MBI Workshop:
Applications of Generalized Networks to Biochemical Reaction Systems

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1 Background
   - Overview
   - Mass Action Systems

2 Classical Results
   - Deficiency Zero Theorem
   - Enzymatic Futile Cycle
   - Generalized Reaction Networks

3 Outlook
   - EnvZ/OmpR Signaling Pathway
   - Wnt Signaling Pathway
   - Summary and Future Work
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Figure: Picture courtesy of Wikipedia.
**Objective:**

Use **network structure** to determine **dynamical properties** of the biochemical reaction systems.

**Protein activation**

\[
\begin{align*}
A + B & \rightarrow 2B \\
B & \rightarrow A
\end{align*}
\]

**Enzymatic futile cycle**

\[
\begin{align*}
S + E & \Leftrightarrow SE \rightarrow P + E \\
P + F & \Leftrightarrow PF \rightarrow S + F
\end{align*}
\]

**EnvZ/OmpR Signaling Pathway**

\[
\begin{align*}
XD & \Leftrightarrow X \Leftrightarrow XT \rightarrow X_p \\
X_p + Y & \Leftrightarrow X_pY \rightarrow X + Y_p \\
XT + Y_p & \Leftrightarrow XTY_p \rightarrow Y
\end{align*}
\]
Mass-Action System

Track concentrations with system of autonomous polynomial ordinary differential equations.

Protein activation model: \((A \text{ inactive, } B \text{ active})\)

\[
\begin{align*}
A + B & \xrightarrow{\alpha} 2B \quad \text{(activation)} \\
B & \xrightarrow{\beta} A \quad \text{(de-activation)}
\end{align*}
\]

Mass-action system:

\[
\begin{align*}
\dot{x}_A &= -\alpha x_A x_B + \beta x_B \\
\dot{x}_B &= \alpha x_A x_B - \beta x_B.
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(activation) (de-activation)

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Enzymatic cycle (e.g. MAPK Cascade):

\[
\begin{align*}
S + E & \xrightleftharpoons[k_2]{k_1} SE \xrightarrow{k_3} P + E \\
P + F & \xrightleftharpoons[k_5]{k_4} PF \xrightarrow{k_6} S + F
\end{align*}
\]

S - substrate  
P - product  
E - kinase  
F - phosphotase

Governed by **mass action system**:

\[
\begin{align*}
\dot{x}_S &= -k_1 x_S x_E + k_2 x_{SE} + k_6 x_{PF} \\
\dot{x}_E &= -k_1 x_S x_E + k_2 x_{SE} + k_3 x_{SE} \\
\dot{x}_{SE} &= k_1 x_S x_E - k_2 x_{SE} - k_3 x_{SE} \\
\dot{x}_P &= k_3 x_{SE} - k_4 x_P x_F + k_5 x_{PF} \\
\dot{x}_F &= -k_4 x_P x_F + k_5 x_{PF} + k_6 x_{PF} \\
\dot{x}_{PF} &= k_4 x_P x_F - k_5 x_{PF} - k_6 x_{PF}.
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\dot{x}_{SE} &= k_1 x_S x_E - k_2 x_{SE} - k_3 x_{SE} \\
\dot{x}_P &= k_3 x_{SE} - k_4 x_P x_F + k_5 x_{PF} \\
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\dot{x}_F &= -k_4 x_P x_F + k_5 x_{PF} + k_6 x_{PF} \\
\dot{x}_{PF} &= k_4 x_P x_F - k_5 x_{PF} - k_6 x_{PF}.
\end{align*}
\]
General mass action system:

\[
\frac{dx(t)}{dt} = \sum_{i=1}^{r} k_i \left( y'_i - y_i \right) \prod_{j=1}^{m} x_j^{y_{ij}}
\]

where, for each reaction,

- \( k_i > 0 \) is the **rate constant**
- \( y'_i - y_i \in \mathbb{R}^m \) is the **reaction vector**
- \( \prod_{j=1}^{m} x_j^{y_{ij}} \) is the **interaction term**

**Polynomial differential equations** arise frequently in mathematical biology! (e.g. infectious disease, ecosystems)
General mass action system:

\[
\frac{dx(t)}{dt} = \sum_{i=1}^{r} k_i \left( y_i' - y_i \right) \prod_{j=1}^{m} x_j^{y_{ij}}
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**Polynomial differential equations** arise frequently in mathematical biology! (e.g. infectious disease, ecosystems)
General properties:

\[
\begin{align*}
\dot{x}_A &= -\alpha x_A x_B + \beta x_B \\
\dot{x}_B &= \alpha x_A x_B - \beta x_B
\end{align*}
\]
General properties:

\[
\begin{align*}
\dot{x}_A &= -\alpha x_A x_B + \beta x_B = 0 \\
\dot{x}_B &= \alpha x_A x_B - \beta x_B = 0
\end{align*}
\]

