Introduction:
Biosensors exploiting communication within genetically engineered bacteria are becoming increasingly important for monitoring environmental changes. Currently, there are a variety of mathematical models for understanding and predicting how genetically engineered bacteria respond to molecular stimuli in these environments, but as sensors have miniaturized towards microfluidics and are subjected to complex time-varying inputs, the shortcomings of these models have become apparent. The effects of microfluidic environments such as low oxygen concentration, increased biofilm encapsulation, and diffusion limited molecular distribution strongly affect rate constants for gene expression not accounted for in previous models.

Materials and Methods:
Standard microbiological techniques were used for culturing *E. coli. E. coli* strain DH5α was used for all cloning. Reporter bacteria were derived from the fully sequenced K-12 strain MG1655 [1]. A microfluidic device made with polydimethylsiloxane (PDMS) was used to house bacteria while providing fresh nutrients and autoinducer N-acyl homoserine lactone (AHL). A range of AHL concentrations was used to produce fluorescent responses (0-30 μM). Fluorescent images were captured once every 10 min and post-processed using MATLAB. The intensity of the pixels within the bacteria chamber was averaged and the background fluorescence was subtracted, yielding relative fluorescence (arbitrary units).

Results and Discussion:
We report a mathematical model that accurately predicts the biological response of the AHL - mediated green fluorescent protein (GFP) expression in reporter bacteria in microfluidic environments by accommodating rate constants. This generalized mass action model considers a chain of biomolecular events from input autoinducer chemical to fluorescent protein expression through a series of six chemical species. We have validated this model against experimental data from our own apparatus as well as prior published experimental results. We measured an average absolute difference between the modeled and measured peak times as 17.1 min, or 9% error. To our knowledge, no other reported model for genetically engineered bacteria has the capability of predicting response peak time for time-varying stimuli. This feature of our model is important for applications in biosensing because the delay between stimulus and peak critically informs stimulus onset time.

Conclusions:
The model accurately captures the response peak time, with 9% error, and somewhat captures the response delay, ramp-up time, response duration, and ramp-down time for time-varying inputs across a range of input concentrations. The model is a substantial improvement, as measured by mean squared error, over prevously reported models for time-constant inputs in microfluidic environments. This model is versatile: applicable to microfluidic or traditional macro-environments; it can be used with time varying (pulse) or constant (step) inputs. This model can serve as a valuable tool in understanding genetically engineered bacteria and improving biosensor design capabilities, opening the door for sensors that adapt to environmental dynamics and communicate with each other.

Reference:
1. Blattner, F.R., et al., *The complete genome sequence of Escherichia coli K-12.* Science, 1997. **277**(5331): p. 1453-&.