Label-free characterization of cell-surface glycans-lectins interactions as test for melanoma development

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Molecular Biophysics – ABC of the puzzle of Life
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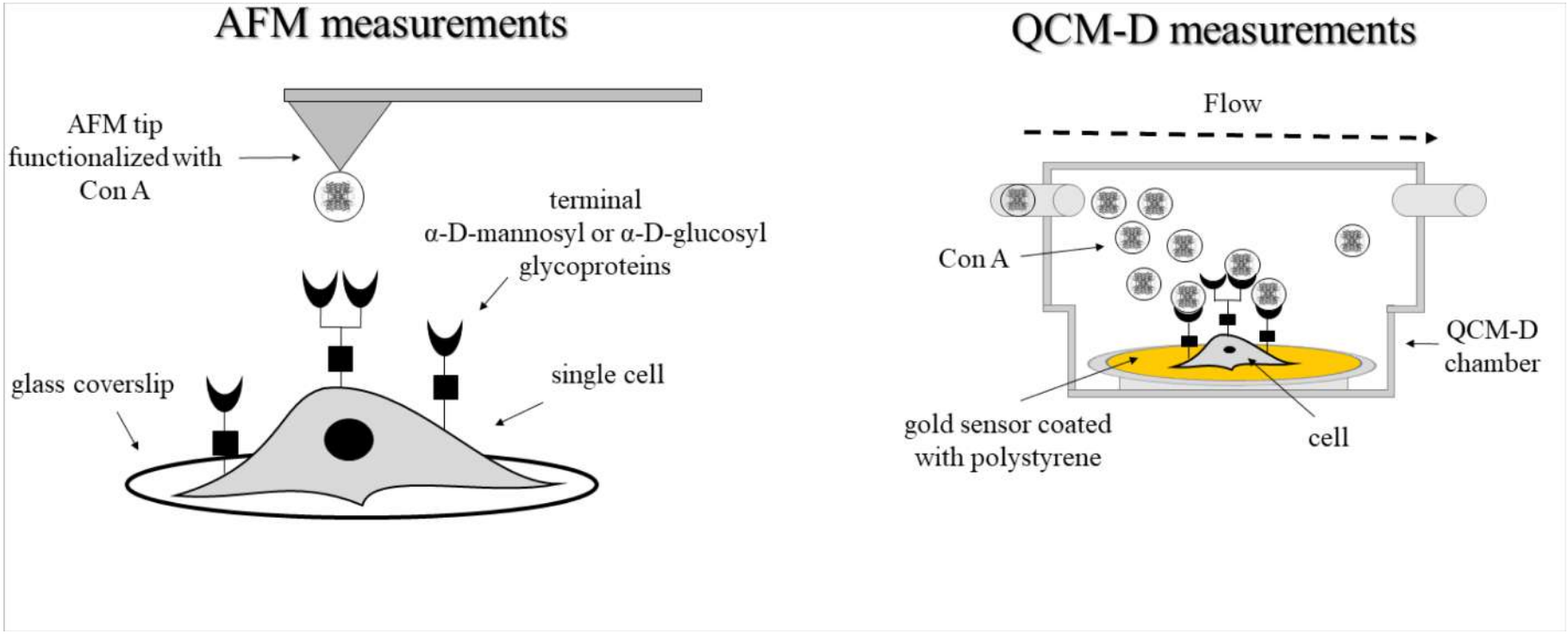
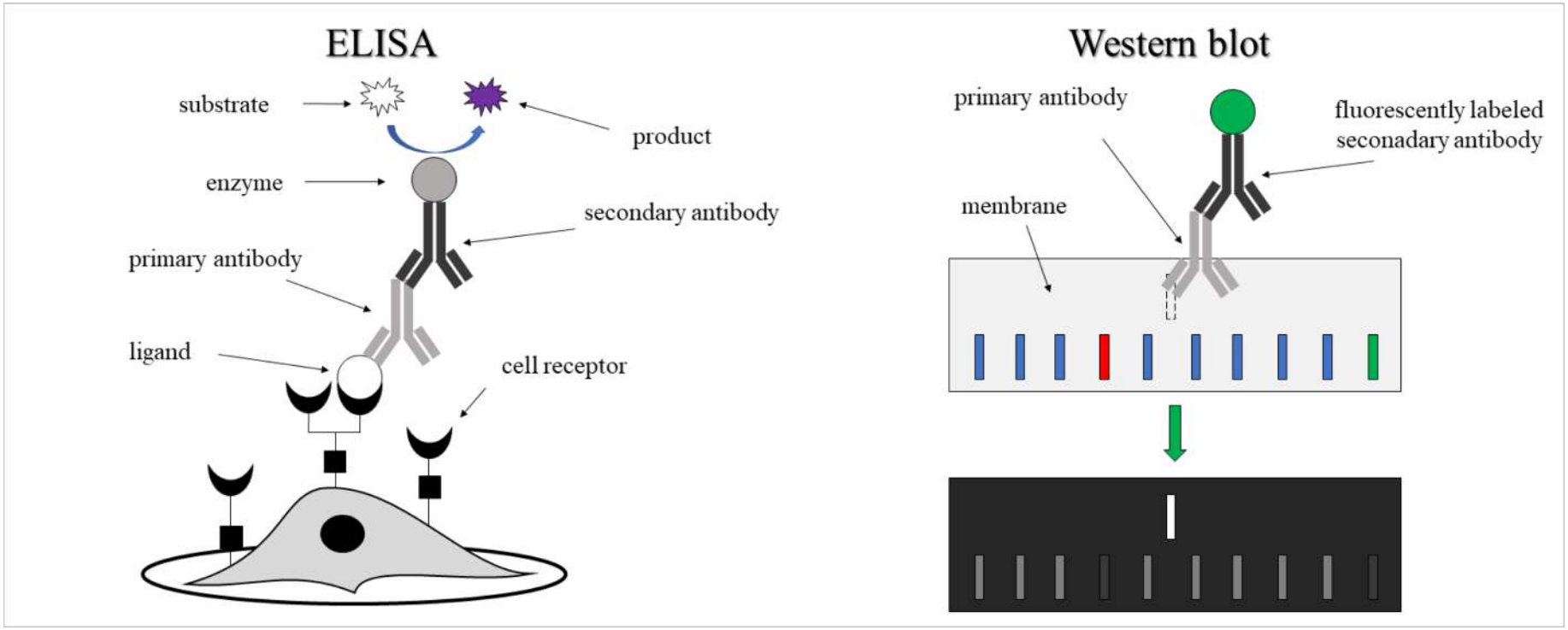
Why to use label-free techniques?



