EXPLORING HYPOTHETICAL METABOLIC UNIVERSES WITH REWIRED COFACTOR COUPLINGS. J. Goldford[1] and D. Segrè[1,2], [1]Bioinformatics Program, Boston University, 44 Cummington Mall, Boston, MA 02215. [2]Department of Biology and Department of Biomedical Engineering, Boston University, 44 Cummington Mall, Boston, MA 02215 (goldford@bu.edu, dsegre@bu.edu).

Introduction: Metabolic networks in present-day life involve several multi-substrate and multi-products reactions. Many of these reactions can be viewed as combinations of pairs of concomitant half-reactions. For example, many energetically unfavorable reactions are coupled with highly exergonic reactions, involving energy currency cofactors (such as X + ATP → Y + ADP + Pi). In many other cases, the coupling involves reducing power cofactors that channel electron to enable balanced redox reactions (e.g., X + NAD(P)H → Y + $NAD(P)^+$ + H^+). While in current metabolic networks (Fig. 1) the task of enabling such coupled reactions is fulfilled by long-term evolved metabolic enzymes with multiple binding sites, very little is known about the emergence and ancient history of these couplings [1].

Here we ask how the capabilities of cellular metabolism would change if cofactors were rewired differently. In particular, we focus on cases where multiple cofactors could similarly satisfy the cellular metabolic requirements. Why, for example, are certain reactions specifically coupled with NADPH or NADH?

We address this question by simulating the physiological consequences of rewiring cofactor networks using genome-scale stoichiometry modeling of metabolism. This approach can help investigate the properties of metabolic networks *in silico*, making it a widely utilized tool with applications ranging from metabolic engineering to personalized medicine. Beyond serving as a platform for systems biology of metabolism, stoichiometry modeling approaches, such as flux balance analysis [2] can be used to generate hypotheses about optimality of metabolic network structure and function [3], e.g. by searching for the set of metabolic rates (fluxes) that maximize a given metabolic objective.

We first re-evaluated the contention that NAD (NADP) is primarily utilized in catabolism (anabolism) by analyzing the usage of cofactor-coupled reactions for catabolic (anabolic) specific objective functions. Whether we classify reactions as catabolic/anabolic based on textbook definitions or the observed role in the model, we found surprisingly little catabolism/anabolism specificity of redox cofactors. In order to assess in a more general way the fitness cost of rewiring NAD/NADP couplings, we next analyzed metabolic network performance properties for many random cofactor-wiring permutations. This random exploration of the fitness landscape across different rewiring schemes can be also searched through optimization algorithms. Specifically, we used mixed-integer linear programming to investigate cofactor rewiring that would lead to maximal growth rate, maximum ATP production or minimal proteome usage. This allows us to ask whether the specific wiring observed in modern-day metabolism is close to predicted optima.

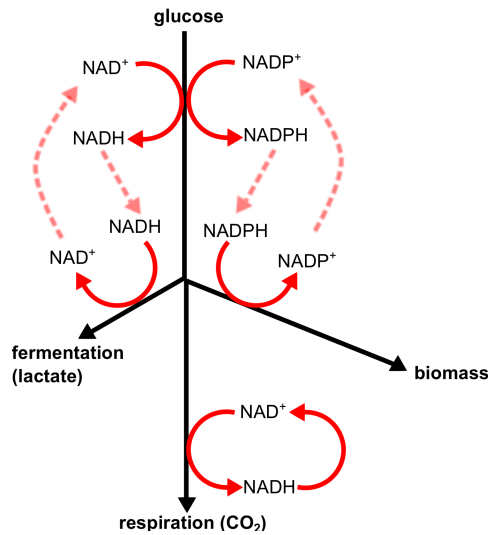

Fig. 1 – An overview of central carbon metabolism and the role of redox cofactors.

Finally, we extended the previous analysis to a more sophisticated model that includes information about the free energy changes of metabolic reactions. This allowed us to test whether usage of specific redox cofactors (NAD vs. NADP) tends to be associated with tightly regulated, highly irreversible reactions, potentially pointing to thermodynamic or regulatory reasons for the observed cofactor specificity.

We suggest that our stoichiometry-based analysis of rewired cofactor couplings in metabolic networks could help explore possible alternative scenarios for the ancient evolution of metabolism.

References: [1] Ebenhöh O, Heinrich R. Bull Math Biol. 2001 Jan;63(1):21-55. [2] Price ND, Reed JL, Palsson BØ. Nat Rev Microbiol. 2004 Nov;2(11):886-97. [3] Riehl WJ, Krapivsky PL, Redner S, Segrè D, PLoS Comput Biol. 2010 Apr 1;6(4):e1000725.