1.1 Kienker Transformations

It is standard to represent Markov models by the *generator matrix*, Q, whose elements, Q_{ij} are the rates of transition from state i to state j if $i \neq j$ and with diagonal elements $Q_{ii} = -\sum_{j\neq i} Q_{ij}$. Then taking $P_{ij}(t)$ to be the probability that the system is in state j at time t given that it was in state i at time 0, we can write

$$\frac{dP}{dt} = PQ \tag{3}$$

with the initial condition $P(0)$ equals the identity matrix. Pairs of states i, j for which Q_{ij} and Q_{ji} are non-zero are linked by an allowed state transition, assumed to be a reversible reaction, and are connected by a line in a reaction diagram. It is also standard, for an aggregated Markov process with aggregates O and C, to partition Q into four submatrices [1–5]: Q_{oo}, Q_{oc}, Q_{co}, and Q_{cc} corresponding respectively to open-to-open transitions, etc.:

$$Q = \begin{pmatrix} Q_{oo} & Q_{oc} \\ Q_{co} & Q_{cc} \end{pmatrix}. \tag{4}$$

We consider regular ergodic Markov processes so that Q has a one dimensional null space. The left null vector w contains the equilibrium probabilities. We denote the right null vector by u, and it contains all ones: $u_i = 1$ for all i. Thus, we have $wQ = 0$ and $Qu = 0$. Sometimes we will write $w = (w_o, w_c)$ where w_o and w_c give the components of the equilibrium corresponding to open and closed states, respectively. Similarly u_o and u_c are column vectors consisting of all ones.

Fredkin and Rice's result [3], that the two dimensional dwell time distributions fully characterize the steady-state data, requires the spectra of Q_{oo} and Q_{cc} to be non-degenerate and the coefficients in the one-dimensional distribution functions (analogous to the α_{ij} in the two-dimensional distribution in Equation 1 in the main text) to all be non-zero. We will refer to these conditions, also assumed by Kienker [5], as the Fredkin-Rice-Kienker (FRK) conditions. Kienker proved that two models with generators Q and $\widetilde{Q}$ satisfying the FRK conditions are equivalent if and only if they can be related by a similarity transformation:

$$\widetilde{Q} = S^{-1} Q S \tag{5}$$

where S is a matrix of the form:

$$S = \begin{pmatrix} S_{oo} & 0 \\ 0 & S_{cc} \end{pmatrix} \tag{6}$$

with

$$Su = u, \tag{7}$$

i.e., rows of S sum to one, which ensures that $\widetilde{Q}u = 0$. Here S_{oo} and S_{cc} are $N_o \times N_o$ and $N_c \times N_c$ matrices respectively. The above imply $\widetilde{Q}_{oo} = S_{oo}^{-1}\, Q_{oo}\, S_{oo}$, and $\widetilde{Q}_{oc} = S_{oo}^{-1}\, Q_{oc}\, S_{cc}$ with similar expressions for $\widetilde{Q}_{co}$ and $\widetilde{Q}_{cc}$. We call similarity transformations of the form given in Eq. 6 and satisfying $Su = u$ "Kienker" transformations.

Theorem 1. *Under the FRK conditions, BKU form is identifiable.*

Proof. With non-degenerate Q_{oo} and Q_{cc}, the Kienker transformation S to BKU form must be constructed from the distinct eigenvectors of Q_{oo} and Q_{cc}. The amplitudes of the various eigenvectors in S are fixed by requiring $Su = u$. Different orderings of these eigenvectors in S simply permute the states and do not alter the model topology or rate constants. □

1.2 Manifest Interconductance Rank Form

To show that MIR form is a valid canonical form, we must show how to transform *almost* any model (*i.e.* except for a set of measure zero) into MIR form. To do this we first put Q_{oc} and Q_{co} into what we call *generalized diagonal* form, in which at most one element in each row and each column of these non-square matrices can be non-zero. The total number of non-zero elements will be R.

Theorem 2. *The off-diagonal blocks Q_{oc} and Q_{co} can be made generalized diagonal by the Kienker transformation with S_{oo} and S_{cc} chosen to diagonalize the products $Q_{oc}Q_{co}$ and $Q_{co}Q_{oc}$, respectively, provided their nonzero eigenvalues are distinct.*

Expressed in equations, if $S_{oo}^{-1} Q_{oc} Q_{co} S_{oo} = \Lambda_{oo}$ and $S_{cc}^{-1} Q_{co} Q_{oc} S_{cc} = \Lambda_{cc}$, where Λ_{oo} and Λ_{cc} are both diagonal matrices, then the theorem states

$$S_{oo}^{-1} Q_{oc} S_{cc} = \Lambda_{oc}$$
$$S_{cc}^{-1} Q_{co} S_{oo} = \Lambda_{co},$$

where Λ_{oc} and Λ_{co} are generalized diagonal non-square matrices. In fact this transformation not only results in Q_{oc} and Q_{co} generalized diagonal, but also their non-zero entries will correspond to reversible transitions: non-zero entries of Q_{co} are in the same locations as the non-zero entries of Q_{oc}^T.

The transformations S_{oo} and S_{cc} that diagonalize $Q_{oc}Q_{co}$ and $Q_{co}Q_{oc}$ are not unique because they have $N_o - R$ and $N_c - R$ dimensional nullspaces respectively. Thus the full transformation to MIR form can be written as a two step operation $S_{\mathtt{MIR}} = S\hat{S}$. $\hat{S}$ is a Kienker transformation applied to diagonalize the $(N_o - R) \times (N_o - R)$ block of Q_{oo} and the $(N_c - R) \times (N_c - R)$ of Q_{cc}.

