A Van-Der-Waals picture for metabolic networks from MaxEnt modeling: inherent bistability & elusive coexistence.

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Abstract. In this work maximum entropy distributions in the space of steady states of metabolic networks are defined upon constraining the first and second moment of the growth rate. Inherent bistability of fast and slow phenotypes, akin to a Van-Der Waals picture, emerges upon considering control on the average growth (optimization/repression) and its fluctuations (heterogeneity). This is applied to the carbon catabolic core of \textit{E coli} where it agrees with some stylized facts on the persisters phenotype and it provides a quantitative map with metabolic fluxes, opening for the possibility to detect coexistence from flux data. Preliminary analysis on data for \textit{E Coli} cultures in standard conditions shows, on the other hand, degeneracy for the inferred parameters that extend in the coexistence region.

1. Introduction

Quantitative modeling cell metabolism would give essential insights on reconstructing cell physiology from molecular mechanisms with wide applications, a quest that shall be addressed within a systemic perspective \cite{1}. Upon modeling cell growth, an approach based on stochiometric constraints under a steady-state hypothesis for the underlying chemical network, complemented by optimality principles, it works in semi-quantitative way at population level in lab conditions \cite{2}, it is straightforward to formulate \cite{3} and computationally fast to implement \cite{4}. However, several phenomena in biology would require to assess single-cell heterogeneity \cite{5} and thus an extension of modeling beyond the population level. Nowadays, recent advancements in microfluidic device can give access to growth rate physiology at single cell level \cite{6}. In particular measures on isogenic populations in identical conditions give rise to growth rate distributions with unavoidable fluctuations \cite{7}, that shall reflect metabolic variability \cite{8}. Both the issue of a systemic perspective and the one of addressing heterogeneity could get interesting insights from statistical mechanics methods. Recent applications of Monte Carlo Markov chain algorithms to integral calculus \cite{9} give rise to the possibility of mapping growth rate distributions into an underlying distribution for the metabolic phenotypes, in the most simple way upon recurring to maximum entropy distributions at fixed average
growth rate in the space of metabolic steady states [10]. These in turns provide quantitative predictions for experimental estimates of metabolic fluxes as well as for their scaling, correlations and fluctuations [11]. Still this variability does not amount to a substantial phenotypic heterogeneity, since a linear constraint on the average growth leads to simple unimodal distributions. On the other hand, motivated by the recent threat of antibiotic resistance development [12], there is a substantial interest on analyzing truly heterogeneous coexistence of persistent phenotypes [13], whose modeling could get interesting insights from physics [14]. In general, a small fraction of bacteria is usually observed to survive an antibiotic treatment, after which they are able to regrown and reestablish an antibiotic-sensitive population, thus indicating that the origin of the resistance of this “persistent” small subpopulation does not rely on genotype mutations. Persister phenotypes are thus known to coexist in cultures in stressed conditions and show an active, yet no (or very slow) growing, metabolism [15]. It has been quantitatively shown that the fitness landscape of E Coli endows inherent bistability of the growth rate [16] and it has been observed that the central carbon core metabolism shall thus endow a bistable phenotypic landscape where slow and fast growing cells sit on wells separated by a watershed barrier whose height and positions are ruled by two phenomenological parameters related to the growth rate and the metabolic flux [17]. In this work a simple phenomenological approach is proposed to model quantitatively bistability and coexistence of fast and slow growing phenotypes for the central catabolic core of E. Coli in terms of maximum entropy distributions. It will be shown that bimodal distributions are retrievec upon constraining the first two moments of the growth rate in the metabolic space, with the two lagrange multipliers in the Boltzmann-Gibbs distribution that play the role of the control parameters that shape the bistable phenotypic landscape akin to a Van-Der-Waals picture. It will be shown that, despite its simplicity, this framework captures stylized facts of the metabolic persistent phenotype, like an active metabolism and increased rate of respiration and carbon dioxide production (per unit of carbon intake) with respect to the fast growing phenotype. This framework would ideally provide a mean to infer elusive coexistence of persisters from flux data and a preliminary analysis from E. Coli cultures in standard conditions will be presented, alongside with conclusions.

