

## Microscopic reversibility or detailed balance in ion channel models

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**Abstract** Mass action type deterministic kinetic models of ion channels are usually constructed in such a way as to obey the principle of detailed balance (or, microscopic reversibility) for two reasons: first, the authors aspire to have models harmonizing with thermodynamics, second, the conditions to ensure detailed balance reduce the number of reaction rate coefficients to be measured. We investigate a series of ion channel models which are asserted to obey detailed balance, however, these models violate mass conservation and in their case only the necessary conditions (the so-called circuit conditions) are taken into account. We show that ion channel models have a very specific structure which makes the consequences true in spite of the imprecise arguments. First, we transform the models into mass conserving ones, second, we show that the full set of conditions ensuring detailed balance (formulated by Feinberg) leads to the same relations for the reaction rate constants in these special cases, both for the original models and the transformed ones.

**Keywords** Microscopic reversibility · Detailed balance · Ion channels · Circuit conditions · Spanning forest conditions

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## 1 Introduction

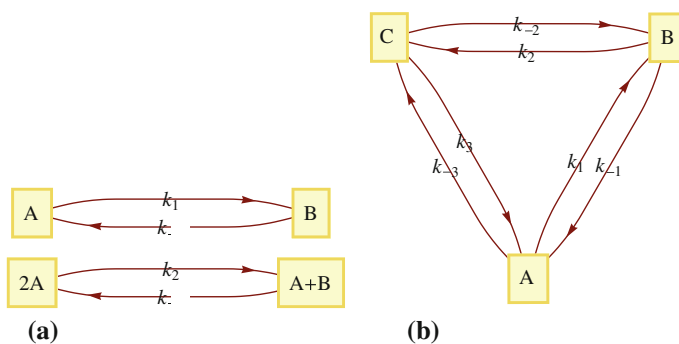
### 1.1 Detailed balancing or microscopic reversibility

At the beginning of the 20th century it was Wegscheider [22] who gave the formal kinetic example  $A \xrightleftharpoons[k_{-1}]{k_1} B \xrightleftharpoons[k_{-2}]{k_2} 2A + B$  to show that in some cases the existence of a positive stationary state alone does not imply the equality of all the individual forward and backward reaction rates in equilibrium: a relation ( $\frac{k_1}{k_{-1}} = \frac{k_2}{k_{-2}}$ ) should hold between the reaction rate coefficients to ensure this. Equalities of this kind will be called (and later exactly defined) as *spanning forest conditions* below. Let us emphasize that violation of this equality does not exclude the existence of a positive stationary state; it exists and it is unique for all values of the reaction rate coefficients, see the details in Sect. 2.4.

A similar statement holds for the reversible triangle reaction in Fig. 1. The necessary and sufficient condition for the existence of such a positive stationary state for which all the reaction steps have the same rate in the forward and backward direction is  $k_1 k_2 k_3 = k_{-1} k_{-2} k_{-3}$ . Equalities of this kind will be called (and later exactly defined) as *circuit conditions* below. (Let us mention that similar conditions were derived by Kolmogoroff [14] for stochastic processes.) Again, violation of this equality does not exclude the existence of a positive stationary state; it exists and is unique for all values of the reaction rate coefficients, see the details in Sect. 2.4.

A quarter of a century after Wegscheider the authors Fowler and Milne [10] formulated in a very vague form a general principle called the *principle of detailed balance* stating that in real thermodynamic equilibrium all the subprocesses (whatever they mean) should be in dynamic equilibrium separately in such a way that they do not stop but they proceed with the same velocity in both directions. Obviously, this also means that time is reversible at equilibrium, that is why this property may also be called *microscopic reversibility*.

A relatively complete summary of the early developments was given by Tolman [19], but see also [1–3, 23].



**Fig. 1** **a** The Wegscheider reaction. **b** The triangle reaction

The modern formulation of the principle accepted by IUPAC [11] essentially means the same: “The principle of microscopic reversibility at equilibrium states that, in a system at equilibrium, any molecular process and the reverse of that process occur, on the average, at the same rate.”

Neither the above document nor the present authors assert that the principle should hold without any further assumptions; for us it is an important hypothesis the fulfilment of which should be checked individually in different models.

It turned out that in the case of chemical reactions this general principle can only hold if both the spanning tree conditions and the circuit conditions are fulfilled. However, it became a general belief among people dealing with reaction kinetics that the circuit conditions alone are not only necessary but also sufficient for all kinds of reactions: Wegscheider’s example proving the contrary was not known well enough. Vlad and Ross [20] drew the conclusions from the Wegscheider example in full generality, but it was Feinberg [9] who gave the definitive solution of the problem in the area of formal kinetics: he clearly formulated, proved and applied the two easy-to-deal-with sets of conditions which together make up a necessary and sufficient condition of detailed balance (for the case of mass action kinetics). In other words, he completed the known necessary condition (the circuit conditions) with another condition (the spanning forest conditions) making this sufficient, as well.

The reason why the false belief is widespread is that in case of reactions with deficiency zero the circuit conditions alone are also sufficient not only necessary, and most textbook examples have deficiency zero.

## 1.2 Ion channel models

Recent papers on formal kinetic models of ion channel gating show that people in this field think that the principle of detailed balance or microscopic reversibility should hold. (However, some authors do not consider the principle of microscopic reversibility indispensable, e. g. Naundorf et al. [17, Supplementary Notes 2, Fig. 3SI(a), page 4] provides a channel model which is not even reversible, let alone detailed balanced.) This may be supported either by a *theoretical* argument: they should obey the laws of thermodynamics, or by a *practical* one: if the principle holds one should measure fewer reaction rate coefficients because one also has the constraints implied by the principle. The second argument seems to be the more important one in the papers by Colquhoun et al. [4,6]. However, the principle is applied in an imprecise way: first, only the necessary part consisting of the circuit conditions is applied, second, the models are formulated in a way that they do not obey the principle of *mass conservation*. In the present paper we transform the models into mass conserving ones, and apply the full set of necessary and sufficient conditions. Our main result is that in classes of models including all the known ion channel examples are compartmental models, therefore they have zero deficiency at the beginning, and being transformed into a mass conserving model they have no circuits, therefore one has only to test the spanning forest conditions. It is not less interesting that the spanning forest conditions obtained for the transformed models are literally the same as the circuit conditions for the original models.

### 1.3 Stochastic models

So far we had in mind only deterministic models (surely not speaking of the general but vague formulation of Fowler and Milne). Turning to stochastic models one possible approach is to check the fulfilment of microscopic reversibility in the following way. Let us suppose we have some measurements on a process, and present the data with reversed time, finally use a statistical test to see if there is any difference. This is an absolutely correct approach and has also been used in the field of channel modeling [18], see also [21].

### 1.4 Outline

The structure of our paper is as follows. Section 2 gives a short summary of the definitions used and presents Feinberg's theorem. In Sect. 3 some usual ion channel models are transformed into realistic models with mass conservation and with the help of a lemma it is shown that *in these special cases* the circuit conditions for the original systems and the spanning forest conditions for the transformed systems lead to exactly the same requirements. The question of the number of free parameters is also discussed here. Finally an outlook and discussion follows in Sect. 4. The formal proof of our main result has been relegated to an Appendix.

Let us also mention that parts of our investigations has been presented in a short, nonrigorous form in [15].

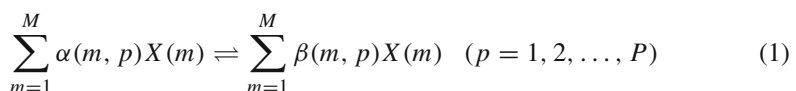
## 2 Tools to be used

### 2.1 Ion channels

There is a difference in electric potential between the interior of cells and the interstitial liquid. An essential part of the system controlling the size of this potential difference is the system of *ion channels*: pores made up from proteins in the membranes through which different ions may be transported via active and passive transport thereby changing the potential difference in an appropriate way. The models of these ion channels are usually described in terms of formal reaction kinetics, thus we have to present these notions first, then we shall be in the position to present a few alternative models of ion channels.

