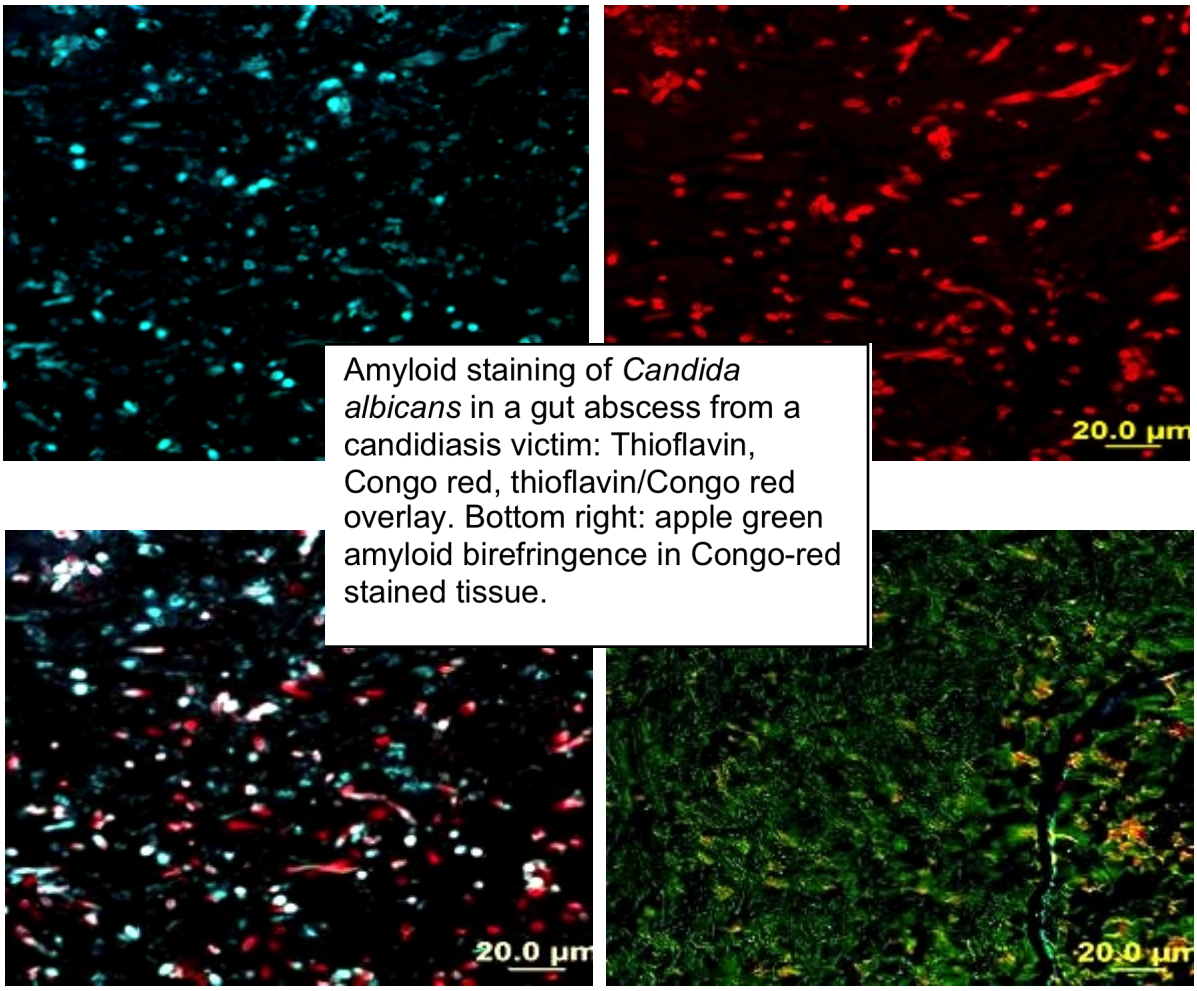
Candida albicans ALS proteins-
AMYLOIDS are our friends (if we are yeasts)

Als proteins bind the yeast to us for commensal and pathological relationships. These cell adhesion proteins have the remarkable ability to bind to an extremely broad number of host molecules, as well as to other Als proteins on other yeasts. The secret is that they form amyloid-like part-crystal patches on the cell surface, so that hundreds or thousands of Als proteins can bind at once. That ability give extremely strong adhesion to thousands of different host proteins.

