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**COMMUNICATION**

# Panorama of DNA hairpin folding observed *via* diffusion-decelerated fluorescence correlation spectroscopy†

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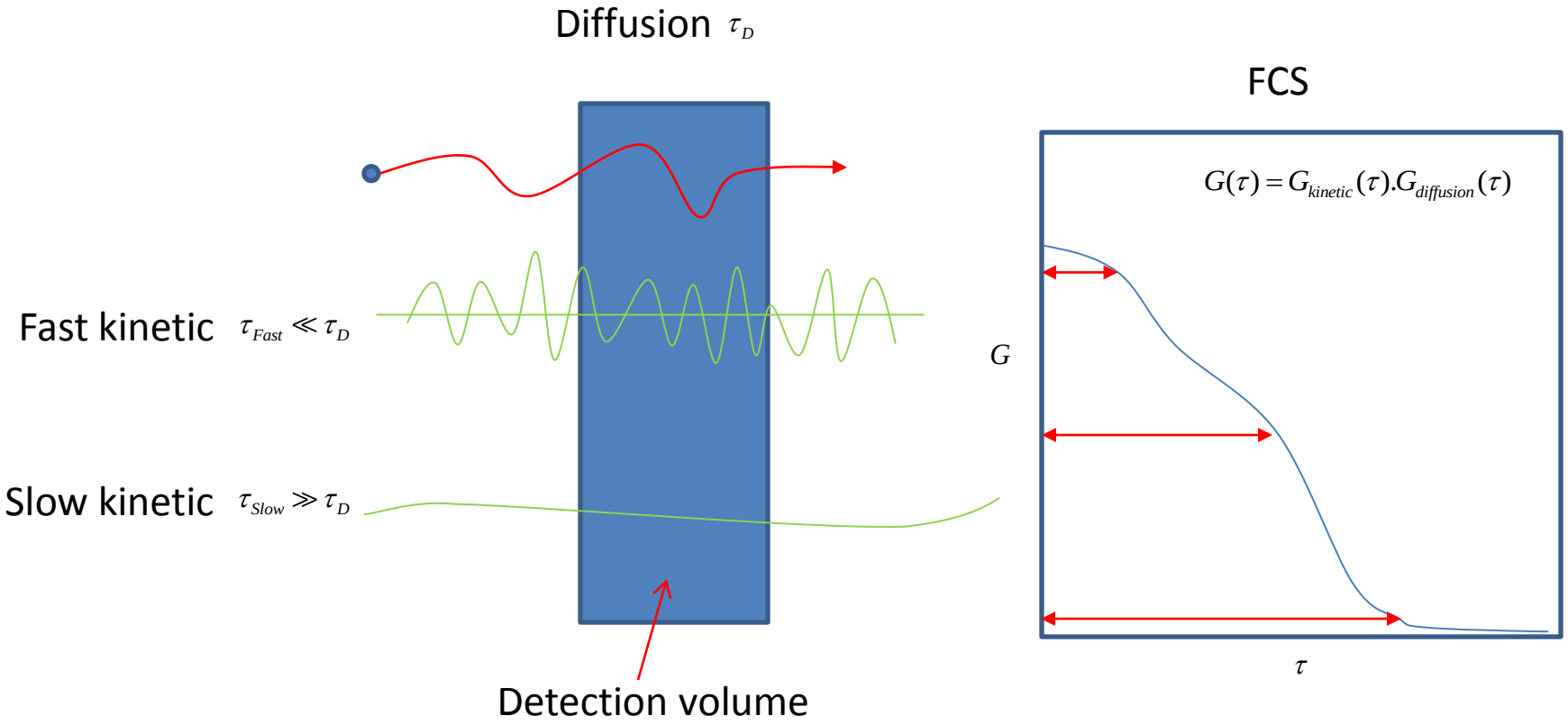
Presented at 2012/09/09
A three state mechanism for DNA hairpn folding characterized by multiparameter FCS

The kinetics of hairpin was proposed by two-state folding model with a relaxation time of tens of microseconds