Why to use label-free techniques?

Label-free techniques do not require the use of markers, they provide direct information

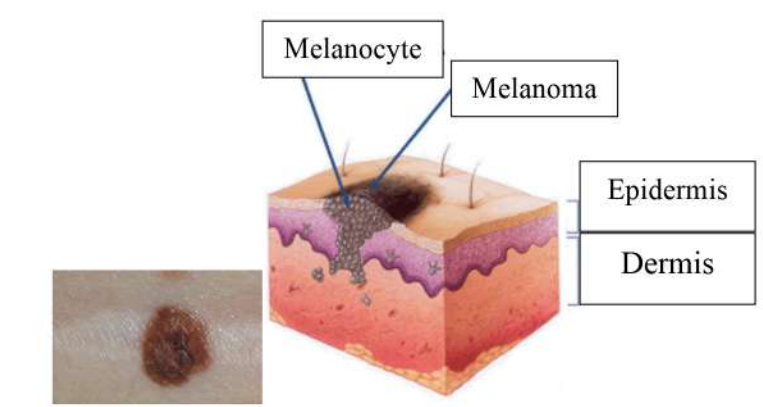


The stages of melanoma development

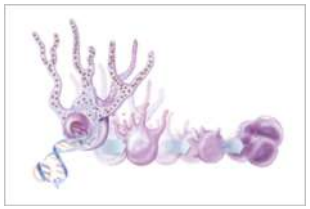
Skin cancer can be cured.

In fact, it is the easiest cancer to cure, provided it is diagnosed and treated early enough.

Everybody should be familiar with the surface of their skin, and conduct regular self-examinations.



Primary melanoma



Metastatic melanoma

World Melanoma Day, 23.05.2019

Targeted Therapy Approach

Direct Detection of a BRAF Mutation in total RNA from Melanoma Cells

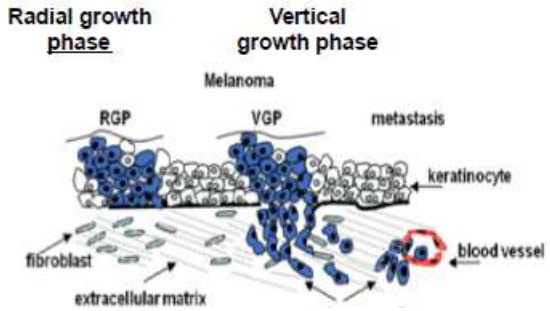
H.P. Lang, NCCR Nano
Univ. Basel, Switzerland

F. Huber et al. Nature Nanotechnology 8, 125 (Feb. 2013)

-Skin cancer (malignant melanoma) is difficult to treat in later stages.

-A single point mutation in a gene (*BRAF*) involved in cell growth, called BRAF V600E, is responsible for unregulated cell growth in 50% of melanoma patients.

-A drug is on the market, called Venurafenib, inhibits the activity of the mutated protein, improves treatment efficacy at later stages of melanoma.

