Elettra
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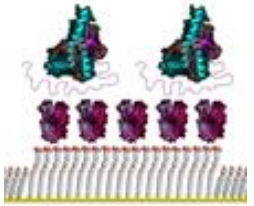


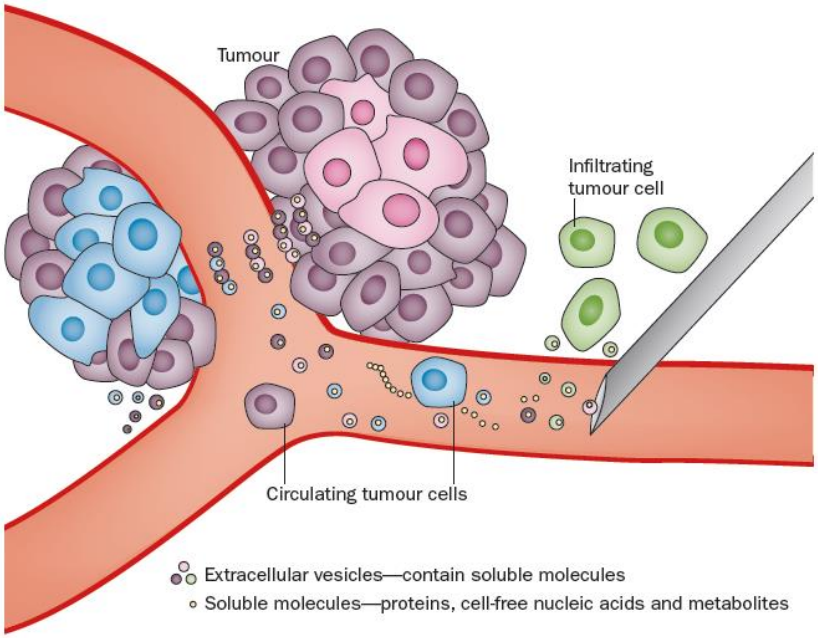
NanoInnovationLab
Elettra Sincrotrone Trieste
http://www.elettra.eu/labs/nanostructure

Nanoscale strategies for high sensitivity liquid biopsy

Loredana Casalis
loredana.casalis@elettra.eu

Bilateral Workshop on Nanotechnology and Nano Application
February 5 - 6, 2020, Ljubljana, Slovenia

Miniaturized sensors for liquid biopsy

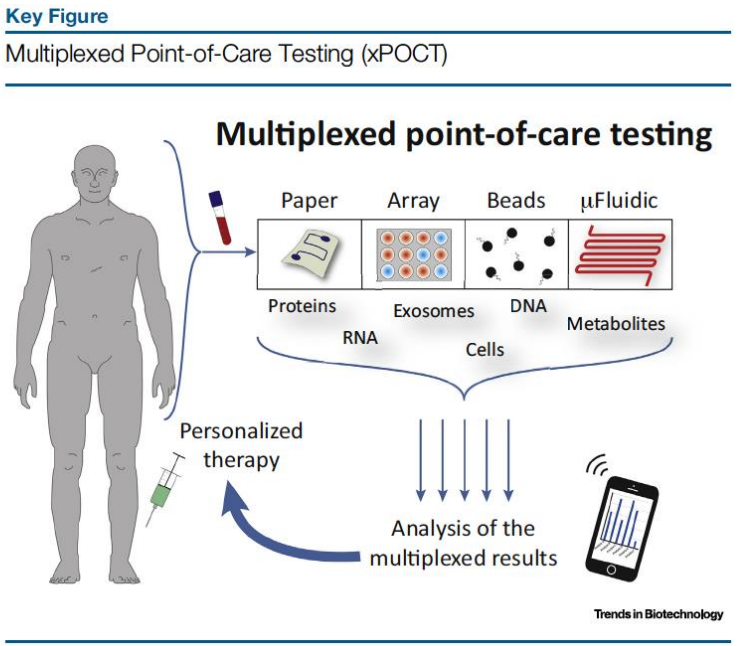


Liquid Biopsy

Both healthy and tumor (diseased) cells transmit information on their state to the bloodstream, either directly (proteins) or as cell signaling intermediate (exosomes, metabolites).

Such information are possible **biomarkers** for **early non-invasive population screening**, cancer diagnosis and prognosis and for **monitoring the response to therapeutic**

Emerging application in resource-limited settings: early and accurate diagnosis



Device equirements:

High sensitivity ---binders!
Reproducibility ---surface functionalization
Low noise --- no aspecific binding
Low cost
Fast

M. Westpahl, Nature Review Neurology, 2015
C. Dincer et al., CellPress 2017

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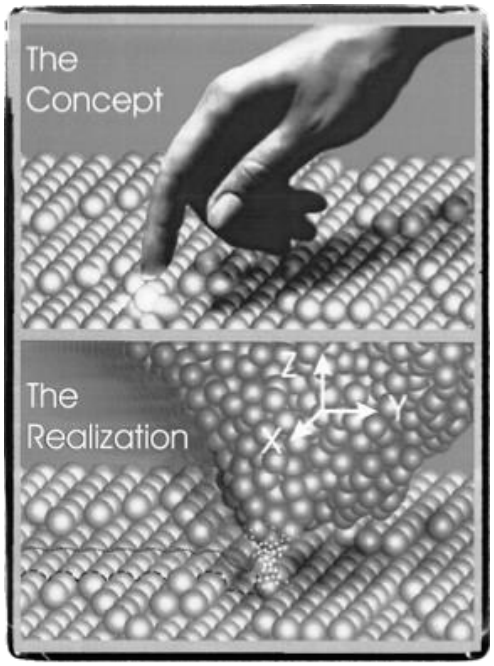
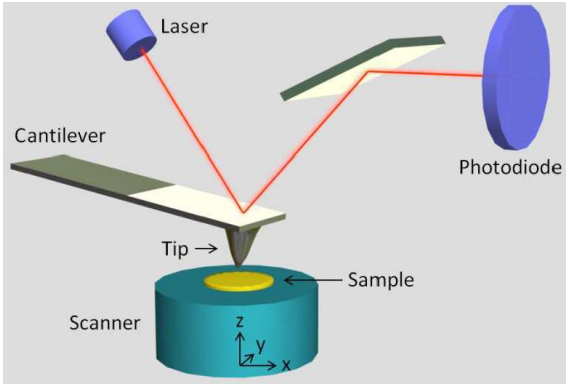
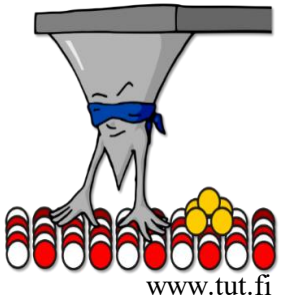
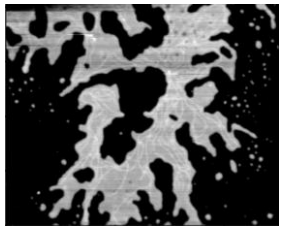
Atomic Force Microscopy Lithography

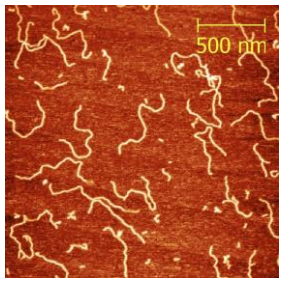


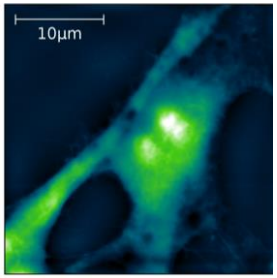


Imaging

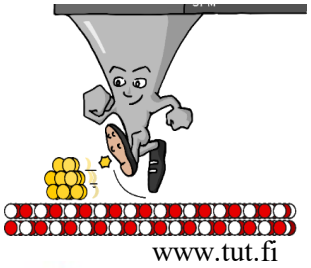


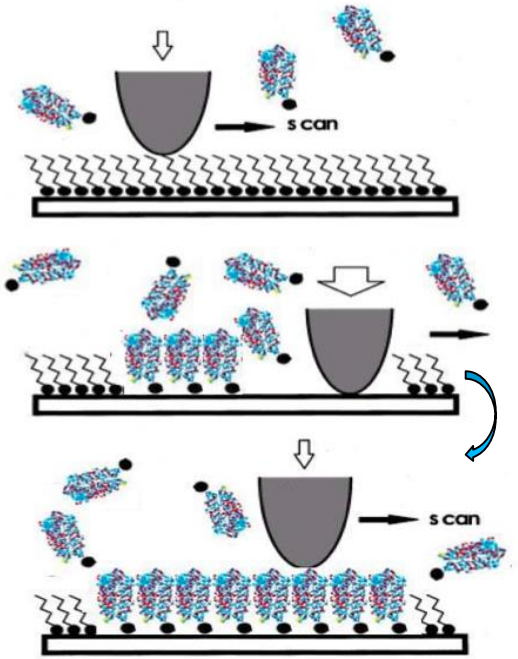




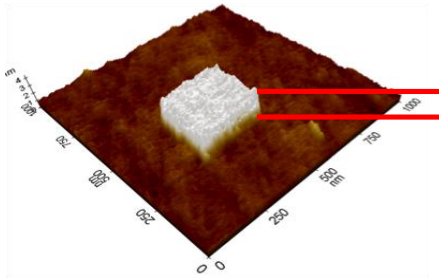


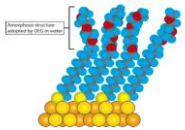
Nanografting for DNA/Protein
Nanoarrays

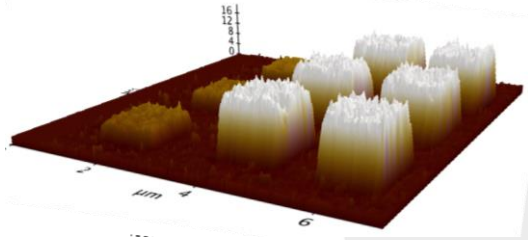




ssDNA into a C11-OEG3 SAM







Nanoarrays

Detection of ECD-Her2 with nanobodies in serum

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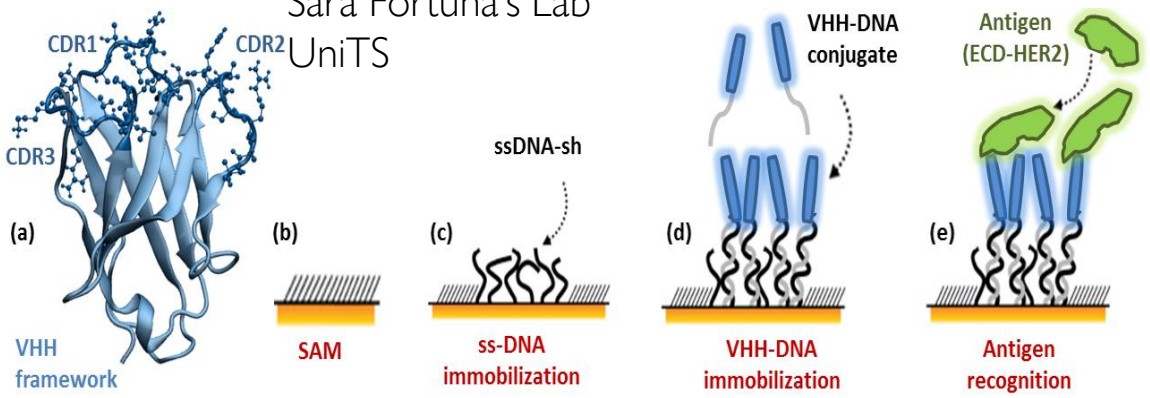
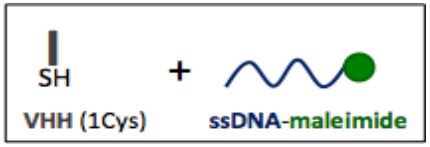
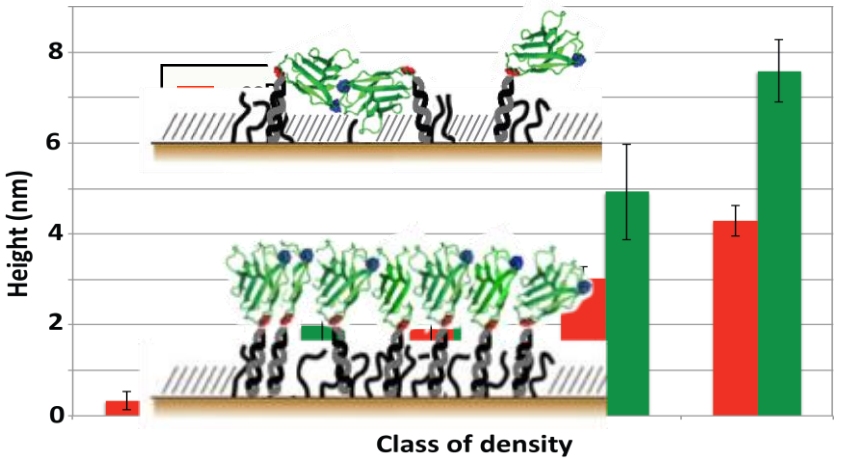
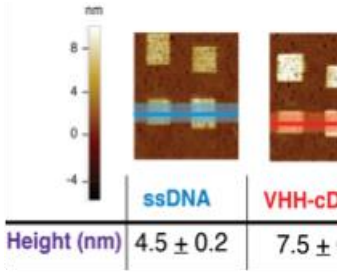
Sara Fortuna's Lab UniTS



