Investigating Cell-Substrate and Cell-Cell Interactions by means of Single-Cell-Probe Force Spectroscopy

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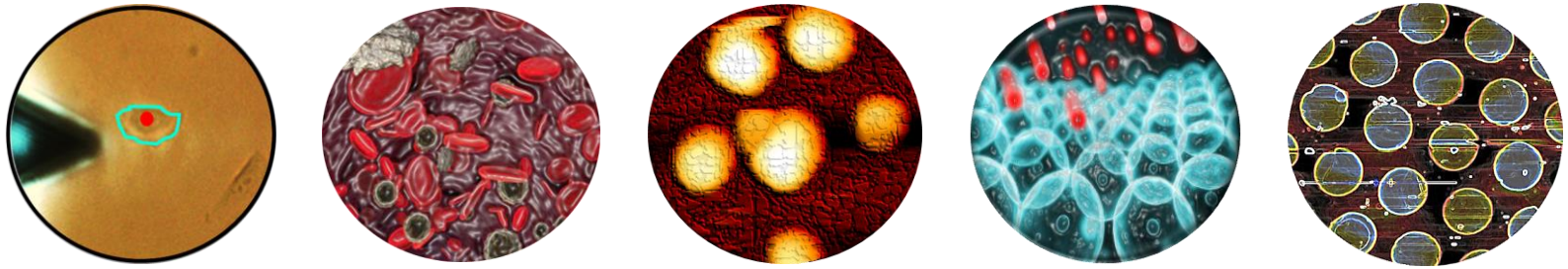
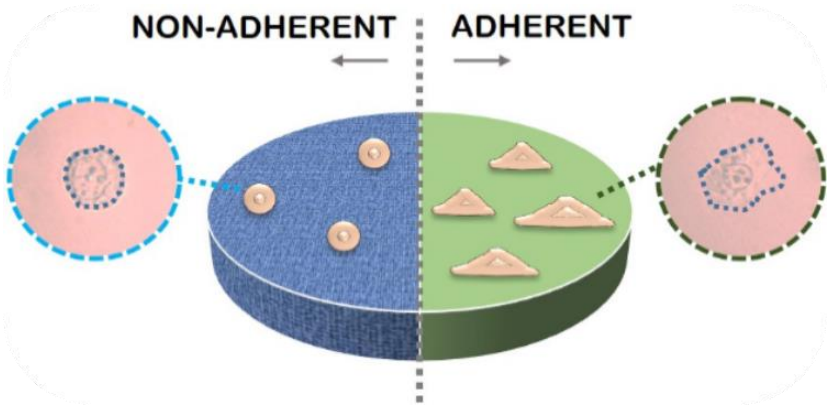
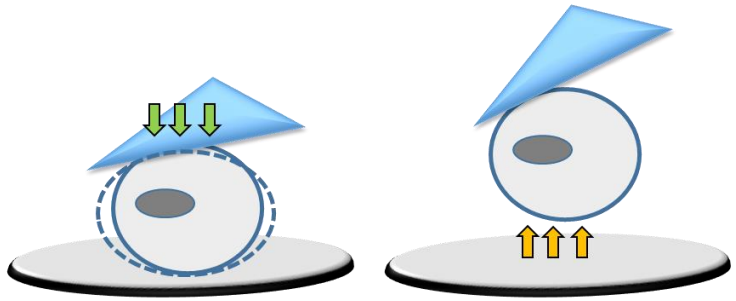


Contents

- Defining Single-Cell-Probe Force Spectroscopy
- Methodology
  - Current techniques
  - New Design: Two half-slide system
- Interacting force analysis
  - Cell-Substrate
  - Cell-Cell
- Conclusions

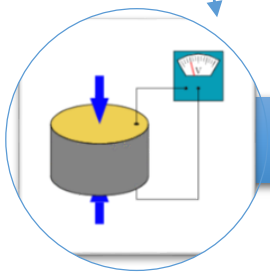




Atomic Force Microscopy

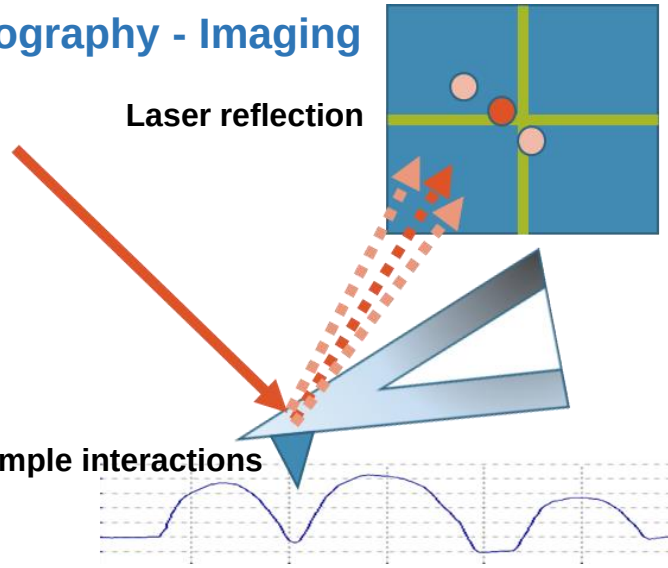
- Born in the 80s (Binnig and Rohrer)
- Scanning Probe Microscopy

