Optimal enzyme profiles in metabolic pathways: principles and simple solutions

Workshop SPT Chemical Reaction Networks

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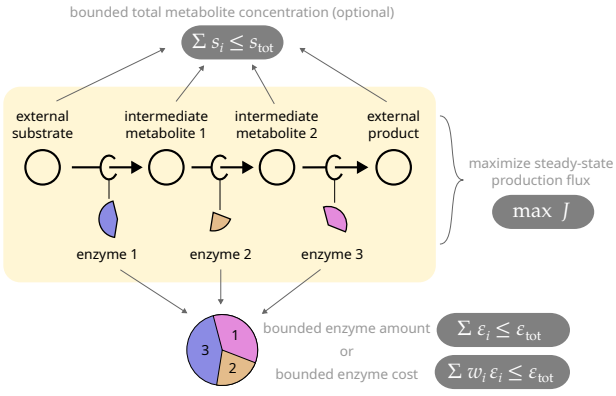
The problem: allocate enzymes to maximize a metabolic pathway flux



Kinetic metabolic models and flux balance models

Kinetic metabolic models describe the metabolic reaction rates v_l depending on enzyme levels ε_l and metabolite concentrations c_i:

$$v_l = \varepsilon_l \; f_l(\mathbf{c})$$

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Metabolite dynamics (for "internal" metabolites i) depends on the rates v_l and on stoichiometric coefficients n_{il}:

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Flux analysis models ignore dynamics and determine steady fluxes $J_l = v_l$ satisfying

$$0 = \sum_l n_{il} \; J_l$$

What is the enzyme cost of a metabolic flux distribution?

Given a stationary flux distribution J_l and remembering the rate laws

$$v_l = \varepsilon_l \; f_l(\mathbf{c}),$$

by solving for the enzyme levels ε_l we obtain the overall enzyme demand

$$\varepsilon_{\text{tot}}(\mathbf{J}) = \sum_l \varepsilon_l = \sum_l \frac{J_l}{f_l(\mathbf{c})}$$

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$$\varepsilon_{\mathrm{tot}}(\mathbf{J}) = \sum_l \varepsilon_l = \sum_l \frac{J_l}{f_l(\mathbf{c})}$$

For fixed concentrations c_i, the enzyme efficiencies $k_l^{\mathrm{app}} = f_l(\mathbf{c})$ are constant and

$$\varepsilon_{\mathrm{tot}}(\mathbf{J}) = \sum_l \frac{J_l}{k_l^{\mathrm{app}}}$$

The demand scales linearly with the fluxes (and hence, with the system's "output flux")

With "trivial" kinetics: enzyme efficiencies are inversely additive

We assume a pathway with stationary flux J and constant efficiencies, $v_l = \varepsilon_l \; k_l^{\mathrm{app}}$. Setting $v_i = J$ and solving for ε_l, we obtain the enzyme demand

$$\varepsilon_{\mathrm{tot}} = \sum_l \varepsilon_l = \sum_l \frac{1}{k_l^{\mathrm{app}}} \cdot J$$

So we can write the pathway flux as

$$J = k_{\mathrm{tot}}^{\mathrm{app}} \, \varepsilon_{\mathrm{tot}}$$

with the effective pathway efficiency

$$k_{\mathrm{tot}}^{\mathrm{app}} = \frac{1}{\sum_l \frac{1}{k_l^{\mathrm{app}}}}$$

The inverse k^{app} are times, and additive like resistances in a series of resistors.