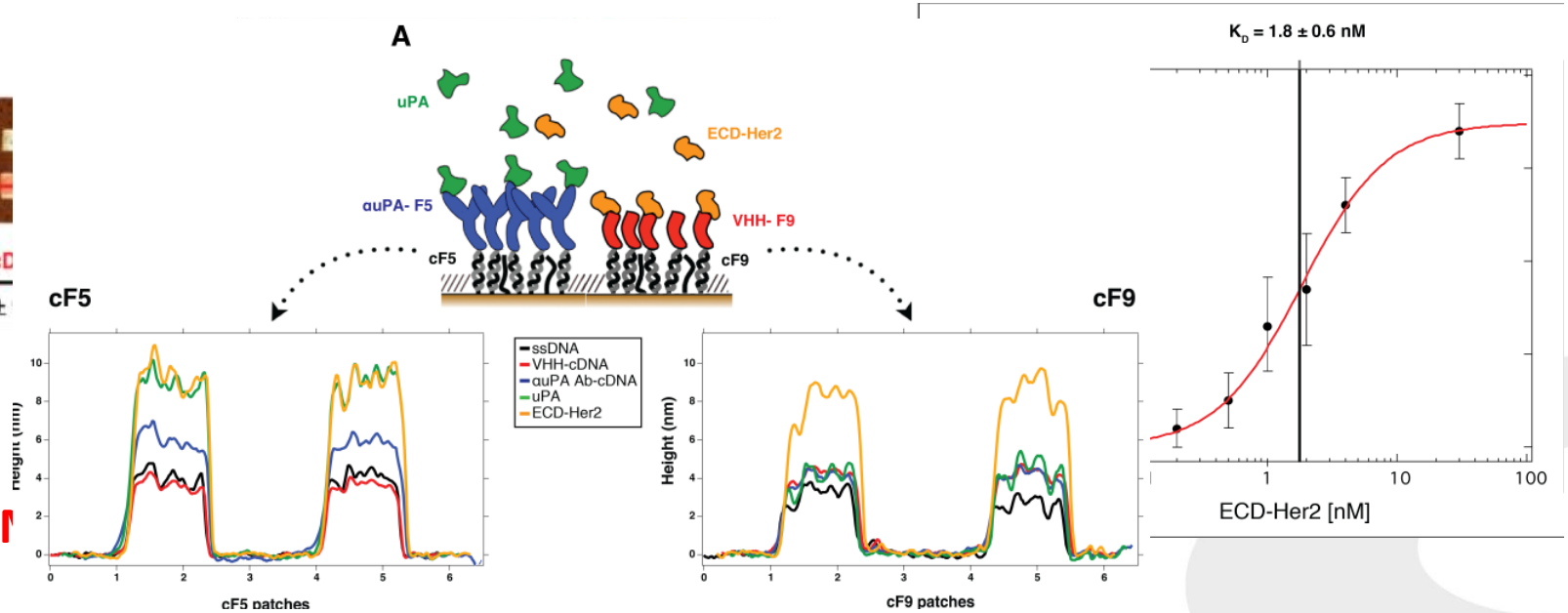




In silico optimized VHH reach affinity binding of 1 nM or better and can be made specific for different splice variants of ERC-HER2, indicator of tumor heterogeneity

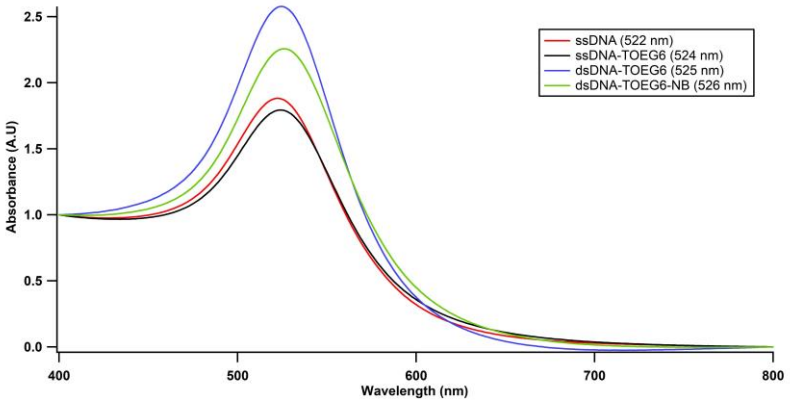


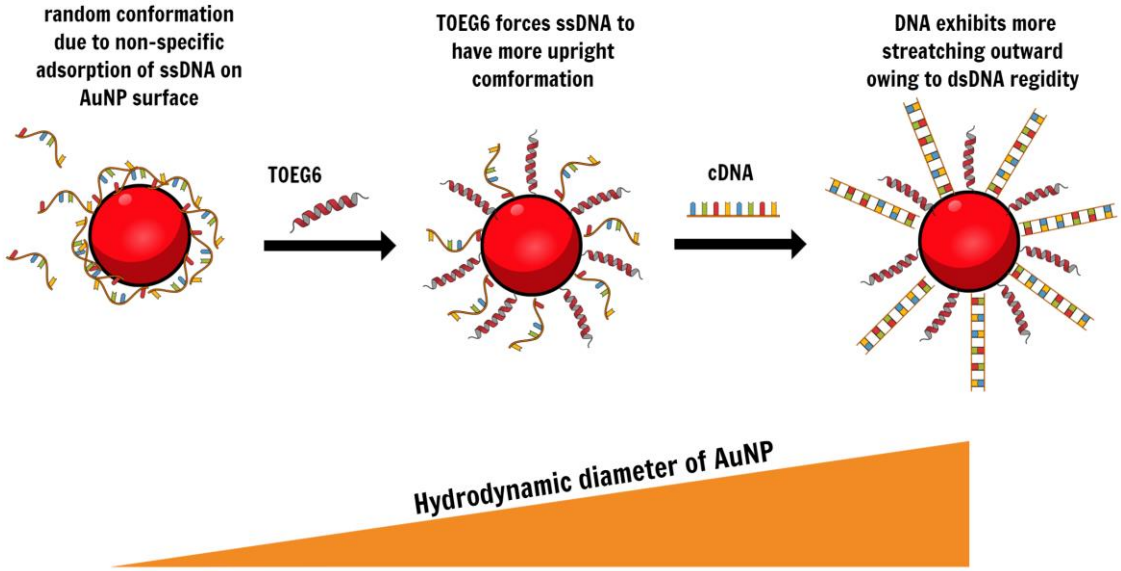
Limit of sensitivity **150 pM**

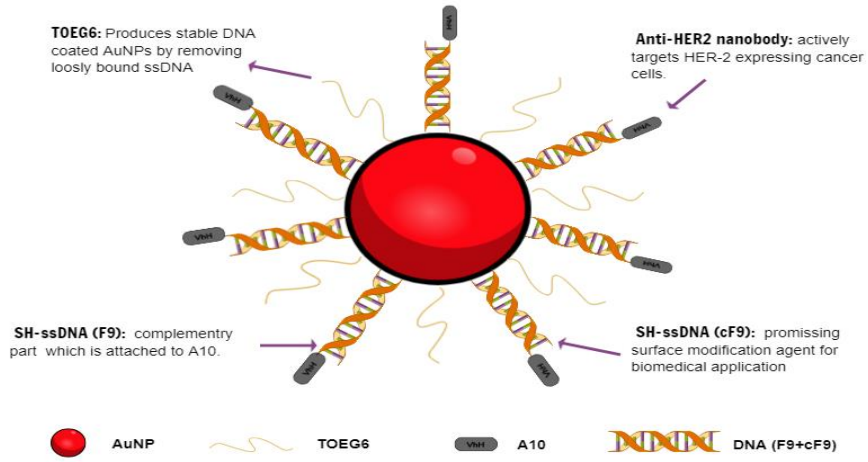


Work in progress: colorimetric assay

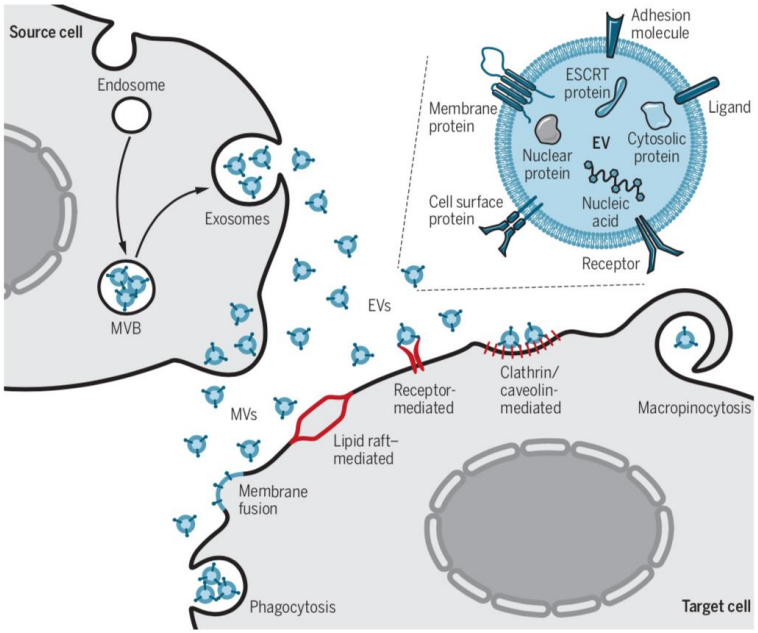
Sample	Z-average (nm)	STD	RSD%
cit@AuNP	23.6	0.5	2.1
ssDNA@AuNPs	24.3	0.5	2.0
ssTOEG6@AuNPs	30.1	0.8	2.6
dsDNA@TOEG6@AuNPs	32.3	1.2	3.6

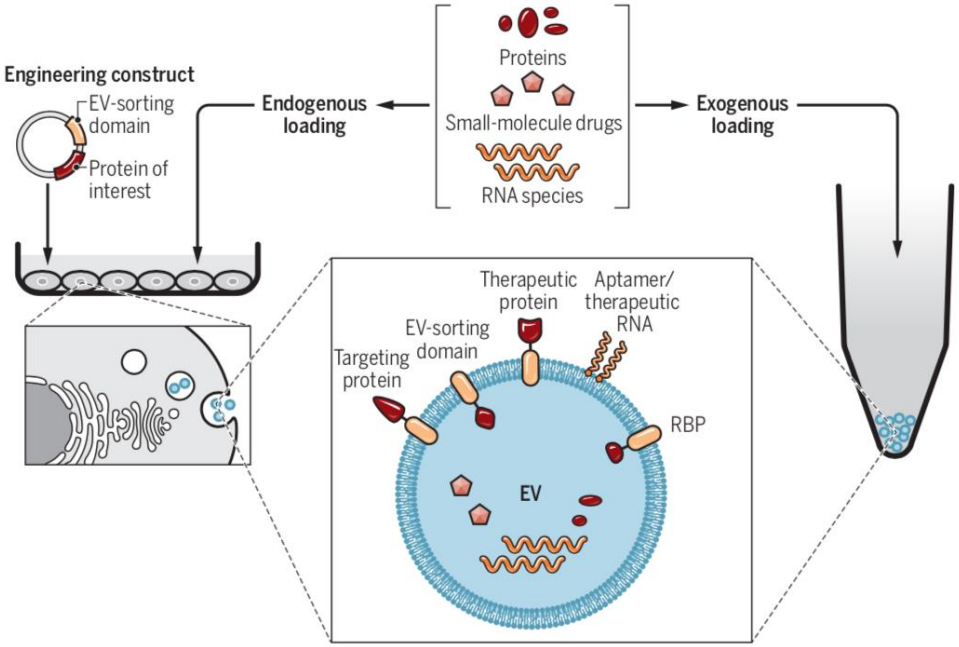






Novel biomarkers: extracellular vesicles

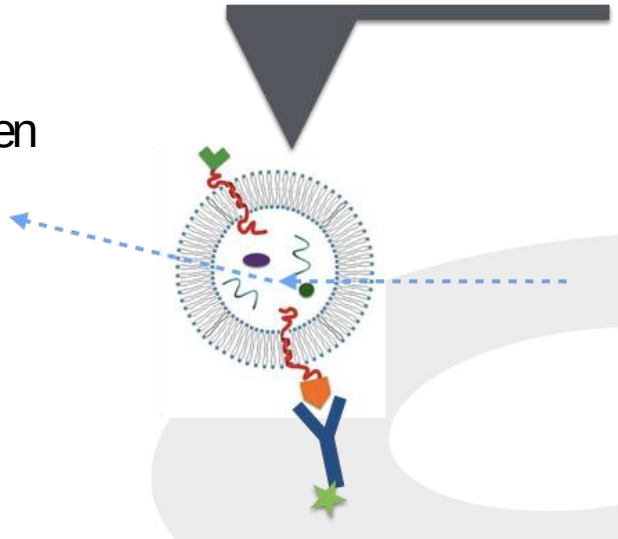




***Wiklander et al.*, Science Trans. Medicine. (2019)**

Extracellular vesicles (EV) are small vesicles ensuring transport of molecules between cells throughout the body; contain specific signatures and have been shown to strongly impact on the fate of recipient cells.

