ARTICLES
https://doi.org/10.1038/s41592-020-0929-2

nature methods

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NATURE METHODS | VOL 17 | OCTOBER 2020 | 1018–1024

Live-cell super-resolved PAINT imaging of piconewton cellular traction forces

Joshua M. Brockman[1,7], Hanquan Su[2,7], Aaron T. Blanchard[1], Yuxin Duan[2], Travis Meyer[1], M. Edward Quach[3], Roxanne Glazier[1], Alisina Bazrafshan[2], Rachel L. Bender[2], Anna V. Kellner[1], Hiroaki Ogasawara[2], Rong Ma[2], Florian Schueder[4,5], Brian G. Petrich[3], Ralf Jungmann[4,5], Renhao Li[3], Alexa L. Mattheyses[6], Yonggang Ke[1,2] and Khalid Salaita[1,2] ✉

Liu Wenjuan

2020.11.05

Repurposing nucleic acids for mechanobiology, synthetic motors, sensors, and therapeutics

Research Members

Biography

Degrees

- Ph.D., Northwestern University 2006

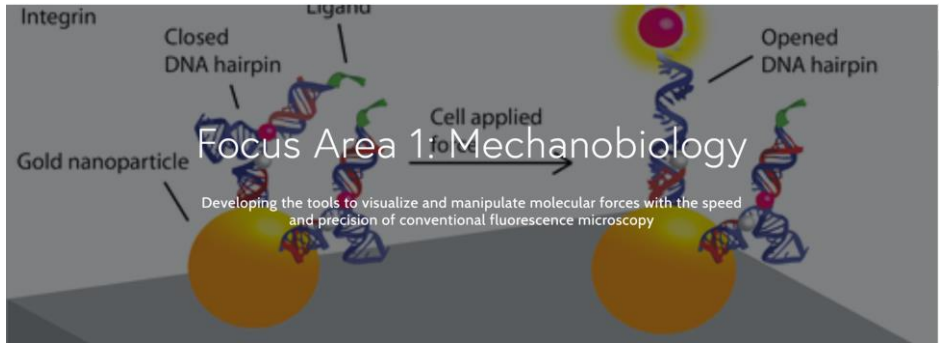
  Studied the electrochemical properties of organic adsorbates patterned onto gold films and developed massively parallel scanning probe lithography approaches

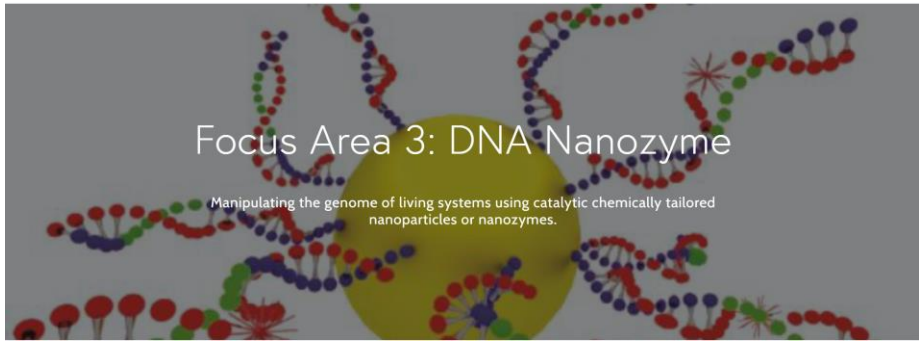
- Postdoctoral Fellowship, University of California, Berkeley, 2006-2009

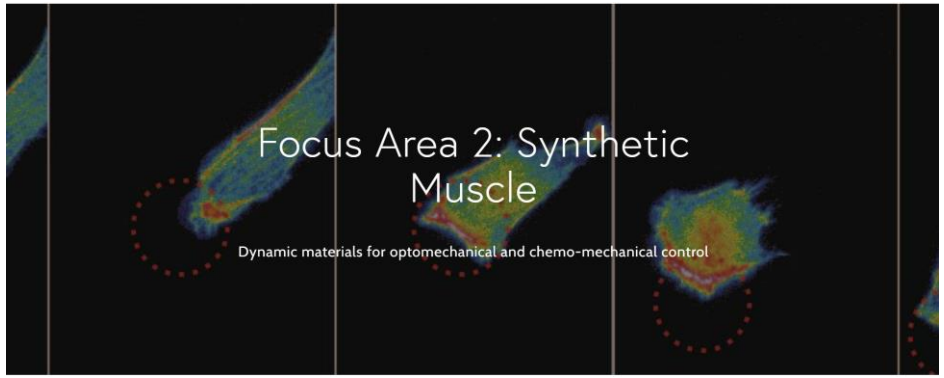
  Investigated the role of receptor clustering in modulating cell signaling

