# Single Molecule Tools to Investigate Terminal Deoxynucleotidyl Transferase (TdT) Mechanisms

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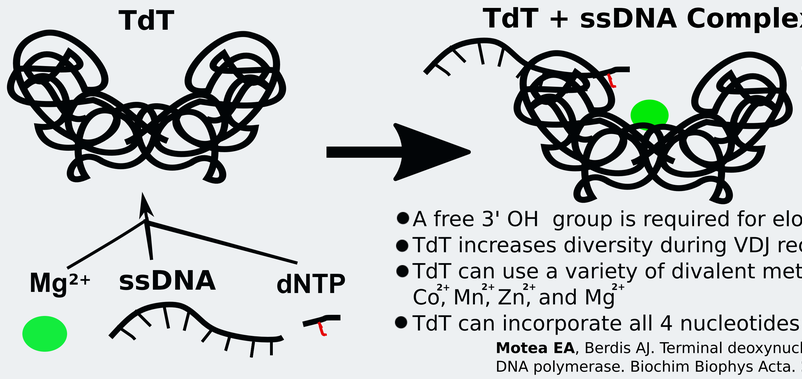
# Overview

Unlike DNA polymerase, the enzyme Terminal Deoxynucleotidyl Transferase (TdT) has the unique ability to elongate single stranded DNA (ssDNA) without a template. Because of this, TdT has potential uses in applications ranging from basic labratory experiments needing ssDNA to gene editing. However, not much has been studied about this particular enzyme, especially it's diffusion kinetics as it elongates ssDNA. Therefore, this study aims to investigate the diffusive coefficient (D) of TdT using two single molecule tools: fluorescence corrrelation microscopy (FCS) with a built confocal microscope and an anti-brownian electrokinetic trap (ABEL-Trap).

# Background

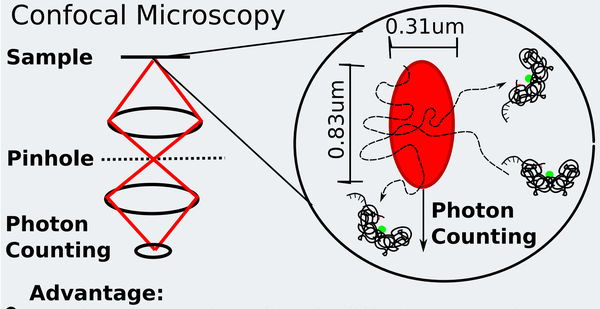
## TdT elongates ssDNA in a template-independent manner




- TdT has a similar structure to DNA polymerase except for a larient loop that is believed to prevent binding to a complementary strand
- A free 3' OH group is required for elongation of ssDNA
- TdT increases diversity during VDJ recombination
- TdT can use a variety of divalent metal ions such as $Co^{2+}$, $Mn^{2+}$, $Zn^{2+}$, and $Mg^{2+}$
- TdT can incorporate all 4 nucleotides at different rates

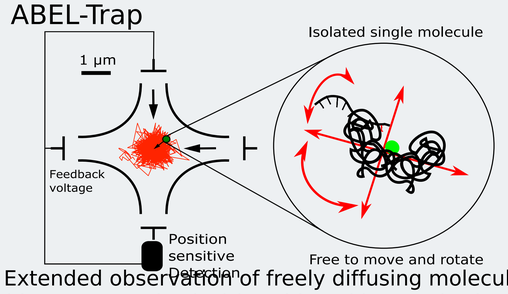
**Motea EA**, Berdis AJ. Terminal deoxynucleotidyl transferase: the story of a misguided DNA polymerase. Biochim Biophys Acta. **2010**;1804(5):1151-1166.