Reversible enzyme kinetics based on Haldane

For a reversible enzyme catalyzed reaction[1]: $S \overset{\mathrm{E}}{\rightleftharpoons} P$

$$v = \varepsilon \cdot \underbrace{\frac{k_+^{\mathrm{cat}}\ s/K_{\mathrm{S}} - k_-^{\mathrm{cat}}\ p/K_{\mathrm{P}}}{1 + s/K_{\mathrm{S}} + p/K_{\mathrm{P}}}}_{k^{\mathrm{app}}}$$

[1] s, p, and ε are the concentrations of S, P, and E

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Haldane further showed that the equilibrium constant satisfies the following relationship:

$$K^{\mathrm{eq}} = \frac{k_+^{\mathrm{cat}}}{k_-^{\mathrm{cat}}} \frac{K_{\mathrm{P}}}{K_{\mathrm{S}}}$$

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The Haldane rate law can be rewritten (Noor et al. [2013]) as[2]:

$$v = \underbrace{\varepsilon \cdot k_+^{\mathrm{cat}}}_{V_{\mathrm{max}}} \cdot \underbrace{\left(1 - e^{\frac{\Delta_r G'}{R\,T}}\right)}_{\eta^{\mathrm{for}}} \cdot \underbrace{\frac{\frac{s}{K_{\mathrm{S}}}}{1 + \frac{p}{K_{\mathrm{P}}} + \frac{s}{K_{\mathrm{S}}}}}_{\eta^{\mathrm{kin}}}$$

[1] s, p, and ε are the concentrations of S, P, and E

[2] where $\Delta_r G' \equiv \Delta_r G'^{\circ} + R\,T \ln(p/s)$ and $\Delta_r G'^{\circ} = -R\,T \ln(K^{\mathrm{eq}})$

Enzyme Cost Minimization: minimize the enzyme demand of a given flux!

Given a pathway with fixed flux and with enzymatic reactions with Haldane rate laws, look for metabolite and enzyme levels that minimize the sum of all enzyme demands.

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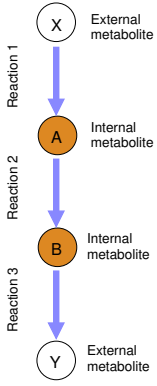
Enzyme demand in one reaction

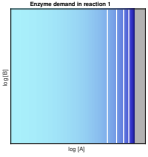
$$\varepsilon = J \cdot \frac{1}{k_+^{\mathrm{cat}}} \cdot \underbrace{\left(1 - e^{\frac{\Delta_r G'}{RT}}\right)^{-1}}_{1/\eta^{\mathrm{for}}} \cdot \underbrace{\frac{1 + \frac{p}{K_{\mathrm{P}}} + \frac{s}{K_{\mathrm{S}}}}{\frac{s}{K_{\mathrm{S}}}}}_{1/\eta^{\mathrm{kin}}}$$

The pathway cost per flux ($\varepsilon_{\mathrm{tot}}/J$) is the sum of demands for all reactions:
a **convex** function of metabolite log-concentrations (Liebermeister and Noor [2015]).

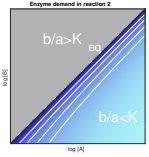
Example: the enzyme demand in a 3-step pathway



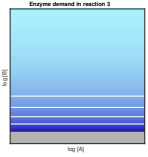
demand in reaction 1



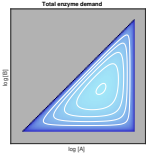
demand in reaction 2

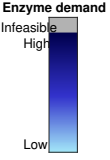


demand in reaction 3



total demand





Can we find analytical solutions to ECM?

Analytical solutions[1] are useful because they:

1. Ensure solution optimality
2. Help find hidden relationships
3. Inspire design principles
4. Suggest mechanisms

[1] Noor and Liebermeister [2024]

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Problem

Kinetic models, where reactions have multi-substrate or Haldane rate laws, do not have known analytical solutions.

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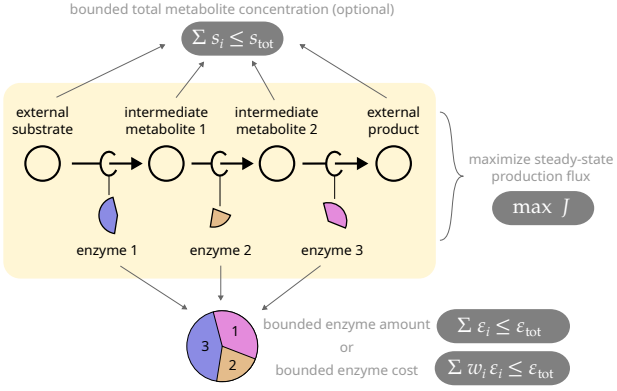
Solution: focus only on unbranched pathways with simplified kinetics.

