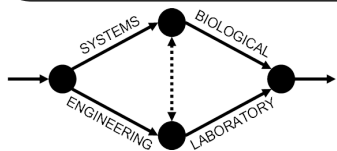
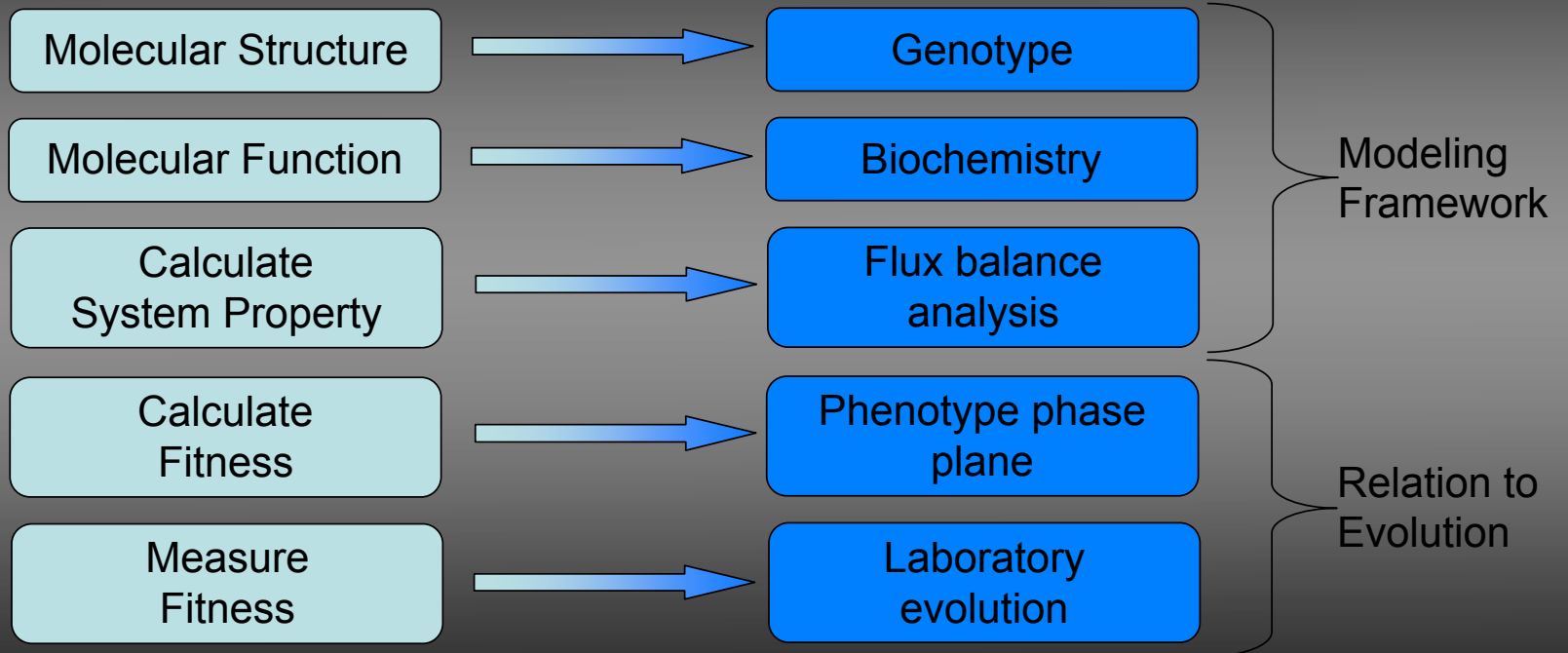


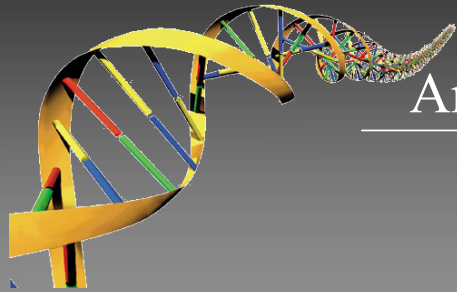
Integration of Metabolic Modeling and Laboratory Evolution

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Department of Chemical and Life Science Engineering