With the labs of Yves Dufrene (U. catholique de Louvain, Belgium), Steve Klotz (U. Arizona), and Jason Rauceo (John Jay College CUNY) we are dissecting how the various domains of these proteins interact with human host proteins and with other fungal and bacterial proteins. These interactions facilitate invasion of the host and colony formation and formation of microbial biofilms that are resistant to anti-fungal treatments. Therefore, interfering with these processes will help fight fungal infections, which actually kill many patients with chronic conditions such as AIDS and leukemia. We have now published over 20 papers showing:

- Amyloid sequences are present and functional in many fungal adhesins
- Amyloid nanodomains on the surface of baker's yeast *S. cerevisiae* and the opportunistic pathogen *C. albicans*
- Amyloid nanodomains form in response to physical force, and that they lead to stronger cell-to-cell binding
- Amyloid nanodomain formation leads to larger and more robust biofilms
- Fungal surface amyloids repress immune response to fungal infections

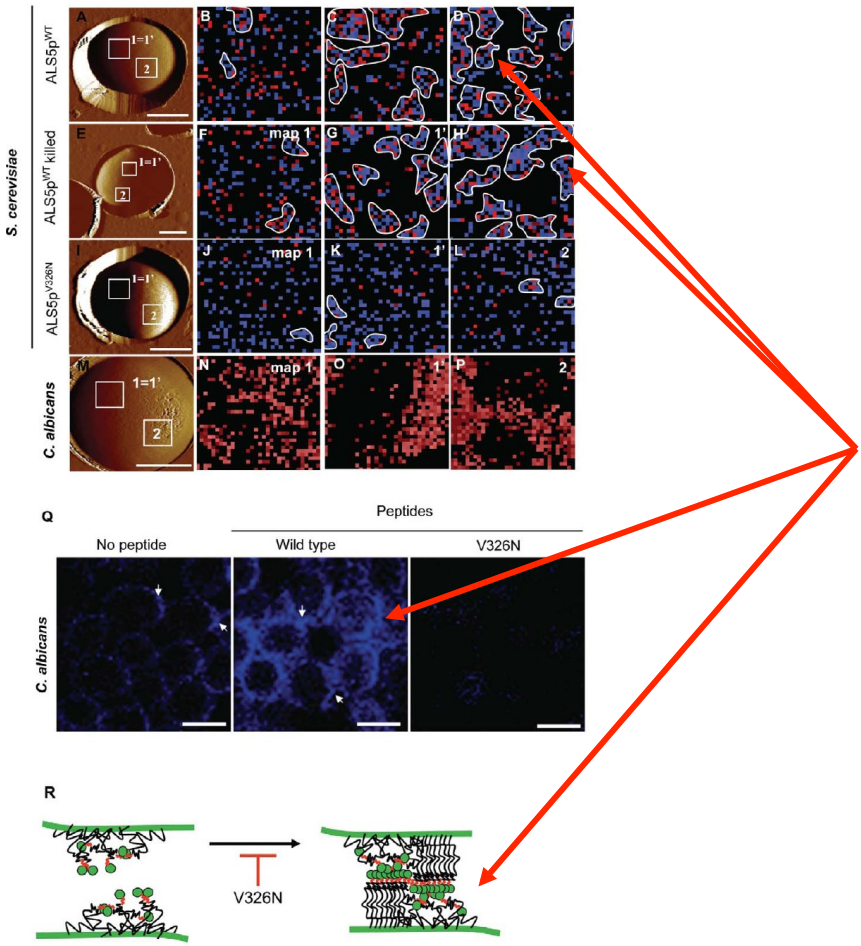
Yeast surface amyloids are detectable in tissue from abscesses, and appear to down-regulate inflammatory responses to fungal infections!



Amyloid staining of *Candida albicans* in a gut abscess from a candidiasis victim: Thioflavin, Congo red, thioflavin/Congo red overlay. Bottom right: apple green amyloid birefringence in Congo-red stained tissue.

Gilchrist, K.B., M.C. Garcia, R. Sobonya, P.N. Lipke and S.A. Klotz. 2012. New features of candidiasis in humans: Amyloid formation by fungi and deposition of Serum Amyloid P component by the host. J. Infect. Dis. *In press.* doi: 10.1093/infdis/jis464

Atomic Force Microscopy in Dr. Dufrene's lab in Louvain, Belgium and confocal microscopy in our lab directly demonstrate clustering of the Als molecules after we pull on a few molecules. This clustering is caused by surface amyloid formation, as shown by studies with inhibitors and mutants.



AFM Surface maps

Amyloid nanodomains

Confocal with thioflavin T amyloid stain

Cartoon!