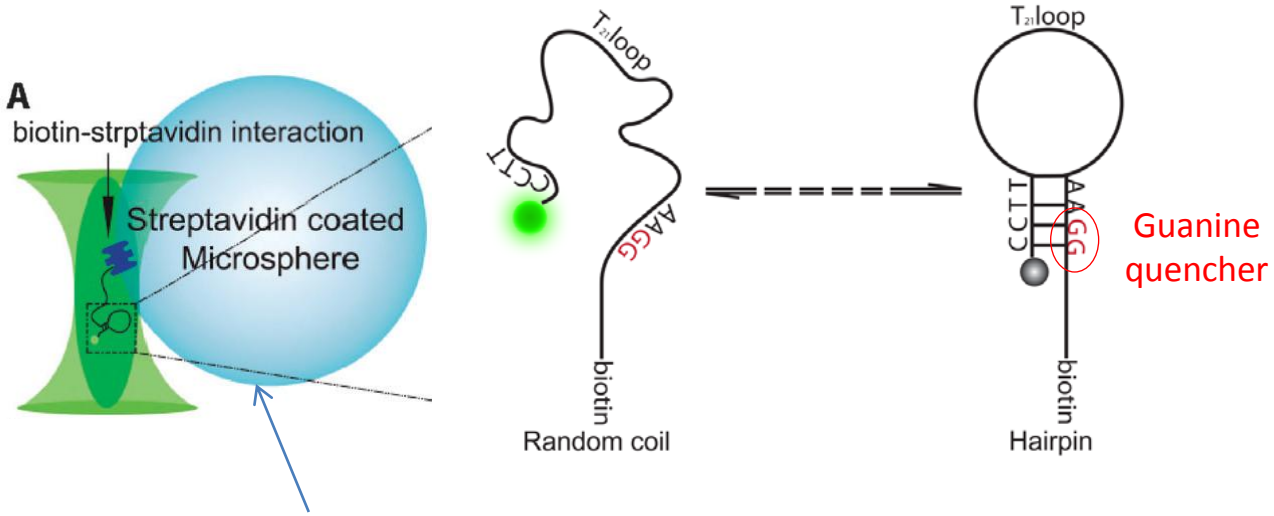
This fast kinetic refers to the relaxation of the intermediate state while the complete folding occurs in the millisecond range




→ Reasonable control $\tau_D$ for each interested kinetic.

-Immobilization $\tau_D = 0$
-Change the object size
-Adjust the viscosity of the solution
-Change the size of detection volume

Change the object size




R=1.87±0.05 μm

*hp4 : 5'*-biotin-TCTCTCTCTCTCTCTCTCTTTTTT GGAA(T)21 TTCC-TMR-3'

