# Stress, Drug resistance and Adhesion: a closer look at the dark side of the wall

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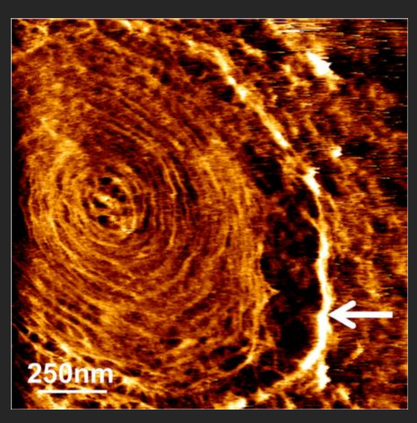
Stress conditions and antifungal drugs induce significant changes in the cell wall composition of yeasts and fungi. They cause modifications of the cell wall molecular architecture, including nature, repartition and attachment of cell wall proteins to the cell surface. **Atomic Force Microscopy (AFM)** is a powerful tool for studying the morphology, nanomechanical and adhesive properties of live microorganisms under physiological conditions. We imaged and measured the biophysical consequences of various stresses on both *S. cerevisiae* and *C. albicans* cell morphology at the nanoscale, focusing on changes in cell surface aspect and characteristics: roughness, elasticity, and adhesive properties.

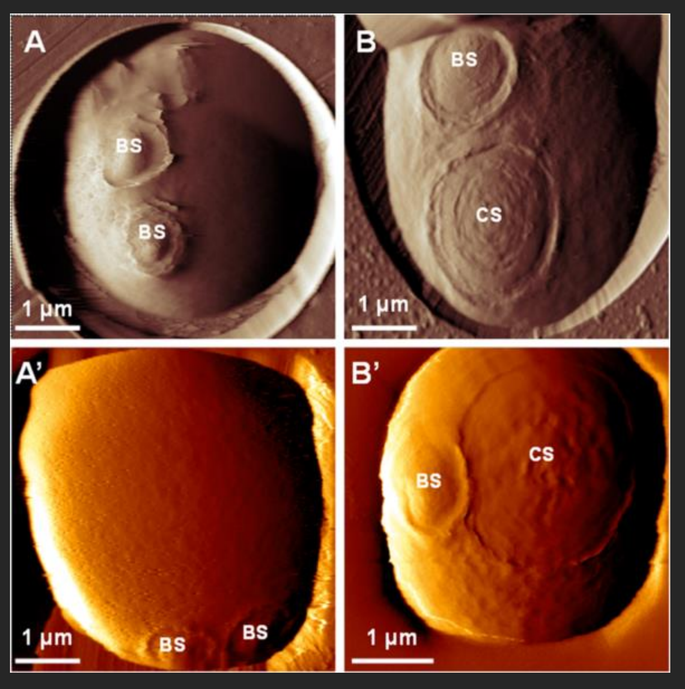
## *Heat Shock*

HS induces formation at the cell surface of a circular structure that takes its origin at a single punctuate source and propagates in a concentric manner to reach a diameter of 2–3 µm [1].

Our results suggest that this singular morphological event occurring at the cell surface is due to a dysfunction in the budding machinery and that this phenomenon is controlled by the CWI pathway.

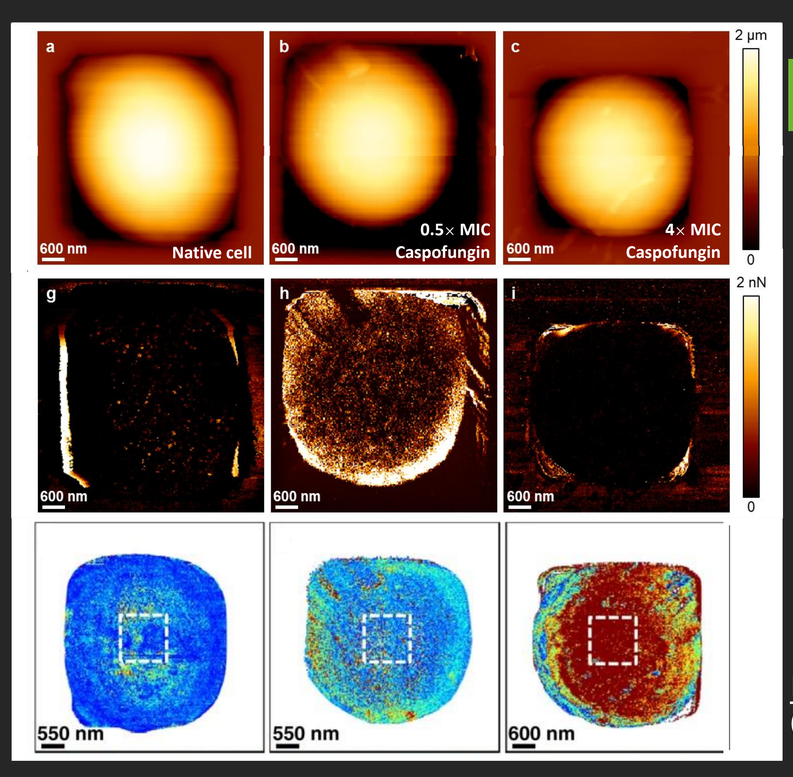



Ultrastructure of the Circular Structure imaged by AFM




AFM deflection images of surface topology of living yeast cell at 30°C (unstressed, A, A') or exposed to heat shock for 1 h at 42°C (B, B'). *BS: Bud scar, CS: circular structure.*

## *Nanoscale effects of Caspofungin on Yeast cell wall*



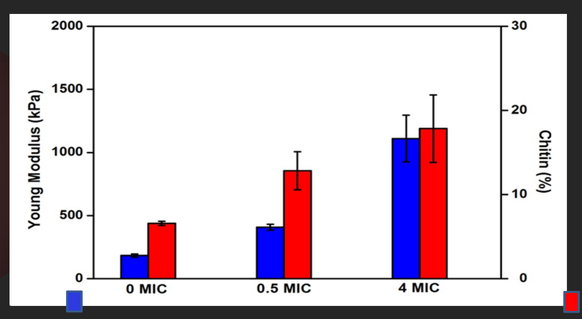

Cell's relief
*(= 3D Image)*

Adhesion Maps

Elasticity maps
(z range = 0.5 MPa)

***Caspofungin induces a deep cell wall remodeling, evidenced by nano-mechanichal properties and dramatic chitin increase[2].***

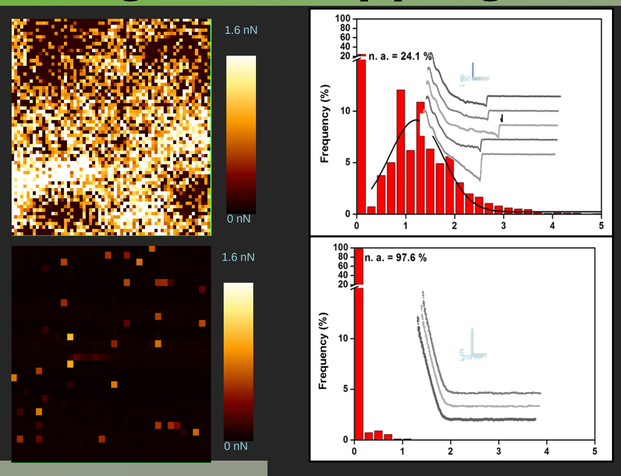
***A low dose of Caspofungin (0.5 x MIC) results in adhesins expression on the cell surface***




## *Measuring and mapping Cellular Adhesion*

*PGA22 Over-expressed*

*Control -*




Adhesion force
Maps of 1µm², corresponding histograms representing the adhesion force repartition, and a few representative force curves.

***Molecular Biology tools and AFM established the contribution of previously uncharacterized PGA proteins to C. albicans adhesion mechanism[3].***

## *Conclusions and outlook*

AFM allowed us to unravel the morphologic effect on yeast cells of Heat Shock, Osmotic Shock, exposure to toxins and drugs. Moreover, using Single Molecule Force Spectroscopy (SMFS) we can now explore the organization of adhesins, map them on the cell surface and quantify their adhesion forces. Altogether, our studies establish the great interest of AFM to explore molecular mechanisms occurring on the cell surface of live fungal cells.

1. Pillet et al. Uncovering by A F M of an original circular structure at the yeast cell surface in response to heat shock. BMC Biology 2014, 12:6
2. Formosa C. et al., (2013), Nanoscale effects of Caspofungin against two yeast species; *S. cerevisiae* and *C. albicans*, AAC, 57, 3498.
3. Cabral V. et al., (2015),Targeted changes of the cell wall proteome influence *C. albicans* ability to form single- and multi-strain biofilms. PloS Pathog. 2014 Dec 11;10(12).