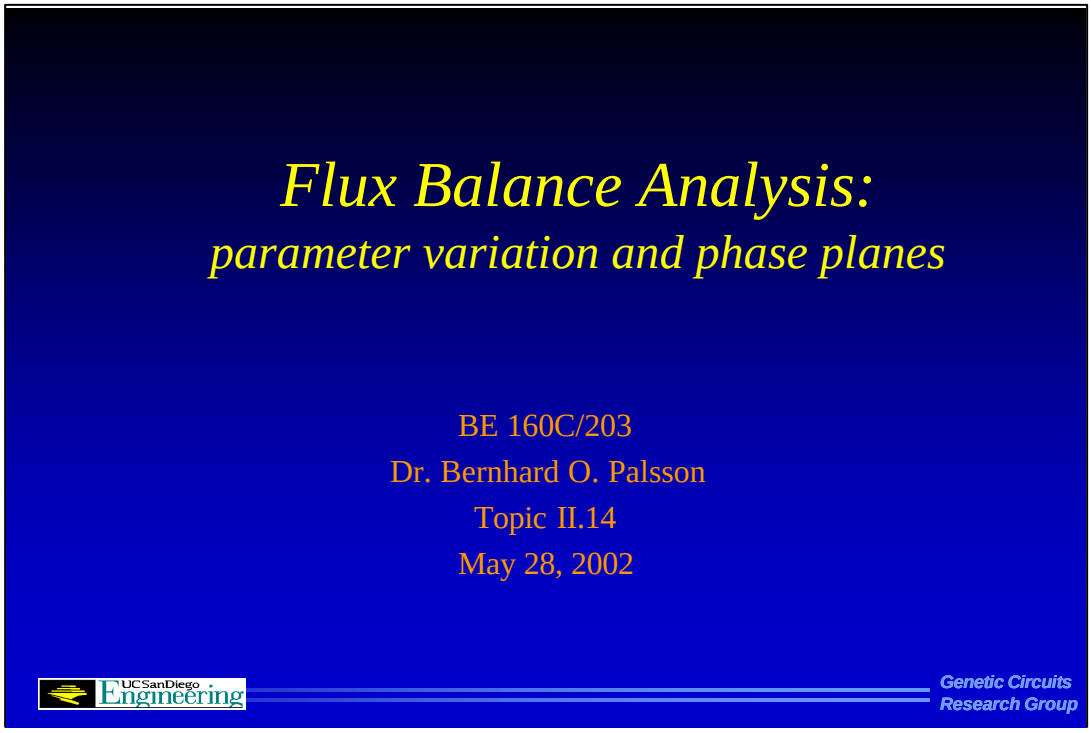


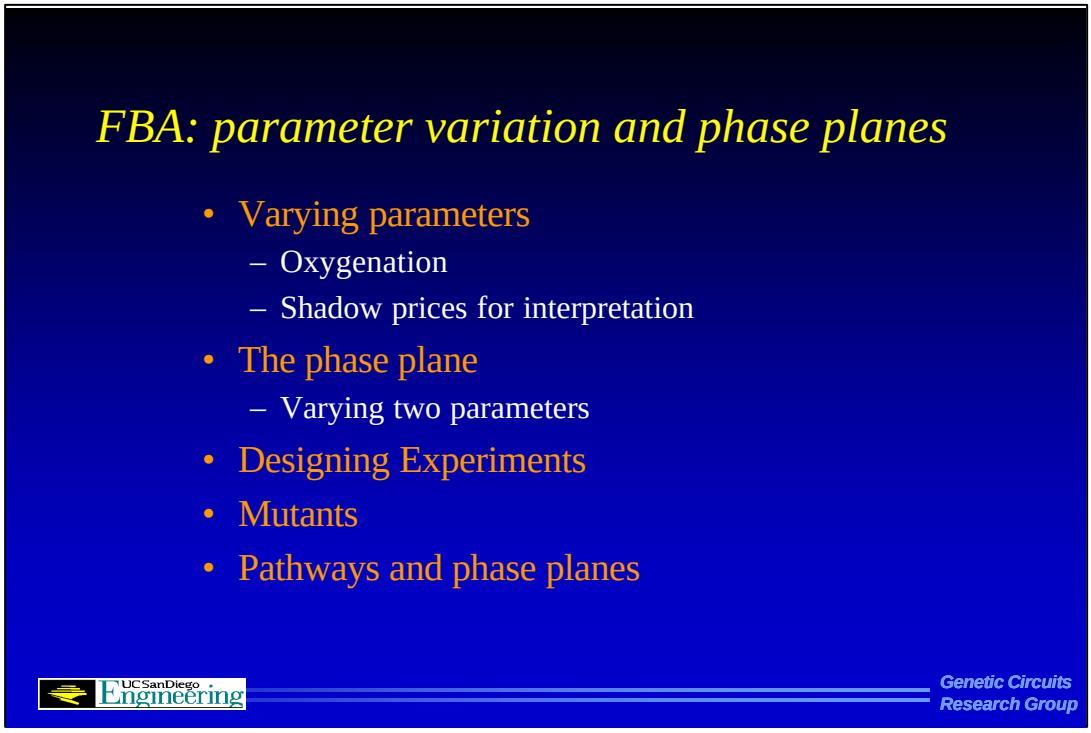
Lecture 13:

Flux Balancing as a Function as Systems Parameters

In the last lecture we saw how flux distributions can be obtained for particular situation. We introduce using linear programming (LP) repeatedly with a continually varying parameter in the flux balance equations and the capacity constraints. Shadow prices are useful in interpreting the results.

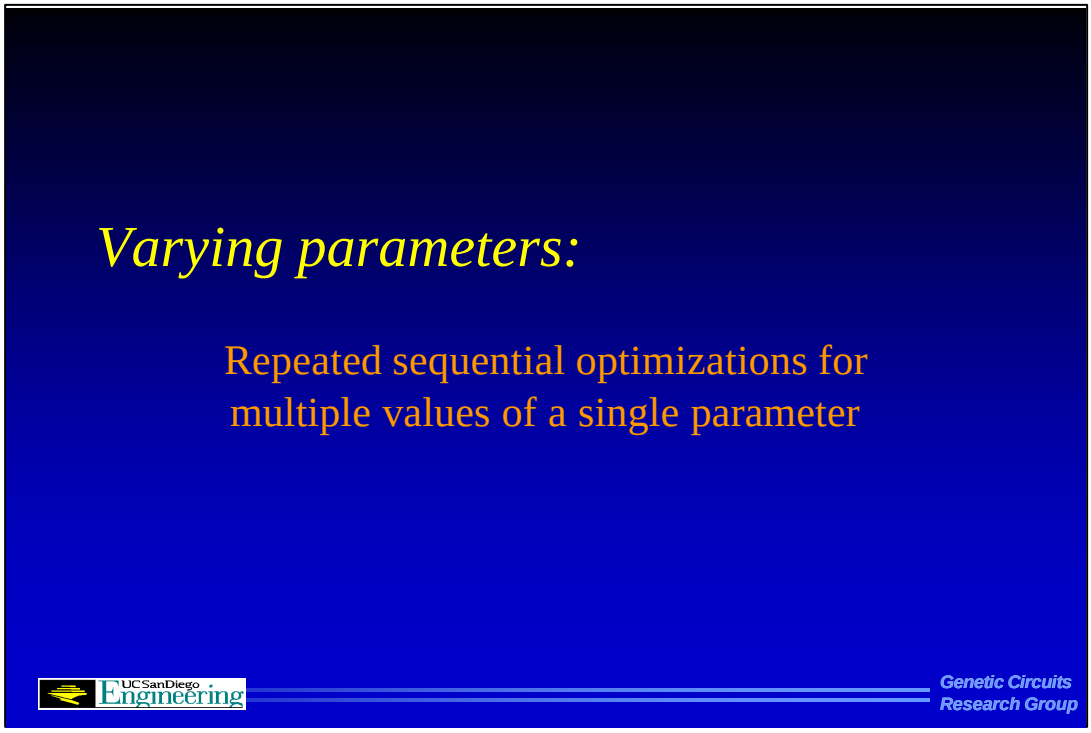
Phase plane analysis is then introduced, where all optimal solutions as a function of two different parameters are displayed. This formalism turns out to be very powerful to interpret a large number of solutions simultaneously. It will also be useful in comparing growth of a strain on different substrates, the consequences of gene deletion, and the design of experiments.

In this lecture we concentrate on the variation in environmental parameters.



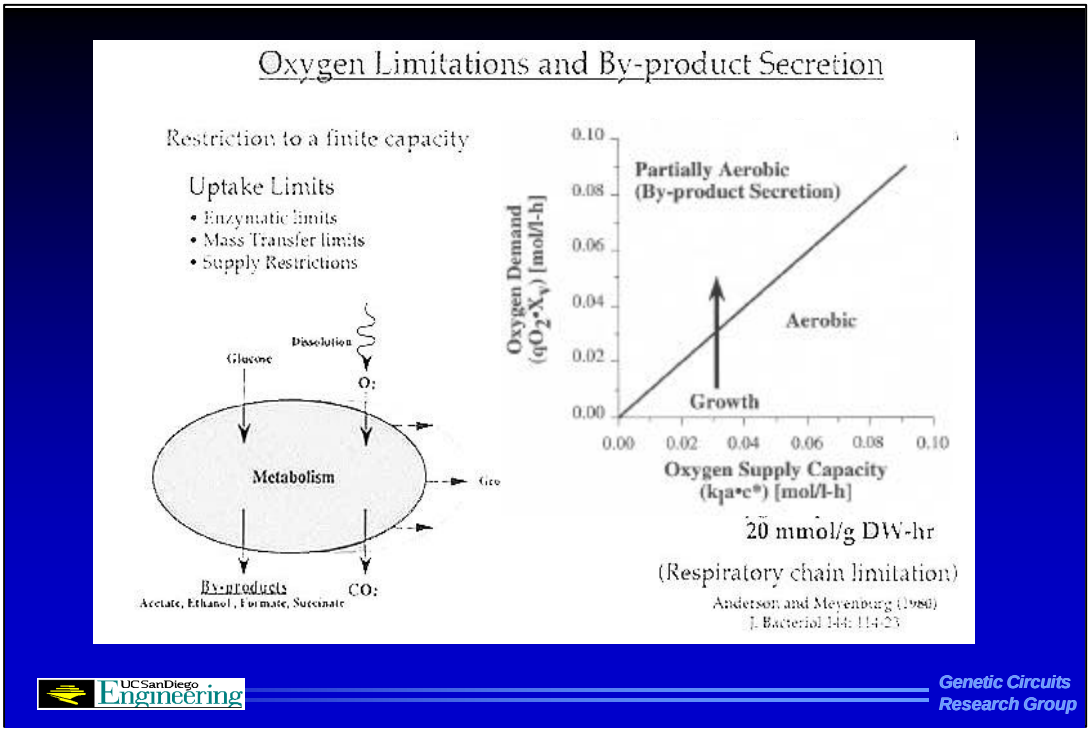
Overview

We begin by continually varying a single parameter and the use of the shadow prices to interpret the discontinuous changes that occur in the optimal solutions as the varying parameter crosses a critical value. This concept is the extended to two parameter variations and the formulation of phase planes.



In a previous lecture we looked at optimal mitochondrial flux maps for different substrates and for constraints on several internal fluxes. These are calculated for a discrete set of conditions.

We may however, be interested in the range of numerical values for a particular parameter. Thus, we can calculate a series of optimal solutions for small incremental changes in a parameter in the system. If the increments are small enough, we effectively get a continuous variation in the parameter of interest.



Example: Reducing Oxygen Availability

When cells grow in the laboratory with abundance of substrate they grow to high densities, eventually outstripping the ability for oxygen to be supplied rapidly enough to support fully aerobic growth. As oxygen becomes limiting, the cells must partially oxidize their substrate and secrete metabolic by-products.

