

<u>**R.González**</u>^{a,*}, **L.Caballero^b**, **F.Melo^b**.

Center for Advanced Interdisciplinary Research in Materials (CIMAT) ^{a,*} D. Ciencia de los Materiales, FCFM, Universidad de Chile. ^bDepartamento de Física, Universidad de Santiago de Chile.

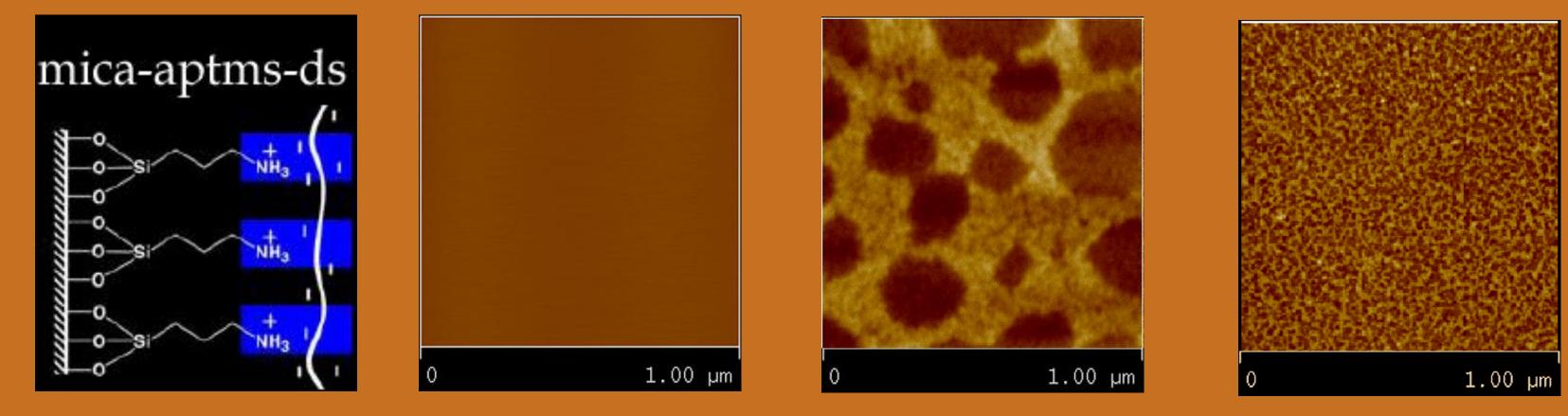
One important problem in biomineralization is the interaction of biological macromolecules with mineral surfaces. The present study investigates the relationship between calcite ionic crystal and the polyanionic oligosaccharide dermatan sulfate (DS), which is present in avian eggshells [1]. To get insight about how DS coordinates on calcite crystal surfaces, template substrates of DS biomolecule were made for calcite nucleation process. First the surface charge density of the substrate is obtained using the microsphere cantilever technique in an atomic force microscope (AFM). Then, the DS substrate surface is activated with CaCl₂ 0.1M and the crystal growth facilitated by adding a supersaturated salt solution. The most favorable sites for nucleation are those able to adsorbe Ca²⁺ ions [2]. Thus, the crystal nucleus orientations have a narrow relationship with substrate DS anionic groups. Crystals of macroscopic dimensions are observed by visible light optic microscopy and SEM analysis allows us to measure and recognize the crystal angles to determine their orientations.