Steady states:

\[
\alpha x_A x_B - \beta x_B = 0 \implies x_B = 0 \text{ or } x_A = \frac{\beta}{\alpha}
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\]

**Steady states:**

\[
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\]

**Invariant Subspace:**

\[
\begin{bmatrix}
\dot{x}_A \\
\dot{x}_B
\end{bmatrix} = \alpha x_A x_B \begin{bmatrix}
-1 \\
1
\end{bmatrix} + \beta x_B \begin{bmatrix}
1 \\
-1
\end{bmatrix}
\]
General properties:

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\begin{align*}
\dot{x}_A &= -\alpha x_A x_B + \beta x_B \\
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Invariant Subspace:

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\end{bmatrix} = \alpha x_A x_B \begin{bmatrix}
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1
\end{bmatrix} + \beta x_B \begin{bmatrix}
1 \\
-1
\end{bmatrix} \in \text{span} \left\{ \begin{bmatrix}
-1 \\
1
\end{bmatrix} \right\}
\]
Figure: State space is partitioned (invariant spaces)
Figure: State space is **partitioned** (invariant spaces)
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Question: Why study the network properties associated with a mass action system?

Results known relating network properties to dynamical properties:

- Existence/number of positive steady states
- Capacity of boundedness and persistence (i.e. non-extinction)
- Long-term dynamical behavior
Deficiency Zero Theorem:

**Assumptions:** Network structure

**Conclusions:** System dynamics

---

**Theorem (Horn, Jackson, Feinberg, 1972 [1, 2, 3])**

Consider a mass action system for which

- the underlying network is weakly reversible; and
- the deficiency is zero.

Then the system has a unique locally stable steady state for every choice of rate constants and every positive stoichiometric compatibility class.
Deficiency Zero Theorem:

Assumptions: Network structure
Conclusions: System dynamics

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Theorem (Horn, Jackson, Feinberg, 1972 [1, 2, 3])

Consider a mass action system for which

- the underlying network is weakly reversible; and
- the deficiency is zero.

Then the system has a unique locally stable steady state for every choice of rate constants and every positive stoichiometric compatibility class.
Example:

\[
\begin{align*}
X_1 & \xrightarrow{k_1} 2X_2 \\
X_2 + X_3 & \xleftarrow{k_3} \xrightarrow{k_2} X_3 \\
X_3 & \xleftrightarrow{k_4 \quad k_5} X_4.
\end{align*}
\]

- Network is \textbf{weakly reversible} (strongly connected)
- Consider \textbf{deficiency} (network parameter):

\[
\delta = n - \ell - s
\]
Example:

Network is **weakly reversible** (strongly connected)

Consider **deficiency** (network parameter):

\[ \delta = n - \ell - s \]
Example:

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- Network is **weakly reversible** (strongly connected)
- Consider **deficiency** (network parameter):

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Example:

\[
\begin{align*}
X_1 & \xrightarrow{k_1} 2X_2 \\
k_3 & \leftrightarrow k_2 \\
X_2 + X_3 & \xleftrightarrow{k_4 \leftrightarrow k_5} X_4.
\end{align*}
\]

- Network is **weakly reversible** (strongly connected)
- Consider **deficiency** (network parameter):

\[
\delta = n - \ell - s = 5
\]
Example:

\[
X_1 \xrightarrow{k_1} 2X_2 \\
X_2 + X_3 \xleftrightarrow{k_2, k_3} X_3 \xleftrightarrow{k_4, k_5} X_4.
\]

- Network is **weakly reversible** (strongly connected)
- Consider **deficiency** (network parameter):

\[
\delta = n - \ell - s = 5 - 2
\]
Example:

\[
\begin{align*}
X_1 & \xrightarrow{k_1} 2X_2 \\
X_2 & \xleftarrow{k_3} X_3 \\
X_2 + X_3 & \xleftrightarrow{k_2} X_3 \\
X_3 & \xleftrightarrow{k_4, k_5} X_4.
\end{align*}
\]

- Network is **weakly reversible** (strongly connected)
- Consider **deficiency** (network parameter):

\[
\delta = n - \ell - s = 5 - 2
\]
Example:

\[
X_1 \xrightarrow{k_1} 2X_2 \\
X_2 \xleftrightarrow{k_2} X_3 \\
X_3 \xleftrightarrow{k_4} X_4.
\]

- Network is **weakly reversible** (strongly connected)
- Consider **deficiency** (network parameter):

\[
\delta = n - \ell - s = 5 - 2 - 3
\]
Example:

\[ X_1 \xrightarrow{k_1} 2X_2 \]
\[ k_3 \leftarrow \downarrow \uparrow k_2 \]
\[ X_2 + X_3 \]

\[ X_3 \xleftrightarrow{k_4 \quad k_5} X_4. \]