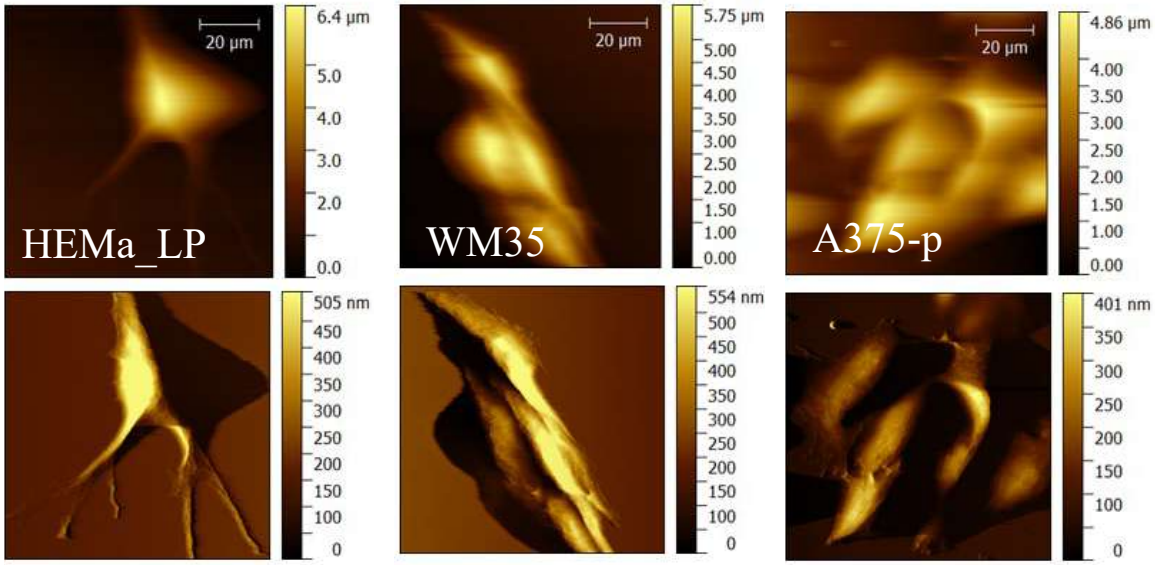


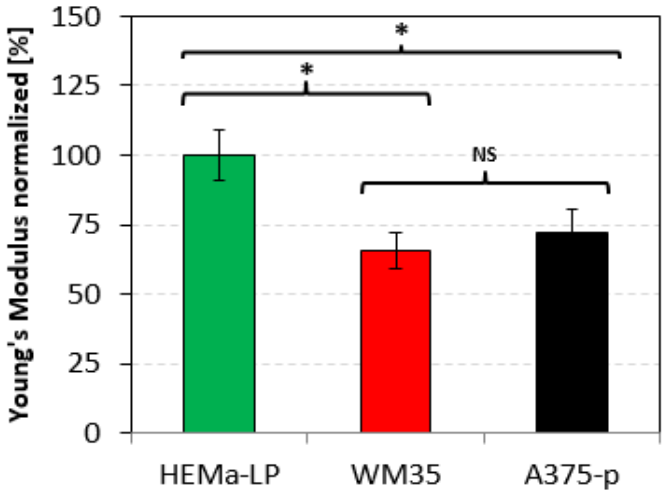
PATLiSci1+2

For more details go to:
https://www.mycancergenome.org/content/disease/melanoma/braf/54/

Mechanical properties of melanocytes & melanoma

AFM





HEMa-LP - melanocytes
WM35 – primary tumor
A375-P – lung metastasis

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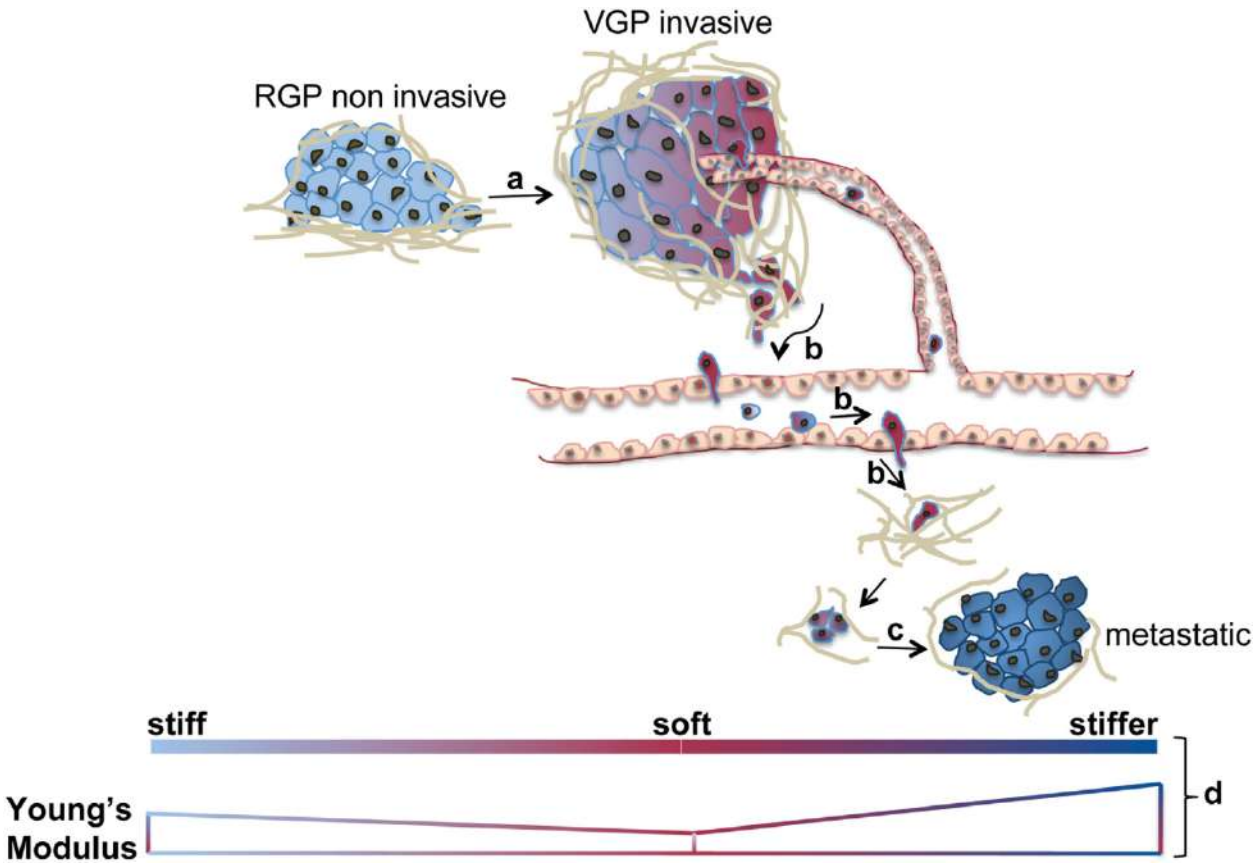
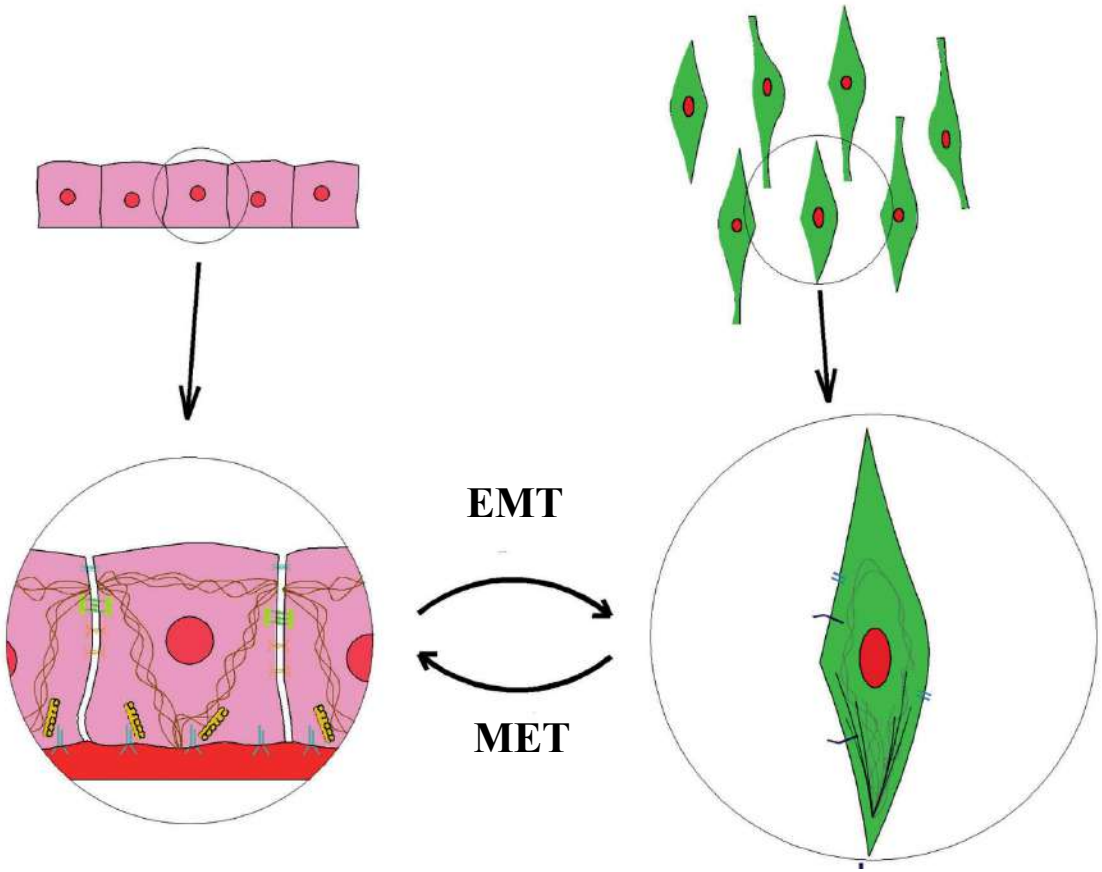
How stiff is metastatic melanoma?



