Imaging integrin tension and cellular force at submicron resolution with integrative tension sensor

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Abstract

Molecular tension transmitted by integrin-ligand bonds is the fundamental mechanical signal in the integrin pathway that plays significant roles in many cell functions and behaviors. To calibrate and image integrin tension with high force sensitivity and spatial resolution, we developed ITS, a DNA-based fluorescent tension sensor. ITS is activated to fluoresce if sustaining a molecular tension, thus converting force to fluorescent signal at the molecular level. Tension threshold for ITS activation is tunable in the range of 10–60 pN that well covers the dynamic range of integrin tension in cells. On a substrate grafted with ITS, integrin tension of adherent cells is visualized by fluorescence and imaged at submicron resolution. ITS is also compatible with cell structural imaging in both live cells and fixed cells. ITS has been successfully applied to the study of platelet contraction and cell migration. This paper details the procedure for the synthesis and application of ITS in the study of integrin-transmitted cellular force.

Abstract

Integrin tension plays important roles in various cell functions. With integrative tension sensor (ITS), integrin tension is calibrated with picoNewton (pN) sensitivity and imaged at submicron resolution.

Keywords

Cell Mechanics; Mechanobiology; Integrin; Integrin tension; Cellular force mapping; Integrin tension sensor

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The authors have nothing to disclose.

INTRODUCTION:

Cells rely on integrins to adhere and exert cellular forces to extracellular matrix. Integrin-mediated cell adhesion and force transmission are crucial for cell spreading^{1, 2}, migration^{3, 4} and survival^{5–7}. In long term, integrin biomechanical signaling also influences cell proliferation^{8–10} and differentiation^{11, 12}. Researchers have developed various methods to measure and map integrin-transmitted cellular forces at the cell-matrix interface. These methods are based on elastic substratum¹³, array of micro-post¹⁴, or atomic force microscopy (AFM)^{15, 16}. Elastic substratum and micro-post methods rely on the deformation of substrates to report the cellular stress and have limitations in terms of spatial resolution and force sensitivity. AFM has high force sensitivity, but it cannot detect force at multi spots simultaneously, making it difficult to map cellular force transmitted by integrins.

In recent years, several techniques were developed to study cellular force at the molecular level. A collection of molecular tension sensors based on poly ethylene glycol^{17, 18}, spider silk peptide¹⁹ and DNA^{20–23} were developed to visualize and monitor tension transmitted by molecular proteins. Among these techniques, DNA was first adopted as the synthesis material in tension gauge tether (TGT), a rupturable linker that modulates the upper limit of integrin tensions in live cells^{22, 24}. Later, DNA and fluorescence resonance transfer technique were combined to create hairpin DNA-based fluorescent tension sensors first by Chen's group²³ and Salaita's group²⁰, respectively. The hairpin DNA-based tension sensor reports integrin tension in real time and has been successfully applied to the study of a series of cellular functions²¹. Afterwards, Wang's lab combined TGT with the fluorophore-quencher pair to report integrin tension. This sensor is named integrative tension sensor (ITS)^{25, 26}. ITS is based on double-stranded DNA (dsDNA) and has broader dynamic range (10–60 pN) for integrin tension calibration. In contrast to hairpin DNA-based sensors, ITS does not report cellular force in real time, but records all historic integrin events as the footprint of cellular force, this signal accumulation process improves the sensitivity for cellular force imaging, making it feasible to image cellular force even with a low-end fluorescence microscope. Synthesis of ITS is relatively more convenient as it is created by hybridizing two single-stranded DNAs (ssDNA).

ITS is an 18 base-paired dsDNA conjugated with a biotin, a fluorophore, a quencher (blackhole quencher 2, BHQ2)²⁷ and a cyclic RGD peptide (Arginylglycylaspartic acid)²⁸ as an integrin peptide ligand (Fig. 1). The lower strand is conjugated with the fluorophore (Cy3 is used in this manuscript. Other dyes such as Cy5 or Alexa series have also been proven feasible in our lab) and the biotin tag, with which ITS is immobilized on a substrate by biotin-avidin bond. The upper strand is conjugated with the RGD peptide and the blackhole quencher which quenches Cy3 with approximately 98% quenching efficiency^{26, 27}. The coating density of ITS on a substrate is around 1100/ μm^2 with the protocol presented in this paper. This is the density we previously calibrated for 18 bp biotinylated dsDNA coated on the neutrAvidin functionalized substrate by following the same coating protocol²⁹. When cells adhere on the substrate coated with ITS, integrin binds ITS through RGD and transmits tension to ITS. The ITS has a specific tension tolerance (T_{tol}) which is defined as the tension threshold that mechanically separates the dsDNA of the ITS within 2 seconds²². ITS rupture by integrin tension leads to the separation of the quencher from the dye that subsequently

emits fluorescence. As a result, the invisible integrin tension is converted to fluorescence signal and cellular force can be mapped by fluorescence imaging.

