Video Article

A Protocol for Using Förster Resonance Energy Transfer (FRET)-force Biosensors to Measure Mechanical Forces across the Nuclear LINC Complex

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Abstract

The LINC complex has been hypothesized to be the critical structure that mediates the transfer of mechanical forces from the cytoskeleton to the nucleus. Nesprin-2G is a key component of the LINC complex that connects the actin cytoskeleton to membrane proteins (SUN domain proteins) in the perinuclear space. These membrane proteins connect to lamins inside the nucleus. Recently, a Förster Resonance Energy Transfer (FRET)-force probe was cloned into mini-Nesprin-2G (Nesprin-TS (tension sensor)) and used to measure tension across Nesprin-2G in live NIH3T3 fibroblasts. This paper describes the process of using Nesprin-TS to measure LINC complex forces in NIH3T3 fibroblasts. To extract FRET information from Nesprin-TS, an outline of how to spectrally unmix raw spectral images into acceptor and donor fluorescent channels is also presented. Using open-source software (ImageJ), images are pre-processed and transformed into ratiometric images. Finally, FRET data of Nesprin-TS is presented, along with strategies for how to compare data across different experimental groups.

Video Link

The video component of this article can be found at https://www.jove.com/video/54902/

Introduction

Force-sensitive, genetically encoded FRET sensors have recently emerged as an important tool for measuring tensile-based forces in live cells, providing insight into how mechanical forces are applied across proteins[1,2,3,4]. With these tools, researchers can non-invasively image intracellular forces in living cells using conventional fluorescent microscopes. These sensors consist of a FRET-pair (donor and acceptor fluorescent proteins, most frequently a blue donor and yellow acceptor) separated by an elastic peptide[3]. In contrast to C- or N-terminal tagging, this sensor is inserted into an internal site of a protein to measure the mechanical force transmitted across the protein, behaving as a molecular strain gauge. Increased mechanical tension across the sensor results in an increased distance between the FRET-pair, resulting in decreased FRET[3]. As a result, the FRET is inversely related to tensile force.

These fluorescent-based sensors have been developed for focal adhesion proteins (vinculin[3] and talin[4]), cytoskeletal proteins (α-actinin[5]), and cell-cell junction proteins (E-Cadherin[6,7], VE-Cadherin[8], and PECAM[8]). The most frequently used and well-characterized elastic linker in these biosensors is known as TSmod and consists of a repetitive sequence of 40 amino acids, $(GPGGA)_8$, which was derived from the spider silk protein flagelliform. TSmod has been shown to behave as a linear elastic nano-spring, with FRET responsiveness to 1 to 5 pN of tensile force[3]. Different lengths of flagelliform can be used to alter the dynamic range of TSmod FRET-force sensitivity[9]. In addition to flagelliform, spectrin repeats[5] and villin headpiece peptide (known as HP35)[4] have been used as the elastic peptides between FRET-pairs in similar force biosensors[4]. Finally, a recent report showed that TSmod can also be used to detect compressive forces[10].

We recently developed a force sensor for the linker of the nucleo-cytoskeleton (LINC) complex protein Nesprin-2G by using TSmod inserted into a previously developed truncated Nesprin-2G protein known as mini-Nesprin2G (**Figure 2C**), which behaves similarly to endogenous Nesprin-2G[11]. The LINC complex contains multiple proteins that lead from the outside to the inside of the nucleus, linking the cytoplasmic cytoskeleton to the nuclear lamina. Nesprin-2G is a structural protein binding to both the actin cytoskeleton in the cytoplasm and to SUN proteins in the perinuclear space. Using our biosensor, we were able to show that Nesprin-2G is subject to actomyosin-dependent tension in NIH3T3 fibroblasts[2]. This was the first time that force was directly measured across a protein in the nuclear LINC complex, and it is likely to become an important tool to understand the role of force on the nucleus in mechanobiology.

The protocol below provides a detailed methodology of how to use the Nesprin-2G force sensor, including the expression of the Nesprin tension sensor (Nesprin-TS) in mammalian cells, as well as the acquisition and analysis of FRET images of cells expressing Nesprin-TS. Using an inverted confocal microscope equipped with a spectral detector, a description of how to measure sensitized emission FRET using spectral unmixing and ratiometric FRET imaging is provided. The output ratiometric images can be used to make relative quantitative force comparisons. While this protocol is focused on the expression of Nesprin-TS in fibroblasts, it is easily adaptable to other mammalian cells, including both cell lines and primary cells. Furthermore, this protocol as it relates to image acquisition and FRET analysis can readily be adapted to other FRET-based force biosensors that have been developed for other proteins.

