SPIKES*mate*

Instruction Manual: Detailed Usage Instructions

01 – Homepage

Navigating to the link provided from the PHAR3311 unit webpage will direct the browser to the SPIKES*mate* website homepage.

Static Elements:
Text Logo: 'SPIKES*mate*'.
Text: 'An educational application to boost proficiency in interpreting competition binding data'.

Interactive Elements:

A: Homepage Button - 'SPIKES*mate*'.
B: Menu Button - '≡'.

A) This button will navigate the browser back to the SPIKES*mate* homepage. This can be clicked from any page in the website and will return the user to the homepage.
B) This button will produce a Sub-Menu of the following 5 buttons:

      i)   **'Development Tool'**
      ii)   **'Self-Assessment Tool'**
      iii)   **'Quiz'**
      iv)   **'$-logK_i$ Reference Table'**
      v)   **'Assumptions of the Model'**

    i)   This button will navigate to the **Development Tool**, which is designed to challenge users to select up to 3 different receptors and densities. Users then select up to 6 ligands,

whose competition binding curves will be displayed according to their $-logK_i$ values and the assumptions of the model.

ii)   This button will navigate to the **Self-Assessment Tool**. This is like the Development Tool but with receptors and densities hidden to challenge the user to determine the receptors and densities of a randomly-generated receptor population. In the Tool, graph co-ordinate values are hidden. When the user clicks to reveal the answer the receptors and densities of the receptor population are revealed.

iii)   The **Quiz** is similar to the Self-Assessment Tool but after the user has determined the receptors and densities they enter the values and click submit. The user will then continue through all questions in the quiz until they have submitted an answer to each of the questions after which the results are displayed complete with a score and the correct answers to each question. Students have an option to write a justification for their answers in a text box.

iv)   Clicking this button produces a pop-up which features some important data on which the tools are based.

-log K$_i$ Reference Table   ✕

Hypothetical ligands have been included in the model to increase its applicability.

Ligand A and Ligand B are examples of ligands that have marked differences in affinities (-log K$_i$ values) for receptor subtypes (not evident with established ligands).

You may nominate affinities (-log K$_i$ values) for Ligands C and D.

Competing Ligand	-log K$_i$ Value				
	M_1	M_2	M_3	M_4	M_5
Established					
Atropine	9.0	8.8	9.3	8.9	9.2
Pirenzepine	8.2	6.5	6.9	7.4	7.2
Methoctramine	6.7	7.7	6.0	7.0	6.3
Darifenacin	7.8	8.0	8.8	7.7	8.0
MT-3	6.7	5.9	6.0	8.1	6.0
S-Secoverine	8.0	7.9	7.7	7.7	6.5
Solifenacin	7.6	6.8	7.9	7.0	7.5
DAU-5884	8.9	7.1	8.9	8.5	8.1
Hypothetical					
Ligand A	9.0	9.0	6.0	6.0	2.0
Ligand B	8.0	5.0	8.0	5.0	8.0
Ligand C	*	*	*	*	*
Ligand D	*	*	*	*	*

CLOSE

The table includes $-logK_i$ values for each of the Established ligands as well as for two Hypothetical ligands, at each of the M_1, M_2, M_3, M_4, M_5 receptor subtypes.

a. In order to close the pop-up either click the Black **'X'** at the top right corner of the table or the **'CLOSE'** button at the bottom right corner of the table

b. This will return navigation to the home page with the pop-up window closed

v) Clicking this button will produce a pop-up which states the assumptions of the competition binding model. Headings/ Paragraphs 1, 2 and 3 describe different assumptions:

a. The assumptions in relation to the law of mass action that describes drug binding to receptors.

b. The second paragraph describes the assumptions associated with competition binding studies

c. The third paragraph describes the assumptions that are model specific

The pop-up can be closed by clicking the black **'X'** at the top right corner of the pop-up window or by clicking the **'CLOSE'** button at the bottom right corner of the pop-up window.

Assumptions of the Model ✕

1. *All of the usual assumptions associated with Law of Mass Action that typically describe drug binding to receptors*
   - All receptors are equally accessible to ligands.
   - All receptors are either free or bound to ligand - i.e. the model ignores any states of partial binding.
   - Neither ligand nor receptor are altered by binding.
   - Binding is reversible.