To demonstrate the application of ITS, here we used fish keratocyte, a widely used cell model for cell migration study^{30–32}, CHO-K1 cell, a commonly used non-motile cell line and NIH 3T3 fibroblast. Co-imaging of integrin tension and cell structures was also conducted.

PROTOCOL:

All methods described here have been approved by the Institutional Animal Care and Use Committee (IACUC, 8–16-8333-I) of Iowa State University.

1. Synthesis of integrative tension sensor

1.1. Customize and order ssDNAs (see the Table of Materials).

NOTE: ssDNA sequences are as follows

Upper strand: /5ThioMC6-D/GGG AGG ACG CAG CGG GCC/3BHQ_2/

Lower strands:

12 pN ITS: /5Cy3/GGC CCG CTG CGT CCT CCC /3Bio/

23 pN ITS: /5Cy3/GGC CCG CTG CGT CC /iBiodT/ CCC

33 pN ITS: /5Cy3/GGC CCG CTG CG/iBiodT/ CCT CCC

43 pN ITS: /5Cy3/GGC CCG C/iBiodT/G CGT CCT CCC

54 pN ITS: /5Biosg/iCy3/GGC CCG CTG CGT CCT CCC

Here /5ThioMC6-D/ represents a thiol modifier C6 S-S (Disulfide) at the 5' end. /3BHQ_2/ represents a blackhole quencher 2 at the 3' end. /3Bio/, /5Biosg/ and /iBiodT/ are biotinylation at the 3', 5' end and on the thymine of internal DNA, respectively. /iCy3/ represents a Cy3 inserted to the internal backbone of ssDNA. These codes are used by the specific commercial supplier, and may be different in other DNA companies.

1.2. Conjugate RGD peptide to the SH-ssDNA-Quencher.

NOTE: Reagents used are phosphate-buffered saline (PBS), sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC), RGD peptide, Tris (2-carboxyethyl) phosphine hydrochloride, also known as TCEP.

1.2.1. Deprotect the thiol modifier on the upper strand DNA using TCEP solution

NOTE: Thiol-modified DNAs ordered from IDT are shipped in oxidized form, with the sulfur atoms protected by a disulfide bond that needs be reduced prior to RGD-DNA conjugation. TCEP is an odorless reduction reagent used for the thiol deprotection.

1.2.1.1. Dissolve 14.3 mg of TCEP-HCl (Tris (2-carboxyethyl) phosphine hydrochloride) in 300 μ L water. Adjust the pH of TCEP solution to 7.2 ~ 7.4 with 1 M NaOH solution (about 150 μ L) using pH test papers. Add pure water to make a final volume 0.5 mL. The final TCEP concentration is 100 mM.

NOTE: TCEP solution is recommended to make fresh every time for DNA thiol deprotection. Avoid stocking TCEP solution for long time.

1.2.1.2. Add 100 μ L of 0.5 M EDTA (Ethylenediaminetetraacetic acid) solution and 400 μ L water to the TCEP solution.

1.2.1.3. Add 10 μ L TCEP+EDTA solution to 20 μ L of 1 mM Thiol-DNA-BHQ2 in PBS. Let the solution react for 30 min in room temperature.

NOTE: The disulfide bond of 5' Thiol Modifier C6 S-S conjugation at this ssDNA will be cleaved by TCEP, leaving the thiol group ready for reaction with maleimide in next steps. The TCEP does not interfere the following thiol-maleimide reaction, thus it is not necessary to passivate TCEP after this step.

1.2.2. Conjugate RGD-NH₂ with sulfo-SMCC

1.2.2.1. Dissolve 5 mg RGD-NH₂ in 100 μ L PBS to obtain 11 mM RGD-NH₂.

1.2.2.2. Add 200 μ L pure water to the tube with 2 mg sulfo-SMCC (pre-measured by the supplier). Smash the sulfo-SMCC solid with the pipette tip and vigorously pipet at the same time to facilitate dissolving SMCC in water. The concentration of sulfo-SMCC will be 23 mM.

NOTE: Because the NHS-ester in sulfo-SMCC is subject to hydrolysis and has a lifetime of a few minutes, this dissolving step should be completed within 1 min to avoid excessive loss of NHS-ester due to hydrolysis. Use pure water to dissolve sulfo-SMCC because its solubility in PBS or other buffers is poor.

