Chapter 20

Analysis of Changes in Nucleosome Conformation Using Fluorescence Resonance Energy Transfer

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Abstract

ATP-dependent nucleosome-remodeling motors use the energy of ATP to alter the accessibility of the underlying DNA. Understanding how these motors alter nucleosome structure can be aided by following changes in histone–DNA contacts in real time. Here, we describe a fluorescence resonance energy transfer-based approach that enables visualization of such changes.

Key words: Histone, ATP-dependent chromatin remodeling, ACF, SNF2h

1. Introduction

The packaging of eukaryotic DNA into chromatin provides a versatile context for regulating access to DNA. A major mode of regulation is through the action of ATP-dependent chromatin-remodeling complexes (1). These complexes catalyze many different types of reactions ranging from moving the nucleosomes *in cis* to exchanging histone components. Fluorescence resonance energy transfer (FRET) is a powerful tool for monitoring changes in distance between two regions of a nucleosome (2–4). In this technique, a donor and an acceptor fluorescent molecule (fluorophores) are attached to two sites of interest. The emission peak of the donor fluorophore must overlap with the excitation peak of the acceptor fluorophore. Excitation of the donor leads to a transfer of energy to the acceptor by dipole–dipole interactions such that the acceptor emits light at its own emission wavelength (2). The higher the efficiency of transfer, the more quenched the emission of the donor and the greater the emission of the acceptor. The transfer efficiency is very sensitive to the distance between the fluorophores as shown in 1.

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Table 1
Fluorescence donor and acceptor properties (see Note 10)

Donor	Excitation (nm)	Emission (nm)	Acceptor	Excitation (nm)	Emission (nm)	~R_0 (Å)
Fluorescein	495	520	Rhodamine	559	583	55
Cy3	550	564	Cy5	648	668	56

$$E = \frac{R_0^6}{R_0^6 + r^6} \quad (1)$$

Ro, also known as the Förster radius, is the distance between the donor and acceptor probe at which the energy transfer is 50% efficient and r is the distance between the fluorophores. Thus, increases or decreases in inter- or intramolecular distances can be monitored by a decrease or increase in FRET efficiency, respectively. Ro is unique to every FRET pair (Table 1).

The histones are labeled at single cysteines, which are introduced by site-specific mutagenesis (e.g., QuikChange from Stratagene). The location of the cysteine mutation is chosen based on the crystal structure of the nucleosome core particle such that it is within the Förster radius from the labeled DNA residue (5, 6). Further, the mutation should not disrupt any histone–DNA interactions. A critical aspect of preparing fluorescently labeled nucleosomes is ensuring high quantum efficiency of the labels. Therefore, it is critical to use a fresh batch of fluorescent dyes when labeling histones. Furthermore, it is important to shield all fluorescently labeled reagents (dyes, DNA, histones, octamers, and nucleosomes) from light exposure throughout all protocols.

Over the last several years, FRET has been extensively used in several studies of chromatin dynamics. This technique has been used to study the process of exchange of core histones with histone variants (7). FRET has also been used to measure rates of spontaneous exposure of nucleosomal DNA sites and the rates of remodeling by the ATP-dependent chromatin-remodeling complex, ACF (8, 9). In all these studies, it was demonstrated that the FRET probes do not interfere with nucleosome integrity.

Here, we describe protocols for FRET measurements with nucleosomes containing a Cy3 dye on one 5′-end of the DNA and a Cy5 dye on histone H2A at position 120, which is mutated to a cysteine (Fig. 1a, H2A-120C). These locations were chosen based on the nucleosome crystal structure such that distance between the dyes is within their Förster radius (Ro) (Table 1). We describe how

