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EXPERIMENTAL STUDY

Reaction time of DNA tweezers

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ABSTRACT

OBJECTIVES AND BACKGROUND: The DNA tweezers is a nanomachine composed of several DNA strands. It can switch between two mechanical states (open and closed), depending on what input is given to the system. In this article, it is the presence of a fuel and/or releasing strand.

METHODS: To check whether the tweezers are in one or another state (or are in transition between those states in either direction) using the method FRET (fluorescence resonance energy transfer). We decided to propose three methods on how to process the reaction time of DNA tweezers, since there was no attempt to measure their time by these methods before (F-test, fitting and comparing the slope of the tangent line, and calculating the work efficiency). A description of used methods and criteria for calculating the reaction time are present in this article.

RESULTS: Our study provided a comparison of statistical calculations of DNA tweezers' reaction time performed by three different methods.

CONCLUSION: The methods we used to calculate the reaction time of DNA tweezers gave very different results. This is because the methods are very different (mainly F-test from fitting) and each of them has its advantages and disadvantages *(Fig. 14, Ref. 25).* Text in PDF *www.elis.sk*

KEY WORDS**:** DNA tweezers, nanomachine, fluorescence resonance energy transfer, reaction time.

Introduction

The field of molecular devices and machines was stimulated by much research over the past two decades (1-6). The developments led to the use of DNA as a designer molecule with an enormous capacity to construct complex and precise nanostructures in 2 and 3 dimensions, primarily due to its self-assembly properties (7). Examples of DNA nanostructures include autonomous DNA walkers (8, 9), DNA origami (10, 11), DNA nanotube (12, 13) or DNA tweezers. DNA tweezers is a DNA-based nanodevice composed of two DNA duplexes connected by a short single strand acting as a flexible hinge (14, 16), which can sense, hold and release target DNA upon specific interaction. The DNA tweezers can assume closed or open conformation depending on whether a "fuel" or a release strand is added to the reaction mixture, through complementary base pairing. These DNA-fuelled molecular nanodevices were first introduced by Yurke et al (14). By adding a "fuel" strand to which the tweezers' ends hybridize, the nanodevice can be closed. A "release" strand, on the other hand, displaces the set strand from the tweezers through branch migration. This frees the tweezers' ends and opens the nanodevice. Our machine was prepared by mixing stoichiometric quantities of three strands, A, B, and C in SPSC buffer (see Material and methods). The structure of a three-strand device, used in this study, with working cycle dynamics is shown in Figure 1. The mechanical work of DNA tweezers, and their relative movements, can be measured using the FRET method, where both the 3´ and 5´ ends are labelled with fluorescent dyes (15). The absorption spectrum of one of the two dyes overlaps the emission spectrum of the other dye. Therefore, given the sufficient proximity of the two fluorophores, when one dye is excited, its emission is absorbed by the other dye. As the distance between the dyes rises, the measured signal of the emitter becomes stronger (17).

In this study, the DNA tweezers' reaction time under different reaction conditions was measured. The opening and closing times of the DNA tweezers tell us how suitable their environment is for their work. Knowing the reaction time is important to determine when the DNA tweezers have completed their work. If DNA tweezers are used for controlled drug delivery, they will not work in their ideal environment (18). Therefore, we decided to induce DNA tweezers with less suitable conditions, when measuring their reaction time to see how this affects them.

The reaction time is defined as time elapsed between the first contact of DNA tweezers with the fuelling (closing reaction time)

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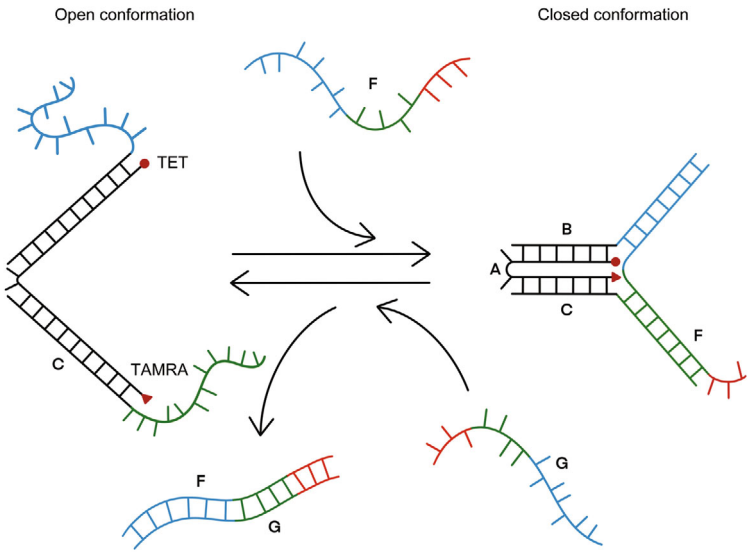

