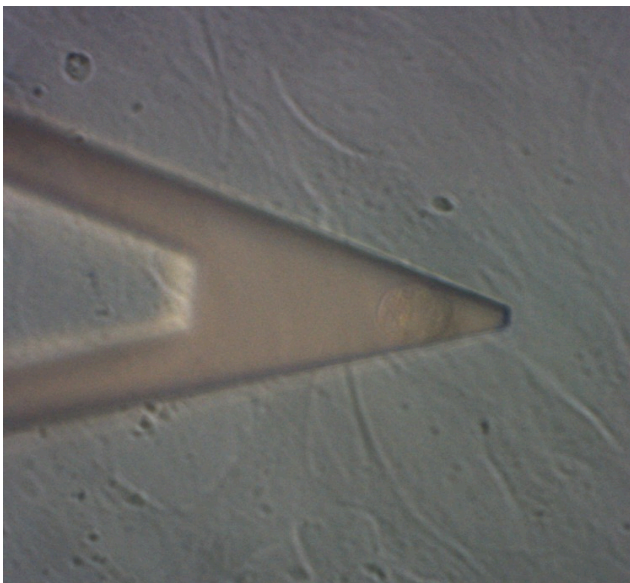
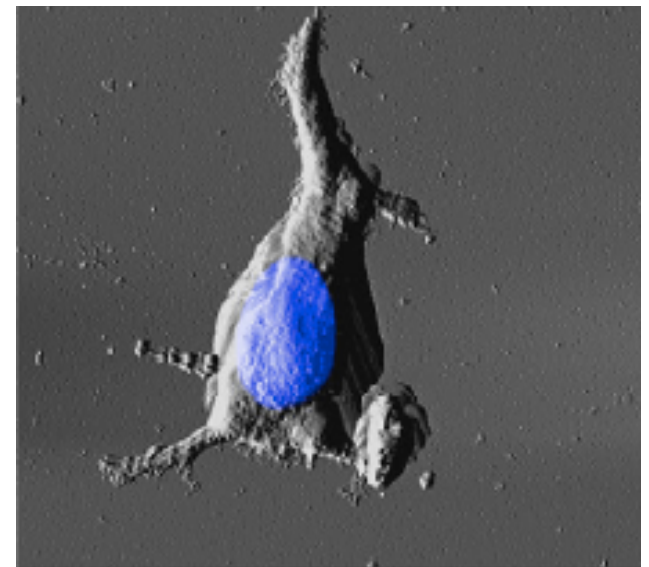


Kraftmessungen an lebenden Zellen

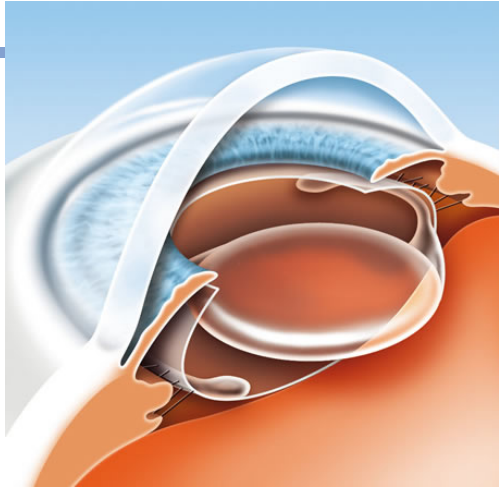
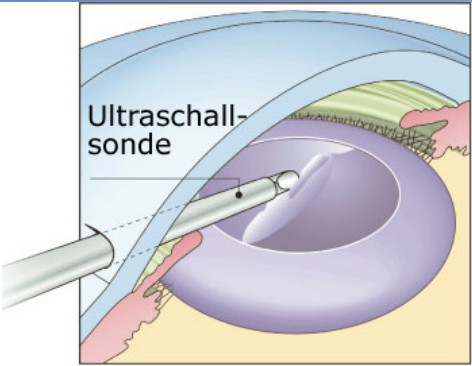
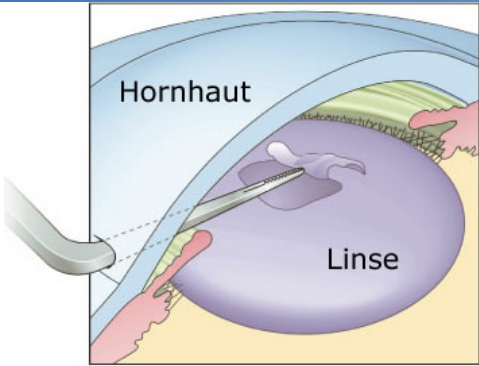


mit dem
Kraftmikroskop

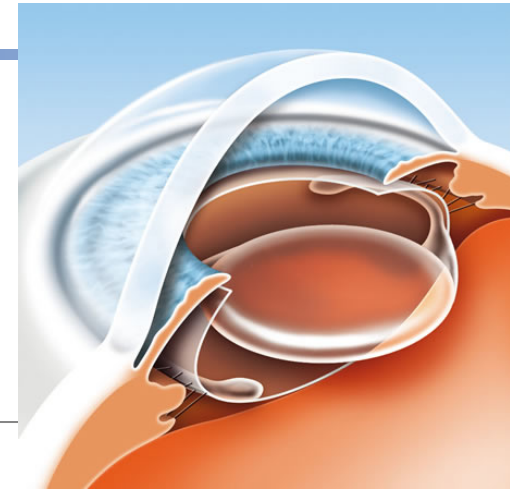
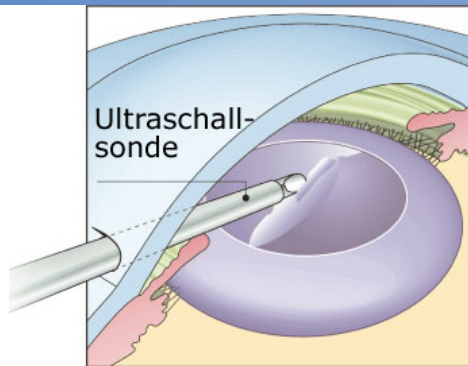
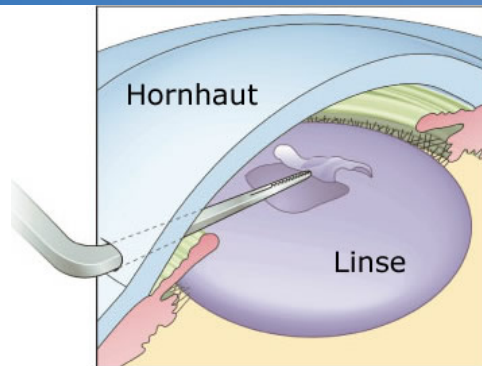
Martin Benoit,
Biophysik,
LS Gaub, LMU



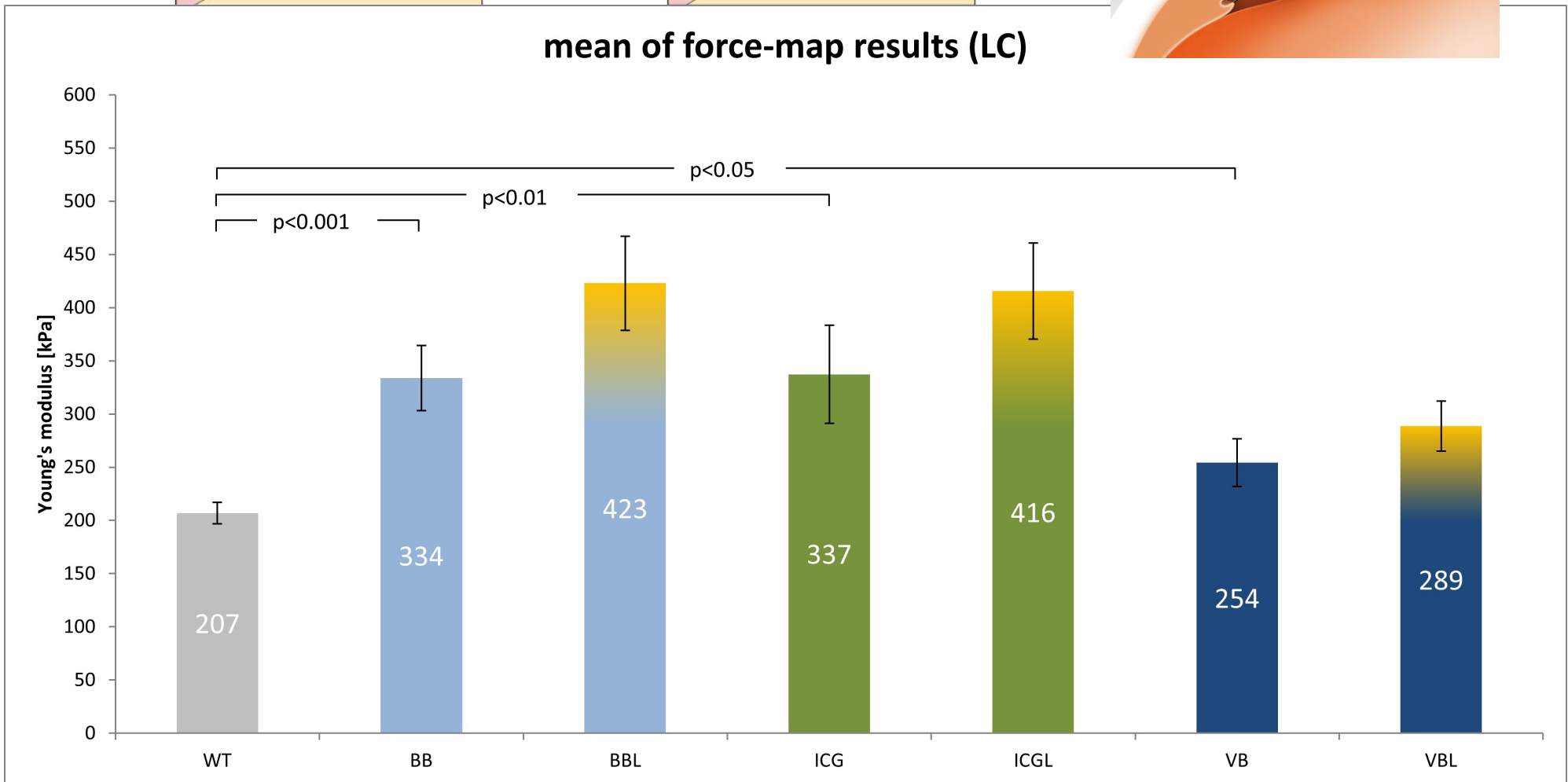
Messung der Steifigkeit der Linsenkapself



Messung der Steifigkeit der Linsenkapsel

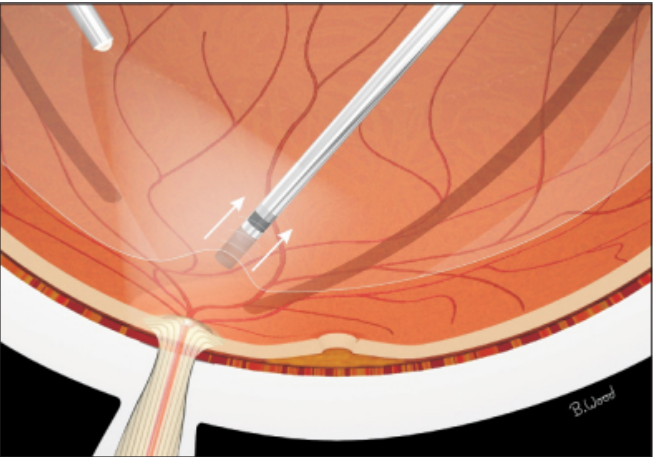
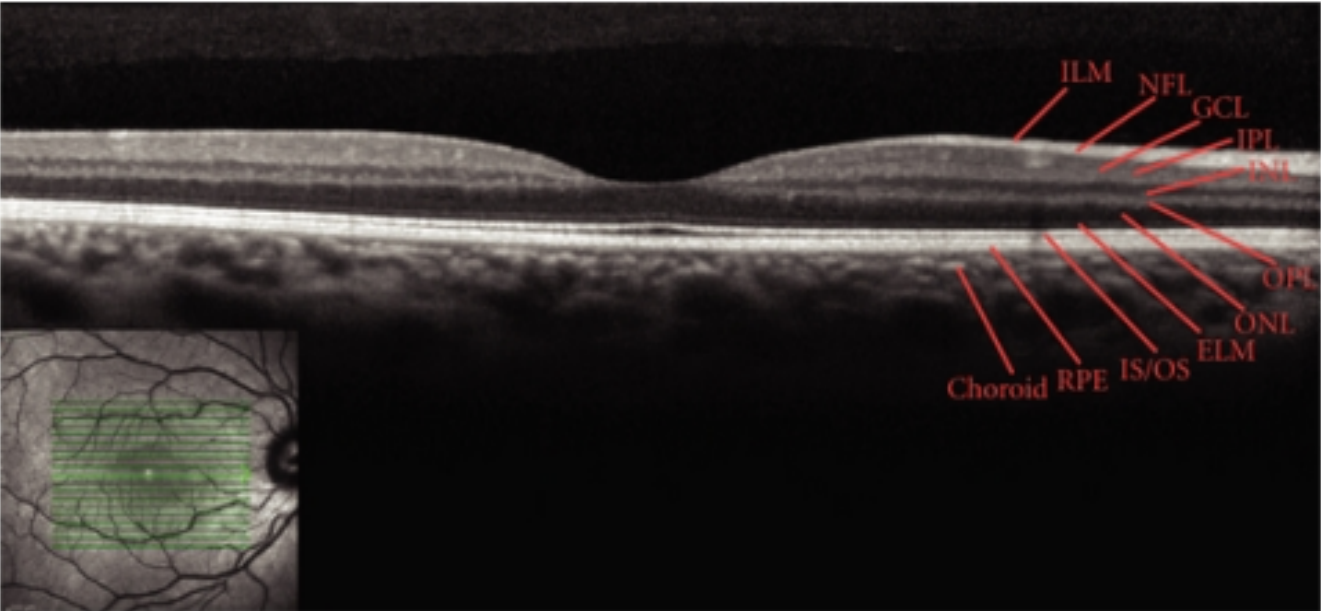
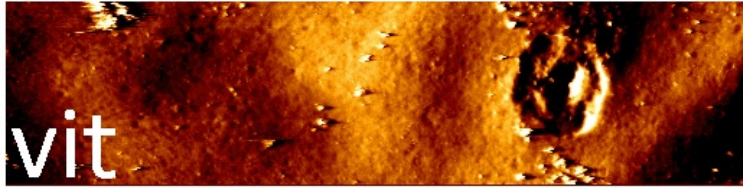
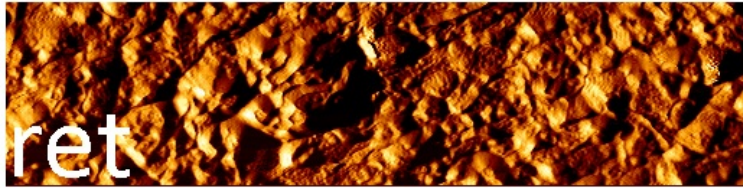


mean of force-map results (LC)



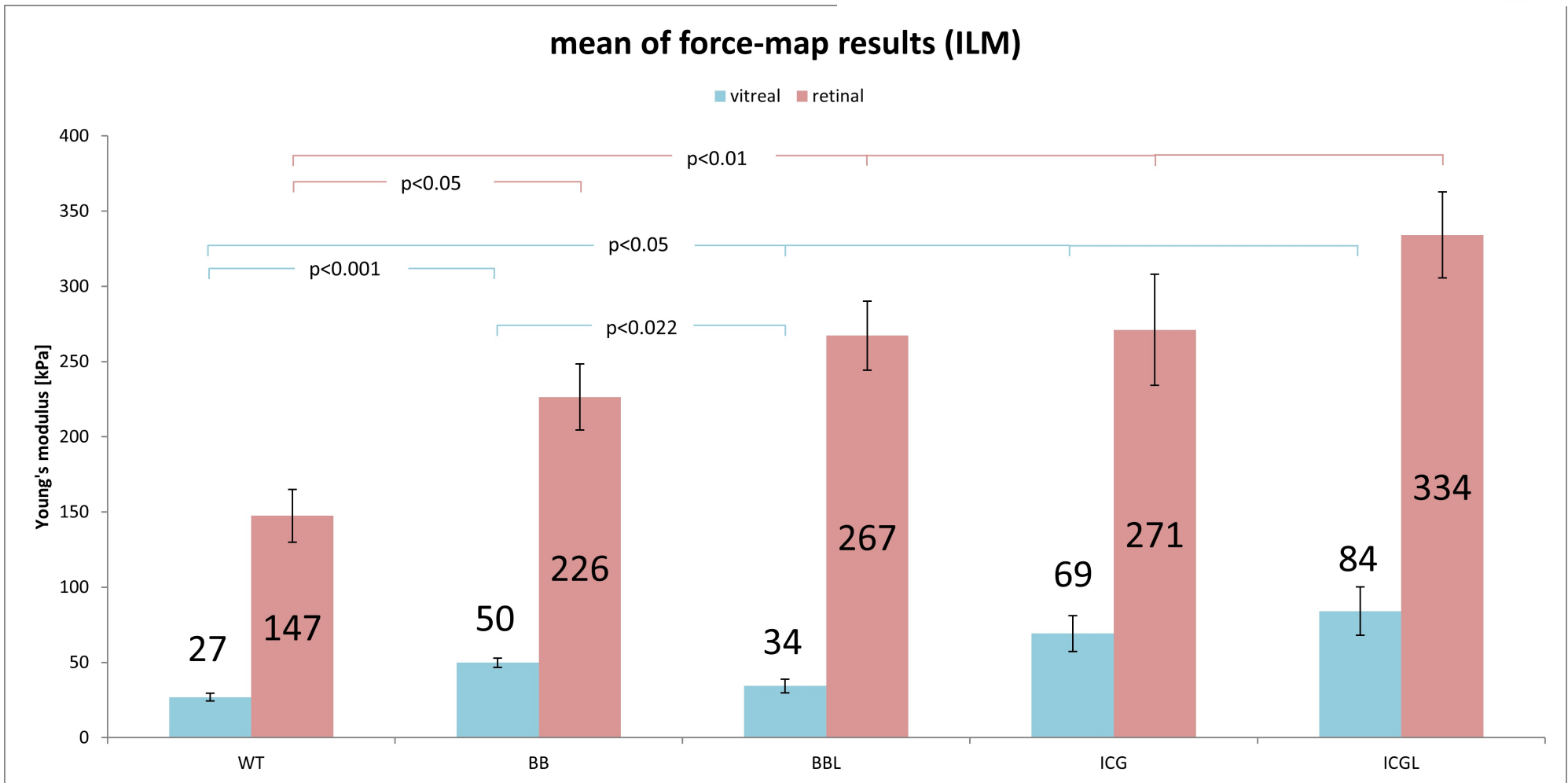
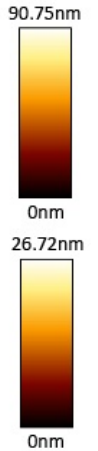
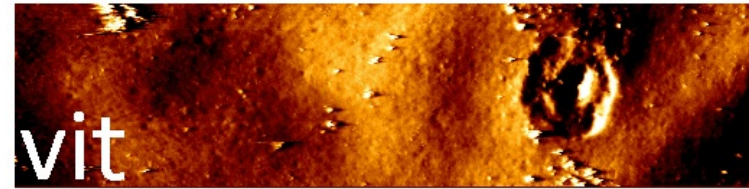
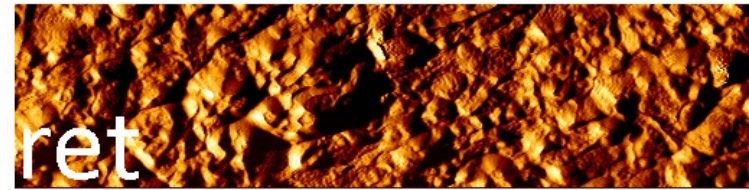
Messung der Steifigkeit der ILM

Stephan Mauell



Messung der Steifigkeit der ILM

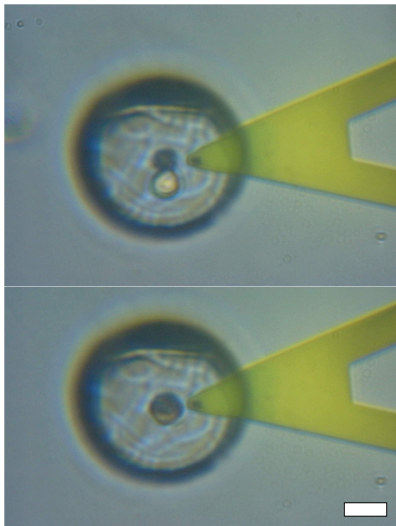
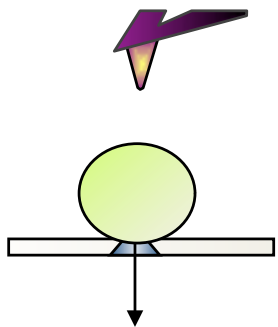
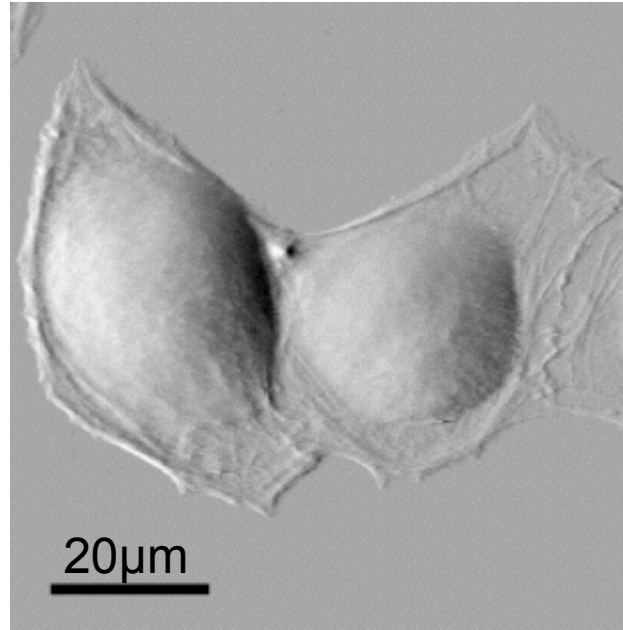
Masterarbeit von
Stephan Mauell



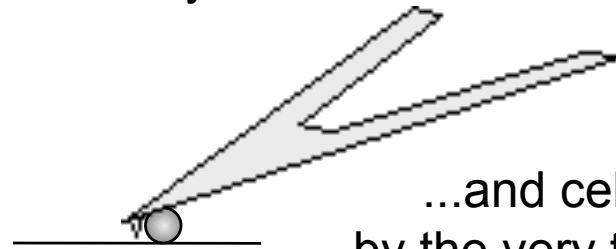
Abbilden von Zellen mit dem Kraftmikroskop

well adherent and spreading cells:

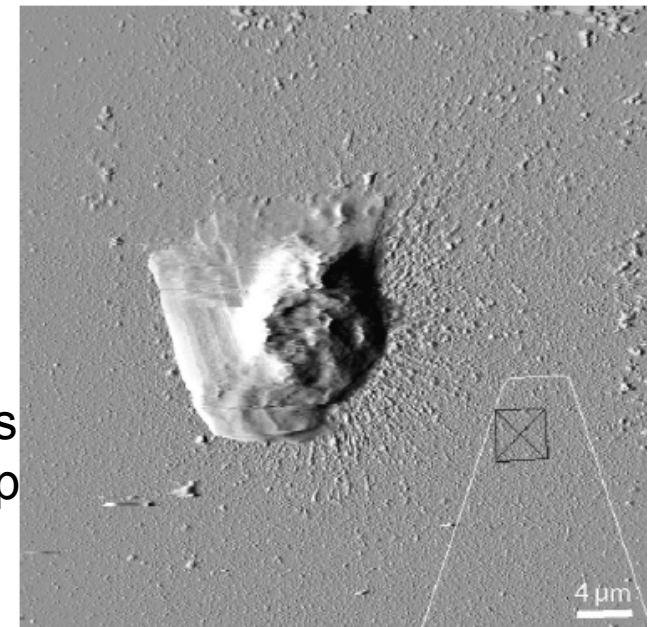
non adherent cells:



imaging force sensors...
...by a cell...



