ANOMALOUS DIFFUSION OF RNA IN THE CYTOPLASM OF HELA CELLS

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INTRODUCTION

Information about the diffusive motion of RNA would provide insights into intracellular structures and functions, as well as gene expression and genetic regulation. We study the motion of individual messenger RNA molecules in the cytoplasm of HeLa cells. When compared to the cytoplasmic motion of synthetic nanoparticles, the analysis of RNA trajectories gives rise to discrepancies that raise questions about specific intracellular interactions.

METHODS

- RNAs were imaged in live HeLa cells via confocal, fluorescent microscopy.
- RNA molecules expressing the MHY9 gene were labeled via MS2 stem loops bound to coat proteins tagged with the HaloTag-JF646 fluorophore.
- Imaging was done using multiple focal planes (usually four) of 0.5 μm in width. Exposure time was 50 ms/plane, yielding a ~200 ms frame rate.
- ImageJ was used (TrackMate plugin) to perform single-particle tracking and obtain trajectories of individual RNA molecules.

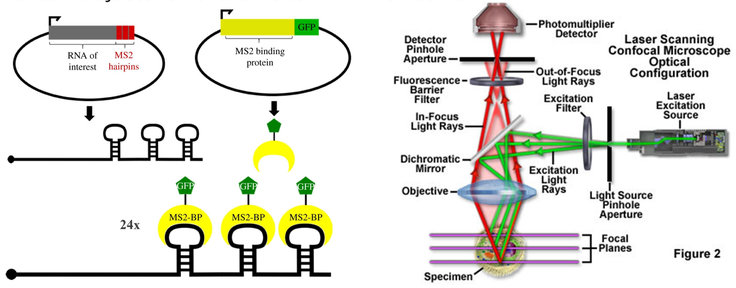
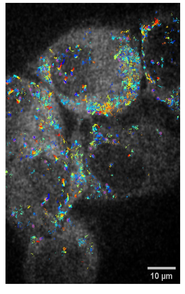
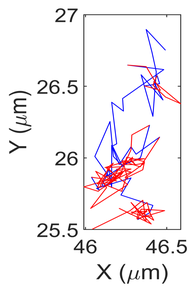


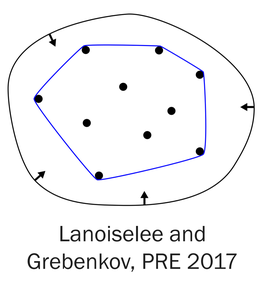
Figure 2

General labeling process (left). Confocal fluorescence microscopy with multiple focal planes (right).

TRACKING / SEGMENTATION







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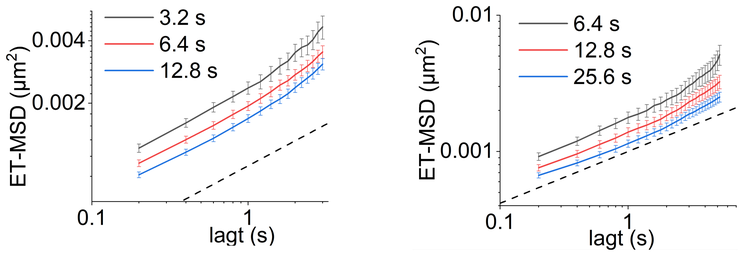
(Left) Trajectories obtained with TrackMate. Each color represents a different trajectory. (Middle) Trajectory segmented using convex hull. Red and blue represent confined and free states, respectively, (Right) Illustration of convex hull method.

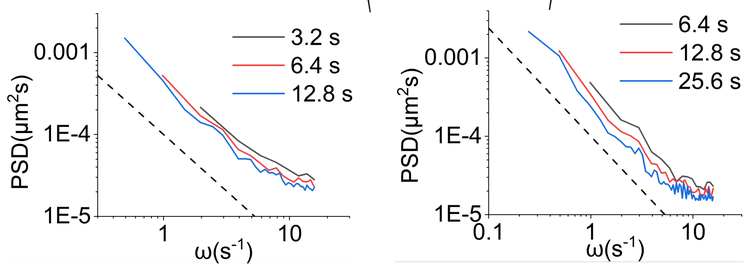
TRAJECTORY ANALYSIS

ET-MSD: $\overline{\delta^2(t_{\mathrm{lag}})} = \frac{1}{T_m - t_{lag}} \int_0^{T_m - t_{lag}} \left[\mathbf{r}(\tau + t_{\mathrm{lag}}) - \mathbf{r}(\tau)\right]^2 \mathrm{d}\tau$



