Marginal novelty of metabolic activity modeling to modulate lifespan and healthspan

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Introduction

Non-technical

“Health” is a nebulous term used liberally in both scientific and laymens audiences. Across both arenas, there is consensus that there can be relatively “good” and “poor” health, and there is tremendous focus on deriving singular drugs, optimal diets, or performant workouts which improve health or extend lifespan. The expectation that one drug can dramatically alter physiology permeates scientific research, but a more “complex” intervention, including diets and lifestyles are more likely to improve health. This goal may first require a commensurately complex analysis of physiology before understanding how to change it. The objective here is to computationally model the entirety of an organism’s physiology and predict interventions to invoke a “healthier” state.

Technical

Among the defining characteristics of life, the activity of all metabolic reactions most intimately represents an organism’s phenotype. While direct observation of all activities is impossible *in vivo*, metabolites and reactions can be depicted as a computational graph (Covert et al., 2001, King et al., 2016). This metabolic graph is then enlivened through computational approaches which enumerate the set of feasible activities. Categorically considered metabolic flux analysis (MFA), these approaches are primarily used in bioengineering (Bordbar et al., 2014). This work repurposes MFA for therapeutic discovery. Many discovery approaches strictly analyze gene activity, protein concentration, or other ‘omic data types, however these top-down approaches neglect deeper characteristics of biology. Metabolic networks exhibit scale-free topologies (Broido and Clauset, 2019), where the connectivities among genes, metabolites, and reactions follow power-law distributions (Figure 1). This information is embedded metabolic graphs, and by accounting for these network properties, MFA presents a bottom-up discovery alternative.

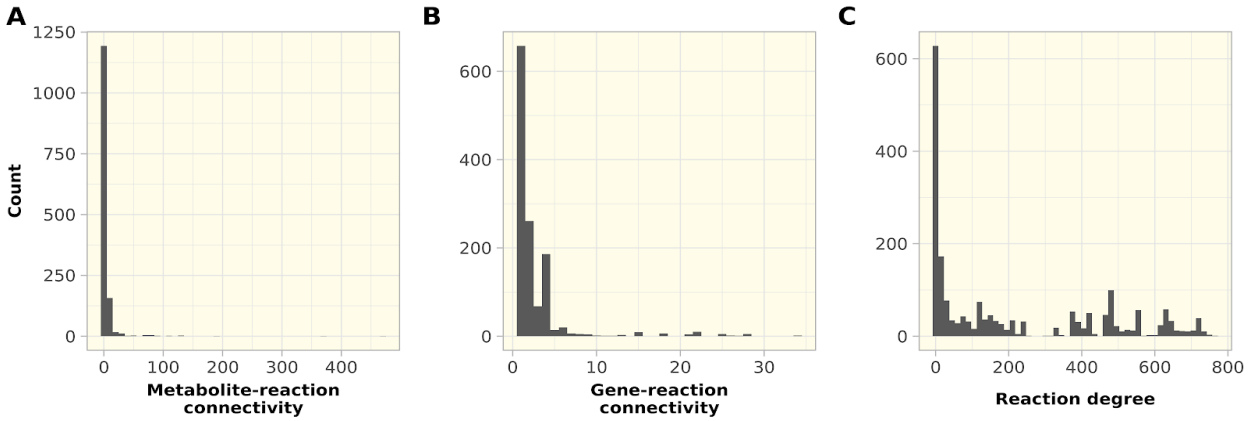

Figure 1. The number of reactions in which a metabolite (A) or gene product (B) participates, and the degree of a reaction itself (C) in a genome-scale metabolic network for *Caenorhabditis elegans* (Yilmaz and Walhout, 2016). These skewed distributions highlight the scale-free nature of biological networks.

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Without additional information, MFA is insufficient to compute fluxes with high biological accuracy. One solution is to introduce a metabolic objective, such as cellular growth, and reformulate MFA into an optimization problem (Feist and Palsson, 2010). This approach is appropriate for bioengineering microbes, however there is no evidence that multicellular eukaryotes exhibit single metabolic objectives or optima. Rather than solving for one optima, the set of all possible flux solutions can be trimmed with experimental data, through a subset of MFA methods termed constraint-based modeling (CBM) (Bordbar et al., 2014). The value in CBM lies in its conceptual simplicity: any data to suggest the reaction rate of an enzyme may serve as a valid constraint, including gene expression, metabolite levels, enzyme structures, or thermodynamics.