1.2.2.3. Add 40 μ L of sulfo-SMCC solution to the 100 μ L RGD-NH₂ solution and incubate it for 20 min.

NOTE: NHS-ester in sulfo-SMCC will react with the amine group in RGD-NH₂, forming amide bond that links RGD and sulfo-SMCC.

1.2.3. Conjugate RGD-SMCC with SH-ssDNA-Quencher.

1.2.3.1. Mix the solutions prepared in 1.2.1 and 1.2.2 and incubate it for 1 h in room temperature. Move the solution to a refrigerator at 4°C and let it further react overnight. The thiol conjugated on upper strand DNA will react with maleimide on sulfo-SMCC, forming thioether group that links RGD with the upper strand DNA.

1.2.4. Perform ethanol precipitation to purify RGD-ssDNA-Quencher from unreacted sulfo-SMCC, RGD and TCEP.

1.2.4.1. Chill 10 mL 100% ethanol in 15 mL Conical tube in a -20 °C freezer for at least 30 min.

1.2.4.2. Mix 3 M NaCl solution, DNA solution and chilled ethanol at a volume ratio: 1: 10: 25. Keep the mixed liquid in a -20°C freezer for 30 min or until DNA is precipitated.

1.2.4.3. Centrifuge the liquid at 10,000 g for 30 min. Discard supernatant.

1.2.4.4. Add PBS to DNA pellet and measure DNA concentration using a spectrometer.

1.2.5. (Optional) Perform DNA electrophoresis to gain higher RGD-ssDNA purity.

Note: With the above protocol, about 70–90% ssDNA will be conjugated with RGD. If higher purity is desired, DNA electrophoresis can also be performed to separate conjugated DNA from unconjugated one.

1.2.5.1. Assemble a vertical electrophoresis system.

1.2.5.2. Prepare 10% polyacrylamide gel solution by mixing 50 mL pure water, 20 mL 40% acrylamide gel, 8 mL 10X TBE (Tris-borate-EDTA) buffer and 800 μL 10% APS (ammonium persulfate).

1.2.5.3. Add 80 μL TEMED (Tetramethylethylenediamine) to the gel solution. Pour the solution to the glass chamber of the electrophoresis system. Insert a single-well comb to create a well on top of the gel. Wait for 10 min till gel is crosslinked.

1.2.5.4. Mix the DNA solution with 100% glycerol at a volume ratio of 1:1. Remove the comb and load DNA the solution to the gel well.

1.2.5.5. Run the gel at a voltage of 200 V. Observe two DNA bands in which the lagging one is the conjugated DNA.

1.2.5.6. Cut the gel band with the conjugated DNA off and dice it to small pieces.

1.2.5.7. Collect the diced gel in two 1.5 mL centrifuge tubes with filters. Add 500 μL PBS to each tube.

1.2.5.8. Put the PBS-soaked gel in a dark place at room temperature for one day. DNA will diffuse out from the gel pieces to the PBS.

1.2.5.9. Collect the DNA solution by centrifuging the tubes at 1000 g for 2 min.

1.2.5.10. Perform another round of ethanol precipitation (step 1.2.4) to collect the DNA.

1.3. Synthesis of ITS by hybridizing RGD-ssDNA-Quencher and dye-ssDNA-biotin

1.3.1. Mix the solutions of the upper strand and the lower strand DNAs at a molar ratio of 1.1:1. Keep the mixture at 4°C overnight to produce the ITS by DNA hybridization.

NOTE: A molar ratio 1.1:1 for DNA hybridization is used instead of 1:1. Excessive RGD-ssDNA-quencher is used to ensure that all dye-ssDNA-biotin will hybridize with RGD-ssDNA-quencher. RGD-ssDNA-quencher without hybridization will be washed off during surface coating and this will not affect ITS surface coating quality.

1.3.2. Aliquot and store ITS solution at -20°C for long term storage.

NOTE: The storage buffer is PBS and storage concentration should be generally above 10 μ M. For regular use, ITS solution can be stored at 4°C for a few weeks without noticeable quality deterioration.

2. Prepare ITS surfaces by immobilizing ITS on glass bottom petri dishes

NOTE: Reagents used are biotinylated bovine serum albumin (BSA-biotin), avidin protein and ITS. Chill all reagents and PBS buffer to around 0°C on ice with an ice bucket.