Tip / lever



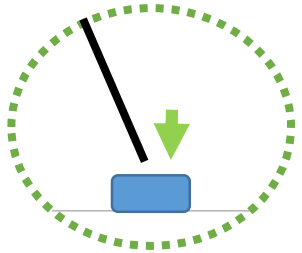
Piezo

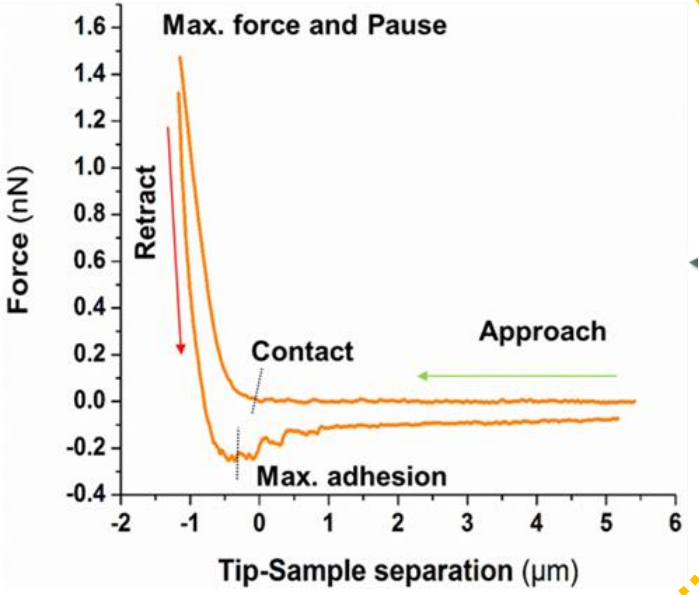
Topography - Imaging



Force Spectroscopy

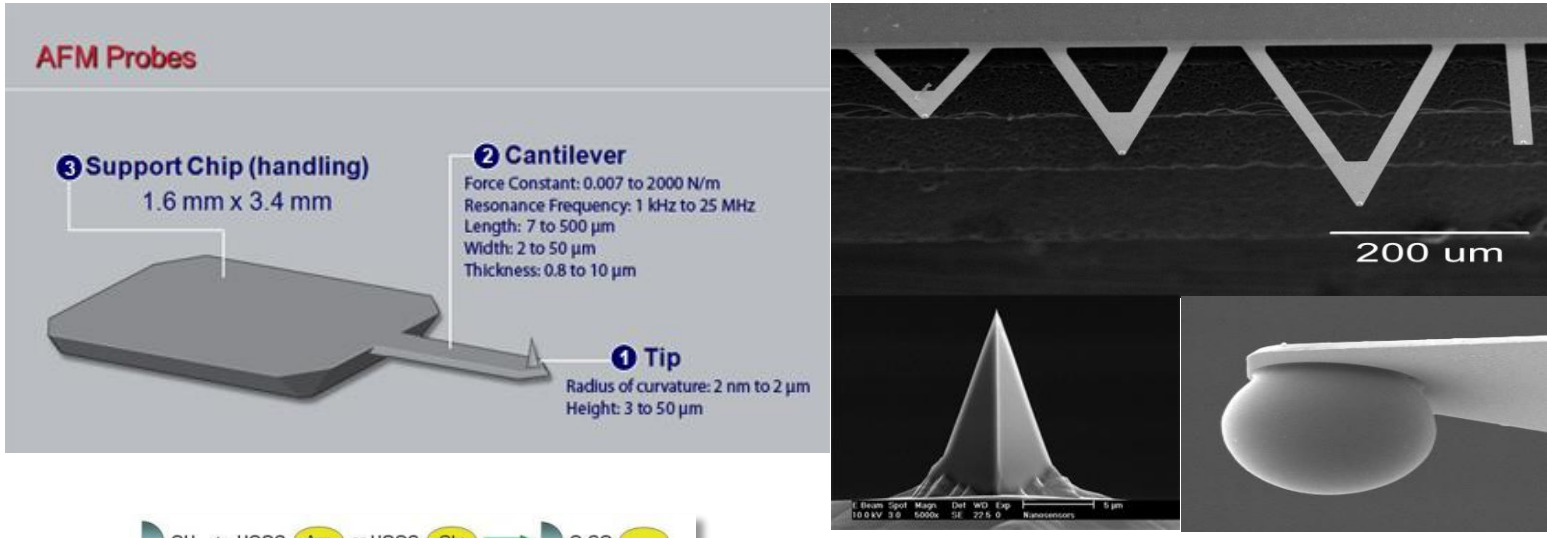
(F-d plots)



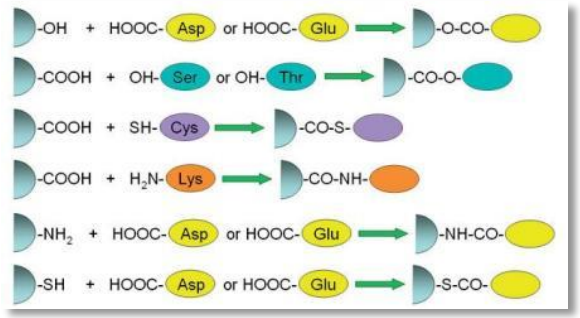


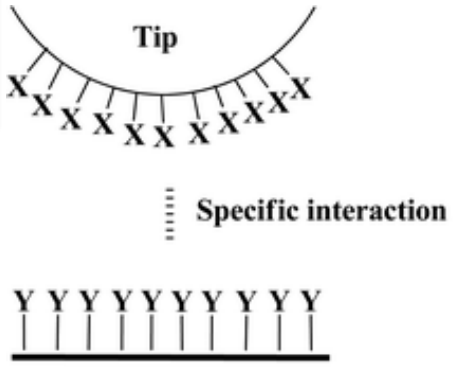
Atomic Force Microscopy

Cantilever selection



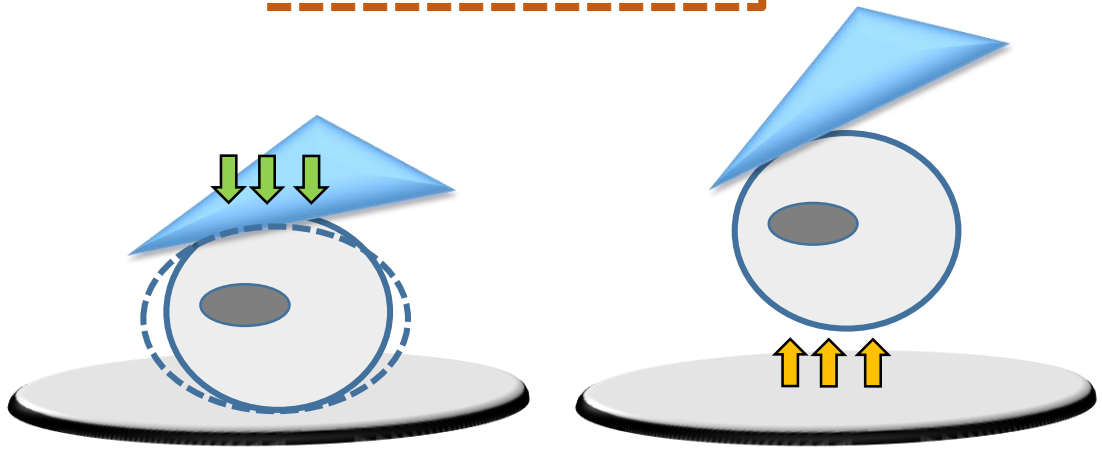
- **Spring constant** – force range (sample)
- **Tip geometry**: pyramidal vs colloidal vs flat
- **Chemical properties** of/on the tip



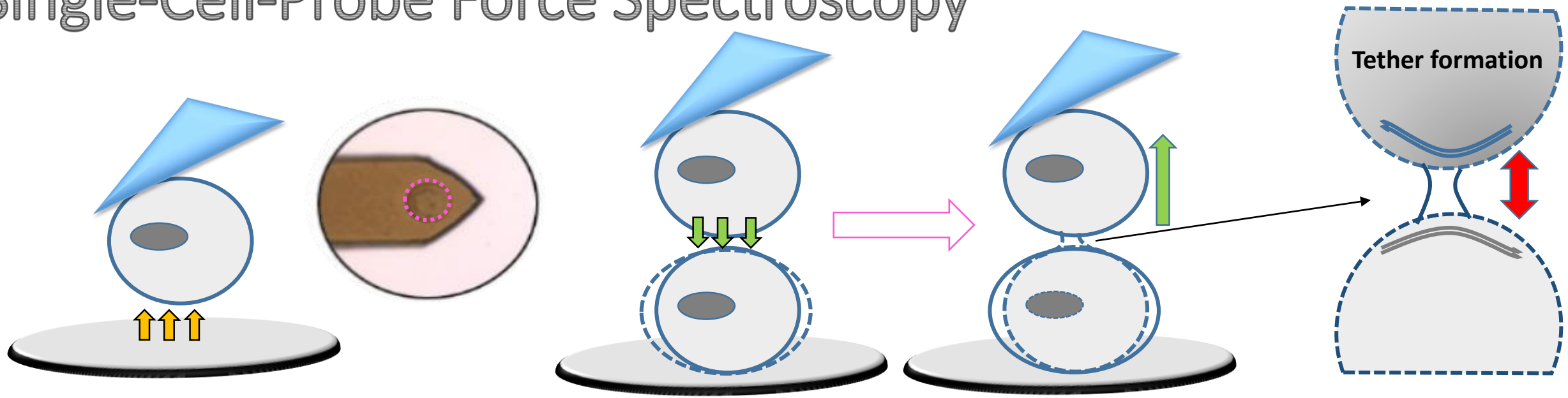


(non) Specific forces

Cell Capturing



Single-Cell-Probe Force Spectroscopy

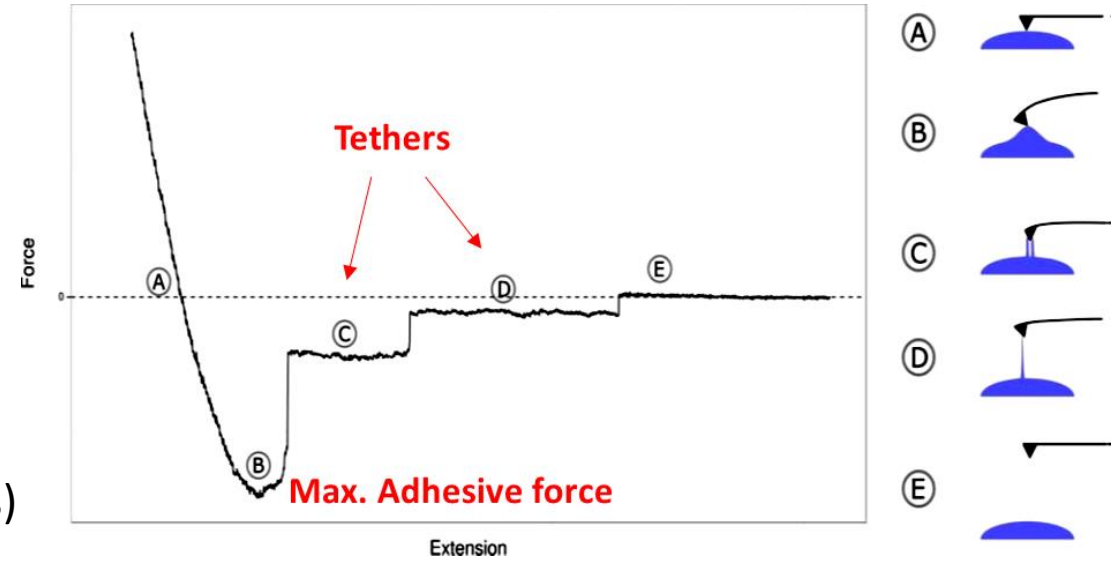


Living cell as measuring probe

Controlled manipulation supported by OM

Interacting Forces: Substrate vs Tissue formation

(Force-distance plots)



Benítez et al. (2017) Microsc. Res Tech.