Useful for diagnostics and therapy.



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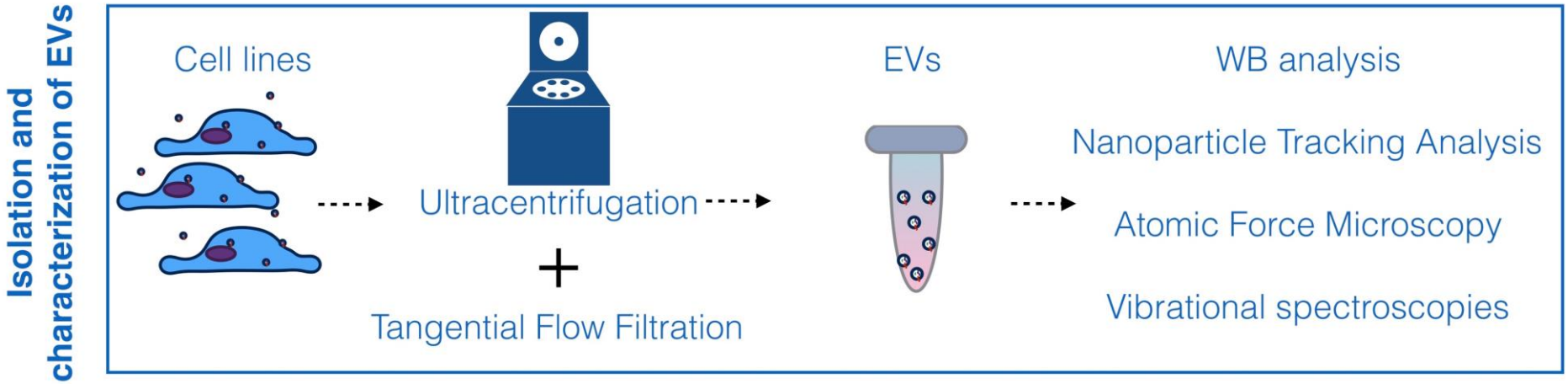
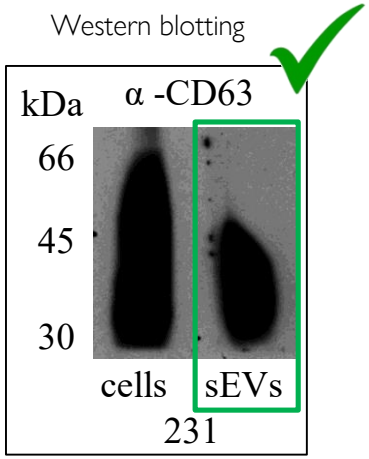
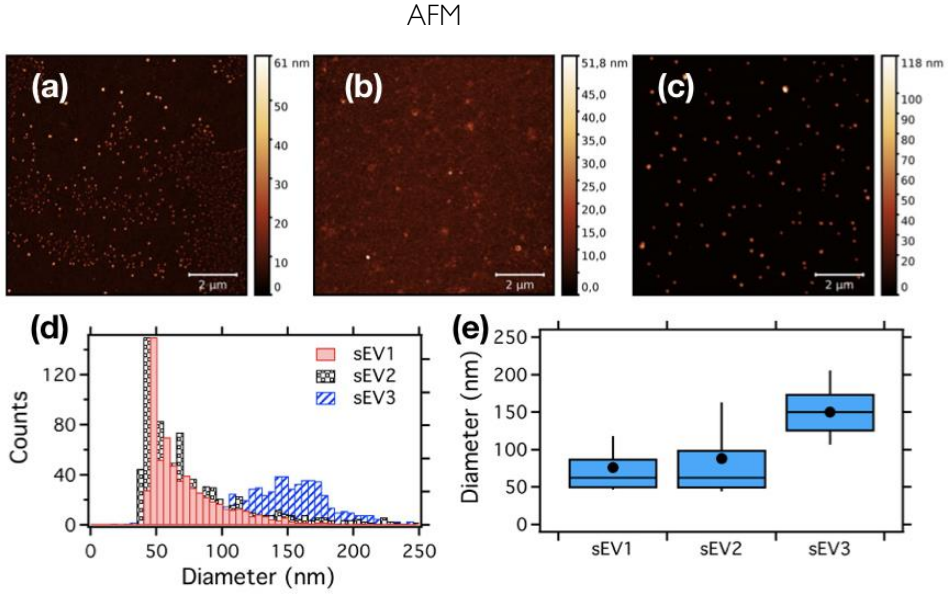
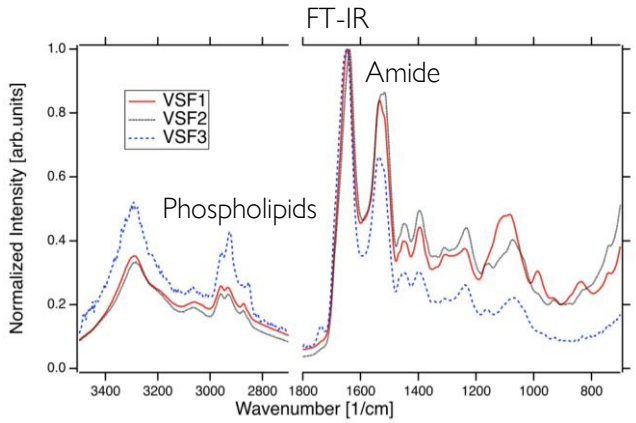
EVs characterization









	FTIR Protein/Lipid ratio	Raman Protein/Lipid ratio
sEV1	14+/-1	14+/-1
sEV2	13+/-1	4+/-1
sEV3	6+/-2	2.9+/-0.7

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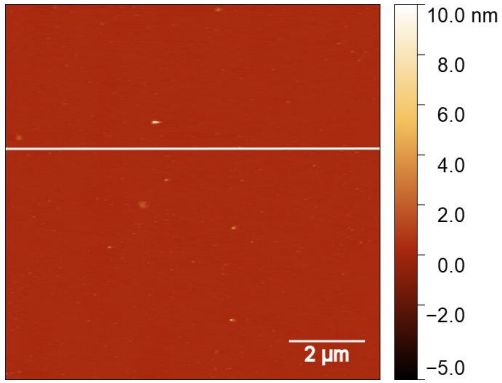
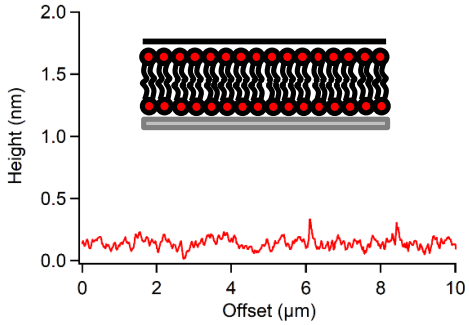
EVs interaction with model membranes: PC lipid bilayer

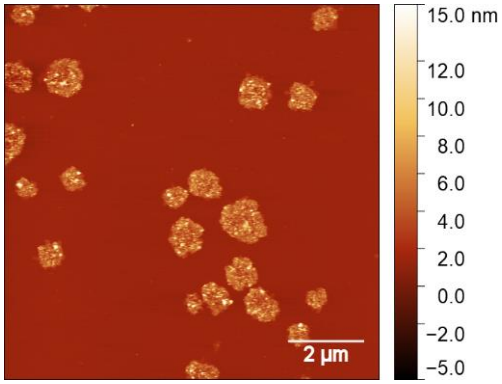
DOPC

+ EVs

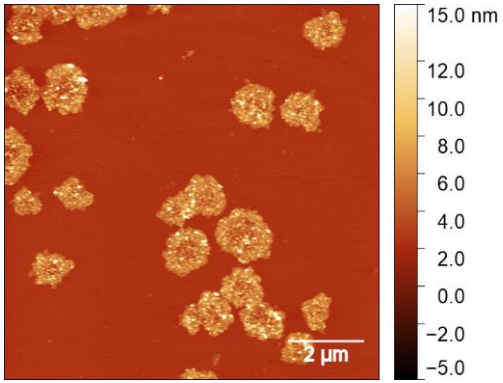




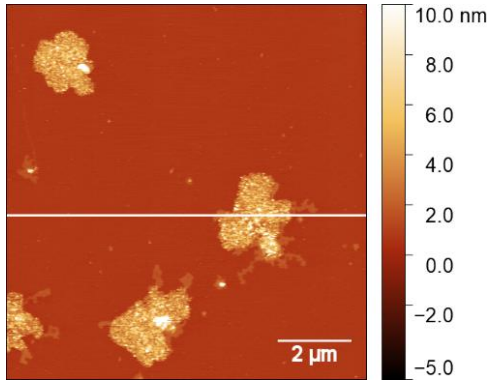
30 min

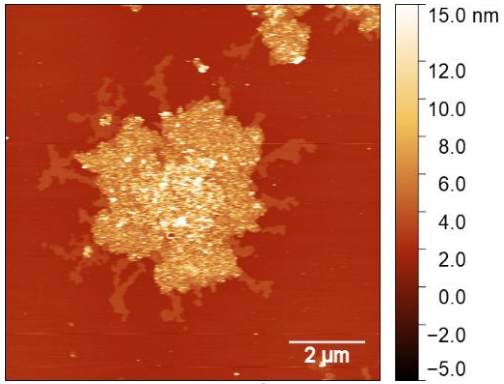


90 min

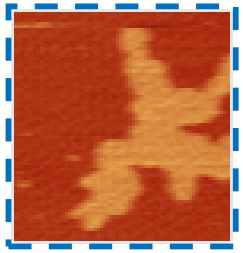


180 min

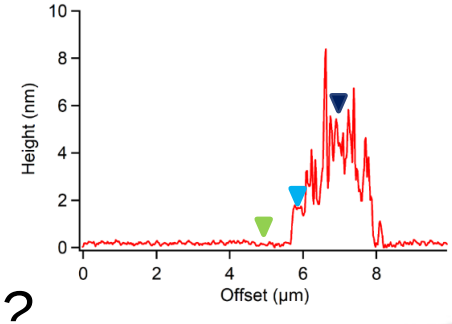




240 min



New lipid phase?