Fig. 1. Schematic depiction of three-strand DNA tweezers and working cycle dynamics. The tweezers are composed of strands A, B and C. Strand A contains a four base pair long region, which functions as a hinge. Strands B and C have domains with bases complementary to the bases on the A strands' arms. The other half of both strands B and C are complementary to a portion of the strand F. Strand F contains a region of nine bases, which exceed the needed length. Strands F and G are complementary to each other. On the left, there are DNA tweezers in an open state conformation. After the introduction of strand F, the tweezers close by bringing both ends of the strands B and C together. Dyes TET (tetrachloro-fluorescein phosphoramidite: and TAMRA (carboxy-tetramethylrhodamine) were connected to the ends of strand A to monitor the operation.

or releasing (opening reaction time) strand and the plateau phase of the signal of closed or open tweezers. A solution containing DNA tweezers was excited by light of a specific wavelength. This was followed by the measurement of light emitted by one of the fluorophores. A time gap between the excitation of the device and acquisition of emitted signal was measured. Measurements were collected at different time points and the acquired fluorescence intensities were plotted as a function of time. The plots show gaps ranging from 3.5 to 6.5 seconds depending on the different adjustments of different measurements. This approach makes it more difficult to determine the approximate time in which the DNA tweezers close or open. Therefore, we tried to find criteria to determine the conditions, under which the tweezers close and open faster or slower. Knowing the reaction time of the DNA tweezers is necessary for considering, when it is possible to manipulate the desired state of the tweezers upon delivery of the signal to induce a change of state. This knowledge can be applied in the development of automated time-controlled tweezers machines. Additionally, if the DNA tweezers are used as target specific drug-delivery machines, reaction time can be used to estimate time of drug release.

Materials and methods

DNA oligonucleotides

The DNA sequences for molecular tweeters were prepared according to Yurke et al (14). All the DNA oligonucleotides were purified by HPLC. The 40-base strand A is labelled at the 5´ end with dye TET (5´-tetrachloro-fluorescein) and at the 3´ end with dye TAMRA (3´-carboxy-tetramethylrhodamine). Strand A has two sections (black), that hybridize to complementary sections of strands B and C (black), and a 4-base single strand region that forms the hinge. The 56-base closing strand F (fuel strand) consists of complementary sections to the dangling ends of B (blue) and C (green), with an additional 8-base overhanging section (red). At this place, interaction with opening strand G (release strand) begins.

Measurement of DNA reaction time

Lyophilised DNA oligonucleotides were dissolved in solution at a concentration 25 µmol.l^{-1} in TE buffer (10 mmol.l^{-1} Tris, pH 8.0, 1 mmol.l^{-1} EDTA). For preparation of DNA tweezers, 2 µl of solution containing strands A, B and were added to 194 µl of SPSC buffer (50 mmol.l^{-1} Na_2HPO_4 and 1 mmol.l^{-1} NaCl at pH 6.5) with pH 6.5. The mix was incubated at room temperature for 50–60 min to allow binding of DNA strands and formation of the DNA tweezers. Fresh solutions were prepared prior to every analysis. Containers with solutions were wrapped in aluminium foil to prevent bleaching of fluorescent dyes. After the DNA tweezers were formed, they were pipetted into a measuring plate. Each solution was compared to its own blank to determine the static state of the measuring device. To measure the performance of DNA tweezers under different reaction conditions,

