**optGpSampler**: An Improved Tool for Uniformly Sampling the Solution-Space of Genome-Scale Metabolic Networks

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**Abstract**

Constraint-based models of metabolic networks are typically underdetermined, because they contain more reactions than metabolites. Therefore the solutions to this system do not consist of unique flux rates for each reaction, but rather a space of possible flux rates. By uniformly sampling this space, an estimated probability distribution for each reaction’s flux in the network can be obtained. However, sampling a high dimensional network is time-consuming. Furthermore, the constraints imposed on the network give rise to an irregularly shaped solution space. Therefore more tailored, efficient sampling methods are needed. We propose an efficient sampling algorithm (called optGpSampler), which implements the Artificial Centering Hit-and-Run algorithm in a different manner than the sampling algorithm implemented in the COBRA Toolbox for metabolic network analysis, here called gpSampler. Results of extensive experiments on different genome-scale metabolic networks show that optGpSampler is up to 40 times faster than gpSampler. Application of existing convergence diagnostics on small network reconstructions indicate that optGpSampler converges roughly ten times faster than gpSampler towards similar sampling distributions. For networks of higher dimension (i.e. containing more than 500 reactions), we observed significantly better convergence of optGpSampler and a large deviation between the samples generated by the two algorithms. **Availability**: optGpSampler for Matlab and Python is available for non-commercial use at: http://cs.ru.nl/~wmechgel/optGpSampler/.

**Introduction**

Modelling metabolic networks helps to unravel the complex machinery of metabolism within the cell. A classic approach is to model the reaction pathways in a dynamic fashion, using detailed kinetic data. For genome-scale models, often involving hundreds or thousands of reactions and metabolites, it is experimentally prohibitive to obtain the kinetic parameters involved. A constraint-based approach has successfully been applied to model and address a wide range of biological questions in the absence of detailed kinetic data [1,2]. By using a steady-state assumption, a first type of constraint dictates that all metabolite concentrations stay constant over time (mass-balance). A second type of constraint limits the flux rate for each reaction (flux-capacity and directionality). The relation between the \( m \) metabolites and \( n \) reactions is described in the \( m \times n \) stoichiometric matrix \( S \). A positive stoichiometric coefficient \( S_{ij} \) means that the metabolite \( i \) is produced by reaction \( j \) and a negative entry indicates that the metabolite is consumed in that reaction. At steady-state, the mass-balance and flux capacity constraints can be formulated as in eq. (1) and inequality (2) respectively.

\[
\frac{d\bar{x}}{dt} = S\bar{v} = 0, \tag{1}
\]

\[
v_{j,\text{min}} \leq v_j \leq v_{j,\text{max}}, \forall j \in \{1 \ldots n\}, \tag{2}
\]

where \( \bar{x} \) and \( \bar{v} \) are vectors of metabolite concentrations and flux rates respectively. Each flux rate \( v_j \) is bounded by inequality constraint (2). Although in some cases the bounds are known from experiments, for most reactions this is not the case and arbitrarily large values are used. Since the matrix \( S \) is a system of linear equations, the constraints in eq.mass-balance and inequality (2) form a bounded convex space [3], containing all possible values of \( \bar{v} \). A major challenge is to characterize the biologically interesting flux distributions among all alternatives. Since the stoichiometric matrix is fixed, the remaining possibility is to add additional constraints or tighten the inequality bounds in (2). This can be done by incorporating measured flux data, which is often laborious, expensive or difficult to obtain, even for a small subset of all reactions.
Many constraint-based methods have been proposed to find flux distributions that are of biological interest. A successful approach is Flux Balance Analysis (FBA) [4], that introduces a biologically relevant objective function and uses linear programming to find a flux distribution that optimizes this objective. FBA proved to be especially useful for cell types with a well-defined objective function, such as maximum growth for unicellular species. Although FBA has successfully been applied to determine possible phenotypes and byproduct secretion in various experimental settings, it is often unable to determine the underlying (internal) flux states [5]. Furthermore, for many objective functions, a wide range of alternative optima exists [6].

