Chapter 3

Single-Molecule FRET Analysis of Helicase Functions

Eli Rothenberg and Taekjip Ha

Abstract

In recent years, advancements in single-biomolecule probing techniques have provided critical information on and greater insight into the nature of biomolecules. Of significance is the application of single-molecule fluorescence resonance energy transfer (smFRET) to probe isolated events and changes at the nanometer scales. In particular, the study of helicases using smFRET has supplied much information regarding the nature and dynamics of these enzymes and provided a toolbox for further investigations. In this chapter we provide a general guide for the construction and execution of single-molecule FRET assays for the study of helicase properties and functionalities.

Key words: Helicase, DNA, single molecule, FRET, binding, translocation, unwinding.

1. Introduction

Single-molecule techniques enable us to uncover specific features otherwise masked by the averaging of the ensemble measurements. Of particular advantage is the single-molecule technique based on FRET (1), which utilizes the energy-transfer interaction between a pair of reporter molecules (FRET pair), donor and acceptor, thus allowing us to probe distance changes on a range of a few nanometers. This approach has proven to be extremely beneficial in probing different functional aspects of various helicases (2–6).

Our studies of DNA helicases' functions have revealed many important characteristics, providing a toolbox for assaying such enzymes. Investigating DNA-binding properties of helicases revealed the binding orientation and mechanism of bacterial Rep helicase (3) and archaeal MCM helicase substrate specificity (5). Studies on the translocation kinetics of single

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Rep (4), PcrA, and UvrD (Jeehae Park, "Reeling in DNA One Base at a Time: Repetitive DNA Looping Coupled with PcrA Helicase Translocation," unpublished work) helicases led to the discovery of unique repetitive shuttling behaviors, revealing specific features of these helicases' translocation mechanisms. Investigations on the unwinding of dsDNA by the bacterial Rep helicase showed unwinding re-initiation (2), while viral NS3 (6) and human Bloom helicases (Jaya and Yodh, "Single-Molecule Study of Bloom Syndrome Helicase Reveals Repetitive Unwinding via Strand-Switching," unpublished work) revealed a repetitive unwinding behavior that disclosed the unwinding mechanisms of these helicases. Further studies of unwinding dynamics of the T7 phage helicase revealed a cooperative unwinding processivity (Manjula Pandey, "Kinetic Coupling of DNA Primase-Helicase and DNA Polymerase Coordinates DNA Replication," unpublished work). The general guidelines for approaching and designing such assays are provided herein.

The design of a suitable smFRET experiment for helicase functionality depends on a number of factors. Initially, the functional aspects that are intended to be probed in the assay need to be defined. These could be divided into three general categories:

1. Binding of the helicase to the DNA substrate.
2. Translocation of the helicase along ssDNA.
3. Unwinding of dsDNA.

The above functionalities are closely intertwined and the outcome of one assay would most likely contribute to the interpretation of another. **Section 3** will provide different assays for probing these functionalities.

When approaching an assay, one should first classify what type of reaction occurs, and construct the experimental probing scheme accordingly. For instance, monitoring single-turnover short-lived reactions, such as a complete unwinding reaction of dsDNA, would require flow-type experiments. In this type of experiment the initiation and progression of the reaction are continuously monitored. In other cases, if the reaction is longer lived, or cyclic, where re-initiation occurs, then data collection may be carried out at different time intervals following the initiation of the reaction.

In this chapter we will provide general protocols for assaying the functions of helicases using smFRET. First the materials and general instrumentation used will be described. We note that a comprehensive guide for the construction of a home-built smFRET setup is not provided here and to the inquisitive reader we recommend Ref. (7) for an excellent tutorial on the topic.

2. Materials

2.1. Reagents

We list the commonly required materials for these experiments. Other necessary reagents that are not listed here are of life sciences grade and may be obtained commercially.

1. Aminopropylsilane (United Chemical Technologies) (store at –20°C).
2. Glucose oxidase (Sigma).
3. Catalase (Roche).
4. Oligonucleotides (IDT technology).
5. Coverslips: VWR 240 × 40 mm, CAT. No. 48393 230 (vwr.com).
6. Standard 3″ × 1″ × 1 mm microscope slides (Fisher).
7. mPEG-SC: MW 5000, 1 g, lot#101-68 (Laysan Bio Incorporated).
8. Biotin-PEG-SC: MW 5000 (Laysan Bio Incorporated).
9. Neutravidin: 31000, 10 mg (http://www.piercenet.com). Prepare a concentration of 5 g/mL in T50 buffer. Store in 4°C.
10. T50 Buffer: 10 mM Tris, pH 8, 50 mM NaCl.
11. Drill bits: Kingsley North Inc.: 1-0500-100 (www.kingsleynorth.com).
12. Permanent double-sided tape (Scotch/3 M).
13. Epoxy, 5-min (http://www.devcon.com/).
14. Tubing for flow experiments. ETT-28 (http://www.weicowire.com/).