Protocol

1. Obtain Nesprin-2G Sensor DNA and Other Plasmid DNA

1. Obtain Nesprin-2G TS (tension sensor), Nesprin-2G HL (headless) control, mTFP1, venus, and TSmod from a commercial source. Propagate all the DNA plasmids and purify them using standard *E. coli* strains, such as DH5-α, as described previously[12,13].

2. Transfect Cells with Nesprin-2G and Other Plasmid DNA

1. Grow NIH 3T3 fibroblasts cells to 70-90% confluence in a 6-well cell culture dish in a standard cell culture incubator with temperature (37 °C) and CO_2 (5%) regulation. For the cell growth medium, use Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum.
2. In a cell culture hood, remove the medium and rinse each well with approximately 1 mL of a reduced-serum cell medium. Add 800 µL of reduced-serum cell medium to each well and place the 6-well chamber in the incubator.
3. Pipette 700 µL of reduced-serum cell medium into a 1.5 mL tube with 35 µL of lipid carrier solution to form the "lipomix". Mix by pipetting. Label the tube with an "L."
4. Gather six 1.5 mL tubes and label them 1 through 6. Pipette 100 µL of reduced-serum cell medium into each tube.
    1. Using the plasmid DNA concentration from step 1, pipette 2 µg of Nesprin 2G-TS into tubes 1 and 2. Pipette 2 µg of Nesprin-HL into tubes 3 and 4. Pipette 1 µg of mTFP into tube 5. Pipette 1 µg of mVenus into tube 6. Do not re-use pipette tips when pipetting different types of DNA.
5. Pipette 100 µL of the lipomix from the "L" tube into each labeled tube (1-6) and mix by repeated pipetting. Use a clean pipette for each tube. Incubate for 10-20 min.
6. Add 200 µL from each labeled tube to a well in the 6-well chamber with 70-90% confluent cells. Label the top of each well with the corresponding DNA added. Place the 6-well chamber in an incubator for 4-6 h.
7. Aspirate the medium and add 1-2 mL of reduced-serum cell medium to rinse. Aspirate the reduced-serum cell medium, add 2 mL of trypsin to each well, and place the 6-well dish in the incubator (5-15 min).
8. While the cells detach in the incubator, coat 6 glass-bottom viewing dishes with a layer of fibronectin at a concentration of 20 µg/mL dissolved in phosphate-buffered saline (PBS). Allow the dishes to coat the surface in the cell culture hood (approximately 20 min).
9. Neutralize the trypsin by adding 2 mL of DMEM once the cells are detached.
10. Transfer the contents of each well to a labeled 15-mL centrifuge tube and spin down at 90 x g for 5 min in a swinging rotor centrifuge. Aspirate the supernatant and re-suspend each cell pellet in 1,000 µL of DMEM by pipette mixing.
11. Aspirate the fibronectin mixture from the glass dishes and pipette 1,000 µL of each cell suspension onto the glass dishes.
12. After the cells settle to the bottom of the glass dishes (~15 min), add another 1 mL of DMEM + 10% FBS + 1% Pen-Strep to each well and place in the cell incubator. Allow the cells to attach and express sensor for at least 18-24 h.
    NOTE: Cells are transfected using commercial cationic lipid transfection reagents (see the **Table of Materials**). Alternatively, stable cell lines can be selected by using a plasmid with a gene conferring resistance to a toxin (the pcDNA plasmids for Nesprin-TS and -HL are available on a DNA repository website (see the **Table of Materials**) provide cells expressing geneticin resistance). Additionally, viral infection methods (lentivirus, retrovirus, or adenovirus) can be utilized to express the sensor in cells that are hard to transfect.
13. In addition to Nesprin-TS, transfect additional cells with the Nesprin HL zero-force control, as described in steps 2.1-2.12; TSmod can also be used as a zero-force control and should exhibit similar FRET to Nesprin-HL.
14. Transfect cells with mTFP1 and venus to generate spectral fingerprints (see step 4).
    NOTE: mTFP1 and venus typically express at higher levels than Nesprin-TS, and as such, lower amounts of DNA may need to be transfected to achieve similar expression levels.

