force-distance curve-based atomic force microscopy

FD-based AFM:
The tool to image and simultaneously map multiple properties of biological systems

Technical Journal Club

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Valeria Eckhardt

Overview

1. Atomic force microscopy (AFM)
   - Brief intro AFM
   - Imaging mode: contact / oscillation
   - Force spectroscopy mode
2. FD-based AFM
3. Applications FD-based AFM
   - Paper 1: Native proteins
   - Paper 2: GPCR-ligand interaction
4. Summary & Outlook

1. Atomic force microscopy (AFM)

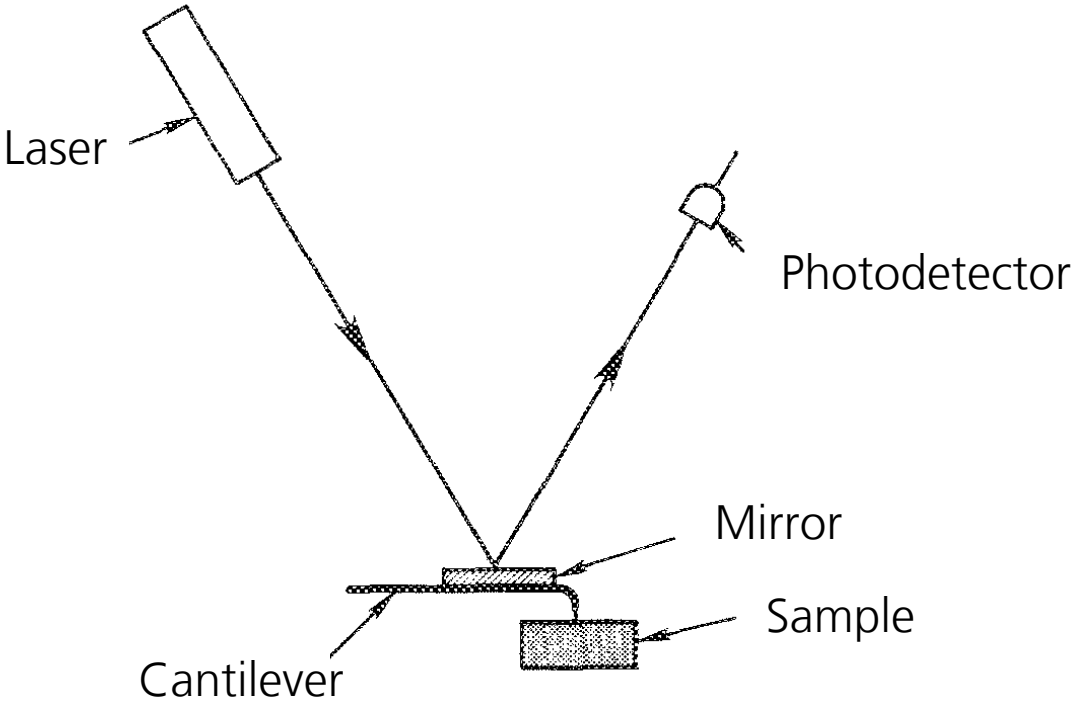
- AFM was invented in 1986 by Gerd Binnig, Calvin Quate and Christoph Gerber

- AFM Imaging mode

  For high-resolution surface imaging

  – Contact mode AFM (l. 0.6 nm v. 0.1 nm)

  – Oscillation mode AFM



Modified from Meyer & Amer, 1988

1. Atomic force microscopy (AFM)

- AFM imaging mode:
  - Advantages of oscillation - vs. contact mode
    1. Reduces contact time, friction and lateral forces
    2. Reduced vertical forces allow imaging of soft materials: DNA, proteins, cells…
  - oscillation mode is the most commonly used AFM method (easy, less invasive)

- AFM imaging mode: no quantification of biological, chemical and physical properties

1. Atomic force microscopy (AFM)

- AFM imaging mode: Contact/ Oscillation
- AFM force spectroscopy mode
  1. Micro- and nanomanipulation
  2. Force spectroscopy mode:
     Quantify inter- and intramolecular interaction forces:
     electrostatic, van der Waals, hydrophobic forces

     Approach & retraction: record Vertical displacement of AFM tip & deflection of the cantilever
     Force-displacement ➔ force-distance curve
     describe dependence of interaction forces between the AFM tip and the sample from the distance
  3. Probe mechanical properties
     Indentation-retraction experiments
     electrostatic properties, deformation, pressure, adhesion

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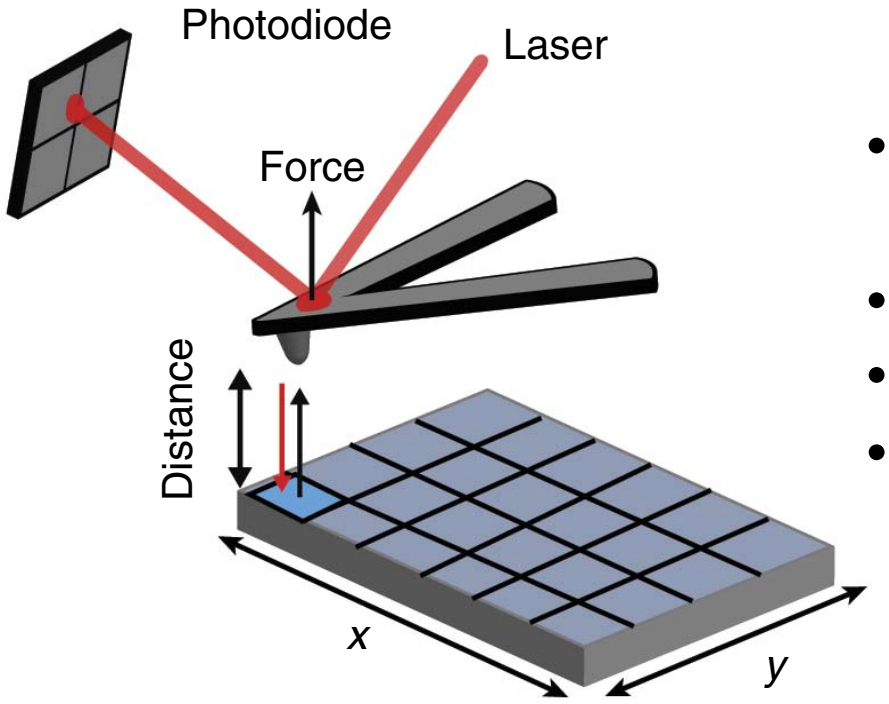
2. FD-based AFM

