L15 Metabolic Engineering

Instructor: Prof. **K. Solomon Ph.D.**
Assistant Professor
Agricultural & Biological Engineering
Laboratory for Renewable Resources Engineering

Fall 2018

Recall...

Parts assembled via variety of techniques

Can be delivered via plasmid or genome

Genomes have properties that are critical for function

2

This lecture

- What is metabolism?
- Why metabolic engineering?
- Review Michaelis-Menten Kinetics
- Metabolic Engineering Strategies

3

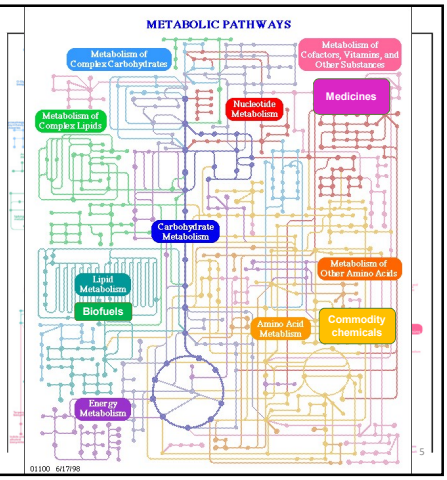
What is metabolism?

Metabolism is the network of reactions cells do to:

- Generate energy
- Create building blocks of biomass
- Protect itself from the environment and compete

4

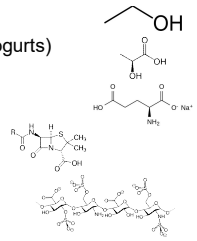
What is metabolism?



Metabolism

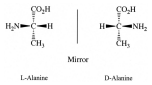
Examples of molecules possible from biology:

- Ethanol (yeast in beer/wine)
- Lactic acid (*Lactobacillus* in yogurts)
- MSG/flavors (*C. glutamicum)*
- Penicillin
- Heparin (anticoagulant)
- Proteins
- methane



Microbial metabolism

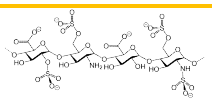
- Microbes make diverse products
- Products are stereospecific
  - ex: L-amino acids only, no D-amino acids
- Use renewable feedstocks



Stereospecificity and wealth of chemistry makes microbes attractive platforms for engineering

Metabolic engineering vs traditional synthesis

Heparin Synthesis

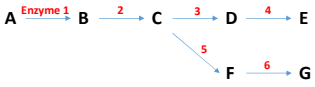


Chemical Synthesis	Enzymatic Synthesis
• Up to 55 steps	• 4 Enzymatic reactions
• Non-stereospecific steps result in unwanted products	• Enzymes are stereospecific

Metabolic engineering

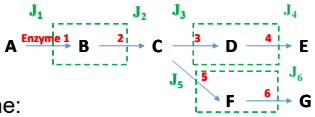
Can represent metabolism with:

- Products formed from metabolic reactions
- Reactions catalyzed by enzymes encoded by genes



9

Metabolic engineering



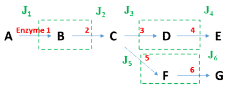
We can define:

- Products/Target Compounds (e.g. E)
- Substrates (reactants) (e.g. A)
- Side Products (e.g. G)
- Intermediates – metabolic nodes between products and substrates (e.g. B, C, D, F)
- Metabolic fluxes (J) – defined as flow of metabolite in or out of arbitrary control volume
  - Typically just reaction rate given by Michaelis-Menten kinetics

What is the relationship between J_2 and J_1?
J_2 and J_5?

10

Metabolic engineering



Goal: Maximize flux towards product

- System is most productive when concentration of intermediates are at steady state

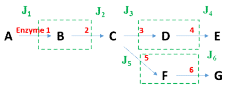
$$\frac{dB}{dt} = \frac{dC}{dt} = \frac{dD}{dt} = \frac{dF}{dt} = 0$$

Why aren't $\frac{dA}{dt}$, $\frac{dE}{dt}$, $\frac{dG}{dt}$ = 0?