Conceptual Overview



Constraint-based Modeling



Annotation →

argE

ppc

Phosphoenolpyruvate + H₂O + CO₂

Oxaloacetate + Phosphate

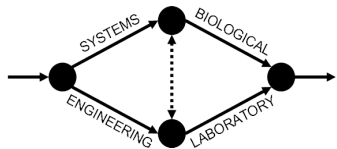
Phosphoenolpyruvate
 H₂O
 CO₂
 Oxaloacetate
 Phosphate

$$\begin{pmatrix} -1 \\ -1 \\ -1 \\ 1 \\ 1 \end{pmatrix}$$

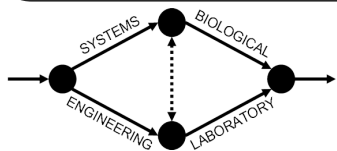
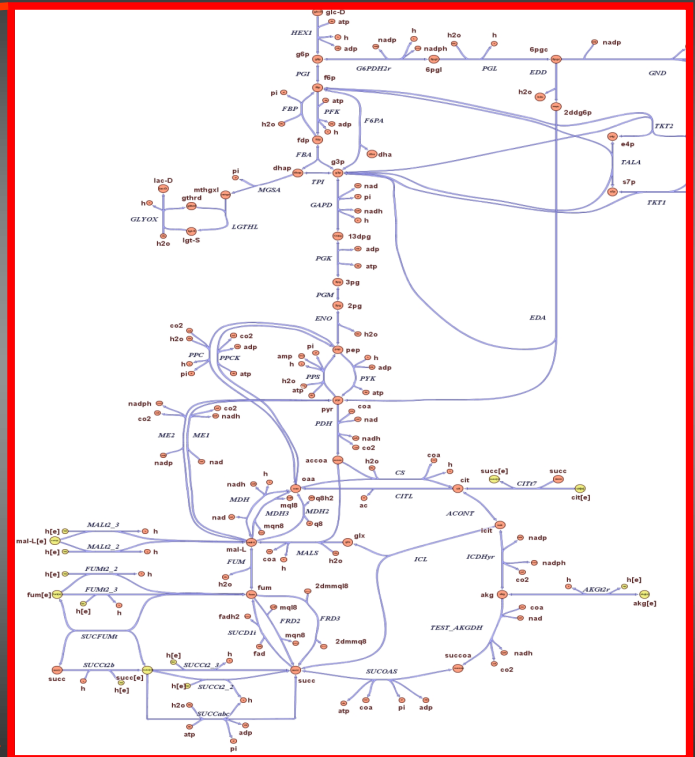
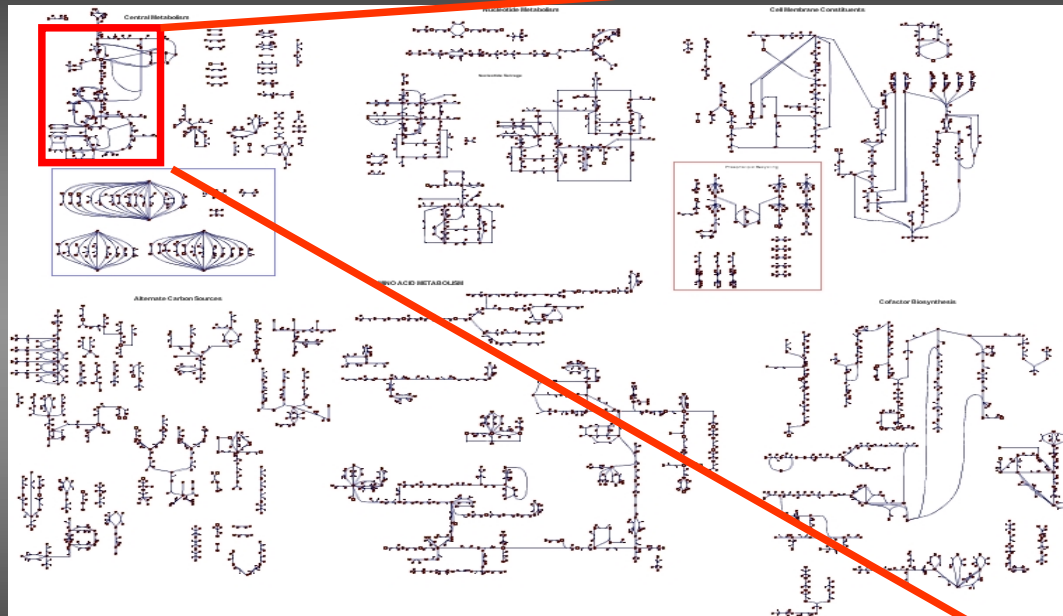
compounds

