Instructions for using simulations that accompany *Binding and Kinetics for Molecular Biologists* by James A. Goodrich and Jennifer F. Kugel

The general instructions that follow below apply to all the simulations cited in the accompanying text *Binding and Kinetics for Molecular Biologists*. Each simulation is listed along with the equation and figure numbers from the respective chapters that best correlate with the simulation. Additional instructions are included for more complicated simulations.

General Instructions

The simulations are named according to the chapter number in which they are cited. In the book, each is referred to where the equations and figures that best correlate with the simulation are explained. All the simulations are Excel files. When you open each simulation in Excel, you must click on "Read Only" because these are protected files and only certain cells can be modified. In addition, you will need to click on "Enable Macros": These files contain macros that are required for the simulations to function.

For optimal viewing on either a Mac or PC, please note the following:

Mac Users

- If the graphs in the spreadsheets initially come up as double images, simply resize the window frame of the spreadsheet. Any double images will then disappear.

PC Users

- If your security setting in your version of Excel is set to “High,” it may disable the macros necessary for using the simulations. If you receive a message requesting you to adjust your security level, this can easily be accomplished in the Excel program by temporarily revising the setting to “Medium” and will be specific to Excel macros. In Excel, on the tool bar, reset the security level as follows:

  Tools > Macro > Security > Security Level = Medium

  Once the setting is at Medium, you will receive a prompt to enable/disable macros upon opening the Excel files.
- Set your view at 85% for optimal viewing.

Each simulation contains a plot(s) on the left and a plot(s) on the right. The *X* and *Y* axes of the plot(s) on the left will automatically adjust when you change the variables in the simulation so that the data are visualized over a useful *X* and *Y* range. You can adjust the *X* axis and/or the *Y* axis on the plot(s) on the right to "zoom in" or "zoom out" to better visualize parts of the curve.

The simulations can be manipulated by changing the numbers in yellow (numbers that are not yellow cannot be changed). In general, these numbers are the variables in the equation(s) that is most relevant to the simulation. All numbers are shown in scientific notation, although numbers can be entered into the cell in any format. After entering values for the yellow numbers, click on "Update Plot" or "Update Axis" and the respective plot will change according to the values you enter-

ed. If you want to return to the original values in a simulation, simply click on "Reset Simulation," typically located below the plots.

When you close a simulation, it will ask if you want to save it. If you choose to save the simulation with your currently entered values, you will need to give it a new name. If the simulation is saved with a new name, all the macros and protected cells will be maintained.

Simulation S1: Fractional Activity

This simulation corresponds to Chapter 1, Illustration 1.4. One of the variables to be entered is K_D, which is explained in Chapter 2; therefore, this simulation may be most useful after reading Chapters 1 and 2. Note that for the red curves, the concentration of AB ([AB]), not the amount of AB, is plotted on the *Y* axes. The gray curves are plots of $[B]_{Free}$ versus $[B]_{Total}$ that help visualize how the relationship between these parameters changes as $[A]_{Total}$ and/or the K_D change (these curves are not shown in Illustration 1.4).

Simulation S2-1: Affinity (General)

This simulation most directly corresponds to Chapter 2, Equations 6 and 9, and Figures 2.4–2.7. This simulation helps visualize the theory behind measuring a K_D: specifically, how plots of [AB] versus $[B]_{Free}$ (blue curves on left) compare to plots of [AB] versus $[B]_{Total}$ (red curves on right) for different values of K_D and $[A]_{Total}$. The simulation can be reset by clicking on the buttons on the upper left to visualize either of the two extremes shown in Figure 2.4: $[A]_{Total} << K_D$ or $[A]_{Total} >> K_D$. The green curves on the right result from plotting $[B]_{Free}$ versus $[B]_{Total}$ to help visualize how the relationship between these parameters changes as $[A]_{Total}$ and the K_D change.

Simulation S2-2: Affinity (K_D)

This simulation most directly corresponds to Chapter 2, Equation 9 and Figure 2.7, but also relates to Figures 2.5 and 2.6.

Simulation S2-3: Dimerization

This simulation corresponds to Chapter 2, Equation 20 and Figure 2.11.

Simulation S3-1: ABC Cooperativity

This simulation corresponds to Chapter 3, Equations 9–11 and Figure 3.4. Enter a value for $K_{D(AB)}$ (corresponding to the blue curve) and for α. The resulting $\alpha K_{D(AB)}$ is calculated for you (displayed in green) and corresponds to the red curve. A value can also be entered for "AB_{max}, ABC_{max}"; these values are the same in this simulation.

Simulation S3-2: Hill Plot

The lower plots in this simulation are Hill plots that correspond to Chapter 3, Equation 16, and Figures 3.6 and 3.7. The calculated slope of the line (n_H) is shown below each plot. The value of n_H will change in the plot on the lower right as the range of the *X* axis changes; this illustrates the point made in Figure 3.6. Plots like those on the top are not shown in Chapter 3. These plots illustrate how the frac-

tions of A, AB, and AB_2 (A + AB + AB_2 = 1) change as the concentration of B is increased. The fractions of A, AB, and AB_2 correspond to the blue, green, and red curves, respectively. This simulation is also useful for investigating the relationships in Tables 3.2 and 3.3 of Chapter 3.

Simulation S4-1: Off Rate (k_{-1})

This simulation corresponds to Chapter 4, Equation 20 and Figure 4.8.

Simulation S4-2: On Rate (k_1)

The upper plots in this simulation correspond to Chapter 4, Equation 27 and Figure 4.10 (note that the *Y* axis is [AB] as opposed to amount of AB). You can enter k_1, k_{-1}, and five different concentrations for B. The simulation will calculate the corresponding k_{obs} and $[AB]_{max}$ values and generate the different colored curves showing plots of [AB] versus time. $[A]_{Total}$ is included as a variable that can be entered because the $[AB]_{max}$ value for each [B] will depend on the value of $[A]_{Total}$. The lower plots correspond to Chapter 4, Equation 25 and Figure 4.11. The *Y* intercepts and slopes of the lines are shown at the bottom of the plots; they will equal the k_{-1} and k_1 values, respectively, entered at the top of the simulation.

Simulation S5: ABC Rates

This simulation corresponds to Chapter 5, Equations 18 and 19, and Figure 5.2. Values for k_1, k_{-1}, k_1', and k_{-1}' can be entered. The *Y* intercepts and slopes of the lines are shown at the bottom of the plots and they will equal the k_{-1} or k_{-1}' and the k_1 or k_1' values, respectively, that you entered at the top of the simulation. The value for α shown in green is calculated from the rate constants and is not a value you can enter. When manipulating the rate constants, the value of α can be monitored to determine how changes in the rate constants correlate to levels of cooperativity.

Simulation S6: Two Step Rates

The upper plot on the right corresponds to Chapter 6, Equation 6 and Figure 6.2. The *Y* intercept and slope of the line are shown; the *Y* intercept equals $1/k_2$. The lower plot on the right corresponds to Chapter 6, Equation 5 and Figure 6.1. The plot on the left corresponds to Chapter 6, Equation 9. You can enter three rate constants and five different concentrations for B. The simulation will calculate the corresponding k_{obs} values and generate the different colored curves showing plots of AB* versus time. The information on the bottom left contains the limitations that must be satisfied for the plots in the simulation to be accurate; these limitations are described in Chapter 6. The values for the ratios shown in green will be recalculated each time you enter a new value for [B]. Ratios shown in green satisfy the limitations. If the value entered for [B] does not satisfy a limitation, the corresponding ratio will turn red.