Supporting Information: Structural conditions on complex networks for the Michaelis-Menten input-output response

Felix Wong[a,b], **Annwesha Dutta**[c], **Debashish Chowdhury**[c], **and Jeremy Gunawardena**[a,1]

[a]Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA; [b]School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, USA; [c]Department of Physics, Indian Institute of Technology, Kanpur 208016, India

In this Supporting Information, we first provide background on the linear framework and then restate and give full proofs of Props. 1-4 from the main text. We also state and prove Prop. S1, which covers a restricted case and give examples falling outside the scope of our results. Finally, Table S1 lists applications in which the Michaelis-Menten formula arises and explains how each of these follows from our results. This tabulation covers all examples known to us with the exception of [1], which is based on a continuum treatment. Concepts and notation are taken from the main text, which should be consulted for more details.

1. Calculating steady states in the linear framework

Further information about the linear framework is available in [2–6]. Given a labelled, directed graph, as described in the main text, if each edge is treated as a chemical reaction under mass-action kinetics, with the label as the rate, then, because an edge has only a single source vertex, the resulting dynamics is linear and can be described in matrix form by,

$$\frac{du}{dt} = \mathcal{L}(G)u\,. \qquad \text{[S1]}$$

Here, G is the graph, u is a vector of component concentrations or state probabilities and $\mathcal{L}(G)$ is the Laplacian matrix of G. This matrix can be thought of as a discrete version of the Laplacian operator [7], so that Eq. S1 is like a discretised diffusion equation. As noted in the main text, in the stochastic setting, Eq. S1 is the master equation, or Kolmogorov forward equation, of the underlying Markov process. Since material is only moved between vertices, there is a conservation law, $\sum_i u_i(t) = u_{tot}$, with $u_{tot} = 1$ in the stochastic setting.

Calculating steady states of Eq. S1 hinges on two results. First, if G is strongly connected, then there is a unique steady state up to a scalar multiple. Second, if G is strongly connected, a representative steady state, $\rho(G)$, is given by the Matrix-Tree Theorem (MTT). To state this, let $\Theta_i(G)$ denote the set of spanning trees rooted at i (Fig. S1). A spanning tree is a subgraph of G which includes each vertex of G (spanning) and has no cycles when edge directions are ignored (tree). It is rooted at i if i has no outgoing edges in the tree. A subgraph is a rooted spanning tree if, and only if, there is a unique directed path to the root from any other vertex. A strongly-connected graph has at least one spanning tree rooted at each vertex. If X is any subgraph of G, such as a spanning tree, let $q(X)$ be the product of all its labels, $q(X) = \prod_{j \xrightarrow{a} k \in X} a$. The MTT states that

$$\rho_i(G) = \sum_{T \in \Theta_i(G)} q(T) \qquad \text{[S2]}$$

is a steady state of Eq. S1, so that $\mathcal{L}(G)\rho(G) = 0$.

If G is strongly connected, then any steady state, u^*, satisfies $u^* = \lambda\rho(G)$ for some scalar λ. It follows from the conservation law that

$$u_i^* = \left(\frac{\rho_i(G)}{\rho_1(G) + \cdots + \rho_N(G)}\right) u_{tot}\,. \qquad \text{[S3]}$$

By following this prescription, Eq. 1 in the main text is easily derived from Fig. 1B in the main text: $\rho_E = k_{-1} + k_2$, $\rho_{ES} = k_1 x$, where $x = [S]$ is the input variable, so that $A = (k_2 u_{tot})$ and $B = (k_{-1} + k_2)/k_1$, with u_{tot} being the total amount of enzyme.

If a graph is reversible, there is a mapping between the spanning trees rooted at any two vertices, $\Phi_{i,j} : \Theta_i(G) \to \Theta_j(G)$, defined as follows. Let $T \in \Theta_i(G)$. Choose the unique path in T from j to the root i and reverse all its edges. This gives a spanning tree rooted at j, $\Phi_{i,j}(T) \in \Theta_j(G)$, for which

Lemma 1. $\Phi_{i,j}$ is a bijection and $\Phi_{i,j}^{-1} = \Phi_{j,i}$.