*polyT: 5'-biotin-TCTCTCTCTCTCTCTCTCTTTTTT TTTT (T)21 TTTT-TMR-3'.*

→ About 50 DNA molecules were attached to each microsphere

Result I: decelerated signal

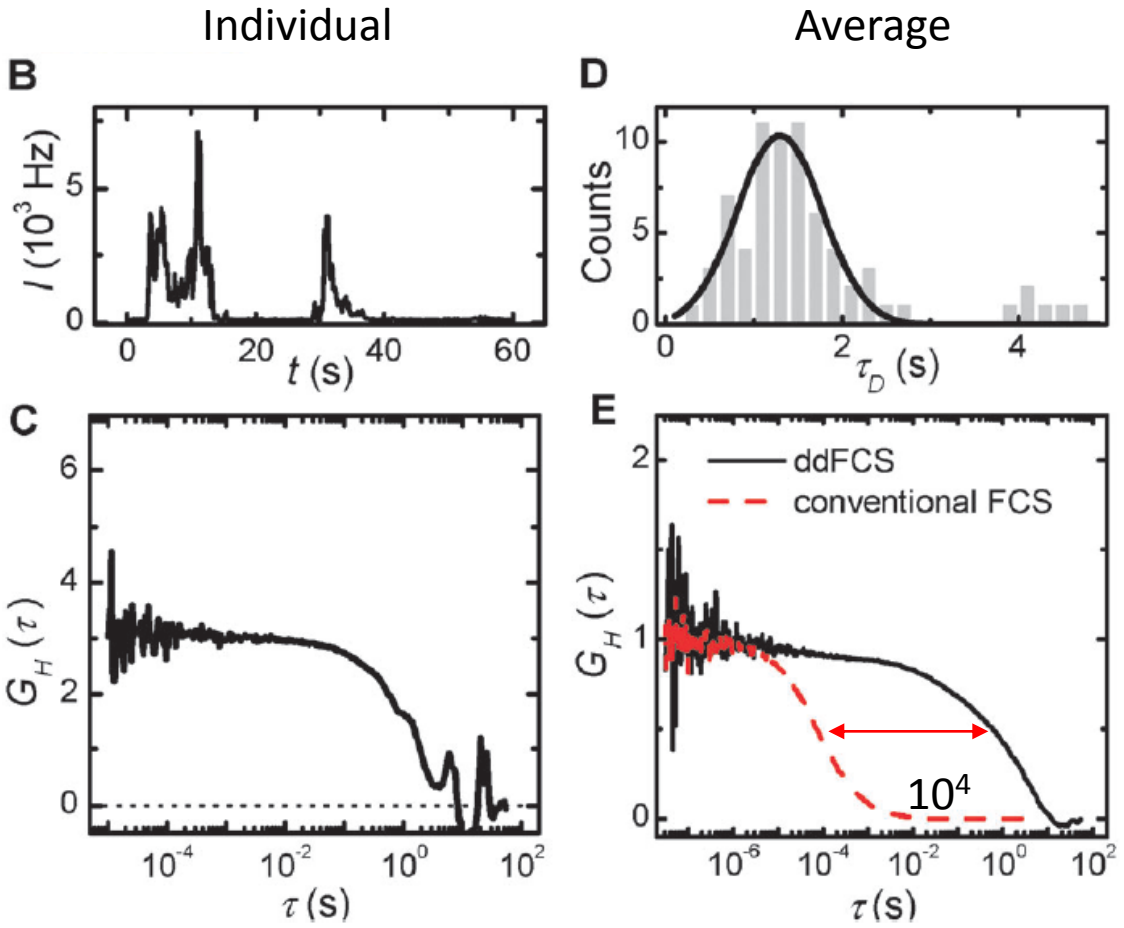
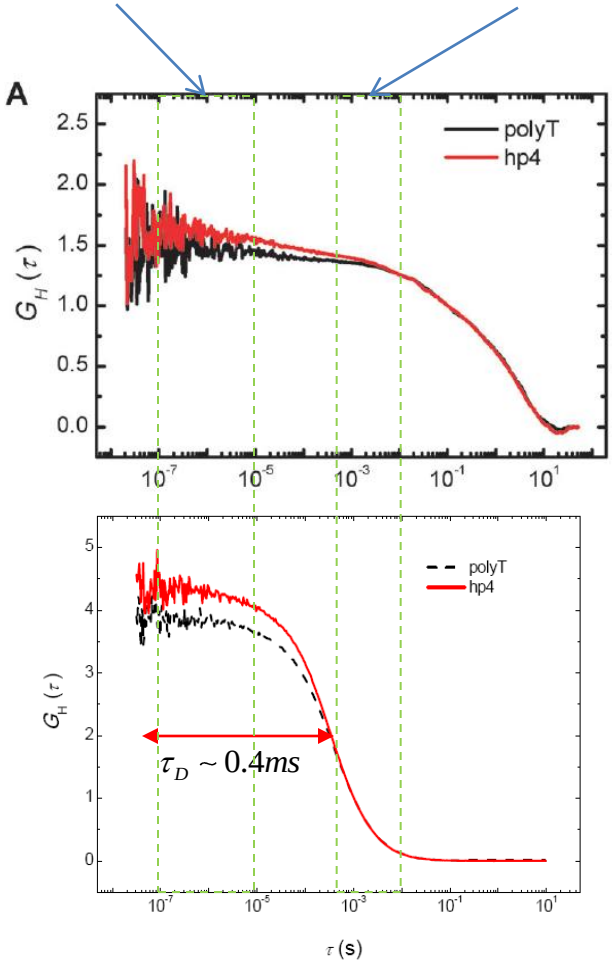



**Fig. 1** Illustration of the features of ddFCS. (A) Schematics of ddFCS; (B) and (C) sample fluorescence-time trace (with bin time of 100 ms for illustration) and corresponding ACF curve; (D) the histogram of diffusion time distribution upon 70 individual ACFs; (E) comparison of ddFCS (solid, black) with the conventional FCS (dash, red) using **polyT**.