2.2. Preparation of Gloxy

1. Put 100 µL of T50 buffer in a 250-µL tube. Add 20 µL of catalase.
2. Add 10 mg of glucose oxidase. Centrifuge at 10,000 rpm for 1 min.
3. Recover the yellow supernatant. Store in 4°C; it is good for 3 weeks.

2.3. Instrumentation

As mentioned above, for a thorough guide on the assembly of homebuilt TIRF-FRET microscope please review Ref. (7).

2.4. Software and Analysis

When analyzing smFRET data, it is best to begin by looking at representative smFRET histograms. These histograms are constructed by averaging the initial 8–10 data points from many smFRET trajectories in the smFRET histograms. For substrates having a well-defined donor–acceptor distance, a second peak will appear corresponding to that distance, as shown in **Fig. 3.1** for a DNA substrate having a separation of seven nucleotides between the donor and acceptor. The donor-only peak serves as the zero FRET mark of the histogram.

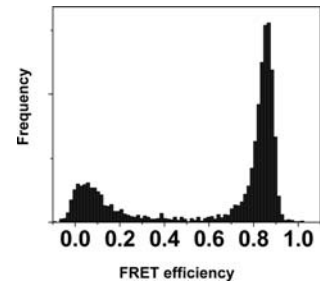

Fig. 3.1. smFRET histogram for the substrates imaged in **Fig. 3.5b**. Two picks are shown in the histogram. The donor-only peak appears at approximately zero FRET efficiency corresponding to substrates having no emitting acceptor, while the highly excited acceptor peak appears at FRET efficiency of about 0.85.

For more in-depth analysis the specific features of single donor/acceptor trajectories should be monitored closely. For instance, **Fig. 3.2** shows two trajectories exhibiting changes: one

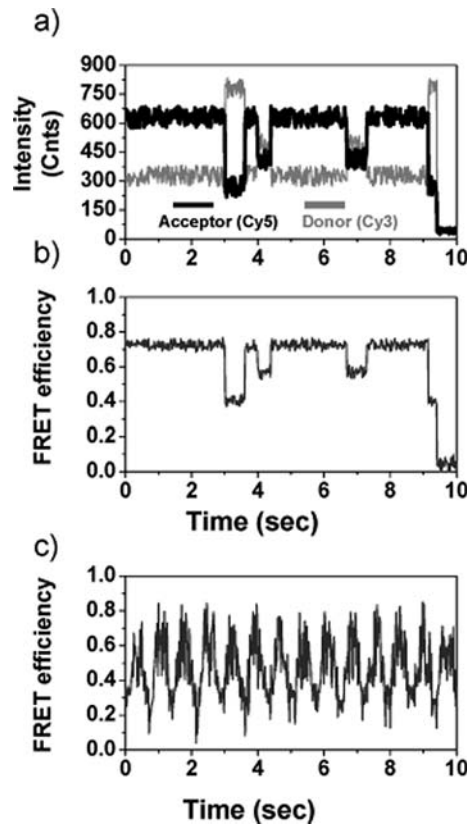

Fig. 3.2. Simulated single-molecule trajectories. **a,–b**. Trajectory of donor (*grey*) and acceptor (*black*) and their corresponding FRET efficiency trajectory, showing several well-defined and long-lived FRET values. **c**. Rapidly and periodically changing FRET efficiency similar to observed repetitive translocation and unwinding.

showing discrete changes with substantial dwell times, while the other showing rapid periodical changes. Analysis of these types of trajectories can provide useful measurements such as dwell times, frequency and magnitude of FRET changes, and intensity of donor and acceptor.

3. Methods

3.1. Coverslips and Slides Preparations and Assembly

Here we provide a basic glass cleaning protocol, though several protocols may be suitable for single-molecule experiments.

3.1.1. Cleaning

1. Rinse and fill the glass container with MilliQ-H_2O. Fill with acetone. Sonicate for 15 min.
2. Fill with 1 M KOH. Sonicate for 20 min. Rinse slides thoroughly with MilliQ-H_2O.
3. Burn the slides with propane torch on each side for 30 s. Burn the coverslips for 1 s on one side. Place back in the container (*see* **Note 1**).

