Flux-balance Analysis:
Determining the capabilities of networks

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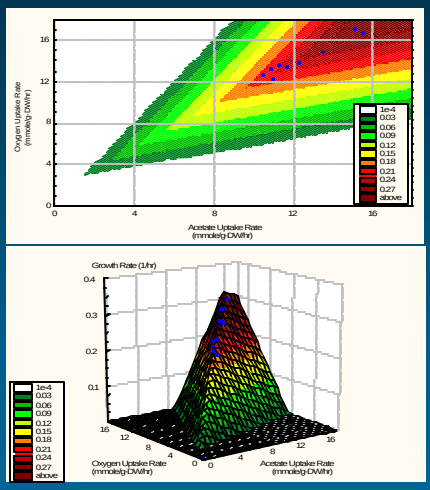
Outline

1) Phase Plane Analysis

2) Capabilities of mitochondrial metabolic network

3) Altering the Genotype: Gene deletions

4) Phase Planes and Experimental Design



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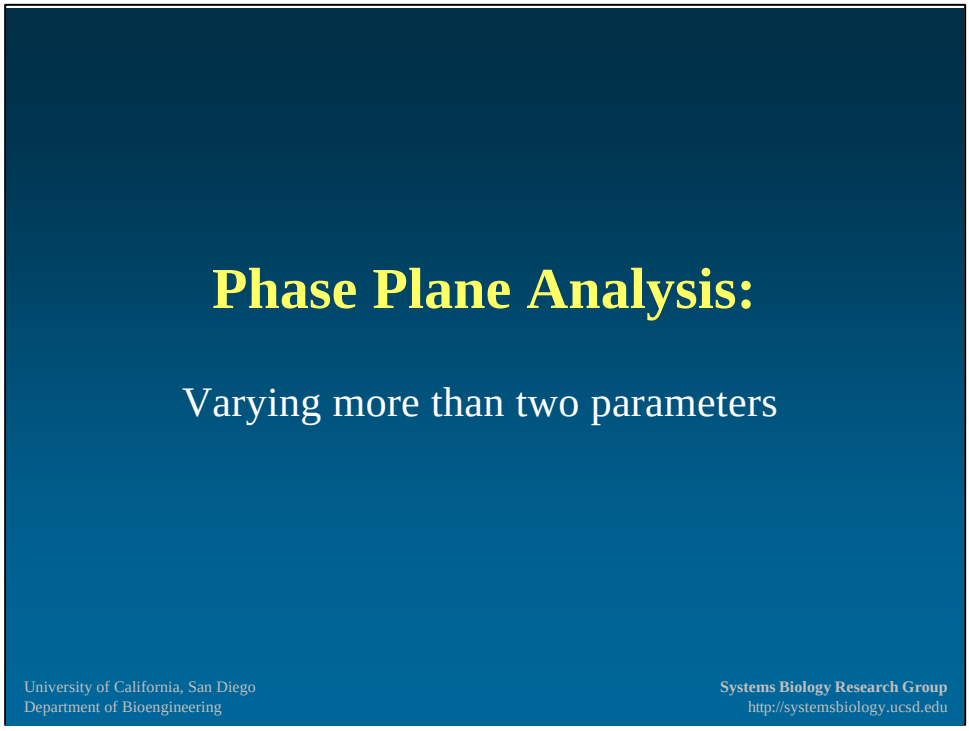
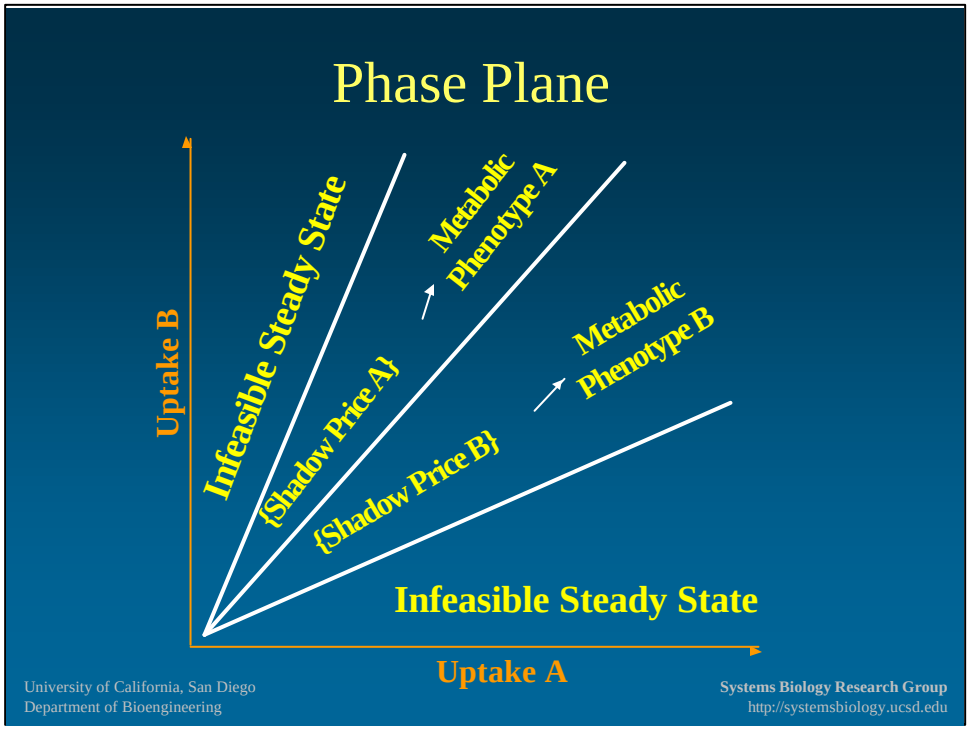


Phenotype Phase Plane Analysis

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.

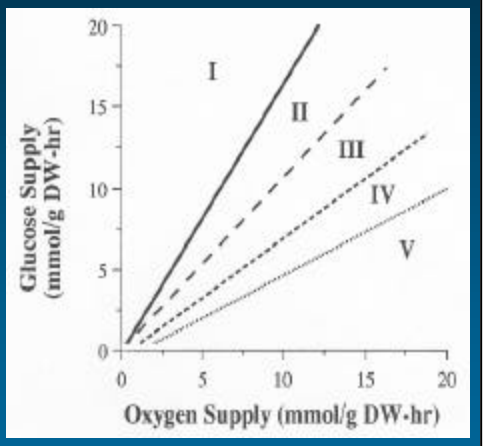
The shadow prices are constant within each region and will be different in the other regions.

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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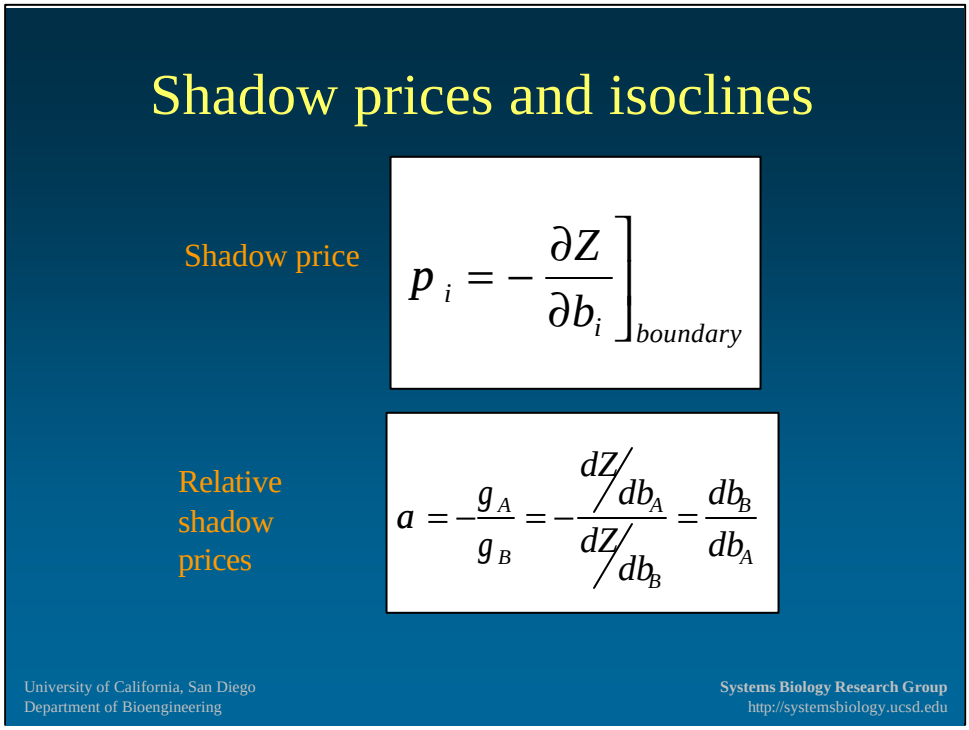
Summary of Phenotype Phase Planes

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.