3. Verify Transfection Efficiency

1. Roughly 18-24 h after completing the transfection, use an inverted fluorescent microscope to examine the efficiency of transfection by comparing the number of fluorescent cells to the total number of cells in view (usually 5-30%).
    1. Use a fluorescent microscope equipped with an excitation frequency near 462 nm (mTFP1) or 525 nm (venus) and an emission filter centered near 492 nm (mTFP1) or 525 nm (venus).
    NOTE: Alternatively, GFP filter sets will capture a combination of mTFP1 and venus emissions and will allow the confirmation of the transfection efficiency.
2. Image live cells within 48 h after transfection.
    1. Alternatively, fix cells in 4% paraformaldehyde (in PBS with calcium and magnesium) for 5 min, store in PBS, and view after 48 h[8].
    NOTE: Beyond 48 h, the signal quality and strength decays. Fixing the cells preserves their state, including the FRET being expressed[8]; however, cells should only be imaged in PBS, as mounting medium may affect FRET[14]. Fixed cells can only be compared to other fixed cells, as there may be a change in expression.
    Caution: Paraformaldehyde is toxic. Wear appropriate personal protective equipment (PPE).

4. Capture Spectral Fingerprints of mTFP1 and Venus Fluorophores for Spectral Unmixing

1. In a cell culture hood, replace the cell medium with imaging medium (HEPES-buffered) supplemented with 10% fetal bovine serum.
2. Place the viewing dishes in a temperature-controlled (37 °C) confocal microscope stage.

3. Place the glass viewing dish with mTFP1-transfected cells over the oil objective at 60X magnification with a numerical aperture of 1.4.
   NOTE: The oil is used on a laser scanning microscope on the 60X objective to closely resemble the refractive index of the glass substrate. The oil is placed on top of the objective lens and comes in direct contact with the glass coverslip.
4. Locate mTFP1 expressing cells with a 458 nm excitation source and an emission bandpass filter centered at 500 nm.
5. With a fluorescent cell in the field of view, select the spectral detection mode ("Lambda Mode" in the software used here) and capture the spectral image (**Figure 3-1**); include all frequencies beyond 458 nm using 10 nm increments (**Figure 3-3**). Select a bright fluorescent region (ROI) on the cell (20-pixel radius).
   NOTE: The spectral shape of the mTFP1-expressing ROI should remain relatively constant across the cell. If the shape varies considerably, re-adjust the laser and gain settings to improve the signal-to-noise ratio.
6. Add the fluorescent ROI mean, normalized intensity to the spectral database by clicking "save spectra to database."
7. Optimize the laser power and gain such that a good signal-to-noise ratio is achieved. Settings will vary for different equipment. Using non-fluorescent, untransfected cells as a background reference, increase the gain and power until the cells are bright, but not beyond the dynamic range of the detector (saturation point). Background cells should not have detectable fluorescence after averaging.
8. Repeat the process for the venus-transfected cells with the following exceptions:
   1. Ensure that the excitation frequency is at 515 nm and that the bandpass filter is centered near 530 nm when locating venus-expressing cells.
   2. In "Spectral Mode," use an excitation frequency of 515 nm instead of 458 nm.

5. Capture Unmixed Images

1. Switch the capturing mode to "Spectral Unmixing."
2. Add the spectral fingerprints of venus and mTFP1 into the unmixing channels (**Figure 3**, Arrow-2).
3. Set the excitation laser back to a 458 nm argon source.
4. Place the Nesprin-TS viewing dish above the 60X oil objective.
5. After focusing on a fluorescent cell with sufficiently bright expression, adjust the gain and laser power to optimize the signal-to-noise ratio.
   NOTE: Since the FRET efficiency of Nesprin-TS is near 20%, the unmixed venus image should be substantially dimmer than the unmixed mTFP1 image. Once an acceptable power and gain setting have been determined iteratively, these parameters must remain constant for all images captured.
6. Capture a minimum of 15-20 images of Nesprin-TS cells with relatively similar brightness and avoid excessive pixel saturation (all saturated pixels are removed during image processing).
7. Repeat the image capturing process with Nesprin-HL cells using identical imaging parameters.

6. Image Processing and Ratio Image Analysis

1. Using open-source ImageJ (FIJI) software (http://fiji.sc/), open native format images using BioFormats Reader.
2. Pre-process the images and compute the ratio images using previously established protocols[15].

Representative Results

Following the protocol above, plasmid DNA was acquired from the DNA repository and transformed into *E. coli* cells. *E. coli* expressing the sensor DNA were selected from LB/Ampicillin plates and amplified in a liquid LB broth. Following the amplification of the vectors, DNA plasmids were purified into TRIS-EDTA buffer using a standard, commercially available DNA isolation kit. Using a spectrophotometer, purified DNA was quantified into a standard concentration of µg/mL (**Figure 1A**, Days 1-2).