### 2.2 Basic definitions of formal kinetics

Let us consider the reversible reaction



with  $M \in \mathbb{N}$  chemical species:  $X(1), X(2), \dots, X(M)$ ;  $P \in \mathbb{N}$  pairs of reaction steps,  $\alpha(m, p), \beta(m, p) \in \mathbb{N}_0$  ( $m = 1, 2, \dots, M$ ;  $p = 1, 2, \dots, P$ ) stoichiometric coefficients or molecularities, and suppose its deterministic model

$$c'_m(t) = f_m(\mathbf{c}(t)) := \sum_{p=1}^P (\beta(m, p) - \alpha(m, p))(w_{+p}(\mathbf{c}(t)) - w_{-p}(\mathbf{c}(t))) \quad (2)$$

$$c_m(0) = c_{m0} \in \mathbb{R}_0^+ \quad (m = 1, 2, \dots, M) \quad (3)$$

describing the time evolution of the concentration versus time functions

$$t \mapsto c_m(t) := [X(m)](t)$$

of the species—is based on mass action type kinetics:

$$w_{+p}(\bar{\mathbf{c}}) := k_{+p} \bar{\mathbf{c}}^{\alpha(\cdot, p)} := k_{+p} \prod_{\mu=1}^M \bar{c}_\mu^{\alpha(\mu, p)} \quad (4)$$

$$w_{-p}(\bar{\mathbf{c}}) := k_{-p} \bar{\mathbf{c}}^{\beta(\cdot, p)} := k_{-p} \prod_{\mu=1}^M \bar{c}_\mu^{\beta(\mu, p)} \quad (p = 1, 2, \dots, P). \quad (5)$$

[(2) is also called the induced kinetic differential equation of the reaction (1).] The number of complexes is the number of different complex vectors among  $\alpha(\cdot, p)$  and  $\beta(\cdot, p)$ , i.e. it is the cardinality of the set

$$\{\alpha(\cdot, p); p = 1, 2, \dots, P\} \cup \{\beta(\cdot, p); p = 1, 2, \dots, P\}$$

and it is denoted by  $N$ . The Feinberg–Horn–Jackson graph (or, FHJ-graph, for short) of the reaction is obtained if one writes down all the complex vectors [or simply the complexes, the formal linear combinations on both sides of (1)] exactly once and connects two complexes with an edge (or two different edges pointing into opposite directions) if there is a reaction step taking place between them. Let us denote the number of connected components of this graph by  $L$ .

The stoichiometric space is the linear subspace of  $\mathbb{R}^M$  generated by the reaction vectors:  $\text{span}\{\beta(\cdot, p) - \alpha(\cdot, p); p = 1, 2, \dots, P\}$ ; its dimension is denoted by  $S$ . Finally, the nonnegative integer  $\delta := N - L - S$  is the deficiency of the reaction (1).

Examples to show the meaning of the definitions follow.

*Example 1* (Simple bimolecular reaction) In the simple reversible bimolecular reaction  $A + B \rightleftharpoons C$  we have  $M = 3, P = 1$ ;  $X(1) = A, X(2) = B, X(3) = C$ ; and the complexes are  $A + B$  and  $C$ , thus the corresponding complex vectors are  $(1, 1, 0)$  and  $(0, 0, 1)$ . As  $N = 2, L = 1, S = 1$ ; the deficiency of the reaction is 0.

*Example 2* (Triangle reaction) In the triangle reaction (Fig. 1) we have  $M = 3, P = 3$ ;  $X(1) = A, X(2) = B, X(3) = C$ ; and the complexes are  $A, B$  and  $C$ , thus the

corresponding complex vectors are  $(1, 0, 0)$ ,  $(0, 1, 0)$  and  $(0, 0, 1)$ . As  $N = 3$ ,  $L = 1$ ,  $S = 2$ ; the deficiency of the reaction is 0.

**Example 3 (Wegscheider)** In the Wegscheider reaction (Fig. 1) we have  $M = 2$ ,  $P = 2$ ;  $X(1) = A$ ,  $X(2) = B$ ; and the complexes are  $A$ ,  $B$ ,  $2A$  and  $A + B$ , thus the corresponding complex vectors are  $(1, 0)$ ,  $(0, 1)$ ,  $(2, 0)$  and  $(1, 1)$ ; therefore the reaction vectors are  $(1, -1)$  and  $(-1, 1)$ . As  $N = 4$ ,  $L = 2$ ,  $S = 1$ ; the deficiency of the reaction is 1.

Let us mention here that it is a boring task with many possibilities of mistake to calculate the characteristic quantities of reactions and this is one of the reasons why a program package `ReactionKinetics.m` is being developed in *Mathematica*. The second example may be prepared for the present purposes as follows.

```
In[1] := << ReactionKinetics`
In[2] := triangle = {A<=>B<=>C<=>A};
In[3] := Column[ReactionsData[triangle]]
species->{A, B, C}
M -> 3
externalspecies->{ }
E -> 0
complexes->{A, B, C}
Out[3] = reactionsteps-> {A->B, B->A, B->C, C->B, C->A, A->C}
R -> 6
variables-> {cA, cB, cC}
alpha -> (
  1 0 0 0 0 1
  0 1 1 0 0 0
  0 0 0 1 1 0
)
beta -> (
  0 1 0 0 1 0
  1 0 0 1 0 0
  0 0 1 0 0 1
)
gamma -> (
  -1 1 0 0 1 -1
  1 -1 -1 1 0 0
  0 0 1 -1 -1 1
)
In[4] := ShowFHJGraph[triangle, {k1, k-1, k2, k-2, k3, k-3},
  VertexLabeling -> True, DirectedEdges -> True]]
```

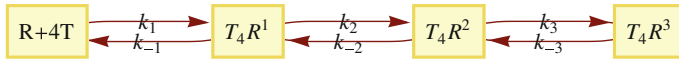
Other

uses of the package are described in the work mentioned above.

### 2.3 Models of ion channels

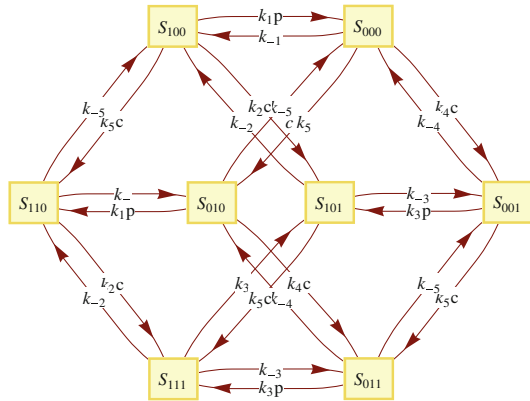
In the models of ion channels the relevant species are receptors and molecules modifying the operation of receptors so as to change the sizes of the pores, thereby decreasing or increasing the quantity of ions flowing through the channels. Altogether there are several hundreds of different types of ion channels in living cells.

One possible model, see Fig. 2, contains receptors, transmitters, and receptor transmitter complexes each with a different conformation having different ion-conductance,



**Fig. 2** The Érdi–Ropolyi model with four transmitters and three different states of the transmitter–receptor complex

**Fig. 3** A model by De Young and Keizer



and these conformations correspond to states in which the channels are between the open and closed states [8].

Another approach, see Fig. 3, might involve multiple types of modifying molecules and complexes, again representing different states of the channels [7]. These are the models we are especially interested in.