Force-distance curve-based atomic force microscopy
FD-based AFM = AFM imaging + AFM force spectroscopy

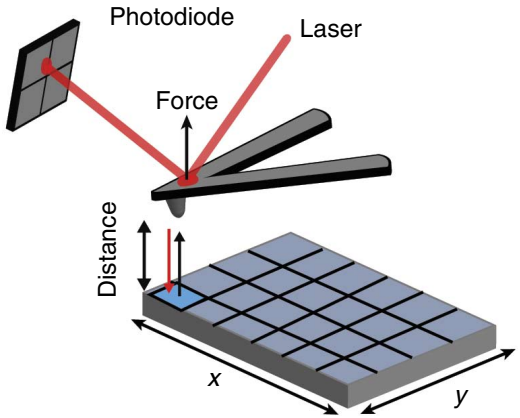


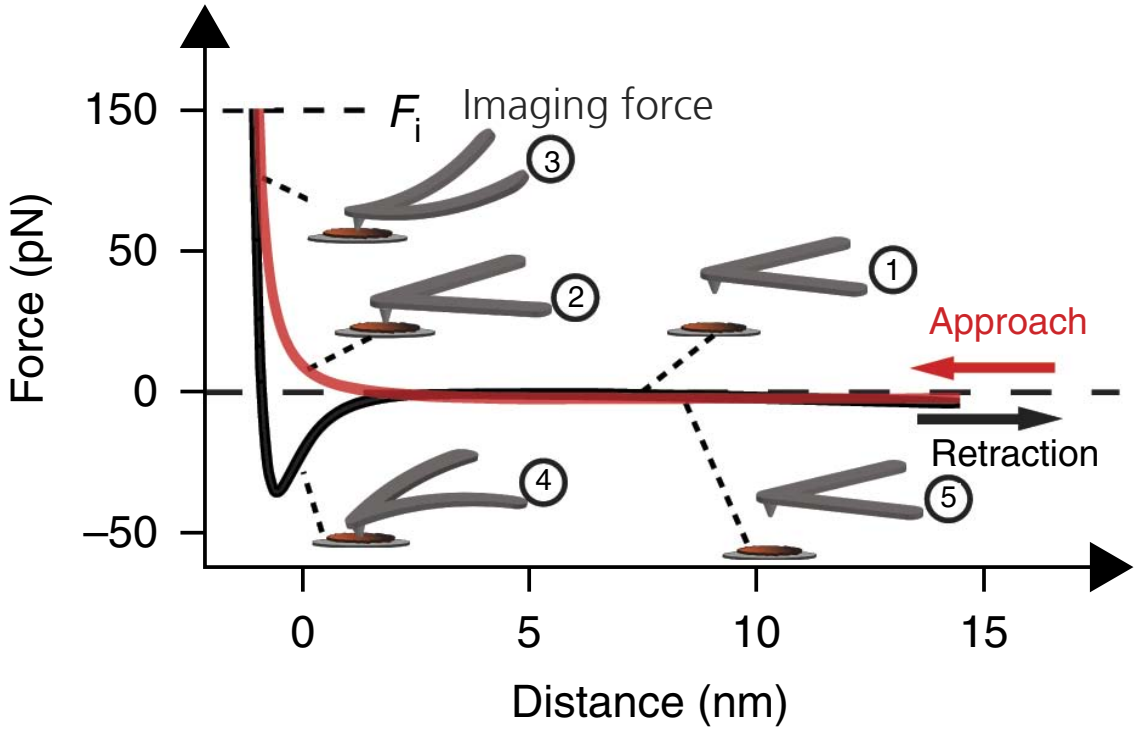
- Lateral resolution 1 nm
- Vertical resolution 0.1 nm

- Oscillating cantilever with Silicon nitride tip
- pixel by pixel manner
- pixel size < 1 nm^2
- The value of every pixel of the final sample topography is determined by the tip-sample distance and the present imaging force.