2. *Assumptions associated with competition binding studies*
   - Only a small fraction of both the labelled and unlabelled ligands has bound, so the free concentration is virtually the same as the added concentration.
   - There is no cooperativity – binding to one binding site does not alter affinity at another site.
   - The experiment has reached equilibrium.
   - Binding is reversible and follows the law of mass action.

3. *Other model-specific assumptions*
   - Analysis is conducted using % Specific Binding (no non-specific binding)
   - [^{3}H-QNB] used in studies was << K_A of ^{3}H-QNB for receptors present, i.e. Cheng-Prusoff equation reduces to $IC_{50} \cong K_i$ for all competing ligands.

CLOSE

There are three main pages accessible from the homepage with links appearing below the SPIKES*mate* title

C: 'Development Tool' Button
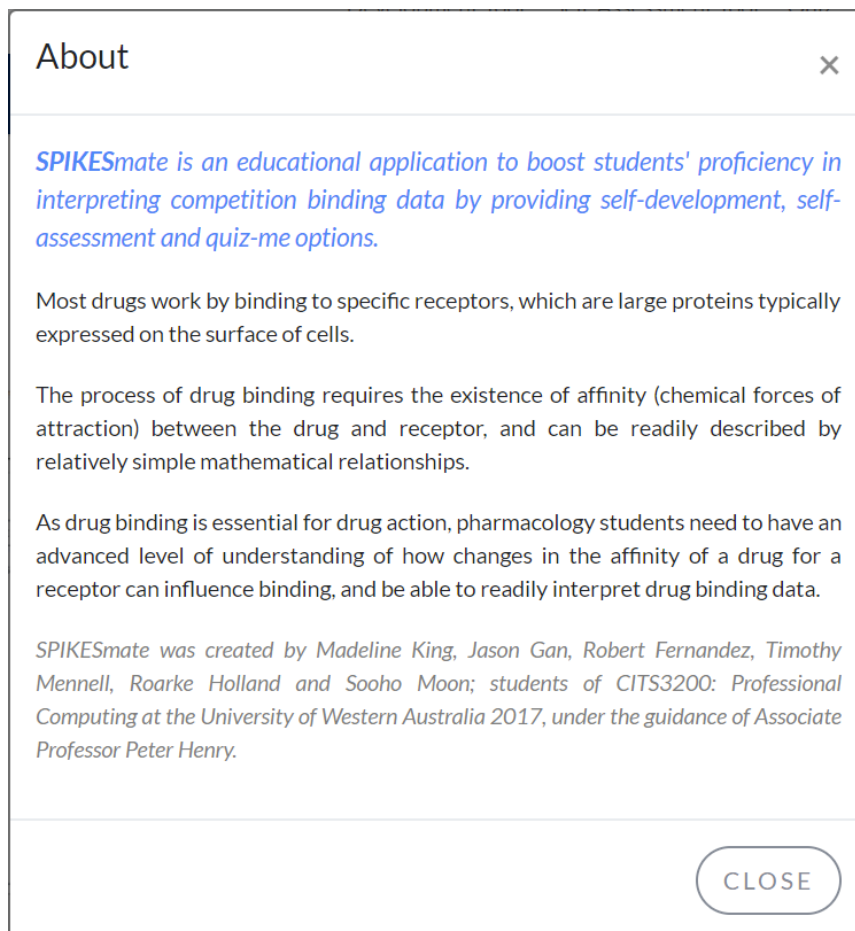**D: 'Self-Assessment Tool' Button**
E: 'Quiz' Button

C) The **Development Tool** button will navigate to the Development Tool page which is an educational tool designed to display competition binding curves based on (a) the receptor subtypes and relative densities selected, and (b) the ligands selected and their $-logK_i$ values. Graph values can also be revealed by tracing the mouse pointer/ cursor over the curves to determine exact values at a given point on each curve. Of value is the potential to change $-logK_i$ values for Ligand C and Ligand D, and to observe the effect on the position and shape of the competition binding curves, in real time.