2. Results

2.1. The Model

A standard approach to model metabolism during cell growth is to consider the underlying chemical network in a well-mixed steady state in the continuum limit, that is approximately valid for timescales slower than diffusion and metabolic turnover. The reaction network is encoded in a matrix of stoichiometric coefficients $S_{i\mu}$ (metabolite $\mu$ in reaction $i$), that linearly relates the time derivative of concentrations $c_\mu$ to enzymatic
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Fluxes $\nu_i$ (mass balance)

$$\dot{c}_\mu = \sum_i S_{i\mu} \nu_i$$  \hspace{1cm} (1)

Upon considering steady states and taking into account reversibility, kinetic limits and medium capacity in terms of flux bounds, a space of feasible states is defined that is geometrically a convex polytope $P$

$$\sum_i S_{i\mu} \nu_i = 0$$  \hspace{1cm} (2)

$$\nu_i \in [\nu_i^{min}, \nu_i^{max}]$$.  \hspace{1cm} (3)

In order to model cell growth, a phenomenological biomass production reaction is added in models, whose flux will be denoted $\lambda$ and whose maximum in given conditions can be evaluated with linear programming.

Here we will consider Maximum Entropy distributions in the space of steady state of a metabolic network upon fixing the first two moments of the growth rate, $\langle \lambda \rangle$ and $\langle \lambda^2 \rangle$. By a standard variational approach [18], constraints on the moments are enforced by Lagrange multipliers $(\beta, \gamma)$ as control parameters in Boltzmann-Gibbs distributions

$$P(\nu) \propto e^{\beta\lambda(\nu) + \gamma\lambda^2(\nu)} \quad \text{if} \quad \nu \in P$$  \hspace{1cm} (4)

whose analysis is developed in the next section.

2.2. A Van-Der-Waals picture

The marginal distribution $q(\lambda)$ of the growth rate from an uniform sampling of the network under exam, the catabolic core of E.coli, has in a good approximation the simplex-like form

$$q(\lambda) \propto (\lambda_{max} - \lambda)^a$$  \hspace{1cm} (5)

where $\lambda_{max}$ is the maximum achievable growth rate, given the conditions (obtainable with linear programming) and $a = D - d - 1$, where $D$ is the dimension of the space and $d$ the dimension of the subspace where $\lambda$ (e.g., $d = 0$ is a vertex). Upon considering the aforementioned constraints on the moments, we have for the marginal distribution of the growth rate $p(\lambda) \propto e^{F_{\beta,\gamma}(\lambda)}$, where the rate function (from now on we assume $\lambda_{max} = 1$ for simplicity of notations) has the form

$$F_{\beta,\gamma}(\lambda) = \beta \lambda + \gamma \lambda^2 + a \log(1 - \lambda) \quad \lambda \in [0, 1]$$  \hspace{1cm} (6)

The extremal points of this function can be analyzed as a function of the control parameters in simple terms. There is a maximum in $\lambda = 0$ (where $F(0) = 0$) iff $F'(0) = \beta - a < 0$. There are other two extremal points where $F'(\lambda_\pm) = 0$, i.e.

$$\lambda_\pm = \frac{2\gamma - \beta \pm \sqrt{(2\gamma + \beta)^2 - 8\gamma a}}{4\gamma}$$  \hspace{1cm} (7)
that are real as soon as $\beta > \sqrt{8\gamma a - 2\gamma}$, and positive if $\gamma > \gamma_c = a/2$, with $\lambda_+$ maximum and $\lambda_-$ minimum. We have qualitatively a picture similar to a Van-Der-Waals fluid, with the two “spinodal” lines ($\gamma > \gamma_c = a/2$)

$$\beta = a$$
$$\beta = \sqrt{8\gamma a - 2\gamma}$$

that mark the onset of probability maxima that refer to respectively, slow (below (5)) and fast (above (6)) growing phenotypes. The curves (5,6) thus define a coexistence region with an equilibrium line given implicitly by the equation $F(0) = 0 = F(\lambda_+)$, where the maxima have equal heights, that marks discontinuous transitions and hysteresis, shrinking at the point ($\gamma_c = a/2, \beta_c = a$). This picture is summarized in the diagram in Fig. 1. Growth rate marginal distributions in particular can be obtained analytically

