Stochastic model of siRNA endosomal escape mediated by fusogenic peptides using Gillespie Algorithm

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Original Research Article

Stochastic model of siRNA endosomal escape mediated by fusogenic peptides

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Dataset link: https://github.com/Nisha1803/Methodology_Endosomal_Escape

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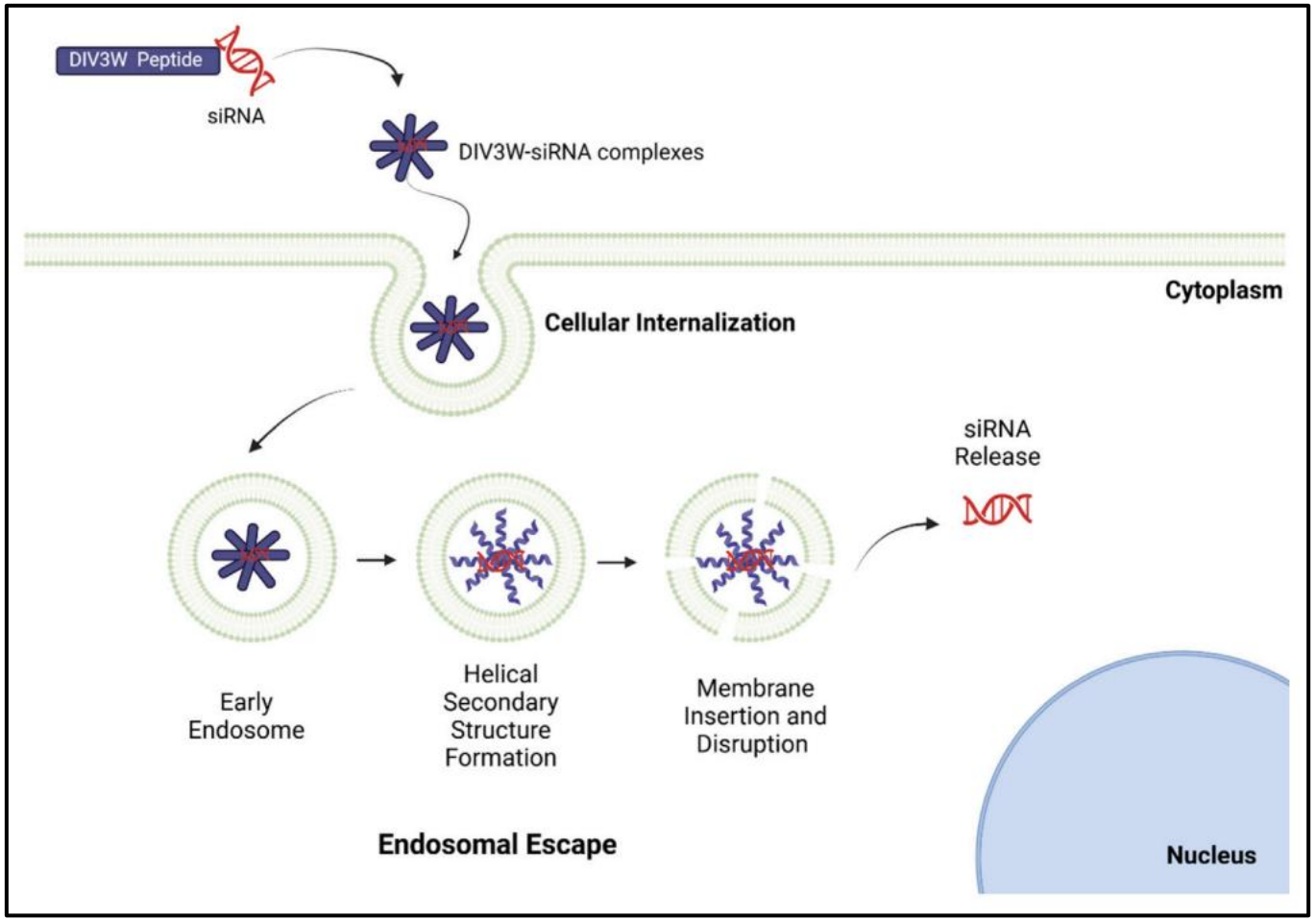
ABSTRACT

Gene silencing via small interfering RNA (siRNA) represents a transformative tool in cancer therapy, offering specificity and reduced off-target effects compared to conventional treatments. A crucial step in siRNA-based therapies is endosomal escape, the release of siRNA from endosomes into the cytoplasm. Quantifying endosomal escape is challenging due to the dynamic nature of the process and limitations in imaging and analytical techniques. Traditional methods often rely on fluorescence intensity measurements or manual image processing, which are time-intensive and fail to capture continuous dynamics. This paper presents a novel computational framework that integrates automated image processing to analyze time-lapse fluorescent microscopy data of endosomal escape, hierarchical Bayesian inference, and stochastic simulations. Our method employs image segmentation techniques such as binary masks, Gaussian filters, and multichannel color quantification to extract precise spatial and temporal data from microscopy images. Using a hierarchical Bayesian approach, we estimate the parameters of a compartmental model that describes endosomal escape dynamics, accounting for variability over time. These parameters inform a Gillespie stochastic simulation algorithm, ensuring realistic simulations of siRNA release events over time. By combining these techniques, our framework provides a scalable and reproducible method for quantifying endosomal escape. The model captures uncertainty and variability in parameter estimation, and endosomal escape dynamics. Additionally, synthetic data generation allows researchers to validate experimental findings and explore alternative conditions without extensive laboratory work. This integrated approach not only improves the accuracy of endosomal escape quantification but also provides predictive insights for optimizing siRNA delivery systems and advancing gene therapy research.

Background & Significance of the problems

The figure shows the complete intracellular pathway of siRNA, including uptake, endosomal processing, and escape into the cytoplasm.



siRNA : Small Interfering RNA

A short RNA molecule (21–23 bases) that silences target genes by degrading their mRNA, preventing gene expression.

This mechanism is known as Gene Silencing.

