

Eric Kernfeld

Summary of Wilkinson’s “Parameter inference for stochastic kinetic models of bacterial gene regulation”, a book chapter in [1].

### Abstract

In this paper, Wilkinson attempts to infer reaction rates for biochemical networks in a setting with discrete observations, missing data, and measurement error. He uses vague priors and likelihood-free MCMC methods within a Bayesian model. He runs four main simulations. The first three iterate through successively more difficult and realistic measurement models, and they show the approach can accurately infer three key reaction rates with a useful precision. The fourth studies a naive model, showing it leads to overconfident, incorrect inferences. All tests are conducted using synthetic data on only one vector of true parameters.

I plan to implement the method in Julia and reproduce the experiments. To honestly test the method, I need to do more simulations, too: what happens when the log-space mean of the prior is not near the true values, or when the true values are somewhere other than Wilkinson’s choice? What happens when all the parameters are unknown, rather than just the three we are interested in?

## 1 Introduction

### 1.1 Summary

This paper develops tools to study bacterial behavior, which is sometimes random: three bacilli in similar environments may act differently. It investigates a possible mechanism for this randomness: fluctuations in biochemical systems that regulate cell metabolism. In this paper and others, these systems are modeled using continuous-time Markov jump processes. This allows for rigorous treatment of stochastic dynamics. The subject of Wilkinson’s paper is parameter inference for these jump processes, which is difficult because in practice the data are partial, discrete-time, and noisy. His paper addresses this problem via Bayesian inference and MCMC.

### 1.2 Bare-Bones Biology

As a motivating case, Wilkinson uses the “decision” of *Bacillus subtilis* whether to become mobile. The paper centers around a gene encoding *flagellin*, which is a protein component of organelles that allow motility. Because biological networks can be disorienting, I’ll outline some of the biological relationships in short sentences in the next paragraph.

The protein *flagellin* helps bacteria move. The protein  $\sigma^D$  promotes *flagellin*. The *fla / che* operon<sup>1</sup> contains many motility-related genes, including the one for  $\sigma^D$ . The protein  $\sigma^A$  and the protein *CodY* both suppress the *fla / che* operon. Thus, they suppress  $\sigma^D$ , and they indirectly suppress *flagellin*. In fact, *CodY* also downregulates *flagellin* directly. This is easiest to digest as a figure.

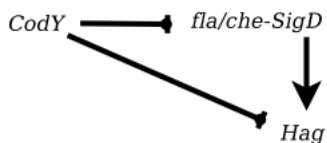


Figure 1: Regulatory relationships. *Hag* is the gene for *flagellin*, while *SigD* encodes  $\sigma^D$ .

The paper focuses on measurements of  $\sigma^D$ . It attempts to infer the rates of binding of *fla/che* repressors, unbinding of *fla/che* repressors, and production of  $\sigma^D$ .

<sup>1</sup>Proteins are complex molecules that take myriad forms and roles within a cell. Operons are the basic transistor-like elements of the genome. In response to an outside stimulus, such as high levels of the sugar lactose, a normally active operon may become inactive or vice versa. By “active”, I mean that the DNA encoded by the operon can be transcribed into RNA; this is the first step in the production of proteins.

## 2 The Model

Since we're simulating a biochemical system, suppose there are  $X_j(t)$  particles of type  $j$  at time  $t$ ,  $j \in \{1 \dots u\}$ . These particles interact via a set of reactions  $\mathcal{R}_i$ ,  $i \in \{1 \dots v\}$ , with  $\mathcal{R}_i$  consuming  $p_{ij}$  particles of type  $j$  and producing  $q_{ij}$  particles of type  $j$ . Let  $R_i(t)$  denote the number of reactions of type  $i$  in  $[0, t]$ . I'll refer to these using the arrays  $X(t)$ ,  $P$ ,  $Q$  and  $R(t)$ . Defining the matrix  $S$  to be  $Q^T - P^T$ , the model says that  $X(t) - X(0) = SR(t)$ . Under some assumptions **EMILY** maybe I should look into these, the different reaction channels evolve independently, and  $R_i(t)$  is a Poisson process with intensity  $c_i \int_0^t \prod_{j=1}^u \binom{X_j(t)}{p_{ij}}$ . The  $c_i$ 's are unknown.