G. Weder et al / Nanomedicine: Nanotechnology, Biology, and Medicine 10 (2014) 141–148

Epithelial-Mesenchymal Transition (EMT)

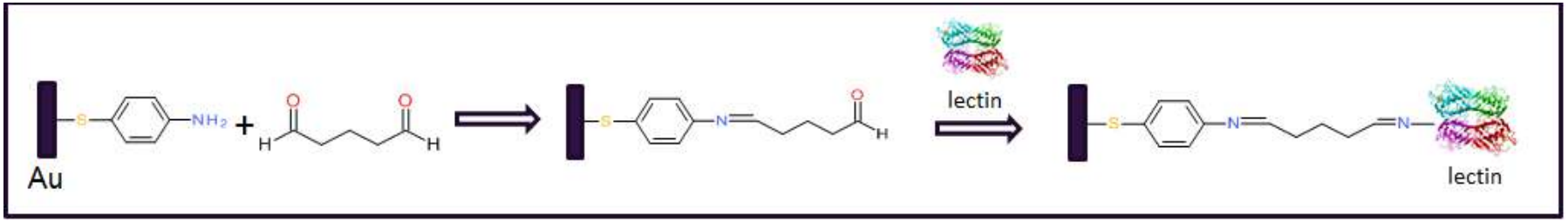
During cancer progression EMT is utilized by cancer cells to develop malignancy.
Evaluation of EMT is based on investigation of glycosylation profile of melanoma.



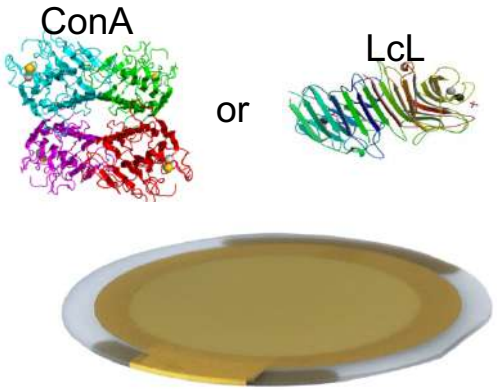
Heerboth et al. Clinical and Translational Medicine (2015) 4:6

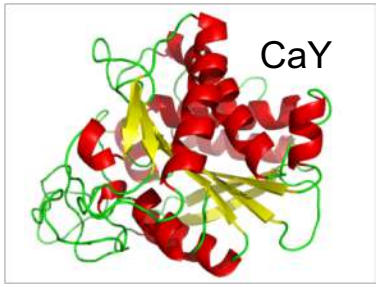
Model ligand-receptor interaction (QCM study)

Surface modification of sensors



Lectins were immobilized on the surface of the QCM-D sensor while free CaY molecules were introduced in the buffer solution