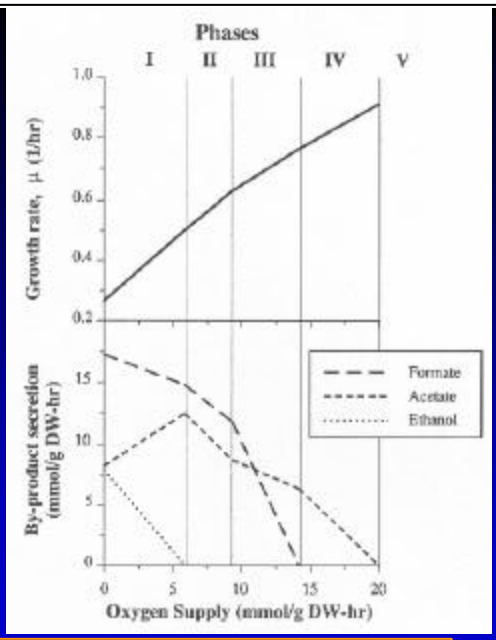
The panel on the left illustrates this problem at the cellular level. At the right this problem is illustrated from a bioprocess viewpoint. The arrow indicates growth of a culture supplied with a constant rate of oxygen. As the culture grows, the oxygen demand increases and passes the line indicating the boundary between aerobic and anaerobic growth.

The following slides were prepared with a reduced *E. coli* model in 1993 (Varma, A&EM), but it illustrates how parameter variations can be used to study problems of fundamental physiological relevance, and practical importance.

Example

In this example we vary the maximum allowable uptake rate of oxygen. The whole range of oxygenation is shown, from fully aerobic conditions to fully anaerobic conditions.

The growth rate is graphed in the upper panel and the by-product secretion rates in the lower.



anaerobic → aerobic

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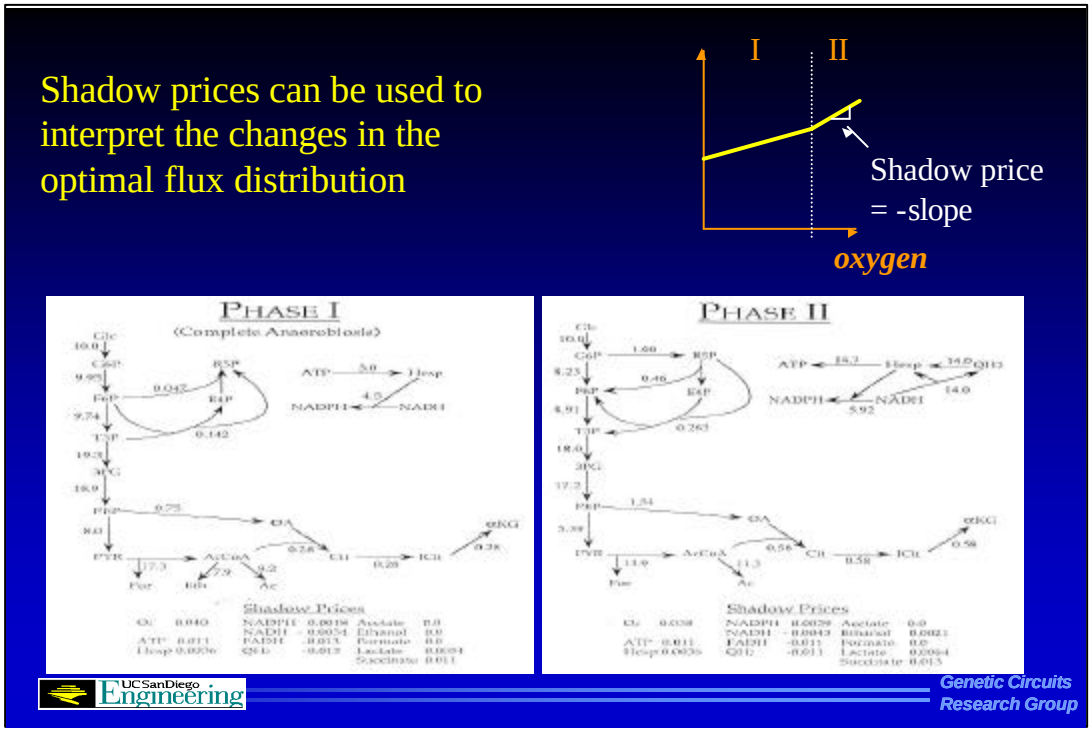
Varying Oxygen Availability

As the dissolution of oxygen cannot keep up with the high volumetric consumption rates at high cell density, the amount available per cell is reduced. Computationally this is represented by lowering the capacity constraints on the oxygen uptake rate.

The results from a series of LP calculations with varying b_{O2} is shown in this slide (glucose uptake rate is fixed at 10mmol Glc/gDW-hr). The optimal growth rate drops as the oxygen uptake rate is reduced, as shown in the upper panel. It does so in piece-wise linear fashion where changes in the slope occur at well defined oxygen uptake rates. This feature naturally divides the range of oxygen uptake rates into distinct phases.

The lower panels shows the secretion rates of metabolic by-products: formate, ethanol and acetate. Each one of these by-products is secreted in a fundamentally different way in each phase. As oxygen is reduced, incomplete oxidation of glucose takes place and metabolic by-products are secreted; acetate is first secreted, then formate, followed by ethanol.

The LP solution in each phase is fundamentally different and the transition from one to another can be interpreted using shadow prices.



Changes In Shadow Prices At Phase Boundaries

The shadow price changes discontinuously at the boundary from one phase to the next. In fact, the change in the shadow price defines the boundary between the phases. The shadow prices basically tell us how the governing constraints on the objective function change and how the base optimal LP solution changes. This change is reflected in a shift in the flux map.

Phase I shown above is for completely anaerobic growth. The shadow prices for oxygen and ATP are negative, indicating that these are constraining factors, since the objective function would increase as more of these compounds are provided to the cell. Some of the redox carriers have positive shadow prices indicating that the cell has a problem with excess redox potential. This is characteristic of anaerobic metabolism.

In Phase I, acetate, ethanol, and formate, all have zero shadow prices, indicating that these intermediates are useless to the cell. Thus they are secreted. Notice that in Phase II, ethanol has a negative shadow price. It thus has value to the cell and is not secreted. In fact the defining difference between the optimal flux maps in phase I and II is the secretion of ethanol. The shadow prices are thus key in interpreting the optimal flux maps and changes in the maps as parameters vary.

Calculations are consistent with experimental data

Anaerobic by-product secretion

Product (mmol/mmol Glc)	Optimal	Experimental
Acetate	0.815	0.75
Ethanol	0.786	0.87
Formate	1.73	1.13
Lactate	0	traces
Succinate	0	0.12
Yield (g DW/mmol Glc)	0.027	0.03

Experimental data from:
Belaich and Belaich (1976)
J. Bacteriol. 125:14-18

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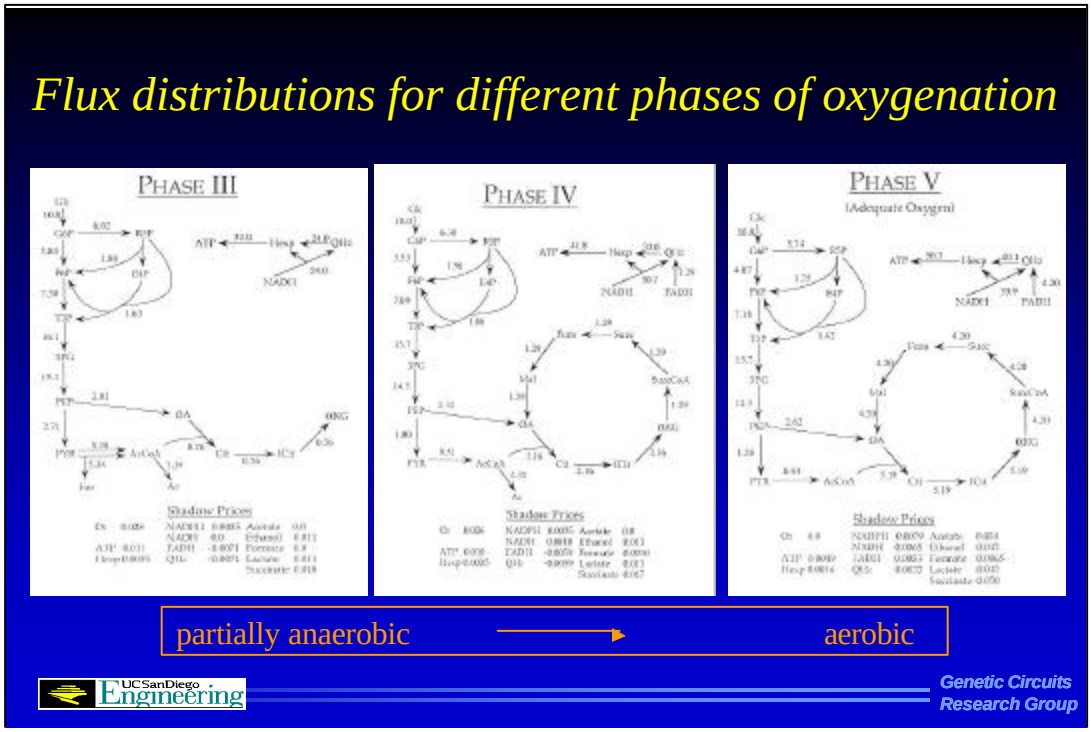
In Silico vs. *In Vivo*

The calculated metabolic secretion rates and the biomass yield can be compared to those experimentally obtained. This table shows that there is quantitative comparison between the *in silico* optimal flux map and metabolic the phenotype that *E. coli* expresses under these conditions.



Other Growth Phases

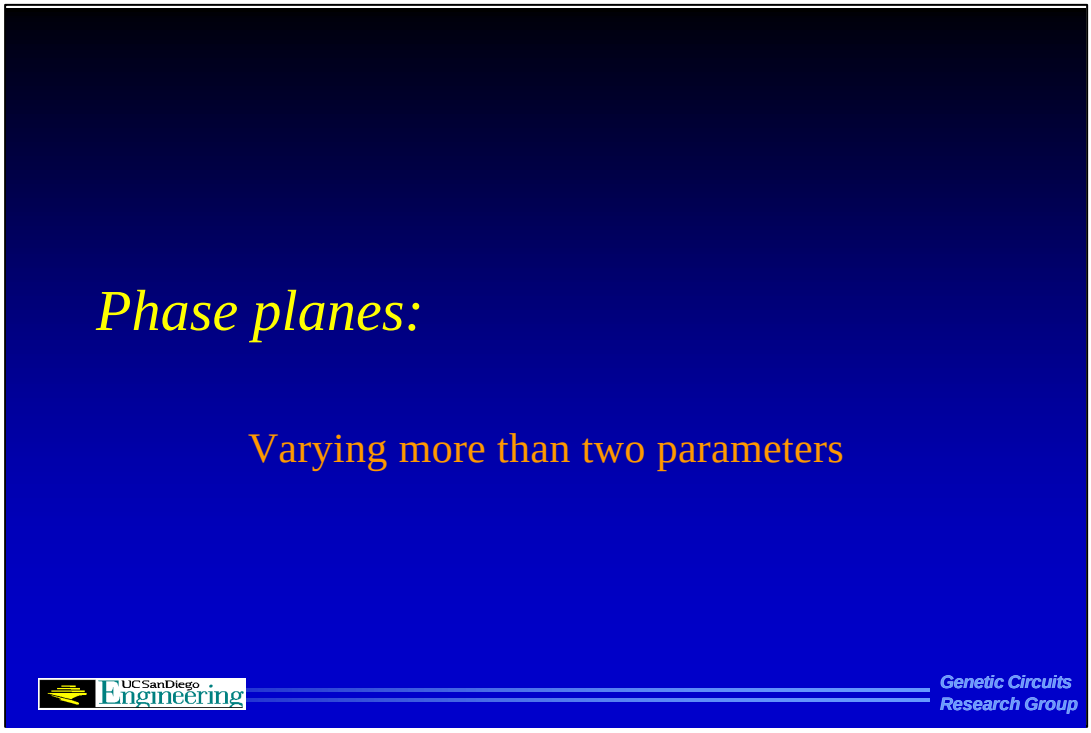
This slide shows the optimal metabolic flux maps for the remaining three phases. Notice that in phase V the shadow price for oxygen is zero, since it is not limiting. More oxygen would not increase the growth rate.

Note that the shadow prices listed in the figures should be multiplied by a -1, the sign convention is reversed.

Notice that the defining difference between Phase IV and V is that the acetate shadow price becomes zero in phase IV. It is thus secreted.

Notice that the systems analysis basically gives an econometric interpretation of cellular metabolism. For any given condition, the best phenotype, or flux solution, is calculated. The shift from one phase to another comes with an interpretation of value of the various components to the cell. These 'values' determine what is secreted and what is limiting.