Applied Methodology

- High cell-cantilever affinity
- Substrate-promoted capture enhancement

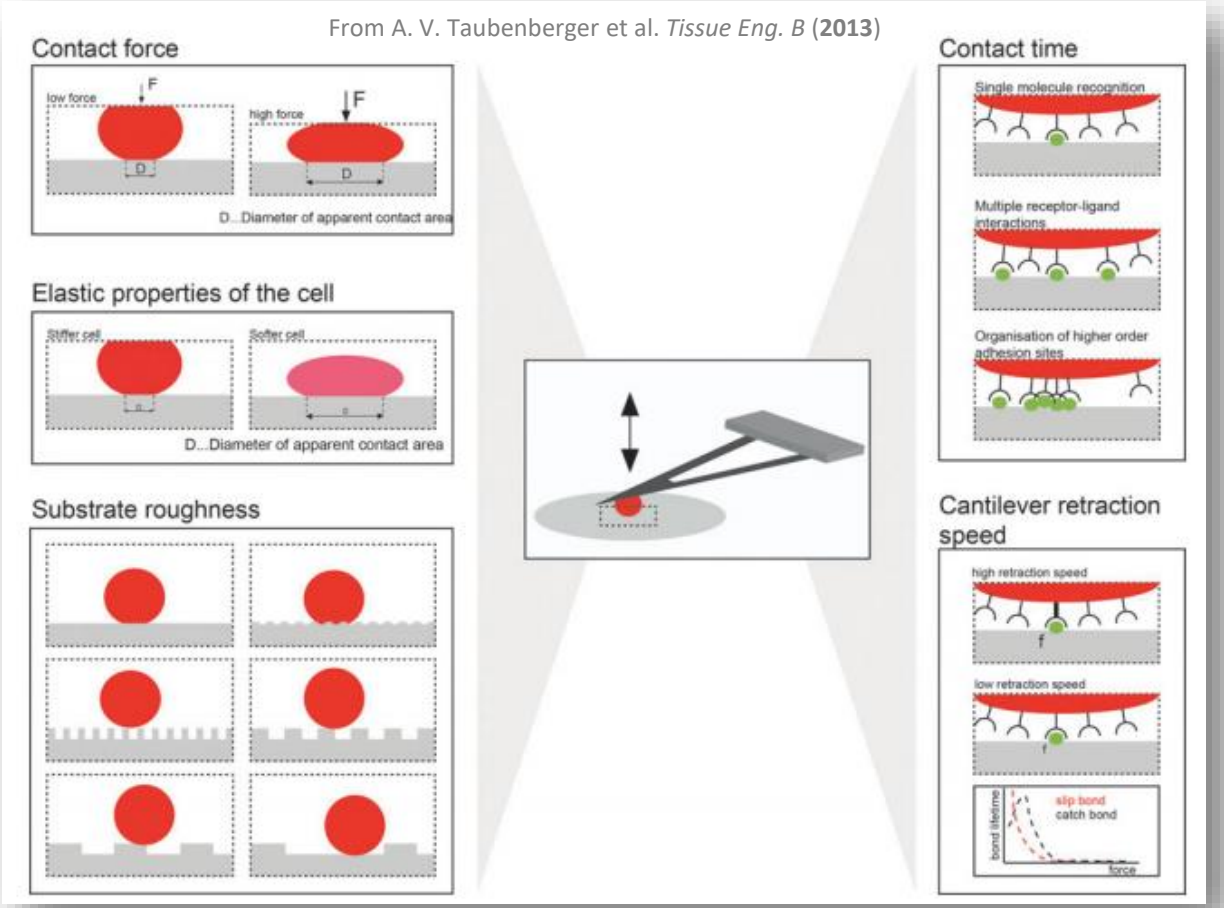
Cantilever Activation

Concavalin A – Carbohydrate groups
Streptavidin – Biotinylated cells
Poly L-Lysine (PLL) – Electrostatic charges
Fibronectin – Cell adhesion receptors
Dopamine (BD Cell Tak) – Polyphenolic proteins

Surface Passivation

BSA – not 100% efficient
PEG (-PLL)

Additional methods
Light-switchable compounds

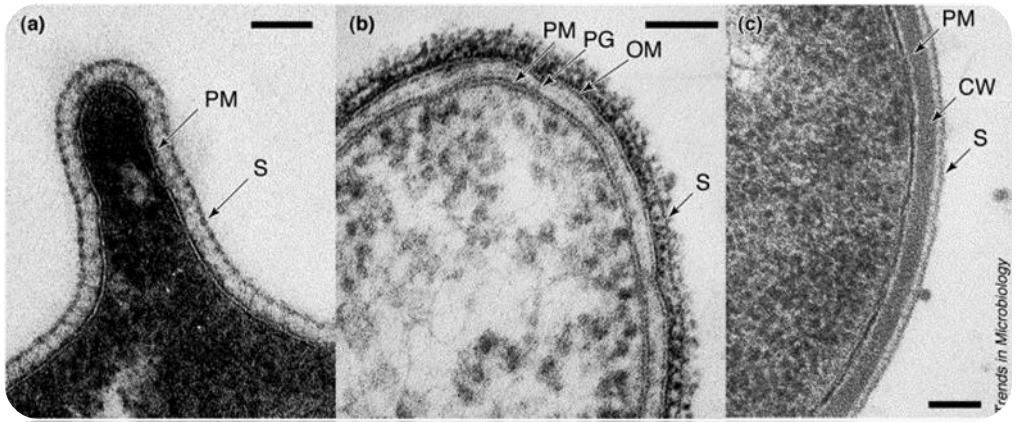


Maintenance of Round structure
Anti-Fouling activity

E. P. Wojcikiewicz et al. J Cell Sci (2003)
P, Panorchan et al. J Cell Sci (2006)
J. Friedrichs et al. Proteomics (2010)
G. Zeng et al. Langmuir (2014)
G. Selhuber-Unkel et al. Biophys J (2008)

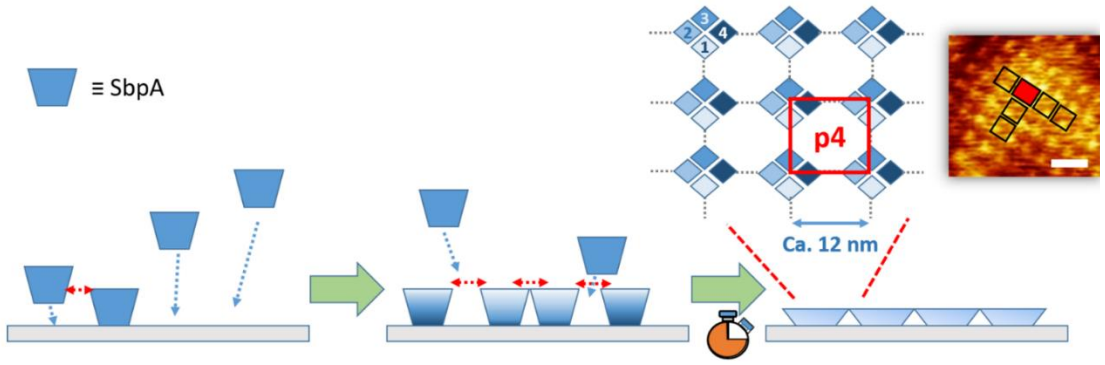
S-layer proteins - SbpA [Lysinibacillus sphaericus]

