## ABSTRACT

In the developing area of synthetic biology, researchers engineer biomolecular circuits within living cells to manipulate and control cellular behaviour for a variety of applications, such as energy production, environmental management, and medical applications. Feedback control strategies, rely on sensors, and efficient estimation strategies are employed to achieve the desired control in biomolecular systems.

RNA thermometers, which are temperature sensors, generate temperature responses with specific thresholds and sensitivities. Researchers have extensively studied the structure and sequences of RNA thermometers, which are mRNAs whose translation rate varies with temperature. The gradual melting of the ribosome-binding site (RBS) at increased temperatures is a key part of this thermosensing process, but the parameters on which the melting of RBS depends is generally unclear. Designing an RNA thermometer that exhibits a precise response, including a specific peak sensitivity and threshold temperature, poses a considerable challenge. We investigated the tradeoffs present in the input-output response of RNA thermometers using mathematical models such as the two-state model and its variants. We found that peak sensitivity and threshold temperature show tradeoffs. To further validate the consistency of the above-mentioned tradeoffs in more realistic models, we used NUPACK, a web-based tool designed for the thermodynamic analysis of nucleic acids. By calculating the melt profiles of synthetic RNA thermometers through this thermodynamics-based server, we found that the results were consistent with the stated constraints. To check for the above trends in the experimental measurements of RNA thermometers, we designed two libraries on either side of the ribosome binding site and experimented with different temperatures. We found that thermometer activities change with temperature and are different for all thermometers. We also found that peak sensitivity and threshold temperature persist in experimental measurements as well.

RNA thermometers have been used as sensors in multiple contexts for gene regulation. However, they can be subject to noise, inaccuracies, or limitations. Like Kalman filters or observers, estimation techniques are required to estimate the true system states.

Estimation plays a crucial role in understanding and analyzing biomolecular systems, as these systems are often complex and difficult to study directly due to their dynamic and intricate interaction. Stochastic differential equation-based models have been used in biomolecular contexts that can have a state-dependent process noise covariance. The choice of the process noise covariance is an important parameter in the design of a Kalman Filter for state estimation, and the theoretical guarantees of updating the process noise covariance as the state estimate changes are unclear. We investigated this issue using the best linear unbiased estimator (BLUE), and Newton's method by minimizing a weighted least squares cost function for the systems where process noise depends on the state. We found that the BLUE-based algorithm was an optimal linear filter for linear system dynamics when the process noise depends on the linear/affine function of state in a square-root-like fashion. We generalized these results by minimizing a positive definite quadratic

function using Newton's method, which removes the restriction on process noise dependent on the state being a linear/affine function of the state. We generalized these results to nonlinear systems with arbitrary process noise dependence, showing that this algorithm minimizes a quadratic approximation to a least-squares cost weighted by the noise covariance. We considered one example to illustrate these results. This study could aid in the manipulation of biomolecular systems, contribute to the development of ideas, and pave the way for future research in synthetic biology and related fields.

Keywords: Biomolecular Systems, RNA Thermometers, Sensitivity, Threshold Temperature, Best Linear Unbiased Estimator, Newton's method, Chemical Langevin Equation (CLE), Mean-Square Error (MSE).