2. FD-based AFM

Force-distance curve-based atomic force microscopy





2. 0 nm = contact point tip-sample

Approach FD curve

1. Noncontact
2. Initial contact
3. Repulsive contact

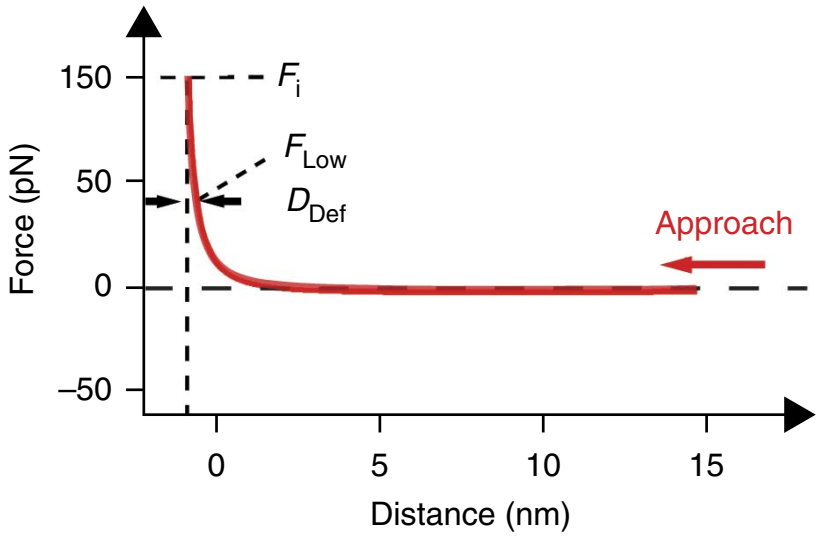
Retracting FD curve

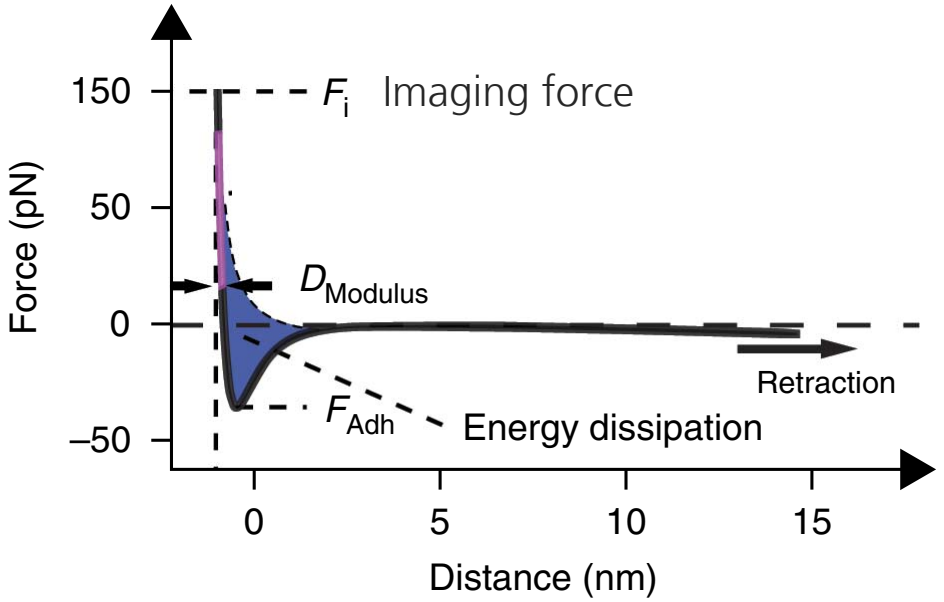
4. Adhesion
5. Noncontact

Pfreudenschuh et al., 2014

2. FD-based AFM

Multiple physical forces can be derived from the approaching and retracting FD curves:





Deformation: $D_{Def} = D_{F_{Low}} - D_{F_i}$

- Mechanical flexibility/stiffness
- Adhesion: minimal force
- Energy dissipation
- Young's modulus

Reduced Young's modulus: $E^* = \frac{3}{4} \times (F_i - F_{Adh}) \times (RD_{Modulus}{}^3)^{-\frac{1}{2}}$

Pfreudenschuh et al., 2014

Multiple physical forces can be derived from the approaching and retracting FD curves:

Physical forces of interactions:

- Coulomb forces
- van der Waals forces
- hydrophobic attraction
- solvation forces

Biochemical forces:

- Covalent bonds
- Ligand-receptor pairs
- Biopolymers
- Nucleic acids
- Membrane and water-soluble proteins
- Cellular membranes
- Lipid bilayers

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Multiparametric high-resolution imaging of native proteins by force-distance curve–based AFM

Moritz Pfreundschuh[1], David Martinez-Martin[1], Estefania Mulvihill[1], Susanne Wegmann[2] & Daniel J Muller[1]

[1]Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland. [2]Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts, USA. Correspondence should be addressed to D.J.M. (daniel.mueller@bsse.ethz.ch).

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Aim: Contour the surface and quantify biophysical and biochemical properties of native proteins at high resolution

Samples: 2 water soluble proteins:

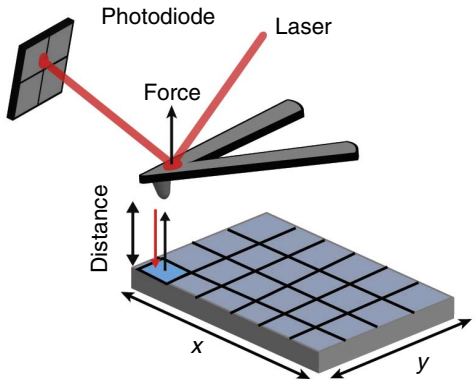
- fibrils of human tau protein, neurofibrillary tangles in AD
  Assess highly variable polymorphic structure and biophysical properties of amyloid-like fibrillar aggregates by FD-b AFM

- Bacteriorhodopsin from the purple membrane of *H.salinarum* (functionally & structurally best studied protein)

3. Applications FD-based AFM

1. Set up the AFM
   - Isolate from acoustic, mechanical and electrical noise (noise analyzers)
   - Equilibrate the AFM: Focus the laser beam onto the tip to adjust the photodiode signal

2. Sample immobilization
3. Cantilever selection
4. Record FD curves
5. FD curve analysis



3. Applications FD-based AFM

1. Set up the AFM
2. Sample immobilization
   - Better hydrophilic supporting surface than hydrophobic
     - Mica: negatively charged at neutral pH, hydrophilic, good electrical insulator
     - HOPG = highly ordered pyrolytic graphite: hydrophobic, good conductor
   - Native membrane proteins should be present in the membrane
   - Glue metal disc, hydrophobic teflon foil, mica sheet
   - Cleave the mica sheet with Scotch tape

3. Applications FD-based AFM

1. Set up the AFM
2. Sample immobilization

Sample preparation

- bacteriorhodopsin preparation:

  Dilute purple membrane stock solution in adsorption buffer to ~10 μg/ml and place ~30 μl of it onto the freshly cleaved mica for 15–30 min

- preparation of tau fibrils:

  Dilute the fibril solution in adsorption buffer

  to a final concentration of ~3 μg ml−1 and place ~10–20 μl of it onto the freshly cleaved mica for 10–20 min

- Remove adsorption buffer, apply imaging buffer and mount sample on AFM. Immerse cantilever in imaging buffer.

3. Applications FD-based AFM

1. Set up the AFM
2. Sample immobilization
3. Cantilever selection

- Soft AFM cantilever to measure interaction forces between single biomolecules, ranging typically from 5 to 250 pN.
- High resonance frequencies >100kHz are needed to detect fast biomolecular interactions.
- Shape and size of the AFM stylus determine the lateral resolution. Sharp styluses with a small tip radius (ca 2 nm) for high resolution.

3. Applications FD-based AFM

1. Set up the AFM
2. Sample immobilization
3. Cantilever selection
   - When measuring the **mechanical flexibility or stiffness**, the spring constant (stiffness) of the cantilever should be similar to that of the sample.
   - Intermediate stiffness ca 0.1N/m
   - Sensitivity for mechanical flexibility is decreased, if stiff cantilevers >1N/m are used.
   - Mechanical properties of biological systems are heterogeneously distributed and can change dynamically.