Suppose  $n$  reactions occur between time 0 and time  $T$ . Suppose the  $i$ th one occurs at time  $t_i$  and suppose it has type  $\nu_i$ . Define  $t_0 \equiv 0$  and  $t_{n+1} \equiv T$ . When it comes to inference, the likelihood follows a competing-hazards model from continuous Markov chain theory<sup>2</sup>.

$$P(\nu, t|c) = \prod_{i=1}^n c_{\nu_i} \prod_{j=1}^u \binom{X_j(t_{i-1})}{p_{\nu_i j}} \exp \left( -c_{\nu_i} \int_0^T \binom{X_j(t)}{p_{\nu_i j}} dt \right)$$

Wilkinson points out that  $\int_0^T \binom{X_j(t_{i-1})}{p_{\nu_i j}} dt$  is tractable; in fact, I derived it as  $\sum_{i=0}^n (t_{i+1} - t_i) \binom{X_j(t_i)}{p_{\nu_i j}}$ . If the number of reactions of type  $k$  is  $r_k$ , then the likelihood becomes

$$P(\nu, t|c) = \prod_{k=1}^u \left\{ c_k^{r_k} \left\{ \prod_{j=1}^u \binom{X_j(t_{i-1})}{p_{kj}} \right\}^{r_k} \exp \left( -c_k \int_0^T \sum_j \binom{X_j(t)}{p_{kj}} dt \right) \right\}.$$

The gamma distribution is conjugate for  $c_k$ , and setting independent priors  $c_k \sim \Gamma(a_k, b_k)$ , the posteriors are independent with  $c_k \sim \Gamma(a_k + r_k, b_k + \int_0^T \sum_j \binom{X_j(t)}{p_{kj}} dt)$ . This is an ideal scenario; missing data make inference more challenging.

## 3 Inference

In Wilkinson's data, not every reaction is recorded. Measurements are intermittent, with error, and only  $\sigma^D$  or a fluorescent reporter gets measured. Conjugate priors cannot be used because computing the likelihood is infeasible. To adapt, the paper modifies methods from [4], so to make the mathematics easier to digest, I'll introduce first the predecessor and only then Wilkinson's method.

Let  $\theta$  include  $c$ , controlling the reaction rates, and  $\tau$ , controlling the scale of measurement error. Let  $x$  denote the true state of the chain, but measured only at discrete times. The likelihood  $P(x|\theta)$  is thought to be intractable. Let  $\mathcal{D}$  be  $x$  measured with error and possibly with missing data for some particle types. If we want to construct a Metropolis-Hastings scheme to sample from  $P(x, \theta|\mathcal{D}) \propto P(\theta)P(x|\theta)P(\mathcal{D}|x, \theta)$  using a proposal  $f(\theta^*, x^*|\theta, x)$ , it works out that the acceptance ratio must be the min of 1 and

$$\frac{f(\theta^*, x^*|\theta, x)}{f(\theta, x|\theta^*, x^*)} \times \frac{P(\theta)}{P(\theta^*)} \times \frac{P(x|\theta)}{P(x^*|\theta^*)} \times \frac{P(\mathcal{D}|x, \theta)}{P(\mathcal{D}|x^*, \theta^*)}.$$

This genre of algorithms builds upon the fact that exact simulations from this model are possible, and also that measurement error helps soften the requirements on where the bridge process should begin and end. The insight from [4] is that one can cancel the intractable term  $\frac{P(x|\theta)}{P(x^*|\theta^*)}$  by constructing a proposal that contains  $\frac{P(x^*|\theta^*)}{P(x|\theta)}$  as a factor. This factor arises if instead of drawing both  $x$  and  $\theta$  from simple out-of-the-box proposals, we draw  $\theta^* \sim f(\theta^*|\theta)$  and compute  $x^*$  via simulation with parameters  $\theta^*$ . In the end result, the

<sup>2</sup>At times, I choose to use equations that stand free of sentences. These I do not punctuate.