In order to obtain an estimated probability distribution of attainable flux values for each reaction in the network, methods based on uniform sampling are used. A fast algorithm for this task is called hit-and-run (HR) [7]. HR collects samples by iteratively choosing a random direction and a random step size in that direction such that the next point also resides in the solution space. For the irregularly shaped solution spaces of metabolic networks the Artificial Centering Hit-and-Run (ACHR) algorithm [8] is better suited, because it is tailored to sample in the elongated directions of the solution space. Partly based on ACHR, the uniform random sampling procedure known as gpSampler is often used to sample metabolic networks and implemented in the COBRA Toolbox [9]. Although these algorithms are often referred to as random uniform samplers, their convergence behaviour in the context of genome-scale metabolic networks has not yet been thoroughly investigated. Besides its irregular shape, the solution space of genome-scale metabolic networks is often high-dimensional, containing hundreds to almost a thousand dimensions as in the human metabolic network reconstruction.

Therefore in this paper we investigate uniform random sampling in the context of metabolic networks. Our contributions are threefold: (1) we introduce an efficient and effective random sampling algorithm which combines the advantages of ACHR and gpSampler; (2) we propose a new measure to quantify the deviation between samples obtained from two independent sampling runs; (3) we perform a thorough analysis on five metabolic network models.

**Methods**

**Uniform Random Sampling**

One of the first attempts to sample the flux states in a metabolic network used a rejection sampling technique [10]. In rejection sampling, the space of interest is enclosed by a regular shape, such as a parallelepiped. Samples are drawn from the uniform distribution over the parallelepiped and rejected if they violate the constraints for the enclosed space of interest. The nice feature of rejection sampling is that the samples are uniformly distributed over the enclosed space. However, fitting a regular shape tightly around the space of interest is hard and often impossible. This means that in higher dimensions, the volume of the enclosing shape grows explosively compared to the volume of the shape of interest [7]. In this case, a very large fraction of the samples have to be rejected, making rejection sampling an inefficient method for genome-scale models.

The hit-and-run (HR) algorithm [7] mitigates this problem, because it samples directly from the solution space (see Fig. 1). Hit-and-run starts from a point \( x_0 \) in the bounded space. It chooses an arbitrary direction \( \bar{u}_1 \) from the uniform distribution on the boundary \( \partial B \) of the unit sphere in \( \mathbb{R}^n \). The distance from \( x_0 \) to the boundary of the solution space in the direction of \( \bar{u}_1 \) determines the maximum distance it can travel. An arbitrary step size \( \lambda_1 \) is selected in the (negative) direction of \( \bar{u}_1 \), such that the sampler does not step out of the constrained space. The next point \( x_2 \) is determined by travelling distance \( \lambda_1 \) in the direction \( \bar{u}_1 \). By iterating this process, HR generates a chain of consecutive sample points. The fact that HR uses only the current point to obtain the next sample makes it a Markov Chain Monte Carlo (MCMC) method [11], which has been shown to converge towards the target distribution (uniform in our setting).

In general, the constraints in metabolic network models lead to a convex space of irregular shape which is elongated for reactions whose flux rates are loosely constrained, and is narrow for tightly constrained fluxes. A consequence of this phenomenon is that many sample points are close to the boundary of the solution space. Since HR chooses a direction \( \bar{u}_1 \) uniformly from all possible directions, this enforces the sampler to perform small steps, so the next generated point is close to the previous one. In practice this prevents the sampler to fully explore the rest of the solution space.

ACHR [8] alleviates the problem of getting trapped in regions close to the boundary because it tries to sample in the elongated directions, thus making larger steps possible. It iteratively generates samples by using an ‘artificial’ centre of the space, which is empirically estimated at each iteration using the points sampled so far. ACHR consists of two phases: warm-up and (main) sampling. In the warm-up phase an arbitrary initial point \( x_0 \) in the solution space is selected to generate a chain \( x_0, \ldots, x_W \) of \( W > n \) points. The requirement \( W > n \) ensures that after the warm-up phase, the set of directions spans \( \partial B \) with probability one [8]. Then the main sampling phase starts from \( x_W \). By iteratively updating the empirical centre and by using a direction from a randomly chosen previous sampled point to this centre, ACHR

![Figure 1. Illustration of hit-and-run](https://plosone.org/doi/10.1371/journal.pone.0086587.g001)
explores elongated directions of the space. Figure 2a illustrates the warm-up and the main sampling phase of ACHR.

