

Single-Molecule Dynamics in Living Cells

Tracking protein diffusion with light-sheet microscopy

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Zusammenfassung

Diese Arbeit verbindet Lichtblattmikroskopie mit einer Hidden-Markov-Analyse, um frei diffundierende und vorübergehend gebundene Zustände einzelner Proteine in lebenden Zellen zu unterscheiden. Für einen Transkriptionsfaktor sind im Mittel 34% der Moleküle gebunden, bei einer mittleren Verweildauer von 1,9 Sekunden.

Abstract

Proteins inside living cells move by diffusion, binding and active transport, and how they move reveals how they work. Single-molecule tracking follows individual labelled proteins, but in the crowded interior of a cell the trajectories are short and noisy. This dissertation combines light-sheet illumination, which reduces background, with a hidden Markov analysis that separates freely diffusing from transiently bound states. Applied to a transcription factor in live human cells, the analysis finds that 34% of molecules are bound at any time, with a mean residence time of 1.9 seconds.

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Chapter 1

Introduction

Much of a cell's regulation depends on proteins finding their targets, which they do by a combination of diffusion and brief binding. Ensemble measurements report averages; single-molecule tracking watches individual molecules and so can separate populations that averages hide [2]. Inside living cells, however, background fluorescence and fast motion make trajectories short. This dissertation improves both the measurement and its analysis.

Chapter 2

Background

For free diffusion in two dimensions, the mean squared displacement grows linearly with lag time τ ,

$$\langle r^2(\tau) \rangle = 4D\tau + 4\sigma^2, \quad (2.1)$$

where D is the diffusion coefficient and σ the localization precision. Light-sheet illumination excites only a thin plane of the sample, reducing out-of-focus background compared with wide-field illumination [1].

Chapter 3

Light-Sheet Tracking and State Analysis

We image cells with a thin light sheet at 100 frames per second and link localizations into trajectories. A two-state hidden Markov model assigns each step to a bound or a free state, with state-specific diffusion coefficients and transition rates estimated by maximum likelihood [3].



Figure 3.1: From movies to binding kinetics.

Chapter 4

Results

Table 4.1 summarizes the fitted states for 8,400 trajectories from 52 cells.

Table 4.1: Fitted diffusive states of the transcription factor.

State	Fraction	D ($\mu\text{m}^2/\text{s}$)
Bound	0.34	0.04
Free	0.66	3.1

The fitted transition rate out of the bound state gives a mean residence time of 1.9 s.

Chapter 5

Conclusion

Light-sheet illumination and state-resolved analysis together make binding kinetics measurable in living cells. Applying the method to proteins with more than two states will require models that select the number of states from the data.

Appendix A

Localization Precision

Localization precision was estimated from immobilized dye molecules as $\sigma = 28$ nm, which enters Equation (2.1) as the static offset.

Bibliography

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