Unraveling the Dual-Mode Impact on Tension Gauge Tethers' Mechanical Stability

Jingzhun Liu[1] and Jie Yan[1,2,3,*]

[1] *Mechanobiology Institute, National University of Singapore, Singapore 117411*
[2] *Department of Physics, National University of Singapore, Singapore 117546*
[3] *Joint School of National University of Singapore and Tianjin University, International Campus of Tianjin University, Binhai New City, Fuzhou, 350207, China*

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Tension Gauge Tethers (TGTs), short DNA segments serving as extracellular tension sensors, are instrumental in assessing tension dynamics in mechanotransduction. These TGTs feature an initial shear-stretch region and an unzip-stretch region. Despite their utility, no theoretical model has been available to estimate their tension-dependent lifetimes ($\tau(f)$), restricting insights from cellular mechanotransduction experiments. We've now formulated a concise expression for $\tau(f)$ of TGTs, accommodating contributions from both stretch regions. Our model uncovers a tension-dependent energy barrier shift occurring when tension surpasses a switching force approximately 13 pN for the recently developed TGTs, greatly influencing $\tau(f)$ profiles. Experimental data from several TGTs validated our model. The calibrated expression can predict $\tau(f)$ of TGTs at 37 degrees Celsius based on their sequences with minor fold-changes.

Tension Gauge Tethers (TGTs) are short segments of double-stranded DNA (dsDNA) that irreversibly dissociate in response to tension. TGTs were initially developed as sensors to measure tension levels transmitted across tension-bearing extracellular receptors, such as integrin, cadherin, and T-cell receptors [1–8]. In these applications, a TGT is incorporated into a tension-transmission pathway via two attachment points on the TGT, which consists of a total of N base pairs. The first point, P_1, is located at the end of one single-stranded DNA (ssDNA) strand, and the second point, P_2, is situated on the opposite strand, M base pairs away from P_1 (see (Fig. 1a)). Therefore, the stretching geometry of a TGT with a specific sequence is entirely defined by N and M, and is henceforth denoted as M/N-TGT ((Fig. 1b)).

A TGT can be divided into two distinct regions: $R_{\rm s}$, which exists between P_1 and P_2 under a shear-stretching geometry, and $R_{\rm u}$, which exists after P_2 under an unzip-stretching geometry (as shown in Fig. 1c). The strand dissociation of a TGT, which depends on the tension, follows a lowest-energy kinetic pathway. This pathway involves sequential breakage of base pairs from the P_1 end until the strands are fully dissociated (as shown in Fig. 1c). The transition state can be indicated by the number of base pairs (n) between the fork and P_1. It is clear that there is a switch in stretching geometry when the fork passes $n = M - 1$.

Our current understanding of the mechanical stability of TGTs remains incomplete. The original description of mechanical stability have been based on the tension tolerance, $T_{\rm tol}$, which is the tension required to dissociate a TGT with a given average lifetime of 2 seconds as per the studies by Wang et al. [1]. Recent research underscores the significance of comprehending the tension-dependent lifetime, $\tau(f)$ [9], which offers a comprehensive understanding of the mechanical stability of TGTs. Despite its importance, no theoretical model has been available to estimate $\tau(f)$ for TGTs based on their sequences and stretching geometry, restricting insights from cellular mechanotransduction experiments.

In this study, we utilized Kramers' kinetic theory to derive a general expression for the TGTs' $\tau(f)$, which has a single model parameter, the effective diffusion coefficient D of DNA fork migration (depicted by red arrows in Fig. 1c) during the transition. Our expression depends on the tension-dependent, one-dimensional energy landscape, which is influenced by various factors, including the DNA sequence, the entropic elasticity of both double-stranded (ds) and single-stranded (ss) DNA, and the stretching geometries involved.