3. Applications FD-based AFM

1. Set up the AFM
2. Sample immobilization
3. Cantilever selection

Mechanical flexibility	Biological system	Exemplification
		Atmospheric pressure: $1x10^5$ Pa= 50 kPa
<1kPa – >100 kPa	Living animal cells	(100kPa = 1 bar) 2-5 bar, bicycle tire
Few MPa – tens of MPa	Protein Membranes	1 Mio Pa, to describe explosions
10 – 500 MPa	Lipid bilayers	
>100 MPa – GPa	Viruses, single-membrane water-soluble and fibrillar proteins	Pressure at which graphite is converted to diamond (6GPa)

3. Applications FD-based AFM

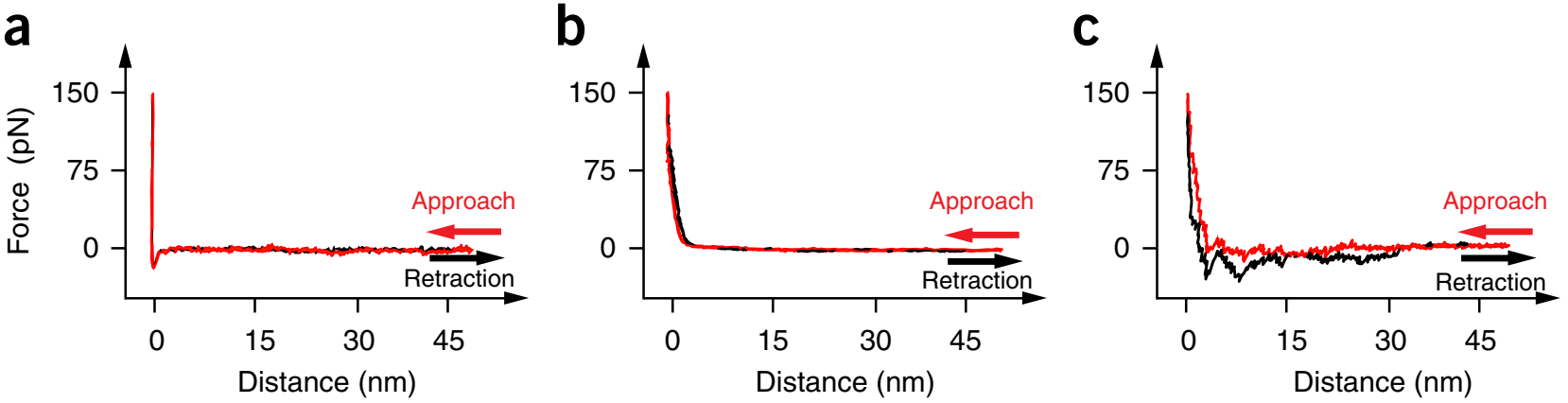
1. Set up the AFM
2. Sample immobilization
3. Cantilever selection
   - Mechanical properties of biological systems are heterogeneously distributed and can change dynamically.

   → Mechanical properties should be precisely assigned to structural details.

   → Functional state at which mechanical measurements were performed should be well defined.

3. Applications FD-based AFM

1. Set up the AFM
2. Sample immobilization
3. Cantilever selection
4. Record and analyze FD curves



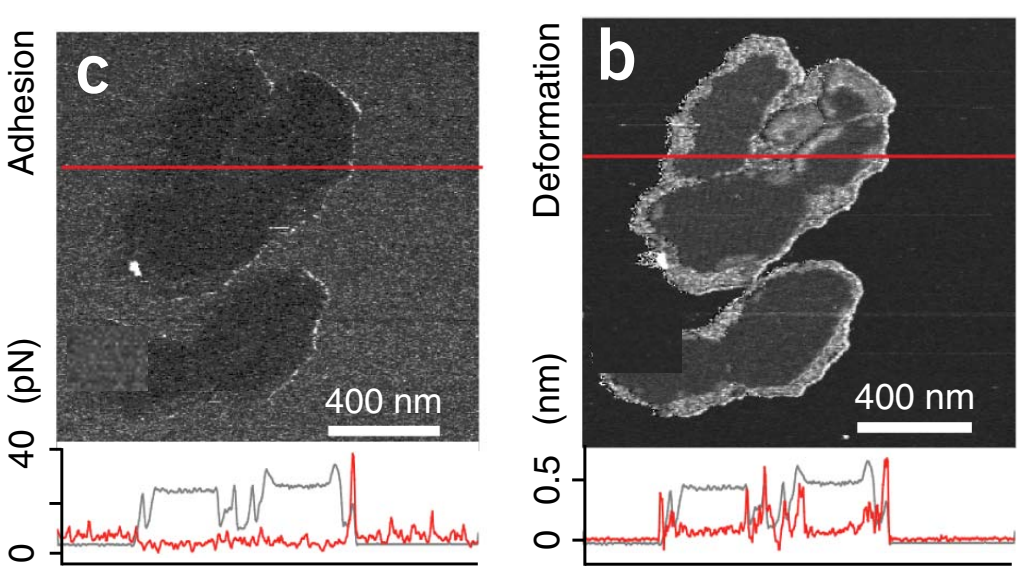
buffer solution and clean mechanical support = mica surface
sharp transition in contact area