- Network is \textbf{weakly reversible} (strongly connected)
- Consider \textbf{deficiency} (network parameter):

\[ \delta = n - \ell - s = 5 - 2 - 3 = 0 \]
Example:

\[ X_1 \xrightarrow{k_1} 2X_2 \]
\[ k_3 \quad \text{↔} \quad k_2 \]
\[ X_2 + X_3 \]
\[ X_3 \xrightleftharpoons[k_4]{k_5} X_4. \]

- Network is **weakly reversible** (strongly connected)
- Consider **deficiency** (network parameter):
  \[ \delta = n - \ell - s = 5 - 2 - 3 = 0 \]
- **Deficiency Zero Theorem** applies!
Mass action system:

\[\begin{align*}
\dot{x}_1 &= -k_1 x_1 + k_3 x_2 x_3 \\
\dot{x}_2 &= 2k_1 x_1 - k_2 x_2^2 - k_3 x_2 x_3 \\
\dot{x}_3 &= k_2 x_2^2 - k_3 x_2 x_3 - k_4 x_3 + k_5 x_4 \\
\dot{x}_4 &= k_4 x_3 - k_5 x_4
\end{align*}\]

- **Steady state manifold** parametrized by

\[E = \{x \in \mathbb{R}_0^4 \mid \ln(x) - \ln(x^*) \in S^\perp\}\]

- **Unique steady state** \(x^*\) within each compatibility class.

- **Asymptotical stability** of \(x^*\) within each compatibility class.
Several powerful network-based results known, e.g.
- Deficiency Zero Theorem (HJ&F, 1972 [1, 2, 3])
- Deficiency One Theorem (Feinberg, 1987 [4])
- Global Attractor Conjecture (Craciun et al., 2009, [5])

Recurring property of interest is weak reversibility.

Minimal dependence on reaction parameters and initial conditions.
Chemical Reaction Network Theory (1972-):

- Several powerful network-based results known, e.g.
  - Deficiency Zero Theorem (HJ&F, 1972 [1, 2, 3])
  - Deficiency One Theorem (Feinberg, 1987 [4])
  - Global Attractor Conjecture (Craciun et al., 2009, [5])

- Recurring property of interest is weak reversibility. (*)

- Minimal dependence on reaction parameters and initial conditions. (*)

- Realistic for biochemical models?
Enzymatic cycle (e.g. MAPK Cascade):

\[
\begin{align*}
S + E & \overset{k_1}{\underset{k_2}{\leftrightarrow}} SE \overset{k_3}{\rightarrow} P + E \\
P + F & \overset{k_4}{\underset{k_5}{\leftrightarrow}} PF \overset{k_6}{\rightarrow} S + F
\end{align*}
\]

- Network is **not weakly reversible**.
- We have \( \delta = n - \ell - s = 1 \neq 0 \).
- Deficiency Zero Theorem does not apply (analogous dynamical result proved by Angeli and Sontag in 2008 [6]).
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- Network is not weakly reversible.
- We have \( \delta = n - \ell - s = 1 \neq 0 \).
- Deficiency Zero Theorem does not apply (analogous dynamical result proved by Angeli and Sontag in 2008 [6]).
Alternative approach:

Perhaps we are considering the **wrong network representation**!

- **Generalized chemical reaction networks** recently introduced (Müller & Regensburger, 2012 [7])

- **Two sets of complexes** for each vertex in the reaction graph:
  1. stoichiometric complexes
  2. kinetic complexes
Example:

\[ X_1 \overset{k_1}{\rightleftharpoons} X_2 + X_3 \]

1. **Stoichiometric complexes:** \( \{X_1, X_2 + X_3\} \)
Example:

\[ X_1 + X_2 \cdots \overset{k_1}{\underset{k_2}{\rightleftharpoons}} X_1 \ \Leftrightarrow \ X_2 + X_3 \cdots 2X_3 \]

1. **Stoichiometric complexes:** \{\(X_1, X_2 + X_3\}\)
2. **Kinetic complexes:** \{\(X_1 + X_2, 2X_3\}\)
Example:

\[ X_1 + X_2 \quad \cdots \quad X_1 \rightleftharpoons^{k_1}_{k_2} X_2 + X_3 \quad \cdots \quad 2X_3 \]

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Generalized mass action system (GMAS):

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\begin{align*}
\dot{x}_1 &= -k_1 x_1 x_2 + k_2 x_3^2 \\
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\dot{x}_3 &= k_1 x_1 x_2 - k_2 x_3^2
\end{align*}
\]
Example:

\[
X_1 + X_2 \quad \cdots \quad X_1 \xrightleftharpoons[1/k_2]{k_1} X_2 + X_3 \quad \cdots \quad 2X_3
\]

1. **Stoichiometric complexes:** \( \{X_1, X_2 + X_3\} \)
2. **Kinetic complexes:** \( \{X_1 + X_2, 2X_3\} \)

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Example:

\[ X_1 + X_2 \cdots X_1 \overset{k_1}{\underset{k_2}{\rightleftharpoons}} X_2 + X_3 \cdots 2X_3 \]

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\end{align*}
\]
Question:
Can we find a **generalized representations** of biochemical reaction systems with better network properties?