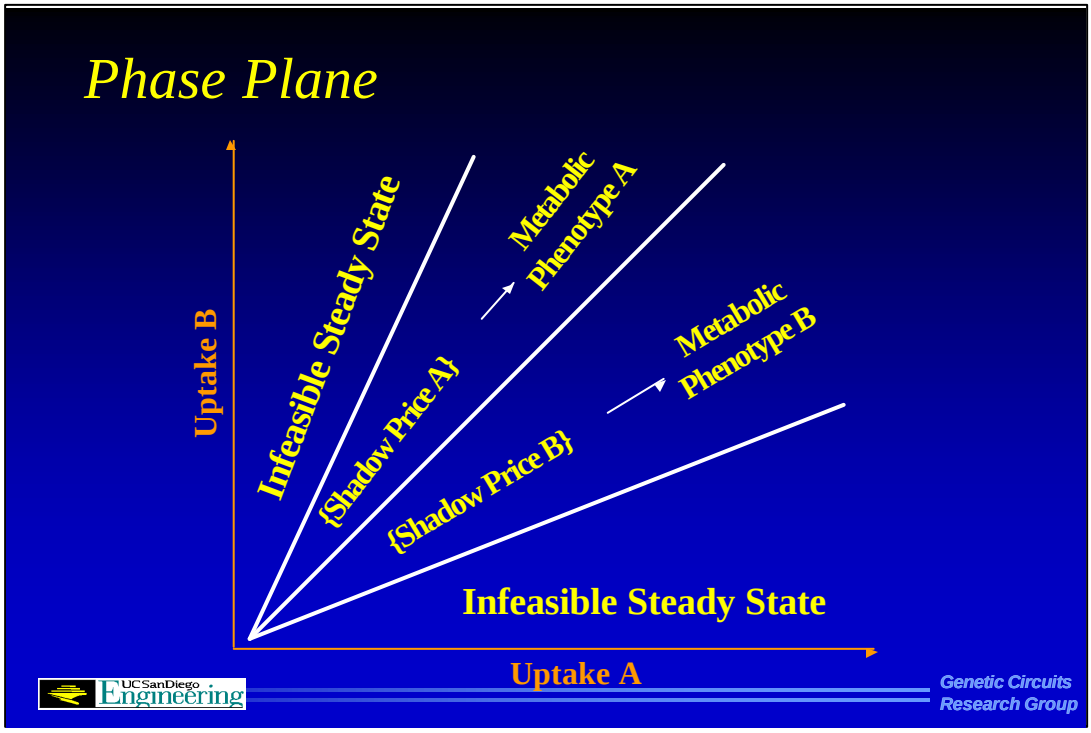
The shadow prices are thus very useful quantities.



Phenotype Phase Plane Analysis

A useful way to extend the study of metabolic genotype-phenotype relation is to use two parameters that describe the growth conditions (such as substrate and oxygen uptake rates) as two axes on an x,y-plane. Then the optimal flux-maps can be calculated for all points in this plane. There are a finite number of fundamentally different optimal metabolic flux maps present in such a plane. The demarcations between the different flux maps are determined from the shadow prices of the metabolites. As we have seen, the shadow prices are sensitivity parameters that are calculated in the dual solution to the LP problem, and can be used to interpret shifts from one optimal flux distribution to another . This procedure leads to the definition of distinct regions in the plane in which the optimal use of the pathways is fundamentally different, corresponding to a different phenotypic behavior. We will denote each phase as:$Pn_{x,y.}$ Where P represents phenotype, n is the number of the demarcated region for this phenotype,and x,y the two uptake rates on the axis of the plane.

This phase plane resembles the phase planes used in physical chemistry, which define the different states (i.e., liquid, gas or solid) of a chemical system depending on the external conditions (e.g., temperature, pressure). The plane that we have just described can thus be called the phenotype phase plane (PhPP) for a given genotype. The construction of the phase plane and its main features will now be described, and then conceptually illustrated with a simple example.



The Phase Plane

Using the shadow prices, we can define a phase plane.

A phase plane is a two dimensional region that is spanned by 2 metabolic fluxes. These fluxes are typically uptake rates, but this isn't required. And then the shadow prices for all the metabolites are calculated for all the points within this space, and lines are drawn to demarcate regions of constant shadow prices.

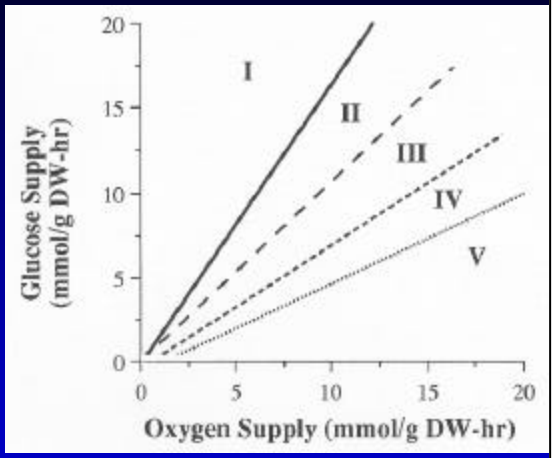
The shadow prices are constant within each region and will be different in the other regions.

Each region typically refers to a different basis solution, which implies a different utilization of the metabolic pathways or a different metabolic phenotype.

Thus, the utilization of the metabolic pathways will be qualitatively different depending on the region of operation within the phase plane.

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



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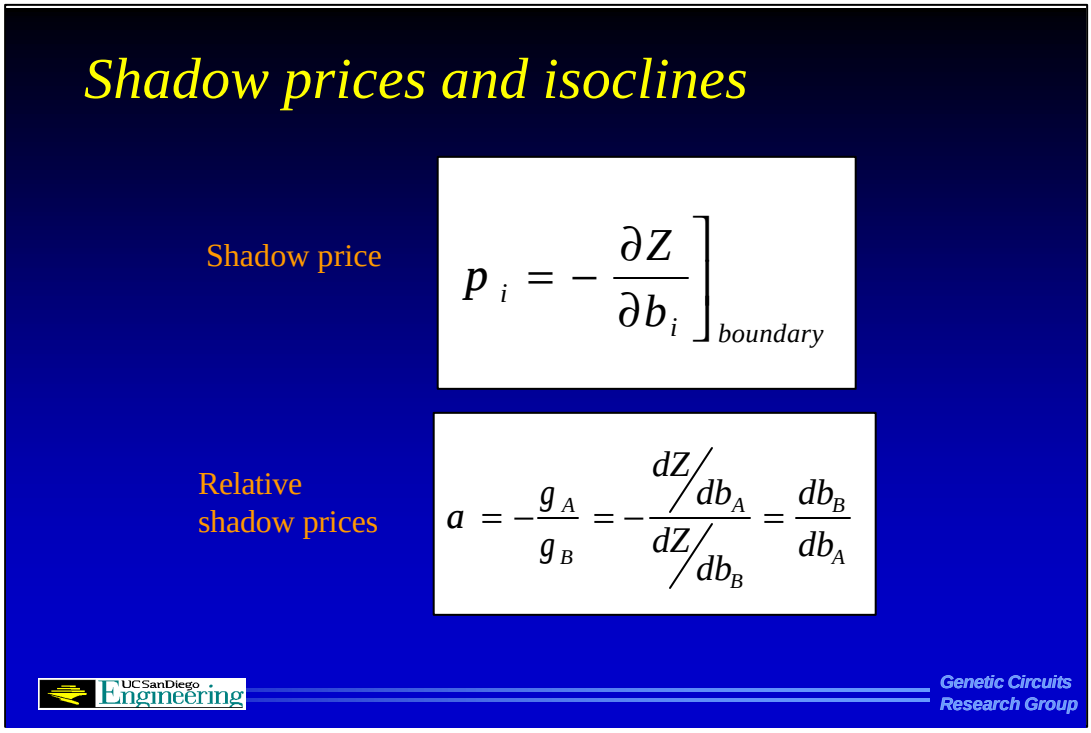
Summary of Phenotype Phase Planes

The example on the right indicates 5 distinct phases when comparing glucose supply to oxygen supply.

Typically, PhPPs are drawn with a carbon source on the x-axis, and oxygen uptake rates on the y-axis.

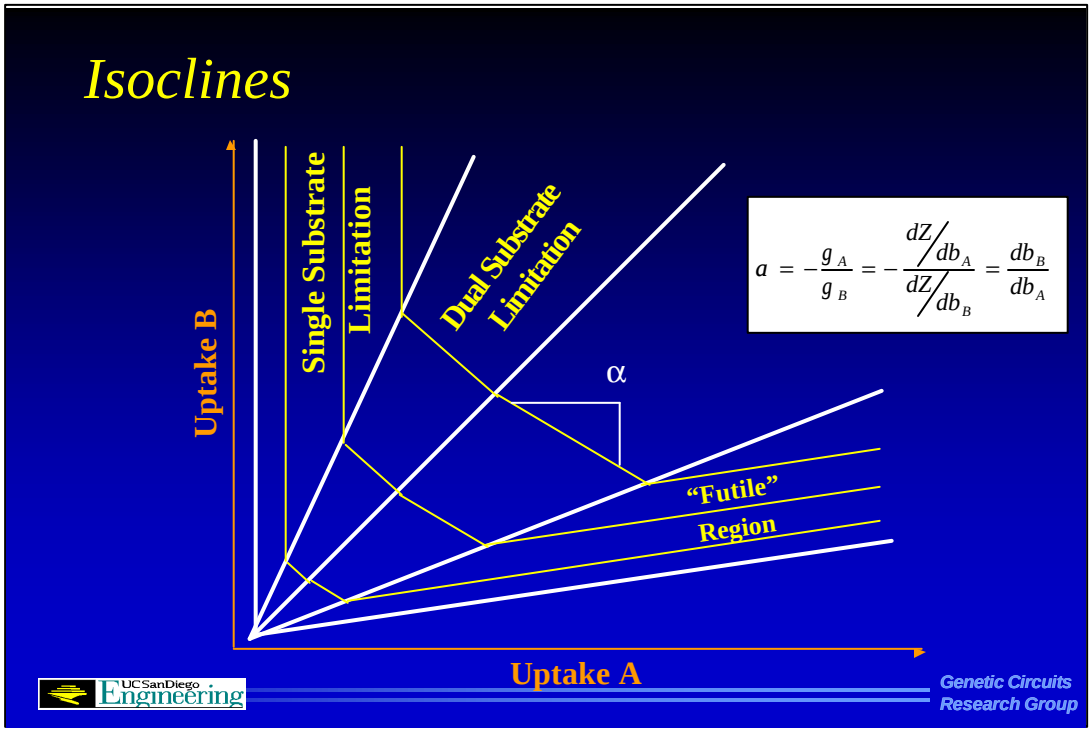


Isoclines

The isoclines represent the combinations of the metabolite uptake rates that will lead to the same value of the objective function. The slope of the isoclines within each region is calculated from the shadow prices; thus, it follows that the slope of the isoclines will be different in each region of the PhPP.

The shadow price is the sensitivity of the objective function (Z) to changes in the availability of metabolites (the **b** vector defines the right hand side of the mass balance constraints). The numerical value on the shadow price can be negative, zero, or positive, depending on the intrinsic value of the metabolite. A ratio of shadow prices between the two external metabolites can be defined.

The negative sign on α is introduced in anticipation of its interpretation. The ratio α is the relative change in the objective function for the two key exchange fluxes. In order for the objective function to remain constant, an increase in one of the exchange fluxes will be accompanied by a decrease in the other and thus we introduce the negative sign on the definition of α. Therefore, the slope of the isoclines (within each region of the PhPP) will be equal to the negative ratio of the x-axis variable shadow price and the y-axis variable shadow price, and this parameter is termed α.