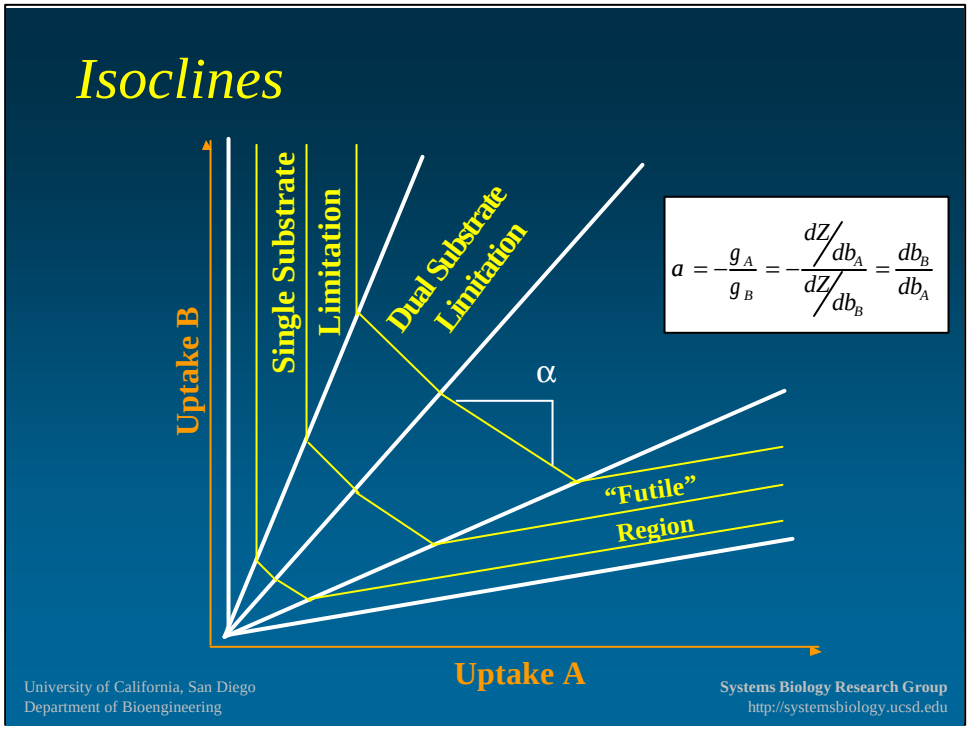


Isoclines

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

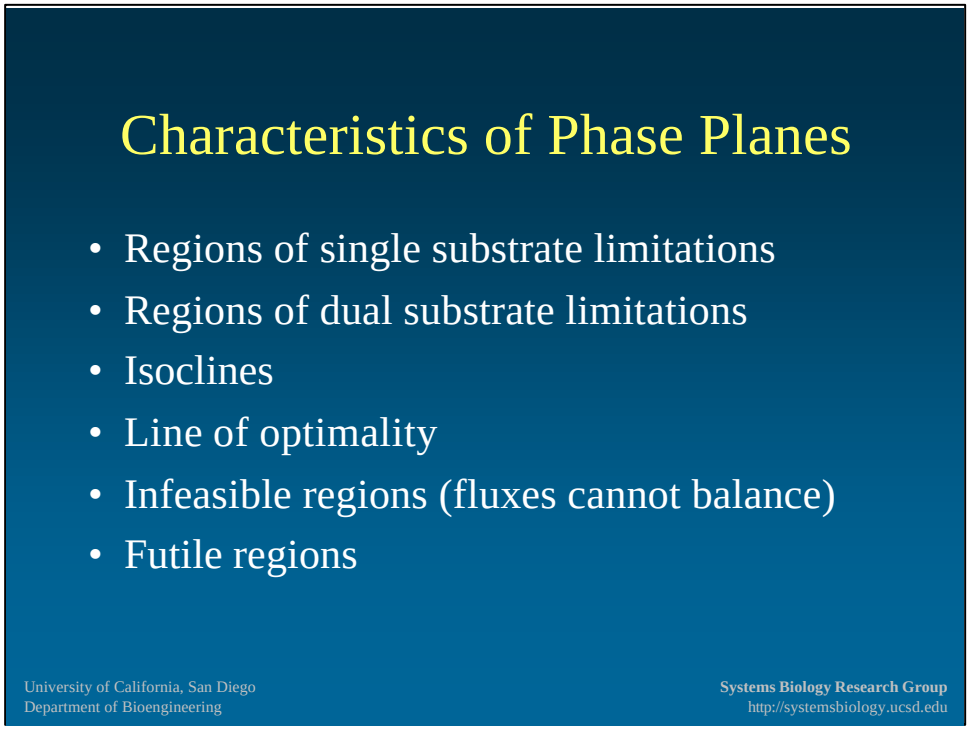
The definition of the shadow prices can be used to determine the slope of the isoclines within each region. Due to the definition of the phase plane, the slope of the isoclines will be constant within each region, however it will be different in the other regions.

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.



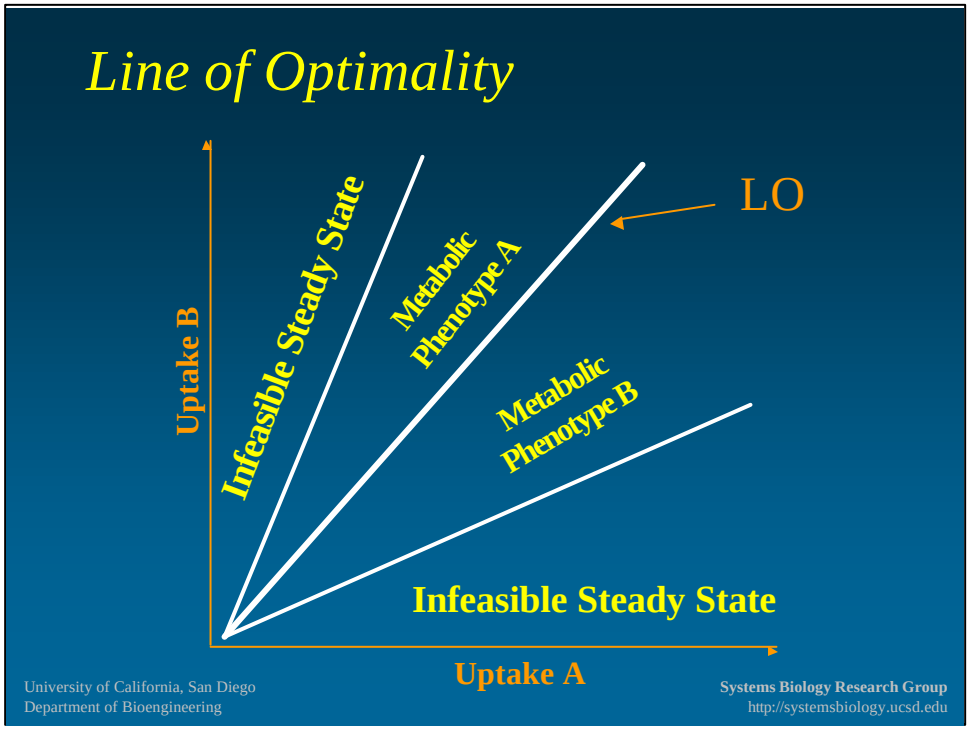
Characteristics of defined phases: The regions of the phase plane can be defined based on the contribution of the two substrates to the overall objective function:

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.

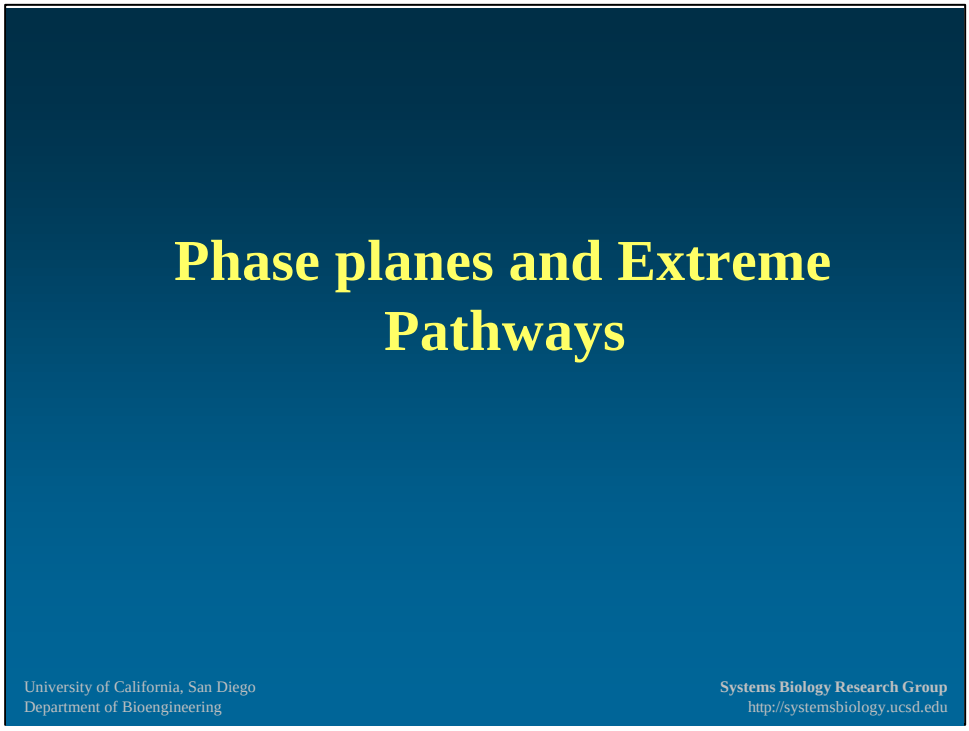


Line of Optimality:

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.

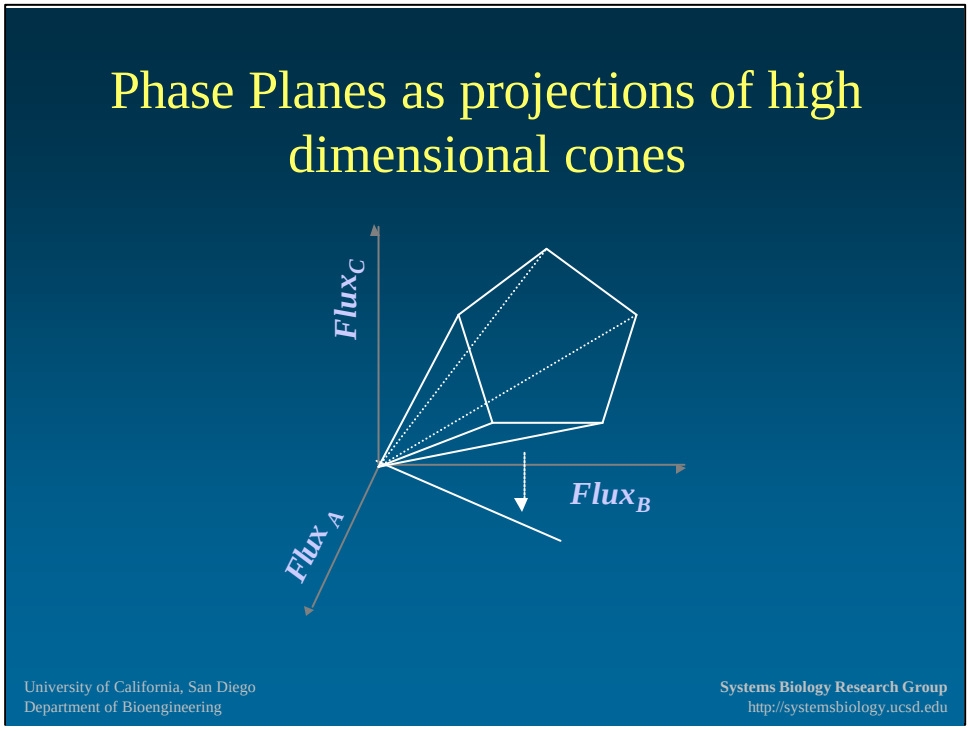
The line of optimality is determined by specifying the uptake rate of the substrate along the x-axis and allowing any value for the flux along the y-axis. LP is then used to calculate the optimal value of the objective as a function of the y-axis flux. Once the objective is determined, the corresponding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



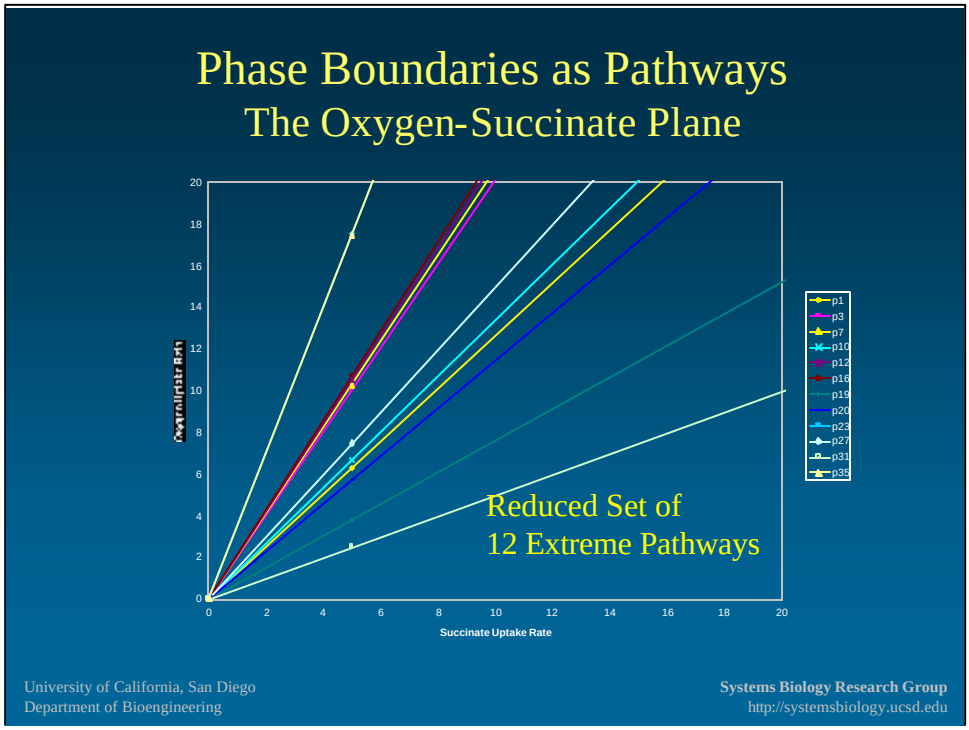
The Relationship Between Phase Planes and Extreme Pathways

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.