fast component
~100 µs

slow component
>1 ms

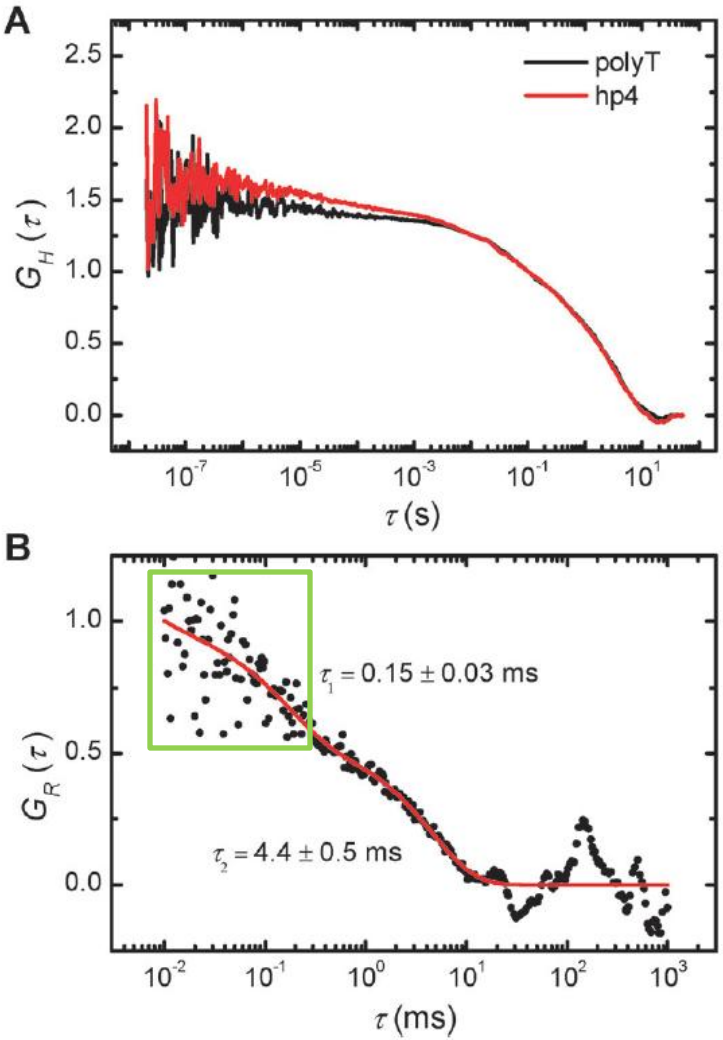


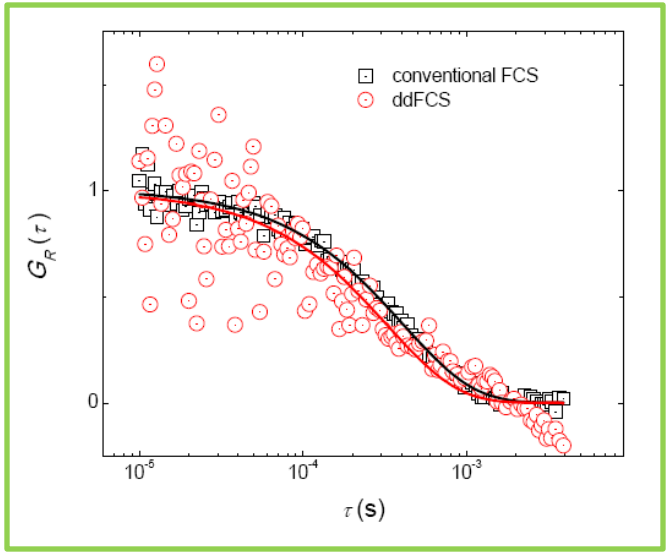

125 mM NaCl

Analysis

$G_R = G_H/G_T$

Reaction Hairpin PolyT

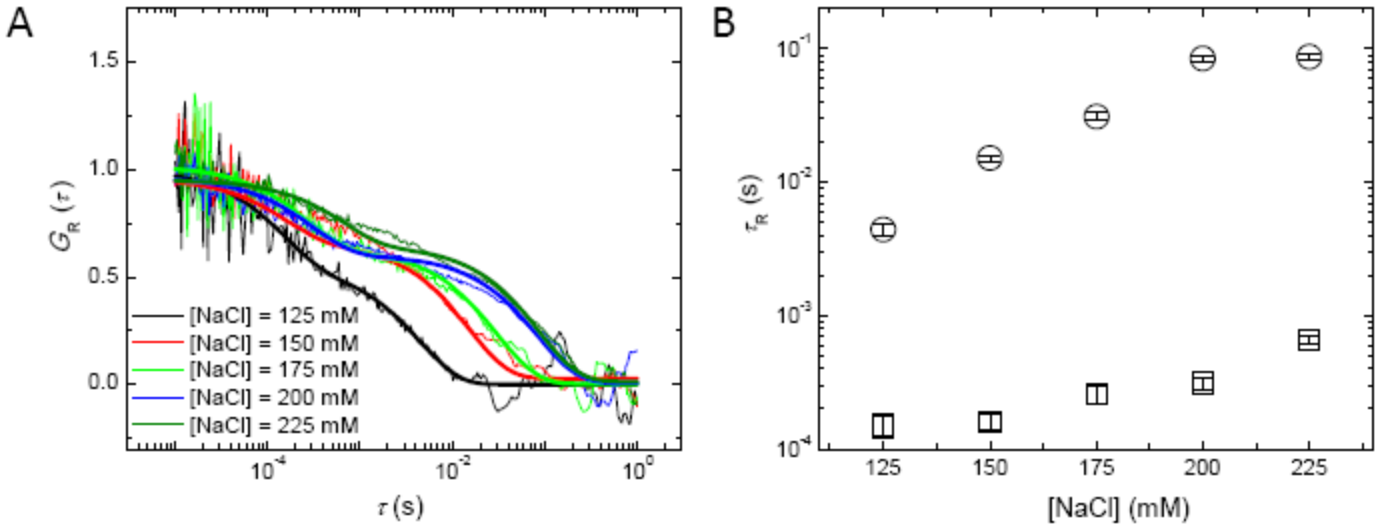



Bead-attached hairpin have the same kinetic with the free one

**Fig. 2** (A) ddFCS data of **polyT** (black) and **hp4** (red); (B) reaction correlation function of **hp4** (dot) and the corresponding fitting (red).

# Result I: Salt concentration dependence



2012/09/09

Cross-correlation function

$$G_C(\tau)-1=\frac{\gamma}{N}\underbrace{\frac{1}{\left(1+\frac{\tau}{\tau_d}\right)}\frac{1}{\sqrt{1+k^2\frac{\tau}{\tau_d}}}}_{G_{Diff}(\tau)}\underbrace{\left(1-T+Te^{-\tau/\tau_T}\right)}_{G_{triplet}(\tau)}\underbrace{\left[1+Be^{-\left(\tau/\tau_R\right)^{\beta}}\right]}_{G_R(\tau)}\underbrace{\exp\left(\frac{-r^2\left(1-\tau/\tau_F\right)}{1+\tau/\tau_d}\right)}_{Flowing}$$

$N$ : number of molecules (one focal volume)

$\gamma$ : geometric correction factor (compair to ideal Gaussian function)

$\tau_d$ : transit time through a focal volume

$\tau_F$ : transit time between two focal volumes $R/V_x$

$r$ : the ratio $R/w$ $T$ : quantum yeild $\tau_T$ : time constant of triplet state

$B$ : amplitude factor $B=K\frac{(1-Q)^2}{(1+QK)^2}$ K: equiblirium distribution F/UnF Q: relative fluorescence intensity

$\tau_R$ : relaxation time of folding and unfolding reaction

$\beta$: strectch parameter.

