Binding kinetics between soluble β-Gal and anti-β-Gal immobilized on chemically nanopatterned surfaces with fractal topography.

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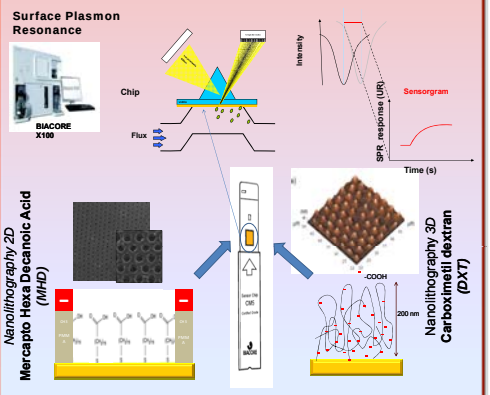
SUMMARY

- The present work was aimed at providing experimental support to the correlation between topographic and kinetic dimensions of protein function we described previously. Through electronic and colloidal nanolithographic techniques we produced surfaces exhibiting a fractal-like pattern with a pre-determined topographic dimension of domains capable to bind proteins in a covalent manner ($Ab_{Anti\beta\text{-}Gal}$).
- These surfaces were used as sensors to reversibly bind β-Gal and enabled the kinetic study of $Ab_{Anti\ \beta\text{-}Gal}$ -β-Gal complex formation by surface plasmon resonance (SPR) spectroscopy. Compared with the behavior of control sensors (homogeneous topography), the Ag-Ab binding kinetics in sensors produced by nanolithography showed higher capacity and broader dispersion of binding sites characterized by a more diffuse attractor in the k_d vs. K_d phase space.

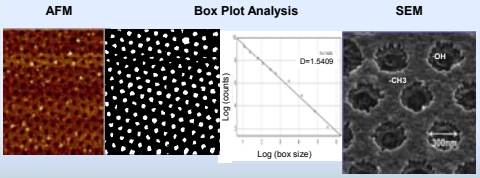
OBJECTIVE

- The aim was to investigate the effect of nanopatterning and fractal types in a protein-protein (Ab_{Anti}β-Gal - β-Gal) interaction kinetics by using nanostructured surfaces with plasmonic resonance properties.

MATERIALS AND METHODS



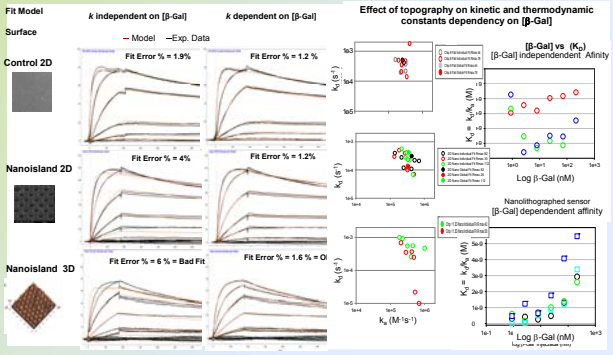
Characterization of nanolithographed substrates



We produced surfaces exhibiting a fractal-like pattern of predetermined fractal dimension in the distribution of domains capable of binding protein in a covalent manner ($Ab_{Anti\beta\ -Gal}$) by applying electronic and colloidal nanolithographic techniques.

RESULTS

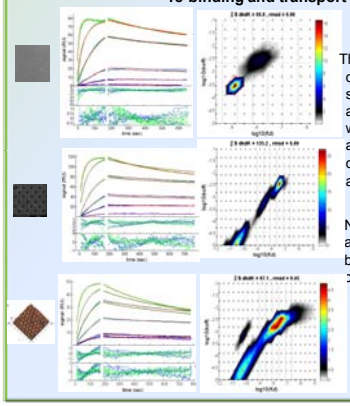
Classic Fit to bi-molecular model



Sensors to reversibly bind β-Gal and enabled the study of $Ab_{Anti\ \beta\text{-}Gal}$ -β-Gal complex formation kinetics by surface plasmon resonance spectroscopy (SPR).

Nanopatterned sensors exhibited β-Gal-dependent values for kinetic constants

Distribution probability of k_d and K_d values in a model that considers effects of re-binding and transport phenomena

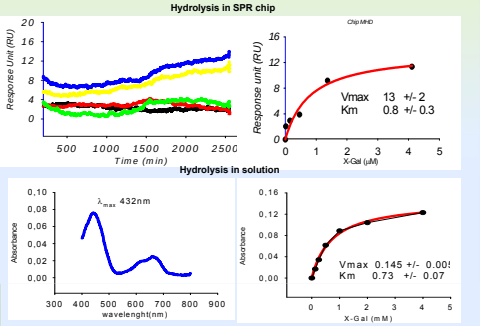


The Ag-Ab binding kinetics showed qualitative differences between control sensors (homogeneous topography) and those produced by nanolithography which evidenced a higher sensitivity and broader dispersion of binding sites characterized by a more diffuse attractor in the phase space k_d vs. K_d.

Nanolithographed surfaces
a)Heterogenity in binding sites
b)↑Sensitivity 2D
c)↓Sensitivity 3D

$$KD = \frac{kd}{ka}$$

Kinetics of X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) hydrolysis in a SPR chip and in solution



We developed a SPR spectroscopic method to detect the kinetics of hydrolysis of a terminal galactose leading to a reaction product that precipitates on the sensor´s surface and interferes with the evanescent field. To our knowledge, this would be the first sucessful SPR application to measure enzyme catalysis.

REFERENCES

Clop EM, Clop PD, Sánchez JM & Perillo MA. *Langmuir*, 24, 10950-10960, 2008.

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