Conformational Dynamics of Telomeric DNA via Single Molecule FRET

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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

AFM

Optical Tweezers

Electronics

Fluorescence

PNAS, 96, 11288, 1999


Biop. J., 87, 3205, 2004

Science, 283, 1676, 1999
Fluorescence Resonance Energy Transfer

Energy Transfer by dipole-dipole interaction between two fluorophores, donor and acceptor

Energy Transfer Efficiency

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

Energy Transfer Range

$R_0 = 50 \text{ Å}$
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

- 532 nm laser
- Mirror
- Dichroic
- Lens
- Sample
- Microscope
- CCD
- Excitation laser
- Prism
- Quartz slide
- Index matching oil
- Evanescent field
- Fluorescence
- Objective
- Long-pass filter
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

Tandem repeat of GGGTTA

D-loop

t-loop

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)

Human G-quadruplex

- Human telomeric DNA: short tandem repeat (GGGTAA)

- Polymorphisms of Human G-quadruplex

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

Conformational Dynamics
Experiments

Sample
G-quadruplex part
5’-Cy5-GGGTTAGGGTTAGGGTTAGGGAGAGGTAAAAGGATAATGGCCACGGTGC-3’-biotin

Complementary stem part
5’-CGCACCGTGCCATTATCCTTT*TACCTCT-3’
(T*: TMR-labeled Thymine)

Immobilization of samples
BSA-biotin & Streptavidin
Heterogeneity of G-quadruplex

At 2 mM K+

Long-lived species

Short-lived species

Mixed species

Dwell time analysis for mixed species

Double exponential fitting

$\tau_1 \sim 110 \text{ sec} \& \tau_2 \sim 15 \text{ sec}$

Heterogeneity!
Titration of K+ at RT

Population histogram

Before transition, the molecule must go through the low FRET

Unfolded single-strand overhang

Time trace at 2mM K+

Before transition, the molecule must go through the low FRET
Dynamics of G-Quadruplex

- G-quadruplex maintains one conformation
- G-quadruplex fluctuates fast
- G-quadruplex maintains one conformation again

or

time
Trend by Concentration at RT

Low Conc. Long-lived Unfolded

All species are mixed at 2 mM K+

High Conc. Long-lived Anti-parallel
Trend by Temperature

2 mM K+

Low Temp.

Long-lived Folded/Unfolded

Short-lived Occurrence

High Temp.

Long-lived Unfolded

100 mM K+

Long-lived Anti-parallel

Long-lived Parallel

Short-lived Occurrence
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-GGGTTAGGGTTAGTGTTAGGGAGAGGTAAAAGGATAATGGCCACGGTGCG-3’-biotin

Complementary stem part
5’-CGCACCGTGGCCATTATCCTT T*TACCTCTCT-3’
(T*: TMR-labeled Thymine)

Guanine → Thymine

Schematic diagram of mutated G-quadruplex
Time Traces of Mutated Sample

- 300 mM
- 100 mM
- 30 mM
- 10 mM

F.I.: Fluorescence Intensity
FRET: Förster Resonance Energy Transfer

Time (sec):
0 20 40 60 80 100 120

Donor (green), Acceptor (red), FRET (blue)
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+)

FRET Efficiency

- Normal AtWhy1
- AtWhy1 N-terminal
- Non-binding
- No protein

New peak appearance

# of molecules

FRET Efficiency
Mutation vs. Wild-type

![Mutation vs. Wild-type](image)

- **Before protein**
  - Mutated G-quadruplex
  - Wild-type G-quadruplex

- **After protein**
  - Mutated G-quadruplex
  - Wild-type G-quadruplex
Schematic Diagram

Wild-type G-quadruplex

Mutated G-quadruplex

AtWhy1

AtWhy1

AtWhy1
4-Quartet G-quadruplex
What is the effect of additional quartet on the conformational dynamics?

4-quartet G-quadruplex: (GGGGTTTT)₃GGGG

► Biologically, this is the telomeric sequences of Oxytricha

► Oxytricha and its telomere model

Time Traces at 2 mM K+

Long-live species are very dominant! (> 99%)
No heterogeneity!
Survival Time Analysis at 2 mM K+

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

**Unfolded survival time**

- $T_1 \sim 363.6$ sec

**Folded survival time**

- $T_1 \sim 690.9$ 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

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.