# Noise Analysis of Confined Cell-free Protein Production

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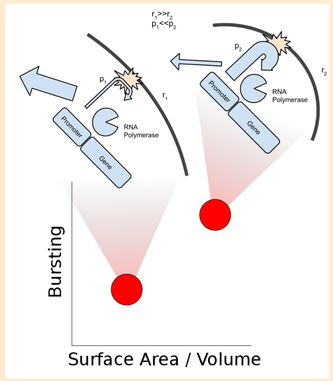
## Introduction

Biological noise from episodic gene expression (bursting) has been observed in all kingdoms of life. The source of bursting is not known, but many sources have been proposed including confinement, crowding, gene looping, chromatin structure, and cellular life cycle[1]. In this paper, we use a cell free synthetic environment to isolate confinement from other potential sources of bursting (Figure 1). Previously, Karig et al. isolated and observed the intrinsic noise of gene circuits in cell-free environments[2]. In these experiments, EGFP was produced by cell-free protein synthesis (CFPS) with S30 E. coli extract inside confined femtoliter PDMS buckets. Here we follow a similar approach, but we look to find spatial correlations[3] that could lead to gene expression bursting. Fluorescence over time for each bucket was recorded during active transcription and translation, and the noise in these processes was isolated and characterized. This measured noise showed non-bursty (Poissonian) expression over the low expression regime, but we have found evidence that higher levels of expression are achieved through bursty processes that are heavily influenced by the degree of confinement.

Figure 1: We hypothesize that as the surface area to volume ratio increases (buckets get smaller) bursting in cell free protein expression will increase. This is possibly due to limited diffusion of translation machinery as wall radii of curvature decrease, and likely leads to an echo effect.



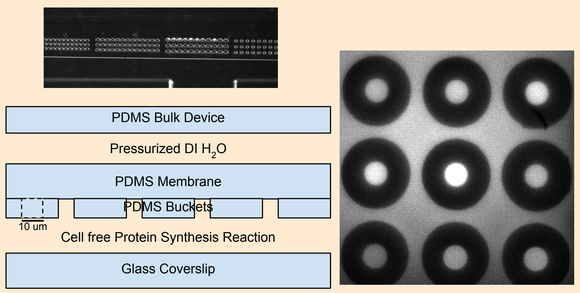
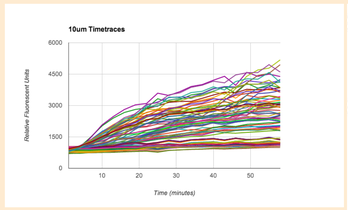




Figure 2: The bucket devices are made of layered Polydimethylsiloxane (PDMS). They include two chambers: one for the cell free reaction, and one for pressurized DI water. The cell free reaction is flowed into the bottom channel between the glass coverslip and the PDMS membrane. Then the water is pressurized to 20 psi, isolating the cell free reaction in the buckets. Right is a fluorescent, grayscale image of the buckets expressing Enhanced Green Fluorescent Protein (eGFP). Top is a brightfield image of the device layout.

## Methods

Cell free reactions (Promega S30 Protein Expression System) were isolated in PDMS cylinders (buckets) with a constant height of ~10 μm and diameters of 10, 5, and 2 μm. The fluorescence of each bucket was measured for one hour. The fluorescence of each bucket is averaged over the entire bucket for each timepoint, producing a time trace for each bucket over the course of the experiment. The time traces include deterministic and stochastic components. The stochastic component is isolated by subtracting the average curve from each trace.



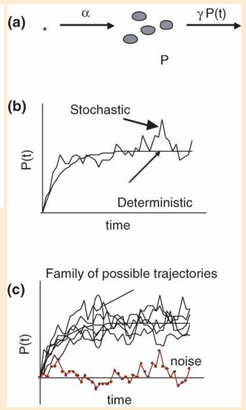




Figure 3: Above are one hour time traces of individual 10 μm buckets from multiple days. Each time trace has a deterministic and stochastic component (as shown to the right in panel b[4]). The deterministic component for bucket expression is be calculated from the average of all buckets of the same day (as shown to the right in panel c[4]). The deterministic component is subtracted from the time trace to isolate the stochastic component.