- 2.1. Load 200 μ L 0.1 mg/mL BSA-biotin in PBS onto the well of a glass bottom petri dish (29 mm diameter with a 14 mm well). Incubate it for 30 min at 4°C in a refrigerator. BSA-biotin is physically adsorbed on the glass surface.
- 2.2. Suck out the solution in the well using a pipette, and add 200 μ L PBS back to the well to wash the surface. Repeat 3 times to complete the washing.
- 2.3. Incubate 200 μ L 50 μ g/mL avidin protein in the well for 30 min at 4°C. Wash it 3 times with PBS. After this step avidin protein binds to BSA-biotin and becomes immobilized on the glass surface.
- 2.4. Incubate 200 μ L 0.1 μ M ITS in the well for 30 min at 4°C. Wash the dish with 3 times PBS. Leave PBS in the well until cell plating. After this step, ITS with the biotin tag binds to avidin protein and becomes immobilized on the surface.

NOTE: During the ITS immobilization. One precaution critical for ITS coating quality and homogeneity is to never dry up the petri dish surface. Keeping all reagents and PBS at 4 °C or around 0°C on ice with an ice bucket helps to reduce the water evaporation rate during washing steps when PBS is drawn out from the well.

3. Cell plating onto ITS surfaces

- 3.1. Culture fish keratocytes
  - 3.1.1. Pluck a piece of fish scale from a fish (goldfish, *Carassius auratus* as the example in the manuscript, other fish species may also work) with a flat-tip tweezer. Gently press the scale onto the well of a glass bottom petri dish, with the inner side of the scale contacting on the glass. Wait for half a minute until the fish scale adheres well on the glass surface of the dish.

Add 1 mL IMDM (Iscove's Modified Dulbecco's Medium) complete medium to fully cover the fish scale. Keep the petri dish in a dark and humid box for 6–48 h at room temperature.

NOTE: IMDM complete medium contains 79% IMDM + 20% fetal bovine serum (FBS) +1% Penicillin. No extra supply of oxygen or CO₂ is needed. A few tissue paper rolls soaked with water can be left in the box to maintain high humidity in the box. Fish keratocytes will migrate out from the fish scale as a sheet of cells after a few hours. This can be observed with a tissue culture microscope. The cells are generally viable within 48 h.

- 3.2. Detach and plate keratocytes cells on ITS surfaces

- 3.2.1.** Remove the medium from the fish scale culturing petridish. Wash the well with cell detaching solution once, and add detaching solution to cover the fish scale and incubate for 3 min.

NOTE: Recipe for the cell detaching EDTA solution: 100 mL 10X HBSS + 10 mL 1M HEPES (PH7.6) + 10 mL 7.5% sodium bicarbonate + 2.4 mL 500 mM EDTA + 1L H₂O. PH is adjusted to 7.4.

- 3.2.2.** Suck out all cell detaching solution and add IMDM complete medium. Disperse keratocytes by gentle pipetting. Adjust the cell solution density to a desired level (10^4 – 10^5 /mL). Plate the cells on the ITS surface (prepared in Step 2). Incubate the cells at room temperature for 30 min before imaging.

NOTE: Cell counting can be done with a hemocytometer after detaching the cells with detaching solution.

NOTE: The cell plating density is relatively low so that keratocytes would have plenty surface area to migrate.

- 3.3.** Plate CHO-K1 cells and NIH 3T3 cells on ITS surfaces

NOTE: Medium for CHO-K1 cells: 89% Ham's F12 + 10% fetal bovine serum (FBS) + 1% Penicillin.

Medium for NIH 3T3 cells: 89% DMEM + 10% calf bovine serum (CBS) + 1% Penicillin.

Culture condition: 37 °C and 5% CO₂.

- 3.3.1.** Culture CHO-K1 cells or NIH 3T3 cells to 80–100% confluency in petridish (or culture flasks).
- 3.3.2.** Warm cell detaching solution and culture medium into 37°C.
- 3.3.3.** Remove all culture medium in petridish with a pipette. Wash the cells with cell detaching solution once. Cover the cells with detaching solution and incubate it in 37°C for 10 min.
- 3.3.4.** Disperse cells by pipetting. Collect cell solution and centrifuge it at 300 x g for 3 min.
- 3.3.5.** Suck out supernatant and add complete medium or serum free medium.
- 3.3.6.** Adjust the cell solution density to a desired level (10^5 – 10^6 /mL). Plate the cells on the ITS surfaces (prepared in Step 2). Incubate the cells at 37 °C for 1–2 h before imaging, or 30 min before video recording.