The process of strand dissociation of a TGT can be understood by considering the random movement of the fork position, with the rates of base pair opening (forward) and formation (backward) depending on the tension. The energy required to open the ith base pair from the ends can be expressed as:

$$\Delta g_{\rm i}(F) = \begin{cases} \varepsilon_{\rm i} - \int_0^F (x_{\rm ss}(f) - x_{\rm ds}(f))df & 1 < i < M \\ \varepsilon_{\rm i} - 2\int_0^F x_{\rm ss}(f)df & M \le i < N \end{cases} \tag{1}$$

Here $x_{\rm ss}(f)$ and $x_{\rm ds}(f)$ are the force-extension curves for 1-nt ssDNA and 1-bp dsDNA, respectively, and $\varepsilon_{\rm i}$ represents the energy cost to open the base pair at zero tension, which is evaluated in the work using the Santa Lucia nearest-neighbour base pair energy data [10].

We calculated tension-dependent energy landscapes for several commonly used TGTs with a given sequence, N, and M (see Fig. 2). For forces below a threshold of $F_{\rm c}^{\rm u} \sim$ 13 pN, the energy profile monotonically increases with the number of opened base pairs until the second last base pair in the TGT is ruptured. However, at higher forces, the energy profile is non-monotonic, with a maximum at

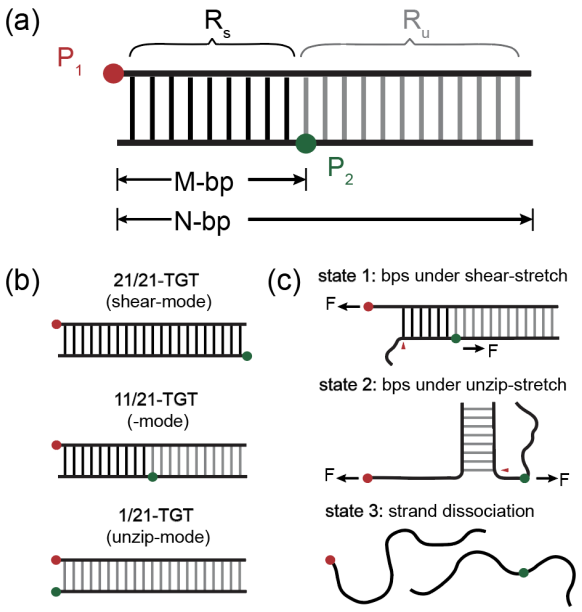

Fig. 1. Illustration of TGTs and the transition pathway for tension-dependent strand dissociation pathway. (a) P_1 (red) and P_2 (green) are two force attaching point on a TGT with a total length of N-bp that divides it into two regions: R_s (black) and R_u (grey) under shear- and unzip-stretching geometry, respectively. A TGT is named as M/N-TGT where M is the base pair distance between P_1 and P_2. (b) Typical design of TGTs with P_2 at different positions (from top to bottom: left-end, middle, and right-end) on DNA strand, named as 21/21-bp, 11/21-bp, and 1/21-bp TGT. (c) The tension-dependent strand dissociation pathway of a TGT via strand peeling from P_1 end.

