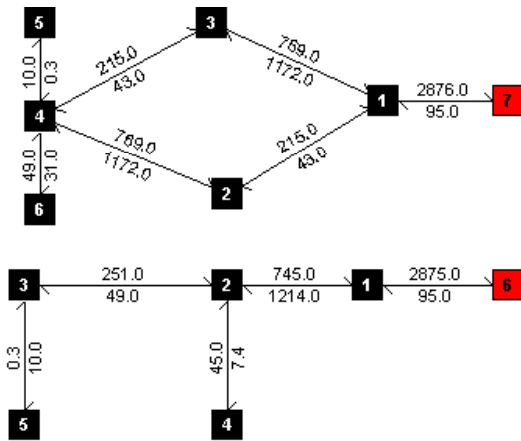


**Glu/Gly- (CTR4) Events: 331,680****Cyclic model results:**

LL: 1,486,470.15  
LL/event: 4.481639

**Closed:** mean, 6.2 ms

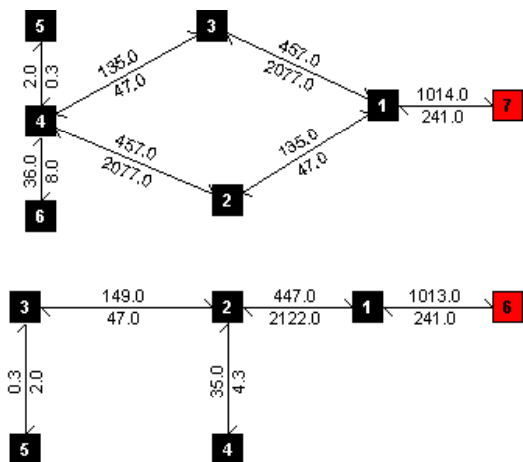
tau (ms)	amp (%)
0.23	47
0.46	0.29
<b>1.73</b>	41
<b>4.49</b>	10
22.7	0.76
2,929	0.16

**Open:** mean, 15.4 ms**Linear model results:**

LL: 1,486,470.92  
LL/event: 4.481641

**Closed:** mean, 6.2 ms

tau (ms)	a (%)
0.23	47
-	-
<b>1.7</b>	41
<b>4.4</b>	10
22	0.78
2,926	0.16

**Open:** mean, 5.4**Glu/Ala (ALA5) Events: 317,143****Cyclic model results:**

LL: 1,303,901.26  
LL/event: 4.1113985

**Closed:** mean, 19 ms

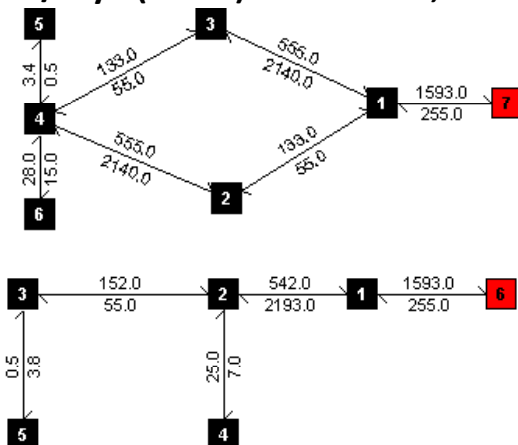
tau (ms)	a (%)
0.29	17
0.37	0.27
<b>4.1</b>	26
<b>12</b>	50
30	5.2
2,861	0.36

**Open:** mean, 4.7 ms**Linear model results:**

LL: 1,303,901.41  
LL/event: 4.1113989

**Closed:** mean: 19 ms

tau (ms)	a (%)
0.29	17
-	-
<b>4.1</b>	26
<b>12</b>	50
30	5.2
2,860	0.36

**Open:** mean, 4.7**SYM /Gly- (SYM4) Events: 226,578****Cyclic model results:**

LL: 987,816.51  
LL/event: 4.3597194

**Closed:** mean, 15 ms

tau (ms)	a (%)
0.24	22
0.35	0.19
<b>3.2</b>	40
<b>9.3</b>	34
41	3.2
2,090	0.44

**Open:** mean 4.8 ms**Linear model results:**

LL: 987,816.55  
LL/event: 4.3597196

**Closed:** mean, 15 ms

tau (ms)	a (%)
0.24	22
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<b>3.2</b>	40
<b>9.3</b>	34
41	3.2
2,090	0.44

**Open:** mean 4.8 ms**Supplementary Methods: Modeling**

For each of the conditions illustrated (Glu/Gly, Glu/Ala and SYM/Gly), one single-channel file was fit separately with the two models illustrated. In each panel: *top*, cyclic model representing independent activation of two subunits: a slow transition ( $C_4-C_3$  and  $C_2-C_1$ , were fixed to be identical with each other) or a fast transition ( $C_3-C_1$  and  $C_4-C_2$ , were constrained to remain equal to each other during fitting); *below*, a sequential model where the slow transition ( $C_3-C_2$ ) occurs before a faster transition ( $C_2-C_1$ ).

Rate constants were estimated for each file with either model; the calculated time constant are illustrated in side panels at right, rate constants are indicated above the respective transition (arrow). LL and LL/event are given for each file, along with the calculated time constants (ms) and relative areas (%) for the closed exponential components. Although the linear model has fewer variables it returned marginally higher LL values, in each of the conditions tested. For this reason, and because the calculated values for exponential components (times and amplitudes) as well as rate constants were similar, we chose the simpler model for our analyses.