Proof. (Theorem 2): Assuming $Q_{oc}Q_{co}$ has distinct non-zero eigenvalues, from $S_{oo}^{-1}Q_{oc}\ Q_{co}S_{oo} = \Lambda_{oo}$, we can have $Q_{co}Q_{oc}Q_{co}S_{oo} = Q_{co}S_{oo}\Lambda_{oo}$. This shows that columns of $Q_{co}S_{oo}$ are eigenvectors of $Q_{co}Q_{oc}$, as are the columns of S_{cc}. Let n and k be the number of non-zero eigenvalues and the algebraic multiplicity of the zero eigenvalue of $Q_{co}Q_{oc}$, respectively. Applying a permutation, we can write Λ_{oo} as:

$$\Lambda_{oo} = \mathrm{diag}(\lambda_1, \lambda_2, \cdots, \lambda_n, \underbrace{0, 0, \cdots, 0}_{k}). \tag{8}$$

Note that $Q_{oc}Q_{co}$ and $Q_{co}Q_{oc}$ have the same non-zero eigenvalues. We assume the nonzero eigenvalues in Λ_{oo} and Λ_{cc} are in the same order (again using post-diagonalization permutations), and thus can write $Q_{co}S_{oo} = S_{cc}\Lambda_{co}$, which implies that $\Lambda_{co} = S_{cc}^{-1}Q_{co}S_{oo}$ is a N_c by N_o block diagonal matrix with the structure:

$$\Lambda_{co} = \begin{pmatrix} D_{co} & \\ & Z_{co} \end{pmatrix}, \tag{9}$$

where D_{co} is a n by n diagonal matrix and $D_{co} = \mathrm{diag}(\alpha_1, \alpha_2, \cdots, \alpha_n)$ and Z_{co} is a $N_c - n$ by k matrix. Similarly, we can have a N_o by N_c block diagonal matrix $\Lambda_{oc} = S_{oo}^{-1}Q_{oc}S_{cc}$ having a structure similar to Λ_{co}, namely $\Lambda_{oc} = \mathrm{diag}(D_{oc}, Z_{oc})$, where $Z_{co}Z_{oc} = 0, Z_{oc}Z_{co} = 0$. If $\mathrm{rank}(Q_{co}) = \mathrm{rank}(Q_{oc}) = n$,* then clearly, $\mathrm{rank}(Z_{co}) = \mathrm{rank}(Z_{oc}) = 0$, i.e., Z_{co} and Z_{oc} are zero matrices. □

If the ranks of Q_{oc} and Q_{co} are not equal, or if some of the λ_i in Eq 8 are equal, the above procedure may not result in generalized diagonal Q_{oc} and Q_{co}. However, in some—but not all—such cases this aim can still be achieved by a suitable transformation on the degenerate subspace or subspaces.

To complete the claim that MIR form is a canonical form, we still need:

Theorem 3. *Except for a set of measure zero, MIR form is identifiable.*

The set of measure zero will be seen to consist of degeneracies and cases where some of the R O–C links have zero rates, causing the true rank to be less than R.

Proof. To prove that MIR form is canonical, we show that the only transformation which transforms an MIR form into MIR form is permutation. Let Q be in MIR form, thus we have:

$$Q_{co}Q_{oc} = \begin{pmatrix} \Lambda & 0 \\ 0 & 0 \end{pmatrix}, \tag{10}$$

*For reversible reactions, the ranks are equal except for a set of measure zero. Under DB the ranks must be equal because $WQ = Q^TW$ implies $W_oQ_{oc} = Q_{co}^TW_c \iff Q_{oc} = W_o^{-1}Q_{co}^TW_c$. Hence, $\mathrm{rank}(Q_{oc})=\mathrm{rank}(Q_{co}^T)=\mathrm{rank}(Q_{co})$.

where Λ is a diagonal block matrix with non-zero and distinct eigenvalues. To diagonalize $Q_{co}Q_{oc}$ that is already diagonal and maintain the non-zero eigenvalues in upper left corner, it is easy to show that the similarity transformation must be in the form:

$$S_{cc} = \begin{pmatrix} p & 0 \\ 0 & X \end{pmatrix}, \tag{11}$$

where p is a permutation matrix (possibly the identity). Column vectors in $\begin{pmatrix} 0 \\ X \end{pmatrix}$ span the nullspace of $Q_{co}Q_{oc}$. X must be a full rank matrix for S_{cc} to be invertible. We partition Q_{cc} in the same way as for S_{cc}, thus we have:

$$\begin{aligned} \widetilde{Q}_{cc} &= S_{cc}^{-1}Q_{cc}S_{cc} && (12)\\ &= \begin{pmatrix} p^T & 0 \\ 0 & X^{-1} \end{pmatrix} \begin{pmatrix} Q_{11} & Q_{12} \\ Q_{21} & \Lambda_{22} \end{pmatrix} \begin{pmatrix} p & 0 \\ 0 & X \end{pmatrix} && (13)\\ &= \begin{pmatrix} p^TQ_{11}p & p^TQ_{12}X \\ X^{-1}Q_{21}p & X^{-1}\Lambda_{22}X \end{pmatrix} && (14) \end{aligned}$$

where

$$\widetilde{Q}_{22} = X^{-1}\Lambda_{22}X. \tag{15}$$

Because Λ_{22} is diagonal, it is clear that the only transformation X that keeps $\widetilde{Q}_{22}$ diagonal is a permutation (cases where eigenvalues of Λ_{22} are non-distinct are part of the set of measure zero). Thus, S_{cc} must be a permutation. The same holds for S_{oo}. □

1.3 Rank and Independent Reactions

We define the number n_I of independent links (or reversible reactions) to be the largest number of links that can be identified, such that none of them share any states as common endpoints. We claim the matrix rank R of Q_{oc}, assumed to equal the rank of Q_{co}, satisfies:

Theorem 4. *Except for a set of measure zero where the reaction rates have linear dependencies,*

$$R = n_I. \tag{16}$$

Proof. Because there are n_I independent entries in Q_{oc}, each in its own row and column, we have $R \geq n_I$. To show $R \leq n_I$, we identify R independent links by the following construction. Because $\mathrm{rank}(Q_{oc}) = R$ we can identify R independent rows of Q_{oc}, and this $R \times N_c$, rank R submatrix must have R independent columns. This identifies an $R \times R$ rank R submatrix of Q_{oc}, which we call A. Expanding the determinant of A by minors, we have for any i:

$$\det(A) = \sum_{j=1}^{R} (-1)^{i+j} A_{ij} M_{ij}, \tag{17}$$

where M_{ij} is a "minor" of A, equal to the determinant of the submatrix of A without the ith row and jth column. $\det(A)$ is non-zero (otherwise A would have rank less than R), so at least one term in the sum must be non-zero. Such a term must have A_{ij} and M_{ij} non-zero, and (i, j) can be identified as an independent link. The same expansion is then carried out replacing A by the $(R-1) \times (R-1)$ rank $R-1$ matrix M_{ij}. Continuing in this way identifies R independent links □.

1.4 Detailed Balance

The DB conditions can be written: $w_i q_{ij} = w_j q_{ji}$. Defining W as the diagonal matrix $W = \mathrm{diag}(w)$ we rewrite the DB condition:

$$WQ = (WQ)^T. \tag{18}$$

We would like to know which Kienker transforms preserve DB. If Q satisfies DB, plugging $\widetilde{Q} = S^{-1}QS$ (with left null vector $\widetilde{w} = wS$ $\widetilde{W} = \mathrm{diag}(\widetilde{w})$) into Eq. 18 and rearranging gives $S^T W S \widetilde{Q} = \widetilde{Q}^T S^T W S$. If $S^T W S$ is diagonal then this equation has the form of Eq. 18 and DB is preserved; furthermore,

$$\widetilde{W} = S^T W S \tag{19}$$

which is seen by multiplying both sides of Eq. 19 on the left by u^T, noting that $u^T S^T = u^T$, $w = u^T W$, and $wS = \widetilde{w} = u^T \widetilde{W}$.

Thus, to show that DB is preserved under a transformation S it is sufficient to show that $S^T W S$ is diagonal. (In fact this condition is also necessary assuming non-degeneracy conditions hold.) First we present the main theorem of this section:

Theorem 5. *If Q is a generator matrix satisfying DB, then the transformation to BKU and MIR forms preserve detailed balance, provided Q satisfies the conditions for identifiability of those forms.*

Proof. Let $\widetilde{Q}$ be the generator after a similarity transformation, i.e., $\widetilde{Q} = S^{-1}QS$. We would like to show, under condition stated in either (1) or (2), that $\widetilde{W}\widetilde{Q} = \widetilde{Q}^T \widetilde{W}$, where $\widetilde{W}$ is a diagonal matrix and its diagonal entries are equilibrium distribution for the resulting model. Here we note that the law of DB $WQ = Q^T W$ can be written as $WS\widetilde{Q} = Q^T WS$. Its partitioned form is:

$$\begin{pmatrix} W_o S_{oo} \widetilde{Q}_{oo} & W_o S_{oo} \widetilde{Q}_{oc} \\ W_c S_{cc} \widetilde{Q}_{co} & W_c S_{cc} \widetilde{Q}_{cc} \end{pmatrix} = \begin{pmatrix} Q_{oo}^T W_o S_{oo} & Q_{co}^T W_c S_{cc} \\ Q_{oc}^T W_o S_{oo} & Q_{cc}^T W_c S_{cc} \end{pmatrix}. \tag{20}$$

We now consider the two canonical forms separately.

BKU Form: In this case, from the above equation (20), we have $W_o S_{oo} \widetilde{Q}_{oo} = Q_{oo}^T W_o S_{oo}$. Since $\widetilde{Q}_{oo}$ is diagonal, it is clear that column vectors of $W_o S_{oo}$ are eigenvectors of Q_{oo}^T. From the similarity transformation, we know that $\widetilde{Q}_{oo} = S_{oo}^T Q_{oo}^T S_{oo}^{-T}$, hence column vectors of S_{oo}^{-T} are eigenvectors of Q_{oo}^T. Since Q_{oo} has distinct eigenvalues, we can write $W_o S_{oo} = S_{oo}^{-T} \Lambda_o$, where $\Lambda = \mathrm{diag}(\alpha_1, \alpha_2, ..., \alpha_{n_o})$, each α_i, for $i = 1, 2, ..., n_o$, is scalar. Similarly, we have $W_c S_{cc} = S_{cc}^{-T} \Lambda_c$. The law of DB $WQ = Q^T W$ can be yet written into $S^T W S \widetilde{Q} = \widetilde{Q}^T S^T W S$, thus $\Lambda \widetilde{Q} = \widetilde{Q}^T \Lambda$, where $\Lambda = S^T W S = \begin{pmatrix} \Lambda_o & 0 \\ 0 & \Lambda_c \end{pmatrix}$. Let u be a column vector with all entries being ones, $u^T \Lambda = u^T S^T W S = u^T W S = wS = \widetilde{w}$, thus $\Lambda = \widetilde{W}$.