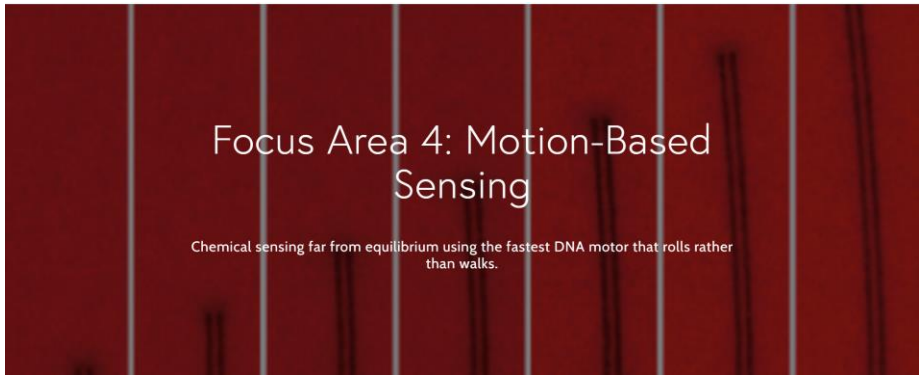
Specific Research Area

- Biophysical, Materials, Nanoscience, Biomolecular Chemistry







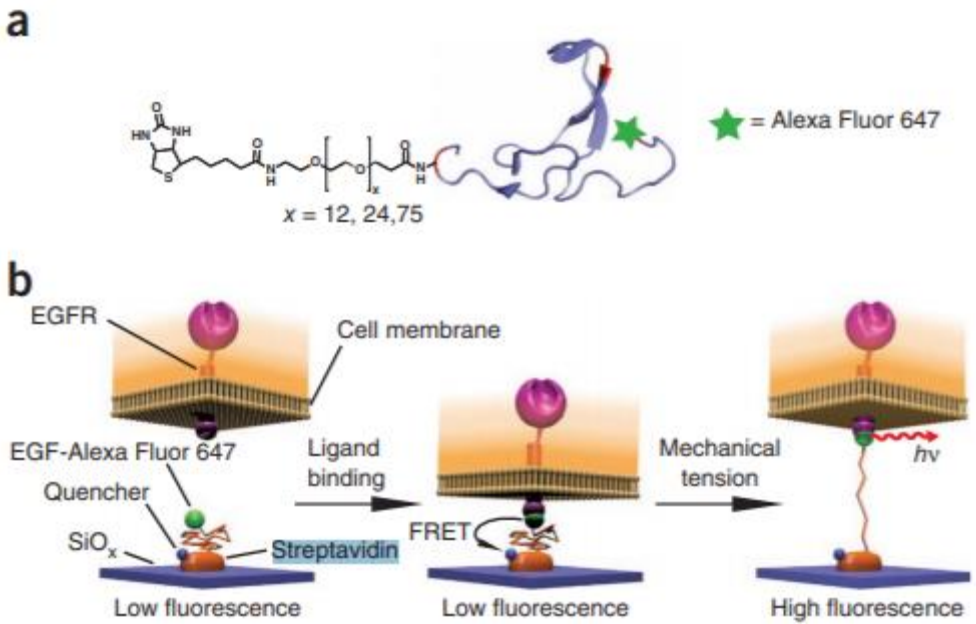


PUBLICATION 1: FIRST TENSION PROBE PAPER

NATURE METHODS | BRIEF COMMUNICATIONS

Visualizing mechanical tension across membrane receptors with a fluorescent sensor

Daniel R Stabley, Carol Jurchenko, Stephen S Marshall & Khalid S Salaita

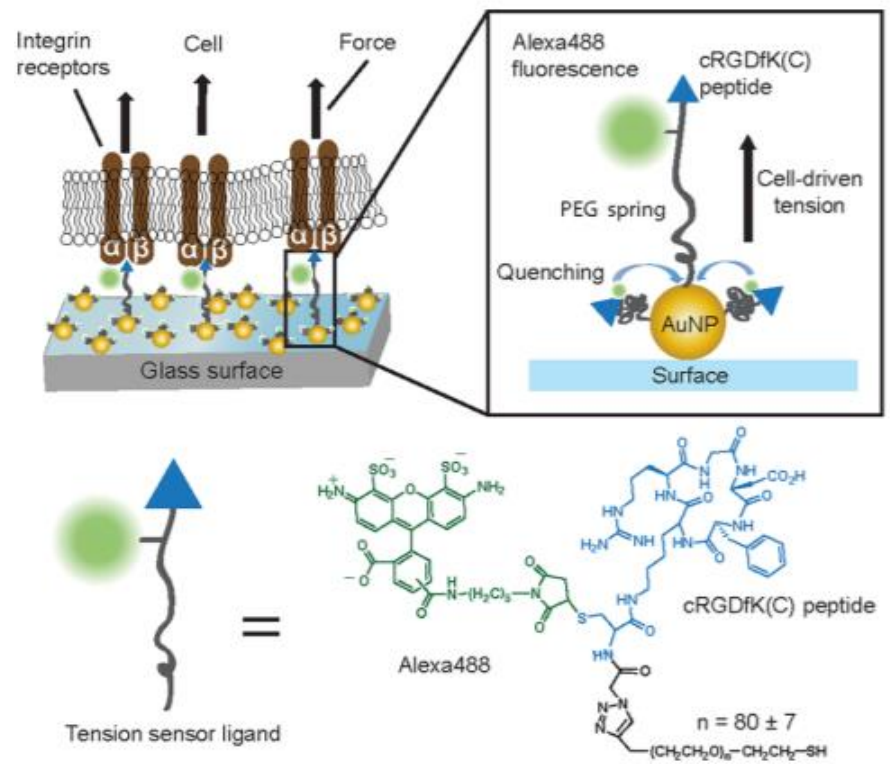


PUBLICATION 2: INTEGRIN FORCES

Journal of the American Chemical Society

Tension Sensing Nanoparticles for Mechano-Imaging at the Living/ Nonliving Interface