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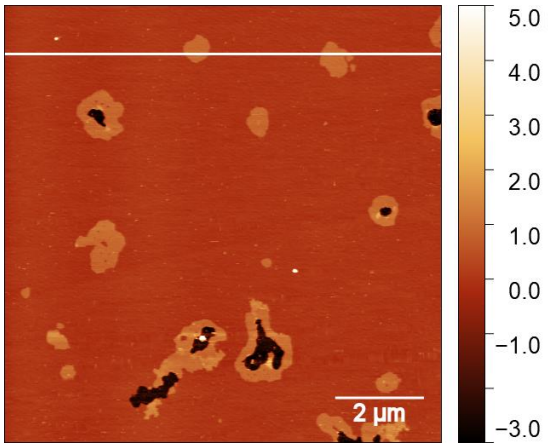
EVs interaction with model membranes: raft-like lipid bilayer

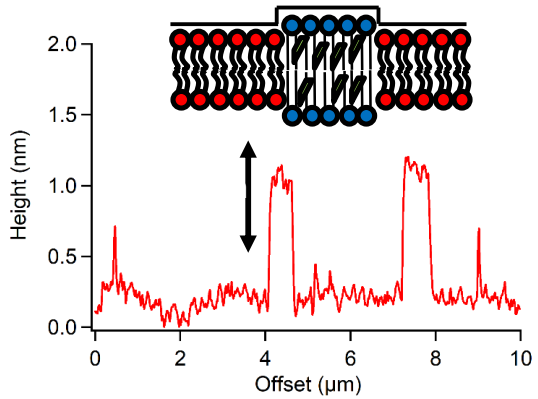
DOPC + SM (2:1) + 5% Chol

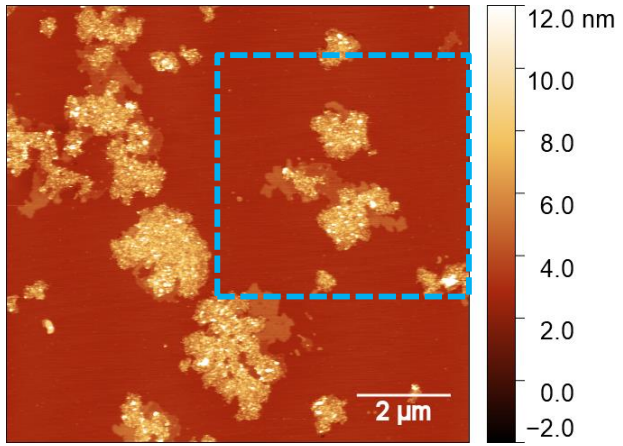
+EVs

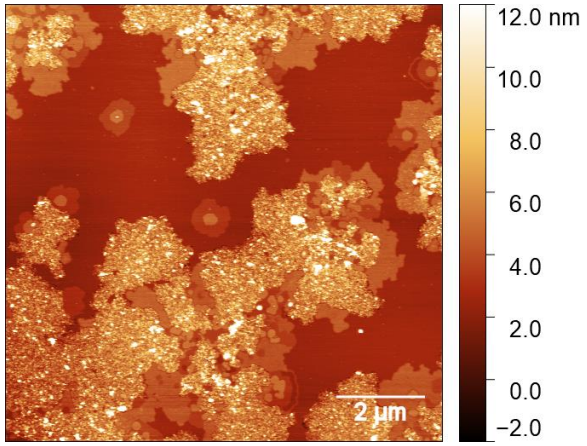
Raft domains (SM+Chol)

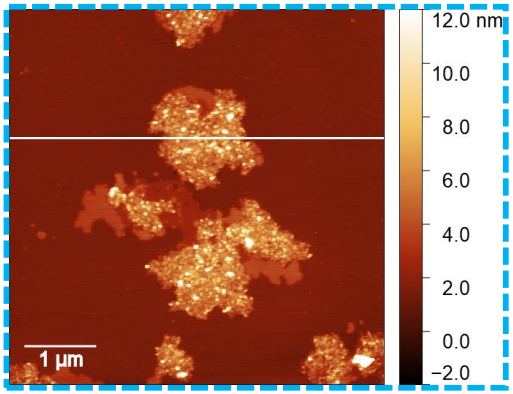
Non-raft phase (DOPC)

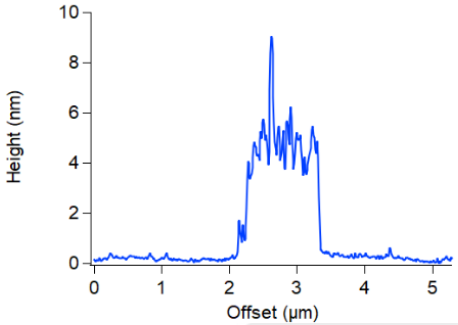












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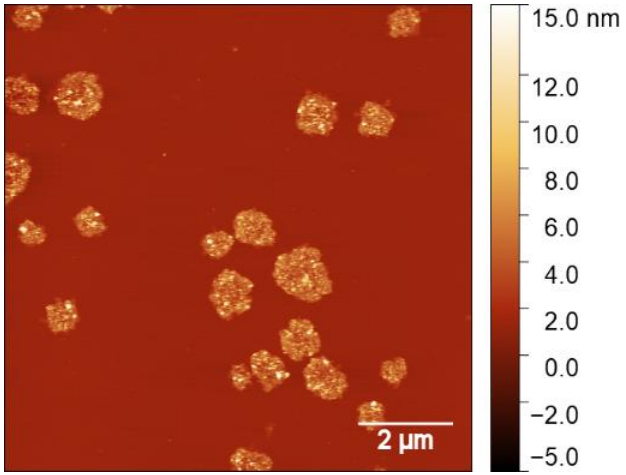
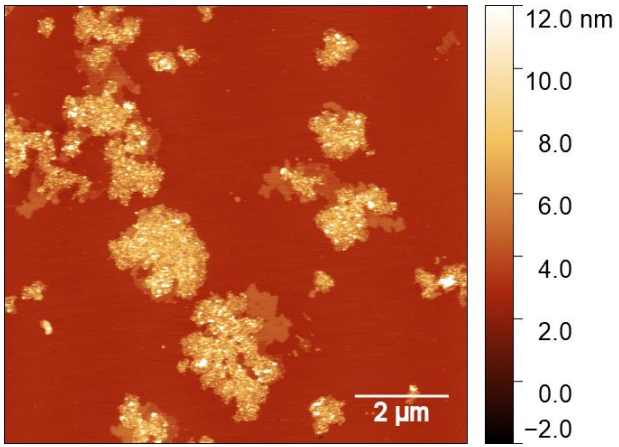
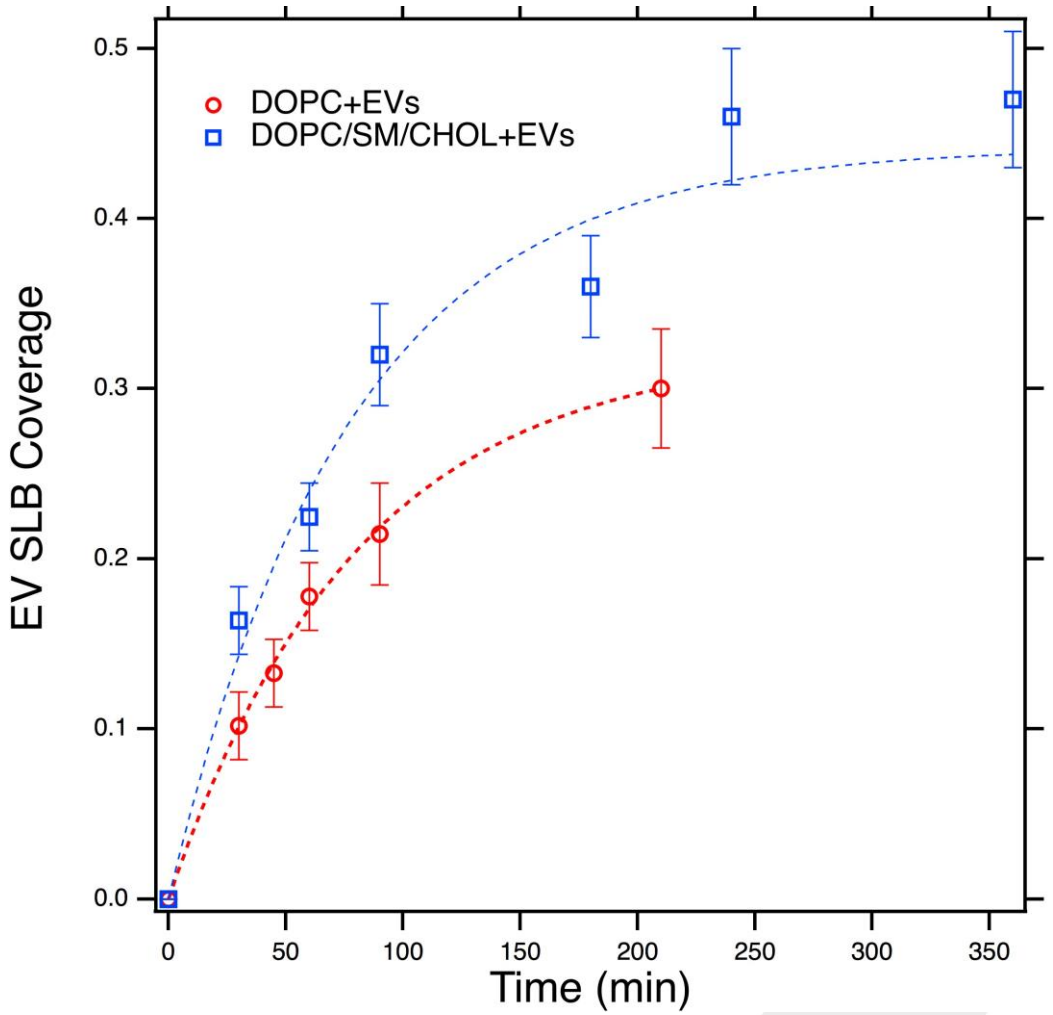
EVs interaction with raft-like bilayer: Comparison with single lipid

DOPC+EVs





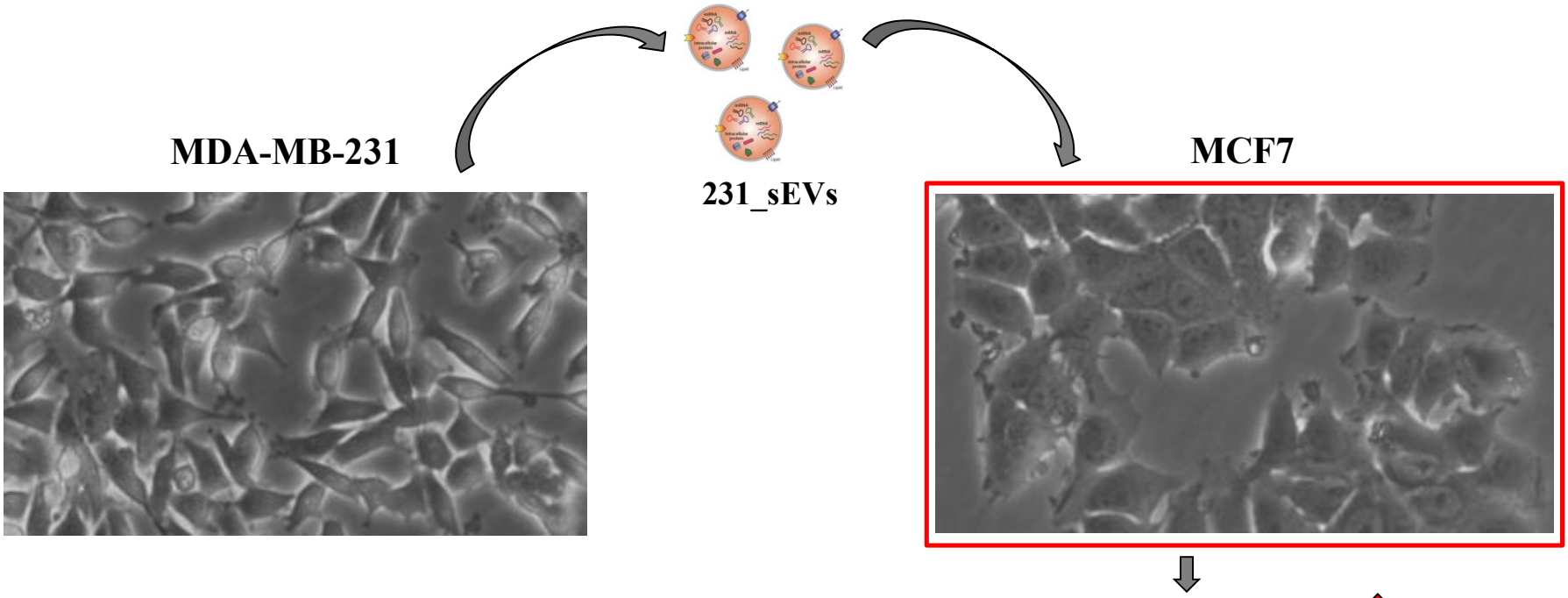
DOPC + SM (2:1) + 5% Chol+EVs



Mechanical response of cells treated with EVs

BREAST CANCER CELL LINE	SUBTYPE	AGGRESSIVENESS
MDA-MB-231	***TNBC (ER-; PR-; HER2-)***	+

BREAST CANCER CELL LINE	SUBTYPE	AGGRESSIVENESS
MCF7	***Luminal A (ER+; PR+; HER2-)***	-

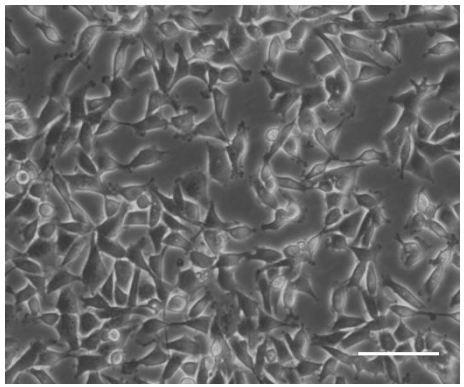


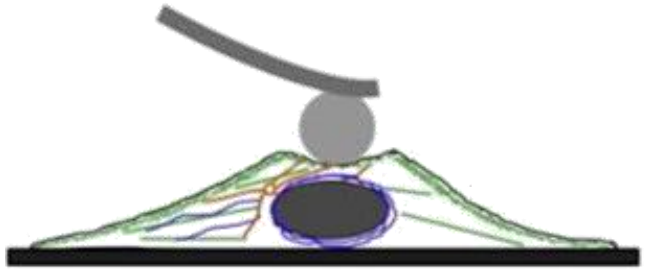
1. **Cell aggressiveness** ⬆
2. **Biomechanical properties?**