Ion channel models are usually required to fulfil the principle of detailed balance both from theoretical and practical points of view. First, thermodynamics is said to require the principle to hold, second, if this principle holds then the number of reaction rate constants to be measured are reduced. Let us turn to the formal definition of detailed balance in the framework given in Sect. 2.2.

### 2.4 Detailed balance: definition and the naïve approach

Within the model exactly defined above we can formulate the property of being detailed balanced [13]. Consider the reaction (1) endowed with mass action kinetics.

**Definition 1** If  $\mathbf{c}_* \in (\mathbb{R}^+)^M$  is such that

$$k_p \mathbf{c}_*^{\alpha(\cdot,p)} = k_{-p} \mathbf{c}_*^{\beta(\cdot,p)} \quad (p = 1, 2, \dots, P), \tag{6}$$

then reaction (1) is said to be *detailed balanced at the stationary point*  $\mathbf{c}_*$ . If the reaction is detailed balanced at all its positive stationary points, then it is *detailed balanced*.

We are especially interested in reactions which are detailed balanced for some choices of the reaction rate constants, and also in the restrictions upon the rate constants which ensure detailed balancing.

*Example 4* (Simple bimolecular reaction) The deterministic model of the reaction  $A + B \xrightleftharpoons[k_{-1}]{k_1} C$  according to Sect. 2.2 can be seen to be (in accord with the usual formulation)

$$\begin{aligned} a' &= -k_1ab + k_{-1}c & b' &= -k_1ab + k_{-1}c & c' &= k_1ab - k_{-1}c \\ a(0) &= a_0 & b(0) &= b_0 & c(0) &= c_0 \end{aligned}$$

which simplifies to

$$\begin{aligned} a'(t) &= -k_1a(t)(a(t) - a_0 + b_0) + k_{-1}(-a(t) + a_0 + c_0) \\ &= -k_1a(t)^2 + (k_1a_0 - k_1b_0 - k_{-1})a(t) + k_{-1}(a_0 + c_0) \\ &= -k_{-1}(Ka(t)^2 - (K(a_0 - b_0) - 1)a(t) - a_0 - c_0). \end{aligned} \quad (7)$$

If the reaction starts from nonnegative initial concentrations  $a_0, b_0, c_0$  for which  $a_0 + c_0 > 0$ , the unique positive (relatively asymptotically stable) equilibrium concentration

$$\begin{aligned} a_* &= \frac{1}{2K}(-1 + K(a_0 - b_0) + r) \\ b_* &= \frac{1 + K(a_0 + b_0 + 2c_0) - r}{K(-1 + K(a_0 - b_0) + r)} \\ c_* &= \frac{1}{2K}(1 + K(a_0 + b_0 + 2c_0) - r) \\ \text{where } K &:= \frac{k_1}{k_{-1}}, r := \sqrt{1 + 2K(a_0 + b_0 + 2c_0) + K^2(a_0 - b_0)^2} \end{aligned}$$

will be attained. The reaction is detailed balanced at this vector of stationary concentrations for all values of the reaction rate coefficients, i. e.  $k_1a_*b_* = k_{-1}c_*$  always holds.

*Example 5* (Triangle reaction) The induced kinetic differential equation of the reversible triangle reaction being

$$\begin{aligned} a' &= -k_1a + k_{-1}b - k_{-3}a + k_3c \\ b' &= k_1a - k_{-1}b - k_2b + k_{-2}c \\ c' &= k_2b - k_{-2}c + k_{-3}a - k_3c \end{aligned}$$

together with the mass conservation relation

$$a(t) + b(t) + c(t) = a_0 + b_0 + c_0 =: m$$

imply that the unique, relatively asymptotically stable vector of positive stationary concentrations—if at least one of the initial concentrations  $a_0, b_0, c_0$  is positive—is as follows.



$$a_* = (k_{-2}k_{-1} + (k_{-1} + k_2)k_3) \frac{m}{d} \tag{8}$$

$$b_* = (k_{-3}k_{-2} + (k_{-2} + k_3)k_1) \frac{m}{d} \tag{9}$$

$$c_* = (k_{-3}k_{-1} + (k_{-3} + k_1)k_2) \frac{m}{d} \tag{10}$$

with  $d := k_{-2}(k_{-1} + k_1) + k_1k_2 + k_{-3}(k_{-2} + k_{-1} + k_2) + k_{-1}k_3 + k_1k_3 + k_2k_3$ .

The reaction is detailed balanced at this vector of stationary concentrations—i. e.

$$k_1a_* = k_{-1}b_* \quad k_2b_* = k_{-2}c_* \quad k_3c_* = k_{-3}a_*$$

if and only if

$$k_1k_2k_3 = k_{-1}k_{-2}k_{-3} \tag{11}$$

holds.

*Example 6* (Wegscheider) The induced kinetic differential equation of the Wegscheider reaction being

$$\begin{aligned} a' &= -k_1a + k_{-1}b - k_2a^2 + k_{-2}ab \\ b' &= k_1a - k_{-1}b + k_2a^2 - k_{-2}ab \end{aligned}$$

which simplifies to

$$\begin{aligned} a' &= -k_1a + k_{-1}(a_0 + b_0 - a) - k_2a^2 + k_{-2}a(a_0 + b_0 - a) \\ &= -(k_2 + k_{-2})a^2 - (k_1 + k_{-1} - k_{-2}(a_0 + b_0))a + k_{-1}(a_0 + b_0). \end{aligned}$$

together with the mass conservation relation

$$a(t) + b(t) = a_0 + b_0 =: m$$

imply that—unless all the initial concentrations are zero—the unique positive (relatively asymptotically stable) stationary concentration vector is as follows.

$$a_* = \frac{k_{-1} + k_1 - k_{-2}m - r}{-2(k_{-2} + k_2)} \tag{12}$$

$$b_* = \frac{k_{-1} + k_1 + k_{-2}m + 2k_2m - r}{2(k_{-2} + k_2)} \tag{13}$$

$$\text{with } r := \sqrt{(k_{-1} + k_1 - k_{-2}m)^2 + 4k_{-1}m(k_{-2} + k_2)}. \tag{14}$$

The reaction is detailed balanced at this vector of stationary concentrations—i. e.

$$k_1a_* = k_{-1}b_*, \quad k_2a_*b_* = k_{-2}b_*^2$$

if and only if

$$\frac{k_1}{k_{-1}} = \frac{k_2}{k_{-2}} \quad (15)$$

holds.

## 2.5 The necessary and sufficient condition of detailed balancing

The necessary and sufficient conditions are formulated in the following way in [9]. Consider the reaction (1) endowed with mass action kinetics.

First suppose that we have chosen an arbitrary spanning forest for the FHJ-graph of the network. It is possible to find a set of  $P - (N - L)$  independent circuits induced by the choice of the spanning forest. For each of these circuits we write an equation which asserts that the product of the rate constants in the clockwise direction and the counterclockwise direction is equal. Thus we have  $P - (N - L)$  equations: the *circuit conditions*.

Next, these equations are supplemented with the  $\delta$  *spanning forest conditions* as follows. Suppose that the edges of the spanning forest has been given an orientation. Then there are  $\delta$  independent nontrivial solutions to the vector equation  $\sum_{(i,j)} a_{ij} \mathbf{v}_{ij} = \mathbf{0}$  where the sum is taken for all reaction steps in the oriented spanning forest and  $\mathbf{v}_{ij}$  is the corresponding reaction step vector. With these  $a_{ij}$  coefficients the spanning forest conditions are

$$\prod k_{ij}^{a_{ij}} = \prod k_{ji}^{a_{ij}}, \quad (16)$$

where  $k_{ij}$  are the corresponding rate coefficients.