2012/09/09

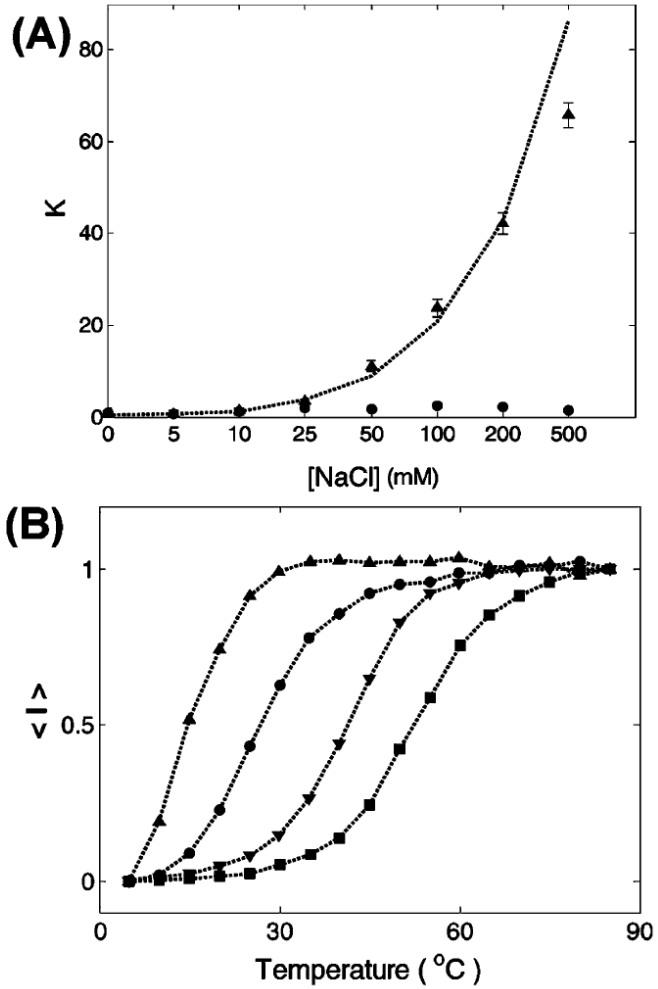



***Figure 3.*** (A) Equilibrium constants of DNA hairpin samples vs NaCl concentration and (B) corresponding melting profiles [data sets with NaCl concentrations of 0 (▲), 25 (●), 100 (▼), and 500 mM (■) are shown]. In panel A, $K_{melt}$ (▲) represents the equilibrium constants evaluated from the melting curves according to eq 12. $K_{FFS}$ (●) represents the equilibrium constants determined from our FCS and PCH analysis. The dotted line in panel A is $K_{melt,3S}$ calculated according to eq 25.

NaCl

$$\bar{N}_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \bar{N}_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \bar{N}_3 \qquad K_1 = \frac{\bar{N}_2}{\bar{N}_1}, K_2 = \frac{\bar{N}_3}{\bar{N}_2}$$

$\bar{N}_1$: refers to the unfolded DNA conformation for which the R6G fluorescence is unquenched

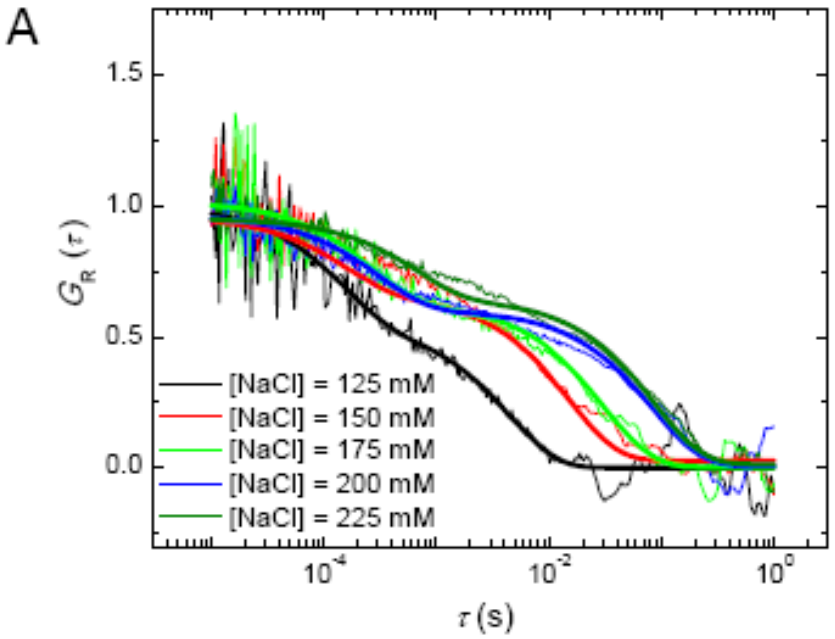
$\bar{N}_2$ : refers to a reaction intermediate that is stable on the sub-millisecond time scale