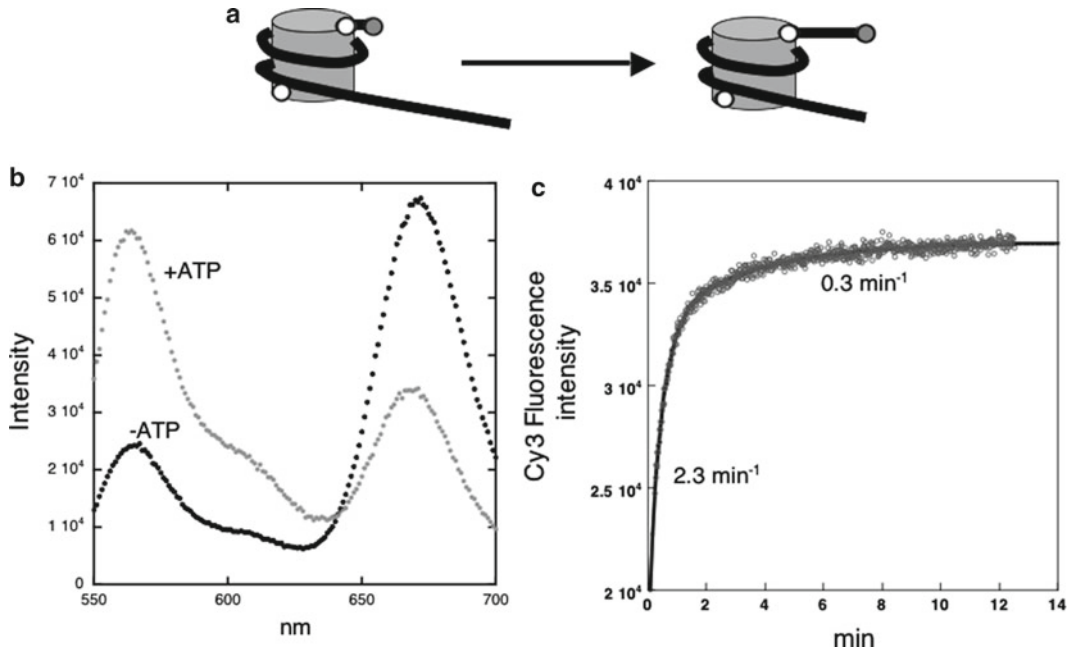

Fig. 1. (**a**) Schematic of a fluorescently labeled nucleosome that is moved toward the center of the DNA upon the action of the ATP-dependent chromatin-remodeling enzyme, SNF2h. The histone octamer is shown as a *cylinder* and the DNA is shown in *black*. The Cy3 dye on the DNA is shown as *filled circle*, and the two Cy5 dyes on each of the two H2A molecules are shown as the *open circles*. Only one of the two Cy5 dyes is close enough to the Cy3 dye to give FRET. (**b**) An emission scan obtained across 500–700 nM after exciting samples at the excitation maximum for Cy3. The samples contain reaction mixtures similar to those described in Subheading 3.4. In the absence of ATP, the Cy3 fluorescence is lower than the Cy5 fluorescence due to FRET (*black line*). After reaction in the presence of ATP, SNF2h moves the nucleosome toward the center as shown in (**a**), which results in an unquenching of Cy3 fluorescence (*grey line*). This increases the distance between the Cy3 and Cy5 dyes and lowers the FRET. As a result, there is unquenching of the Cy3 dye fluorescence and a reduction of the FRET-induced fluorescence of the Cy5 dye. (**c**) The kinetics of remodeling can be measured by following the unquenching of the Cy3 fluorescence as a function of time. In this particular plot, the data is best fit by two exponentials resulting in a fast rate constant (2.3 min^{-1}) and a slower rate constant (0.3 min^{-1}).

to measure the effects of nucleosome remodeling by SNF2h, the ATPase subunit of the human ACF complex. The specific buffer conditions for the remodeling reaction can be altered as needed for other remodeling enzymes.

2. Materials

2.1. Site-Specific Labeling of Histones with Fluorescent Dyes

1. Lyophilized histones with single cysteines (4).
2. Cy5-mono maleimide.
3. Anhydrous dimethylformamide (DMF).
4. Tris(2-carboxyethyl) phosphine hydrochloride (TCEP), 0.5 M, pH 7.
5. 2-mercaptoethanol.

6. Labeling buffer, pH 7.3: 20 mM Tris–HCl, pH 7.0, 7 M guanidinium HCl, 5 mM EDTA.
7. Centrifugal filter device for sample concentration, such as Microcon-10 (Millipore).
8. SYPRO-Red (Molecular Probes).

