Conformational Dynamics of Telomeric DNA via Single Molecule FRET

D. S. Kim

School of Physics, Seoul Nat'l Univ., South Korea

Nov. 2nd 2005

Contents

1. Introduction
    Single Molecule Spectroscopy & FRET
    Telomere & G-quadruplex
2. Conformation Dynamics
    Human Telomeric DNA
    Mutated Human Telomeric DNA
    Protein Binding
    4-quartet G-quadruplexes
3. Summaries

Why Single Molecule?

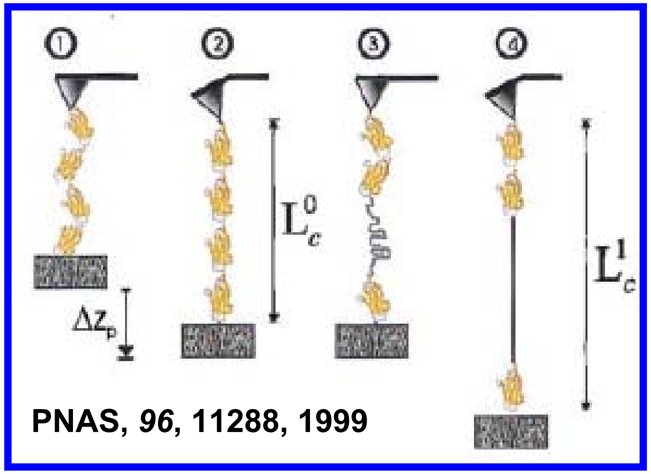
- ***Remove the ensemble average effect***
  - ► Construction of a frequency histogram of the actual distribution of values for an experimental parameter
  - ► Give more information than standard ensemble measurements

- Observation of ***exact dynamics*** between conformational states ***without synchronization***

Single Molecule Spectroscopy

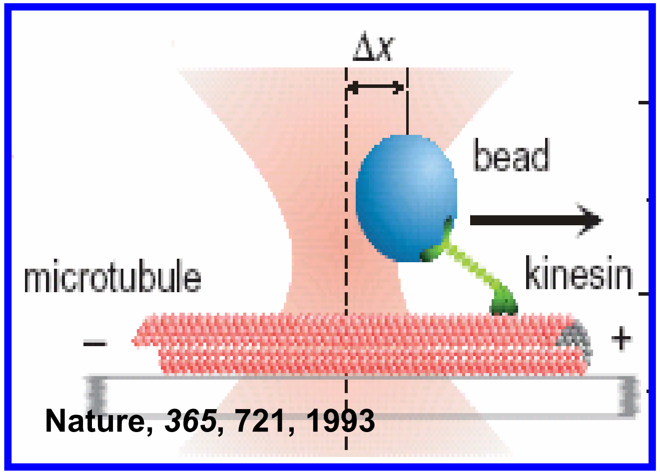
UPL
Seoul National University

AFM



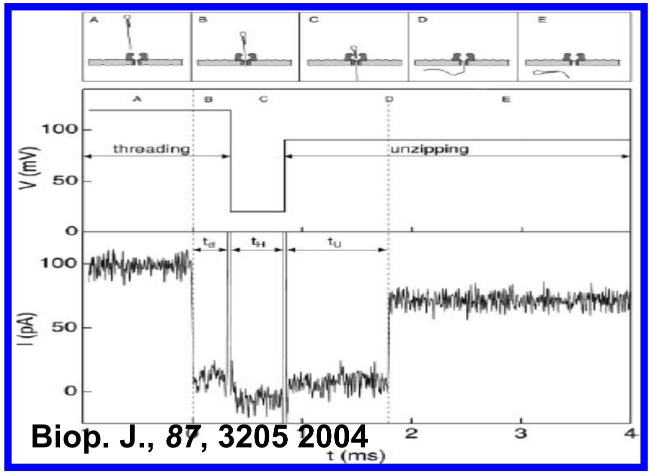
PNAS, *96*, 11288, 1999

Optical Tweezers



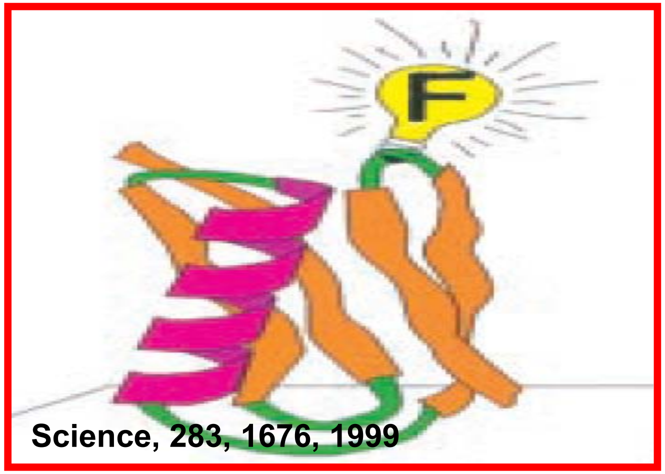
Nature, *365*, 721, 1993

Electronics



Biop. J., *87*, 3205 2004

Fluorescence



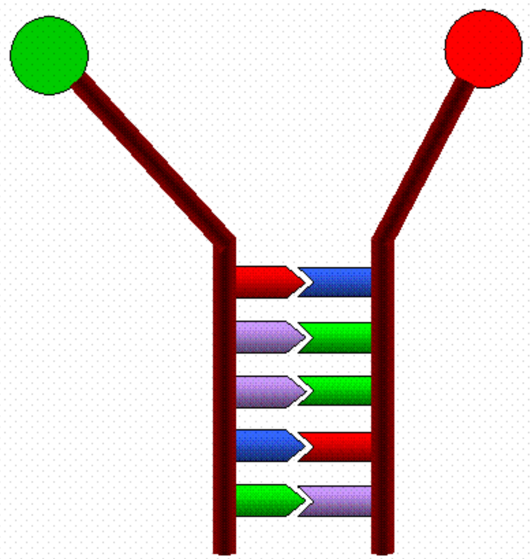
Science, 283, 1676, 1999

FRET

Fluorescence Resonance Energy Transfer

Energy Transfer by dipole-dipole interaction

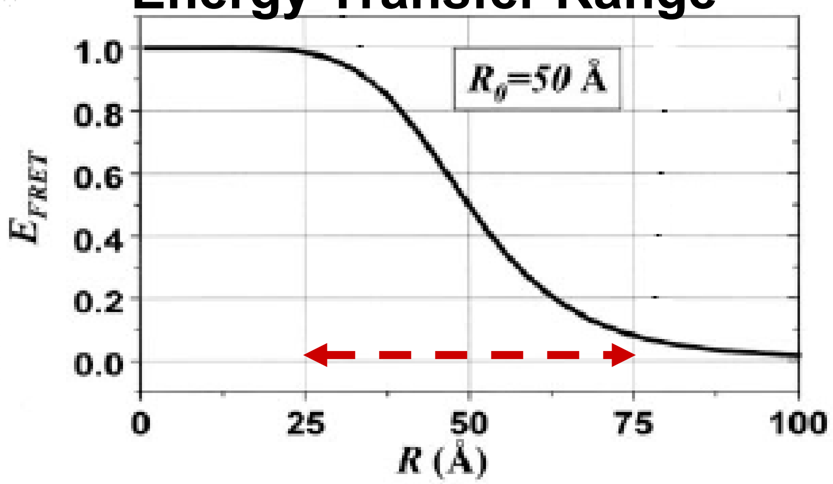
between two fluorophores, donor and acceptor



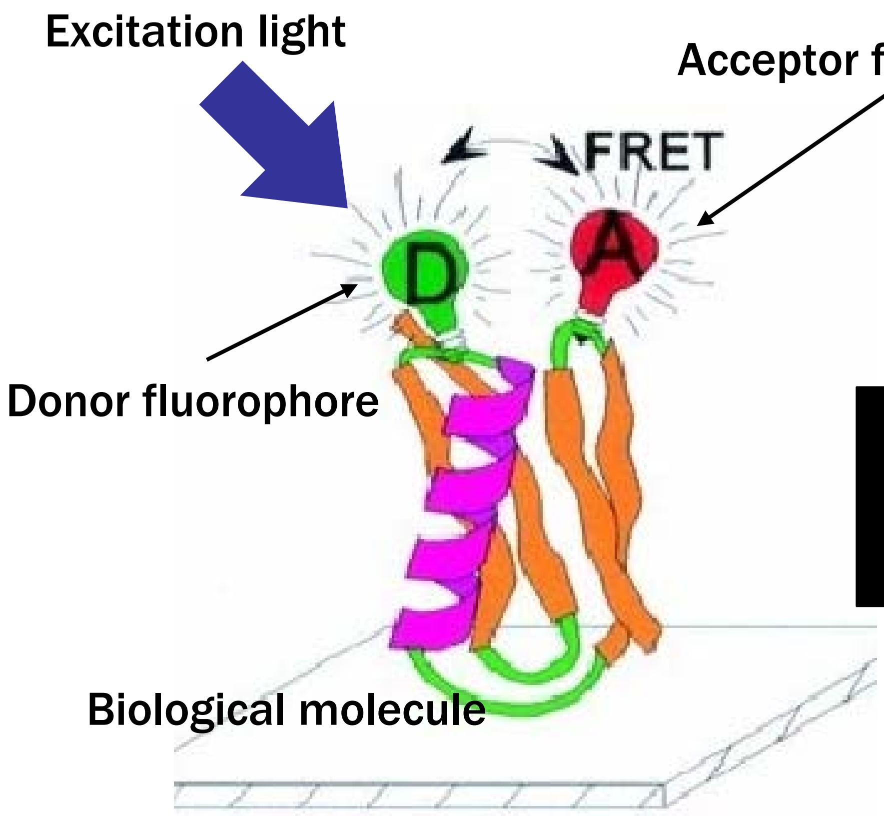
Energy Transfer Efficiency

$$E = \frac{1}{1+(R/R_0)^6} \sim \frac{I_a}{I_a + I_d}$$

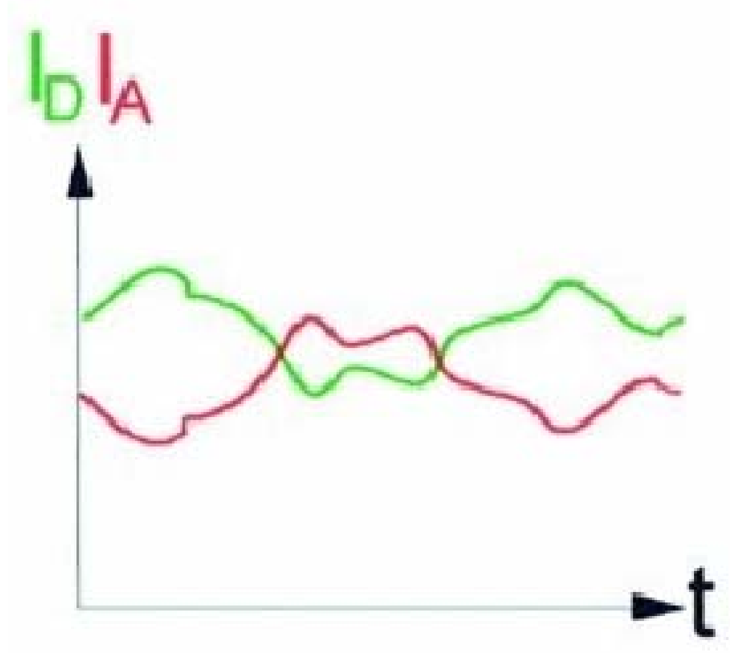
Energy Transfer Range



Single Molecule FRET (smFRET)