MIR form: In this case, from Eq. 20, we have:

$$\begin{aligned} W_o S_{oo} \widetilde{Q}_{oc} &= Q_{co}^T W_c S_{cc} \\ W_c S_{cc} \widetilde{Q}_{co} &= Q_{oc}^T W_o S_{oo}, \end{aligned}$$

thus $W_o S_{oo} \widetilde{Q}_{oc} \widetilde{Q}_{co} = Q_{co}^T W_c S_{cc} \widetilde{Q}_{co} = Q_{co}^T Q_{oc}^T W_o S_{oo}$. Since $\widetilde{Q}_{oc} \widetilde{Q}_{co}$ is diagonal and has distinct non-zero eigenvalues, the column vectors of $W_o S_{oo}$ are either eigenvectors of matrix $Q_{co}^T Q_{oc}^T$ or in the null space which could be eigenvector of zero eigenvalue or zero vector. We also have $\widetilde{Q}_{oc} \widetilde{Q}_{co} = S_{oo}^T Q_{co}^T Q_{oc}^T S_{oo}^{-T}$, thus it is clear that columns of S_{oo}^{-T} are also eigenvectors of $Q_{co}^T Q_{oc}^T$ or in the null space. We can write $W_o S_{oo} = S_{oo}^{-T} \Lambda$. If $Q_{co}^T Q_{oc}^T$ is full rank, it is clear that Λ is diagonal. If $Q_{co}^T Q_{oc}^T$ is less than full rank, according to what has been shown in (1), Λ is still a diagonal matrix because S_{oo} also diagonalizes the part of Q_{oo}, which has the dimension of $N_o - \mathrm{rank}(Q_{co}^T Q_{oc}^T)$. □

$\widetilde{Q}$ may have negative entries on the off-diagonals and thus is strictly speaking not the generator for a Markov process but for a generalized Markov process [6] with negative reaction rates. With DB we have the following corollary:

Corollary 1. *If S preserves DB then all components of $\widetilde{w} = wS$ are positive.*

This is so because if $\widetilde{W} = S^T W S$ is diagonal then each $\widetilde{W}_{ii}$ is just the dot product of the the ith column of $W^{1/2}S$ with itself.

1.5 Real and Complex Rates

If DB holds, the eigenvalues of Q must all be real. This is true because Eq. 18 also implies that $W^{1/2} Q W^{-1/2}$ is symmetric, and this must have the same spectrum as Q. When the spectrum is real, the diagonalizations can be carried out by real S, and the resulting rates will be real.

If Q does not obey DB, a transformation to canonical form may result in rates that are complex numbers. Histograms of gating statistics may show oscillations caused by complex eigenvalues in Q_{oo} or Q_{cc}. In such cases BKU and MIR forms will have complex rates, but Larget's form [7] will not. However, Larget's form will have negative rates, and it is possible with negative rates to avoid complex rates in the other forms without adding new parameters.

In the complex versions of BKU and MIR forms, diagonal values will appear in complex conjugate pairs. Two by two Kienker transformations that mix the corresponding pairs of states can obtain real Qs, at the expense of relaxing the form to being block diagonal with 2×2 blocks. In fact there are algorithms for finding this block diagonalization without using complex numbers [8]. No new parameters are added, because in this form the diagonal values in each block can be made equal, and the off-diagonal elements will be negatives of each other.

When fitting data to canonical form, especially in systems where DB violation my be suspected, it would be wise to either allow complex rates or to repeat fits with different numbers of pairs of diagonal elements replaced by 2×2 blocks with these constraints. The first option may in practice reduce to the second, because it is unclear how to evaluate a non-real likelihood that might arise if the eigenvalues are not in complex conjugate pairs.

1.6 Rank 1

Definition 1. *Given two topologies T_1 and T_2, if for almost all models having topology T_1, there is an equivalent model having topology T_2, and vice versa, then T_1 and T_2 are equivalent topologies.*

"Almost all" means except for a set of measure zero; this is motivated by the models with degenerate Q_{oo} or Q_{cc} matrices that do not satisfy the FRK conditions. When detailed balance is violated, such models often violate the otherwise strict equivalence between two topologies. For example, Q_{oo} may not be diagonalizable, making transformation to BKU form impossible. However, models having statistics arbitrarily close to the original model can still be found in BKU form.

With this definition we can state simply:

Theorem 6. Rank one equivalence*: All rank 1 topologies with fixed N_o and N_c are equivalent.*

Proof. We know that we can transform from almost any model to both MIR form (Theorem 2) and BKU forms [5]. If we can show we can transform almost any rank 1 model with one of these canonical forms to any rank 1 topology, then we can transform between any two rank 1 topologies using canonical form as an intermediate, which will prove the theorem.

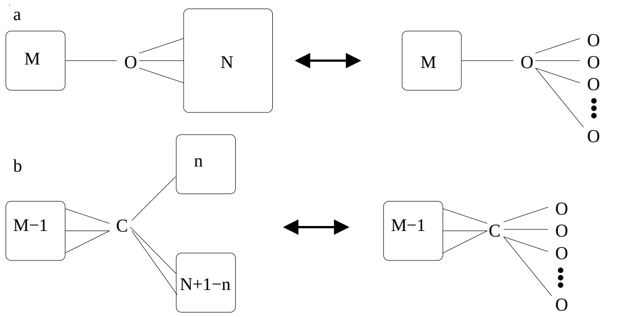

Fig. 7. The two cases discussed in the proof of Theorem 6. The boxes represent arbitrary topologies containing the number of states given inside the box (n or N for O states, M for C states). Both cases on the left are equivalent to having the Os in canonical form, as shown on the right.

Many rank 1 topologies have loops, but such topologies are never identifiable. Given a rank 1 topology containing one or more loops, we can always choose to transform to a special case of that topology where rates of some reactions are zero, so that no loops remain. This allows us to henceforth consider only those topologies that do not contain loops.