4. Imaging, video recording and real-time integrin tension mapping.

- 4.1.** Quasi real-time imaging of integrin tension in live cells.
- 4.1.1.** Incubate keratocytes on ITS surfaces (prepared in step 2) for 30 min at room temperature, or incubate CHO-K1 cells or NIH 3T3 cells for 1 h on ITS surfaces in an incubator at 37°C

- 4.1.2.** Mount the petridish on a fluorescence microscope stage. Perform cellular force imaging with an exposure time of 1 second. The magnification is 40X or 100X.
- 4.1.3.** Take a video on ITS images with a frame interval of 20 s for keratocytes, or 1–2 min for CHO-K1 cells or NIH 3T3 cells.
- 4.1.4.** Use MATLAB or image analysis tools to subtract a previous frame of the ITS video from a current frame to compute the ITS signal newly produced in the latest frame interval. The newly produced ITS signal represents the integrin tension generated in the latest frame interval, therefore reporting cellular force in a quasi real-time manner.
- 4.2.** Co-imaging of integrin tension and cellular structure in immunostained cells.
- 4.2.1.** After step 3.2.2 or step 3.3.6, perform immunostaining to mark the target structural proteins.

NOTE: Use different dyes to avoid the fluorescence crosstalk between integrin tension imaging and cell structural imaging. In this manuscript, Cy5 fluorophore was used for phalloidin and Alexa488 was used for vinculin staining. The concentration of both primary and secondary antibody is 2.5 µg/mL. The concentration for phalloidin is 1.5 units/µL.

- 4.2.2.** Perform multi-fluorescent channel imaging to acquire both integrin tension map and cell structural imaging.

REPRESENTATIVE RESULTS:

With ITS, integrin tension map of fish keratocytes was captured. It shows that a keratocyte migrates and generates integrin tension at two force tracks (Fig 2A). Resolution of the force map was calibrated to be 0.4 microns (Fig 2B). High integrin tension concentrates at the rear margin (Fig 3A). ITS also shows different specific patterns of different cells. A non-motile cell, NIH-3T3, forms specific integrin tension pattern (Fig 3B) quite different from that of the fast migrating keratocyte.

With immunostaining, focal adhesions and integrin tension of fish keratocyte were co-imaged (Fig 4A). The relation between integrin tension and cell structure in keratocyte was studied in details in the reference²⁵. Integrin tension and stress fibers in CHO-K1 cells were also co-imaged (Fig 4B). These experiments indicate that ITS enables the co-imaging of cell adhesion force and cell structures simultaneously under similar imaging settings, therefore facilitating the study of cell structure-force interplay.

DISCUSSION:

ITS is a highly accessible yet powerful technique for cellular force mapping in terms of both synthesis and application. With all materials ready, ITS can be synthesized within one day. During experiments, only three steps of surface coating are needed prior to cell plating. Recently we further simplified the coating procedure to one step by directly linking ITS to bovine serum albumin which enables direct physical adsorption of ITS to glass or

polystyrene surfaces³³. ITS brings the fluorescent intensity of cellular force signal to a comparable level of cellular structural imaging. The convenient synthesis and sample preparation, modest requirement for microscopy and high sensitivity for cellular force make ITS a robust and reliable technique for the study of cell mechanics. In this paper, we presented a comprehensive procedure for ITS synthesis and application.

Conjugating RGD ligand with ssDNA is the most critical procedure in ITS synthesis. As mentioned in PROTOCOL section, the NHS ester on sulfo-SMCC is not stable in water. The longer it takes to dissolve sulfo-SMCC in water, the more amount of sulfo-SMCC loses NHS ester to hydrolysis and becomes “dud”, which conjugates with DNA through the maleimide-thiol reaction but fails to conjugate with RGD-NH₂. As a result, a big portion of DNA molecules will be occupied by the dead sulfo-SMCC and cannot link with RGD, leading to a low yield of ssDNA-RGD. For this reason, dissolving sulfo-SMCC in water should be completed quickly, generally within one minute. It is also recommended to check the yield of ssDNA-RGD with electrophoresis. If the yield is higher than 80%, purification is generally unnecessary. Otherwise, DNA purification by polyacrylamide gel is recommended. The recovery rate of DNA by polyacrylamide gel is about 60–80 %.