If a steady state, u^*, is one of thermodynamic equilibrium, so that detailed balance holds, then, as explained in the main text, the graph must be reversible and each pair of reversible edges is independently in flux balance, with $\ell(i \to j)u_i^* = \ell(j \to i)u_j^*$. Eq. S2 then simplifies dramatically. Choose any path of reversible edges from a reference vertex, taken by convention to be vertex 1, to vertex i, $1 = i_1 \rightleftarrows i_2 \rightleftarrows \cdots \rightleftarrows i_p = i$, and let $\mu_i(G)$ be the product of the label ratios along the path,

$$\mu_i(G) = \prod_{k=1}^{p-1} \kappa(i_k \to i_{k+1})\,. \qquad \text{[S4]}$$

It is a consequence of detailed balance that $\mu_i(G)$ does not depend on the choice of path from 1 to i. Indeed, detailed balance is equivalent to the *cycle condition*, which states that, for any cycle of reversible edges in G, the product of labels going clockwise around the cycle equals the product going counterclockwise. The independence of $\mu_i(G)$ from the chosen path follows immediately. Accordingly, if $T \in \Theta_i(G)$, then by taking the unique path in T from 1 to i, we see that $q(T) = \mu_i(G)q(\Phi_{i,1}(T))$. Summing over all $T \in \Theta_i(G)$ and using Lemma 1, it follows from Eq. S2 that $\rho_i(G) = \mu_i(G)\rho_1(G)$, so that $\rho(G)$ is a scalar multiple of $\mu(G)$. Eq. S3 then simplifies, with $\mu(G)$ taking the place of $\rho(G)$. Since $\kappa(i \to j)$ can be interpreted in terms of the free energy difference between i and j, this corresponds to the prescription of equilibrium statistical mechanics and the denominator of Eq. S3 becomes the partition function (for the grand canonical ensemble under our assumptions regarding the "slow" components). Note, however, that Eq. S3 also holds away from equilibrium, so its denominator provides a nonequilibrium partition function.

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[1] To whom correspondence should be addressed. E-mail: jeremy@hms.harvard.edu

2. Proof of Proposition 1

Proposition 1. Let G be a reversible graph with monomial ratios at thermodynamic equilibrium. If G is partitioned by V_0 and V_1 so that $\deg \kappa(i \to j) = 1$ for all edges splitting V_0 from V_1 and $\deg \kappa(i \to j) = 0$ for all non-splitting edges, then all vertices in V_1 satisfy the MM formula. Conversely, if some vertex satisfies the MM formula and $V_1 \subseteq \nu(G)$ consists of all such vertices, then $V_0 = \nu(G) \backslash V_1$ and V_1 form a partition of G for which $\deg \kappa(i \to j)$ has the same properties. In either direction, all vertices with the MM formula have the same denominator.

Proof. Let i be any vertex in the graph and consider any path of reversible edges from 1 to i, $1 = i_1 \rightleftarrows i_2 \rightleftarrows \cdots \rightleftarrows i_p = i$, as for Eq. S4. By hypothesis and Eq. S4, the quantity $\mu_i(G)$ is a monomial in x. Its degree can be calculated as follows. Recall that, by convention, $1 \in V_0$. If $i \in V_0$, then, since the path must return to V_0, if there is a reversible edge, $i_j \rightleftarrows i_{j+1}$, in which $i_j \to i_{j+1}$ separates V_0 from V_1, then it must eventually be followed by a reversible edge $i_k \rightleftarrows i_{k+1}$ in which $i_k \to i_{k+1}$ separates V_1 from V_0. By hypothesis, $\deg(\kappa(i_j \to i_{j+1})) = 1$ and $\deg(\kappa(i_k \to i_{k+1})) = -1$, so that the contribution of x cancels in Eq. S4. Hence, $\deg(\mu_i(G)) = 0$. If $i \in V_1$, a similar argument shows that $\deg(\mu_i(G)) = 1$.

Now recall from §1 that the representative steady state, $\rho(G)$, as given by the Matrix-Tree Theorem (Eq. S2), satisfies $\rho(G) = \mu(G)\rho_1(G)$. Hence, $\mu(G)$ can be used in place of $\rho(G)$ to calculate a steady state in Eq. S3, so that,

$$u_i^* = \left(\frac{\mu_i(G)}{\mu_1(G) + \cdots + \mu_N(G)} \right) u_{tot} \, . \tag{S5}$$

If $i \in V_1$, then it follows from the argument above that $\mu_i(G) = A_i x$ and $\mu_1(G) + \cdots + \mu_N(G) = Cx + D$, where $A_i, C, D \in \mathbb{R}_{>0}$ are independent of x. It follows from Eq. S5 that $u_i^* = u_{tot}(A_i/C)x/(x + (D/C))$, which is the MM formula, with the denominator being independent of i.