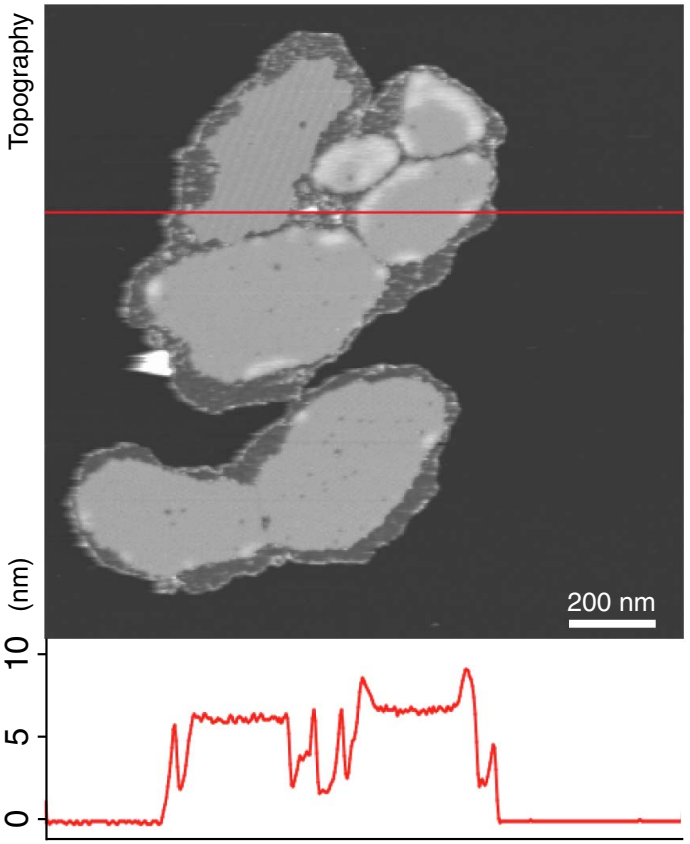
buffer solution and a clean and mechanically flexible sample
smooth transition in contact area

buffer solution with contaminated AFM stylus or mica surface

4. Record & analyze FD curves

densely packed patches of
Bacteriorhodopsin, lipid bilayer





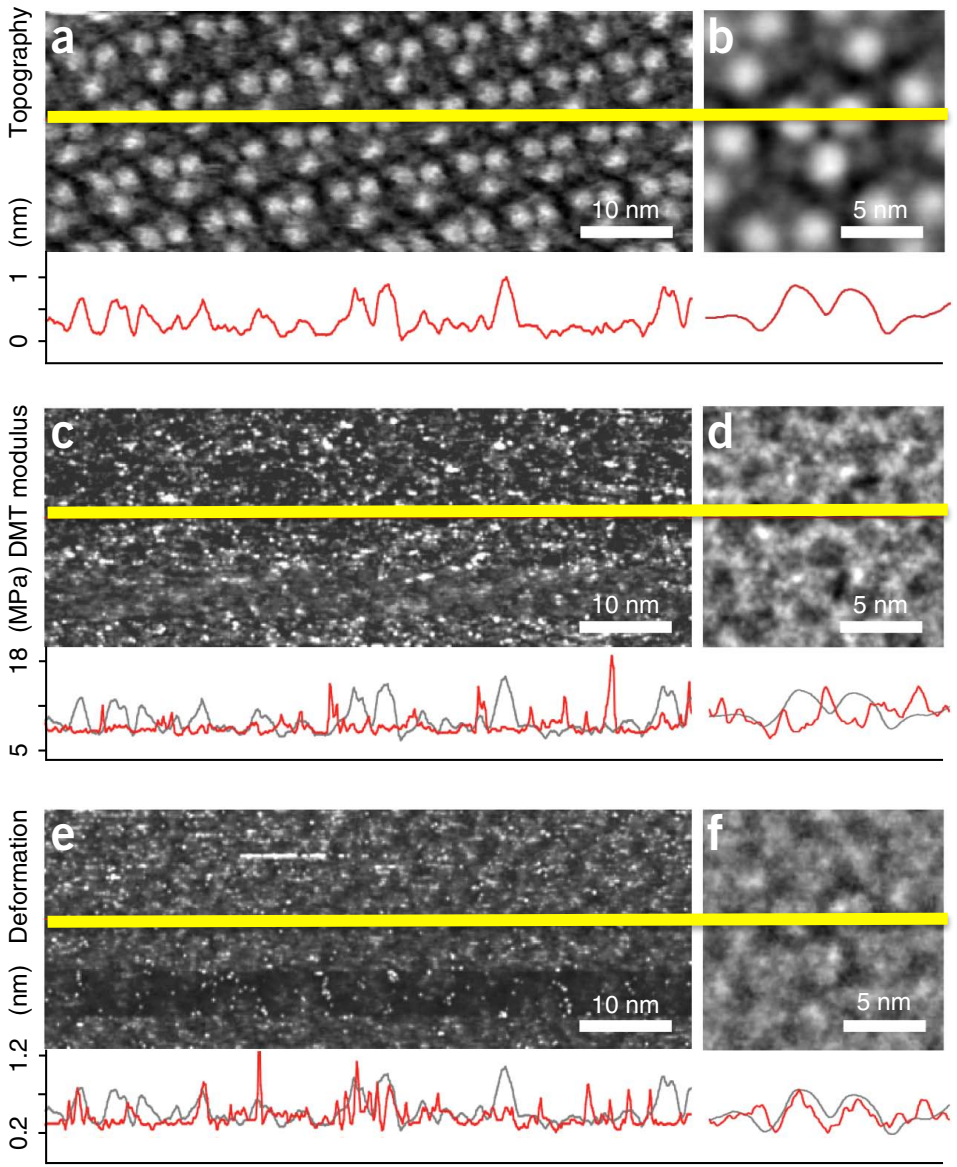
imaging force of 140 pN, a cantilever amplitude of 40 nm, a frequency of 2 kHz and a scanning frequency of 1 Hz per line

4. Record & analyze FD curves

Topography

Young's modulus:
force needed to
stretch / compress
the sample

Deformation



imaging force of 45 pN, a cantilever amplitude of 14 nm,
a frequency of 2 kHz and a scanning frequency of 0.77 Hz per line

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Imaging G protein–coupled receptors while quantifying their ligand-binding free-energy landscape