TNBC = Triple Negative Breast Cancer
sEVs = Small-Extracellular Vesicles

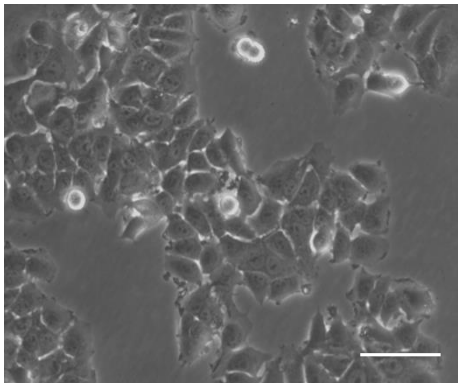
Mechanical response of cells treated with EVs

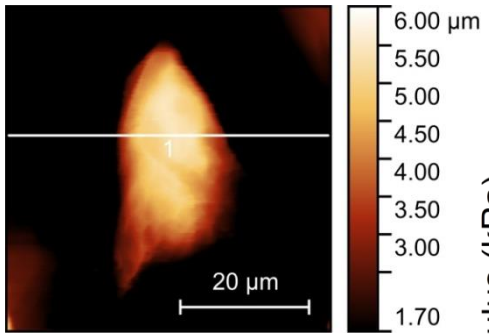
MDA-MB-231

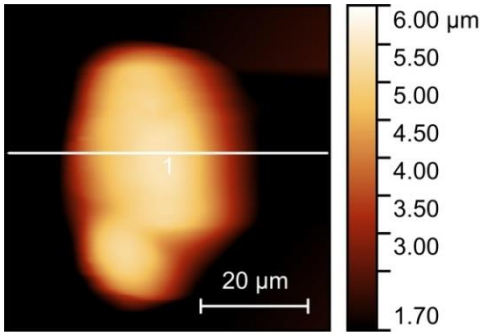


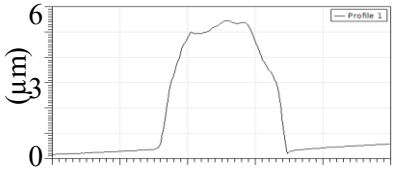


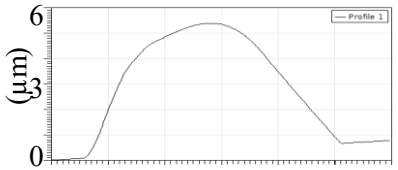
MCF7

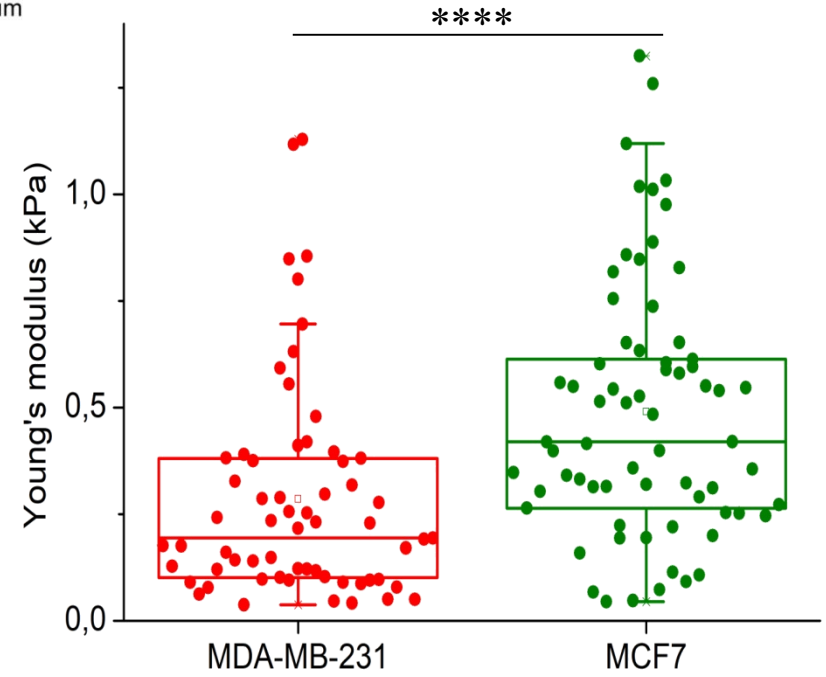








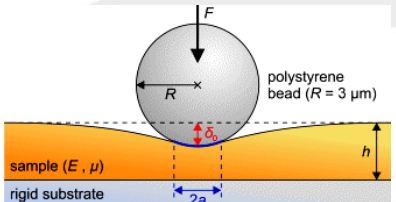




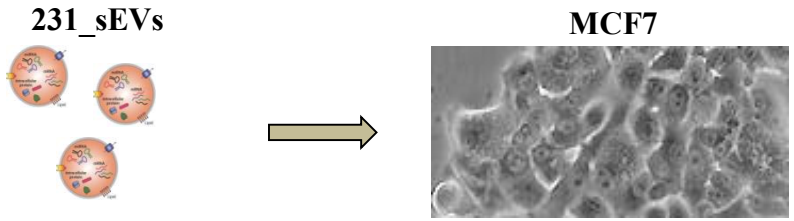
NT-MDT Smena
Tip: CSG01 (k = 0.006N/m)
Silica sphere D = 20µm
Fitting: Hertzian Model
Sample manteined in PBS1x buffer (30 cells and 2 sample analyzed for each cell line)
****= P-value < 0.0001 (Wilkcoxon test)

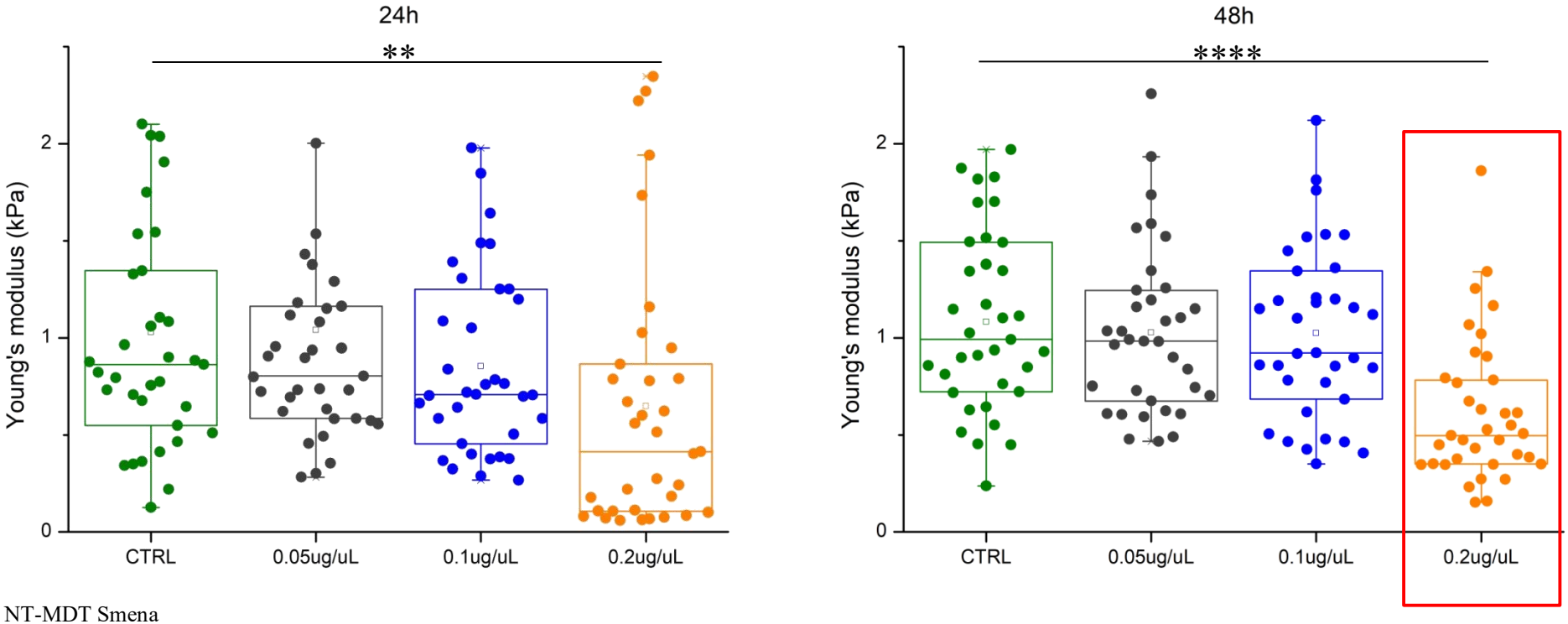
$$F = \frac{4}{3}\frac{E}{1-\mu^2}\sqrt{R\delta_0^3}$$

F ... applied force
R ... radius of the probe
δ_0 ... indentation of the sample
E ... elastic modulus
μ ... POISSON's ratio



Mechanical response of cells treated with EVs





NT-MDT Smena
Tip: CSG01 (k = 0.012N/m)
Silica sphere D = 20µm
Fitting: Hertzian Model
**= P-value < 0.01 ****= P-value < 0.0001 (Wilkcoxon test)

Huang et al. 2019, Am J Trasl Res.
Wang et al. 2019, Stem Cell Res Ther.