Figure 7. Force-induced adhesion nanodomains in single live cells. (A) AFM topographic image (scale bar: 2 μm), in buffer, showing wild-type *S. cerevisiae* cells expressing V5-tagged Als5p^{WT} proteins. (B) Adhesion force map (1 μm ×1 μm) recorded with an anti-V5 tip on a given target area of the native cell that was never subjected to force (maps #1, recorded on the square shown in (A). Blue and red pixels correspond to forces smaller and larger than 150 pN, respectively, thus to V5-tagged Als5p^{WT} recognition and unfolding. (C) Second adhesion force map (1 μm × 1 μm) recorded on the same target area (map #1'). The heterogeneous distribution of coloured pixels, which represents the detection of single Als5p^{WT} molecules documents the formation of nanoscale clusters (outlined in white). (D) Adhesion force map (1 μm × 1 μm) recorded on a remote area (map #2) localized several hundred nanometers away from the first map (see squares in A). (E–H) Same sequence of data as in A–D obtained on heat-killed *S. cerevisiae* cells expressing Als5p^{WT}. (I–L) Same sequence of data as in A–D obtained on *S. cerevisiae* expressing ALS5p^{V326N}. (M) AFM

J.J Heinisch, P.N. Lipke, A. Beaussart, S. El Kirat Chatel, V. Dupres, D. Alsteens, and Y.F. Dufrene. 2012. Atomic force microscopy–looking at mechanosensors on the cell surface. J. Cell Sci. *In press.*

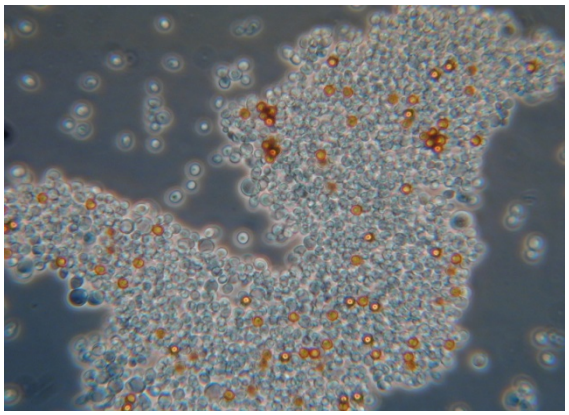
Lipke, P.N., M.C. Garcia, D. Alsteens, C.B. Ramsook, S.A. Klotz and Y.F. Dufrêne. 2012. Strengthening relationships: amyloids create adhesion nanodomains in yeasts. Trends Microbiol. 20: 59-65.

Alsteens, D., C.B. Ramsook, P.N. Lipke, and Y.F. Dufrene. 2012. Unzipping of a functional microbial amyloid. ACS Nano *in press*. DOI: 10.1021/nn3025699

Garcia, M.C., J.T. Lee, C.B Ramsook, D. Alsteens, Y.F Dufrene, and P.N. Lipke. 2011. A role for amyloid in cell aggregation and biofilm formation. PLoS One 6: e17632. doi:10.1371/journal.pone.0017632

Alsteens, D., M.C. Garcia, P.N. Lipke, and Y. Dufrene* (*co-corresponding authors). 2010. Force-induced formation and propagation of adhesion nanodomains in living fungal cells. Proc. Natl. Acad Sci., USA 107:20744-9. *[featured "In This Issue"]*

Als proteins bind to many mammalian proteins (coated on the brown-gold beads in this picture) then cause the yeast to stick to each other. The binding causes a conformational change, which results in increased fluorescence by ANS, a dye that binds to hydrophobic regions of proteins.

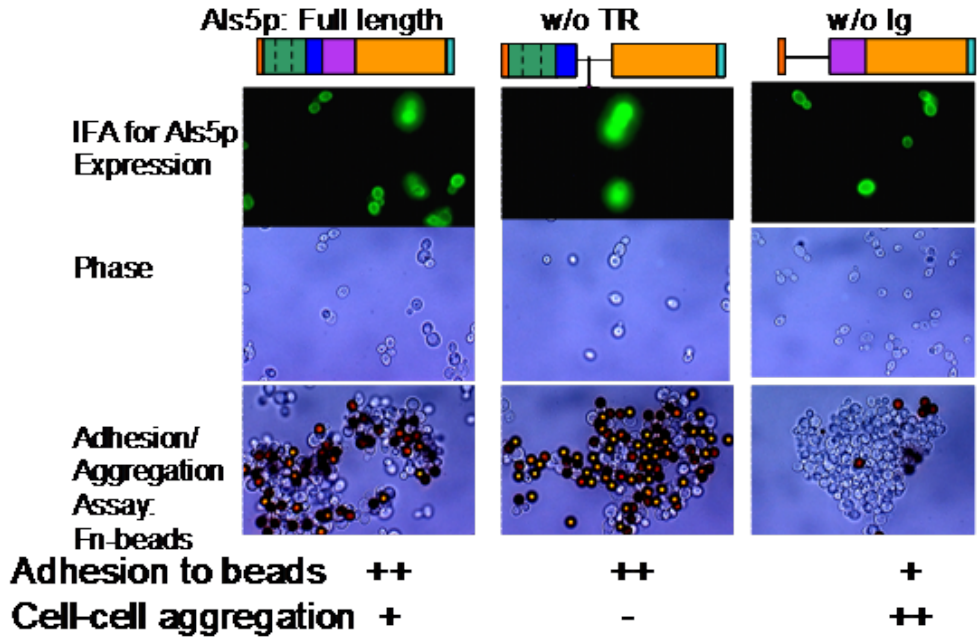


Rauceo, J., N.K. Gaur, S.A. Klotz, K.-G. Lee, and P.N. Lipke. 2004. Global Cell-surface Conformational Shift Mediated by a *Candida* Adhesin. *Infect. Immun.* 72: 4948-4955.