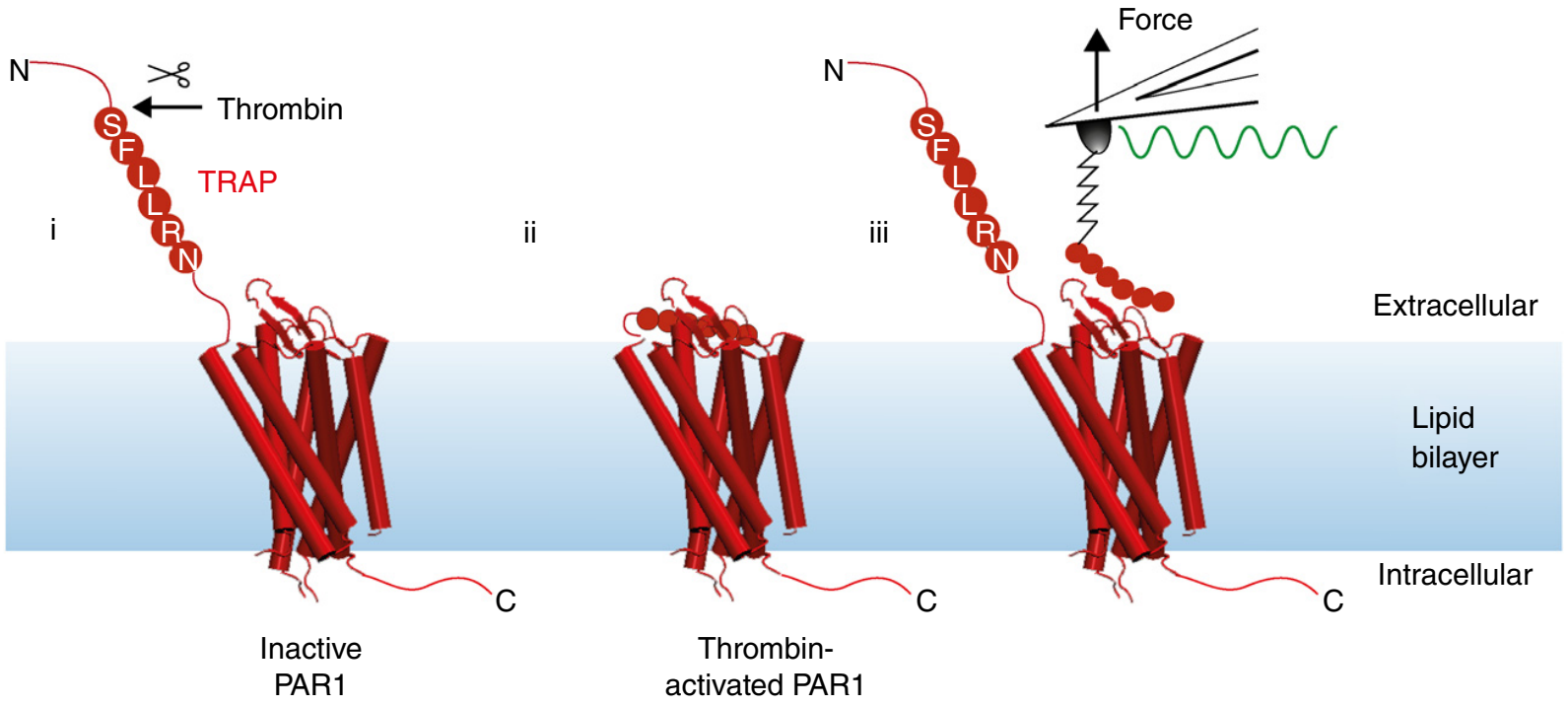
David Alsteens[1,5], Moritz Pfreundschuh[1,5], Cheng Zhang[2,4], Patrizia M Spoerri[1], Shaun R Coughlin[3], Brian K Kobilka[2] & Daniel J Müller[1]

Aim: image single native GPCRs in membranes and quantify their dynamic binding strength to native and synthetic ligands

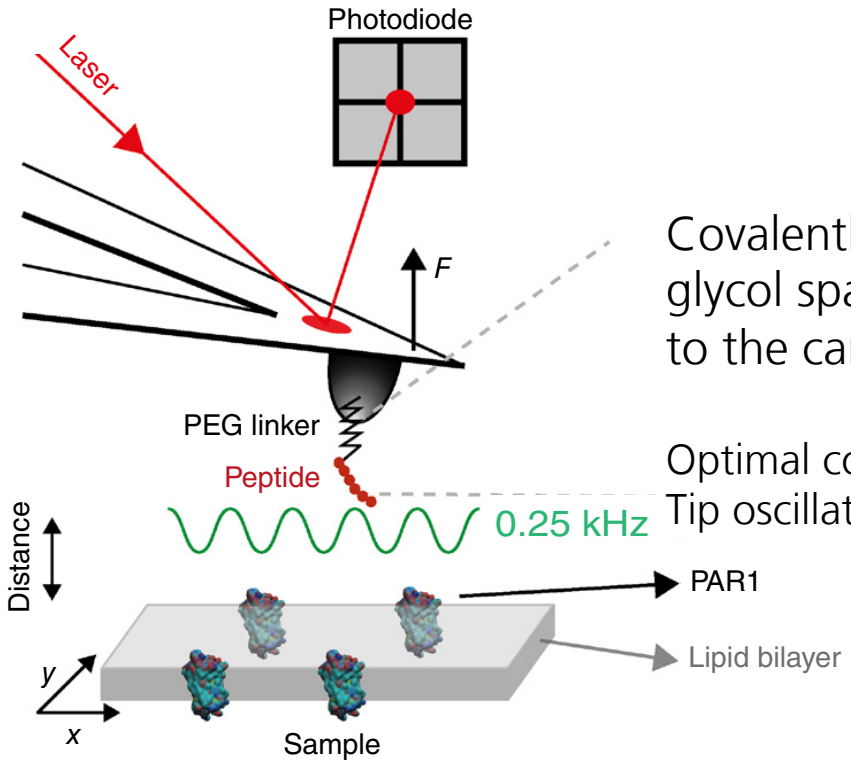
GPCR PAR1: Protease-activated receptor-1
Receptor for thrombin, important in coagulation cascade

GPCR PAR1: Protease-activated receptor-1

- Receptor for thrombin, important in coagulation cascade
- TRAP: Thrombin receptor-activating peptide, binds to heptahelical bundle
- Quantify how tethered ligands bind PAR1