Phase Plane With Objective Function Isoclines

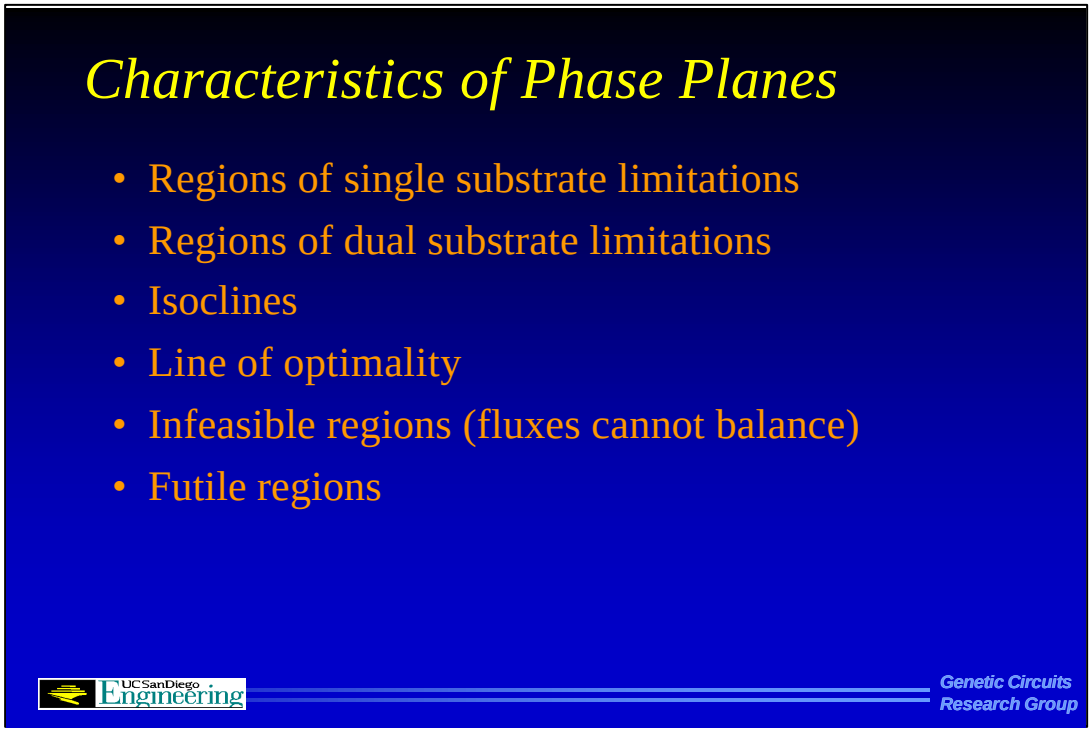
The definition of the shadow prices can be used to determine the slope of the isoclines within each region. Due to the definition of the phase plane, the slope of the isoclines will be constant within each region, however it will be different in the other regions.

We can draw isoclines for the objective function on the phase plane. The Isoclines are defined as the lines that will provide the same value of the objective function as the parameters on the X and Y axes are changed.

For example, as you follow this line, the objective function (here taken as growth rate) will be constant.

The state of the metabolic network can be classified by the value of alpha.

For example, a negative slope as shown here indicates dual substrate limitation. Isoclines can also be horizontal or vertical, and this corresponds to single substrate limitation. These situations occur when the shadow price for one of the substrates goes to zero, and thus has no value to the cell. Finally, the sign can change on one of the shadow prices, this will cause the isoclines to have a positive slope. This indicates a situation where one of the substrates is in excess and is actually inhibitory toward the cell. This defines a "futile" region.



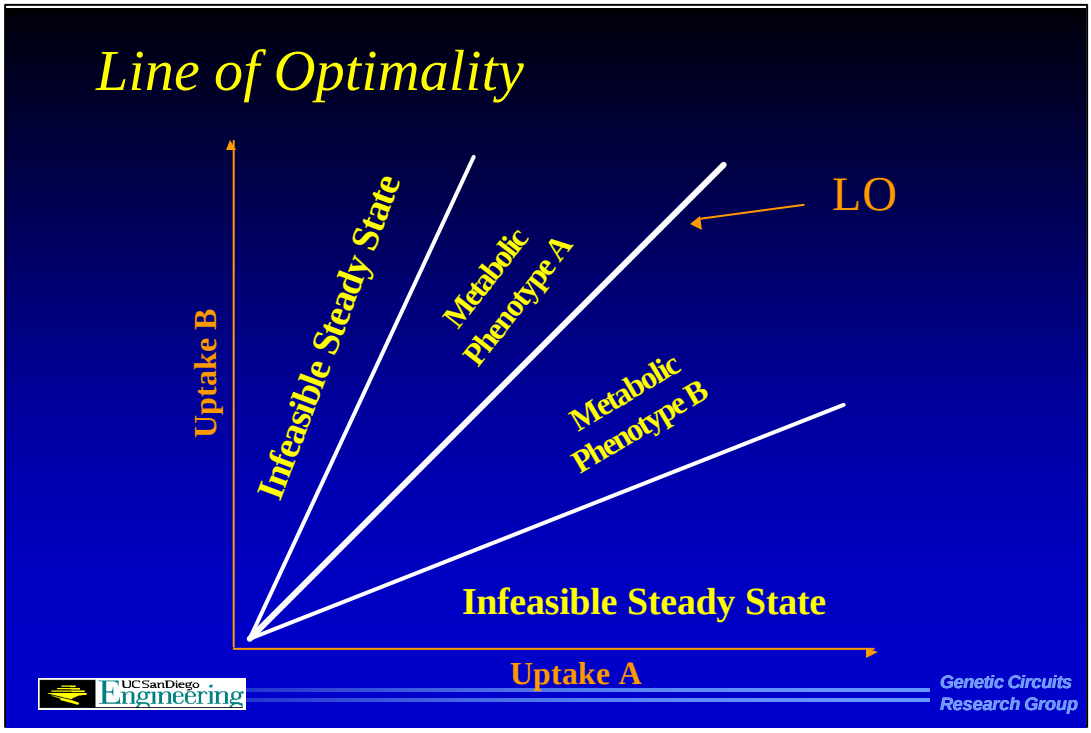
Characteristics of defined phases: The regions of the phase plane can be defined based on the contribution of the two substrates to the overall objective function:

In regions where the α value is negative, there is dual limitation of the substrates. Based on the absolute value of α , the substrate with a greater contribution toward obtaining the objective (here considered to be biomass production) can be identified. If the absolute value of α is greater than unity, the substrate along the x-axis is more valuable toward obtaining the objective, whereas if the absolute value of α is less than unity, the substrate along the y-axis is more valuable to the objective.

The regions where the isoclines are either horizontal or vertical are regions of single substrate limitation, the α value in these regions will be zero or infinite, respectively. These regions arise when the shadow price for one of the substrates goes to zero, and thus has no value to the cell.

Regions in the PhPP can also have a positive α value; these regions are termed “futile” regions. In these regions one of the substrates is inhibitory toward obtaining the objective function, and this substrate will have a positive shadow price. The metabolic operation in this region is wasteful, in that it consumes substrate that it does not need, and is thus unavailable for later utilization.

Finally, due to stoichiometric limitations, there are infeasible steady state regions in the PhPP. If the substrates are taken up at the rates represented by these points, the metabolic network is not able to obey the mass, energy, and redox constraints while generating biomass. The operation of the metabolic network can only transiently operate in such a region.

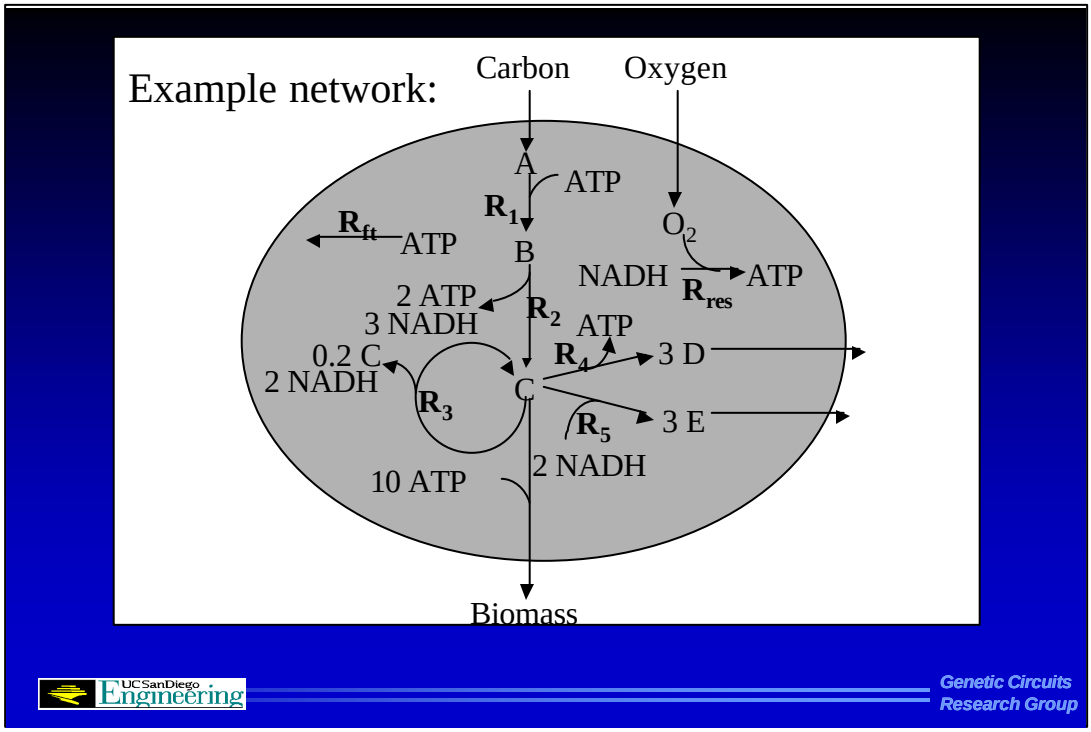


Line of Optimality:

The line of optimality is defined as the line representing the optimal relation between the two metabolic fluxes corresponding to the axis of the PhPP. For aerobic growth, this line is interpreted as the optimal amount of oxygen to be taken up to allow for the complete oxidation of the substrate.

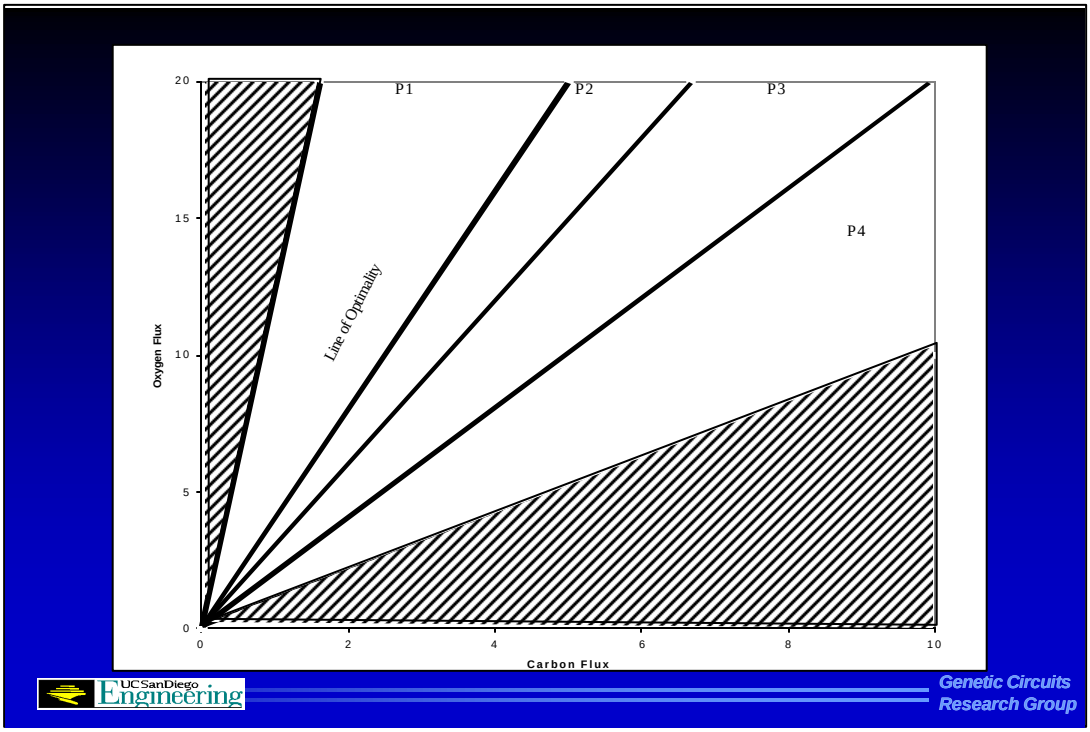
The line of optimality is determined by specifying the uptake rate of the substrate along the x-axis and allowing any value for the flux along the y-axis. LP is then used to calculate the optimal value of the objective as a function of the y-axis flux. Once the objective is determined, the corresponding flux value for the y-axis is used to plot the line of optimality (LO).