The sequence of ACHR iterates is not a Markov chain, due to the dependence of directions on prior iterates. Thus the sequence of iterates is not guaranteed to converge to a uniform distribution [8].

ACHR is at the core of gpSampler [9], a popular sampling algorithm for metabolic network analysis. Figure 2b illustrates how gpSampler works. The highly irregular shape of the solution space of metabolic networks makes a uniform direction choice on \( L_B \) a poor choice. Therefore, in order to generate a number of \( T \) samples, gpSampler's warm-up phase uses linear programming in a two parts procedure. In part (1) \( 2n \) warm-up points are generated by consecutively minimizing and maximizing the flux rate of each reaction. In part (2) the remaining \( T - 2n \) warm-up points are generated by assigning random weights to the fluxes that should be optimized. The optimal solutions (and thus the warm-up points) for a linear program reside on the boundary of the constrained space [12]. Often, this causes the allowed step sizes to become very small, which makes it hard to move away from the boundary. Therefore, gpSampler uses a linear transformation to 'pull' the warm-up points more into the interior of the solution space. Then, each moved warm-up point is used in the main sampling phase to generate \( T \) separate chains of length \( k \). The user provided step count parameter \( k \) determines the number of ACHR iterates between the starting point \( \tilde{x}_W \) and the end point \( \tilde{x}_W + k \) of each chain. Finally, the \( T \) end points of the chains are returned as samples.

**optGpSampler**

We propose to combine a part of the warm-up phase of gpSampler and ACHR. The resulting algorithm is called optGpSampler (see Table 1. Algorithm 1). First, \( 2n \) warm-up points are generated...
generated using part (1) of gpSampler’s warm-up procedure by successively minimizing and maximizing the flux through each reaction. We do not generate $T$ warm-up points as in gpSampler because running a linear program on a large network is much more time-consuming than random sampling. Moreover, although the weight vector for the linear program is randomly chosen, the constraints in eq. (1) and inequality (2) often lead to the same or a similar optimal solution. This could bias the starting points and directions choice of our sampler.

The sampling phase of optGpSampler is similar to that of ACHR (see Fig. 2c), but only selects a sample at each $k$ iterates. Furthermore, instead of generating one chain of consecutive sample points, optGpSampler exploits $p$ processors and generates $p$ chains in parallel. In practice, the desired number of sample points $T$ is much larger than the number of available processors $p$. This makes the length of the chains generated by optGpSampler a factor of $T/p$ longer than those generated by gpSampler.

We implemented optGpSampler in C++ and used Armadillo [13], a fast linear algebra library. OpenMpi [14] was utilized to start a separate chain on $p$ processor cores in parallel. Interfaces for Matlab and Python enable users to easily sample existing models with optGpSampler or integrate our sampler in new methods.

### Experiments

#### Datasets

We benchmarked gpSampler and optGpSampler on five publicly available reconstructions of genome-scale metabolic networks [14]. All reactions and associated metabolites that could not carry a flux were removed from the models prior to the sampling. The network size remaining after this preprocessing step, i.e. the number of $m$ metabolites and $n$ reactions is given between brackets. In ascending size order, we used E. coli central metabolism (68, 87), C. thermocellum iSR432 (288, 351), S. cerevisiae iND750 (479, 631), E. coli iAF1260 (1032, 1532) and H. sapiens recon 1 (1587, 2469).

#### Evaluation

We assessed efficiency and quality performance of gpSampler and optGpSampler. Efficiency was measured using runtime. Quality performance in our context amounts to measure the capability of a sampler to effectively generate points uniformly distributed in the solution space. Since both gpSampler and optGpSampler have no theoretical convergence guarantees, we use two methods: empirical convergence diagnostics and a new method called xy-deviation.

### Empirical Convergence Diagnostics

Empirical convergence diagnostics are used to test whether the sampled distribution converges towards a stationary one. There is not always good agreement between different convergence diagnostic methods [16]. Therefore we use the following three convergence diagnostics tests: Gelman and Rubin [17], Geweke [18] and Heidelberger and Welch (HW) [19]. They are available in the Convergence Diagnosis and Output Analysis (CODA) Toolbox for MCMC [20].