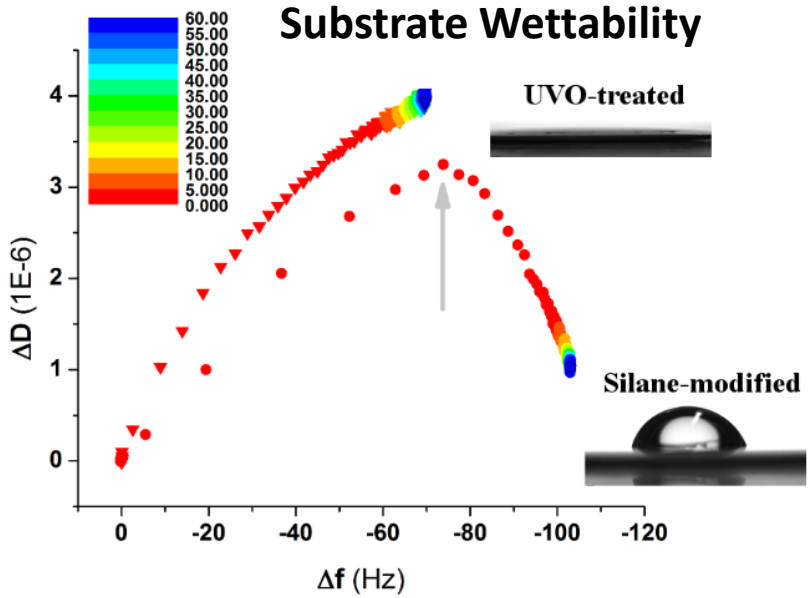
- Common in bacterial cell envelope (Archaea, Gram +/-)
- Identical proteins or glycoproteins and built via self-assembly.
- Different lattice symmetry: *oblique* (p1, p2), ***square* (p4)** or *hexagonal* (p3, p6) depending of the protein subunits involved.
- Thickness between 5 and 25 nm
- Recrystallization after **isolation and purification**
- Successfully tested on diverse 2D and 3D interfaces

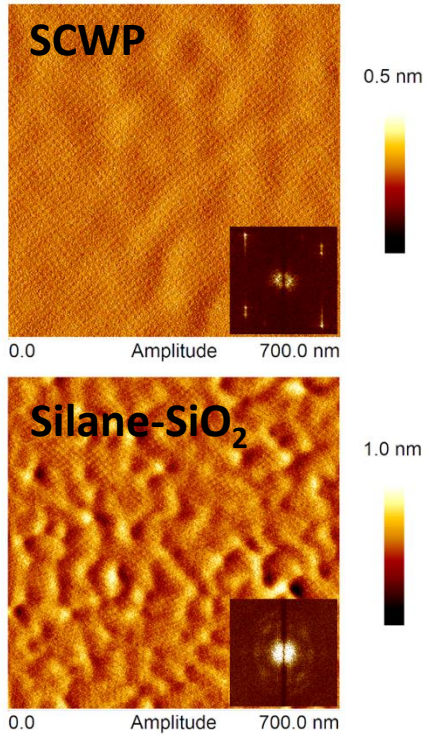


Electron micrographs of thin sections *of (a) an* archeon (*Sulfolobus acidocaldarius*), (b) a Gram-negative bacterium (*Aeromonas salmonicida*) and (c) a Gram-positive bacterium (***Bacillus thuringiensis***), all of which possess a crystalline cell surface layer (S-layer). Abbreviations: CW, Gram-positive cell wall; OM, outer membrane; PG, peptidoglycan layer; PM, plasma membrane; **S, S-layer.** Scale bar = 50 nm.

U. B. Sleytr, T. J. Beveridge. *Trends in Microbiology* (**1999**) 7 (6), 253-260





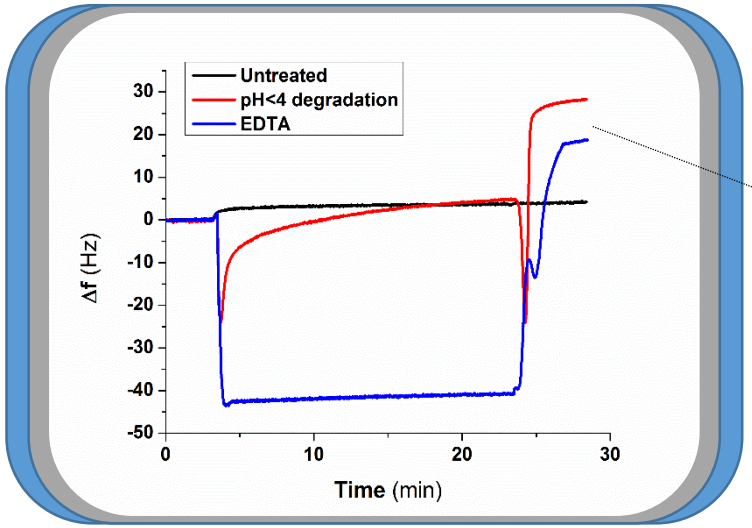


J. Iturri et al. *Beilstein J Nanotechnol* (**2017**)
J. Iturri et al. Col. Surf. A (**2016**) in press

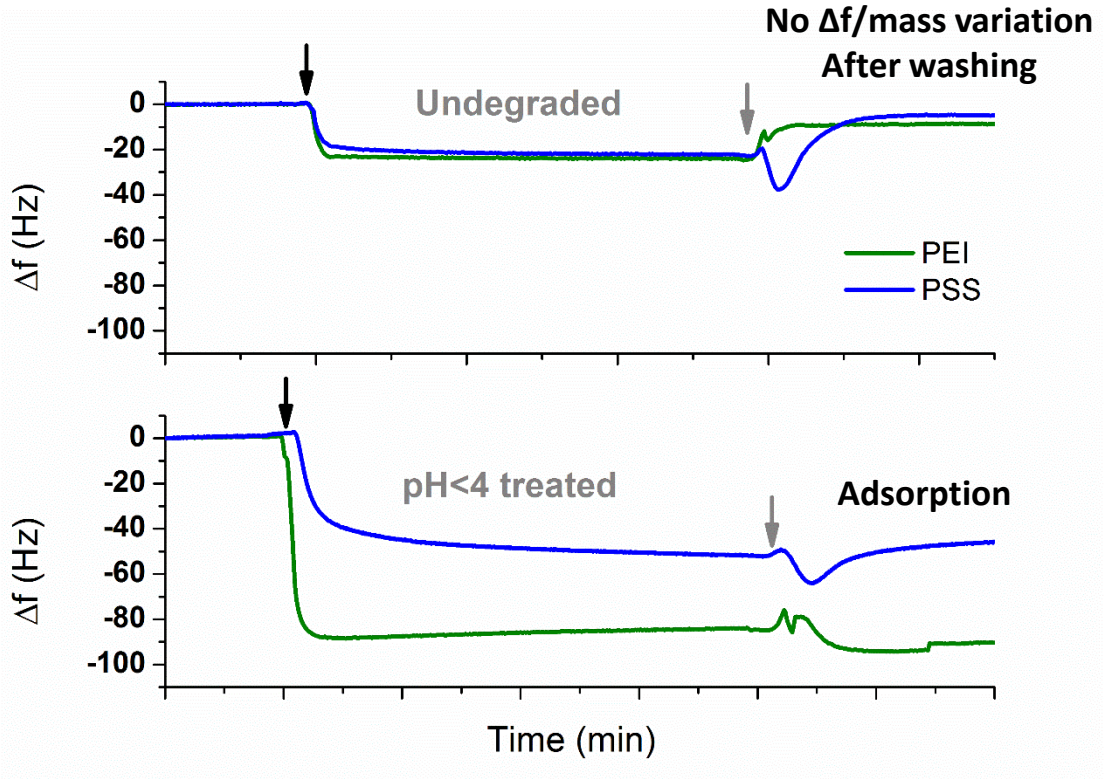
S-layer proteins - SbpA [Lysinibacillus sphaericus]

