

sHaRPer: algorithm that acquires kinetic information from slow transitions in smFRET measurements

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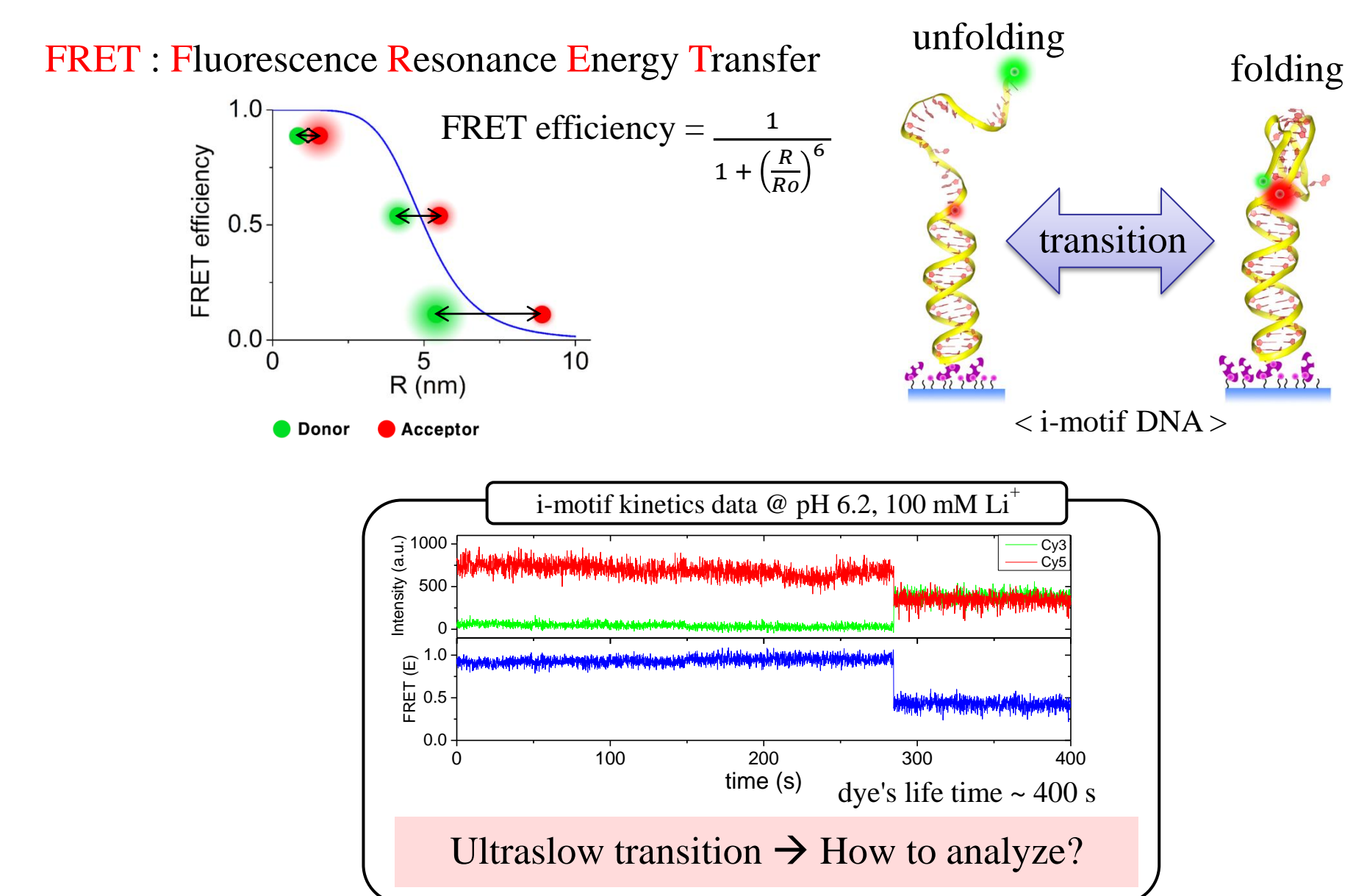
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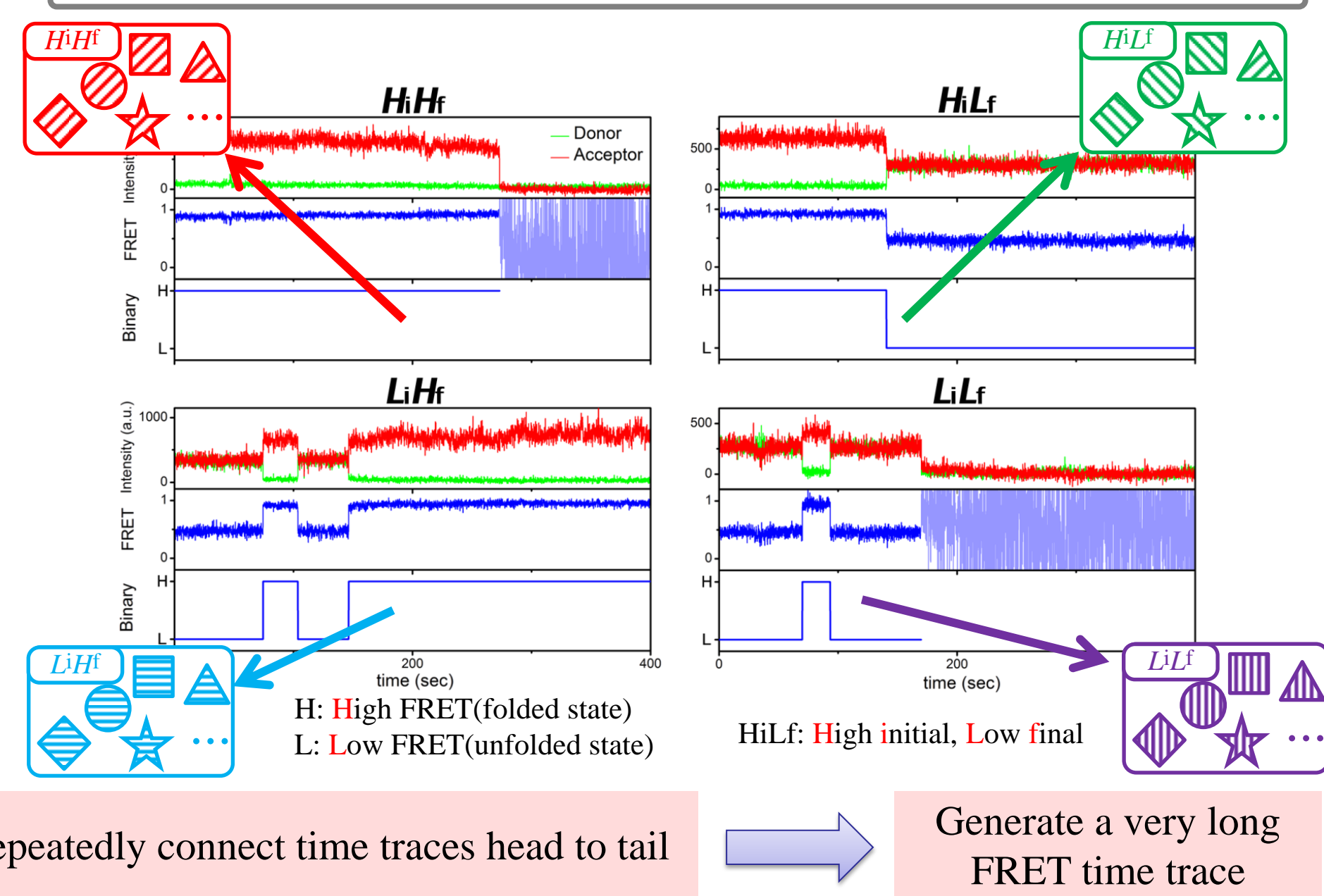
Abstract

