Supplemental material

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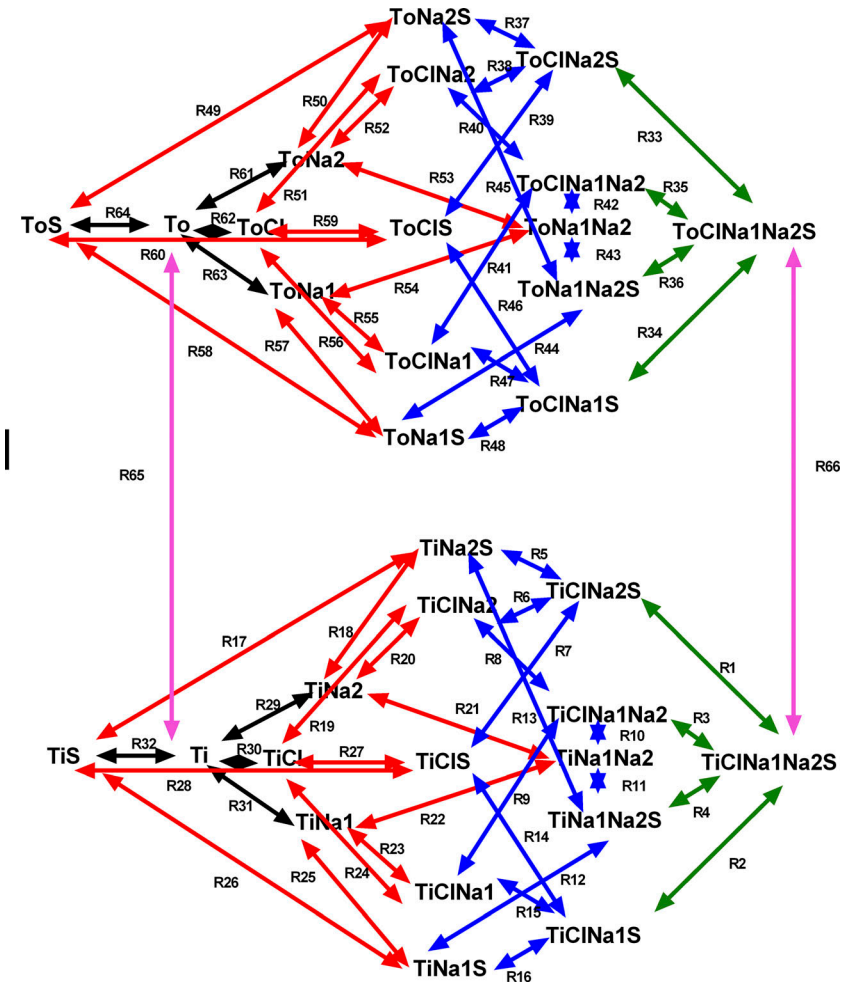

Figure S1. **Kinetic scheme for GlyT1.** The rate constants are indicated Table S1. Substrate and cosubstrates are allowed to bind to the transporter in random order. Cooperativity is incorporated by the use of a Coop. This factor is multiplied with the corresponding dissociation rates. z is indicated in Fig. 7. To reduce the number of states in the GlyT1 model, we allowed for filling of the Na3 site by sodium only after three ligands had already bound to the transporter. For a better overview, specific colors were assigned to reactions depending on the load of the states, i.e., black indicates one cargo (the substrate or one of the cosubstrates) bound to the transporter; red, binding of a second cargo; blue, binding of a third cargo; green, binding of the fourth cargo. Note that double arrows look like star-shaped symbols due to space limitations in the scheme.