Reconsider the **enzymatic futile cycle**:

\[
(N1) \begin{cases} 
S + E \iff SE \rightarrow P + E \\
P + F \iff PF \rightarrow S + F 
\end{cases}
\]
Question:

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Reconsider the **enzymatic futile cycle**:

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(N1) \begin{cases} 
S + E \rightleftharpoons SE \rightarrow P + E \\
\quad (+F) \\
\end{cases} \quad \begin{cases} 
P + F \rightleftharpoons PF \rightarrow S + F \\
\quad (+E) \\
\end{cases}
\]
Can we find a **generalized representations** of biochemical reaction systems with better network properties?

Reconsider the **enzymatic futile cycle**:

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\begin{align*}
(N1) \quad & S + E \iff SE \rightarrow P + E \quad (+F) \\
& P + F \iff PF \rightarrow S + F \quad (+E)
\end{align*}
$$

$$
\begin{align*}
(N2) \quad & S + E + F \iff SE + F \\
& PF + E \iff P + E + F
\end{align*}
$$
Question:

Can we find a **generalized representations** of biochemical reaction systems with better network properties?

Reconsider the **enzymatic futile cycle**:

\[
(N1) \begin{cases}
S + E \Leftrightarrow SE \rightarrow P + E & (+F) \\
P + F \Leftrightarrow PF \rightarrow S + F & (+E)
\end{cases}
\]

\[
(N2) \begin{cases}
S + E + F \Leftrightarrow SE + F \\
PF + E \Leftrightarrow P + E + F
\end{cases}
\]
Question:
Can we find a generalized representations of biochemical reaction systems with better network properties?

Reconsider the enzymatic futile cycle:

\[
(N1) \begin{cases}
S + E \rightleftharpoons SE \rightarrow P + E \\
P + F \rightleftharpoons PF \rightarrow S + F
\end{cases} ( + F ) \\
( + E )
\]

\[
(N2) \begin{cases}
S + E + F \rightleftharpoons SE + F \\
PF + E \rightleftharpoons P + E + F
\end{cases}
\]
Network is **weakly reversible** and **deficiency zero** but we still need to consider the **kinetic complexes**:

\[
S + E + F \rightleftharpoons SE + F
\]

\[
\uparrow \quad \downarrow
\]

\[
PF + E \rightleftharpoons P + E + F
\]

Corresponding generalized mass action system has **exactly** the same governing dynamical equations!
Network is **weakly reversible** and **deficiency zero** but we still need to consider the **kinetic complexes**:

\[
\begin{align*}
S + E & \quad \cdots \quad S + E + F \Leftrightarrow SE + F \quad \cdots \quad SE \\
\uparrow & \quad \quad \downarrow \\
PF & \quad \cdots \quad PF + E \Leftrightarrow P + E + F \quad \cdots \quad P + E
\end{align*}
\]

Corresponding generalized mass action system has **exactly** the same governing dynamical equations!
There are now two graphs, \textit{stoichiometric} and \textit{kinetic}:

\[
\begin{align*}
\text{(S)} \quad \left\lbrace \begin{array}{c}
S + E + F \iff SE + F \\
\uparrow \quad \downarrow \\
PF + E \iff P + E + F
\end{array} \right. \\
\text{(K)} \quad \left\lbrace \begin{array}{c}
S + E \iff SE \\
\uparrow \quad \downarrow \\
PF \iff P + F
\end{array} \right.
\end{align*}
\]

\text{Generalized mass action system:}

\[
\dot{x} = \sum_{i=1}^{r} k_i (y_i' - y_i) \prod_{j=1}^{m} x_j^{\tilde{y}_{ij}}
\]

Note one-to-one correspondence between nodes in \textbf{(S)} and \textbf{(K)}!
There are now two graphs, *stoichiometric* and *kinetic*:

\[
\begin{align*}
\text{(S)} & \quad S + E + F \rightleftharpoons SE + F \\
& \quad \uparrow \quad \downarrow \\
& \quad PF + E \rightleftharpoons P + E + F \\
\text{(K)} & \quad S + E \rightleftharpoons SE \\
& \quad \uparrow \quad \downarrow \\
& \quad PF \rightleftharpoons P + F
\end{align*}
\]