Experimental Procedures

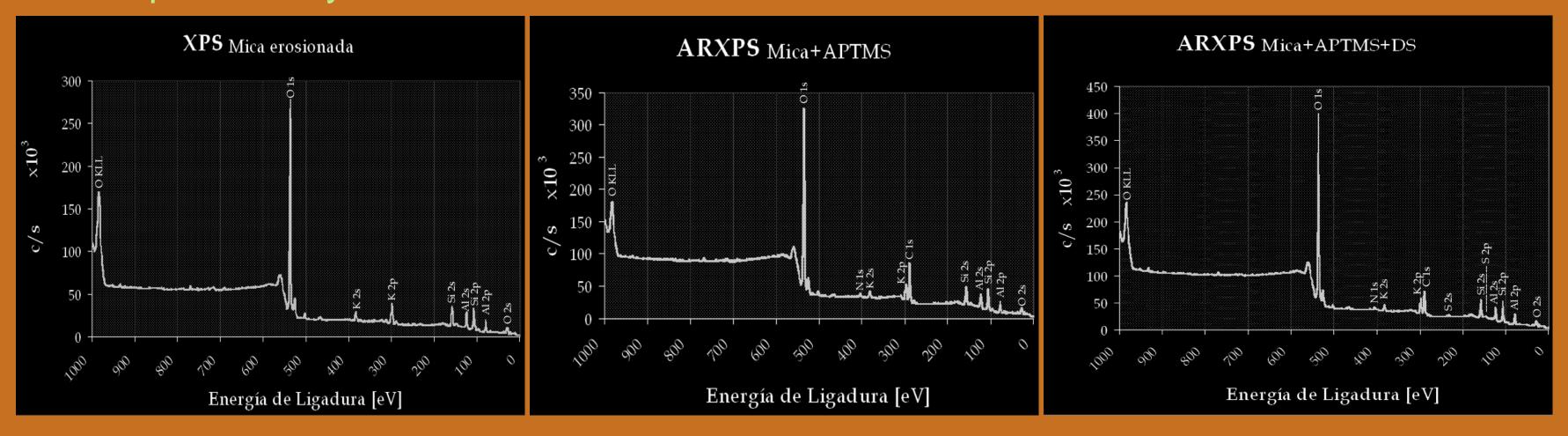
To attach firmly DS molecules to a mica substrate, a 3-aminopropyltrimethoxysilane (APTMS) self-assembled monolayer were made. This molecules binds covalently with

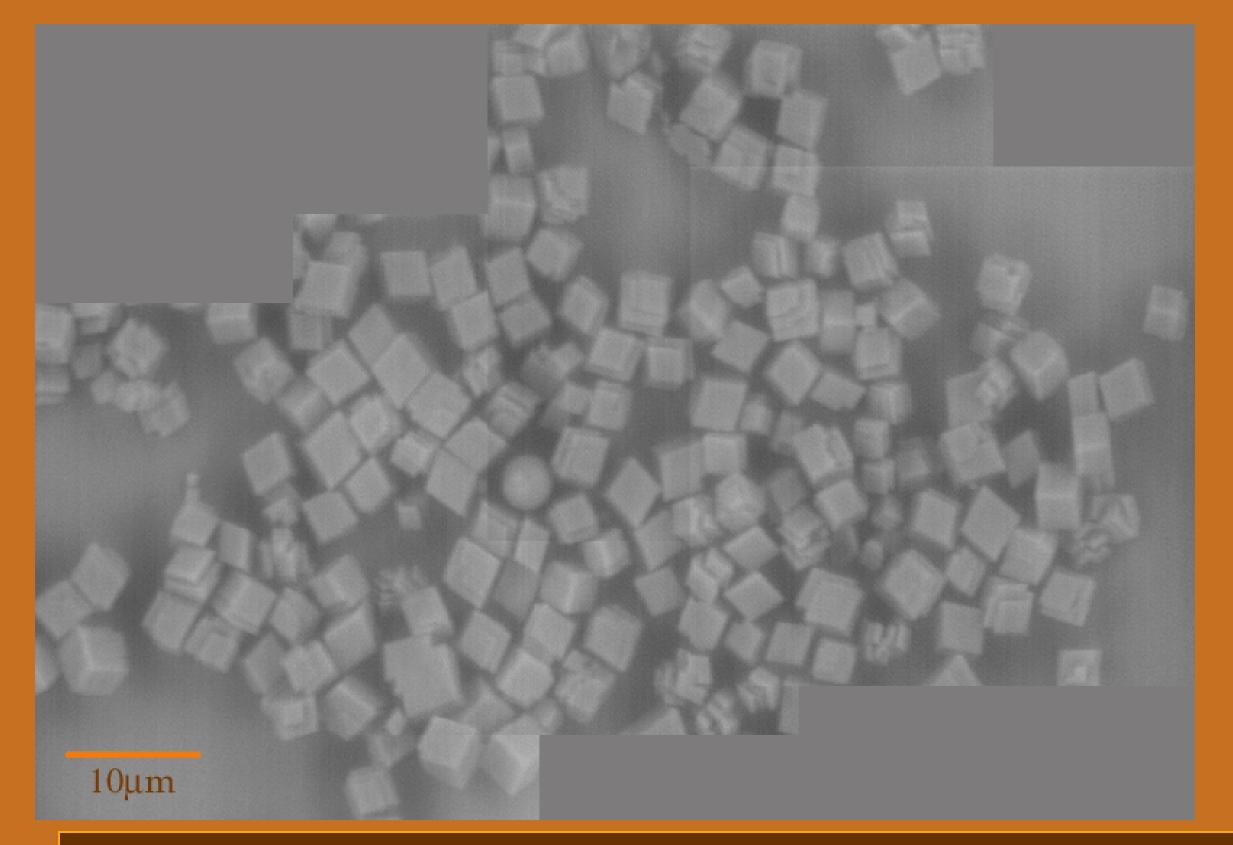
Once the DS substrate is prepared it is activated with a solution of $CaCl_2$ [6] that growth as soon as a supersaturated solution of salts is added [7]. The crystalline phases and orientations are analized using XRD, AFM, SEM and BSE.