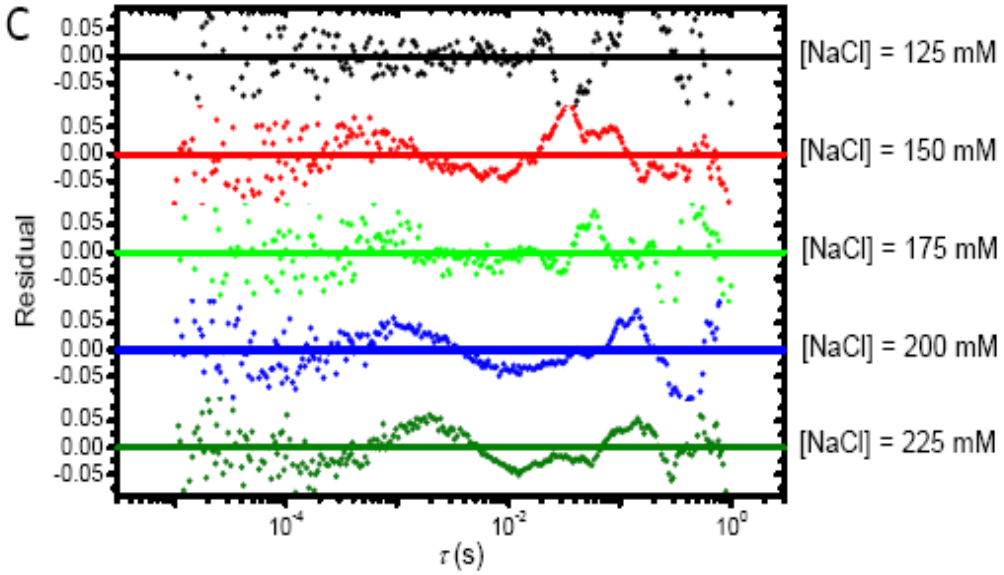
$\bar{N}_3$ : is fully folded DNA hairpin

- The relaxation time of the slow, full folding process should depend on the NaCl concentration.

- The fast process should be less affected

# Discrete exponentials




Two exponentials

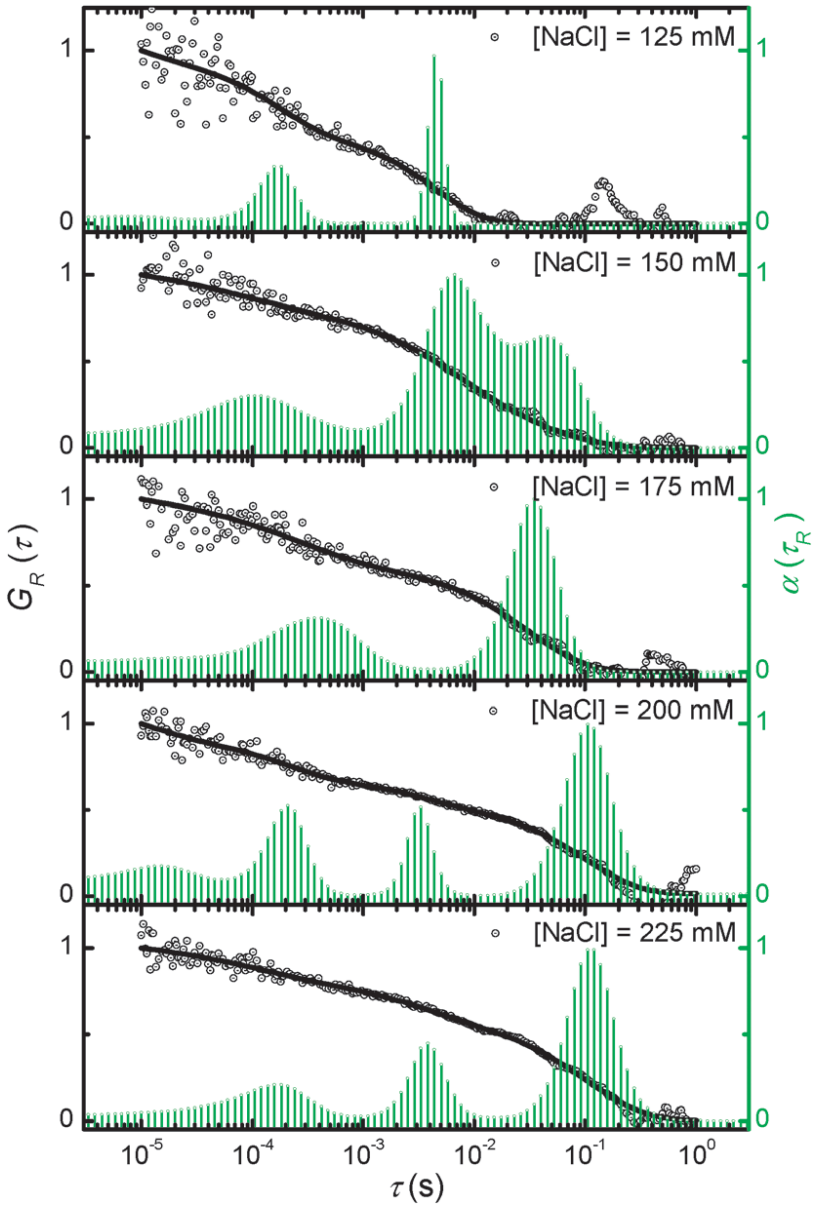
$$G(\tau) \sim \left( \alpha_1 e^{-\frac{\tau}{\tau_1}} + \alpha_2 e^{-\frac{\tau}{\tau_2}} \right)$$