2.2. Preparation of Histone Octamer from Purified Core Histones

1. Lyophilized histones H2B, H3, and H4.
2. Cy5-labeled histone H2A (prepared in Subheading 3.1).
3. Unfolding buffer, 50 ml: 20 mM Tris–HCl, pH 7.5, 7 M guanidinium HCl, 10 mM DTT (add just prior to use).
4. Refolding buffer, 3.5 l: 10 mM Tris–HCl, pH 7.5, 2 M NaCl, 1 mM EDTA, 5 mM 2-mercaptoethanol (add just prior to use).
5. Dialysis membranes (6–8 kDa cutoff) preboiled in Milli-Q water and cooled to room temperature.
6. Superdex 200 HR 10/30 column (GE, Amersham) or equivalent.
7. Centrifugal filter device for sample concentration, such as Microcon-10 (Millipore).
8. 0.22 μm Millipore Ultrafree centrifugal filter or equivalent.
9. Wash buffer: 50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA, 1 mM benzamidine HCl (add just before use).

2.3. Large-Scale PCR Preparation of Fluorescently Labeled DNA

1. 96-well PCR plates.
2. Forward primer labeled with cy3 at 5′ end (HPLC purified).
3. 10× PCR buffer: 100 mM Tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 5% Tween 20.
4. 2 mM dNTPs.
5. Taq polymerase.
6. OakRidge centrifuge tubes or equivalent.
7. Large gel electrophoresis apparatus.
8. 3 M sodium acetate, pH 5.0.
9. 200 proof ethanol.
10. Native DNA-loading buffer: 20% glycerol, 20 mM Tris–HCl, pH 7.5.
11. TE buffer: 10 mM Tris–HCl, pH 8.0, 1 mM EDTA.
12. 50-ml Conical tube filter units.
13. 5% acrylamide, 0.5× TBE mini gel.
14. 10-ml syringe without needle.
15. DNA of known concentration (*preferably DNA used to assemble nucleosomes*).

16. Ethidium bromide (EtBr).
17. Scanning device, such as Typhoon Scanner.

2.4. Measurement of Remodeling End Points

1. 15 mM $MgCl_2$.
2. 10 mM ATP–$MgCl_2$.
3. BC100: 20 mM HEPES, pH 7.9, 100 mM KCl, 0.2 mM EDTA, 20% glycerol.
4. Nucleosome buffer: 20 mM Tris–HCl, pH 7.5, 1 mM EDTA, 20% glycerol, 0.1% NP-40.

2.5. Measurement of Remodeling Kinetics

1. BC100 with FLAG peptide: BC100: 20 mM HEPES, pH 7.9, 100 mM KCl, 20% glycerol, 0.2 mM EDTA, 1 mg/ml FLAG peptide.
2. Nucleosome buffer: 20 mM Tris–HCl, pH 7.5, 1 mM EDTA, 20% glycerol, 0.1% NP-40.
3. BC100 without glycerol: 20 mM HEPES, pH 7.9, 100 mM KCl, 0.2 mM EDTA.
4. 100 mM $MgCl_2$.
5. 120 mM Mg.ATP.
6. BC100: 20 mM HEPES, pH 7.9, 100 mM KCl, 0.2 mM EDTA, 20% glycerol.
7. 15 mM $MgCl_2$.

3. Methods

3.1. Site-Specific Labeling of Histones with Fluorescent Dyes

The method described here is for labeling of H2A-120C with Cy5 but can be applied to any histone and maleimide combination. The histones must have single cysteine mutations incorporated in the location of interest, for example by using QuikChange mutagenesis. Location of labeling can be determined based on the crystal structure of the nucleosome (5, 6). Maleimide chemistry is then used to specifically label the cysteines. All steps in this protocol are carried out at room temperature and shielded from light.

1. Dissolve 0.5 mg of *H2A-120C* in 0.4 ml of labeling buffer (~0.1 mM final) at room temperature (see Note 1).
2. Add 1 μl of 0.5 M TCEP to the reaction (~1.25 mM final).
   This step reduces the cysteines, so they can be modified.
3. Incubate at room temperature for 2 h (shield from light by covering the entire rack of tubes with foil and storing in a drawer).
4. Add 12.5 μl of 100 mM Cy5-maleimide (~3 mM final) (see Note 2).