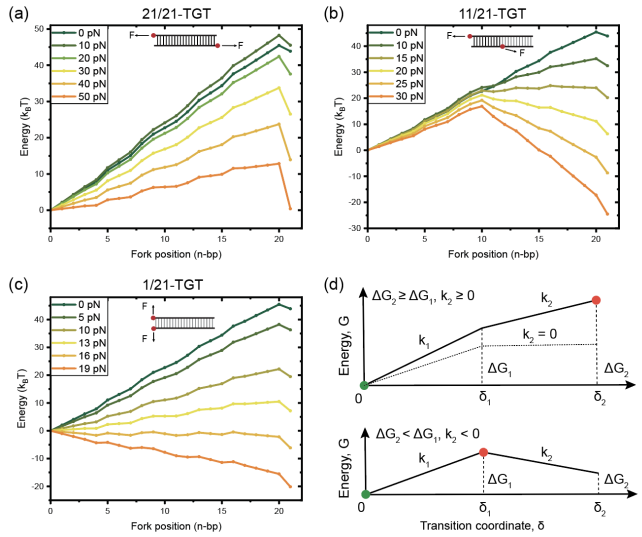

Fig. 2. Tension-dependent energy landscape of a series of 21-bp TGT with different stretching modes (5'-GTGTCGTGCCTCCGTGCTGTG-3'): (a) 21/21-TGT in shear-stretch mode, (b) 11/21-TGT in dual-mode, and (c) 1/21-TGT in unzip-stretch mode. The transition distance, the x-axis, is indicated by the fork position n. The tension-dependent energy landscapes of these TGTs are plotted over a wide force range from zero to a force that the dissociation state have much lower free energy than the hybridized state. (d) Illustration of the simplified energy landscapes of the 11/21-TGT with 10-bp in shear-mode and 11-bp in unzip-mode.

the base pair position $n = M - 1$. Our analysis suggests a tension-dependent switch in the energy barrier. Note that the threshold tension $F_\mathrm{c}^\mathrm{u} \sim 13$ pN is critical because it represents the tension at which the opening and closing rates of a base pair are equal under the unzip-stretching geometry.

It can be seen from Figure 2 that for TGTs with the sequence 5'-GTGTCGTGCCTCCGTGCTGTG-3' ($N = 21$), at a given tension, the energy landscape can be approximated by a piecewise linear function:

$$U(\delta) = \begin{cases} k_1\delta & 0 < \delta < \delta_1 \\ k_2(\delta - \delta_1) + \Delta G_1 & \delta_1 \le \delta < \delta_2 \end{cases} \qquad (2)$$

Here, δ is a force-independent transition coordinate, chosen to be the number of dissociated base pairs during transition. The energy landscape is composed of two straight lines with different slopes, each defined by four geometric parameters. The first set of parameters, δ_1 and ΔG_1, describe the number of dissociated base pairs and energy difference between the fully hybridized state ($n = 1$) and the state at fork position $n = M - 1$. The second set of parameters, δ_2 and ΔG_2, describe the difference between the fully hybridized state ($n = 1$) and the ruptured state ($n = N$) (as shown in Fig. 2d).

The four parameters are not independent variables; rather, they are directly derived from the values of M, N, and the tension-dependent base pair energy data Δg_i. Specifically, they are calculated as follows: $\delta_1 = M - 1$, $\delta_2 = N$, $\Delta G_1 = \sum_1^{M-1} \Delta g_\mathrm{i}$, and $\Delta G_2 = \sum_1^{N} \Delta g_\mathrm{i}$. ΔG_1 and ΔG_2 are normalized into a dimensionless quantity by dividing by $k_\mathrm{B}T$, where k_B is the Boltzmann constant and T is the temperature. The slopes of the two lines are determined by the corresponding parameters: $k_1 = \frac{\Delta G_1}{\delta_1}$ and $k_2 = \frac{\Delta G_2 - \Delta G_1}{\delta_2 - \delta_1}$, respectively.

We examine $\tau(f)$ of a TGT, $k(f)$, based on the energy landscape Eq. 2 within the framework Kramer's kinetic theory [11]. Following the derivation reported previously [12, 13], it can be shown that (SI-III):

$$\tau(f) \approx \frac{e^{\Delta G_1}/k_1 + (e^{\Delta G_2} - e^{\Delta G_1})/k_2}{Dk_1}, \qquad (3)$$

where the single model parameter D is the effective diffusion coefficient for fork migration during the transition process. The above formula has assumed sufficiently high value ΔG_1 to meet the pre-equilibration condition imposed by the Kramer's kinetic theoretical framework [11].