The proof is by induction on the number of states. We will start by holding fixed the number of Cs, and call it M, and use induction on N, the maximum number of Os for which all rank 1 topologies are equivalent to the topologies with the Cs unchanged, and the Os in canonical form. (Since we can transform between BKU and MIR forms, they are certainly equivalent.) Clearly $N \geq 1$ because the Os are already in canonical form when there is only one. Now we show that if the equivalence to canonical form for the Os holds for some N then it also holds for $N+1$, implying it must hold for any number of Os.

There are two cases to consider with rank 1: either the Cs links directly to only one O, or there is a gateway C that links to more than one as shown in Figs 1.6a and b. Because we assume there are no loops, the latter case allows us to identify two separate O subnetworks, of sizes n and $N+1-n$, as shown in Fig 1.6b, where n is the number of Os linked to one of the Os linked to the gateway C. $N+1-n$ and n are both no greater than N, so both subnetworks must be equivalent to BKU form. To transform from almost any BKU-form model to the desired topology, we apply the Kienker transformation (which exists by induction) on the first n Os in BKU form that will create the first subnetwork, and then apply the Kienker transformation on the next $N+1-n$ Os that will yield the second subnetwork.

In the former case (Fig 1.6a), the "gateway" O that connects to the C also connects to a subnetwork of N Os. To transform from almost any MIR-form model to the desired topology, we consider the transformation that would take $N_o = N, N_c = 1$ BKU form to the size N subnetwork connected to a C rather than an O. By induction this transformation is possible, and when applied to the N Os leaving the gateway O and the C network unchanged, it transforms from MIR form model to the desired network.

This proves that for rank 1, all topologies with fixed N_o and the same topology for the Cs are equivalent. The same must be true with O and C interchanged. Clearly by the definition, equivalence is transitive. So if T_o is the O part of the topology, and T_c is the C part, $(T_o, T_c) \cong (T'_o, T_c) \cong (T'_o, T'_c)$ implies $(T_o, T_c) \cong (T'_o, T'_c)$; taking the T'_o and T'_c to be MIR form shows that any rank 1 topology is equivalent to MIR form. □

The induction proves that it is possible to transform between any two rank 1 topologies by a series of transformations on various sub-networks, where each transformation takes the sub-network into BKU or MIR form. Using the following result, it follows that if detailed balance holds, no negative rates will be introduced when transforming between rank 1 topologies.

Theorem 7. *Rank 1 models that satisfy detailed balance have positive rate constants in both BKU and MIR forms.*

Proof: for MIR form: Because Q is negative semidefinite, and remains so after any similarity transformation, all its diagonal elements must always be less than or equal to zero. For rank 1, all but two rows of Q have only two entries in MIR form, and each row sums to zero, implying all rates in those rows must be non-negative. Of the two remaining rows, let us first consider the one that includes one row of Q_{oo} and the one non-zero entry in Q_{oc}. The off-diagonal entries in this row of Q_{oo} are positive because by DB $W_o Q_{oo} = Q_{oo}^T W_o$ and thus each entry equals a positive number times one of the rates already shown to be non-negative. The entry in Q_{oc}, when multiplied by the equilibrium probability of being in the corresponding open state, gives the steady state flux from the open to closed aggregates. This is an experimentally observable quantity that must be conserved when transforming between equivalent models. Thus, if the original model had all non-negative rate constants, the transformed Q_{oc} entry will be non-negative (and positive assuming ergodicity) as well. The same arguments apply to the remaining row of Q.

for BKU form: Again write $Q_{oc} = v_o v_c^T$ and likewise $Q_{co} = x_c x_o^T$. From the definition of DB $WQ = Q^T W$, and defining W_o and W_c to be the open and closed diagonal blocks of W, we can state $W_c Q_{co} = Q_{oc}^T W_o$. Thus, $W_c x_c x_o^T = (v_o v_c^T)^T W_o$ so that $x_c x_o^T = W_c^{-1} v_c v_o^T W_o$. Both sides of this equation are rank 1 matrices formed by outer products of two vectors. Therefore the vectors must be equal up to a scalar factor γ, so

$$x_c = \gamma W_c^{-1} v_c, \tag{21}$$

and $x_o^T = \gamma^{-1} v_o^T W_o$. Recall that the rows of Q must sum to zero, and in BKU form Q_{oo} is diagonal with negative entries. Thus, each row of Q_{oc} must sum to a positive value. The sum of the ith row can be expressed as $v_{oi} v_c^T u_c$. Thus, each entry of v_o must have the same sign as $v_c^T u_c$. Likewise each entry of x_c must have the same sign as $x_o^T u_o$. Then the entries of v_c all have the same sign as well by equation 21, since all the diagonal entries of W are positive. Thus, all entries in Q_{oc} have the same sign, and they sum to a positive value, thus they are all positive. The same is true of Q_{co}.

Theorem 8. *In BKU form, all rank 1 models have $2N_O N_C$ nonzero rate constants.*

Proof: A any rank one matrix can be written as an outer product of two vectors so that $Q_{oc} = v_o v_c^T$ and likewise $Q_{co} = x_c x_o^T$. If any component of any of these vectors were zero, then either an entire row or column of Q_{OC} or Q_{CO} would be all zeros, which would violate ergodicity. To see this note that an entire row or column of Q_{OC} or Q_{CO} being zero implies a row or column of Q being all zeros except for the diagonal element. If a row of Q is zero except for the diagonal element then there is an absorbing state. If a column of Q is zero except for the diagonal element then there is an unreachable state.

1.6.1 None of C-O-O-C, O-C-C-O, and C-O-C-O are equivalent to each other.

To see this note that C-O-O-C and O-C-C-O are both subtopologies of the $R = N_O = N_C = 2$ MIR form topology. Thus, if C-O-O-C were equivalent to O-C-C-O it would violate MIR form identifiability because MIR form would contain pairs of equivalent models different from the exceptions in the MIR form identifiability proof. If C-O-C-O is not equivalent to C-O-O-C then it is not equivalent to O-C-C-O by symmetry.

