Progress Report
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National Science Foundation

Dr. Bernhard Palsson and Dr. George Church

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Engineering

- Goal #1 (@UCSD) – Automate the generation of metabolic genotypes *in silico*
- Goal #2 (@UCSD) – Develop algorithms and computer software to study properties and capabilities of genotype
- Goal #3 (@Harvard) – Experimental testing of computed metabolic functions
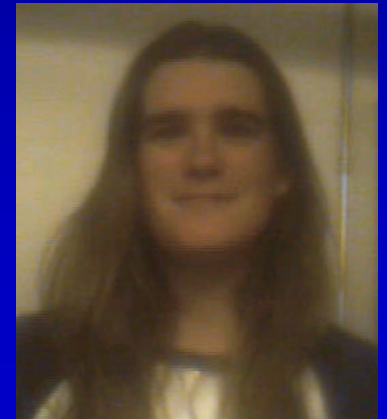
Time Period: October 1, 1998 – October 1, 2001
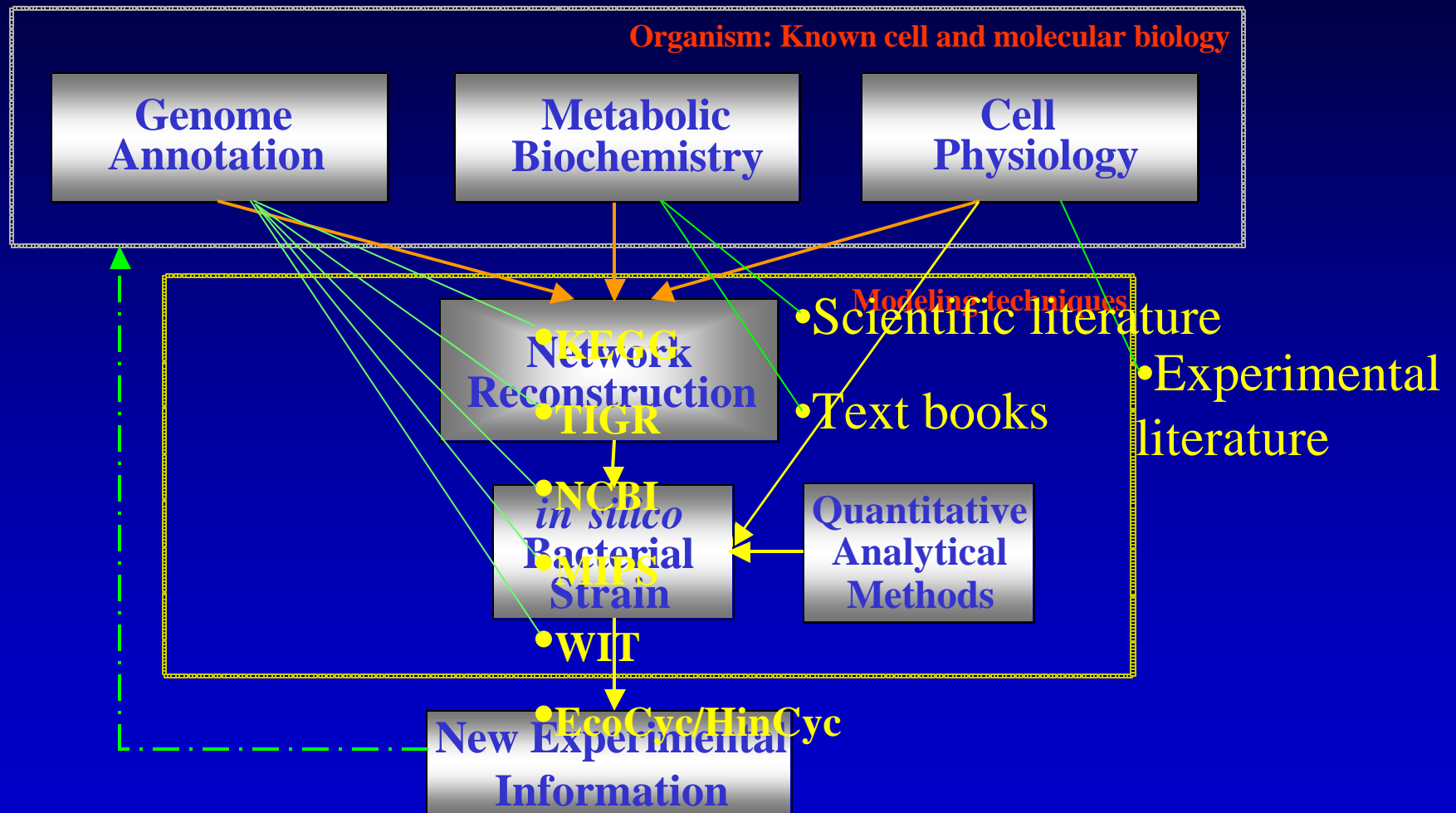
tudents Funded @UCSD

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Research Group

Goal #1:
Reconstructing Metabolic Networks

Organism	E.coli K-12	H.influenzae Rd	H. Pylori 26695
Genome Characteristics			
Genome Size (Mb)	4.6	1.83	1.66
Total Genes	4288	1743	1590
Completion Date	1997	1995	1997
In Silico Strain Properties			
Total Metabolic Genes	660	400	290
Metabolic Reactions	697	412	272
Metabolites	442	367	332

In Silico Metabolic Reconstruction

Organism: Known cell and molecular biology

Genome Annotation **Metabolic Biochemistry** **Cell Physiology**

Modeling techniques

- Scientific literature
- Text books
- Experimental literature

Network Reconstruction

- KEGG
- TIGR
- NCBI
- MIPS
- WIT
- EcoCyc/HinCyc

in silico **Bacterial Strain**

Quantitative Analytical Methods

New Experimental Information

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Lessons Learned

• Reconstruction cannot be automated – it needs significant human evaluation and judgment

• Diverse skills set needed: bioinformatics (relatively easy); know-how of biochemistry and organism physiology. Team effort may be needed

• Left with some ambiguity; but a small % of total ORFs and metabolic map

• Hard for a single person – need independent audits

• Can help with ORF assignments (e.g. HP1017, putative amino acid permease)

