Spin Glass Theory of Interacting Metabolic Networks

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We cast the metabolism of interacting cells within a statistical mechanics framework considering both, the actual phenotypic capacities of each cell and its interaction with its neighbors. Reaction fluxes will be the components of high-dimensional spin vectors, whose values will be constrained by the stoichiometry and the energy requirements of the metabolism. Within this picture, finding the phenotypic states of the population turns out to be equivalent to searching for the equilibrium states of a disordered spin model. We provide a general solution of this problem for arbitrary metabolic networks and interactions. We apply this solution to a simplified model of metabolism and to a complex metabolic network, the central core of the E. coli, and demonstrate that the combination of selective pressure and interactions define a complex phenotypic space. Cells may specialize in producing or consuming metabolites complementing each other at the population level and this is described by an equilibrium phase space with multiple minima, like in a spin-glass model.

Cellular metabolism is defined as the network of chemical reactions that transforms raw materials from the environment into useful products to the cell. It provides cells with the energy and the building blocks demanded for most biological functions [1]. Although each type of cell has its own metabolic network [2], many reactions and pathways are conserved across species, and their specific role in metabolism is well characterized. Prominent examples are glycolysis, the pentose-phosphate pathway and oxidative phosphorylation, which form the backbone of the network of metabolic reactions of most cells.

The recent annotation of genome scale metabolic networks [3] lead the study of metabolism to a new level of abstraction and computational difficulty, opening the doors to its quantitative understanding beyond the independent pathway approximation. In this context, algebraic approaches [4, 5], Linear Programming [6] and statistical analysis [7–12] have become standard tools of the community.

Much less understood is the role of metabolism in the interaction between nearby cells. The importance of these interactions has been known for many years because they drive the appearance of several diseases [13–16]. More generally, cells compete for nutrients, and at the same time they produce by-products that could be either toxic [17], alternative sources of energy [18, 19], or alter the environment [20]. This results necessarily in direct or indirect relations between the metabolism of different cells [21]. However, most of the theoretical work on interacting metabolic networks has been characterized by i) the study of competing/cooperating populations after drastic simplifications of their metabolism [22, 26], and ii) the study of the interaction between two individual cells with identical or nearly identical complex metabolism [8–10, 27, 28]. When populations of cells are described by complex metabolic networks, interacting between them or with the environment, simulations have been the main research tool [29–32]. An analytic approach to deal with the interaction of complex cells is currently absent from the literature.

The main purpose of this paper is to build this theory developing an analogy with the physics of classical disordered systems. We treat the reaction rates of the metabolism in each cell as the components of a continuous spin vector $\vec{v}$ bounded by thermodynamic constrains $b_r \leq v_r \leq u_r$ for each reaction $r$ and representing kinetic limitations of the fluxes in the physiological context of the cell, such as irreversibility. These fluxes must also satisfy the stoichiometric relations of the metabolism in stationary state, $S\vec{v} = \vec{b}$, where $S$ is the stoichiometric matrix and $\vec{b}$ represents the exchange fluxes with the environment. These constraints define a polytope that contains the possible metabolic states of the cells.

In the absence of interactions it is often considered that each cell $i$ tries to maximize its own utility function, $-E_i = \sum_r h_r v_{r,i}$, where the choice of $h_r$ defines the utility function of the cell, and the minus sign accommodates the physical convention that energy is minimized. Usually, the $h_r$ stand for the growth rate [33], ATP production, or maximization of the rate of synthesis of a particular product of interest [34], etc. The maximization of this utility function subject to the stoichiometric constraints constitutes an optimization problem easily solved by linear programming techniques [35]. However, only in specific situations, such as simple bacteria growing in rich media, cells should be studied as if they optimize an individual utility function. Cells in a community such as a tissue or a culture share and exchange metabolic products from and with the help of the environment. In this case, a proper description of the whole system must consider a modified utility function that takes explicitly
these interactions into account.

