Metabolic Flux Analysis in Mitochondria

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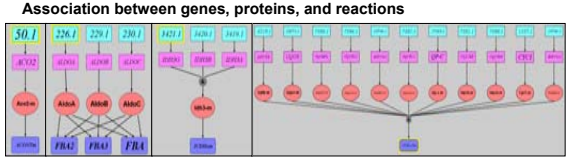
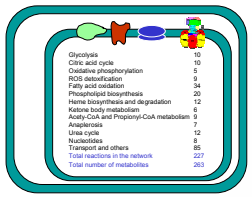
Abstract

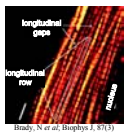
The emergence of high-throughput data has enabled the study of mitochondria as systems. We have reconstructed and characterized the human mitochondrial metabolic network based on proteomic and biochemical data. Linear programming and Monte Carlo sampling methods were applied to identify candidate steady states consistent with the imposed physiological and chemical observations. Analysis of equivalent optimal flux distributions, calculated with respect to each of the three metabolic functions, identified a group of flux distributions that are highly correlated, and thus are likely to be physiological relevant. Samples of steady-state flux distributions showed that the experimentally observed reduced activity of pyruvate dehydrogenase in diabetic and ischemic patients could be a result of stoichiometric constraints, and may not necessarily require enzymatic inhibition. Application of isotopomer data from isolated mouse hearts identified the fate of perfused [U-$^{13}C_6$]glucose and [U-$^{13}C_3$]pyruvate and flux redistribution at key substrate branch points.

The mitochondrial network

Association between genes, proteins, and reactions







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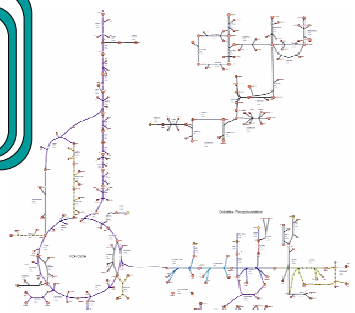
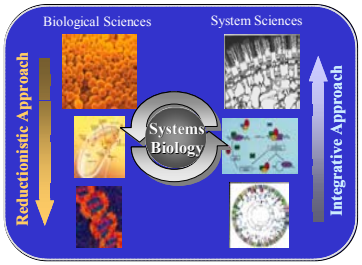
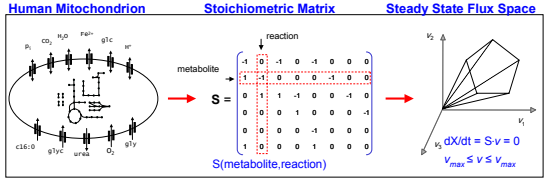


Methods

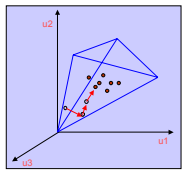
The constraint-based approach for analyzing reconstructed networks involves the application of a series of constraints arising from reaction stoichiometry, thermodynamics, enzymatic capacities, and regulatory and isotopomer balance constraints when they are available.



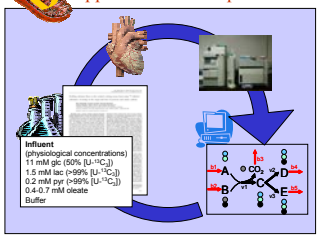
Analysis of equivalent optimal solutions

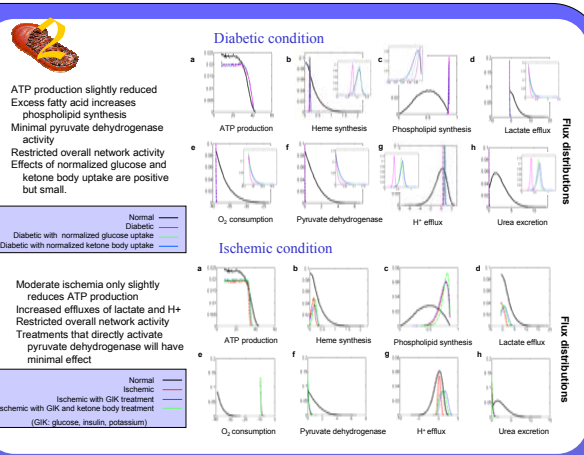


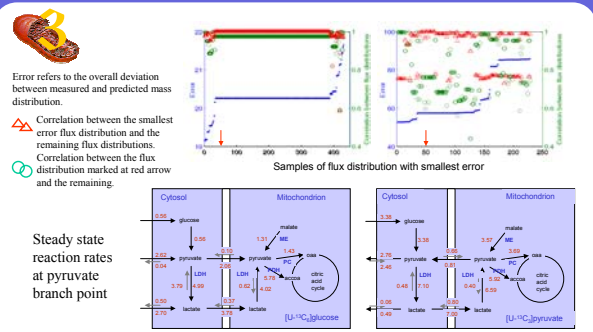
Monte Carlo sampling of solution space



Application of isotopomer data



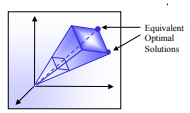




Results

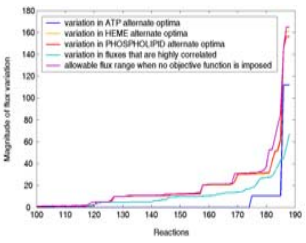
Metabolic objective functions

ATP production	Z = -1 ATP -1 H_2O + 1 ADP + 1 P_i + 1 H^+
Phospholipid biosynthesis	Z = 0.18 cardiolipin + 0.34 phosphatidylethanolamine + 0.43 phosphatidylcholine
Heme biosynthesis	Z = protoheme



	ATP	Heme	Phospholipids
Total number of optima	4	8,288	21,863
Average reactions used per optimal solution	57.5	78.7	99.1
Reactions with variable fluxes	15	114	114
Reactions with constant (non-zero) fluxes	47	14	21
Reactions always used (largest correlated set)	53	35	56
Reactions never used	127	61	54
Number of feasible extreme points	9,160	13,000	50,012

Reaction participation among equivalent optimal flux distributions calculated with respect to the three metabolic objective functions. Optima refer to the extreme points of the solution space that achieve the optimal value for the objective function, whereas feasible extreme points only satisfy the constraints of the linear programming problem.



Conclusions

1. An *in silico* framework integrating multiple datasets is useful for studying mitochondrial metabolism under normal and stress conditions, and provides a basis for assessing effects of potential disease treatments.
2. Metabolic flux profiles with isotopomer data can uncover details about substrate utilization, substrate redistribution at network branch points, and quantitative information about enzyme activity.

References

1. Vo TD, Greenberg HJ, Palsson BO. 2004. Reconstruction and functional characterization of the human mitochondrial metabolic network based on proteomic and biochemical data. J Biol Chem 279(38):39532-40.
2. Thiele I, Price ND, Vo TD, Palsson B. 2004. Candidate metabolic network states in human mitochondria: impact of diabetes, ischemia, and diet. J Biol Chem. in press