Single-molecule FRET is one of the most powerful and widely used biophysical techniques in biological sciences. It, however, has certain limitations such as weak signal and limited measurement time intrinsic to single-molecule fluorescence measurements. Despite several ameliorative measures taken to increase measurement time, it is nearly impossible to acquire meaningful kinetic information for systems whose characteristic transition times are comparable to or longer than measurement times limited by the finite lifetime of fluorescent dye. We report a scheme called sHaRPer (serialized Handshaking Repeated Permutation with end removal) to alleviate the aforementioned kinetic issues of single-molecule analysis and address the accuracy and reliability of average kinetic information acquired with sHaRPer by systemically varying (simulated) experimental parameters. Application of the sHaRPer method to single-molecule data, however, has a potential risk of distorting the time constants of individual kinetic phases if the data are described with kinetic partitioning. To this end, we provide a criterion to decide in what condition such distortion is expected. Our sHaRPer method is a useful way to handle single-molecule data with slow transition dynamics. This study provides a practical guide to use sHaRPer..

Motivation

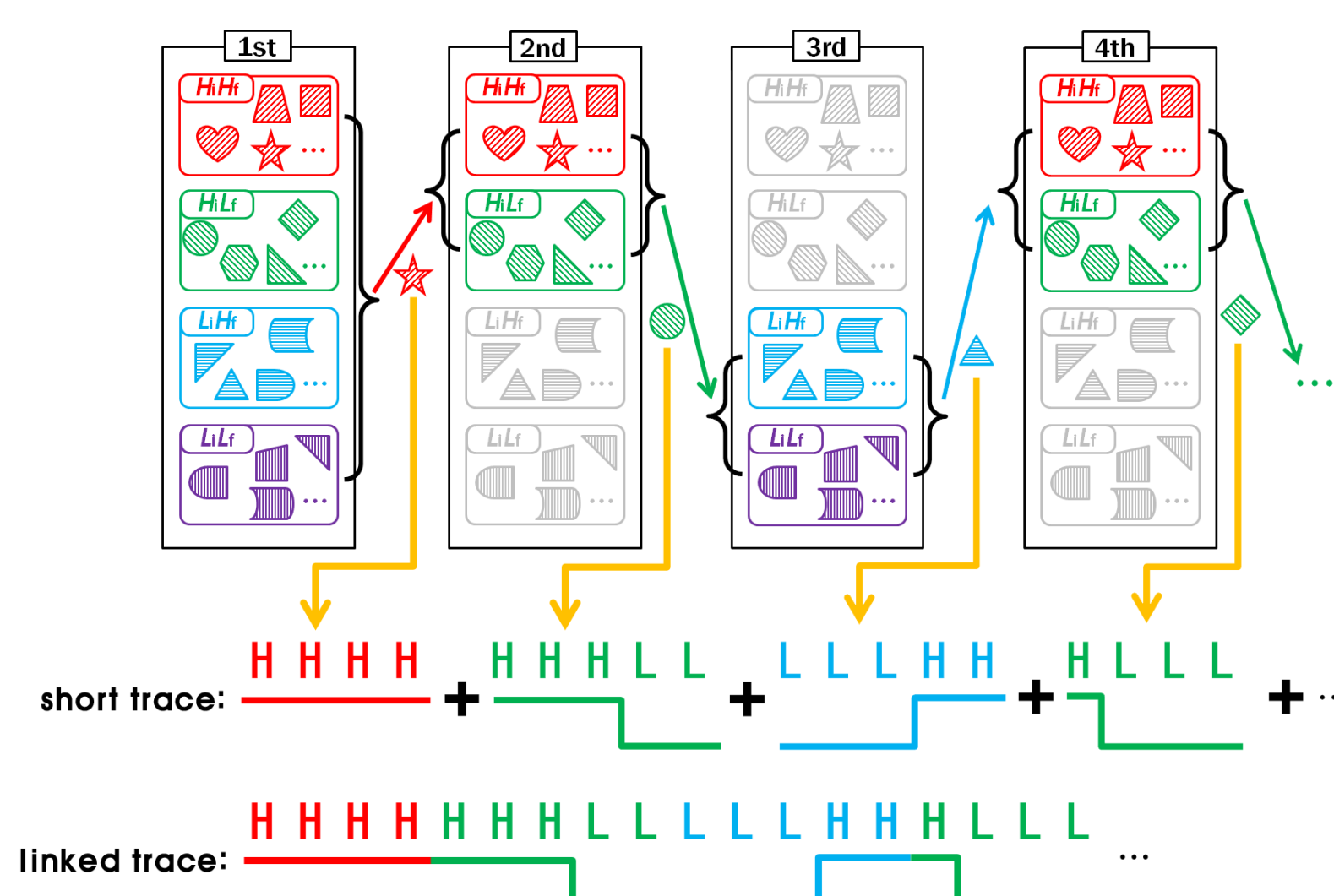


Four possible types of FRET time traces

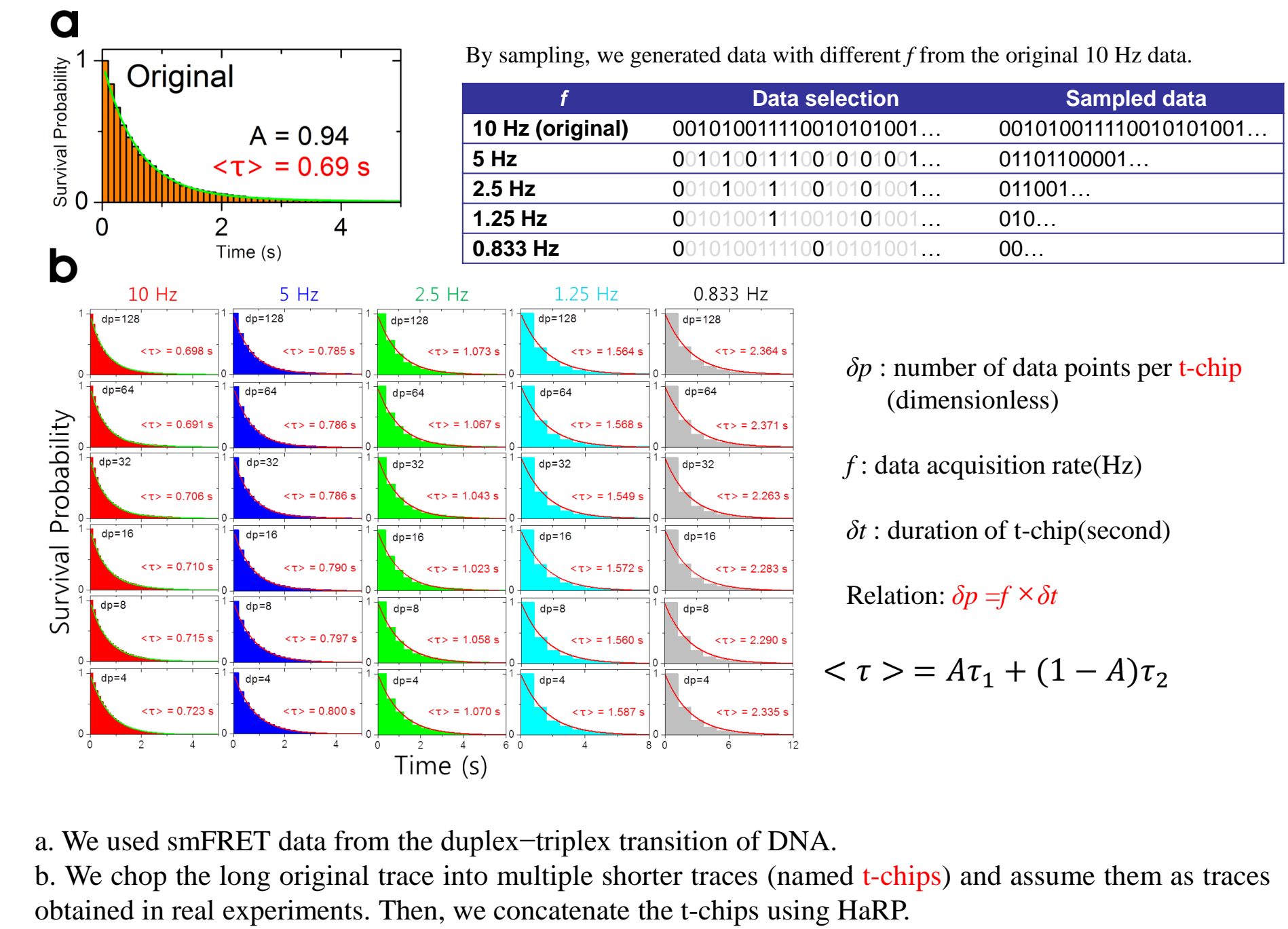


HaRP

Schematics of HaRP (Handshaking Repeated Permutation)