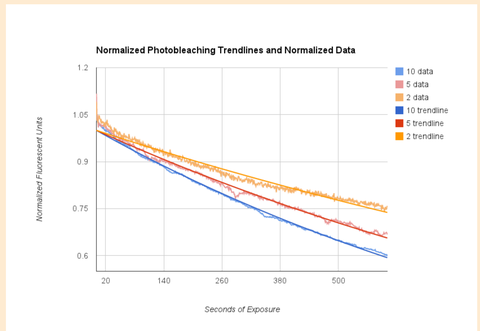



Figure 4: Photobleaching has only a slight effect on fluorescent intensities during experiments. Maximum of 400 seconds of exposure during experiments.

| | 10 um | 5 um | 2 um |
|---|---|---|---|
| Exponential Time Constants | 1196.25 | 1424.61 | 1973.25 |
| Half-life (seconds of exposure) | 829.18 | 987.47 | 1367.76 |

## Results

We measured expression noise ($CV^2$=variance in expression/mean expression level squared) in 137 different buckets with diameters of 2, 5, and 10 μm and equal heights of about 10 μm. We observe the transient for EGFP expression in each bucket is around one hour. In a Poissonian system, the mean and variance of the noise are equal. This leads to $CV^2 \propto 1/\text{protein abundance}$, where noise decreases as expression level increases. In the CFPS system, the burst size, number of protein produced from each mRNA transcript, factors into the noise as well. $CV^2 \propto \text{burst size/protein abundance}$. For all bucket sizes at the low end of expression, we observed the noise decreasing as expression level increased, as would be expected for Poissonian expression. However, the magnitude of the noise in this regime was smaller in the more confined buckets (Fig. 5), indicating that translational efficiency is affected by confinement. Conversely at higher expression levels, there were strong deviations from Poissonian expression that we believe are caused by increased level of transcriptional bursting. These deviations from Poissonian behavior were found to be affected by bucket size (Fig. 5), and we hypothesize that greater levels of confinement lead to more pronounced expression burstiness.

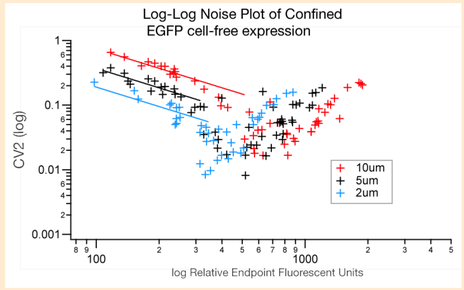



Figure 5: Log-log noise plot of confined protein expression shows three distinct lines of constant burst size (number of protein molecules per mRNA transcript) at least for lower abundances. Larger buckets show a larger burst size. At higher abundances, transcriptional bursting increases the noise of expression above a pure Poissonian process.

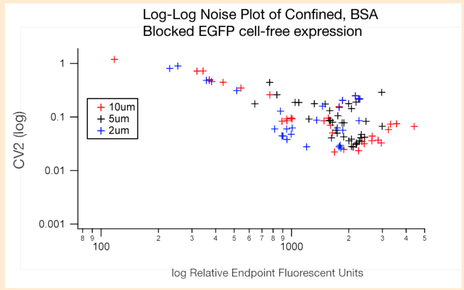
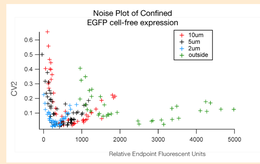



Figure 6: The log-log noise plot of BSA blocked protein expression shows an increase in the noise level at higher abundances. The walls of the devices in these experiments are 'blocked' with Bovine Serum Albumin (BSA) to inhibit protein adsorption (and possible protein inactivation). These results indicate a shift to isoquants of a higher burst size and an increase in expression.

## Discussion

Our results indicate decreased translational efficiency and increased transcriptional burst rates in buckets with greater spatial confinement. Decreased translational bursting is indicated by the horizontal shifts of the curves to lower abundances with constant noise levels. Increased transcriptional bursting is indicated by the increase of noise at higher abundance levels. This effect is seen in Figure 7. The confinement data is on the left and a pure Poissonian process is on the right. At higher abundance levels a Poissonian process has decreased noise. The confined reactions show increased noise at higher abundances, indicating increased transcriptional bursting. Confinement likely leads to spatial correlations between transcriptional machinery and the gene promoter, leading to repeated reinitiation of transcription. This hypothesis is being validated against computer models of the confined CFPS reaction.



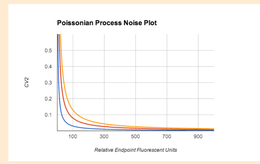




Figure 7: Comparison between noise data from the confined cell free reactions (left) and a pure Poissonian process (right). In a Poissonian process, standard deviation equals the mean ($\sigma^2=\mu$) and $CV^2$ is the inverse of the mean ($CV^2=1/\mu$). In a bursty process, $CV^2$ is proportional to the burst rate, b, also, so $CV^2 \propto b/\mu$. Note the confined data appears Poissonian at lower abundances (with varying burst rates as shown by the isoquant lines), but at higher abundances the confined data deviates from the Poissonian process and noise increases.

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## References

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