With all these the widely-accepted *necessary* conditions (the circuit conditions) are complemented with the spanning forest conditions to form a set of *necessary and sufficient* conditions for detailed balancing in mass action systems of arbitrary complexity.

**Theorem 1** (Feinberg) *The reaction (1) is detailed balanced for all those choices of the reaction rate constants which satisfy the  $P - (N - L)$  circuit conditions and the  $\delta$  spanning forest conditions.*

*Remark 1* The circuit conditions are called *spanning tree method* in [6].

*Remark 2* There are three interesting special cases.

1. For a reversible mass action system which has a deficiency of zero, the circuit conditions *alone* become necessary and sufficient for detailed balancing. The reason why the circuit conditions were generally accepted as sufficient as well, is that a large majority of models are of zero deficiency. This case is exemplified by the triangle reaction.
2. For networks with no nontrivial circuits, that is, in which there are just  $N - L$  reaction pairs and so  $P - (N - L) = 0$ , the circuit conditions are vacuous.

Therefore, the spanning forest conditions *alone* are necessary and sufficient for detailed balancing. The example by Wegscheider belongs to this category.

3. Finally, if a reversible network is circuitless and has a deficiency of zero, both the circuit conditions and the spanning forest conditions are vacuous. The system is detailed balanced (or fulfils the principle of microscopic reversibility), regardless of the values of the rate constants. Such is a compartmental system with no circles in the FHJ-graph, the simple bimolecular reaction or the érdi–Ropolyi model.

### 3 The main result

#### 3.1 Our strategy

Let us denote by  $M, P, \delta, N, L, S, K$  and  $M', P', \delta', N', L', S', K'$  the number of species, the number of (half) reaction steps, the deficiency, the number of complexes, the number of linkage classes, the dimension of the stoichiometric space (i.e., the number of independent reaction steps) and the number of independent cycles respectively in the original and in the transformed system.

All the investigated original (not mass-conserving) ion channel models are formally compartmental systems which means that each complex consists of a single species and all species are different. Therefore all these models are of deficiency zero. Thus, in order to check detailed balancing it is enough to test the circuit conditions, and this is what the authors in [4, 6] do.

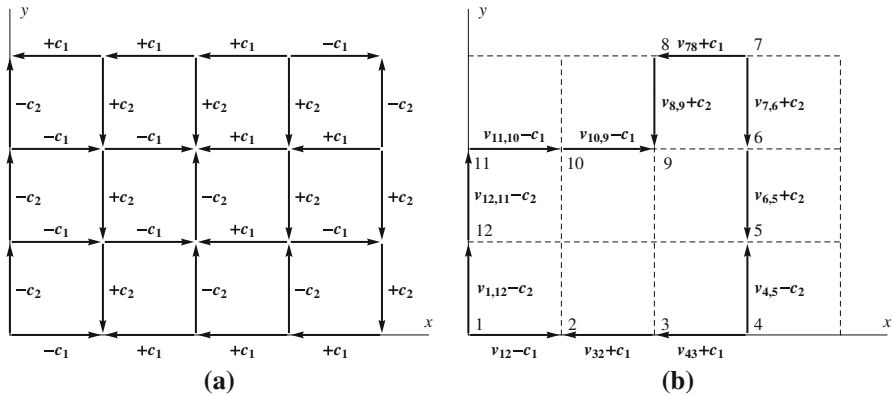
What we propose is to transform these models into a mass-conserving model in such a way as to reflect the same physical reality. The transformed models have the following properties.

1. There is no cycle in the transformed system.
2.  $S = S'$
3.  $N' - L' - S' = \delta' = K$
4. The circuit conditions in the original system are equivalent to the spanning forest conditions in the transformed system.

This transformation is constructed in the Appendix for a large class of systems—those with rectangular grids as FHJ-graphs—containing all the special cases we have met up to now.

#### 3.2 Lemma

Consider a directed graph whose edges and vertices are the edges and vertices of a planar rectangular grid. Suppose that the graph has  $n$  vertices and that to each vertex  $j$  we assign a  $\mathbf{y}_j$  vector in  $\mathbf{R}^{n+2}$  such that these vertex vectors are linearly independent. Let  $\mathbf{c}_1$  and  $\mathbf{c}_2$  be vectors in  $\mathbf{R}^{n+2}$  such that they are linearly independent of each other and of each  $\mathbf{y}_j$ . Let us denote by  $e_{ij}$  the directed edge of the graph from vertex  $i$  to vertex  $j$  and to each  $e_{ij}$  edge let us assign the  $\mathbf{v}_{ij} = \mathbf{y}_j - \mathbf{y}_i$  vector. Let us define the  $\mathbf{u}_{ij}$  vectors in the following way. If  $e_{ij}$  is directed in the positive or negative direction in relation to the  $x$  axis then  $\mathbf{u}_{ij} := \mathbf{v}_{ij} - \mathbf{c}_1$  or  $\mathbf{u}_{ij} := \mathbf{v}_{ij} + \mathbf{c}_1$ , respectively. Similarly,



**Fig. 4** Rectangular grid with directed edges

if  $e_{ij}$  is directed in the positive or negative direction in relation to the  $y$  axis then  $\mathbf{u}_{ij} := \mathbf{v}_{ij} - \mathbf{c}_2$  or  $\mathbf{u}_{ij} := \mathbf{v}_{ij} + \mathbf{c}_2$ , respectively (Fig. 4b). Fig. 4a is only to show the transformations. Let us denote by  $\text{span}\{\mathbf{v}_{ij}\}$  the subspace generated by the  $\mathbf{v}_{ij}$  vectors.

**Lemma 1** *Under these conditions the following statements hold.*

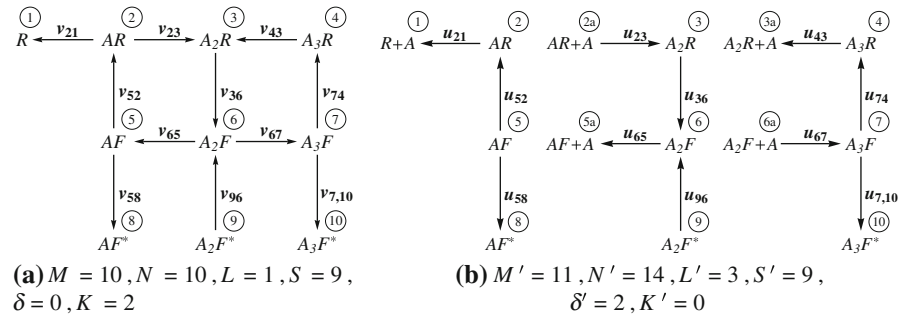
1. Along each directed circle in the graph,  $\sum a_{ij} \mathbf{v}_{ij} = \sum a_{ij} \mathbf{u}_{ij} = \mathbf{0}$  where  $a_{ij} := 1$  if the edges of the graph and the circle are directed in the same way and  $a_{ij} := -1$  otherwise.
2. The dimension of  $\text{span}\{\mathbf{v}_{ij}\}$  and  $\text{span}\{\mathbf{u}_{ij}\}$  is  $n - 1$ .

*Proof 1.* Since the  $\mathbf{v}_{ij}$  vectors are the differences of the corresponding vertex vectors, it is obvious that along a directed circle, the sum of the  $\mathbf{v}_{ij}$  vectors is  $\mathbf{0}$ . It is enough to show that the  $\mathbf{c}_1$  and  $\mathbf{c}_2$  vectors disappear in the sum of the  $\mathbf{u}_{ij}$  vectors. In order to see this, first assume that along a directed circle we change the direction of the  $e_{ij}$  edges so that each is directed clockwise. In this case it is obvious that the sum of the  $\mathbf{c}_1$  and  $\mathbf{c}_2$  vectors is zero since the number of the “ $+c_1$ ” and “ $+c_2$ ” vectors is equal to the number of the “ $-c_1$ ” and “ $-c_2$ ” vectors, respectively. Then, changing the original directions back, the sign of the  $\mathbf{c}_1$  and  $\mathbf{c}_2$  vectors changes twice and thus they will not appear in the sum.