Capabilities of Mitochondrial Metabolic Network

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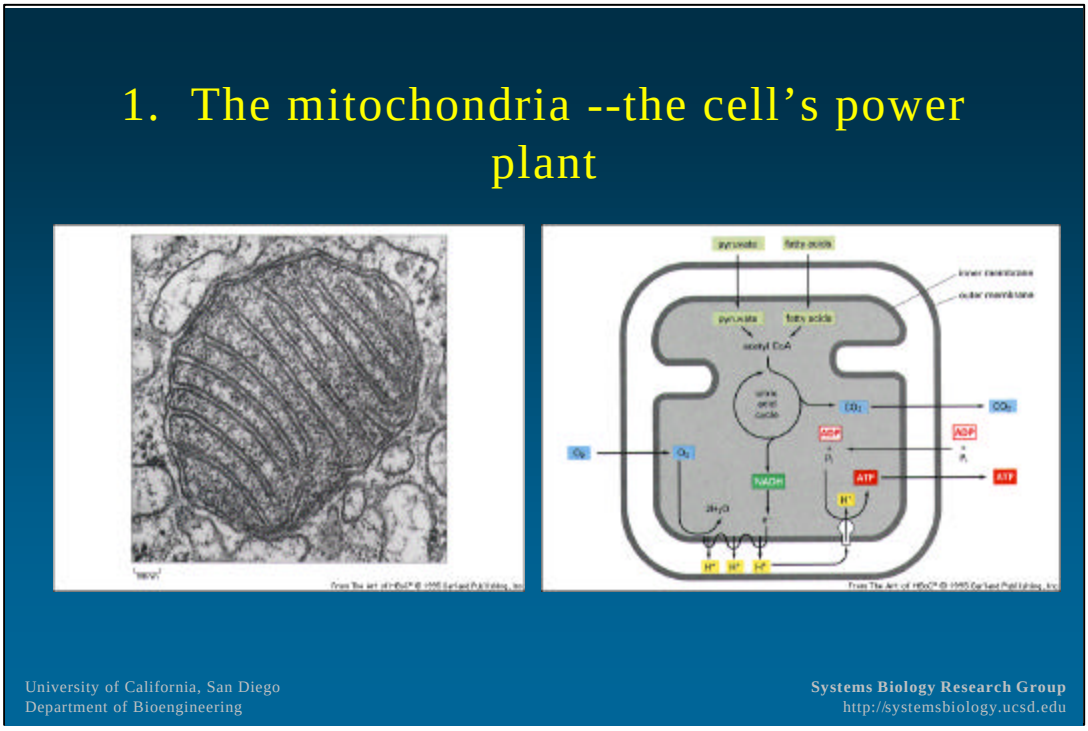
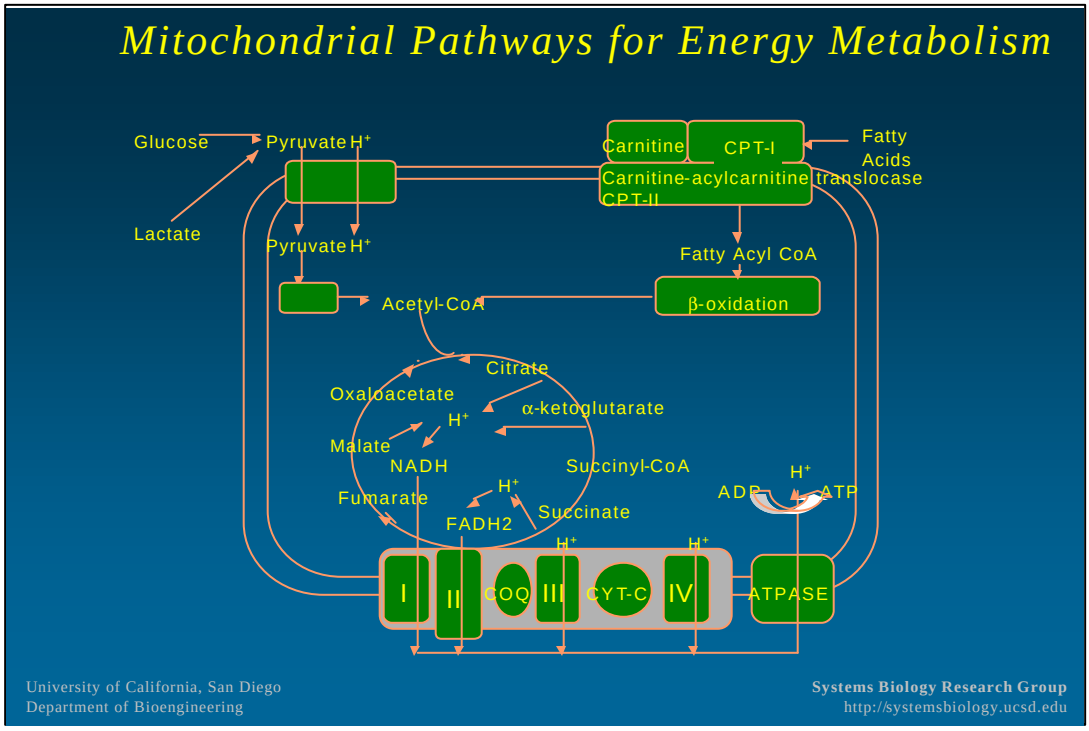


THE MITOCHONDRIA

Cellular respiration, and consequently mitochondrial metabolism, has a significant role in the functions of aerobic organs. In systems such as the heart, this process supports myofilament contraction, transmembrane ion and intracellular calcium cycling. Normal well-perfused myocardium generates more than 90% of its ATP by oxidative metabolism and less than 10% by anaerobic glycolysis. Mitochondrial metabolism also plays a critical role in the function of other organs, such as the liver and the brain, where the impaired functioning of mitochondria has been implicated in several neurological disorders.

THE OBJECTIVE OF MITOCHONDRIAL FUNCTION

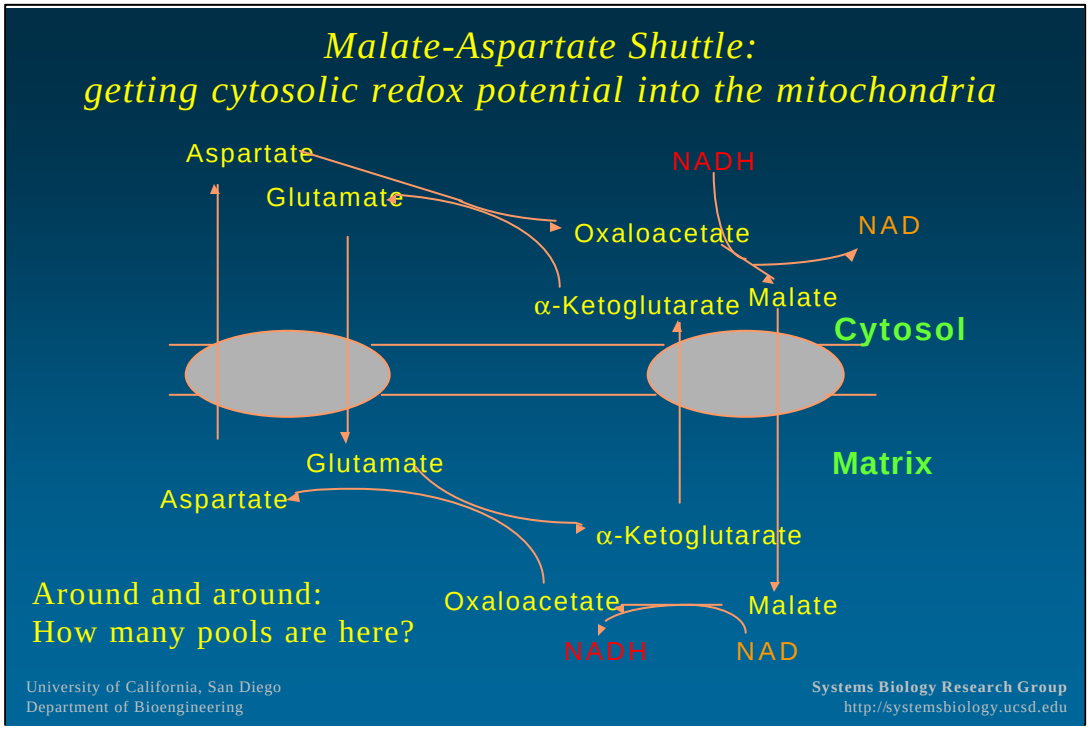
The flux balance model requires an objective which the cell or the organelle is attempting to achieve. For this analysis, maximizing the production of ATP is chosen as the objective function. Cairns *et al*. have considered the functional basis for control of mitochondrial oxidative phosphorylation in different organs for rat mitochondria. ATP production in the brain and heart mitochondrial systems were found to use more oxygen, but produce ATP at a faster rate than liver systems. They attribute these qualities based on the thermodynamic degree of oxidative coupling. The general conclusion is that maximizing the rate of energy production, rather than maintaining thermodynamic efficiency is the important characteristic of mitochondria in physiological systems. This conclusion supports the postulate that the objective of mitochondrial metabolism is the maximization of ATP production.



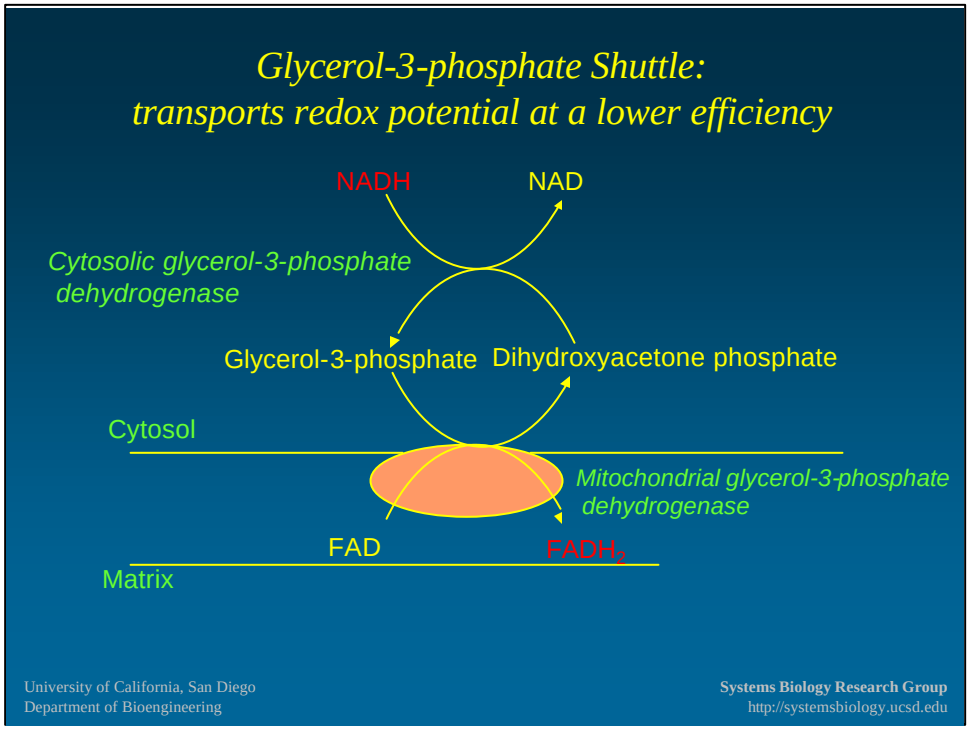
THE MITOCHONDRIAL MODEL

The flux balance model for mitochondria presented here, comprises the glycolytic pathway, the TCA cycle and the electron transport system. The pentose phosphate pathway has not been included, since its activity is believed to be quite low for mitochondrial related functions. The oxidative metabolism of substrates takes place in the mitochondria; thus the substrates, metabolites and cofactors must cross the selectively permeable membrane that separates the mitochondrial space from the cytosolic space. The reactions comprising the TCA cycle occur in the mitochondrion and produce the reduced coenzymes, NADH and $FADH_2$, that transfer electrons to oxygen in a regulated manner. Shuttles play an important role in transporting reducing equivalents generated in the cytosol into the mitochondrion.