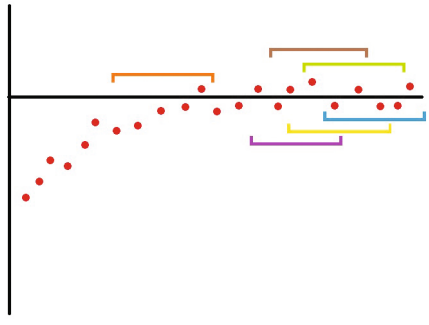

Fig. 2. The use of the F-test in determining reaction time of the DNA tweezers' opening. (Note that a similar approach applies for closing). Red dots represent acquired data points obtained during the measurement. The black horizontal line represents the plateau phase. The blue bracket represents the reference data to which other data points are compared. The data, which are compared to the data in blue brackets are in green, yellow, brown, and purple brackets. Data points in the orange bracket have values that reject the null hypothesis of F-test. This suggests the beginning of the plateau phase.

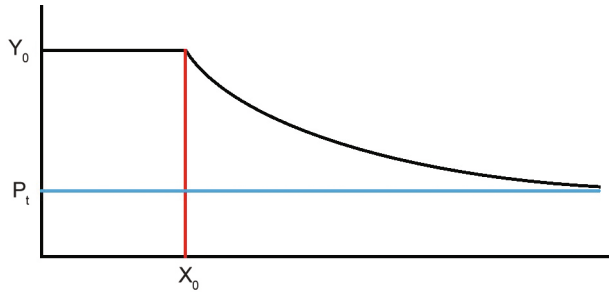

Fig. 3. Illustration of the fitting function: Plateau followed by single-phased exponential decrease.

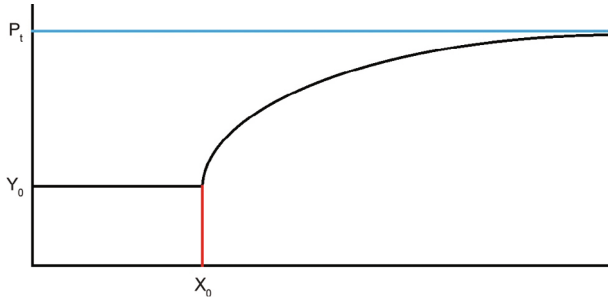

Fig. 4. Illustration of the fitting function: Plateau followed by single-phased exponential increase. The weakness of this method is that it is challenging to determine the experimental cut-off value for calculation of opening and/or closing reaction time.

adjustments to solutions and/or measuring apparatus were made to fulfil the set requirements. For example, $CoCl_2.6H_2O$ was added in different concentrations to the solution of DNA tweezers to see how it affects performance of the tweezers. The measurement itself was conducted as follows: At first, the performance of DNA tweezers was measured without any additions. To monitor the effect of an added chemical, this chemical was added in required concentration and volume to the solution of DNA tweezers. Tested substance was added also into corresponding DNA tweezers blank to avoid any side effects caused by the fluorescence of added substance. This new solution was measured again to determine whether there is any interference between the used chemical and fluorescent dye. Subsequently, the fuel strand was added to close the tweezers and the closing time was measured. Finally, the releasing strand was added to open the DNA tweezers and the opening phase was measured. To analyse the acquired data, Microsoft Excel 2007 and GraphPad Prism 5 were used.

F-test used for calculation of DNA tweezers´ reaction time

The DNA tweezers' reaction time was analysed using the F-test. The last 30 obtained data points were used for the analysis (Fig. 2). These data points correspond to the plateau phase of the measurements. The number 30 was picked because the difference between neighbouring values was quite large (in the case of 500 and more data points – the actual values are shown in the Figure 5 and 30 is the maximum recommended number of entries per data set to be analysed by F-test. It is usually difficult to pick one specific value that would correspond to the beginning of the plateau phase, but it rather depends on an interval where this value could lie. This interval can be relatively large.

Fitting of the acquired data using the particular function for calculation of DNA tweezers´ reaction time

Next, the DNA tweezers' reaction time calculation involves fitting of the acquired data using a function described below. For the closing phase of DNA tweezers, the function is:

y = {Y0 x<X0 Pt+Y0-Pt ×e-k × (x-X0) x≥X0

The graphical representation is depicted in Figure 3. For the opening phase, the function is:

y = {Y0 x<X0 Pt+- Y0×(1-e-k × x-X0) x≥X0

The graphical representation is depicted in Figure 4.