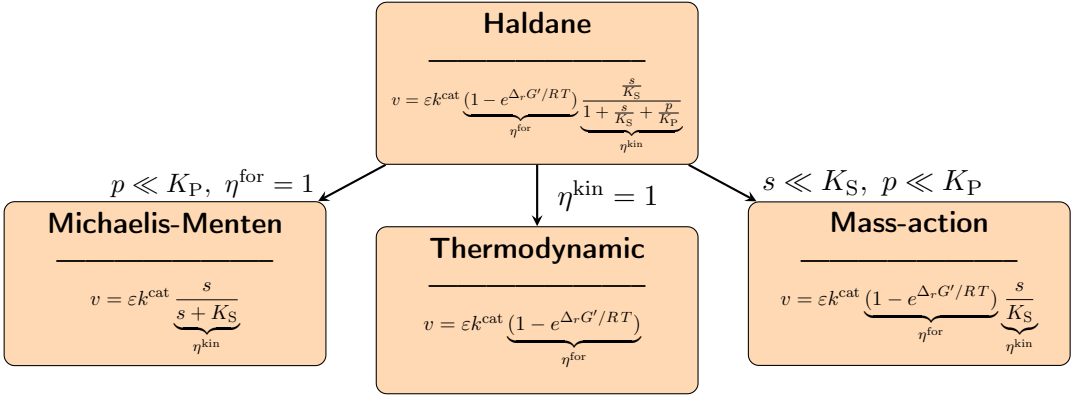
[1] Noor and Liebermeister [2024]

We come back to our full optimality problem

Instead of Enzyme Cost Minization (minimizing enzyme at given fluxes):
Maximize the pathway flux with a bound on enzyme (and possible on metabolite) levels!



The factorized Haldane rate law and some simplifications



The "mass-action" approximation

Haldane:

$$v = \varepsilon \cdot \frac{k_+^{\mathrm{cat}}\ s/K_{\mathrm{S}} - k_-^{\mathrm{cat}}\ p/K_{\mathrm{P}}}{1 + s/K_{\mathrm{S}} + p/K_{\mathrm{P}}}$$

If we assume:

$$s \ll K_{\mathrm{S}},\ p \ll K_{\mathrm{P}}$$

Therefore:

$$v = \varepsilon\ \cdot\ \left(\underbrace{k_+^{\mathrm{cat}}/K_{\mathrm{S}}}_{k_+}\ \cdot\ s\ \ -\ \ \underbrace{k_-^{\mathrm{cat}}/K_{\mathrm{P}}}_{k_-}\ \cdot\ p \right)$$

Unbranched pathway with "mass-action" kinetics

$$\mathsf{S}_0 \overset{\mathrm{v}_1}{\rightleftharpoons} \mathsf{S}_1 \overset{\mathrm{v}_2}{\rightleftharpoons} \ldots \overset{\mathrm{v}_n}{\rightleftharpoons} \mathsf{S}_n$$

$$\forall i \quad J = \varepsilon_i \cdot (k_i \; s_{i-1} - k_{-i} \; s_i)$$

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Optimized flux solution[2]

$$J^* = \varepsilon_{\mathrm{tot}} \cdot \frac{s_0 - s_n/K^{\mathrm{eq}}}{\left(\sum_i \sqrt{\gamma_i}\right)^2}$$

where $\gamma_i \equiv \frac{\prod_{j=i+1}^{n} k_j}{\prod_{j=i}^{n} k_{-j}}$ and $K^{\mathrm{eq}} \equiv \frac{\prod_{j=1}^{n} k_j}{\prod_{j=1}^{n} k_{-j}}$

[2] Waley [1964], Burns [1971]