Genome-wide fluxes, or fluxomics, have yet to inform therapeutic discovery. Among the 'omics, fluxomics possesses the greatest discovery potential, for two main reasons. First, flux analysis operates on the underlying structure of metabolism, and this structure is the foundation to integrate any other form of molecular data. Second, flux most intimately represents an organism's active phenotype, and captures more information than reductionist physiological measures, such as glucose or total lipid concentrations. This deep representation not only redefines physiology, but unlocks a redefinition of therapeutics. A therapeutic can now be defined not by its impact on glucose levels in a diabetic patient, but by its impact on the patient's metabolic signature. The major logical leap of this redefinition is the selection of a therapeutic which shifts the metabolic signature towards health, or away from disease. While this technology predicts drugs to mimic youthful metabolism, its application is also evident for chronic and complex diseases, including diabetes and obesity.

Results

MFA corroborates prior anti-aging chemical and genetic lifespan modulators in *C elegans*

Metabolic flux, as both a novel representation of physiology and mechanism of therapeutic discovery, demands proof-of-concept and validation. To this end, 60 publicly available *C elegans* expression datasets were identified with a clear comparative experimental design. These designs included exposures to drugs, diets, genetic manipulations, environments, and age comparisons (the study designs for these 60 samples were studied and assigned to one of these categories of intervention). The data was then fit to CBM on the iCEL1273 genome-scale metabolic model (Yilmaz and Walhout, 2016), adopting flux-fitting approach developed by Braunstein et al, 2017, and converted to a differential metabolic flux between experimental and control groups, within experiments. Intra-experimental calculations mitigates concerns of inter-experimental variance. These high-dimensional differential flux vectors were then compressed onto a low-dimensional "map" using t-Distributed Stochastic Neighbor Embedding (t-SNE). This map addresses limitations of machine learning in high dimensions, and facilitates cross-experiment comparisons. Unlike principal components analysis, t-SNE is stochastic and primarily used for visualization. However, these embeddings do not assume linearities, capture deeper latent structures, and act as a hypothesis generator. From these embeddings, the drug that most closely mimics the metabolic flux of a young worm is Rapamycin (Figure 2). Rapamycin has been shown to extend lifespan across multiple organisms (Bitto et al., 2015), and this finding suggests the utility of genome-level flux to characterize model organismal physiology and predict metabolic modulators..

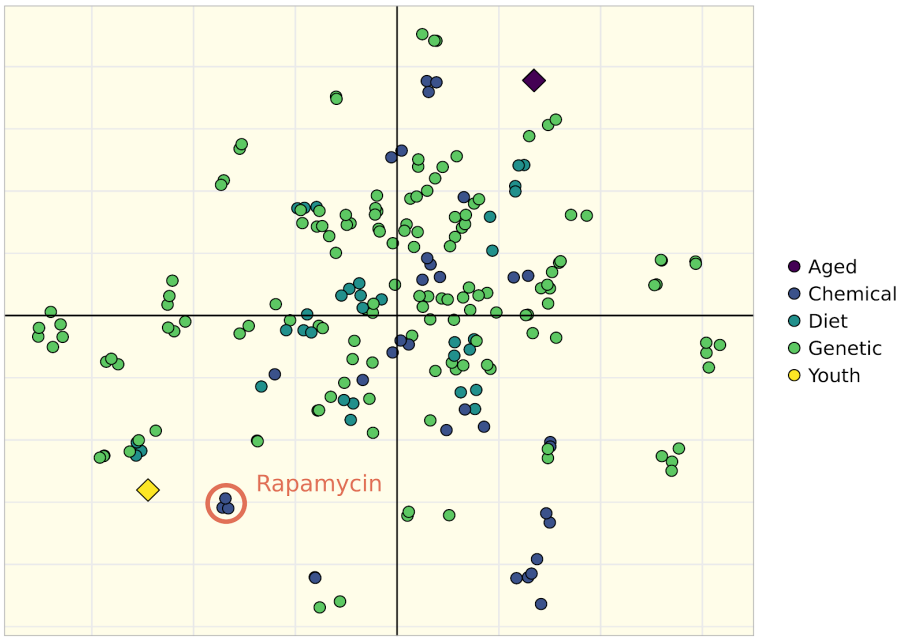

Figure 2. Low-dimensional representation of differential metabolic flux across experimental designs in *C elegans*. Axes represent the first two embeddings from t-SNE. 39 of the initial 60 experiments were selected for this plot, which categorically fell under interventions of age, genetic (knockouts and knockdowns), diet, or chemical. Young (yellow) and old (dark blue) worms noted with diamonds.

Additional interventions which mimic young *C elegans* include genetic knockouts of *osm-7, osm-8,* and *osm-11*. All three knockouts are known to induce stress resistance (Rohlfing et al., 2016). Bifidobacterium animalis subsp. lactis CECT 8145, a probiotic shown to decrease obesity in worms (Martorell et al., 2016), rats (Carreras et al., 2018) and humans (Pedret et al., 2018), also mimics young *C elegans* metabolic phenotypes. Interventions which induce an “aged” metabolic phenotype include a *daf-12* knockout, which has been previously established (Fisher et al., 2006), and silver nanoparticles, known to be toxic.