Examples / Applications

- Used to study gene functions
- Used to reduce or overcome drug resistance
- Used to suppress or inhibit cancer cell activity

Endocytosis

After siRNA enters the cell, it becomes trapped inside an endosome. siRNA must escape from the endosome to reach the cytosol and perform gene silencing. This release process is called Endosomal Escape.

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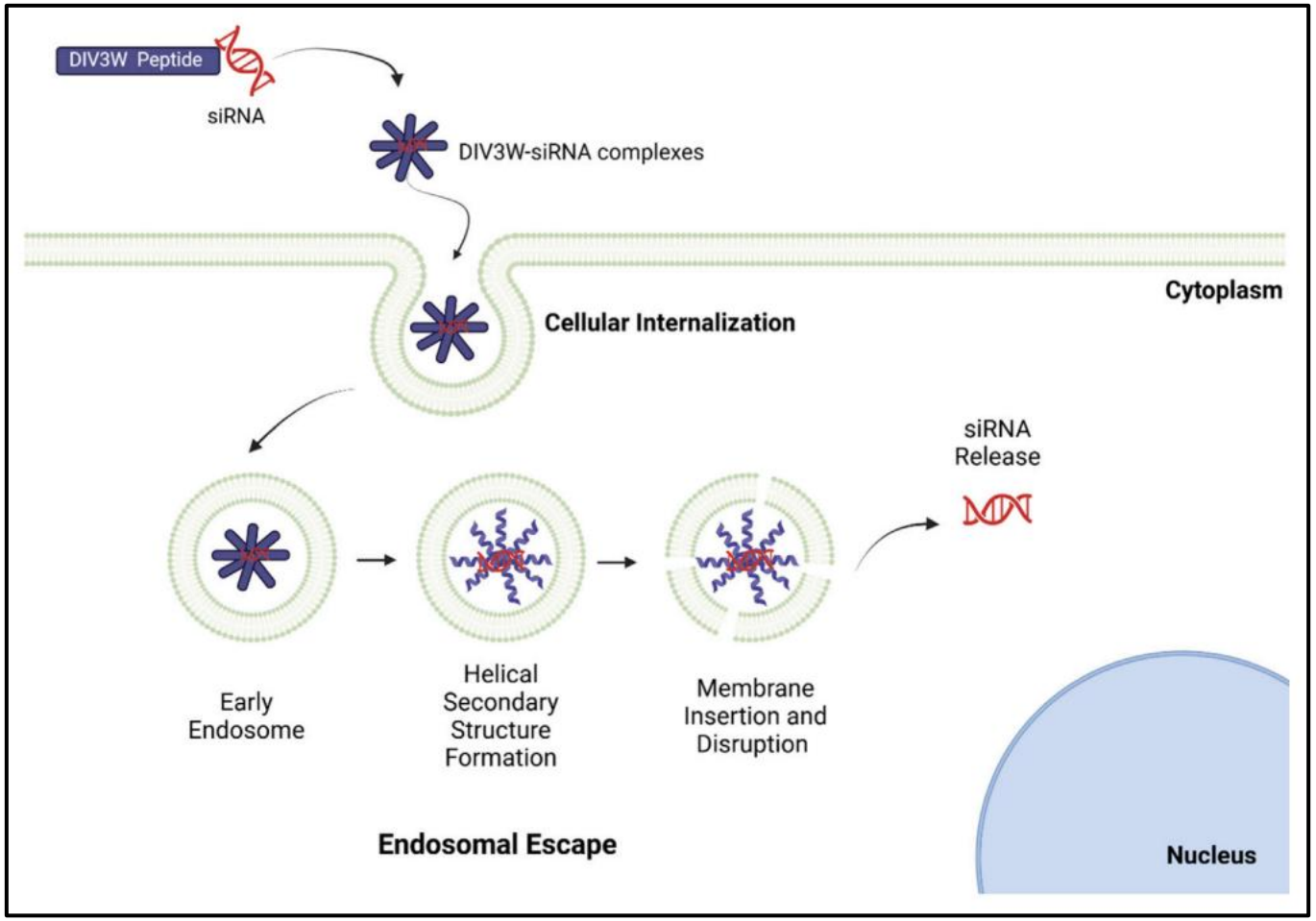


Fusogenic Peptide (DIV3W)

DIV3W is a pH-responsive fusogenic peptide that undergoes structural changes under acidic conditions.

In this study, it is used as a delivery cargo to facilitate the transport of siRNA into the cells.

Mechanism of Endosomal Escape

The endosome has an acidic pH, which triggers DIV3W to shift into a helical structure. This helical form allows DIV3W to insert into the endosomal membrane, creating pores or causing membrane disruption.

As a result, siRNA can escape into the cytoplasm, where it proceeds to carry out Gene Silencing.