## Single molecule tools to measure diffusion of molecules



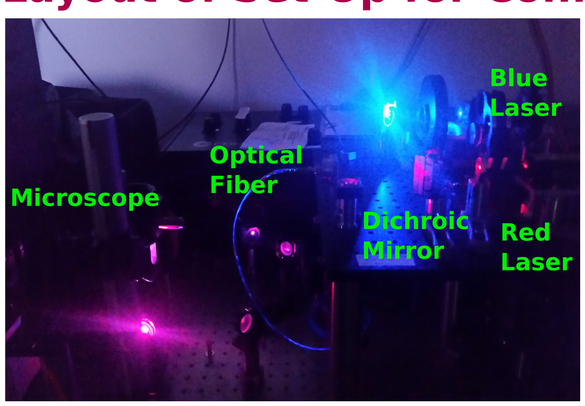

**Advantage:**
- Enables observation of freely diffusing molecules using fluorescence labels (no immobilization)

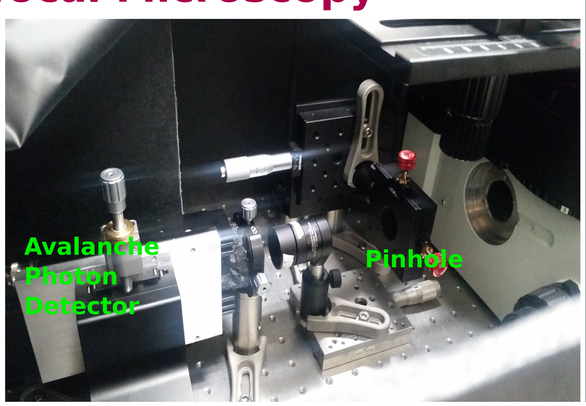
**Disadvantage:**
- Observation time for one molecule is diffusion limited (typically ~1ms)




Extended observation of freely diffusing molecules (down to 1nm in size) is achieved by monitoring the molecule's position using fluorescence, and applying feedback electric fields after each detected photon to move the molecule back towards the center of the observation volume.

# Layout of Set-Up for Confocal Microscopy




## FCS measures a molecule's diffusion coefficient from its autocorrelation function

The time for the autocorrelation function to decay to half its maximum value is about the same as the duration of the measured signal. From a molecule's fluorescence, autocorrelation can be used to measure the average dwell time of the molecule in the laser's confocal volume.

**Measured Autocorrrelation**

$$G(\tau) = \frac{\sum_t I(t)I(t-\tau)}{(\sum_t I(t))^2} - 1$$

G(τ) - autocorrelation at some time lag τ
I(t) - measured signal at some time t

**Single Diffusive Species Model**

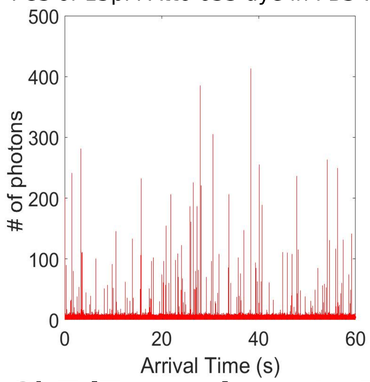
$$G(\tau) = G(0)(1+\frac{\tau}{t_D})^{-1}(1+\frac{\tau}{k^2 t_D})^{-1/2}$$

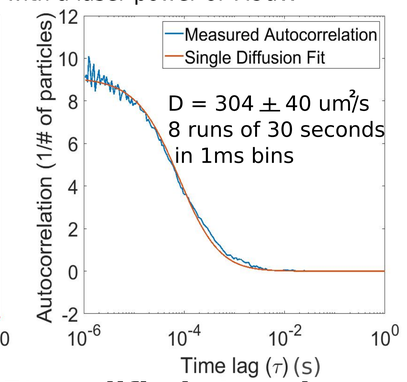
$k = w_{xy}/w_z$ is the ratio of the confocal beam widths
$t_D = \frac{w_{xy}^2}{4D}$ is the diffusion time