Dissolve a fresh batch of Cy5-mono maleimide *in anhydrous DMF. Usually, all dissolved dye is used up. Leftover dissolved dye is discarded.*

5. Incubate at room temperature for 3 h (shield from light by covering with foil and keeping in a drawer).
6. Stop the reaction by adding beta-mercaptoethanol to 80 mM final concentration.
7. Save a small aliquot to run on a gel. At this stage, the reaction can be stored overnight if needed (shield from light and store at room temperature).
8. To remove most of the free dye from the labeled histone, concentrate the histone in a Microcon-10 or similar filter concentration device at room temperature three times. Each time, concentrate to approximately one-third the original volume, and dilute back down to original volume with labeling buffer. Use a new filter every time. Save the flow through until certain that histones are retained on top of the Microcon (see Note 3).
9. Following the Microcon treatment, run a sample on a 15% polyacrylamide separating gel and stain with Coomassie or SYPRO (for quantification) (see Note 4). Dilute 1 μl of sample into 50 μl of water before adding SDS loading dye and load ~15 μl of the diluted histone on the gel. This dilution step is required because the guanidium in the reaction mixture can cause precipitation with SDS. Load equal volumes of sample from before and after the concentration treatment to determine the yield. To further avoid precipitation, load the sample directly onto the gel after heating to 95°C (do not spin down). Use an imaging system, such as Molecular Dynamics Typhoon, to quantify the two bands. Since the concentration of the histone before concentration is known, this can be used to determine the concentration of the histone afterward by comparing the fluorescence signals for the two samples. The gel can also be stained using SYPRO red as an independent way of quantifying the histone concentration (see Note 5).
10. At this stage, the Microcon-treated histones should be shielded from light and can be stored at room temperature overnight if needed.
11. Octamers can then be assembled by mixing appropriate amounts of the labeled histone in its guanidinium buffer with other histones dissolved in the unfolding buffer (see refs. 10, 11).

3.2. Preparation of Histone Octamer from Purified Core Histones

This method describes the assembly of histone octamer using a labeled histone subunit and other nonlabeled subunits (adapted from (10, 11)). The octamer is folded by dialysis against high salt

and purified over an FPLC column. The H3/H4 tetramer is difficult to resolve from the octamer on the column; therefore, it is important to use a slight molar excess of H2A/H2B to make sure that octamer formation goes to completion.

1. Dissolve each lyophilized histone to a concentration of 2 mg/ml in unfolding buffer. Pipette up and down to dissolve. Do not vortex.
2. Allow unfolding to proceed for at least 30 min at room temperature and for not more than 3 h.
3. Mix the four histones so that the molar ratio of H2A:H2B:H3:H4 is 1.2:1.2:1:1 (see Note 6).
4. Adjust the final protein concentration of the mix to 1 mg/ml total protein using unfolding buffer.
5. For volumes up to ~8 ml of mixed protein, dialyze in 6–8 kDa cutoff dialysis bag three times against 1 l of refolding buffer in the cold room. At least one dialysis should be overnight. The rest should be at least 3 h each. For large-scale assemblies, scale up the dialysis buffer proportionately.
6. After the final dialysis, spin down sample to remove any precipitated material. Save the supernatant for loading on the column.
7. Begin washes and equilibration of Superdex 200 HR 10/30 column the day before. A wash step with 2-column volumes of water is required if the column has been stored in 20% ethanol or another similar storage buffer. Then, equilibrate with at least 2-column volumes of refolding buffer (24 ml × 2).
8. Concentrate the assembly to ~200 or 500 μl in a Microcon 10 or other concentration device. Save the flow through until it is verified that histones have been retained in the concentrating chamber.
9. Filter the concentrated sample using a 0.22-μm Millipore Ultrafree or equivalent centrifugal filter and load on the column (save ~5 μl for running on a gel). Run the buffer at 0.5 ml/min and collect 0.5-ml fractions (see Note 7).
10. Run the flow through, load and peak fractions on a gel, and pool bona fide octamer fractions that contain equimolar H2A, H2B, H3, and H4. Concentrate to greater than 1 mg/ml using a Microcon 10 or other concentration device.
11. Determine octamer concentration by SYPRO staining using BSA standards.
12. Flash freeze in liquid N_2 and store at −80°C. The octamer is stable at −80°C for at least 6 months.