3.1.2. Aminosilanization and PEGylation

1. Pour 150 mL of MeOH into the flask. Add 7.5 mL of acetic acid (glacial) with a glass pipette. Add 1.5 mL of aminopropylsilane by a glass pipette into the flask and mix well.
2. Pour the mixture quickly into both slide and coverslip containers. Incubate for 10 min on bench, sonicate for 1 min, and then incubate on bench for an additional 10 min. After completion of aminosilanization, rinse coverslips and slides with MeOH and water and dry them with N_2(g). Place the coverslips inside tip boxes. Place slides inside tip boxes (*see* **Note 2**).
3. Measure 1–2 of biotin-PEG (for five slides) and place inside a 1.5 mL tube.
4. Measure 40 mg of mPEG and put it in the same tube.
5. Add 320 μL of the PEGylation buffer (10 mL MilliQ-H_2O + 84 mg sodium bicarbonate) and mix gently with pipette. Centrifuge for 1 min at 10,000 rpm.
6. Drop 70 μL of it on each slide. Gently place a coverslip on the top of each slide (be careful not to create bubbles). Place boxes in a dark, well-leveled location. Incubate for 2–3 h.
7. After the incubation period, disassemble the slides, rinse them thoroughly with MilliQ-H_2O, and dry completely with N_2(g). Store and assemble the slides according to your application. Always store them in the dark (*see* **Note 3**).

3.1.3. Assembly of Slides and Coverslips into Flow Chambers

The number of channels in the flow chamber depends on the desired experiments and applications. The holes should be drilled in the glass prior to cleaning, using a Dremel and diamond-coated drill bits. For a single experiment or flow experiments, a single diagonal channel would suffice, as shown in **Fig. 3.3**, while for running several experiments sequentially or simultaneously several channels may be used (**Fig. 3.4**).

1. Place the slide on a stable surface with the PEG-coated surface facing up.
2. Place two strips of double-sided tape on the slide bordering a single diagonal channel between the drilled flow holes (*see* **Fig. 3.3** and **Note 5**).
3. For assembly of multiple channels, place parallel strips of double-sided tape as spacers between channels (refer to **Fig. 3.4**).
4. Gently place the coverslip on top of the slide. Cut double-sided tape around the coverslip and carefully peel off the unsandwiched portions.
5. Reinforce double-sided adhesion by carefully pressing tape regions through the coverslip (using a pipette tip). Seal open regions of the channels with 5-min epoxy (*see* **Note 4**).

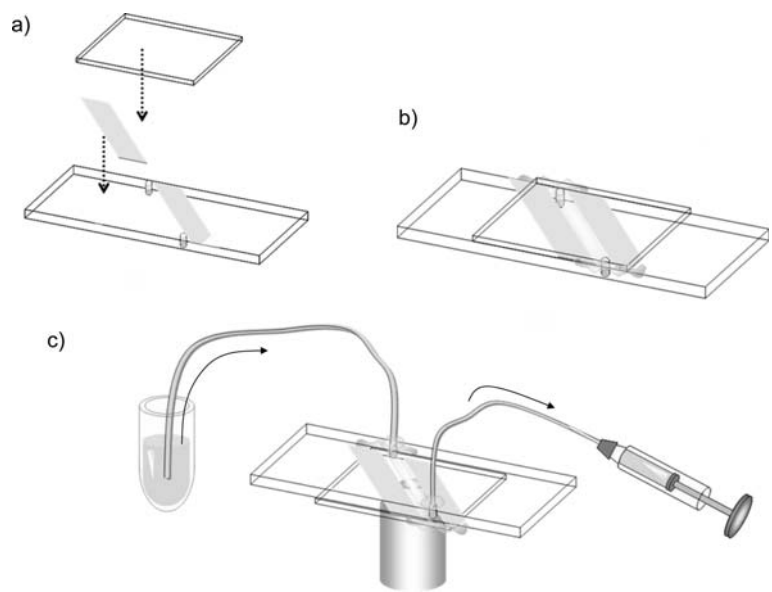

Fig. 3.3. Assembly of single-channel diagonal flow chamber (*see* text for protocol). **a**. After drilling the holes and cleaning, the double-sided tape and coverslip is placed. **b**. The channel is filled with buffer and sealed with epoxy. **c**. The flow apparatus is assembled and the chamber is placed on the microscope.