...and cells
by the very tip

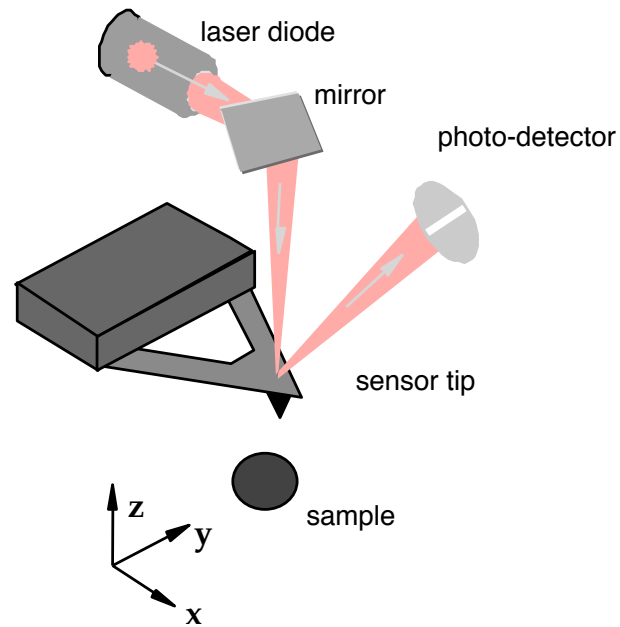


nanjion

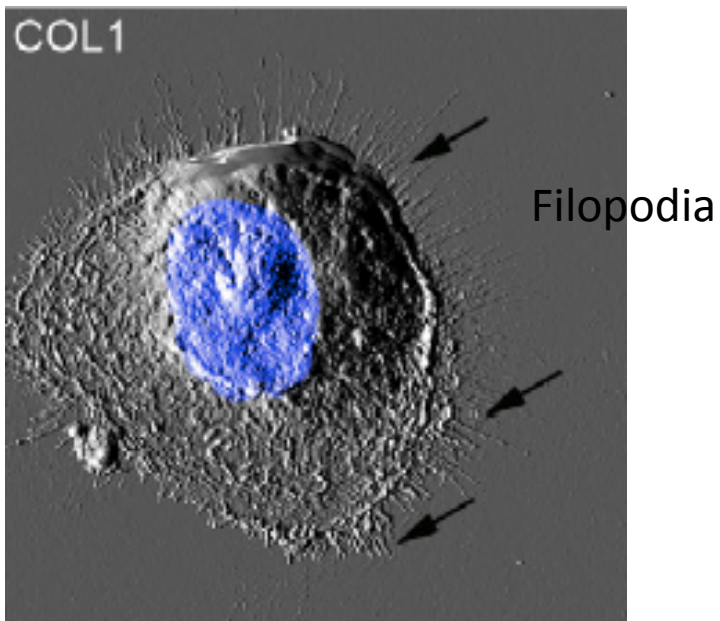
Nanon Technologies GmbH

Adhäsionskraftmessungen an lebenden Zellen

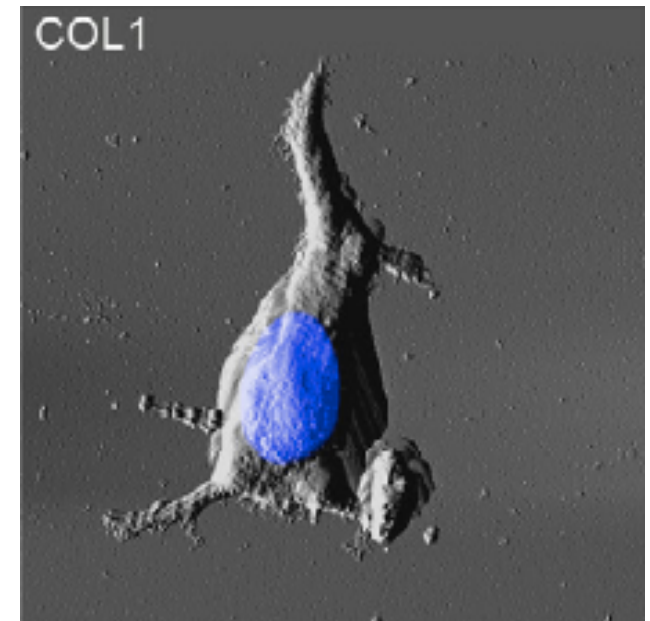
Doktorarbeit von
Ediz Sarıısık



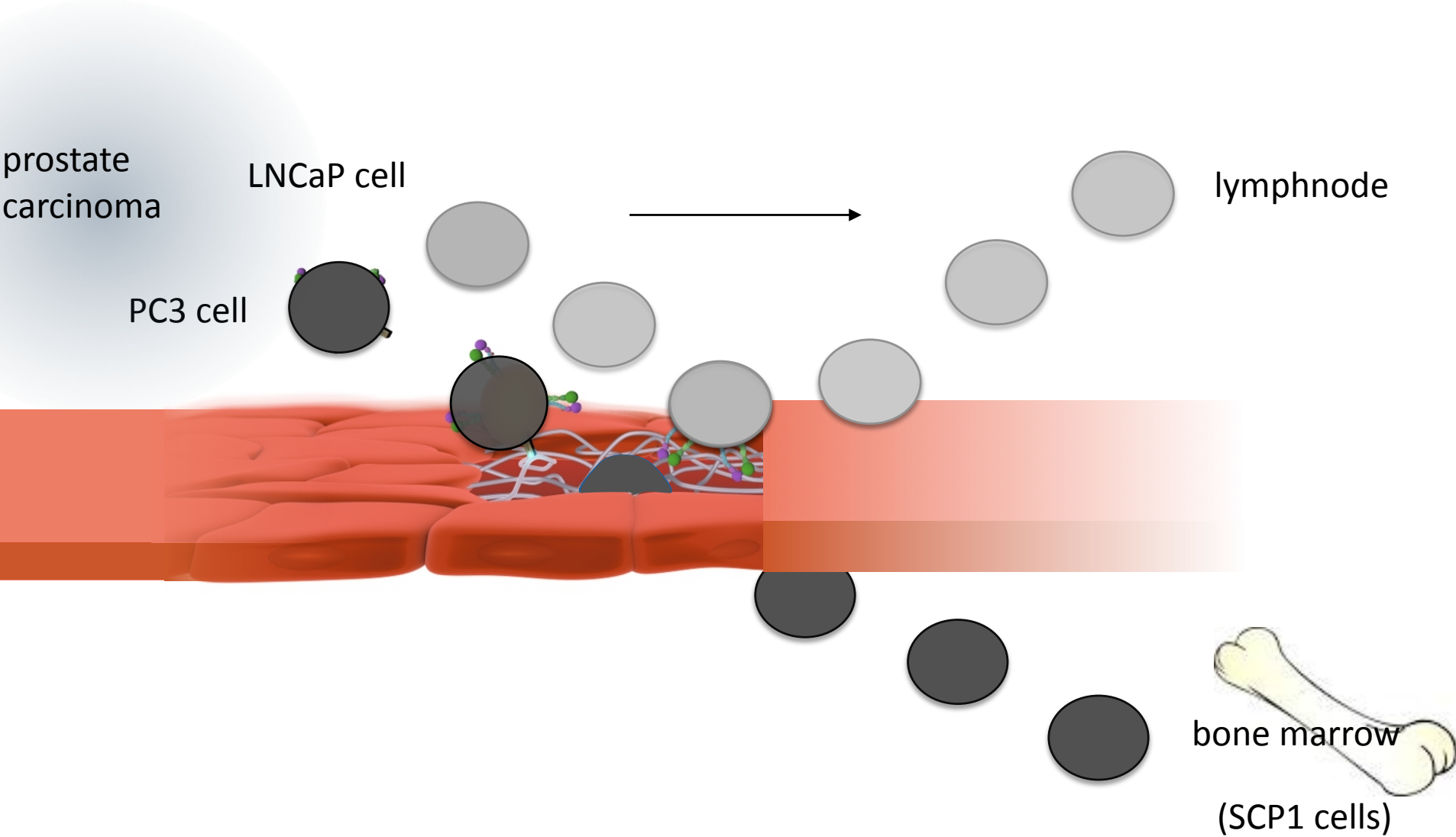
prostate cancer
PC3 cell



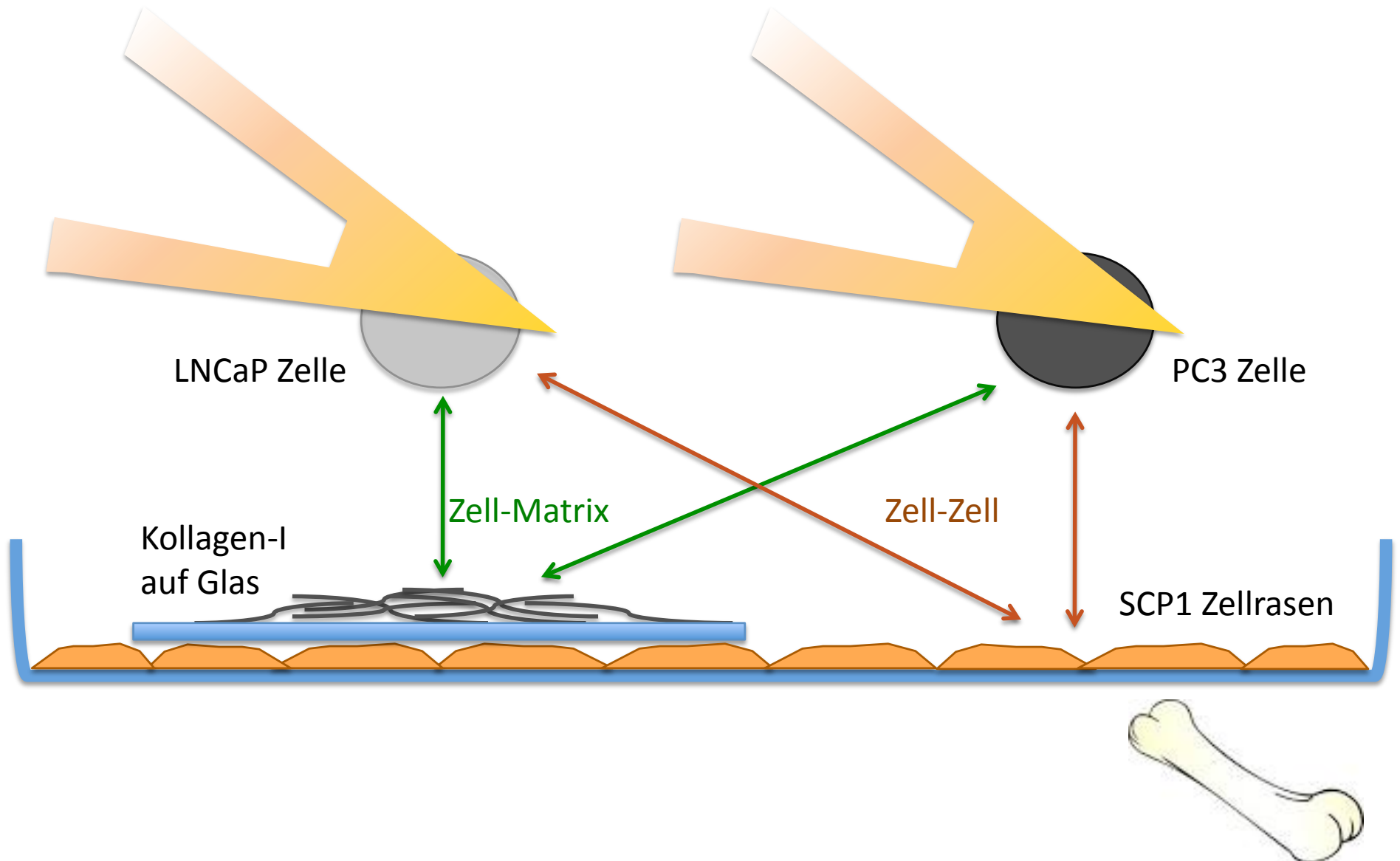
prostate adenocarcinoma
LNCaP cell



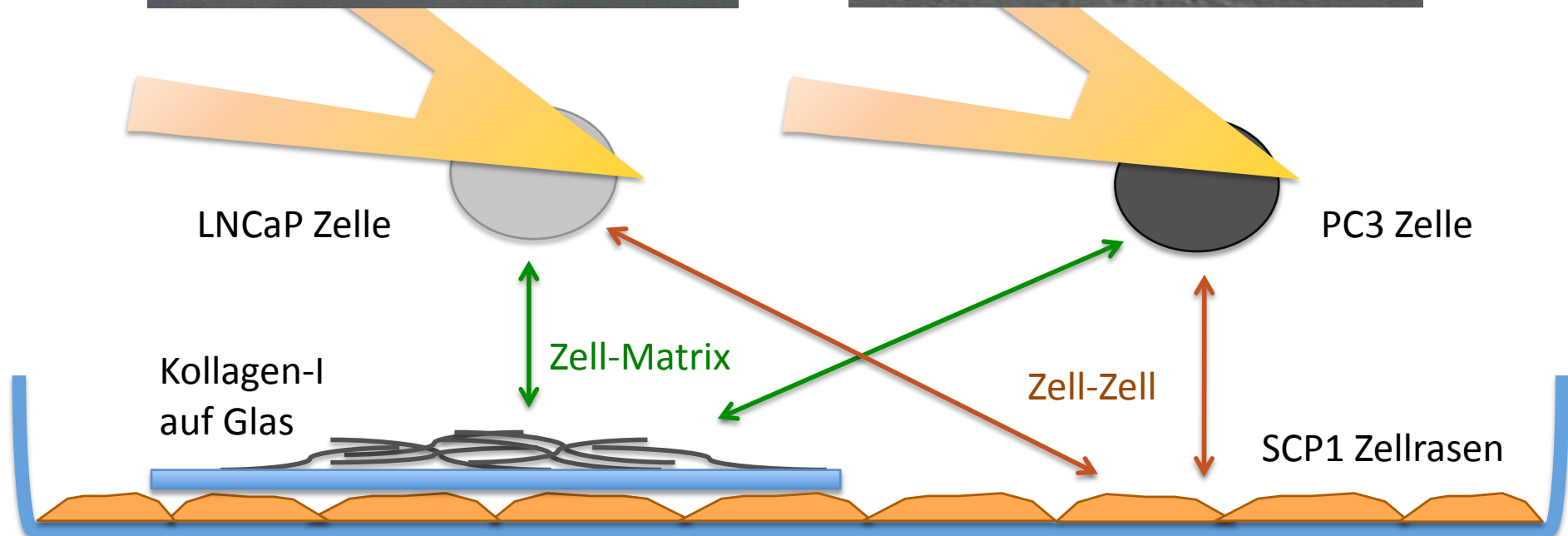
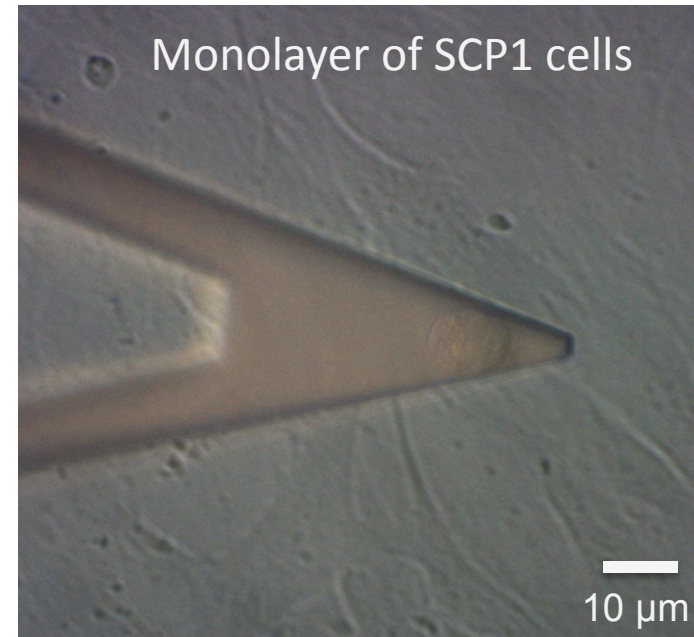
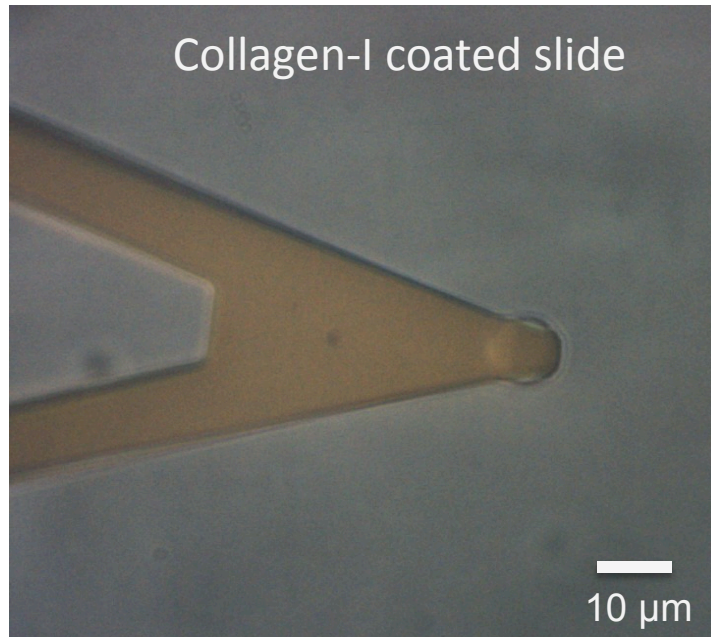
Knochenmarksmetastasen und Zelladhäsion