• Gives a good basis for integrative analysis

• For integrative analysis, modeling experience and math skills are required

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Goal #2:
Flux Balance Analysis (FBA) Program

Inputs

FBA Algorithm

Outputs

Stoichiometric
Matrix, [S]
+
Capacity
constraints
+
Linear
Programming
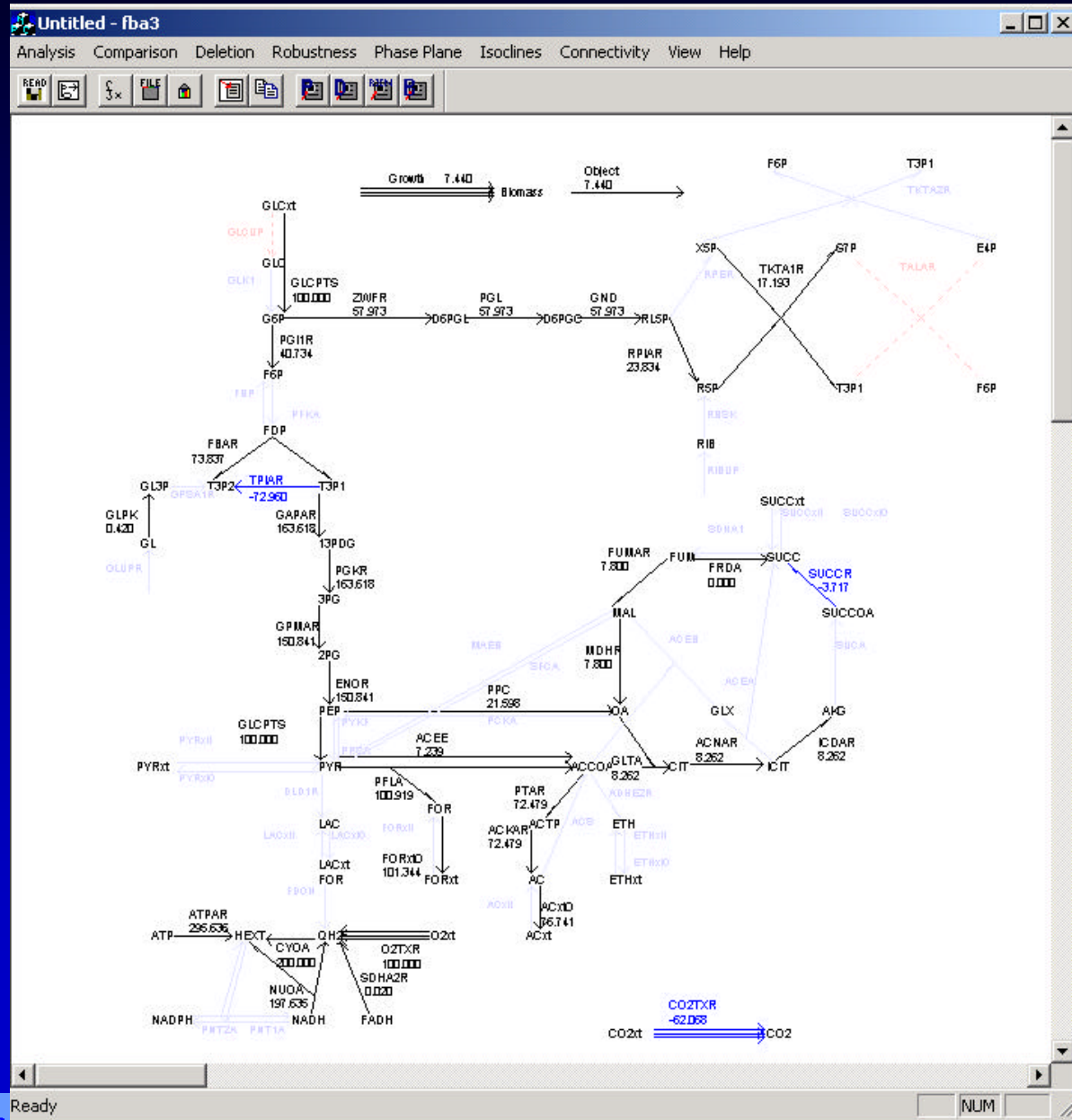
Flux Distribution and Flux Map

Robustness Analysis

Phenotypic Phase Plane (PhPP)