Unclear insights from MFA in mammalian systems

This approach was then applied to *Mus musculus* expression data. 80 available liver expression datasets were identified with a clear comparative experimental design. These designs included exposures to drugs, diets, genetic manipulations, and their combinations. The flux vectors generated from these datasets using the iMM1415 genome-scale metabolic model (Sigurdsson et al., 2010) were then compressed using Uniform Manifold Approximation (UMAP) after an initial PCA dimensionality reduction. This approach was chosen instead of tSNE, as tSNE produced a balanced, but largely uniform distribution of embeddings without any clear clustering. A modicum of clustering was observed, however the study designs within some of these clusters are inconsistent. For instance, the studies highlighted in Figure 3A include fasting, rapamycin treatment, and KRAP knockout (which produces a HFD phenotype resistance, Fujimoto et al., 2009), all of which are shown to be metabolically beneficial or longevity-inducing. However this cluster also includes knockouts for Pten and Sav1, which accelerate fatty liver development and increases risk of liver cancer (Jeong et al., 2018). For references, the accession expression references within cluster A include GSE46582, GSE13583, GSE94797, and GSE10503. All four studies in the cluster highlighted in Figure 3B produce a fatty liver phenotype either genetically, through HDAC knockouts, or through a high fat diet. The accession expression references within cluster B include GSE49386,

GSE66711, GSE48331, and GSE40899. While the phenotypic similarity within this cluster is encouraging, its proximity to a cluster with pro-metabolic or longevity-inducing phenotypes casts doubt on the utility of metabolic modeling to characterize metabolic states in more complex organisms, at least through these embedding approaches.

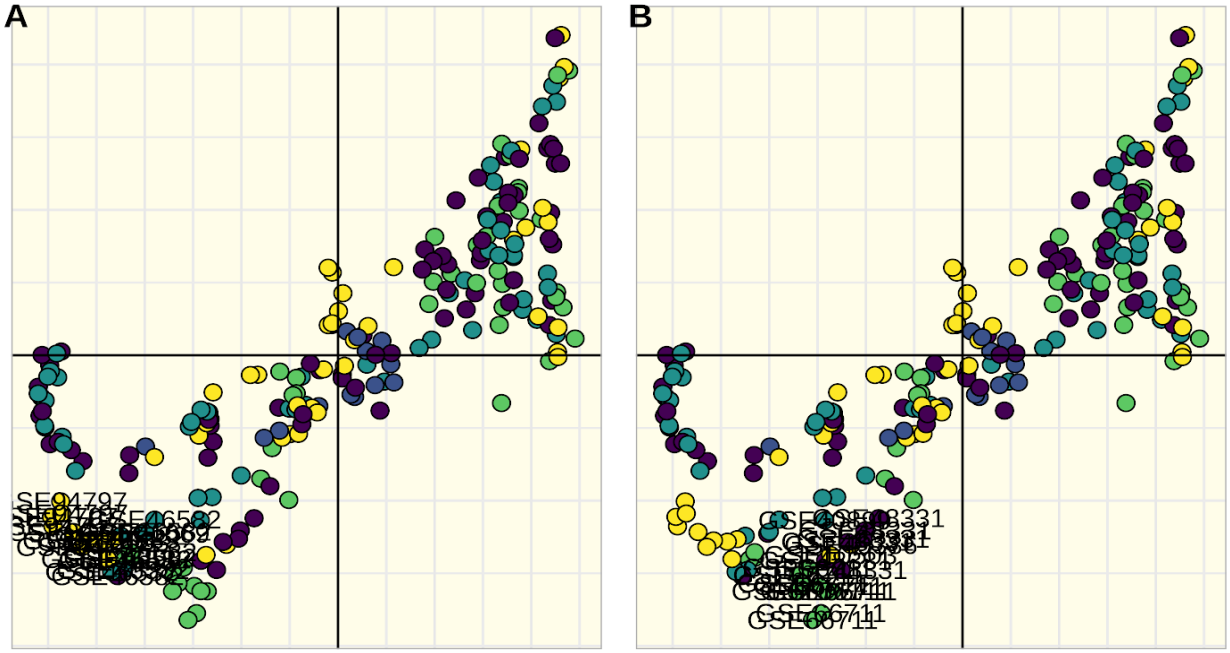

Figure 3. Low-dimensional representation of differential metabolic flux across experimental designs in *Mus musculus*. Axes represent the first two embeddings from UMAP for 80 experiments representing 700 samples. Color is applied by study, demonstrating the variance of flux profiles within and across studies.