To synthesize reasonable amount of RGD-ssDNA-BHQ₂, the starting amount of thiol-ssDNA-BHQ₂ should be at least 20 nanomole (1 mM×20 μL). Note that DNA modifications significantly reduce the of the ssDNA ordered from DNA companies. For example, if 1000 nanomole ssDNA with two modifications is ordered, the guaranteed yield may only be 20 nanomole. If this is the case, order at least 1000 nanomole ssDNA for the RGD-ssDNA conjugation. Researchers can also design their own sequences for ITS constructs. Normally we keep the GC content in the ITS higher than 70% to improve the dsDNA thermal stability. DNA sequence analysis can be performed to minimize the probability of forming self-hairpin, self-hybridization, stable secondary structure, etc. The analysis tool is available on the website of Integrated DNA Technologies. In the manuscript, the upper strand DNA is the same for the ITS with different T_{tol} . We vary the biotin location at the lower strand DNA to achieve different T_{tol} . However, varying the location of RGD on the upper strand can be also applied to tune the T_{tol} of ITS. The calculation of T_{tol} for different dsDNA geometry is available in the references^{22, 34}, especially for the dsDNA in the unzipping mode^{35, 36} and the shear mode^{37, 38}.

Although ITS surfaces record all integrin tensions taking place in the past, by recording a video of the cellular force map consecutively, one can compute the integrin tension produced in the latest frame interval by subtracting the previous frame from a current frame to generate the ‘quasi real-time cellular force map’. The frame interval in the ITS video varies depending on the cell types, usually 20 s for fast migrating cells and 1–2 min for stationary cells. The frame subtraction method reports the quasi real-time integrin tension while retaining the high sensitivity for integrin tension imaging due to the signal accumulation effect. Photobleaching is minimal between two consecutive images and therefore has no observable influence to the frame subtraction method.

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REFERENCES:

1. Price LS, Leng J, Schwartz MA, Bokoch GM Activation of Rac and Cdc42 by Integrins Mediates Cell Spreading. *Molecular Biology of the Cell*. 9 (7), 1863–1871 (1998). [PubMed: 9658176]
2. Cavalcanti-Adam EA, Volberg T, Micoulet A, Kessler H, Geiger B, Spatz JP Cell Spreading and Focal Adhesion Dynamics Are Regulated by Spacing of Integrin Ligands. *Biophysical Journal*. 92 (8), 2964–2974 (2007). [PubMed: 17277192]
3. Huttenlocher A, Horwitz AR Integrins in cell migration. *Cold Spring Harbor perspectives in biology*. 3 (9), a005074 (2011).
4. Hood JD, Cheresch DA Role of integrins in cell invasion and migration. *Nature Reviews Cancer*. 2 (2), 91–100 (2002). [PubMed: 12635172]
5. Giancotti FG Integrin signaling: specificity and control of cell survival and cell cycle progression. *Current Opinion in Cell Biology*. 9 (5), 691–700 (1997). [PubMed: 9330873]
6. Illario M et al. Integrin-Dependent Cell Growth and Survival Are Mediated by Different Signals in Thyroid Cells. *The Journal of Clinical Endocrinology & Metabolism*. 88 (1), 260–269 (2003). [PubMed: 12519863]
7. Aoudjit F, Vuori K Integrin Signaling in Cancer Cell Survival and Chemoresistance. *Chemotherapy Research and Practice*. 2012, 1–16 (2012).
8. Hou S, Isaji T, Hang Q, Im S, Fukuda T, Gu J Distinct effects of $\beta 1$ integrin on cell proliferation and cellular signaling in MDA-MB-231 breast cancer cells. *Scientific reports*. 6, 18430 (2016). [PubMed: 26728650]
9. Shankar G, Davison I, Helfrich MH, Mason WT, Horton MA Integrin receptor-mediated mobilisation of intranuclear calcium in rat osteoclasts. *Journal of cell science*. 105 (Pt 1) (1), 61–8 (1993). [PubMed: 7689577]
10. Moreno-Layseca P, Streuli CH Signalling pathways linking integrins with cell cycle progression. *Matrix Biology*. 34, 144–153 (2014). [PubMed: 24184828]
11. Gómez-Lamarca MJ, Cobreros-Reguera L, Ibáñez-Jiménez B, Palacios IM, Martín-Bermudo MD Integrins regulate epithelial cell differentiation by modulating Notch activity. *Journal of cell science*. 127 (Pt 21), 4667–78 (2014). [PubMed: 25179603]
12. Wang H, Luo X, Leighton J Extracellular Matrix and Integrins in Embryonic Stem Cell Differentiation. *Biochemistry insights*. 8 (Suppl 2), 15–21 (2015). [PubMed: 26462244]
13. Schwarz US, Soiné JRD Traction force microscopy on soft elastic substrates: A guide to recent computational advances. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*. 1853 (11), 3095–3104 (2015). [PubMed: 26026889]
14. Xie T, Hawkins J, Fibrosis SY Methods in Molecular Biology. (2017).
15. Radmacher M Studying the Mechanics of Cellular Processes by Atomic Force Microscopy. *Methods in Cell Biology*. 83, 347–372 (2007). [PubMed: 17613316]
16. Charras GT, Lehenkari PP, Horton MA Atomic force microscopy can be used to mechanically stimulate osteoblasts and evaluate cellular strain distributions. *Ultramicroscopy*. 86 (1–2), 85–95 (2001). [PubMed: 11215637]
17. Miller JS, Shen CJ, Legant WR, Baranski JD, Blakely BL, Chen CS Bioactive hydrogels made from step-growth derived PEG-peptide macromers. *Biomaterials*. 31 (13), 3736–43 (2010). [PubMed: 20138664]
18. Legant WR, Miller JS, Blakely BL, Cohen DM, Genin GM, Chen CS Measurement of mechanical tractions exerted by cells within three-dimensional matrices. *Nature methods*. 7 (12), 969 (2010). [PubMed: 21076420]
19. Brenner MD et al. Spider Silk Peptide Is a Compact, Linear Nanospring Ideal for Intracellular Tension Sensing. *Nano letters*. 16 (3), 2096–102 (2016). [PubMed: 26824190]