The Gelman and Rubin test is multivariate: it returns a so called $R$ value (with $R \geq 1.0$). $R$ values smaller than 1.2 indicate convergence, with values closer to 1.0 indicating better results. The other two tests are univariate. The Geweke test returns a z-value for each reaction, with lower values indicating better convergence. The HW test returns whether a sample distribution for a given reaction converged (value = 1) or not (value = 0).

### Table 1. Algorithm 1

| Input: $S$: the solution space; $T$: sample count; $k$: step count; $p$: number of processors; |
| output: $P$: sequence of $T$ sampled points; |
| /* Warm-up phase */ |
| 1 Generate $2n$ warm-up points as in part (1) of gpSampler’s warm-up phase; |
| /* Sampling-phase */ |
| 2 $P = \langle \rangle$; |
| 3 $L = \langle Tk/p \rangle$; |
| 4 for $i = 1$ to $p$ do |
| 5 $x_0 = \langle \rangle$ a point randomly chosen from the $2n$ warm-up points; |
| 6 for $j = 1$ to $k$ do |
| 7 $x_0 = \langle \rangle$ point generated from $x_{i-1}$ by performing one iterate of the ACHR sampling phase; |
| 8 if $j \mod k = 0$ then |
| 9 $P = \langle P, x_0 \rangle$; |
| 10 end |
| 11 end |
| optGpSampler($S, T, k, p$). |

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xy-deviation

In general, a larger step count gives a sample distribution that is closer to the target distribution, at the expense of a longer runtime. Therefore, we introduce a measure called xy-deviation that quantifies how samples generated by sampler $x$ using a given step count $k_1$ deviate from those generated by sampler $y$ using a much larger step count $k_2$. Specifically, a small deviation indicates that the sample distribution generated by sampler $x$ converged empirically to the target distribution of $y$.

Given samplers $x$ and $y$, step counts $k_1$ and $k_2$, with $k_2$ very large ($k_2 = 5000$ in our experiments), the number $c$ of runs, and the number $T$ of samples, xy-deviation is computed as follows.

1. Perform $c$ runs of sampler $x$ with step count $k_1$, and $c$ runs of sampler $y$ with step count $k_2$, where each run generates $T$ samples.
2. Sort the points in each run (e.g., in increasing order), producing $c$ sorted chains of points for sampler $x$ and $y$: $\langle x_{b,j}^i \rangle$, $\langle y_{b,j}^i \rangle$, $b \in \{1, \ldots, T\}$, $j \in \{1, \ldots, n\}$, $i \in \{1, \ldots, c\}$.
3. For each reaction $j$, normalize the flux rates: we divide $x_{b,j}^i$ (resp. $y_{b,j}^i$) by the difference between the upper and lower bounds of $v_j$.

\[
X_{b,j}^i = \frac{x_{b,j}^i}{v_{j,\text{max}} - v_{j,\text{min}}}, \quad Y_{b,j}^i = \frac{y_{b,j}^i}{v_{j,\text{max}} - v_{j,\text{min}}}, \quad i \in \{1, \ldots, c\}, j \in \{1, \ldots, n\}, b \in \{1, \ldots, T\}.
\]

4. Compute the mean chain of the $c$ runs of $y$:

\[
Y_b^j = \frac{1}{c} \sum_{i=1}^c Y_{b,j}^i, \quad j \in \{1, \ldots, n\}, b \in \{1, \ldots, T\}.
\]

5. Our assumption is that chains generated by sampler $x$ that converge will have small deviation from the ’average-chain’ generated by $y$. For each reaction $j$, we measure such deviation by computing the mean absolute deviation over the chains generated by $x$ from $Y_b^j$:

\[
D_j = \frac{1}{c \times T} \sum_{i=1}^c \sum_{b=1}^T |X_{b,j}^i - Y_b^j|, \quad j \in \{1, \ldots, n\}.
\]

Our choice to use a mean deviation over the standard deviation is motivated by the fact that the mean deviation is more efficient than the standard deviation in the realistic situation where some of the measurements are in error, and more efficient for distributions other than perfect normal [21]. Note that the range of $D_j$ is $[0,1]$, and can be expressed as a percentage, which makes it convenient to compare deviations across different reactions.
6. Then the \( xy \)-deviation over \( c \) chains with respect to \( k_1 \) and \( k_2 \) is defined as the average of the reaction deviations:

\[
D = \frac{1}{n} \sum_{j=1}^{n} D_j
\]

Small values of \( xy \)-deviation indicate convergence of \( x \) to \( y \). In the experiments we analysed self-deviation (\( xx \)-deviation) and cross-deviation (\( xy \)-deviation, with \( x \) and \( y \) different samplers) for different values of \( k_1 \).