3.3. Large-Scale PCR Preparation of Fluorescently Labeled DNA

The following protocol is for the amplification of a 202-bp DNA fragment on a 10-ml scale. The protocol uses the 601 template (8) and a Cy3-labeled forward primer. This protocol can be adapted to amplify DNA fragments from any template using any 5′-labeled primer. For amplification on a larger scale, simply adjust the PCR protocol proportionally. A general PCR protocol is used with the modifications outlined below. The amplified product is then gel purified and precipitated with EtOH. Keep exposure to light at a minimum by shielding samples whenever possible.

1. Set up PCR as follows:

10× buffer (with 15 mM Mg^{2+})	1× final
Fluorescent forward primer	0.5 μM final
Reverse primer	0.5 μM final
dNTP	0.2 mM final
601 template	0.1 ng/μl final
Taq polymerase	0.05 U/μl final
H_2O (Milli-Q)	Adjust to 10 ml
Total volume	10 ml

2. Mix all components on ice in a 15-ml falcon tube.
3. Add 100 μl PCR mix in each well of a 96-well plate using the 12-channel multi-pipettor and seal.
4. Place in thermal cycler and use 35 cycles (sample program):

195°C	4 min
95°C	45 s
58°C	30 s
72°C	1 min
Loop to step 2, repeat 35 times	
72°C	10 min
4°C	

5. Confirm amplification by running 5 μl of sample on a 5% 1× TBE mini-acrylamide gel next to a 100-bp ladder. Stain with EtBr. A good yield is ~50 ng/μl.
6. Transfer the PCR product into a 30-ml Oak Ridge tube.
7. Add one-tenth volume 3 M sodium acetate, pH 5.0, and three volumes of 200 proof ethanol.
8. Incubate at −20°C over night (or for 3 h if time is limiting) to precipitate DNA.

9. Spin down DNA at 4°C at 25,000 × *g* for 20 min.
10. Carefully remove supernatant and allow the DNA pellet to air dry for ~30 min. There is no need to wash the pellet at this stage (see Note 8).
11. Redissolve pellet in ~400 μl native DNA-loading buffer (no dye).
12. Earlier in the day, pour a 5% acrylamide (29:1 acrylamide:bis) and 1× TBE gel (16 cm long × 40 cm wide). Use a comb with a large well (12 cm wide).
13. Load the DNA into the large well. Load xylene cyanol and bromophenol blue dyes in an adjacent smaller well for tracking. For a 200-bp fragment, run the gel at 100–150 V until the bromophenol blue has reached the bottom of the gel.
14. Open the gel plates such that the gel remains on one of the two plates. Cover with plastic film wrap and then aluminum foil to shield from light.
15. Take off the foil and view the DNA band under a short-range handheld UV lamp in the darkroom. Cut out the desired band using a clean razor blade. Exposure to UV should be kept to a minimum.
16. Plunge the gel piece through a 10-ml syringe (no needle) into 15-ml tube. This step crushes the gel matrix.
17. Add ~10 ml TE buffer, pH 8.0, wrap in aluminum foil, and rock overnight at room temperature. This step extracts the DNA from the gel.
18. Pour gel and TE buffer mixture over a 50-ml conical-top filter unit, attach to a vacuum, and collect the flow through. DNA is in the flow through.
19. Transfer flow through to an Oak Ridge tube and repeat steps 7–10, this time washing the pellet with cold 70% ethanol.
20. After it is dry, dissolve pellet into ~50–100 μl TE buffer, pH 8.0.
21. Quantify by measuring absorbance at 260 nm. The DNA can be stored at −20°C till further use.
22. The purified labeled DNA and labeled octamer can then be assembled into mononucleosomes as described in 10, 11 (see Chapters 15 and 21).
23. Nucleosomes are purified from free DNA over a 10–30% glycerol gradient containing 0.1% NP40 to stabilize the nucleosomes (12) (see Chapter 21, Subheading 3.1.4). A Microcon 100 is used for subsequent concentration of the nucleosomes as the size of the micelles formed by NP40 is ~90 kDa.
24. Nucleosomes can be quantified according to steps 23–25 below. These steps are also applicable to quantification of

nonlabeled nucleosomes. Incorporation of DNA into nucleosomes quenches ethidium bromide fluorescence of DNA by 2.5-fold. This value is used when quantifying nucleosome concentration. DNA is stained with EtBr and scanned on a Typhoon or similar scanning device.