It is possible to calculate the tangents' value *a* in y = a×x+b by using derivation. As the absolute value of *a* decreases, the slope reaches the plateau phase. It is obvious from the character of the fitting function that *a* will never reach zero. Therefore, a value has to be picked in order to make a comparison. For example, in our measurements we picked the value –3 for the closing and 3 for the opening phase. That means that for the closing phase, we determined that the time at which *a* surpassed –3 when analysing the closing phase of DNA tweezers, was the time when plateau was reached. The same logic applies to the opening phase, except the value *a* had to get smaller than 3. This approach helped us to assess, under which circumstances the tweezers close or open faster.

Calculating the work efficiency of DNA tweezers

This method is based on the fitting functions described in method 2; however, the critical value that *a* has to reach is calculated from linear fitting of the last 30 acquired values. The weakness of this method is that sometimes the linear fit of the last 30 values yields a number that is unreachable for the tangents to surpass or get lower than. For example, the linear fit of the last 30 values of a graph of opening tweezers can have a decreasing tendency due to bleaching of the fluorescent dye. In this case it is impossible to reach negative values.

Results

Examples of favourable results

As a good example, we chose to measure the performance of the ABC-FG system dissolved in 100 μmol.l^{-1} KI solution. The measurements were plotted on a graph (Fig. 5).

Closing phase

We determined that the plateau phase was reached at 792 s using method 1. The fuel strand was added at 454 s, so the reaction time was calculated as 338 ± 90 s.

Using method 2, we calculated the function as:

y = 30303+43659-30303e-0.01493t-454

The determination coefficient of the fitting function is r2 = 0.9636.

Applying the time derivation on *y* we got the function:

y = -199×405 e-0.01493t-454

Inserting a different time as *t* into the derivation we found that critical value of tangent a = -3 was surpassed at *t* = 738 s. Therefore, the reaction time was calculated to be 284 s. Using method 3, we fitted the last 30 values of the data of the closing phase. The function of the linear fit is:

y = -2.6548t+32664

The tangent surpassed critical value a = - 2.6548 at 744 s. Therefore, the calculated reaction time was 290 s. The closing phase with different calculated values of *t* is in Figure 6 and the fitting function is in Figure 7.

Opening phase

We determined that the plateau phase was reached at 1788 s using method 1. The releasing strand was added at 1468 s, so the reaction time was 320±90 s because of the F-test properties.

Using method 2, we calculated the function of the fit:

y = 29833+56335-29833×(1-e-0.02219t-1468)

The determination coefficient of fitting function is r2 = 0.9892.

Applying the time derivation on *y*, we got the function:

y = 588.079 e-0.02219t-1468

We found that critical value of tangent a = 3 was reached at t = 1494 s by inserting a different time as *t* into the derivation. The reaction time was 24 s.

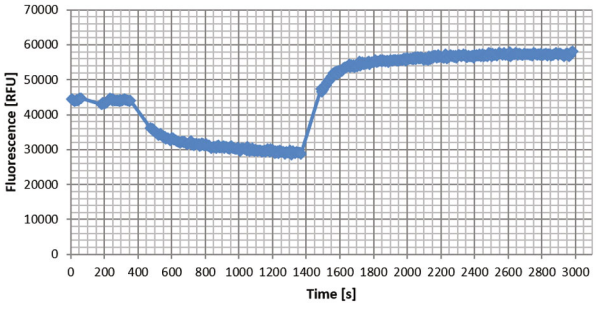

Fig. 5. The whole cycle of closing and opening of DNA tweezers from ABC-FG system. 2 µl of solution of KI with concentration of 100 µmol.l^{-1} is present.