reactions →

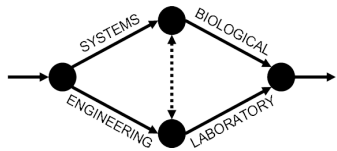
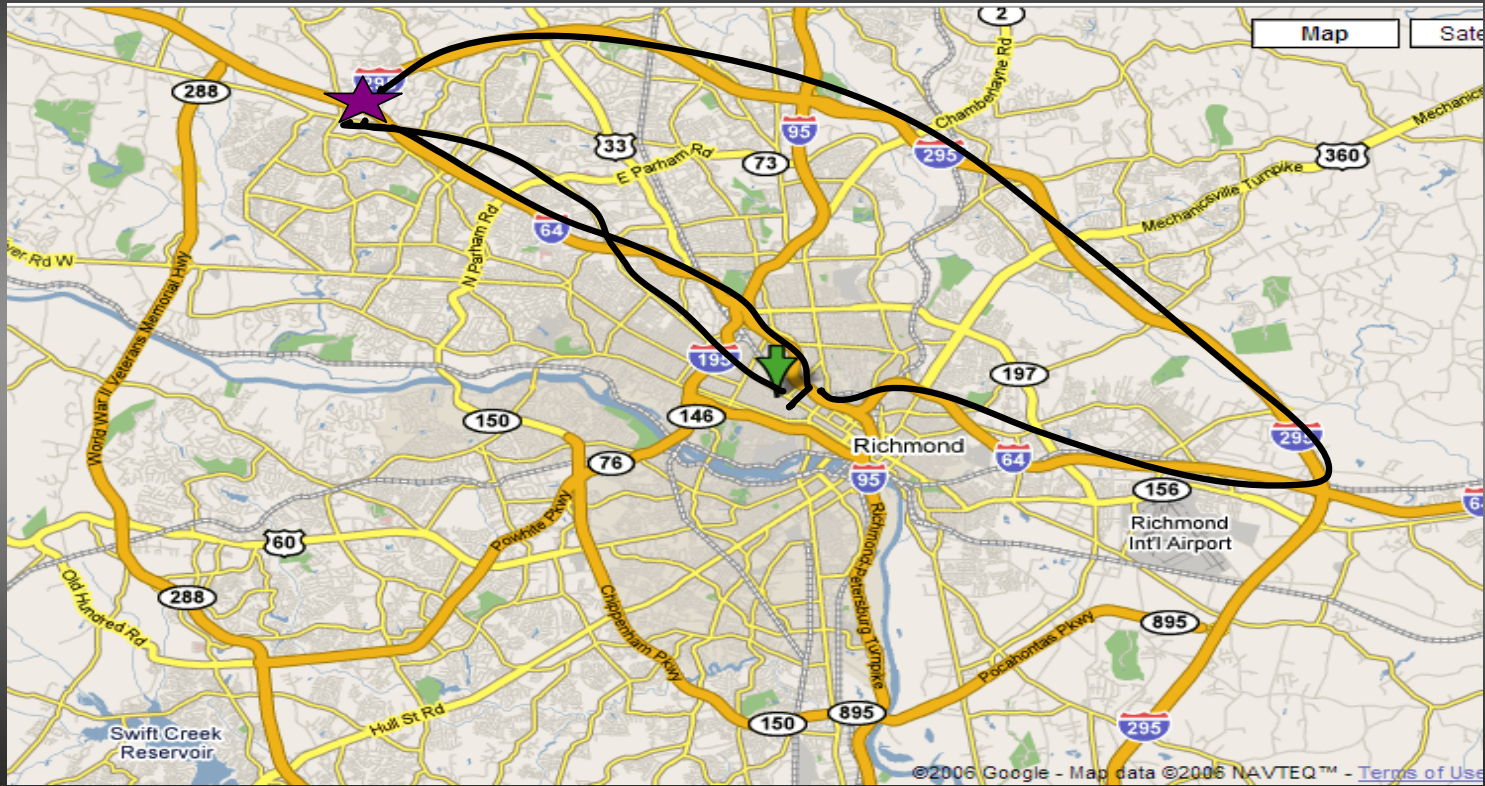
| | v_i |
|---|-------|
| A | -a |
| B | 0 |
| C | -c |
| D | 0 |
| E | +e |
| F | 0 |
| G | 0 |
| H | +h |