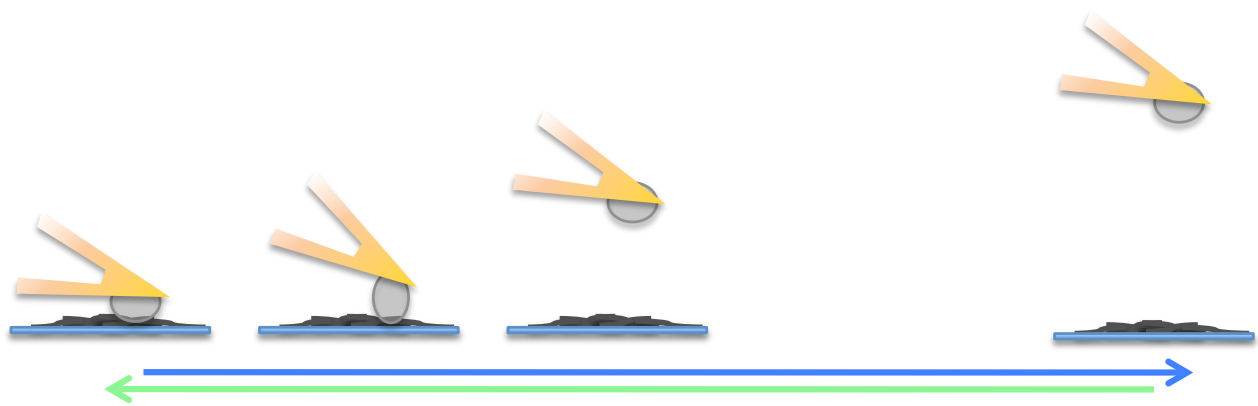
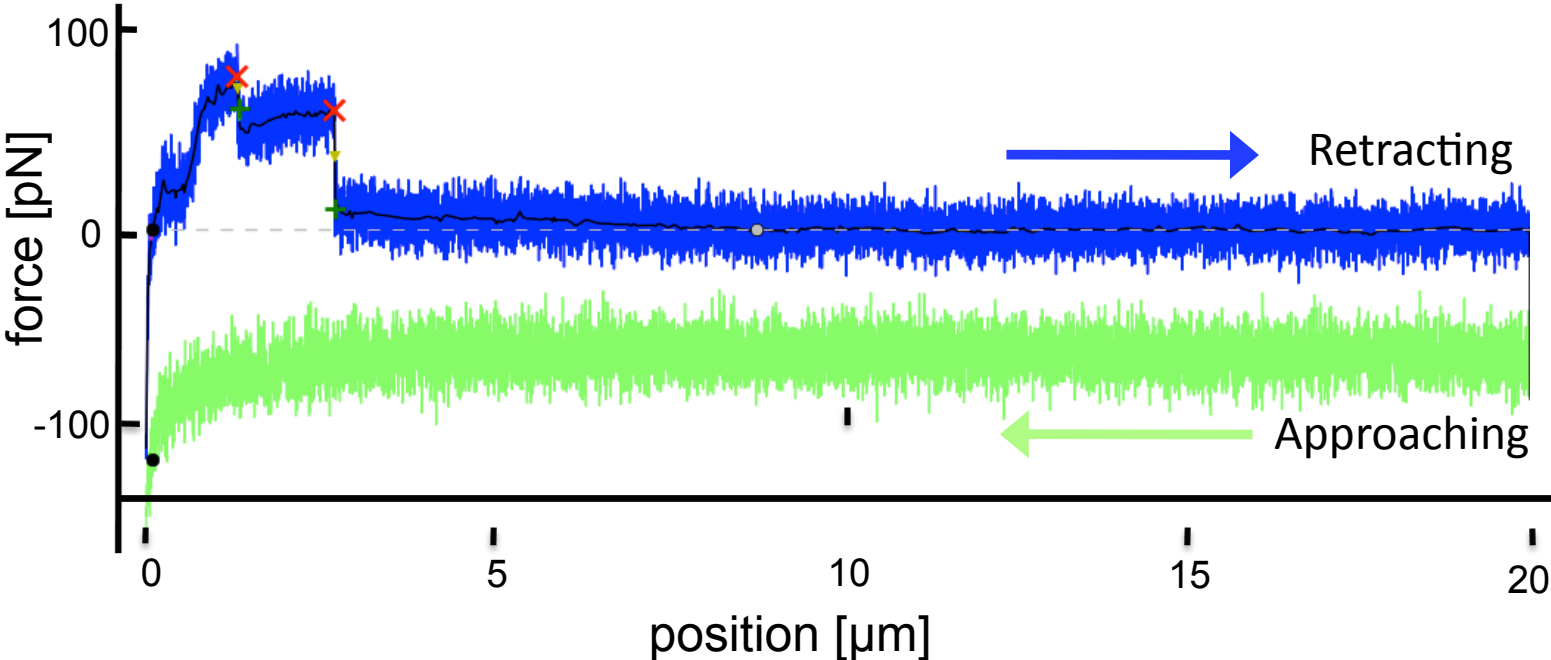
Aufbau des Experiments



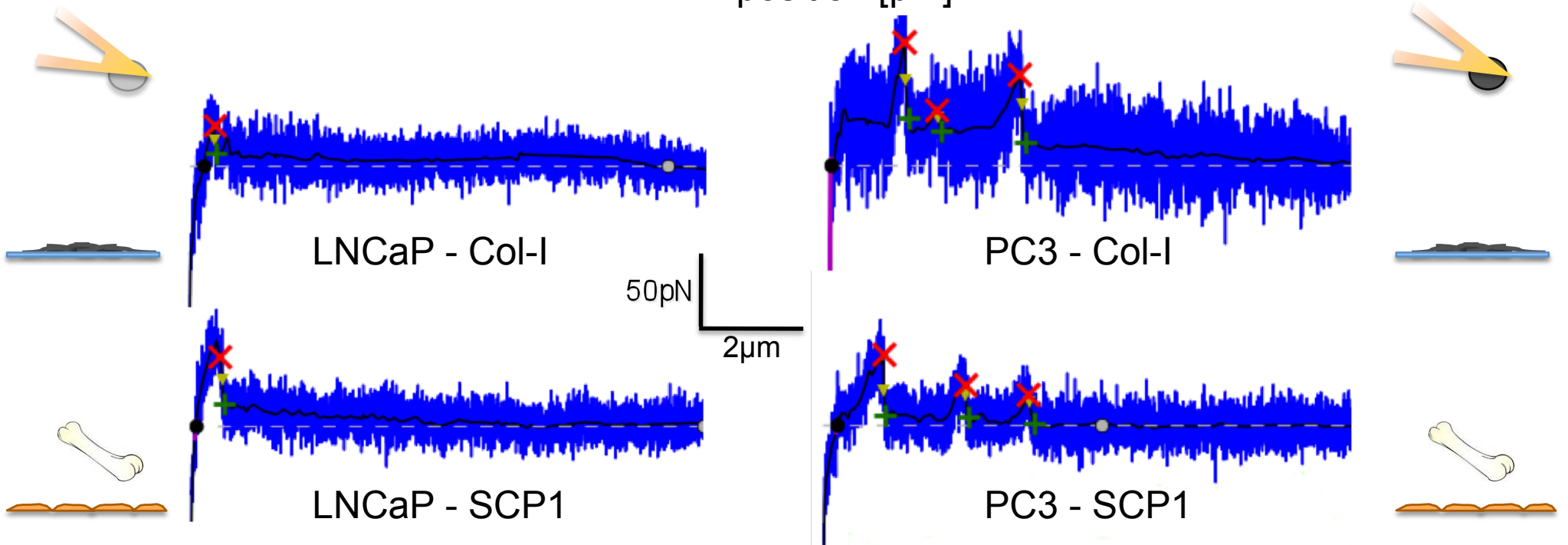
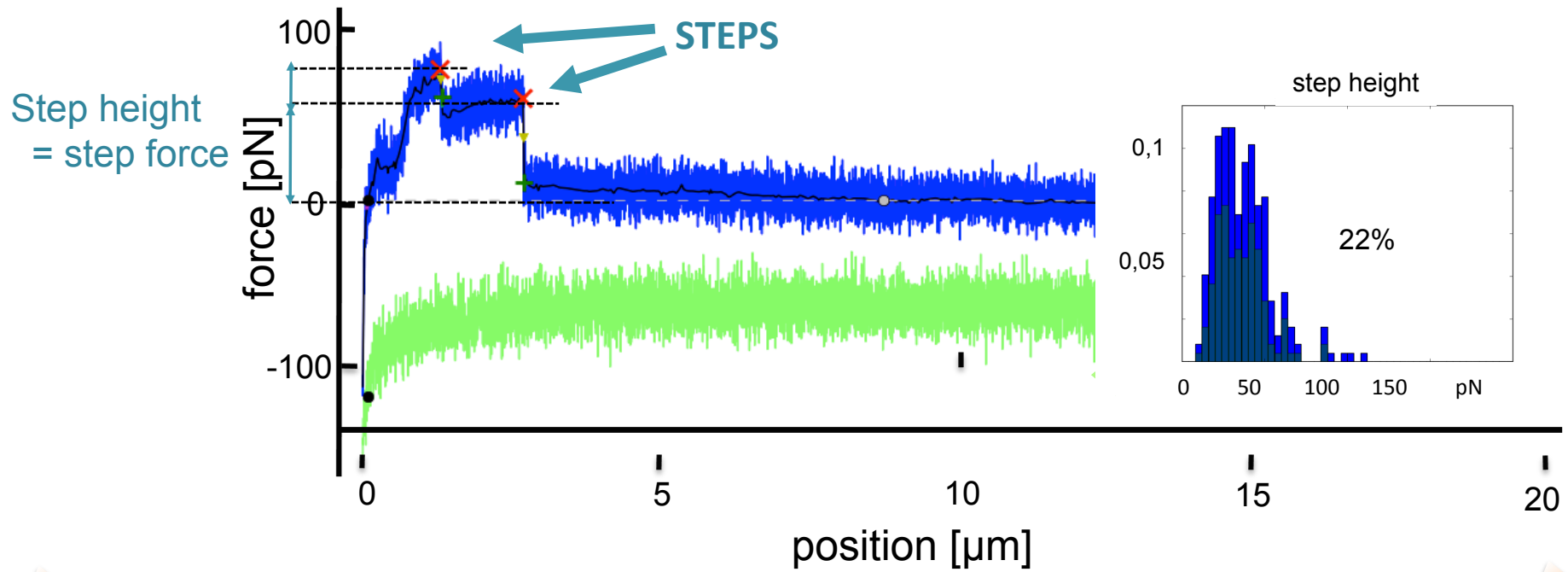
Aufbau des Experiments



Eine typische Kraftkurve



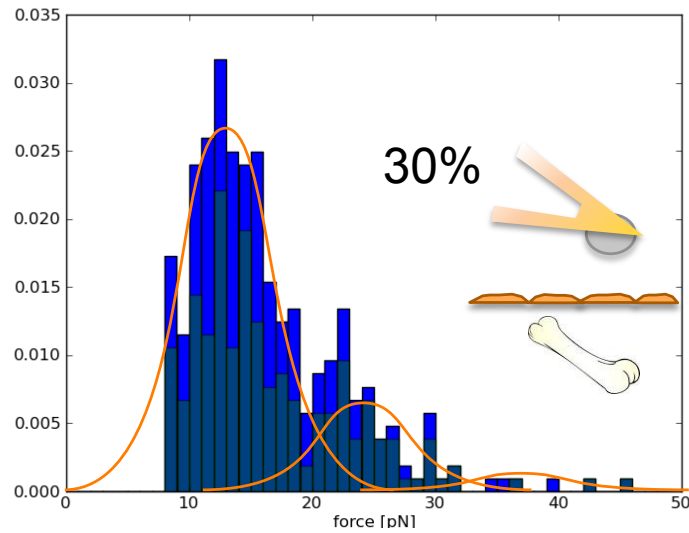
Typische Kraftkurven



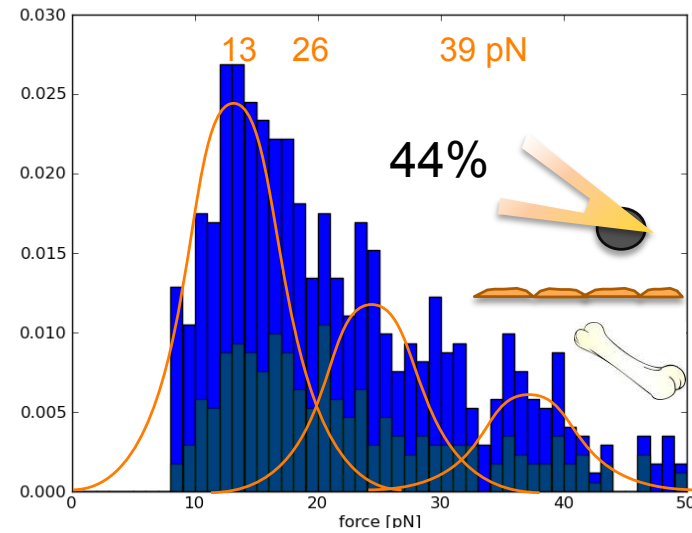
Auswertung der Kraftkurven

Adhesion rates and force histograms

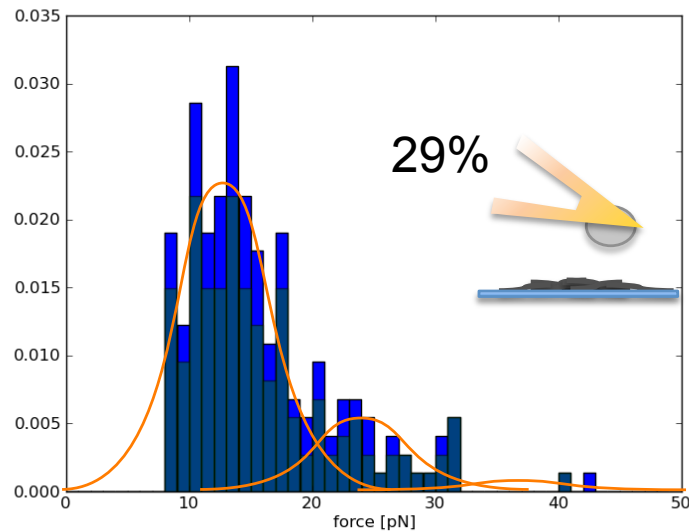
LNCaP - SCP1



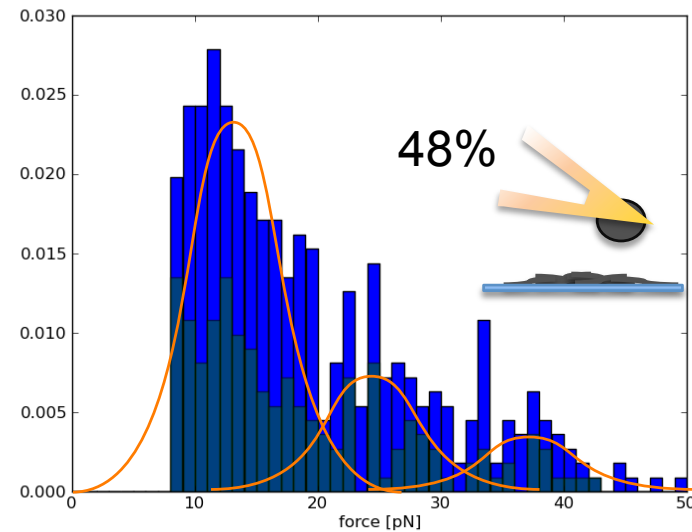
PC3 - SCP1



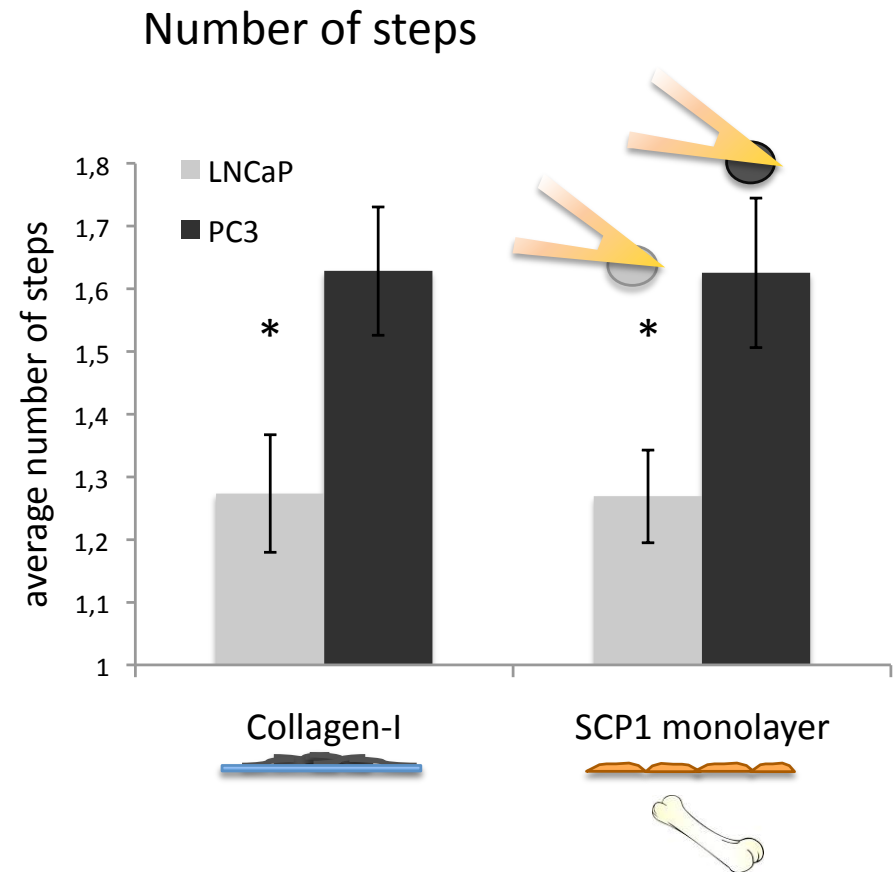
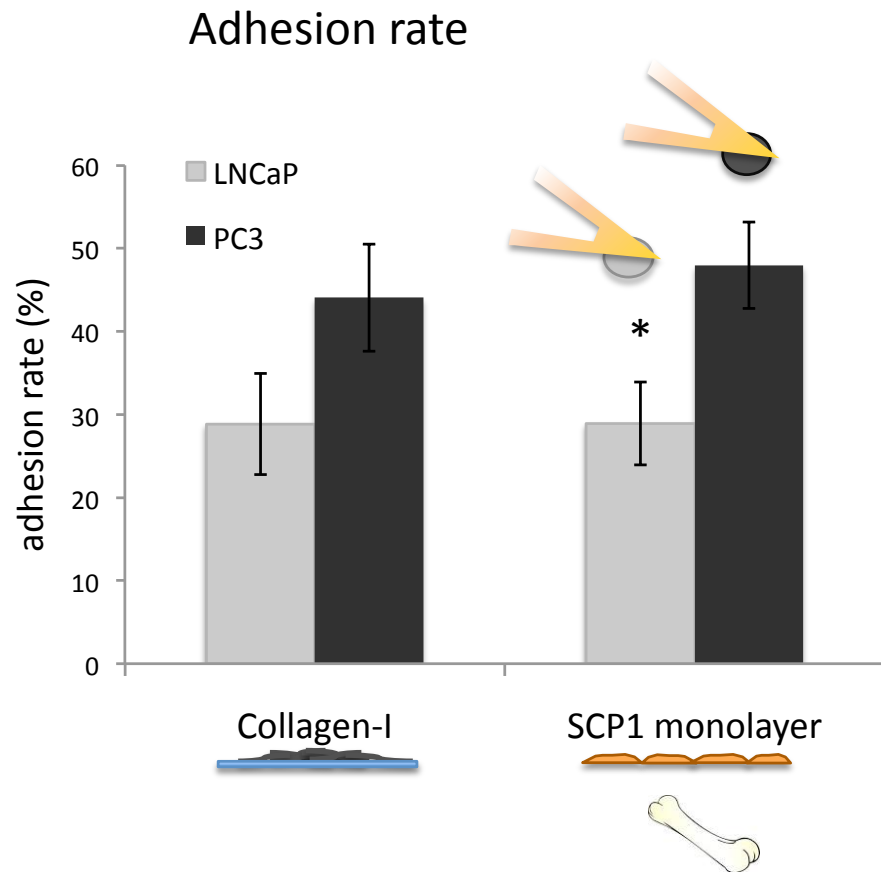
LNCaP - Col-I