Schematic diagram of smFRET

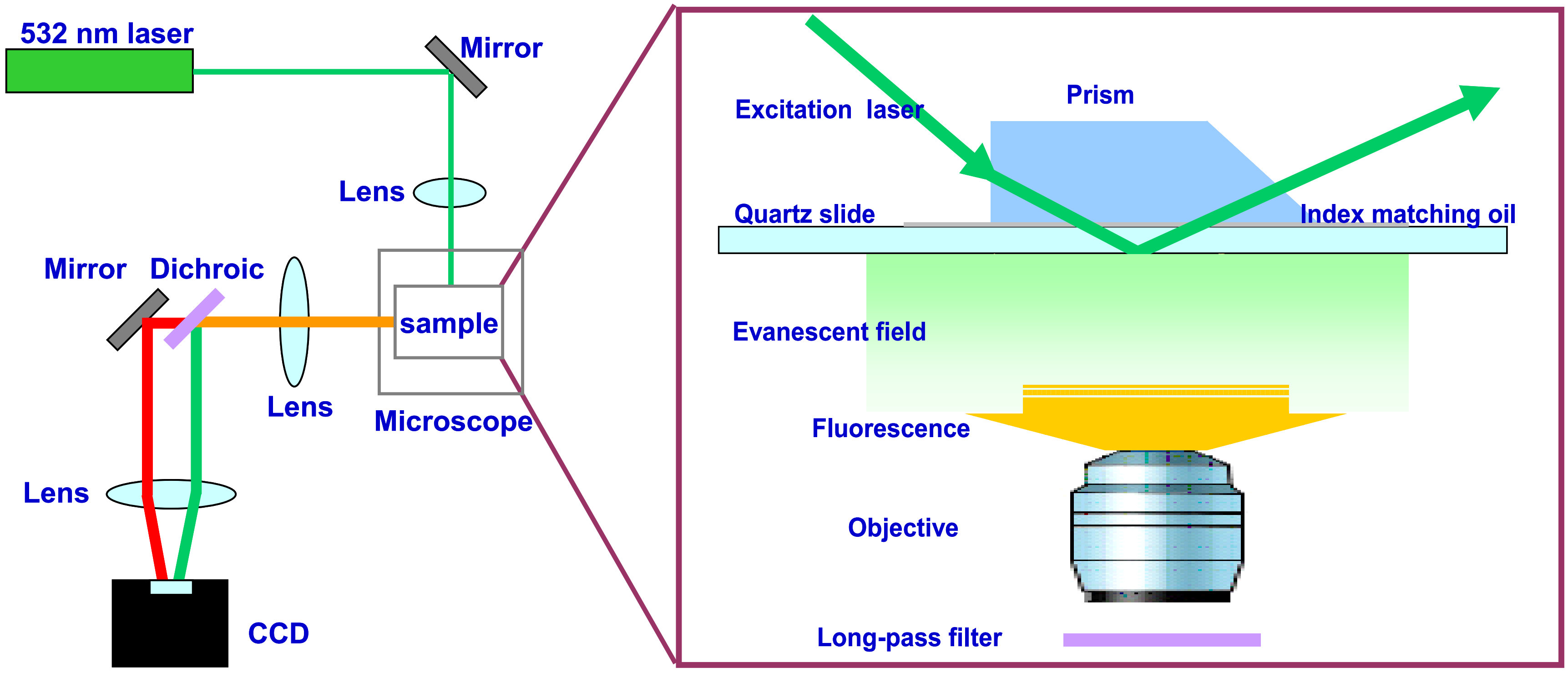


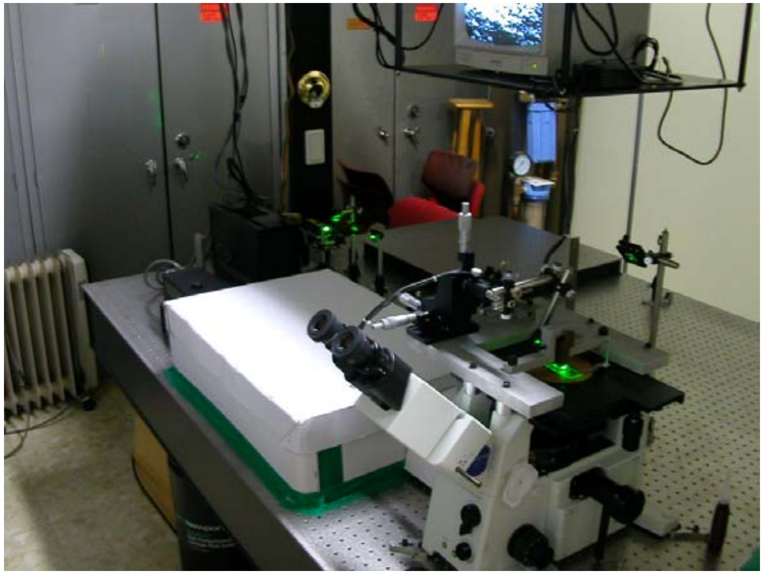
Fluorescence intensity change by conformational changes of a single molecule