Optimal Value of Objective Function

Gene Deletions

FBA – Choosing an Objective Function
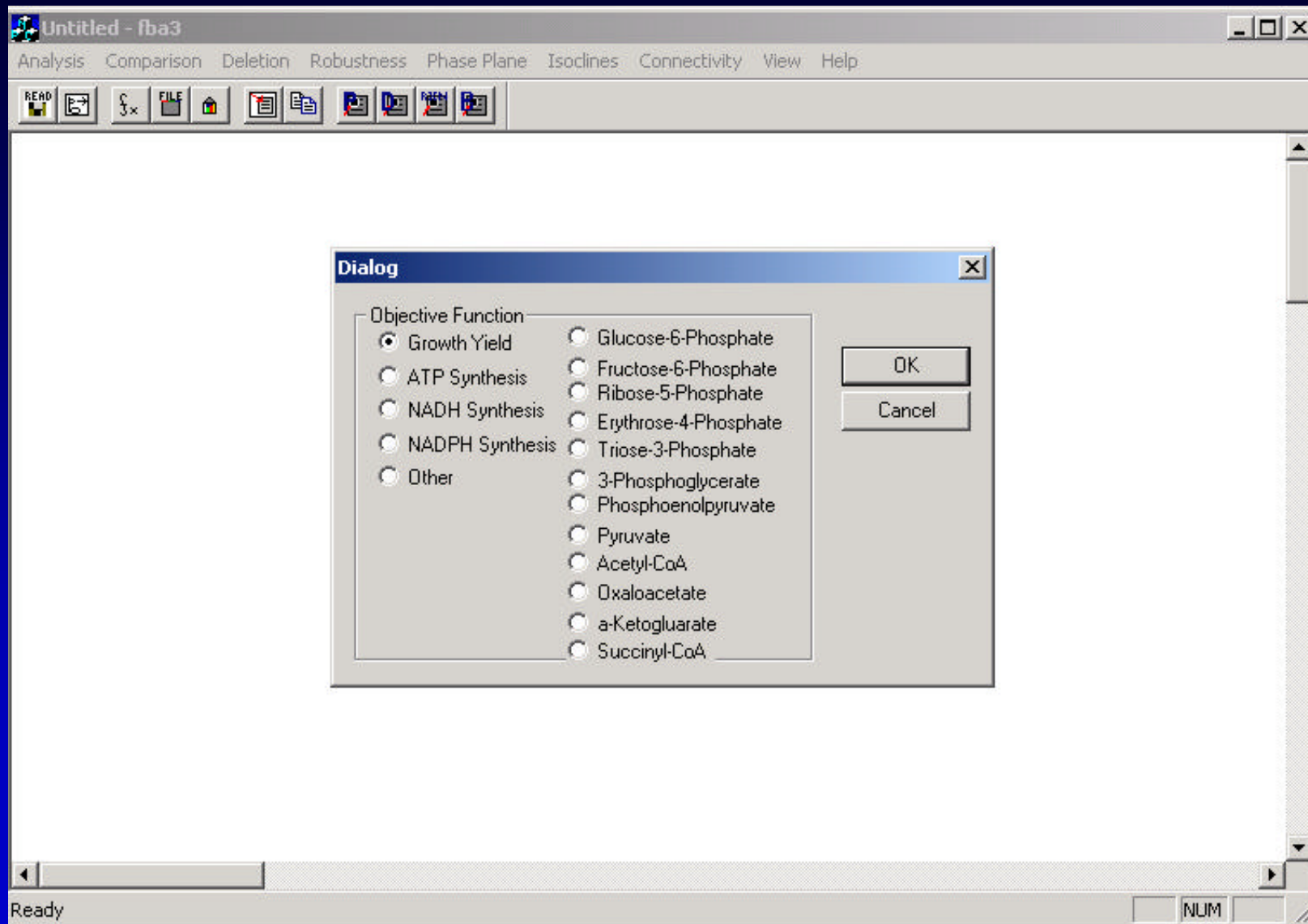