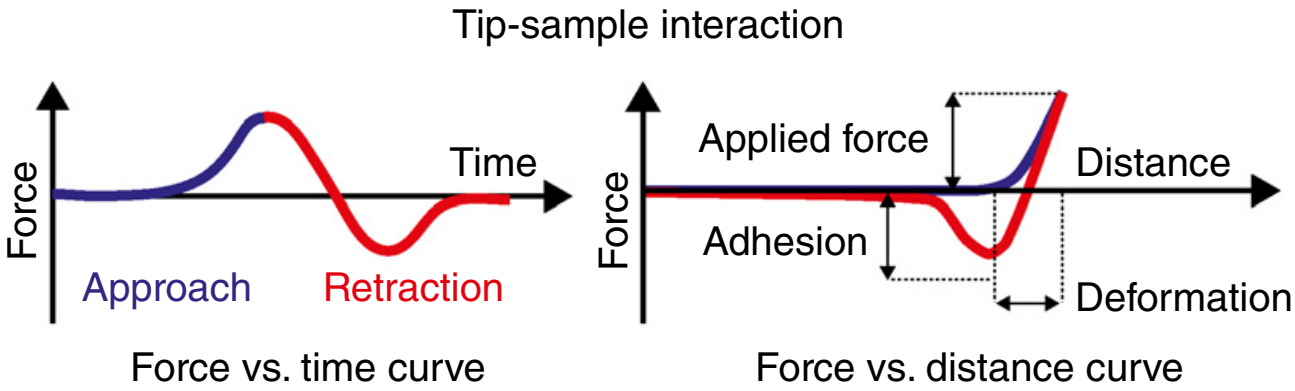
- Functionalize the AFM tip with the TRAP: physiological condition
- If brought to contact, ligand and receptor can bind
- Retraction breaks the specific bond, the required force is measured by the deflecting AFM cantilever



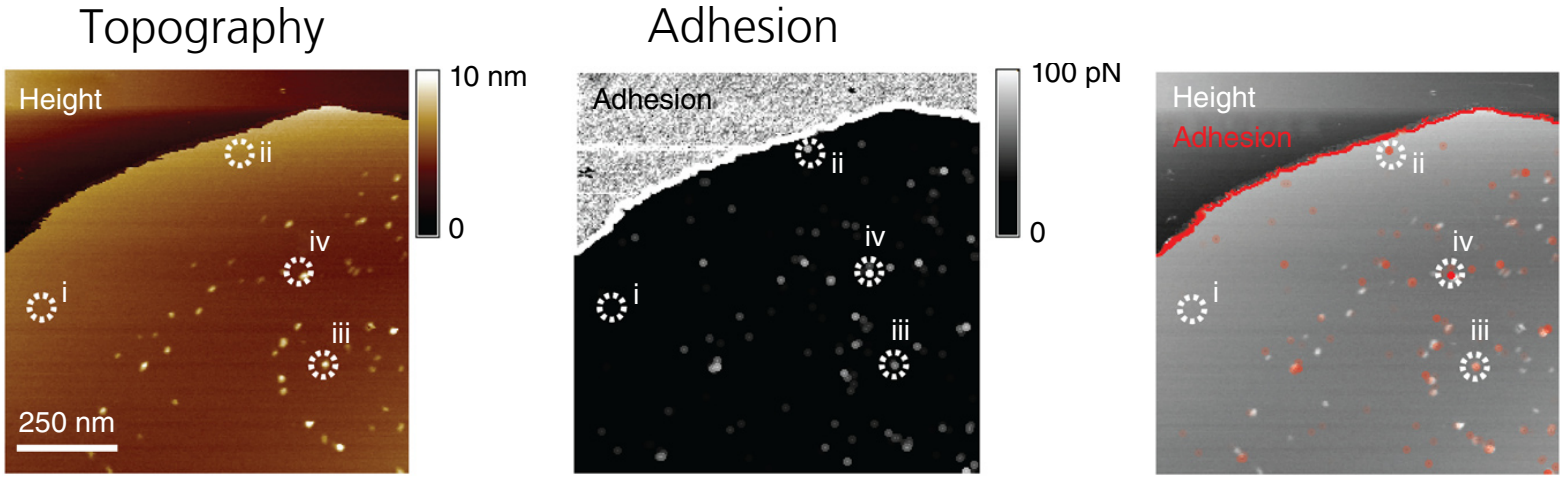
Covalently bind TRAP to PEG poly ethylen glycol spacer, which is chemically attached to the cantilever

Optimal conditions:
Tip oscillation at 0.25 kHz & amplitudes of 50 nm

Sample: human PAR1 in proteoliposomes

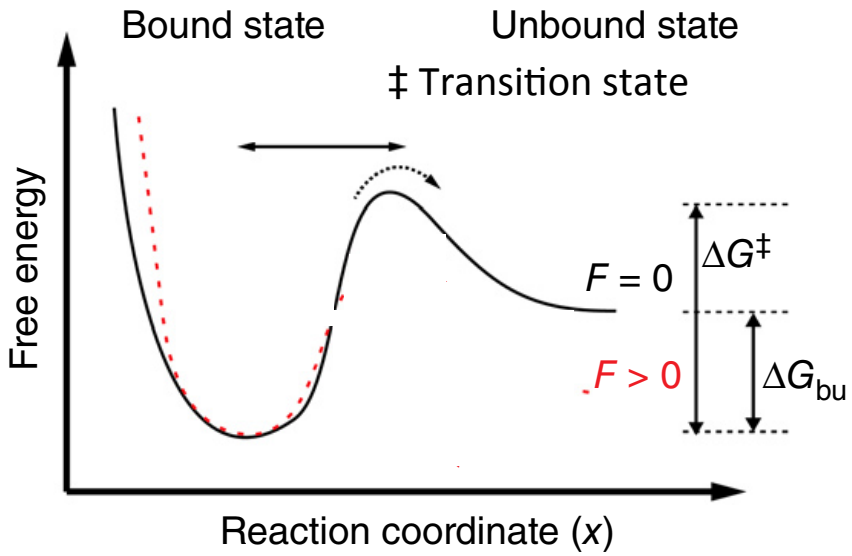


- Adhesion: Minimal force of the retraction FD curve
- Deformation: The difference between two applied imaging forces



- Topography: Membrane patches protrude 4.5 nm +/- 0.7 nm
- Topography = Height measurement is congruent with adhesion map