Using NIH3T3 fibroblasts, cells were grown to 70-90% confluence in a 6-well plastic cell culture dish. To insert plasmid DNA into the NIH3T3 cells, a lipid-mediated plasmid transfection was performed. Commercially available transfection reagent (see the **Table of Materials**) was used as the lipid vessel for DNA. Two replicates of the Nesprin-TS and Nesprin-HL sensors were transfected into 4 cell wells (**Figure 1B**). In preparation for FRET imaging, single fluorophore constructs of mTFP1 and venus were transfected into NIH3T3 cells (**Figure 1B**). Following transfection, cells were transferred to glass-bottom viewing dishes compatible with high magnification, inverted confocal microscopes.

Before proceeding to confocal imaging, a simple inverted wide-field fluorescent microscope was used to verify the transfection efficiency. Using a 10X objective with a numerical aperture of 0.25, many cells in a typical field-of-view expressed the Nesprin-TS (**Figure 2**). Generally, the sensor localized around the nuclear envelope, consistent with the endogenous localization of Nesprin-2G[2]. The no-force control sensor, Nesprin-HL, followed a similar expression pattern[2].

After the confirmation of the transfection efficiency of Nesprin sensors TS and HL, live cells expressing single fluorophore constructs (mTFP1 or venus) were imaged with an inverted confocal microscope. Using the spectral detection mode, mTFP1 and venus spectral fingerprints were recorded in the spectral unmixing database (**Figure 3**, Arrow-2 and **Figure 4A**). Following fingerprinting, the capture parameter was switched to unmixing mode, with the mTFP1 and venus spectra loaded as the components (**Figure 4D**). Raw images were acquired as spectrally resolved image stacks (**Figure 4**).

Finally, the spectrally resolved image stacks were imported into ImageJ open-source software for processing. The images were pre-processed, and ratio images were computed using established procedures[15].

Since the FRET ratio is a relative measurement, every experiment must compare two or more conditions. Additionally, because laser and camera detector settings can influence FRET, all conditions should be acquired in the same imaging session. Frequently, we use a force-insensitive headless or tailless control, which represents a zero-force control, as a base-line for our experiments. Because FRET ratio images can be noisy, it is often necessary to take multiple images (5-20) for each condition. For conditions where there are large differences in FRET, this will be visually discernable in the FRET ratio images. The most dramatic difference in FRET for the Nesprin tension sensor was between unpatterned and elongated cells (**Figure 5**). Although there is variation between cells, the change in FRET between the two conditions is so dramatic that further analysis may not be required. However, other conditions often have minimal changes in FRET; they may not be visually discernable between individual images, but rather require the data to be analyzed in aggregate. To determine if these changes are significant, the median FRET ratio for each image is computed and visually expressed as a set of histograms. By comparing histograms between conditions, it becomes easier to see subtler changes in FRET between experimental groups. In **Figure 6A** and **6B**, the FRET histograms of each individual cell are represented by a color in the stacked histogram. Calyculin A-treated cells (**Figure 6B**) exhibit a leftward-shifted FRET histogram relative to cells treated with myosin inhibitor ML7 (**Figure 6A**).