Challenges

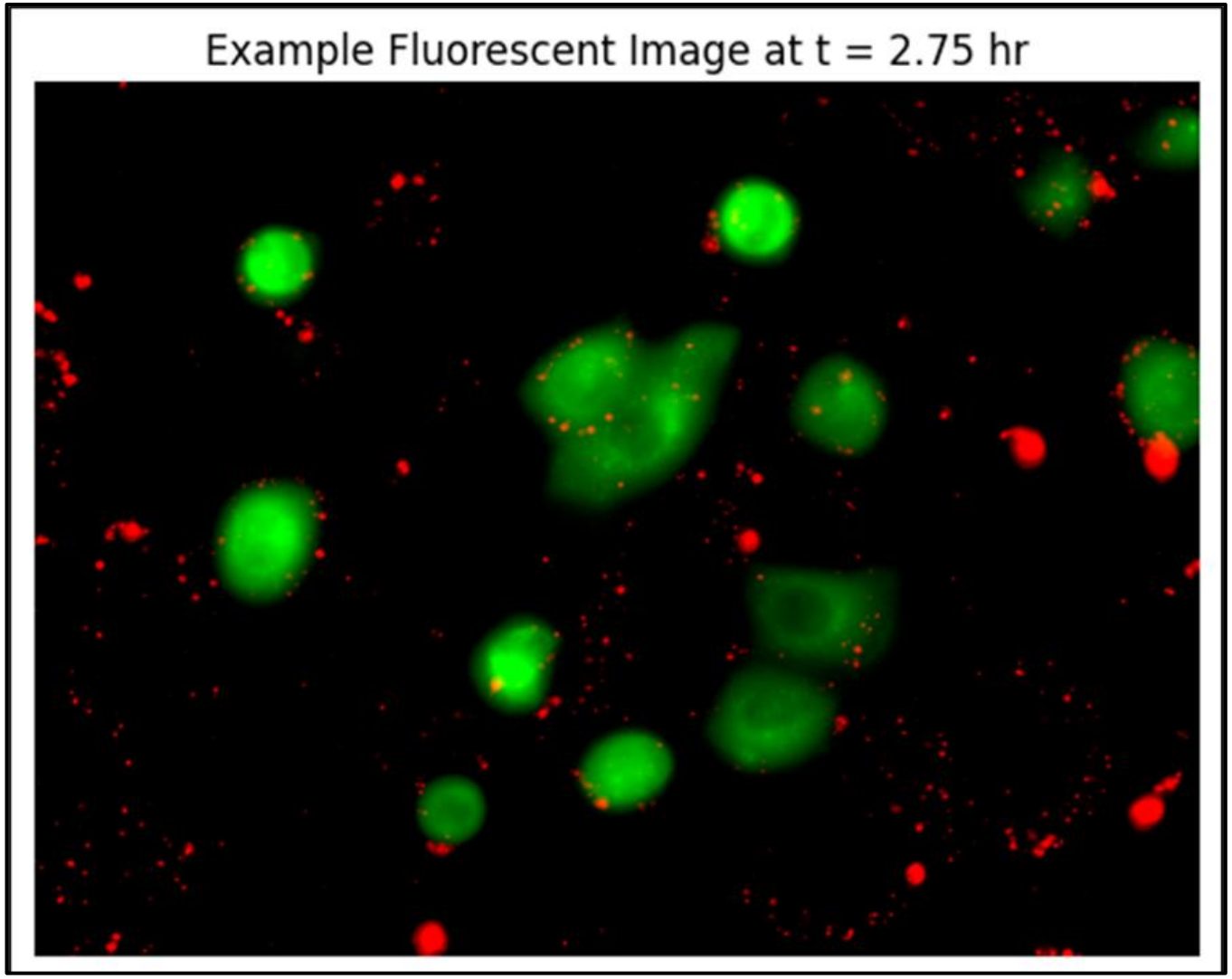
Fluorescence microscopy is **noisy** and only provides **discrete snapshots**, resulting in limited temporal resolution of endosomal escape.

Challenges in Image-Based Quantification

Fluorescence-intensity analysis is inconsistent and noise-sensitive, while endosomal escape occurs at different times in each endosome. This stochastic behavior makes reaction rates difficult to estimate from intensity measurements alone.

Green fluorescence highlights the endosomes of the cell.

Red fluorescence marks the localization of siRNA.