Conversely, let $V_1 \subseteq \nu(G)$ be the set of those vertices of G which have the MM form. By hypothesis, $V_1 \neq \emptyset$. Choose $i \in V_1$, so that, by hypothesis, $u_i^* = A_i x/(x + B_i)$, where $A_i, B_i > 0$ and may potentially depend on i. Omitting the symbol G for convenience, we can rewrite Eq. S5 above to yield,

$$\frac{\mu_1}{\mu_i} + \cdots + \frac{\mu_N}{\mu_i} = \frac{u_{tot}}{A_i} + \frac{B_i u_{tot}}{A_i x} \, . \tag{S6}$$

Note that each summand on the left is a monomial with a degree. Letting $x \to \infty$, we see that no summand on the left has positive degree and some summands have degree 0. Let $U_1 \subseteq \nu(G)$ contain those indices k for which $\deg(\mu_k/\mu_i) = 0$, so that $U_1 \neq \emptyset$, and let $U_0 = \nu(G) \backslash U_1$. Multiplying Eq. S6 by x and taking the limit $x \to 0$, we see that $U_0 \neq \emptyset$ and that $\deg(\mu_k/\mu_i) = -1$ for all $k \in U_0$. In other words, if $\deg(\mu_i) = r$, then $\deg(\mu_k) = r$ if $k \in U_1$ and $\deg(\mu_k) = r - 1$ if $k \in U_0$. It now follows from Eq. S5 above, dividing above and below by x^{r-1}, that U_1 contains exactly those vertices satisfying the MM formula, so that $U_1 = V_1$ and so also $U_0 = V_0$. Hence, neither V_0 nor V_1 is empty and so V_0, V_1 is a partition of G. Note also that, since $\deg(\mu_1) = 0$ and $1 \in V_0$, it must be that $r = 1$, which shows the consistency with the first part of the argument above.

Now choose any edge $i \to j$ in G. If this edge is non-splitting then $\deg(\mu_i) = \deg(\mu_j)$, with the common value being 0 or 1 depending on whether the edge lies in V_0 or V_1, respectively. Since a path of reversible edges from 1 to i may be extended by $i \rightleftarrows j$ to j, it follows from Eq. S4 that $\deg(\kappa(i \to j)) = 0$. If $i \to j$ separates V_0 from V_1, then $\deg(\mu_i) = 0$ and $\deg(\mu_j) = 1$, so that, by a similar argument, $\deg(\kappa(i \to j)) = 1$. Since the conditions of the first part of the Proposition are satisfied, all vertices satisfying the MM formula must also have the same denominator. This completes the proof.

3. Proof of Proposition 2

Proposition 2. Let G be a reversible graph with monomial labels that is x-acyclic. If G is partitioned by V_0 and V_1 so that $\deg \kappa(i \to j) = 1$ for all edges splitting V_0 from V_1 and $\deg \kappa(i \to j) = 0$ for all non-splitting edges, then all vertices in V_1 satisfy the MM formula. Conversely, if some vertex satisfies the MM formula and $V_1 \subseteq \nu(G)$ consists of all such vertices, then $V_0 = \nu(G) \backslash V_1$ and V_1 form a partition of G for which $\deg \kappa(i \to j)$ has the same form. In either direction, all vertices with the MM formula have the same denominator.

Proof. Let i be any vertex in G. Since each label is a monomial in x by hypothesis, so too is $q(X)$ for any subgraph X of G and it follows from the Matrix-Tree Theorem (Eq. S2) that the quantity $\rho_i(G)$ is a sum of monomials in x. We show first that $\rho_i(G)$ is itself a monomial in x. Let $T \in \Theta_i(G)$ be any spanning tree rooted at i and let $n(T) = \deg q(T)$. If T is the only spanning tree rooted at i, then G must itself be a tree, which, as noted in the main text, always satisfies detailed balance, so the result follows from Prop. 1. Otherwise, choose another tree $T' \in \Theta_i(G)$. If $n(T') \neq n(T)$ then there must be an edge, $k \to l$, with $\deg(\ell(k \to l)) \neq 0$, which is found on only one of the trees. If $k \to l$ is appended to the other tree, it must necessarily lie on a proper cycle. But then, since $\ell(k \to l)$ depends on x, G is not x-acyclic. Hence, $n(T)$ does not depend on $T \in \Theta_i(G)$. It follows from Eq. S2 that $\rho_i(G)$ is a monomial in x, as claimed.