11

Metabolic engineering

Mass balances around branch points at steady state are useful



- Balance of C:

$$\frac{dC}{dt} = Influx - Outflux$$

$$= J_2 - J_3 - J_5 = 0$$

$$J_2 = J_3 + J_5$$

$$J_2 = J_3 + J_5$$

$$\frac{k_{cat2}[E_2]_0[B]}{K_{m2} + [B]} = \frac{k_{cat3}[E_3]_0[C]}{K_{m3} + [C]} + \frac{k_{cat5}[E_5]_0[C]}{K_{m5} + [C]}$$

Fluxes given by Michaelis-Menten kinetics

12

Review: Michaelis-Menten Kinetics

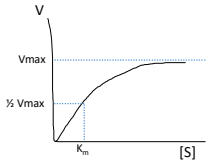
$E + S \underset{k_r}{\overset{k_f}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$

E = Enzyme
S = Substrate
ES = Enzyme-substrate complex
k_f = Forward rate constant
k_r = Reverse rate constant
k_{cat} = Catalytic rate constant (turnover number)
Molecules of S converted to P per second

13

Review: Michaelis-Menten Kinetics

$$V = \frac{dP}{dt} = V_{max}\frac{[S]}{K_m + [S]} = k_{cat}[E_0]\frac{[S]}{K_m + [S]}$$



- V = reaction rate
- [S] = concentration of substrate S
- V_{max} = maximum rate achieved by system
  - At saturating substrate concentration
- K_m = Substrate concentration when rate is ½ Vmax

14

Review: Michaelis-Menten Kinetics

$$V = k_{cat}[E]_0\frac{[S]}{K_m + [S]}$$

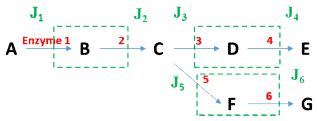
Reaction order:

[S] << K_m : **1st order** $V \approx k_{cat}[E]_0\frac{[S]}{k_m}$
Rate varies linearly with [S]

[S] >> K_m : **0th order** $V \approx k_{cat}[E]_0$
Rate approaches V_{max}
V_{max} = All E bound to S

15

Example 1

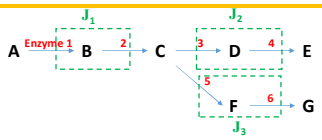


$K_{m,enz1}$ = 0.25 μM
[A] = 0.10 μM

How does one improve production of B?

16

Example 1



$K_{m,enz1}$ = 0.25 μM
[A] = 0.10 μM

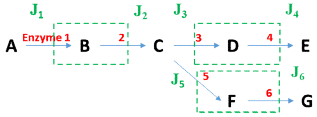
1st Order
$V = Vmax\frac{[A]}{k_m}$

How does one improve production of B?

[A] < K_m
Rate is ~ linear in [A]
Increase [A] to increase rate

17

Example 2

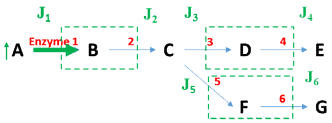


$K_{m,enz1}$ = 0.25 μM
[A] = 5.0 μM

How does one improve production of B?

18

Optimizing product formation



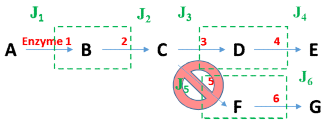
How do you optimize product formation (E)?

1. **Push flux from A**
   - Increase [A]
   - Increase $[E_1]_T$ (upregulate enzyme 1)

$V = k_{cat}[E_1]_0\frac{[A]}{k_m + [A]}$

19

Optimizing product formation



How do you optimize product formation (E)?

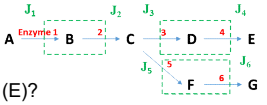
$$\frac{k_{cat2}[E_2]_T[B]}{K_{m2} + [B]} = \frac{k_{cat3}[E_3]_T[C]}{K_{m3} + [C]} + \frac{k_{cat5}[E_5]_T[C]}{K_{m5} + [C]}$$

2. **Pull flux from branch point (C)**
   - Increase $[E_3]_T$ – overexpression
   - Decrease $[E_5]$ – downregulate or knockout

20

Optimizing product formation

$$\frac{k_{cat2}[E_2]_T[B]}{K_{m2}+[B]} = \frac{k_{cat3}[E_3]_T[C]}{K_{m3}+[C]} + \frac{k_{cat5}[E_5]_T[C]}{K_{m5}+[C]}$$



How do you optimize product formation (E)?