2. Let us choose a spanning tree in the graph consisting of  $n - 1$  of the  $e_{ij}$  edges. Then the corresponding  $\mathbf{v}_{ij}$  vectors are linearly independent and since the  $\mathbf{c}_1$  and  $\mathbf{c}_2$  vectors are independent of them, the corresponding  $\mathbf{u}_{ij}$  vectors are also linearly independent. □

**Remark 3** It is trivial that the statements of the lemma remain true if either  $\mathbf{c}_1$  or  $\mathbf{c}_2$  is the zero vector, or, if the graph contains edges that are not part of a circle.

**Remark 4** The statements of the lemma are also true for graphs consisting of  $k$ -dimensional grids ( $k \geq 3$ ), see Figs. 7 and 8a, b as an illustration for the three-dimensional case.



**Fig. 5** A model for  $\alpha\beta$  glycine channels where  $A$  represents an agonist, and  $R, F$  and  $F^*$  denotes the resting states, flipped states and open states of the receptor, respectively

### 3.3 Examples

In the next three examples, the left side of the figure shows the original system and the right side of the figure shows the transformed system with an oriented spanning forest. Both systems are reversible, the arrows show a direction needed to write down the spanning forest conditions. The choice of the numbering of the species as well as the direction of the reaction vectors is arbitrary but in both systems they are chosen correspondingly.

*Example 7* The system in Fig. 5 can be found in [4]. The meaning of the species is as follows: The core of the system is obviously a rectangle, the additional parts do not mean an extra problem as the reader can easily verify it. The original system consists of  $M = 10$  species,  $N = 10$  complexes,  $L = 1$  linkage class and it contains two circles while the transformed system contains one more species,  $A$ , there are  $N' = 14$  complexes,  $L' = 3$  linkage classes and it is circuitless. In order to compare these systems easily, in both cases let us number the species in the same way and let  $A$  be the last one, that is,

$$X(1) := R, X(2) := AR, X(3) := A_2R, \dots, X(10) := A_3F^*, X(11) := A.$$

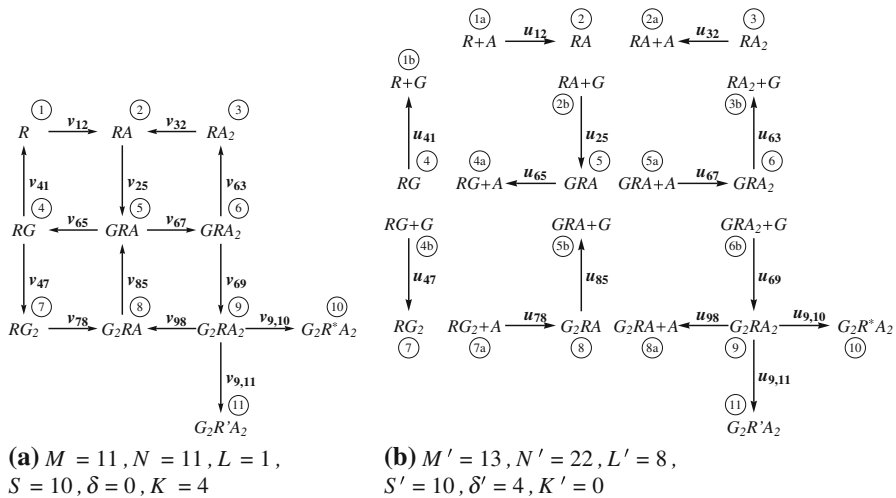
Let us assign a vector  $\mathbf{y}_i \in \mathbb{R}^{11}$  to the  $i$ th species so that  $y_{i,j} = 1$  if  $i = j$  and  $y_{i,j} = 0$  if  $i \neq j$  where  $i, j = 1, \dots, 11$  and let  $\mathbf{a} := \mathbf{y}_{11}$ .

The complex vectors in Fig. 5a are  $\mathbf{y}_1, \mathbf{y}_2, \dots, \mathbf{y}_{10}$  and the corresponding reaction vectors are  $\mathbf{v}_{21} = \mathbf{y}_1 - \mathbf{y}_2, \mathbf{v}_{23} = \mathbf{y}_3 - \mathbf{y}_2, \dots, \mathbf{v}_{7,10} = \mathbf{y}_{10} - \mathbf{y}_7$ . The dimension of  $\text{span}\{\mathbf{v}_{21}, \mathbf{v}_{23}, \dots, \mathbf{v}_{7,10}\}$  is  $S = 9$ . Thus, the deficiency of this system is  $\delta = N - L - S = 0$ . It means that the circuit conditions are necessary and sufficient for detailed balancing. The circuit conditions along circles 2365 and 4367 are

$$k_{23}k_{36}k_{65}k_{52} = k_{32}k_{25}k_{56}k_{63}$$

$$k_{43}k_{36}k_{67}k_{74} = k_{34}k_{47}k_{76}k_{63}$$

The complexes in Fig. 5b are numbered as 1, 2, 2a, ..., 10 and the complex vectors are  $\mathbf{y}'_1 = \mathbf{y}_1 + \mathbf{a}, \mathbf{y}'_2 = \mathbf{y}_2, \mathbf{y}'_{2a} = \mathbf{y}_2 + \mathbf{a}, \dots, \mathbf{y}'_{10} = \mathbf{y}_{10}$ . The reaction vectors are



**Fig. 6** A model containing two binding sites for the agonists  $A$  and  $G$

$\mathbf{u}_{21} = \mathbf{v}_{21} + \mathbf{a}, \mathbf{u}_{23} = \mathbf{v}_{23} - \mathbf{a}, \mathbf{u}_{43} = \mathbf{v}_{43} + \mathbf{a}, \mathbf{u}_{52} = \mathbf{v}_{52}, \mathbf{u}_{36} = \mathbf{v}_{36}, \mathbf{u}_{74} = \mathbf{v}_{74}, \mathbf{u}_{65} = \mathbf{v}_{65} + \mathbf{a}, \mathbf{u}_{67} = \mathbf{v}_{67} - \mathbf{a}, \mathbf{u}_{58} = \mathbf{v}_{58}, \mathbf{u}_{96} = \mathbf{v}_{96}, \mathbf{u}_{7,10} = \mathbf{v}_{7,10}$ . The lemma can be applied to this system with  $\mathbf{c}_1 = \mathbf{a}$  and  $\mathbf{c}_2 = \mathbf{0}$ . The dimension of  $\text{span}\{\mathbf{u}_{21}, \mathbf{u}_{23}, \dots, \mathbf{u}_{7,10}\}$  is also  $S' = 9$ . Thus, the deficiency is  $\delta' = 14 - 3 - 9 = 2$ . Since this system is circuitless, there are two equations according to the spanning forest conditions that ensure detailed balancing. Along the circles ‘2365’ and ‘3476’ in both systems,  $\mathbf{v}_{23} + \mathbf{v}_{36} + \mathbf{v}_{65} + \mathbf{v}_{52} = \mathbf{u}_{23} + \mathbf{u}_{36} + \mathbf{u}_{65} + \mathbf{u}_{52} = \mathbf{0}$  and  $\mathbf{v}_{43} + \mathbf{v}_{36} + \mathbf{v}_{67} + \mathbf{v}_{74} = \mathbf{u}_{43} + \mathbf{u}_{36} + \mathbf{u}_{67} + \mathbf{u}_{74} = \mathbf{0}$ . Since each coefficient of the  $\mathbf{u}_{ij}$  vectors in the above linear combinations is 1,

$$k'_{23}k'_{36}k'_{65}k'_{52} = k'_{32}k'_{63}k'_{56}k'_{25}$$

$$k'_{43}k'_{36}k'_{67}k'_{74} = k'_{34}k'_{63}k'_{76}k'_{47}$$

which are equivalent to the circuit conditions.