Antifouling activity vs Controlled degradation

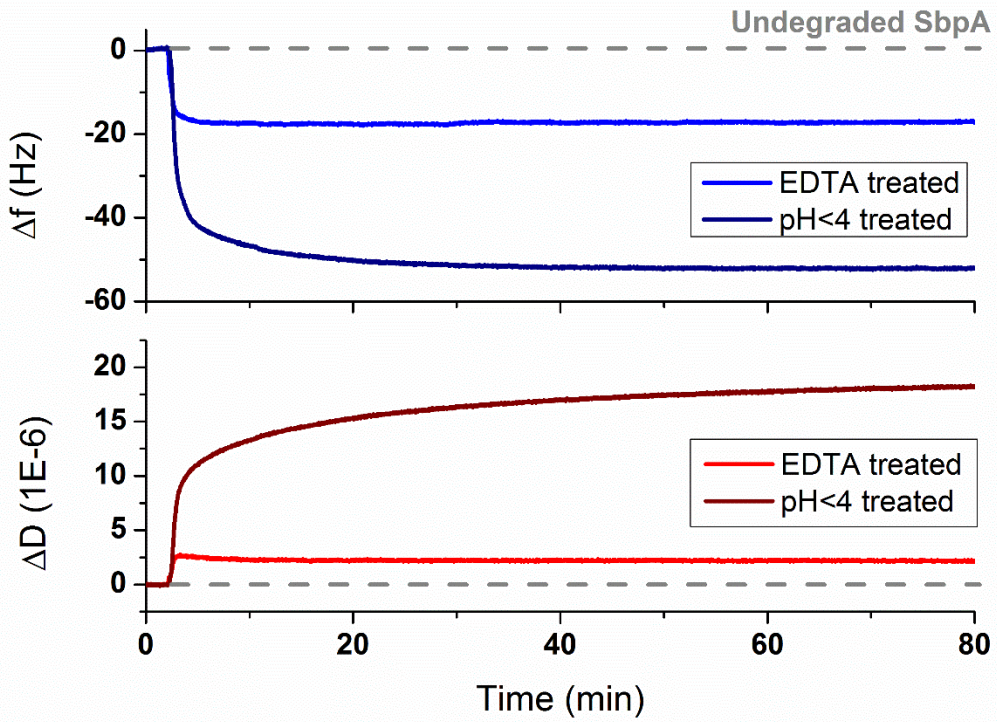
- **EDTA** as Ca^{2+} chelating agent
- Full structural disruption at pH < pI (**Citrate buffer**)



Protein release monitoring



Proteins/Polyelectrolytes

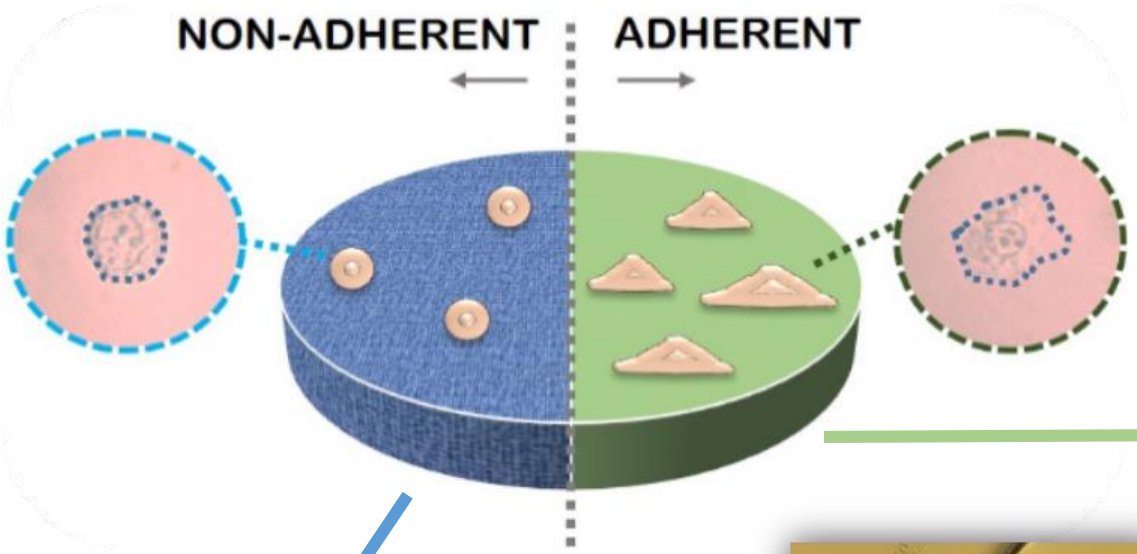


Cell adsorption

J. Iturri et al. *in preparation* (**2017**)

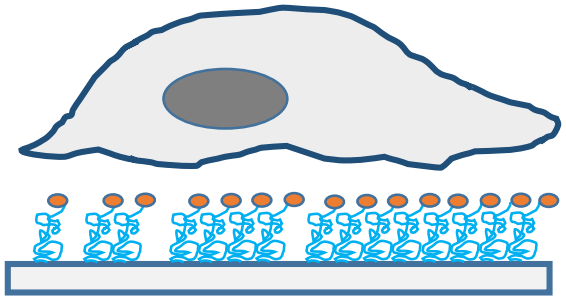
Developed Methodology

- **2x** precision cut half-covers
  (allows individual modification)
- Supportive cover slide underneath
- MCF-7 cell line