Now consider any two vertices i, j in G and let $T \in \Theta_i(G)$. Recalling from Lemma 1 that the map, $\Phi_{i,j} : \Theta_i(G) \to \Theta_j(G)$ is bijective, and letting $\wp$ denote the unique directed path from j to i, we see that,

$$n(T) = n(\Phi_{i,j}(T)) + \sum_{k \to l \in \wp} \deg(\kappa(k \to l)) \, . \tag{S7}$$

If i and j belong to the same subset of the partition into V_0 and V_1, then the number of edges in $\wp$ which split V_0 from V_1 must equal the number of edges in $\wp$ which split V_1 from V_0. By hypothesis, the former edges satisfy $\deg \kappa(k \to l) = 1$ and the latter edges satisfy $\deg \kappa(k \to l) = -1$. Hence, the summation term in Eq. S7 must be zero. Since this holds for any $T \in \Theta_i(G)$, we see that $\deg \rho_i(G) = \deg \rho_j(G)$, as long as i and j are both in the same subset of the partition. Let $p = \deg \rho_1(G)$, so that $\deg \rho_i(G) = p$ for all $i \in V_0$. A very similar argument to the one above shows that, if $i \in V_1$, then $\deg \rho_i(G) = p + 1$.

It follows that, if $i \in V_1$, we can write $\rho_i(G) = A_i x^{p+1}$ and $\rho_1(G) + \cdots + \rho_N(G) = Cx^{p+1} + Dx^p$, where $A_i, C, D \in \mathbb{R}_{>0}$ do not depend on x. By Eq. S3, $u_i^* = u_{tot} A_i x^{p+1}/(Cx^{p+1} + Dx^p)$. Dividing above and below by Cx^p, we see that u_i^* assumes the form of Eq. 1 in the main text, with the denominator being $x + D/C$ which is independent of i. Hence, any vertex in V_1 satisfies the MM formula with the same denominator.

Conversely, let $V_1 \subseteq \nu(G)$ be the set of those vertices of G which have the MM form. By hypothesis, $V_1 \neq \emptyset$. Consider any $i \in V_1$. By hypothesis, $u_i^* = A_i x/(x + B_i)$, where $A_i, B_i > 0$ and may depend on i. Omitting the symbol G for convenience, we can rewrite Eq. S3 as,

$$\frac{\rho_1}{\rho_i} + \cdots + \frac{\rho_N}{\rho_i} = \frac{u_{tot}}{A_i} + \frac{B_i u_{tot}}{A_i x} \,. \qquad \text{[S8]}$$

Since G is x-acyclic by hypothesis, we know from the argument in the first paragraph of the proof that $\rho_i(G)$ is a monomial in x for any i in G, so that the same is true for any summand on the left hand side of Eq. S8. Using a very similar argument as in the proof of Prop. 1 above, of letting $x \to \infty$ in Eq. S8 and multiplying both sides of Eq. S8 by x and letting $x \to 0$, we see that $V_0 = \nu(G)\backslash V_1 \neq 0$, so that V_0, V_1 is a partition of G, and that $\deg \rho_i(G) = p$ if $i \in V_0$ and $\deg \rho_i(G) = p + 1$ if $i \in V_1$.

Consider now any edge $k \to i$ in G. Choose $T \in \Theta_i(G)$. If $k \to i$ is not in T, we can remove the unique edge in T that is outgoing from k and append the edge $k \to i$ in its place. The resulting subgraph is still a spanning tree that is rooted at i. Hence, we can always choose $T \in \Theta_i(G)$ so that that $k \to i$ is an edge in T. It follows that $q(T) = \kappa(k \to i)q(\Phi_{i,k}(T))$, where $\Phi_{i,k}(T) \in \Theta_k(G)$. Hence,

$$\deg q(T) = \deg(\kappa(k \to i)) + \deg q(\Phi_{i,k}(T)) \,.$$

If $k \to i$ is a non-splitting edge, then k and i belong to the same subgraph in the partition and we know from the argument above that $\deg q(T) = \deg \rho_i(G) = \deg \rho_k(G) = \deg q(\Theta_{i,k}(T))$. Hence, $\deg \kappa(k \to i) = 0$, as required. If $k \in V_0$ and $i \in V_1$ then, by a similar argument, $\deg q(T) = p{+}1$ and $\deg q(\Phi_{i,k}(T)) = p$ so that, $\deg \kappa(k \to i) = 1$, also as required. Since the conditions of the first part of the Proposition are satisfied, all vertices satisfying the MM formula must have the same denominator. This completes the proof.