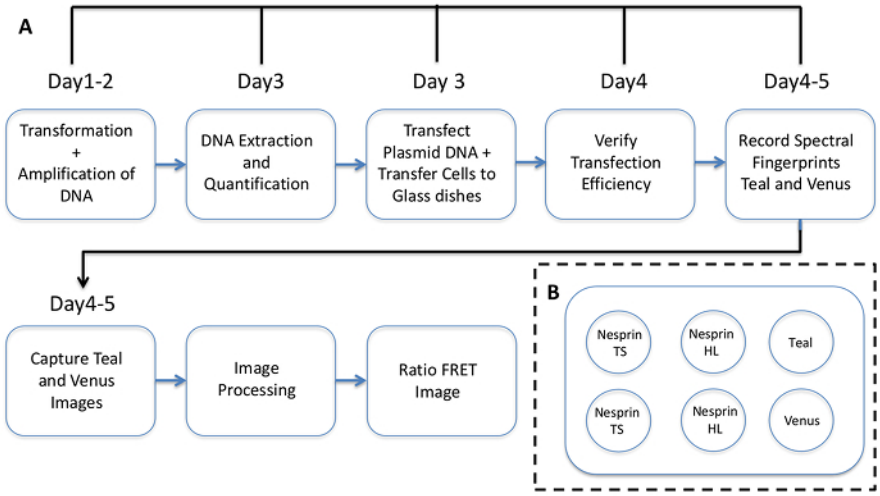

Figure 1: Experimental Time-based Flow Chart. (**A**) Starting with acquired sensor plasmid DNA, *E. coli* vectors are transformed with DNA, selected, and amplified (Days 1-2). From concentrated *E. coli* LB broth, plasmid DNA is isolated and quantified (Day 3). Using purified DNA plasmids, NIH3T3 fibroblasts are transfected in a six-well format (**B**) and transferred onto glass-bottom dishes that are compatible with an inverted confocal microscope (Day 3). To verify the success of the transfection, cells are viewed under a widefield fluorescent microscope equipped with the appropriate filter sets (Day 4). Contingent upon a successful transfection, cells are imaged on a confocal microscope equipped with a spectral detector. Using spectral unmixing, mTFP1 and venus channels are separated during the acquisition and saved as spectrally resolved image stacks (Days 4-5). Lastly, images are preprocessed in ImageJ, and the ratio-images are computed. Please click here to view a larger version of this figure.

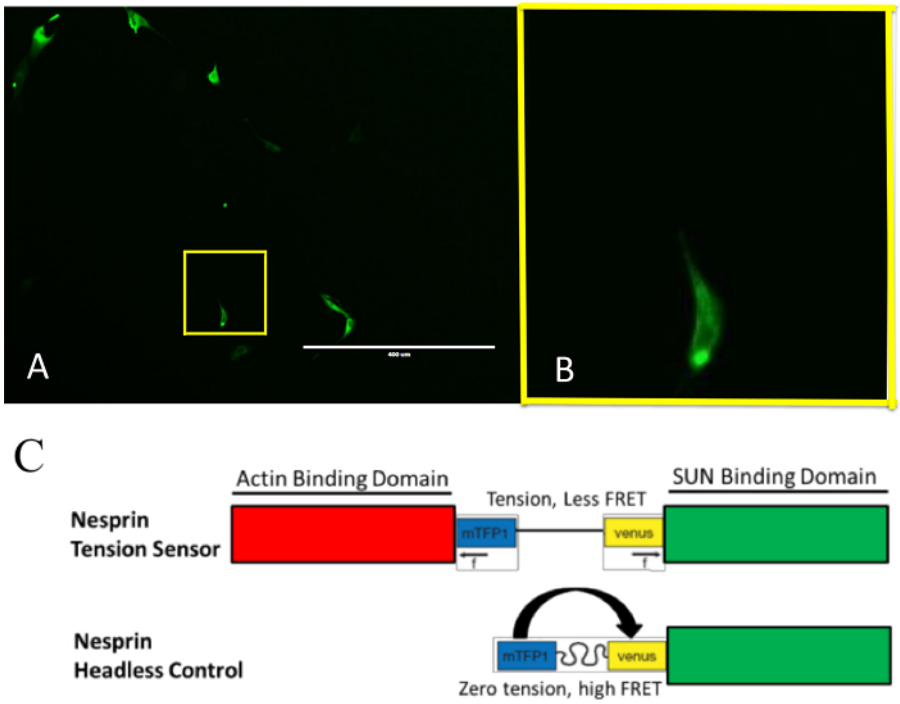

Figure 2: A Representative Image of Transfected NIH3T3 Fibroblasts. (**A**) 10X magnification of transfected NIH3T3 fibroblasts expressing Nesprin-TS using a GFP filterset. (**B**) An exploded view of the bounding box from part A. Sensor expression follows the nuclear envelope and often extends into the cytoplasm. (**C**) Nesprin-TS and -HL Control and how they bind to the actin and SUN binding domains. Please click here to view a larger version of this figure.

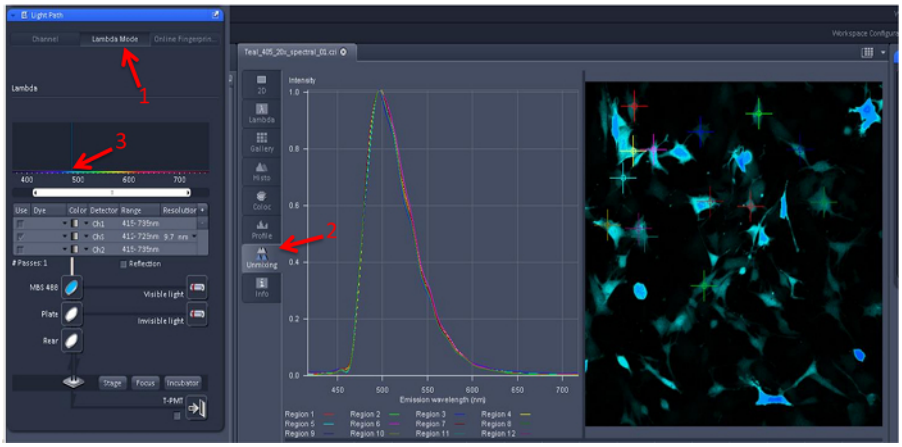

Figure 3: Spectral Fingerprinting Tutorial Using Confocal Software. Normalized emission spectra obtained from 20-pixel radii in the highlighted regions-of-interest delineated by colored crosshairs. (Arrow-1) Spectral detection mode to capture the image. (Arrow-2) "Spectral Unmixing" setting to add normalized spectra to the spectral database. (Arrow-3) The excitation frequency. Please click here to view a larger version of this figure.