**Generalized mass action system:**

\[
\dot{x} = \sum_{i=1}^{r} k_i (y'_i - y_i) \prod_{j=1}^{m} x_{ij}^{\tilde{y}_{ij}}
\]

Note one-to-one correspondence between nodes in (S) and (K)!
There are now two graphs, **stoichiometric** and **kinetic**:

\[ \begin{align*}
(S) & \quad \begin{cases}
S + E + F & \Leftrightarrow SE + F \\
PF + E & \Leftrightarrow P + E + F
\end{cases} \\
(K) & \quad \begin{cases}
S + E & \Leftrightarrow SE \\
PF & \Leftrightarrow P + F
\end{cases}
\end{align*} \]

**Generalized mass action system:**

\[
\dot{x} = \sum_{i=1}^{r} k_i \left( y_i' - y_i \right) \prod_{j=1}^{m} x_j^{\tilde{y}_{ij}}
\]

Note one-to-one correspondence between nodes in \((S)\) and \((K)\)!
We have the following properties:

1. Steady states are complex-balanced (in generalized network)
2. Characterization: $$\tilde{K}_i x^{\tilde{y}_j} - \tilde{K}_j x^{\tilde{y}_i} = 0$$
3. Parametrization: $$\ln(x) - \ln(x^*) \in \tilde{S}^\perp$$
4. $$\tilde{K}_i, \tilde{y}_i, \text{ and } \tilde{S}^\perp$$ come from generalized network!

**Stability** and **uniqueness** of steady states are lost (ongoing work)
1 Background
- Overview
- Mass Action Systems

2 Classical Results
- Deficiency Zero Theorem
- Enzymatic Futile Cycle
- Generalized Reaction Networks

3 Outlook
- EnvZ/OmpR Signaling Pathway
- Wnt Signaling Pathway
- Summary and Future Work
What we know so far:

- **GMAS theory** is developing (Müller & Regensburger, [7, 8]).
- Process for generating GMAS known as network translation (Johnston, [9, 10])
What we know so far:

- **GMAS theory** is developing (Müller & Regensburger, [7, 8]).
- Process for generating GMAS known as **network translation** (Johnston, [9, 10])

What happens in actual biochemical networks?

- Generalized network can be **well connected** (great!)
- Generalized network can be **crowded** (resolvable?)
- Generalized network can be **sparse** (???)
EnvZ/OmpR signaling pathway: (Shinar & Feinberg, 2010 [11])

\[
\begin{align*}
XD & \Leftrightarrow X \rightarrow XT \rightarrow X_p \\
X_p + Y & \rightarrow X_pY \rightarrow X + Y_p \\
XT + Y_p & \rightarrow XTY_p \rightarrow XT + Y \\
XD + Y_p & \rightarrow XDY_p \rightarrow XD + Y
\end{align*}
\]

where $X = \text{EnvZ}$, $Y = \text{OmpR}$, $D = \text{ADP}$, $T = \text{ATP}$, and $p = \text{phosphate group}$. 
EnvZ/OmpR signaling pathway: (Shinar & Feinberg, 2010 [11])

\[
\begin{align*}
XD & \Leftrightarrow X \rightarrow XT \rightarrow X_p \quad (+XD + XT + Y) \\
X_p + Y & \rightarrow X_pY \rightarrow X + Y_p \quad (+XD + XT) \\
XT + Y_p & \rightarrow XTY_p \rightarrow XT + Y \quad (+XD + X) \\
XD + Y_p & \rightarrow XDY_p \rightarrow XD + Y \quad (+X + XT)
\end{align*}
\]

where $X = \text{EnvZ}$, $Y = \text{OmpR}$, $D = \text{ADP}$, $T = \text{ATP}$, and $p = \text{phosphate group}$. 
**EnvZ/OmpR signaling pathway:** (Shinar & Feinberg, 2010 [11])

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XD + Y_p & \rightarrow XDY_p \rightarrow XD + Y
\end{align*}
\]

where $X = \text{EnvZ}$, $Y = \text{OmpR}$, $D = \text{ADP}$, $T = \text{ATP}$, and $p = \text{phosphate group}$. 
Generalized reaction network:

\[
\begin{align*}
(N4) \quad & 2XD + XT + Y \rightleftharpoons XD + X + XT + Y \longrightarrow XD + 2XT + Y \\
& \quad \uparrow \quad \updownarrow \\
& \quad X + XT + XDY_p \quad XD + X + XTY_p \quad XD + XT + X_p + Y \\
& \quad \downarrow \quad \uparrow \quad \updownarrow \\
& \quad XD + X + XT + Y_p \leftarrow XD + XT + X_p Y
\end{align*}
\]
**Generalized reaction network:**

\[
\begin{align*}
(\text{N4}) \quad \left\{ 
\begin{array}{l}
2XD + XT + Y & \iff XD + X + XT + Y & \longrightarrow & XD + 2XT + Y \\
X + XT + XD Y_p & & & XD + X + XT Y_p \\
& & & XD + XT + X_p + Y \\
& & & XD + X + XT + Y_p & \iff & XD + XT + X_p Y
\end{array}
\right.
\end{align*}
\]