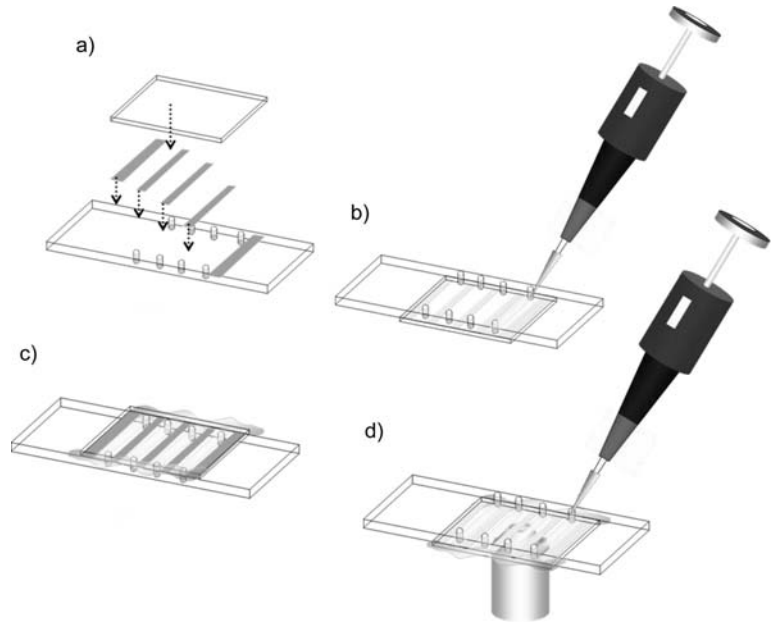

Fig. 3.4. Assembly of multichannel flow chamber (*see* text for protocol). **a**. After drilling the holes and cleaning, small strips of double-sided tape are placed as spacers and the coverslip is placed on *top*. **b**. The channel is filled with buffer and (**c**) sealed with epoxy. **d**. Buffer is added with a pipette directly to the channel and imaged with the microscope.

6. For flow channel assembly, cut two pieces of tubing, approximately 20–30 each. Carefully insert a syringe needle into the end of one of the tubes.
7. Insert the two tubes directly into the drilled holes in the glass.
8. Verify flow through the channel. Insert the loose end of one tube into a tube containing buffer. Pull buffer through the channel using the syringe. Seal the tubing-holes connections using 5-min epoxy (refer to **Fig. 3.3**).

3.2. Sample Preparation

Checking for surface integrity and nonspecific binding is a prerequisite for the reliability of surface-tethered experiments. Please refer to **Fig. 3.5** for an example of nonspecific labeled protein binding and specific binding of DNA substrates.

3.2.1. Nonspecific Binding

1. Image surface.
2. Add 1 nM of Cy3-labeled DNA and image the surface. If image does not contain fluorescent spots resulting from nonspecifically bound DNA, use T50 buffer to wash off channels with 8 channel volumes (*see* **Note 6**).
3. To determine the level of nonspecific binding by protein, add protein labeled with Cy3 or Cy5 at similar concentrations as intended to be used in the experiments (at least 1 nM) and

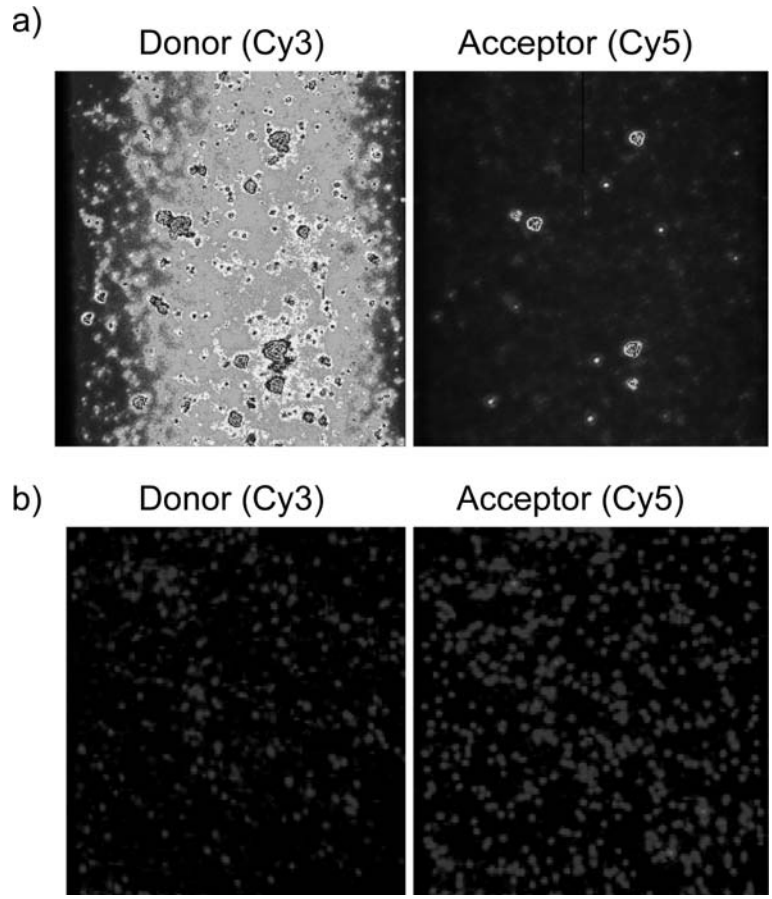

Fig. 3.5. Nonspecific and specific binding in TIRF-FRET imaging. **a**. Nonspecific binding of Cy3 (donor) labeled protein. The *left*-donor channel is saturated. **b**. Specific binding with optimal coverage of a DNA substrate exhibiting high FRET. The *right*-acceptor channel shows a number of spots corresponding to the fluorescence of Cy5 molecules.

image the surface. **Figure 3.5a** shows an example of high levels of nonspecifically bound Cy3-labeled protein. If no fluorescent spots resulting from nonspecific binding are observed, use at least 8 channel volumes of T50 buffer to wash off each channel.