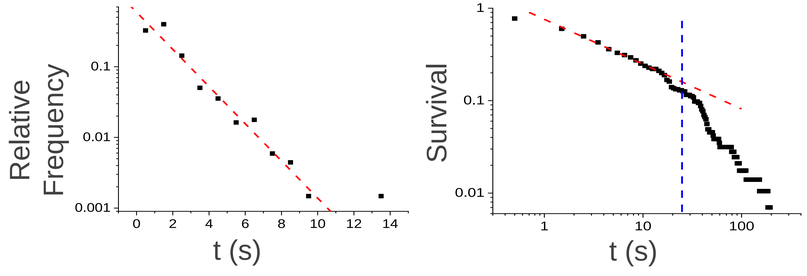
$\langle \overline{\delta^2(\tau, T_m)} \rangle \sim \frac{\tau^\beta}{T_m^{1-\alpha}} \sim \frac{\tau^{0.55}}{T_m^{0.44}}$ → $\alpha = 0.56$ | $\beta = 0.55$

PSD: $S_T(\omega) = \left\langle \left| \frac{1}{T} \int_0^T e^{i\omega t} X(t) dt \right|^2 \right\rangle$



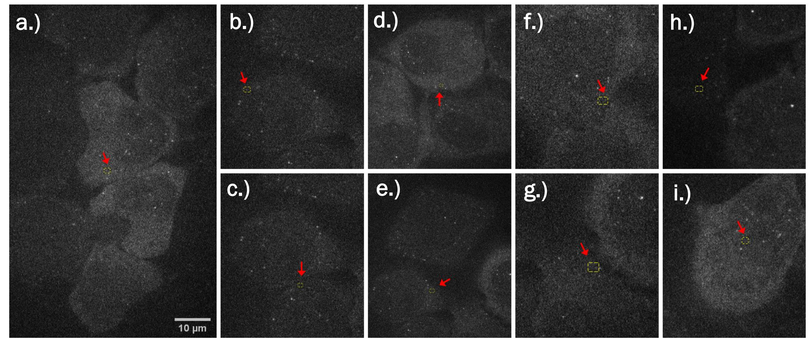
$\langle S(\omega, T_m) \rangle \sim \frac{1}{T_m^{1-\alpha} \omega^{1+\beta}} \sim \frac{1}{T_m^{0.47} \omega^{1.45}}$ → $\alpha = 0.53$ | $\beta = 0.45$

ET-MSD plots (top) for trajectories with a minimum of 12.8 s (left) and 25.6 s (right) durations. Varying diffusion coefficients for different observation times indicates aging and a lack of ergodicity. PSD (bottom) also exhibits aging.



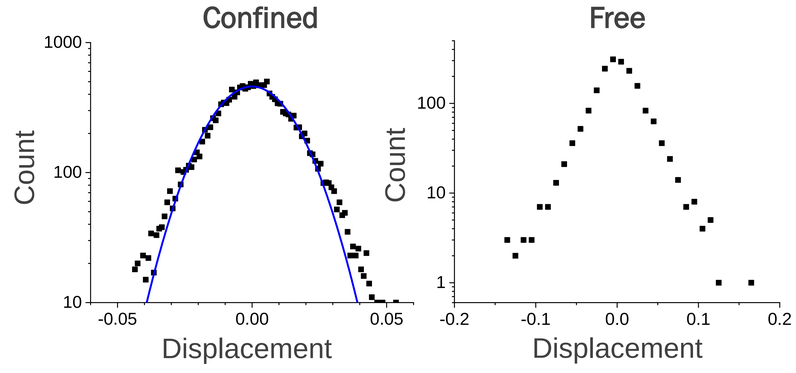
Sojourn times for free (left) and confined (right) states, showing exponential and power-law distributions, respectively.

LONG FREE STATES



(**a-g**) 78% of trajectories containing long free states (> 8 s) are located at or near the cell membrane.

DISPLACEMENTS



Distribution of displacements for free and confined states. Free state exhibits a Laplace distribution, indicating heterogeneous diffusivity.

CONCLUSIONS

- RNAs exhibit subdiffusion, showing ergodicity breaking and dependence on observation time. Trajectories switch between free and confined states with an order of magnitude difference in diffusivity.
- Trajectories that remain in the free state for long times are found to exist near the edges of cells, farther from the nucleus and closer to the membrane.

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