Supporting Information

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SI Text

The MinOver$^+$ algorithm. From an abstract viewpoint, the problem to be solved consists in finding the largest value $\rho > 0$ for which a nonzero flux vector $\mathbf{s} = \{s_i \geq 0\}$ exists such that

$$c^\mu \equiv \sum_{i=1}^{N} s_i(a_i^\mu - \rho b_i^\mu) \geq 0, \qquad \forall \mu \in \{1, \ldots, P\}, \quad \textbf{[s1]}$$

where i labels reactions and ranges from 1 to N, μ labels reagents and runs from 1 to P, whereas $\{a_i^\mu\}$, $\{b_i^\mu\}$ denote output and input stoichiometric coefficients, respectively. The general strategy used by MinOver$^+$ consists in (*i*) finding a suitable flux configuration for some value $\rho < \rho^*$, and (*ii*) increasing $\rho \to \rho + \Delta\rho$ and using the solution just obtained as the initial guess for locating a new solution. Note that Eq. **s1** is trivially verified by all flux vectors for $\rho = 0$.

Given the input/output matrix and a fixed value $\rho = \bar{\rho}$, a flux vector satisfying Eq. **s1** is found by iterating the following scheme (see ref. 1 for a proof of convergence and an estimate of convergence times):

1. Initialize the flux vector $\mathbf{s}$ to a random vector $\mathbf{s^{(0)}} \neq \mathbf{0}$.
2. Evaluate the constraints.

$$c^\mu \equiv \sum_{i=1}^{N} s_i^{(0)} (a_i^\mu - \bar{\rho} b_i^\mu), \qquad \forall \mu. \quad \textbf{[s2]}$$

3. Find $\mu_0 = \arg\min_\mu c^\mu$, i.e., the value of μ such that c^μ is minimum.
4. (a) If $c_0^\mu \geq 0$ then $\mathbf{s}^{(0)}$ is a solution. Increase $\bar{\rho}$ by $\Delta\rho$ and go to 2.

   (b) If $c_0^\mu < 0$ then update the fluxes as follows:

$$s_i^{(0)} \to s_i^{(1)} = \max\{0,\ s_i^{(0)} + \eta(b_i^{\mu_0} - \rho a_i^{\mu_0})\}, \qquad \forall i = 1, \ldots, N \quad \textbf{[s3]}$$

and go to 2.

The factor η in Eq. **3** is a real number between 0 and 1 and may speed up the convergence when the algorithm works close to ρ^*.

We define the unsatisfiable region as the one where the algorithm can't find a solution within a certain maximum computational time. ρ^* can then be approximated (within $\Delta\rho$) by the higher value for which a solution has been found. Alternatively, it can be computed by extrapolation from a convergence-time versus ρ plot.

Uniform Sampling. An important question to address is whether the above algorithm samples the solution space uniformly, i.e., whether it is ergodic. Indeed, even if the solution space of this problem is flat and there aren't local minima or energy barriers to overcome, is still possible that the algorithm has some intrinsic bias that limits its evolution on a certain subspace. Unfortunately, there is no general way to test the ergodicity of an algorithm. We show here that sampling is achieved for a 3-dimensional problem (similar low-dimensional tests confirm these results).

We consider the system of $n = 3$ fluxes, with the sole constraint: $c = s_1 + s_2 - s_3 \geq 0$. The constraint corresponds to a metabolite produced by reactions 1 and 2 and consumed by reaction 3. Running the algorithm 100 times starting from different initial random fluxes, one finds that the maximum growth rate is $\rho = 0.999$ with $c = 0.000$. In Fig. S1, we show the 100 final solutions scaled by the factor $S = s_1^2 + s_2^2 + s_3^2$ to project the solutions on the unitary sphere. This solution space is clearly sampled uniformly.