3.2.2. Surface Tethering of DNA Constructs

1. In a 1.5 mL tube, add 960 μL of T50 buffer and 40 μL of Neutravidin stock, giving a final concentration of 0.2 mg/mL. Add 100 μL of Neutravidin solution to each channel. Incubate for 1 min.
2. Wash off Neutravidin with 400 μL T50 buffer (4–6 channel volumes).
3. Add 100 μL of 30 pM biotinylated DNA (*see* **Note 7**). Wait for 5 min and wash off with 400 μL T50 buffer.

4. Image surface and obtain the density of fluorescent spots. If the number of spots is sufficient, the channel is now ready for the experiments. Refer to **Fig. 3.1** for an example of desired density of spots for a high FRET substrate (*see* **Note 8**).

3.2.3. Helicase Activity Assays

As discussed earlier, the three main activity assays would be helicase–DNA binding, ssDNA translocation, and dsDNA unwinding. The experimental components for which concentrations should be optimized for kinetic analysis include helicase, hydrolyzable nucleotides and analogues, and salt (*see* **Note 9**).

The various helicase functionality assays differ mainly in the choice of FRET pair and substrates that will be used, which consequently will define the measured functionality. Besides the various substrates and FRET pairs, the manner by which the experiments are executed is similar. Hence, we will provide some choices for substrates to be used in each type of assay, followed by a general assay that can be used to probe each of the pairs/substrates.

The total volume of the imaging/reaction buffer to be added to the channel is typically 100 μL (*see* **Note 10**). In a flow-type experiment, data recording is started several seconds prior to the flow of imaging/reaction buffer. For recording data, initially take three long (1000 frames each) consecutive movies followed by ten short movies (30–50 frames each). The longer movies will provide information on single-molecule dynamics over time while the shorter movies will provide data on multiple molecules, which can be used to construct statistically significant fluorescence intensity and FRET histograms.

3.2.4. Imaging Buffer

All single-molecule reactions will be carried out in imaging buffer with an oxygen scavenger system (gloxy/catalase) to reduce the photobleaching rate and BME for reduced blinking. Buffer is made immediately before addition to the channel.

1. In a 250 μL tube prepare 98 μL of reaction buffer already containing the desired reactants (helicase, ATP, etc.) and 0.4% beta-D-glucose. Add 1 μL of Gloxy. Add 1 μL of BME (*see* **Note 11**). Mix by pipetting up and down several times (being careful to avoid forming bubbles) and add to the channel.
2. Capture data.

3.3. Binding Assay with Labeled DNA and Protein

3.3.1. Binding Assays

To verify binding and gain structural and organizational information on DNA-bound helicase, the FRET between a labeled helicase and the DNA substrate is monitored. The choice of which will serve as donor and which as acceptor would depend on the binding stoichiometry and labeling efficiency of the helicase. **Figure 3.6** shows typical substrates for binding assays. The fork substrate in **Fig. 3.6a** may be used to monitor the distance

between the tails, which may be drawn together or restricted due to binding of a helicase (**Fig. 3.6a** (ii)). In the substrate shown in **Fig. 3.6b**, the distance between the end and the junction is monitored and may display a change upon binding. An example of such a change is shown in **Fig. 3.6b** (iii), where binding of ssoMCM helicase to such a substrate with a tail of 40 nt shows a shift in the peak in the smFRET histograms. **Figure 3.6c** shows a scheme for binding assays that involves measuring FRET between the labeled DNA and the labeled helicase. In this assay no FRET will be detected unless the helicase binds the DNA substrate. By using various DNA substrates or a helicase labeled in different locations, the resultant smFRET histograms may provide domain orientation and structural information, as illustrated in **Fig. 3.6c** (i)–(iii).