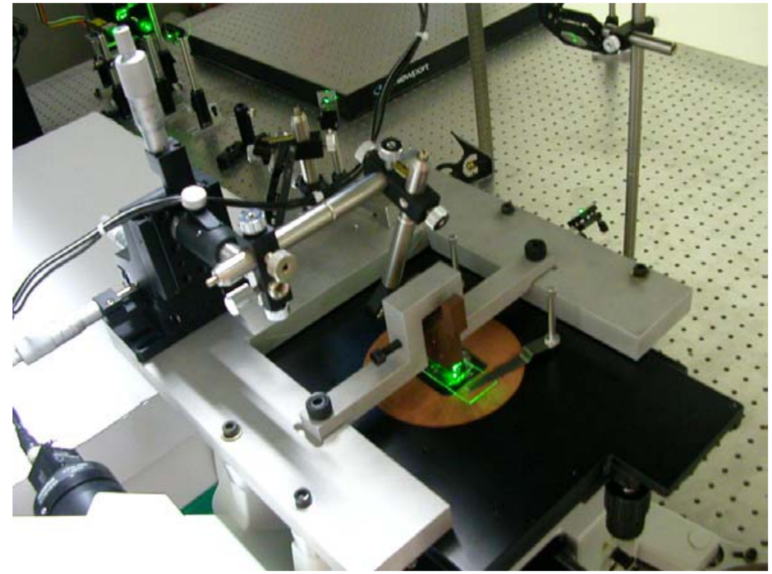
S. Weiss, Science, *283*, 1676, 1999

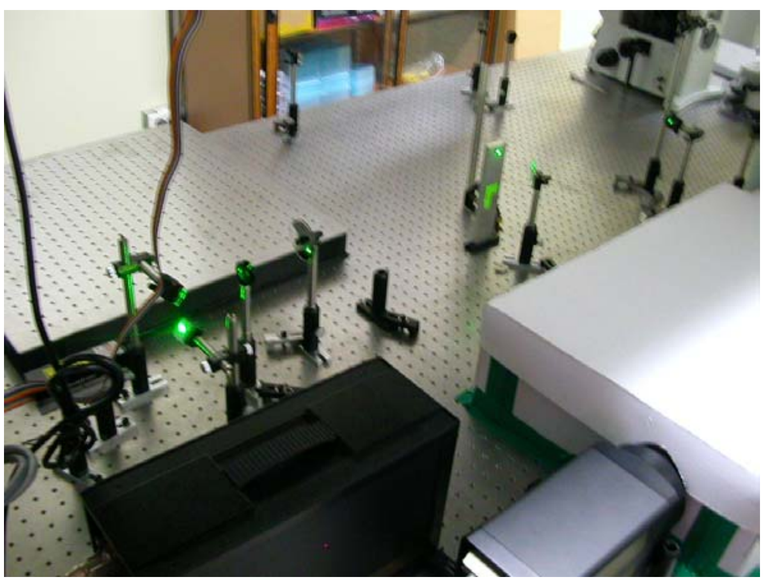
Experimental Setup for smFRET

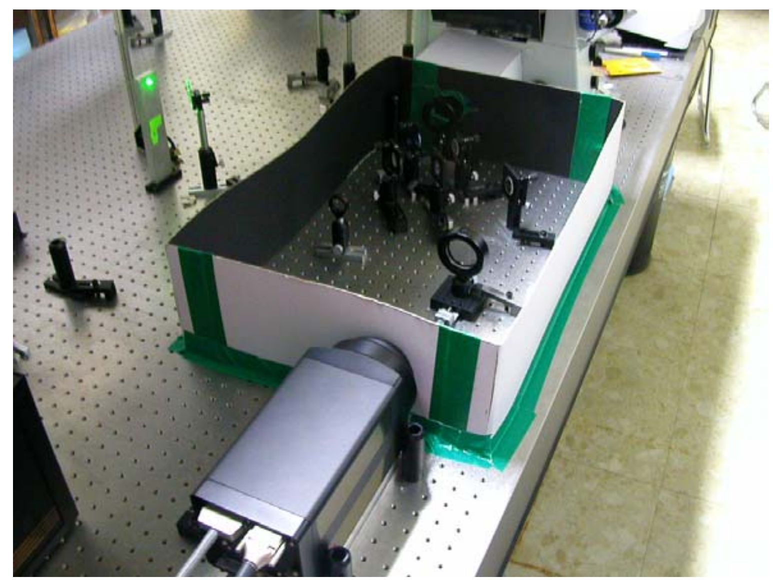
Prism-type TIR











Telomere

&

G-quadruplex

Telomere

Definition

The end regions of the chromosomes

- non-coding DNA: tandem repeat of short sequences
- associated proteins

Function of telomere

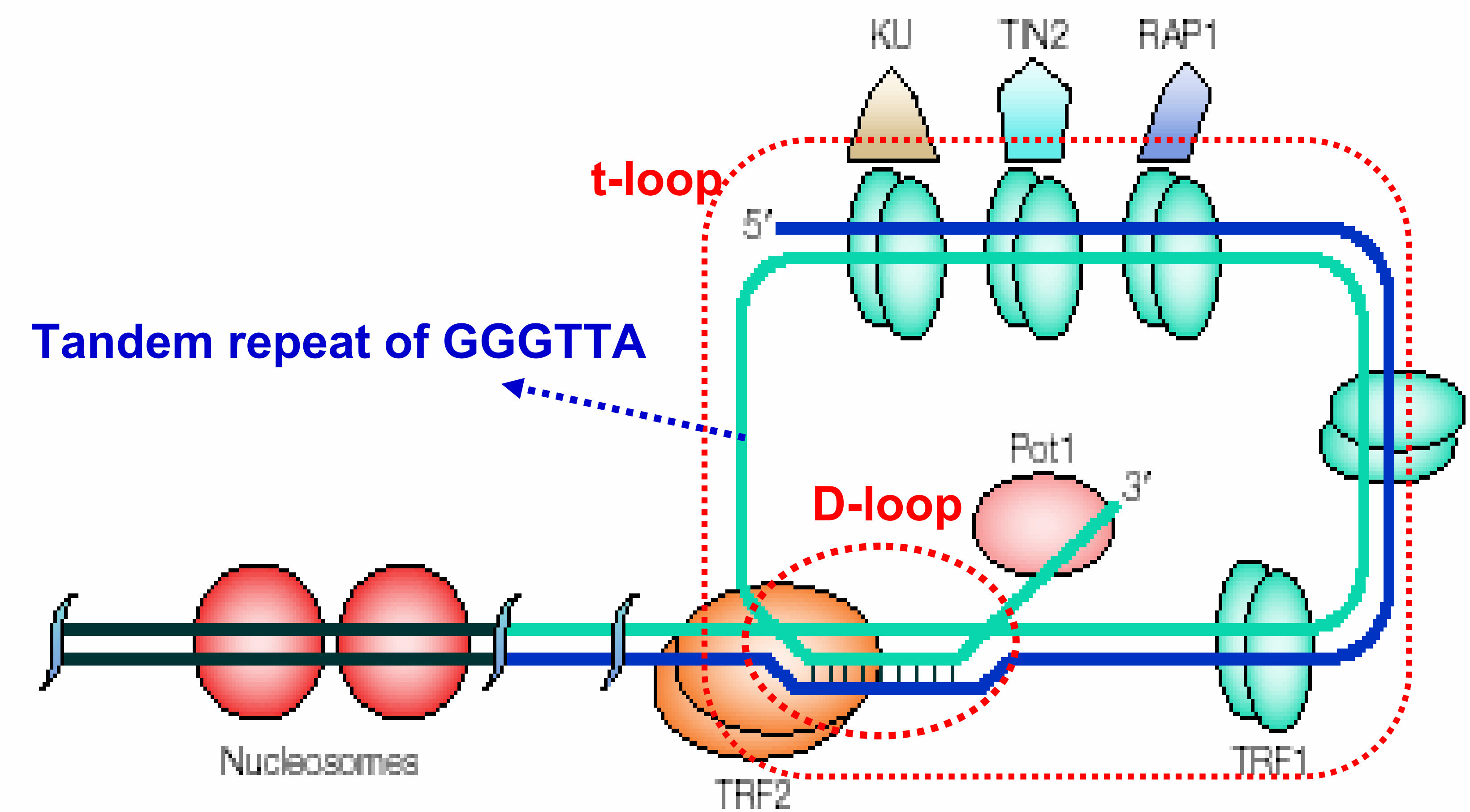
Protection of the chromosome ends from degradation

- end-to-end fusion
- damage by exo-nucleases

Shortened as the cell division goes on

- ***Apoptotic process***

Human Telomere Structure

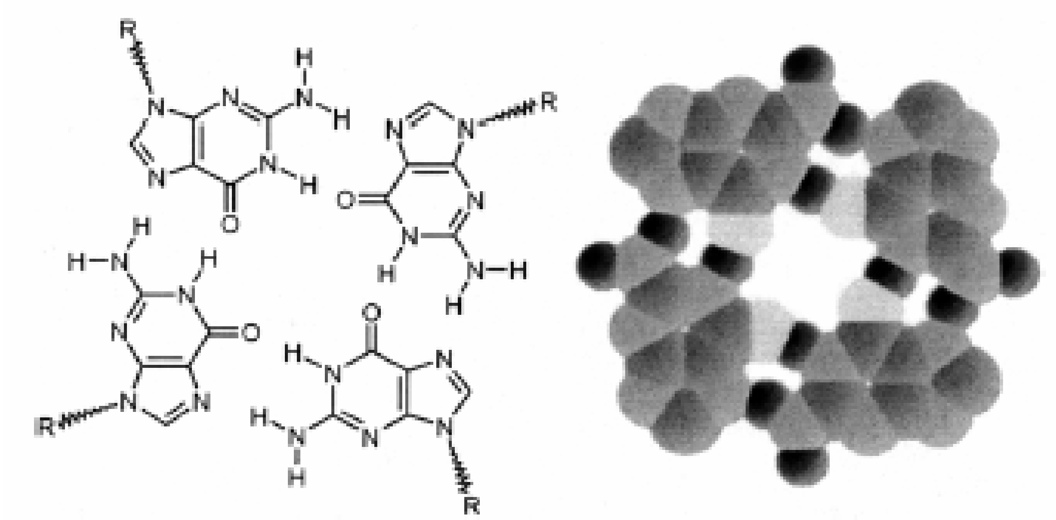


S. Neidle & G. Parkinson, Nature Drug Discovery, *1*, 383, 2002

Guanine Tetrad (G-quartet)

A square co-planar bonding of 4 guanine bases, where each base is both the donor and acceptor of 2 hydrogen bonds with its neighbors

- Hoogsteen bonding



Guanine tetrad (G-quartet)

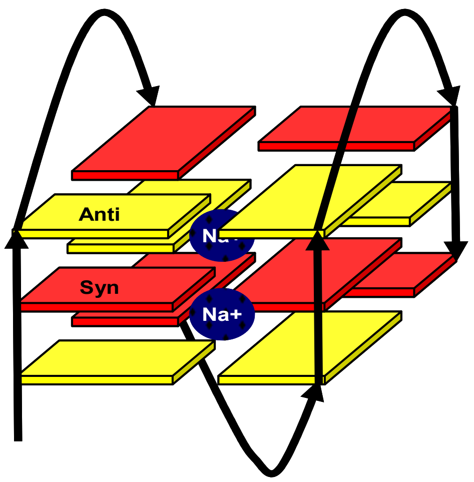
T. Simonsson, Biol. Chem., *382*, 621, 2001

Human G-quadruplex

- **Human telomeric DNA: short tandem repeat (GGGTTA)**
- **Polymorphisms of Human G-quadruplex**

Anti-parallel

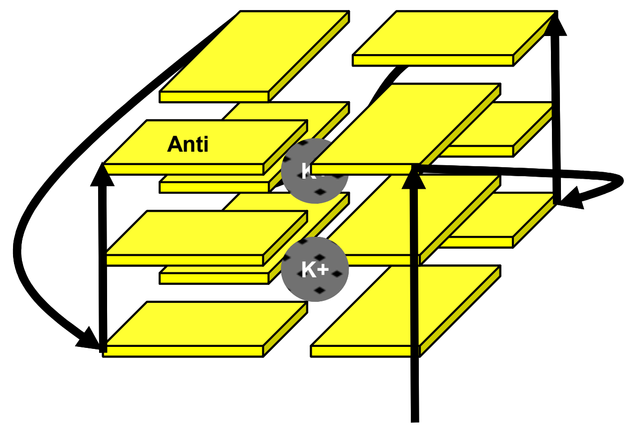
Na+ based conformation from NMR



Y. Wang *et al.* Structure, *1*, 263, 1993

Parallel

K+ based conformation from x-ray crystallography



G. N. Parkinson *et al.*, Nature, *417*, 877, 2002

Conformational Dynamics

Experiments

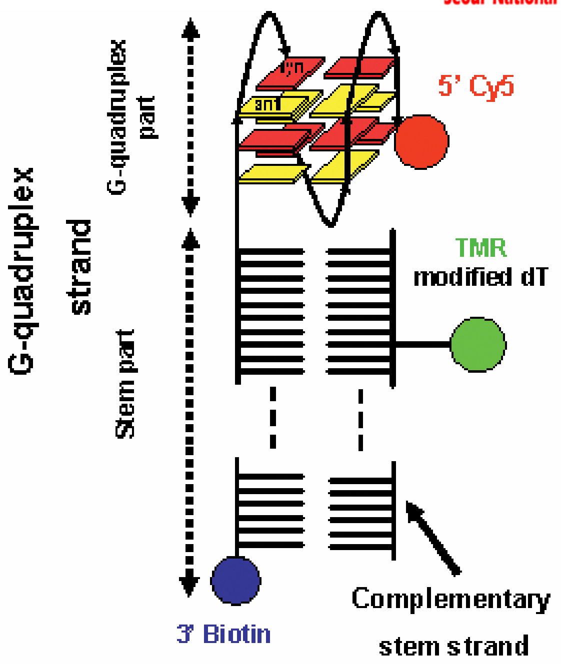
Sample

G-quadruplex part

5’-**Cy5-GGGTTAGGGTTAGGGTTAGGG**
AGAGGTAAAAGGATAATGGCCACGGTGCG-
3’-**biotin**

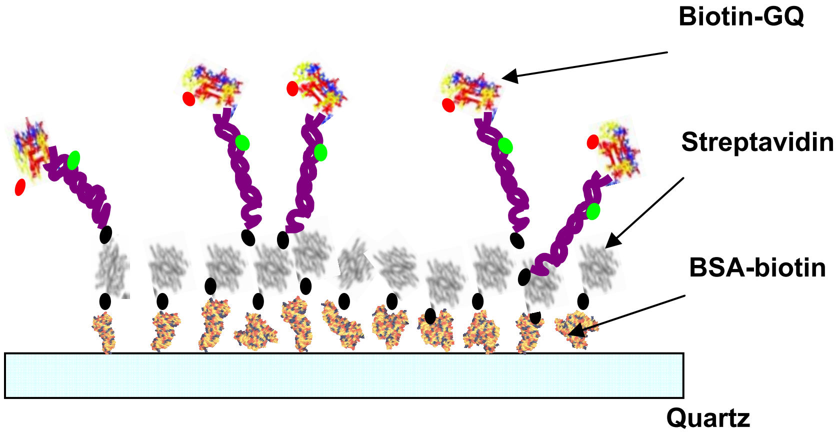
Complementary stem part

5’-CGCACCGTGGCCATTATCCTT
T*TACCTCT-3’
(T*: TMR-labeled Thymine)