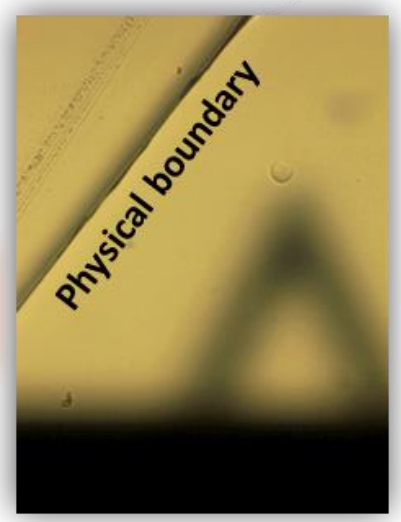


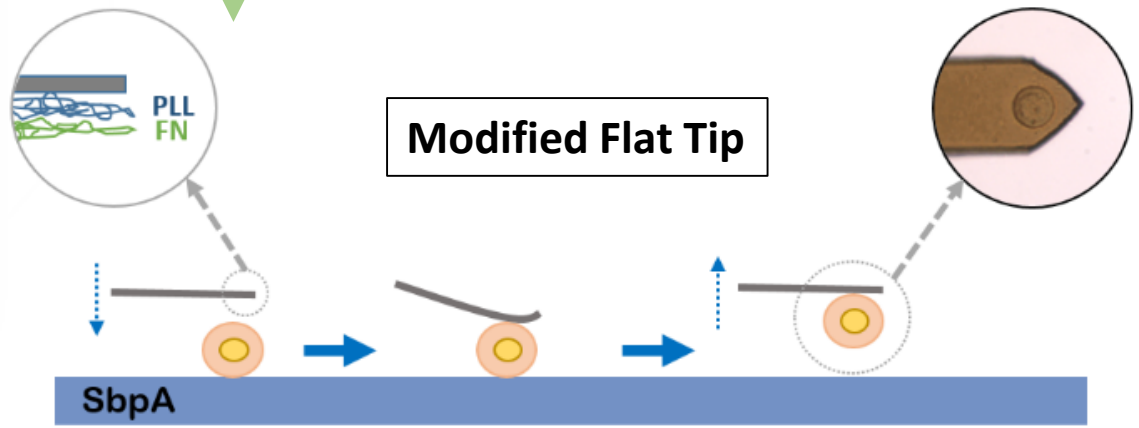
Fibronectin

Specific binding to integrins



Bacterial Crystalline S-layer

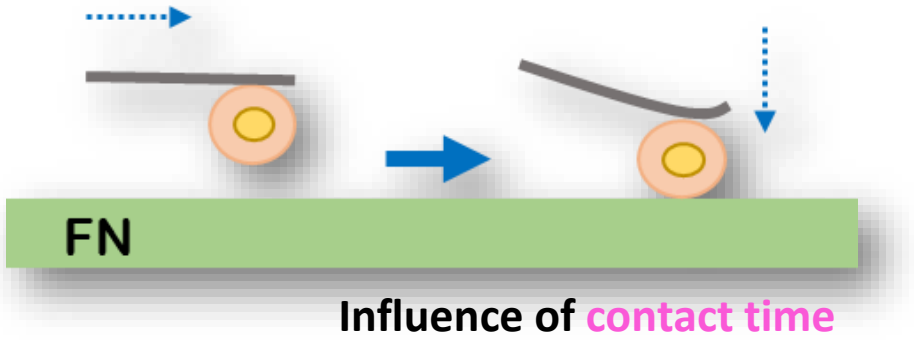




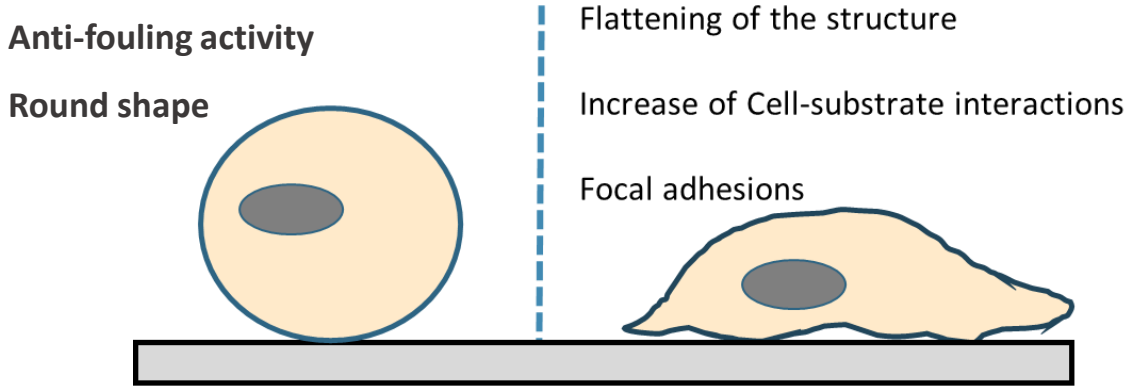
Modified Flat Tip

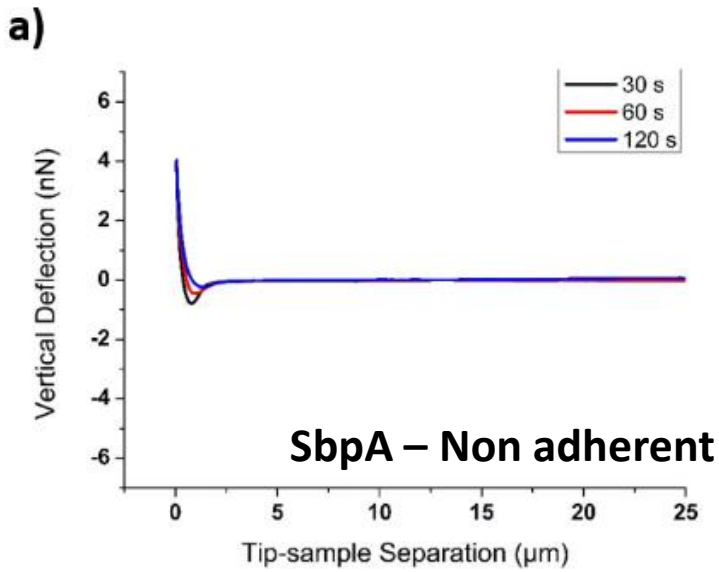
A. Moreno-Cencerrado et al. *Microsc Res Tech* (**2017**)