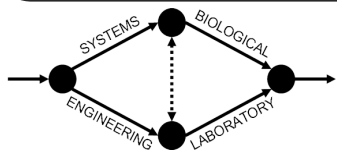
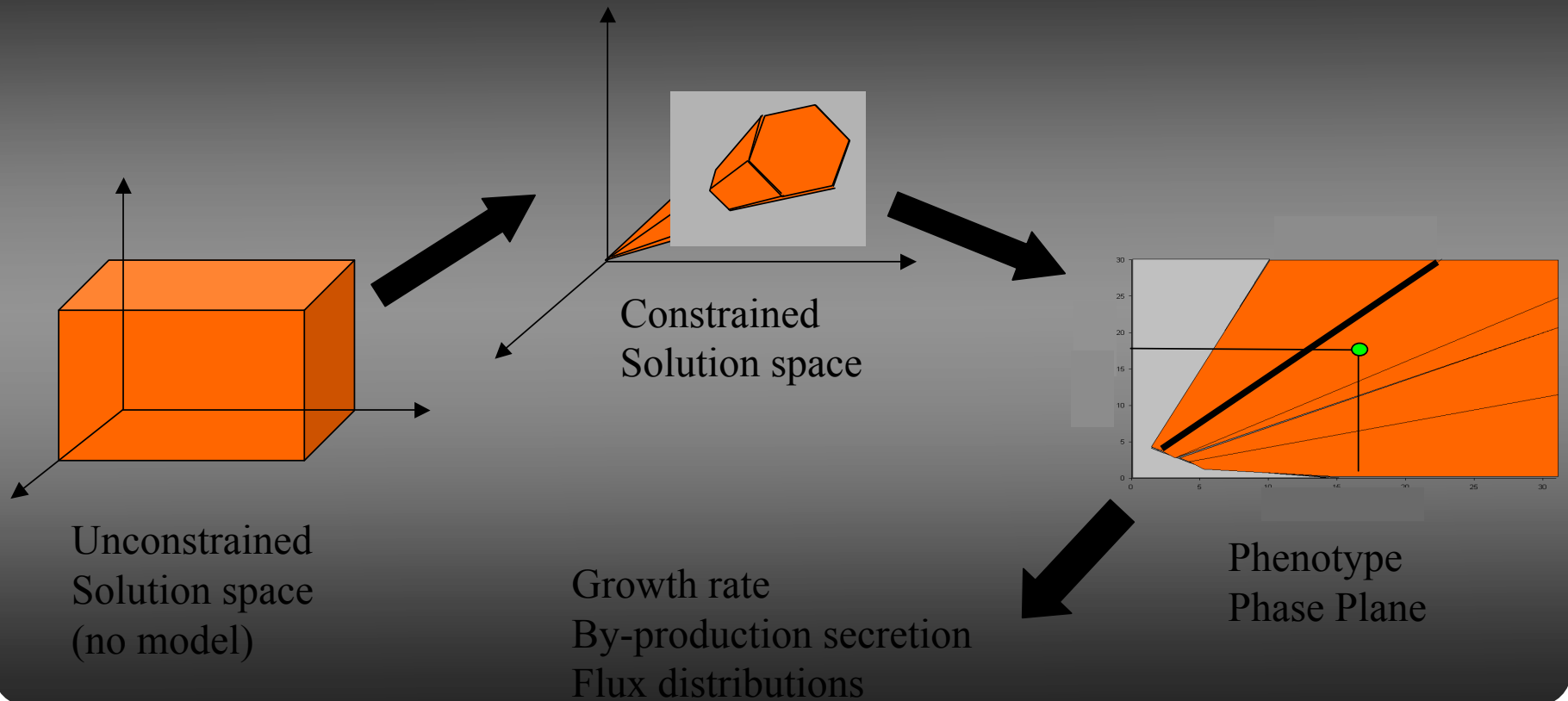
Escherichia coli Metabolic Network