Unbranched pathway with "thermodynamic" kinetics

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$$J = \varepsilon_i \; k_i^{\mathrm{cat}} \left(1 - e^{\Delta_r G_i'/RT}\right)$$

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Optimized flux (approximated) solution[3]

$$J^* \approx \varepsilon_{\mathrm{tot}} \cdot \frac{1}{a}\left(1 - \exp\left(\frac{a}{b}\,\frac{\Delta_r G'_{\mathrm{tot}}}{RT}\right)\right)$$

where $a \equiv \sum_j \frac{1}{k_j^{\mathrm{cat}}}$, $b \equiv \sum_j \frac{1}{\sqrt{k_j^{\mathrm{cat}}}}$, and $\Delta_r G'_{\mathrm{tot}} \equiv \sum_j \Delta_r G'_j$

[3] Noor and Liebermeister [2024]

Unbranched pathway with Michaelis-Menten kinetics

$$\mathsf{S}_0 \underset{}{\overset{\mathrm{v}_1}{\rightleftharpoons}} \mathsf{S}_1 \overset{\mathrm{v}_2}{\rightleftharpoons} \ldots \overset{\mathrm{v}_n}{\rightleftharpoons} \mathsf{S}_n$$

$$\forall i \quad J = \varepsilon_i \cdot k_i^{\mathrm{cat}} \, \frac{s_{i-1}}{s_{i-1} + K_{\mathrm{i}}}$$

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Optimizing J would require infinite metabolite concentrations!
Therefore we add a total metabolite constraint: $\sum_i s_i \leq s_{\mathrm{tot}}$

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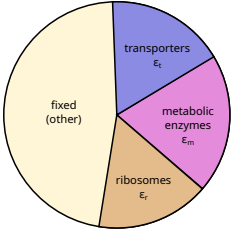
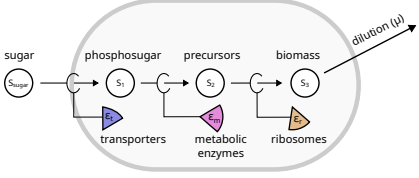
Optimized flux solution[4]

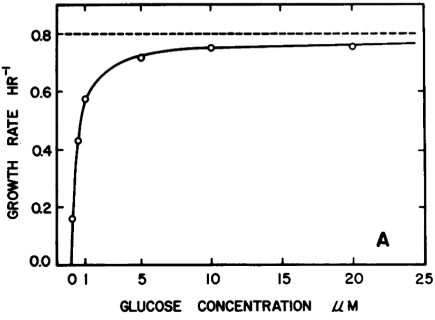
$$J^* = \varepsilon_{\mathrm{tot}} \cdot \frac{1}{\sum_j 1/k_j^{\mathrm{cat}} + \left(\sum_j \sqrt{K_{\mathrm{j}}/k_j^{\mathrm{cat}}}\right)^2 \Big/ s_{\mathrm{tot}}}$$

[4] Noor and Liebermeister [2024]

An application of simple pathway models: a model of a growing cell







Monod curve for *E. coli* on glucose (Shehata and Marr [1971]).

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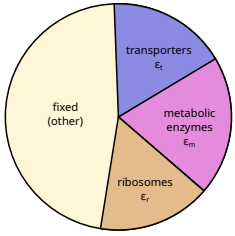

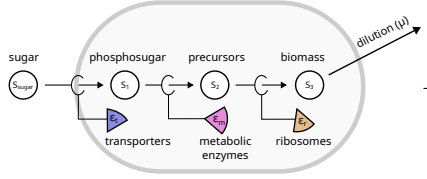

Assume:

- **Coarse-graining:** 3 enzymatic processes

  $$\mathrm{S_{sugar}} \overset{\varepsilon_t}{\rightleftharpoons} \mathrm{S_1} \overset{\varepsilon_m}{\rightleftharpoons} \mathrm{S_2} \overset{\varepsilon_r}{\rightleftharpoons} \mathrm{S_3} \overset{\mu}{\longrightarrow}$$