silane group to mica surface, and leave their amino groups to the upper end [3]. The amino groups remain positively charged until pH<6, here DS molecules are deposited because of their negative charges at pH>4



The x-ray photoelectron spectroscopy is used to verify that the substrate was formed in the expected way.





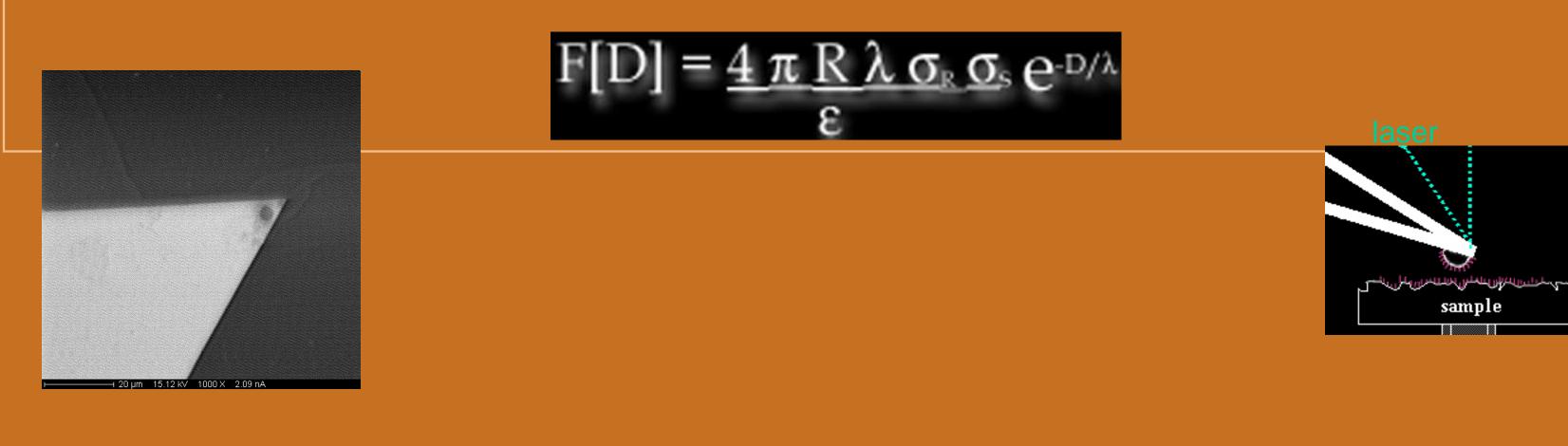
Conclusions:

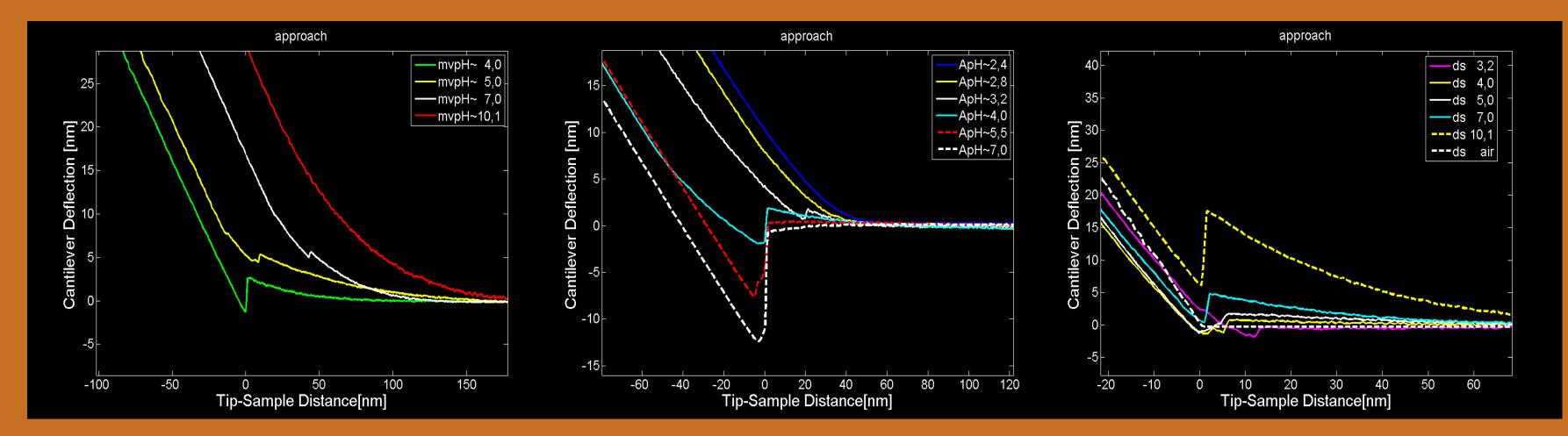
Precipitation of CaCO3 on mica presents rhombohedric calcite crystal nucleation with preferential direction in the <104> face.

5.00 µm

The APTMS surface does not favor crystal nucleation, amorphous structures are observed, that attribute to be of calcium carbonate (vaterite) and sodium