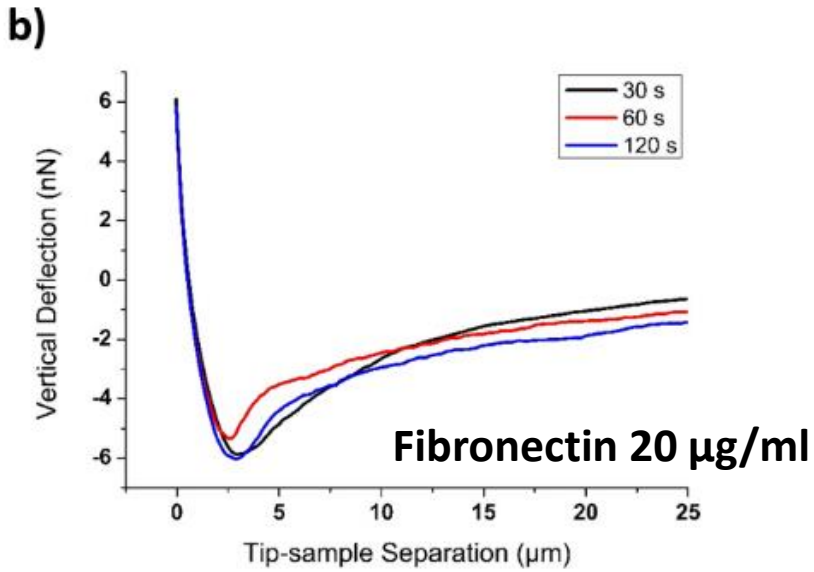
Results - Cell to surface: SbpA vs FN

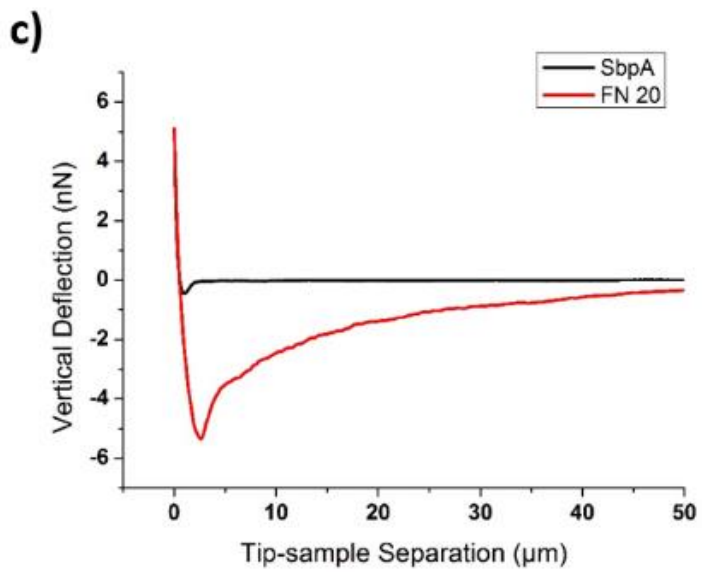


Influence of contact time





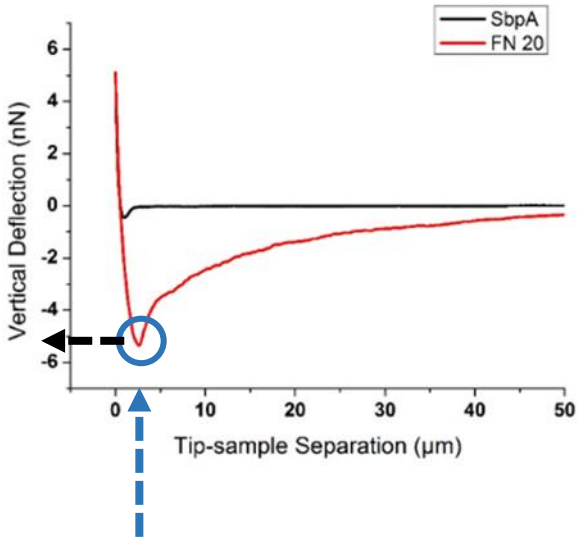


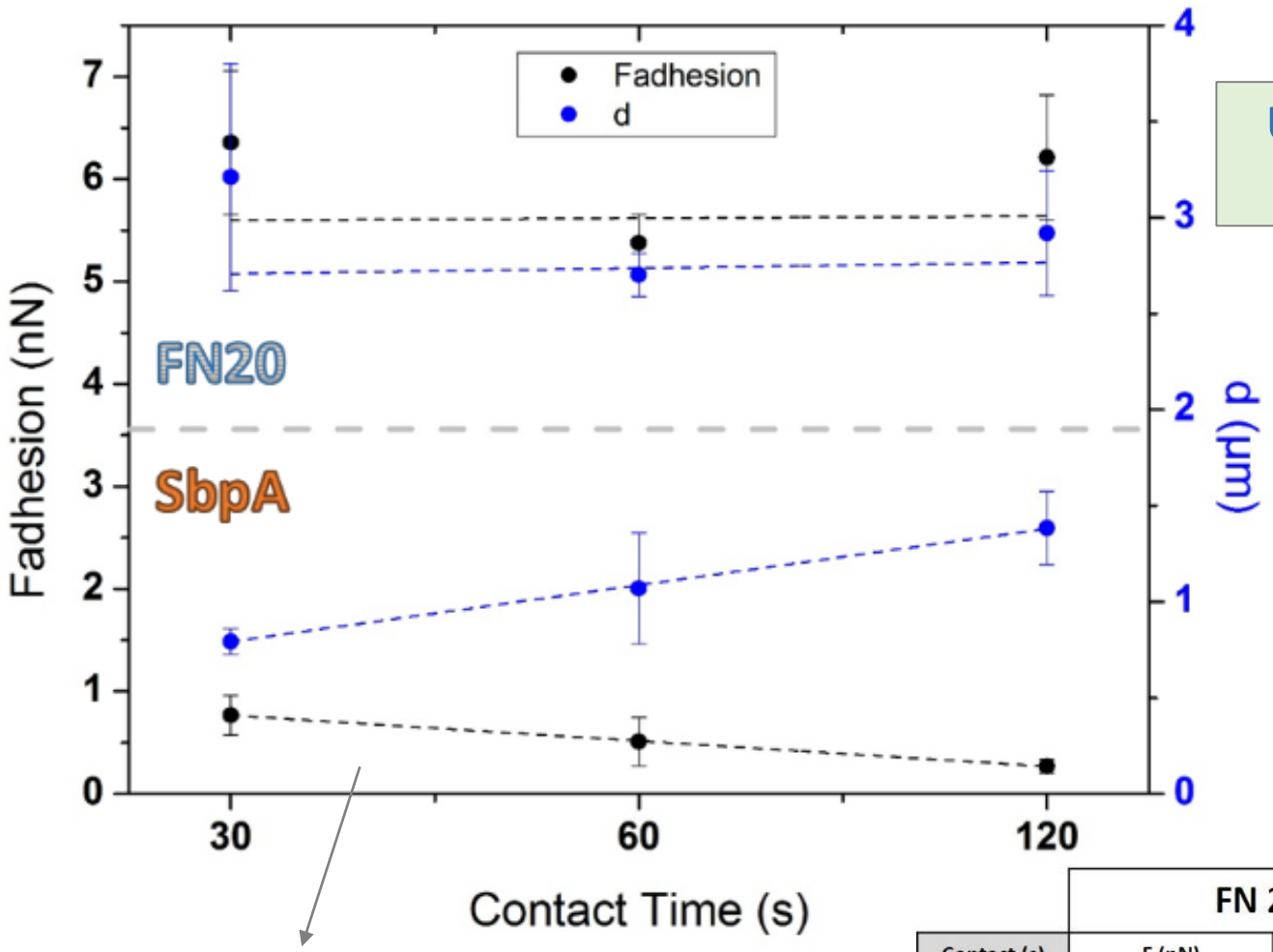


Very low MCF7-SbpA adhesive force

A. Moreno-Cencerrado et al. *Microsc Res Tech* (**2017**)

Results - Cell to surface: SbpA vs FN





Unaltered MCF7-FN adhesive forces

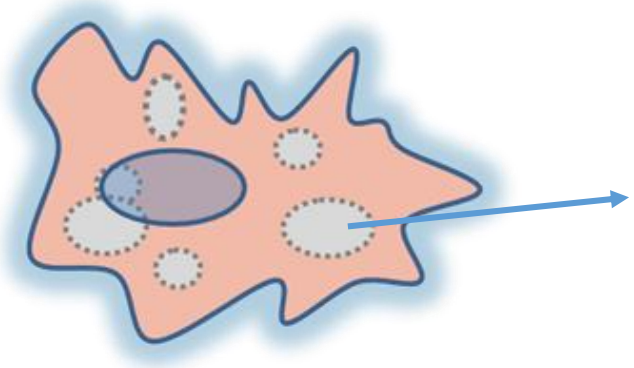
	FN 20		SbpA	
Contact (s)	F (nN)	d (µm)	F (nN)	d (µm)
30	6.357 ± 0.699	3.211 ± 0.591	0.768 ± 0.193	0.793 ± 0.066
60	5.375 ± 0.280	2.701 ± 0.112	0.509 ± 0.236	1.070 ± 0.289
120	6.213 ± 0.609	2.918 ± 0.324	0.268 ± 0.067	1.384 ± 0.190

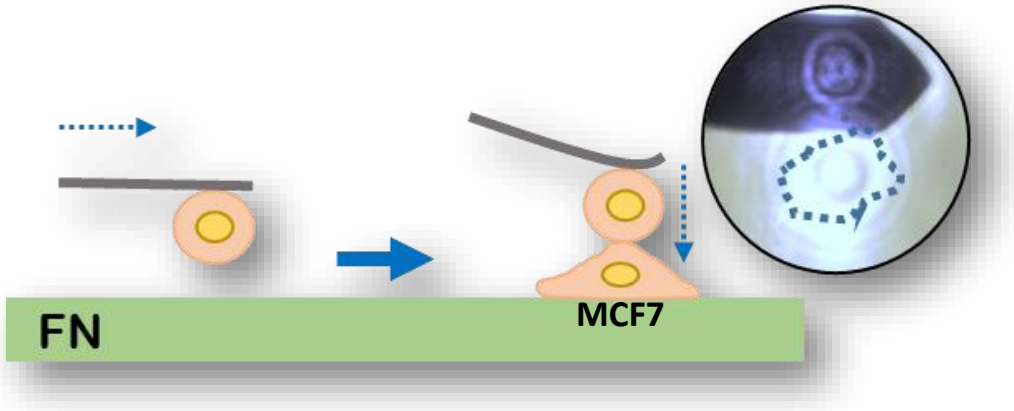
Low MCF7-SbpA adhesive force for longer contacts

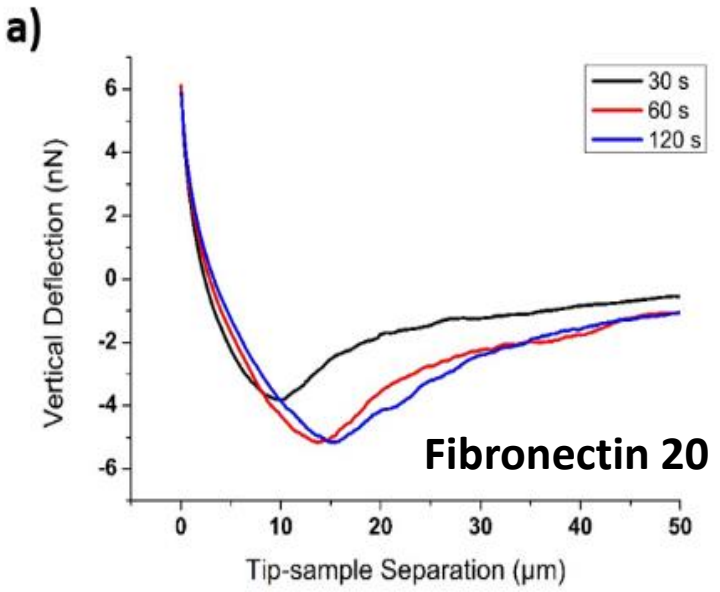
A. Moreno-Cencerrado et al. *Microsc Res Tech* (**2017**)

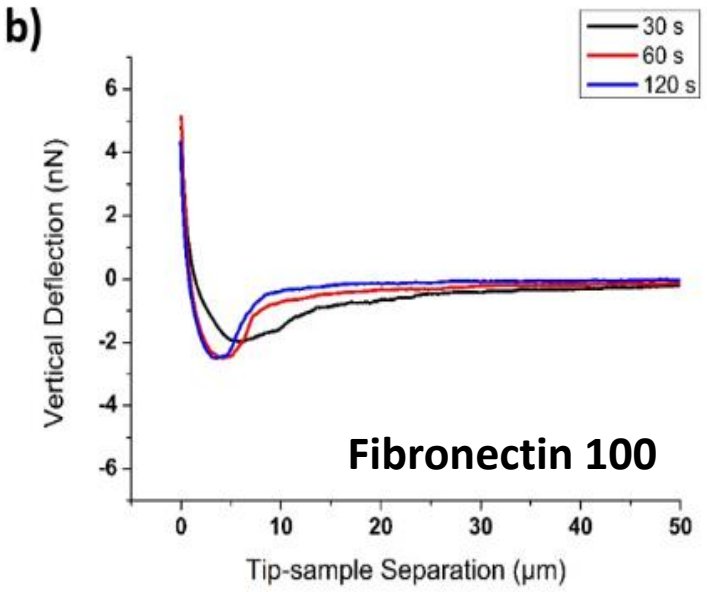
Results - Cell to cell: FN 20 vs 100

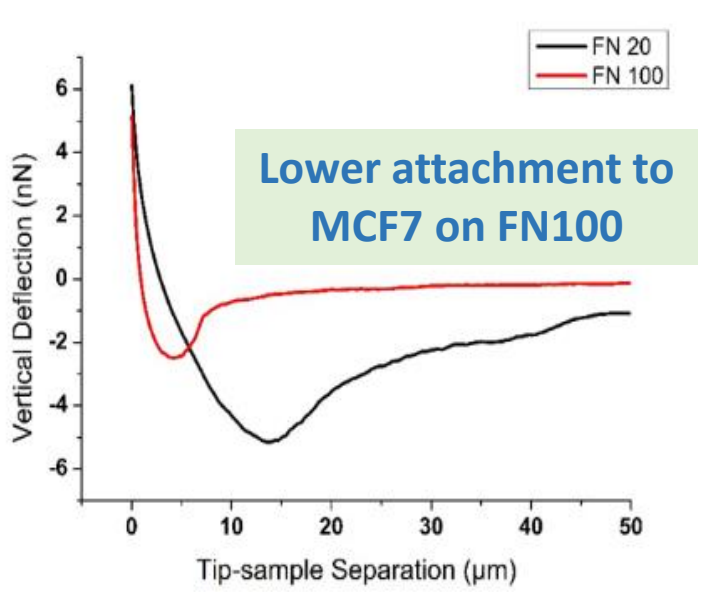
Higher concentration promotes spreading & focal adhesions