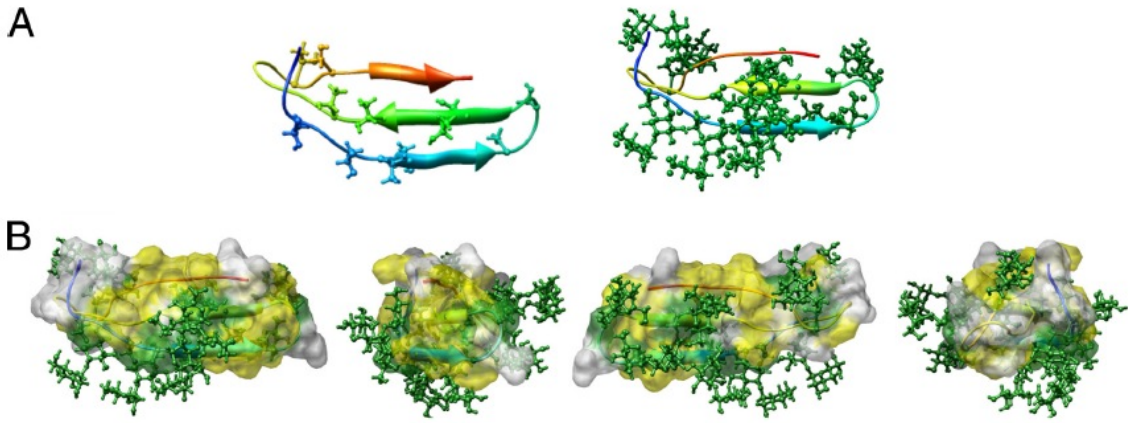
Gaur NK, Klotz SA, Henderson RL. Overexpression of the Candida albicans ALA1 gene in Saccharomyces cerevisiae results in aggregation following attachment of yeast cells to extracellular matrix proteins, adherence properties similar to those of Candida albicans. Infect Immun. 1999 Nov;67(11):6040-7.

Klotz, S.A., N.K. Gaur, D.F. Lake, V. Chan, J. Rauceo, and P.N. Lipke. 2004. Degenerate Peptide Recognition by *Candida albicans* Adhesins Als5p and Als1p. *Infect. Immun.* 72: 2029-2034

The Ig-like (green) and TC (blue) and TRR regions (purple) participate in this binding and yeast aggregation.



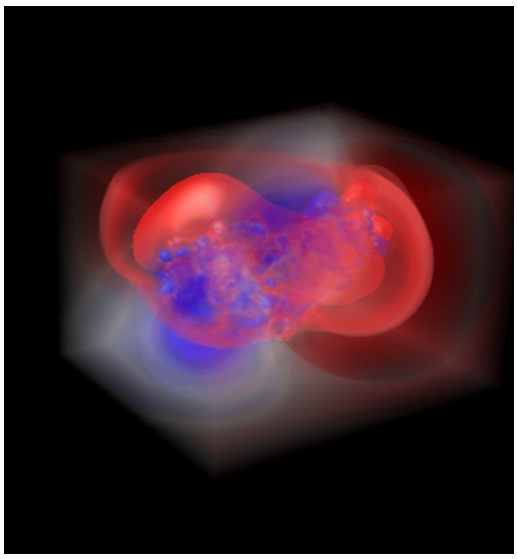
Rauceo JM, De Armond R, Otoo H, Kahn PC, Klotz SA, Gaur NK, Lipke PN. 2006 Threonine-rich repeats increase fibronectin binding in the Candida albicans adhesin Als5p. Eukaryot Cell. 5:1664-73.



A model of a tandem repeat domain from:

*Frank, A.T., C.B. Ramsook, H.N. Otoo, C. Tan, G. Soybelman, J.M. Rauceo, N.K. Gaur, Stephen A. Klotz, and P.N. Lipke. 2010. Structure and Function of Glycosylated Tandem Repeats from *Candida albicans ALS* Adhesins. Eukaryot. Cell 9: 405-414. *[Cover illustration]*

The hydrophobic exposed surfaces (yellow) bind to plastic and each other. O-glycans are colored green.



An image of the electrostatics of a tandem repeat, used as the cover illustration for the journal Eukaryotic Cell