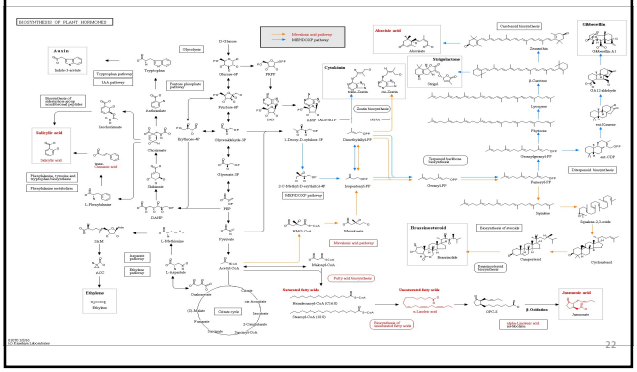
3. **Improve kinetics of enzymes (e.g. Enz 3)**
   - Replace enzyme with homolog of similar function but superior kinetics
     - Databases: Genbank, KEGG, MetaCyc – info on sequences, metabolism of various organisms
     - Kinetic info in BRENDA
   - Reduce diffusional barriers
     - e.g. introduce substrate channeling where desired pathway is physically co-localized in complex or scaffold
       - e.g. Cellulosome for cellulose hydrolysis

http://www.weizmann.ac.il/Biomolecular_Sciences/Bayer/research-activities/biofuels

21

KEGG (Kyoto Encyclopedia of Genes and Genomes)

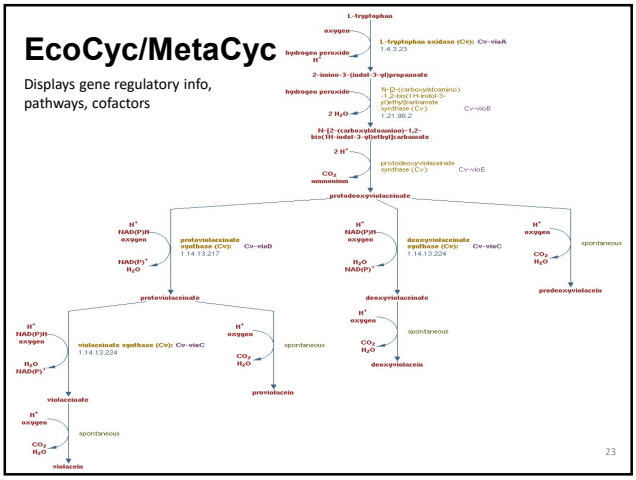
Displays chemistries



22

EcoCyc/MetaCyc

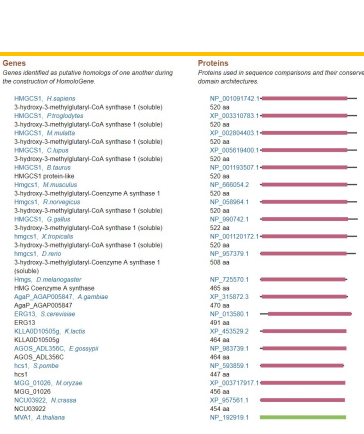
Displays gene regulatory info, pathways, cofactors



23

GenBank

Homolog search



24

BRENDA (Braunschweig Enzyme Database)

Kinetic information

KM VALUE [mM]	SUBSTRATE	ORGANISM	UNIPROT	COMMENTARY	LITERATURE	IMAGE
0.5 - 1.16	2-deoxy-D-glucose	⊞ 4 entries				
0.07 - 3.05	ATP	⊞ 36 entries				
5	CTP	Homo sapiens	-	MgCTP2-, hexokinase I, at 37°C, pH 8.1	640237	
10 - 157.7	D-fructose	⊞ 3 entries				
0.5 - 1.5	D-glucosamine	⊞ 3 entries				
0.032 - 3.8	D-glucose	⊞ 15 entries				
0.07 - 4.6	D-mannose	⊞ 3 entries				
16.6	ITP	Homo sapiens	-	MgITP2-, hexokinase I, at 37°C, pH 8.1	640237	
5	UTP	Homo sapiens	-	MgUTP2-, hexokinase I, at 37°C, pH 8.1	640237	
additional information	additional information	Homo sapiens	-	-	640245	-

