

With our softer we are modeling the complete actin.myosin ATPase cycle. We can see the occupancy of each of the intermediates in the ATPase cycle for some values of rate constants at different actin concentration. Choosing a set of rate constants for study depends upon having a good estimate of the steady-state ATPase data and a complete set of the rate constants for the cycle depicted. Complete parameter estimation methods are described in Mijailovich et al., 2017.

For each equilibrium constant  $K_i = k_i/k_{-i}$  we have  $k_i$  and  $k_{-i}$ , the forward and reverse rate constants. Defining any two of the three parameters experimentally is sufficient; the third can be calculated from the first two.

Follow these steps:

- 1) Set a values for all rate constants

Field	Value	Unit
k+A:	10.75	1/(uMs)
k+Pi:	15.95	1/(uMs)
k+D:	1000.0	1/s
k-A:	1000.0	1/s
k-Pi:	1.59e-4	1/s
k-D:	27.80	1/s
KA:		1/uM
KPi:		1/uM
KD:		
k+D*:	59.0	1/s
k+T:	10.05	1/(uMs)
k+T*:	1543	1/s
k-D*:	354.0	1/s
k-T:	3.349e3	1/s
k-T*:	10.0	1/s
KD*:		
KT:		1/uM
KT*:		
k+T**:	1000.0	1/s
k+H:	12.47	1/s
k+Ah:	12.63	1/s
k-T**:	1.0	1/(uMs)
k-H:	1.4	1/s
k-Ah:	0.2	
KT**:		uMs
KH:		
KAh:		1/s

Rate Constants Used for Modeling the ATPase Cycle for HC-WT  $\beta$ -S1  
(example from Mijailovich et al., 2017)

- 2) Set a values for all concentration

Concentration od Myosin:	1	<input checked="" type="radio"/>	<input type="radio"/>	0.1	to	10000	4	/decade
Concentration od Actin:	3	<input checked="" type="radio"/>	<input type="radio"/>	0.1	to	10000	4	/decade
Concentration od Pi:	0.0	<input checked="" type="radio"/>	<input type="radio"/>	0.1	to	10000	4	/decade
Concentration od ADP:	0.0	<input checked="" type="radio"/>	<input type="radio"/>	0.1	to	10000	4	/decade
Concentration od ATP:	5000	<input checked="" type="radio"/>	<input type="radio"/>	0.1	to	10000	4	/decade

- 3) There is the option to select the folder that will be registered output files. If the folder does not indicate the output results will be stored in C: \ Users \ Documents \ ATPase Results

4) Also, the results will be shown in the table on the right side. Data in the table can be copied.

The screenshot shows the ATPase software interface. On the left, there are various parameter settings for kinetic constants (k+A, k+P, k+D, etc.) and concentrations of Myosin, Actin, Pi, ADP, and ATP. A 'Run' button is located at the bottom left. On the right, a table displays simulation results. A red circle highlights the table, and a red arrow points to the 'Run' button.

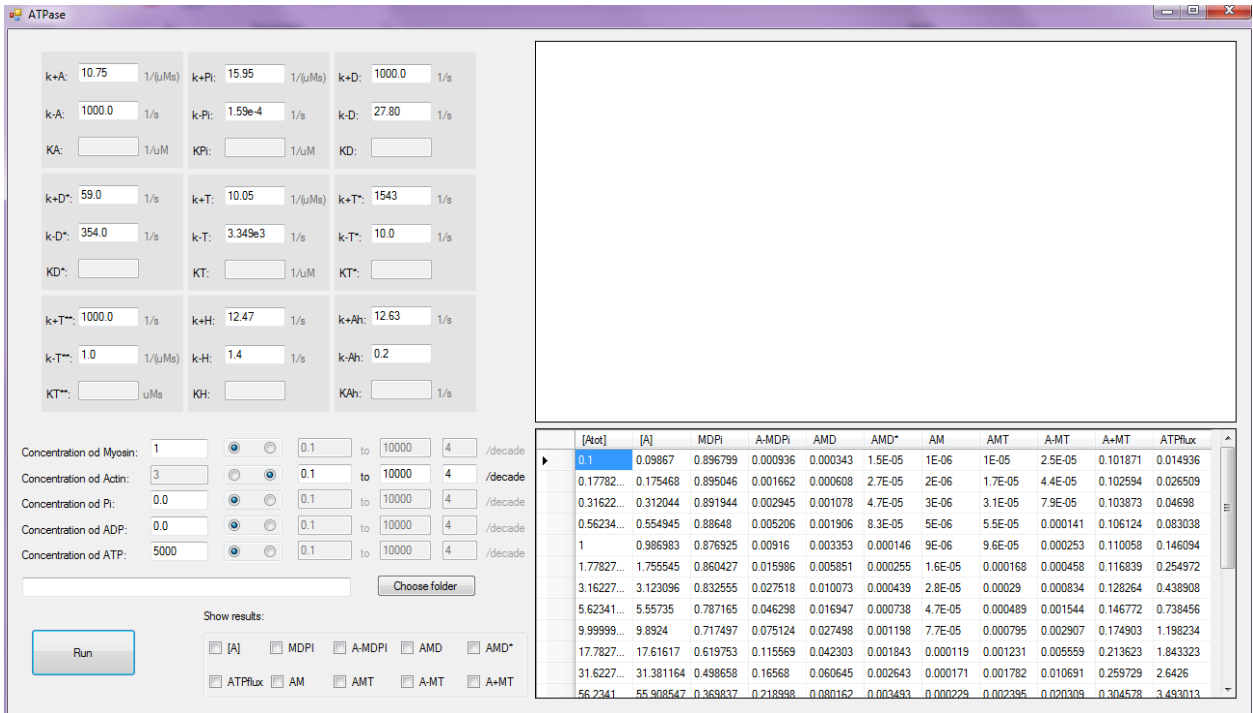
[A]	MDPi	A-MDPi	AMD	AMD*	AM	AMT	A-MT	A+MT	ATPflux
118.215339	0.223216	0.279711	0.102385	0.004461	0.000301	0.003179	0.044424	0.342322	4.461389

5) The results for fixed values of concentration cannot be drawn on charts. But we can use value range and number of points in the range for any concentration. For the beginning you can put for example  $[A_{tot}] = 0.1 \mu\text{M}$  to  $100 \text{ mM}$ ,  $4/\text{decade}$ , and  $[M_{tot}] = 1 \mu\text{M}$ ,  $[\text{ATP}] = 5\text{mM}$ ,  $[\text{Pi}] = [\text{ADP}] = 0$ .

This image shows a close-up of the concentration settings in the ATPase software. The settings are as follows:

- Concentration of Myosin: 1, range 0.1 to 10000, 4 /decade
- Concentration of Actin: 3, range 0.1 to 10000, 4 /decade
- Concentration of Pi: 0.0, range 0.1 to 10000, 4 /decade
- Concentration of ADP: 0.0, range 0.1 to 10000, 4 /decade
- Concentration of ATP: 5000, range 0.1 to 10000, 4 /decade

6) If we run simulation in the table will be display values for different concentrations of actin



7) Now we can display the results on a graph choosing what data we want. We can see the occupancy of each of the intermediates in the ATPase cycle for different values of actin concentration.