Yang Liu, Kevin Yehl, Yoshie Narui, and Khalid Salaita*



nature COMMUNICATIONS

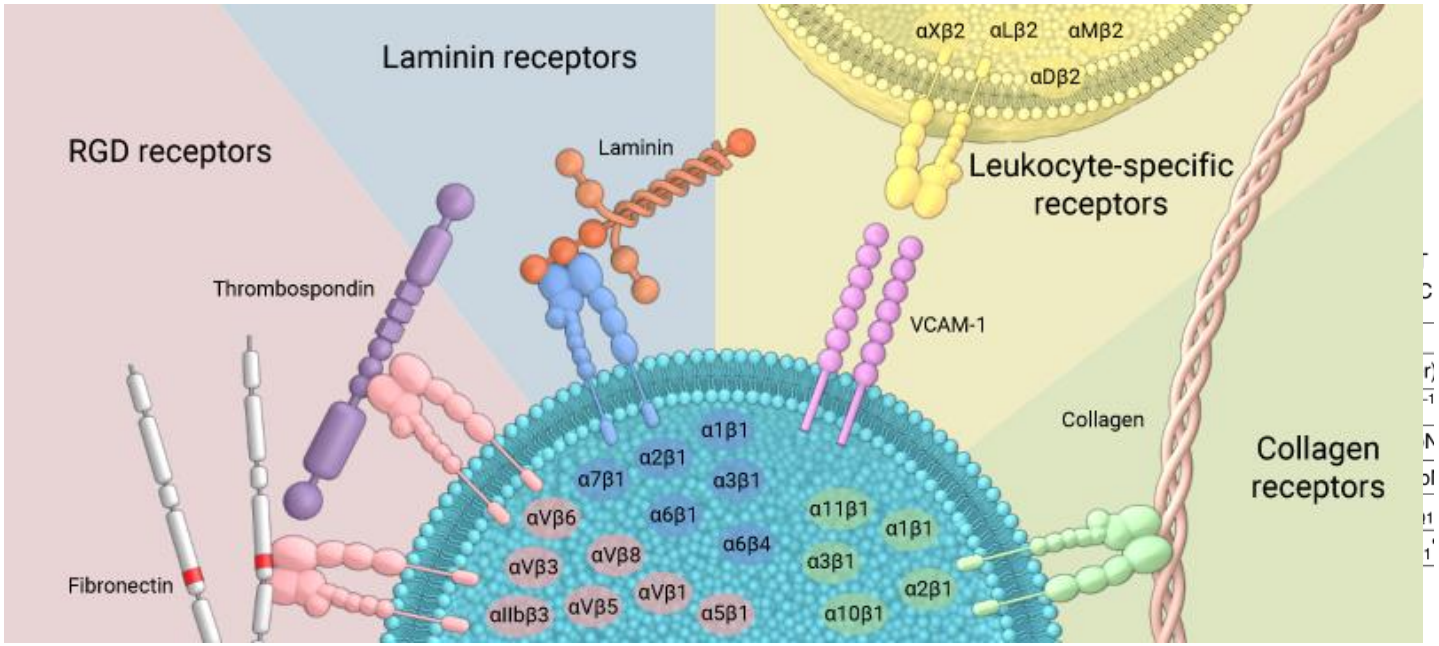
ARTICLE

Received 8 Jul 2014 | Accepted 6 Sep 2014 | Published 24 Oct 2014

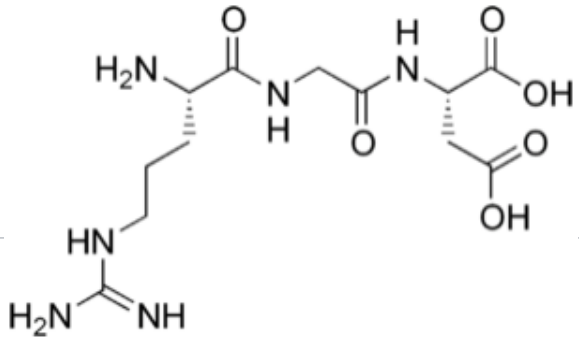
DOI: 10.1038/ncomms6167

DNA-based digital tension probes reveal integrin forces during early cell adhesion

Yun Zhang[1], Chenghao Ge[2], Cheng Zhu[2,3,4] & Khalid Salaita[1,2]



RGD肽 [编辑]



维基百科，自由的百科全书

RGD肽（全称**精甘天冬氨酸肽**，英语：**RGD peptide**或**Arginylglycylaspartic acid**）是由L-精氨酸、甘氨酸和L-天冬氨酸组成的三肽序列，是细胞识别中的常用元件[1]，因而作为研究细胞识别常用的生化工具。

RGD肽是一种细胞粘附序列，可以模仿细胞粘附蛋白并与整合素αVβ3结合，并可用于组织工程中的合成支架，以增强细胞附着，模仿体内(*in vivo*)的条件。[2]

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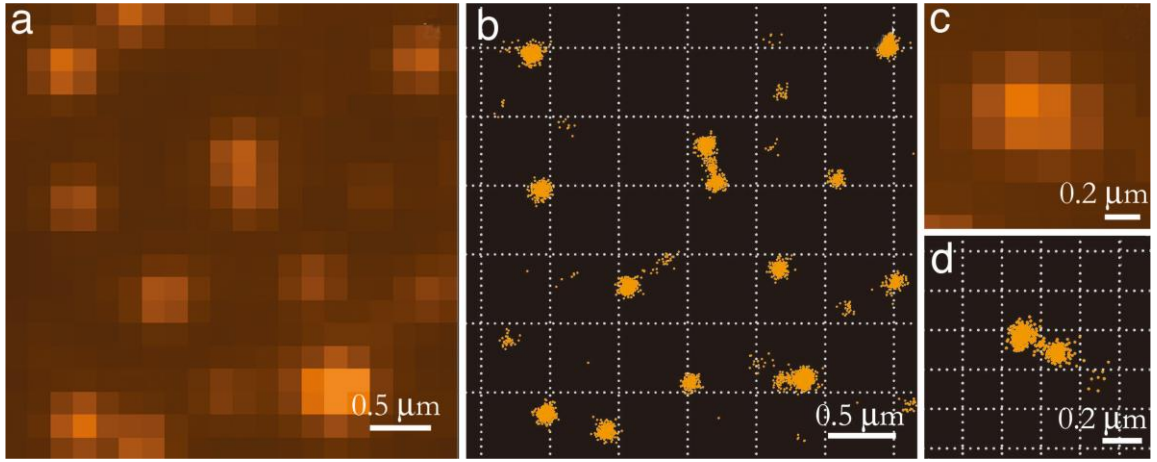
➢ About PAINT

PAINT: point accumulation for imaging in nanoscale topography	A method in which the 'on' state is generated by the binding of something to the structure to be imaged, and the 'off' state by free diffusion or another dark (bleached) state.