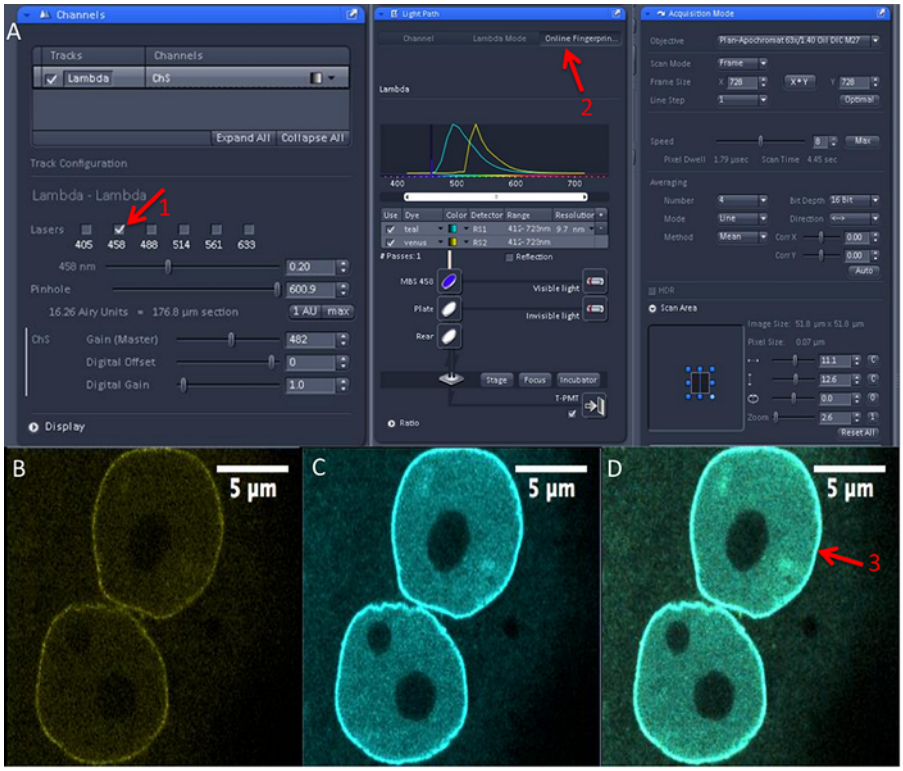

Figure 4: Live Spectral Unmixing and Output of Confocal Software. (**A**) Critical imaging parameters to reproduce live, unmixed images. (Arrow-1) The excitation frequency. (Arrow-2) Live spectral unmixing mode. (**B**) Nuclei image in unmixed venus channel. (**C**) Nuclei image in unmixed mTFP1 channel. (**D**) Combined image of venus and mTFP1. (Arrow-3) The nuclear membrane of the cell where Nesprin-TS is being expressed. Please click here to view a larger version of this figure.