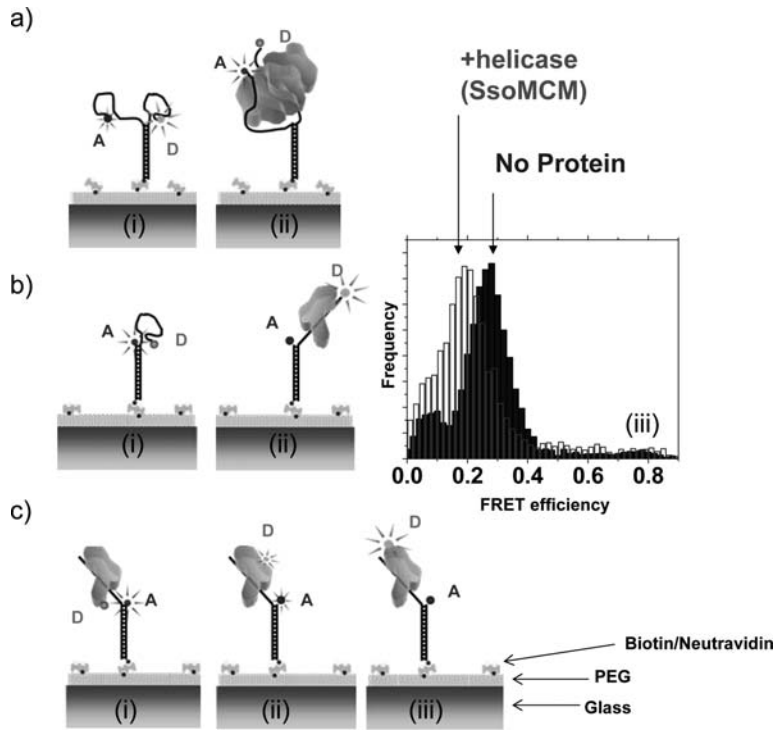

Fig. 3.6. DNA substrates for binding assays. **a**. (*i*),(*ii*) End-labeled fork substrate for monitoring the change between the single-stranded ends. **b**. (*i*),(*ii*) End- and junction-labeled substrate for monitoring distance change between junction and tail, and helicase loading on the tail. (*iii*) FRET change induced by loading of helicase on a substrate as shown on the *left*, having a tail of 40 nt. Blue histogram represents substrate when no protein is present. Red histogram shows a distinct shift to *lower* FRET in the histogram after helicase was added, representing helicase-induced stretching of the DNA. **c**. Substrate for probing binding of labeled helicase.

3.3.2. Translocation Assays

Translocation can be monitored in three principal assays, illustrated in **Fig. 3.7**. First, the distortion of the ssDNA track resulting from the translocation can be probed. This is accomplished by monitoring the FRET change along the track itself using a FRET pair on ends of the track, as illustrated in **Fig. 3.7a**. Since translocation involves a relative motion of the helicase to the DNA substrate, measuring the FRET change between the moving helicase and a fixed point on the DNA track may provide information on translocation speed and step size. **Figure 3.7b** shows the scheme for such assays. In this assay, no FRET will be observed prior to binding of the helicase. Finally, probing helicase's conformational changes associated with translocation and hydrolysis can be done by using a helicase labeled with both donor and acceptor and an unlabeled DNA (*see* **Notes 7** and **8**), as illustrated in **Fig. 3.7c**.

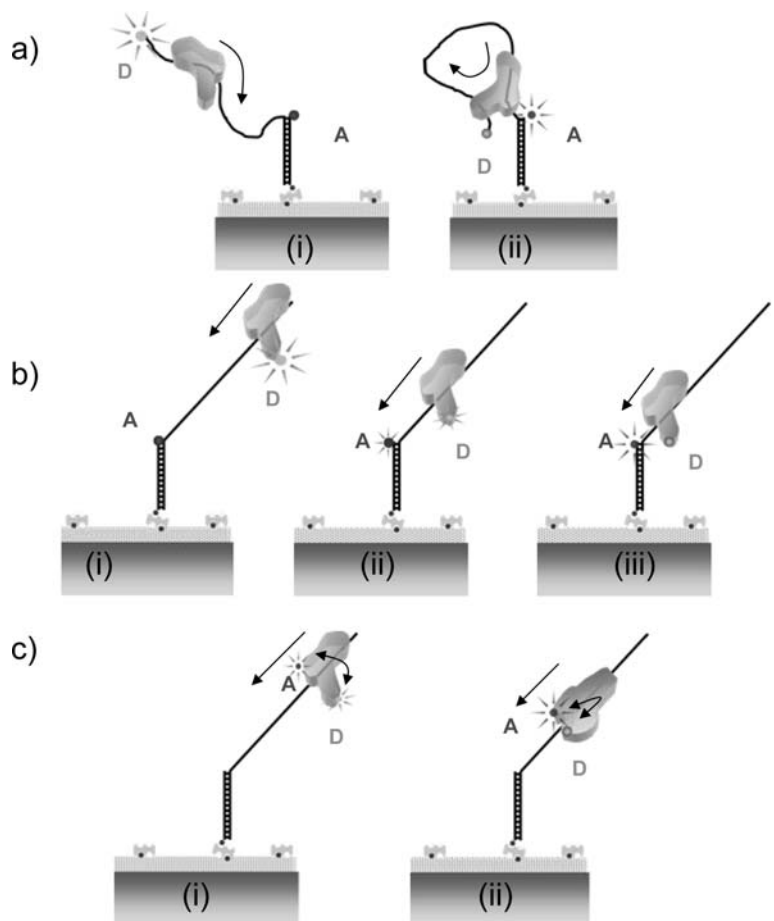

Fig. 3.7. DNA substrates for translocation assays. **a**. (*i*)–(*iii*) End- and junction-labeled substrate to monitor track distortion and end recapture. **b**. (*i*)–(*iii*) End-labeled substrate to probe translocation of labeled helicase along the track. **c**. (*i*)–(*iii*) Unlabeled substrates to probe conformational changes of helicase labeled with donor and acceptor.