25. Run a few different amounts of nucleosomes on 5% acrylamide gel. The nucleosomes off the gradient already contain ~20% glycerol and can be loaded directly onto the gel. Do not use any dye.
26. Load 3–4 DNA amounts between 10 and 100 ng to generate a standard curve. Use loading buffer without dye. A 100-bp ladder and bromophenol blue can be loaded in a separate well for tracking. Run gel until dye reaches one-fourth of the way from the bottom of the gel.
27. Stain gel with EtBr and scan using the Typhoon or similar scanning device in the EtBr channel. Use the DNA standard to calculate the amount of nucleosomal DNA. This value must be adjusted for the 2.5× quenching effect of EtBr when staining nucleosomal DNA. 1 mole of nucleosomes contains 1 mole of nucleosomal DNA.

3.4. Measurement of Remodeling End Points

This protocol allows measurement of the final remodeling-induced FRET changes (Fig. 20.1b). We describe here how to measure the effects of nucleosome remodeling by SNF2h, the ATPase subunit of the human ACF complex.

1. Keep exposure of fluorescently labeled nucleosomes to a minimum.
2. Use all appropriate protocols and precautions when using your particular fluorometer. We use an ISS K2 fluorometer.
3. Prepare two reactions on ice, each 80 µl, as outlined below. One reaction contains ATP (+ATP) and the other does not (–ATP). The specific reaction volume depends on the size of the cuvette:

   +ATP reaction

   (a) 16 µl 15 mM $MgCl_2$ (3 mM final)

   (b) 16 µl 10 mM ATP–$MgCl_2$ (2 mM final obtained by mixing equimolar ATP and $MgCl_2$)

   (c) 16 µl 50 nM nucleosomes in nucleosome buffer (10 nM final)

   (d) 32 µl 300 nM SNF2h in BC100 (180 nM final) (see Note 9)

   –ATP reaction

   (a) 16 µl 15 mM $MgCl_2$ (3 mM final)

   (b) 16 µl water

(c) 16 µl 50 nM nucleosomes in nucleosome buffer (10 nM final)

(d) 32 µl 300 nM SNF2h in BC100 (180 nM final) (see Note 9)

4. Mix each reaction well and incubate at 30°C for 30 min. Then, transfer sample to the cuvette and place inside fluorometer.
5. Excite sample at the excitation wavelength of the donor fluorescent label (Cy3) and take an emission scan that spans the emission wavelengths of the donor and acceptor (Fig. 1b) (see Note 10).

3.5. Measurement of Remodeling Kinetics

Either donor unquenching or acceptor quenching can be measured in real time. The data shown in Fig. 1c follows Cy3 unquenching of the nucleosome shown in Fig. 1a as a function of SNF2h remodeling. Reactions are initiated by addition of ATP, and data collected at 30°C for at least 10 min and sampled once per second.

1. Prepare the ATP mix (40 µl):
   (a) 16 µl BC100 with 1 mg/ml FLAG peptide (Sigma)
   (b) 8 µl Nucleosome buffer
   (c) 8 µl BC100 without glycerol
   (d) 6 µl 100 mM $MgCl_2$
   (e) 2 µl 120 mM Mg–ATP
2. Set up the reaction mix on ice, keep in the dark (80 µl):
   (a) 16 µl 15 mM $MgCl_2$ (3 mM final)
   (b) 16 µl BC100 buffer
   (c) 16 µl 37.5 nM nucleosomes in nucleosome buffer (5 nM final)
   (d) 32 µl 1.125 µM SNF2h in BC100 with 1 mg/ml FLAG (300 nM final)
3. Immediately after addition of the enzyme in step 2, separately incubate both the ATP stock and reaction mix at 30°C for 5 min in the dark.
4. Add the 80 µl reaction mix to a cuvette that has been prewarmed to 30°C in the fluorometer and perform an emission scan as described in Subheading 3.4.
5. Start collecting time-course data of the 80 µl reaction mix. After verifying that the fluorescence intensity is stable over at least 10 s, pause the data collection and manually mix in the 40 µl of prewarmed ATP mix. Mix well, but avoid introducing air into the sample. Then, resume data recording. The time elapsed between the initiation of mixing and resumption of data acquisition should be accurately recorded; as this time-lag is needed when fitting the data (usually, about 10–15 s).