INTERACTING CELLS

In practice we assume that a collection of cells interacts through a term of the form: \( V_{ij} = - \sum_r J_{rij} \bar{v}_r \bar{v}_j \), where the sum over \( r \) is done over a set of exchange reactions. If \( J_{rij} > 0 \), a state where both cells \( i \) and \( j \) carry a similar flux on reaction \( r \) is favored. For example, if reaction \( r \) represents consumption of a nutrient such as glucose, then there is an evolutionary pressure favoring competition between cells \( i \) and \( j \) for this nutrient. On the contrary if \( J_{rij} < 0 \), cells \( i \) and \( j \) tend to carry opposite fluxes on reaction \( r \). If reaction \( r \) represents an exchange of a byproduct, such as lactate, it means then that cell \( i \) tends to produce lactate and cell \( j \) to consume it (or vice versa), establishing a so called lactate shuttle [22, 36–38]). In this case we say that cells cooperate.

Combining the utility functions of individual cells with the terms arising from these pairwise interactions gives rise to the following energy function for the population of cells,

\[
H(\{v\}) = - \sum_{r,i} h_i \bar{v}_r - \sum_{i<j} \sum_r J_{rij} \bar{v}_r \bar{v}_j
\]

which favors states where individual cells attempt to maximize their own utility functions but also try to form favorable interactions with neighboring cells. Ideally we seek a configuration of cells that minimizes \( H(\{v\}) \).

In our approach cells are not necessarily identical, stochastic fluctuations in the synthesis of proteins [39], evolutionary processes [21] or local fluctuations in the concentration of raw materials [40] forces the cell to explore different metabolic states and not only “optimal” ones. We assume that these metabolic states are distributed according to a Boltzmann distribution:

\[
P(\{\bar{v}\}) = \frac{1}{Z[J]} \exp(-\beta H(\{v\}))
\]

provided that the constraints \( l_b \leq \bar{v}_r \leq u_b \) and \( S \bar{v} = \bar{b} \) are satisfied, where \( Z \) is a normalization constant known in the physics literature as the partition function of the problem, and the parameter \( \beta \) quantifies the strength of the stochastic fluctuations that drive cells away from the preferred configurations. In absence of interactions \( \beta \) has been interpreted as an equilibration time-scale in the dynamics of a logistic growth model [28]. In a more general setting, \( \beta \) was also connected to the mutation rate (or more generally the rate of cell differentiation) in simple dynamic evolutionary models [27]. In general, when \( \beta \to 0 \), the exponential term is irrelevant and the properties of the problem are defined by the solution space fixed by the stoichiometric constraints (a polytope) [3 [10 [41 42]). In the opposite limit, when \( \beta \to \infty \), and in absence of interactions, cells minimize their own \( E_i \) subject to the stoichiometric constraints. This solution, known in the literature as flux-balance analysis (FBA) [8], lies in a vertex of the polytope and can be found by efficient linear programming techniques [35].

This way to write the problem allows to explore the solution space of interacting metabolic networks using precise mathematical terms. In addition, this analogy between reaction fluxes and continuous spin variables enlarges the family of disordered systems and makes possible the use of the whole arsenal of concepts, techniques, and approximations already developed in their study in the analysis of metabolic interacting cells [43].

MEAN FIELD SOLUTION

To model an heterogeneous population we assume that the couplings \( J_{rij} \) are drawn from a normal distribution with mean \( J_r \) and variance \( \Delta_r \). For simplicity we take the \( h_i = h_r \) as fixed values to be specified below. The physics of the problem is summarized by a free energy density, defined by \( f[J] = -\langle 1/N \rangle \log Z[J] \) that must be averaged over the disorder of the couplings. We denote this operation by an overbar, \( \bar{f} = \frac{1}{N} \log Z[J] \). To perform the average of the log, we can use the replica trick [43], \( \log Z = \lim_{\alpha \to 0} \frac{1}{\alpha} \log Z^\alpha \) (Details are provided in the Supplementary Material). We quote here only the final result:

\[
\bar{f} = -\sum_{r,\alpha} \frac{J_r^2}{2} \bar{m}_{\alpha r} - \sum_{\alpha<\beta} \sum_r \frac{\Delta_r^2}{2} q_{\alpha \beta}^2 - \sum_{\alpha,\beta} \frac{\Delta_r^2}{4} \zeta_{\alpha \beta}
\]