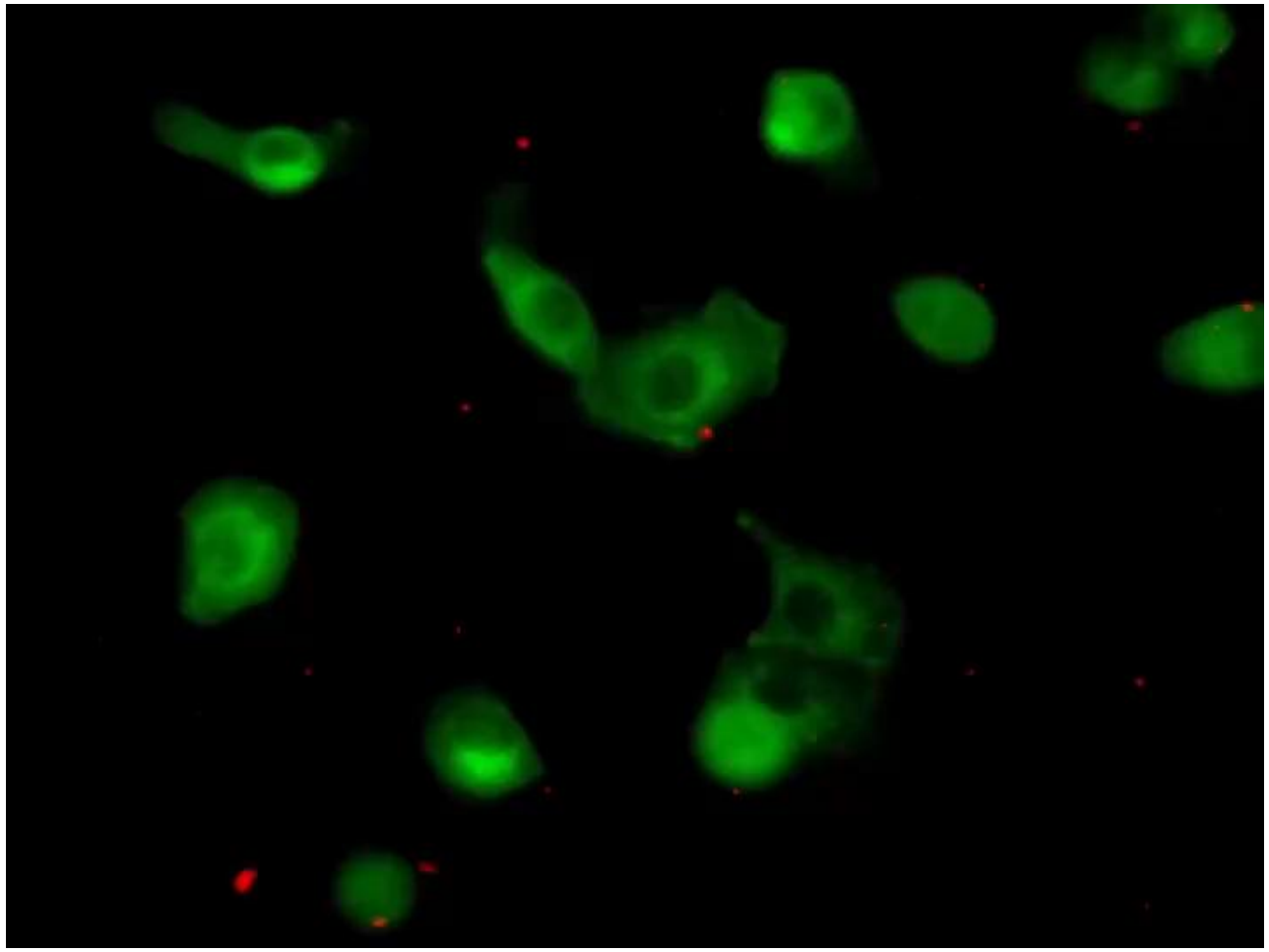


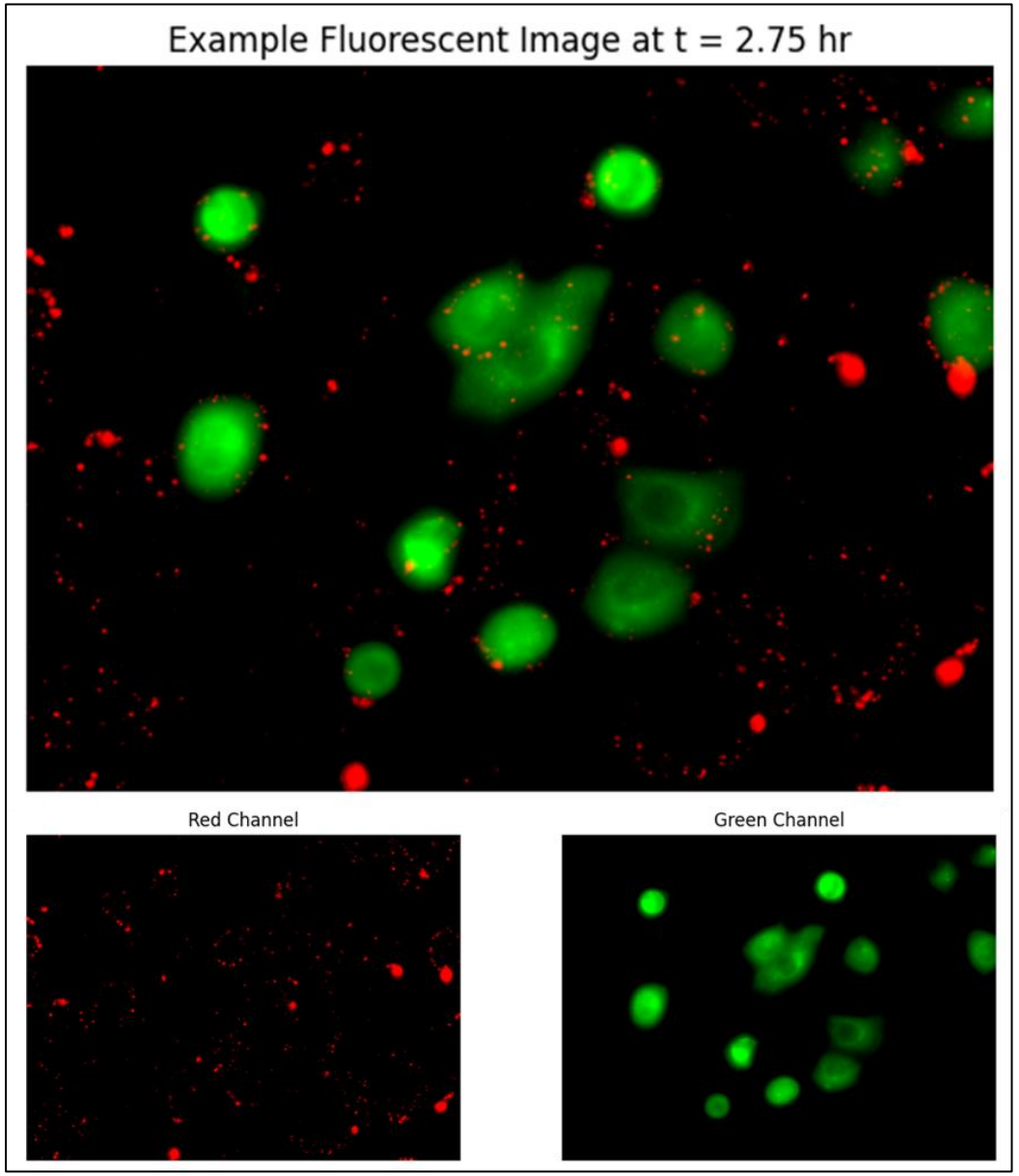
Research objective

- Bayesian Inference is used to estimate the distribution of reaction rates, capturing uncertainty and time-dependent variability in the system.
- The Gillespie Algorithm is employed to simulate the stochastic transitions between compartments, enabling realistic modeling of endosomal escape dynamics.

Methods & Simulation Algorithm

Time-lapse Fluorescent images in 4 hours, every 5 minutes





Methods & Simulation Algorithm

The table summarizes pixel-based quantification of siRNA in each compartment

Image	Red inside Green	Red outside Green	Total Red Count	Endosomal Escape (%)	EFP_in (%)	Endosomal Entrapment (%)
T0001	480	3390	3870	3.89	4.44	0.55
T0002	414	2797	3211	3.21	3.69	0.48
T0003	575	4640	5215	5.33	5.99	0.66
T0004	806	5738	6544	6.59	7.52	0.93

Encapsulated Fusogenic Peptide siRNA (EFP_{in})

Fraction of siRNA located **inside** endosomes (red overlapping green).

$$\mathrm{EFP_{in}} = \frac{\mathrm{Red_{inside\ green}}}{\mathrm{Total\ Red}} \times 100\%$$

Endosomal Escape (EE)

Fraction of siRNA that has escaped from endosomes (red outside green).