Nature Methods, 2009, 24

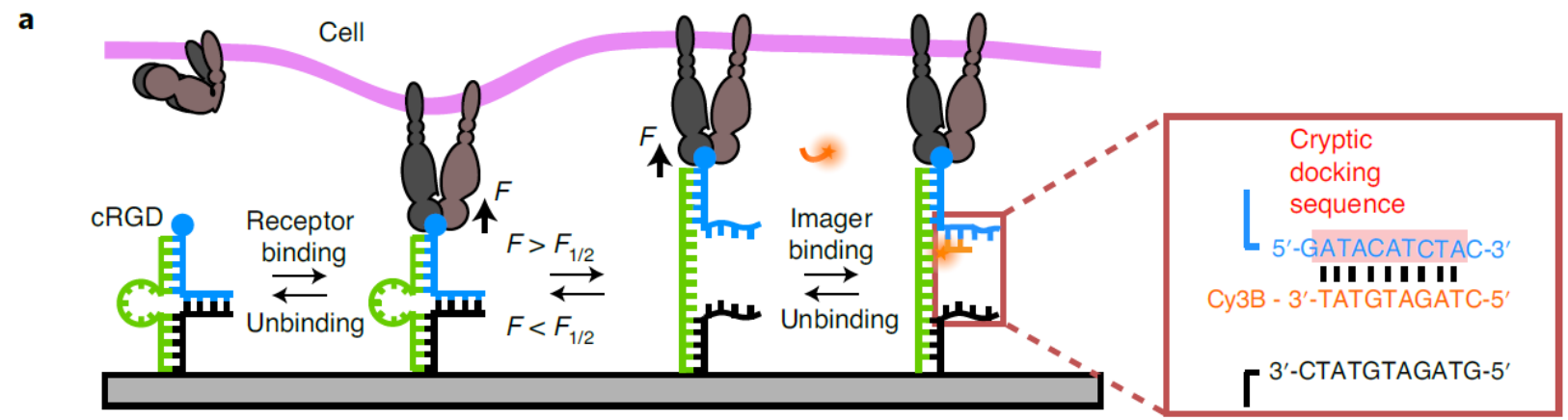


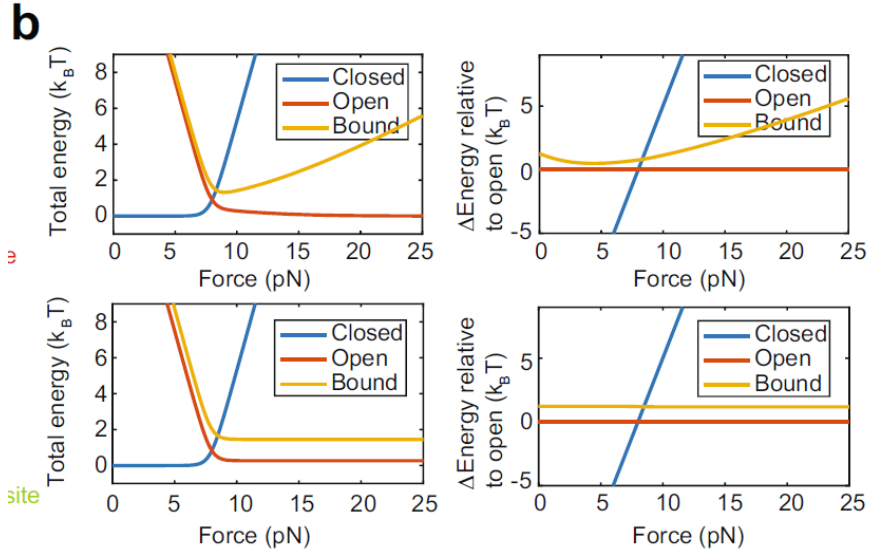
PNAS, 2006, 18911

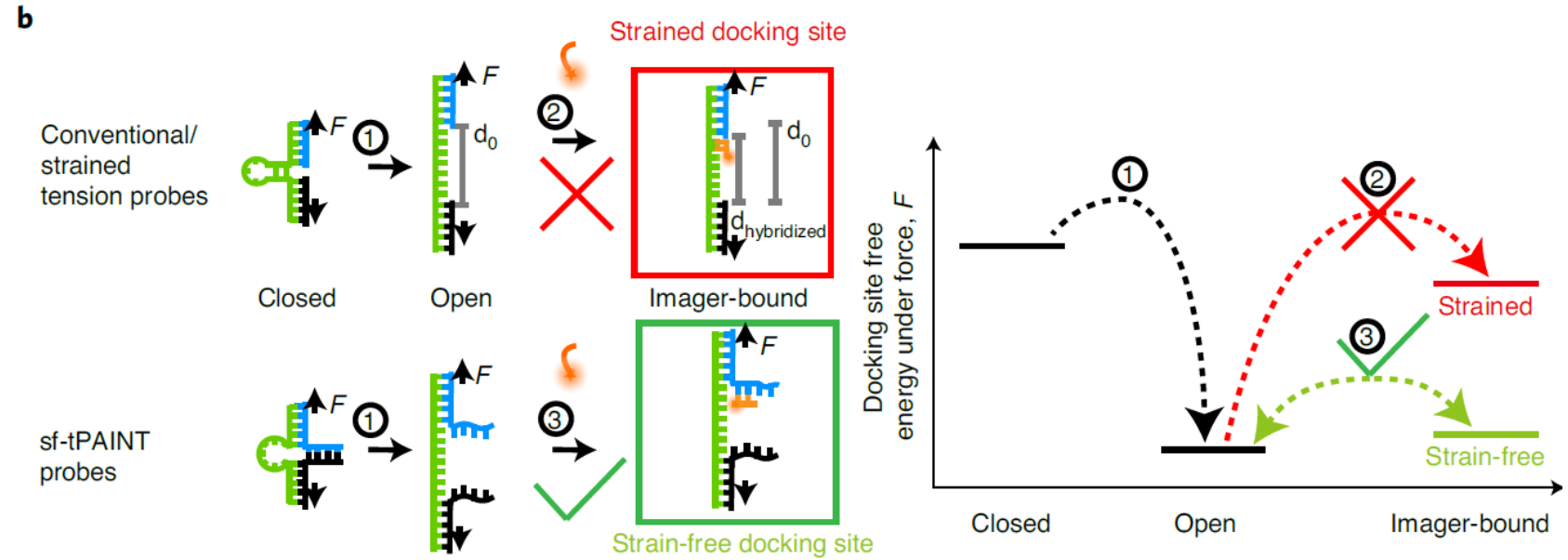
2014, DNA-PAINT

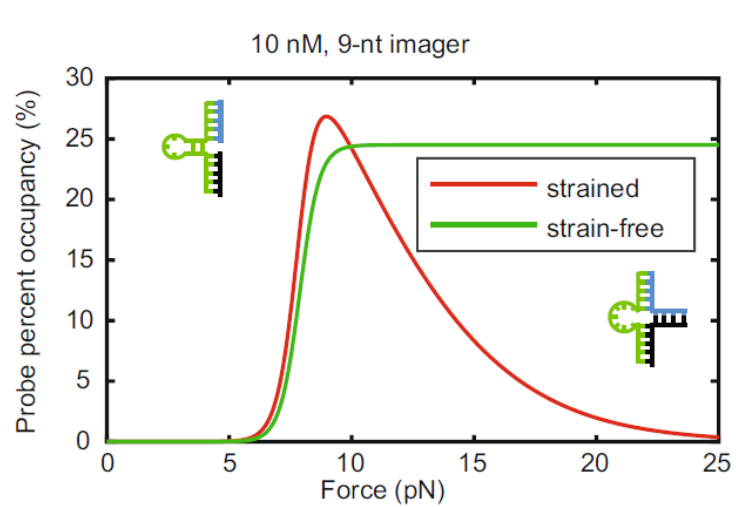
qPAINT

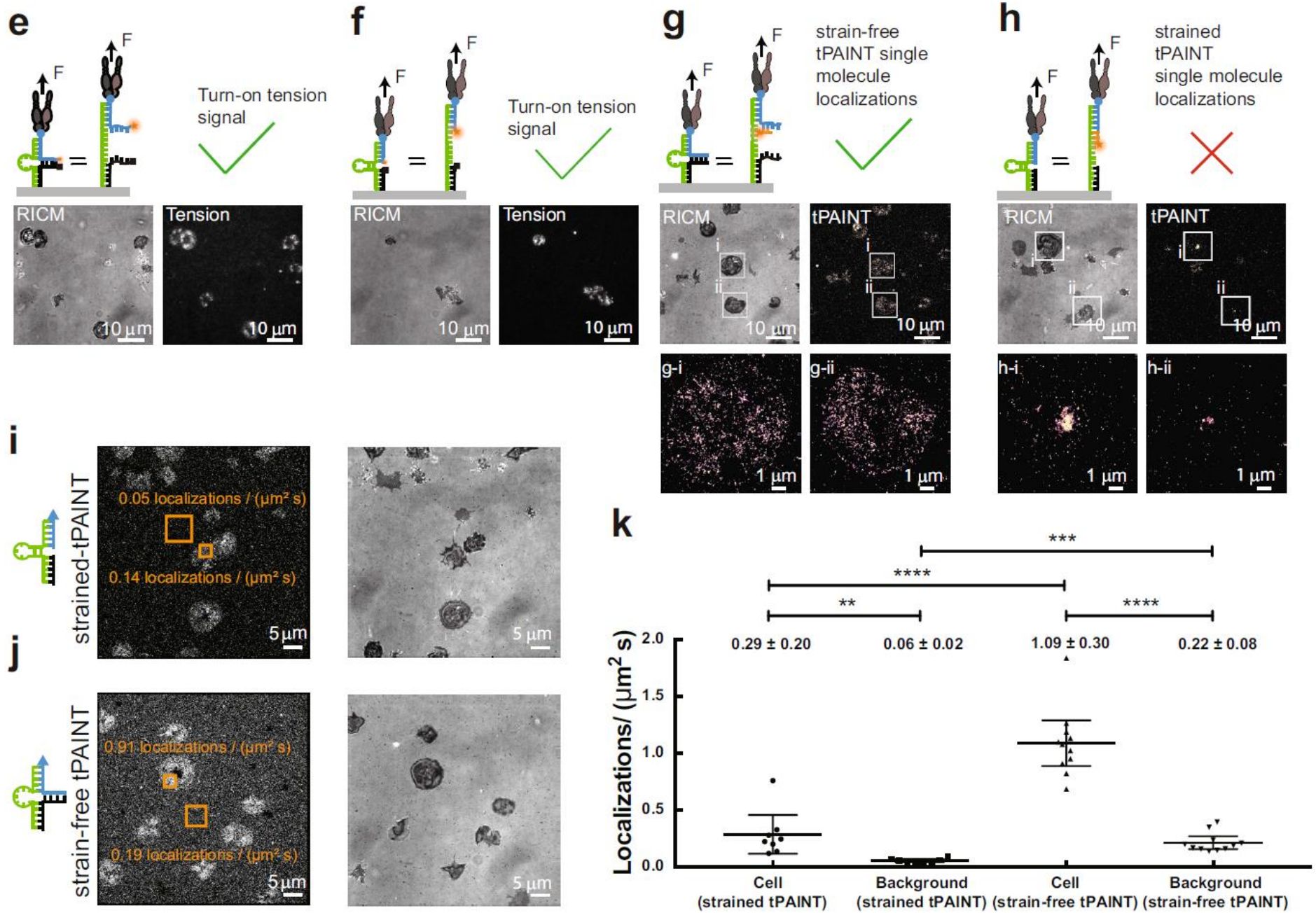
……

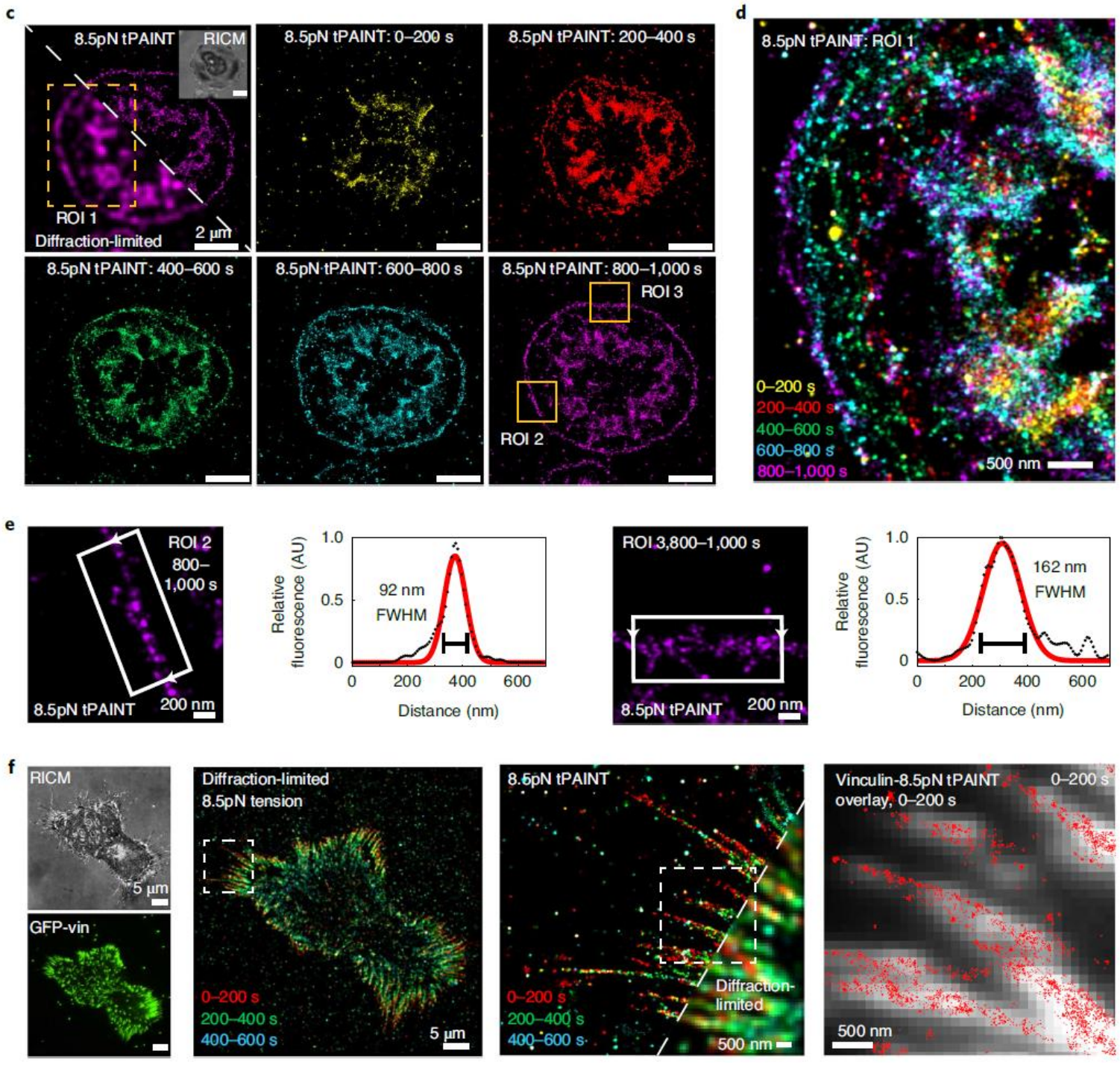

a
Cell
cRGD
Receptor binding
Unbinding
F
F > F1/2
F < F1/2
Imager binding
Unbinding
Cryptic docking sequence
5′-GATACATCTAC-3′
Cy3B - 3′-TATGTAGATC-5′
3′-CTATGTAGATG-5′

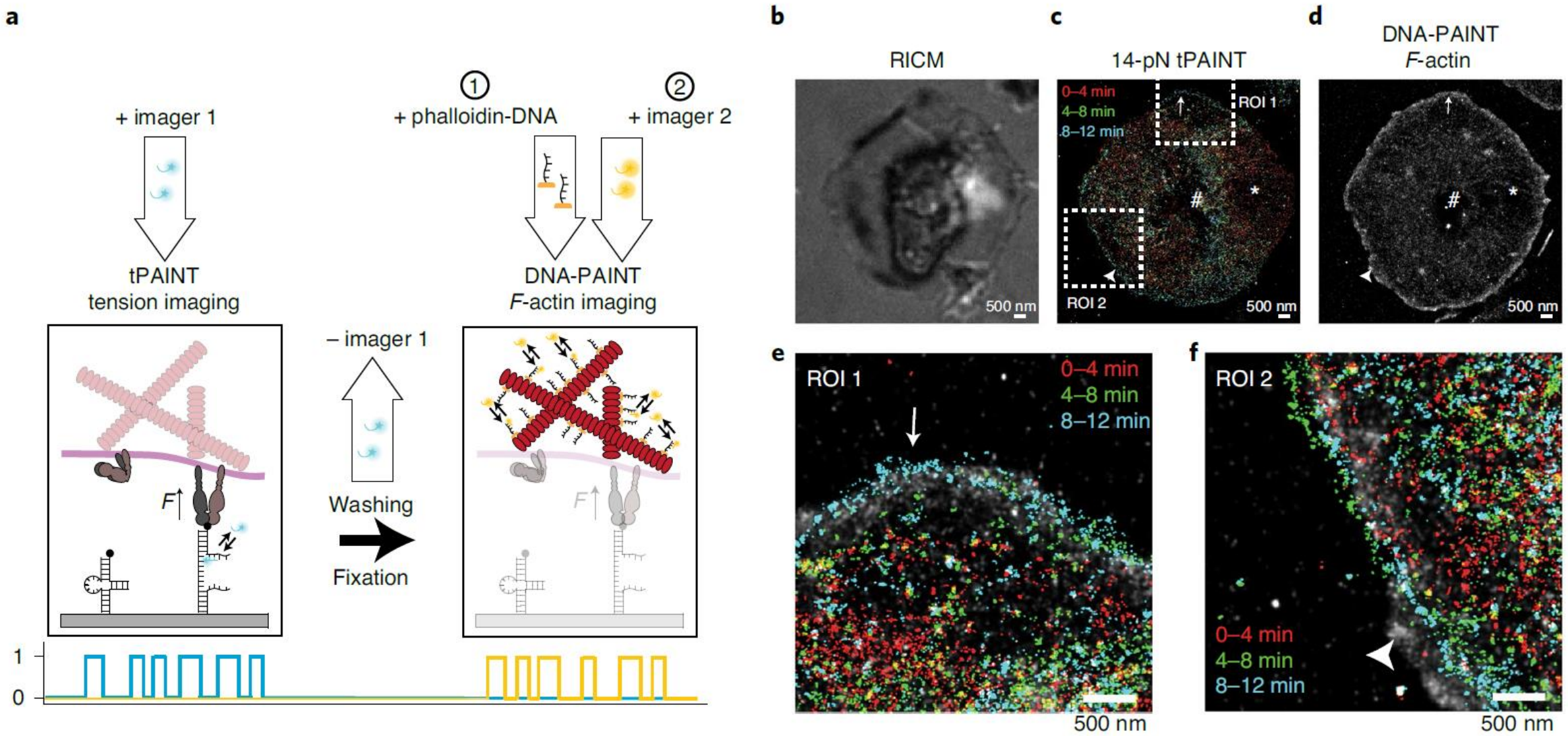

b
Total energy (kBT)
ΔEnergy relative to open (kBT)
Force (pN)
Closed
Open
Bound

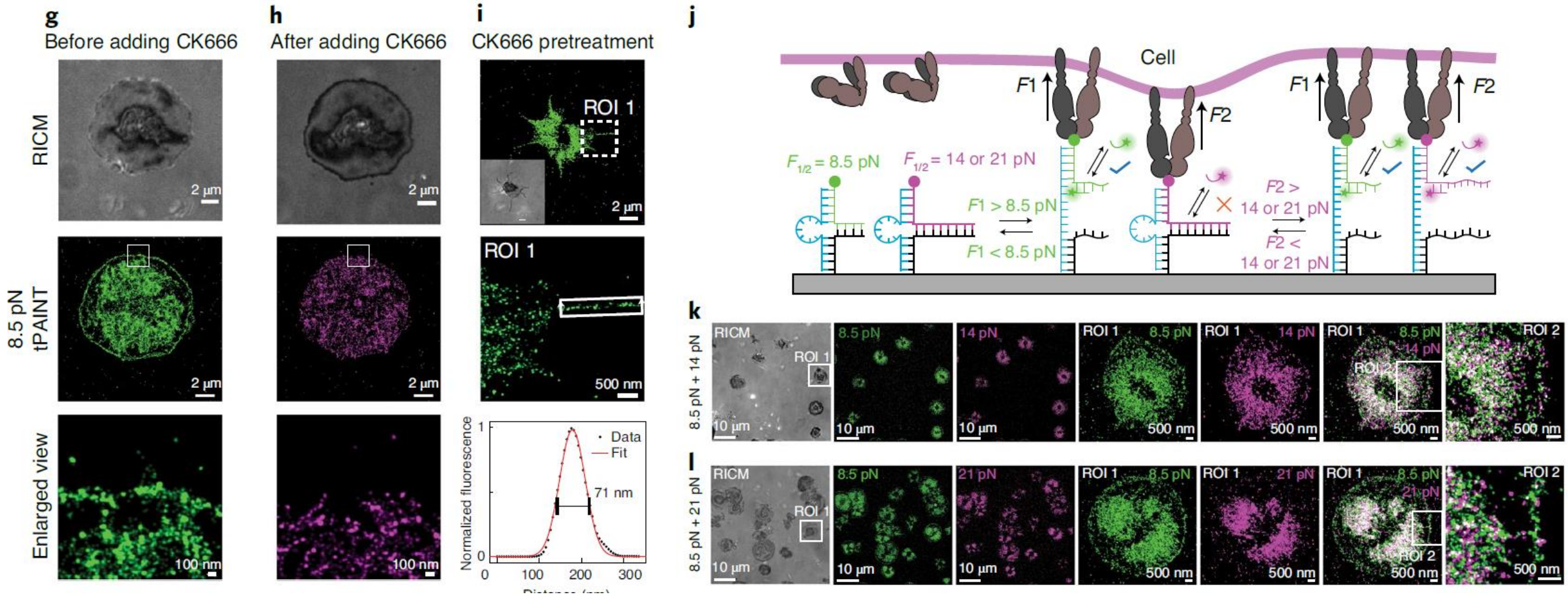

b
Strained docking site
Conventional/ strained tension probes
sf-tPAINT probes
Closed
Open
Imager-bound
Strain-free docking site
d0
dhybridized
Docking site free energy under force, F
Strained
Strain-free
Closed
Open
Imager-bound

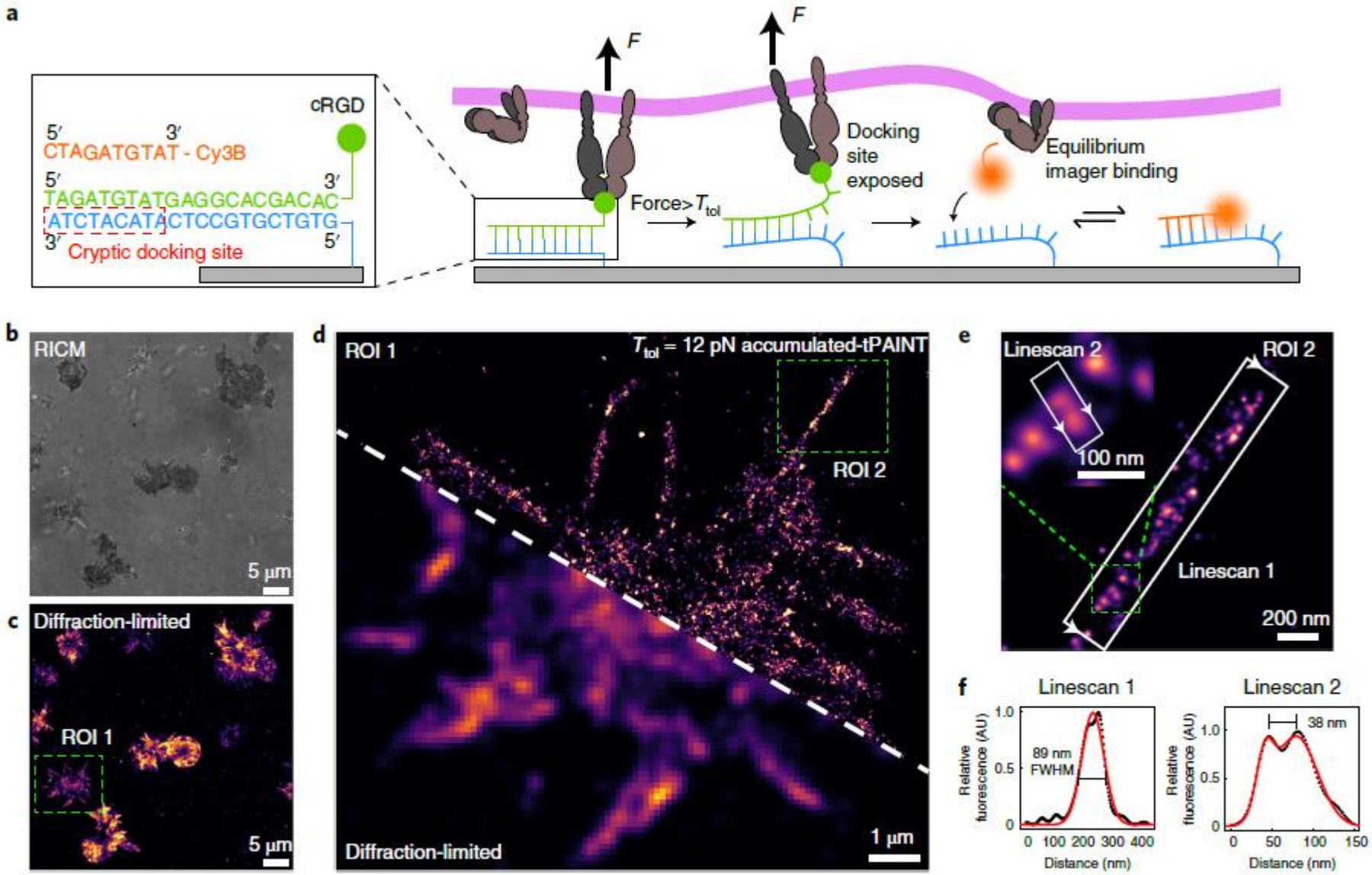

c
10 nM, 9-nt imager
Probe percent occupancy (%)
Force (pN)
strained
strain-free

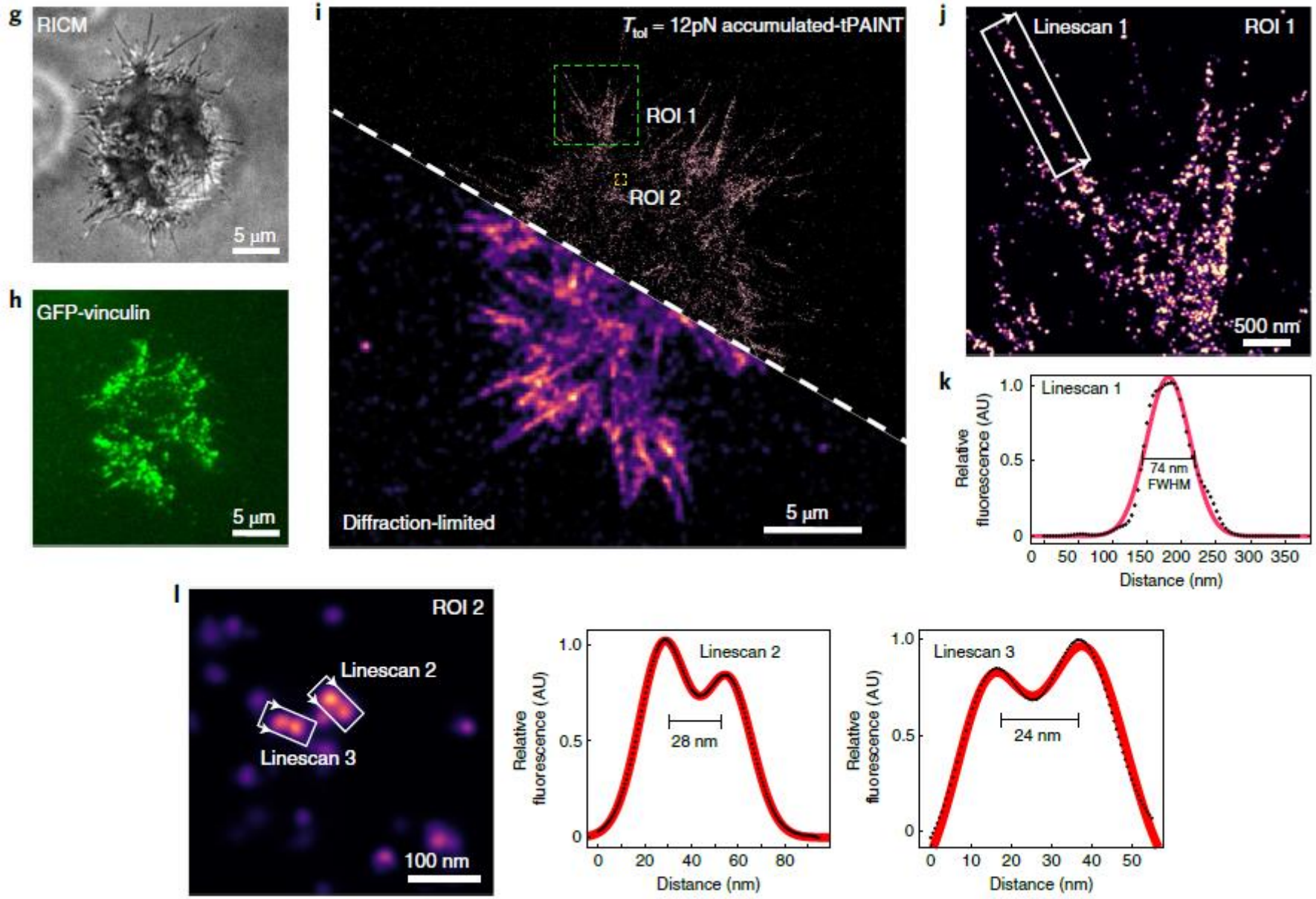

e
F
F
Turn-on tension signal
RICM
Tension
10 µm
10 µm
f
F
F
Turn-on tension signal
RICM
Tension
10 µm
10 µm
g
F
F
strain-free tPAINT single molecule localizations
RICM
tPAINT
i
ii
10 µm
10 µm
g-i
g-ii
1 µm
1 µm
h
F
F
strained tPAINT single molecule localizations
RICM
tPAINT
i
ii
10 µm
10 µm
h-i
h-ii
1 µm
1 µm
i
strained-tPAINT
0.05 localizations / (µm² s)
0.14 localizations / (µm² s)
5 µm
5 µm
j
strain-free tPAINT
0.91 localizations / (µm² s)
0.19 localizations / (µm² s)
5 µm
5 µm
k
***
****
**
****
0.29 ± 0.20
0.06 ± 0.02
1.09 ± 0.30
0.22 ± 0.08
Localizations/ (µm² s)
2.0
1.5
1.0
0.5
0.0
Cell (strained tPAINT)
Background (strained tPAINT)
Cell (strain-free tPAINT)
Background (strain-free tPAINT)


c
8.5pN tPAINT
RICM
ROI 1
Diffraction-limited
2 μm
8.5pN tPAINT: 0–200 s
8.5pN tPAINT: 200–400 s
8.5pN tPAINT: 400–600 s
8.5pN tPAINT: 600–800 s
8.5pN tPAINT: 800–1,000 s
ROI 3
ROI 2
d
8.5pN tPAINT: ROI 1
0–200 s
200–400 s
400–600 s
600–800 s
800–1,000 s
500 nm
e
ROI 2
800–
1,000 s
8.5pN tPAINT
200 nm
Relative fluorescence (AU)
1.0
0.5
0
92 nm
FWHM
0
200
400
600
Distance (nm)
ROI 3,800–1,000 s
8.5pN tPAINT
200 nm
Relative fluorescence (AU)
1.0
0.5
0
162 nm
FWHM
0
200
400
600
Distance (nm)
f
RICM
5 μm
GFP-vin
Diffraction-limited
8.5pN tension
0–200 s
200–400 s
400–600 s
5 μm
8.5pN tPAINT
0–200 s
200–400 s
400–600 s
Diffraction-limited
500 nm
Vinculin-8.5pN tPAINT
overlay, 0–200 s
0–200 s
500 nm


a
+ imager 1
tPAINT
tension imaging
F
– imager 1
Washing
Fixation
1
+ phalloidin-DNA
+ imager 2
DNA-PAINT
F-actin imaging
F
1
0
b
RICM
500 nm
c
14-pN tPAINT
0–4 min
4–8 min
8–12 min
ROI 1
ROI 2
500 nm
d
DNA-PAINT
F-actin
500 nm
e
ROI 1
0–4 min
4–8 min
8–12 min
500 nm
f
ROI 2
0–4 min
4–8 min
8–12 min
500 nm


g
Before adding CK666
h
After adding CK666
i
CK666 pretreatment
RICM
8.5 pN tPAINT
Enlarged view
2 μm
100 nm
ROI 1
500 nm
Normalized fluorescence
Data
Fit
71 nm
Distance (nm)
j
Cell
F1
F2
F1/2 = 8.5 pN
F1/2 = 14 or 21 pN
F1 > 8.5 pN
F1 < 8.5 pN
F2 > 14 or 21 pN
F2 < 14 or 21 pN
k
8.5 pN + 14 pN
RICM
ROI 1
ROI 2
8.5 pN
14 pN
10 μm
500 nm
l
8.5 pN + 21 pN
21 pN


a
cRGD
5′ 3′
CTAGATGTAT - Cy3B
5′ 3′
TAGATGTATGAGGCACGACAC
ATCTACATACTCCGTGCTGTG
3′ 5′
Cryptic docking site
F
F
Docking site exposed
Force>T_{tol}
Equilibrium imager binding
b
RICM
5 µm
c
Diffraction-limited
ROI 1
5 µm
d
ROI 1
T_{tol} = 12 pN accumulated-tPAINT
ROI 2
Diffraction-limited
1 µm
e
Linescan 2
ROI 2
100 nm
Linescan 1
200 nm
f
Linescan 1
Relative fluorescence (AU)
1.0
0.5
0
89 nm FWHM
0 100 200 300 400
Distance (nm)
Linescan 2
Relative fluorescence (AU)
1.0
0.5
0
38 nm
0 50 100 150
Distance (nm)


g
RICM
5 μm
h
GFP-vinculin
5 μm
i
T_{tol} = 12pN accumulated-tPAINT
ROI 1
ROI 2
Diffraction-limited
5 μm
j
Linescan 1
ROI 1
500 nm
k
Linescan 1
Relative fluorescence (AU)
74 nm FWHM
Distance (nm)
l
ROI 2
Linescan 2
Linescan 3
100 nm
Linescan 2
28 nm
Relative fluorescence (AU)
Distance (nm)
Linescan 3
24 nm
Relative fluorescence (AU)
Distance (nm)