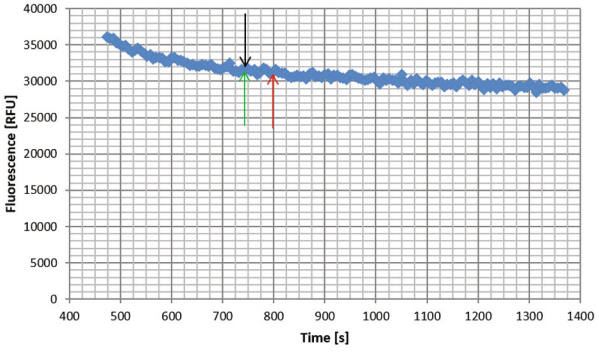

Fig. 6. Closing phase of DNA tweezers from ABC-FG system. 2 µl of solution of KI with concentration of 100 µmol.l^{-1} is present. The arrows point to values where the plateau phase begins according to method 1 (red), method 2 (green) and method 3 (black).

Using method 3, we fitted the last 30 values of the data of closing phase. The function of the linear fit was:

y = 0.4662t+55924

The tangent reached value a = 0.4662 at 1506 s. The calculated reaction time was 44 s. The opening phase with different calculated values of *t* is in Figure 8 and the fitting function is in Figure 9.

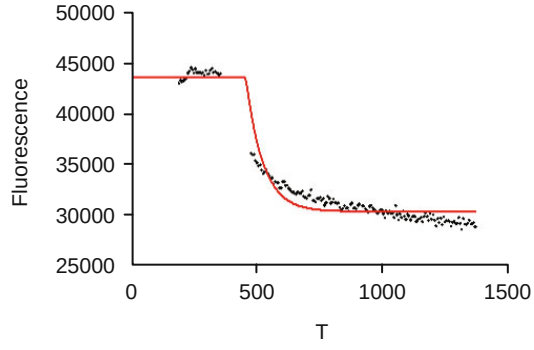

Fig. 7. Fitting function (red) of closing phase of DNA tweezers for the ABC-FG system. Black dots represent the measured data. 2 µl of solution of KI with concentration of 100 µmol.l^{-1} is present. T – time (s).

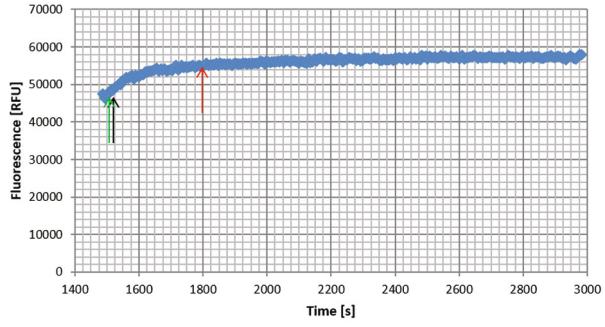

Fig. 8. Opening phase of DNA tweezers from ABC-FG system. 2 µl of solution of KI with concentration of 100 µmol.l^{-1} is present. The arrows point to values where the plateau phase begins according to method 1 (red), method 2 (green) and method 3 (black).

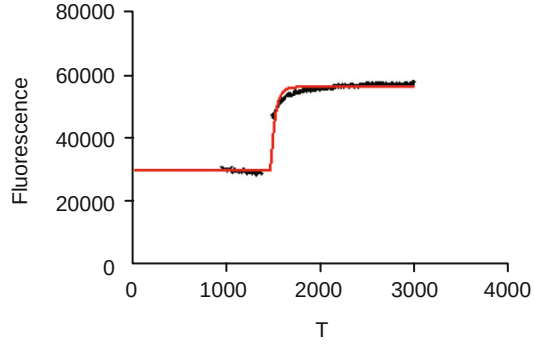

Fig. 9. Fitting function (red) of opening phase of DNA tweezers for the ABC-FG system. Black dots represent the measured data. 2 µl of solution of KI with concentration of 100 µmol.l^{-1} is present. T – time (s).

Examples of unfavourable results

We chose to measure the performance of the ABC-FG system dissolved in 100 µM NaOH solution as not a suitable reaction solution. The measurements were plotted on a graph (Fig. 10).

Closing phase

Using method 1 we determined that the plateau phase was reached at 1071 s. The fuel strand was added at 394 s, so the reaction time was 677 ± 67.5 s because of the characteristics of F-testing.

Using method 2, we calculated the function as:

y = 13161+43759-13161e-0.004034t-394

The determination coefficient of the fitting function is r2 = 0.9914.