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EXOTHERA

Pietro Parisse

Beatrice Senigagliesi

Fabio Perissinotto

Sara Fortuna

Hendrik Vondracek

Elena Ambrosetti
(now at Karolinska Institutet)

Luciana Gneo

Elettra Sincrotrone Trieste

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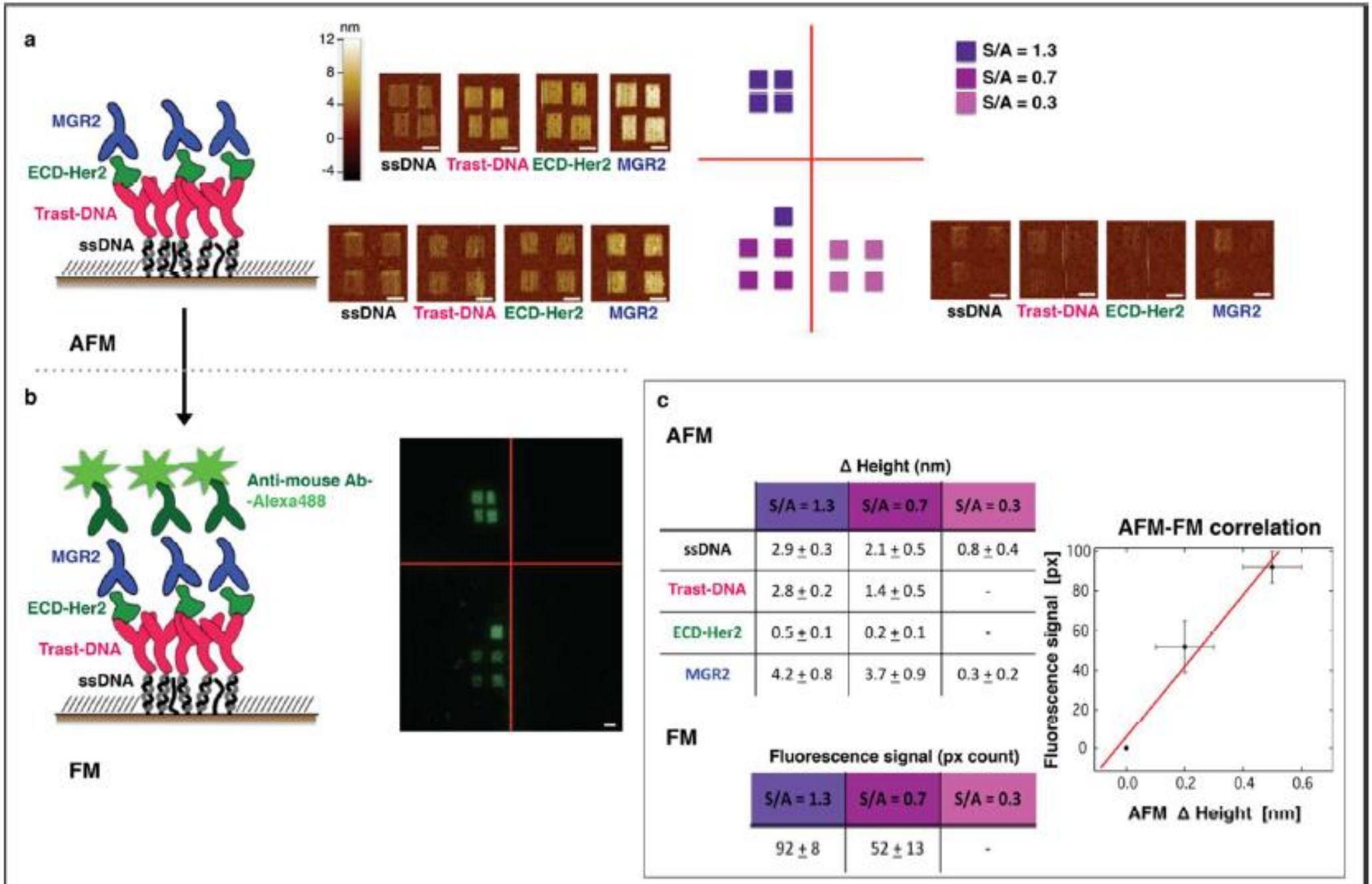
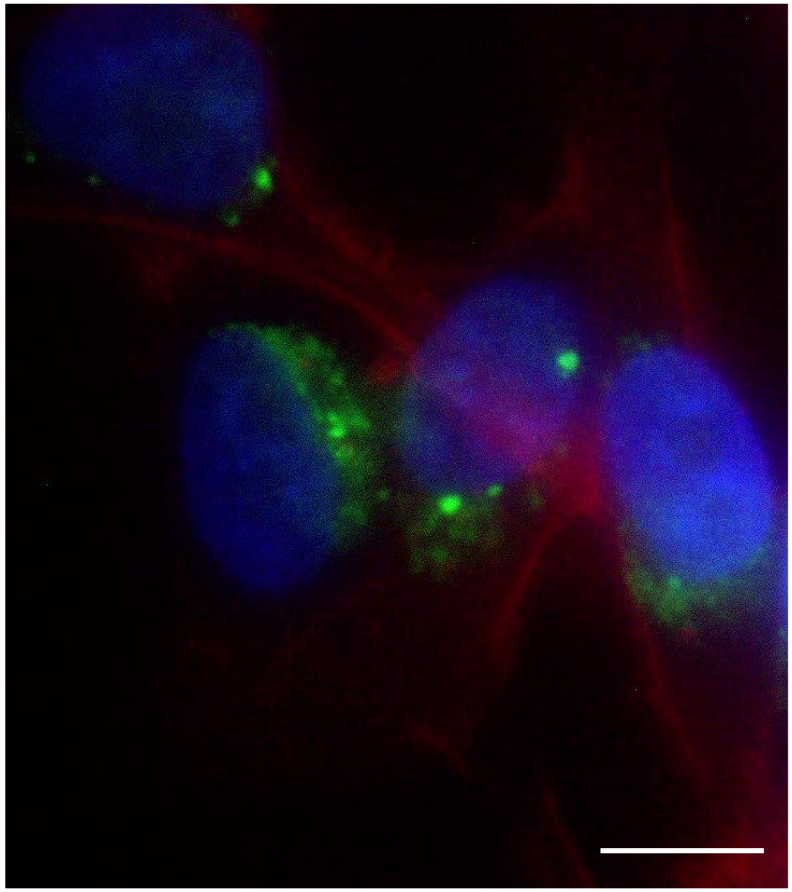
Nanoarrays

Detection of ECD-Her2 with nanobodies

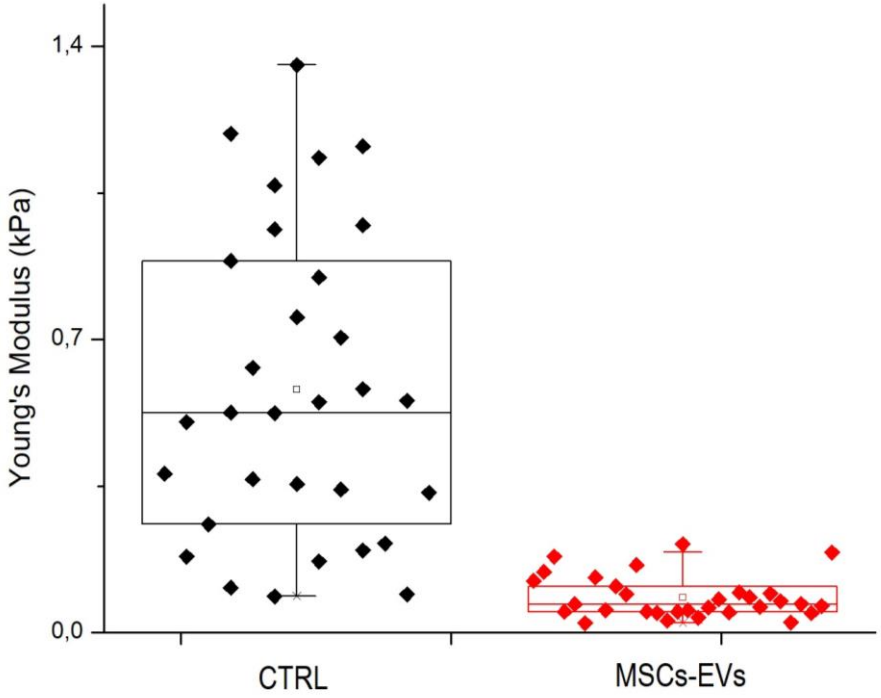


Mechanical response of cells treated with EVs



DAPI = Nuclei
DiO = EVs
Phalloidin = Actin

Scale bar = 10 µm



MSCs
mesenchimal stem cells from
Umbilical cord

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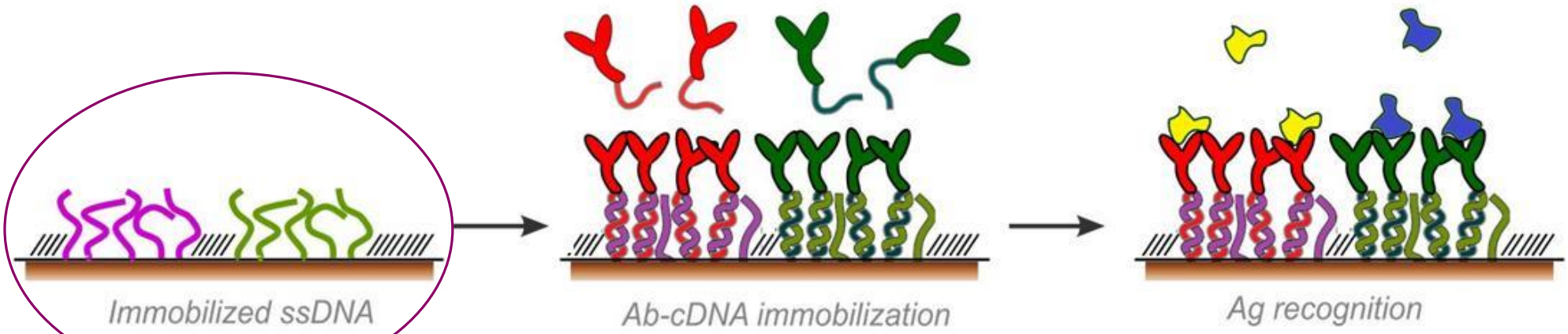
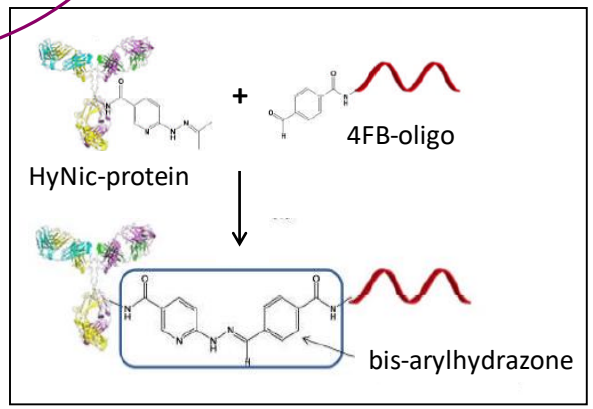
Protein Nanoarrays: DNA directed immobilization of DNA-barcoded protein binders





DNA-antibodies Conjugation: "click chemistry"

PROs
- different Abs on the same surface → Multiplexing protein nanoarray
- small volume detection system
- label-free detection system

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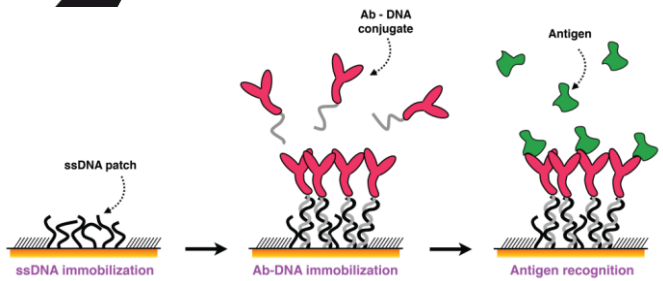
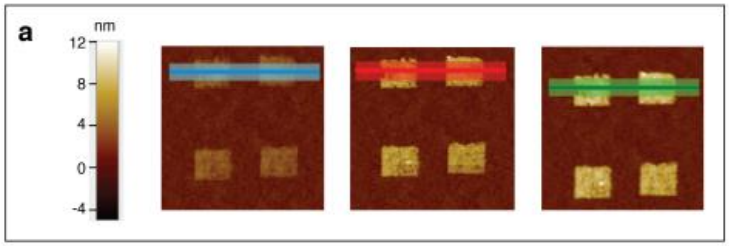
Nanoarrays