Lastly, an additional assay that we wish to include in conjunction with the binding assays is a helicase binding-stability/dissociation assay (**Section 3.3.5**). This assay is complementary to the binding assays (**Sections 3.3.1 and 3.3.4**) and may be done in the same flow chamber immediately following binding experiments. Here, *after* binding is verified, the channel is washed with buffer to remove excess helicase in solution and then the changes in FRET associated with dissociation are monitored.

3.3.3. Unwinding Assays

The principal role of helicase is the unwinding of dsDNA by harvesting energy from nucleotide hydrolysis, resulting in separation of dsDNA into two strands of ssDNA. Generally, unwinding is best approached by monitoring FRET changes on dually labeled DNA in the vicinity of the duplex region of the DNA substrate, and unlabeled helicase, as illustrated in **Fig. 3.8**. The substrates for unwinding may be partial duplexes, having a free tail for the loading of the helicase (**Fig. 3.8a** (iii),(iv)), or forked having both tails (**Fig. 3.8a** (i),(ii)). The substrate may be orientated relative to the surface through tethering, either via the blunt end of the duplex (**Fig. 3.8a** (i)–(iii)) or in reverse orientation via the ssDNA tail such that the duplex is away from the surface (**Fig. 3.8a** (ii)–(iv)). The

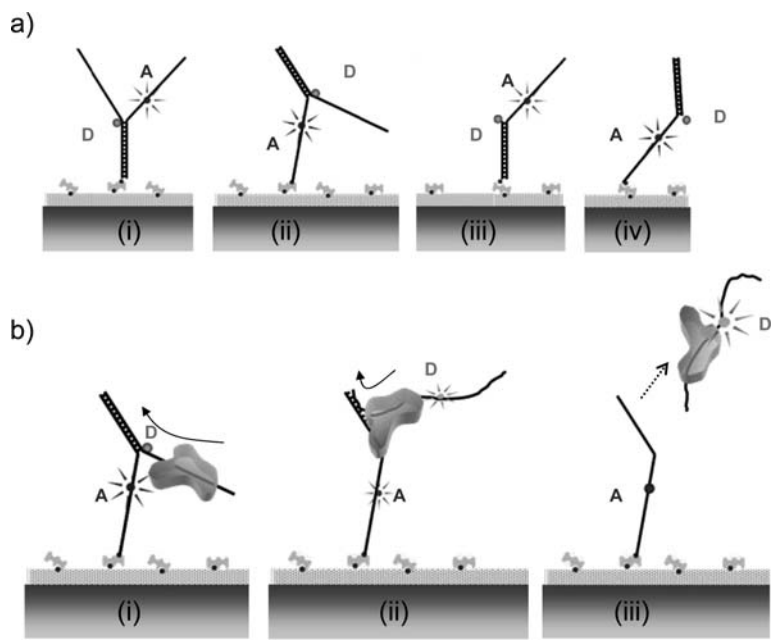

Fig. 3.8. DNA substrates for unwinding assays. **a**. (*i*)–(*iv*) Partial duplex substrates for unwinding, with either single tails (*iii*),(*iv*) or forked (*i*),(*ii*) structures with the substrate being either directly tethered to the surface through its duplex blunt end or tethered via a ssDNA tail, away from the surface (i.e., reverse orientation). **b**. (*i*)–(*iii*) Scheme for unwinding where donor strand disengages from substrate after unwinding.

biotin surface-tethered strand should be labeled with Cy5 such that if full unwinding occurs, as illustrated in **Fig. 3.8b** (i)–(iii), the Cy3 donor-labeled strand would be released from the substrate and the fluorescence signal will diminish.

3.3.4. Helicase General Activity Assay

This assay is to be customized according to the desired functionality and probing scheme, as specified in **Sections 3.3.1, 3.3.2, and 3.3.3** (also *see* **Note 12).**

1. Check nonspecific binding properties of the surface (**Section 3.2.1**) – if a labeled protein is to be used, measure nonspecific binding of labeled protein.
2. Attach substrate of interest (depending on desired activity, *see* **Sections 3.3.1, 3.3.2, and 3.3.3**).
3. Place all reagents (helicase, ATP, gloxy, etc.) in a covered ice bucket.
4. Measure FRET of substrate alone as a control – add imaging buffer and take ten short movies (20–40 frames) and three long ones (1000 frames).
5. Prepare reaction buffer (*see* **Note 12**), add and take ten short movies (20–40 frames) and three long ones (1000 frames).
6. If the studied reaction can be re-initiated, change the concentration of one of the reaction variables and repeat step 5.