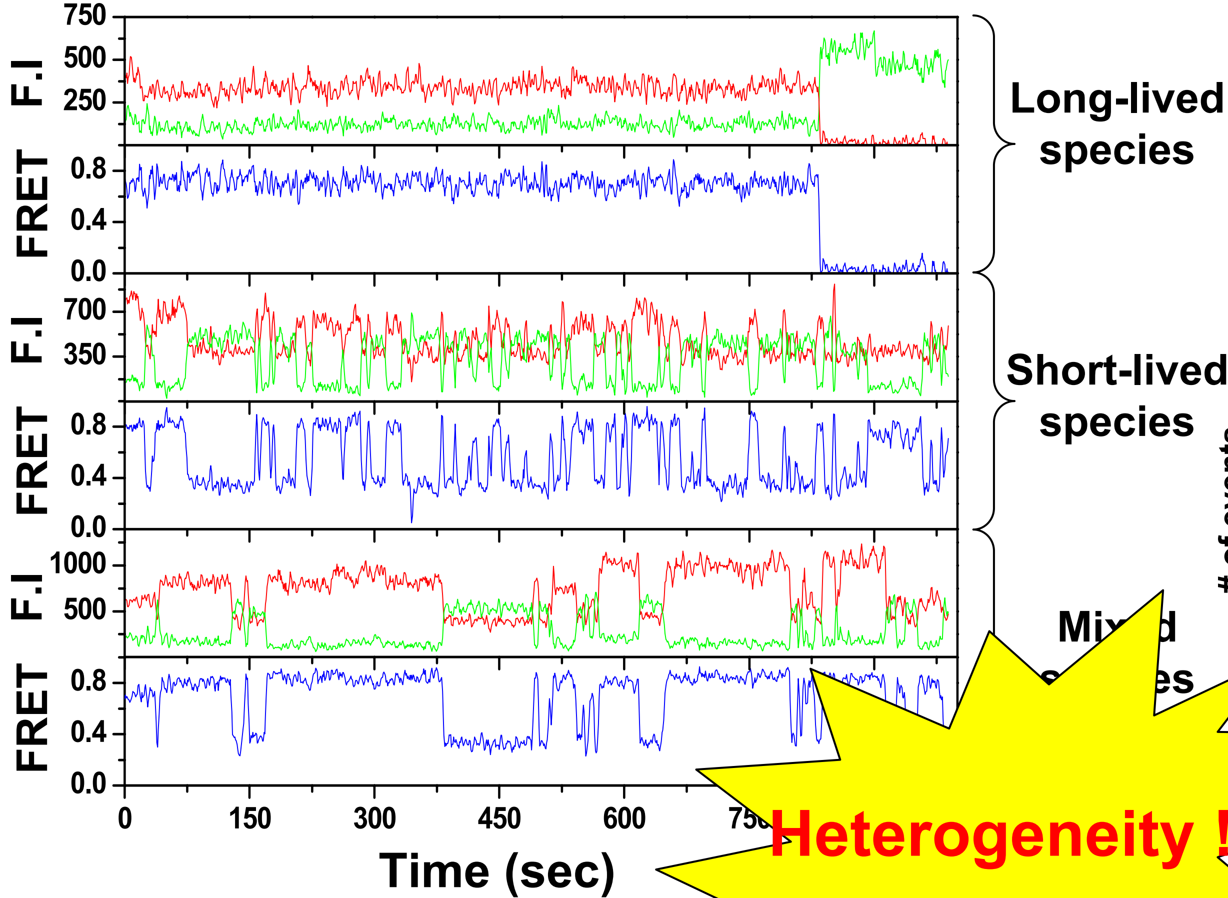
Immobilization of samples

BSA-biotin & Streptavidin

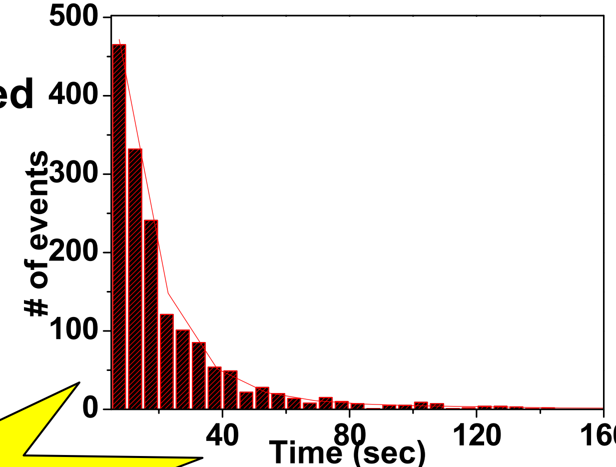


Heterogeneity of G-quadruplex

At 2 mM K+



Dwell time analysis for mixed species



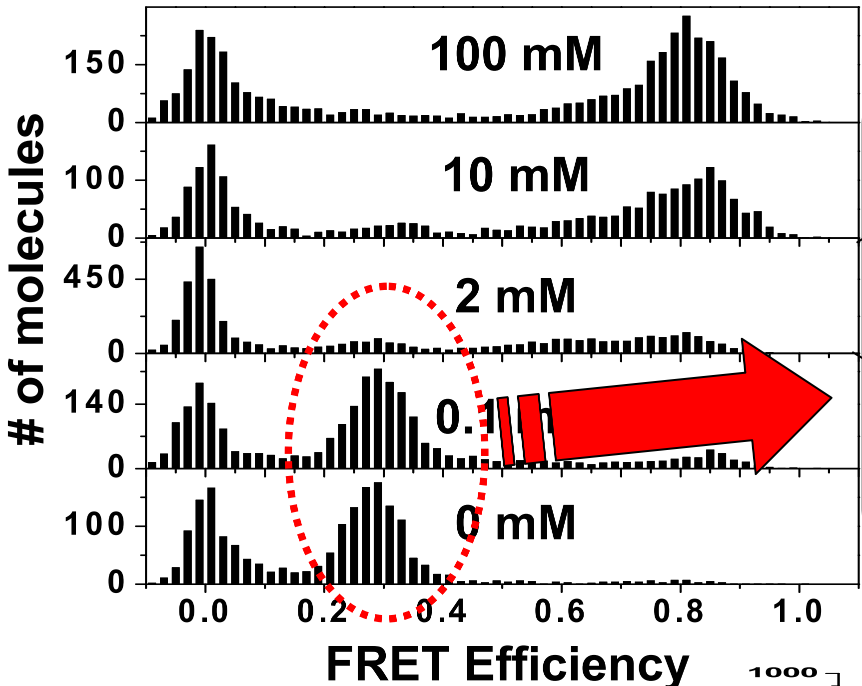
Double exponential fitting

$t_1 \sim 110$ sec & $t_2 \sim 15$ sec

Heterogeneity !

Titration of K+ at RT

Population histogram



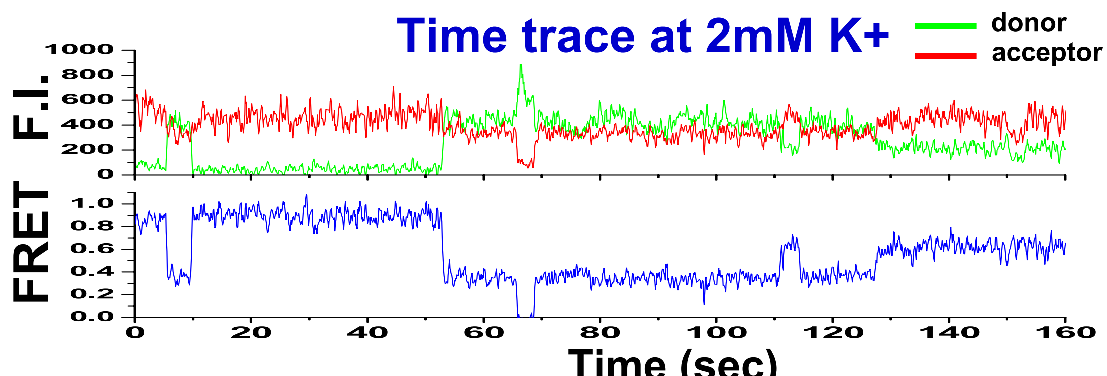
Before transition, the molecule must go through the low FRET