**Results**

We used the \( gPpSampler \) implementation in the COBRA Toolbox. Results of extensive experiments are given in supplementary material. Tables S1–S5 in File S1 provide results for all the runtime experiments performed. Figures S1–S6 in File S1 provide the results of the convergence diagnostic tests and \( xy \)-deviation. We visualized how a small or large \( xy \)-deviation translates to a similar or (highly) dissimilar sample distribution in figures S7–S11 in File S1.

**Efficiency**

We sampled all networks four times using \( T = 10, 50 \) and \( 100 \) thousand samples, with step count \( k = 50 \). Both \( gPpSampler \) and \( optGpSampler \) were executed in parallel mode on an AMD desktop computer using \( p = 4 \) processor cores in parallel. Efficiency results, summarized in Table 2, show that \( optGpSampler \) is roughly 6 to 40 times faster than \( gPpSampler \).

**Quality**

To assess the quality of the results, we performed four independent runs with each sampler. Each run collected \( T = 50,000 \) samples and was repeated for six different step counts \( k \in \{50,250,500,1000,2500,5000\} \).

We ran all convergence diagnostics with the default settings in the CODA package. The convergence tests results were averaged over the four runs. A univariate test for a sampler outputs a vector of length \( n \). Significance of the difference between \( gPpSampler \) and \( optGpSampler \) was assessed by applying the Wilcoxon signed-rank test to the corresponding vectors. The results for the Gelman and Rubin test indicated convergence for all experiments, in disagreement with results of the Geweke and HW tests.

The obtained chains were also used to compute the \( xy \)-deviation for the above given step count \( k_1 \), and \( k_2 = 5000 \). All
sample chains were compared by the average of the four chains obtained at the highest step count of \( k = 5000 \).

**Small networks (less than 500 reactions).** On the *E. coli central metabolism* network, the Geweke test returned relatively low z-values for both *gsSampler* and *optGpSampler* (see figure S1 in File S1). Results of the HW test indicated that *optGpSampler* converged rapidly, at \( k = 50 \) step count. Both the Geweke and HW tests showed that *gsSampler* needs a step count close to 500 to converge. Results of xy-self-deviation demonstrate a large deviation of samples generated by *gsSampler* with a small step count from those generated with a higher step count. In general, results showed that *optGpSampler* with a low step count generates samples that are both close to those generated using a higher step count with the same sampler and to those generated by *gsSampler* with a higher step count.

On the *C. thermocellum* IR432 network, the HW test showed significantly higher convergence rates for *optGpSampler* (see Fig. 3). Both the Geweke and HW tests showed that *optGpSampler* converged at step counts bigger than 1000. The situation for *gsSampler* is different: the values of the HW test dropped at step count 250 and then increased significantly when the step count reached 5000. This indicates two distinct points of convergence, one at small step values and one at large values. This behaviour can be explained by the way *gsSampler* collects its samples. It starts at a warm-up point and uses \( k \) iterates of the ACHR algorithm to obtain a sample point. It repeats this process, each time starting from a different warm-up point (see Fig. 2b). These warm-up points turn out to be close to each other, as a consequence of the linear programming procedure used. Thus *gsSampler* often starts the sampling from the same area of the solutions space. In higher dimensions, a small step count can prevent *gsSampler* to ‘travel’ far from the warm-up points. In this case the small step count causes *gsSampler* to show a bias towards the regions close to the warm-up points and the sampling chain converges towards these regions. The large xy-deviation (see Figure 4) shows that the samples collected are different from those sampled at a higher step count.