The LO defines the optimal utilization of the metabolic pathways without limitations on the availability of the substrates.



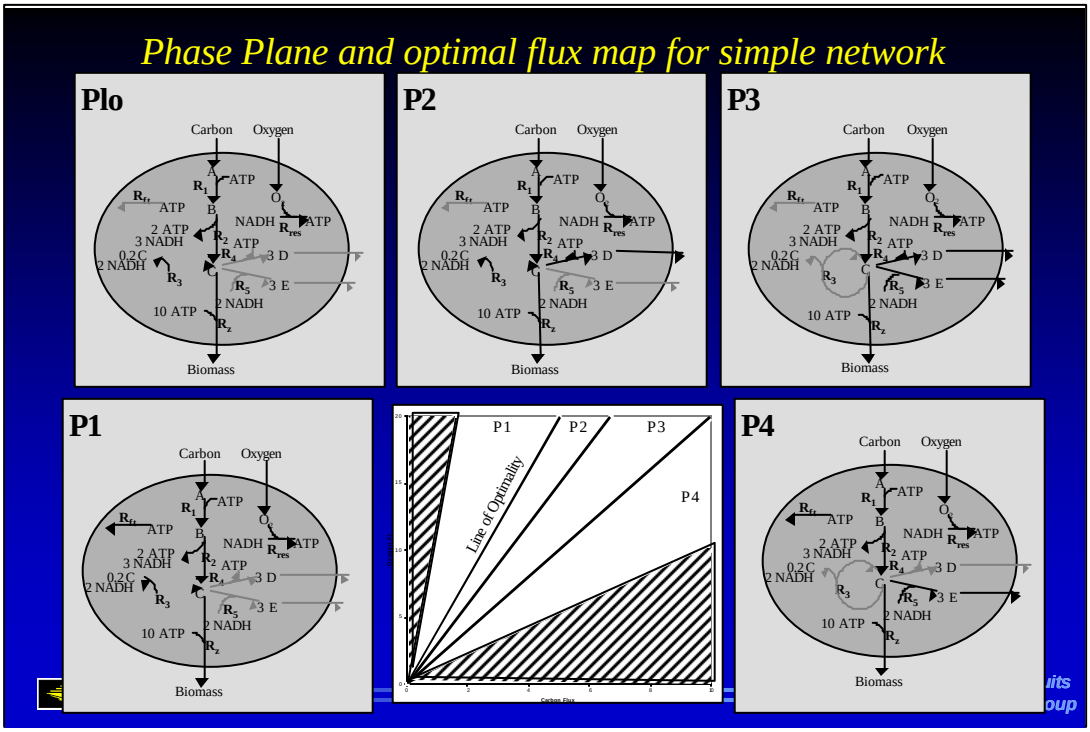
Example

To illustrate these concepts, we now present an example of a hypothetical metabolic system. This network utilizes a single carbon source, which it metabolizes to a single biosynthetic precursor, **C**. This precursor is converted into biomass, via $\mathbf{R_z}$ (the objective function), and to two different metabolic by-products, **D** and **E**. An electron acceptor, oxygen, is also included in this example. This electron acceptor can be used to convert redox potential into high-energy phosphate bonds, $\mathbf{R_{res}}$. Additionally, there is a reaction, $\mathbf{R_3}$, which consumes 0.2 **C** to generate NADH. Finally, one reaction, $\mathbf{R_{ft}}$, represents futile cycles that hydrolyze ATP.



The methods presented above were used to calculate the PhPP for this hypothetical metabolic system. The PhPP and the qualitative flux maps for each phase are shown the next slide. P1 is the futile region where the electron acceptor is provided in excess. The metabolic network dissipates the excess electron acceptor taken up by the cell by increasing the flux in $\mathbf{R_3}$, which generates NADH but also oxidizes the precursor, **C**. Additionally, the futile cycle reaction $\mathbf{R_{ft}}$ is utilized to eliminate the excess ATP produced. The upper limit of P1 occurs when the entire biosynthetic precursor produced is oxidized to eliminate the excess electron acceptor, and thus no biomass can be generated.

The shaded regions indicate no growth, or no biomass generation. These are the "infeasible steady states."



The metabolic flux map of this system is also shown for conditions on the line of optimality (LO). The LO is a special case of P1; this is the point where the electron acceptor is no longer in excess and the futile cycle flux is zero (Table 1--next slide). The LO represents the optimal utilization of this example metabolic network to produce biomass. The qualitative flux map indicates that under conditions defined by the LO there is no metabolic by-product production and futile cycle flux equals zero.

The next distinct flux map for this hypothetical metabolic network is found in region P2. In P2 a reduced metabolic by-product (**D**) is secreted from the cell. The shadow price for the metabolite **D** in this system is zero in region P2, and the utilization of the metabolic pathways in this region is fundamentally different than in P1, Plo, P3, and P4. The metabolic pathway for the production and secretion ($\mathbf{R_4}$) of **D** is turned on under the conditions defined in this region, and the excess redox potential is eliminated through the secretion of **D**.

The utilization of the metabolic network in P3 is fundamentally different than in P2. In P3, the cyclic reaction $\mathbf{R_3}$ is not utilized, and thus redox potential production is reduced. Both of the reduced metabolic by-products are secreted (**D** and **E)** as sinks for redox potential. Thus, in this region, both of these metabolites will have a shadow price equal to zero.

Finally, in P4, the futile cycle reaction is utilized, and all the metabolic by-

Shadow prices for simple network

Table 1: Shadow price of the metabolites from the example shown in Figure 1.

	Carbon	A	B	C	D	E	O_2	NADH	ATP
P1	-1.30	-1.30	-1.30	-1.00	-0.33	-0.40	**0.10**	-0.10	
Plo	-0.90	-0.90	-0.93	-0.67	-0.21	-0.27		-0.07	-0.03
P2	-0.21	-0.21	-0.30	-0.09		-0.04	-0.17	-0.01	-0.09
P3	-0.05	-0.05	-0.14	-0.09			-0.23	**0.05**	-0.09
P4	**0.50**	**0.50**	**0.50**	-1.00	-0.33		-0.50	**0.50**	

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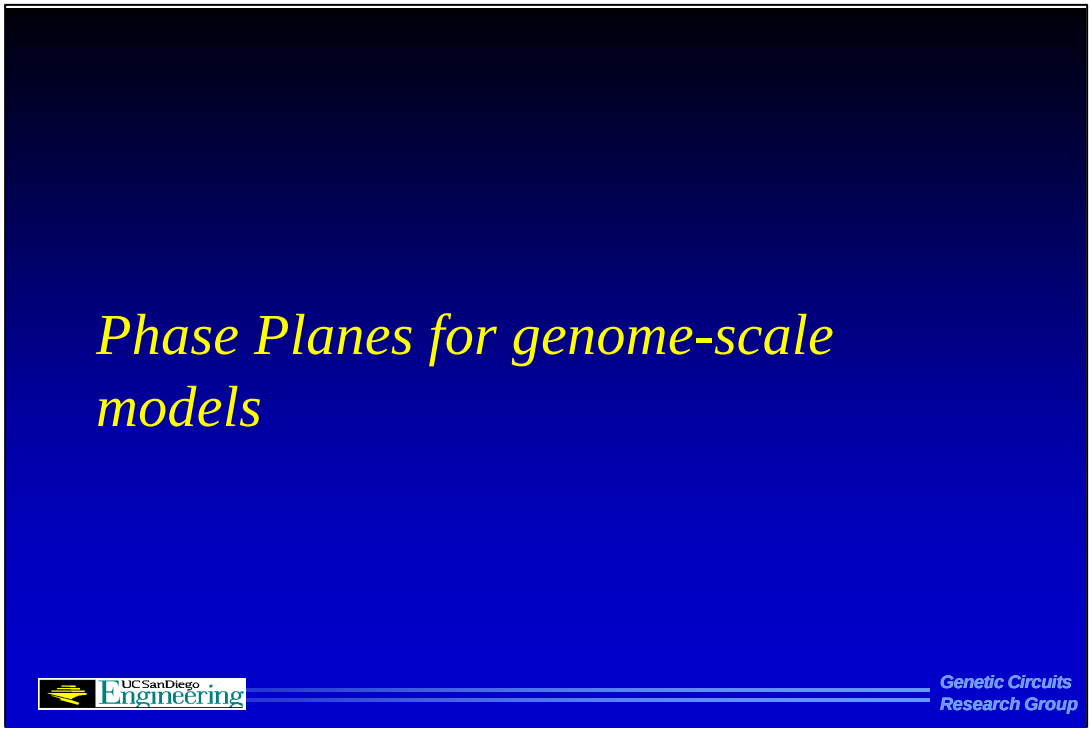
Shown here are a table of the shadow prices in the different phase plane regions. Numbers shown in bold are positive and zero shadow prices are left as blank spaces in the table.

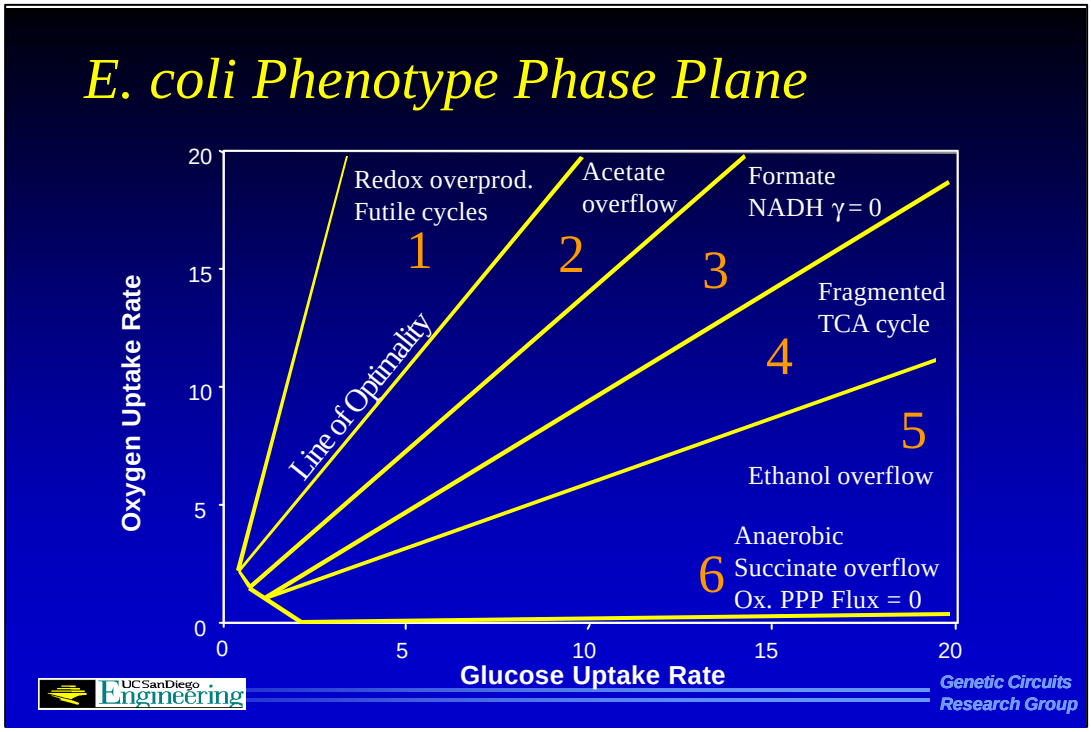
This simple example illustrates the utility of the PhPP in the interpretation of the metabolic physiology of the system. It clearly shows that the optimal phenotypes are condition dependent, and that a finite number of qualitatively different optimal phenotypes can be derived from a single genotype.

End of example



Genome Scale Models

Phase planes for genome scale models can be computed even though the stoichiometric matrices that represent a whole organism may be large. In this class we can only solve 100 X 100 stoichiometric matrix, so none of these examples will be used for homework purposes.