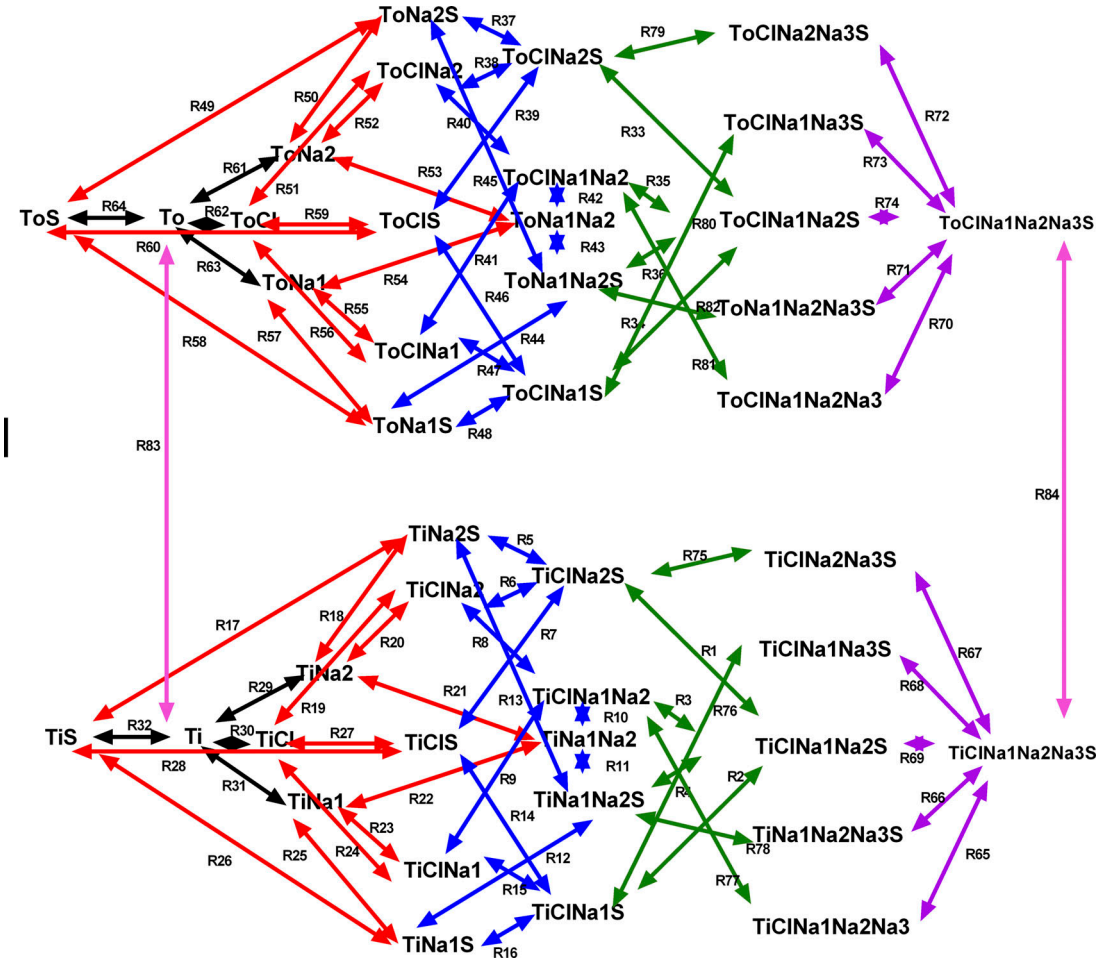

Figure S2. **Kinetic scheme for GlyT2.** The rate constants are indicated in Table S2. Substrate and cosubstrates are allowed to bind to the transporter in random order. Coop is multiplied with the corresponding dissociation rates. z is indicated in Fig. 7. To reduce the number of states in the GlyT2 model, we allowed for filling of the Na3 site by sodium only after three ligands had already bound to the transporter. The same color coding was applied as in Fig. S1; purple indicates the binding of the fifth cargo (one of the cosubstrates or the substrate). Note that double arrows look like star-shaped symbols due to space limitations in the scheme.