The reactions, which make up the model, are divided into three sets, based on whether they occur in the cytosol or in the mitochondria, or in transporting an intermediate across the mitochondrial membrane. The glycolytic reactions take place in the cytosol. The reactions in the TCA cycle and the electron transport system take place in the mitochondrial matrix. This compartmentalization of reactions in an organelle leads to the need for special reaction sequences, or shuttles, to transport reducing equivalents generated in the cytosol into the mitochondria. There are two such shuttles that have been found to be active in mitochondria.

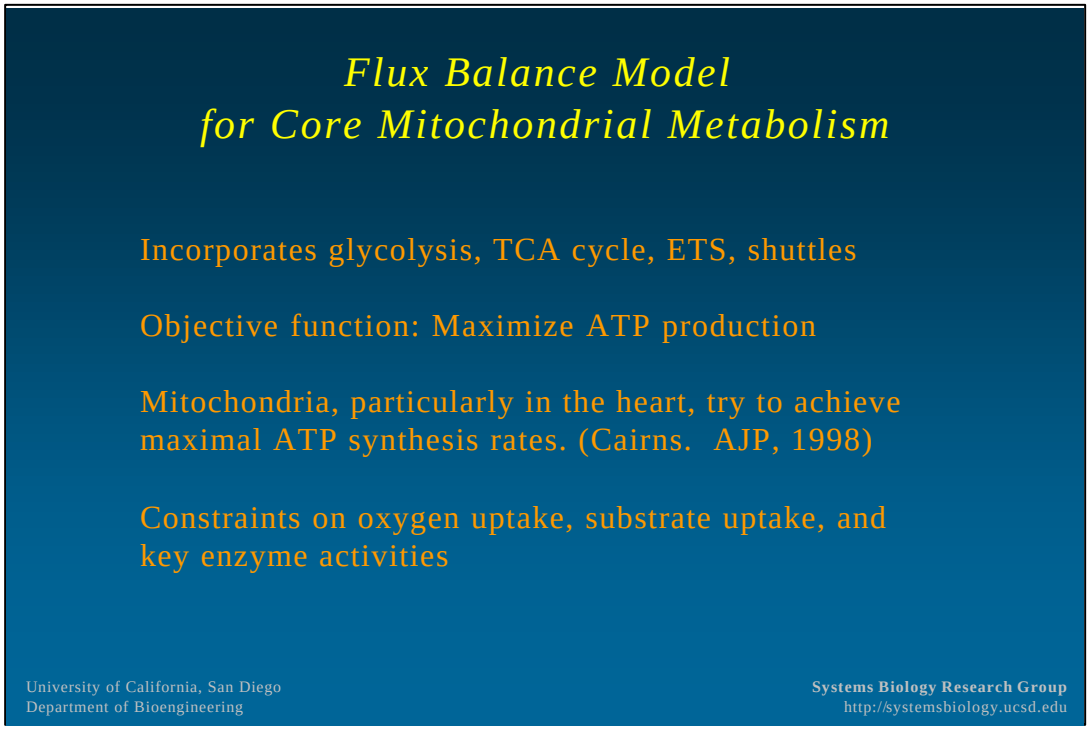


The ubiquitous malate-aspartate shuttle transports external NADH into the mitochondria. The coordinated exchange of malate and α-ketoglutarate with aspartate and glutamate by respective antiporters is a key feature of the shuttle. The malate dehydrogenase reaction functions in opposite directions in the cytosol and in the matrix. The cytosolic reaction forms malate from oxaloacetate and involves the oxidation of NADH. Malate is in turn transported into the matrix along with concomitant efflux of α-ketoglutarate. Malate is used to form oxaloacetate in the matrix and NADH is formed in this reaction. The cycle is completed by the transamination reaction involving oxaloacetate, α-ketoglutarate, glutamate and aspartate.



An alternate shuttle that occurs in the skeletal muscle and brain is the glycerol-3-phosphate shuttle. Here, the reducing equivalents from NADH are delivered as $FADH_2$ into the mitochondria. Thus, the energy yield is reduced compared to the malate-aspartate shuttle. There are two dehydrogenases involved in this shuttle, the cytosolic and mitochondrial glycerol-3-phosphate dehydrogenase. The cytosolic dehydrogenase reduces dihydroxyacetone phosphate to glycerol-3-phosphate using NADH and the mitochondrial dehydrogenase mediates the reverse reaction using FAD.

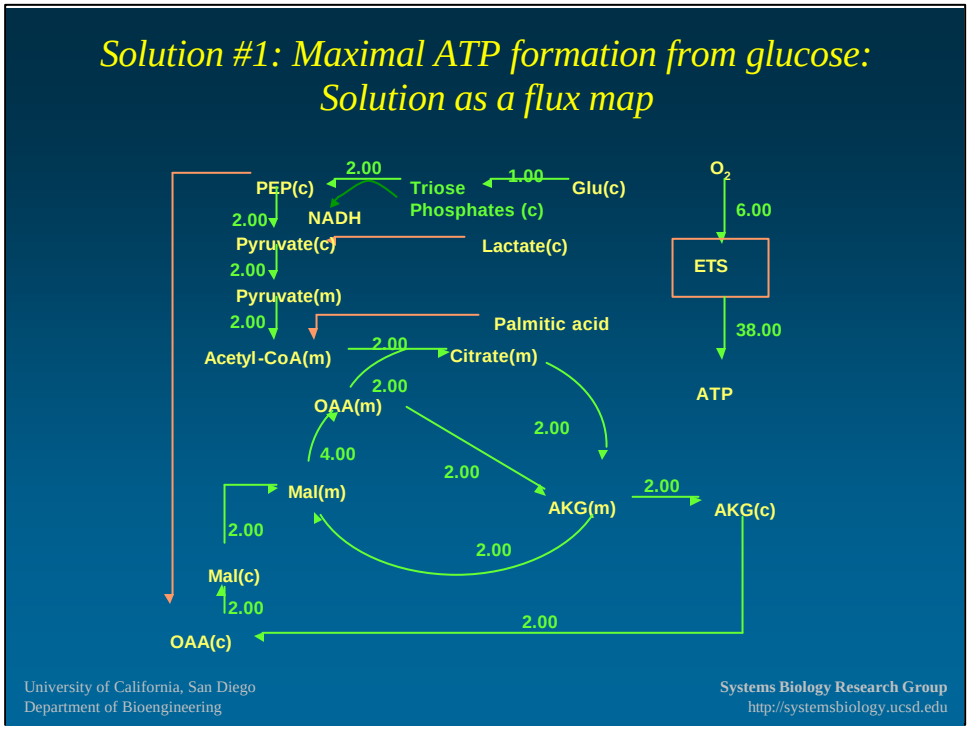
Additionally, fluxes for substrate input and intermediate secretion are also included in the model. The substrate input fluxes are constrained for the different situations that will be studied.



Summary of Mitochondrial Model

The model is specified, including the reactions, the objective function, and constraints.

A metabolic map can be written and flux distributions displayed.

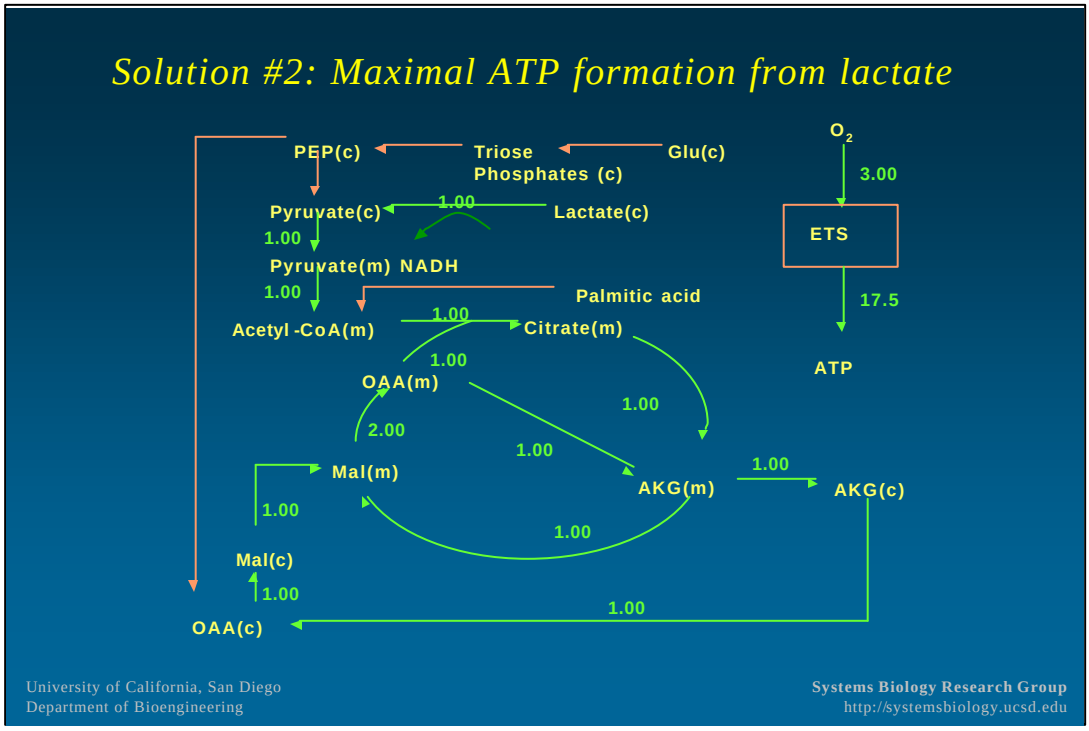


Studying Substrate Preferences

The flux distributions for optimal production of ATP, shown in the next few slides, were determined under conditions where the substrate uptake is restricted to 1 mole/unit time/mass dw. That input is normalized to unity.