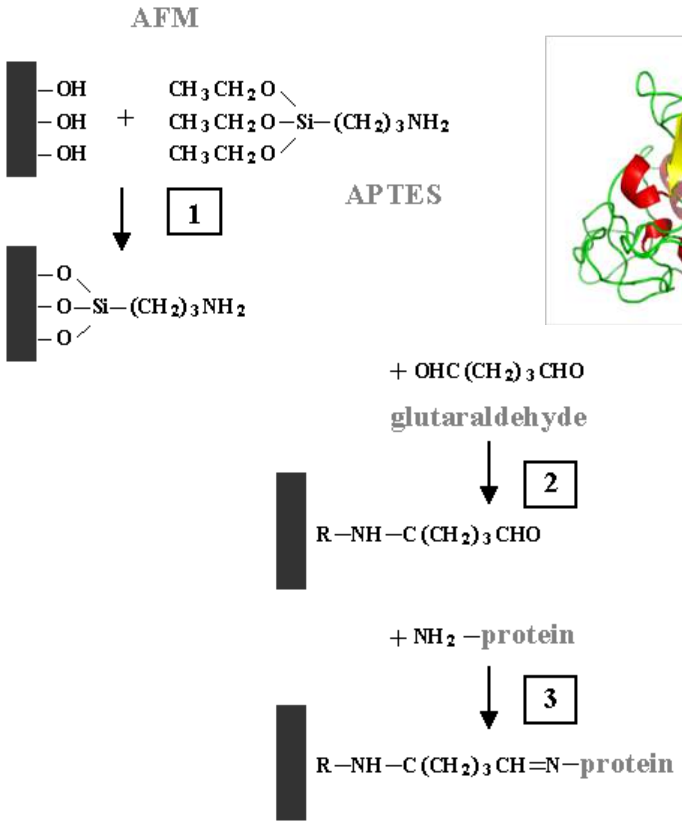


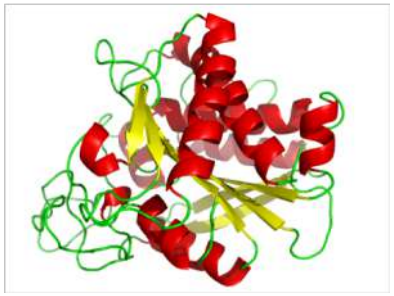


E. Senkara, T.Kobiela et al., Biosensors and Bioelectronics, 36 (2012) 103-109

Model ligand-receptor interaction (AFM study)

Surface modification of AFM cantilevers





E. Senkara, T.Kobiela et al., Biosensors and Bioelectronics, 36 (2012) 103-109

Model ligand-receptor interaction (QCM & AFM study)

Summary of the results

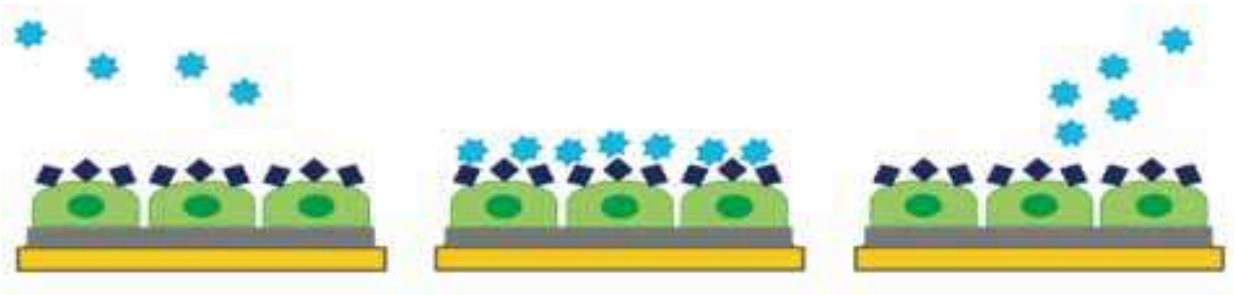
Method	*AFM*		*QCM-D*			
Parameter	k_{off} [s^{-1}]	x_b [Å]	k_{on} $\times 10^4$[$M^{-1}s^{-1}$]	k_{off} [s^{-1}]	K_a $\times 10^6$[M^{-1}]	ΔG [kJ/mol]
Con A	0.036 ± 0.005	2.28 ± 0.04	6.26 ± 0.10	0.026 ± 0.002	2.25 ± 0.10* 2.34 ± 0.05**	-36.40 ± 0.11* -36.34 ± 0.06**
LcL	0.045 ± 0.006	1.86 ± 0.04	5.21 ± 0.20	0.054 ± 0.002	1.92 ± 0.08* 1.06 ± 0.07**	-35.85 ± 0.10* -34.37 ± 0.14**

* *Values obtained by the Langmuir equation*
** *Values obtained by the relaxation time contant*

E. Senkara, T.Kobiela et al., Biosensors and Bioelectronics, 36 (2012) 103-109

QCM of melanoma surface glycans - lectin interactions

Characterization of complex lectin-cell kinetics



Schematic representation of the interaction between the lectin Con A (light blue stars) and cell surface glycans (dark blue squares).
Association and dissociation are monitored in real-time by the QCM-based cell biosensor