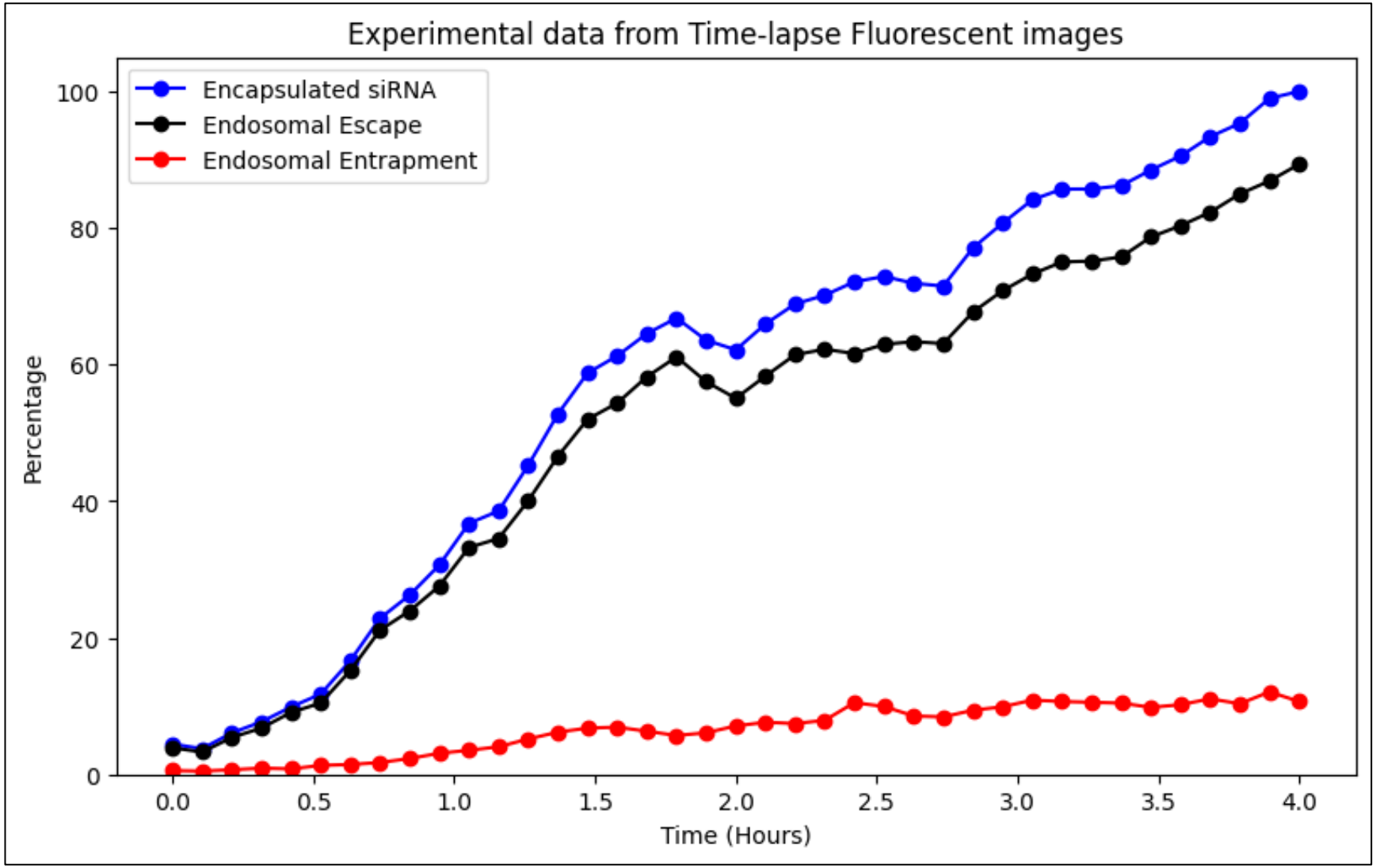
$$\mathrm{EE} = \frac{\mathrm{Red_{outside\ green}}}{\mathrm{Total\ Red}} \times 100\%$$

Endosomal Entrapment (EFP_{trap})

Remaining siRNA still trapped inside endosomes.

$$EPF_{trap} = EFP_{in} - EE$$

Methods & Simulation Algorithm



This plot shows the temporal evolution of each process.
As time increases, all three measurements rise accordingly.
This indicates that more siRNA enters the endosomes and subsequently escapes over time.

In this work,
the **Gillespie Algorithm** is used to simulate these stochastic events.

Methods & Simulation Algorithm

Endosomal Escape Compartment Model

$FP + siRNA \xrightarrow{\mu} EFP_{in}$ The binding interaction between the fusogenic peptide (FP) and siRNA to facilitate entry into the endosome.

$EFP_{in} \xrightarrow{\sigma} EE$ Endosomal Escape

$EFP_{in} \xrightarrow{d_{exit}} EFP_{trap}$ The entrapment and degradation within the endosome.

$siRNA \xrightarrow{\omega} \emptyset$ The degradation of siRNA is neglected in this work (in this work, $\omega = 0$)

where μ is endocytosis rate

Q σ is escaping rate

d_{exit} is entrapment rate

Methods & Simulation Algorithm

Gillespie Algorithm

A simulation method designed for **stochastic systems.** Its key feature is that it does not use **fixed time steps**; instead, it simulates one reaction event at a time by randomly determining which reaction occurs and when it occurs. This produces event trajectories that closely reflect the true behavior of the system.

In this work, the Gillespie algorithm is used to **simulate** the endosomal escape process because reactions inside individual endosomes **do not occur simultaneously**.
A stochastic simulation is therefore the most accurate way to capture these dynamics, allowing us to reflect the inherent randomness of intracellular reactions.

Reaction Rate Calculation at Time Step t_n

$$\frac{dEFP_{in}(t_n)}{dt} = \frac{EFP_{in}(t_{n+1}) - EFP_{in}(t_n)}{\Delta}$$

$$\frac{dEE(t_n)}{dt} = \frac{EE(t_{n+1}) - EE(t_n)}{\Delta}$$

$$\frac{dEFP_{trap}(t_n)}{dt} = EFP_{in}(t_n) - EE(t_n)$$

$$\text{where } \Delta \text{ is } 5 \text{ min from } 0 \ - 4 \text{ hours}$$

These rates estimated from fluorescence data are later used as parameters $\mu, \sigma,$ and d_{exit} in the Gillespie simulation.

Methods & Simulation Algorithm

Bayesian Hierarchical Modeling for Reaction Rates

Why Bayesian?