Applying the time derivation on y we got the function:

y = -123.432 e-0.004034t-394

We found that critical value of tangent a = -3 was surpassed at t = 1323 s by inserting different time as *t* into derivation. Therefore, the reaction time was calculated to be 924.5 s.

Using method 3, we fitted the last 30 values of the data of the closing phase. The function of the linear fit is:

y = 4.7894t+32664

The tangent surpassed critical value a = 4.7894 cannot be surpassed by inserting different *t* values in the equation:

y = 13161+43759-13161e-0.004034t-394

In this case, it was not possible to determine the reaction time using this method. The closing phase with different calculated values of t in Figure 11 and the fitting function is in Figure 12.

Opening phase

Using method 1, we determined the plateau phase was reached at 1938 s. The releasing strand was added at 1409.5 s, so the reaction time was 528.5 ± 67.5 s because of the F-test properties.

Using method 2, we calculated the function of the fit:

y = 15970+53289-15970×(1-e-0.004539t-1409.5)

The determination coefficient of fitting function is r2 = 0.993.

Applying the time derivation on *y*, we got the function:

y = 214.781 e-0.004539t-1409.5

We found that critical value of tangent a = 3 was reached at t = 2352 s by inserting a different as *t* into the derivation. The reaction time was calculated to be 942.5 s.

Using method 3 we fitted the last 30 values of the data of closing phase. The function of the linear fit is:

y = 3.9376t-43776

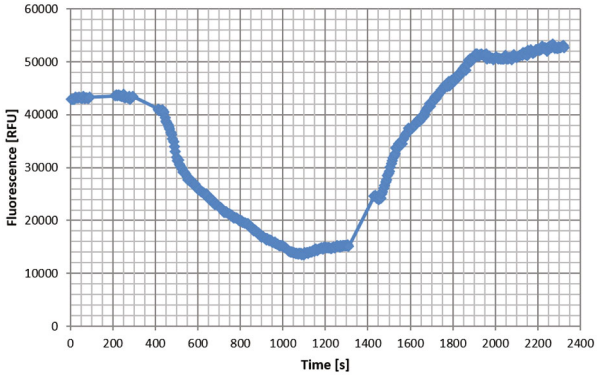

Fig. 10. The whole cycle of closing and opening of DNA tweezers from ABC-FG system. 2 µl of solution of NaOH with concentration of 100 µmol.l^{-1} is present.

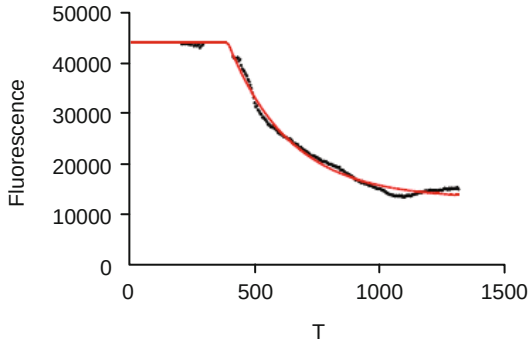

Fig. 12. Fitting function (red) of closing phase of DNA tweezers for the ABC-FG system. Black dots represent the measured data. 2 µl of solution of NaOH with concentration of 100 µmol.l^{-1} is present. T – time (s).

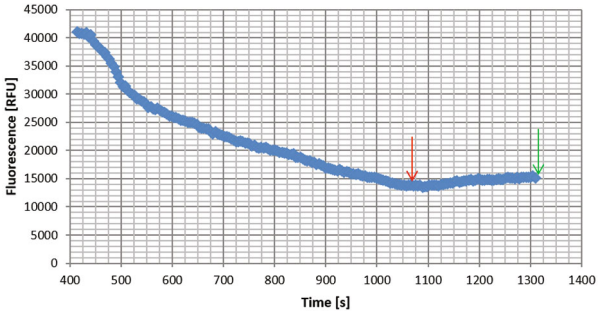

Fig. 11. Closing phase of DNA tweezers from ABC-FG system. 2 µl of solution of NaOH with concentration of 100 µmol.l^{-1} is present. The arrows point to values where plateau phase begins according to method 1 (red), method 2 (green). Method 3 is not given.