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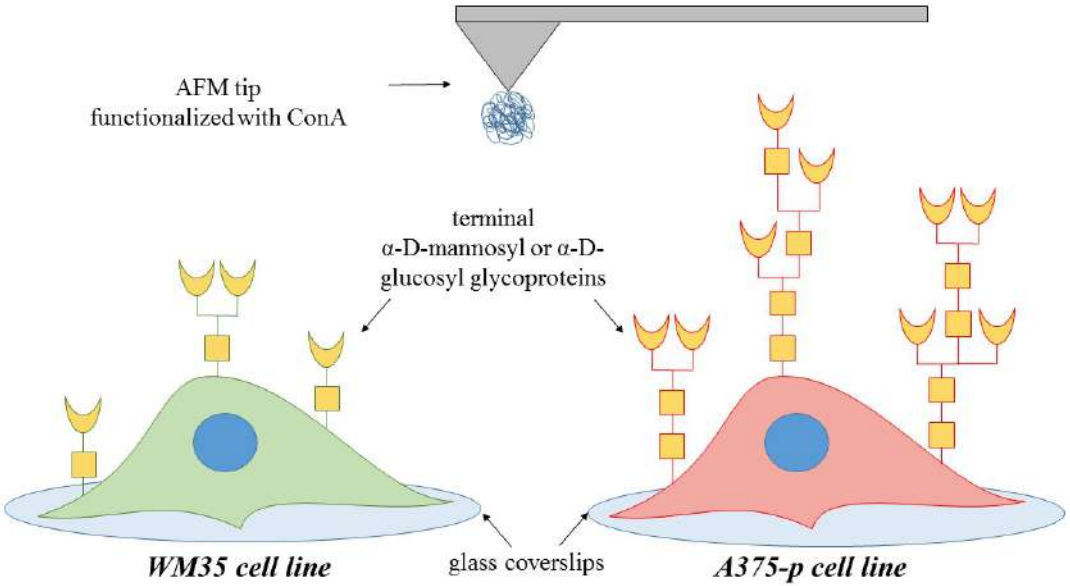
AFM of melanoma surface glycans

Probing cell surface glycans with ConA



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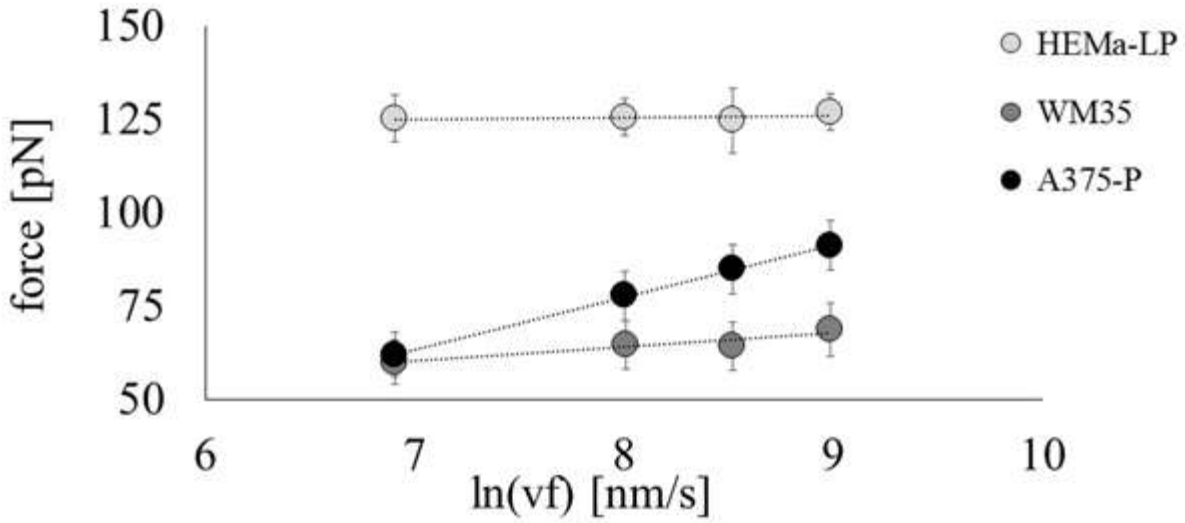
AFM of melanoma surface glycans - lectin binding

System spring constant

When the receptors are present on the surface of cells immobilized on the substrate, the system spring constant reflecting the elasticity of both the cell and the molecular bond has to be considered.
To determine k_s, the average force values were plotted against the corresponding values of the retraction velocity (vf), assuming that $r_f = k_s \times v_f$