Here we show that the C-O-C-O $\neq$ C-O-O-C. The generator matrix, Q for C-O-C-O is of the form:

$$Q = \begin{pmatrix} x & 0 & x & x \\ 0 & x & 0 & x \\ x & 0 & x & 0 \\ x & x & 0 & x \end{pmatrix} \tag{22}$$

while the generator Q' for $COOC$ is of the form

$$Q' = \begin{pmatrix} x & x & x & 0 \\ x & x & 0 & x \\ x & 0 & x & 0 \\ 0 & x & 0 & x \end{pmatrix}. \tag{23}$$

Here the "x's" represent nonzero elements. So we're looking for a similarity transformation from Q to Q' with row sum of unity. First not that Q_{cc} and Q'_{cc} are both diagonal. This means that S_{cc} is either the identity or the permutation operator. We treat only the identity case.

In this case we have that $S_{cc} = I$ so that $Q'_{oc} = S_{oo}^{-1} Q_{oo}$. Note that

$$Q_{oc} = \begin{pmatrix} q_{11}^{oc} & q_{12}^{oc} \\ 0 & q_{22}^{oc} \end{pmatrix} \tag{24}$$

and that

$$Q'_{oc} = \begin{pmatrix} q'^{oc}_{11} & 0 \\ 0 & q'^{oc}_{22} \end{pmatrix}. \tag{25}$$

We also have:

$$Q_{co} = \begin{pmatrix} q_{11} & 0 \\ q_{21}^{co} & q_{22}^{co} \end{pmatrix} \tag{26}$$

and

$$Q'_{co} = \begin{pmatrix} q'^{co}_{11} & 0 \\ 0 & q'^{co}_{22} \end{pmatrix}. \tag{27}$$

The transformation from Q_{oo} to Q_{oc} doesn't put any constraints on S_{oo}, which we write:

$$S_{oo} = \begin{pmatrix} x & 1-x \\ y & 1-y \end{pmatrix} \tag{28}$$

so that

$$S_{oo}^{-1} = \frac{1}{x-y} \begin{pmatrix} 1-y & x-1 \\ -y & x \end{pmatrix}. \tag{29}$$

The off-diagonal elements of Q'_{oc} and Q'_{co} are all zero so that

$$\begin{aligned} 0 = q'^{oc}_{12} &= (S_{oo}^{-1} Q_{oc})_{12} \\ &= \frac{1}{x-y}((1-y)q_{12}^{oc} + (x-1)q_{22}^{oc} \\ 0 = q'^{oc}_{21} &= (S_{oo}^{-1} Q_{oc})_{21} = \frac{1}{x-y}(-y q_{11}^{oc}) \\ 0 = q'^{co}_{12} &= (Q_{co} S_{oo})_{12} = (1-x) q_{11}^{co} \\ 0 = q'^{co}_{21} &= (Q_{co} S_{oo})_{21} = x q_{21}^{co} + y q_{22}^{co}. \end{aligned}$$

The above set of four equations for x and y is overdetermined. It is easy to check that it does not have a solution.

1.7 C-O-O-C Has Negative Rates in BKU Form

Here we prove that every physical C-O-O-C model when transformed into BKU form has negative rates.

The blocks of the generator of C-O-O-C have the following structure:

$$Q_{oo} = \begin{pmatrix} q_{11}^{oo} & q_{21}^{oo} \\ q_{21}^{oo} & q_{22}^{oo} \end{pmatrix} \tag{30}$$

$$Q_{oc} = \begin{pmatrix} q_{11}^{oc} & 0 \\ 0 & q_{22}^{oc} \end{pmatrix} \tag{31}$$

$$Q_{co} = \begin{pmatrix} q_{11}^{co} & 0 \\ 0 & q_{22}^{co} \end{pmatrix} \tag{32}$$

$$Q_{cc} = \begin{pmatrix} q_{11}^{cc} & 0 \\ 0 & q_{22}^{cc} \end{pmatrix}. \tag{33}$$

Note that Q_{cc}, Q_{co}, and Q_{oc} are all diagonal. So that S_{cc} is the identity matrix. To show that the equivalent BKU form model has negative rates for every physical C-O-O-C model it suffices to show that $\widetilde{Q}_{oc} = S_{oo} Q_{oc}$ has negative rates. Note that all elements of the generator for C-O-O-C are non-negative except for the diagonal ones of Q_{oo} of Q_{cc}. Thus, Q_{oc} is a positive diagonal matrix. Thus, to show that $\widetilde{Q}_{oc}$ has at least one negative entry it suffices to show that S_{oo} has at least one negative entry. Note now that since S_{oo} diagonalizes Q_{oo} it must be comprised of the eigenvectors of Q_{oo}:

$$Q_{oo} \begin{pmatrix} \psi_{1i} \\ \psi_{2i} \end{pmatrix} = \lambda_i \begin{pmatrix} \psi_{1i} \\ \psi_{2i} \end{pmatrix}. \tag{34}$$