6. After the Cy3 signal stops increasing with time, the reaction is complete. At this point, a final emission scan can be performed to ensure the proper change in donor and acceptor fluorescence intensities.

The final concentrations for the reaction are as follows: 12 mM HEPES, pH 7.9, 4 mM Tris, pH 7.5, 60 mM KCl, 3 mM $MgCl_2$, 0.32 mM EDTA, 12% glycerol, 0.02% NP-40, 0.4 mg/ml FLAG peptide, 2 mM Mg–ATP, 5 nM nucleosomes, 300 nM SNF2h.

3.6. Fitting Models to the Data and Interpretation

Data are best fit by single or multiple exponentials using a program, such as Kaleidagraph or MATLAB (Fig. 1c). Variables include rate constants as well as fluorescence values for the starting material, any intermediates, and the end product. The fluorescence intensity obtained from the fit at time = zero should be very close to the value obtained from dilution of the reaction mix with the ATP mix. In the above experiment, the fluorescence intensity at time = zero should equal ~2/3 the fluorescence intensity before addition of ATP (80 μl diluted to 120 μl final). The first data point that is collected may differ significantly from the initial value as the reaction may have proceeded partially between manual mixing and data acquisition. Thus, one must also correct for this lag at every time point.

The rate constants obtained from fitting the data only represent the change in fluorophore distances that is detectable by FRET, which is based on the Förster radius of the fluorophores. The fit of data to multiple exponentials may indicate the presence of intermediates in the remodeling reaction or the presence of two populations of nucleosomes, which react with different rates (Fig. 1c). While the absolute value of FRET cannot be used to determine the exact distances between dyes due to orientation effects, this technique can reliably detect changes in distance between dyes.

4. Notes

1. A good control is to perform the labeling in parallel with H2A lacking any cysteines to ensure that labeling is specific to the single cysteine.
2. If fluorescein 5-maleimide or tetramethylrhodamine (TMR) maleimide are being used instead, these two dyes should be dissolved in anhydrous DMSO (100 mM final).
3. Each concentration step takes approximately 30 min for 0.4 ml. The free dye passes through the Microcon filter, but the histone does not, so at the end the free dye concentration is reduced by ~27-fold.
4. The labeled histone may migrate more slowly than unlabeled histone if labeled with fluorescein or rhodamine. The Cy dyes do not alter the mobility significantly.

5. The SYPRO gel can also be used to estimate the concentration of the labeled histone by comparison with a relevant and previously quantified histone standard.
6. A slight excess of H2A and H2B is used to ensure complete octamer formation. This is because octamer and H2A/H2B dimer can be easily resolved on the Superdex200 column, but the H3/H4 tetramer cannot be easily resolved from the octamer (M.W.: H2A – 13,960 Da, H2B – 13,774 Da, H3 – 15,273 Da, H4 – 11,236 Da).
7. For this particular column, the octamer should elute at between ~12 and 13 ml and the dimer between ~15 and 16 ml.
8. Do not discard supernatant until you have confirmed DNA recovery. Unlabeled DNA has a clear pellet that is harder to visualize by eye. Fluorescent labels on DNA result in a colored pellet that is clearly visible for this scale PCR. If no pellet is visible, respin the supernatant and if pellet is still not visible repeat the precipitation.
9. The specific buffer conditions for the remodeling reaction can be altered as needed for other remodeling enzymes.
10. While the excitation maximum for Cy3 is 550 nm, we often excite at 515 nm, which is the smaller excitation peak of Cy3, so as to minimize direct excitation of Cy5.

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