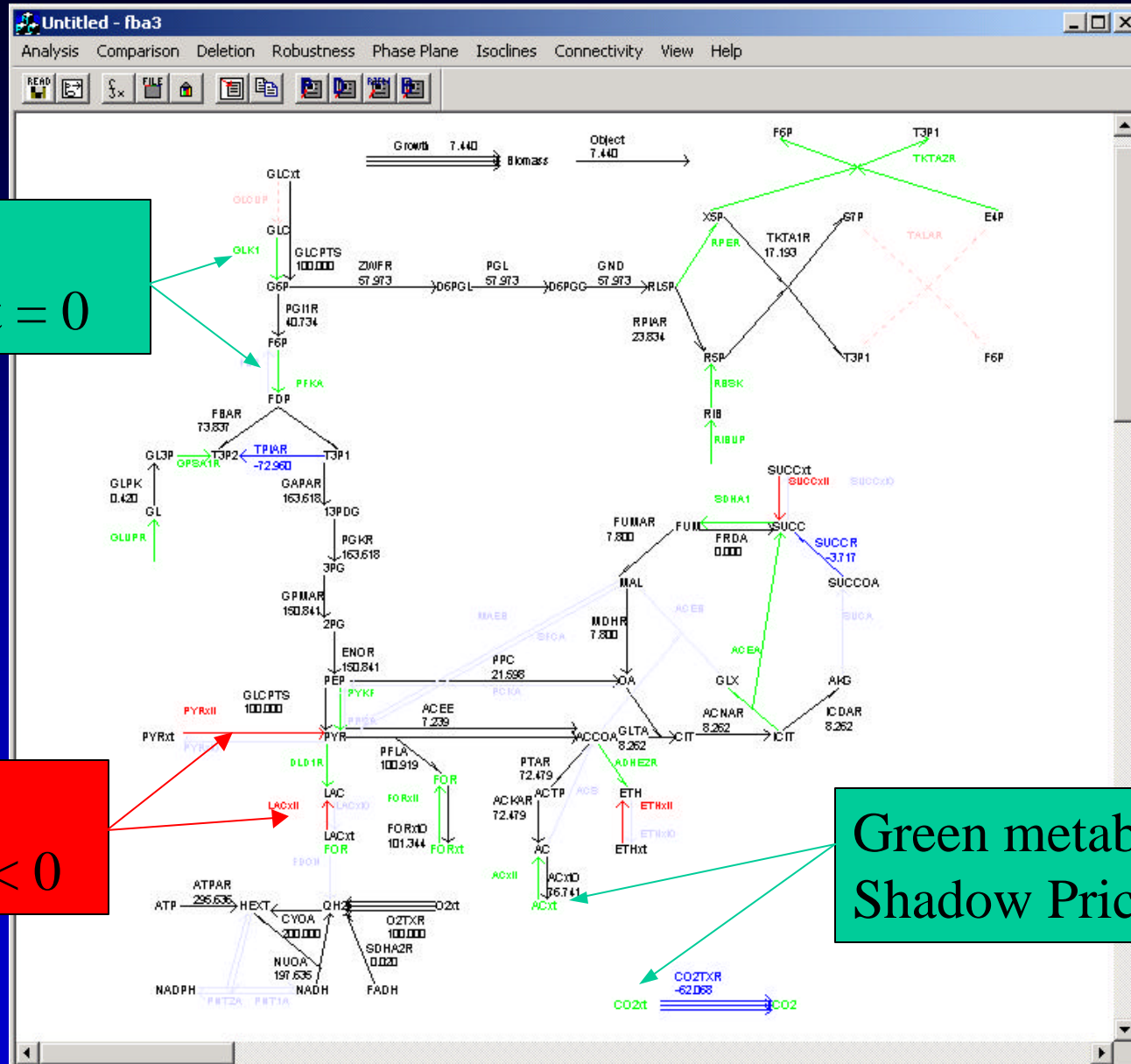# FBA – The Flux Map Display With Fluxes
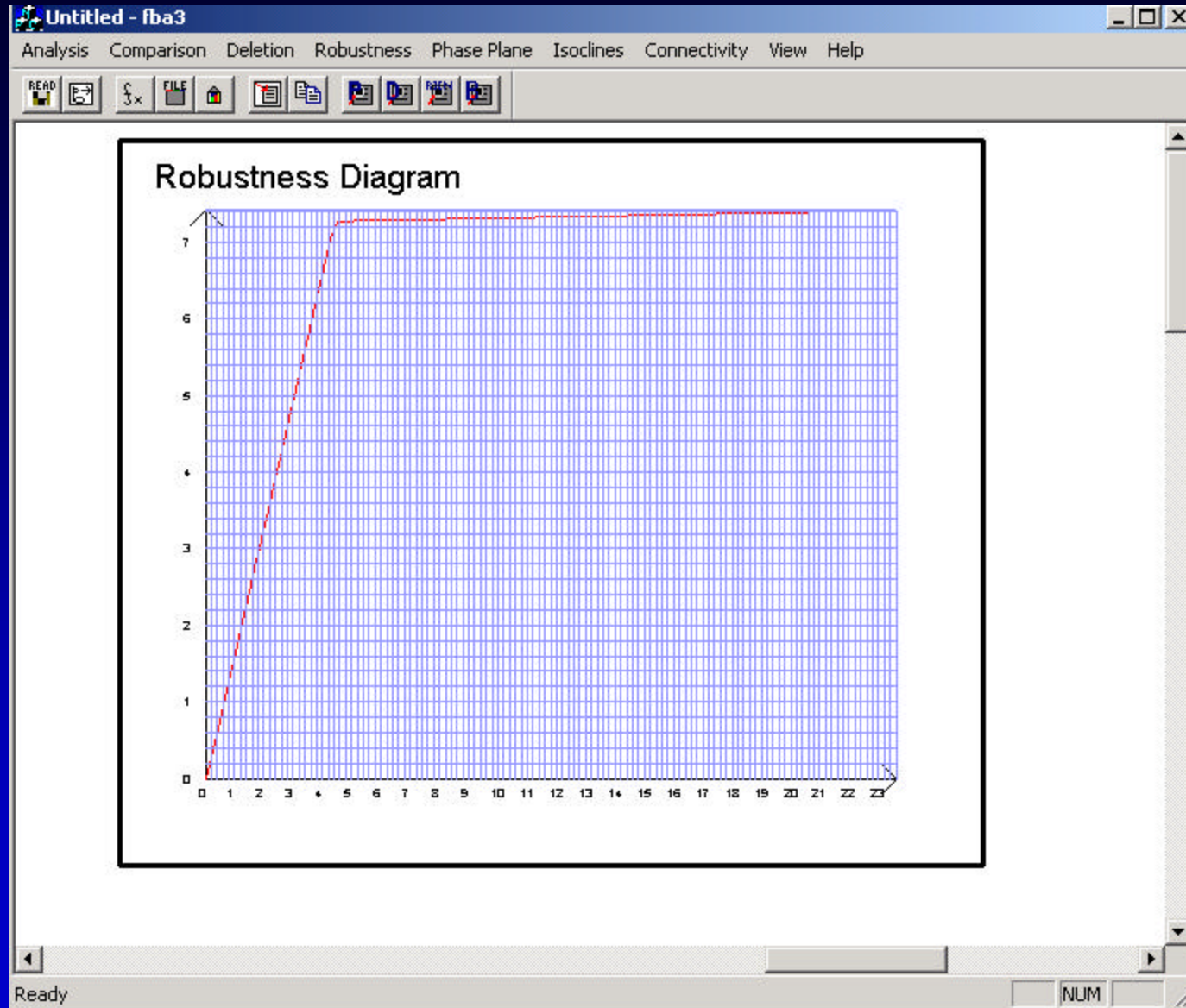
FBA – Shadow Prices and Reduced Costs