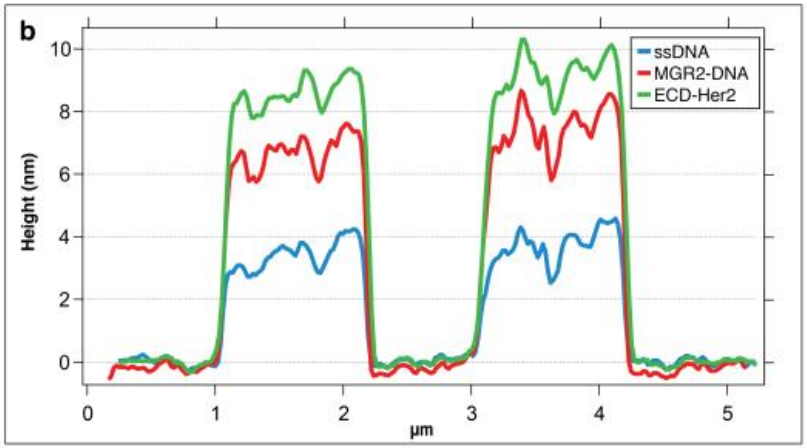
Detection of ECD-Her2 with mouse Ab



MGR2, MGR3 monoclonal antibodies,
non cross reactive

MGR2





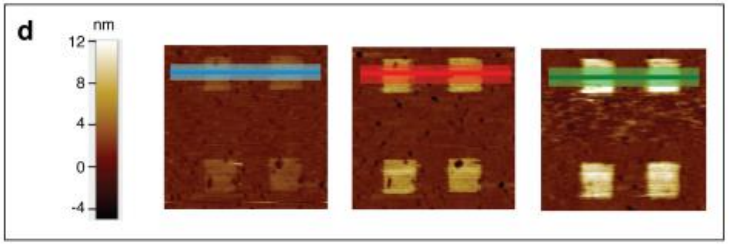
c

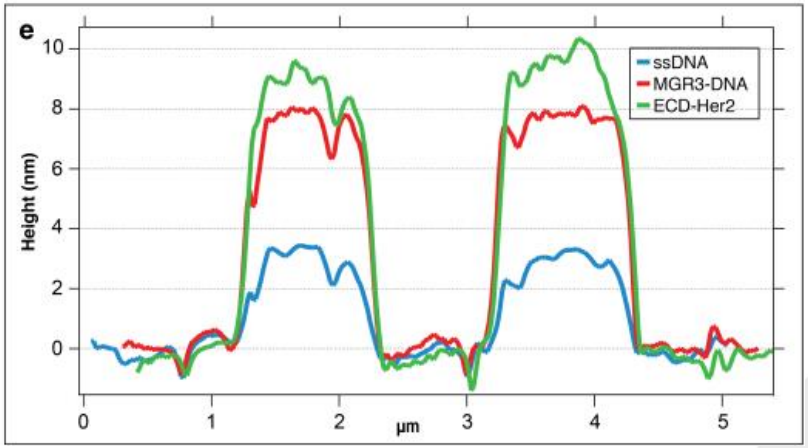
	ssDNA	MGR2-DNA	ECD-Her2
Height (nm)	3.5 ± 0.1	7.6 ± 0.4	9.4 ± 0.7

Limit of sensitivity

1 nM

MGR3





f

	ssDNA	MGR3-DNA	ECD-Her2
Height (nm)	2.6 ± 0.6	7.8 ± 0.4	8.9 ± 0.3

3 nM

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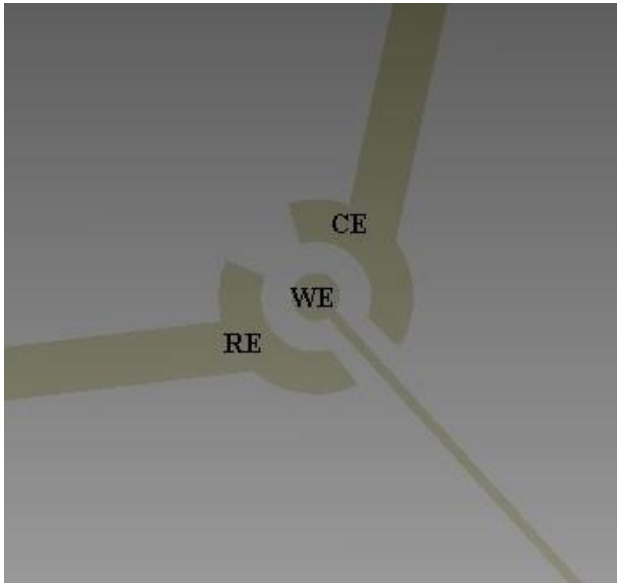
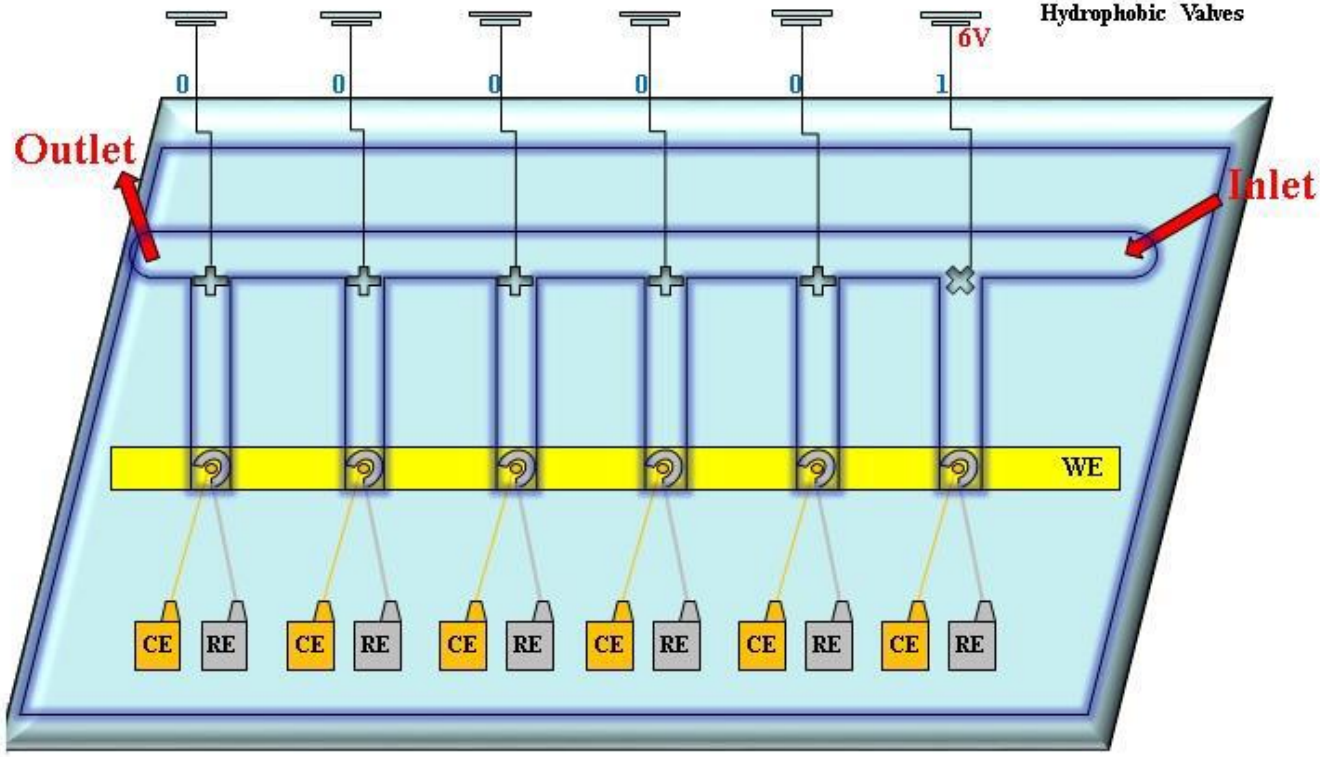
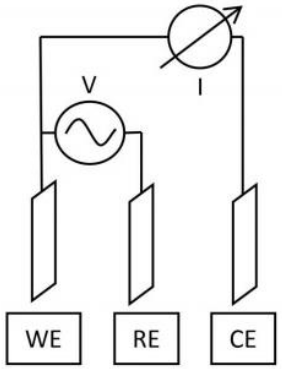
Miniaturized capacitive immunosensors