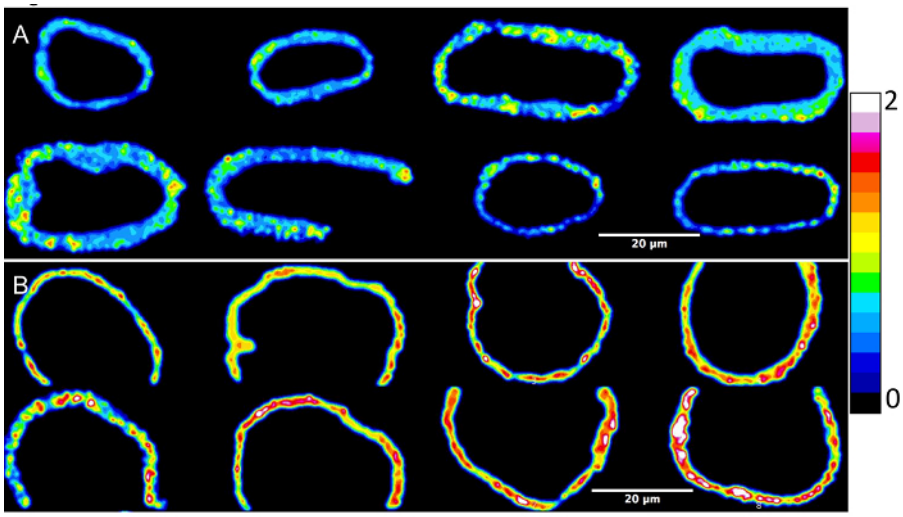

Figure 5: Nesprin-TS FRET ratio images in patterned and unpatterned Madin-Darby Canine Kidney (MDCK) cells. MDCK cells that stably express Nesprin-TS were imaged on 10 µm-wide fibronectin lines (**A**) or unpatterned polydimethylsiloxane (PDMS) membranes (**B**). Nuclear membranes were visually masked and processed into FRET ratios. The colored scale bar represents the FRET ratio amplitude for unpatterned and patterned nuclei. Please click here to view a larger version of this figure.

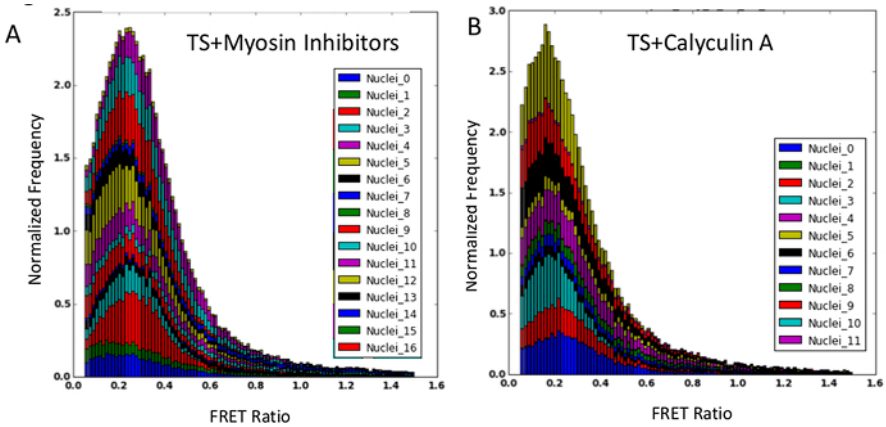

Figure 6: Processed Ratiometric FRET Images from Nesprin-TS-Expressing Cells and Histogram Analysis of Aggregated Data. (**A**) A representative, normalized stacked histogram of FRET ratio images of Nesprin-TS treated with ML7 myosin inhibitor, color-coded by individual cell nuclei. (**B**) A representative, normalized stacked histogram of FRET ratio images of Nesprin-TS treated with Calyculin A, an activator of cell contractility. The legend correlates each analyzed nucleus to its location on the histogram. Please click here to view a larger version of this figure.

Discussion

A method and demonstration of live cell imaging of mechanical tension across Nesprin-2G, a protein in the nuclear LINC complex, was outlined above. Prior to this work, various techniques, such as micropipette aspiration, magnetic-bead cytometry, and microscopic laser-ablation, have been used to apply strain on the cell nucleus and to measure its bulk material properties[16,17,18]. However, until our recent work, no studies had directly shown that tensile forces are transferred directly onto an LINC complex protein in live cells[2].

Previously, our laboratory demonstrated that a modified version of Nesprin-2G, known as mini-Nesprin2G, could be engineered to express a FRET-force probe known as TSmod (**Figure 2C**)[2]. TSmod was previously shown to dynamically measure tensile forces in live cells and in various load-bearing proteins[3,4,6,8]. It was further shown that mini-Nesprin-2G was indistinguishable from endogenous Nesprin-2G with respect to its localization, known effects on the cytoskeleton, and ability to rescue nuclear positioning[11,19]. Using the mini Nesprin-2G protein with the TSmod (Nesprin-TS), it was shown that NIH3T3 fibroblasts expressing the sensor have a baseline level of tension relative to Nesprin-HL, which cannot bind to actin[2]. Furthermore, the modulation of cellular contractility using inhibitors or activators of myosin can be detected by Nesprin-TS[2].

There are a number of critical steps in this protocol. Confusing Nesprin-TS with Nesprin-HL (DNA or expressing cells) is not readily detected, as both similarly localize to the nuclear membrane, and will lead to confusing results. Since Nesprin-HL is a no-force control, the measured FRET should be higher than Nesprin-TS on average. To be cautious, sequencing isolated DNA constructs and carefully labeling cells is advised. Second, it is important to carefully optimize the detector gain and laser power on the confocal microscope to improve the signal-to-noise ratio and to minimize bleaching. To properly measure FRET, these parameters must remain constant during the image acquisition. Furthermore, optimizing the detector gain or power for dim or very bright expressing cells is suboptimal, because median expressing cells will tend to fall out of the dynamic range of the detector. Certain cells that express a high concentration of sensor tend to fluoresce in the nucleoplasm and endoplasmic reticulum, making it difficult to resolve the nuclear membrane, which is presumably the more interesting region with regards to force. Selecting cells with slightly lower expression levels tends to resolve this issue of sensor localization.