![Figure 1. Phase diagram of metabolic network states in the plane ($\gamma, \beta$) of the two lagrange multipliers constraining the first two moments of the growth rate ($a = 20$). Red dashed lines: “spinodal” lines that mark the onset of probability peaks for slow and fast growing phenotypes. Black full line: “equilibrium” curve where the frequencies of fast and slow phenotypes are equal.](image)

by Legendre transform as we show in fig 2, where a comparison with Monte Carlo computations on the network under exam (see materials and methods section ) is shown. More in general, to each point of the phase diagram it corresponds a given distribution of metabolic fluxes, and in next section a simple analysis will be performed for a point inside the coexistence region for the model under exam, that is the central carbon metabolism of E Coli in glucose limited aerobic conditions and low dilution.
2.3. Some examples of flux distributions at coexistence

We consider the point \((\beta = 0, \gamma = 50)\) in the coexistence region of the phase diagram. In Fig 3 we show scatter plots of a) the rate of oxygen consumption, b) carbon dioxide production and c) ATP synthase flux, all relative to the glucose uptake and scattered with the respect to the growth rate (relative to the maximum achievable one). In qualitative agreement with stylized facts, the slow growing phenotype results metabolically active as indicated by comparable level of ATP synthase flux with respect to the fast growing phenotype (and increased variance). Further, the level of oxygen consumption and carbon dioxide production of the slow growing phenotype is higher with respect to the fast growing phenotype, clearly indicating this as a diversion of the carbon source intake from the biomass. However the absolute rate of glucose uptake of fast and slow growing phenotypes are comparable within this simple framework, at odds with experiments with induced persistence where slower cells intake less carbon units.
Figure 3. Scatter plots of a) the rate of oxygen consumption, b) carbon dioxide production and c) ATP synthase flux, relative to the glucose uptake and scattered with the respect to the relative growth rate, for a point ($\gamma = 50, \beta = 0$) in the phase diagram in fig. 1. From numerical Monte Carlo calculations on the catabolic core of *E.Coli*.

The flux distributions obtained at varying ($\beta, \gamma$) can be then compared to experimental data in order to infer coexistence of fast and slow growing phenotypes as we stress in the following section.

2.4. Coexistence in standard conditions?

Here it is examined the problem of inferring the parameters ($\beta^*, \gamma^*$) that maximize the likelihood of data. For simplicity we consider a Gaussian approximation, i.e. if we have a subset of $K$ experimental fluxes, averages and variances, $\nu_{i,\text{exp}}, \sigma_{i,\text{exp}}$ we look at the $\chi^2$ as a function of the parameters ($\beta, \gamma$) that is

$$\chi^2(\beta, \gamma) = \sum_i^K \frac{(\nu_{i,\text{exp}} - \langle \nu_i \rangle_{\beta,\gamma})^2}{\sigma_{i,\text{exp}}^2 + \sigma_{i,\beta,\gamma}^2}$$

A preliminary analysis has been performed to data for *E.Coli* cultures in standard conditions (see materials and methods section). In Fig. 4 it is shown the $\chi^2(\beta, \gamma)$ heat-map comparing data and predicted flux distributions in the plane ($\beta, \gamma$). The inference correctly predict growing phenotypes, but with a certain level of degeneracy for the
Figure 4. $\chi^2(\gamma, \beta)$ heat-map comparing predicted flux distributions in the plane $(\gamma, \beta)$ and data referring to E Coli core metabolism for cultures grown in glucose limited aerobic conditions with growth/dilution rates below 0.4h$^{-1}$, averages over 35 technical replicates, control experiments retrieved from the database [19]. Dashed line: $80 = \beta + 1.5\gamma$.

inferred parameters, with low values of the $\chi^2$ that extend in the coexistence region. More precisely a line of $\chi^2$ minima develops approximately given by $80 = \beta + 1.5\gamma$.