where the trace is over all the replicated flux variables \( v_{\alpha r}^r \), respecting the stoichiometric constrains in each replica, \( \alpha = 1, \ldots, n \). The effective Hamiltonian coupling replicas is given by:

\[
H_{\text{eff}} = -\sum_{r,\alpha} h_r v_{\alpha r}^r - \sum_{r,\alpha} J_r v_{\alpha r}^r m_{\alpha r}^r - \sum_r \sum_{\alpha<\beta} \frac{\Delta_r^2}{2} v_{\alpha r}^r v_{\beta r}^\beta q_{\alpha \beta}^2 - \sum_r \frac{\Delta_r^2}{4} (v_{\alpha r}^r)^2 \zeta_{\alpha r}
\]

and the parameters \( m_{\alpha r}^r, q_{\alpha \beta}^r, \zeta_{\alpha r} \) must be chosen to extremize this expression. Differentiating gives the following set of coupled equations:

\[
m_{\alpha r}^r = \langle v_{\alpha r}^r \rangle, \quad q_{\alpha \beta}^r = \langle v_{\alpha r}^r v_{\beta r}^\beta \rangle, \quad \zeta_{\alpha r} = \langle (v_{\alpha r}^r)^2 \rangle
\]

where \( \langle \ldots \rangle \) denotes an average with weight exp (\( -H_{\text{eff}}(\{v\}) \)).

A crucial step is to propose an ansatz to the form of the parameters \( m_{\alpha r}^r, q_{\alpha \beta}^r, \zeta_{\alpha r} \) that solve these equations. Since the replicas are indistinguishable, a natural assumption is that the extremization respects this symmetry, and therefore that \( m_{\alpha r}^r = m_r, q_{\alpha \beta}^r = q_{r \beta}, \zeta_{\alpha r} = \zeta_r \) are independent of the replica index. This is known as the replica symmetric (RS) ansatz, which we assume throughout the
rest of the paper. In short, \( q \) quantifies the metabolic fluctuations between populations with different realizations of the disorder and \( \zeta \) the fluctuations in the fluxes between individuals of a population with the same disorder.

To first explore this formalism in a manageable model, we considered a simplified metabolic network consisting of three reactions with fluxes \( v_i, i = 1, 2, 3 \) satisfying the constraint \( v_1 + v_2 + v_3 = 0 \), \(-1 \leq v_1 \leq 0\), and \( 0 \leq v_2 \leq 1 \). The fluxes \( v_i, i = 1, 2, 3 \) can be interpreted as glucose consumption \( (v_1) \), respiration \( (v_2) \), and lactate secretion/consumption \( (v_3) \), respectively. It has two metabolic modes: the respiration mode with \( v_3 \leq 0 \) and the fermentation mode with \( v_3 \geq 0 \). To model the contribution of these modes to energy production in the cell, we set \( h_2 = -h_1 = h = 1 \), \( h_3 = 0 \) and \( \beta = 1 \). This simplified model has been instrumental in the biological understanding of the Warburg effect \(^{44}\), where fast growing cancer cells engage in the apparently wasteful activity of secreting copious amounts of lactate even in the presence of oxygen \(^{40,45,48}\). We assume that cells are coupled by the byproduct of the third reaction only, so that \( J_i = \Delta_i = 0 \) for \( i = 1, 2 \). In this case, the RS solution simplifies to:

\[
f = -\frac{J}{2} m^2 - \frac{\Delta^2}{4} (\zeta^2 - q^2) + \int_{-\infty}^{\infty} \frac{dt}{\sqrt{2\pi}} e^{-t^2/2} \ln \text{Tr} \exp(-\mathcal{H}^{RS})
\]

where

\[
\mathcal{H}^{RS} = h(v_1 - v_3) + (J m + \Delta \sqrt{q^2}) (v_1 + v_2) - \frac{\Delta^2}{2} (v_1 + v_2)^2 (\zeta - q)
\]

and \( m, q, \zeta \) are such that \( f \) is extremized. Upon differentiation the set of coupled equations for the order parameters takes the form:

\[
m = \langle v_3 \rangle, \quad q = \langle v_3 \rangle^2, \quad \zeta = \langle v_3^2 \rangle
\]

where the angle brackets denote a Boltzmann average with Hamiltonian \( \mathcal{H}^{RS} \) at fixed \( t \), and the overbar denotes a Gaussian average over \( t \). Alternatively, \(^{8}\) can be interpreted as giving the statistics of flux \( v_3 \) for a given cell, with the angle brackets denoting a Boltzmann average at fixed disorder and the overbar the average over the disorder.