Road Map – getting from A to B



Flux Balance Analysis



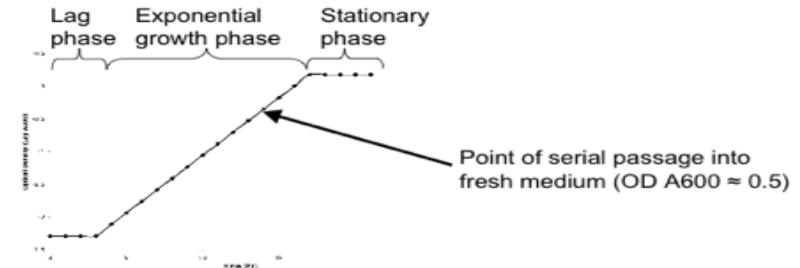
Adaptive Evolution

Serial passage during exponential growth

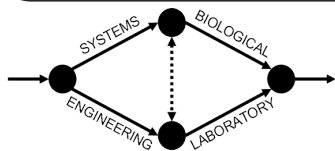
Stable growth rate achieved at end of evolution

Cells frozen throughout evolution for phenotype testing

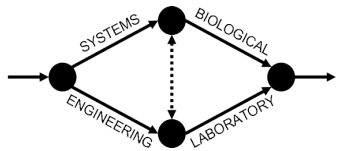
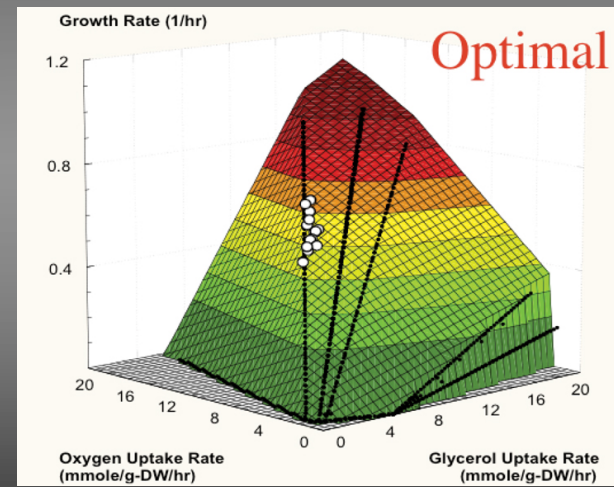
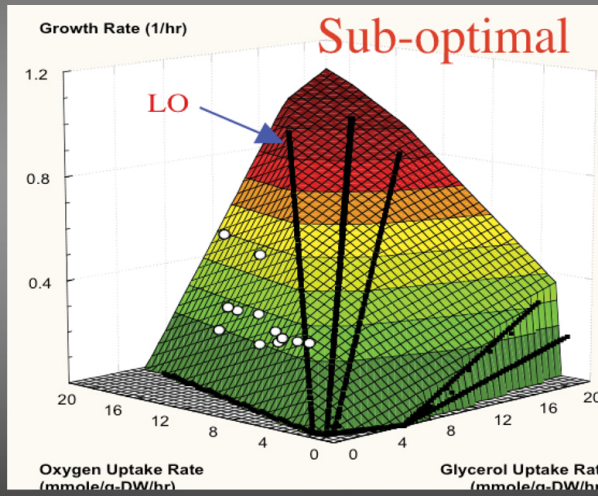
Single Batch Culture