That is

$$S_{oo} = \begin{pmatrix} \psi_{11} & \psi_{12} \\ \psi_{21} & \psi_{22} \end{pmatrix}, \begin{pmatrix} \alpha_1 & 0 \\ 0 & \alpha_2 \end{pmatrix} \tag{35}$$

where the amplitudes (α_i) are chosen so that Eq 7 is satisfied. (*i.e.* the rows of S_{oo} sum to 1). We need only prove that S_{oo} has at least one negative entry. To do this it suffices to show that ψ_{21}/ψ_{11} and ψ_{12}/ψ_{22} have opposite sign. The eigenvalue equations can be written:

$$\begin{aligned} (q_{11}^{oo} - \lambda_1)\psi_{11} + q_{12}^{oo}\psi_{21} &= 0 \quad (36) \\ q_{21}^{oo}\psi_{12} + (q_{22}^{OO} - \lambda_2)\psi_{22} &= 0 \quad (37) \end{aligned}$$

so that

$$\frac{\psi_{21}}{\psi_{11}} = \frac{-(q_{11}^{oo} - \lambda_1)}{q_{12}^{oo}}$$

and

$$\frac{\psi_{12}}{\psi_{22}} = \frac{-(q_{22}^{oo} - \lambda_2)}{q_{21}^{oo}}. \tag{38}$$

Note that $q_{11}^{oo} + q_{22}^{oo} = \lambda_1 + \lambda_2$ so that $(q_{11}^{oo} - \lambda_1) = -(q_{22}^{oo} - \lambda_2)$ so that

$$\frac{\psi_{21}}{\psi_{11}} = \frac{(q_{22}^{oo} - \lambda_2)}{q_{12}^{oo}}, \tag{39}$$

from which it follows that that ψ_{21}/ψ_{11} and ψ_{12}/ψ_{22} have opposite sign (since q_{12}^{oo} and q_{21}^{oo} are positive by assumption).

1.8 Identifiability and Equivalence of all 4-link Topologies with $N_o = N_c = R = 2$.

Here we prove the assertion that the four topologies shown in Fig. 6 of the main text are identifiable and equivalent. The topologies shown in Fig. 6a and b are the BKU and MIR canonical forms, respectively. Thus, they are identifiable and equivalent. By symmetry if topology 6d is identifiable then topology 6c is identifiable. If topology 6d is equivalent to topology 6a then so is 6c, and then it would follow that all four topologies are equivalent to each other. Thus, we have only to prove that topology 6d is identifiable and that it is equivalent to 6a.

1.8.1 Identifiability

The blocks of the generator for topology 6d have the following structure:

$$\begin{aligned} Q_{oo} &= \begin{pmatrix} q_{11}^{oo} & 0 \\ 0 & q_{22}^{oo} \end{pmatrix} \\ Q_{oc} &= \begin{pmatrix} q_{11}^{oc} & 0 \\ q_{21}^{oc} & q_{22}^{oc} \end{pmatrix} \\ Q_{co} &= \begin{pmatrix} q_{11}^{co} & q_{12}^{co} \\ 0 & q_{22}^{co} \end{pmatrix} \\ Q_{cc} &= \begin{pmatrix} q_{11}^{cc} & q_{12}^{cc} \\ q_{21}^{cc} & q_{22}^{cc} \end{pmatrix}. \end{aligned} \tag{40}$$

Note now that the preservation of the topology implies that

$$\begin{aligned} \widetilde{Q}_{oo} &= \begin{pmatrix} \tilde{q}_{11}^{oo} & 0 \\ 0 & \tilde{q}_{22}^{oo} \end{pmatrix} \\ \widetilde{Q}_{oc} &= \begin{pmatrix} \tilde{q}_{11}^{oc} & 0 \\ \tilde{q}_{21}^{oc} & \tilde{q}_{22}^{oc} \end{pmatrix} \\ \widetilde{Q}_{co} &= \begin{pmatrix} \tilde{q}_{11}^{co} & \tilde{q}_{12}^{co} \\ 0 & \tilde{q}_{22}^{co} \end{pmatrix} \\ \widetilde{Q}_{cc} &= \begin{pmatrix} \tilde{q}_{11}^{cc} & \tilde{q}_{12}^{cc} \\ \tilde{q}_{21}^{cc} & \tilde{q}_{22}^{cc} \end{pmatrix}. \end{aligned} \tag{41}$$

Note that Q_{oo} and $\widetilde{Q}_{oo}$ are diagonal so that any transformation from the topology 6d to itself must preserve that diagonality. This means that S_{oo} is either the identity or it is a permutation of the first row and column with the second row and column. We treat the identity case here. The permutation case is similar.

The closed-closed transitions do not constrain S_{cc} which thus can be written:

$$S_{cc} = \begin{pmatrix} x & 1-x \\ y & 1-y \end{pmatrix}. \tag{42}$$

Now we check to see if the forms of the interconductance transition matrices Q_{oc} and Q_{co} constrain S_{cc}. We have:

$$\widetilde{Q}_{oc} = Q_{oc} S_{cc} \tag{43}$$

so that

$$\begin{pmatrix} \tilde{q}_{11}^{oc} & 0 \\ \tilde{q}_{21}^{oc} & \tilde{q}_{22}^{oc} \end{pmatrix} = \begin{pmatrix} q_{11}^{oc} & 0 \\ q_{21}^{oc} & q_{22}^{oc} \end{pmatrix} \begin{pmatrix} x & 1-x \\ y & 1-y \end{pmatrix}. \tag{44}$$

To maintain the zero then we must have $x = 1$ (unless $q_{11}^{oc} = 0$ which is both measure zero and non-ergodic). To this point then we have determined that

$$S_{cc} = \begin{pmatrix} 1 & 0 \\ y & 1-y \end{pmatrix} \tag{45}$$

so that

$$S_{cc}^{-1} = \frac{1}{1-y} \begin{pmatrix} 1-y & 0 \\ -y & 1 \end{pmatrix} \tag{46}$$

and now

$$\widetilde{Q}_{co} = S_{cc}^{-1} Q_{co} \tag{47}$$

so that

$$\begin{pmatrix} \tilde{q}_{11}^{co} & \tilde{q}_{12}^{co} \\ 0 & \tilde{q}_{22}^{co} \end{pmatrix} = \frac{1}{1-y} \begin{pmatrix} 1-y & 0 \\ -y & 1 \end{pmatrix} \begin{pmatrix} q_{11}^{co} & q_{12}^{co} \\ 0 & q_{22}^{co} \end{pmatrix}.$$

To preserve the 0 element we must have $y = 0$ (or q_{11}^{co} which is measure zero and nonergodic). Thus, we find that

$$S_{cc} = I \tag{48}$$

Similar arguments lead to S_{oo} and S_{cc} being permutations. Thus, the topology 6d is identifiable, as asserted in the body.