4. Proof of Proposition 3

Proposition 3. Let G be a strongly connected graph with monomial labels. If G is partitioned into V_0 and V_1 so that $\deg \ell(i \to j) = 1$ for all edges with $i \in V_0$ and $\deg \ell(i \to j) = 0$ for all edges with $i \in V_1$, then all vertices in V_1 satisfy the MM formula with the same denominator.

Proof: Suppose $i \in V_1$. Choose any tree $T \in \Theta_i(G)$. Since T is spanning, each vertex $j \in V_0$ is in T and has a unique outgoing edge on the path in T from j to i. Recall from Eq. S2 that $q(T)$ is the product of the labels on the edges of T. By hypothesis, $\deg q(T) = m$, where m is the number of vertices in V_0. It follows from Eq. S2 that $\rho_i(G) = x^m c_i$, where $\deg c_i = 0$. Now suppose $i \in V_0$. By a similar argument, $\rho_i(G) = x^{m-1} c_i$. Hence, the steady-state input-output response, as given by Eq. S3, becomes, for any vertex $i \in V_1$,

$$u_i^* = \frac{x^m c_i}{x^{m-1}(\sum_{k \in V_0} c_k) + x^m(\sum_{l \in V_1} c_l)} \,. \qquad \text{[S9]}$$

This yields Eq. 1 of the main text upon cancelling x^{m-1}, with the denominator being independent of i. This completes the proof.

5. Proof of Proposition 4

Proposition 4. Let G be a strongly connected graph that is partitioned by V_0 and $V_1 = \{j\}$, with V_1 containing only a single vertex, j, which is a sink. Suppose that all edges to and from j have monomial labels for which $\deg \ell(i \to j) = d$ and $\deg \ell(j \to i) = d - 1$ and that, furthermore, $\ell(i \to j)$ does not depend on i. Then u_j^* satisfies the MM formula.

Proof. If $j \to l$ is an outgoing edge to the sink vertex, j, let G_l denote the subgraph of G obtained by removing from G all other outgoing edges from j and keeping $j \to l$. Note that G_l is connected, since it retains the incoming edges to j, but may not be strongly connected. If S is any subset of vertices, let $\Theta_S(G)$ denote the set of spanning trees rooted at the vertices of S, so that $\Theta_S(G) = \cup_{i \in S} \Theta_i(G)$. The proof hinges on characterising the set $\Theta_{V_0}(G_l)$. Since, by hypothesis, every vertex in V_0 has an incoming edge to j, and there is an edge $j \to l$ in G_l, it is easy to see that G_l has at least one spanning tree that is rooted at $l \in V_0$, so $\Theta_{V_0}(G_l) \neq \emptyset$.

Note first that any spanning tree of G that is rooted at $i \in V_0$ has a unique outgoing edge from j. Hence, we have the decomposition,

$$\Theta_{V_0}(G) = \bigcup_{j \to l \in G} \Theta_{V_O}(G_l) \,, \qquad \text{[S10]}$$

with the sets on the right-hand side being pairwise mutually disjoint. Second, there is a map, $\zeta : \Theta_{V_0}(G_l) \to \Theta_j(G_l)$ defined as follows. Choose $T \in \Theta_{V_0}(G_l)$ and suppose that T is rooted at $i \in G_l$. There is a unique directed path in T from j to i, which must start with the edge $j \to l$, since that is the only outgoing edge from j in G_l. Remove $j \to l$ from T and adjoin the edge $i \to j$, which exists because j is a sink vertex. It is clear that the resulting subgraph, $\zeta(T)$, is a spanning tree of G_l that is now rooted at j. Now choose $T \in \Theta_j(G_l)$. There is a unique directed path in T from l to j, which terminates in some incoming edge to j, say $i \to j$. Note that i may be the same as l. Remove $i \to j$ from T and adjoin the edge $j \to l$. It is clear that the resulting subgraph is a spanning tree of G_l that is rooted at $i \in V_0$ and that this operation is inverse to ζ. Hence, ζ defines a bijection between $\Theta_{V_0}(G_l)$ and $\Theta_j(G_l)$. Furthermore, for any $T \in \Theta_{V_0}(G_l)$,

$$\ell(l \to j)q(T) = \ell(j \to l)q(\zeta(T)) \,. \qquad \text{[S11]}$$

Summing up Eq. S11 over all $T \in \Theta_{V_0}(G_l)$ and using the fact that ζ is a bijection, we find that,

$$\ell(l \to j)\left(\sum_{i \in V_0} \rho_i(G_l)\right) = \ell(j \to l)\rho_j(G_l) \,. \qquad \text{[S12]}$$