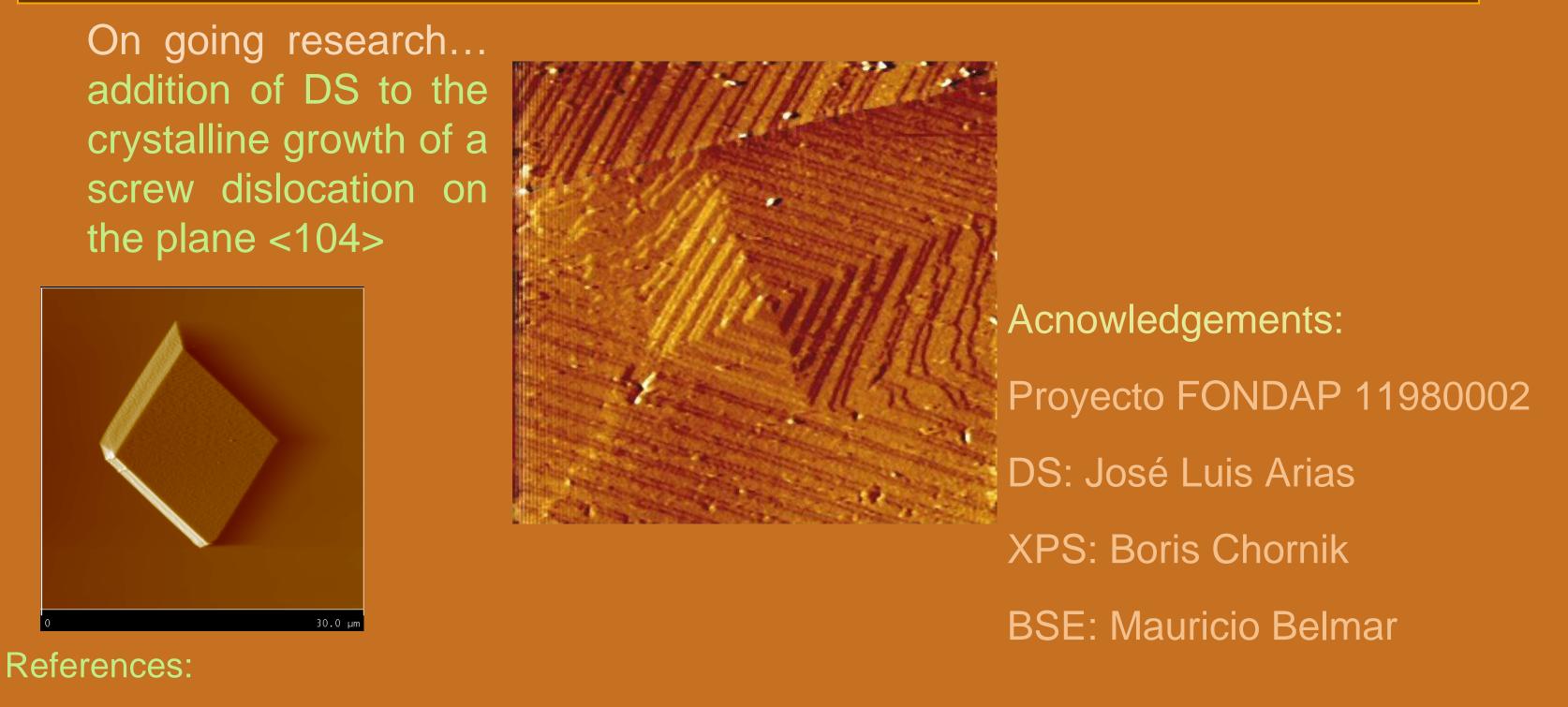
To verify the change in the surface of the substrate covered by a molecule (none, APTMS, DS) we faced an amorphous silicon dioxide (glass) microsphere [4] covered with a similar molecule against the covered mica, then depending on pH the ionizable groups, of the molecules of interest, have different charge states producing a repulsive electrostatic force, in opposition to a weak attractive van der Waals force, measured by the AFM by force spectroscopy experiments [5]. This measurement is done in each stage during the substrate formation.





chloride. The crystals that nucleates in the direction <104>, are assumed to be directly grown from the mica surface.

In the presence of DS, crystal conglomerates are observed and in addition rhombohedric crystals in different directions that the usual one on mica.



[1] J.L.Arias et al J. Mater. Chem. 10(2004)1039 "Sulfated Polymers in biological mineralization: a plausible source for bio-inspired engineering"

[2] L. Addadi et al. Proc. Natl Acad. Sci USA 84 (1987) 2732-2736 "A Chemical Model for the Cooperation of Sulfates and Carboxylates in Calcite Crystal Nucleation: Relevance to Biomineralization"

[3] L. Ng et al. J. Struct. Biol. 143 (2003) 242-257 "Individual cartilage aggrecan macromolecules and their constituent glycosaminoglycans visualized via atomic force microscopy"

[4] S.H.Behrens et al. J. of Chem. Phys. 115(2001) 14, 6716-6721 "The Charge of Glass and Silica Surfaces"[5] W.F.Heinz et al Nanotechnology 17(1999)143-150 "Spatially resolved force spectroscopy of biological surfaces using the atomic force microscope"

[6] J. Aizenberg et al. J. Am. Chem. Soc. 121 (1999) 4500-4509 "Oriented Growth of Calcite Controlled by Self-Assembled Monolayer of functionalized Alkanethiols Supported on Gold and Silver"
[7] H.H. Teng et al. Geochimica et Cosmochimica Acta 64(2000)13, 2255-2266 "Kinetics of calcite growth: Surface processes and relationships to macroscopic rate laws"