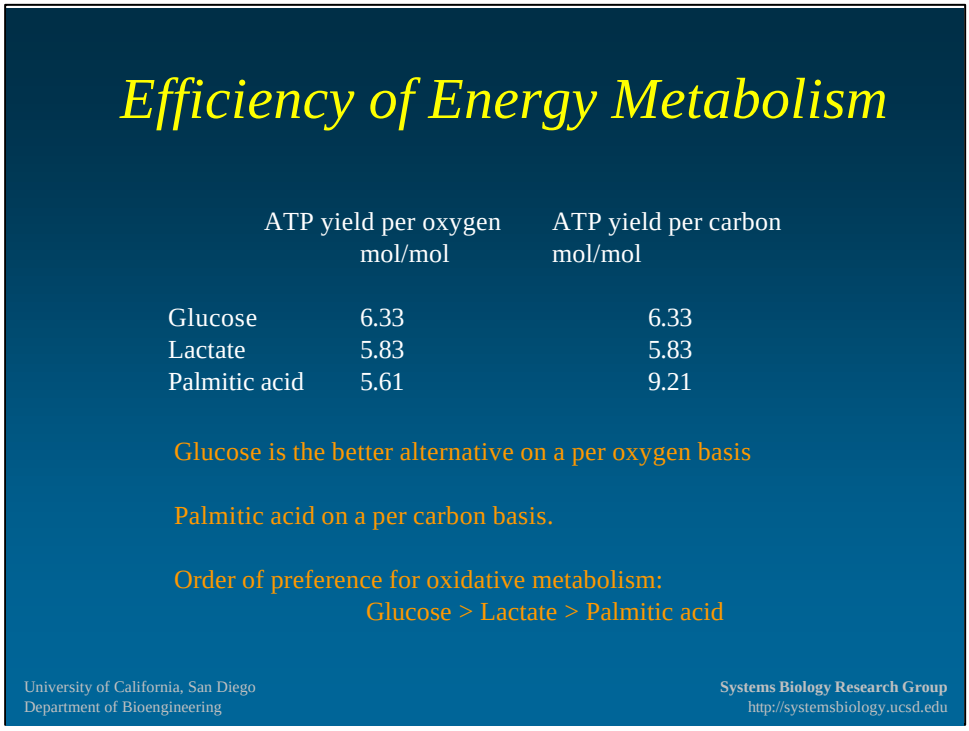
The calculation of optimal flux maps was carried out for three different substrates (glucose, lactate and palmitic acid). Shown on this slide is the metabolic flux map for optimal production of ATP from glucose. The following two slides show the results from the other substrates. Arrows shown in green are fluxes that are active and arrows in orange are fluxes that are not utilized in the solution.

The complete utilization of 1 mole of glucose results in the formation of 38 ATP with the concomitant utilization of 6 moles of O_2. The utilization of 1 mole of lactate forms 17.5 ATP with the utilization of 3.0 moles of O_2 and the utilization of 1 mole of palmitic acid (a 14-C fatty acid) produces 129 ATP, but requires 23 moles of O_2. Glucose is the preferred substrate compared to lactate and palmitic acid when the oxygen flux is restricted and all three substrates are made available. Since glucose is the best substrate for producing maximum ATP per mole of oxygen consumed, ATP synthesis from glucose is the optimal



Optimal Flux Map For Lactate

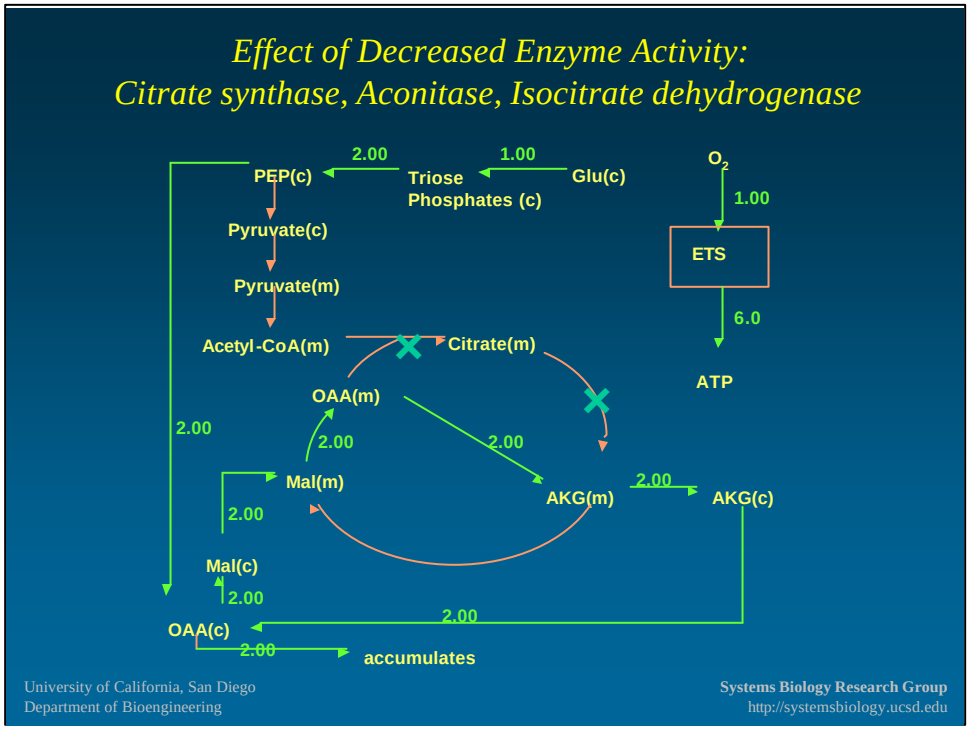
The optimal production of ATP from lactate is shown in this slide. Note that the conversion of lactate to pyruvate produces NADH and NADH is then shuttled into the mitochondria via the malate-aspartate shuttle and the redox equivalents processed by the ETS to produce ATP.



Summary of Results

This table compares the three substrates in terms of the ATP yield on a per oxygen basis and a per carbon basis. While the fatty acid provides more ATP on a per carbon basis, the cost in terms of oxygen consumption (or uptake) makes the fatty acid a sub-optimal alternative to the other substrates. The reduced costs contain information on the contribution of each flux to the optimal solution. The input fluxes for lactate and for fatty acid reflect reduced costs of -17.5 and -129.00, when glucose is the energy source. Since this is the number of ATP that can be synthesized from either substrate, these fluxes represent an alternate option for ATP synthesis. Therefore, if the oxygen uptake flux increases by 3 units and the glucose uptake flux remains constant, the uptake of 1 unit of lactate will yield an additional 17.5 units of ATP.

The malate-aspartate shuttle is active when glucose or lactate are the substrates for energy metabolism. When glycolysis is active, the glycerol-3-phosphate shuttle is a secondary option for transporting reducing equivalents into the mitochondrion from the cytosol. This exchanges one molecule of cytosolic NADH for one mitochondrial $FADH_2$ and is therefore an inferior option for maximizing ATP production. The shadow price for NADH is 3.00 and that for $FADH_2$ is 2.00. Thus, ATP synthesis from glucose yields 36 ATP if the glycerol-3-phosphate shuttle is utilized (rather than 38 ATP). The shuttles are not required during fatty acid oxidation, since the reducing equivalents are entirely derived from the TCA cycle.

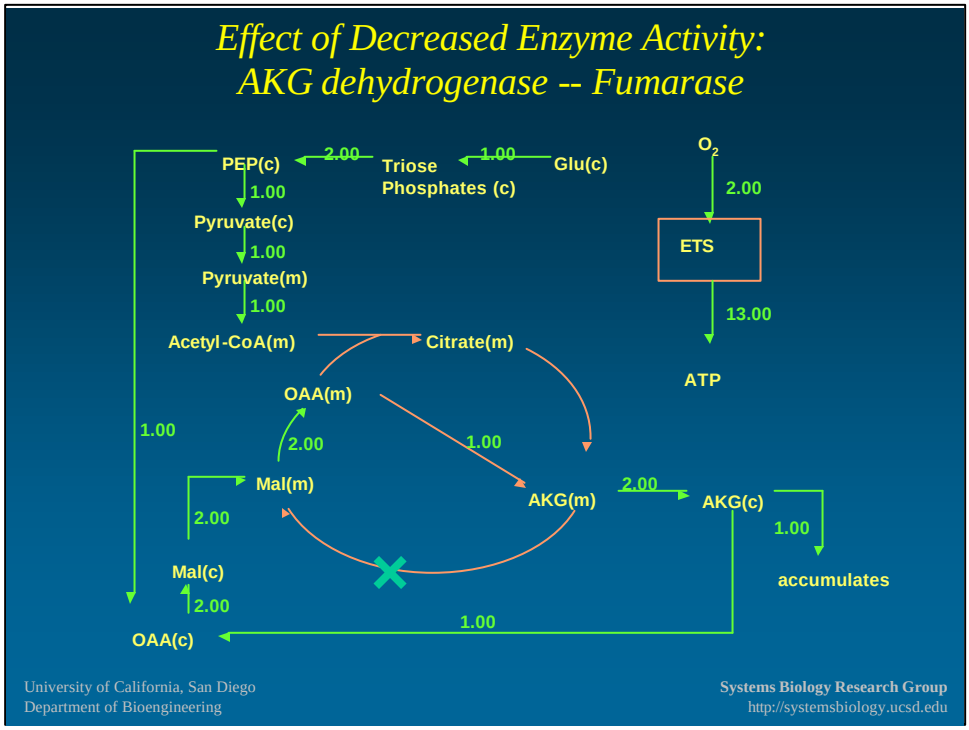


ALTERATIONS IN ENZYME ACTIVITY:

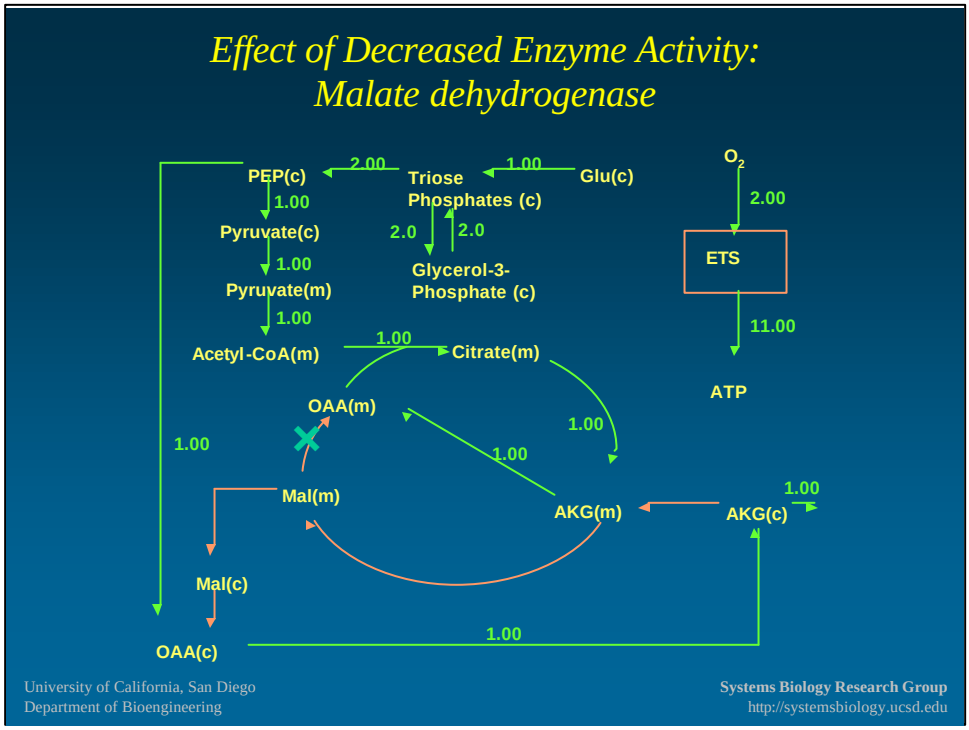
Studying pathological situations using constraints

The FBA model can be used to evaluate the systemic effect of reduced enzyme activity. Mathematically, this is equivalent to setting a constraint on a specific flux (i.e. v_{AKGDH}, the flux through α- ketoglutarate dehydrogenase) to be either zero or some specific value (i.e., $a_i = b_i =$ 0, or a specific value). Alterations in the activity of different enzymes involved in energy metabolism can be investigated under glucose limitations to simulate enzyme defects.