- **Controlled parameter:** s_{sugar}
- **Constraint:** $\varepsilon_t + \varepsilon_m + \varepsilon_r = \varepsilon_{\mathrm{tot}}$
- **Optimization:** maximize growth rate (μ)
- **Rate laws:** mass-action, thermodynamic, or Michaelis-Menten[a]
- **Not included:** metabolite dilution by growth

[a] **Extra constraint:** $s_{\mathrm{tot}} \geq s_1 + s_2 + s_3$

Cell model example (1): mass-action kinetics

$$\mu = \varepsilon_{\mathrm{tot}} \cdot \frac{s_0 - s_n/K^{\mathrm{eq}}}{\left(\sum_i \sqrt{\gamma_i}\right)^2}$$

where

$$\gamma_t \equiv \frac{K_{\mathrm{M;t}}}{k_{\mathrm{t}}^{\mathrm{cat}}} \cdot K_r^{\mathrm{eq}} \cdot K_m^{\mathrm{eq}} \cdot K_t^{\mathrm{eq}}$$

$$\gamma_m \equiv \frac{K_{\mathrm{M;m}}}{k_{\mathrm{m}}^{\mathrm{cat}}} \cdot K_r^{\mathrm{eq}} \cdot K_m^{\mathrm{eq}}$$

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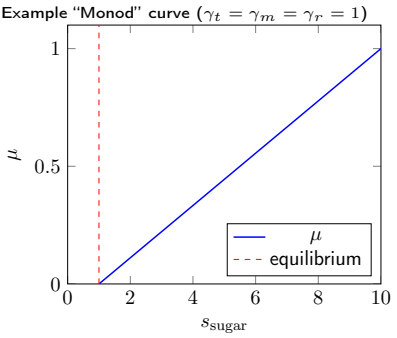

Cell model example (2): "thermodynamic" kinetics

$$\mu \approx \varepsilon_{\mathrm{tot}} \cdot \frac{1}{a}\left(1 - e^{-\frac{a}{b}\cdot \ln\left(s_{\mathrm{sugar}}\frac{K^{\mathrm{eq}}}{s_3}\right)}\right)$$

where

$$a \equiv \frac{1}{k_{\mathrm{t}}^{\mathrm{cat}}} + \frac{1}{k_{\mathrm{m}}^{\mathrm{cat}}} + \frac{1}{k_{\mathrm{r}}^{\mathrm{cat}}}$$

$$b \equiv \frac{1}{\sqrt{k_{\mathrm{t}}^{\mathrm{cat}}}} + \frac{1}{\sqrt{k_{\mathrm{m}}^{\mathrm{cat}}}} + \frac{1}{\sqrt{k_{\mathrm{r}}^{\mathrm{cat}}}}$$

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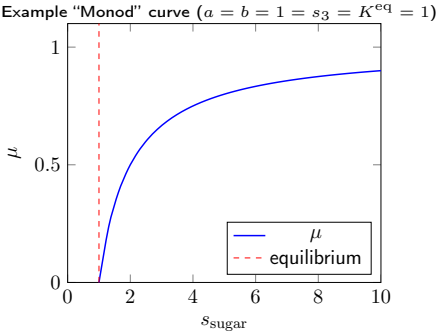
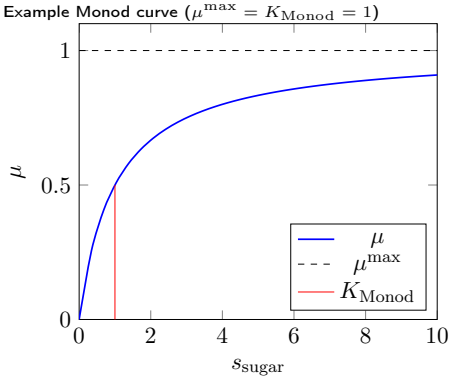


Cell model example (3): Michaelis-Menten kinetics

$$\mu = \mu^{\max} \cdot \frac{s_{\text{sugar}}}{s_{\text{sugar}} + K_{\text{Monod}}}$$