Free energy landscape of the receptor: Assess ligand binding free energy

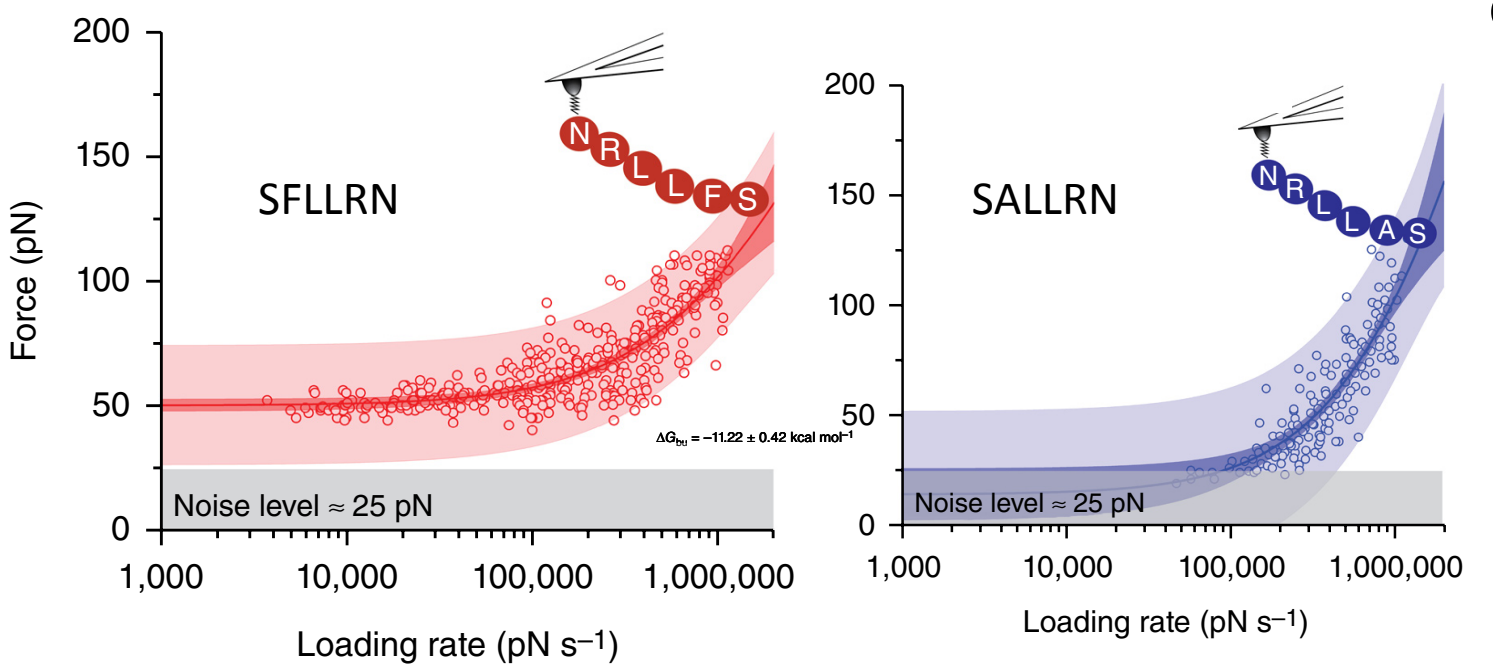


Activation free energy to cross the transition state

free-energy difference between bound & unbound state

- external force stressing a bound, reduces the activation energy barrier towards unbound state
- Apply external forces and measure how much is needed to break the ligand-PAR1 bond

- Force required to separate the ligand from PAR1 is plotted against the loading rate
- Loading rate: describes the force applied over time

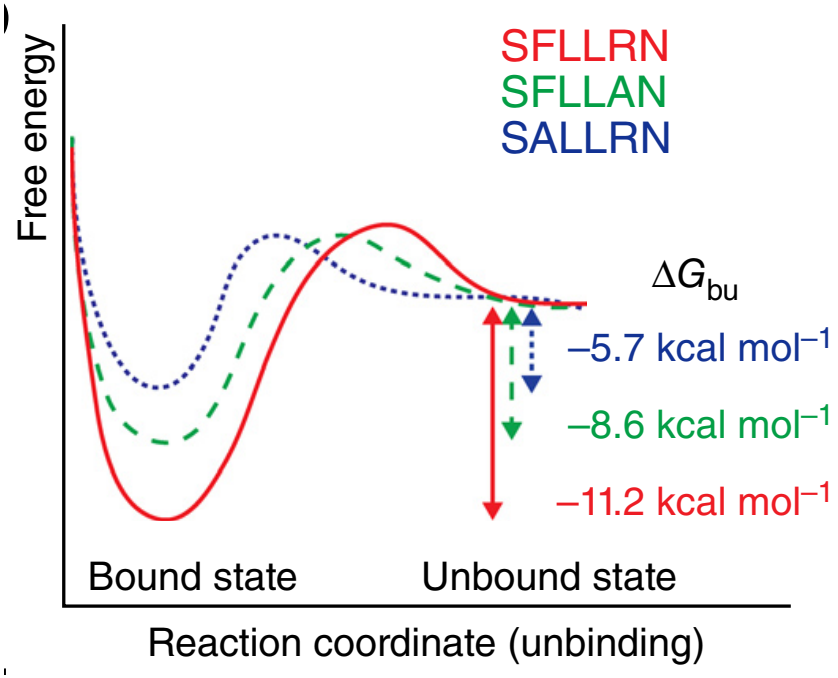


ΔGbu = –11.22 ± 0.42 kcal mol–1

ΔGbu = –5.73 ± 3.76 kcal mol–1

- SFLLRN: Phenylalanine and Arginine important for specificity
- Replace by Alanine: SALLRN and SFLLAN
- SALLRN: ΔGbu = –5.73 ± 3.76 kcal mol–1
- SFLLAN: ΔGbu = –8.61 ± 0.82 kcal mol–1
- reduced free energy difference, abolished high-affinity binding

Free energy binding landscape of three different peptide-ligands



- Free energy difference between the ligand-bound and unbound state
- Less energy needed to break bond
- Lower affinity to PAR1

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FD-based AFM allows to:

- Structurally quantify the physical (mechanical), chemical and biological properties of native proteins
- Is applicable for most membrane and water-soluble proteins.
- Image human PAR1 in proteoliposomes at high resolution and simultaneously quantify their dynamic binding strength to different ligands.

4. Summary & Outlook

- Limitation:
- Limited to the characterization of single native proteins in vitro
- Outlook:
  - Characterize single proteins in their native environment of the living cell or tissue to determine how proteins work in the cellular context and how cells control proteins to function as required.
  - Image cells at subnanometer resolution