Table S1. **GlyT1 model**

Reactions (GlyT1 model)	Forward rate (s^{-1} or $s^{-1}*M^{-1}$)	Backward rate (s^{-1})
R1: TiClNa2S <-> TiClNa1Na2S	$3*10^6$	10^3
R2: TiClNa1S <-> TiClNa1Na2S	$3*10^6$	10^3
R3: TiClNa1Na2 <-> TiClNa1Na2S	$6*10^6$	200
R4: TiNa1Na2S <-> TiClNa1Na2S	10^6	10^4
R5: TiNa2S <-> TiClNa2S	10^6	10^4* Coop
R6: TiClNa2 <-> TiClNa2S	$6*10^6$	200*Coop
R7: TiClS <-> TiClNa2S	$3*10^6$	10^3*Coop
R8: TiClNa2 <-> TiClNa1Na2	$3*10^6$	10^3*Coop
R9: TiClNa1 <-> TiClNa1Na2	$3*10^6$	10^3*Coop
R10: TiNa1Na2 <-> TiClNa1Na2	10^6	10^4* Coop
R11: TiNa1Na2 <-> TiNa1Na2S	$6*10^6$	200*Coop
R12: TiNa1S <-> TiNa1Na2S	$3*10^6$	10^3*Coop
R13: TiNa2S <-> TiNa1Na2S	$3*10^6$	10^3*Coop
R14: TiClS <-> TiClNa1S	$3*10^6$	10^3*Coop
R15: TiClNa1 <-> TiClNa1S	$6*10^6$	200*Coop
R16: TiNa1S <-> TiClNa1S	10^6	10^4*Coop
R17: TiS <-> TiNa2S	$3*10^6$	10^3*Coop
R18: TiNa2 <-> TiNa2S	$6*10^6$	200*Coop
R19: TiCl <-> TiClNa2	$3*10^6$	10^3*Coop
R20: TiNa2 <-> TiClNa2	10^6	10^4* Coop
R21: TiNa2 <-> TiNa1Na2	$3*10^6$	10^3*Coop
R22: TiNa1 <-> TiNa1Na2	$3*10^6$	10^3*Coop
R23: TiNa1 <-> TiClNa1	10^6	10^4* Coop
R24: TiCl <-> TiClNa1	$3*10^6$	10^3*Coop
R25: TiNa1 <-> TiNa1S	$6*10^6$	200*Coop
R26: TiS <-> TiNa1S	$3*10^6$	10^3*Coop
R27: TiCl <-> TiClS	$6*10^6$	200*Coop
R28: TiS <-> TiClS	10^6	10^4*Coop
R29: Ti <-> TiNa2	$3*10^6$	10^3*Coop
R30: Ti <-> TiCl	10^6	10^4*Coop
R31: Ti <-> TiNa1	$3*10^6$	10^3*Coop
R32: Ti <-> TiS	$6*10^6$	200*Coop
R33: ToClNa2S <-> ToClNa1Na2S	$3*10^6$	10^3
R34: ToClNa1S <-> ToClNa1Na2S	$3*10^6$	10^3
R35: ToClNa1Na2 <-> ToClNa1Na2S	$6*10^6$	200
R36: ToNa1Na2S <->ToClNa1Na2S	10^6	10^4
R37: ToNa2S <->ToClNa2S	10^6	10^4*Coop
R38: ToClNa2 <->ToClNa2S	$6*10^6$	200*Coop
R39: ToClS <-> ToClNa2S	$3*10^6$	10^3*Coop
R40: ToClNa2 <-> ToClNa1Na2	$3*10^6$	10^3*Coop
R41: ToClNa1 <-> ToClNa1Na2	$3*10^6$	10^3*Coop
R42: ToNa1Na2 <-> ToClNa1Na2	10^6	10^4*Coop
R43: ToNa1Na2 <-> ToNa1Na2S	$6*10^6$	200*Coop
R44: ToNa1S <-> ToNa1Na2S	$3*10^6$	10^3*Coop