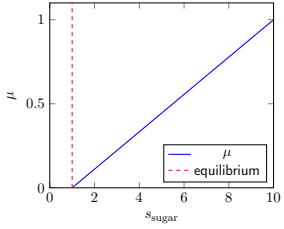
$$\mu^{\max} \equiv \frac{\varepsilon_{\text{tot}}}{\frac{1}{k_{\text{t}}^{\text{cat}}} + \frac{1}{k_{\text{m}}^{\text{cat}}} + \frac{1}{k_{\text{r}}^{\text{cat}}} + \left(\sqrt{\frac{K_{\text{M;m}}}{k_{\text{m}}^{\text{cat}}}} + \sqrt{\frac{K_{\text{M;r}}}{k_{\text{r}}^{\text{cat}}}}\right)^2 \frac{1}{s_{\text{tot}}}}$$

$$K_{\text{Monod}} \equiv \frac{K_{\text{M;t}}/k_{\text{t}}^{\text{cat}}}{\frac{1}{k_{\text{t}}^{\text{cat}}} + \frac{1}{k_{\text{m}}^{\text{cat}}} + \frac{1}{k_{\text{r}}^{\text{cat}}} + \left(\sqrt{\frac{K_{\text{M;m}}}{k_{\text{m}}^{\text{cat}}}} + \sqrt{\frac{K_{\text{M;r}}}{k_{\text{r}}^{\text{cat}}}}\right)^2 \frac{1}{s_{\text{tot}}}}$$



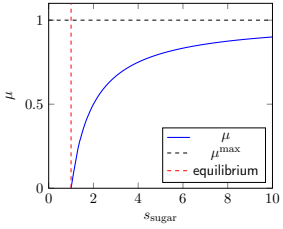
Summary of three cell models

Mass-action



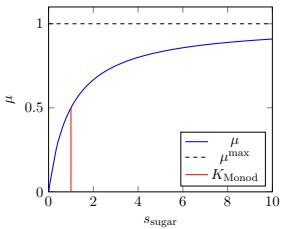
$$\mu = a \cdot s_{\text{sugar}} \cdot \left(1 - \frac{s_3}{s_{\text{sugar}}\ K^{\text{eq}}}\right)$$

Thermodynamic



$$\mu \approx \mu_{\max} \cdot \left(1 - \left(\frac{s_3}{s_{\text{sugar}}\ K^{\text{eq}}}\right)^b\right)$$

Michaelis-Menten



$$\mu = \mu_{\max} \cdot \frac{s_{\text{sugar}}}{s_{\text{sugar}} + K_{\text{Monod}}}$$

Take-home messages

- Enzyme Cost Minimization is a **convex** problem

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- For some special cases, we provide **analytical** solutions

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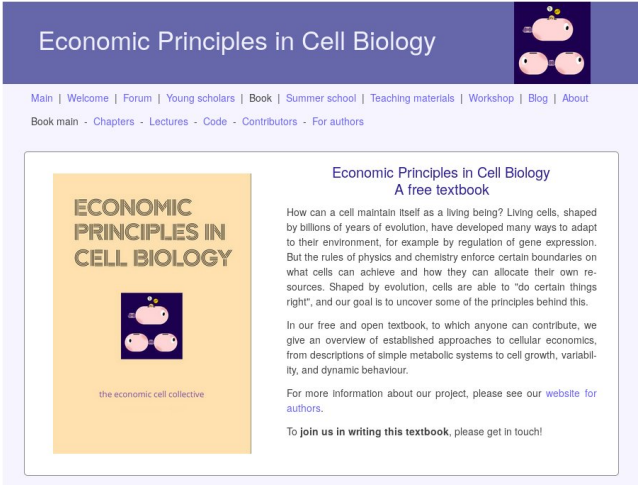
- For some special cases, we provide **analytical** solutions
- We can use these results to build **corse-grained** cell models

Activities around Economic Principles in Cell Biology



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  1st Thursday of each month

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For more information, see https://principlescellphysiology.org

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