Multi-exponentials

$$G(\tau) \sim \sum_i \alpha_i e^{-\frac{\tau}{\tau_i}}$$

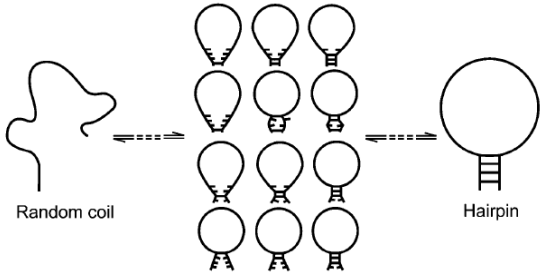
Maximum Entropy Method (MEM).

# Results




125 - 175 mM NaCl: two major kinetic components ~200 ms and ~5ms
The faster process less affected; the slower process more affected

up to 200 mM: one more kinetic component was found



# Resume

-Interested technique that slowdown the diffusion process without introducing any effect on the kinetic of the DNA hairpins.

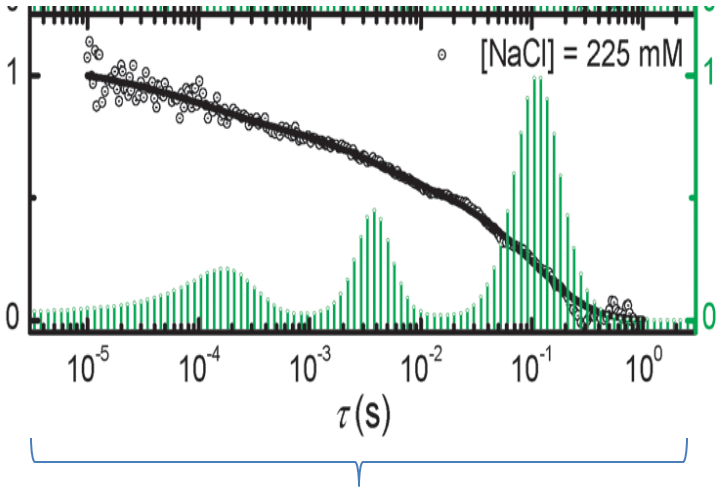
-The relaxation time of full hairpin folding is on the order of ms instead of μs.

-There are multiple intermediates with different relaxation times.

-MEM is a reasonable way to analyze precisely multi-exponential distributions.

$$G(\tau) \sim \sum_{i}^{N} \alpha_i e^{-\frac{\tau}{i\Delta\tau}}$$

$$S = -\sum_{i}^{N} \alpha_i \log(\alpha_i)$$




N x $\Delta\tau$

Star at

$$S_{\max} = -\log\left(\frac{1}{N}\right) \text{ while } \alpha_i = \frac{1}{N}$$

$$\chi^2 = \frac{\frac{1}{N}\sum_{i=1}^{N}\left[G_{simul}(t_i) - G_{data}(t_i)\right]^2}{G_{data}(t_i)^2}$$