Supplementary Information for Kinetic schemes for post-synchronized single molecule dynamics

Chunlai Chen, Michael J. Greenberg, Joseph M. Laakso, E. Michael Ostap, Yale E. Goldman, and Henry Shuman

We provide a program to simulate post-synchronized ensemble averages as described in our accompanying paper. Our intention is to provide readers with (a) an interactive tool to simulate the specific biological examples provided in the paper, and (b) a simple program that is useful for modeling post-synchronized averages for their unique experimental systems. The program is designed to run in MATLAB version 7.5.0 or later. Two files are required to run the program (*.m and *.fig), and we provide two versions of each file that implement the program with rate constants and amplitudes for simulation of the data provided in Figures 1 and 2. The detailed schemes used for the simulations can be found in Fig. S2.

Installing and running the program:

1. Place both postsynch_XXX.fig and postsynch_XXX.m in the same directory (XXX is either ribosome or myosin, depending on which case you want to simulate).

2. Open MATLAB.

3. Open postsynch_XXX.m. Press the green run button (or F5) to initialize the program.

Simulating synchronized ensemble averages:

1. To simulate the synchronized ensemble averages for a given kinetic scheme, enter the transition rate constants into the transition matrix.

For example, in the reaction c → d, the rate of transition would be entered in the box in column 3, row 4.

***For the purposes of this simulation, each enzymatic cycle is assumed to be isolated from previous or subsequent cycles. Each such cycle in an experimental trace is denoted “an event”. Isolation of events is accomplished by requiring reactions to terminate in a “Separator State”. To properly isolate single molecule events, the transition rates out of the Separator State must be made much slower than the slowest non-zero transition rate. This restriction for the MATLAB program does not apply to the procedure and equations in the main text.

***Note, this program requires all 5 programmed states to be populated in the simulation. If you wish to simulate a model that requires fewer than 5 states, you will need to create "virtual" states that:

a. have amplitudes equal to the final state in your model,
b. have exit transition rates that are much faster than the fastest real transition rate in the cycle,
c. have exit (or backwards) rates from each virtual state that are irreversible.

Fulfilling these three criteria will ensure that these virtual states will not contribute appreciably to the final solution, but will enable the program to run without changing the internal code.

2. To set the amplitude of each state (i.e., the fluorescence level or the step size), enter the amplitude of each state in the boxes labeled State a, State b, etc.

3. Select the transitions to be used for synchronization of the time-forward and time-reversed averages. For example, in the case of myosin, the time-forward averages would be synchronized by the transition into AM.ADP (State a) and the time-reversed averages would be synchronized by the (forward) transition out of AM (State b, c or d [the latter two being virtual states]).

4. In the "Value for Extensions of Time-Forward Averages," set the value of the extensions used to compute the ensemble time-forward averages (i.e., the ensemble averages that are synchronized at the event beginnings). This is usually the amplitude of the final state before the Separator State. For example, when the synchronized time-forward averages for myosin (Fig. S2) are synchronized on the transition into the AM.ADP state, the extensions would be equal to the amplitude of the AM state (State c). In special cases, the extension does not need to be equal to the final event state.

Similar guidelines apply for picking the value for extensions of the time-reversed averages. If the events are synchronized on the (forward) transition from AM to M.ATP, the extensions would have the amplitude of the AM.ADP state (State a).

For reactions containing an equilibrium between states with different amplitudes (such as in case of the ribosome (Fig. S2)), the amplitude of the extension is given by the weighted average of the amplitudes of the equilibrated states.

5. Select the total amount of time plotted.

6. Press simulate. The ensemble averages, as well as the mole fractions of the intermediate states will appear.

***Check the eigenvalues of the system. If the eigenvalues are degenerate (i.e., 2 or more eigenvalues have the same value) or imaginary, then the linear algebraic solution used in this simulation is not well defined. In this case, the user might need to use a differential equation solver.