Now note that any spanning tree of G that is rooted at j cannot contain an outgoing edge from j. Therefore, for any outgoing edge $j \to l$, $\Theta_j(G_l) = \Theta_j(G)$, so that $\rho_j(G_l) = \rho_j(G)$. Furthermore, by hypothesis, $\ell(l \to j)$ does not depend on l. Hence, summing up Eq. S12 over all incoming edges to the sink, $l \to j$, and using Eq. S10, we find that,

$$\ell(l \to j)\left(\sum_{i \in V_0} \rho_i(G)\right) = \left(\sum_{l \to j \in G} \ell(j \to l)\right)\rho_j(G) \,. \qquad \text{[S13]}$$

By hypothesis, $\deg \ell(l \to j) = d$ and $\deg \ell(j \to l) = d - 1$. Hence, we can rewrite Eq. S13 as,

$$\sum_{i \in V_0} \rho_i(G) = \frac{M \rho_j(G)}{x} \,, \qquad \text{[S14]}$$

where M does not depend on x. It follows from Eq. S3 that,

$$u_j^* = \frac{\rho_j(G) u_{tot}}{\left(\sum_{i \in V_0} \rho_i(G)\right) + \rho_j(G)} = \frac{x u_{tot}}{x + M} \,,$$

which has the Michaelis-Menten form, as required. This completes the proof.

6. MM away from equilibrium, with irreversible edges

We note here a simple result that does not fall under the scope of Props. 1-4. It covers certain examples, as noted in Table S1, but has very restrictive conditions.

Proposition S1. Let G be a strongly connected graph with monomial labels. If G is partitioned into V_0 and V_1 so that there exists only one edge, $k \to l$, splitting V_0 from V_1, with $\deg \ell(k \to l) = 1$, and $\deg \ell(i \to j) = 0$ for all other edges, then all vertices in V_1 satisfy the MM formula with the same denominator.

Proof. Choose any vertex i in G and any tree $T \in \Theta_i(G)$. By hypothesis, $\deg q(T) = 1$ if $k \to l \in T$ and $\deg q(T) = 0$ if $k \to l \notin T$. Hence, $\rho_i(G) = a_i x + b_i$, where $\deg a_i = \deg b_i = 0$. Since, by hypothesis, $k \to l$ is the only way to reach vertices in V_0 on a spanning tree rooted at a vertex in V_1, $\deg q(T) = 1$ for all such trees. Hence, whenever $i \in V_1$, $\rho_i(G) = a_i x$ and $b_i = 0$. On the other hand, $k \to l$ is outgoing from k and so cannot be on any spanning tree rooted at k. Hence, $\rho_k(G) = b_k$ with $a_k = 0$. The result follows from Eq. S3.

7. Examples of other graphs

Fig. S1 shows two graphs which lie beyond the scope of Props. 1-4 and S1 and contain vertices having the MM form. Fig. S1A shows a reversible graph which does not satisfy the conditions of Prop. 1 because it is away from equilibrium, nor the conditions of Prop. 2 because it is not x-acyclic. Similarly, it does not satisfy the labelling conditions of Props. 3-4 and S1. The quantities $\rho_k(G)$ can be calculated by the MTT in Eq. S2, using the rooted spanning trees shown in Fig. S1A. We find that,

$$\begin{aligned} \rho_1(G) &= be^2 + ce^2 + bex + cex \\ \rho_2(G) &= abe + ace + abx + bdx \\ \rho_3(G) &= bde + cde + acx + cdx \\ \rho_4(G) &= aex + dex + ax^2 + dx^2 \,, \end{aligned}$$

so that, by Eq. S3,

$$\begin{aligned} u_4^* &= \left(\frac{\rho_4(G)}{\rho_1(G) + \rho_2(G) + \rho_3(G) + \rho_4(G)} \right) u_{tot} \\ &= \frac{u_{tot}(a+d)(e+x)x}{(a+d)(e+x)x + (b+c)(a+d+e)(e+x)} \\ &= \frac{u_{tot}(a+d)x}{(a+d)x + (b+c)(a+d+e)} \,. \end{aligned}$$

This has the Michaelis-Menten form (Eq. 1 of the main text), with $A = u_{tot}$ and $B = (b+c)(a+d+e)/(a+d)$.