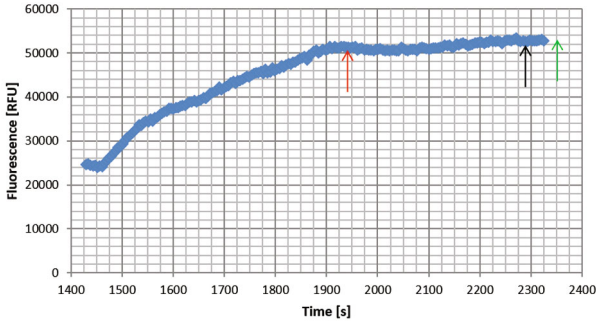

Fig. 13. Opening phase of DNA tweezers from ABC-FG system. 2 µl of solution of NaOH with concentration of 100 µmol.l^{-1} is present. The arrows point to values where the plateau phase begins according to method 1 (red), method 2 (green) and method 3 (black).

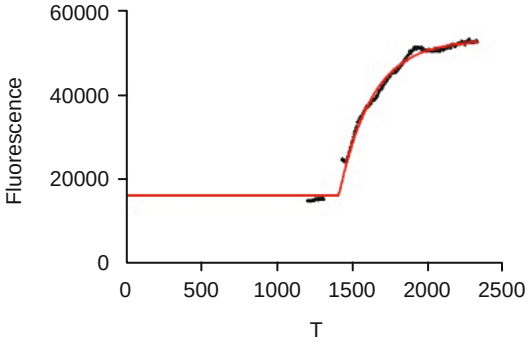

Fig 14. Fitting function (red) of opening phase of DNA tweezers for the ABC-FG system. Black dots represent the measured data 2 µl of solution of NaOH with concentration of 100 µmol.l^{-1} is present. T – time (s).

The tangent reached value a = 3.9376 at 2293.5 s. The calculated reaction time was 884 s. The opening phase with different calculated values of *t* is in Figure 13 and the fitting function is in Figure 14.

Discussion

Here we report the study on FRET-labelled DNA hairpin folding transitions using optical tweezers combined with fluorescence microscopy. The reaction time of DNA tweezers of the ABC-FG system in presence of KI solution using method 1 was 338±90s in the closing phase. In case of calculations using method 2, the reaction time was 284s and 290s by method 3. In the opening phase, method 1 showed the reaction time to be 320±90s because of the F-test properties. The reaction time was determined to be 24s in fitting function and 44s in method 3. We decided to show the limitations of these types of calculation methods, and therefore also describe unfavourable results. The reaction time was measured in NaOH solution and in the closing phase was 677±67.5s, 924.5s using the second method. In case of the third method, the tangent surpassed critical value 4.7894 and could not be surpassed by inserting different *t* values in the equation, thus the reaction time could not be determined. In the opening phase, 528.5±67.5s was the reaction time of method 1 calculations, 942.5s in method 2 and 884s in method 3.

For instance, Gong et al (2015), reported a FRET-based platform for the monitoring of miR-141 from human prostate cancer cells (19). Two years ago, Lertanantawong et al (2019), presented a biosensing platform using DNA-tweezers based on FRET that is suitable for multiplex detection in one-pot assay (20). We have proposed three methods and performed in different measurements, but were not able to conclude which method, or combination of methods, is the best fit to determine the reaction time of opening or closing DNA tweezers. Studying how macromolecules fold and undergo conformational changes is crucial to understand the underlying mechanisms of biological processes.

Conclusion

In summary, we constructed DNA tweezers, whose functionality was tested by measuring their reaction times under various conditions. The conformation changes or reaction time of DNA tweezers between two defined states, the open and closed state, can be controlled by fuel or realizing strand. The fluorophore/quencher decorated on the DNA tweezers and the conformation changes were observed by FRET. We speculate that a combination of different methods may be the most suitable for assessment of the reaction time characteristics, since it provides the best view of the sloping, scatter and other characteristics of the signal acquired. However, we cannot compare the suitability of individual methods because we do not have enough measurements for the same conditions, and thus not being able to statistically evaluate them. DNA is a great candidate for drug delivery, microsurgery, nanomaterial components and many other applications (21–25). We believe this work will lead to the development of other novel DNA nanomachines to execute diverse tasks inside living cells by customizing specific DNA components.

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