Low FRET

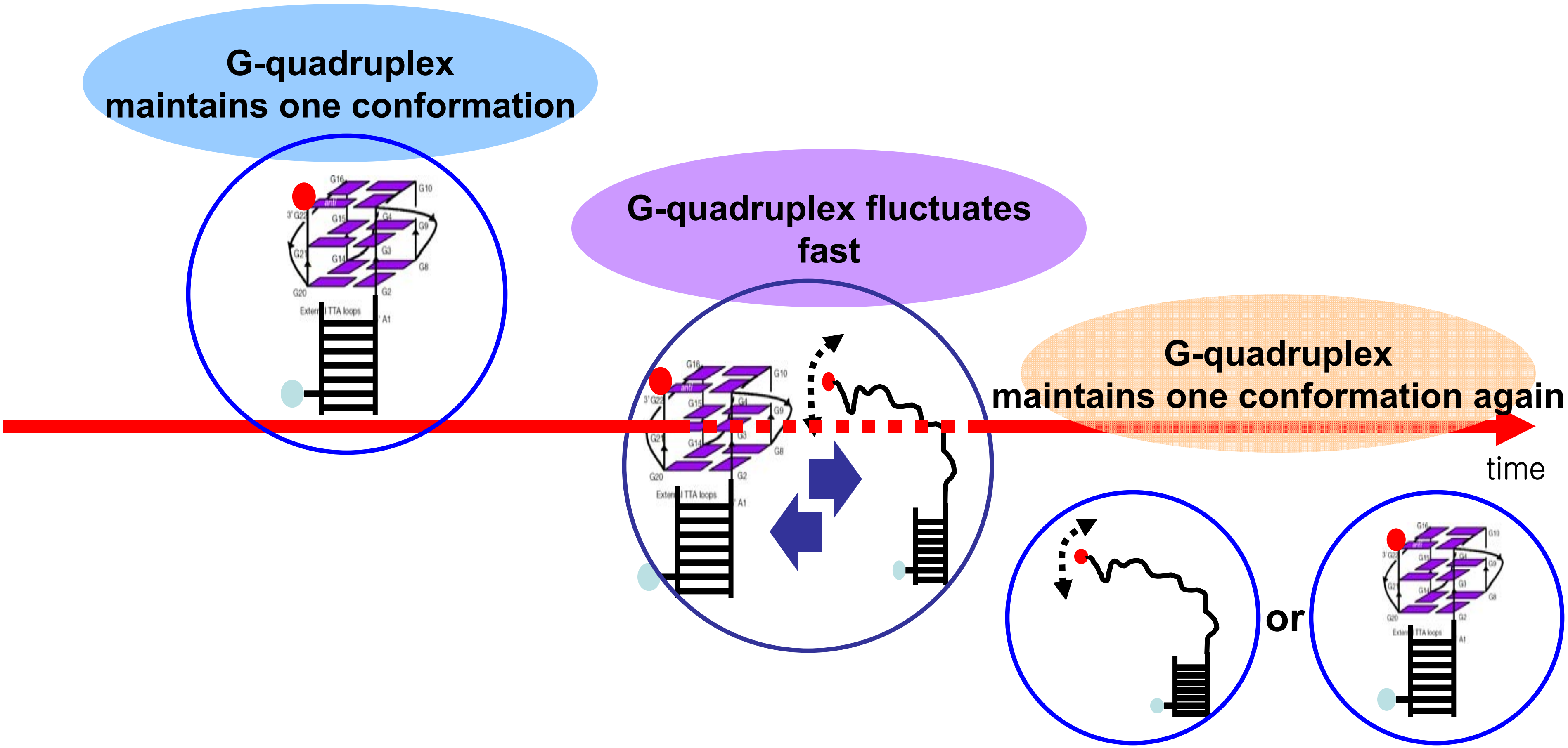
▼

Unfolded single-strand overhang

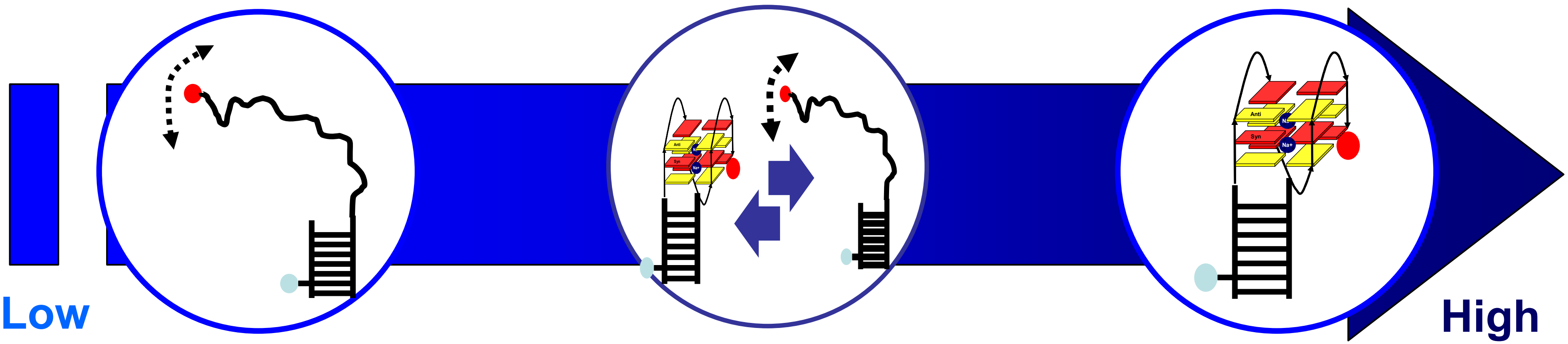
Time trace at 2mM K+



Dynamics of G-Quadruplex



Trend by Concentration at RT



Low Conc.

Long-lived Unfolded

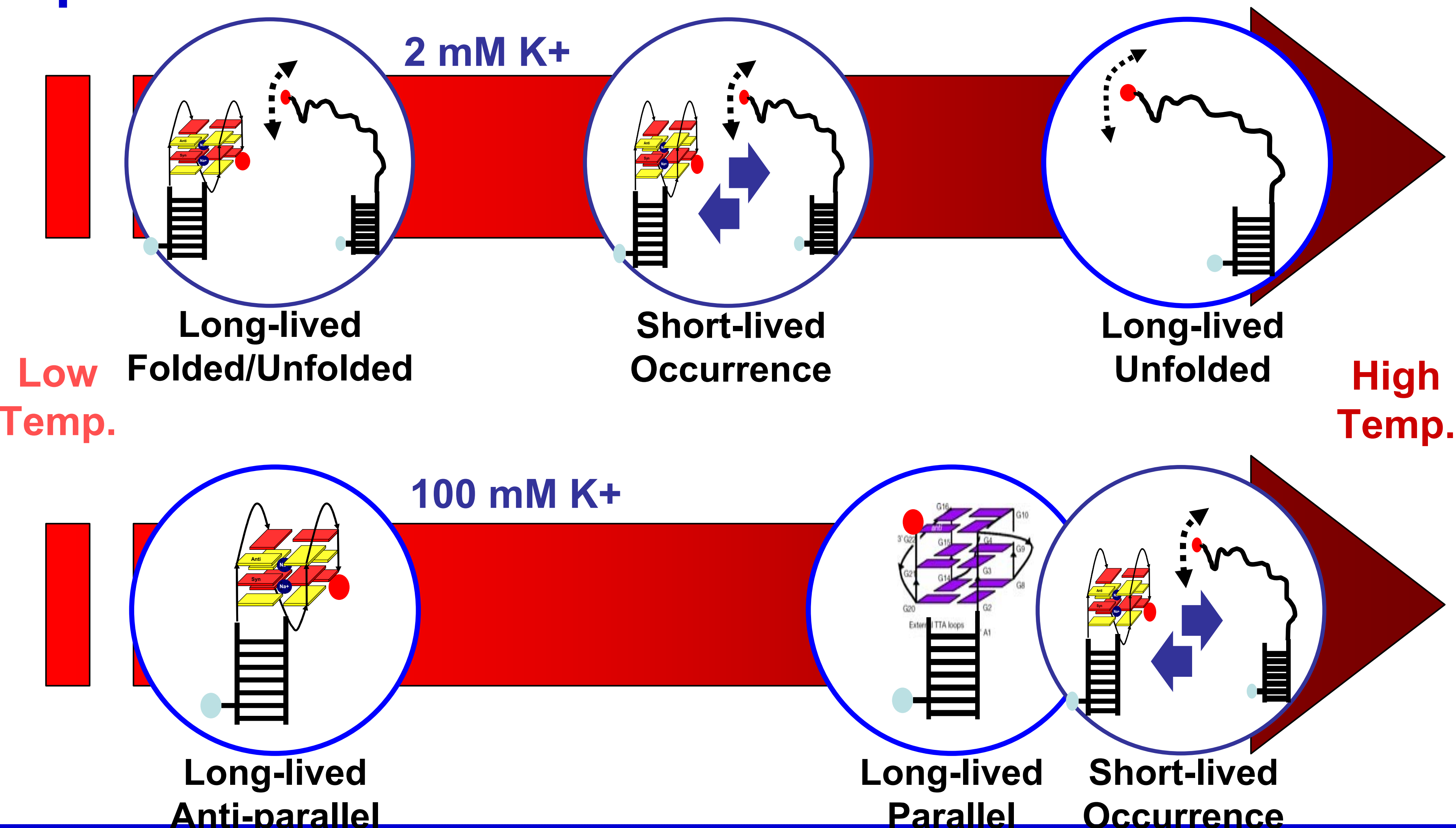
All species are mixed

at 2 mM K+

Long-lived Anti-parallel

High Conc.

Trend by Temperature



Mutated Human G-quadruplex

Why Mutation?