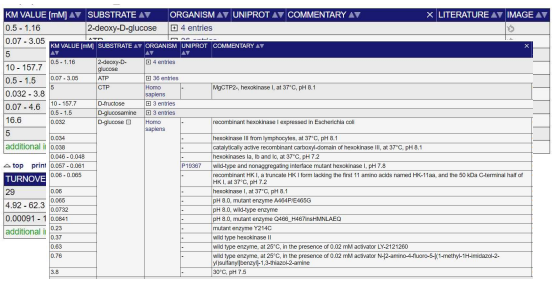
top print hide 32 entries ⊞

TURNOVER NUMBER [1/s]	SUBSTRATE	ORGANISM	UNIPROT	COMMENTARY	LITERATURE	IMAGE
29	2-deoxy-D-glucose	Homo sapiens	-	wild-type enzyme	672188	
4.92 - 62.3	ATP	⊞ 10 entries				
0.00091 - 101	D-glucose	⊞ 19 entries				
additional information	additional information	⊞ 2 entries				-

25

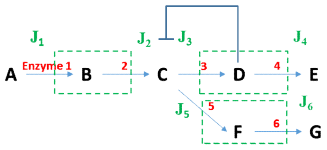
BRENDA

Kinetic information



26

Optimizing product formation



How do you optimize product formation (E)?

4. **Introduce feedback to detect presence of key intermediates and control activity accordingly**
   - e.g. if D is essential for product but toxic to cell, want to keep [D] as high as is safe

27

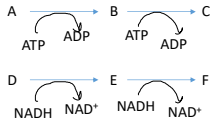
Can also improve production with fermentation optimization

- Cofactors are used by many reactions
  - E.g. ATP, NADH, NADPH, $FADH_2$, etc

  What are the functions of these cofactors?

- Altering production pathways will affect cofactor pools and thus cell health and growth

  Ideal situation: Production = Consumption



Energy intensive pathway
- Can increase aeration

Pathway needs reduced co-factors
- Make process anaerobic

28

We can also engineer *non-natural* products

Enzymes are ***promiscuous***

- will catalyze the same chemistry for similar molecules.
  - e.g. Alcohol dehydrogenase (ADH) reducing acetylaldehyde to EtOH (C_2) or proponaldehyde to propanol (C_3)

- Given starting substrate and final product, we can identify synthesis pathway (enzymes) required. (***Retrobiosynthesis***)

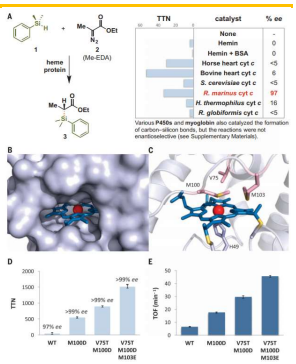
- Can use databases to identify candidates to screen

29

Enzyme promiscuity

- Directed evolution
- Cytochrome P450
  - Natural oxygenase activity
  - Mutated to incorporate Si

First biological C-Si bonds evolved in lab



Kan et al, 2016 Science 354: 1048-1051

30

Metabolic engineering summary

Regulation of pathways critical to:

- Maximize product formation
- Minimize impact on host processes (e.g. cofactor balance)
- Prevent build-up of toxic intermediates

Achieved by:

- **Push/Pull** - Expression of enzymes at appropriate levels (promoters, RBS, term)
- **Feedback** - Programmable control of expression (biological feedback through inducible/repressible promoters or independent control)
- **Homologs** - Identifying enzymes with sufficient activity
- **Process optimization**

31

Metabolic engineering

Engineering host may be required

- To reduce product/intermediate toxicity
- Remove native regulation of enzymes
- Reduce consumption of product

32

Next time

- Practical examples: journal club

33