3. Materials and methods

The network analyzed is the catabolic core of a network reconstruction of E. Coli metabolism [20], that includes glycolysis, pentose phosphate pathway, Krebs cycle, oxidative phosphorylation and several nitrogen catabolic reactions. The underlying polytope of steady states in glucose limited aerobic conditions turns out to be a $D = 23$—subdimensional variety in a space with $N = 86$ (number of reactions) dimensions. States can be efficiently sampled with an hit-and-run Monte Carlo Markov chain upon handling of ill conditioning due to heterogeneous scales [21], with rounding ellipsoids, for which a method due to L.Lovazs has been implemented [22]. The bias due to the Boltzmann-Gibbs factor enforcing the moments constraint has been implemented with a Metropolis-Hastings rejection rule for the proposed step during the hit-and-run dynamics [23]. The flux data refer to E. Coli cultures grown in glucose limited minimal medium in aerobic conditions at low dilution/growth rates below 0.4h$^{-1}$, i.e. the threshold for the acetate switch. The sample consists of 35 technical replicates collected from control experiments retrieved in the database [19] (same dataset analyzed in [11]).
4. Conclusions

In this work it has been shown that a simple phenomenological approach in which the first two moments of the growth rate in the space of steady states of a metabolic network are constrained can be used to model quantitatively bistability and coexistence of fast and growing cells in heterogeneous cell populations. This shows that bistability and coexistence can be an inherent feature of the central carbon core upon assuming control of the level of the average growth rate (optimization/repression) and its fluctuations (heterogeneity), providing in turn a quantitative map with metabolic flux distributions.

It has been shown that this simple framework can account for stylized facts on persisters phenotypic heterogeneity, in particular their active metabolism at the level of ATP production, oxygen consumption rate and carbon dioxide production. This simple framework, however fails to predict lower level of carbon uptakes by the slow growing phenotype as observed experimentally. This last experimental fact refers however to stressed conditions, something that could be added phenomenologically by constraining the average covariance of biomass and uptakes, $\langle u\lambda \rangle$ (with a corresponding term in the Boltzmann-Gibbs weight $\propto e^{\delta\lambda u}$). Such Maximum Entropy approach can be further used to infer from flux data elusive coexistence and a preliminary analysis on data about E Coli cultures grown in glucose limited aerobic minimal medium show degeneracy of parameters that extends in the coexistence region. This would require further investigations upon complementing flux data with measures of single cell growth physiology, in particular rare events and in microfluidic devices where slower persisters are not taken over. Finally it is worth to mention that in general a Maximum Entropy approach shows that constraining the first two moments of any flux in the metabolic space can lead to a Van-Der-Waals picture with coexistence and bistability for the underlying flux, in turn providing an effective way to model single cell heterogeneity for any secondary metabolites production.

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References

[1] H Kacser, J Burns. The control of flux. In Symp. Soc. Exp. Biol., volume 27, pages 65–104, 1973.
[2] RA Majewski and MM Domach. Simple constrained-optimization view of acetate overflow in e. coli. Biotechnology and bioengineering, 35(7):732–738, 1990.
[3] Leonid Vitalievich Kantorovich. A new method of solving of some classes of extremal problems. In Dokl. Akad. Nauk SSSR, volume 28, pages 211–214, 1940.
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[4] George Dantzig. *Linear programming and extensions*. Princeton university press, 2016.

[5] Steven J Altschuler and Lani F Wu. Cellular heterogeneity: do differences make a difference? *Cell*, 141(4):559–563, 2010.

[6] Ping Wang, Lydia Robert, James Pelletier, Wei Lien Dang, Francois Taddei, Andrew Wright, and Suckjoon Jun. Robust growth of escherichia coli. *Current biology*, 20(12):1099–1103, 2010.