$$F = \frac{kT}{x_b} \ln v_f + \frac{kT}{x_b} \ln \frac{k_s x_b}{k_{off} kT}$$



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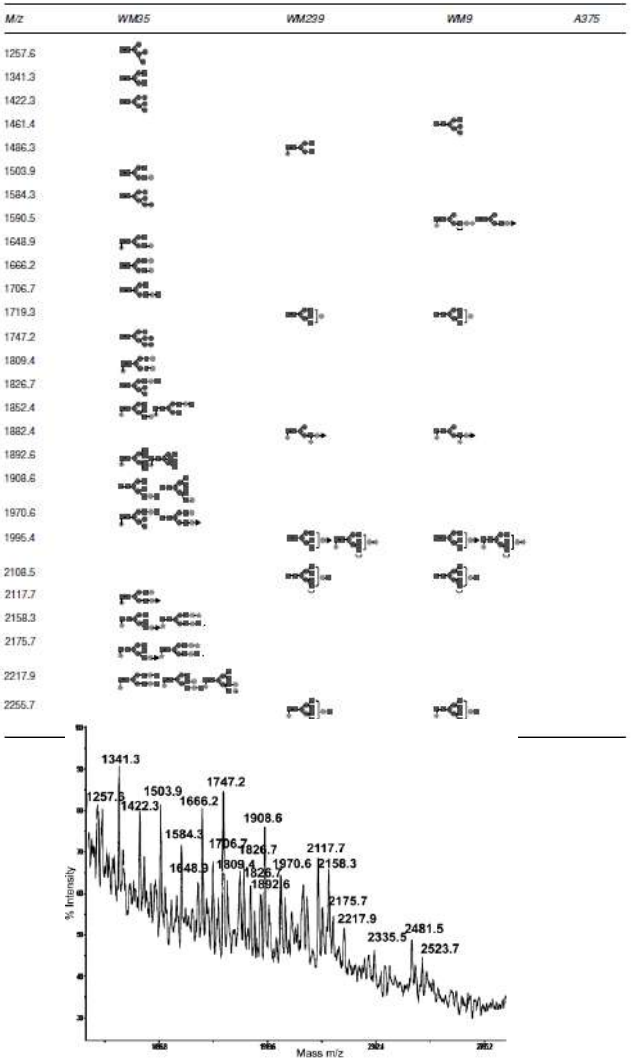
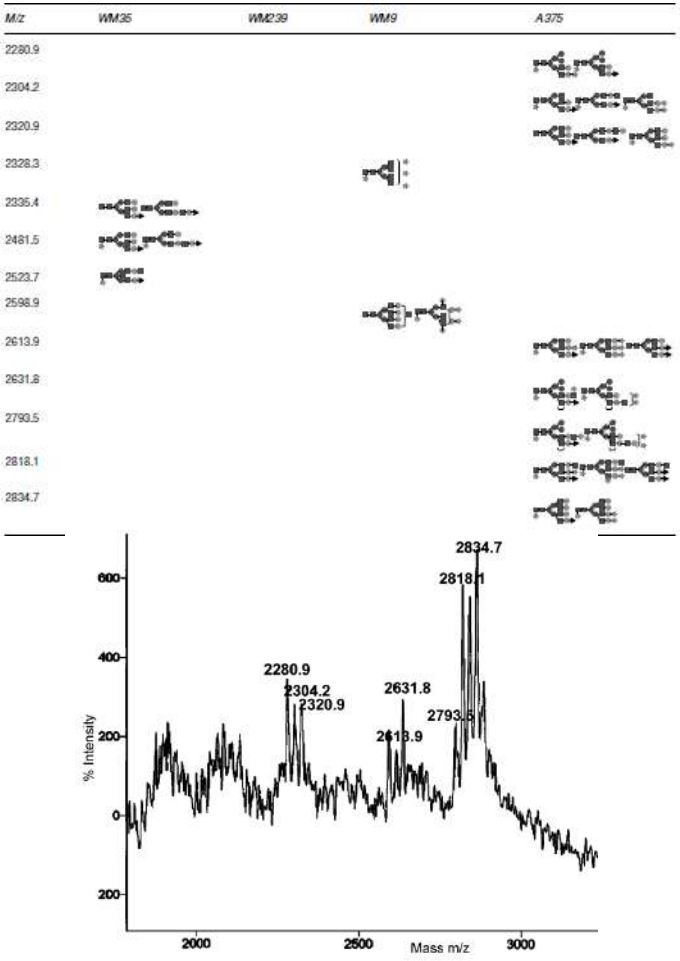
AFM of melanoma surface glycans - lectin binding

Hypothetical oligosacharide structures observed in MALDI MS spectra of melanoma glycans