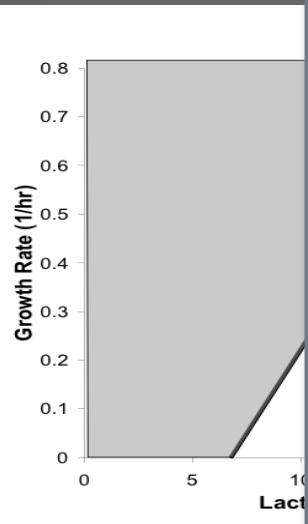
Fong et al., Genome Research 2005



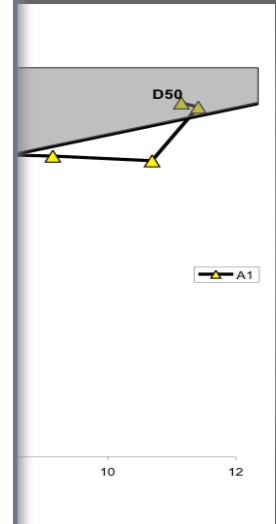
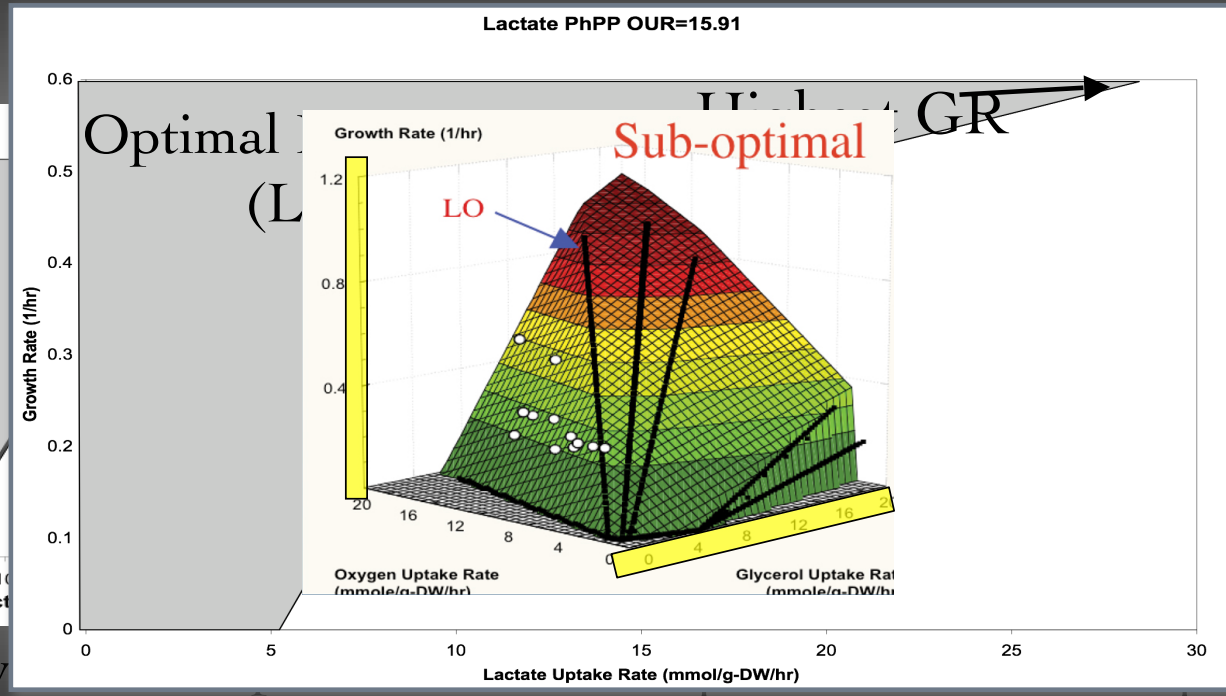
3-D Phase Plane



Fitness - Growth rate or efficiency?