20. Zhang Y, Ge C, Zhu C, Salaita K DNA-based digital tension probes reveal integrin forces during early cell adhesion. *Nature Communications*. 5, 5167 (2014).
21. Liu Y et al. DNA-based nanoparticle tension sensors reveal that T-cell receptors transmit defined pN forces to their antigens for enhanced fidelity. *Proceedings of the National Academy of Sciences of the United States of America*. 113 (20), 5610–5 (2016). [PubMed: 27140637]
22. Wang X, Ha T Defining Single Molecular Forces Required to Activate Integrin and Notch Signaling. *Science*. 340 (6135) (2013).
23. Blakely BL et al. A DNA-based molecular probe for optically reporting cellular traction forces. *Nature Methods*. 11 (12), 1229–1232 (2014). [PubMed: 25306545]
24. Wang Y, Wang X Integrins outside focal adhesions transmit tensions during stable cell adhesion. *Scientific Reports*. 6 (1), 36959 (2016). [PubMed: 27845380]
25. Wang Y et al. Force-activatable biosensor enables single platelet force mapping directly by fluorescence imaging. *Biosensors and Bioelectronics*. 100, 192–200 (2018). [PubMed: 28915383]
26. Zhao Y, Wang Y, Sarkar A, Wang X Keratocytes Generate High Integrin Tension at the Trailing Edge to Mediate Rear De-adhesion during Rapid Cell Migration. *iScience*. 9, 502–512 (2018). [PubMed: 30472533]
27. Crisalli P, Kool ET Multi-Path Quenchers: Efficient Quenching of Common Fluorophores. *Bioconjugate Chemistry*. 22 (11), 2345–2354 (2011). [PubMed: 22034828]
28. Mondal G, Barui S, Chaudhuri A The relationship between the cyclic-RGDfK ligand and $\alpha v\beta 3$ integrin receptor. *Biomaterials*. 34 (26), 6249–6260 (2013). [PubMed: 23702147]
29. Wang X et al. Integrin Molecular Tension within Motile Focal Adhesions. *Biophysical Journal*. 109 (11), 2259–2267 (2015). [PubMed: 26636937]
30. Euteneuer U, Schliwa M Persistent, directional motility of cells and cytoplasmic fragments in the absence of microtubules. *Nature*. 310 (5972), 58–61 (1984). [PubMed: 6377086]
31. Kucik DF, Elson EL, Sheetz MP Cell migration does not produce membrane flow. *The Journal of cell biology*. 111 (4), 1617–22 (1990). [PubMed: 2211827]
32. Mueller J et al. Load Adaptation of Lamellipodial Actin Networks. *Cell* (2017).
33. Sarkar A, Zhao Y, Wang Y, Wang X Force-activatable coating enables high-resolution cellular force imaging directly on regular cell culture surfaces. *Physical Biology*. 15 (6), 065002 (2018).
34. Mosayebi M, Louis AA, Doye JPK, Ouldrige TE Force-Induced Rupture of a DNA Duplex: From Fundamentals to Force Sensors. *ACS Nano*. 9 (12), 11993–12003 (2015). [PubMed: 26575598]
35. Bockelmann U, Essevaz-Roulet B, Heslot F Molecular Stick-Slip Motion Revealed by Opening DNA with Piconewton Forces. *Physical Review Letters*. 79 (22), 4489–4492 (1997).
36. Krautbauer R, Rief M, Gaub HE Unzipping DNA oligomers. *Nano Letters*. 3 (4), 493–496 (2003).
37. de Gennes P-G Maximum pull out force on DNA hybrids. *Comptes Rendus de l'Académie des Sciences - Series IV - Physics*. 2 (10), 1505–1508 (2001).
38. Hatch K, Danilowicz C, Coljee V, Prentiss M Demonstration that the shear force required to separate short double-stranded DNA does not increase significantly with sequence length for sequences longer than 25 base pairs. *Physical Review E*. 78 (1), 011920 (2008).