- Fluorescence-derived increments are noisy and sparse, making direct rate estimation unreliable
- Bayesian inference provides posterior distributions for each reaction rate and reflects uncertainty
- Supports time-varying parameters, essential for dynamic endosomal escape

Hierarchical Model Structure

Global hyperpriors represent overall trends

Local parameters per time step

Likelihoods from observed increments

Posterior sampled using MCMC by PyMC

Posterior to Simulation Input

Extract posterior means at each time point:

μ_estimates[t] = uptake rate

σ_estimates[t] = escape rate

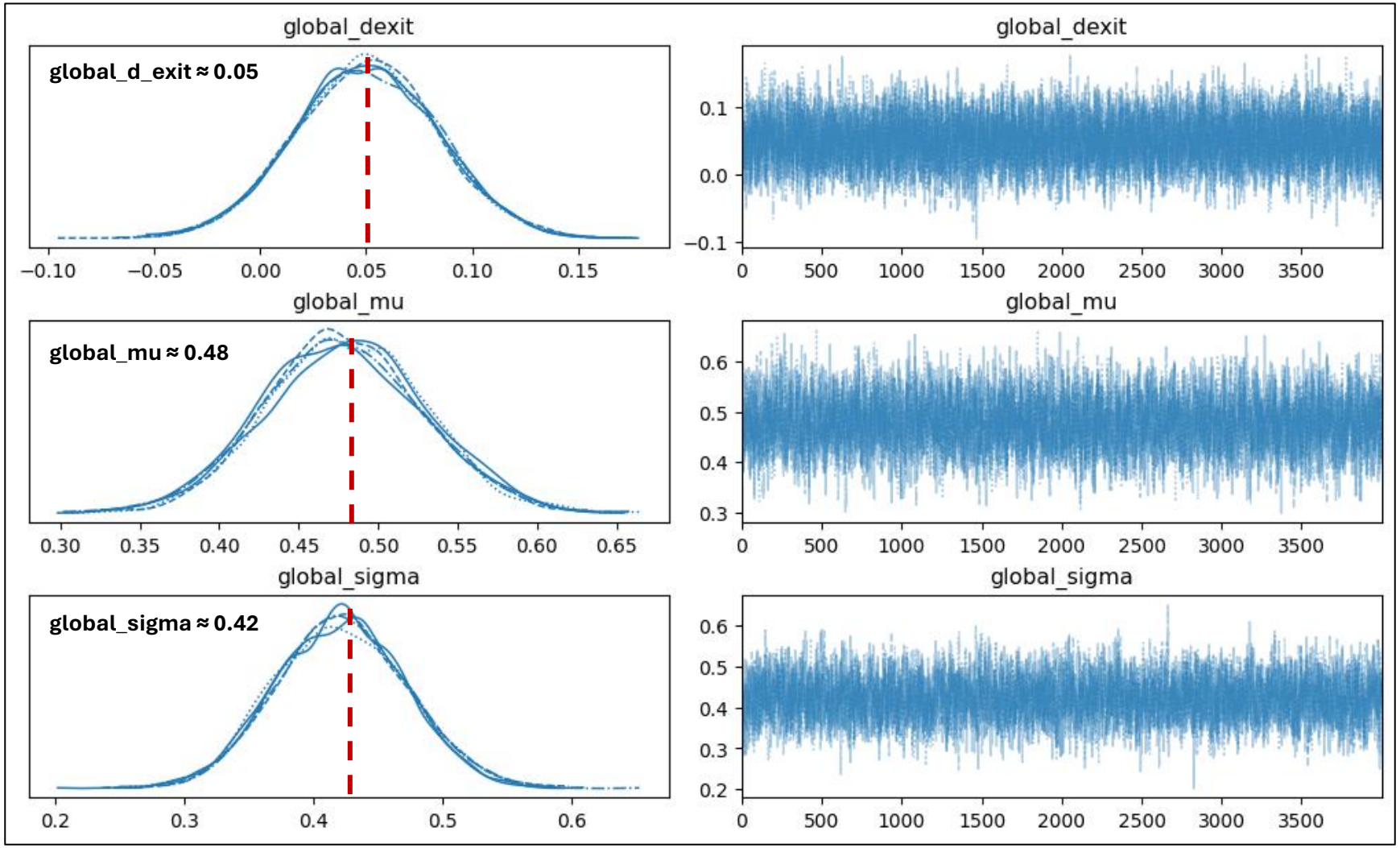
d_exit_estimates[t] = trapping/degradation rate

These become time-dependent reaction rate functions.

Used as inputs to the Gillespie Algorithm to simulate stochastic endosomal escape