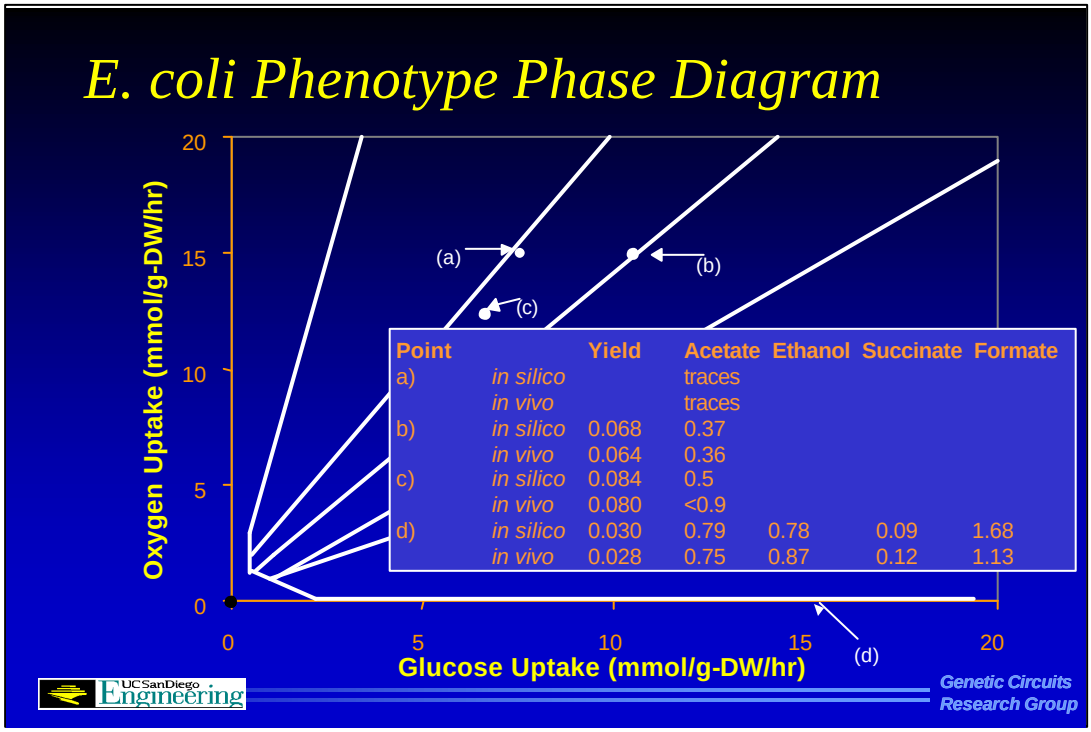


The *E. coli* Glucose-Oxygen Phase Plane

This is the phenotype phase plane for *E. coli.* It represents all the qualitative optimal metabolic phenotypes of *E. coli* as a function of the glucose and oxygen uptake rate.

It is shown that there are 6 distinct phases or phenotypes.

- Region 1 is a futile region, where redox potential is overproduced.
- Adjacent to this region is the line of optimality. It represents the optimal relation between the glucose and oxygen uptake rate.
- Region 2 is the acetate overflow region, and acetate is optimally secreted as a metabolic by-product.
- In Region 3, formate is optimally secreted.
- In Region 4, the TCA cycle does not operate cyclically, and serves the role of biosynthetic precursor production.
- In Region 5, ethanol is secreted as a metabolic by- product.
- Finally, Region 6 is the completely anaerobic phase, with succinate secreted as a metabolic by-product.



In Silico* vs. *In Vivo

The *in silico* results were compared to independently obtained experimental observations.

Comparison with experimentally determined metabolic behavior of the wild type strain is noted on the Phase Plane

This figure depicts several points for which the biomass yield, by-product production, and the uptake rates of glucose and oxygen were measured.

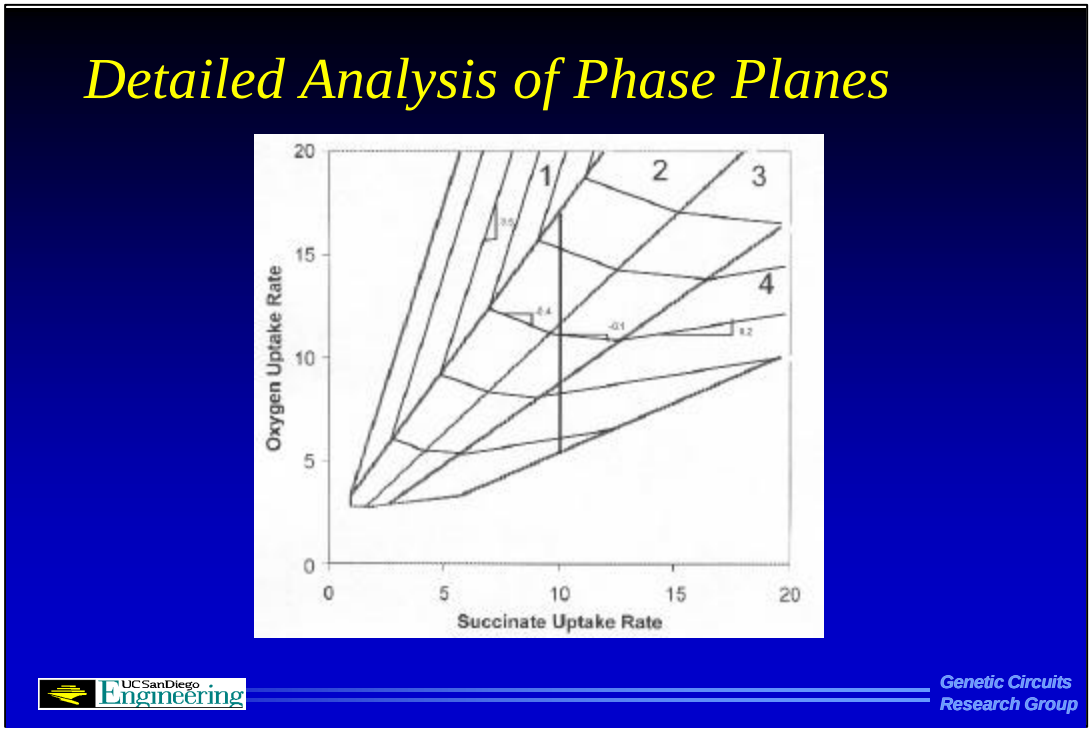
The *in silico* predictions were obtained by using the experimentally determined uptake rates as input into the simulation.

The *in silico* predictions were all consistent with the experimental data, and the points in the phase plane are noted on this diagram.

For example, point (a) was experimentally determined to be the point at which acetate is secreted by the cell when the glucose uptake was increased.

Points (b) and (c) were also in quantitative agreement with experimental data.

Additionally, one anaerobic point was compared to the in silico predictions, and the biomass yield, the production of acetate, ethanol, formate, and succinate, were found to be in quantitative agreement with the simulations.



The Succinate Phase Plane in *E. coli*.

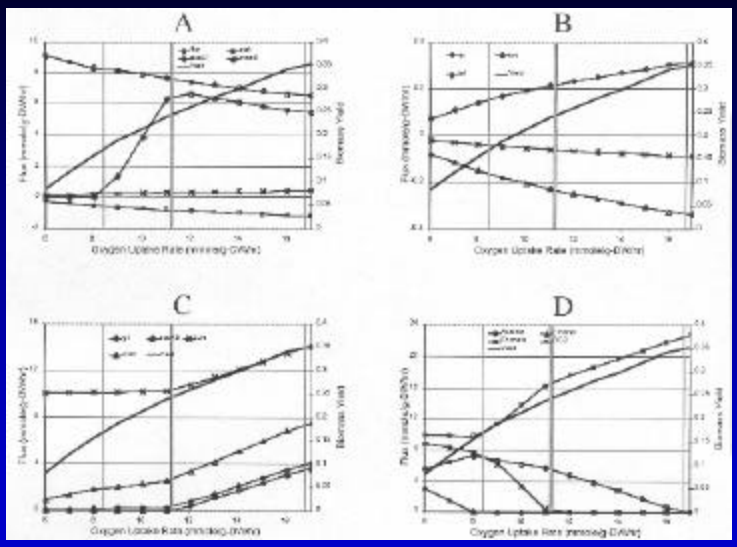
This slide shows the details of the succinate phase plane in the genome scale *E. coli* model. It shows all the phases of the phase plane and the isoclines in them. As we have seen in previous slides, the optimal use of the metabolic map is different in each phase. In other words, the flux maps are fundamentally different in each one of those phases. This slide also shows a straight line at a constant glucose uptake rate, and varying oxygen uptake rates. We can calculate the variation of any internal flux in the network as a function of oxygen concentration along that line. Such calculations are shown in the next slide.

The succinate-oxygen PhPP contains four distinct metabolic phenotypes. There are several distinguishing characteristics of the succinate-oxygen PhPP. First, the PhPP illustrates that the cell is unable to utilize succinate as the sole carbon and energy source for growth in an microaerobic environment. Secondly, the PhPP has two futile regions: one where oxygen is in excess (Phase 1), and another where the carbon source is in excess (Phase 4). The succinate-oxygen PhPP also contains two additional dual substrate limitation regions.

$P1_{succ,oxy}$ is an oxygen excess futile region. Additional carbon is consumed to eliminate the oxygen that is provided in excess. Therefore, the operation of the metabolic network in this region is wasteful because carbon is oxidized to eliminate the oxygen, and is unavailable for biomass production. The availability of high-energy phosphate bonds is not limiting growth in the futile region, and the α value in this region is 3.5. Thus, the stoichiometric coupling of oxygen and succinate in this region is 7:2.

On the LO, (separating $P1_{succ,oxy}$ from $P2_{succ,oxy}$) the optimal utilization of the metabolic pathways involves the cyclic operation of the TCA cycle, no flux in the oxidative branch of the PPP, the utilization of the malic enzyme to produce pyruvate and NADPH, and CO_2 production as the only metabolic by-product.

Detailed Analysis of Phase Planes



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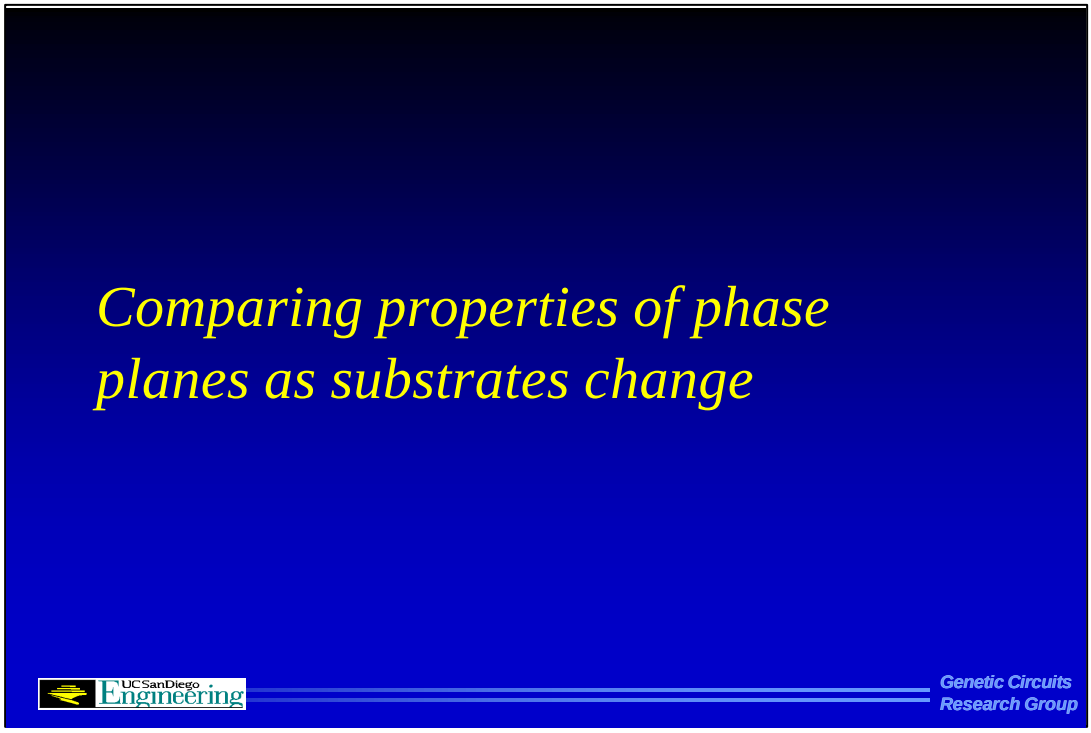
The effect of decreased oxygen availability on metabolic fluxes was examined with the succinate uptake rate was set to constant value ($b_{succinate\_uptake}$ = 10 mmole/g-DW/hr). The vertical lines in the four panels separate the four phases.