Figure 1: Schematics of ITS

ITS is a dsDNA decorated with a RGD ligand, a fluorescence quencher, a dye and a biotin tag. The dye (green filled circle) is quenched by the quencher. Under integrin tension, the dsDNA is ruptured and Cy3 is freed from quenching and emits fluorescence that can be detected by fluorescent microscopy.

Figure 2. Integrin tension map of fish keratocyte and submicron resolution of ITS

A. During fast migration, a keratocyte consistently generates integrin tension map in two force tracks.

B. Distance between the two force track is typically 40 μm . Spatial resolution of cellular force map imaged by ITS is around 0.4 μm . Linear profile of Cy3 fluorescent intensity of the area marked by a short red line in the lower left panel of Fig. 2B is calculated for the calibration of resolution in cellular force imaging. The result is shown in the lower right panel and fitted with a Gaussian curve.

Scale bar: 10 μm .

Figure 3. Real-time integrin tension map of fish keratocyte and NIH-3T3.

A. A fish keratocyte and its integrin tension map.

(Upper left) A motile fish keratocyte.

(Lower left) Integrin tension map of the keratocyte reported by ITS.

(Upper right) Real-time integrin tension was obtained by frame subtraction. It shows that the newly generated integrin tension is co-localized with the cell rear edge.

(Lower right) Integrin tension map (green) and real-time integrin tension (magenta) are presented in one merged figure. Scale bar: 10 μm.

B. A NIH-3T3 fibroblast and its integrin tension map.

(Upper left) An NIH-3T3 fibroblast.

(Lower left) Integrin tension map of the NIH-3T3 fibroblast. Integrin tension was generated in a stripe pattern.

(Upper right) Real-time integrin tension shows that newly generated integrin tension mainly forms at cell peripheral region.

(Lower right) Integrin tension map (green) and real-time integrin tension (magenta) are presented in one merged figure.

Scale bar: 10 μm.

Figure 4. Co-imaging of integrin tension and cell structure.

A. Co-imaging of integrin tension, vinculin and F-actin in a keratocyte. The keratocyte was fixed and immunostained to report vinculin and F-actin.

B. Co-imaging of integrin tension, vinculin and F-actin in a CHO-K1 cell.

Scale bar: 10 μ m.

Materials

Name of Reagent/Equipment	Company	Catalog Number	Comments/Description
BSA-biotin	Sigma-Aldrich	A8549	Customer designed. DNA sequence is shown in PROTOCOL section
Neutravidin	Thermo Fisher Scientific	31000	
Streptavidin	Thermo Fisher Scientific	434301	
upper strand DNA	Integrated DNA Technologies	N/A	Customer designed. DNA sequences are shown in PROTOCOL section.
lower strand DNA	Integrated DNA Technologies	N/A	Customer designed. DNA sequences are shown in PROTOCOL section.
sulfo-SMCC	Thermo Fisher Scientific	A39268	
Cyclic peptide RGD with an amine group	Peptides International	PCI-3696-PI	
IMDM	ATCC	62996227	spectrometer
FBS	ATCC	302020	
Penicillin	gibco	15140122	
TCEP	Sigma-Aldrich	C4706	Device for electroporesis
200 uL petri dish	Cellvis	D29-14-1.5-N	
NanoDrop 2000	Thermo Scientific	N/A	
SE410 Tall Air-Cooled Vertical Protein Electrophoresis Unit	Hofer	SE410-15-1.5	Primary antibody for vinculin immunostaining
CHO-K1 cell line	ATCC	CCL-61	
NIH/3T3 cell line	ATCC	CRL-1658	
Anti-Vinculin Antibody	EMD Millipore	90227	Secondary antibody for vinculin immunostaining
Goat anti-Mouse IgG (H+L) Supercloonal™	Invitrogen	A28175	
Alexa Fluor™ 647 Phalloidin	Invitrogen	A22287	
Eclipse Ti	Nikon	N/A	microscope