D) The **Self-Assessment Tool** is identical to the Development Tool only that the receptors and their respective densities are hidden. The goal of this tool is for each student to learn to identify receptors and densities based on student-selected ligands (including custom ligands) and their graphed curves. Note that in this mode, graph curve values are not revealed as this would trivialise the identification of the receptors in question.

E) The **Quiz** is similar to the Self-Assessment Tool in that receptors and densities are hidden and that the graph co-ordinate values are not displayed but there are several program-selected practice questions which form a quiz that can be attempted by a user. Once an answer has been submitted for all of the questions, results are published including scores and a list of the correct answers (and associated competition binding curves.

F: 'About' Button
G: 'Instructions' Button
H: 'Contact' Button

F) This About section pop-up describes the project and it's key contributors and IP holders. Interactivity is featured in the **'X'** close button, and **'CLOSE'** button

About   ✕

SPIKESmate is an educational application to boost students' proficiency in interpreting competition binding data by providing self-development, self-assessment and quiz-me options.

Most drugs work by binding to specific receptors, which are large proteins typically expressed on the surface of cells.

The process of drug binding requires the existence of affinity (chemical forces of attraction) between the drug and receptor, and can be readily described by relatively simple mathematical relationships.

As drug binding is essential for drug action, pharmacology students need to have an advanced level of understanding of how changes in the affinity of a drug for a receptor can influence binding, and be able to readily interpret drug binding data.

SPIKESmate was created by Madeline King, Jason Gan, Robert Fernandez, Timothy Mennell, Roarke Holland and Sooho Moon; students of CITS3200: Professional Computing at the University of Western Australia 2017, under the guidance of Associate Professor Peter Henry.

(CLOSE)

G) Development Tool, Self-Assessment Tool and Quiz instructions appear as well as **'X'** close, and **'CLOSE'** button

Instructions   ✕

Development Tool

Self-Assessment Tool

Quiz

(CLOSE)

Contact details for the unit co-ordinator including title, name and relation to the webpage are listed
- **Location** for the unit co-ordinator (physical staff address for Academic Enquiries)
- **Email address** provided

Contact   ✕

Found a bug in our application?
Or simply wanted to give some feedback?
Please don't hesitate to let us know!

Peter Henry
Associate Professor
The University of Western Australia

Room 1.34, M Block,
School of Biomedical Sciences,
QEII Medical Centre

peter.henry@uwa.edu.au

CLOSE

02 – Development Tool

SPIKES*mate*

Development Tool   Self-Assessment Tool   Quiz   -log K$_i$ Reference Table   Assumptions of the Model

Receptor

	M$_1$	M$_2$	M$_3$	M$_4$	M$_5$
Subtypes	☑	☐	☑	☐	☐
Relative Density (%)	50		50		

Ligands

-log K$_i$ Values

	M$_1$	M$_2$	M$_3$	M$_4$	M$_5$
1. Atropine ▾	9	8.8	9.3	8.9	9.2
2. Pirenzipine ▾	8.2	6.5	6.9	7.4	7.2
3. Methoctromine ▾	6.7	7.7	6	7	6.3
4. Ligand A ▾	9	9	6	6	3
5. Ligand C ▾	7	3.2	6.3	5.1	7.6
6. Ligand D ▾	8	9.1	8.4	6.0	5.2

Competition Binding Curves

Specific Binding (%) vs log [Ligand] (M)

A: Receptor Module

i) **Subtypes**
- a total of 1 or 2 or 3 receptors can be selected.
- any given receptor can be selected by clicking on the radio button (A 'TICK' is shown in the box) below the required receptor; M_1, M_2, M_3, M_4, M_5
- 1, 2 or 3 receptors can be selected at any given time. The application will prevent the user from selecting more than 3 receptors at once.

ii) **Relative Density (%)**
Depending on the number of receptors selected, the density can be specified as follows (Density is always an integer between 1% and 100% inclusive)
1 Receptor Selected: Default 100%, 100% (Hard-Coded)
2 Receptors Selected: Default 50%, 50%
Total of 100%: $(x, y \,|\, 100 \geq x \geq 10, 100 \geq y \geq 0, x + y = 100)$
3 Receptors Selected: Default 33.3%, 33.3%, 33.3%
Total of 100%: $(x, y, z \,|\, 100 \geq x \geq 0, 100 \geq y \geq 0, 100 \geq z \geq 0, x + y + z = 100)$