In region $P2_{succ,oxy}$, biomass production is limited by the availability of both succinate and oxygen. The absolute value of α in this region is small (0.4), and oxygen has over twice the value (contribution to biomass production) as succinate on a per mole basis. The α value indicates that the availability of oxygen is more important than succinate toward maximizing the objective function (cell growth) under these conditions. In $P2_{succ,oxy}$, the acetate production, pyruvate dehydrogenase, and the malic enzyme fluxes were increased as the oxygen uptake rate was decreased. The other fluxes in the central metabolic pathways were decreased with the decreased oxygen exchange flux in $P2_{succ,oxy}$. The availability of redox potential and high-energy phosphate bonds are limiting the ability of the cell to generate biomass, as indicated by the negative shadow price for these metabolites ().

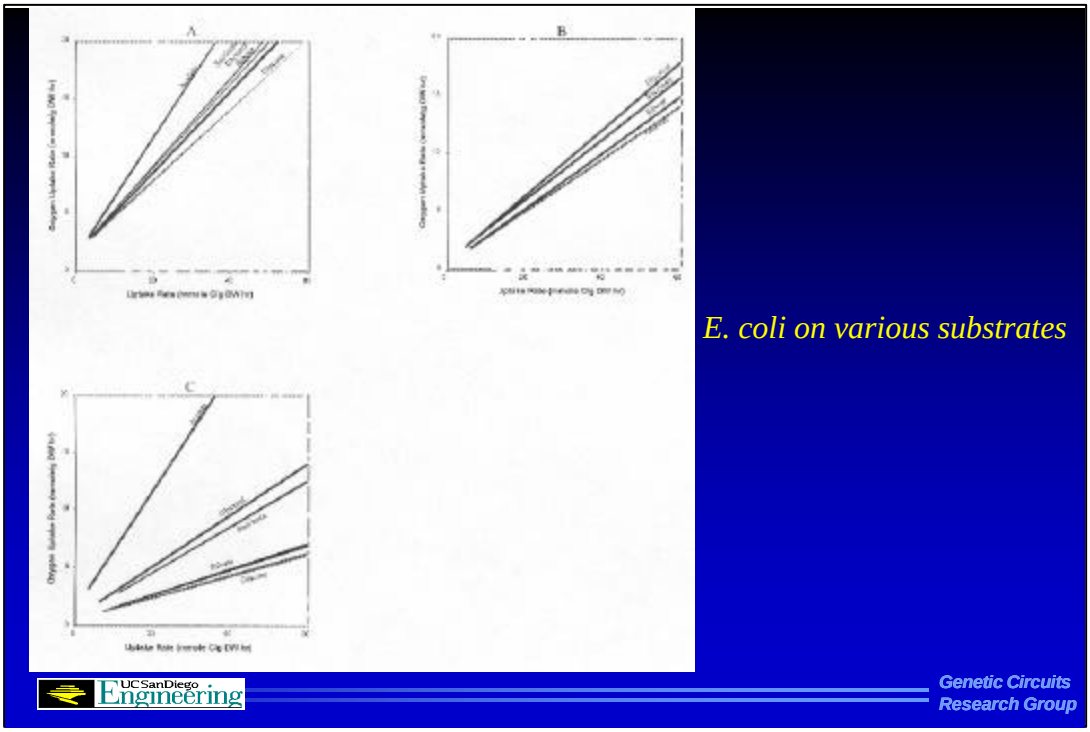
In region $P3_{succ,oxy}$ the value of succinate to the cell's ability to generate biomass is lower than in $P2_{succ,oxy}$ This is due to the low oxygen availability to the cell, and the reduced cellular efficiency of converting succinate to biomass. The reduced value of succinate is reflected in the α value in this region (-0.1). Thus, the isoclines are nearly horizontal, which indicates that growth is almost solely limited by the availability of oxygen. In $P3_{succ,oxy}$, additional NADH has no value to the cell, the malic enzyme flux is further increased, and the pyruvate dehydrogenase flux is decreased with the carbon directed toward the formation of formate. Furthermore, it can be seen that the TCA cycle does not function cyclically.

$P4_{succ,oxy}$ is a futile region where succinate is in excess. The succinate that is taken up beyond the demarcation is not utilized for biomass production, rather, cellular resources must be devoted to the elimination of this excess succinate. This relation is quantitatively observed by the positive slope of the isoclines. The ability of the metabolic network to eliminate excess redox potential limits cellular growth in this region. Although the over-production of redox potential is limiting biomass formation, additional high-energy phosphate bonds are desirable as indicated by the shadow prices. The redox constraints facing cellular growth in this region lead to the production of ethanol as part of the optimal metabolic flux distribution. However, the *in silico* analysis does not predict the formation of ethanol in any region other than the futile region.



Use of Phase Planes for Detailed Analysis

As we have seen in the previous slides, we can calculate phase planes and produce experimental results. These results cumulatively indicate that *E. coli* does grow optimally on the substrates tested. With this in hand, we can actually calculate the phase planes for a range of substrates and compare them.

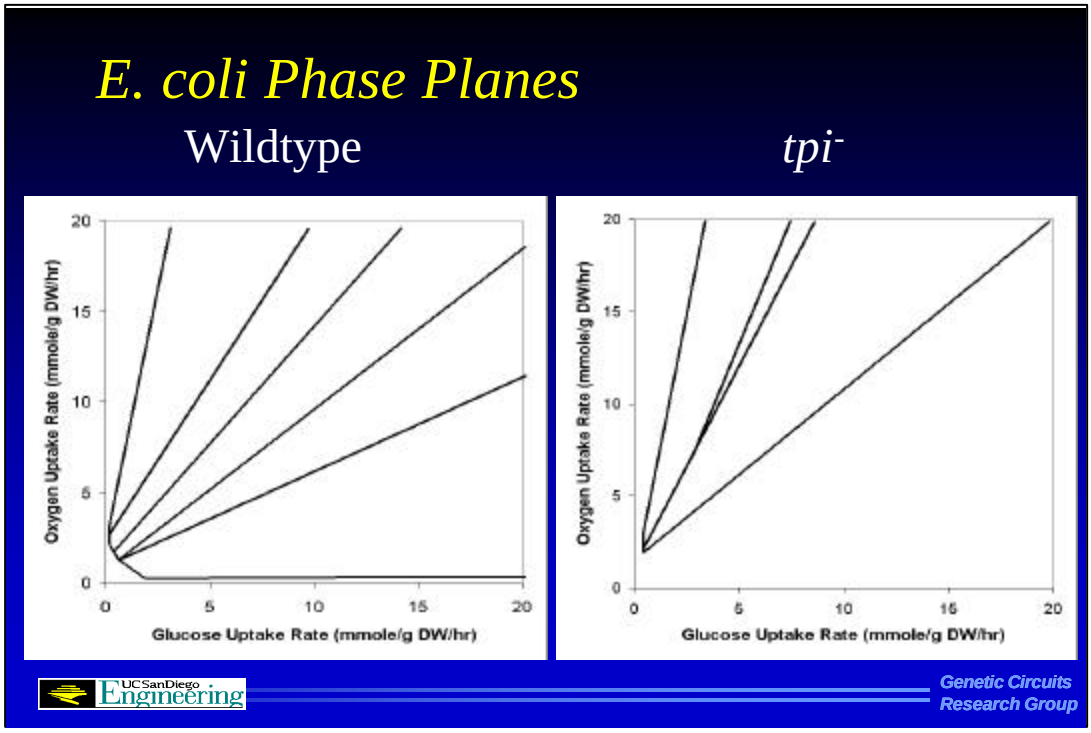


Comparing Phase Planes

This slide shows the comparison of three properties of phase planes for many different substrates. These calculations are all done for the genome scale model of *E. coli*. Panel A shows the lines of optimality for a number of substrates. Panel B shows the demarcation between phase two and three where redox metabolism becomes a challenge for the cell. Finally, panel C shows the demarcation below which ethanol is secreted by the cells. Quantitative experiments and interpretations result from comparison of this numerical data.

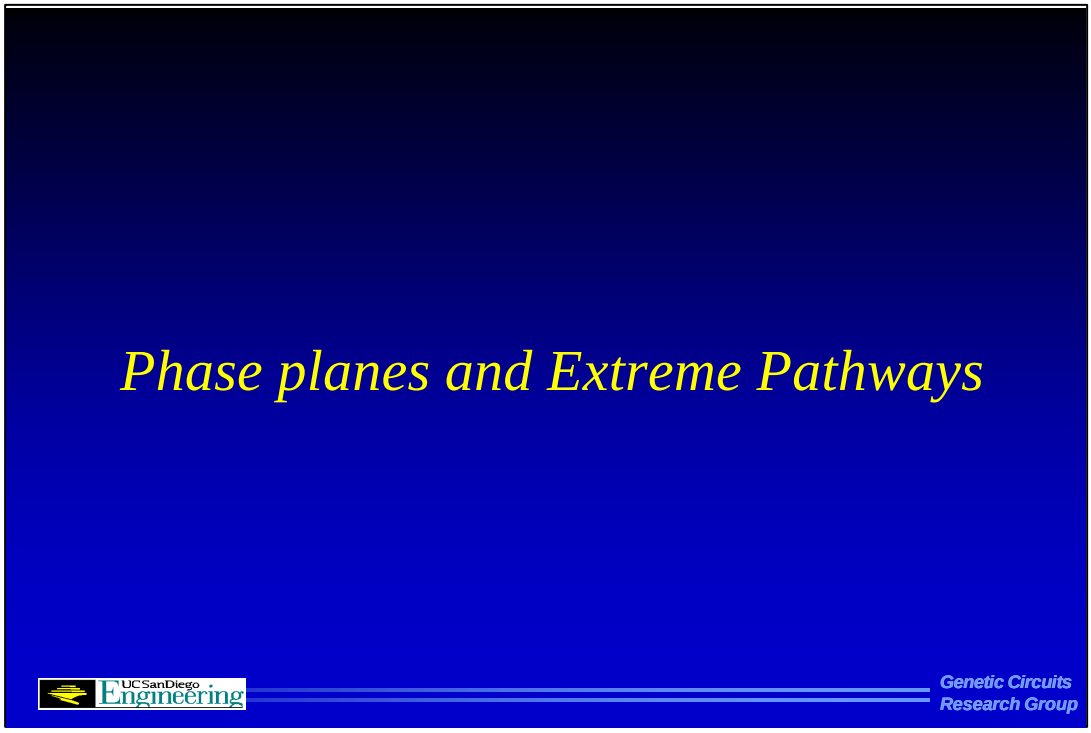
Phase Plane Analysis of Gene Deletions

As with analysis of multiple substrates, we can calculate phase planes for a wild-type strain and we can calculate a phase plane for a mutant or a knockout from that wild-type strain. Comparing these phase planes allow us then to directly compare what we expect to be optimal growth properties of the wild-type strain and how they will be altered by knocking out the gene.