When citrate synthase, aconitase or isocitrate dehydrogenase are inactive (fluxes are constrained to zero), the ATP yield drops significantly and the model predicts the accumulation of oxaloacetate. The predicted flux distribution for these cases is shown in the slide. The glucose input flux was maintained at 1 unit, in order to compare with a normally functioning network. The carbon flux is partially cycled through PEP carboxylase and pyruvate dehydrogenase.

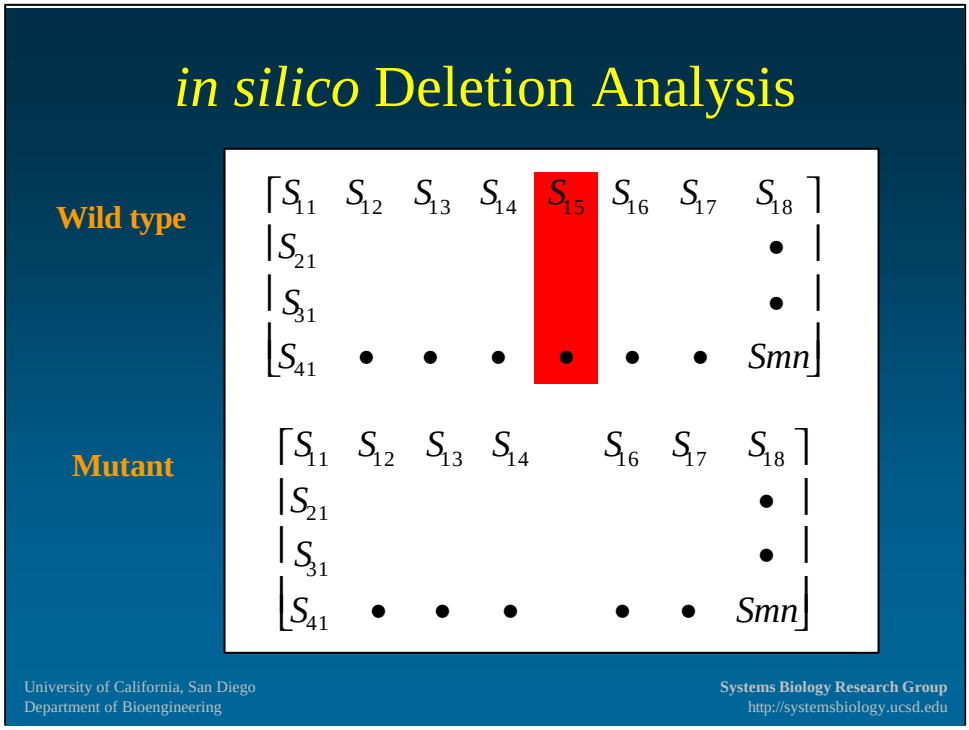


When any of the of enzymes from α- ketoglutarate dehydrogenase to fumarase is inactive, the TCA cycle is not completely functional. Excess carbon in this situation is secreted as α- ketoglutarate. An excess of α- ketoglutarate, has in fact been identified as a feature of mitochondrial diseases involving mutations in the genes that code for these enzymes. The ATP yield is again significantly lower than the optimal value. The flux distribution for this scenario is shown above.



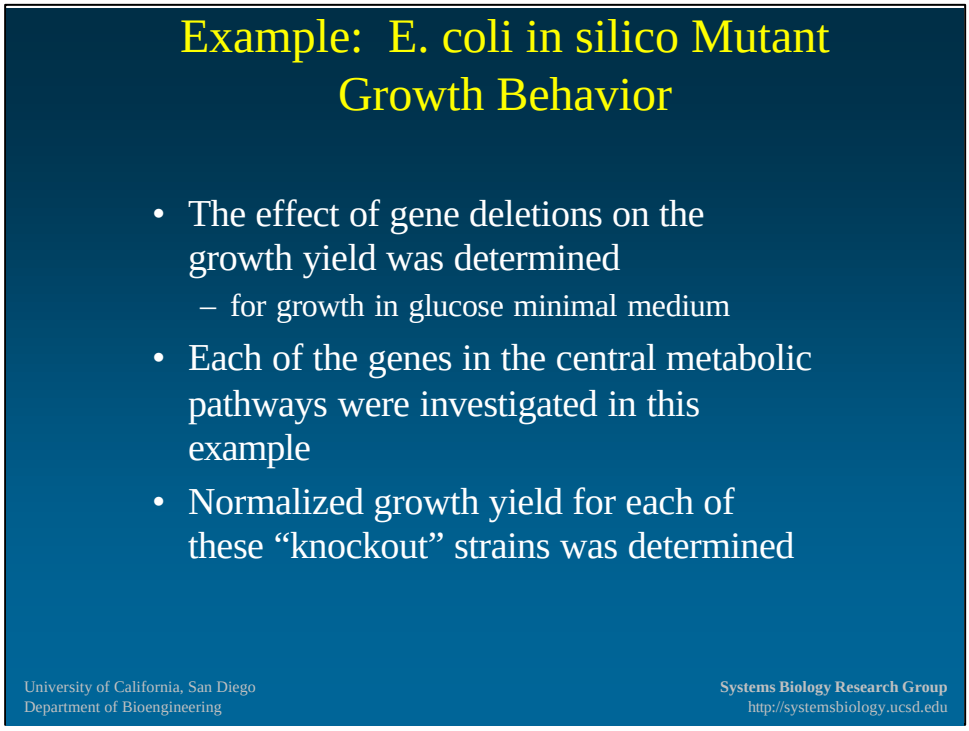
The activity of the malate dehydrogenase enzyme also affects the ATP production in an adverse manner and predicts the accumulation of α-ketoglutarate. Interestingly, the effect of malate dehydrogenase is limited until the metabolic flux is reduced to less than 50% of the normal activity. The flux distributions predicted in this situation are different depending on the activity. The slide shows the flux distribution when the malate dehydrogenase flux is completely inactive. Here the malate-aspartate shuttle functions in the reverse direction as compared to the normal case. This results in less ATP production. Malate dehydrogenase is active in both the cytosol as well as the mitochondria, and a loss in the activity of either or both isozymes leads to the same predicted phenotype.

Altering the Genotype:
Gene deletions



If a gene is deleted (or "knocked-out"), the cell will no longer be able to synthesize the protein product that the gene encodes. If this gene product is a metabolic enzyme, the cell will no longer be able to support the reaction that the particular enzyme catalyzed. Thus, a column (or columns, in the event of a gene product that catalyzes more than one reaction) in the stoichiometric matrix will be eliminated in the knock-out strain of the cell. FBA can then be performed for the "mutant" stoichiometric matrix, the results of which can be compared to those of the wild-type strain.

An example of this kind of analysis is provided in the following slides.

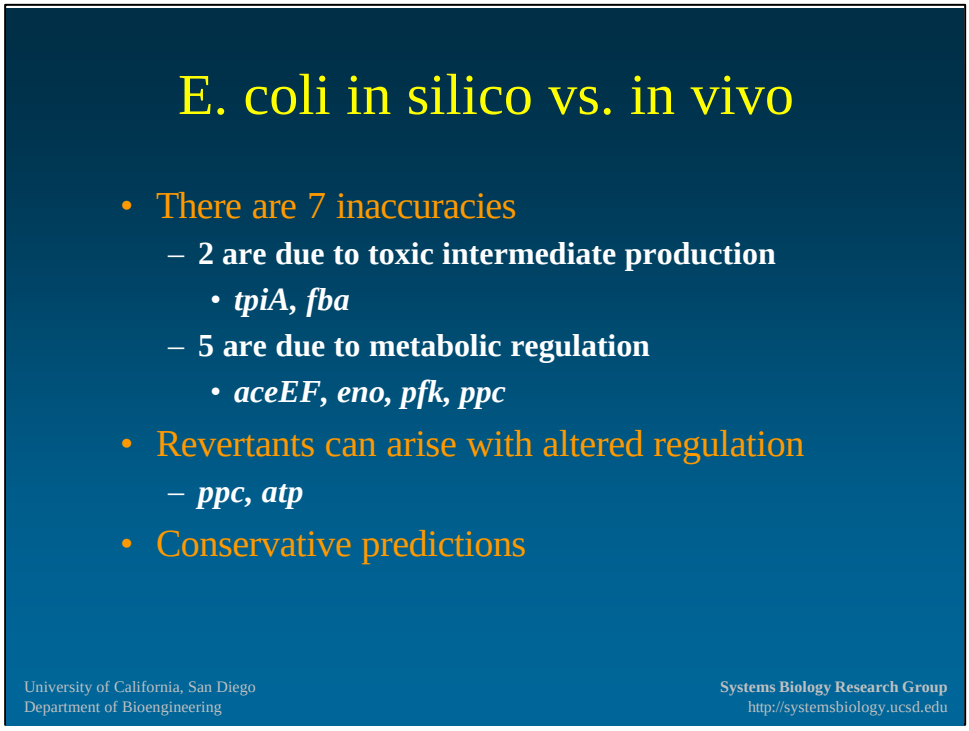


EXAMPLE case of a gene deletion study. Here the genes associated with core metabolism are studied.

For each gene, a new matrix is formed without the column(s) that represent reactions that the gene product is involved in. Then the optimal growth rate, or yield can be calculated without these column(s). The optimal performance for the reduced matrix are then compared to those of the full matrix. To get a common frame of reference, the growth performance of all the reduced matrices (in silico mutants, or knock-outs) are compared to those of the full matrix (or the in silico wild-type).

This slide shows the results of gene deletions in *E. coli* central intermediary metabolism; maximal biomass yields on glucose for all possible single gene deletions in the central metabolic pathways were calculated. The optimal value of the mutant objective function (Z_{mutant}) compared with the wild-type objective function (Z), where Z is the objective function for cellular growth, as defined in Lecture 10. The results were generated in a simulated aerobic environment with glucose as the carbon source. The transport fluxes were constrained as follows: the glucose influx was capped at 10 mmol/g DCW/hr, and the oxygen influx was limited to 15 mmol/g DCW/hr. The maximal yields were calculated by using FBA with the objective of maximizing growth. The biomass yields are normalized with respect to the results for the full metabolic genotype. The genes marked "lethal" resulted in no growth when they were deleted. Those marked "retarding" represent gene deletions that reduced the maximal biomass yield to less than 95% of the *in silico* wild type. "Redundant" genes refer to those whose deletion resulted in no significant (i.e., 5% or less) reduction in growth yield. Those genes to the right in the figure represent genes that were unused in the base solution.

This table presents a comparison of the predicted mutant growth characteristics from the gene deletion study (presented in the previous slide) to published experimental results with single mutants. Results are scored as + or – meaning growth or no growth determined from *in vivo/in silico* data. The discrepancies (highlighted in red) are described in the next slide.



