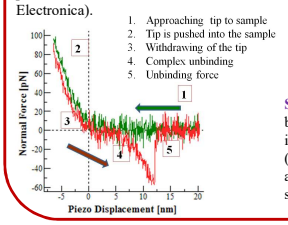
RECENT PROGRESS IN MOLECULAR RECOGNITION IMAGING OF PROTEIN SYSTEMS AT THE NANOSCALE LEVEL

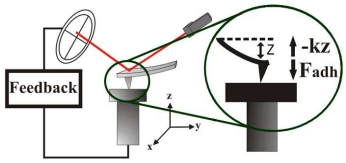
Carlos Marcuello[1,2,*], Anabel Lostao[1,2,3,*]

[1] *Instituto de Nanociencia y Materiales de Aragón (INMA), CSIC-Universidad de Zaragoza, 50009 Zaragoza. Spain*
[2] *Laboratorio de Microscopías Avanzadas (LMA), Universidad de Zaragoza, 50018 Zaragoza. Spain .* [3] *Fundación ARAID, 50018 Zaragoza. Spain*
*Corresponding authors: **cmarcuel@unizar.es; aglostao@unizar.es***

ATOMIC FORCE MICROSCOPY (AFM)

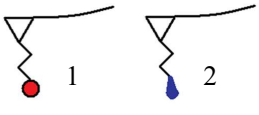
AFM is unique in providing 3-D images of biological structures in a near-physiological environment with nanometer resolution **[1]**. AFM measurements were performed with a Cervantes FullMode SPM (Nanotec Electronica).

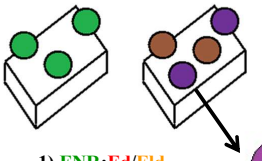




Single Molecule Force Spectroscopy (SMFS) measures the interaction forces between a ligand molecule attached to the AFM tip and a receptor molecule immobilized on a surface. At a given loading rate hundreds of force-distance (Fz) curves are taken (left). Rupture events are processes of stochastic nature and their analysis allows calculating the unbinding force corresponding to a single complex and other parameters of the transition state.

MOLECULAR RECOGNITION IMAGING (MRI) BY JUMPING MODE (JM)



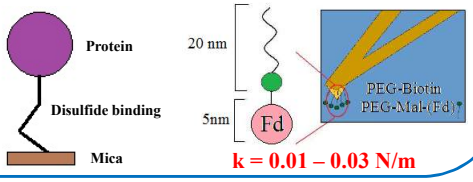


Images were obtained with **JM** using functionalized tips. JM takes a Fz on every point of the sample surface but only the **maximum adhesion** value and **topography data** are collected on each pixel image. In order to prevent non-desire unspecific forces between tip and sample **very low forces are applied** creating a **double repulsive layer (REDL) regime [2].** Thus tip-sample adhesion maps turn into molecular recognition images due to the correlation with topography maps obtaining not only qualitative but also quantitative information of the intermolecular interactions. The goal is improve the sensitivity of previous studies distinguishing between different proteins and considering the proper protein orientation on a sample.

1) FNR:Fd/Fld redox complexes

2) streptavidin/avidin: biotin system

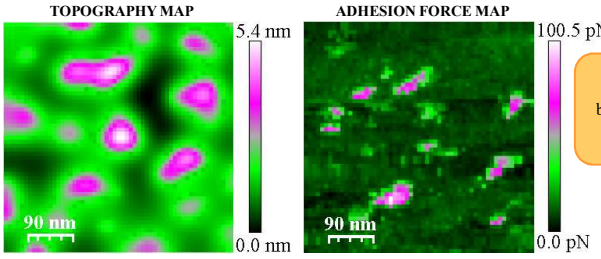
IMMOBILIZATION TIP / SURFACE STRATEGIES



k = 0.01 – 0.03 N/m

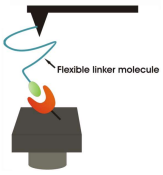
1) MOLECULAR RECOGNITION IMAGING EVIDENCES THE OPTIMUM ORIENTATION OF FNR_C TOWARD FD

FNR:FD BINDING POCKET PROTECTED DURING LABELLING PROCEDURE

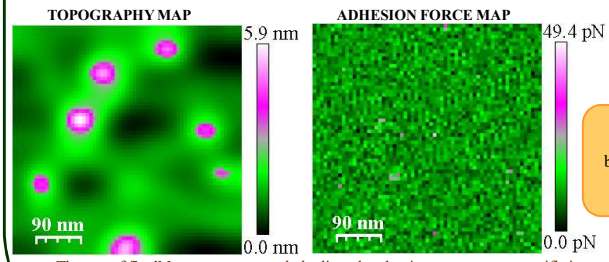


GOOD CORRELATION between topography and adhesion force maps **[2]**

The peaks in the adhesion maps are due to molecular recognition events **[2].**
Similar results were found with functionalized Fld tips **[ref chemphyschem 2015]**