The most important role of G-quadruplexes

► ***Protection of telomere***

One way for protection

► ***Prevention from the binding of external proteins*** with G-quadruplexes

Comparison between wild-type and mutated G-quadruplexes

► **Confirmation of the role of G-quadruplexes**

► **Observation of the protein binding mechanism**

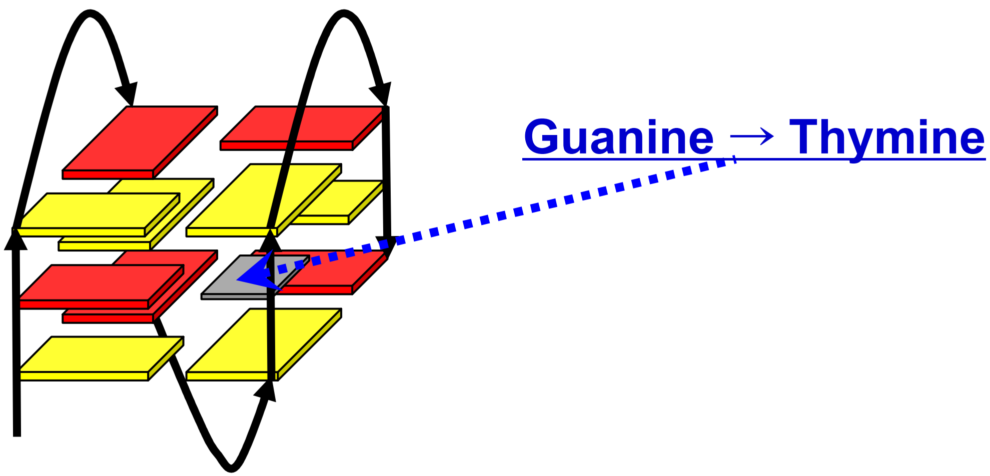
Single-base Mutation

Single-base mutated G-quadruplex part

5'**-Cy5-GGGTTAGGGTTAGTGTTAGGG**
AGAGGTAAAAGGATAATGGCCACGGTGCG-3'**-biotin**

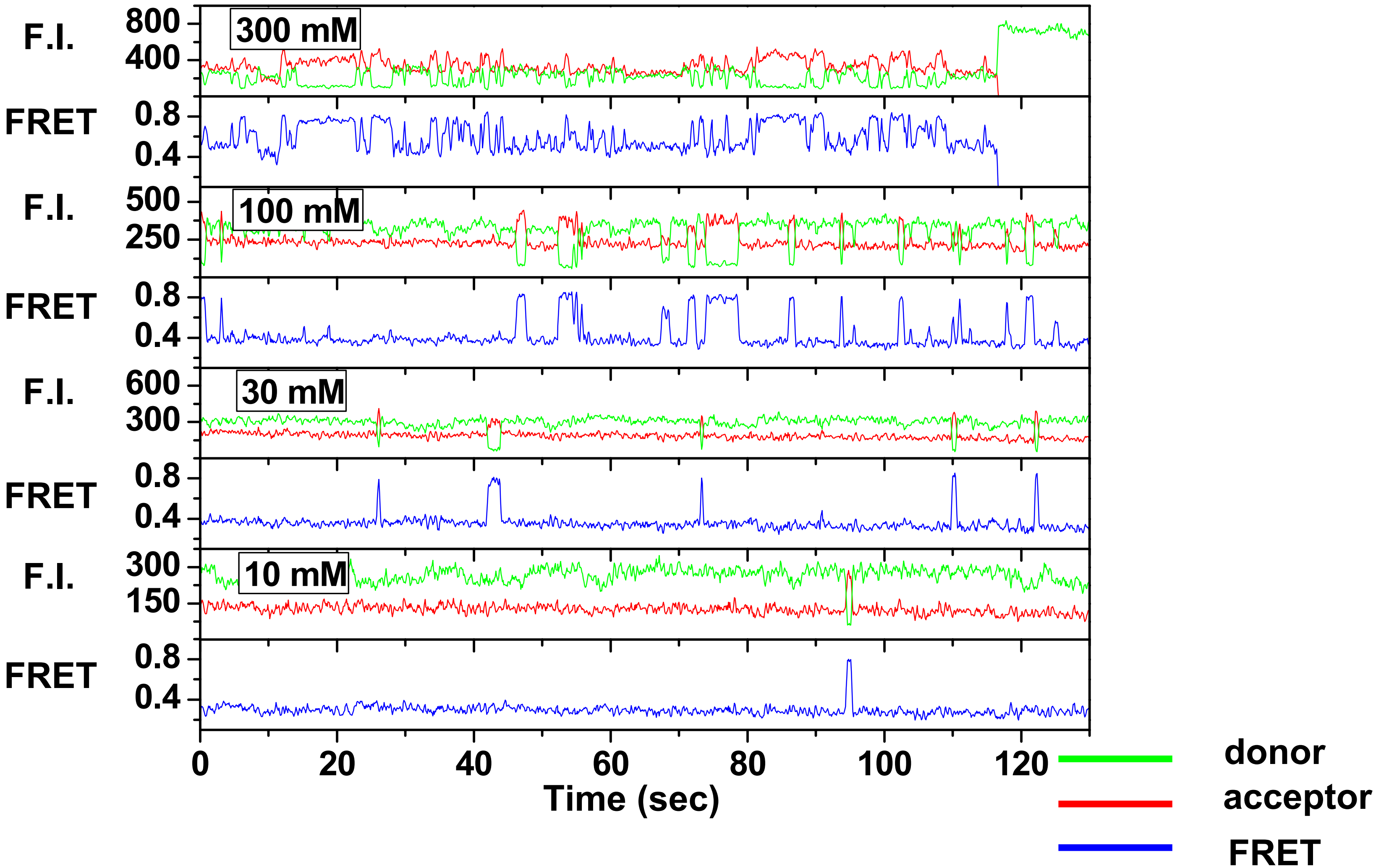
Complementary stem part

5'**-**CGCACCGTGGCCATTATCCTT **T***TACCTCT-3'
(T*: TMR-labeled Thymine)



Schematic diagram of mutated G-quadruplex

Time Traces of Mutated Sample



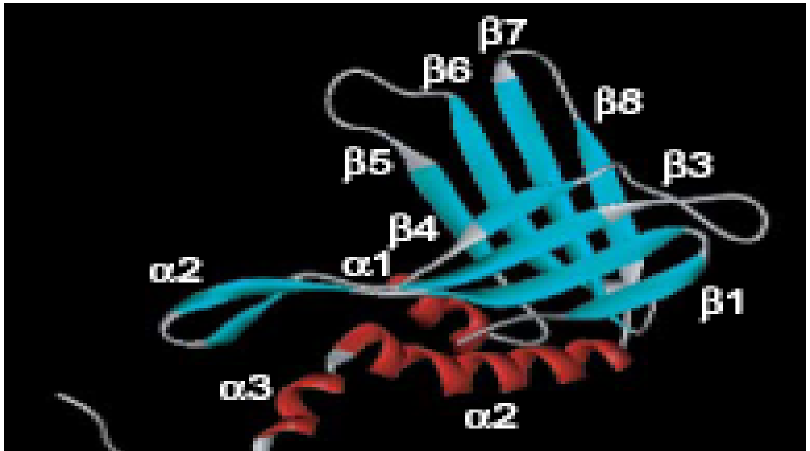
Telomere Binding Protein

AtWhy1 (One of Whirly protein family)

1. One of plant transcription factors for defense gene regulation
2. Single stranded binding protein
3. ***Binding with telomeric DNA***

► **The exact binding mechanism is not revealed yet.!**

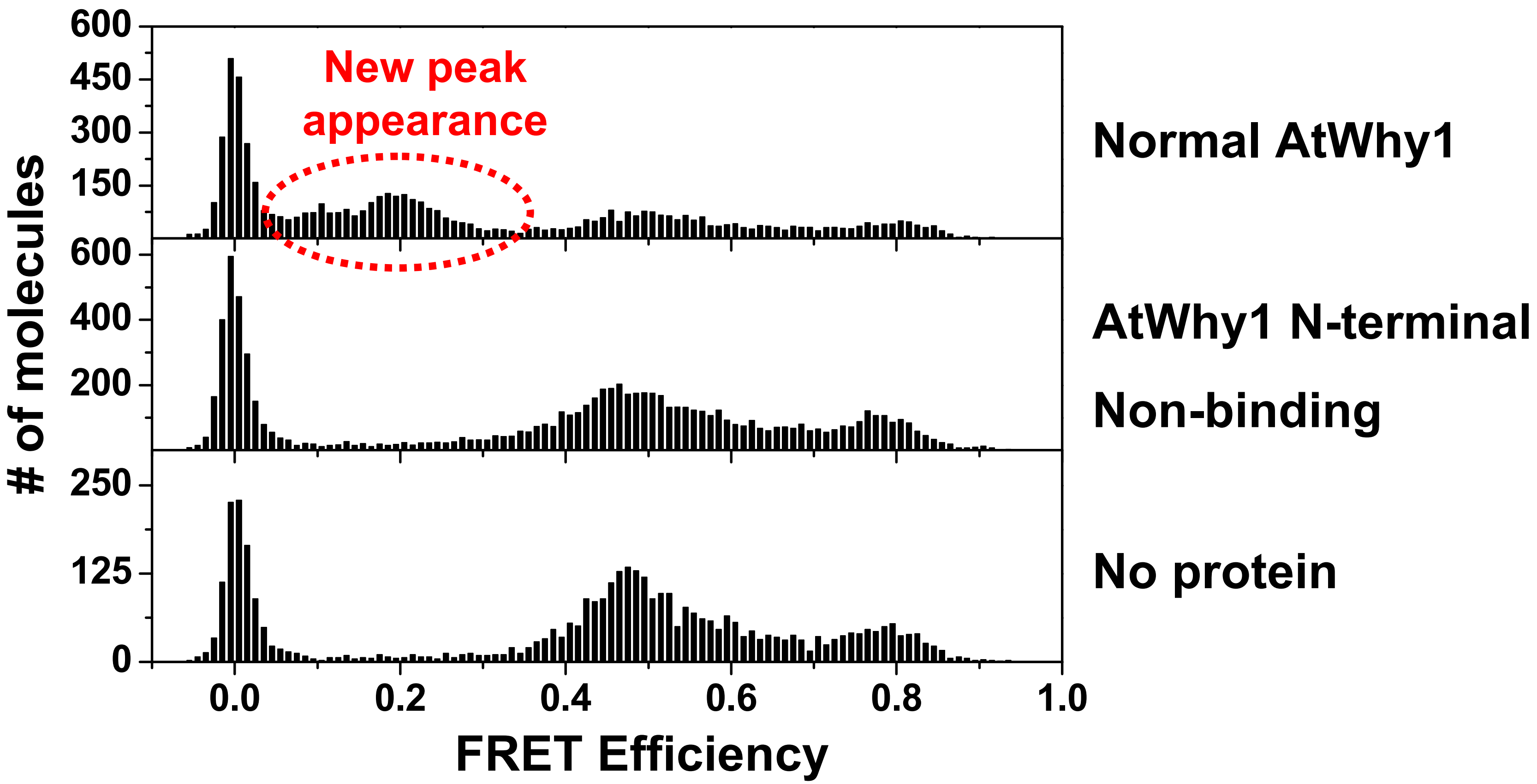
※ The sample is obtained from I. K. Chung in Yonsei Univ.