Results Bayesian Inference

Posterior Distributions of Global Reaction Parameters



Density Plot

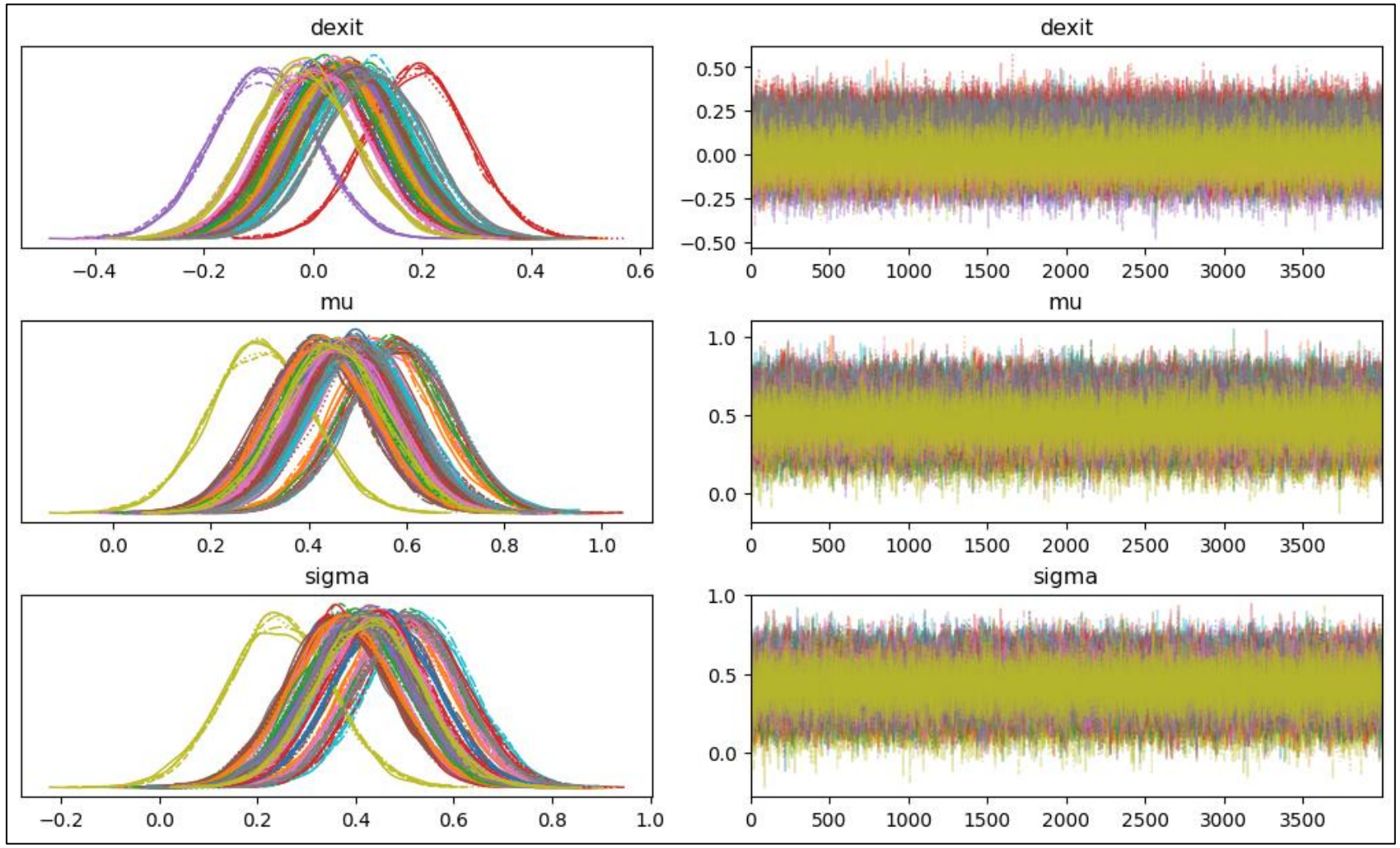
Trace Plot

- Global parameters describe overall system behavior.
- Posterior distributions show good mixing and stable estimates.
- Serve as hyperparameters guiding local, time-dependent rates.
- Provide constraints to stabilize inference under noisy data.

Results Posterior Distributions of Local Reaction Parameters

Local parameters represent time-dependent reaction rates at each time step.

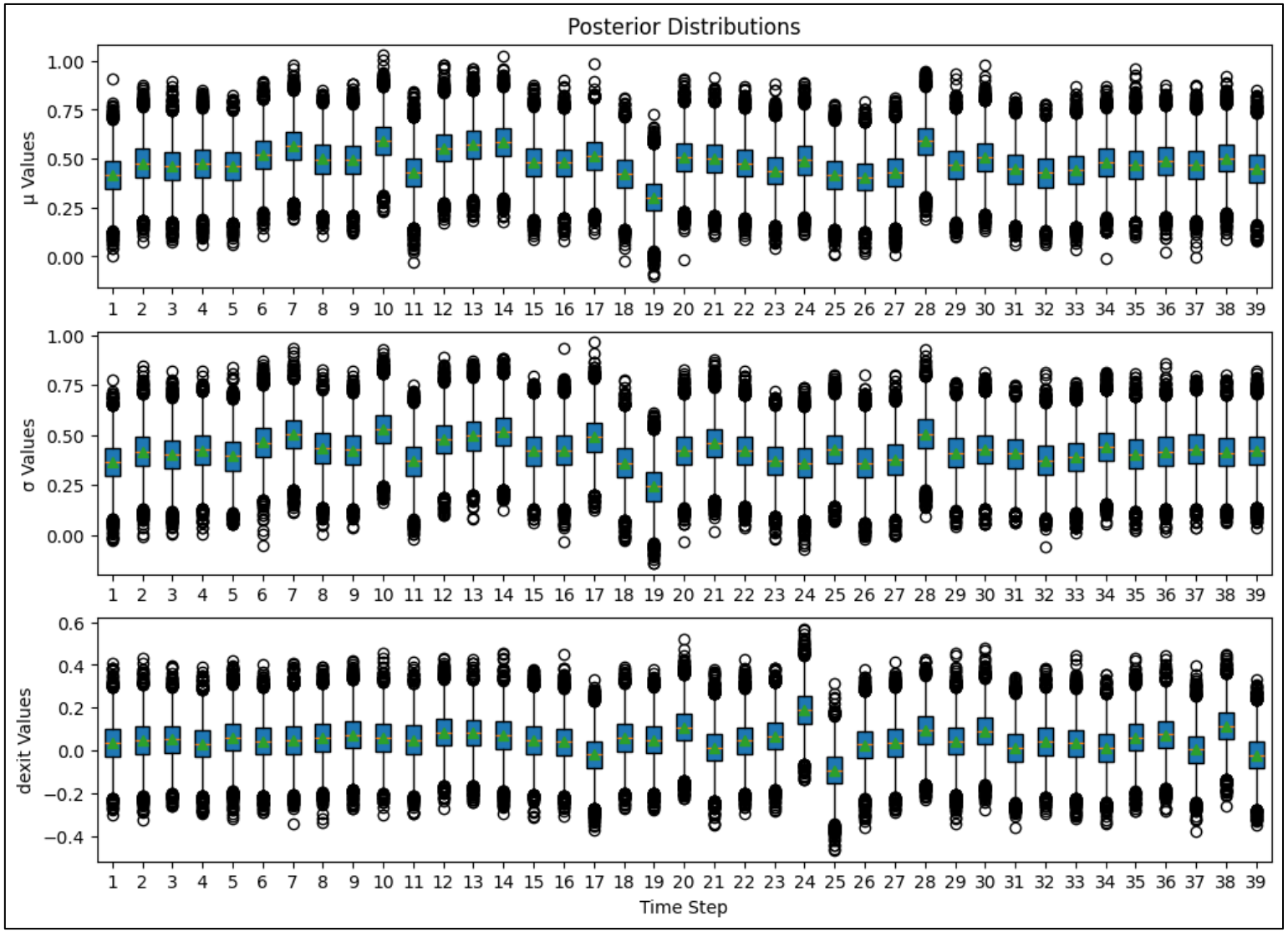
Each curve shows the posterior distribution for that time point.