Green flux:
Reduced Cost = 0

...ed flux:
...educed Cost < 0

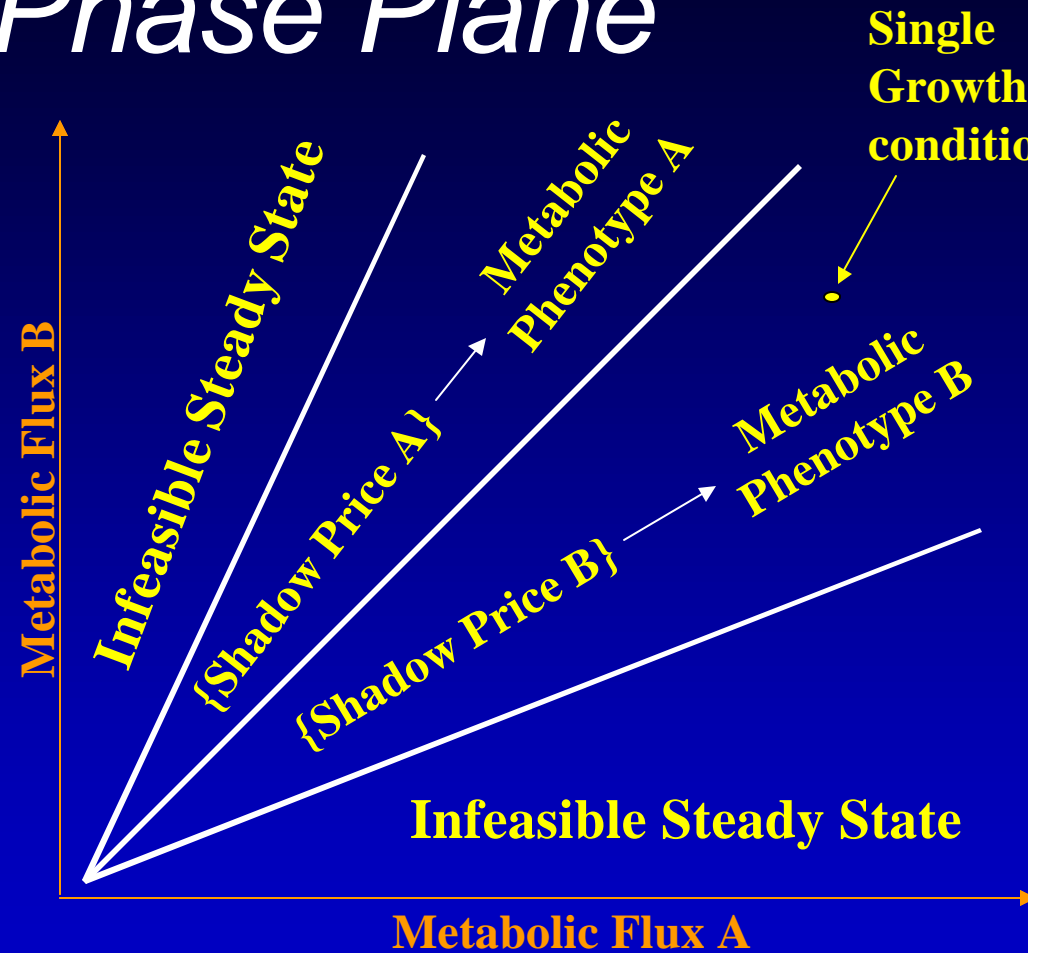Green metabolites:
Shadow Price = 0

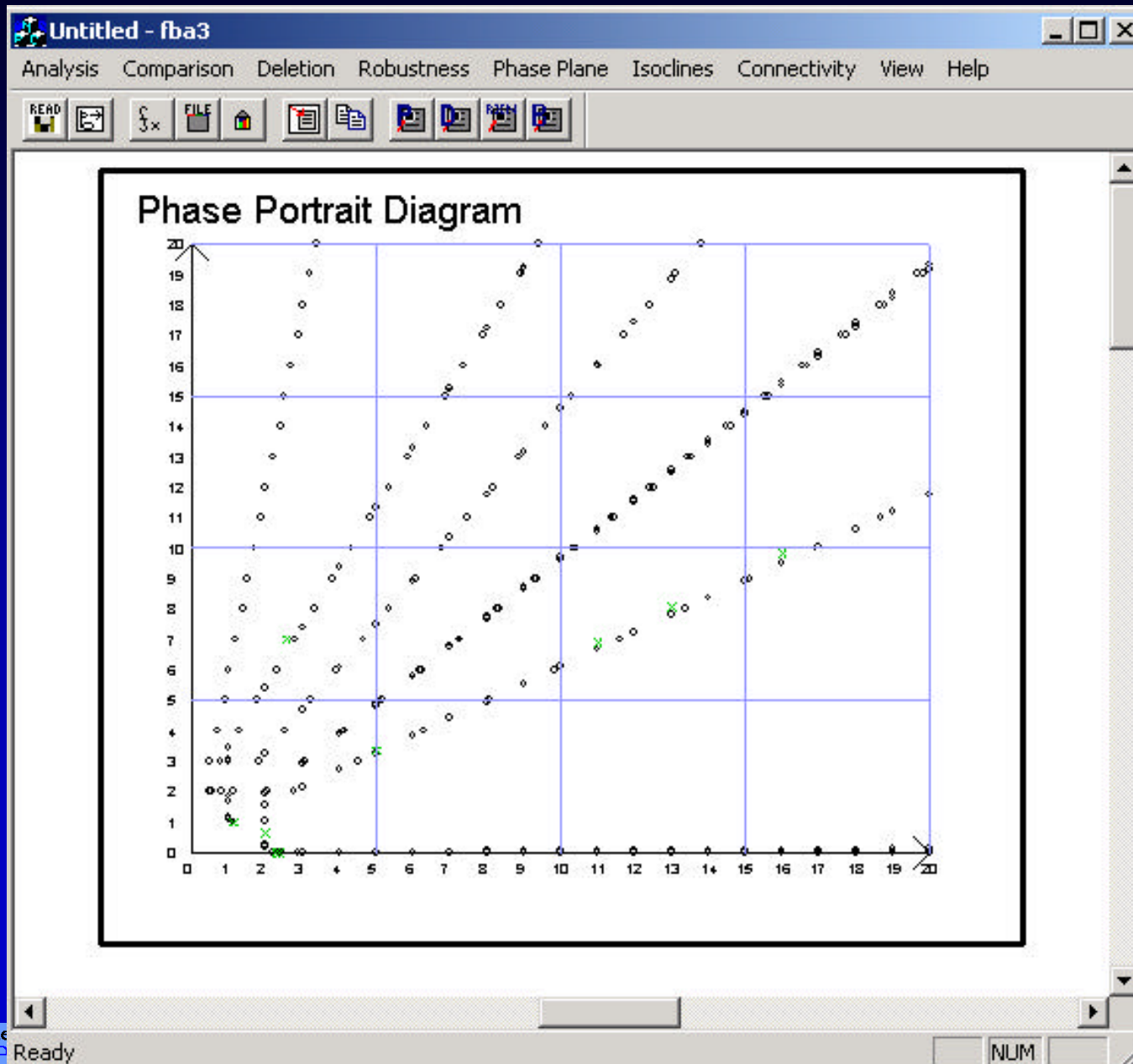# FBA – "Robustness" for RPI Enzyme

Phenotype Phase Plane

2-dimensional region

- Spanned by 2 metabolic fluxes
  - Typically uptake rates
- Shadow prices (metabolite value) are calculated
- lines to demarcate regions of constant shadow price
- By definition, metabolic pathway utilization is different in each region of the phase plane