This model exhibits three distinct phases, depending on the interactions between the cells, \( (J, \Delta) \). Figure 1 shows the types of solutions obtained. When \( m > 0 \) \((m < 0)\), the population exhibits an overall net flux of production (consumption) of the product of the third reaction, the ferromagnetic state. But it is also possible that cells arrange themselves in such a way that the average flux \( v_3 \) balances, with no bias for production or consumption at the level of the population. In this case \( m = 0 \), and the parameter \( q \) allows us to draw a further distinction. If \( q = 0 \), each cell in the population has an average flux of \( v_3 = 0 \), a bona-fide paramagnetic state. On the other hand, if \( q > 0 \), although the net flux over the entire population is zero, different cells in the population will have positive or negative average values of \( v_3 \). This is analogous to the spin glass phase of statistical mechanics \(^{43}\). Biologically, cells spontaneously specialize into different metabolic phenotypes that cooperate through the exchange of the byproduct.

A more realistic setting, usually exploited to study the metabolism of bacteria, is the core metabolic network of the \( E. \ coli \). This network consists of 72 metabolites and 87 reactions \(^{49}\). For simplicity we assume here that the cells are only positively coupled by the exchange of acetate. This may happen in long-term cultures \(^{50}\) where interactions mediated by acetate, such as cross-feeding polymorphisms appear \(^{51}\), or when spatial structure influences the evolution of cooperative and competitive phenotypes \(^{51}\).

For large metabolic networks like this, the computation of the Trace in \(^{3}\) becomes computationally challenging. It is a multivariate integral over a high-dimensional polytope that can be approximately solved using the Expectation Propagation algorithm (see Supplementary Materials) \(^{8,27,32}\). We simulated this network with its original flux bounds \(^{49}\) and with mean couplings of \( J = 10 \) in the acetate exchange flux and with \( h = 10 \) for the Biomass synthesis reaction, accounting for an evolutionary pressure towards fast growth. These parameter values are chosen to be on the same order as flux values in the default units of the original network. Finally we set \( \beta = 1 \).

To illustrate how the metabolic profile of the population is affected by the interactions in Figure 2 we plot the average distribution function characterizing selected fluxes of the network for different values of the param-
FIG. 2: *E. coli* core metabolic network. Population flux histograms for selected reactions of the network, for different values of the disorder average (*J*) and fluctuations (∆). From top to bottom, glucose, acetate, citrate, lactate and biomass.

FIG. 3: *E. coli* core metabolic network. (a) Expected metabolic variance between samples with different realizations of disorder. (b) Expected metabolic variance in population with a given realization of the disorder.

parameters of the model (see Supplementary Materials). As reference, we plot in the first column the distribution of these fluxes in the absence of interactions. In a presence of interaction, for a weakly disordered system, Δ = 2 (second column), cells start to produce more acetate, at the expense of a lower biomass production. In this case, energetic metabolism is down-regulated in the majority of cells. This is exemplified by the reduction in the production of citrate, a reaction of the Krebs cycle, and the fermentation to lactate.

As the disorder increases (third column), Δ = 10, interactions mediated by acetate become less dominant in the metabolic fate of the cells, which begin to divert resources towards other pathways at the expense of acetate production. Glucose consumption spreads towards lower velocities, but citrate synthase has a higher flux indicating that energy is being produced by efficient metabolic routes. Then, Lactate production and the growth rate increase, making the culture more biosynthetically active than independent cells in the leftmost column. Therefore, while at low disorder cells focus on the production of acetate, higher disorder shifts the population towards the default metabolic modes of generating energy and biomass synthesis.