At tensions much greater than F_c^u, $\Delta G_1 \gg \Delta G_2$ and so $\tau(f) \approx \frac{1/k_1 - 1/k_2}{Dk_1} e^{\Delta G_1}$, indicating that the lifetime is determined by the barrier ΔG_1. On the contrary, at tensions much smaller than F_c^u, $\Delta G_1 \ll \Delta G_2$ and so $\tau(f) \approx \frac{1/k_2}{Dk_1} e^{\Delta G_2}$, indicating that the lifetime is determined by ΔG_2. The result indicates a switch of the energy barrier from ΔG_1 to ΔG_2 when the tension decreases from below to above F_c^u.

To evaluate the predictive capabilities of the model for dual-mode TGTs containing both shear-stretch and unzip-stretch regions at various forces, we conducted experiments for five TGTs, 11/11-TGT, 11/16-TGT, 11/21-TGT, 15/21-TGT, and 20/21-TGT (Fig. 3a). The first three TGTs share the same shear-stretch region, but differ in the length of their unzip-stretch regions, while the last three TGTs have the same overall length but feature different separations of shear-stretch and unzip-stretch regions (Fig. 3b).

For each TGT, the average lifetimes were determined by analyzing at least 30 force-jump cycles for each target tension, using data from three individual detectors. As tension increases, the lifetime of all these TGTs decreases, indicating that they become less stable under higher tension. As depicted in Fig. 3c and d, the model can reasonably fit the data obtained from all the TGTs, with fitting values of D over a range from 2.4×10^8 s^{-1} to 1.6×10^9 s^{-1}, by introducing a small constant energy correction $\sigma = 0.25\ k_B T$ to account for any inaccuracy in tension-dependent base pair energy. As the contour length of a dsDNA base pair is $l_{ds} = 0.34$ nm, the effective diffusion coefficient D can be converted to $D' = l_{ds}^2 \cdot D$ with the unit of nm$^2 \cdot$s^{-1}.The value of D' is over the range from 2.8×10^7 nm$^2 \cdot$ s^{-1} to 1.8×10^8 nm$^2 \cdot$ s^{-1}, which is in the same order of magnitude as reported in previous studies [14, 15].

Collectively, these findings indicate that the single-parameter model (Eq. 3) can be applied to various TGTs with differing lengths, diverse stretch region-to-unzip region ratios, and both lower ($f < f_u^c$) and higher ($f > f_u^c$) forces. Although the fork diffusion constant D fitting values vary among TGTs, the fold differences remain within one order of magnitude. These disparities in the fitting values of D can be attributed to inaccuracies in estimating tension-dependent base pair energies (SI-IV).

By utilizing an average value of approximately 6.4×10^8 s^{-1}, we were able to estimate $\tau(f)$ for six 21-bp TGTs that were investigated in previous cell experiments [1–8] (as depicted in Figure 4). The plot reveals highly different tension-dependent lifetime profiles due to the different positions of P_2. When P_2 is located near the middle of the DNA segment, the profile shows a clear change of slope slightly above the value of $f_c^u \approx 13$ pN. Since the highly diverse $\tau(f)$ profiles result from changes in P_2 that alter the lengths of the shear-stretch and unzip-stretch regions, this strongly suggests differential contributions from both regions to the tension-dependent lifetime of TGTs.