**Note:**

Network is weakly reversible and deficiency zero **BUT** we still have to assign kinetic complexes.
Stoichiometric and kinetic graphs:

\[
\begin{align*}
2XD + XT + Y & \iff XD + X + XT + Y \rightarrow XD + 2XT + Y \\
\text{(S)} & \\
X + XT + XDY_p & \quad XD + X + XTY_p & XD + XT + X_p + Y
\end{align*}
\]

\[
\begin{align*}
XD & \iff X \rightarrow XT \\
\text{(K)} & \\
XDY_p & \quad XTY_p & X_p + Y
\end{align*}
\]

\[
\begin{align*}
\left\{ XD + Y_p \right\} & \quad \{ XT + Y_p \} \leftarrow X_p Y
\end{align*}
\]

Mapping between complexes is not one-to-one \(\Longrightarrow\) crowded network.
Stoichiometric and kinetic graphs:

\[
\begin{align*}
\text{(S)} & \quad \begin{cases}
2XD + XT + Y \rightleftharpoons XD + X + XT + Y \quad \rightarrow \quad XD + 2XT + Y \\
X + XT + XDY_p \quad XD + X + XTY_p \quad XD + XT + X_p + Y \\
XD + X + XT + Y_p \leftarrow XD + XT + X_p Y
\end{cases}
\end{align*}
\]

\[
\begin{align*}
\text{(K)} & \quad \begin{cases}
XD \rightleftharpoons X \quad \rightarrow \quad XT \\
XDY_p \quad XTY_p \quad X_p + Y \\
\{XD + Y_p\} \quad \{XT + Y_p\} \leftarrow X_p Y
\end{cases}
\end{align*}
\]

Mapping between complexes is not one-to-one $\implies$ crowded network.
Question:
When can overlaps in kinetic complexes be “resolved”?

Overlap may be resolved if generalized network satisfies:

1. a subspace inclusion condition; and
2. a technical graph theoretical condition.

Steady states may be characterized after a rescaling of the rates!
Network is **resolvable** (Johnston, [9]) at steady state

\[
x_{XD} \cdot x_{Y_p} = \left(\frac{x_{XD}}{x_{XT}}\right) x_{XT} \cdot x_{Y_p} = \left(\frac{k_2 k_4}{k_1 k_3}\right) x_{XT} \cdot x_{Y_p}
\]

**Final restructured (and reweighted) reaction network:**

\[
(N4) \begin{cases}
2XD + XT + Y \xrightleftharpoons[2]{k_1} XD + X + XT + Y \xrightarrow{k_3} XD + 2XT + Y \\
X + XT + XDY_p \xleftarrow[k_9(k_2 k_4/k_1 k_3)]{k_10} X + X + XTY_p \\
XD + X + XT + Y_p \xleftarrow[k_6]{k_5} XD + XT + X_p Y
\end{cases}
\]
Network is **resolvable** (Johnston, [9]) at steady state

\[ x_{XD} \cdot x_{Y_p} = \left( \frac{x_{XD}}{x_{XT}} \right) x_{XT} \cdot x_{Y_p} = \left( \frac{k_2 k_4}{k_1 k_3} \right) x_{XT} \cdot x_{Y_p} \]

**Final restructured (and reweighted) reaction network:**

\[(N4) \begin{cases} 
2XD + XT + Y \xrightarrow{k_1, k_2} XD + X + XT + Y \xrightarrow{k_3} XD + 2XT + Y \\
X + XT + XDY_p \xrightarrow{k_7} XD + X + XTY_p \xrightarrow{k_4} XD + XT + X_p + Y \\
XD + X + XT + Y_p \xleftarrow{k_6} XD + XT + X_p Y
\end{cases}\]
Network is **resolvable** (Johnston, [9]) at steady state

\[ x_{XD} \cdot x_{Y_p} = \left( \frac{x_{XD}}{x_{XT}} \right) x_{XT} \cdot x_{Y_p} = \left( \frac{k_2 k_4}{k_1 k_3} \right) x_{XT} \cdot x_{Y_p} \]

**Final restructured (and reweighted) reaction network:**

\[
\begin{align*}
(N4) \quad \begin{cases}
2XD + XT + Y & \xrightarrow{k_1} XD + X + XT + Y & \xrightarrow{k_3} XD + 2XT + Y \\
X + XT + XDY_p & \xrightarrow{k_9} XD + X + XTY_p & \xrightarrow{k_5} XD + XT + Xp + Y \\
XD + X + XT + Y_p & \xrightarrow{k_6} XD + XT + XpY
\end{cases}
\end{align*}
\]