3.3.5. Helicase Dissociation Assay

1. For binding assays, following each of the above additions, wash channel using 400 μL T50 buffer. Add imaging buffer and image. Take two long and ten short movies.
2. Repeat, after each concentration, addition in the above assays. Further suggestion regarding assays and analysis are provided in **Notes 13** and **14.**

4. Notes

1. Heated coverslips tend to bend; therefore, pass the coverslips quickly through the flame.
2. The layer of PEG solution in between the glass tends to dry; incubate in vapor-saturated environment (empty pipette tip box with a flooded floor).
3. Can be stored in –20°C for extended periods. Prior to assembly, let it thaw and reach room temperature.
4. Prior to sealing sides with epoxy, carefully add 100 μL of T50 to the channels. This is done so epoxy will not fill the channel. Apply epoxy to the sides using a pipette tip and let it dry.

5. Wash with two additions of 200 μL for 50 μL channel volume. When adding solution to a channel, pipette carefully so no bubbles are formed; place a folded Kimwipe on the exit hole to absorb flow-through.
6. For some substrates adjusting the buffer pH to the range of 8–8.5 may lower nonspecific binding, particularly for ssDNA.
7. The concentration of substrate to be added varies and would depend on labeling efficiency of the substrates and the actual amount of biotin and Neutravidin on the PEG surface; typically the concentration range of 30–200 pM yields a sufficient number of surface-bound fluorescence spots.
8. If the initial number of spots is low (due to reasons given in **Note 7**), gradually increase the concentration of substrate until the desired number of fluorescent spots is reached.
9. T50 buffer with 10 mM of added Mg is typically required for helicase–DNA binding (magnesium acetate or $MgCl_2$). Alternatively enzymatic activity buffer, such as NEBuffer 4 (New England Biolabs), may be used.
10. In the case of narrow channels or if protein is scarce, smaller quantities can be used as long as the concentration of reactants remains the same.
11. BME does not provide a good blinking suppressor in the case of a FRET pair along rigid dsDNA regions. One can use buffer containing TROLOX for blinking suppression (8).
12. For each of the functionalities that are being probed, there exist matrixes of variable quantities. For binding assays, salt and helicase concentration should be titrated. For translocation and unwinding assay, salt, helicase, and hydrolyzable nucleotide concentration should be titrated. Accordingly, for each of these variables a separate control is required. For instance, in translocation and unwinding assays, prior to adding the helicase to the reaction buffer, a control containing imaging buffer plus hydrolyzable nucleotides should be performed in order to ensure that the substrate FRET is not affected.
13. Careful and methodological data analysis is the principal part in quantifying and interpreting the resulting smFRET data. For specific IDL and Matlab code requests, the readers are encouraged to contact the authors for their availability.
14. We note that the assays provided here *must* be optimized depending on the helicases that are being investigated. Optimizing the assays and constructing appropriate DNA substrates should depend on general helicase characteristics such as dissociation constants and effective concentration, helicase oligomeric forms, DNA footprint, directionality,

and processivity. We recommend that prior to expediting smFRET experiments, one should perform fluorimeter activity assays in the bulk, with the same (or similar) DNA constructs as are intended for the smFRET measurements.

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References

1. Ha T., Enderle T., Ogletree D. F., Chemla D. S., Selvin P. R., and Weiss S. (1996) Probing the interaction between two single molecules: Fluorescence resonance energy transfer between a single donor and a single acceptor. *Proc. Natl. Acad. Sci. USA* **93**, 6264–6268.
2. Ha T., Rasnik I., Cheng W., Babcock H. P., Gauss G. H., Lohman T. M., and Chu S. (2002) Initiation and re-initiation of DNA unwinding by the *Escherichia coli* Rep helicase. *Nature* **419**, 638–641.
3. Rasnik I., Myong S., Cheng W., Lohman T. M., and Ha T. (2004) DNA-binding orientation and domain conformation of the *E-coli* Rep helicase monomer bound to a partial duplex junction: Single-molecule studies of fluorescently labeled enzymes. *J. Mol. Biol.* **336**, 395–408.
4. Myong S., Rasnik I., Joo C., Lohman T. M., and Ha T. (2005) Repetitive shuttling of a motor protein on DNA. *Nature* **437**, 1321–1325.
5. Rothenberg E., Trakselis M. A., Bell S. D., and Ha T. (2007) MCM fork substrate specificity involves dynamic interaction with the 5′ tail. *J. Biol. Chem.* **282**, 34229–34234.
6. Myong S., Bruno M. M., Pyle A. M., and Ha T. (2007) Spring-loaded mechanism of DNA unwinding by hepatitis C virus NS3 helicase. *Science* **317**, 513–516.
7. C. Joo and T. Ha (2008) Ch 2. "Single-Molecule FRET with Total Internal Reflection Microscopy" In, "Single Molecule Techniques: A Laboratory Manual", Eds. Pauls R. Selvin and Taekjip Ha. Cold Spring Harbor Laboratory Press, New York, ISBN 978-087969775-4, pp. 507.
8. Rasnik I., McKinney S. A., and Ha T. (2006) Nonblinking and longlasting single-molecule fluorescence imaging. *Nat. Methods* **3**, 891–893.