Discussion

Metabolic flux analysis is an established approach to engineer microbes and yeast for industrial chemical, therapeutic, and food and beverage production. This work applied these methods to more complex biological systems to generate hypotheses for therapeutic discovery or for chemical agents, diets, and regimens which might mimic youthful or metabolically healthy phenotypes. The integration of publicly available *C elegans* expression data with CBM yielded metabolic flux signatures which corroborate prior work in longevity research. Anti-aging phenotypes served as an accessible proof-of-concept for this approach, however presents a more challenging objective in mammalian systems. To this end, a broader search of physiological mimetics was extended to diets and fasting regimens. The murine flux predictions did not yield clear hypotheses for fasting mimetics, with antithetical clusterings of both fasted and fatty liver phenotypes. Two possible explanations here include the constraints in CBM, where gene expression may be insufficient to generate realistic bounds of fluxes. The other main reason may be due to an incomplete and inaccurate metabolic model. As an analogy, estimating the rate of traffic on a complete map of streets and highways is considerably easier than if the map were half complete. Since this work, new murine metabolic models have been created, and may advance the predictive ability of this approach. Until accurate maps of such complex biological systems can be created, the utility of metabolic modeling may continue to reside in simpler systems.

References

Bitto A, Wang AM, Bennett CF, Kaeberlein M. Biochemical Genetic Pathways that Modulate Aging in Multiple Species. Cold Spring Harb Perspect Med. 2015;5(11).

Bordbar A, Monk JM, King ZA, Palsson BO. Constraint-based models predict metabolic and associated cellular functions. Nat Rev Genet. 2014;15(2):107-20.

Braunstein A, Muntoni AP, Pagnani A. An analytic approximation of the feasible space of metabolic networks. Nat Commun. 2017;8:14915.

Broido AD, Clauset A. Scale-free networks are rare. Nat Commun. 2019;10(1):1017.

Carreras NL, Martorell P, Chenoll E, Genovés S, Ramón D, Aleixandre A. Anti-obesity properties of the strain Bifidobacterium animalis subsp. lactis CECT 8145 in Zücker fatty rats. Benef Microbes. 2018;9(4):629-641.

Covert MW, Schilling CH, Famili I, et al. Metabolic modeling of microbial strains in silico. Trends Biochem Sci. 2001;26(3):179-86.

Feist AM, Palsson BO. The biomass objective function. Curr Opin Microbiol. 2010;13(3):344-9.

Fisher AL, Lithgow GJ. The nuclear hormone receptor DAF-12 has opposing effects on Caenorhabditis elegans lifespan and regulates genes repressed in multiple long-lived worms. Aging Cell. 2006;5(2):127-38.

Fujimoto T, Miyasaka K, Koyanagi M, Tsunoda T et al. Altered energy homeostasis and resistance to diet-induced obesity in KRAP-deficient mice. PLoS One 2009;4(1):e4240

Jeong SH, Kim HB, Kim MC, Lee JM et al. Hippo-mediated suppression of IRS2/AKT signaling prevents hepatic steatosis and liver cancer. J Clin Invest 2018 Mar 1;128(3):1010-1025.

King ZA, Lu J, Dräger A, et al. BiGG Models: A platform for integrating, standardizing and sharing genome-scale models. Nucleic Acids Res. 2016;44(D1):D515-22.

Martorell P, Llopis S, González N, et al. Probiotic Strain Bifidobacterium animalis subsp. lactis CECT 8145 Reduces Fat Content and Modulates Lipid Metabolism and Antioxidant Response in Caenorhabditis elegans. J Agric Food Chem. 2016;64(17):3462-72.

Pedret A, Valls RM, Calderón-pérez L, et al. Effects of daily consumption of the probiotic Bifidobacterium animalis subsp. lactis CECT 8145 on anthropometric adiposity biomarkers in abdominally obese subjects: a randomized controlled trial. Int J Obes (Lond). 2018.

Rohlfing AK, Miteva Y, Hannenhalli S, Lamitina T. Genetic and physiological activation of osmosensitive gene expression mimics transcriptional signatures of pathogen infection in C. elegans. PLoS One 2010 Feb 2;5(2):e9010.

Sigurdsson, MI, Jamshidi, N, Steingrimsson, E, Thiele, I, Palsson, BT. A detailed genome-wide reconstruction of mouse metabolism based on human Recon 1. BMC Syst. Biol. 2010;4.

Yilmaz LS, Walhout AJ. A Caenorhabditis elegans Genome-Scale Metabolic Network Model. Cell Syst. 2016;2(5):297-311.