But many networks **DO NOT** satisfy these technical conditions...
Shuttled Wnt Signaling Pathway: (Gross et al. [12])

\[
\begin{align*}
(1) \quad & E_i \leftrightarrow E_a \leftrightarrow E_a^{(n)}, \quad P^{(n)} + G_i^{(n)} \leftrightarrow G_a^{(n)}, \quad D_i \leftrightarrow D_i^{(n)} \\
& D_a + E_a \leftrightarrow D_a E_a \rightarrow D_i + E_a \\
(2) \quad & D_i + F_a \leftrightarrow D_i F_a \rightarrow D_a + F_a \\
& D_a^{(n)} + E_a^{(n)} \leftrightarrow D_a^{(n)} E_a^{(n)} \rightarrow D_i^{(n)} + E_a^{(n)} \\
& D_i^{(n)} + F_a^{(n)} \leftrightarrow D_i^{(n)} F_a^{(n)} \rightarrow D_a^{(n)} + F_a^{(n)} \\
& D_a + P \leftrightarrow D_a P \rightarrow D_a, \quad P \leftrightarrow P^{(n)} \\
& D_a^{(n)} + P^{(n)} \leftrightarrow D_a^{(n)} P^{(n)} \rightarrow D_a^{(n)}, \quad \emptyset
\end{align*}
\]
Shuttled Wnt Signaling Pathway: (Gross et al. [12])

\[
\begin{align*}
(1) & \quad E_i \xleftrightarrow{\text{input}} E_a \xrightarrow{} E_a^{(n)}, \quad P^{(n)} + G_i^{(n)} \xleftrightarrow{} G_a^{(n)}, \quad D_i \xleftrightarrow{} D_i^{(n)} \\
& \quad D_a + E_a \xleftrightarrow{} D_aE_a \xrightarrow{} D_i + E_a \\
(2) & \quad D_i + F_a \xleftrightarrow{} D_iF_a \xrightarrow{} D_a + F_a \\
& \quad D_a^{(n)} + E_a^{(n)} \xleftrightarrow{} D_a^{(n)}E_a^{(n)} \rightarrow D_i^{(n)} + E_a^{(n)} \\
(3) & \quad D_i^{(n)} + F_a^{(n)} \xleftrightarrow{} D_i^{(n)}F_a^{(n)} \rightarrow D_a^{(n)} + F_a^{(n)} \\
& \quad D_a + P \xleftrightarrow{} D_aP \xrightarrow{} D_a, \quad P \xleftrightarrow{} P^{(n)} \\
& \quad D_a^{(n)} + P^{(n)} \xleftrightarrow{} D_a^{(n)}P^{(n)} \rightarrow D_a^{(n)}, \quad \emptyset
\end{align*}
\]
Kinetic Graph:

(1) $E_i \iff E_a \iff E_a^{(n)}$, \quad $P^{(n)} + G_i \iff G_a$, \quad $D_i \iff D_i^{(n)}$

(2) \begin{align*}
D_a + E_a & \iff D_a E_a \\
D_i F_a & \iff D_i + F_a
\end{align*}

(3) \begin{align*}
D_a^{(n)} + E_a^{(n)} & \iff D_a^{(n)} E_a^{(n)} \\
D_i^{(n)} F_a^{(n)} & \iff D_i^{(n)} + F_a^{(n)}
\end{align*}

(4) \begin{align*}
\left\{ \begin{array}{c} P \\ P + D_a \end{array} \right\} & \iff \left\{ \begin{array}{c} P^{(n)} \\ P^{(n)} + D_a^{(n)} \end{array} \right\} \\
D_a P & \iff \emptyset \iff D_a^{(n)} P^{(n)}
\end{align*}
Kinetic Graph:

(1) \[ E_i \iff E_a \iff E_a^{(n)}, \quad P^{(n)} + G_i \iff G_a, \quad D_i \iff D_i^{(n)} \]

(2) \[
\begin{align*}
D_a + E_a & \iff D_aE_a \\
D_iF_a & \iff D_i + F_a
\end{align*}
\]

(3) \[
\begin{align*}
D_a^{(n)} + E_a^{(n)} & \iff D_a^{(n)}E_a^{(n)} \\
D_i^{(n)}F_a^{(n)} & \iff D_i^{(n)} + F_a^{(n)}
\end{align*}
\]

(4) \[
\begin{align*}
P & \iff P + D_a \\
P + D_a & \iff P^{(n)} + D_a^{(n)}
\end{align*}
\]

\[
\begin{align*}
D_aP & \iff \emptyset & \emptyset & \iff D_a^{(n)}P^{(n)}
\end{align*}
\]
PROBLEM!