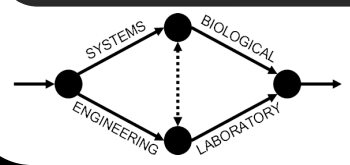


Optimal yield



Optimal yield

Fong et al. 2003 *J. Bacteriology*



Deletion strains

ack

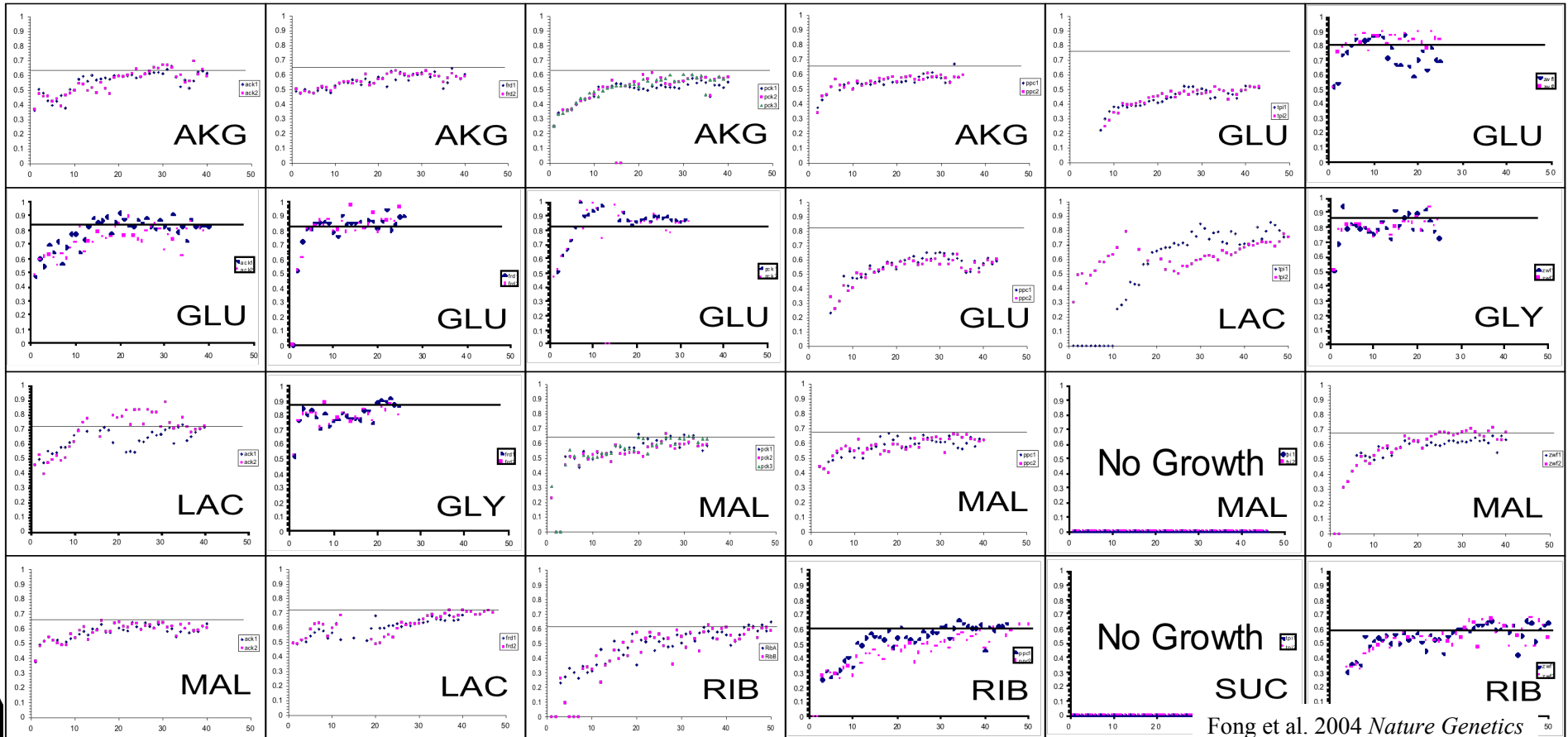
frd

pck

ppc

tpi

zwf



Fong et al. 2004 *Nature Genetics*

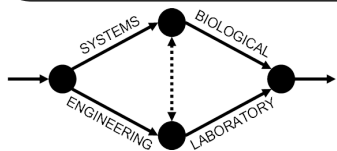
More Detail Please!

Pathway

- Obtain transcriptomic or proteomic data
 - Mixed-integer linear programming with model
- Metabolic network corresponding to experimental state

Reaction

- Genome re-sequencing
 - Bioinformatic and molecular biology to determine effects
- Characterization of enzymatic changes



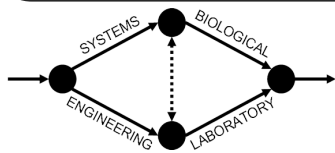
Mapping Transcriptomic or Proteomic data

| | Detected | Not detected |
|--------------|----------|--------------|
| Nonzero flux | | |
| Zero flux | | |

$$\begin{aligned}
 & \max_{v, y^+, y^-} \left(\sum_{i \in R_H} (y_i^+ + y_i^-) + \sum_{i \in R_L} y_i^+ \right) \\
 & s.t. \\
 & \bar{S} \cdot v = 0 \tag{1} \\
 & v_{min} < v < v_{max} \tag{2} \\
 & v_i + y_i^+ (v_{min,i} - \epsilon) \geq v_{min,i}; i \in R_H \tag{3} \\
 & v_i + y_i^- (v_{max,i} + \epsilon) \leq v_{max,i}; i \in R_H \tag{4} \\
 & v_{min,i} (1 - y_i^+) \leq v_i \leq v_{max,i} (1 - y_i^-); i \in R_L \tag{5} \\
 & v \in R^m \\
 & y_i^+, y_i^- \in [0, 1]
 \end{aligned}$$