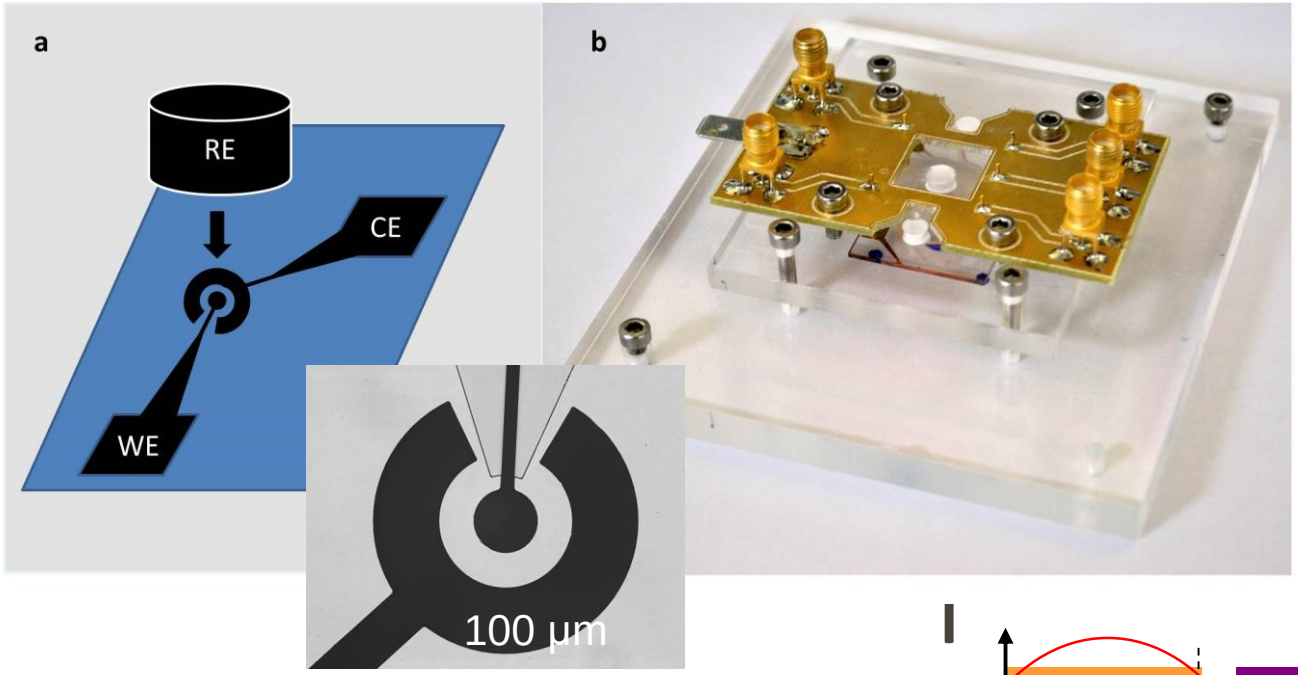
ECD-HER2 detection in human serum

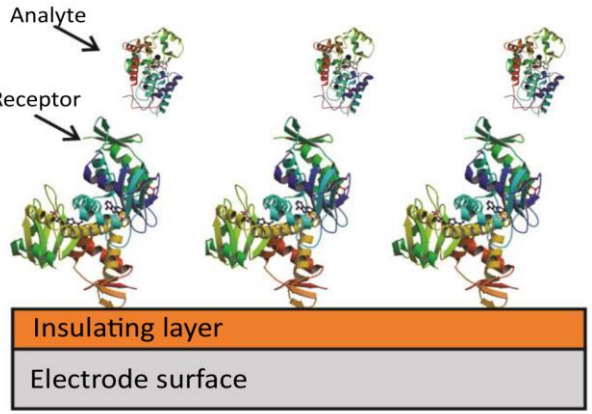


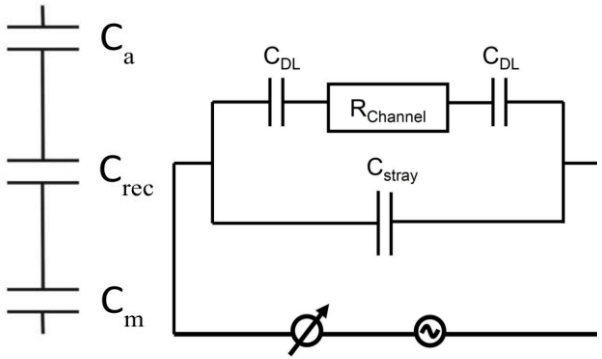


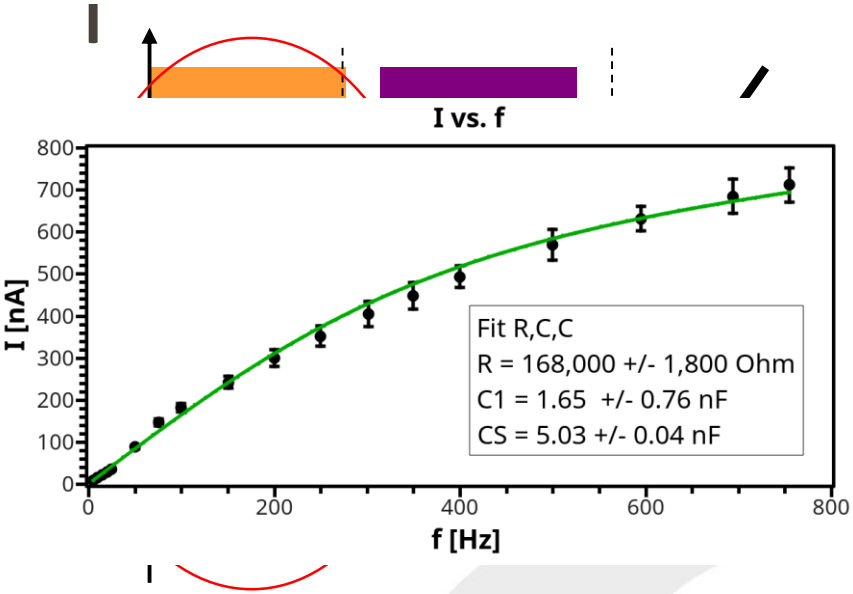
Miniaturized capacitive immunosensors











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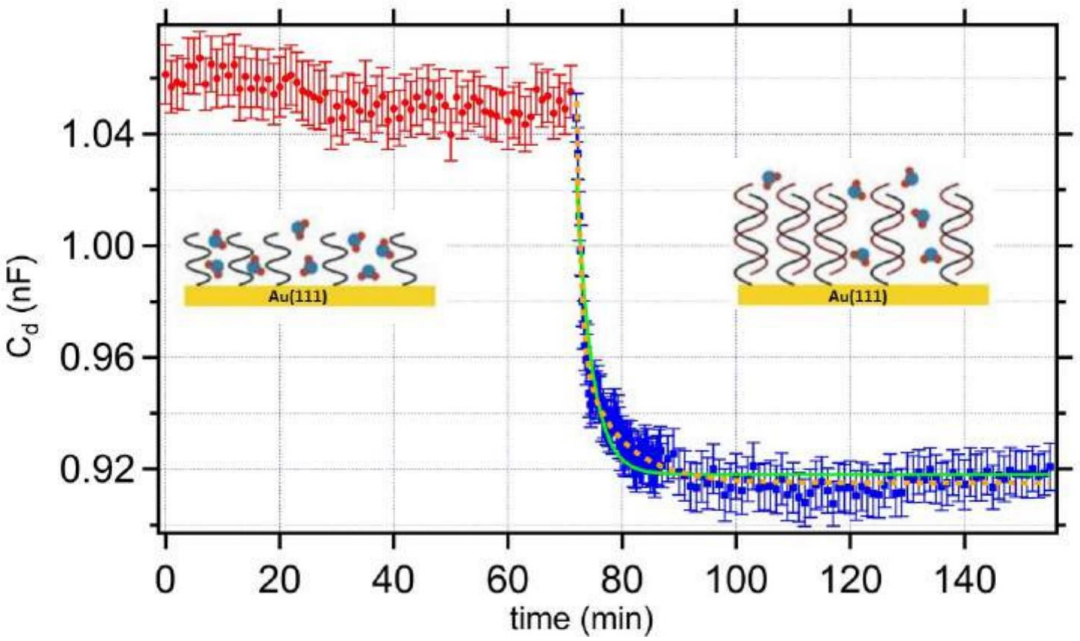
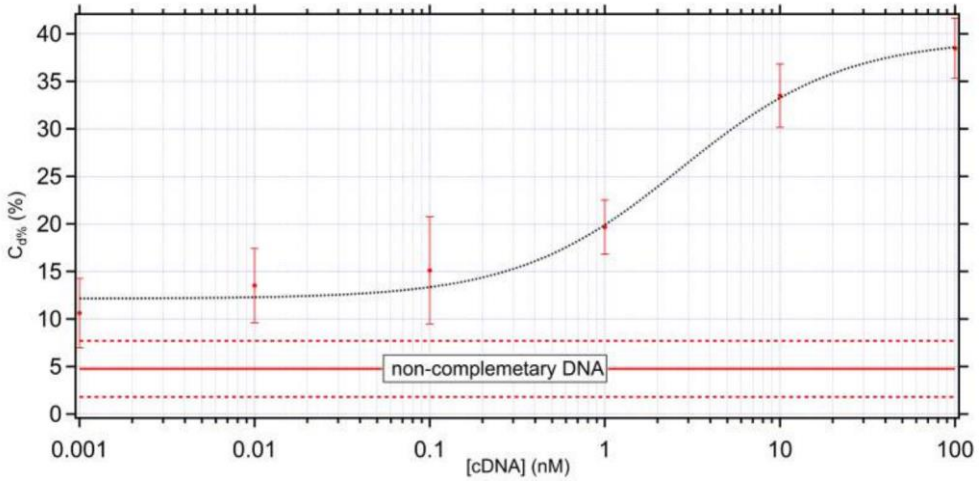
Miniaturized capacitive immunosensors

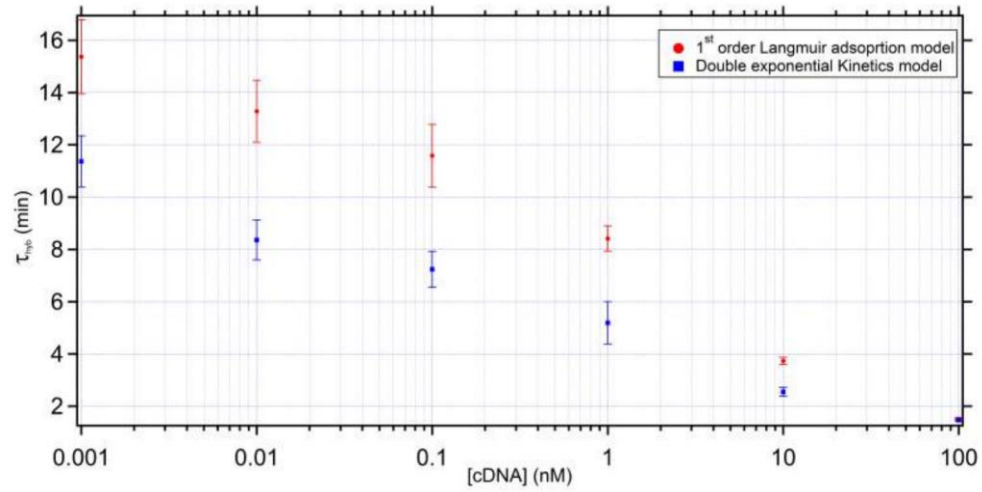
DNA hybridization



ACS Sensors 2016, 1, 1003



Variation of Cd w.r.t. c_{conj}



Hybridization time τ_d w.r.t. c_{conj}

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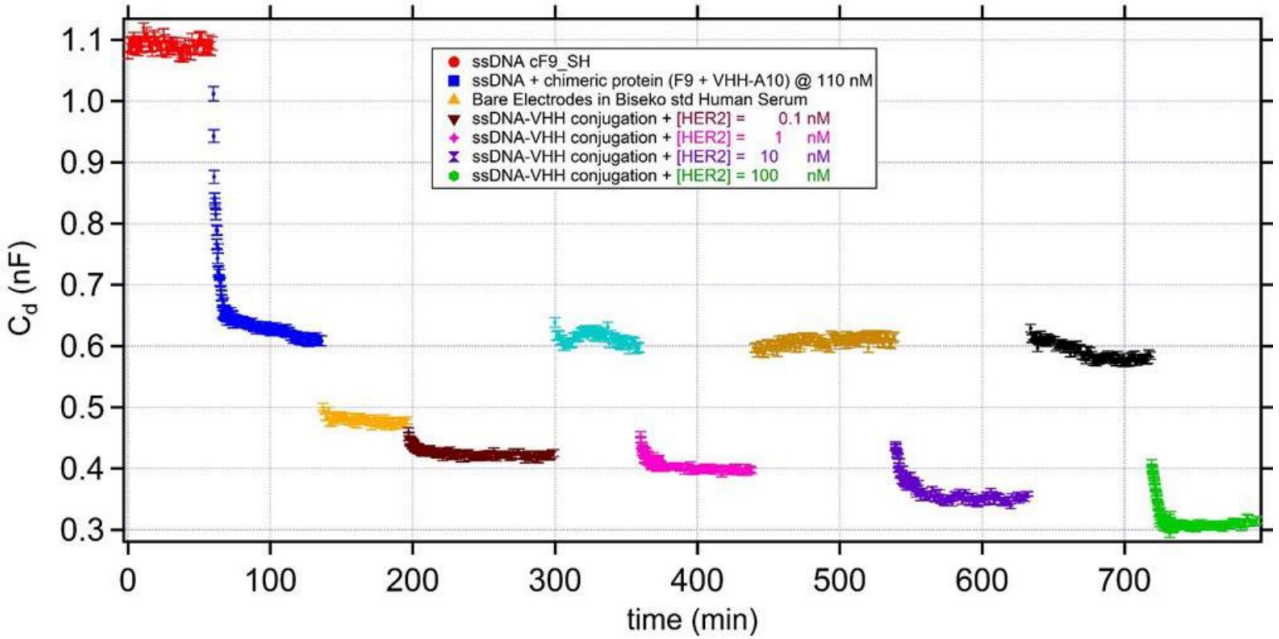
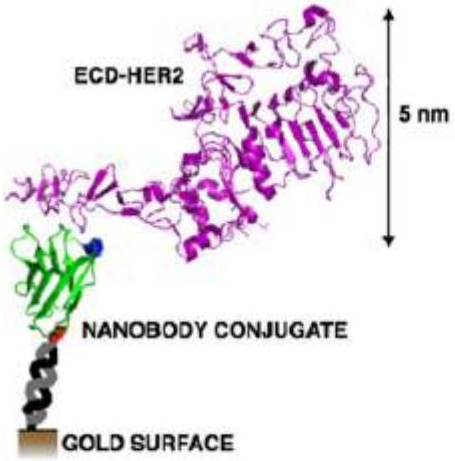
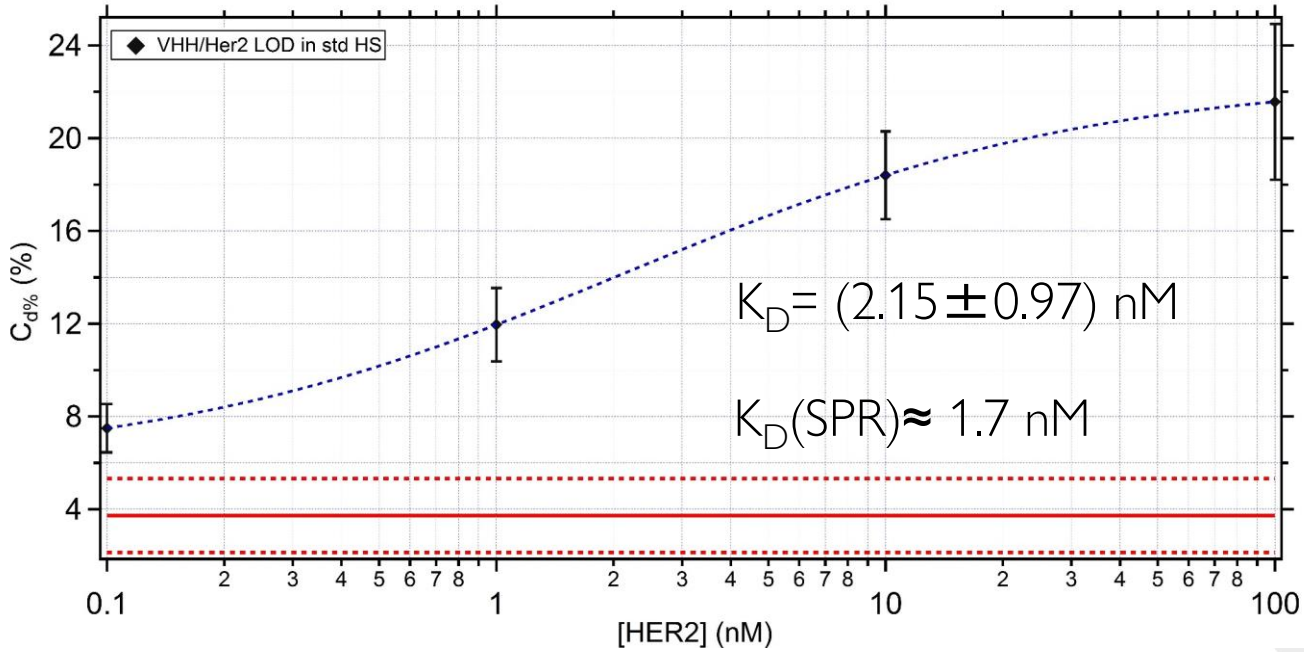
Miniaturized capacitive immunosensors

ECD-HER2 detection in human serum