PC3 - Col-I



Auswertung der Kraftkurven

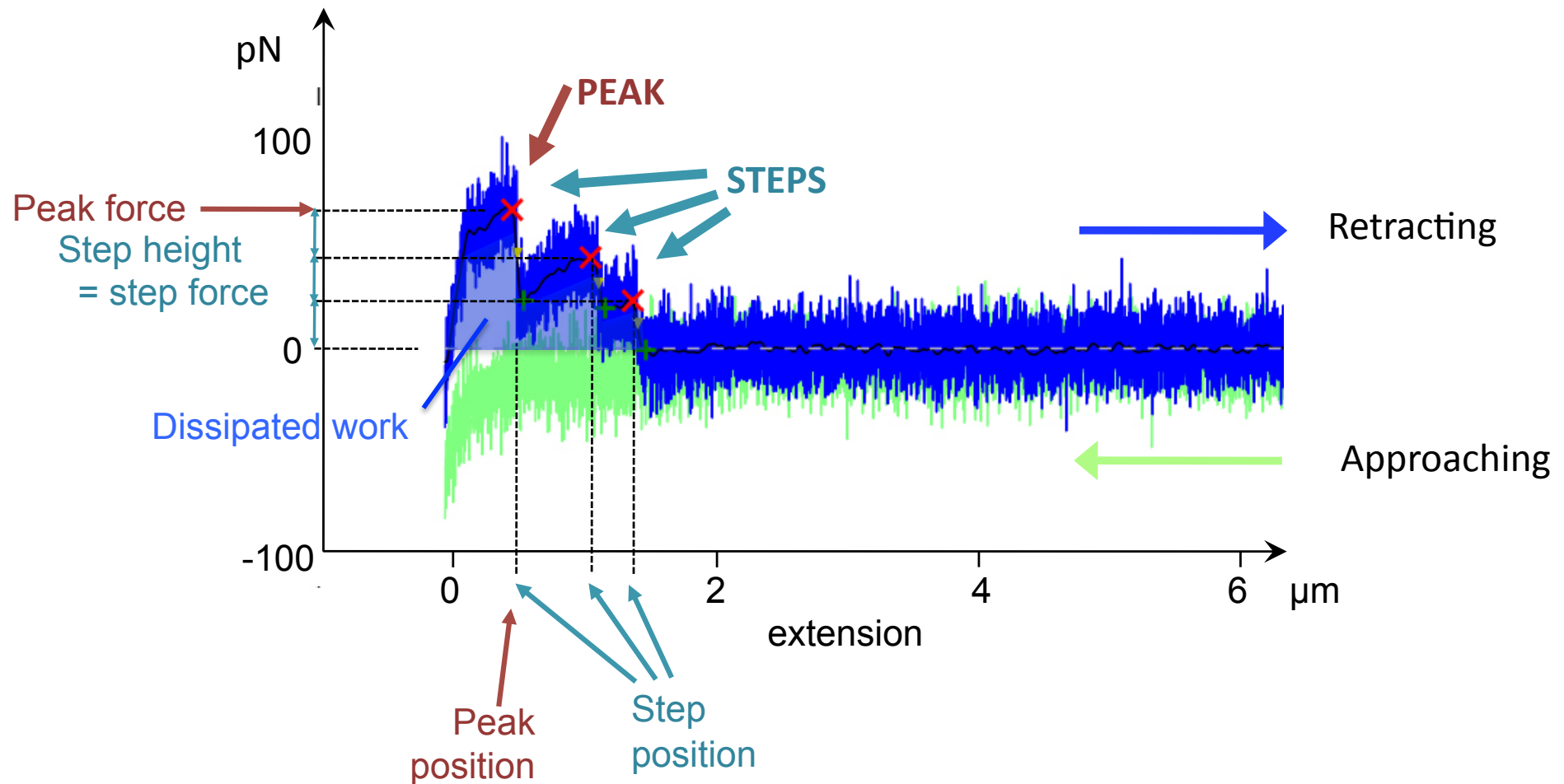


Auswertung der Kraftkurven

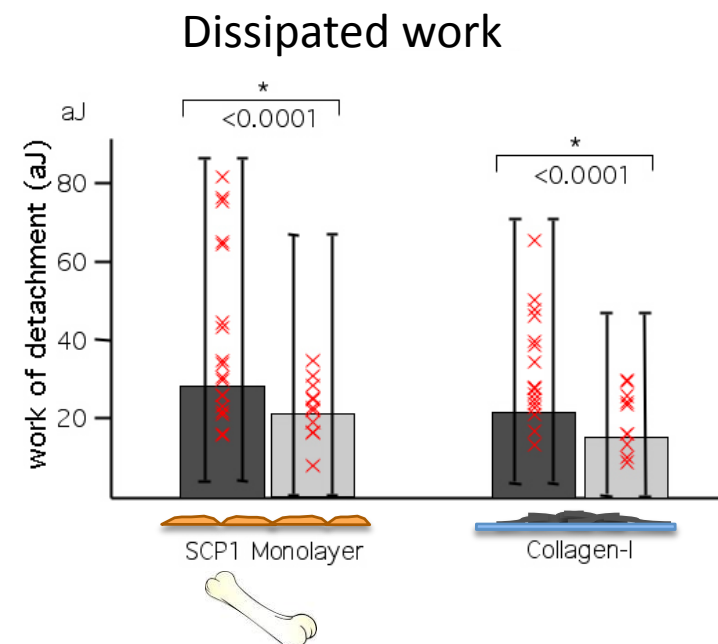
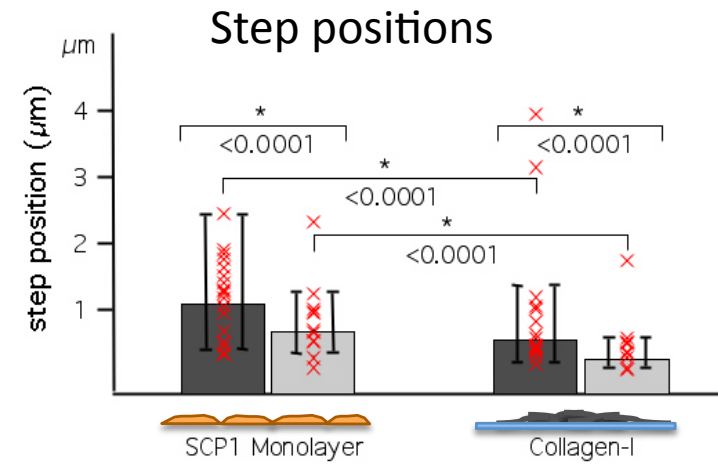
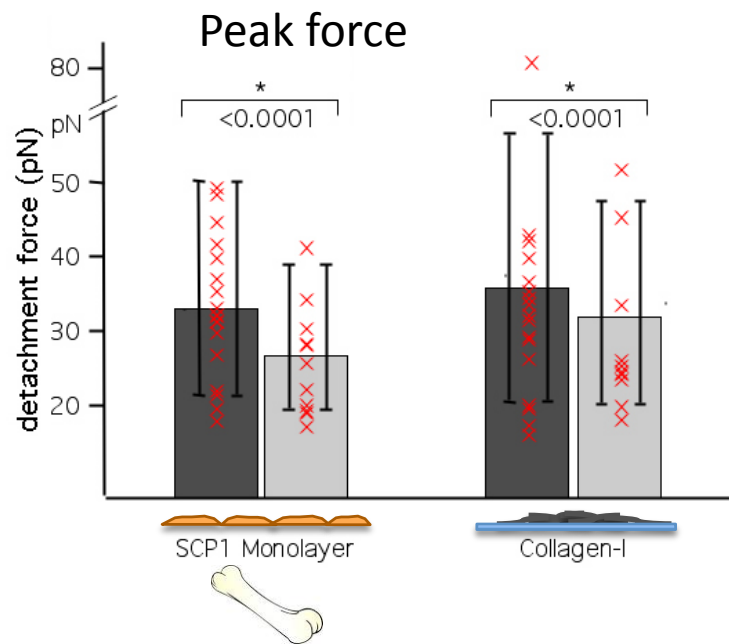
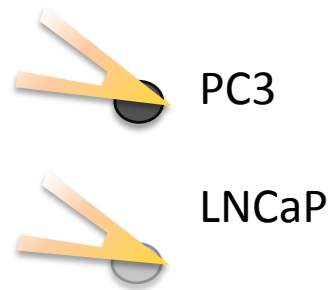
Peak force

Step/peak positions

Dissipated work



Auswertung der Kraftkurven



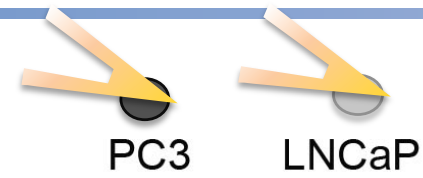
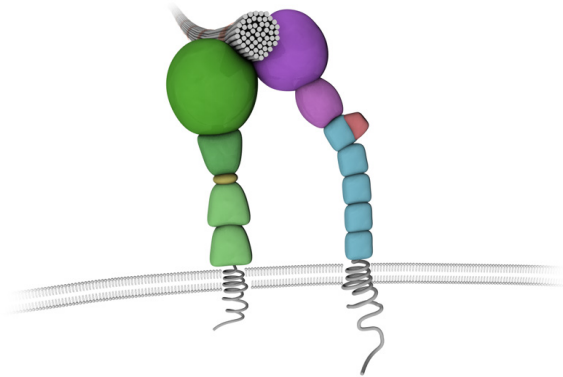
Zusammenfassung der Kraftmessungen



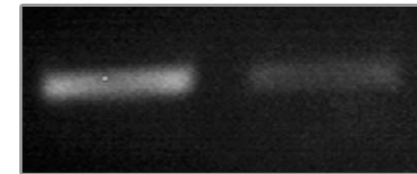
Messgröße	PC3	LNCaP
Adhäsionsrate	+	-
Anzahl der Kraftstufen	+	-
Maximalkraft	+	-
Höhe der Kraftstufen	±	±
Stufenposition	+	-
dissipierte Arbeit	+	-

Beide Zelltypen verhalten sich auf Kollagen genauso wie auf Knochenmark

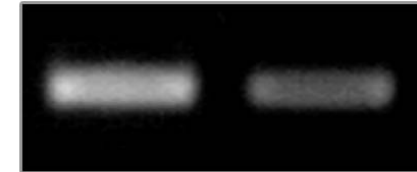
Die Integrin Familie der Adhäsionsmoleküle...



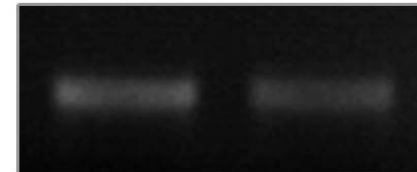
$\alpha 1$
(241 bp)



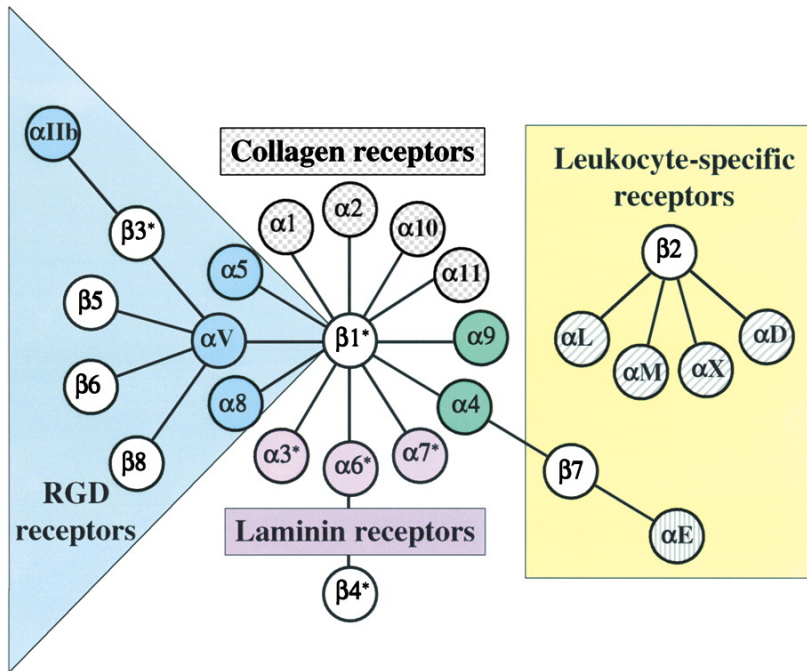
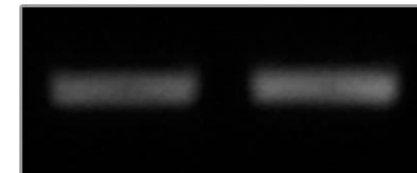
$\alpha 2$
(217 bp)



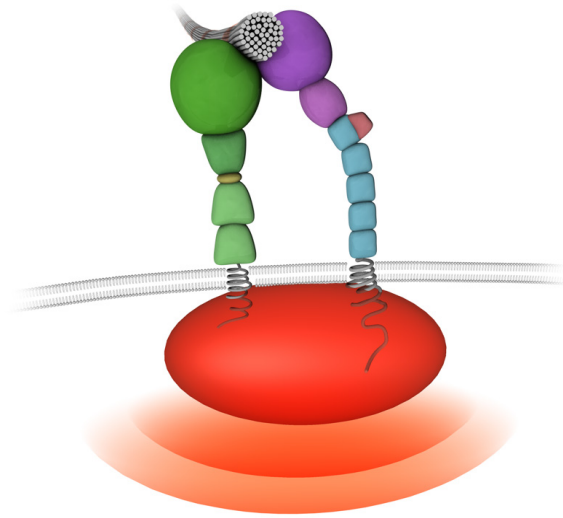
$\beta 1$
(322 bp)



GAPDH
(181 bp)

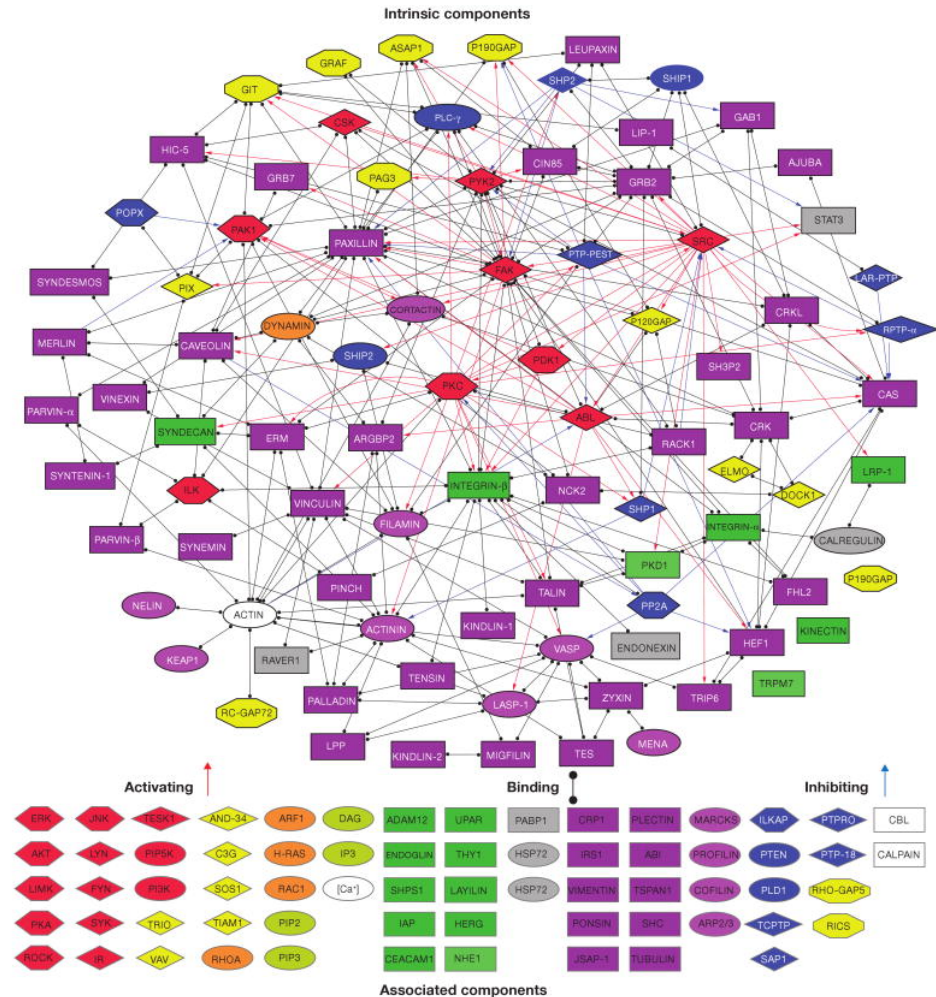


Integrine vermitteln Informationen in die und aus der Zelle (signaling)



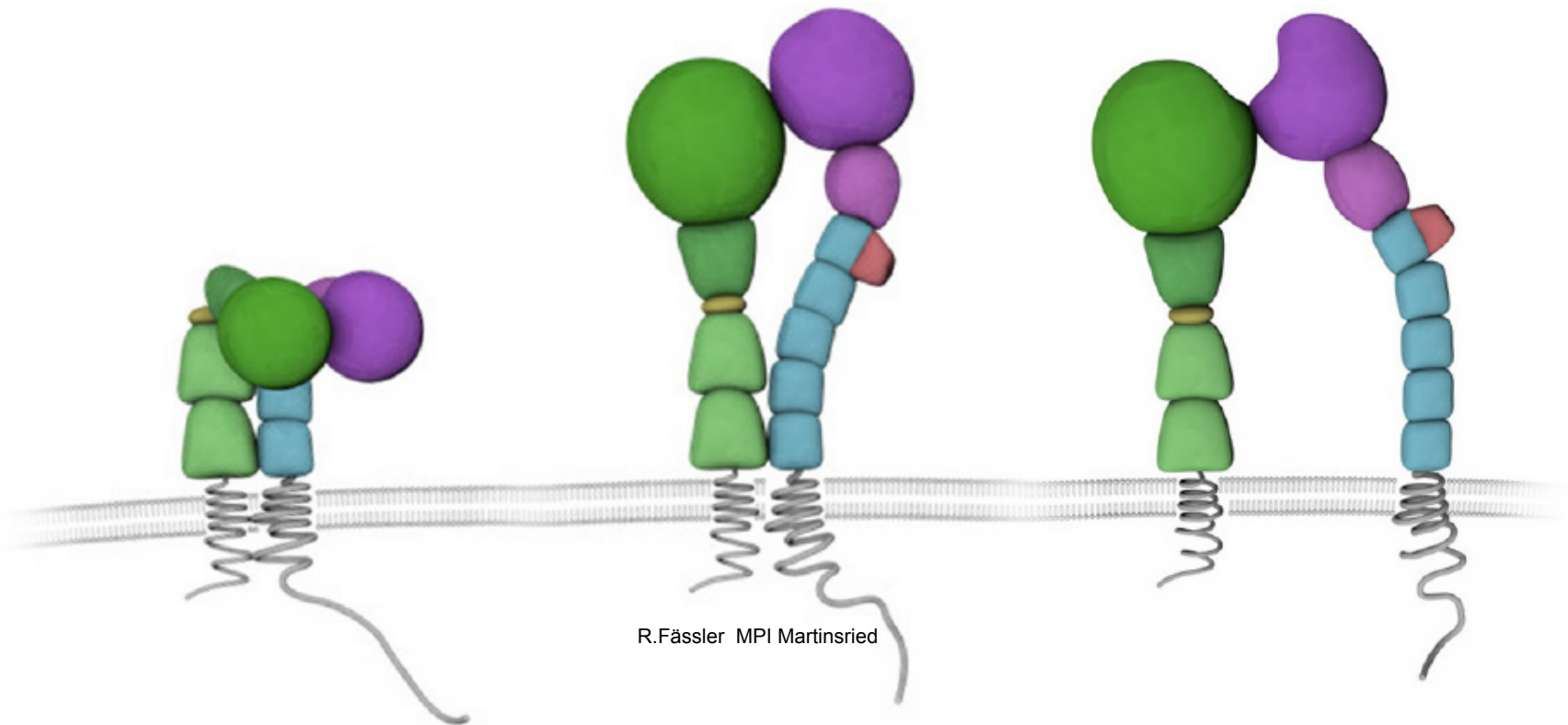
Zellwachstum
Zellwanderung
Zellpolarisation

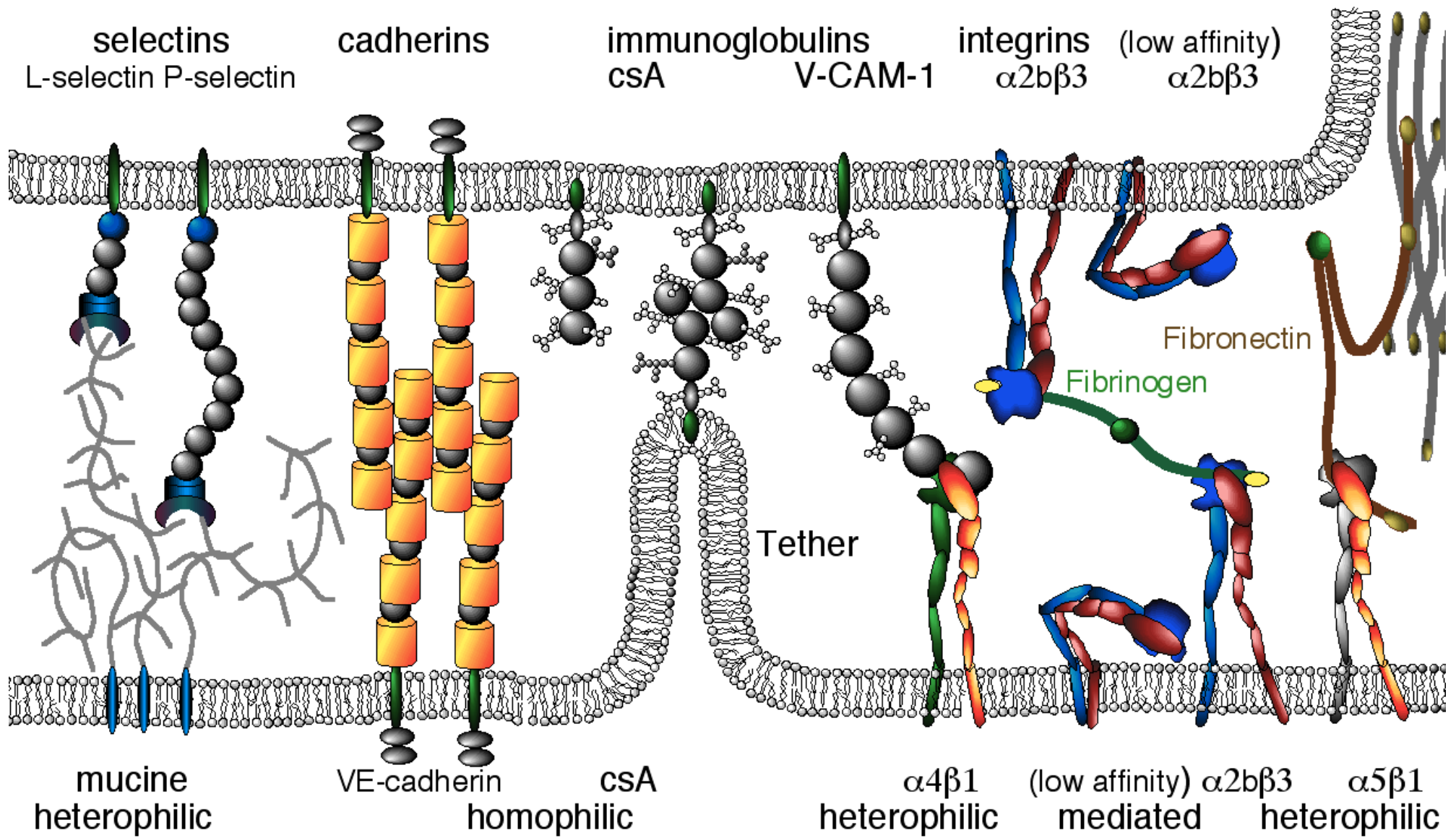
Zelldifferenzierung
Zellteilung
überlebenswichtig