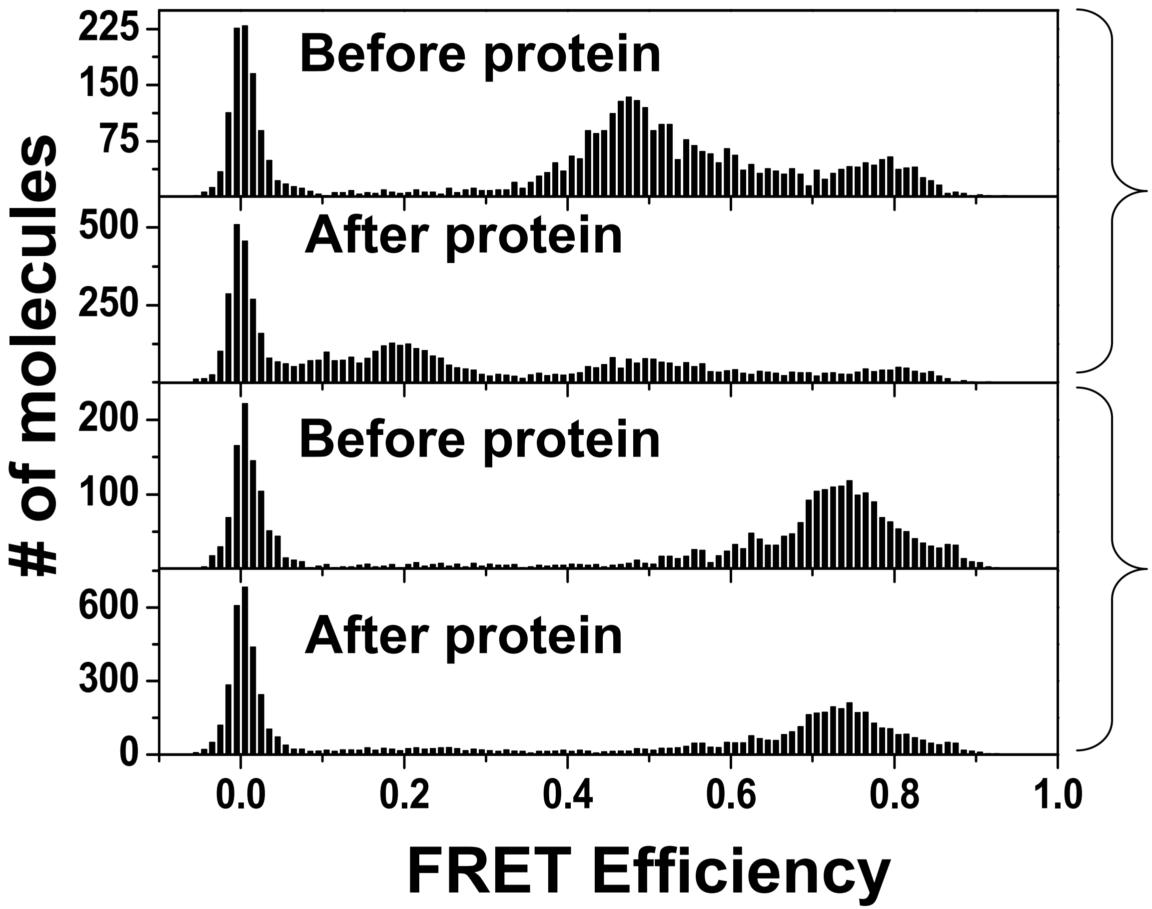
3D structure of Whirly protein

D. Desveaux et al.,Trends in Plant Sci., *10*, 95, 2005

Protein Binding (200 mM K+)