[7] Andrew S Kennard, Matteo Osella, Avelino Javer, Jacopo Grilli, Philippe Nghe, Sander J Tans, Pietro Cicuta, and Marco Cosentino Lagomarsino. Individuality and universality in the growth-division laws of single e. coli cells. *Physical Review E*, 93(1):012408, 2016.

[8] Daniel J Kiviet, Philippe Nghe, Noreen Walker, Sarah Boulineau, Vanda Sunderlikova, and Sander J Tans. Stochasticity of metabolism and growth at the single-cell level. *Nature*, 2014.

[9] V Turcin. On the computation of multidimensional integrals by the Monte Carlo method. *Th Probab Appl*, 16:720–724, 1971.

[10] Daniele De Martino, Fabrizio Capuani, and Andrea De Martino. Growth against entropy in bacterial metabolism: the phenotypic trade-off behind empirical growth rate distributions in e. coli. *Physical biology*, 13:036005, 2016.

[11] Daniele De Martino, Anna Andersson, Tobias Bergmiller, Călin C Guet, and Gašper Tkačik. Statistical mechanics for metabolic networks during steady-state growth. *arXiv preprint arXiv:1703.01818*, 2017.

[12] Vanessa M DCosta, Christine E King, Lindsay Kalan, Mariya Morar, Wilson WL Sung, Carsten Schwarz, Duane Froese, Grant Zazula, Fabrice Calmels, Regis Debruyne, et al. Antibiotic resistance is ancient. *Nature*, 477(7365):457–461, 2011.

[13] Nathalie Q Balaban, Jack Merrin, Remy Chait, Lukasz Kowalik, and Stanislas Leibler. Bacterial persistence as a phenotypic switch. *Science*, 305(5690):1622–1625, 2004.

[14] Rosalind Allen and Bartlomiej Waclaw. Antibiotic resistance: a physicists view. *Physical biology*, 13(4):045001, 2016.

[15] Oliver Kotte, Benjamin Volkmer, Jakub L Radzikowski, and Matthias Heinemann. Phenotypic bistability in escherichia coli’s central carbon metabolism. *Molecular systems biology*, 10(7):736, 2014.

[16] J Barrett Deris, Minsu Kim, Zhongge Zhang, Hiroyuki Okano, Rutger Hermsen, Alexander Groisman, and Terence Hwa. The innate growth bistability and fitness landscapes of antibiotic-resistant bacteria. *Science*, 342(6162):1237435, 2013.

[17] Jakub Leszek Radzikowski, Silke Vedelaar, David Siegel, Álvaro Darío Ortega, Alexander Schmidt, and Matthias Heinemann. Bacterial persistence is an active σs stress response to metabolic flux limitation. *Molecular Systems Biology*, 12(9):882, 2016.

[18] Edwin T Jaynes. Information theory and statistical mechanics. *Physical review*, 106(4):620, 1957.

[19] Zhengdong Zhang, Tie Shen, Bin Rui, Wenwei Zhou, Xiangfei Zhou, Chuanyu Shang, Chenwei Xin, Xiaoguang Liu, Gang Li, Jiansi Jiang, et al. Cecafdb: a curated database for the documentation, visualization and comparative analysis of central carbon metabolic flux distributions explored by 13c-fluxomics. *Nucleic acids research*, page gku1137, 2014.

[20] Jeffrey D Orth, Ronan MT Fleming, and Bernhard Ø Palsson. Reconstruction and use of microbial metabolic networks: the core escherichia coli metabolic model as an educational guide. *EcoSal plus*, 4(1), 2010.

[21] Daniele De Martino, Matteo Mori, and Valerio Parisi. Uniform sampling of steady states in metabolic networks: Heterogeneous scales and rounding. *PLoS ONE*, 10(4):e0122670, 2015.

[22] László Lovász. *An algorithmic theory of numbers, graphs and convexity*, chapter 2, page 59. SIAM, 1984.

[23] W Keith Hastings. Monte carlo sampling methods using markov chains and their applications. *Biometrika*, 57(1):97–109, 1970.