Single Growth conditic

Infeasible Steady State

Metabolic Flux B

{Shadow Price A}

Metabolic Phenotype A

{Shadow Price B}

Metabolic Phenotype B

Infeasible Steady State

Metabolic Flux A

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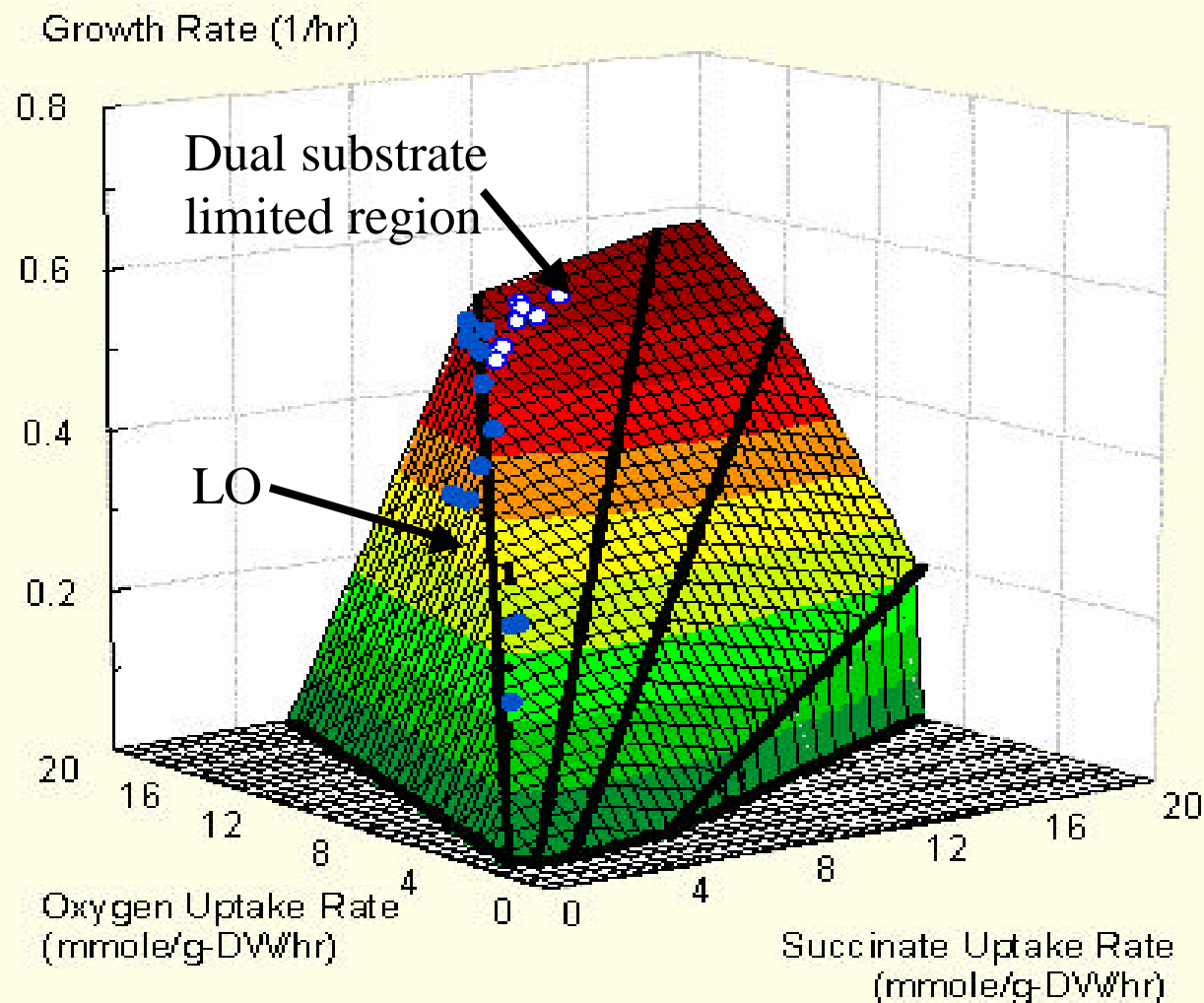# FBA – Phenotypic Phase Plane (PhPP)