This network is crowded and the crowded vertex cannot be resolved!

- We would still like to characterize the steady state set.
- Preliminary results suggest considering subnetworks:
  - Stoichiometry balances across crowded vertices
  - Steady state ideal found by balancing subnetworks
Kinetic Graph:

\[ E_i \iff E_a \iff E_a^{(n)}, \quad P^{(n)} + G_i \iff G_a, \quad D_i \iff D_i^{(n)} \]

\[ D_a + E_a \iff D_a E_a \]

\[ D_i F_a \iff D_i + F_a \]

\[ \{ \begin{array}{l} P \\ P + D_a \end{array} \} \iff \{ \begin{array}{l} P^{(n)} \\ P^{(n)} + D_a^{(n)} \end{array} \} \]

\[ D_a P \iff \emptyset \iff D_a^{(n)} P^{(n)} \]
**Kinetic Graph:**

\[
\begin{align*}
\text{(1)} & \quad E_i & \rightleftharpoons E_a & \rightleftharpoons E_a^{(n)}, \quad P^{(n)} + G_i & \rightleftharpoons G_a, \quad D_i & \rightleftharpoons D_i^{(n)} \\
\text{(2)} & \quad D_a + E_a & \rightleftharpoons D_a E_a \\
\text{(3)} & \quad D_a^{(n)} + E_a^{(n)} & \rightleftharpoons D_a^{(n)} E_a^{(n)} \\
\text{(4)} & \quad \left\{ \begin{array}{c} P \\ P + D_a \end{array} \right\} & \rightleftharpoons \left\{ \begin{array}{c} P^{(n)} \\ P^{(n)} + D_a^{(n)} \end{array} \right\} \\
\end{align*}
\]
Kinetic Graph:

(1) \[ E_i \rightleftharpoons E_a \rightleftharpoons E_a^{(n)}, \quad P^{(n)} + G_i \rightleftharpoons G_a, \quad D_i \rightleftharpoons D_i^{(n)} \]

(2) \[
\begin{align*}
D_a + E_a & \rightleftharpoons D_a E_a \\
D_i F_a & \rightleftharpoons D_i + F_a
\end{align*}
\]

(3) \[
\begin{align*}
D_a^{(n)} + E_a^{(n)} & \rightleftharpoons D_a^{(n)} E_a^{(n)} \\
D_i^{(n)} F_a^{(n)} & \rightleftharpoons D_i^{(n)} + F_a^{(n)}
\end{align*}
\]

(4) \[
\begin{align*}
\{ P \} & \rightleftharpoons \{ P^{(n)} \} \\
\{ P + D_a \} & \rightleftharpoons \{ P^{(n)} + D_a^{(n)} \} \\
D_a P & \rightarrow \emptyset \leftarrow D_a^{(n)} P^{(n)}
\end{align*}
\]
Kinetic Graph:

(1) \[ E_i \rightleftharpoons E_a \rightleftharpoons E_a^{(n)}, \quad P^{(n)} + G_i \rightleftharpoons G_a, \quad D_i \rightleftharpoons D_i^{(n)} \]

(2) \[ \begin{align*}
D_a + E_a & \rightleftharpoons D_a E_a \\
D_i F_a &= \rightleftharpoons D_i + F_a
\end{align*} \]

(3) \[ \begin{align*}
D_a^{(n)} + E_a^{(n)} & \rightleftharpoons D_a^{(n)} E_a^{(n)} \\
D_i^{(n)} F_a^{(n)} &= \rightleftharpoons D_i^{(n)} + F_a^{(n)}
\end{align*} \]

(4) \[ \begin{align*}
\{ P \} & \rightleftharpoons \{ P^{(n)} \} \\
\{ P + D_a \} & \rightleftharpoons \{ P^{(n)} + D_a^{(n)} \} \\
D_a P & \rightarrow \emptyset \rightarrow D_a^{(n)} P^{(n)}
\end{align*} \]
Summary of Approach:

- Correspond a biochemical reaction network to a **generalized network** with better structure (weak reversibility).

- Generalized network has **two sets of complexes** for each vertex (Müller & Regensburger, 2012 [7]).

- Method of **network translation** makes such a correspondence (Johnston, 2014 [9]).

- Recent work also **algorithmizes** the process of network translation (Johnston, 2015 [10]).
Future Work:

- Develop generalized mass action system theory to include:
  
  1. **More varied behaviors** (e.g. multistationarity, persistence, steady state stability, long-term dynamics, etc.)

  2. **Non-traditional embeddings** of kinetic complexes in the reaction graph (e.g. crowded and sparse embeddings)

- Expand scope of application (Conradi and Shiu, 2014 [13])
Thank you!
Selected Bibliography:


