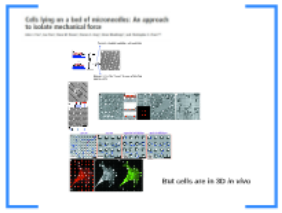
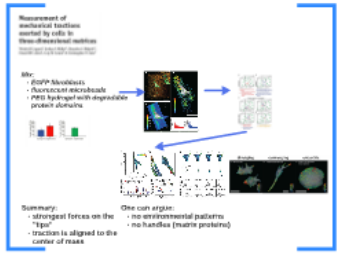
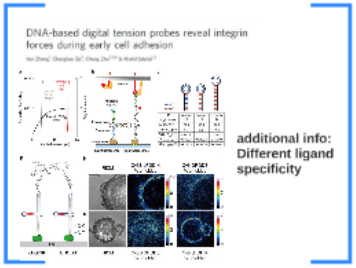
Tension probes: reporting cellular traction forces

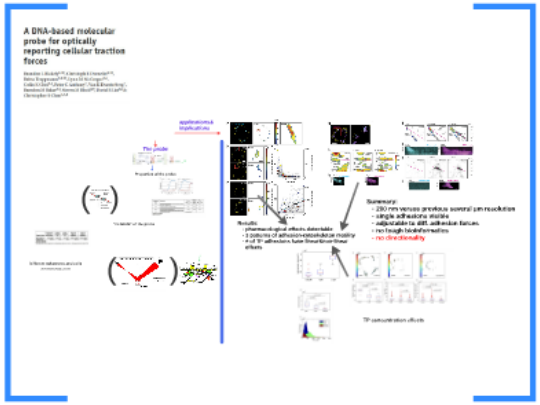
Cell adhesion is the critical interaction between cells and the ECM.

- **Focal adhesions occur by the clustering of integrins &**
- **Contraction of the corresponding actin cytoskeleton**
- **Cells probe the mechano-chemical environment**
- **~mechano-chemical sensors**
- **Adhesions influence signaling in e.g. proliferation-differentiation**







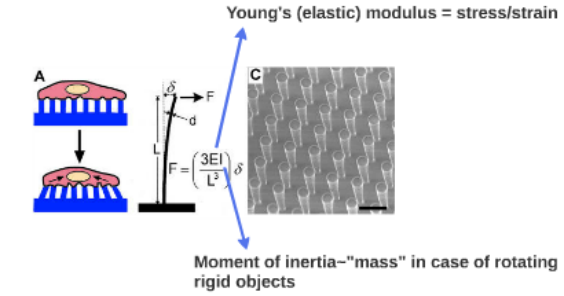


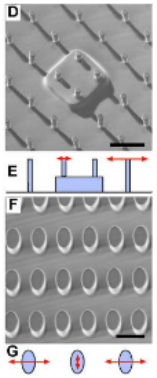
Thank you for your attention!

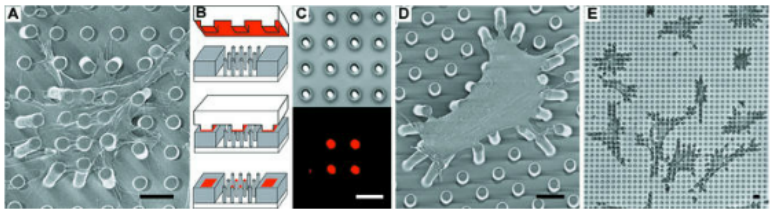
Daniel Kirschenbaum
Technical Journal Club
03.03.2015

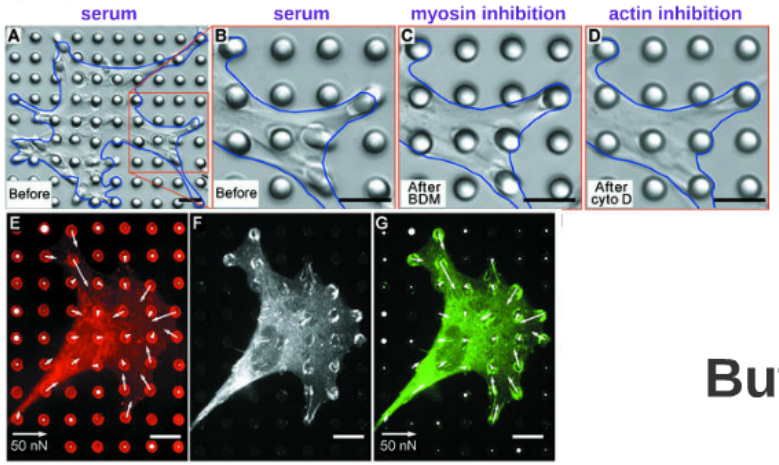
Cells lying on a bed of microneedles: An approach to isolate mechanical force

John L. Tan*, Joe Tien*, Dana M. Pirone*, Darren S. Gray*, Kiran Bhadriraju*, and Christopher S. Chen*†‡



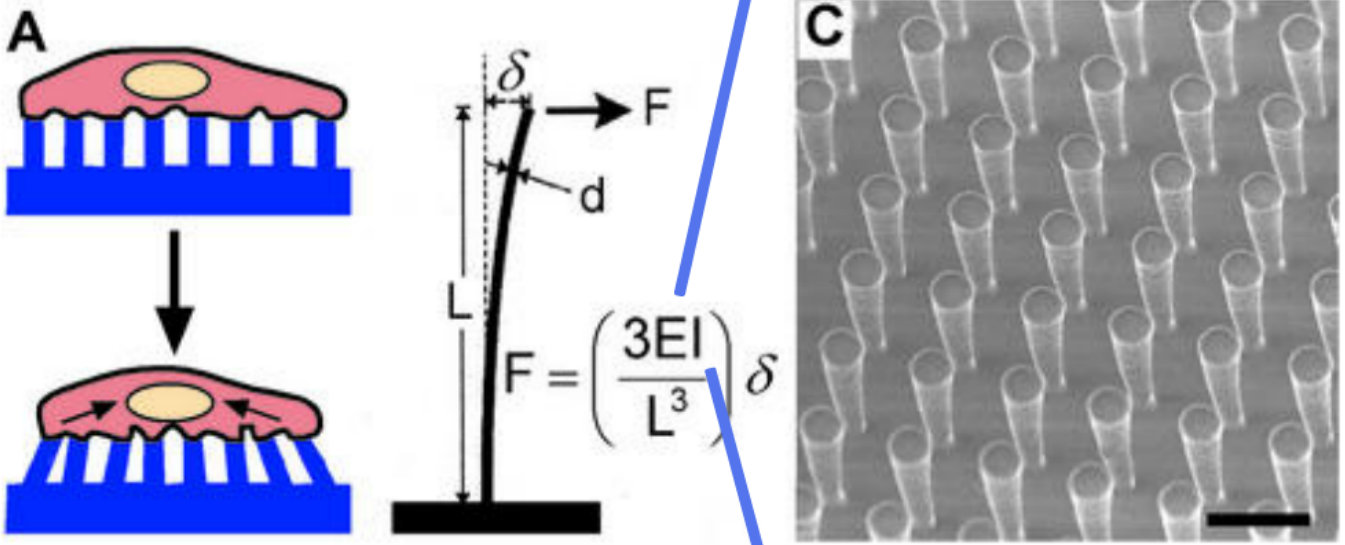




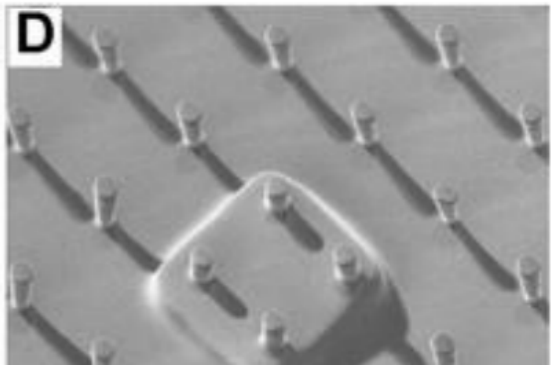


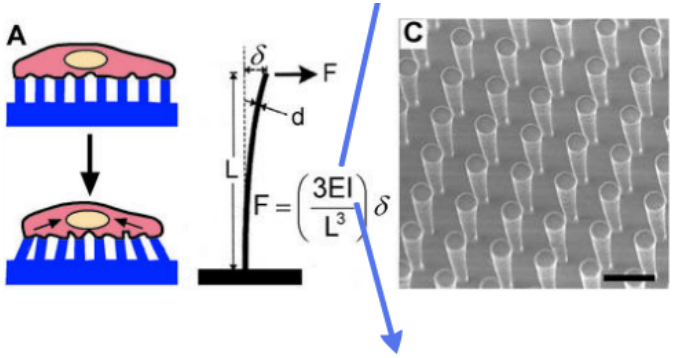
But cells are in 3D *in vivo*

Young's (elastic) modulus = stress/strain

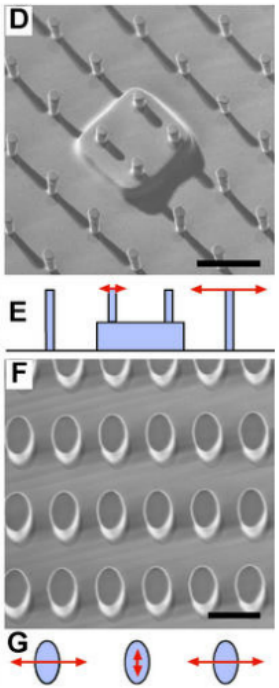


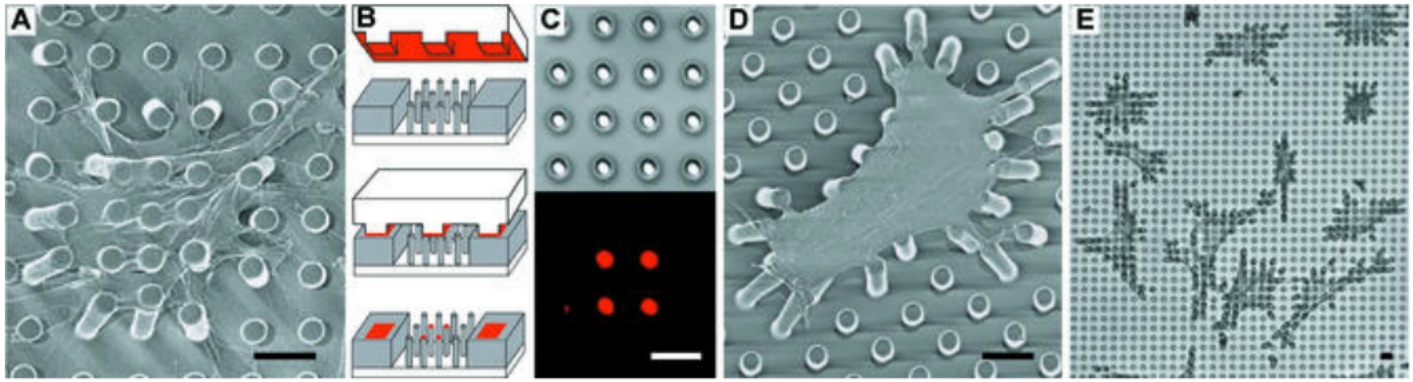
Moment of inertia~"mass" in case of rotating rigid objects

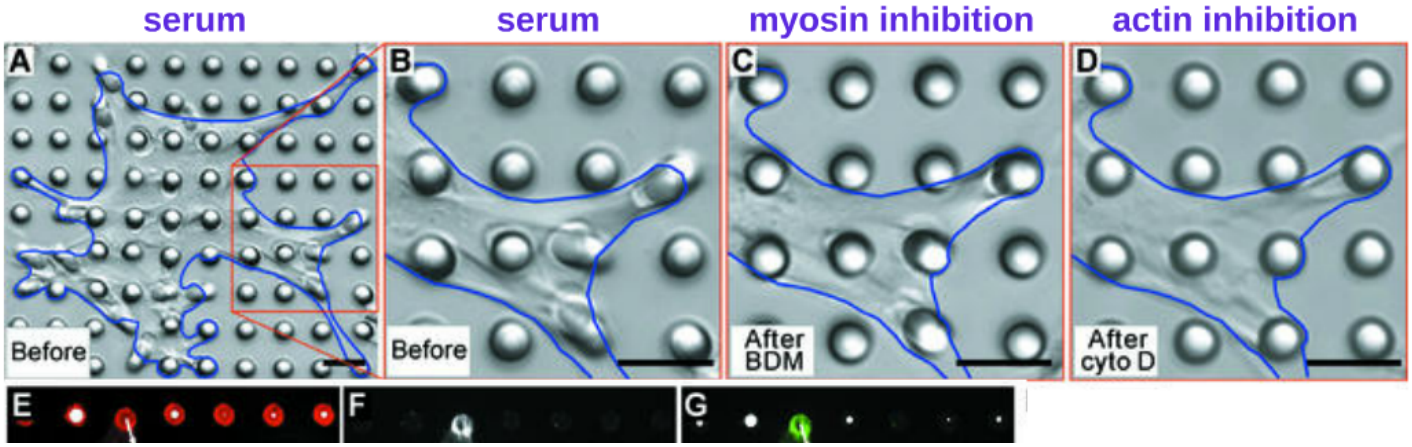


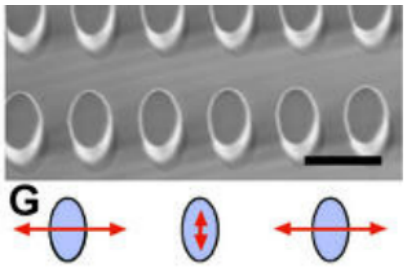


Moment of inertia~"mass" in case of rotating rigid objects







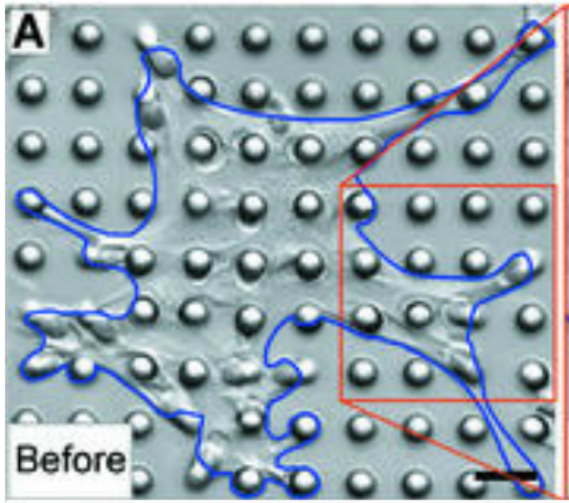


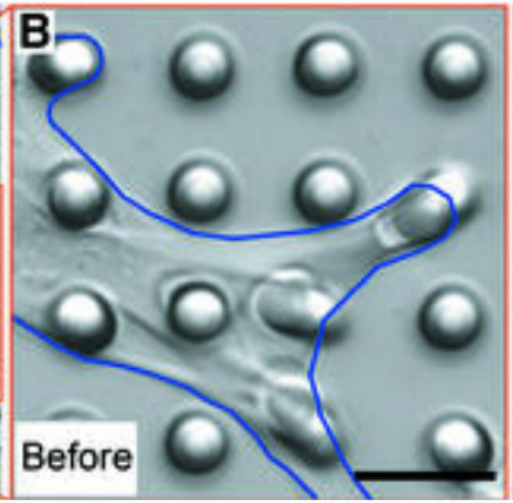
serum

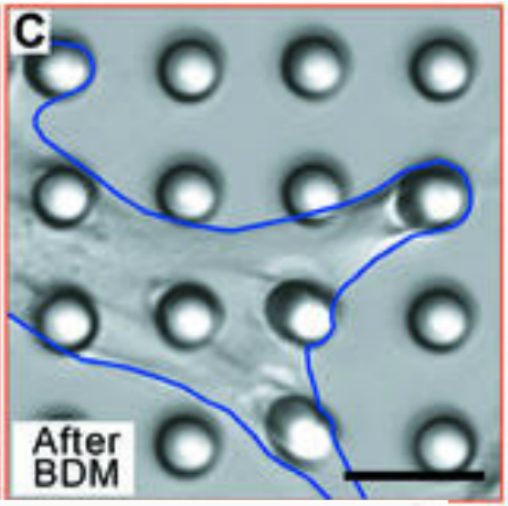
serum

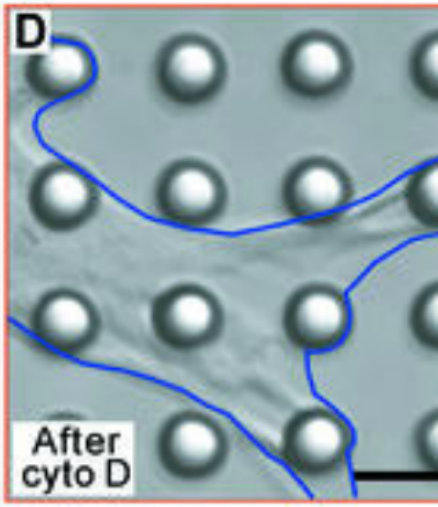
myosin inhibition

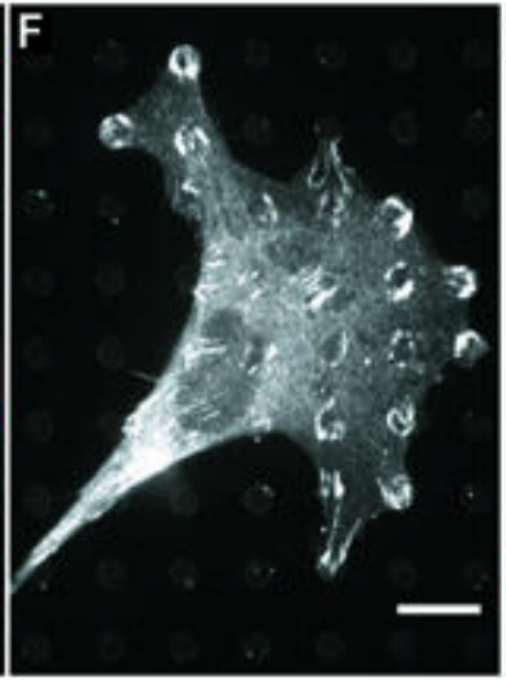
actin inhibition

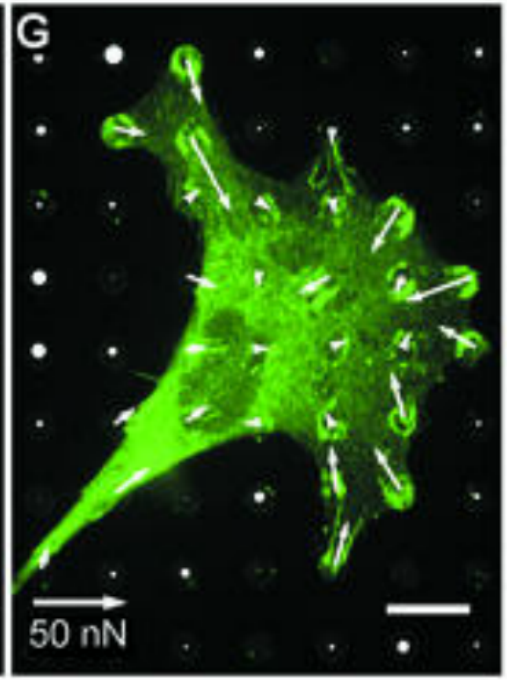












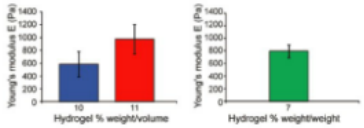
B

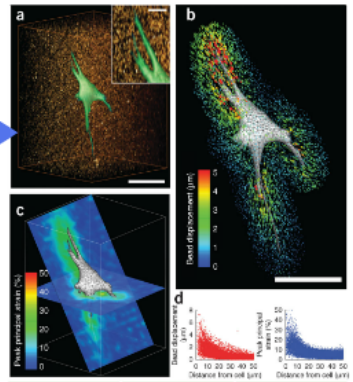
Measurement of mechanical tractions exerted by cells in three-dimensional matrices

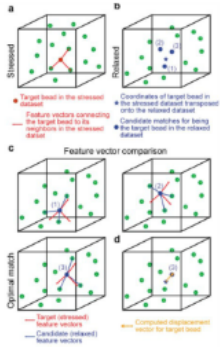
Wesley R Legant[1], Jordan S Miller[1], Brandon L Blakely[1], Daniel M Cohen[1], Guy M Genin[2] & Christopher S Chen[1]

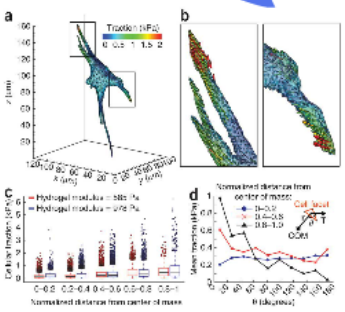
Mix:

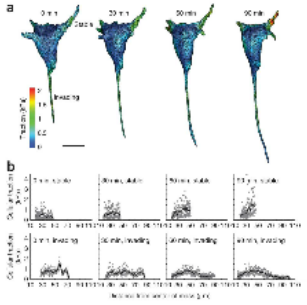
- *EGFP fibroblasts*
- *fluorescent microbeads*
- *PEG hydrogel with degradable protein domains*

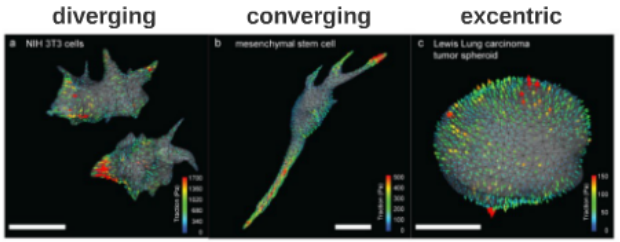












Summary:

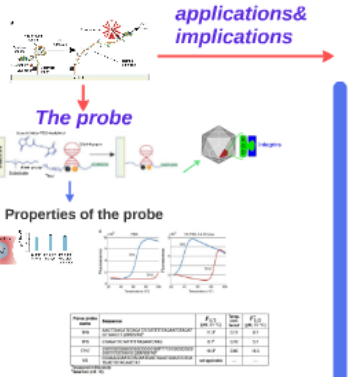
- strongest forces on the "tips"
- traction is aligned to the center of mass

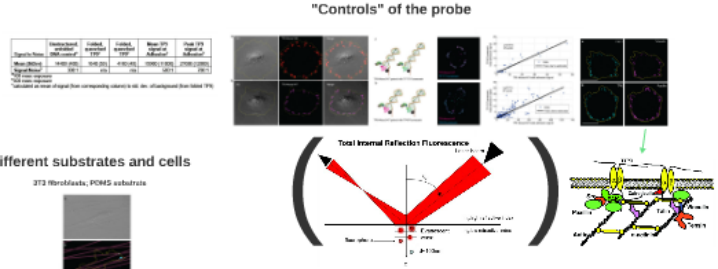
One can argue:

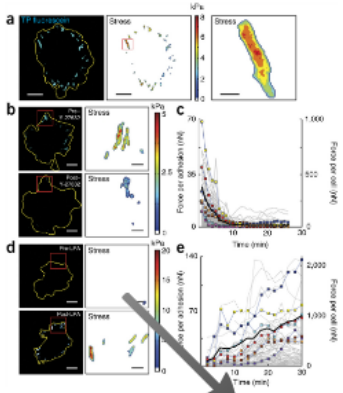
- no environmental patterns
- no handles (matrix proteins)

A DNA-based molecular probe for optically reporting cellular traction forces

Brandon L Blakely[1,10], Christoph E Dumelin[2,10], Britta Trappmann[3,4,10], Lynn M McGregor[5,6], Colin K Choi[3,4], Peter C Anthony[7], Van K Duesterberg[7], Brendon M Baker[3,4], Steven M Block[8,9], David R Liu[5,6] & Christopher S Chen[1,3,4]

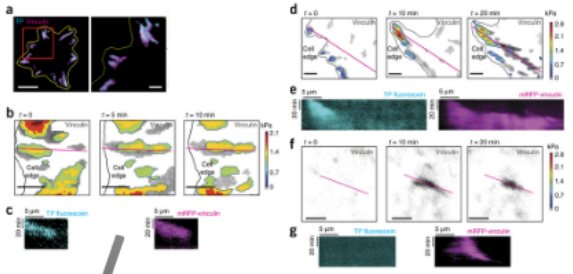






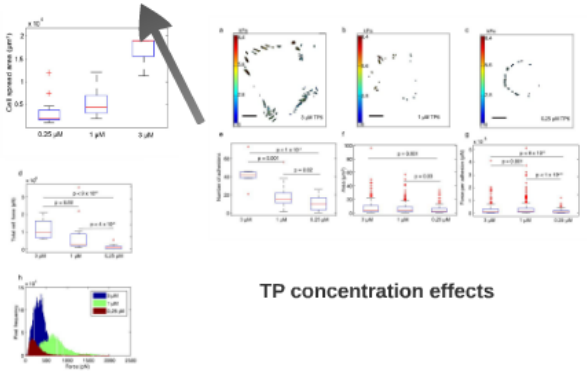
Results:

- pharmacological effects detectable
- 3 patterns of adhesion-cytoskeleton motility
- # of TP adhesions have linear&non-linear effects



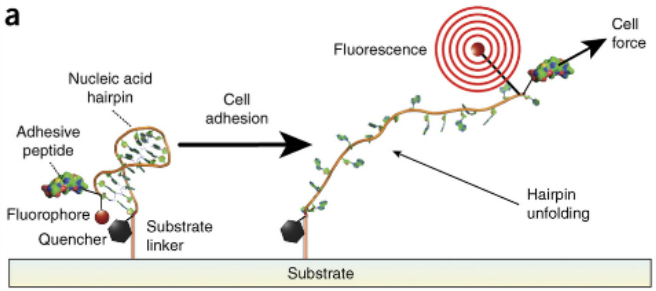
Summary:

- 200 nm versus previous several µm resolution
- single adhesions visible
- adjustable to diff. adhesion forces
- no tough bioinformatics
- no directionality

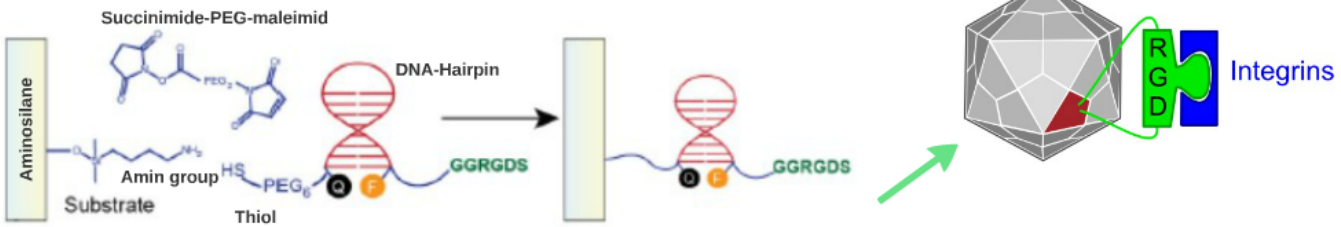


TP concentration effects

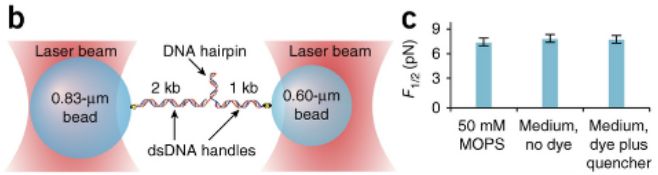
applications& implications

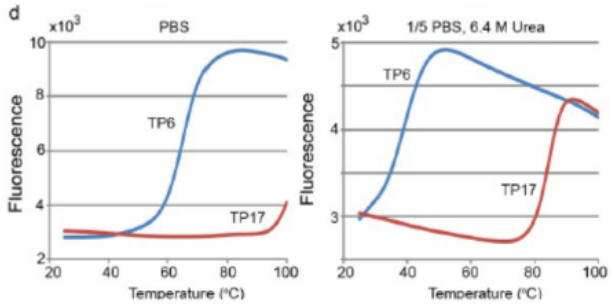


The probe



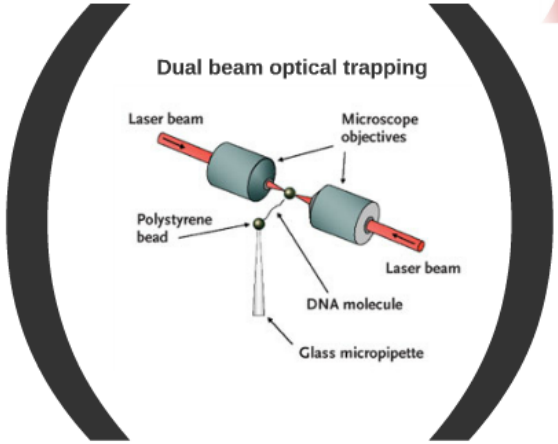
Properties of the probe

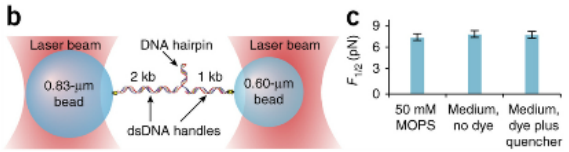


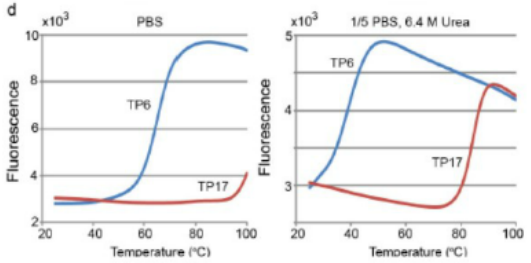


Force probe name	Sequence	$F_{1/2}$ (pN; 21 °C)	Temp. corr. factor	$F^*_{1/2}$ (pN; 37 °C)
	AAGTTAACATCTAGATTCTATTTTAGAATCTAGAT			

Properties of the probe







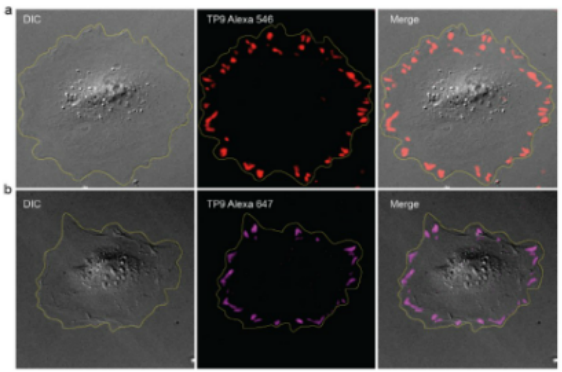
Force probe name	Sequence	$F_{1/2}$ (pN; 21 °C)	Temp. corr. factor	$F^{*}_{1/2}$ (pN; 37 °C)
TP9	*AAGTTAACATCTAGATTCTATTTTTAGAATCTAGAT GTTAACTT* (20R25/T4)[b]	11.3[a]	0.77	8.7
TP6	*CTAGATTCTATTTTTAGAATCTAG*	8.1[a]	0.70	5.7
TP17	*CGCCGCGGGCCGGCGCGCGGTTTTCCGCGCGCC GGCCCGCGGCG* (20R100/T4)[b]	19.3[b]	0.86	16.5
US	CGGAAGGAATGTAGAATGAGTGAGTGGATCGTGA TGACTGTACAACTAT	not applicable	—	—

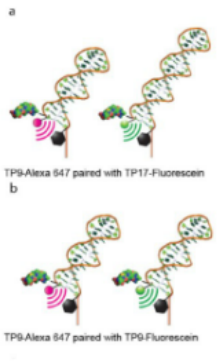
[a]measured in this study
[b]data from (ref. 16)

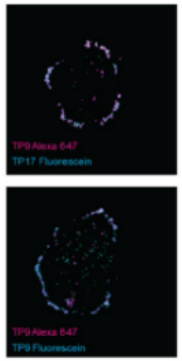
"Controls" of the probe

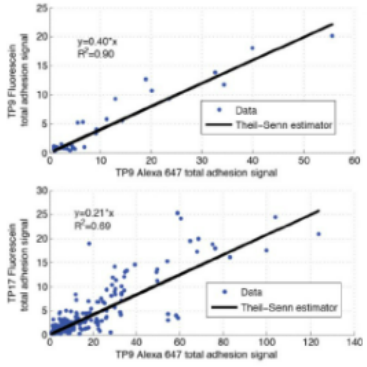
n TP9 nal at esion[b]	Peak TP9 signal at Adhesion[b]
(11000)	27600 (12000)
500:1	700:1

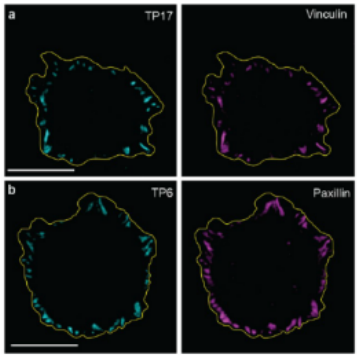
background (from folded TP9)



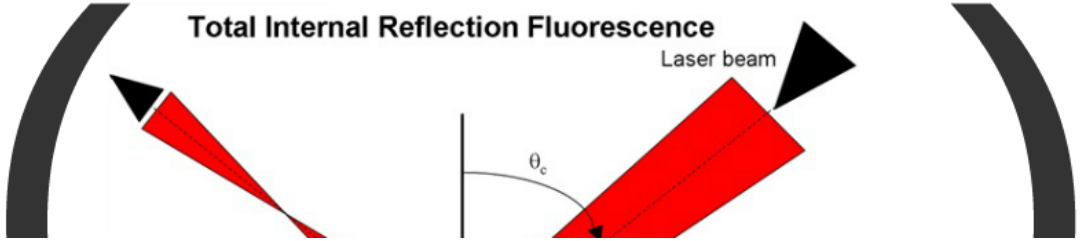


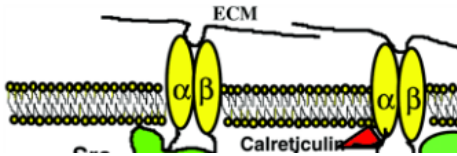




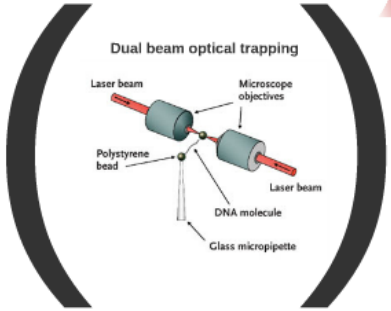


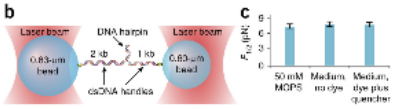
cells

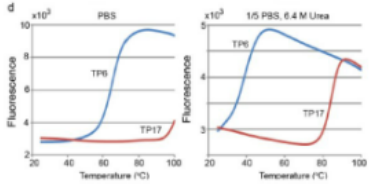




Properties of the probe







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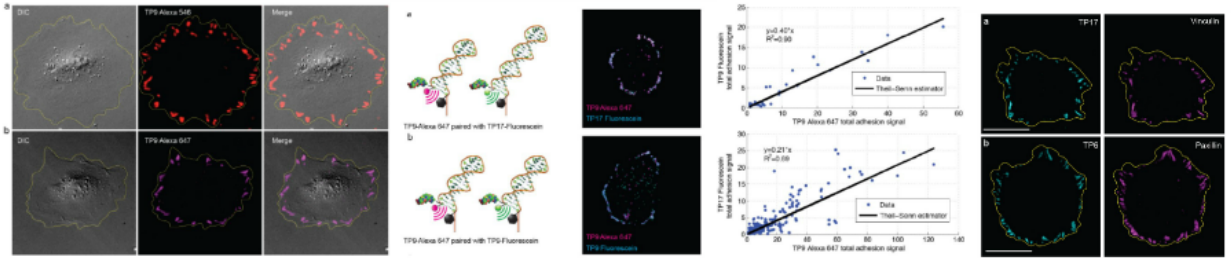
US	CGGAAGGAATGTAGAATGAGTGAGTGGATCGTGA TGACTGTACAACTAT	not applicable	—	—

[a]measured in this study
[b]data from (ref. 16)

"Controls" of the probe

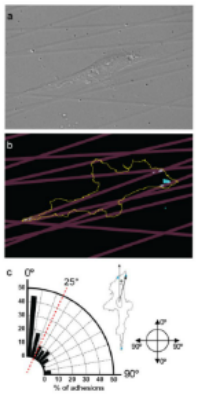
Signal to Noise	Unstructured, unfolded DNA control[a]	Folded, quenched TP9[a]	Folded, quenched TP9[b]	Mean TP9 signal at Adhesion[b]	Peak TP9 signal at Adhesion[b]
Mean (StDev)	14400 (400)	1640 (50)	4160 (40)	19900 (11000)	27600 (12000)
Signal:Noise[c]	300:1	n/a	n/a	500:1	700:1

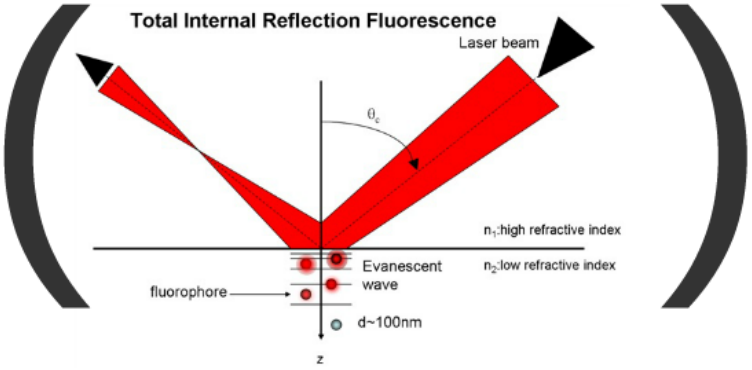
[a]100 msec exposure
[b]500 msec exposure
[c]calculated as mean of signal (from corresponding column) to std. dev. of background (from folded TP9)

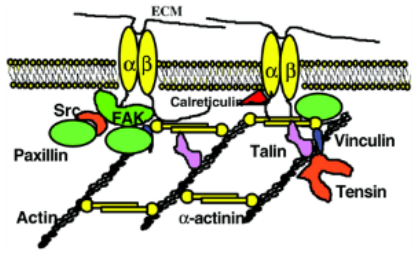


Different substrates and cells

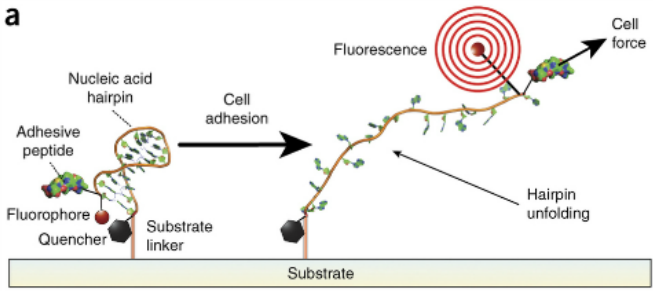
3T3 fibroblasts; PDMS substrate



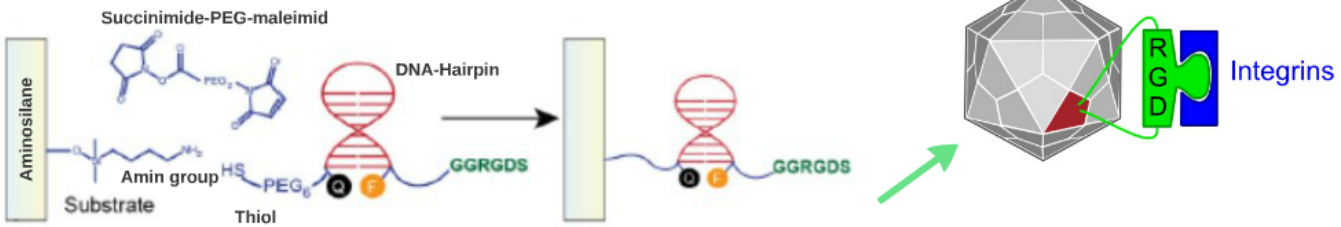




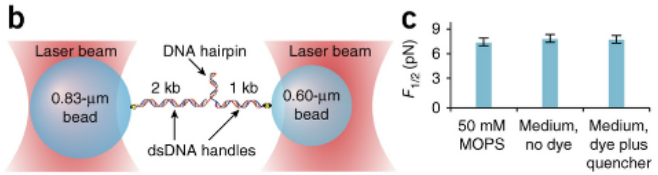
applications& implications

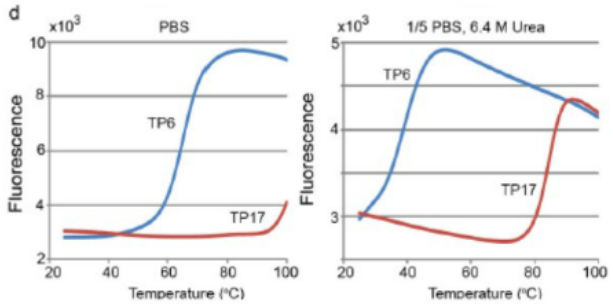


The probe

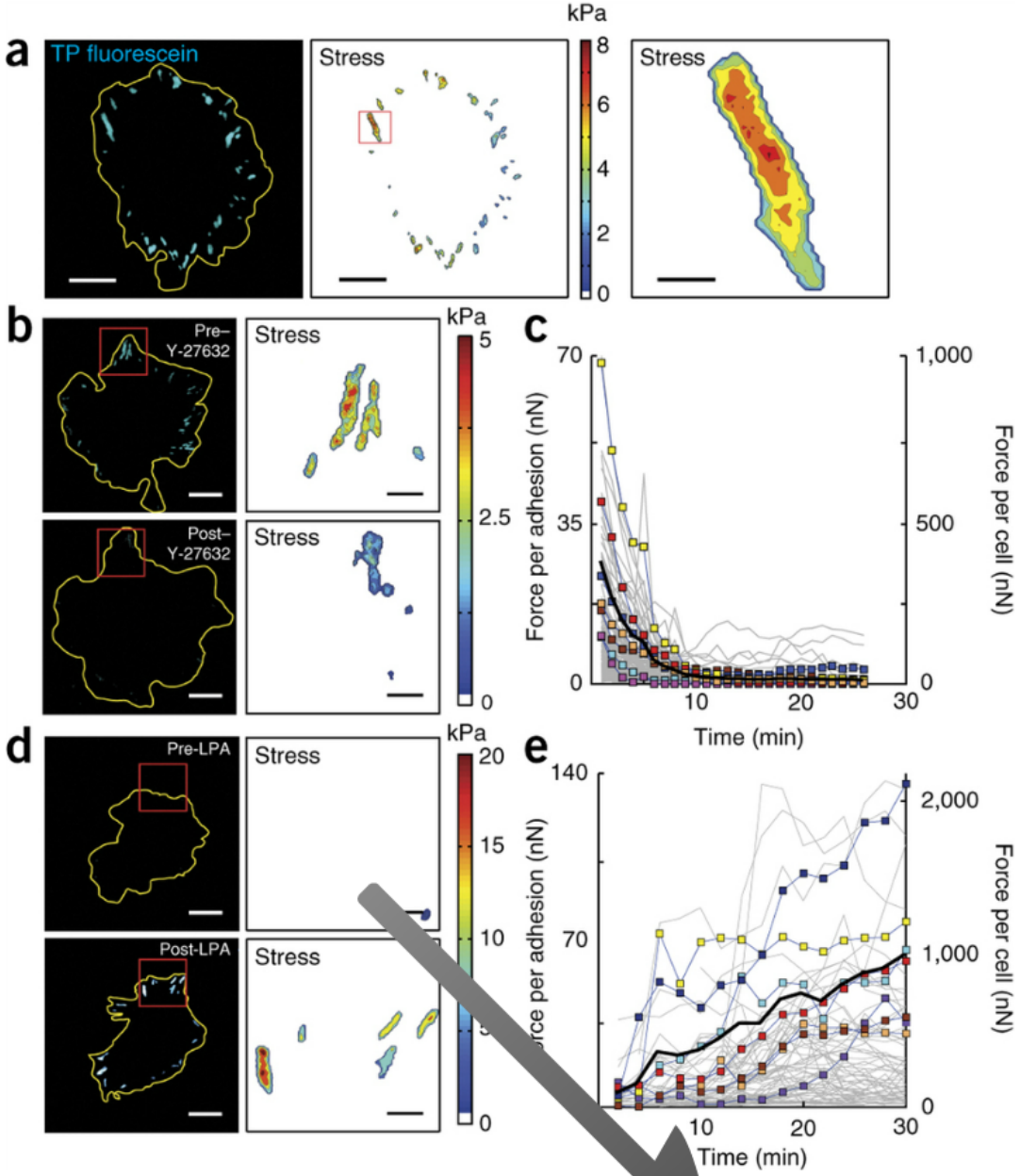


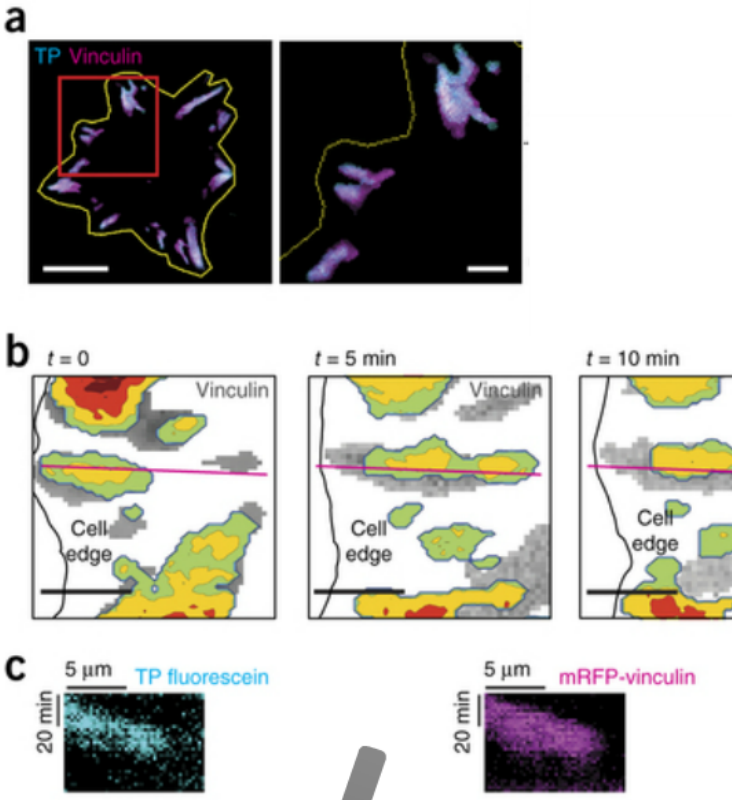
Properties of the probe





Force probe name	Sequence	$F_{1/2}$ (pN; 21 °C)	Temp. corr. factor	$F^*_{1/2}$ (pN; 37 °C)
	AAGTTAACATCTAGATTCTATTTTAGAATCTAGAT			



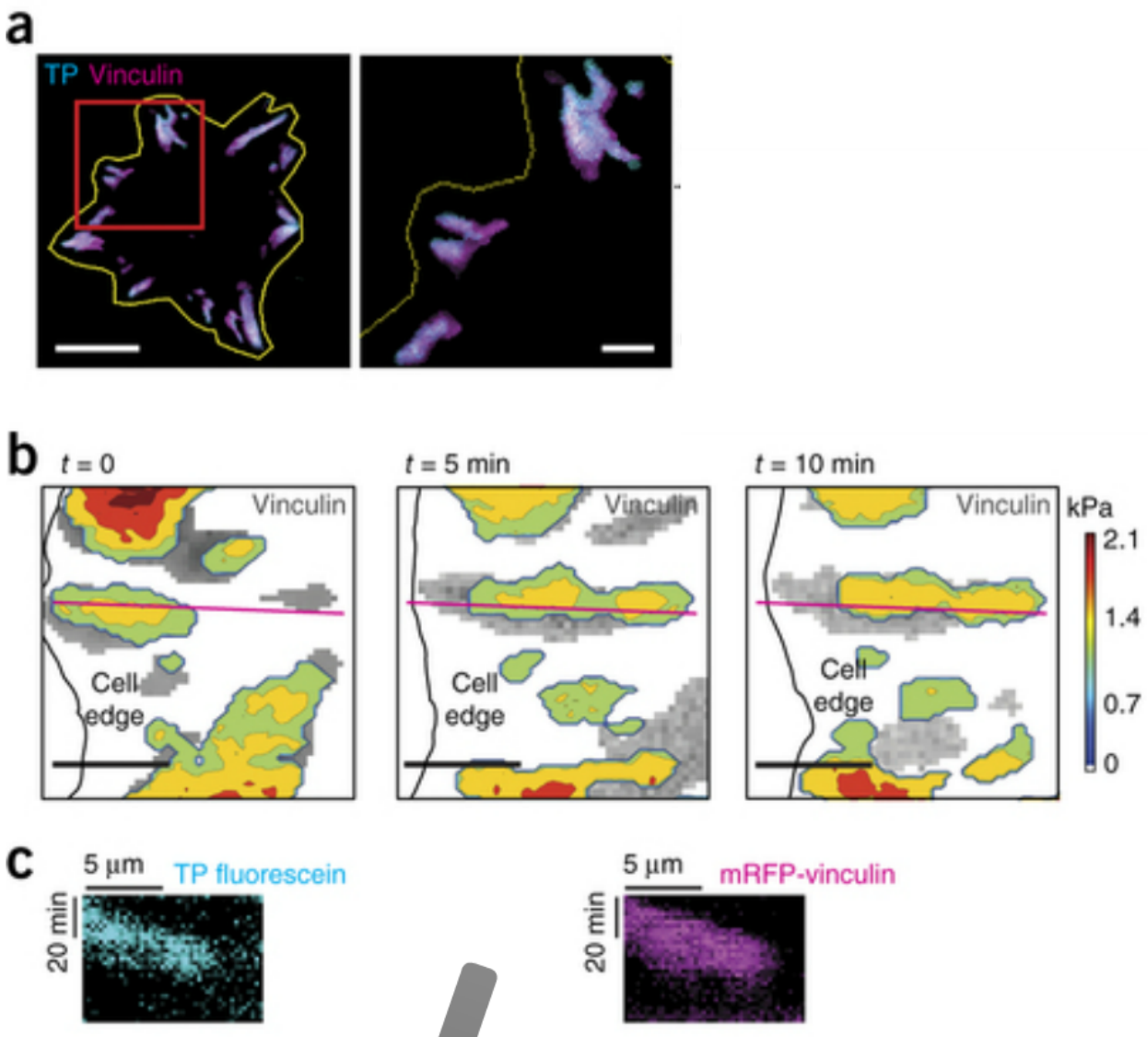


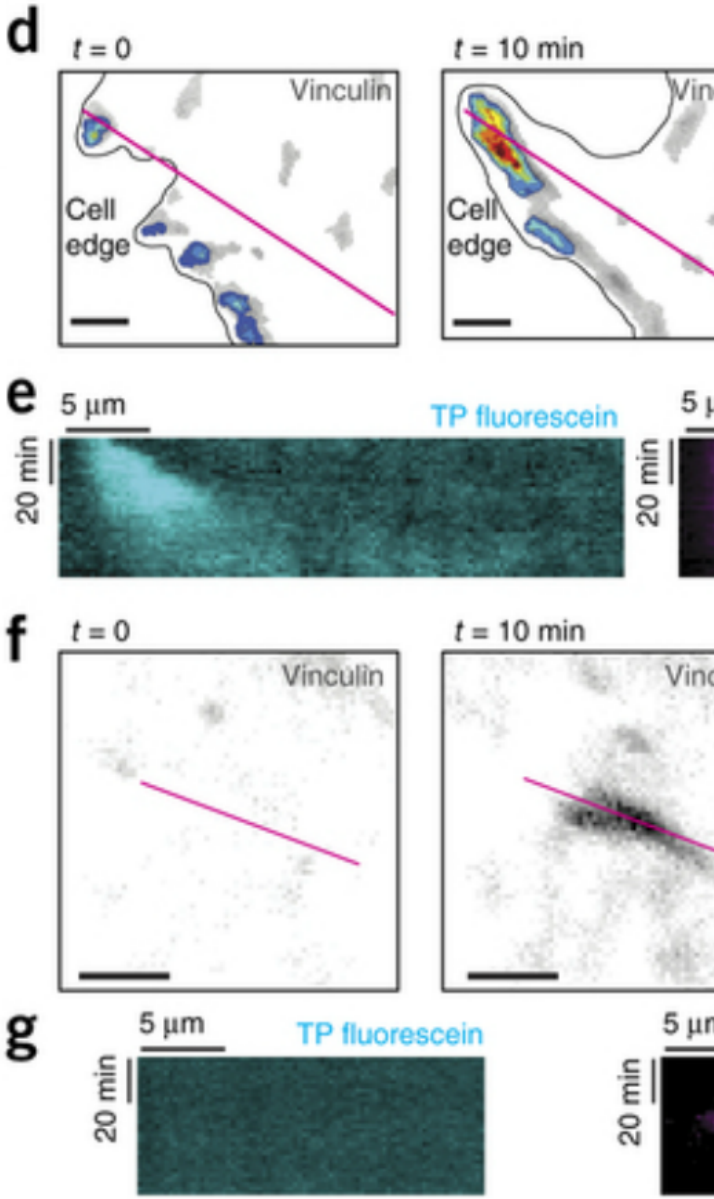
Results:

- pharmacological effects detectable
- 3 patterns of adhesion-cytoskeleton motility
- # of TP adhesions have linear&non-linear

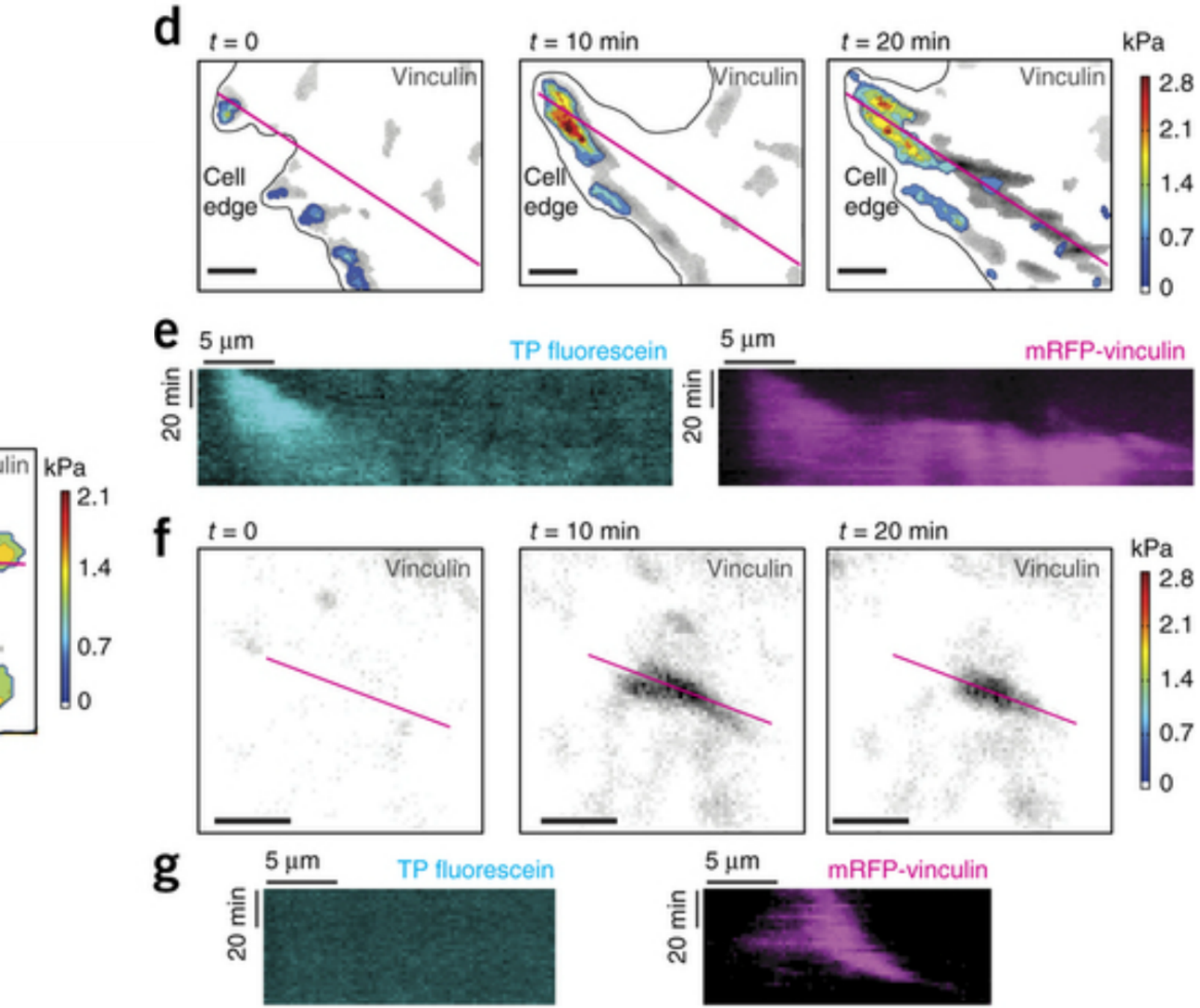
Summa

- 200
- sing
- adju
- no t
- no c


a
TP Vinculin
b
t = 0
t = 5 min
t = 10 min
Vinculin
Cell edge
kPa
2.1
1.4
0.7
0
c
5 µm
TP fluorescein
20 min
5 µm
mRFP-vinculin
20 min


d
t = 0
t = 10 min
Vinculin
Cell edge
e
5 µm
TP fluorescein
20 min
f
t = 0
t = 10 min
Vinculin
g
5 µm
TP fluorescein
20 min

Summary:


d
t = 0
Vinculin
Cell edge
t = 10 min
Vinculin
Cell edge
t = 20 min
Vinculin
Cell edge
kPa
2.8
2.1
1.4
0.7
0
e
5 μm
TP fluorescein
20 min
5 μm
mRFP-vinculin
20 min
f
t = 0
Vinculin
t = 10 min
Vinculin
t = 20 min
Vinculin
kPa
2.8
2.1
1.4
0.7
0
g
5 μm
TP fluorescein
20 min
5 μm
mRFP-vinculin
20 min

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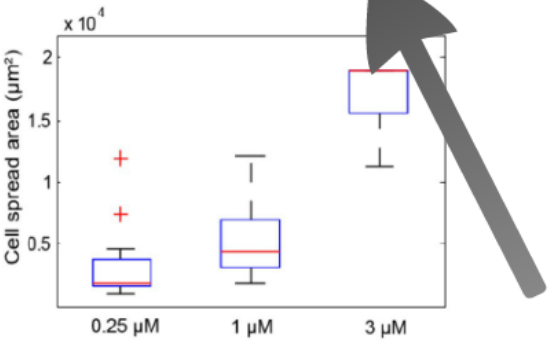
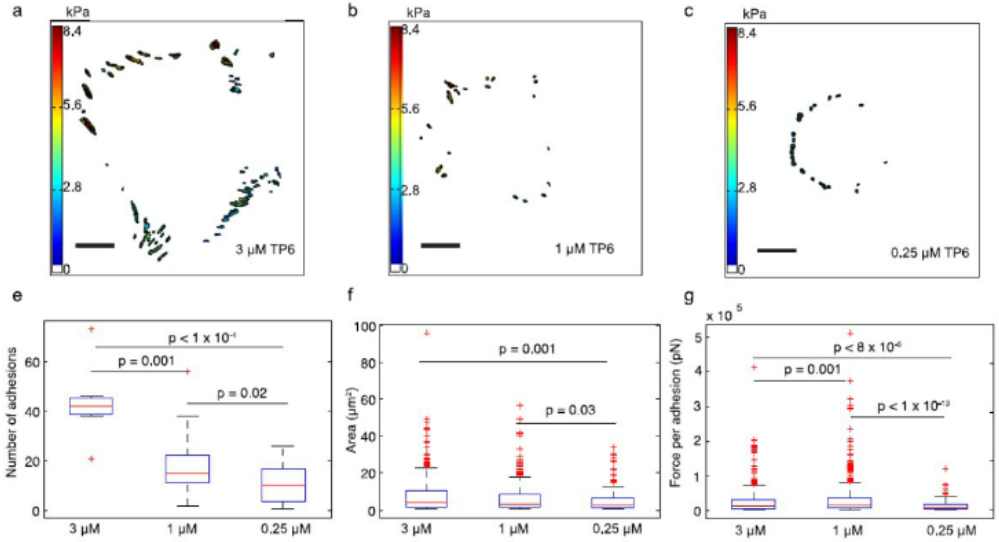
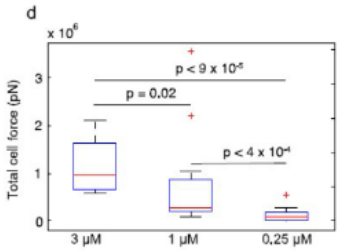
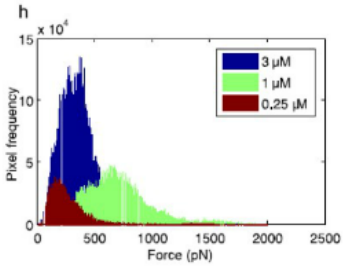


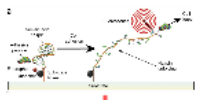






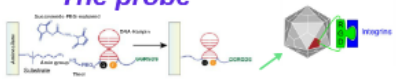
TP concentration effects

erg[7],
5,6 &

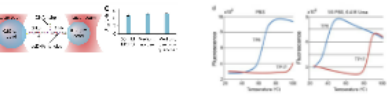


applications&
implications

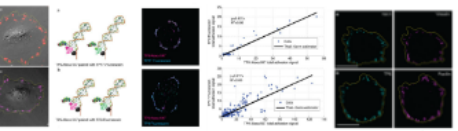
The probe

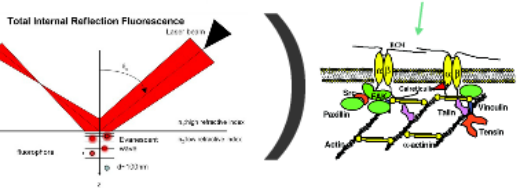


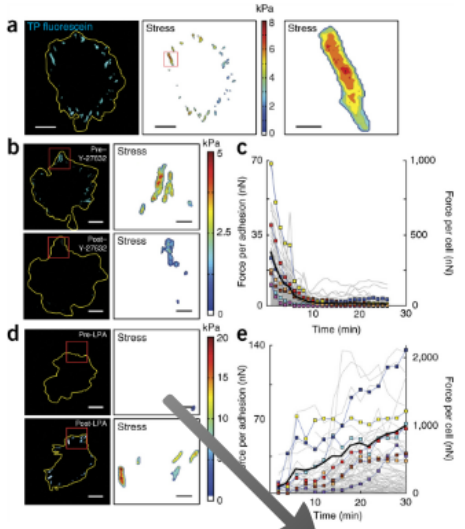
Properties of the probe



ontrols" of the probe







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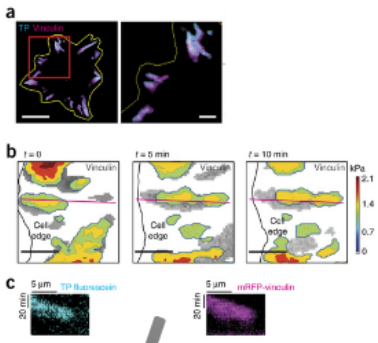

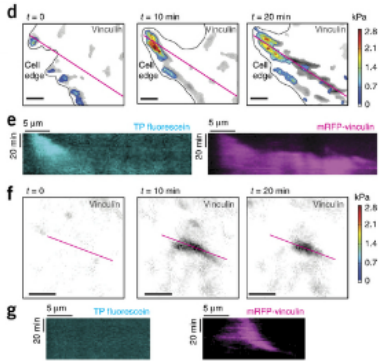

Summary:

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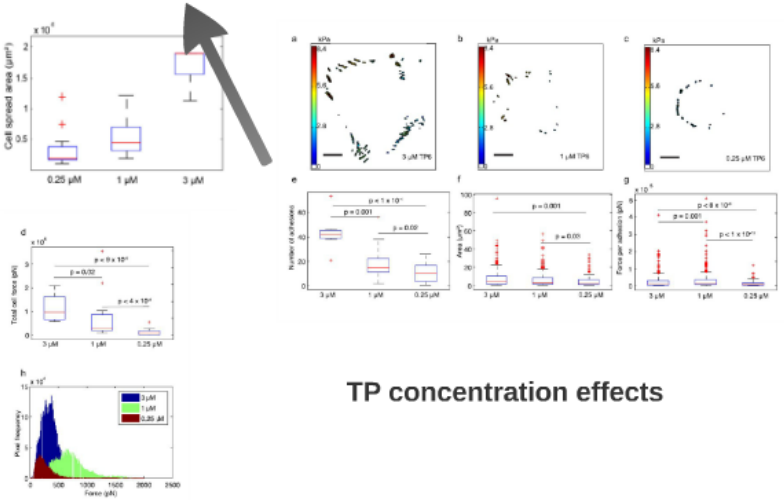
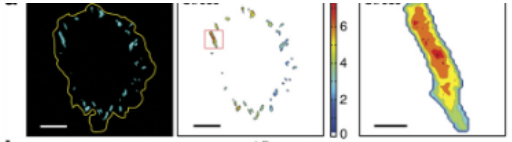
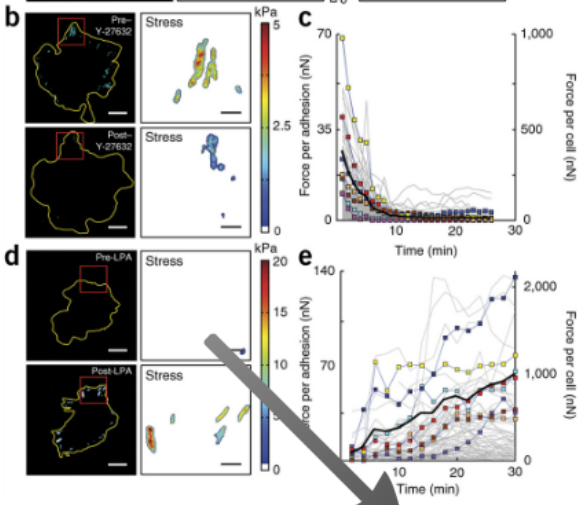
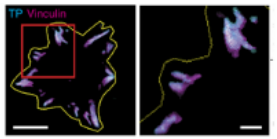
- single adhesions visible
- adjustable to diff. adhesion forces
- no tough bioinformatics
- no directionality

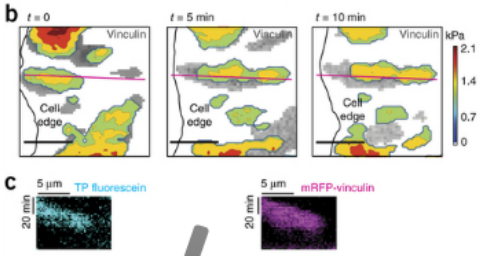


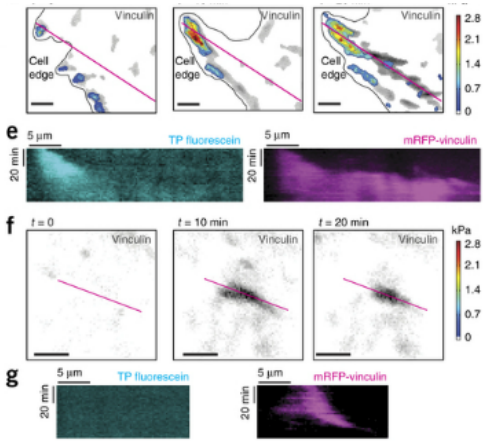
TP concentration effects











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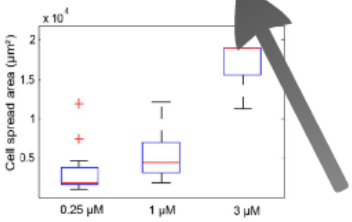
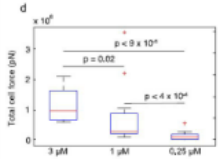
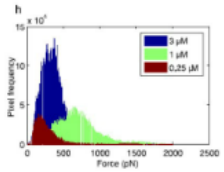
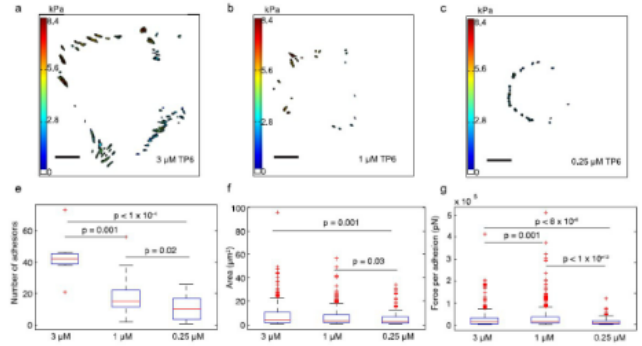






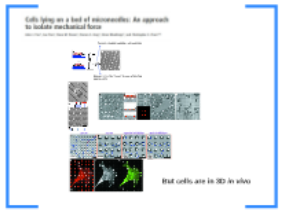


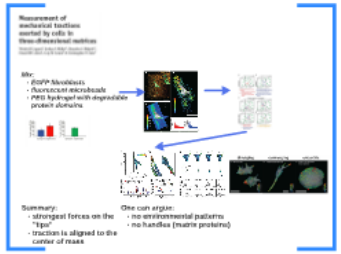
TP concentration effects

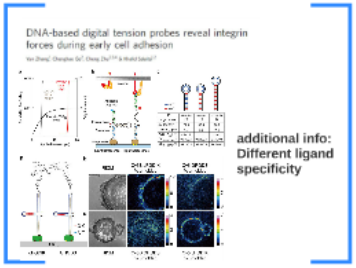
Tension probes: reporting cellular traction forces

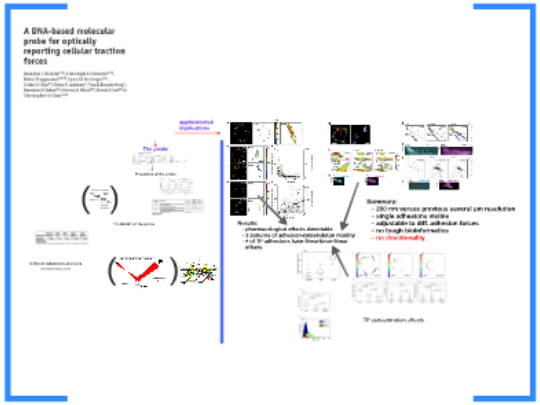
Cell adhesion is the critical interaction between cells and the ECM.

- **Focal adhesions occur by the clustering of integrins &**
- **Contraction of the corresponding actin cytoskeleton**
- **Cells probe the mechano-chemical environment**
- **~mechano-chemical sensors**
- **Adhesions influence signaling in e.g. proliferation-differentiation**







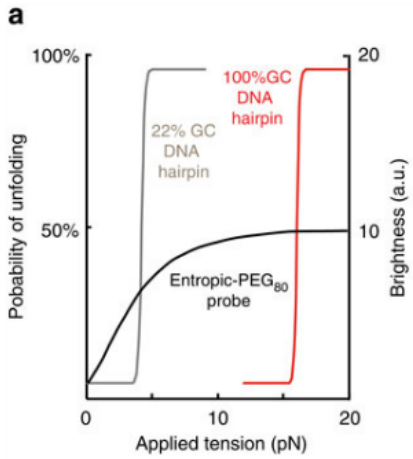


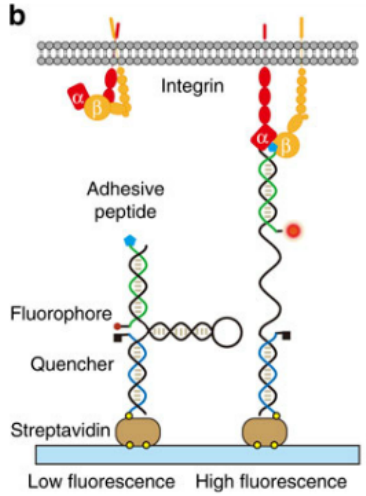
Thank you for your attention!

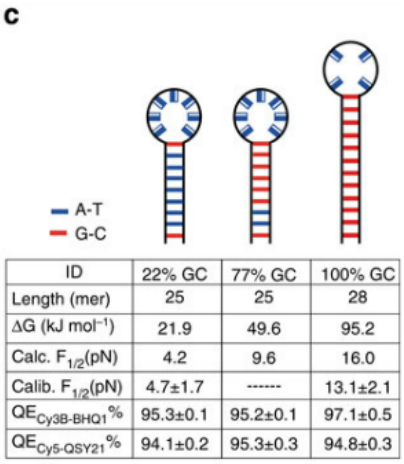
Daniel Kirschenbaum
Technical Journal Club
03.03.2015

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

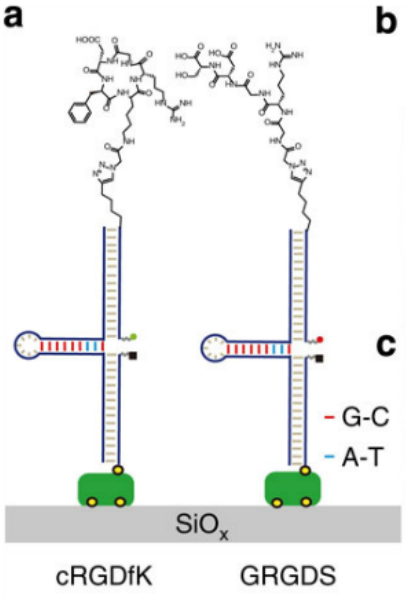
Yun Zhang[1], Chenghao Ge[2], Cheng Zhu[2,3,4] & Khalid Salaita[1,2]

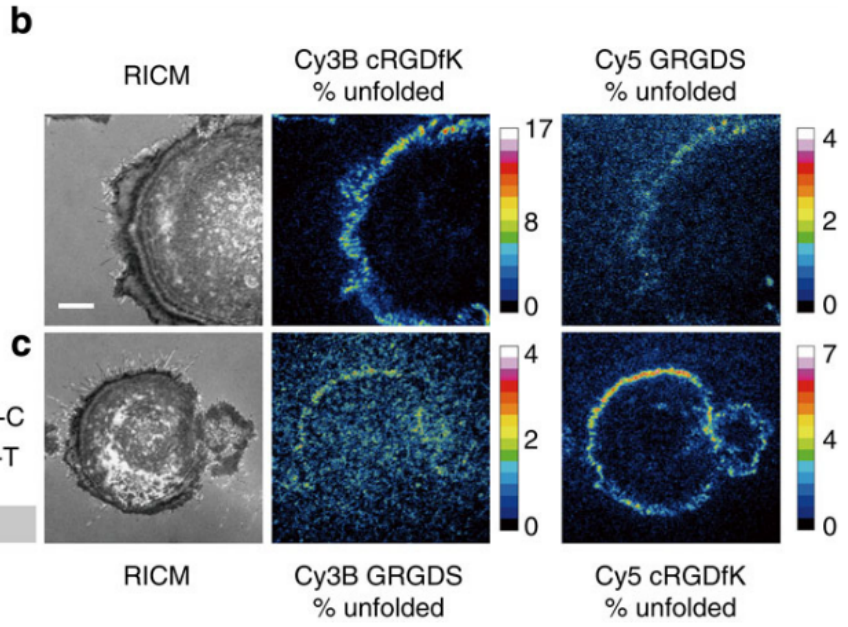






ID	22% GC	77% GC	100% GC
Length (mer)	25	25	28
ΔG (kJ mol^{-1})	21.9	49.6	95.2
Calc. $F_{1/2}$(pN)	4.2	9.6	16.0
Calib. $F_{1/2}$(pN)	4.7±1.7	------	13.1±2.1
$QE_{Cy3B\text{-}BHQ1}$%	95.3±0.1	95.2±0.1	97.1±0.5
$QE_{Cy5\text{-}QSY21}$%	94.1±0.2	95.3±0.3	94.8±0.3





additional info: Different ligand specificity