If three receptor subtypes have been selected, then the combined value must again be 100. In this case, receptor densities should always be entered Left Receptor first, i.e. the left-most active receptor density must be specified first. Once this has been done either of the remaining two densities can be entered and the final density will automatically be calculated. For example, if Receptor 1 has density 60% and Receptor 2 is specified as 30% density, Receptor 3 will automatically be given 10% density. Similarly, if Receptor 1 has density 50% and Receptor 3 has density 40% then Receptor 2 will automatically be given 10% density. This is how the problem of 2 unknown variables is managed.

B: Ligands

Entries 1, 2, 3, 4, 5, 6 are drop down boxes allowing the user to select from a list of 12 ligands
- The row of numeric values to the right of each of the drop downs represent the $-logK_i$ values for each of the selected ligands
- Selectable ligands include 8 Established ligands as well as 4 Hypothetical ligands. Whilst the $-logK_i$ values of all 8 Established ligands and 2 of the Hypothetical ligands (ligand A and Ligand B) are pre-set, the remaining 2 Hypothetical ligands (Ligand C and Ligand D) require the user to specify the required $-logK_i$ values.
- Based on each of the $-logK_i$ values specified for the custom ligands the number entered will be corrected to be constrained to the domain of numbers permissible by the module. The lowest or minimum legal value is 2 whilst the highest or maximum legal value is 11 so that any user input will produce and populate a number in this field within the domain of real numbers between 2 and 11 inclusive. This legal domain will be enforced for any numeric value entered into the text fields.
- The values for any respective value can be derived from the lookup table accessible from any page on the website through the '≡' button

C: Competition Binding Curves

The binding curves provide a graphical representation of the binding curves given the input data for receptors, $-logK_i$ values, ligands and graph range (typically 2 to 11)
The **Specific Binding %** is specified as the vertical range for the graph along the y axis (0 to 100%)
The log **[Ligand](M)** are specified as the horizontal range for the graph along the x axis (-12 to -2)

Note that the different colours produced for each curve correspond to the colours associated with each ligand specified in the ligand module.
 - The curves can be traced such that the value of the (x,y) coordinates of any point on the curve can be found by hovering the cursor over them producing a read out of each curve's value in their respective colours at any point traced on the graph.
- Note that the two options at the top right corner of the graph window allow the user to toggle between displaying co-ordinate data for a single curve or all graphed curves simultaneously