Subsequent Interpretation of *E. coli* PhPP Using Shadow Prices and the Flux Map
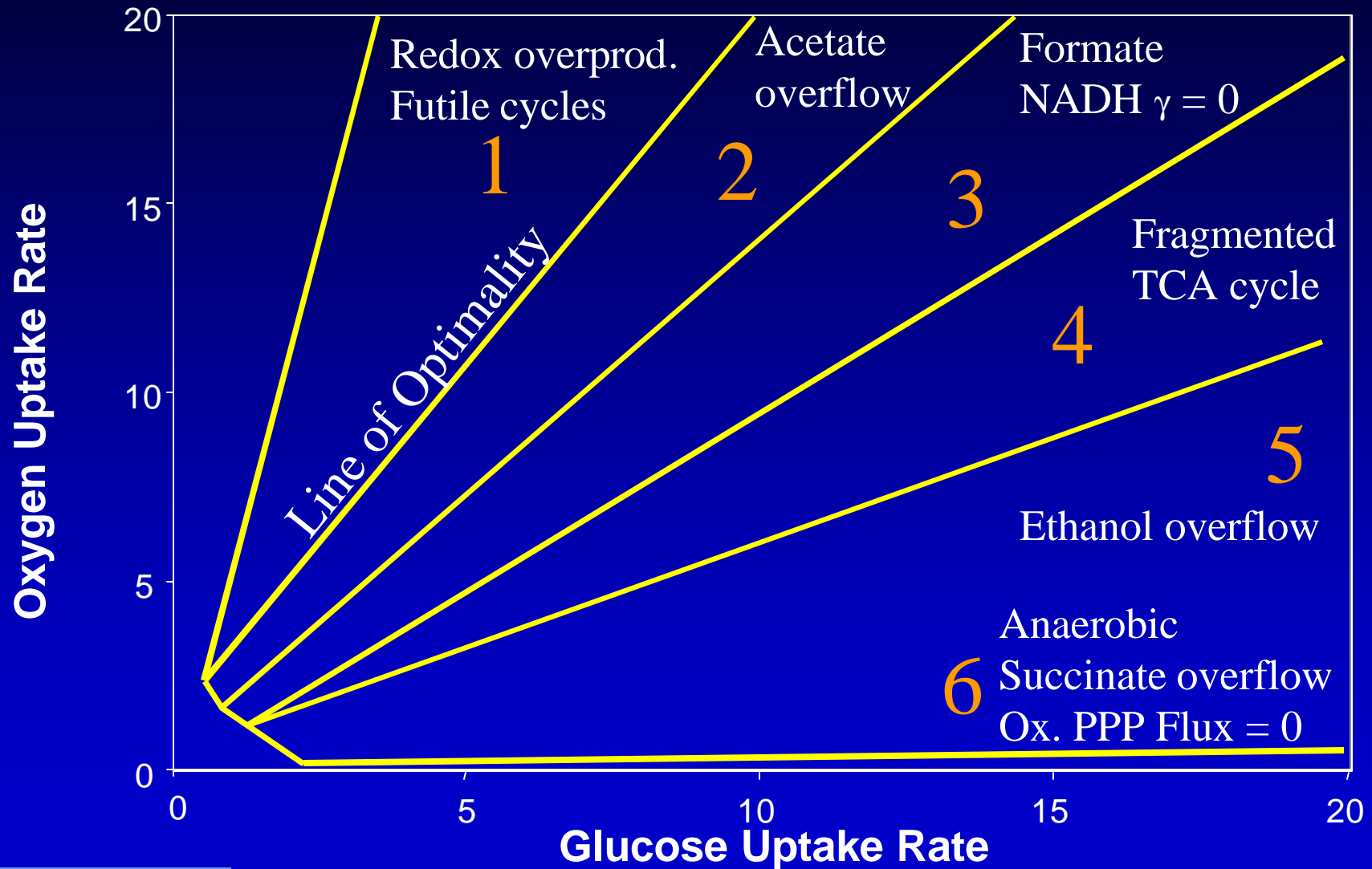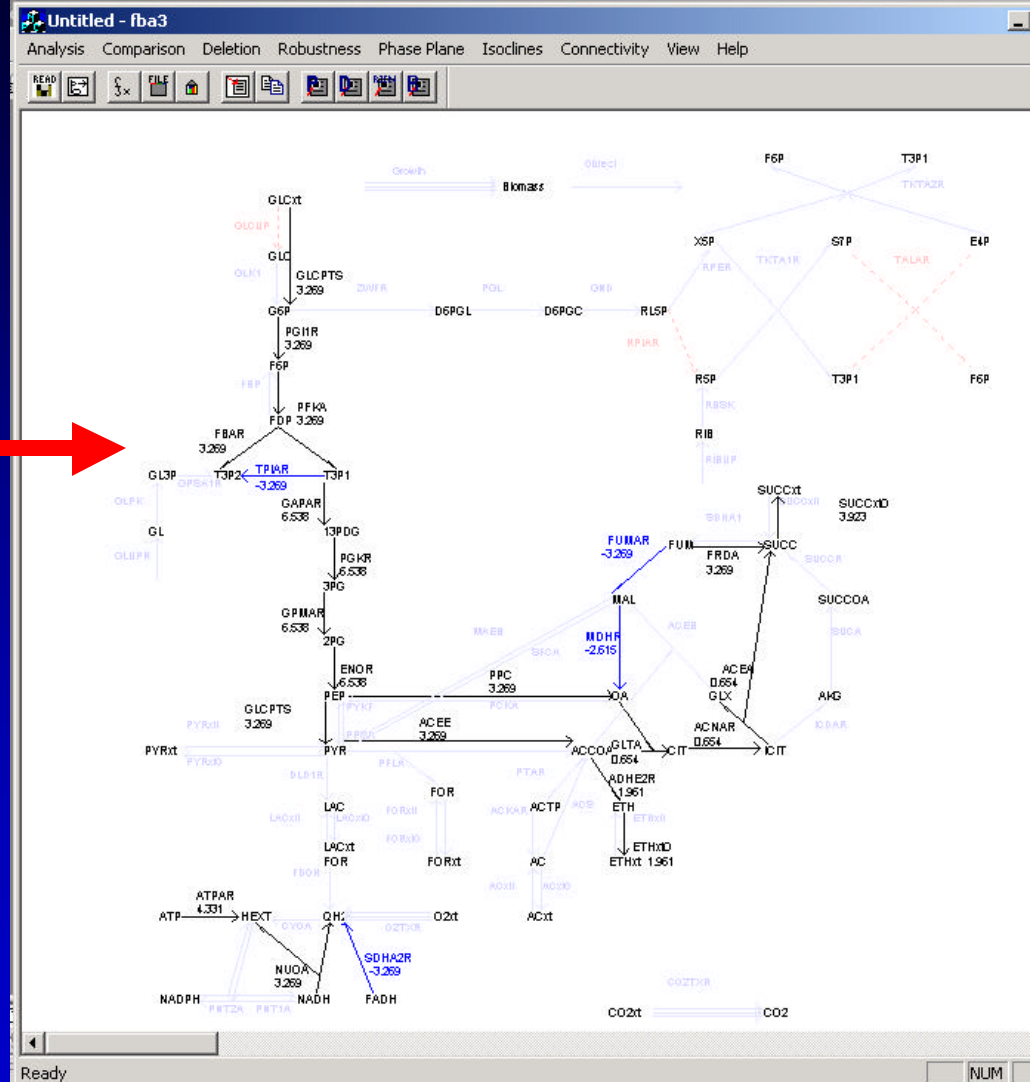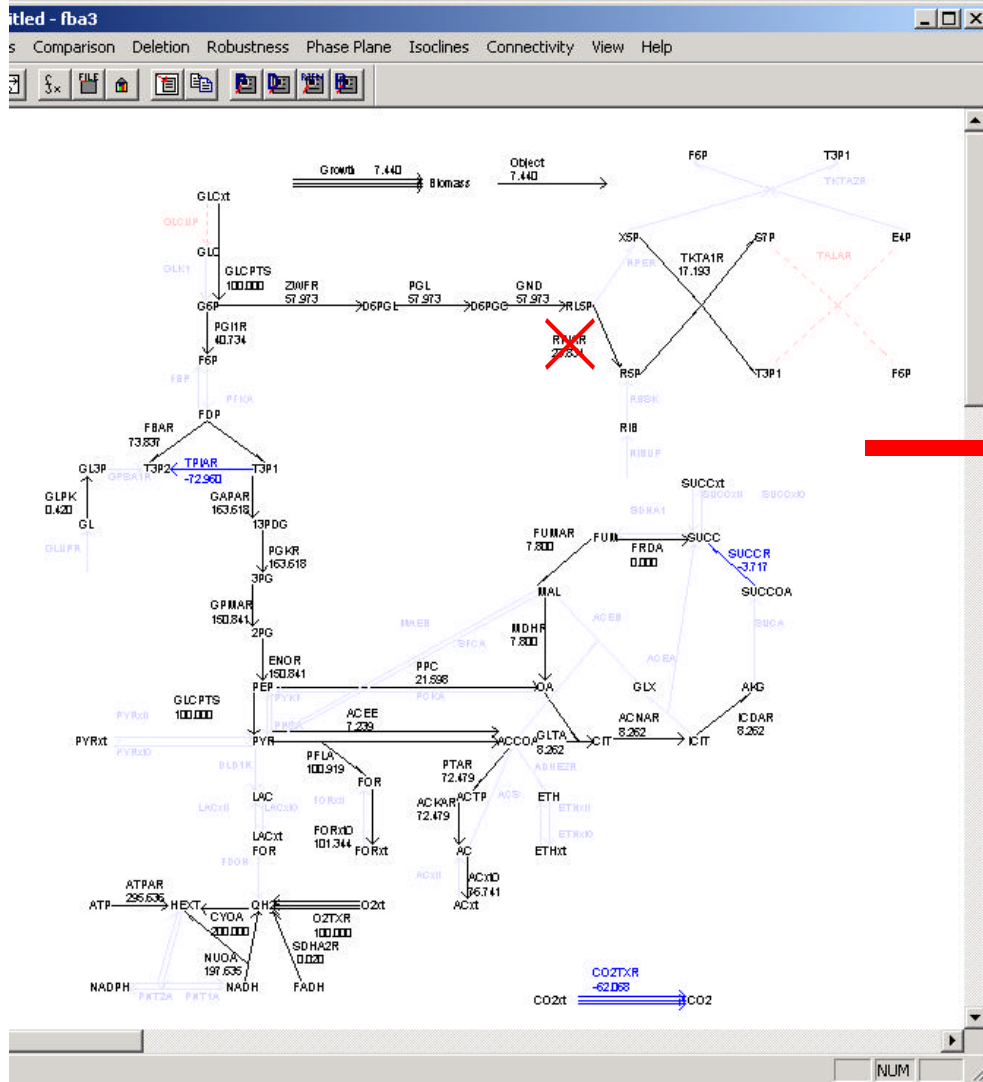
Succinate Phenotype Phase Plane
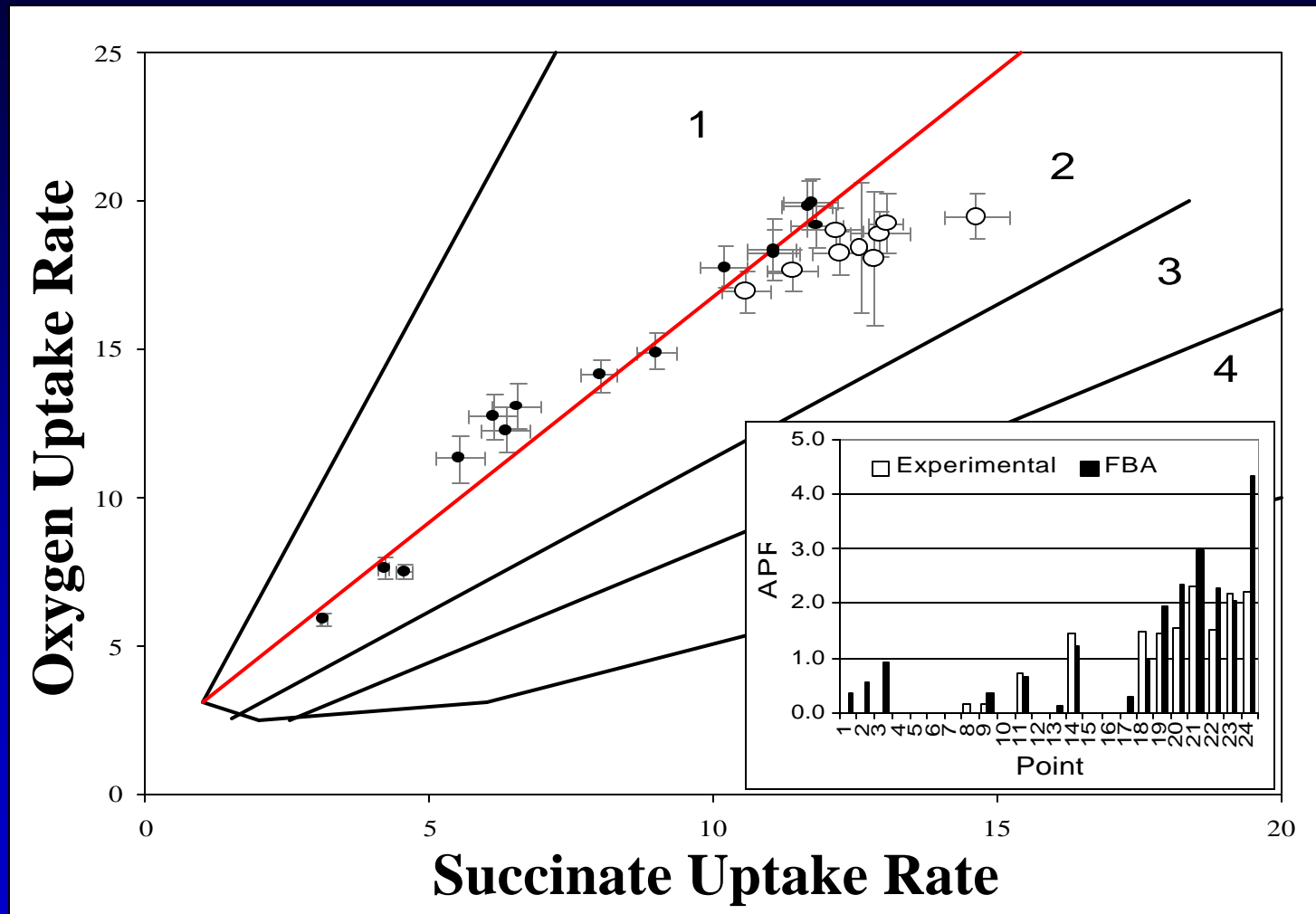
Succinate 3-D Phenotype Phase Plane

RPI Enzyme Deletion

Summary

- Pathway reconstruction is semi-automated, and it will be some time before it can be fully automatic

- A prototype "research grade" FBA program has been constructed and is operational

- Full *in silico* and experimental evaluation of knock-outs can now commence