*Remark 5* Let us observe that the equivalence of the circuit conditions in the original system and the spanning forest conditions in the transformed system follows from the first statement of the lemma, that is, along each circle the  $\mathbf{v}_{ij}$  vectors and the correspondingly chosen  $\mathbf{u}_{ij}$  vectors satisfy the same linear equalities. If, say, instead of circle ‘4367’ we choose circle ‘234765’ then  $\mathbf{v}_{23} - \mathbf{v}_{43} - \mathbf{v}_{74} - \mathbf{v}_{67} + \mathbf{v}_{65} + \mathbf{v}_{52} = \mathbf{u}_{23} - \mathbf{u}_{43} - \mathbf{u}_{74} - \mathbf{u}_{67} + \mathbf{u}_{65} + \mathbf{u}_{52} = \mathbf{0}$ . The corresponding circuit condition in Fig. 5a is

$$k_{23}k_{34}k_{47}k_{76}k_{65}k_{52} = k_{32}k_{25}k_{56}k_{67}k_{74}k_{43}$$

and the equivalent equation from the spanning forest condition in Fig. 5b is  $k'_{23}(k'_{43})^{-1}(k'_{74})^{-1}(k'_{67})^{-1}k'_{65}k'_{52} = k'_{32}(k'_{34})^{-1}(k'_{47})^{-1}(k'_{76})^{-1}k'_{56}k'_{25}$ .

*Example 8* The system in Fig. 6 can be found in [16]. Figure 6a shows the original system where there are  $M = 11$  species and Fig. 6b shows the transformed system

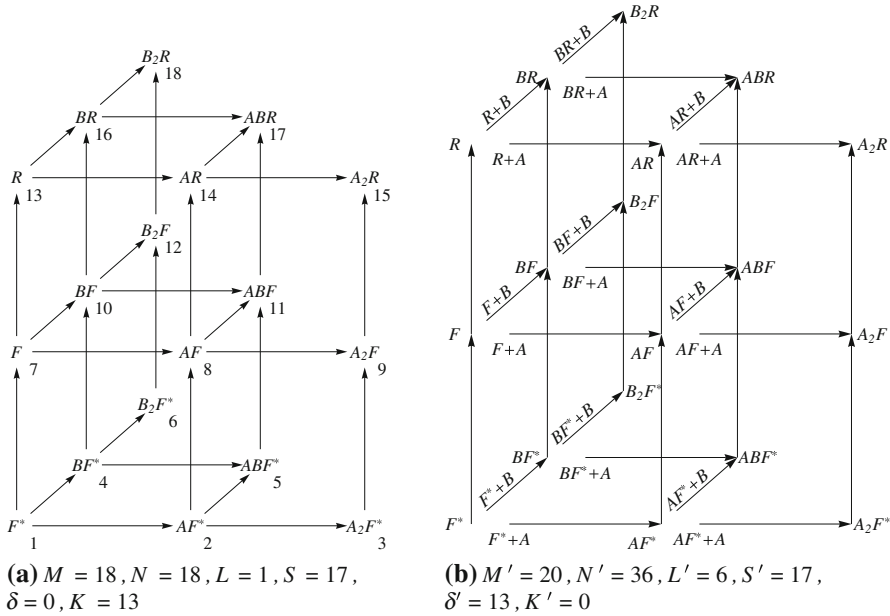


Fig. 7 A model with two binding sites for the agonists A and B

where there are two more species, A and G. Again, let us number the species in the same way as in Fig. 5 and let A and G be the last two, that is,

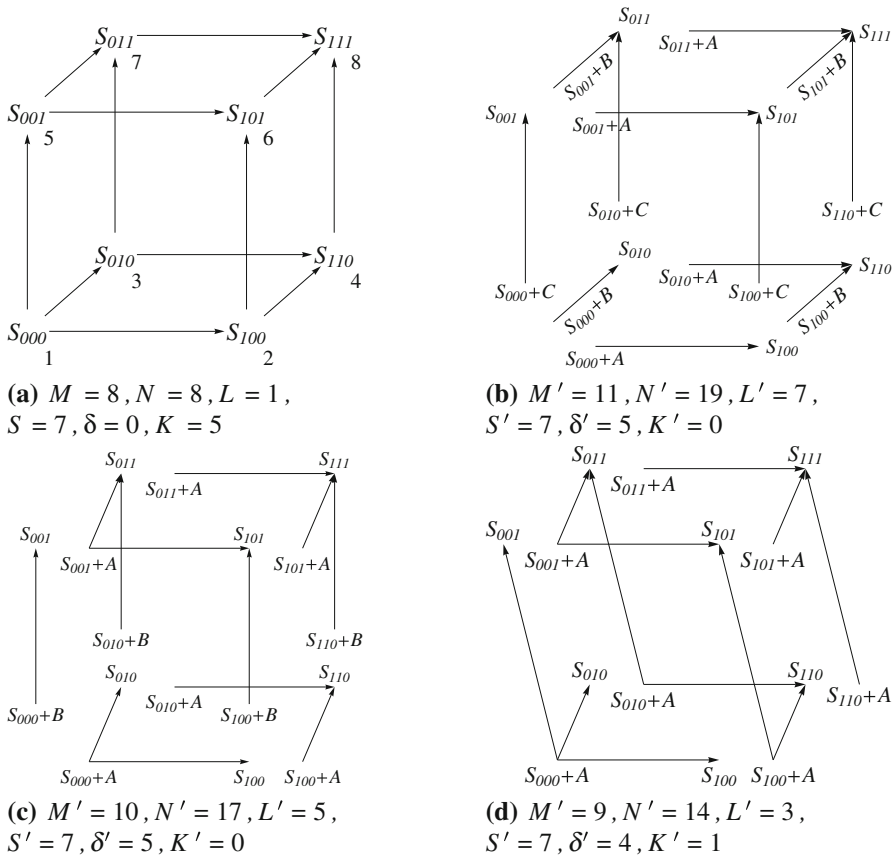
$$X(1) := R, X(2) := RA, \dots, X(11) := G_2R'A_2, X(12) := A, X(13) := G.$$

Let us assign a vector  $\mathbf{y}_i \in \mathbb{R}^{13}$  to the  $i$ th species so that  $\mathbf{y}_{i,j} = 1$  if  $i = j$  and  $\mathbf{y}_{i,j} = 0$  if  $i \neq j$  where  $i, j = 1, \dots, 13$ . With  $\mathbf{a} := \mathbf{y}_{12}$  and  $\mathbf{g} := \mathbf{y}_{13}$ , the corresponding reaction vectors of the transformed system are  $\mathbf{u}_{12} = \mathbf{v}_{12} - \mathbf{a}, \mathbf{u}_{32} = \mathbf{v}_{32} + \mathbf{a}, \mathbf{u}_{41} = \mathbf{v}_{41} + \mathbf{g}, \mathbf{u}_{25} = \mathbf{v}_{25} - \mathbf{g}, \dots, \mathbf{u}_{98} = \mathbf{v}_{98} + \mathbf{a}, \mathbf{u}_{9,10} = \mathbf{v}_{9,10}, \mathbf{u}_{9,11} = \mathbf{v}_{9,11}$ . Thus, the lemma can be applied so that  $\mathbf{c}_1 = \mathbf{a}, \mathbf{c}_2 = \mathbf{g}$  and the  $x$  and  $y$  axes are directed in the '147' and '123' direction, respectively.