Mutation vs. Wild-type



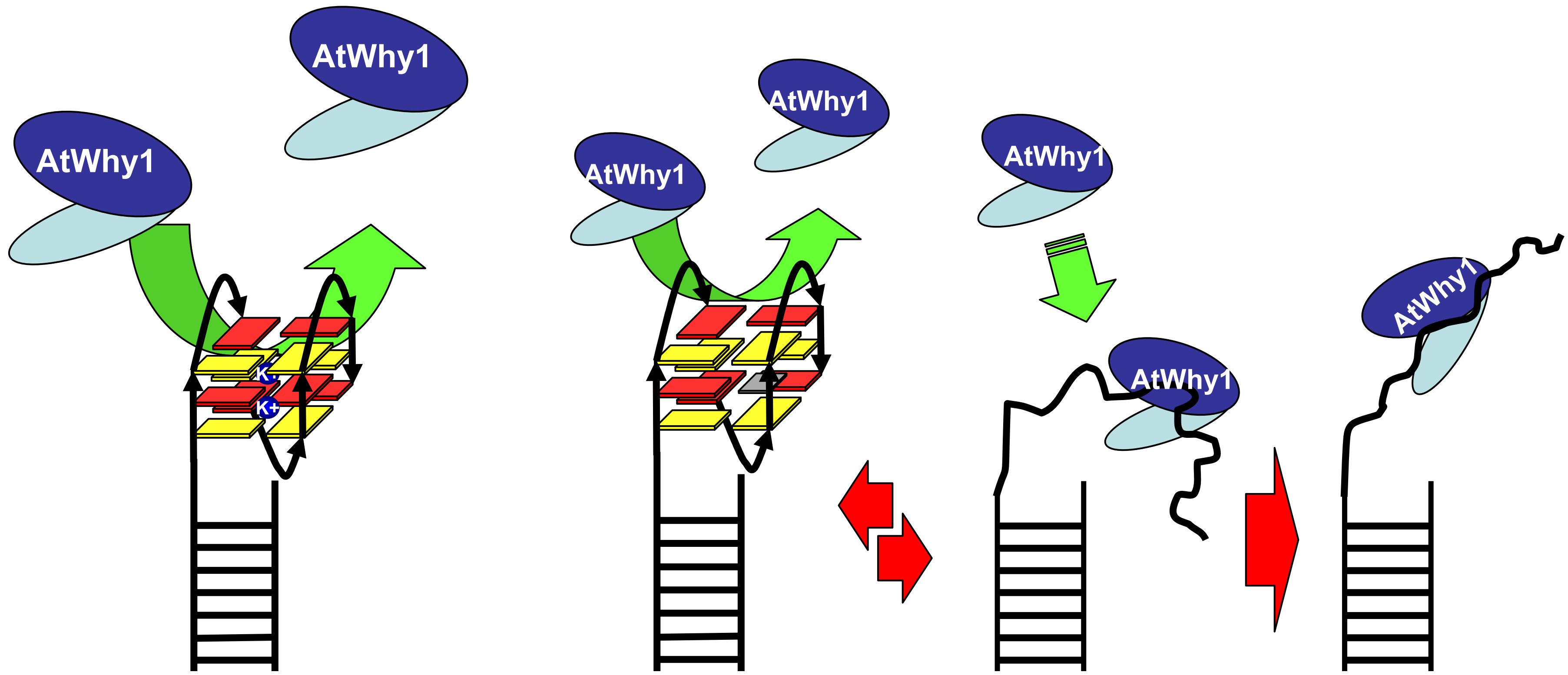
Mutated G-quadruplex

Wild-type G-quadruplex

Schematic Diagram

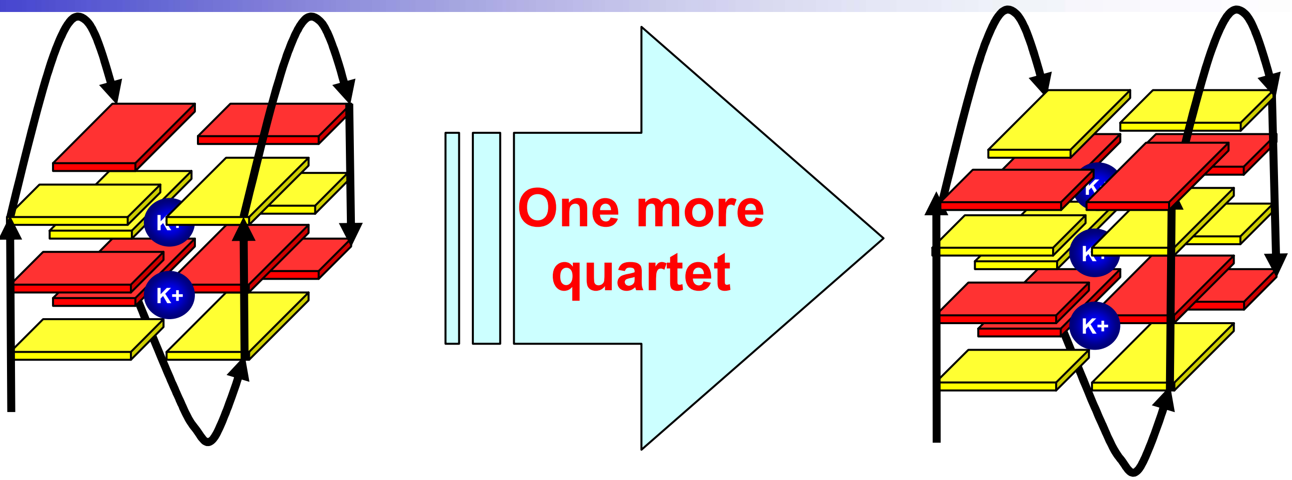
Wild-type G-quadruplex

Mutated G-quadruplex



4-Quartet G-quadruplex

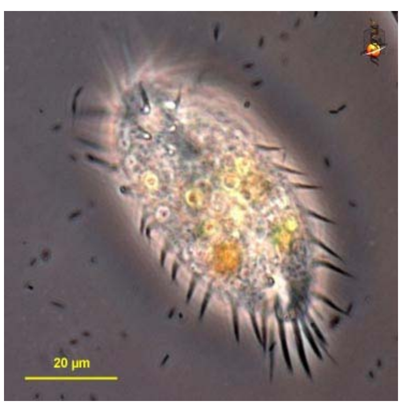
4-quartet G-quadruplex



What is **the effect of additional quartet** **on the conformational dynamics?**

4-quartet G-quadruplex: (GGGGTTTT)$_3$GGGG

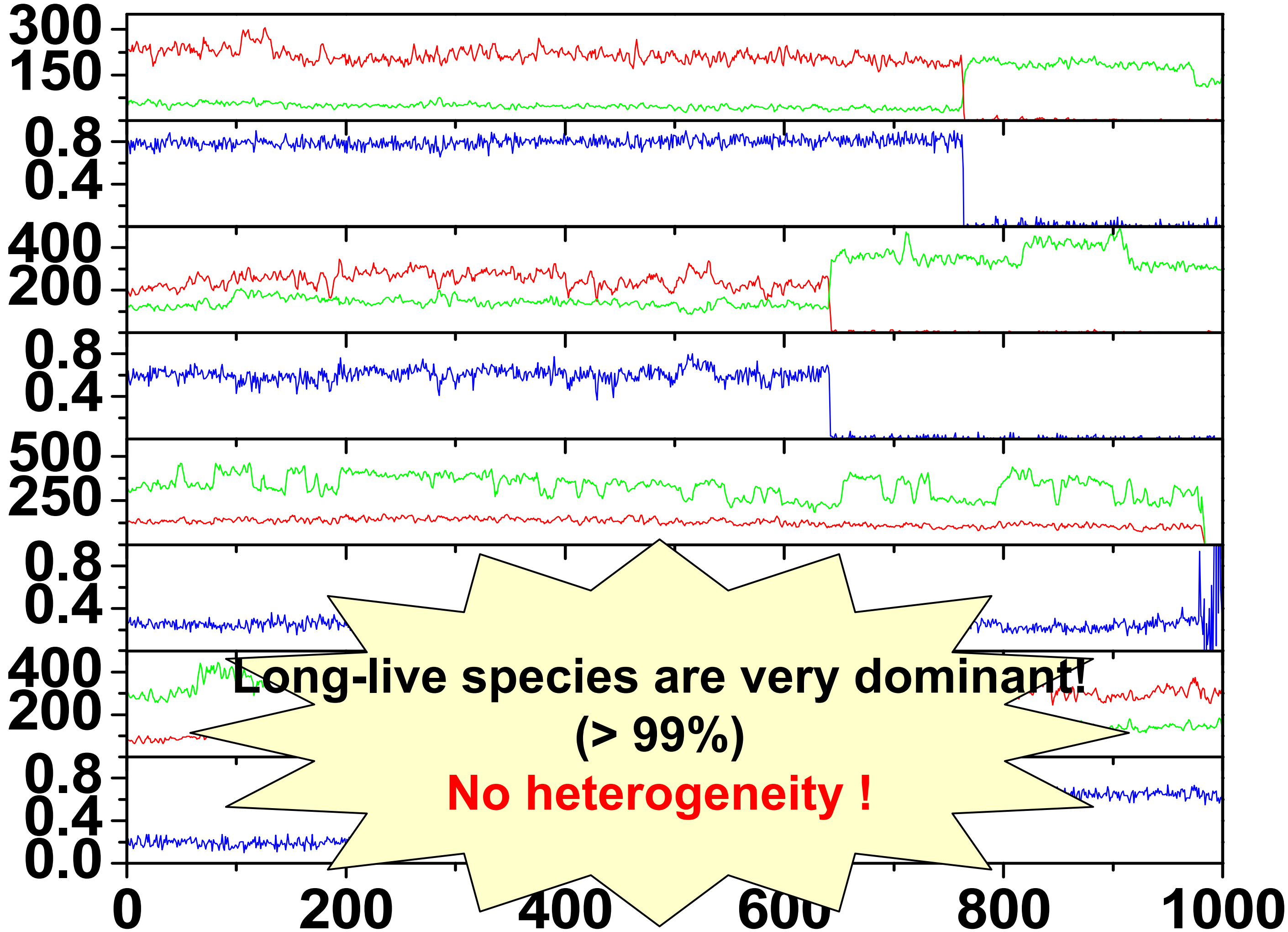
► Biologically, this is the ***telomeric sequences of Oxytricha***



► ***Oxytricha*** **and its telomere model**

C.H.Kang et al., Nature, *356*,126, 1992

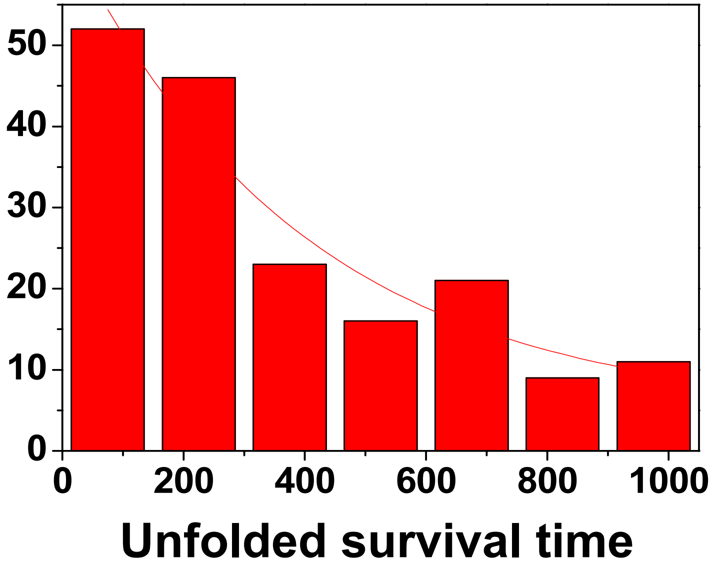
Time Traces at 2 mM K+



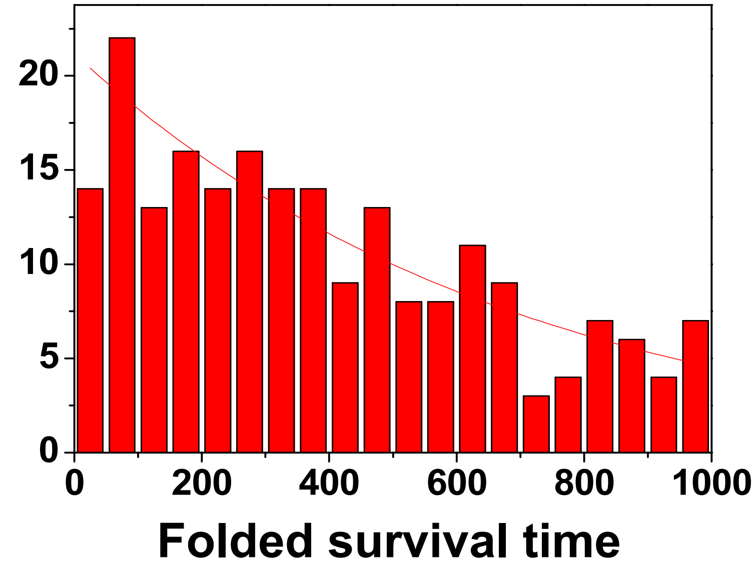
Survival Time Analysis at 2 mM K+

- **Survival Time Analysis**

Measure how long the molecules survive before transition or photo-bleaching



$T_1 \sim 363.6$ sec

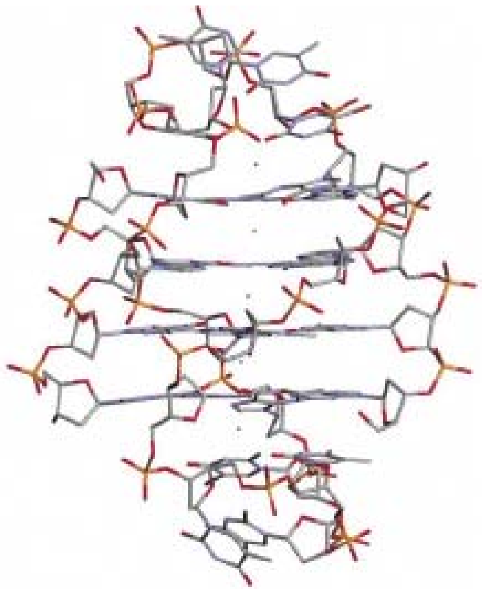


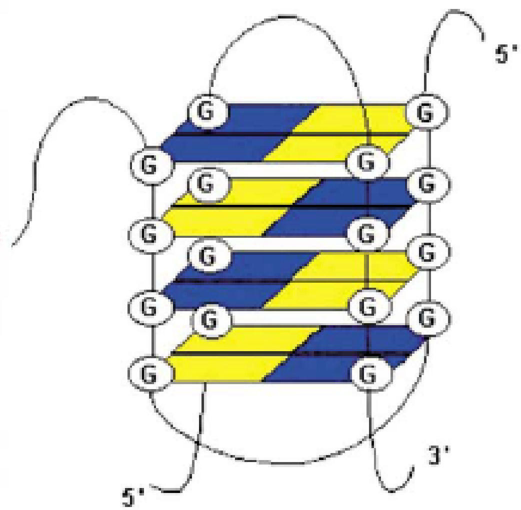
$T_1 \sim 690.9$ sec

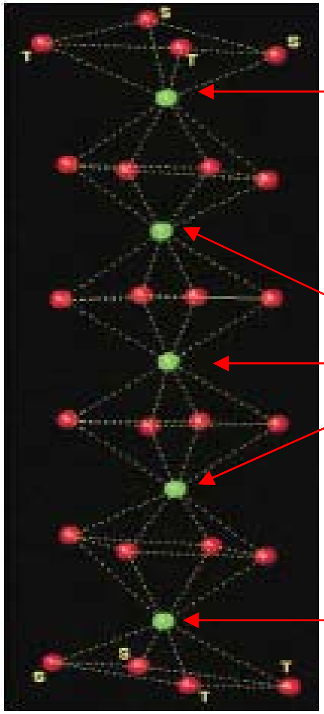
This dwell time is at least 5 fold larger than for the human telomeric DNA.

Crystal Structure of Oxytricha's Telomere

K+ formation of two d(GGGGTTTTGGGG)s







Loop Binding

Between quartets

Loop Binding

S. Haider, G. N. Parkinson, & S. Neidle, JMB, 320, 189~200 (2002)

Coordination bonding of *more than two K+ ions*
→ higher stability than human telomeric DNA
► High stability of Oxytricha's telomeric DNA

Summaries

Via single molecule FRET

- Human telomeric DNA.
  - Confirm the coexistence of ***parallel and anti-parallel*** conformations.
  - ***Heterogeneity*** of conformational dynamics.
- Mutated human telomeric DNA.
  - Short-lived folded species are dominant.
  - Easy to bind with single-strand protein.
    - ► ***G-quadruplex is a protective structure of telomere.***
- 4-quartet G-quadruplex.
  - More stable than 3-quartet G-quadruplex.
    - ► Coordination bonding of more than 2 cations.
    - ► ***High stability of Oxytricha's telomere.***