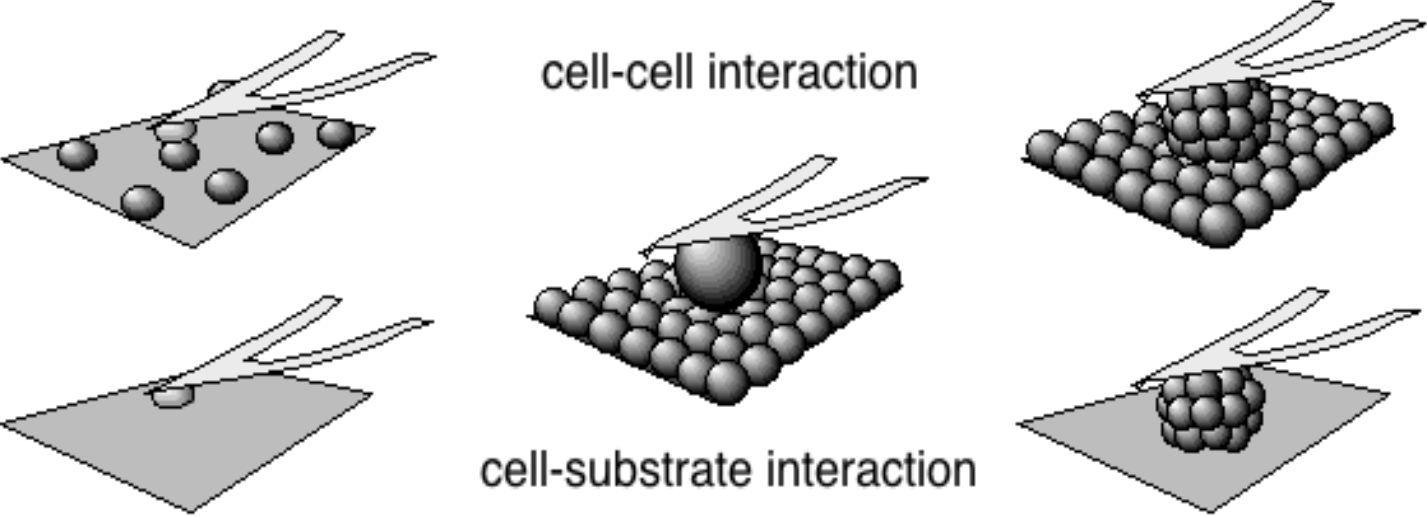
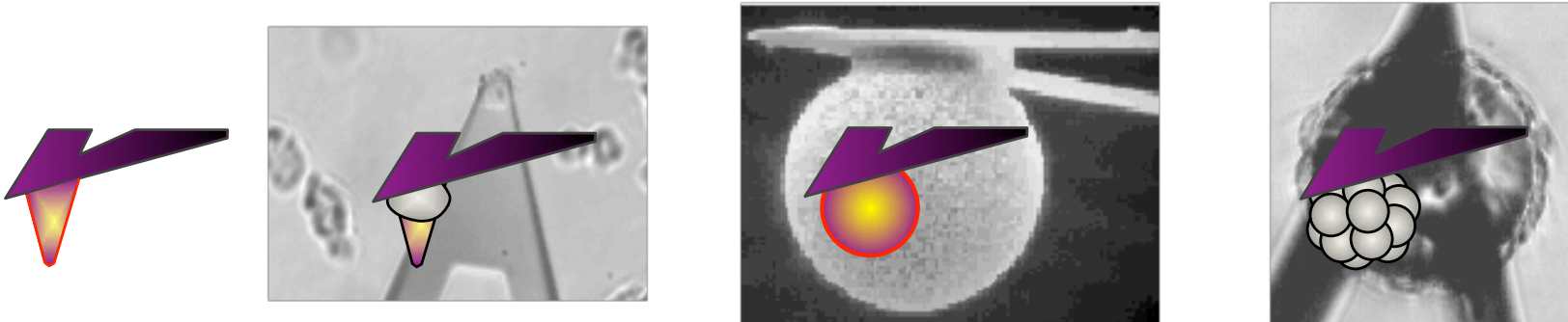
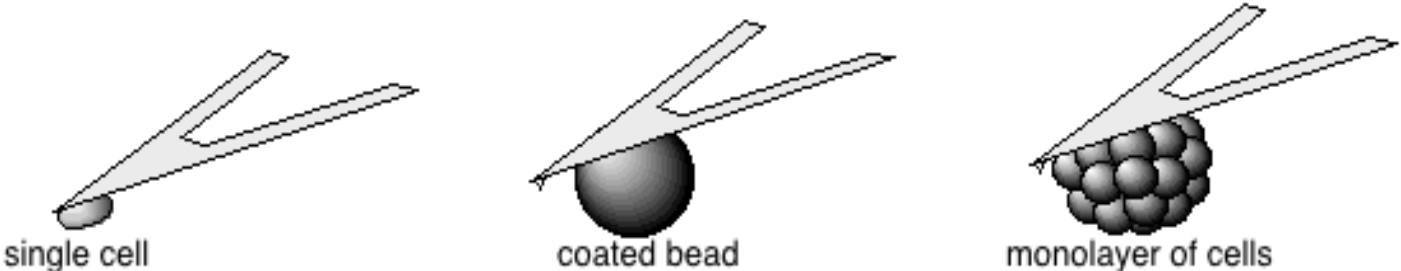
Die Integrin Familie der Adhäsionsmoleküle...

Masterarbeit von
Julian Hartmann : “Single molecule force measurements of integrin $\alpha_v\beta_3$ and $\alpha_5\beta_1$ interactions with fibronectin“



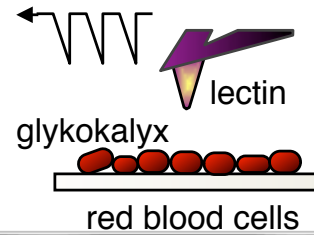


Kraftsensormodifikationen für Zelladhäsionsmessungen

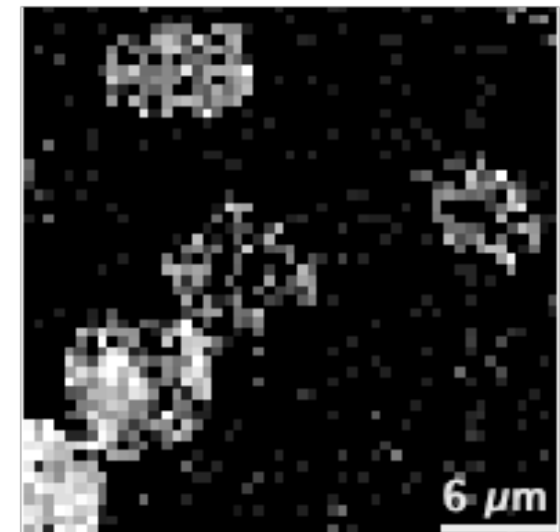
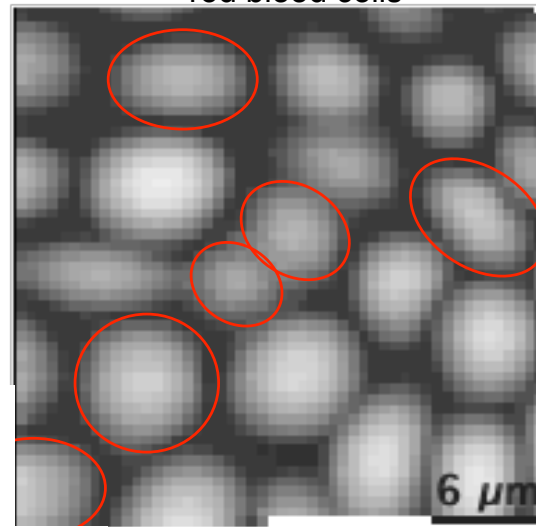
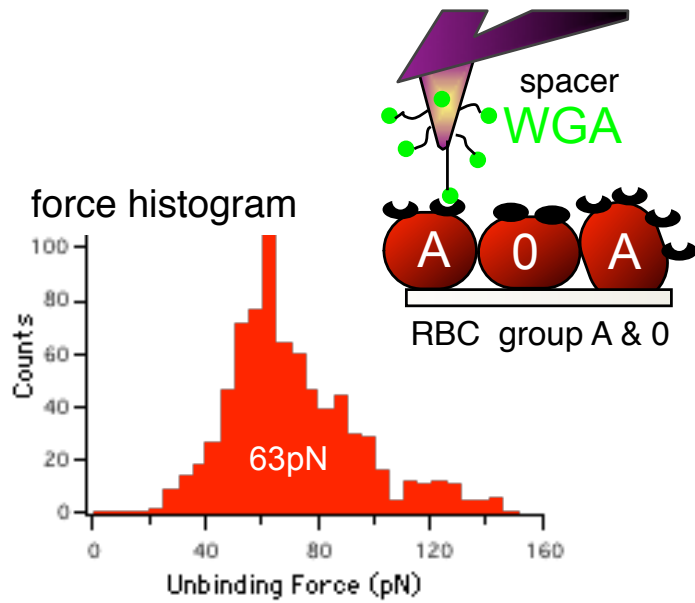


Einzelmolekülkraftmessungen an Blutzellen

Doktorarbeit von
Jochen Müller

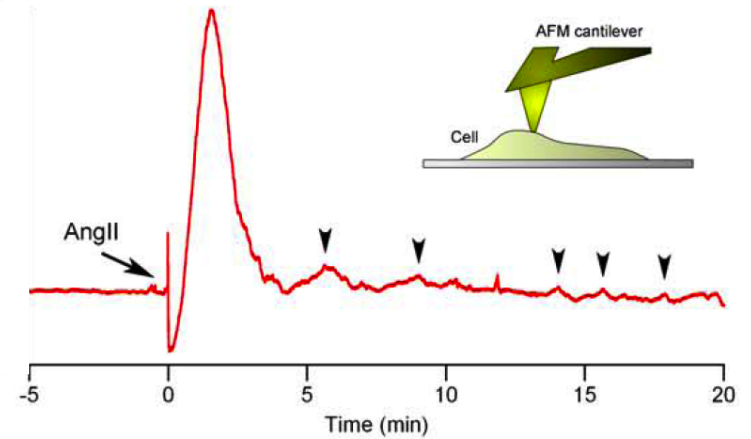


“cell sorting” by adhesion force

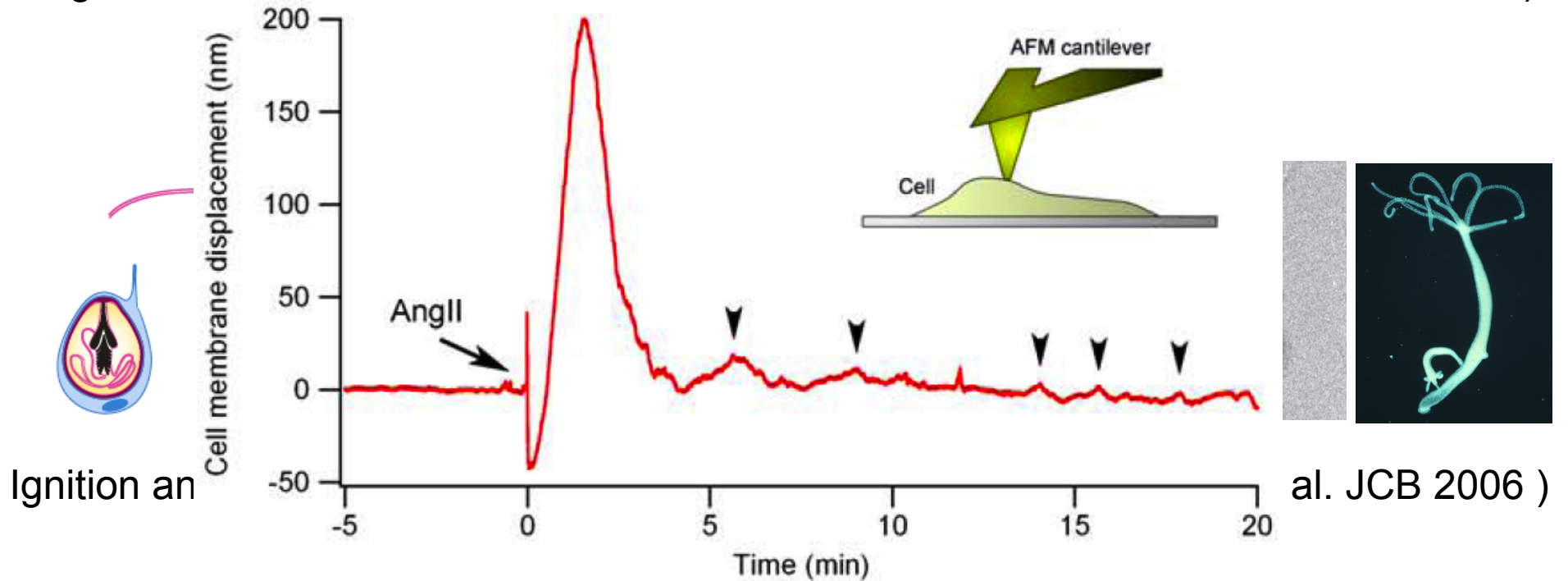


Methods (tin modifications):

AFM Messungen zur Zellmechanik



Angiotensin II **signaling** to the actin-myosin meshwork (Cuerrier et al. EJP 2008)



Nesselkapseln: Nematocysten

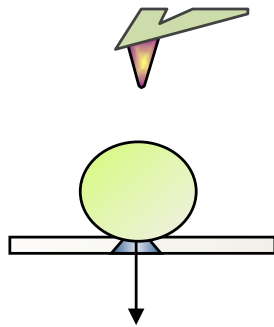
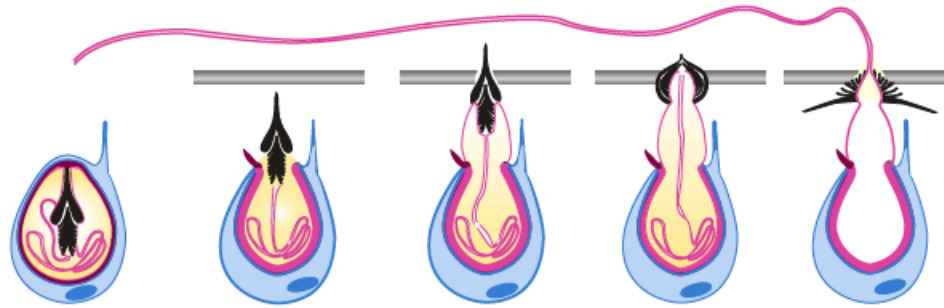


Frischwasserpoly: Hydra

AFM Messungen zur elektrisch stimulierten Zellmechanik

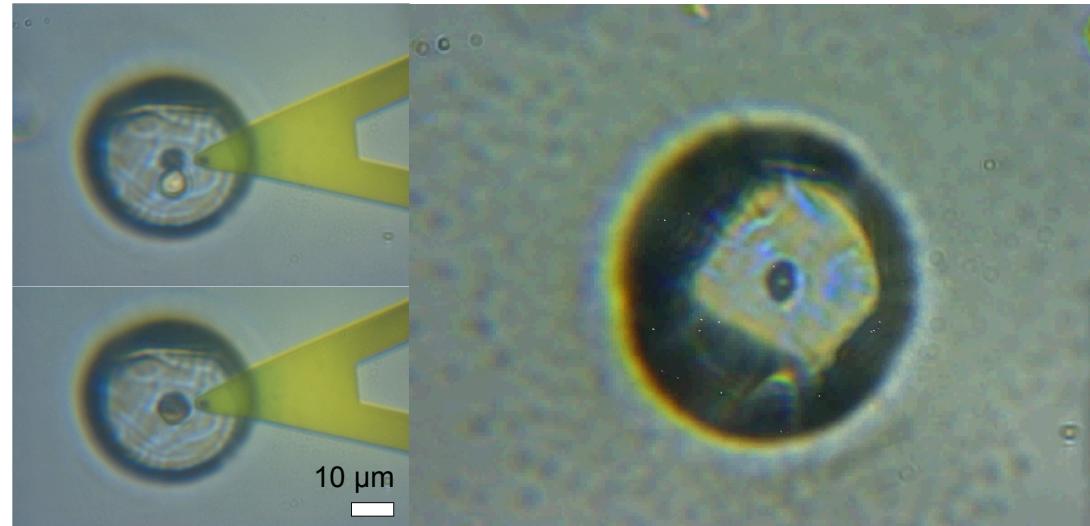
Nesselkapseln:
Nematocysten

150bar!



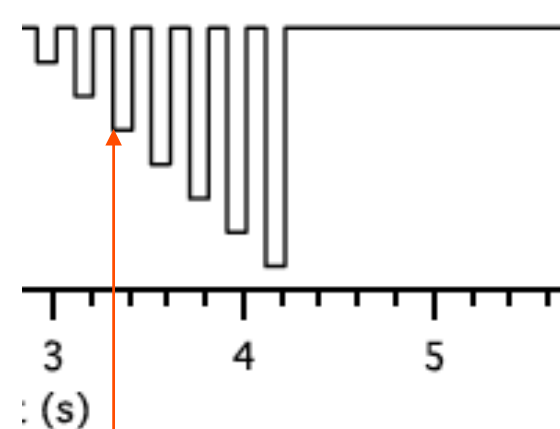
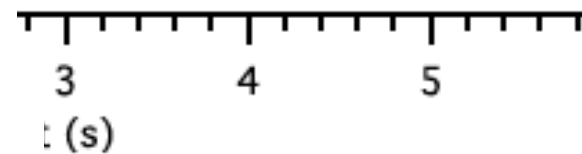
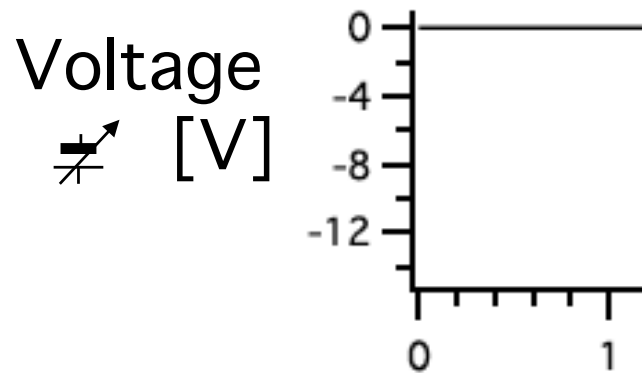
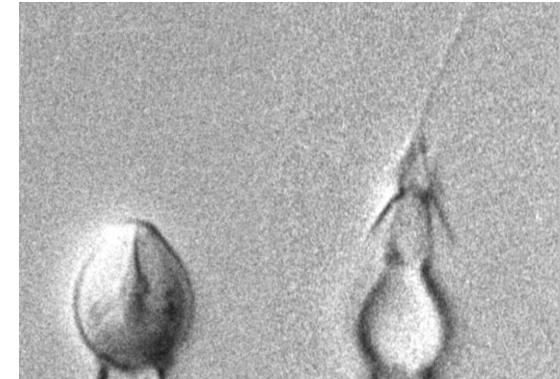
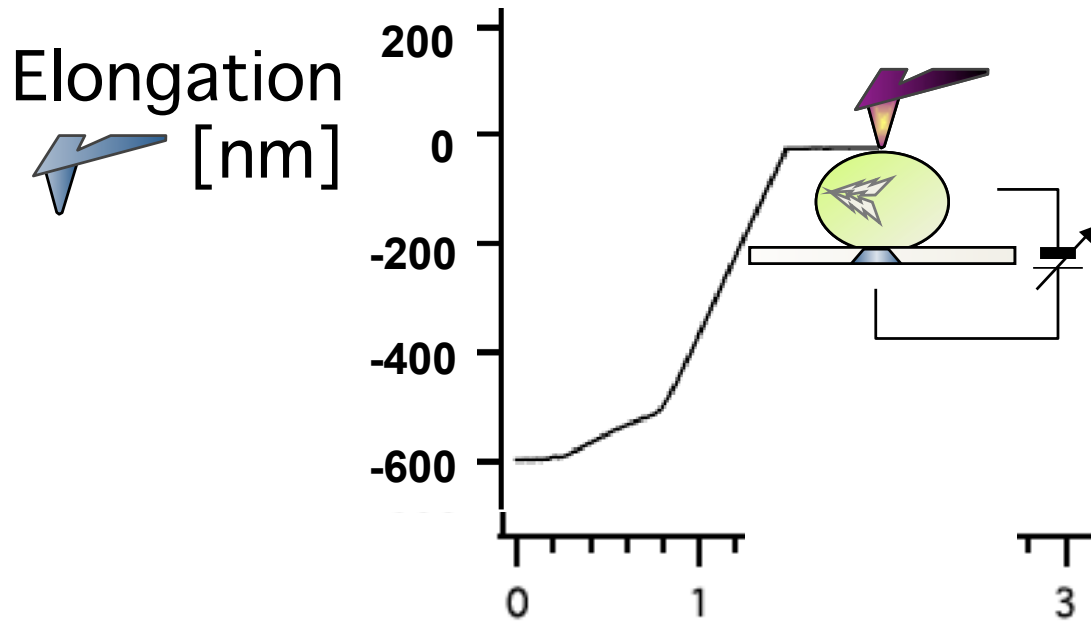
nanjion

Nanon Technologies GmbH



AFM Messungen zur elektrisch stimulierten Zellmechanik

“explosion” in the “patch clamp AFM”



“ignition”

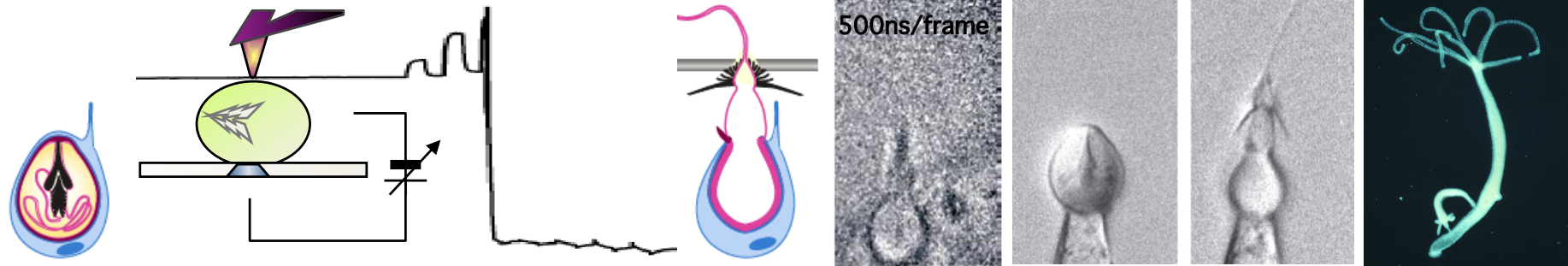
AFM Messungen zur Zellmechanik

planares Patch-Clamp

Niels Fertig und Jan Behrends (2000)

klassisches Patch-Clamp

Erwin Neher und Bert Sakmann (1976)



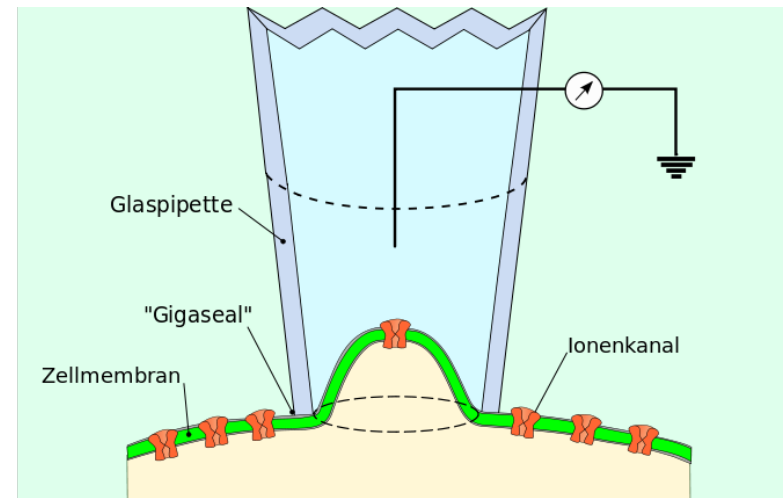
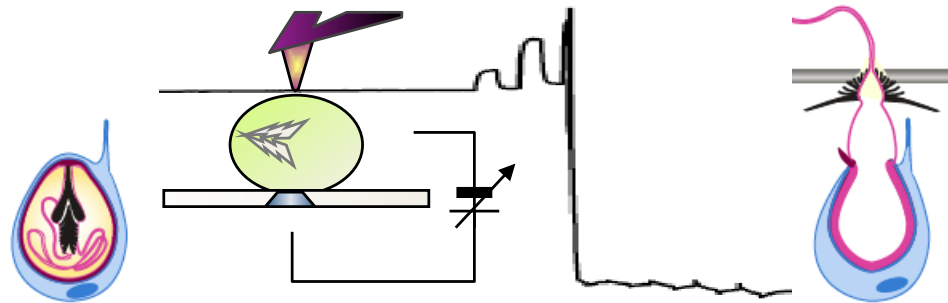
AFM Messungen zur Zellmechanik

planares Patch-Clamp

Niels Fertig und Jan Behrends (2000)

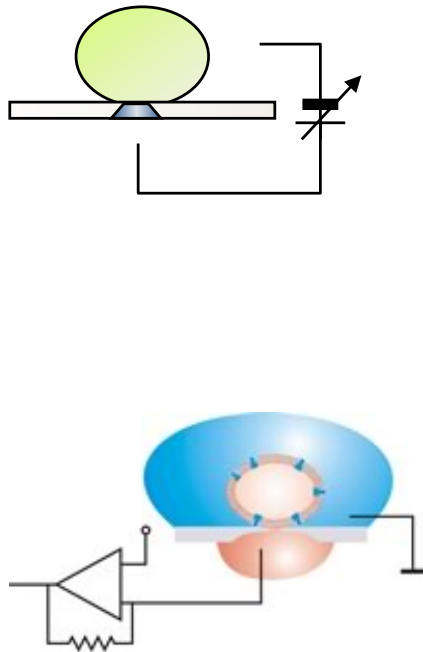
klassisches Patch-Clamp

Erwin Neher und Bert Sakmann (1976)

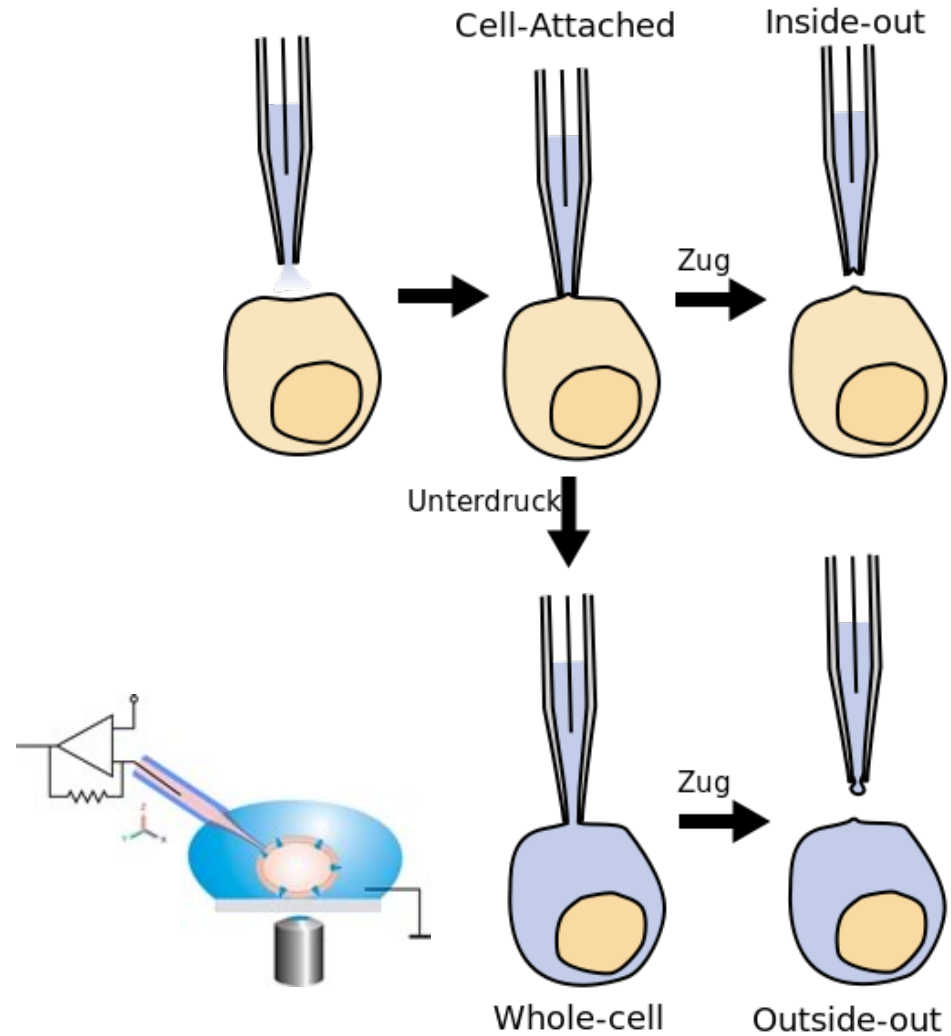


AFM Messungen zur Zellmechanik

planares Patch-Clamp
Niels Fertig und Jan Behrends (2000)



klassisches Patch-Clamp
Erwin Neher und Bert Sakmann (1976)

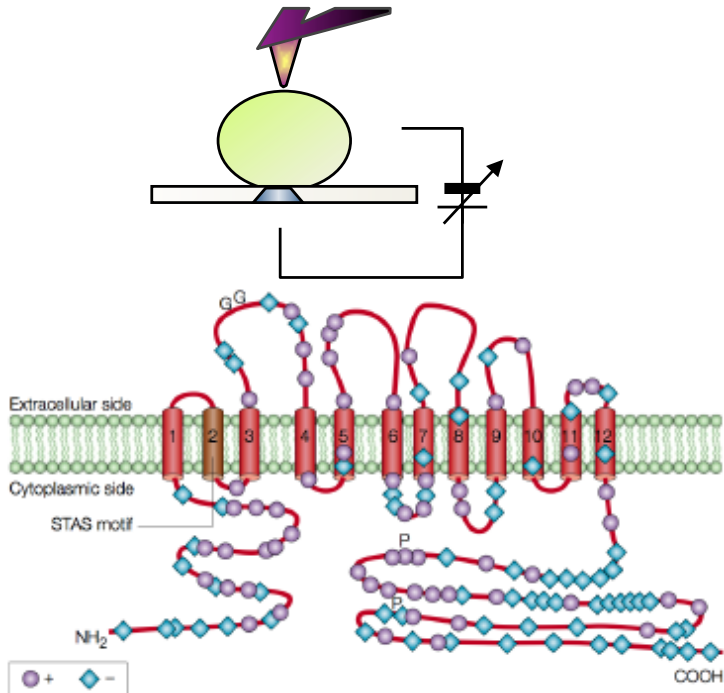


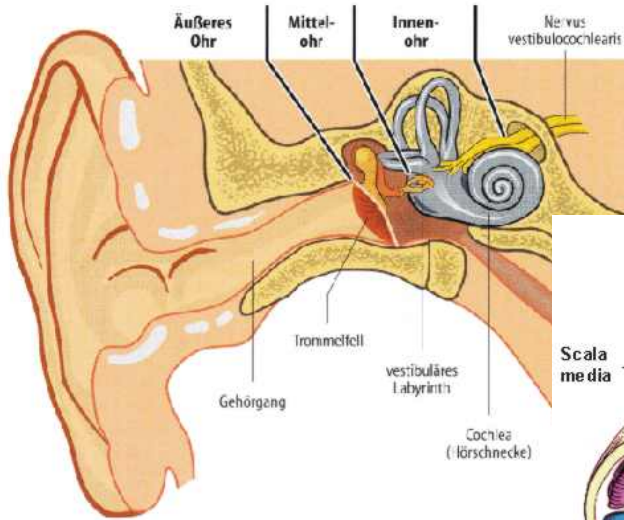
Prestin, ein Spannungsgetriebener Membranmotor

planares Patch-Clamp
Niels Fertig und Jan Behrends (2000)

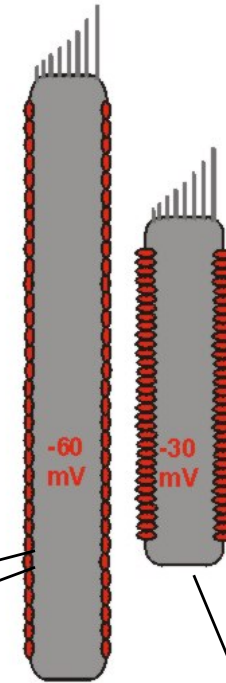
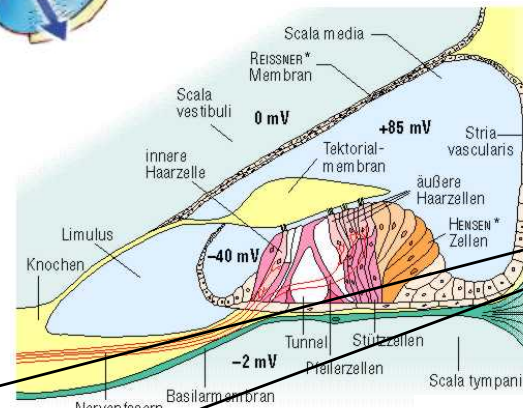
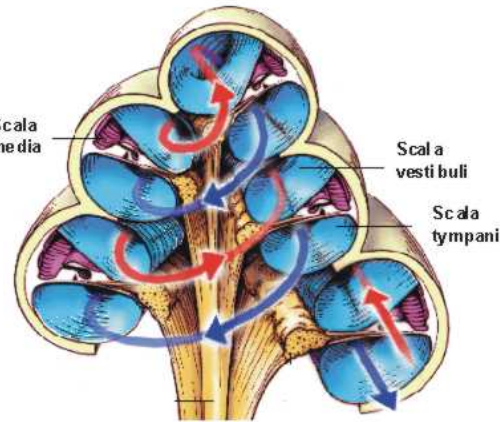
klassisches Patch-Clamp
Erwin Neher und Bert Sakmann (1976)

In Kombination mit Kraftmikroskopie

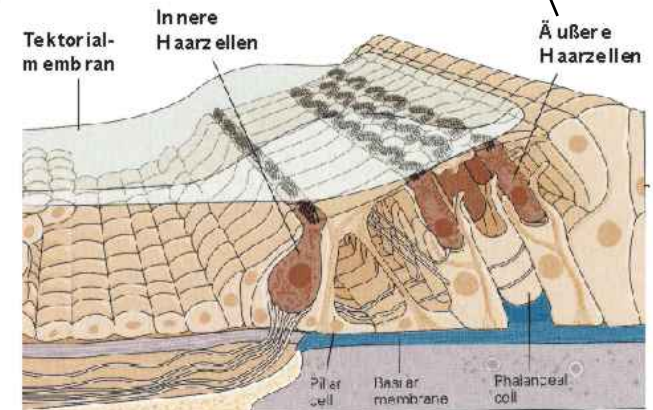
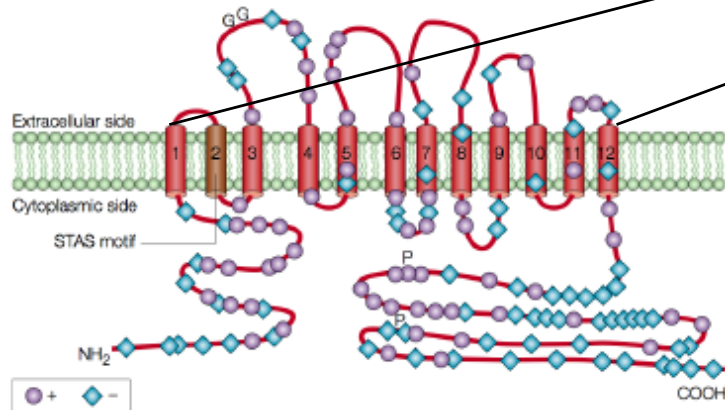




ein Motor zur Verstärkung der Hörleistung

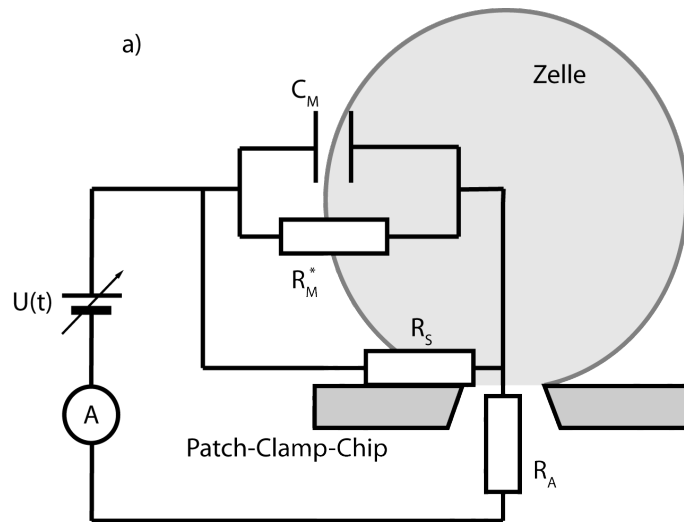


Prestin,

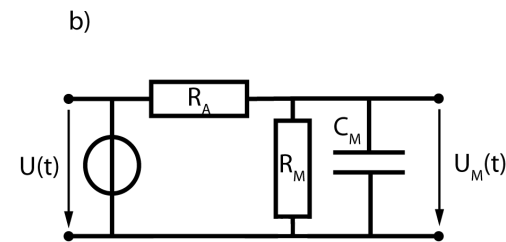


Elektrisches Ersatzschaltbild einer Zelle

Patch-Clamp

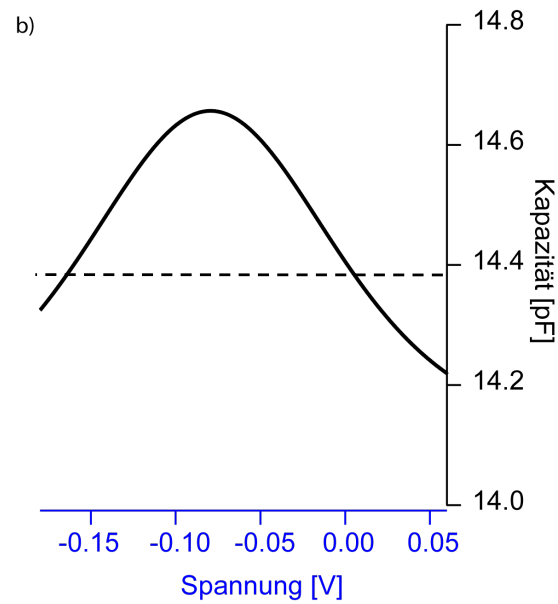
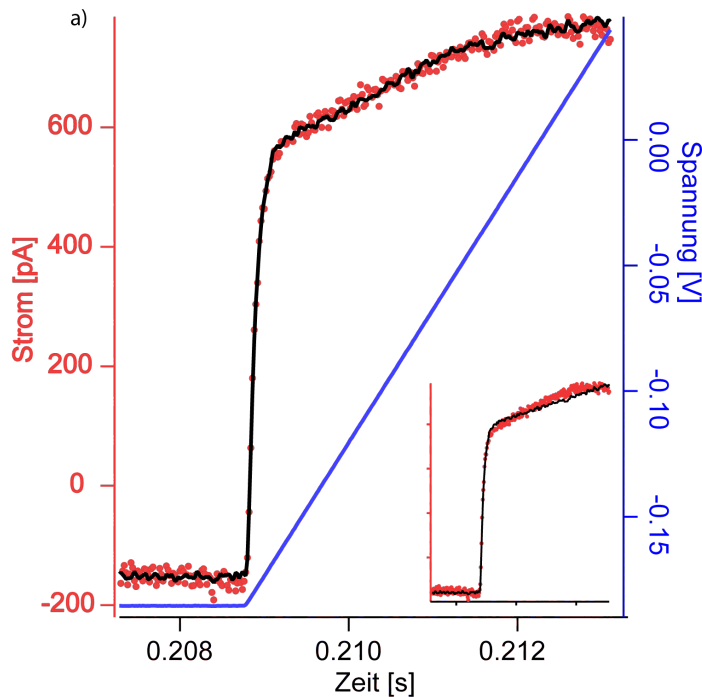
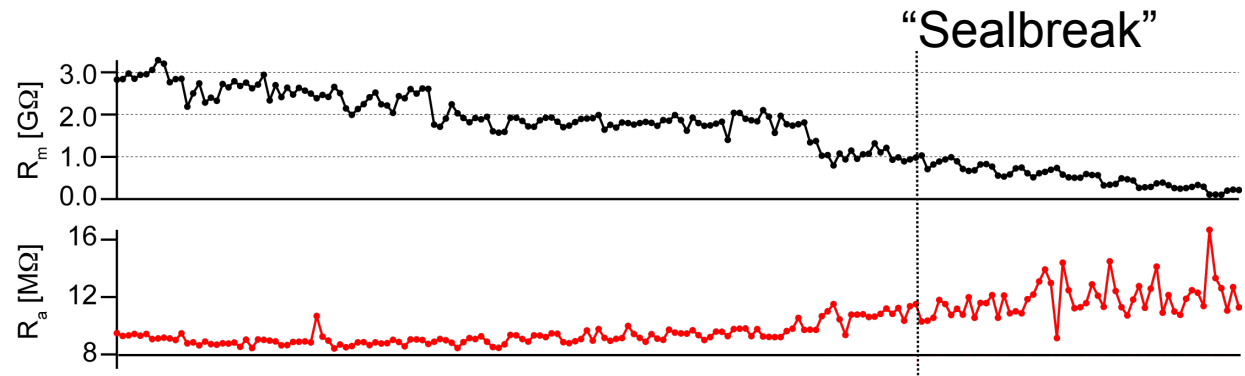
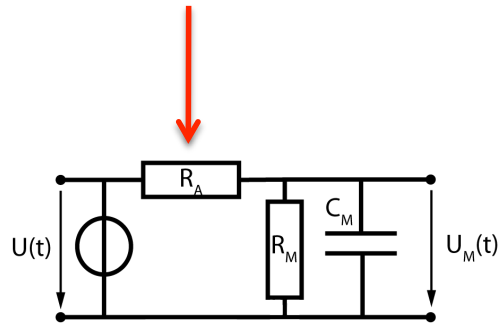


(a)
Abdichtwiderstand R_S (seal) ,
Membrankapazität C_M ,
Membranwiderstand R_M^* und
Zugangswiderstand R_A (access).



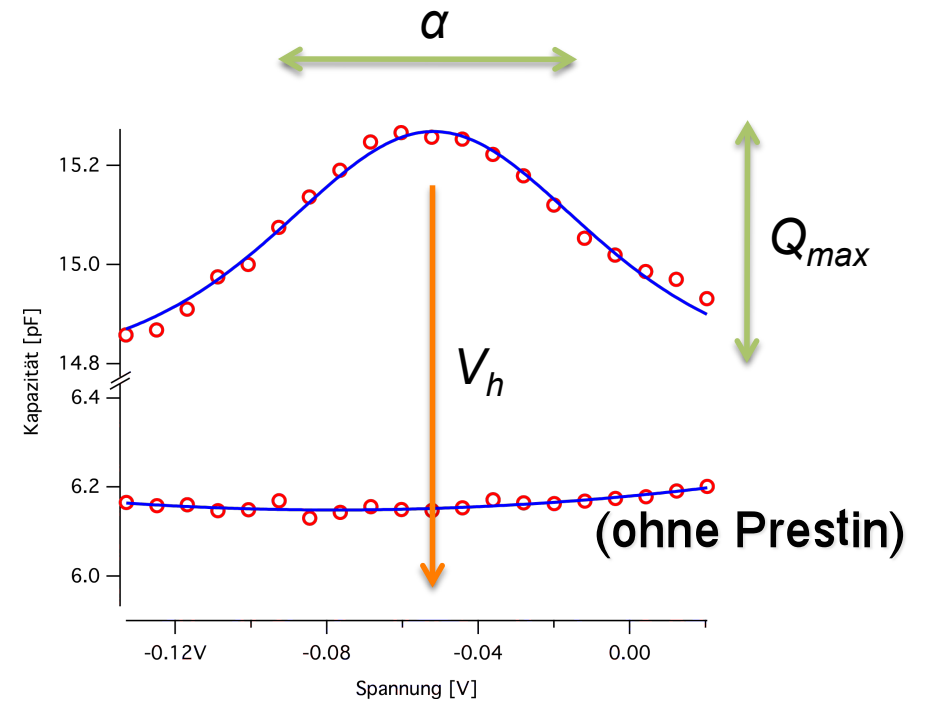
(b)
Zur Vereinfachung werden R_M^* und R_S
zusammengefasst zu R_M .

Bestimmung der elektrischen Kenngrößen einer Prestin Zelle

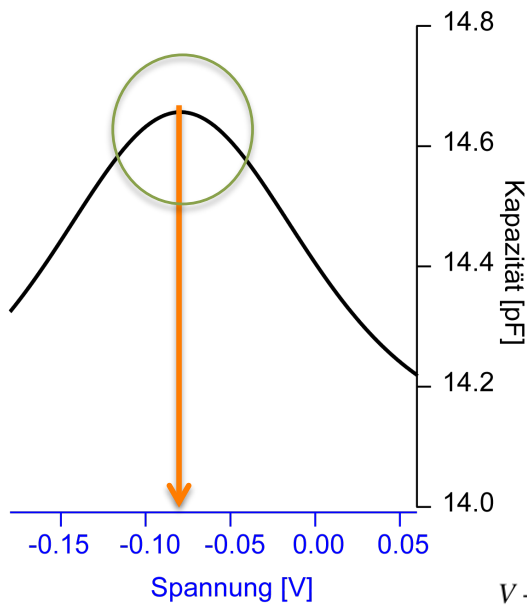
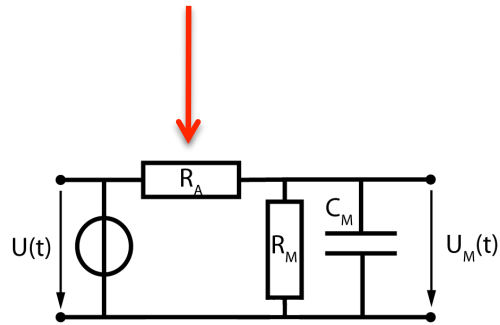


Bestimmung der kapazitiven Nichtlinearität einer Prestinzelle

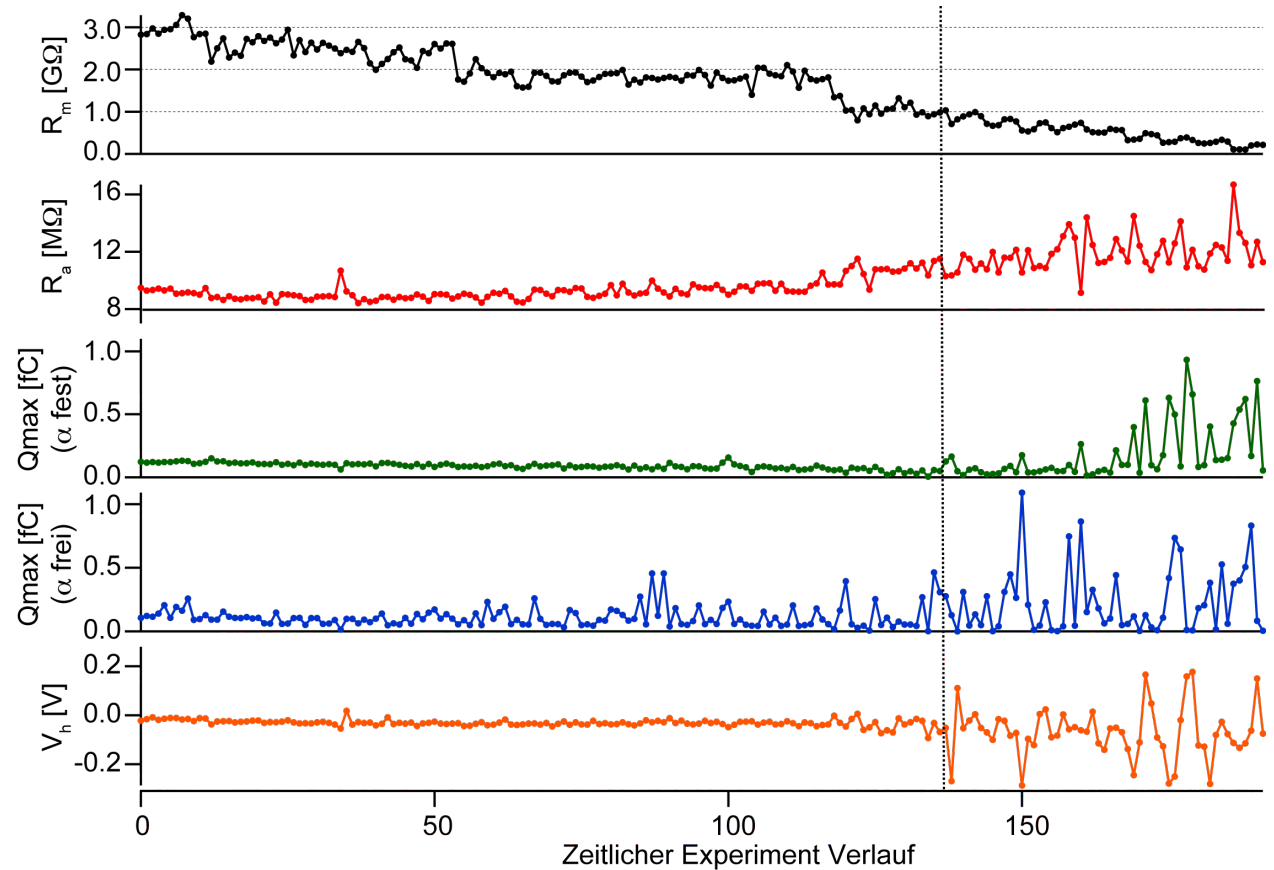
$$C(V) = C_0 + Q_{\max} \frac{e^{-\frac{V-V_h}{\alpha}}}{\alpha \left(1 + e^{-\frac{V-V_h}{\alpha}} \right)}$$



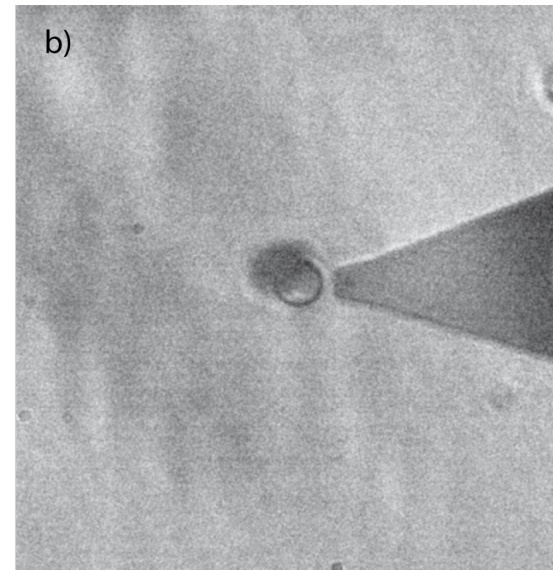
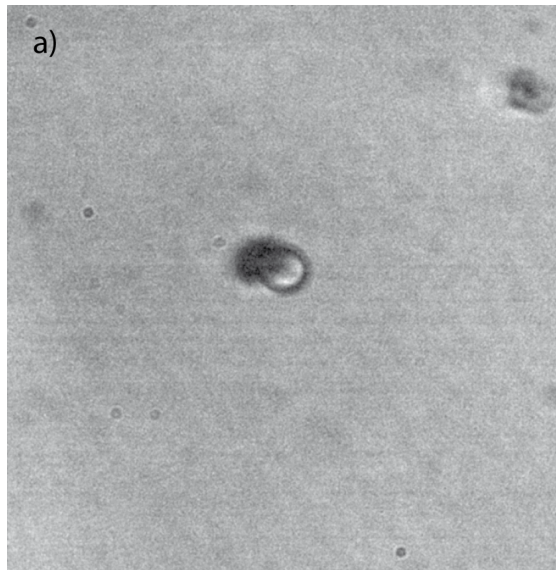
Bestimmung der kapazitiven Nichtlinearität einer Prestinzelle



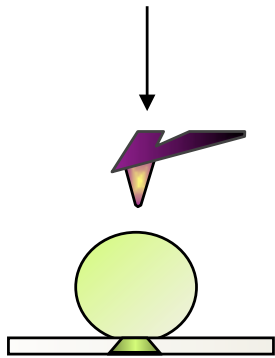
$$C(V) = C_0 + Q_{\max} \frac{e^{-\frac{V-V_h}{\alpha}}}{\alpha \left(1 + e^{-\frac{V-V_h}{\alpha}} \right)}$$



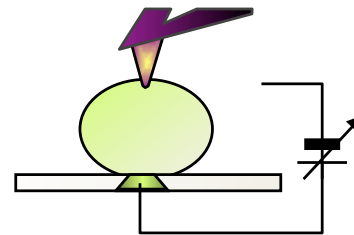
Bestimmung der Mechanik einer Prestinzelle mit dem AFM



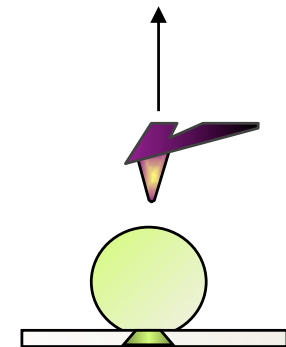
Bestimmung der Mechanik einer Prestinzelle mit dem AFM



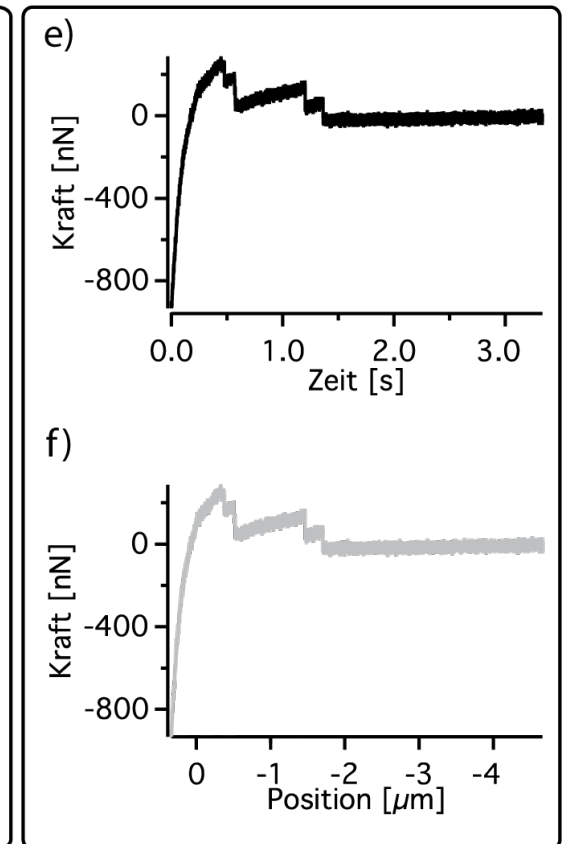
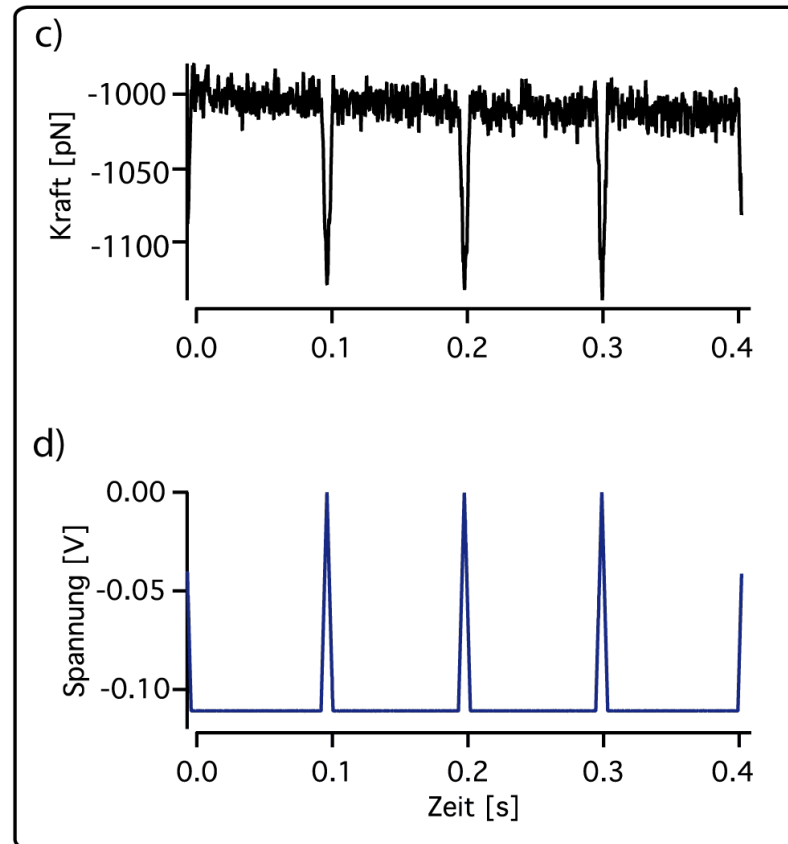
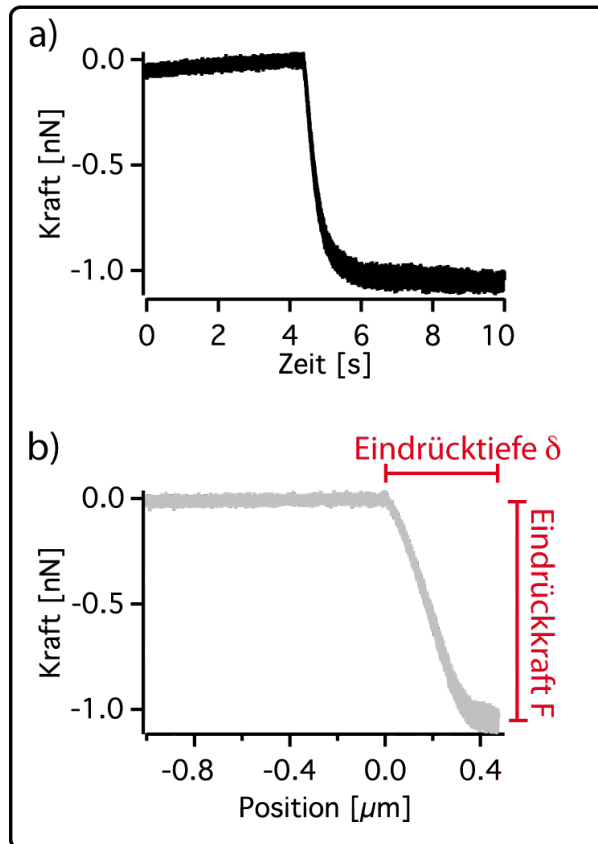
Kontaktierung



Pulsphase



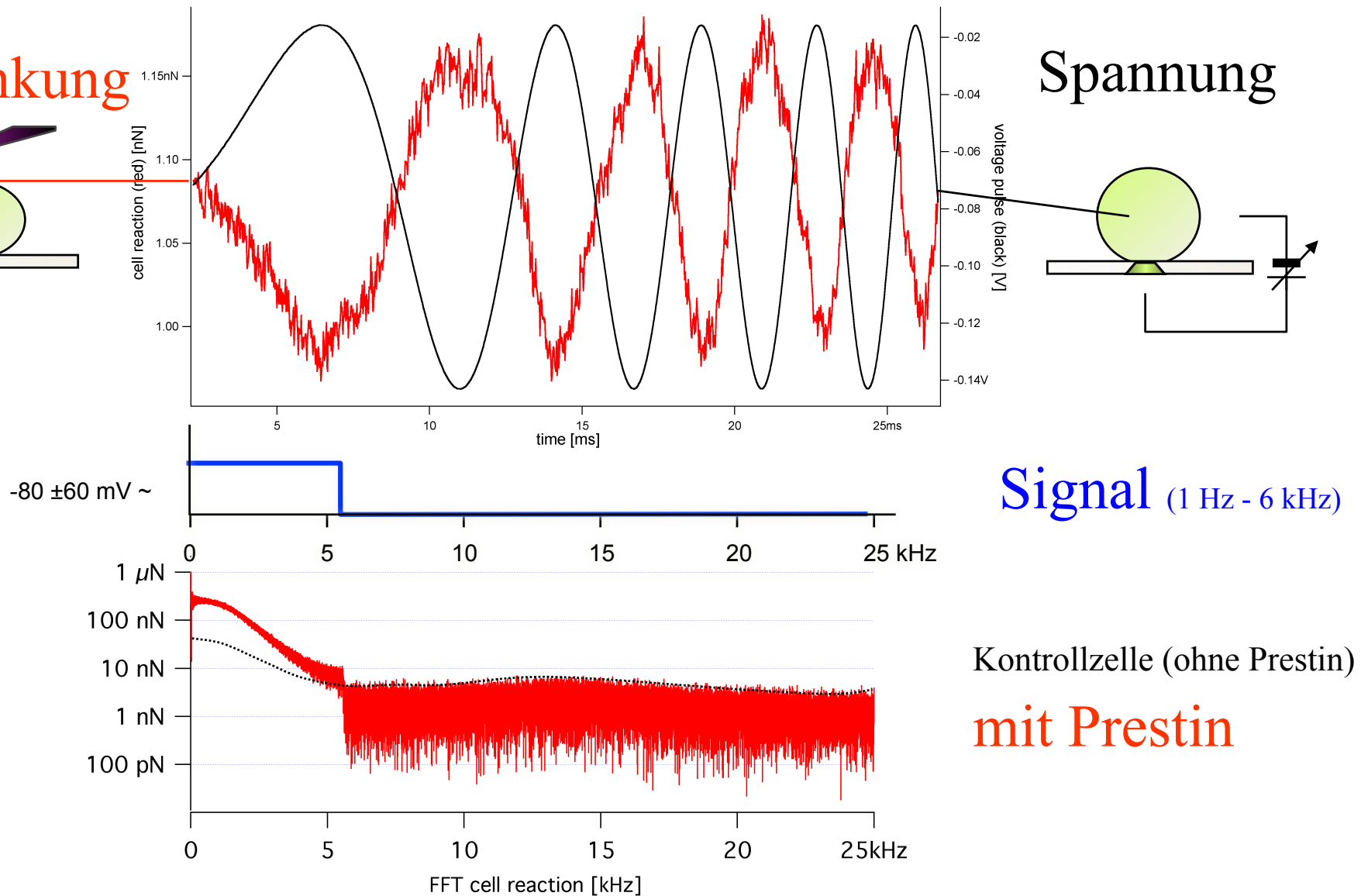
Rückzug



Frequenzgang einer Prestinzelle im Patch-Clamp-AFM

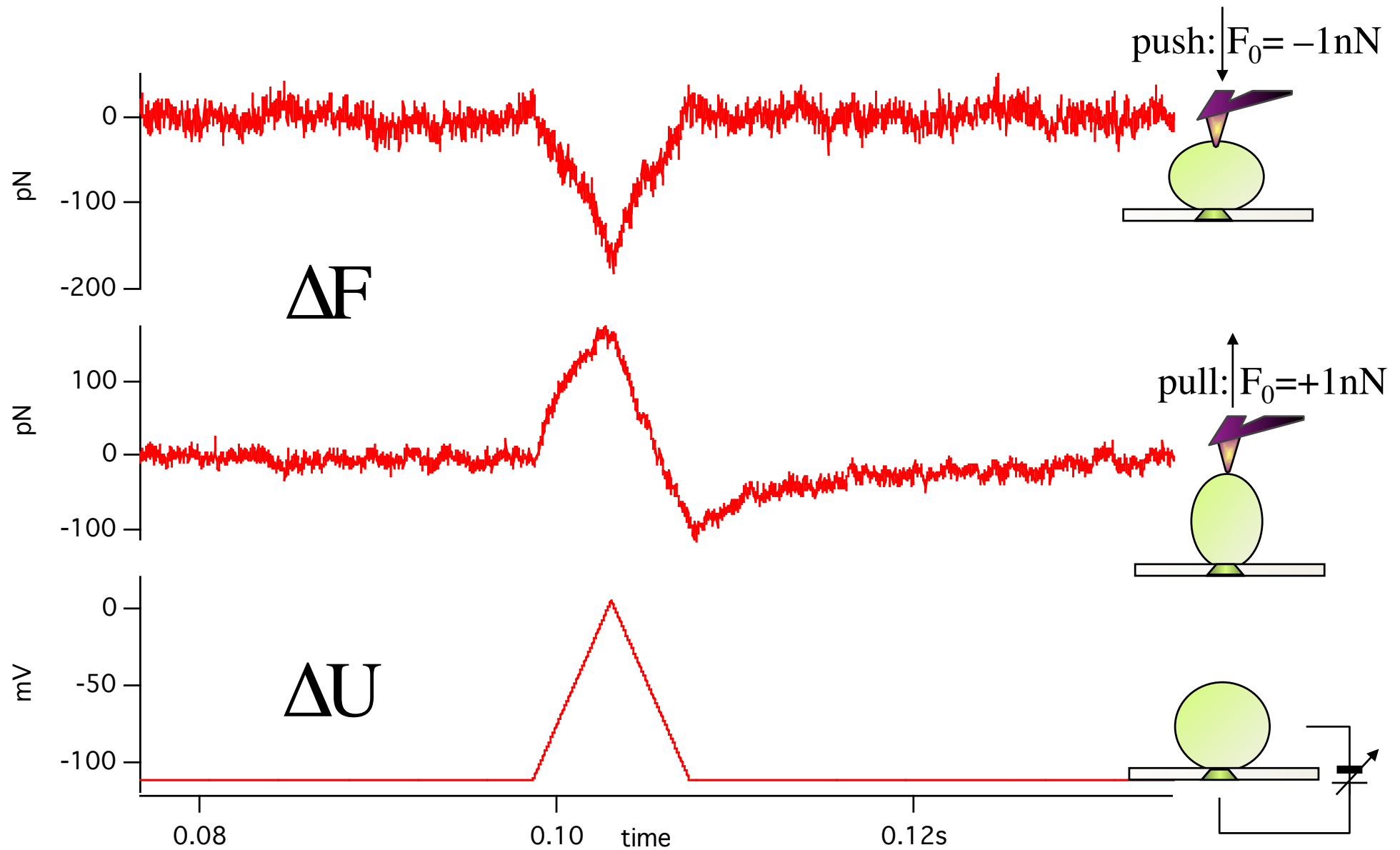
Auslenkung

Spannung



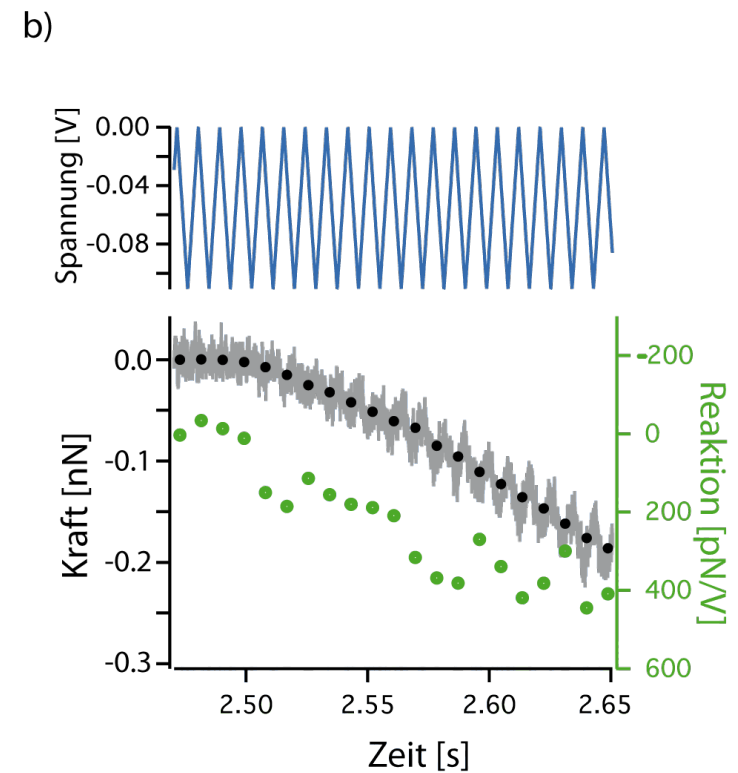
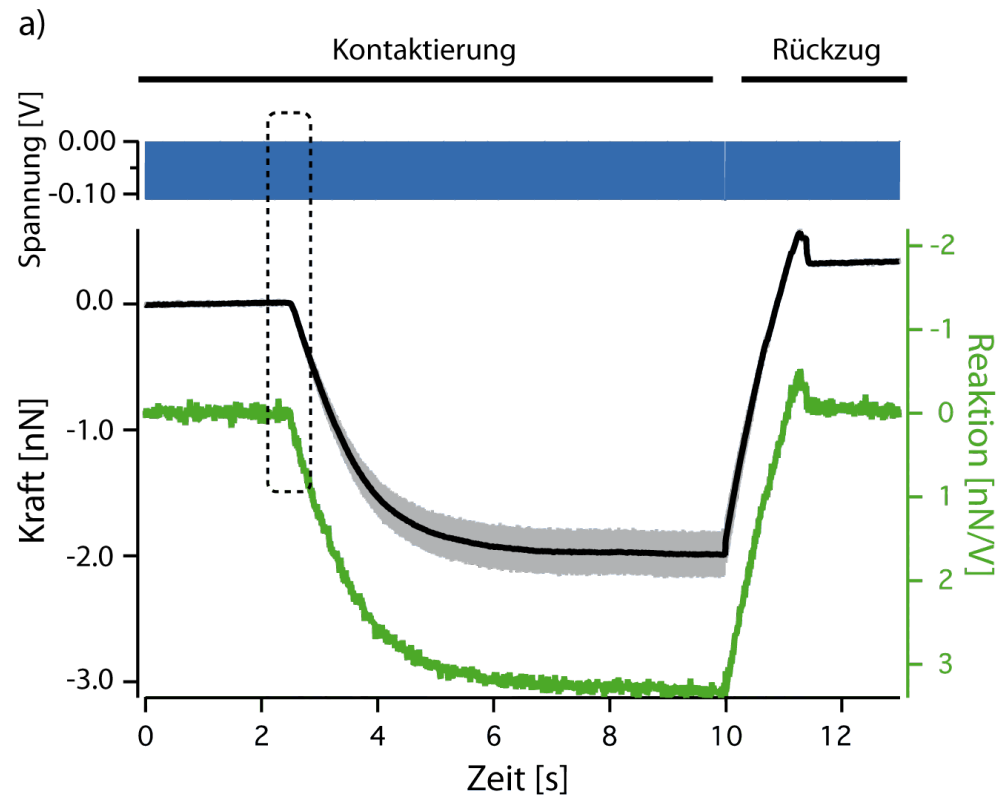
Bestimmung der Mechanik einer Prestinzelle mit dem AFM

Prestin at work

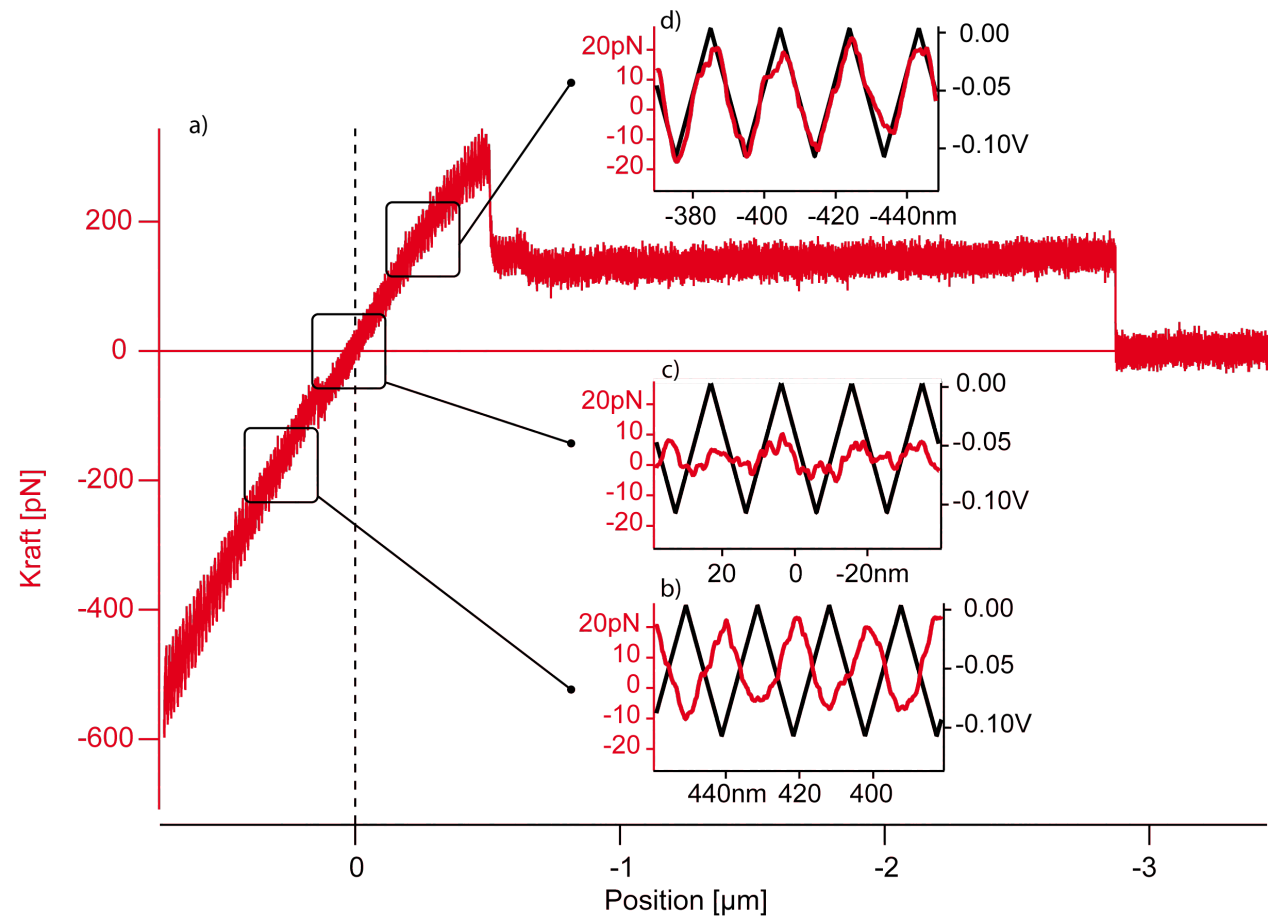
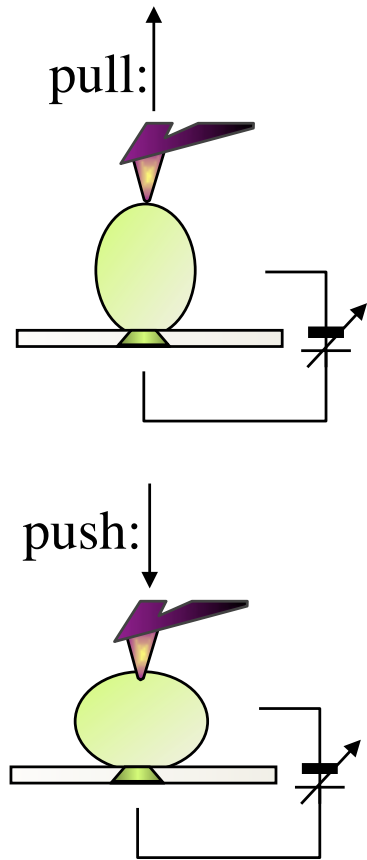


Bestimmung der Mechanik einer Prestinzelle mit dem AFM

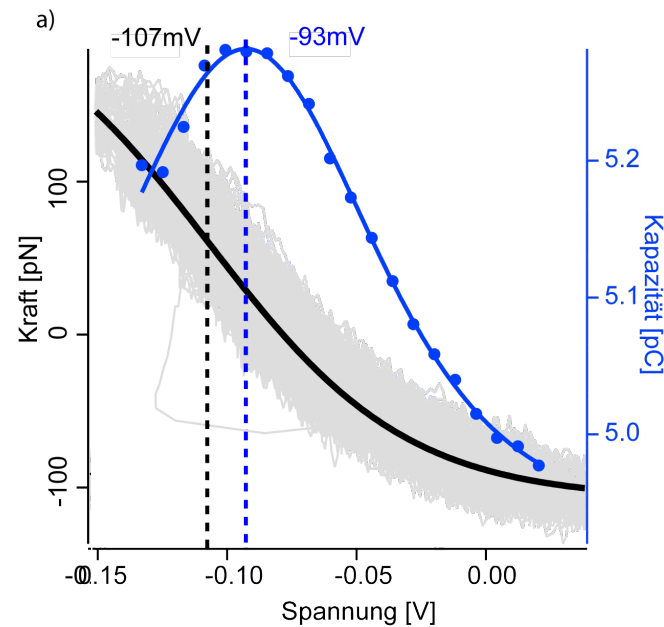
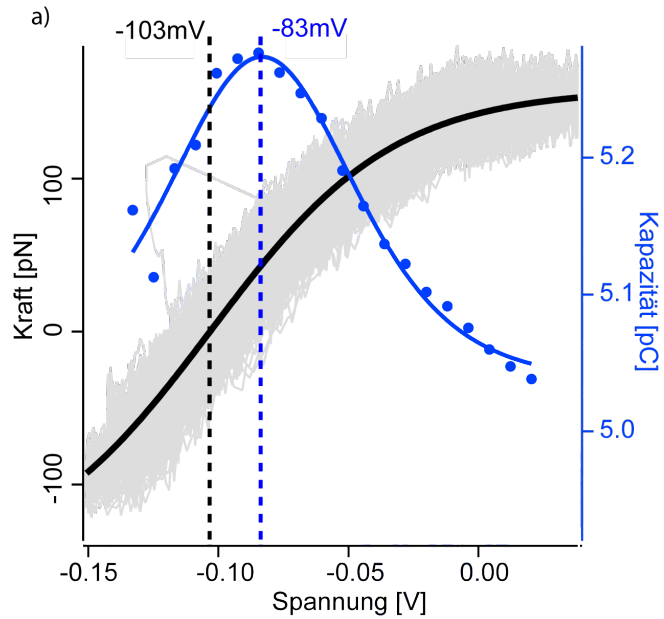
Kontinuierlicher Stimulationspuls zur Ermittlung der Abhängigkeit von der Kontaktkraft



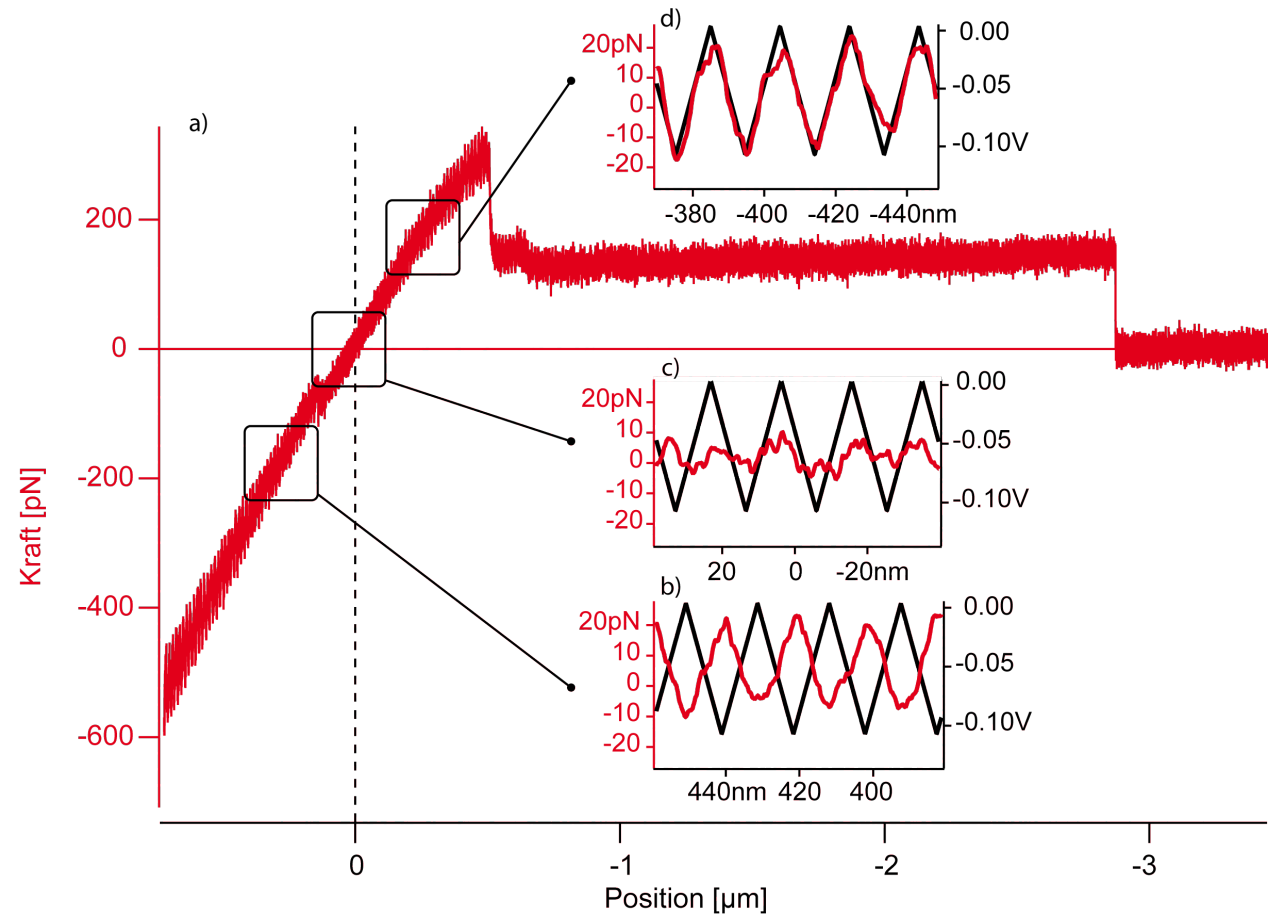
Bestimmung der Mechanik einer Prestinzelle mit dem AFM



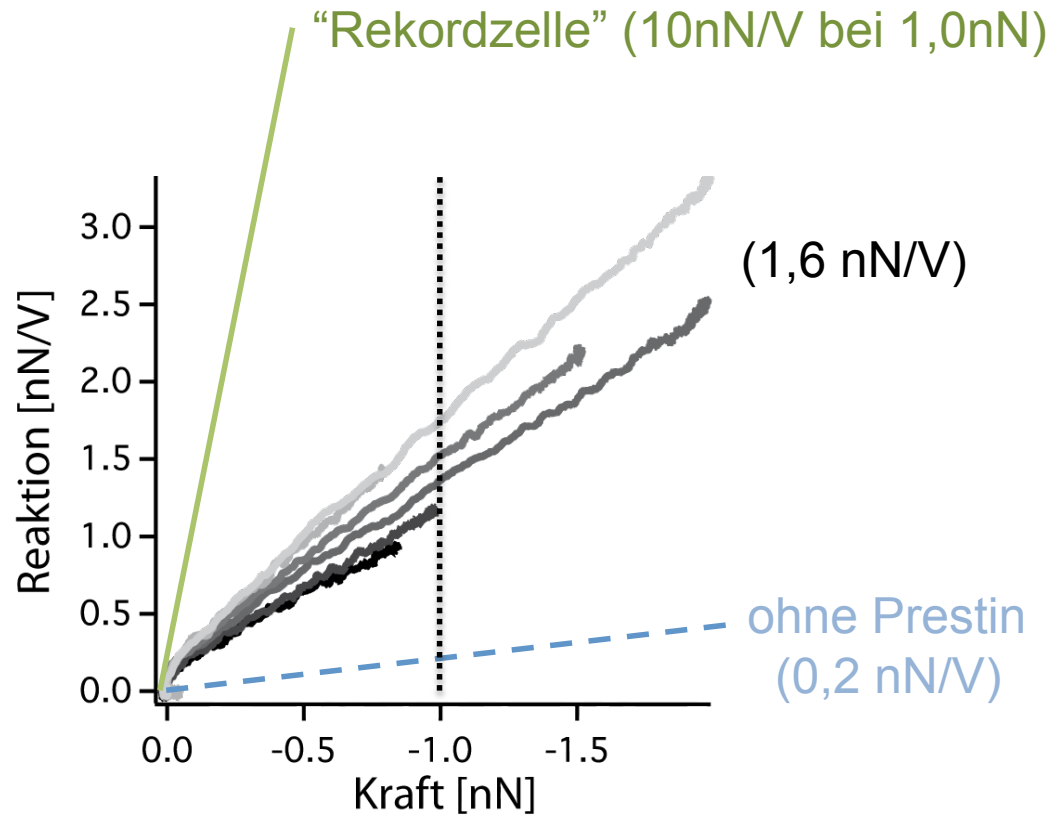
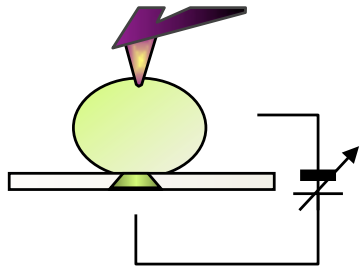
Bestimmung der Mechanik einer Prestinzelle mit dem AFM



Sigmoidaler Verlauf der Prestinreaktion entspricht fast dem nichtlinearen Verlauf der Kapazität.



Prestin ist ein mechanischer 8 (bis 20!)-fach Verstärker



Danke an meine hilfreichen Mitarbeiter, Kollegen und Unterstützer

Cell mechanics:

Angiotensin: Ch.Cuerrier & M.Grandbois (Uni Sherbrooke, Canada) 

P-Pili:  R.Lugmaier, S.Schedin (Uni Umeå, Sweden)

Hydra:  S. Mauell, S.Özbek & Th.Holstein (Uni Heidelberg) 

Cell adhesion force measurements:

Method:  W. Dettmann, M.Thie (Uni Witten/Herdecke)

(Dicty:  D.Gabriel & G.Gerisch (MPI Martinsried) 

(Leukocytes:  J.Schmitz, K.Gottschalk (uulm))

Integrins:  S.Baumann & J.Hartmann, H.Schiller & R.Fässler (MPI Martinsried)

 K.Legate, S.Massberg (DHZ-TU Munich)

 E.Sarıisik, D.Docheva (Imu), H.Clausen-Schaumann (FU Munich)

Patch-ClampAFM: S.Scheuer, E.Pamir, N.Fertig (nanion Munich) 

Cell culture & TEM: A.Kardinal, T. Nicolaus, S. Kempter (LSRädler, Munich)

Electronics & REM: Ch. Holopirek, S. Manus & Ph. Altpeter (LS Kotthaus, Munich)

: H.E.Gaub

Danke an meine hilfreichen Mitarbeiter, Kollegen und Unterstützer

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Cell adhesion

Method:

(Dicty:

(Leukocytes:

Integrins:

Patch-Clamp

Cell culture & TEM: A. Karo

Electronics & REM: Ch. Ho

: H.E. Gaub



(Herdecke) (Martinsried))

Fässler (MPI Martinsried)

naumann (FU Munich)

ch)) (LSRädler, Munich)

Altpeter (LS Kotthaus, Munich)