ratio of interest simplifies:

$$\begin{aligned}
& \frac{f(\theta^*, x^* | \theta, x)}{f(\theta, x | \theta^*, x^*)} \times \frac{P(x | \theta)}{P(x^* | \theta^*)} \times \frac{P(\theta)}{P(\theta^*)} \times \frac{P(\mathcal{D} | x, \theta)}{P(\mathcal{D} | x^*, \theta^*)} \\
&= \frac{f(\theta^* | \theta)}{f(\theta | \theta^*)} \times \frac{P(x^* | \theta^*)}{P(x | \theta)} \times \frac{P(x | \theta)}{P(x^* | \theta^*)} \times \frac{P(\theta)}{P(\theta^*)} \times \frac{P(\mathcal{D} | x, \theta)}{P(\mathcal{D} | x^*, \theta^*)} \\
&= \frac{f(\theta^* | \theta)}{f(\theta | \theta^*)} \times \frac{P(\theta)}{P(\theta^*)} \times \frac{P(\mathcal{D} | x, \theta)}{P(\mathcal{D} | x^*, \theta^*)}.
\end{aligned}$$

To simplify further, use an independence sampler with the prior as a proposal, and the calculation reduces to  $\frac{P(\mathcal{D} | x, \theta)}{P(\mathcal{D} | x^*, \theta^*)}$ .

If the measurement error is small, or  $\mathcal{D}$  is high-dimensional, or both, this scheme leads to very high rejection rates, so it is not usable. Instead, you can break down  $X$  and  $\mathcal{D}$ , adding only one data point at a time. The exact approach is best understood by referring to Algorithm 1. In broad strokes, the procedure alternates between generating large samples from  $P(\theta, x_{t_{1:i}} | \mathcal{D}_{t_{1:i}})$ , done via likelihood-free MCMC, and folding in new data (incrementing  $i$ ). To be clear, instead of running MCMC just once, in this method runs another five million steps through the sampler *for every time point in the dataset*. It isn't as bad as it sounds: for a sampler that runs only once, the chain will not mix without a sophisticated proposal, and constructing a usable proposal is often linear in the size of the data anyway.

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**Algorithm 1:** Wilkinson's sequence of MCMC Samplers

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Given a hidden continuous-time Markov process  $\{x_t\}_{t=0}^T$  with:

Unknown parameters  $\theta$

Known initial state  $x_0$

Data points  $\mathcal{D}_{t_i}$  at times  $t_i, i \in \{1, \dots, I\}$

A simple, tractable error model  $P(\mathcal{D}_{t_i} | x_{t_i}, \theta)$

A simulator for paths of  $x$  given  $\theta$  the process

A big array  $B_0$  (Wilkinson uses length 1,000,000) of samples from a prior on  $\theta, x_0$

Empty arrays  $B_i$  of the same length

For each time point (for  $i \in \{1, \dots, I\}$ ), fill  $B_i$  with samples from this Metropolis Hastings scheme:

Initialize  $(\theta, x_{t_i})$

Until  $B_i$  is full:

Draw  $(\theta^*, x_{t_{i-1}}^*)$  from  $B_{i-1}$  or a KDE of its contents (note  $t_0 \equiv 0$ )

Using  $(\theta^*, x_{t_{i-1}}^*)$ , simulate up to  $x_{t_i}^*$ , the state at time  $t_i$

Set  $A = \min(1, \frac{P(\mathcal{D}_{t_i} | x_{t_i}^*, \theta^*)}{P(\mathcal{D}_{t_i} | x_{t_i}, \theta)})$



With probability  $A$ , overwrite  $(\theta, x_{t_i})$  with  $(\theta^*, x_{t_i}^*)$

If the number of times through this loop exceeds 1000 ( for the burn-in) and equals one modulo five (for the thinning), add  $(\theta, x_{t_i})$  to  $B_i$