1. De Martino A, Martelli M, Monasson R, Perez Castillo I (2007) Von Neumann's expanding model on random graphs. *J Stat* P05012.
2. Reed JL, Vo TD, Schilling CH, Palsson BØ (2003) An expanded genome-scale model of *Escherichia coli* K-12 (iJR904 GSM/GPR). *Genome Biol* 4:R 54.1–R54.12.

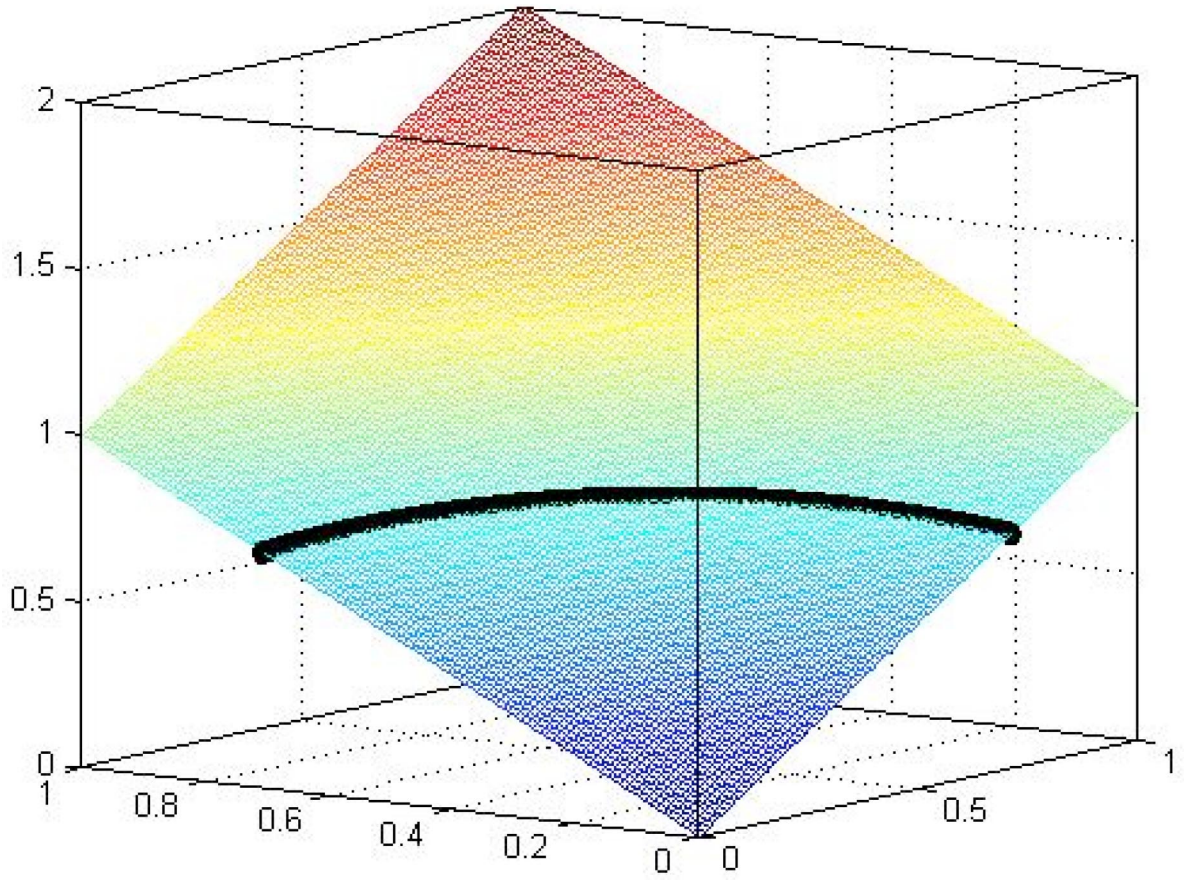

Fig. S1. Each point on the unitary sphere corresponds to a flux vector satisfying $s_1 + s_2 - s_3 \geq 0$.