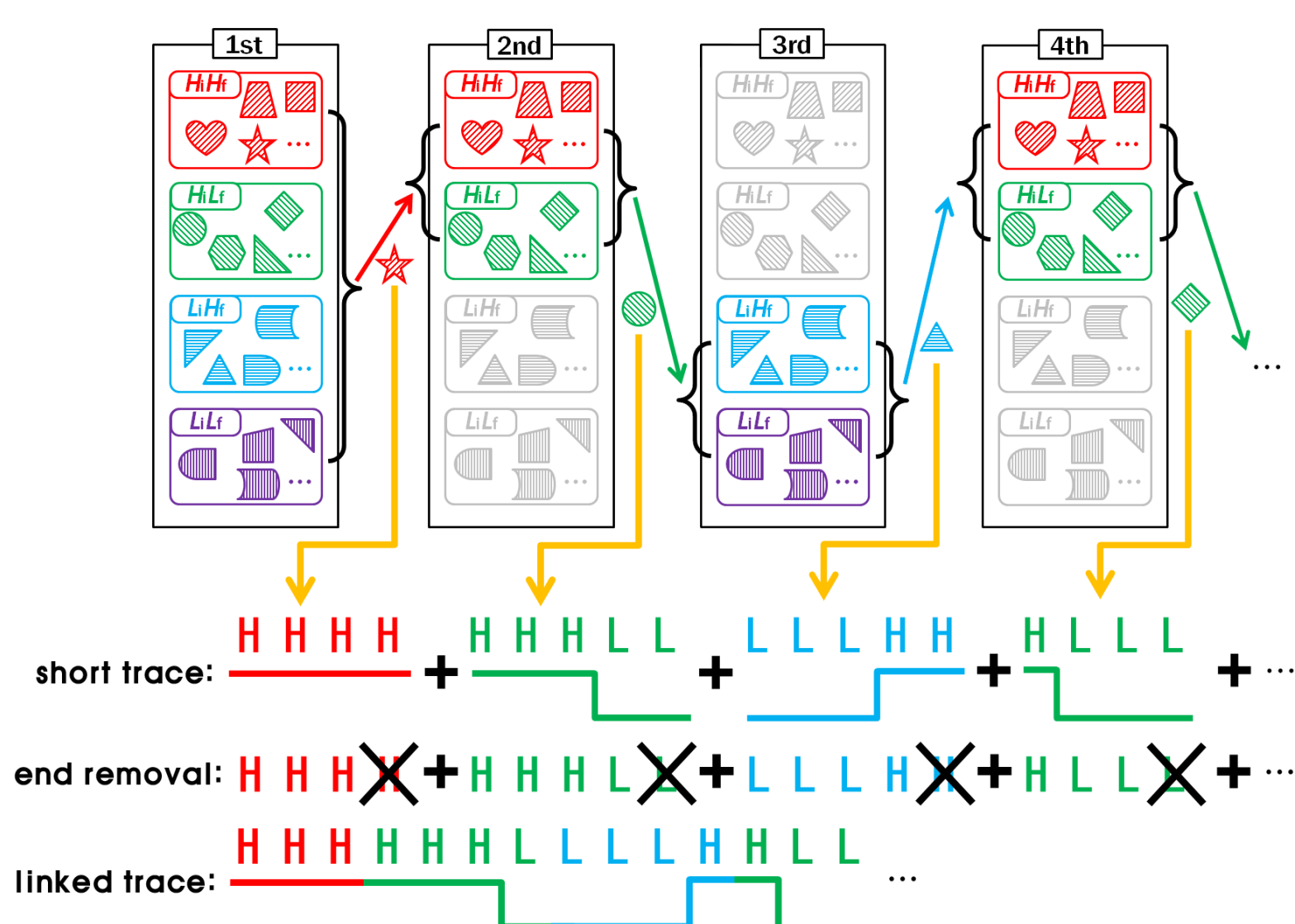


HaRP TEST

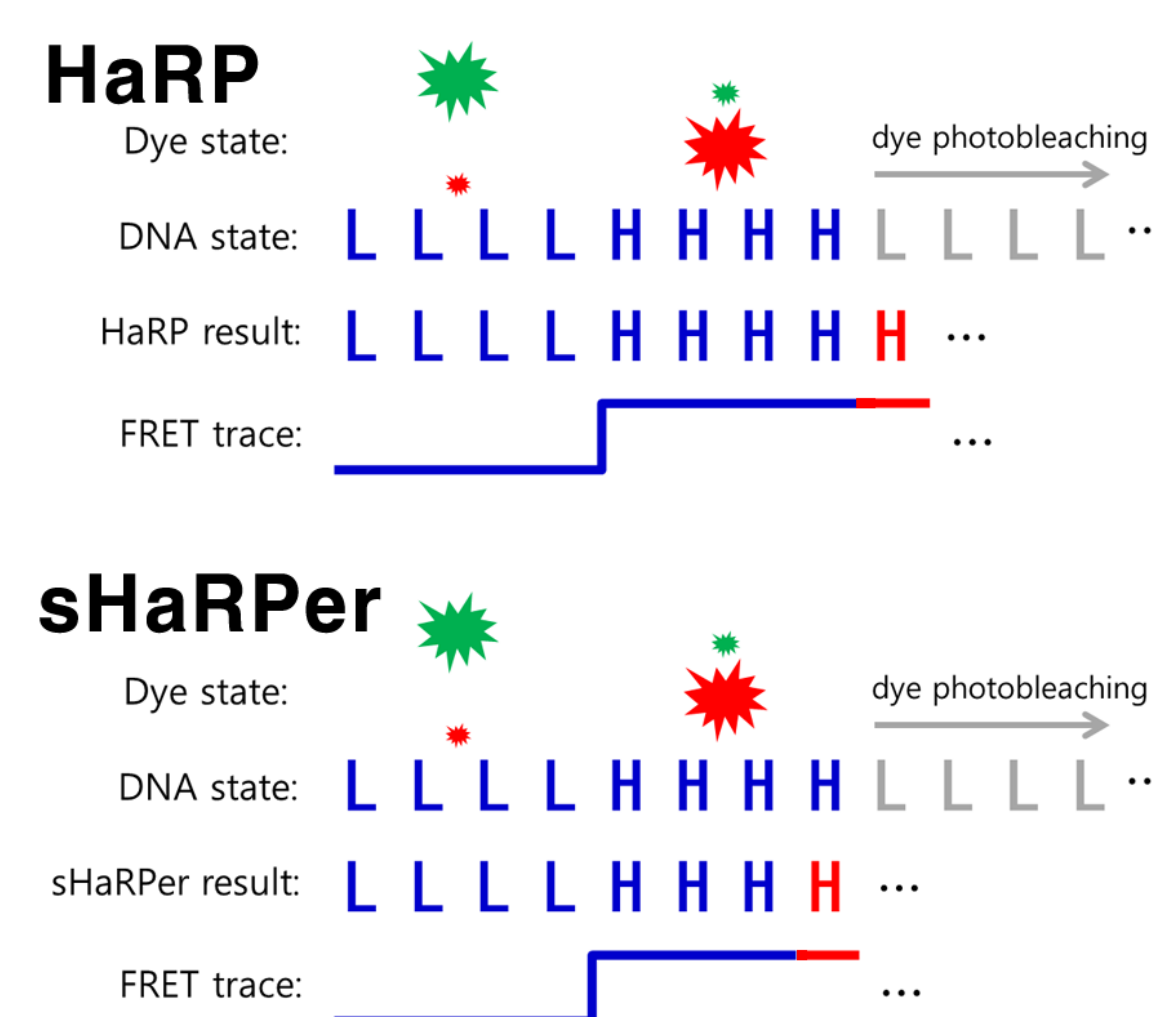


sHaRPer

Schematics of sHaRPer (serialized Handshaking Repeated Permutation with end removal)