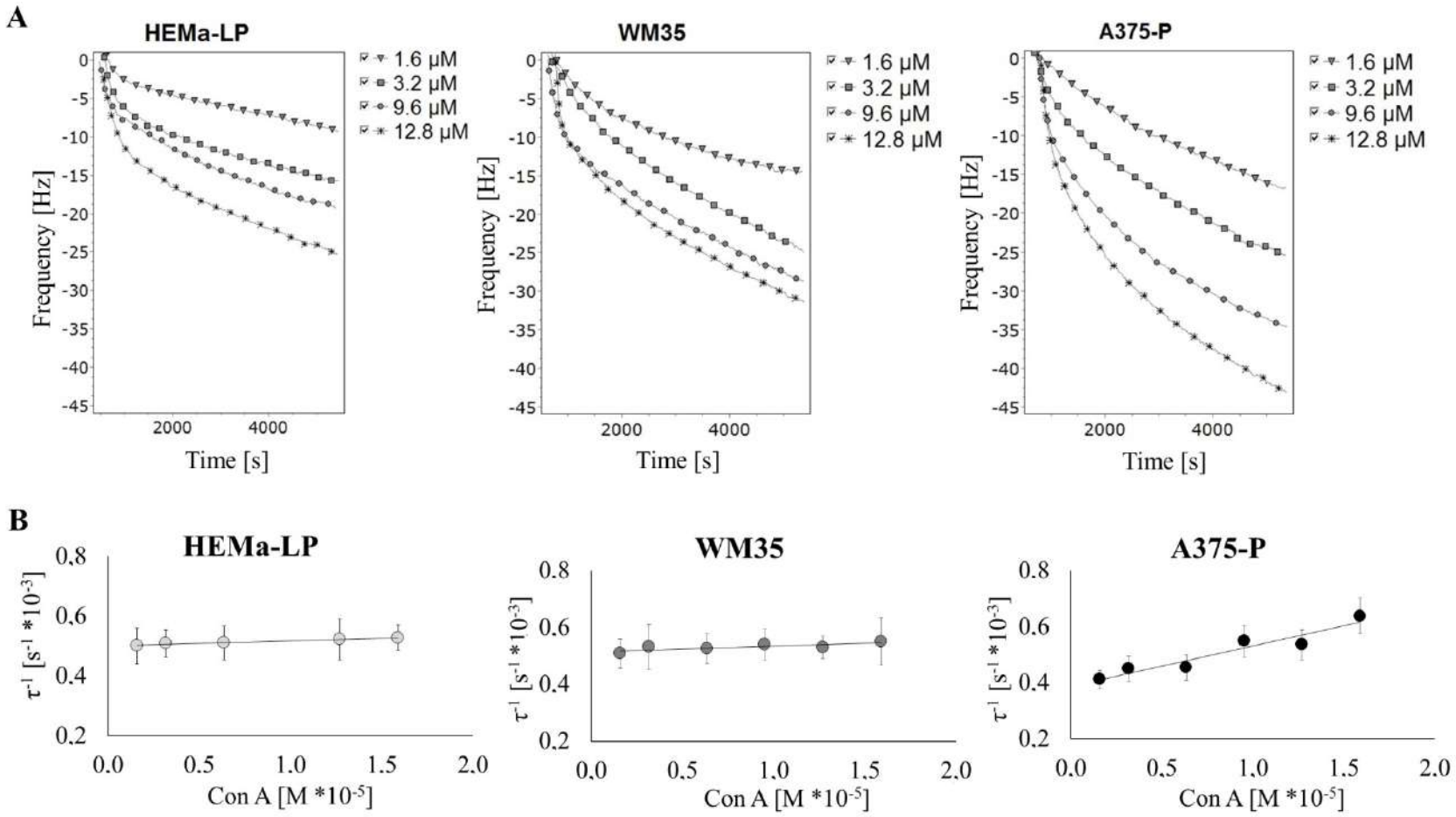




Ciolczyk-Wierzbicka et al., Glycoconjugate Journal 20, 483–492, 2004

QCM of melanoma surface glycans - lectin binding

Dissociation rate constant determination



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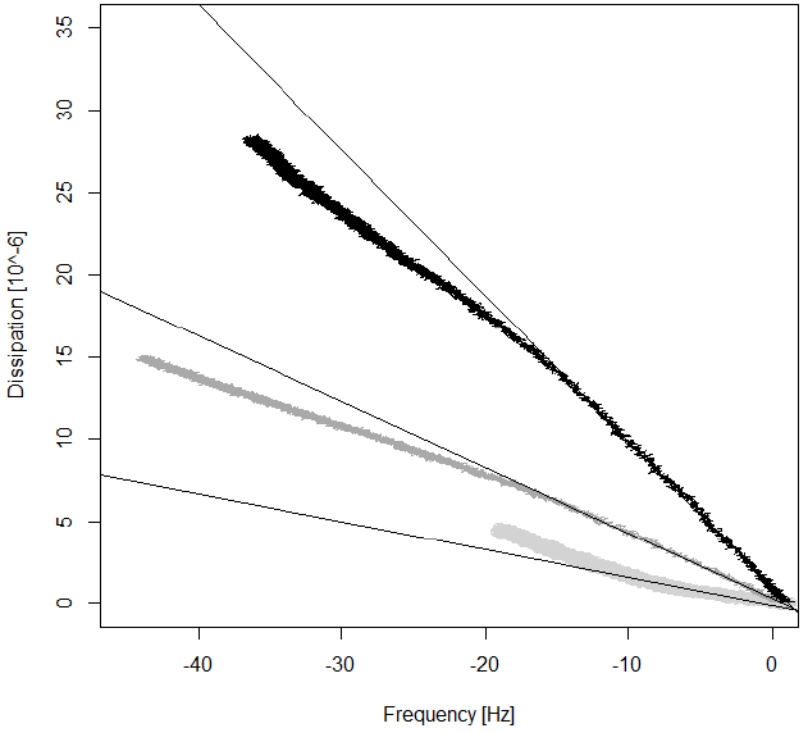
Real-time determination of a cell metastatic potential

Plotting D versus f

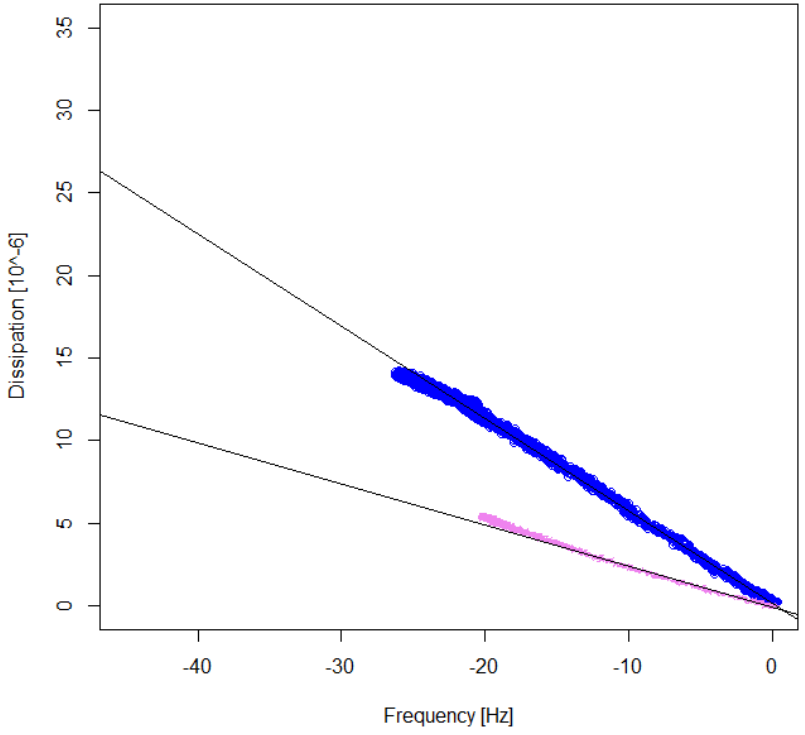
commercial cell lines

melanocytes, primary tumor, metastatic



cells isolated from biopsies

primary tumor, metastatic



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