7. To save the output (i.e., the time-forward averages, time-reversed averages, and reaction intermediates), enter the full destination path, followed by the file name and the extension .xls (e.g. C:\Synchronized files\output.xls will generate a file named output.xls in the Synchronized files folder). The file is readable in Excel with text headings for each column.

***Note, you must press simulate before you can save the output.

Troubleshooting and Tips:

General tip: If the program does not run, check the main MATLAB console to see if an error occurred.

1. The ensemble averages look incorrect.

    - Ensure that the transition rates out of the Separator State are much slower than the slowest non-zero transition rate.
    - Ensure that there is at least one finite entry and exit transition-rate into and out of each state.
    - Check the eigenvalues. If the eigenvalues are imaginary or degenerate, then the linear algebraic solution used in this simulation is not well defined.
    - Check that the extensions are chosen as described above.

2. Simulation files are not saved.

    - Check the path name. Ensure that the path is complete and that the file name ends with the extension ".xls".
    - Make sure that you press simulate before pressing save.
    - Users have reported difficulty saving files using networked versions of MATLAB. The cause of this problem is unknown and a solution is not currently available. Use a single-user version.

3. If you want to probe substeps before the final transition in the event:

    - Set the amplitudes of all states after the desired transition and the extension to the same value as the state after the probed transition

4. If you want to model more or fewer states.

    - To probe more states, the user will need to expand the program.
    - To probe fewer states, create virtual states with amplitudes equal to the final state. If the transition rates from these states are much faster than the fastest transition rate in the reaction cycle (and irreversible), they will not be significantly populated in the solution.

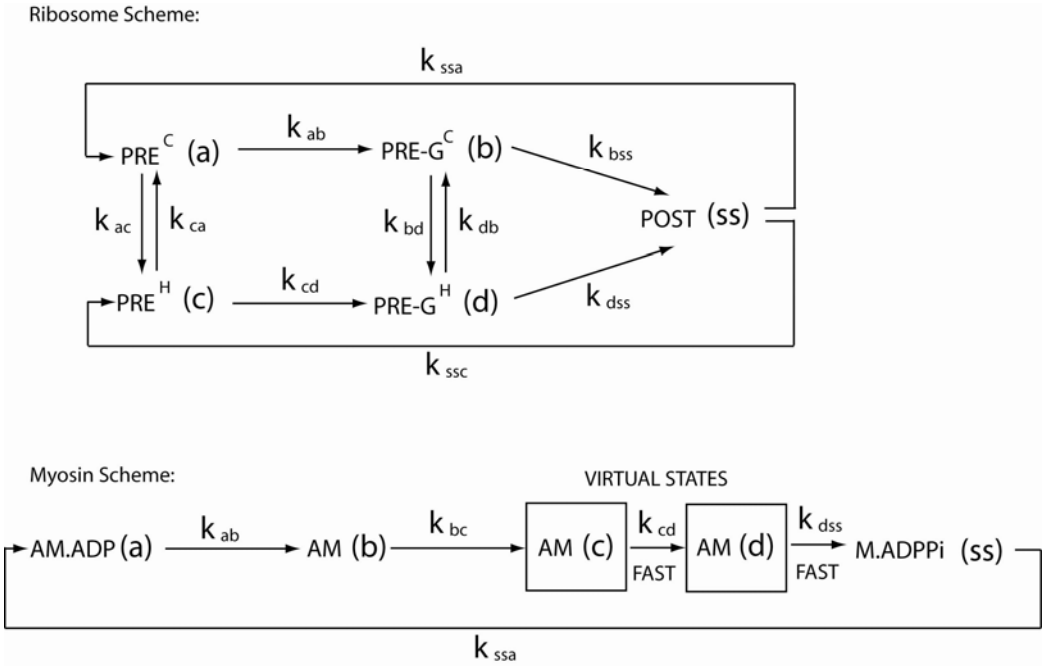

Figure S2: Schemes used in computer simulations for the ribosome and myosin. The state names are shown in parentheses and the transition rates are listed above the arrows. Note that the simulation of myosin required the creation of two virtual states, as described in the supplemental text above.