ANALYSIS OF INACCURATE IN SILICO PREDICTIONS

•There are 7 discrepancies between the in silico and in vivo results. These differences are due to basically two reasons:

- •1. Toxic intermediate production
- •2. Missing regulation of metabolism or gene expression

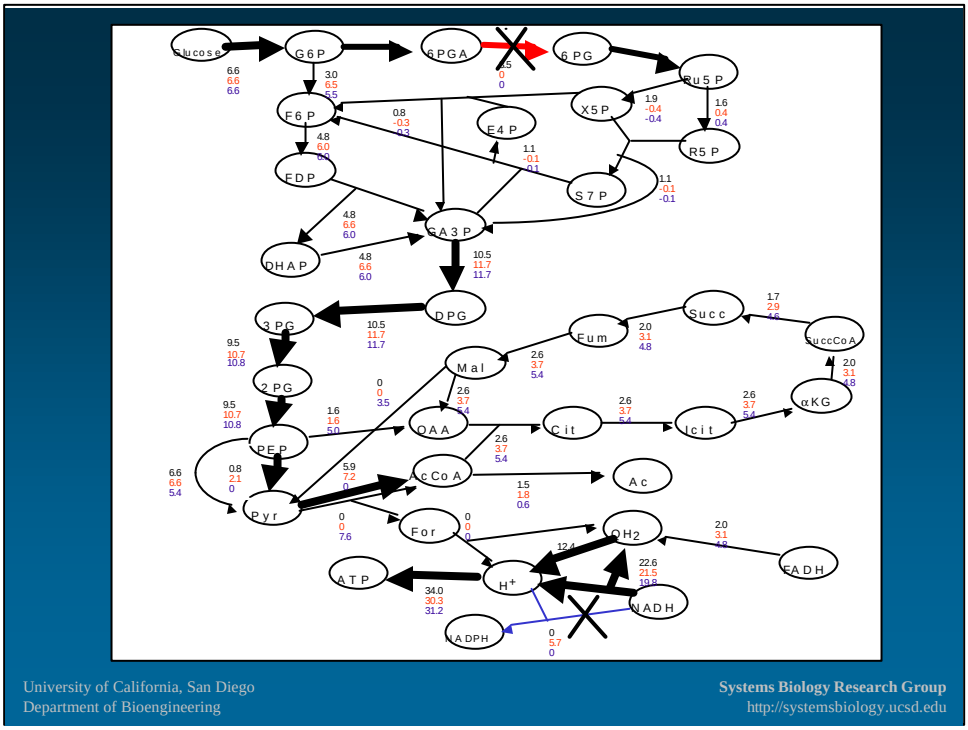
•For example, when the *tpiA* and *fba* gene products are removed, it is thought that the cell produces a toxic intermediate, and this prevents the cell from growing. This can not be predicted using FBA since no concentrations are calculated.

•Also, when the enolase gene is removed from the system, the experimental data suggests that this cell is unable to grow, whereas the *in silico* cell is able to grow. Upon further examination, it is seen that the *in silico* cell is able to grow by synthesizing and degrading an amino acid, something that the cell is unlikely to do.

•Interestingly, it has been observed that revertants can spontaneously arise with altered gene expression. For example, ATPase mutants have been shown to not grow on succinate, however, this metabolic model predicts that they theoretically can. It has been recently reported that the ATPase deletion strains were unable to grow due to a transport deficiency, and revertants arose after about a week that do grow on succinate, at yields near the theoretical maximum.

Such deletion analyses can be used to identify critical reactions in the network. The reactions highlighted in red in this map of central metabolism correspond to genes whose deletions are lethal to the cell growing in glucose minimal medium.

It is also possible to systematically test the growth (*in silico*) of *E. coli* on glucose minimal medium for all pair-wise combinations of double deletions. The double deletions marked as blue are found to be redundant; those marked yellow retard cell growth; and those marked in red are predicted to be lethal. The scarcity of “red” double-knockouts emphasizes the overall robustness within the central metabolic network in *E. coli*.



RE-ROUTING OF METABOLIC FLUXES

The deletion of a gene can generate a alternative optimal use of the metabolic network. This slide shows the optimal fluxes through the core pathways for a single and a double mutant.

- (Black) Flux distribution for the complete gene set.
- (Red) *zwf*- mutant. Biomass yield is 99% of the results for the full metabolic genotype.
- (Blue) *zwf- pnt* mutant.

Biomass yield is 92% of the results for the full metabolic genotype. The solid lines represent enzymes that are being utilized, with the corresponding flux value noted. The fluxes are for a glucose uptake rate of 6.6 mmol glucose/hr/g-DW and an oxygen uptake rate of 12.4 mmol oxygen/hr/g-DW .

Phase Planes and Experimental Design

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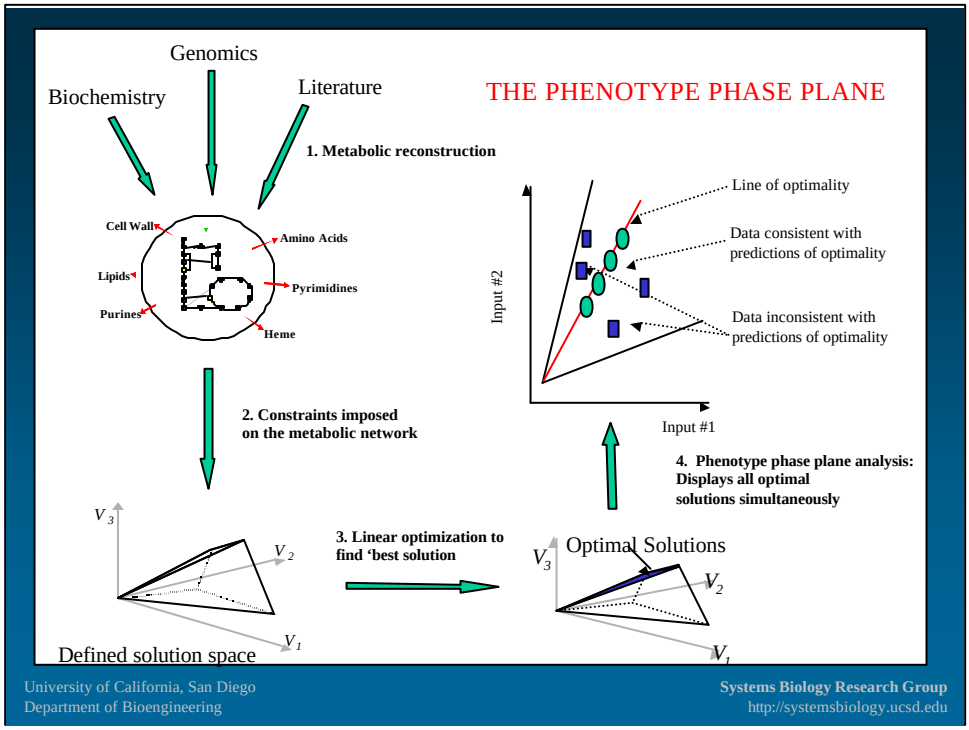
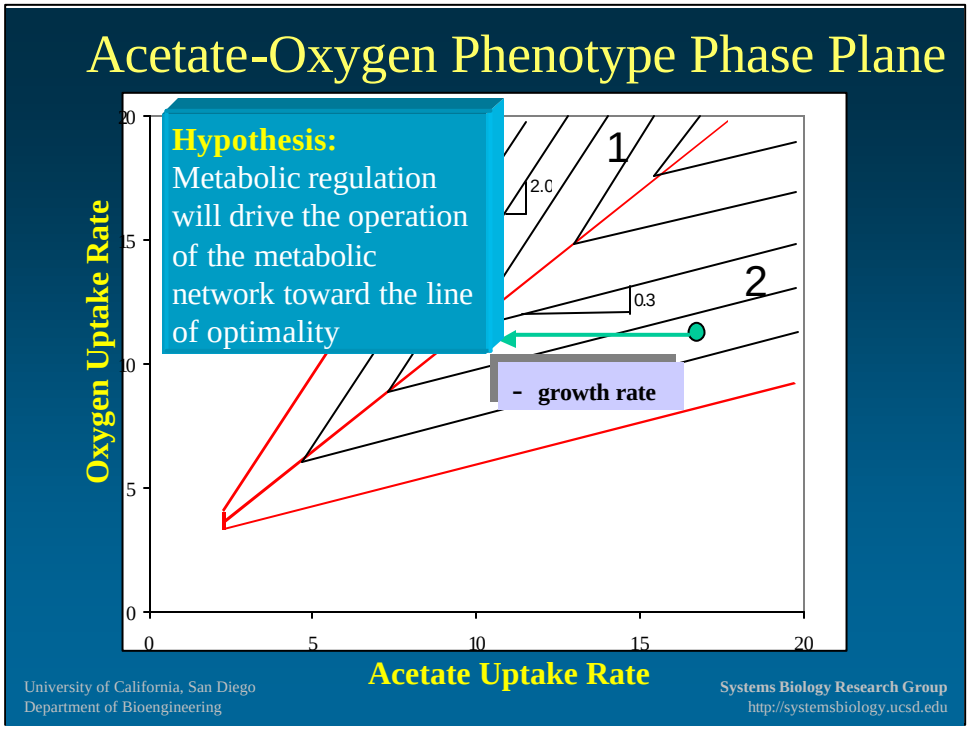


Combining Modeling with Experimental Data

This slide presents an overview of the coupling of *in silico* modeling and experimental data. This is a review of the constraints-based modeling approach with the addition of how it can be used to interpret experimental data using phenotype phase plane analysis.



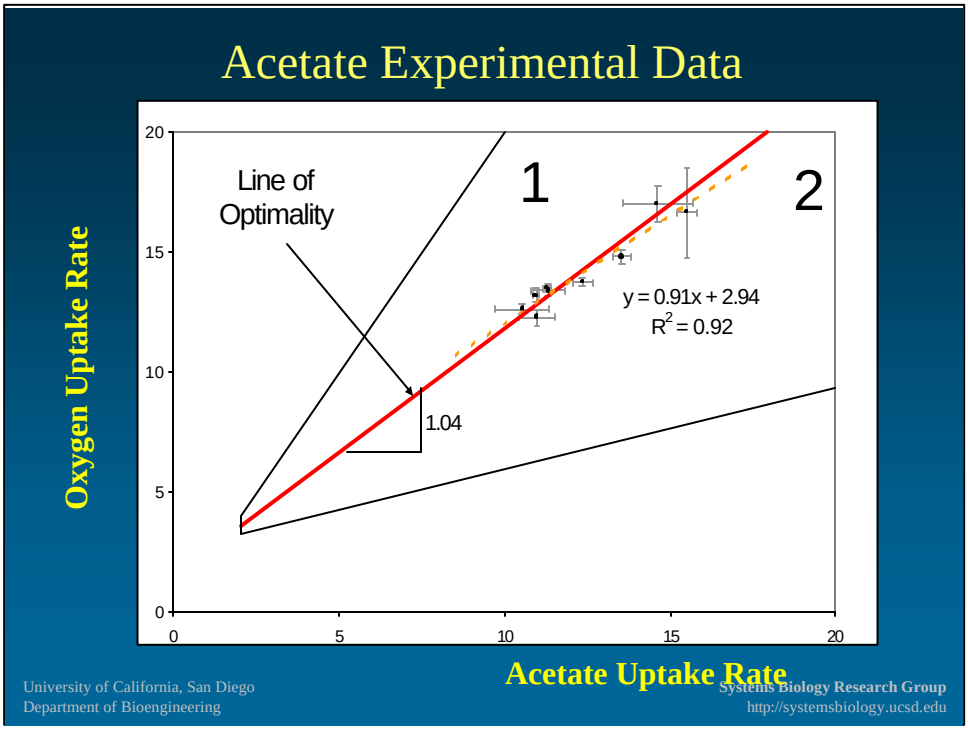
Interpreting the Phase Plane:

Using isoclines

This slide describes the acetate-oxygen phenotype phase plane for E. coli.

It can be seen that there are 2 distinct regions. We have also drawn the isoclines on this figure, and it can be seen that the isoclines have a positive slope in both regions. This means that they are unstable -- it is advantageous for the organism to move the the edge of the region

The optimal growth occurs at the line separation the two phases, the so-called line of optimality.