However, a more complex picture emerges looking into the order parameters of the model, defined in the same way as in the simple network solved above. In Fig. 3(a) we show the dependence of *q* − *m*2 with ∆. For small ∆, the disorder has no effect on the behavior of the population and the sample to sample fluctuations in the average acetate flux of a given cell vanish. For ∆ ≈ 3, a phase transition occurs and sample to sample fluctuations become important. On the other hand, Fig. 3(b) shows the expected metabolic variance in the acetate flux of a cell for a given instance of the disorder. If ∆ is small, this variance is negligible. Above the phase transition, the variances become positive, different cells use acetate in different ways and the interpretation of the third column in Fig. 2 should be done with care. This is an average over the disorder and, in this phase, does not represent the typical behavior of a population in a particular sample. It misses the actual heterogeneity of the population. As in the simplified model, in the statistical mechanics jargon, for ∆ > 3 the system is in a spin glass phase.

DISCUSSION

We provide a general scenario to deal with interacting cells taking into account the real complexity of their metabolism. Within this scenario, cells may compete for the same specific nutrients, and by-products of one cell can be used by or be toxic to others. This competition or cooperation is defined by the interaction between the cells and by their actual metabolic capabilities. The later are fixed by the biochemical constraints and imposed by the stochiometric matrix, i.e. the flux conservation within the cell.

We solved the problem within a mean field approximation mimicking a structureless environment. This solution is summarized in full generality (within the RS formalism as the saddle point of Eq. (3) (see also the Supplementary Materials). To first explore its implications we started studying a very simple metabolic network, representing in a highly ideal manner the two major metabolic modes of a mammalian cell: fermentation and respiration. We showed that three qualitatively distinct phases are very well defined. A disordered (paramagnetic phase), in which the interaction between the
OCCUPANCY OF STATES IN THE FLUX POLYTOPE

\[ \Delta = 0 \]

\[ \Delta \neq 0 \]

FIG. 4: Stoichiometric and reversibility constrains define a high-dimensional polytope of feasible metabolic fluxes for each cell, here represented as a 2-dimensional polygon. In absence of interactions (\( \Delta = 0 \)), the distribution of cells within this space has a peak at the maximum growth rate. As \( \beta \to \infty \) the cells concentrate sharply at this peak (FBA), while if \( \beta = 0 \) the cells diffuse over the entire space uniformly (UNIFORM) [27, 28]. When cells interact (\( \Delta \neq 0 \)), there might be multiple peaks which collectively define the ground state of the model. At \( \beta \to \infty \) the cells concentrate sharply at these peaks with different masses. At \( \beta = 0 \) we recover the uniform distribution, while for \( 0 < \beta < \infty \), the cells form diffuse clouds around the peaks.

Once the cells are fixed in a tissue, or a culture, mean-field approximations give a general clue about the general physics behind the model of interest, but may fail to catch the whole richness of a problem. This, for example, was certainly the case for second order phase transitions [43] and we expect that something similar may happen in this scenario. For these kinds of problems, statistical physics rests mainly on simulations and we follow a similar approach here modeling a two dimensional collection of interacting cells. In the Supplementary Materials we show results for simulations of our toy model in a finite dimensional lattice. Again, we obtain a rich picture, consistent with our mean-field calculations. The metabolism of the cells within the tissue may have a very disordered structure (paramagnetic or spin glass) or may be organized in an anti-ferromagnet structure, in which by-products of one cell are used by neighboring ones. Such a scenario has been observed in cancer tissues exchanging metabolites with stromal cells [22, 38], and also in healthy tissues such as the brain, where astrocytes exchange lactate with neurons [14, 37, 54].

In conclusion, the general picture emerging from our results can be summarized as follows (see Fig. 4). In absence of interactions or selective pressure, cells distribute uniformly over the space of metabolic states allowed by physico-chemical constrains. The selective pressure tends to favor a concentration of cells near the state of maximum fitness, which in this case corresponds to the FBA solutions [27, 28]. But when selective pressure is combined with the interaction between the cells, the population may reach equilibrium states where the cells concentrate in different points of the phenotypic space. Different cells may specialize in different functions establishing relations of cooperation or competition between them.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

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The authors declare no competing financial interests.
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