Table S1. **GlyT1 model (*Continued*)**

Reactions (GlyT1 model)	Forward rate (s^{-1} or $s^{-1}*M^{-1}$)	Backward rate (s^{-1})
R45: ToNa2S <-> ToNa1Na2S	$3*10^6$	10^3*Coop
R46: ToClS <-> ToClNa1S	$3*10^6$	10^3*Coop
R47: ToClNa1 <-> ToClNa1S	$6*10^6$	200*Coop
R48: ToNa1S <-> ToClNa1S	10^6	10^4*Coop
R49: ToS <-> ToNa2S	$3*10^6$	10^3*Coop
R50: ToNa2 <-> ToNa2S	$6*10^6$	200*Coop
R51: ToCl <-> ToClNa2	$3*10^6$	10^3*Coop
R52: ToNa2 <-> ToClNa2	10^6	10^4*Coop
R53: ToNa2 <-> ToNa1Na2	$3*10^6$	10^3*Coop
R54: ToNa1 <-> ToNa1Na2	$3*10^6$	10^3*Coop
R55: ToNa1 <-> ToClNa1	10^6	10^4*Coop
R56: ToCl <-> ToClNa1	$3*10^6$	10^3*Coop
R57: ToNa1 <-> ToNa1S	$6*10^6$	200*Coop
R58: ToS <-> ToNa1S	$3*10^6$	10^3*Coop
R59: ToCl <-> ToClS	$6*10^6$	200*Coop
R60: ToS <-> ToClS	10^6	10^4*Coop
R61: To <-> ToNa2	$3*10^6$	10^3*Coop
R62: To <-> ToCl	10^6	10^4*Coop
R63: To <-> ToNa1	$3*10^6$	10^3*Coop
R64: To <-> ToS	$6*10^6$	200*Coop
R65: To <-> Ti	210	210
R66: ToClNa1Na2S <-> TiClNa1Na2S	70	70

Cooperativity factor, *Coop = 50.

Table S2. **GlyT2 model**

Reactions (GlyT2 model)	Forward rate (s^{-1} or $s^{-1}*M^{-1}$)	Backward rate (s^{-1})
R1: TiClNa2S <-> TiClNa1Na2S	10^6	10^4*Coop
R2: TiClNa1S <-> TiClNa1Na2S	10^3	300*Coop
R3: TiClNa1Na2 <-> TiClNa1Na2S	10^6	100*Coop
R4: TiNa1Na2S <-> TiClNa1Na2S	10^6	10^4*Coop
R5: TiNa2S <-> TiClNa2S	10^6	10^4*Coop
R6: TiClNa2 <-> TiClNa2S	10^6	100*Coop
R7: TiClS <-> TiClNa2S	10^3	300*Coop
R8: TiClNa2 <-> TiClNa1Na2	10^4	100*Coop
R9: TiClNa1 <-> TiClNa1Na2	10^3	300*Coop
R10: TiNa1Na2 <-> TiClNa1Na2	10^6	10^4*Coop
R11: TiNa1Na2 <-> TiNa1Na2S	10^6	100*Coop
R12: TiNa1S <-> TiNa1Na2S	10^3	300*Coop
R13: TiNa2S <-> TiNa1Na2S	10^4	100*Coop
R14: TiClS <-> TiClNa1S	10^4	100*Coop
R15: TiClNa1 <-> TiClNa1S	10^6	100*Coop
R16: TiNa1S <-> TiClNa1S	10^6	10^4*Coop
R17: TiS <-> TiNa2S	10^3	300*Coop
R18: TiNa2 <-> TiNa2S	10^6	100*Coop
R19: TiCl <-> TiClNa2	10^3	300*Coop
R20: TiNa2 <-> TiClNa2	10^6	10^4*Coop
R21: TiNa2 <-> TiNa1Na2	10^4	100*Coop
R22: TiNa1 <-> TiNa1Na2	10^3	300*Coop
R23: TiNa1 <-> TiClNa1	10^6	10^4*Coop
R24: TiCl <-> TiClNa1	10^4	100*Coop
R25: TiNa1 <-> TiNa1S	10^6	100*Coop
R26: TiS <-> TiNa1S	10^4	100*Coop
R27: TiCl <-> TiClS	10^6	100*Coop
R28: TiS <-> TiClS	10^6	10^4*Coop
R29: Ti <-> TiNa2	10^3	300*Coop
R30: Ti <-> TiCl	10^6	10^4*Coop
R31: Ti <-> TiNa1	10^4	100*Coop
R32: Ti <-> TiS	10^6	100*Coop
R33: ToClNa2S <-> ToClNa1Na2S	10^4	100*Coop
R34: ToClNa1S <-> ToClNa1Na2S	10^3	300*Coop
R35: ToClNa1Na2 <-> ToClNa1Na2S	10^6	100*Coop
R36: ToNa1Na2S <->ToClNa1Na2S	10^6	10^4*Coop
R37: ToNa2S <-> ToClNa2S	10^6	10^4*Coop
R38: ToClNa2 <-> ToClNa2S	10^6	100*Coop
R39: ToClS <-> ToClNa2S	10^3	300*Coop
R40: ToClNa2 <-> ToClNa1Na2	10^4	100*Coop
R41: ToClNa1 <-> ToClNa1Na2	10^3	300*Coop
R42: ToNa1Na2 <-> ToClNa1Na2	10^6	10^4*Coop
R43: ToNa1Na2 <-> ToNa1Na2S	10^6	100*Coop
R44: ToNa1S <-> ToNa1Na2S	10^3	300*Coop