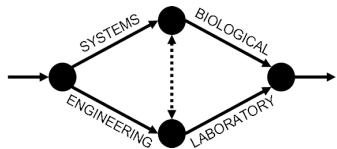
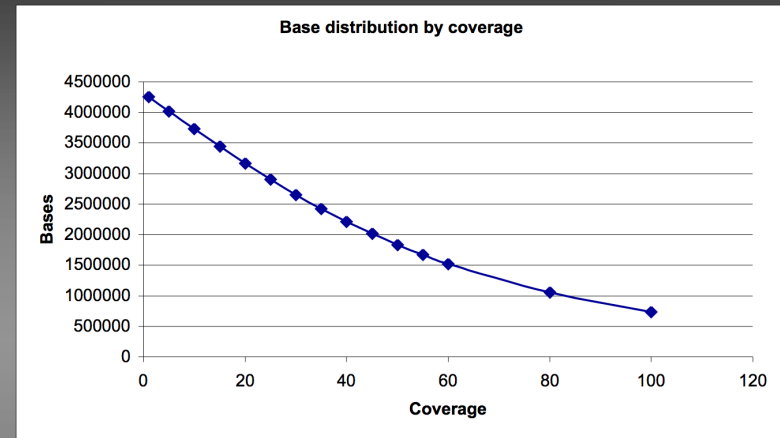
Schlomi et al 2008 *Nature Biotech.*

*lower bound, biomass flux > 0



Illumina Genome Re-sequencing

- Range of Fold coverage: 0-1000+
- Average Fold coverage: 56x
- 1st pass analysis on fragments with unique alignment with maximum of one mismatch



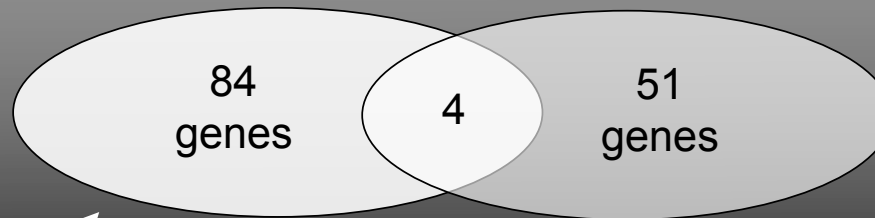
Sample Re-sequencing Results

pta-adhE strains (5)

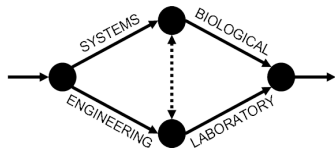
| Strain | Criteria | | |
|--------|----------|--------|-----------|
| | Fold | % call | Mutations |
| 1 | 25 | 85 | 18 |
| 2 | 10 | 85 | 13 |
| 3 | 20 | 85 | 11 |
| 4 | 30 | 100 | 35 |
| 5 | 30 | 100 | 22 |

pta-pfk strains (3)

| Strain | Criteria | | |
|--------|----------|--------|-----------|
| | Fold | % call | Mutations |
| 1 | 5 | 85 | 17 |
| 2 | 5 | 85 | 15 |
| 3 | 5 | 85 | 27 |

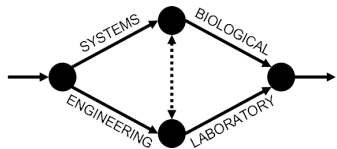


2 mutations in multiple strains



Potential Applications

- Computational strain design (couple multiple objectives)
- Evolution-produced stable strains
- Increase cellulase expression/activity



Research Group

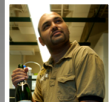


Post-doc: Seth Roberts, Ph.D.

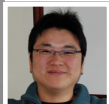


Lab manager: Cindy Lovelace

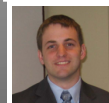
Graduate students:



Advait Apte



Yu Deng



Chris Gowen



Oscar Martin



George McArthur

Undergraduates:

Joe Alvin

Craig Alberg

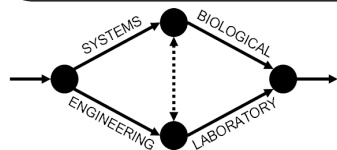
Adam Bower

Kevin Bussing

Richard Clay Crenwelge IV

Maria McClintock

Afton Trent



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• NASA – AISR