- Wide spreads reflect measurement noise and biological variability.
- Trace plots indicate good mixing and stable MCMC sampling.

Results

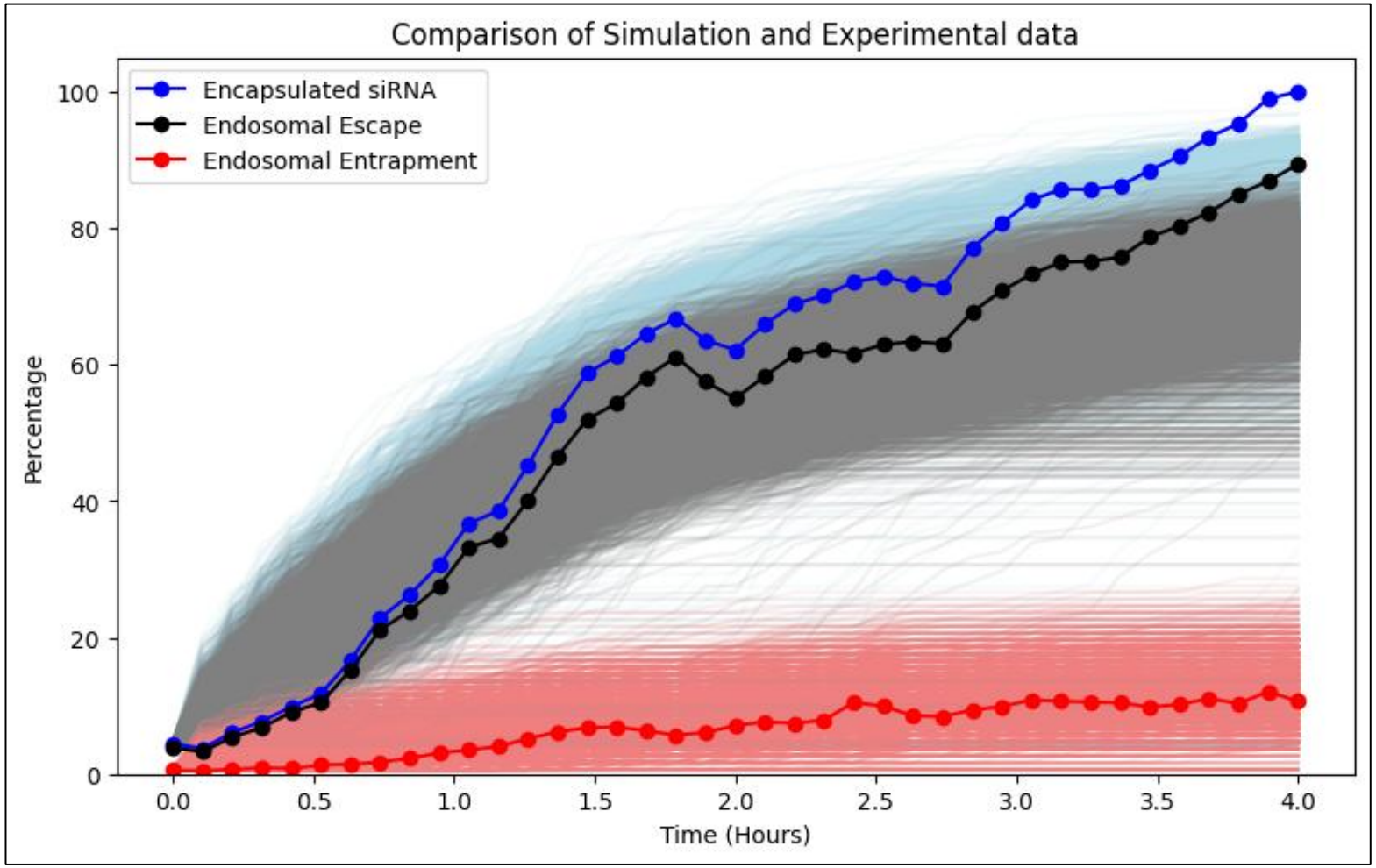
Time-Dependent Posterior Reaction Rates



- All parameters vary over time, reflecting dynamic intracellular processes
- Wide distributions indicate uncertainty from fluorescence measurements
- Posterior means from each time point will be used as time-dependent reaction rates in the Gillespie simulation

Results Comparison of Simulation and Experimental Results

This graph shows the Gillespie simulation results (shaded lines) compared with the observed trajectories of EFP_{in}, Endosomal Escape, and EFP_{trap}.



- Simulation closely follows the experimental trends.
- Posterior-driven rates capture uptake, escape, and release behaviors.
- The spread of simulated curves reflects the stochastic nature of endosomal escape.
- Overall, the model reproduces observed cellular dynamics well.

Discussion & Conclusions

Discussion

- Bayesian inference successfully extracted time-dependent reaction rates from noisy fluorescence data.
- Global–local hierarchical structure stabilized parameter estimation across all time points.
- Gillespie simulation using posterior-derived rates reproduced experimental dynamics realistically.
- Model captured both the trend and stochastic variability of the endosomal escape process.

Conclusion

- Using Bayesian inference to estimate reaction-rate trajectories, followed by Gillespie simulation, allowed us to reconstruct the dynamic behavior of endosomal escape.
- This combined probabilistic–stochastic approach provides a powerful framework for studying complex intracellular processes and can be adapted to other delivery systems or biological pathways.

References

[1] Yadav, N., Boulos, J., Alexander-Bryant, A., & Cook, K. (2025). Stochastic model of siRNA endosomal escape mediated by fusogenic peptides. Mathematical Biosciences, 109476.

Code availability

https://github.com/kiiraatii/SCPY562-Mini_Project_Finale.git

Thank you for your Attention