# Results

## 1) FCS can record photon counts for single molecules diffusing in solution

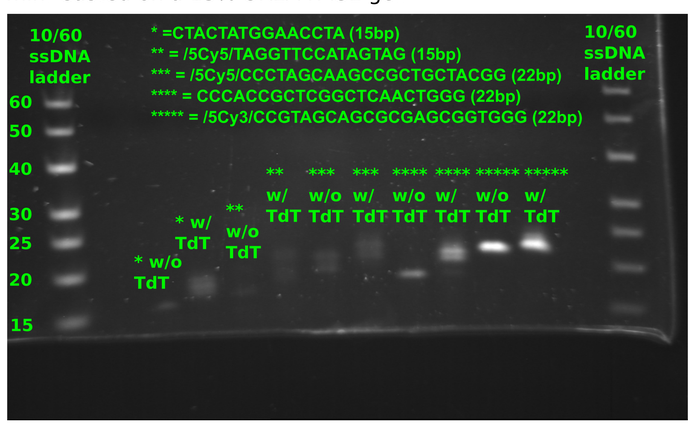
FCS of 15pM Atto 633 dye in PBS with a laser power of 7.9uW



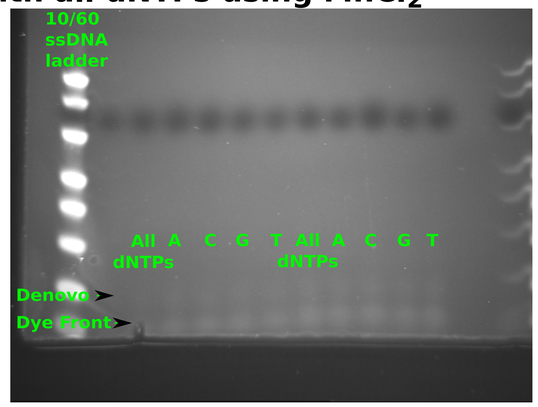




## 2) TdT can elongate 5' modified strands

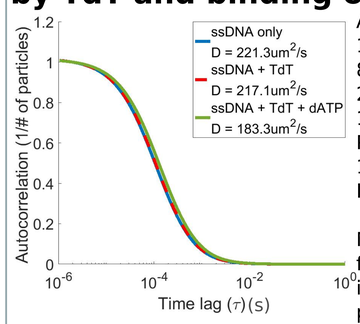
A reaction of 10 pmol ssDNA, 82nM TdT, 100uM dCTP, 250uM $CoCl_2$was used with 10x TdT buffer and incubated at 37°C for 30 min. Casted on a 15% UREA-PAGE gel.




## 2) TdT can do Denovo Synthesis with all dNTPs using $MnCl_2$

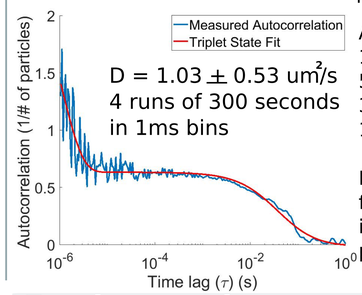



## 4) FCS measures ssDNA elongated by TdT and binding of TdT + ssDNA




A reaction of:
1.25nM of ** ssDNA
82nM of TdT
250uM of $MgCl_2$
100uM dATP
PBS for final volume = 100uL
Incubated at 37°C for 5 min

Measured at a 2um depth from the glass-PBS interface with a laser power of 79nW




A reaction of:
1.25nM of ** ssDNA
5.75uM of TdT
330uM of $MgCl_2$
1.66X TdT Buffer

Measured at 15um depth from the glass-solution interface with a laser power of 7.9uW

## Further work

- Measuring the point spread function at different depths to accurately measure the diffusion coefficient
- Using the ABEL-Trap to test processtivity vs. distributive elongation and run length of TdT

# Acknowledgements

Quan Wang and Hugh Wilson for help with building the set-up and conducting experiments, the whole Wang Lab for fruitful discussion, and OURSIP for funding.