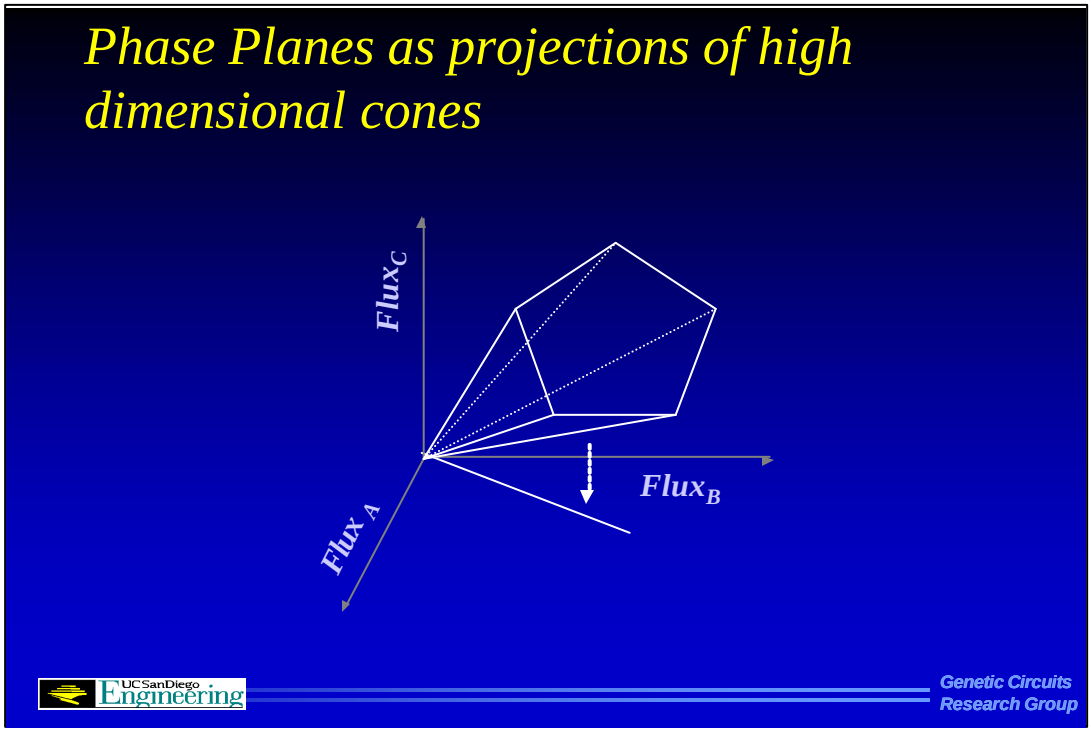
The *tpi* Knockout

This slide shows the comparison of the glucose-oxygen phase plane for *E. coli* K12, the wild-type to the *tpi* knockout triosphosphate isomerase. The enzyme encoded by *tpi* is a key enzyme in glycolysis and seriously disturbs metabolism in the lower part of the glycolytic pathway if lost. The phase plane shown on the right differs from the one on the left considerably. Now there are large regions in the phase plane where growth is not enabled, the line of optimality has shifted, and so forth. Clearly these two phase planes can be used for quantitative and insightful experimental design of the phenotypic consequences of a knockout.



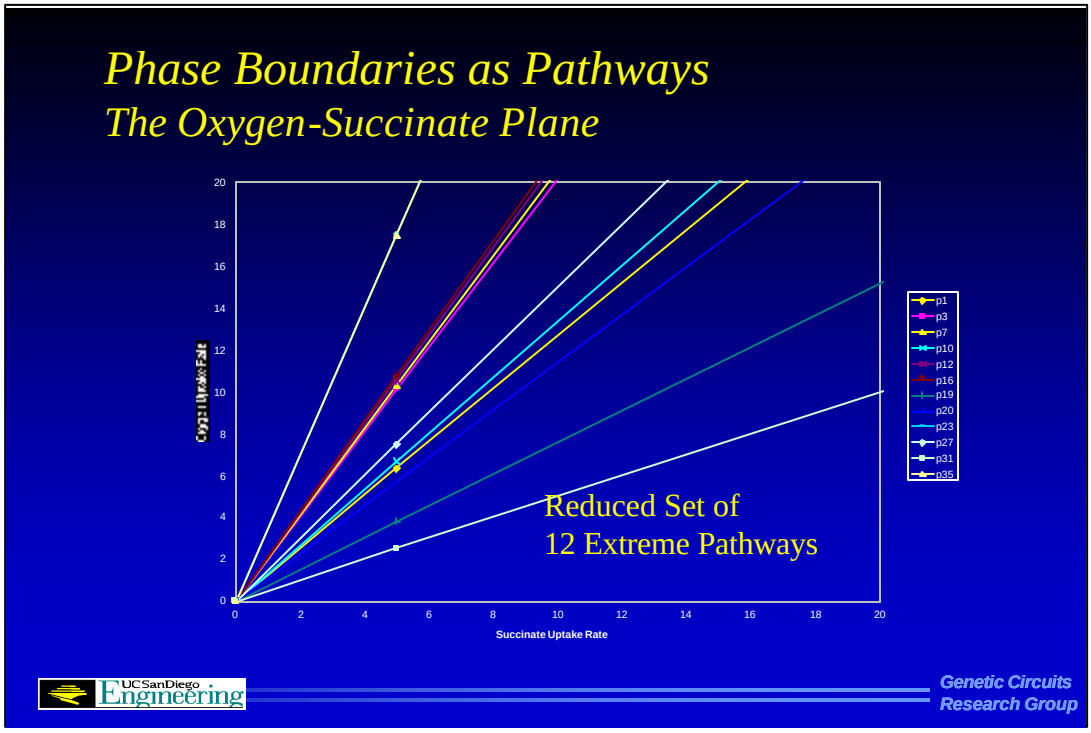
The Relationship Between Phase Planes and Extreme Pathways

In previous lectures we covered the topic of extreme pathways as the generating vectors for cones in high-dimensional spaces. It turns out that there is a close relationship between these extreme pathways and what is shown in the phase plane.



Projections of Extreme Pathways

This slide illustrates the projection of the edge of a cone onto a 2-dimensional space. The 2-dimensional space would be formed by the two uptake fluxes or any other two fluxes of interest, and the vector corresponding to the edge is drawn in that particular 2-dimensional phase plane. If that edge corresponds to an extreme pathway that is physiologically meaningful, and the cell positions itself close to it, then the data will project onto the phase plane very close to that line. This indeed corresponds to the line of optimality shown in the numerous slides before this. The line of optimality is an edge on the cone in a high dimension.

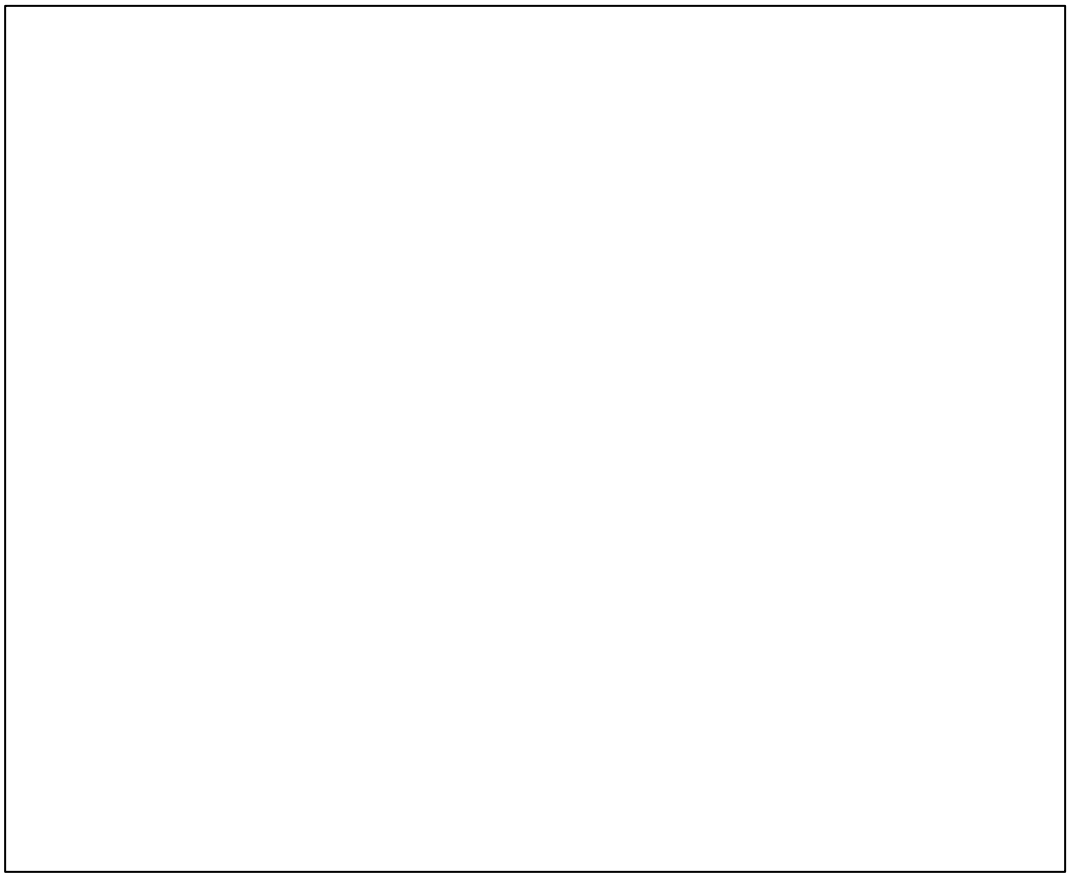


The Oxygen-Succinate Extreme Pathways in the Phase Plane

This slide shows the projection of a number of extreme pathways calculated from the core *E. coli* model with succinate as the carbon source. We see that all these pathways form a straight line in the phase plane. One of these pathways corresponds to the line of optimality, and it in return corresponds to the extreme pathway with the highest biomass yield.

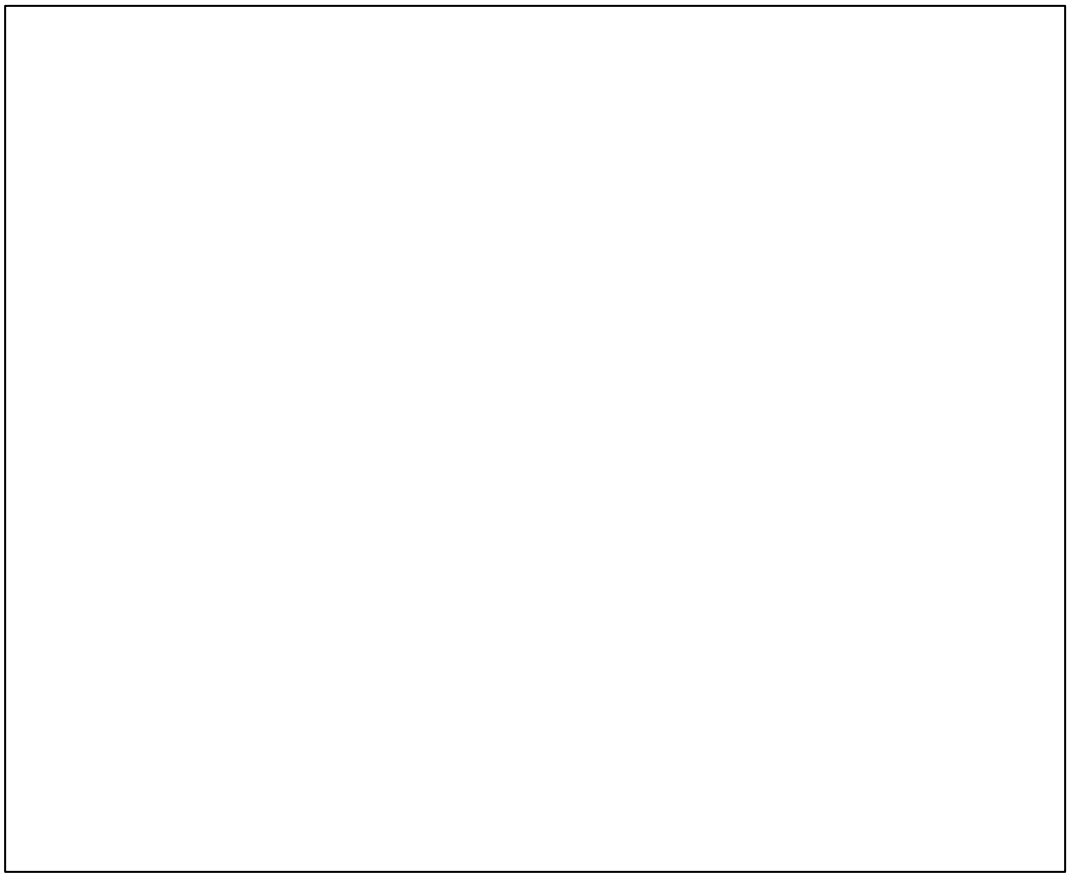
Summary

- A series of LP can be solved to represent a continuous variation in a parameter of interest
- Shadow prices are critical in interpreting the results of such calculations
- All possible combinations of the values of two parameters leads to the definition of a phase plane
- The phase plane can be used to study the genotype-phenotype relationship and to design experiments
- The boundaries in the phase plane are edges on the polyhedral cone projected into the plane. Thus, the boundaries represent systemic pathways



Future challenges

- Volumes of cones
- Intersections of cones
- Regulation and removal of edges of cone
- Continuous cell culture data
- Shifts in growth conditions
  - Compare flux maps and expression profiles
- Detailed analysis of mutant function



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