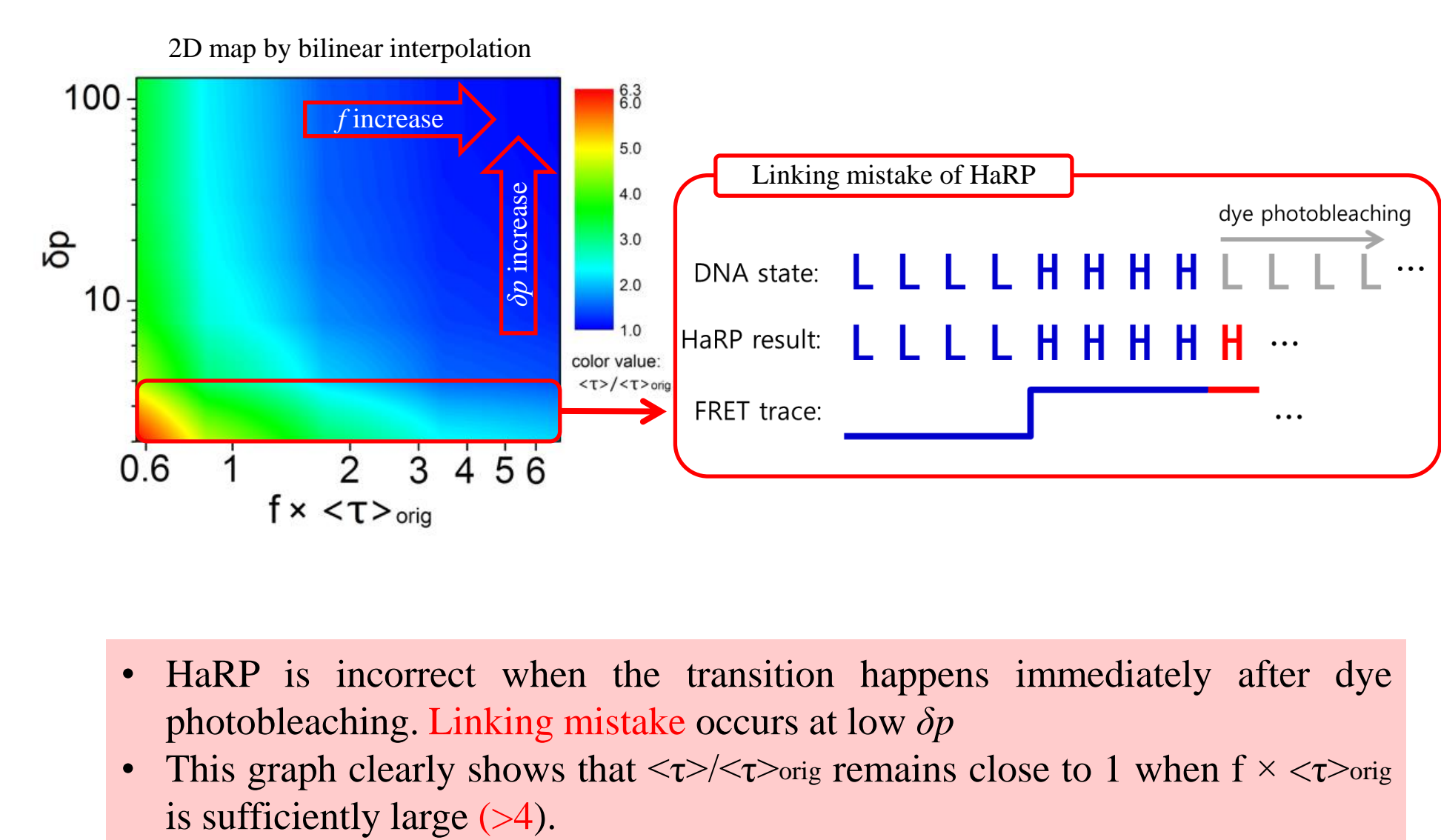


Difference between HaRP and sHaRPer



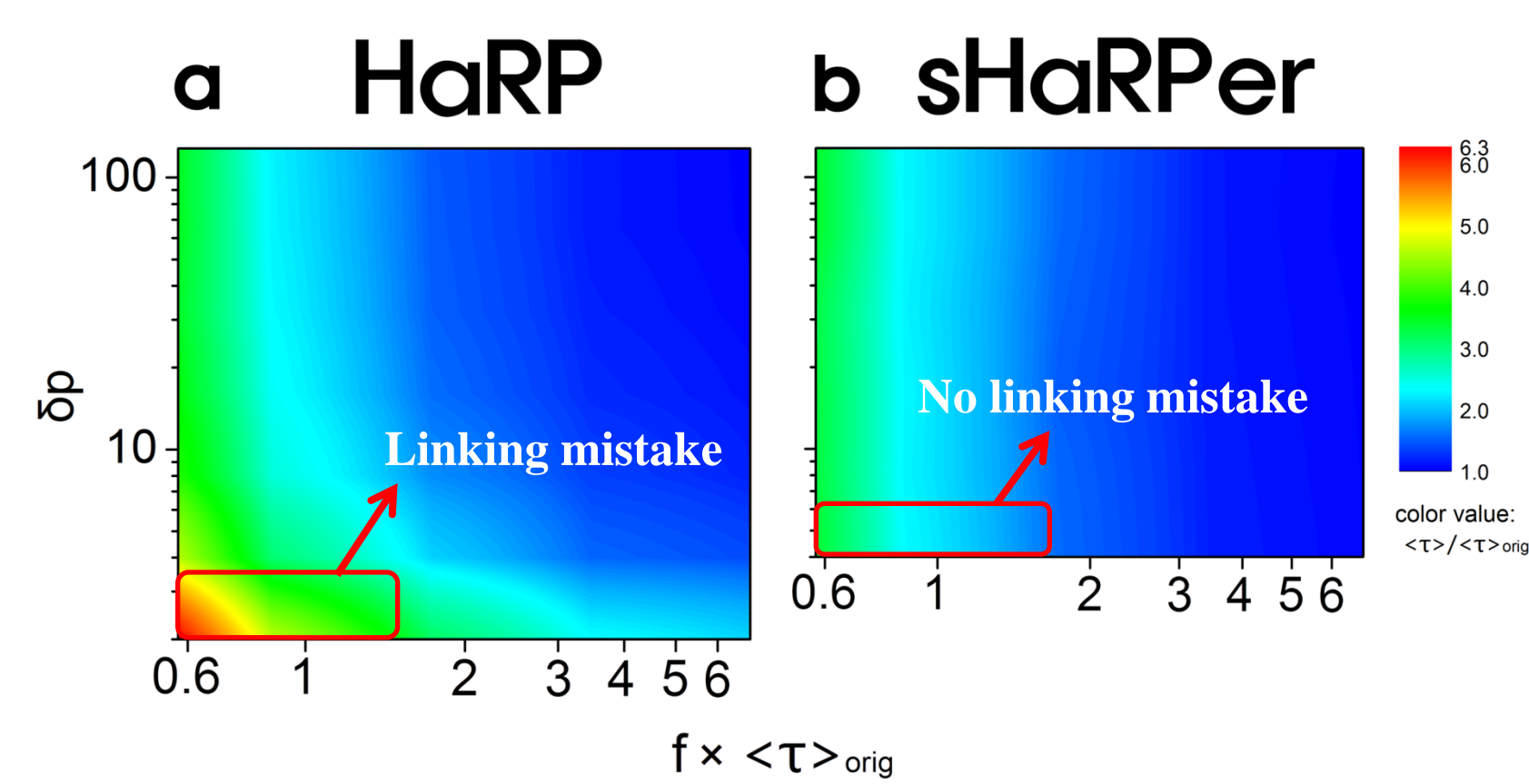
The sHaRPer scheme in which the last data point is **deleted** and the **information** of the last point is used to select a next trace.