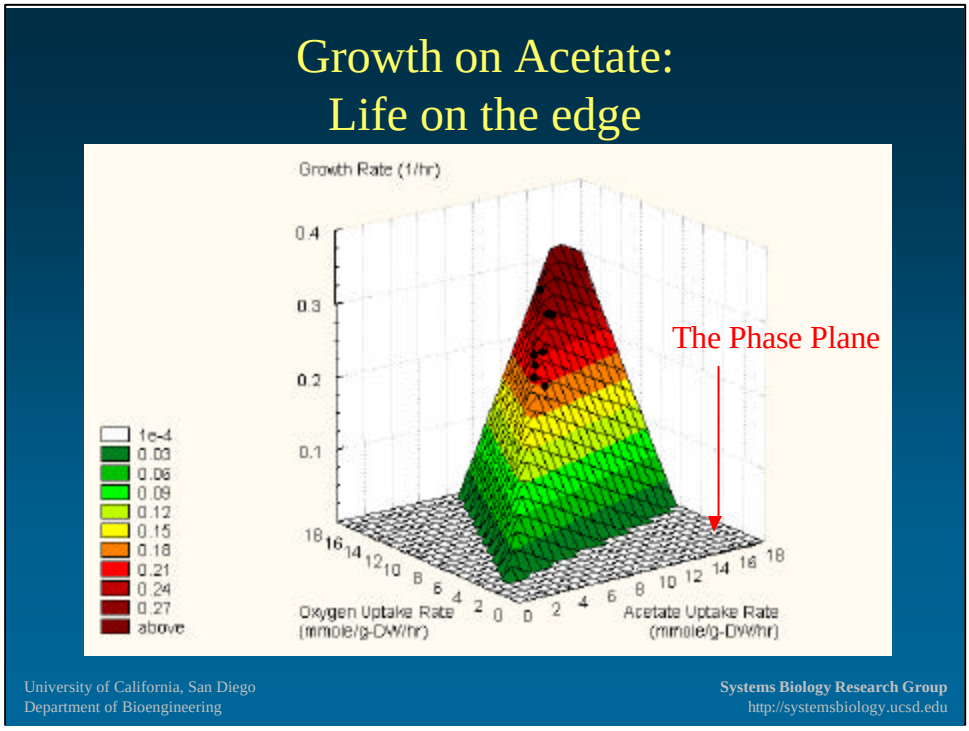


Experimental Test of Hypothesis

Batch growth experiments were performed allowing oxygen uptake rate to vary, and controlling the acetate uptake rate by the acetate concentration. The growth rate and any secreted metabolic byproducts were measured.

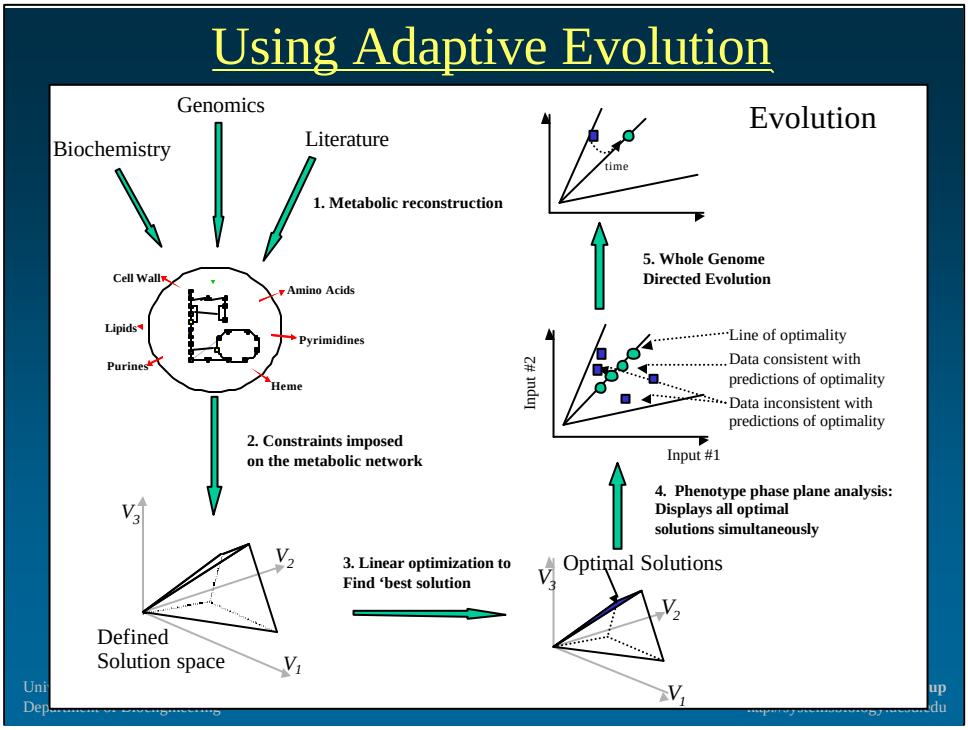
The experimental data points were plotted on the the phenotype phase plane and the results are shown in this slide. It can be seen that the points define a line with a correlation coefficient of 0.92, and the slope of the line is within 15% of the *in silico* derived line of optimality.

The operation of the metabolic network with acetate as the carbon source appears to be restricted to a single line, which is defined *in silico* as the line of optimality.



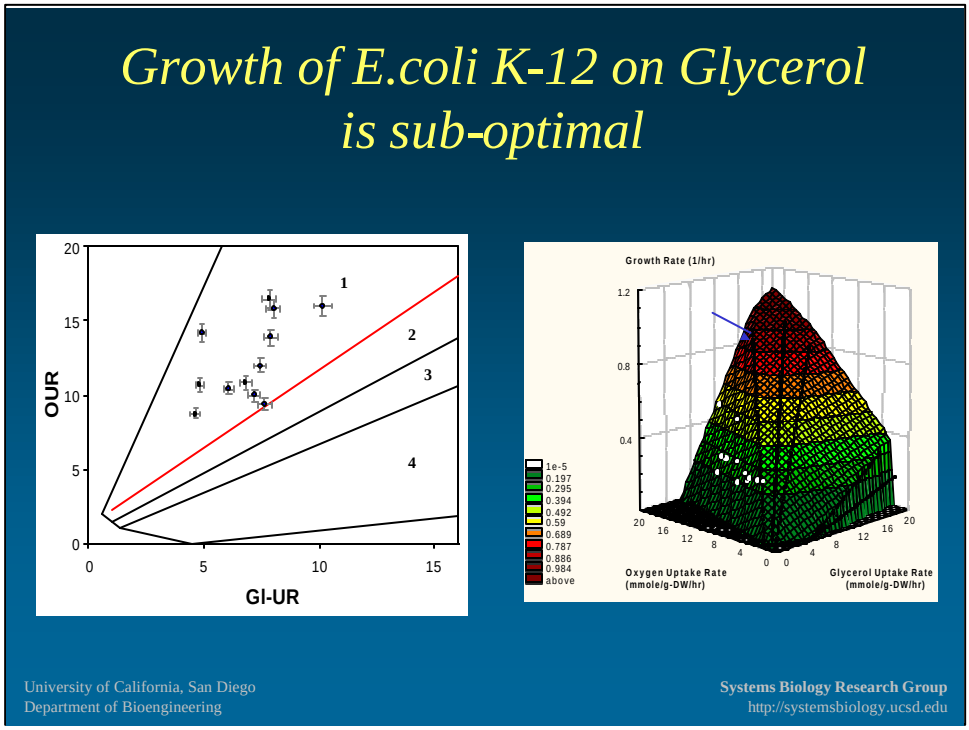
3-D Phase Planes

Phenotype phase planes are normally visualized in two dimensions with the substrate uptake rate and oxygen uptake rate as the two axes. The specific growth rate of the cells can be added as a third dimension to help further elucidate the functional state of the cells.



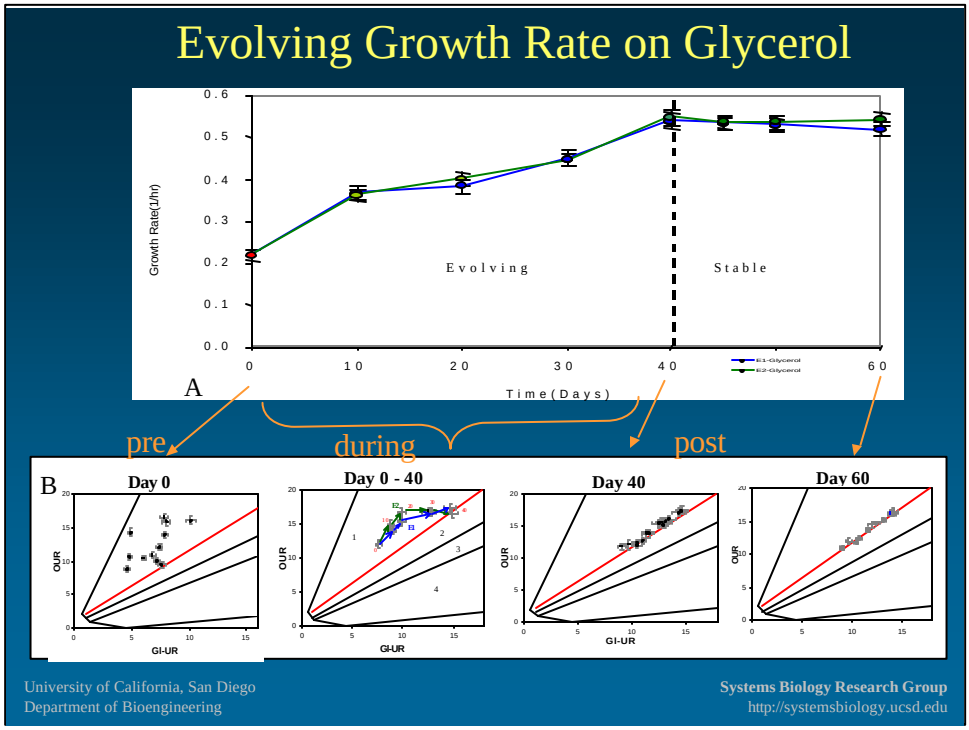
Adaptive Evolution

Adding to the scheme showed earlier, this slide shows how adaptive evolution can be added as an experimental program to more fully complement the predictions made by the *in silico* model. For cells that do not function along the line of optimality in their native state, allowing the cells to evolve may cause them to shift their functionality to operate along the line of optimality.



Wild type Glycerol phenotype

The wild type K-12 strain of *E.coli* was grown on M9 minimal medium supplemented with glycerol, and the glycerol uptake rates (Gl-UR) and oxygen uptake rates (OUR) were measured. Testing was done at various glycerol concentrations and temperatures. All *E.coli* tested operated in region 1 of the phenotype phase plane.



Adaptive evolution on glycerol

Since the wild type K-12 strain was shown to operate in region 1 of the phase plane, the cells were evolved on 2g/L glycerol medium to determine if the cells would evolve towards the line of optimality (shown in red). Figure A shows the increase in growth rate for the cells as they were evolving. A stable growth rate was reached at day 40 of the evolution. Figure B shows the metabolic phenotype of the cells at various points throughout evolution. Again, at day 0 the cells operated in region 1 and over the forty days of evolution, the cells modified their functionality to operate on the line of optimality. Subsequent testing of the culture at day 60 showed that the cells continued to function on the line of optimality.

Summary

- The phase plane can be used to study the genotype-phenotype relationship and to design experiments
- FBA can be used to perform insightful calculations for reconstructed metabolic networks
- For the skeleton mitochondria model we can rank the value of substrates and assess the consequences of enzymopathies
- Changes in internal model parameters (changes in the genotype) can be studied using FBA
- The effects of gene deletions are condition dependent. Null phenotypes can thus be misleading
- *E. coli* operate on the line of optimality when grown on acetate, succinate or malate.
  - *E. coli* utilizes its metabolic network to optimize for growth.

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