While ratiometric spectral imaging is a relatively simple and quick way to measure FRET, it does not output units of FRET efficiency due to acceptor signal bleed-through[20]. Without units of FRET efficiency, it is not possible to convert the FRET ratio index into a calibrated force measurement. Due to this limitation, only relative quantitative force comparisons can be made. FRET efficiency can be determined by more complicated methods, such as fluorescence-lifetime imaging microscopy (FLIM) or acceptor photobleaching. Absolute force (at the pN level) can be estimated from FRET efficiency, as previously described[3,8].

In contrast to previous methods to measure the mechanical properties of the nucleus or the displacement of the nucleus based on externally applied forces, measuring the FRET from Nesprin-TS has the advantage of non-invasively probing tension on a component of the nuclear membrane[16,17,18]. The sensor outputs a fluorescent signal that is correlated with the strain on individual Nesprin-2G molecules. In contrast, micropipette aspiration requires the use of delicate tools when working with live cells. Confocal laser ablation causes local cellular damage and requires a pulsed laser source. Magnetic twisting cytometry transfers forces onto the nuclear membrane via the cytoskeleton and cannot be used to measure forces on specific proteins, unless it were to be coupled with the Nesprin-2G tension sensor.

While it has been demonstrated that actin-based tensile forces are transferred to Nesprin-2G, a component of the LINC complex, it remains unknown how much tensile or compressive forces are exerted onto other structural LINC proteins, such SUN-1 or SUN-2. Future work will be directed at cloning FRET-force probes into these putative load-bearing LINC proteins to further elucidate nuclear mechanics with protein-level resolution. Another issue that presents itself is that changes in FRET may be unrelated to changes in tension but rather reflect either changes in Nesprin-2G oligomerization or conformational changes of Nesprin-2G. Changes in cellular pH can influence the fluorescence of the sensor and change the measured FRET. The Nesprin-2G HL sensor represents a good control for some of these changes (as FRET changes observed with this sensor are most likely unrelated to force), and intermolecular FRET control constructs can be developed to assess oligomerization (see below). Additionally, the expression of nesprin-2G TS is driven by a CMV promoter, which results in overexpression, and this high level of expression may potentially induce biological artifacts. Recent advances with clustered regularly interspaced short palindromic repeats (CRISPR) have allowed for homology-directed repair (HDR) approaches to insert larger DNA sequences into the genome[21]. This approach could be used

to modify endogenous Nesprin-2G (or other proteins) to include TSmod. This would provide expression of the force sensor using the appropriate endogenous promotor, reducing the potential for overexpression artifacts.

TSmod and other elastic FRET probes can also be used to develop new sensors for other proteins, which could then be used in a similar protocol as that described in this article. A major concern with the development of any new force sensor is determining the internal insertion site for the FRET sensor. First, the site of insertion must be in a region of the protein subjected to mechanical load. These regions can be identified by examining where cytoskeletal-connected proteins interact with the protein. Second, the insertion of the large (~50 kDa) TSmod must not disrupt the biological function of the protein being studied. A previously developed "mini" form of Nesprin-2G showed that actin-binding CH domains bridged to the SUN-binding KASH domain were sufficient for Nesprin-2G nuclear movement in wounded monolayers[11,19], suggesting that the insertion of TSmod would likely not disrupt protein function. Confirmation of the biological function of the sensor requires a functional assay for protein function (in which the force sensor can be shown to rescue a specific function in cells depleted of endogenous protein). Third, changes in protein oligomerization can alter the FRET between proteins (termed intermolecular FRET[22]), which would result in FRET changes that are not related to force. A careful analysis of this often requires specialized intermolecular FRET constructs that specifically report oligomerization.

FRET-based force sensors have enabled the spatial and temporal analysis of mechanical forces with protein-specific resolution. Although this protocol details the use of the Nesprin-2G tension sensor, it is applicable to a number of existing sensors available for proteins in focal adhesions and cell-cell junctions. The use of these sensors for Nesprin-2G and other load-bearing proteins will provide significant insight into cell mechanics and mechanobiology.

Disclosures

The authors have nothing to disclose.

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