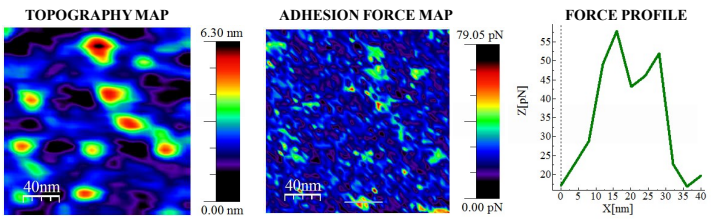
FNR AND FD RANDOMLY TAGGED



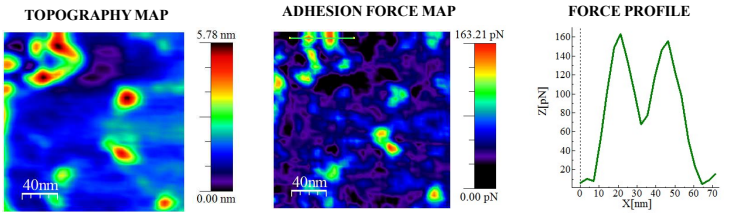
BAD CORRELATION between topography and adhesion force maps **[2]**

The use of **flexible spacers** to attach the ligand at the tip removes non-specific interactions improving the measurement of the specific interaction forces. Operating in JM, topography and adhesion images are obtained simultaneously. When operating **JM in repulsive regime**, adhesion images become MRI images. Images obtained using PEG-maleimide-Fd modified tips [2].

2) MOLECULAR RECOGNITION IMAGING FOR AVIDIN AND STREPTAVIDIN SAMPLES

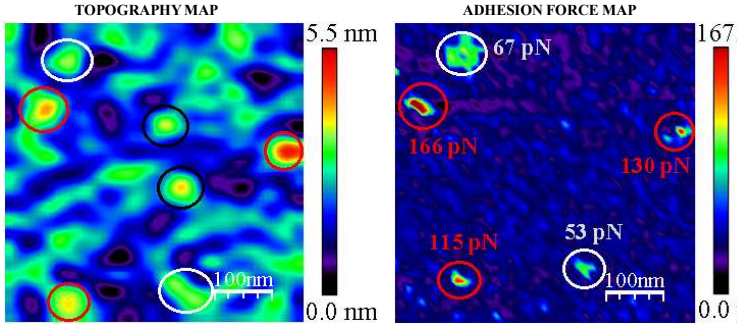


AVIDIN SAMPLES: Unbinding forces for **avidin:biotin** complexes between **40-85 pN (loading rate 1 nN/s)**



STREPTAVIDIN SAMPLES: Unbinding forces for **streptavidin:biotin** complexes between **105-175 pN (loading rate 1 nN/s)**

DISCRIMINATION BETWEEN AVIDIN AND STREPTAVIDIN RECEPTORS IN A SAMPLE MIXTURE



After statistical analysis (N=200 entities) the ratio of identified protein features was **42.0 %** and **46.5 %** for **streptavidin** and **avidin** proteins, respectively. Only **11.5 %** of protein features were of an **unknown** nature (loading rate 1 nN/s) **[4]**.

These findings are not surprising since the mica surfaces were incubated with an equimolecular hybrid amount of strept(avidin) proteins.

RED CIRCLES = Streptavidin protein molecules
WHITE CIRCLES = Avidin protein molecules
BLACK CIRCLES = Unknown protein features

CONCLUSIONS

Jumping Mode is a suitable AFM method to get quantitative information correlating the topography maps with the maximum tip-sample adhesion forces. Working at specific operation parameters, **applying very low forces in a repulsive regime, adhesion images become MRI maps**, resulting in a novel method of **protein identification**. Additionally, a strategy was developed to immobilize in an oriented manner the proteins without altering their functionality (enzymatic assays). This methodology allowed to achieving a high efficiency in the formation of bonds over that of approaches in SMFS for Fd-tips, 61% on FNR_c versus a 17% on FNR_t (0.34 nN, R 19.5 nN/s). This allowed to obtain well-correlated force maps regarding topography based on specific interactions. By other side, mixture samples were scanned with biotinylated tips and found the conditions were avidin and streptavidin molecules could be identified through the intermolecular force with biotin. The identification of protein receptors may open the development of very sensitive biosensors based on force measurements able to identify analytes at the single molecule level.

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