Example 9 The system in Fig. 7 can be found in [5]. Again, let us number the species as shown in Fig. 7a, that is

$$X(1) := F^*, X(2) := AF^*, \dots, X(18) := B_2R, X(19) := A, X(20) := B.$$

and let us assign a vector  $\mathbf{y}_i \in \mathbb{R}^{20}$  to the  $i$ th species so that  $\mathbf{y}_{i,j} = 1$  if  $i = j$  and  $\mathbf{y}_{i,j} = 0$  if  $i \neq j$  where  $i, j = 1, \dots, 20$ . Similarly as in the previous two cases, let  $\mathbf{v}_{ij}$  and  $\mathbf{u}_{ij}$  respectively denote the reaction vectors in the original and in the transformed system (these are the differences of the corresponding complex vectors) and let  $\mathbf{a} := \mathbf{y}_{19}$  and  $\mathbf{b} := \mathbf{y}_{20}$ . Then,  $\mathbf{u}_{ij} = \mathbf{v}_{ij} - \mathbf{a}, \mathbf{u}_{ij} = \mathbf{v}_{ij} - \mathbf{b}$  or  $\mathbf{u}_{ij} = \mathbf{v}_{ij}$  if  $\mathbf{u}_{ij}$  and  $\mathbf{v}_{ij}$  correspond to an edge in the graph parallel to the '12', '14' or '17' directions, respectively. The lemma can be used here with  $\mathbf{c}_1 = \mathbf{a}, \mathbf{c}_2 = \mathbf{b}$  and  $\mathbf{c}_3 = \mathbf{0}$ .



**Fig. 8** One receptor with three binding sites

*Example 10* Consider the system in Fig. 8a where there is one receptor with three binding sites, and the different states of the sites are denoted by  $S_{ijk}$ . The next three figures show three possible transformation of this system in the following cases. Figure 8b shows the transformed versions of the system in Fig. 8a in the case when there are three different atoms,  $A, B, C$ , binding to the three sites. Figure 8c shows the transformed version of the system in Fig. 8a in the case when there are two different atoms,  $A, B$ , binding to the three sites. This is the De Young and Keizer model, and again, the transformed system does not contain a circle. In the interesting theoretical case when there is only one atom,  $A$ , binding to each of the three sites, the transformed system contains a circle, this can be seen in Fig. 8c. It can be verified easily that the five circuit conditions in the original system are equivalent to the five spanning forest conditions in the systems in Fig. 8b, c and are also equivalent to the four spanning forest conditions and one circuit condition in the system in Fig. 8d.



### 3.4 On the number of free parameters

We would also like to make some comments on one of the statements in Appendix 2 of [6]. According to this, the number of free parameters can be determined as follows. Suppose that we have a system with

- $N$  complexes,
- $R$  rate coefficients (as parameters),
- and  $C$  constraints (the sum of the number of the microscopic reversibility constraints and the number of arbitrary constraints—independent of the microscopic reversibility constraints and of each other—to be imposed on some of the rate coefficients).

The number of free parameters will then be equal to  $R - \rho$ , where  $\rho$  is the rank of an  $C \times N$  matrix,  $A$ .

Recall from [9] that a reversible mass action system is detailed balanced if and only if the rate constants satisfy the  $P - (N - L)$  circuit conditions and the  $\delta$  spanning forest conditions where the system has  $P$  reaction pairs,  $N$  complexes,  $L$  linkage classes, and  $S$  is the rank of the stoichiometric space, and  $\delta$  is the deficiency of the network. Using these notations, it can be written that the number of unknowns  $R$  equals  $2P$ , and the number of independent constraints  $C$  equals

$$Q + (P - (N - L)) + \delta = Q + P - S, \quad (17)$$

where  $Q$  denotes the number of (further independent) external constraints to be imposed on some of the rate coefficients. In [9], only  $P - (N - L) + \delta = P - S$  is considered to be the number of constraints and in [6], the deficiency is not taken into account in this sum. Thus, our Eq. (17) is a common generalization of the equations by Feinberg and Colquhoun et al.

## 4 Discussion, open problems

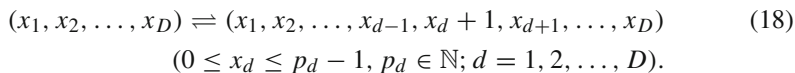
We have provided a method to transform the most common ion channel models into a model where mass-conservation is taken into account. Using the theorem by Feinberg we have also shown that the heuristic method happens to lead to the same results, in spite of the fact that it is based on imprecise assumptions.

All the original models in question have a rectangular grid structure with zero deficiency, and all the transformed models have a deficiency equal to the number of independent circuits in the original model. To put it another way, the sum of deficiency and the number of independent circuits is invariant under our transformation. The natural question arises if the same consequences can be drawn with nonzero deficiency (and nonzero number of independent circuits, respectively) and what can be said about reactions having an FHJ-graph of different structure. The widest possible generalization of detailed balance has been presented by [12] of which the biological applications are yet missing.

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## Appendix: Reactions of rectangular grid structure

Let us consider a special class of reversible compartmental systems with species constructed from  $D \in \mathbb{N}$  different atoms, say,  $G^1, G^2, \dots, G^D$ , sitting on a receptor which will be omitted as it plays no role in the calculations. Let us represent the species  $G_{x_1}^1 G_{x_2}^2 \dots G_{x_D}^D$  by the vector  $(x_1, x_2, \dots, x_D) \in \mathbb{N}_0^D$ , and suppose (this is the speciality of the system) that we only have the following reaction steps *in terms of the atomic representation of the species*:



This means that the Feinberg–Horn–Jackson graph (FHJ graph) of the reaction is a rectangular grid in the first orthant with  $\prod_{d=1}^D (p_d + 1)$  vertex.

Such kind of reactions are often used when modeling ion channels see Fig. 8 or [7].

Realizing that atoms are not conserved in the above reaction, we try to improve it by constructing a model without this fault but reflecting the same physical reality. In order to do so we have to introduce  $D$  new, single-atom species,  $G^d$  ( $d = 1, 2, \dots, D$ ) and the new reaction steps



where  $\mathbf{e}_d$  is the  $d$ th element of the standard base.

To test if a general reaction is detailed balanced or not one has to write down  $\delta$  number of circuit conditions and  $K$  number of spanning forest conditions in terms of the reaction rate constants which form a set of necessary and sufficient conditions together.

If we are interested in detailed balancing of the first reaction (18) we should rather transform it to (19) and have only the spanning forest conditions. The astonishing fact, however, is that for these special reactions not only the number of conditions are the same, but the conditions themselves, as well.

Let us use the following notations:

- $N$  : the number of complex vectors (the number of vertices)
- $P$  : the number of reaction pairs (the number of edges)
- $L$  : the number of linkage classes (the number of connected components)
- $S$  : the dimension of the stoichiometric space  
(the number of independent reaction steps)

$\delta$  : the deficiency  
 $K$  : the number of independent circuits

To get some experience with this kind of systems we summarize the essential characteristics of these systems in two and three dimensions and then formulate and prove the general formula.

**Statement 1** If  $D = 2$  then the formulas for system (18) (left column) and system (19) (right column) are the following:

$$\begin{array}{ll} N = (p + 1)(q + 1) & N' = 2(p + q) + 3pq \\ L = 1 & L' = p + q + pq \\ S = N - 1 & S' = S \\ \delta = N - L - S = 0 & \delta' = N' - L' - S' = K \\ K = P - (N - L) = pq & K' = P' - (N' - L') = 0 \end{array}$$

where  $P = P' = p(q + 1) + (p + 1)q$ .