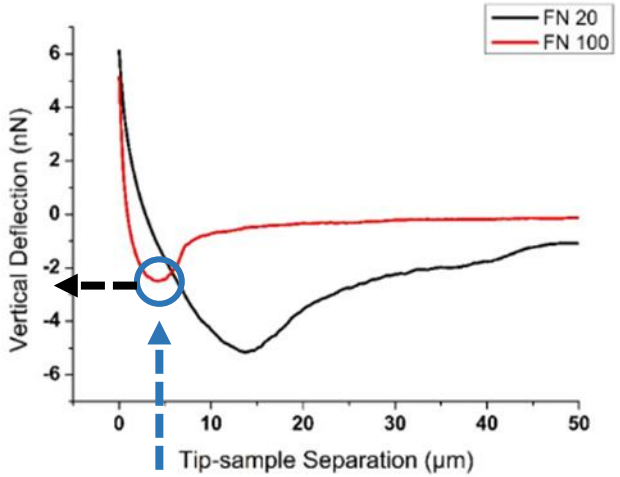


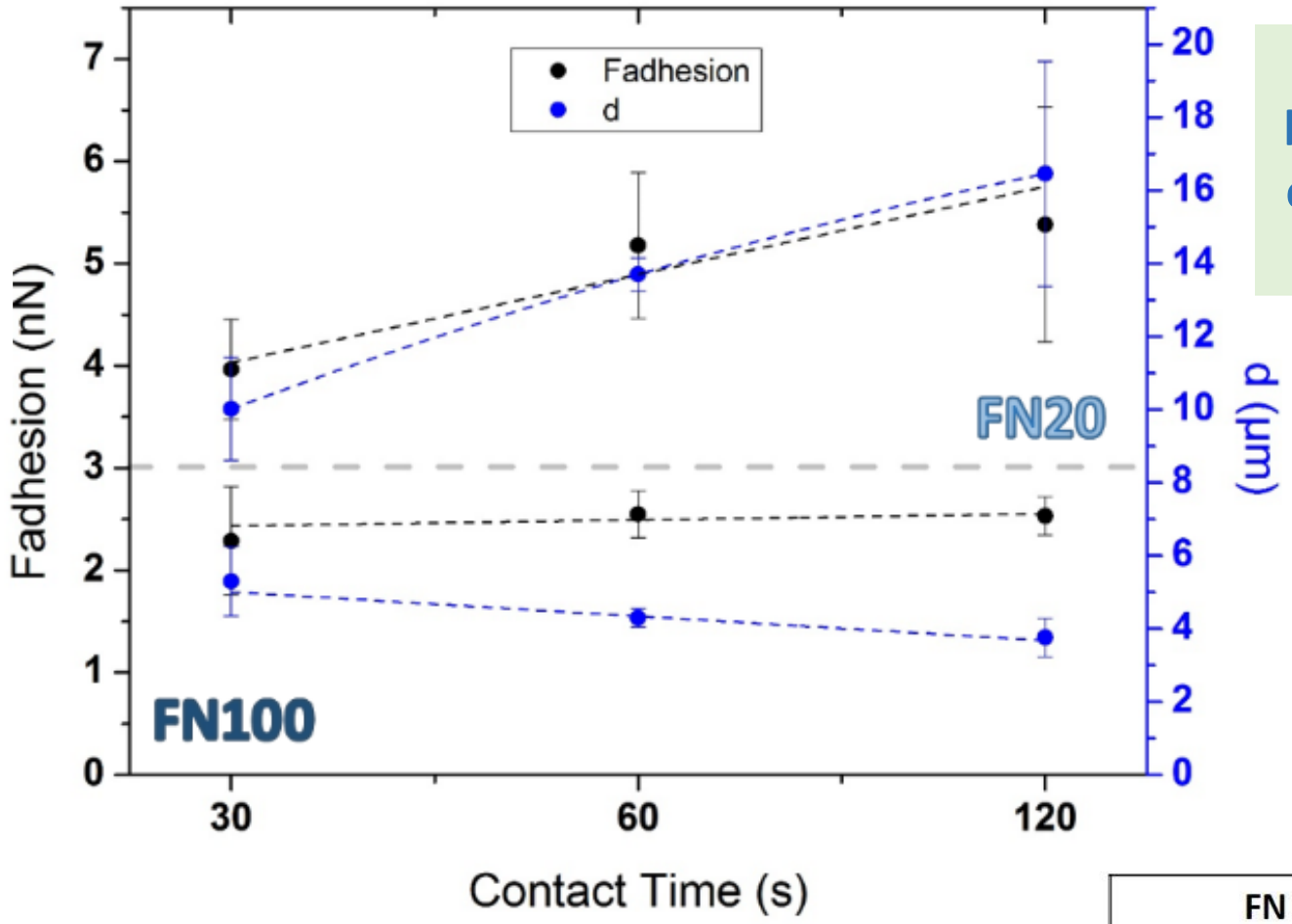


Lower attachment to MCF7 on FN100

Higher affinity for the surface induces hardening of the cell membrane
More limited cell-cell interaction

Results - Cell to cell: FN 20 vs 100





Stronger cell-cell binding for "rounder" cells under increasing contact times.

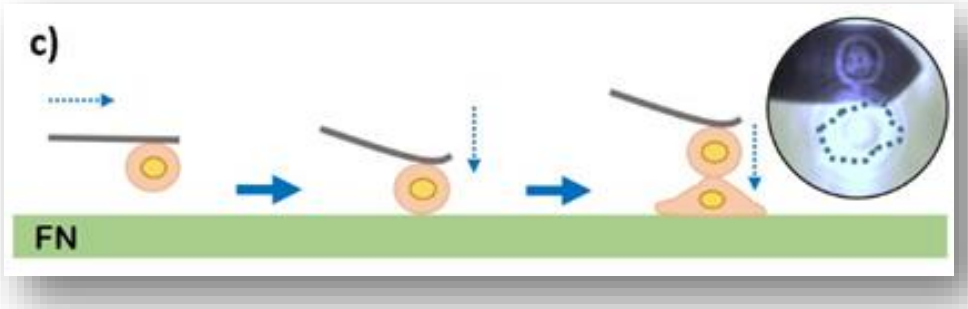
Higher surface affinity induces weak MCF7-MCF7 contact

	FN 100		FN 20	
Contact (s)	F (nN)	d (µm)	F (nN)	d (µm)
30	2.289 ± 0.528	5.303 ± 0.956	3.965 ± 0.490	10.019 ± 1.411
60	2.549 ± 0.230	4.301 ± 0.254	5.179 ± 0.713	13.704 ± 0.446
120	2.534 ± 0.186	3.769 ± 0.530	5.382 ± 1.150	16.462 ± 3.082

A. Moreno-Cencerrado et al. *Microsc Res Tech* (**2017**)

Conclusions

- Successful S-layer based procedure for Single-Cell-Probe
- MCF-7:
  - Low affinity for SbpA proven vs Specific FN
  - Substrate-supported Cell: Increasing surface affinity, lowered cell-cell
- Infinite possibilities: Cell lines / Substrates…
- Tissue formation studies, pathologies, Influence of drugs/nutrients…..



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