03 – Self-Assessment Tool
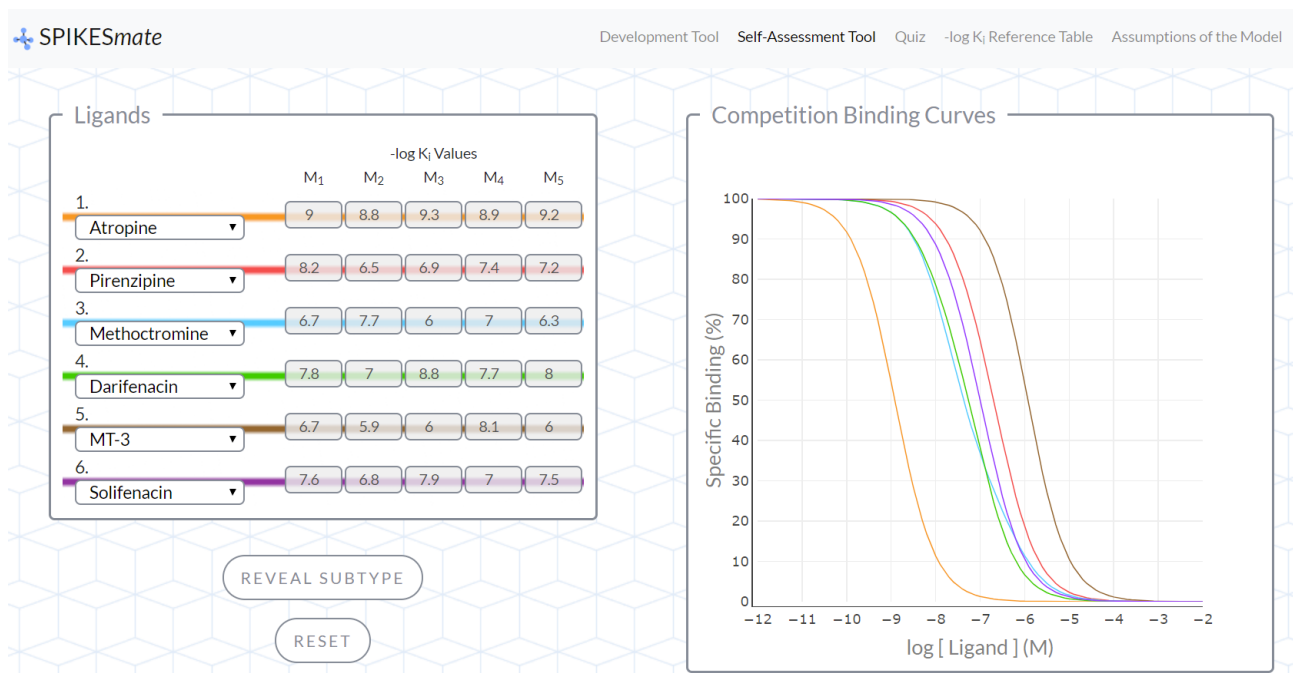
A: Ligands
As in section 02 – Development Tool

B: Interactive Buttons
I: 'Reveal Subtype'
II: 'Reset'
   i)   This reveals the hidden receptors and their relative densities
   ii)   This button 'resets' the system and randomly generates a new receptor/ density combination

C) Competition Binding Curves.
As in section 02 – Development Tool
However the curves cannot be traced in this instance and hovering over the curves will not reveal the X, Y co-ordinate pairs, as this would trivialise the determination of the receptors and their densities.

04 – Quiz
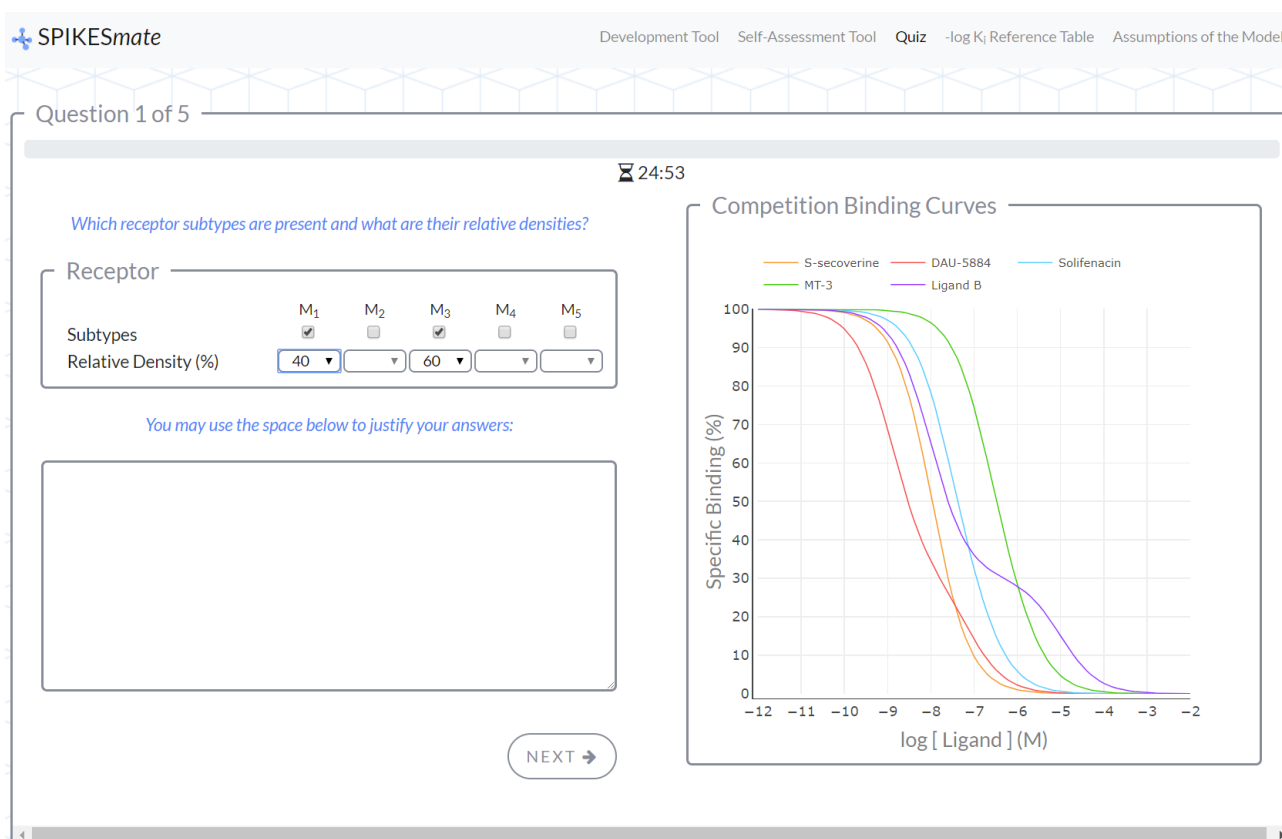
Quiz Start Page:
This page describes the quiz, which will follow. Possible answers, marking system and answer entry are all explained. The quiz is both timed and limited to 5 questions.
START QUIZ: Will begin the interactive quiz

A: Receptor Module
I) Subtypes
Here, students analyse the presented competition binding curves using the SPIKES approach and record the answer (Subtypes, Relative density) using the boxes provided in the Receptor box.
Students can access the $-logK_i$ Reference Table at anytime o help solve the question.
- either 1 or 2 receptors can be selected in total.
- any given receptor can be selected by clicking on the corresponding radio button (A mark is shown in the box) below the required receptor M_1, M_2, M_3, M_4, M_5
- 1 or 2 receptors can be selected at any given time. The module will prevent the user from selecting more than 2 receptors.
- note that only 2 receptors are ever required as the difficulty level associated with having 3 concurrent receptors goes beyond the competency expected of a Level 3 pharmacology student.

II) Relative Density (%)
As in section 02 – Development Tool

B: Text Box
A text box is provided for students to provide a formal answer to each question, as would be required in an examination.

C: Interactive Buttons
I: 'Next Question'
II: 'Submit'
   i)   This button submits the entered receptor density combination and records this answer for interpretation later in the results section
   ii)   This button only appears whilst completing the last question. It completes the quiz.

D: Competition Binding Curves.
As in section 02 – Development Tool
This time, as no ligand table is provided, a legend is displayed which labels the ligand associated with each line colour. This is essential for determining the correct receptors and their respective densities.

E: Review
The review page appears after all quiz questions have been answered.
At the top of the review page is the score determined for the quiz attempted
The table below shows each question number, the answer provided by the user and the correct answer for the given problem.
This enables students to recognise where they have selected the correct answer and where they haven't and shows them what the correct answer was in the given instance.
Students can click on a particular answer (e.g. an incorrect one) and will be taken to a question Review page that displays side-by-side the competition binding curves related to (a) the students (incorrect) answer and (b) the original curves. The student's text box, if used in answering the question, will also be presented in this review page.

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Development Tool   Self-Assessment Tool   Quiz   -log K$_i$ Reference Table   Assumptions of the Model

Review

Score: 0/5

CLICK QUESTION TO REVIEW

Questions	You Answered	Correct Answer	Mark
1	M1 50% M3 50%	M2 50% M3 50%	✖
2	M1 50% M2 50%	M3 40% M1 60%	✖
3	M3 60% M4 40%	M4 100%	✖
4	M4 30% M5 70%	M1 40% M2 60%	✖
5	M2 20% M4 80%	M4 30% M3 70%	✖

RESTART

RESTART: This resets the quiz