If  $D = 3$  then the formulas are

$$\begin{array}{ll} N = (p + 1)(q + 1)(r + 1) & N' = 2(p + q + r) + 3(pq + pr + qr) + 4pqr \\ L = 1 & L' = (p + q + r) + (pq + pr + qr) + pqr \\ S = N - 1 & S' = S \\ \delta = 0 & \delta' = K \\ K = pq + pr + qr + 2pqr & K' = 0 \end{array}$$

where  $P = P' = p(q + 1)(r + 1) + (p + 1)q(r + 1) + (p + 1)(q + 1)r$ , see Fig. 8a, b as an illustration.

**Theorem 2**

1. The essential characteristics of reactions (18) (with its FHJ-graph as a rectangular grid) and (19) are as follows.

$$\begin{array}{ll} N = \prod_{d=1}^D (p_d + 1) & N' = \sum_{k=1}^D (k + 1)p_{d_1} p_{d_2} \dots p_{d_k} \\ L = 1 & L' = \sum_{k=1}^D p_{d_1} p_{d_2} \dots p_{d_k} \\ S = N - 1 & S' = S \\ \delta = 0 & \delta' = K \\ K = \sum_{k=2}^D (k - 1)p_{d_1} p_{d_2} \dots p_{d_k} & K' = 0 \end{array}$$

where each sum is taken with the restrictions  $1 \leq d_1 < d_2 < \dots < d_k \leq D$ .

2. The circuit conditions for reaction (18) are exactly the same as the spanning tree conditions for reaction (19).

*Proof* In both systems the number of edges can be calculated as

$$\begin{aligned}
 P = P' &= p_1(p_2 + 1) \dots (p_D + 1) + (p_1 + 1)p_2(p_3 + 1) \dots (p_D + 1) + \dots + \\
 &\quad + (p_1 + 1) \dots (p_{D-1} + 1)p_D \\
 &= \sum_{k=1}^D k p_{d_1} p_{d_2} \dots p_{d_k} \quad (1 \leq d_1 < d_2 < \dots < d_k \leq D)
 \end{aligned}$$

The number of independent circuits in a graph can be calculated as  $K = P - (N - L)$ . Thus, using that  $N = 1 + L'$ , we obtain the formula for  $K$ :

$$\begin{aligned}
 K &= P - (N - L) = P - N + 1 = P - L' \\
 &= \sum_{k=1}^D k p_{d_1} p_{d_2} \dots p_{d_k} - \sum_{k=1}^D p_{d_1} p_{d_2} \dots p_{d_k}
 \end{aligned}$$

The formulas for  $N'$  and  $L'$  follow from the following observation: in the graph of the transformed system the number of components consisting of one edge (and two vertices) is  $p_1 + p_2 + \dots + p_D$ ; the number of components consisting of two edges (and three vertices) is  $p_1 p_2 + p_1 p_3 + \dots + p_{D-1} p_D$ ; etc.; the number of components consisting of  $D$  edges (and  $D + 1$  vertices) is  $p_1 p_2 \dots p_D$ . The equality  $S = S'$  and the equivalence of the circuit conditions and spanning forest conditions follow from the  $D$  dimensional version of the lemma. Finally, using that  $S' = S = N - 1 = L'$ ,  $\delta' = N' - L' - S'$  and  $K' = P' - (N' - L')$ , we obtain the formulas for  $\delta'$  and  $K'$ .  $\square$

## References

1. R.A. Alberty, Principle of detailed balance in kinetics. *J. Chem. Educ.* **81**(8), 1206–1209 (2004)
2. R.K. Boyd, Detailed balance in chemical kinetics as a consequence of microscopic reversibility. *J. Chem. Phys.* **60**(4), 1214–1222 (1974)
3. R.K. Boyd, Detailed balance in nonequilibrium theories of chemical kinetics. *J. Chem. Phys.* **61**(12), 5474–5475 (1974)
4. V. Burzomato, M. Beato, P.J. Groot-Kormelink, D. Colquhoun, L.G. Sivilotti, Single-channel behavior of heteromeric  $\alpha 1\beta$  glycine receptors: An attempt to detect a conformational change before the channel opens. *J. Neurosci.* **24**(48), 10924–10940 (2004)
5. D. Colquhoun, Why the Schild method is better than Schild realised. *Trends Pharmacol. Sci.* **28**(12), 608–614 (2007)
6. D. Colquhoun, K.A. Dowsland, M. Beato, A.J.R. Plested, How to impose microscopic reversibility in complex reaction mechanisms. *Biophys. J.* **86**(6), 3510–3518 (2004)
7. G.W. De Young, J. Keizer, A single-pool inositol 1,4,5-triphosphate-receptor-based model for agonist-stimulated oscillations in  $\text{Ca}^{2+}$  concentration. *Proc. Natl. Acad. Sci. USA* **89**, 9895–9899 (1992)
8. P. Érdi, L. Ropolyi, Investigation of transmitter–receptor interactions by analyzing postsynaptic membrane noise using stochastic kinetics. *Biol. Cybern.* **32**(1), 41–45 (1979)
9. M. Feinberg, Necessary and sufficient conditions for detailed balancing in mass action systems of arbitrary complexity. *Chem. Eng. Sci.* **44**, 1819–1827 (1989)
10. R.H. Fowler, E.A. Milne, A note on the principle of detailed balancing. *Proc. Natl. Acad. Sci. USA* **11**, 400–401 (1925)
11. V. Gold, K.L. Loening, A.D. McNaught, P. Shemi, *IUPAC Compendium of Chemical Terminology*, 2nd edn (Blackwell Science, Oxford, 1997)

12. A.N. Gorban, G.S. Yablonsky, Extended detailed balance for systems with irreversible reactions. *Chem. Eng. Sci.* **66**, 5388–5399 (2011)
13. F. Horn, R. Jackson, General mass action kinetics. *Arch. Ration. Mech. Anal.* **47**, 81–116 (1972)
14. A. Kolmogoroff, Zur Umkehrbarkeit der statistischen Naturgesetze. *Mathematische Annalen* **113**, 766–772 (1936)
15. I. Nagy, B. Kovács, J. Tóth, Detailed balance in ion channels: applications of Feinberg’s theorem. *React. Kinet. Catal. Lett.* **96**(2), 263–267 (2009)
16. R. Nahum-Levy, D. Lipinski, S. Shavit, M. Benveniste, Desensitization of NMDA receptor channels is modulated by glutamate agonists. *Biophys. J.* **80**, 2152–2166 (2001)
17. B. Naundorf, F. Wolf, M. Volgushev, Unique features of action potential initiation in cortical neurons. *Nature* **440**, 1060–1063 (2006)
18. B.S. Rothberg, K.L. Magleby, Testing for detailed balance (microscopic reversibility) in ion channel gating. *Biophys. J.* **80**(6), 3025–3026 (2001)
19. R.C. Tolman, The principle of microscopic reversibility. *Proc. Natl. Acad. Sci. USA* **11**, 436–439 (1925)
20. M.O. Vlad, J. Ross, Thermodynamically based constraints for rate coefficients of large biochemical networks. *Syst. Biol. Med.* **1**(3), 348–358 (2009)
21. M. Wagner, J. Timmer, The effects of non-identifiability on testing for detailed balance in aggregated Markov models for ion-channel gating. *Biophys. J.* **79**(6), 2918–2924 (2000)
22. R. Wegscheider, Über simultane Gleichgewichte und die Beziehungen zwischen Thermodynamik und Reaktionskinetik homogener Systeme. *Zsch. phys. Chemie* **39**, 257–303 (1901/1902)
23. E.P. Wigner, Derivations of Onsager’s reciprocal relations. *J. Chem. Phys.* **22**(11), 1912–1915 (1954)