The result of the HaRP TEST

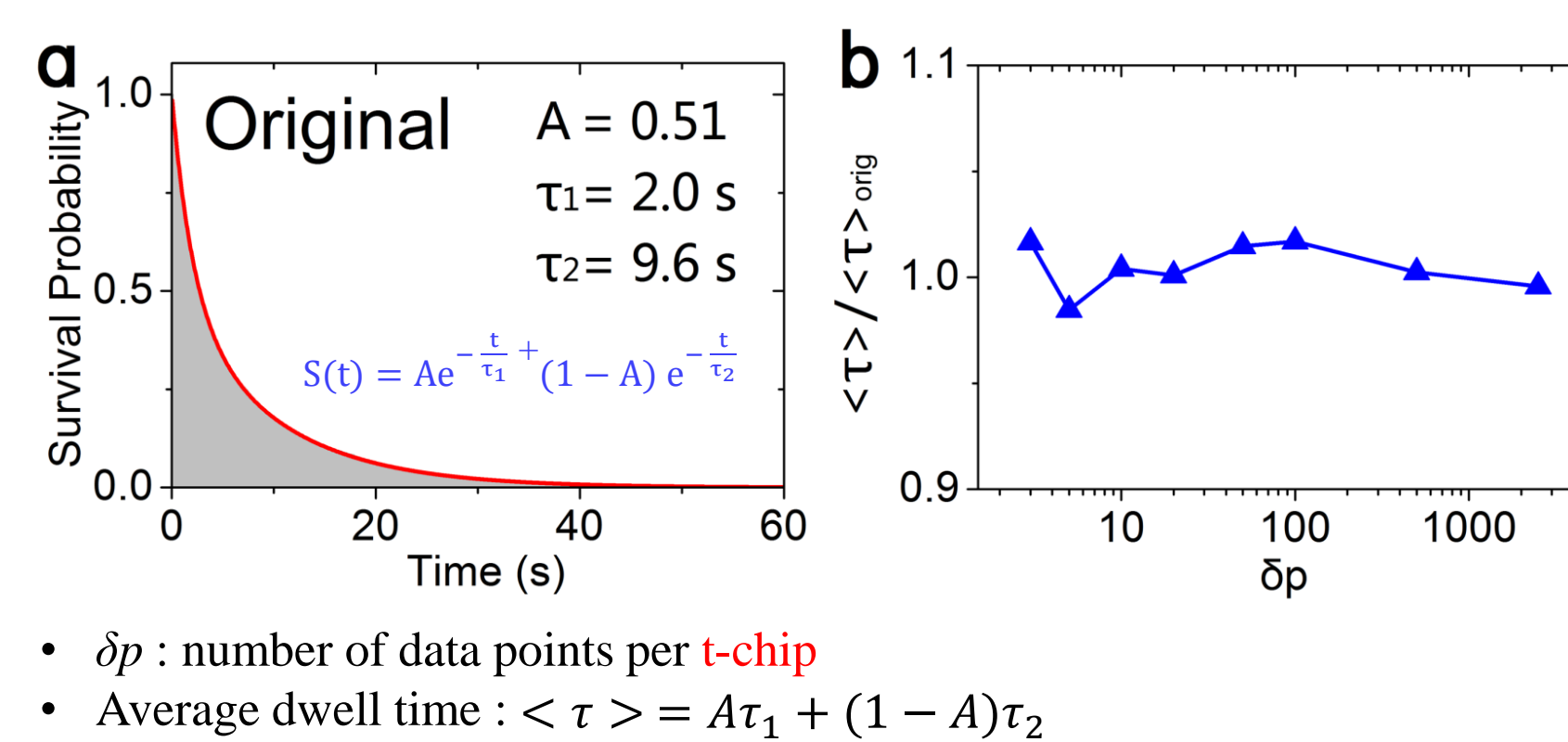


Result & Discussion

sHaRPer is better than HaRP



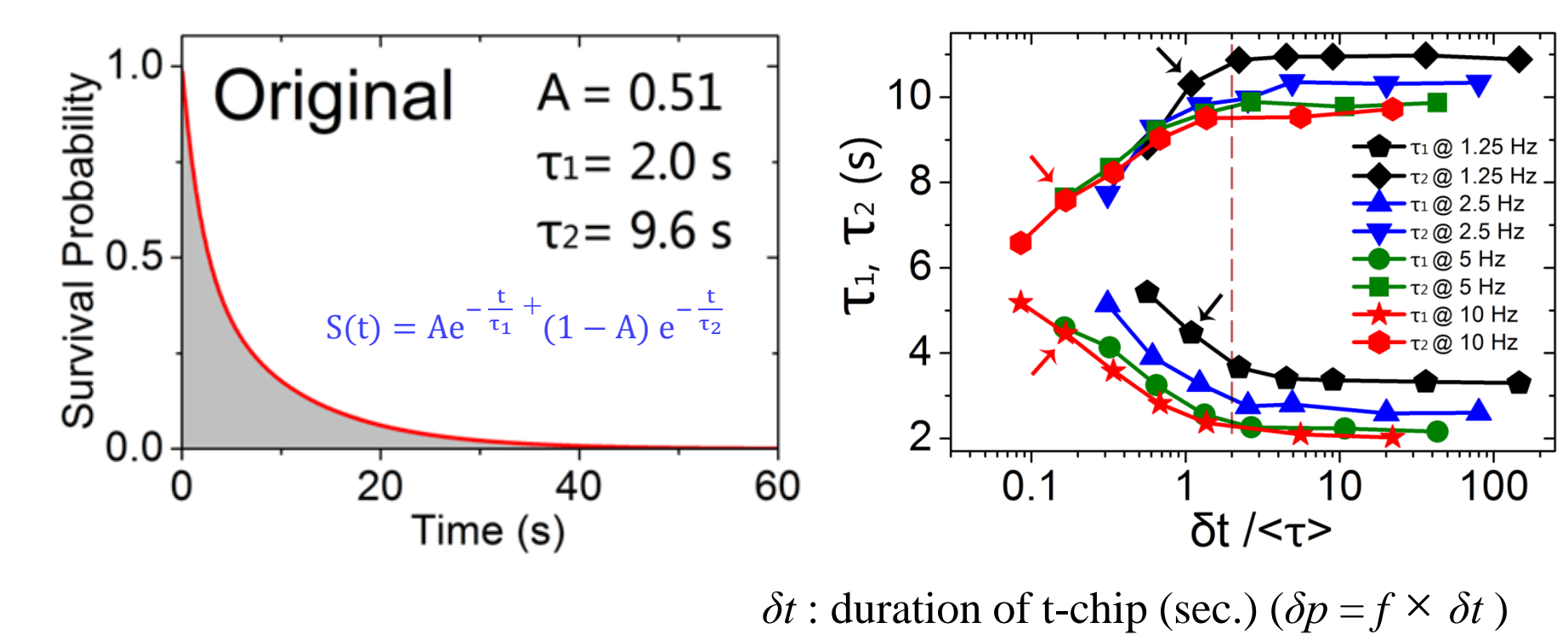
Testing sHaRPer using 'simulation data' (A~0.5)



a. Survival probability of the high FRET state in a simulation trace

b. $\langle \tau \rangle$ by sHaRPer remains **almost constant** for all δp

Kinetic heterogeneity can be degraded if the data acquisition time is considerably short



- Degradation of kinetic heterogeneity is related to δt .
- To prevent the kinetic heterogeneity from being degraded, δt is 2 times greater than $\langle \tau \rangle$.

Summary

- The sHaRPer method is viable means to obtain kinetic information from systems with slow kinetics.
- The sHaRPer method is a universal remedy for average dwell time
- To gain a reliable kinetic information, data acquisition rate($=f$) should be 4 times larger than the average transition rate ($f > 4 / \langle \tau \rangle$).
- To prevent the kinetic heterogeneity from being degraded, δt is 2 times greater than the average dwell time ($\delta t > 2 \langle \tau \rangle$).

Reference

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