The results for *gsSampler* at large step count (\( k = 5000 \) steps) again indicates convergence. In this case the small xy-deviation indicates that an extended region is covered by the samples. Since *optGpSampler* does not restart at a warm-up point, it is better able to ‘escape’ from the regions near the warm-up points because it effectively uses much longer chains. Therefore, its convergence and xy-deviation results are better.

**Large networks (at least 500 reactions).** The convergence and results for the *S. cerevisiae* ND7350 (see figure S3 in File S1) and especially the *E. coli* iAF1260 network in Fig. 5 showed an even more surprising result. For these larger models, the convergence results for *gsSampler* declines when the step count is increased. For *optGpSampler*, we still observed an increasing convergence performance for the yeast network, although a much larger step count (\( k = 2500 \)) was required. The more stable results for the larger *E. coli* iAF1260 network could be an effect of the minimum glucose setting of this network, which significantly reduces the attainable flux states. The xy-deviation results in Fig. 6 indicate that the samplers give completely different sampling results. The self-deviation shown in Fig. 6, reveals a small variability within the four independent runs for each sampler. This means that both samplers give relatively stable results, and thus that the deviation results observed between the samplers must be due to the difference between the algorithms.

Finally, the results for the human network reconstruction (see figures S5 and S6 in File S1) indicate that *gsSampler* converges already at low \( k \). Although we believe that the sample distributions indeed converged in this case, it seems unlikely that they represent a uniformly distributed sample. First, the high dimensionality of almost a thousand and the declining results for the Geweke test make this unlikely. Next, the huge deviation with samples obtained by *optGpSampler* indicate a non-uniform distribution, especially since we saw that *optGpSampler* performs better on the smaller networks. The convergence results for *optGpSampler* seem more realistic, with a relatively large z-value and a HW test result that indicates that around 60% of the sampled flux distributions converged.

**Discussion**

We proposed a new algorithm for uniform sampling of the steady-state solution space of metabolic networks. Our algorithm also implements ACHR, but in a different manner than the state-of-the-art sampling method for metabolic networks (*gsSampler*). We compared the runtimes with those of *gsSampler*, and showed its superior efficiency. We investigated empirical convergence using different diagnostics and showed faster convergence of *optGpSampler* on the two smaller networks studied. Moreover, by using the here introduced xy-deviation measure, we compared the sampled distributions. For smaller networks, the samples obtained by *optGpSampler* using a small step count are close to those obtained with a high step count by both *optGpSampler* and *gsSampler*.

On three large networks the convergence performance of *gsSampler* diminishes when the step count increases. We hypothesized that the approach *gsSampler* takes by starting each sample chain at a warm-up point, together with the high dimensionality of the solution space, restrains its ability to move from the vicinity of these warm-up points. Because our method continues the ACHR procedure from the last collected point, it effectively uses much larger step counts. Therefore, *optGpSampler* is more likely to escape the regions near the warm-up points, leading to a better sampling result.

For the larger networks, results showed that the convergence observed at lower step counts does not reflect a convergence towards the target distribution, since the sample distributions deviate significantly from those generated using a large step count. Therefore, especially larger networks should be sampled with a high step count.

To the best of our knowledge there is no method to assess whether samples are truly uniformly random distributed in a convex space of unknown shape. Since the chains generated by ACHR are not Markov chains, asymptotic convergence guarantees also do not hold for both *gsSampler* and *optGpSampler*. Therefore convergence results should be interpreted with caution. The accelerated convergence of ACHR towards a uniform distribution was demonstrated by [8] for convex polytopes of known shape. However it remains uncertain to what extent the samples obtained by ACHR for the irregular solution space of metabolic networks are truly uniformly distributed. As expected, our experiments indicate that convergence results deteriorate when the dimensionality of the solution space increases, and that for the large genome-scale metabolic networks, using a large step count is advisable.

We envisage the provided implementation of *optGpSampler* will be beneficial to constraint-based metabolic network analysis, as it provides an efficient and versatile algorithm for sampling the irregular solution space of metabolic networks.

**Supporting Information**

File S1  Runtimes, convergence results and xy-deviation for the other metabolic models considered. (PDF)
Acknowledgments

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Author Contributions

Conceived and designed the experiments: WM EM. Performed the experiments: WM. Analyzed the data: WM EM. Wrote the paper: WM MH EM.

References