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In any M-H scheme, the acceptance probability is either 1 or the proposal ratio times the posterior ratio. For step  $i$ , the proposal ratio  $\times$  posterior ratio can be expanded as follows:

$$\frac{P(x_{t_i}^* | x_{t_{i-1}}^*, \theta^*, \mathcal{D}_{t_{i-1}})}{P(x_{t_i} | x_{t_{i-1}}, \theta, \mathcal{D}_{t_{i-1}})} \times \frac{P(x_{t_{i-1}}^*, \theta^* | \mathcal{D}_{t_{i-1}})}{P(x_{t_{i-1}}, \theta | \mathcal{D}_{t_{i-1}})} \times \frac{P(x_{t_{i-1}}^*, \theta^* | \mathcal{D}_{t_{i-1}})}{P(x_{t_{i-1}}, \theta | \mathcal{D}_{t_{i-1}})} \times \frac{P(\mathcal{D}_{t_i} | x_{t_i}, \theta)}{P(\mathcal{D}_{t_i} | x_{t_i}^*, \theta^*)}.$$

 This shows how the compact form in Algorithm 1 arises. The Markov model would allow us to include or omit red terms at  $x_{t_{i-1}}^*$ .  Note that using a KDE does not affect the ratio, because usually the kernel  $K(\cdot | \cdot)$  is symmetric, so the extra term  $\frac{K(\theta^* | \theta)}{K(\theta | \theta^*)}$  is just 1.

## 4 Alternative Methods

- Faster alternatives, not exact; these approximate the process using a continuous-state diffusion process satisfying a certain SDE [5] or a linear approximation to the solution of the SDE [6].
- An alternative method, exact and billed as “scalable.” This paper uses MCMC, but with a parallelized approximation algorithm to help generate good proposals [7]. The tandem discussion of likelihood free MCMC and approximate Bayesian computation is similar to [8]. This paper [9] also centers on improving MCMC proposals (it has a particularly nice set of references in the introduction). There is yet more related work embedding approximations inside of MCMC samplers [10, 11].

## 5 Experiments, Extensions and Further Checks

### 5.1 Experiments

Wilkinson runs four main simulations. All tests are conducted using synthetic data on only one vector of true parameters. The first three iterate through successively more difficult and realistic measurement models:

- First experiment: observe  $\sigma^D$  directly
- Second experiment: observe  $Hag$  rather than  $\sigma^D$
- Third experiment: observe only

The fourth shows that a naive model, assuming the fluorescent reporter protein is proportional to the protein of interest, leads to strong and incorrect claims in the posterior probabilities.

#### 5.1.1 Details needed to reproduce experiments

Wilkinson describes a twelve-reaction regulatory model for these chemicals, spelling it out in table 1, and he lists three scientifically important reaction rates that, for tests of the inference method, will be treated as a “ground truth.” The prior distributions cover 4 orders of magnitude, and they are uniform on a log scale. The experiment assumes  $D_t$  is the number of molecules observed with Gaussian error of standard deviation 10 molecules. The initial state of the cell is assumed known **EMK: but does he specify it?**, and observations occur every 5 minutes (300 seconds) for 2 hours (7200 seconds).

### 5.2 Further checks

Some authors [12] claim that reaction rates range over seven orders of magnitude. Wilkinson’s prior covers only four orders of magnitude. What happens when the log mean of the prior is not near the true values? In general, where does the prior information come from?

Wilkinson also treats parameters as known except for the three parameters of interest. How well does this method work when the rest of the reaction rates must be inferred, or when the measurement error must be inferred?

### 5.3 Extensions

Wilkinson mentions many areas for future work in terms of biological applications for this method. In terms of new methodology, he mentions that in order to process data on batches of cells, further work is needed on scalable methods, and to integrate data from microarrays or RNA-seq, the model would need to be extended.

I am no expert on dynamical systems, but I would add that in isolation, parameter inference does not seem to contribute much to our understanding of these systems. A modeling endeavor of this sort should culminate in successful predictions; what will Wilkinson predict here? Systems with multiple stable modes corresponding to bacterial behaviors? Previously unknown biochemical interactions? Fruitful sites to target antibiotics that disrupt bacterial metabolism? **EMK: Update: another of Wilkinson’s papers says**

that parameter inference is essential to larger modeling projects [5]. There is a discussion of this reverse engineering in [13].

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