1.8.2 Equivalence

Here we have to prove that topology 6d is equivalent to topology 6a. Clearly every topology of the form 6d can be diagonalized (except for a set of measure zero. Thus we need only show that every topology of the form 6a can be put into the form 6d. So we need to show the existence of a similarity transformation that does that. We let $\widetilde{Q}$ be the generator for topology 6d and Q be the generator for the topology 6a.

$$\begin{aligned} \widetilde{Q}_{oo} &= \begin{pmatrix} \tilde{q}_{11}^{oo} & 0 \\ 0 & \tilde{q}_{22}^{oo} \end{pmatrix} \\ \widetilde{Q}_{oc} &= \begin{pmatrix} \tilde{q}_{11}^{oc} & 0 \\ \tilde{q}_{21}^{oc} & \tilde{q}_{22}^{oc} \end{pmatrix} \\ \widetilde{Q}_{co} &= \begin{pmatrix} \tilde{q}_{11}^{co} & \tilde{q}_{12}^{co} \\ 0 & \tilde{q}_{22}^{co} \end{pmatrix} \\ \widetilde{Q}_{cc} &= \begin{pmatrix} \tilde{q}_{11}^{cc} & \tilde{q}_{12}^{cc} \\ \tilde{q}_{21}^{cc} & \tilde{q}_{22}^{cc} \end{pmatrix} \end{aligned} \tag{49}$$

$$
\begin{aligned}
Q_{oo} &= \begin{pmatrix} q^{oo}_{11} & 0 \\ 0 & q^{oo}_{22} \end{pmatrix} \\
Q_{oc} &= \begin{pmatrix} q^{oc}_{11} & q^{oc}_{12} \\ q^{oc}_{21} & q^{oc}_{22} \end{pmatrix} \\
Q_{co} &= \begin{pmatrix} q^{co}_{11} & q^{co}_{12} \\ q^{co}_{21} & q^{co}_{22} \end{pmatrix} \\
Q_{cc} &= \begin{pmatrix} q^{cc}_{11} & 0 \\ 0 & q^{cc}_{22} \end{pmatrix}
\end{aligned}
\tag{50}
$$

Since $\widetilde{Q}_{oo}$ and Q_{oo} are both diagonal S_{oo} is either the identity or a permutation. We treat the identity case here. The permutation case is similar. Since S_{cc} takes the diagonal Q_{cc} into the full $\widetilde{Q}_{cc}$ that transformation does not constrain S_{cc} so that we again write:

$$
S_{cc} = \begin{pmatrix} x & 1-x \\ y & 1-y \end{pmatrix}. \tag{51}
$$

We now look at the transformation between $\widetilde{Q}_{oc} = Q_{oc}S_{cc}$. We have then that:

$$
\begin{pmatrix} \tilde{q}^{oc}_{11} & 0 \\ \tilde{q}^{oc}_{21} & \tilde{q}^{oc}_{22} \end{pmatrix} = \begin{pmatrix} q^{oc}_{11} & q^{oc}_{12} \\ q^{oc}_{21} & q^{oc}_{22} \end{pmatrix} \begin{pmatrix} x & 1-x \\ y & 1-y \end{pmatrix}.
$$

To obtain the zero in $\widetilde{Q}_{oc}$ we must have:

$$
q^{oc}_{11}(1-x) + q^{oc}_{12}(1-y) = 0 \tag{52}
$$

so that

$$
x = 1 + \frac{q^{oc}_{12}}{q^{oc}_{11}}(1-y) = a - by \tag{53}
$$

Now we have that S^{-1}_{cc} is given by:

$$
S^{-1}_{cc} = \frac{1}{x-y} \begin{pmatrix} (1-y) & -(1-x) \\ -y & x \end{pmatrix}. \tag{54}
$$

We now look at the transformation between $\widetilde{Q}_{co} = S^{-1}_{cc}Q_{co}$. We have then that:

$$
\begin{pmatrix} \tilde{q}^{co}_{11} & \tilde{q}^{co}_{21} \\ 0 & \tilde{q}^{co}_{22} \end{pmatrix} = \frac{1}{x-y} \begin{pmatrix} (1-y) & -(1-x) \\ -y & x \end{pmatrix} \begin{pmatrix} q^{co}_{11} & q^{co}_{12} \\ q^{co}_{21} & q^{co}_{22} \end{pmatrix}.
$$

To obtain the zero in $\widetilde{Q}_{CO}$ we must have:

$$
q^{co}_{21}x - q^{co}_{11}y = 0 \tag{55}
$$

so that

$$
x = \frac{q^{co}_{11}}{q^{co}_{21}} y = cy \tag{56}
$$

so that

$$
y = \frac{a}{b+c} \tag{57}
$$

where

$$
\begin{aligned}
a &= 1 + \frac{q^{oc}_{12}}{q^{OC}_{11}} && (58) \\
b &= \frac{q^{oc}_{12}}{q^{oc}_{11}} && (59) \\
c &= \frac{q^{co}_{11}}{q^{co}_{21}}. && (60)
\end{aligned}
$$

Thus, the transformation is well defined provided $c \neq 1$. Thus, we have shown that every BKU model in topology $6a$ can be transformed into a model with topology $6d$. Thus, the two topologies are equivalent.

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