the lab bench “binding sites” become saturated, the card curve levels off at 13 bound cards, and the fit to the Hill-Langmuir equation is spoiled. After all, ligands and proteins diffuse through three-dimensional space and bind to one another reversibly, unlike cards laid on a bench. This makes molecular equilibrium probability densities⁹ very different, and more complicated, than the mathematics of the saturation game described in the Supporting Information.

185 A more realistic pedagogical approach to stochastic protein ligand binding may be made through computer simulations,¹⁰ but the strength of the saturation game lies in its simplicity rather than its accuracy. The playing cards through which it conveys its message are familiar and tangible manifestations of chance. Like dice games,^{11, 12} card games can teach students how rules emerge from randomness, allowing them to connect their own experience of luck with the molecular world.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available as a Microsoft Word file on the ACS Publications website
190 at DOI: 10.1021/acs.jchemed.XXXXXXX. It contains an analysis of the mathematics of the saturation
game, the student handout, and the pre-lab MCQs.

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