Fig. S1B shows a graph with irreversible edges which does not satisfy the conditions of Props. 3 and S1 because it cannot be partitioned correctly or the conditions of Prop. 4 because neither vertex 1 nor vertex 2 are sinks. We find that,

$$\begin{aligned} \rho_1(G) &= x^2 \\ \rho_2(G) &= x^2 \\ \rho_3(G) &= x^2 + ax \,, \end{aligned}$$

so that, by Eq. S3,

$$\begin{aligned} u_1^* = u_2^* &= \left(\frac{\rho_1(G)}{\rho_1(G) + \rho_2(G) + \rho_3(G)} \right) u_{tot} \\ &= \frac{u_{tot} x^2}{x(3x + a)} \\ &= \frac{u_{tot} x}{3x + a} . \end{aligned}$$

This has the Michaelis-Menten form (Eq. 1 of the main text), with $A = u_{tot}/3$ and $B = a/3$.

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References	Network and MM formula and additional details	Prop.
[8]	Eq. 6 and section titled “Theory of Adsorption on Plane Surfaces”	1
[9]	Eq. 4 and Section 1	1
[10–12]	Fig. 1B and Eq. 3 in [10]; Fig. 10.5A and D and Table 10.1A and D in [11]; Fig. 6A and C and Table 2A and C in [12]	1
[13]	Eqs. 1 and 2	1
[14]	Eqs. 3 and 4 and SI Section 2	1
[15]	Eqs. 5 and 26	1
[12, 16–19]	Eqs. 28 and 30 in [16]; Eqs. 2.4 and 4.4 in [17]; Fig. 2a and Eq. 21 in [18]; Fig. 2 and Eq. 1 in [19]; Fig. 6E and Table 2E in [12] (assume that $\delta_i \to \infty$ in [16] and [17] throughout, and see Fig. S2 and assume that $(k_{-1i} + k_{2i})/k_{1i} = (k_{-1j} + k_{2j})/k_{1j}$, $\alpha_{ij} = \alpha_{ji}$, and $\beta_{ij} = \beta_{ji}$; here and below, the indices i and j range over all enzyme conformations, $1, \cdots, n$)	1
[20]	Fig. 1 and Eq. 13 (assume $k_{\mathrm{s}}, k_{\mathrm{h}} \ll k_L \equiv k_P^+[P] + k_D^+[D] + k_P^- + k_D^-$, $k_{D1}^\pm = k_{D2}^\pm \equiv k_D^\pm$, $k_{P1}^\pm = k_{P2}^\pm \equiv k_P^\pm$, and that the ATP concentration is small)	1
[16, 17]	Eqs. 28 and 30 in [16]; Eqs. 2.4 and 4.4 in [17] (see Fig. S2 and Fig. 3C of the main text, and assume that the catalytic rates, k_{2i}, are much greater than the interconversion rates, β_{ij})	2
[16, 17, 21]	Eqs. 28 and 30 in [16]; Eqs. 2.4 and 4.4 in [17]; Eqs. 2.1 and 4.1 in [21] (assume that $\delta_i \to \infty$ in [21] throughout, and see Fig. S2 and Fig. 3C of the main text and assume that only the β_{ij} are extremely small)	2
[16, 17, 21]	Eqs. 28 and 30 in [16]; Eqs. 2.4 and 4.4 in [17]; Eqs. 2.1 and 4.1 in [21] (see Fig. S2 and Fig. 3D of the main text, and assume that only the α_{ij} are extremely small)	2
[22]	Fig. 1 and Eq. 5	S1
[10]	Fig. 1A and Eq. 3	S1
[23]	Eq. 58 and Table 2	S1
[24]	Eq. 2 and Schemes 1 and 2	S1
[16, 17, 21]	Eqs. 28 and 30 in [16]; Eqs. 2.4 and 4.4 in [17]; Eqs. 2.1 and 4.1 in [21] (see Fig. S2 and Fig. 3E of the main text, and assume that the α_{ij} are much greater than all the other rates)	3
[10–12]	Fig. 1C and D and Eq. 3 in [10]; Fig. 10.5B, C, and E and Table 10.1B, C, and E in [11]; Fig. 6B and D and Table 2B and D in [12]	3
[25]	Fig. 2 and Eq. 28 (assume either ADP or P concentration is zero)	3
[26]	Eqs. 12 and 14 (assume the concentration of substrate B to be small and fixed)	3
[26]	Eqs. 32 and 36	3
[27–29]	Fig. 3 and Eq. 20 in [27]; Fig. 1 and Eq. 4 in [28]; Fig. 3 and Eq. 24 in [29]	3
[30, 31]	Fig. 1 and Eq. 11 in [30]; Fig. 2 and Eqs. 1-5 and 19 in [31]	3
[23]	Eqs. 29, 32, 44, and Table 2	3
[32]	Fig. 2 and Eq. 24 (assume P concentration is zero)	3
[33]	Fig. 2 and Eq. 13 (reduce to the cycle $\mathcal{F}$ and assume the limit of small ADP and P concentrations)	3
[34]	Eq. 1 and Eq. J in Appendix S3	3
[16, 17, 21]	Eqs. 28 and 30 in [16]; Eqs. 2.4 and 4.4 in [17]; Eqs. 2.1 and 4.1 in [21] (see Fig. S2 and Fig. 3F of the main text, and assume that $k_{1i} = k_{1j}$ and the β_{ij} are much greater than the other rates)	4

Table S1. Applications of Propositions 1-4 and S1 to the contexts identifed in Table 1 of the main text.

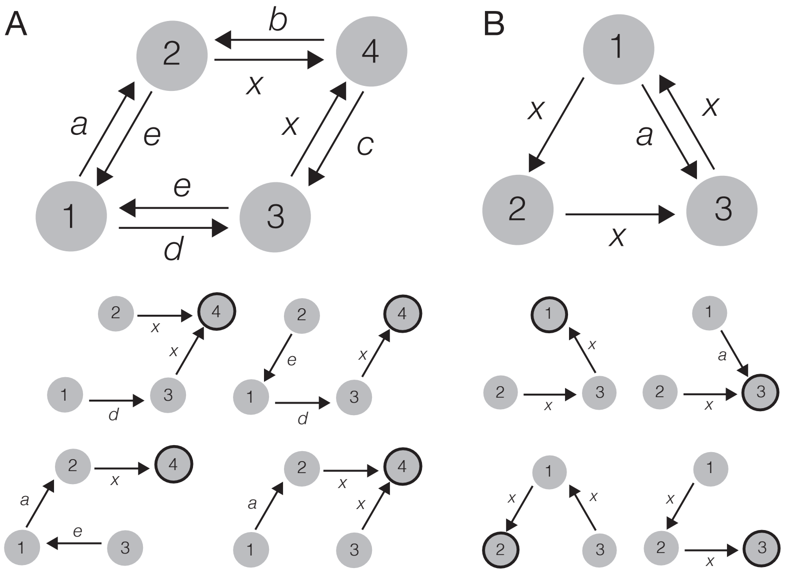

Fig. S1. Graphs having the MM formula but falling outside the scope of Props. 1-4 and S1. See the text above for further details. (*A*) A reversible graph for which vertex 4 satisfies the MM formula. The spanning trees rooted at vertex 4 (circled) are shown below. The spanning trees rooted at other vertices can be obtained by the method of Lemma 1. (*B*) An irreversible graph for which vertices 1 and 2 satisfy the MM formula. All rooted spanning trees are shown below, with the root vertices circled.

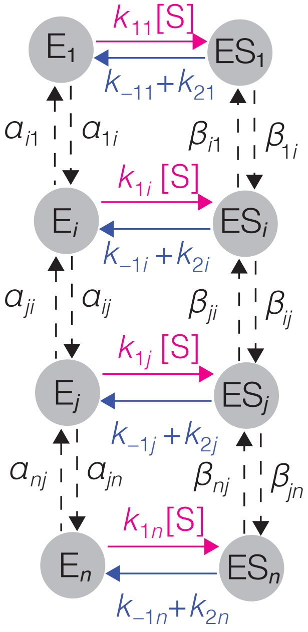

Fig. S2. A model of single-molecule enzyme kinetics, as considered in [16, 17, 21]. The free enzyme is denoted by E, the substrate by S, the enzyme-substrate complex by ES and distinct conformations by subscripts i and j running from 1 to n. Note that there are no self-loops, so that, effectively, $\alpha_{ii} = \beta_{ii} = 0$ for all i. Various parametric regimes of this model, as listed in Table S1, give rise to the MM formula, with substrate concentration being the input variable, $x = [S]$. The graphs arising from these parametric regimes, which fall under the scope of Props. 1-4, are shown in Fig. 3 of the main text.