Table S2. **GlyT2 model (*Continued*)**

Reactions (GlyT2 model)	Forward rate (s^{-1} or $s^{-1}*M^{-1}$)	Backward rate (s^{-1})
R45: ToNa2S <-> ToNa1Na2S	10^4	100*Coop
R46: ToClS <-> ToClNa1S	10^6	10^4*Coop
R47: ToClNa1 <-> ToClNa1S	10^6	100*Coop
R48: ToNa1S <-> ToClNa1S	10^6	10^4*Coop
R49: ToS <-> ToNa2S	10^3	300*Coop
R50: ToNa2 <-> ToNa2S	10^6	100*Coop
R51: ToCl <-> ToClNa2	10^3	300*Coop
R52: ToNa2 <-> ToClNa2	10^6	10^4*Coop
R53: ToNa2 <-> ToNa1Na2	10^4	100*Coop
R54: ToNa1 <-> ToNa1Na2	10^3	300*Coop
R55: ToNa1 <-> ToClNa1	10^6	10^4*Coop
R56: ToCl <-> ToClNa1	10^4	100*Coop
R57: ToNa1 <-> ToNa1S	10^6	100*Coop
R58: ToS <-> ToNa1S	10^4	100*Coop
R59: ToCl <-> ToClS	10^6	100*Coop
R60: ToS <-> ToClS	10^6	10^4*Coop
R61: To <-> ToNa2	10^3	300*Coop
R62: To <-> ToCl	10^6	10^4*Coop
R63: To <-> ToNa1	10^4	100*Coop
R64: To <-> ToS	10^6	100*Coop
R65: TiClNa1Na2Na3 <-> TiClNa1Na2Na3S	10^6	100
R66: TiNa1Na2Na3S <-> TiClNa1Na2Na3S	10^6	10^4
R67: TiClNa2Na3S <-> TiClNa1Na2Na3S	10^4	100
R68: TiClNa1Na3S <-> TiClNa1Na2Na3S	10^3	300
R69: TiClNa1Na2S <-> TiClNa1Na2Na3S	10^5	1000
R70: ToClNa1Na2Na3 <-> ToClNa1Na2Na3S	10^6	100
R71: ToNa1Na2Na3S <-> ToClNa1Na2Na3S	10^6	10^4
R72: ToClNa2Na3S <-> ToClNa1Na2Na3S	10^4	100
R73: ToClNa1Na3S <-> ToClNa1Na2Na3S	10^3	300
R74: ToClNa1Na2S <-> ToClNa1Na2Na3S	10^4	100
R75: TiClNa2S <-> TiClNa2Na3S	10^5	1,000*Coop
R76: TiClNa2S <-> TiClNa1Na2S	10^4	100*Coop
R77: TiClNa1Na2 <-> TiClNa1Na2Na3	10^5	1,000*Coop
R78: TiNa1Na2 <-> TiNa1Na2Na3S	10^6	100*Coop
R79: ToClNa2S <-> ToClNa2Na3S	10^4	100*Coop
R80: ToClNa2S <-> ToClNa1Na2S	10^4	100*Coop
R81: ToClNa1Na2 <-> ToClNa1Na2Na3	10^4	100*Coop
R82: ToNa1Na2 <-> ToNa1Na2Na3	10^4	100*Coop
R83: To <-> Ti	300	300
R84: ToClNa1Na2Na3S <-> TiClNa1Na2Na3S	300	300

Cooperativity factor, *Coop = 8.