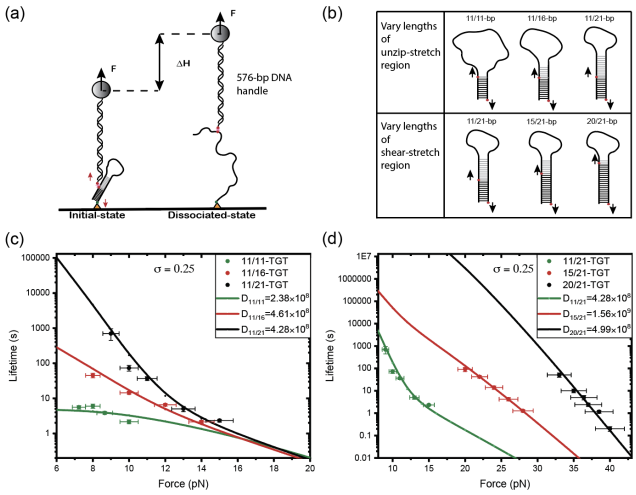

Fig. 3. Single-molecule quantification of the tension-dependent lifetime of five different TGTs. (a) Schematic of the designed DNA detector tethered between a glass surface and a superparamagnetic bead. The TGT is stretched through a thiol anchored on glass surface and a biotin linked to a 576-bp DNA handle attached on the superparamagnetic bead. (b) The stretching geometry of our examined TGTs with varying lengths in unzip- or shear-stretch regions. (c) The tension-dependent lifetime of 11/11-, 11/16-, and 11/21-TGT with different lengths in unzip-stretch region. (d) The tension-dependent lifetime of 11/21-, 15/21-, and 20/21-TGT with different lengths in shear-stretch region.

Overall, the findings suggest that, at high forces ($f > f_c^u$), the transition rate is primarily influenced by the energy barrier's height at position $M-1$. Additionally, the unzip-stretch region has a marginal impact on the transition rate at forces slightly higher than f_c^u. At lower forces ($f < f_c^u$), both the shear and unzip-stretch regions contribute to the tension-dependent lifetime $\tau(f)$. The significantly increased lifetime in the lower force range is a direct result of the higher energy cost of breaking each base pair, the switched energy barrier, and the longer diffusion distance. Further details on the differential contributions from shear- and unzip-stretch regions at low or high force regions are provided in Supplementary Informations (SI-V and VI).

In summary, through the analysis of the energy

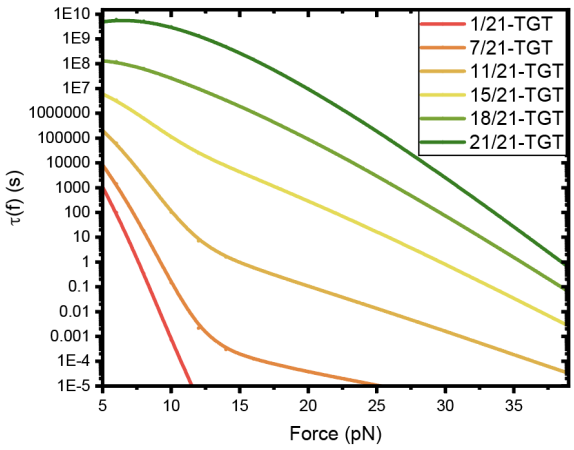

Fig. 4. The tension-dependent lifetime of several 21-bp TGTs with different stretching geometry. As the length of shear-stretch region ($M-1$) of a TGT increases, it becomes more stable and has a longer lifetime. The dashed line represents the critical force value $F_c^u \sim 13$ pN, below which $\tau(f)$ is mainly determined by the unzip-stretch region, and above which it mainly depends on the shear-stretch region.

landscape of dual-mode TGTs and the application of Kramer's kinetic theory, we have derived a simple analytical expression, $\tau(f)$, for which the fork diffusion constant serves as the single model parameter. The energy parameters, ΔG_1 and ΔG_2, are calculated from the DNA sequences using the Santa Lucia nearest-neighbour base pair energy data and the force-extension curves of the ds and ssDNA. The expression was calibrated at a temperature of 37 °C, which aligns with the conditions of most cell experiments. The calibrated expression yielded a range of fork diffusion constants between 2.4×10^8 s^{-1} and 1.6×10^9 s^{-1} for five different TGTs with varying lengths and shear-to-unzip base pair ratios. As a result, this calibrated expression of $\tau(f)$ can be broadly applied to TGTs based on their sequences and stretching geometries, with minor fold-changes, facilitating their use in cell mechanotransduction studies conducted at 37 degrees Celsius.

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* Email: phyyj@nus.edu.sg

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