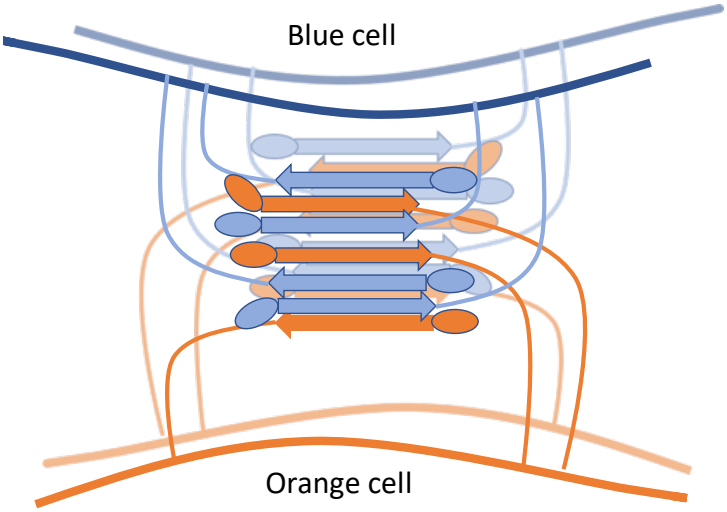
Candida albicans ALS proteins-
AMYLOIDS are our friends (if we are yeasts)

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. These biofilms can help clarify beer and wine
- Fungal surface amyloids repress immune response to fungal infections
- Amyloid-like bonds form between cells to make a tight bond that leads to self-self reconition and exclusion of unrelated cells from biofilms. The illustration below is a model for how amylpid interactions could form between two cells: a blue cell on top and an orange cell on the bottom



Dehullu, J., C. Valotteau, P. Herman-Bausier, M. Garcia-Sherman, M. Mittelviefhaus, J.A. Vorholt, P.N. Lipke*, and Y.F. Dufrêne* (*co-corresponding authors). 2019. Homophilic adhesion by *Candida albicans* Als proteins is mediated by amyloid-like interactions between cells. Nano Letters 19, 3846-3853 https://doi.org/10.1021/acs.nanolett.9b01010

Dehullu, J., J.A. Vorholt, P.N. Lipke, and Y.F. Dufrêne. 2019. Fluidic force microscopy captures amyloid bonds between microbial cells. *Trends Microbiol. 2019 Jul 1. pii: S0966-842X(19)30156-8. doi:*

10.1016/j.tim.2019.06.001

- Fungal surface amyloids repress immune response to fungal infections

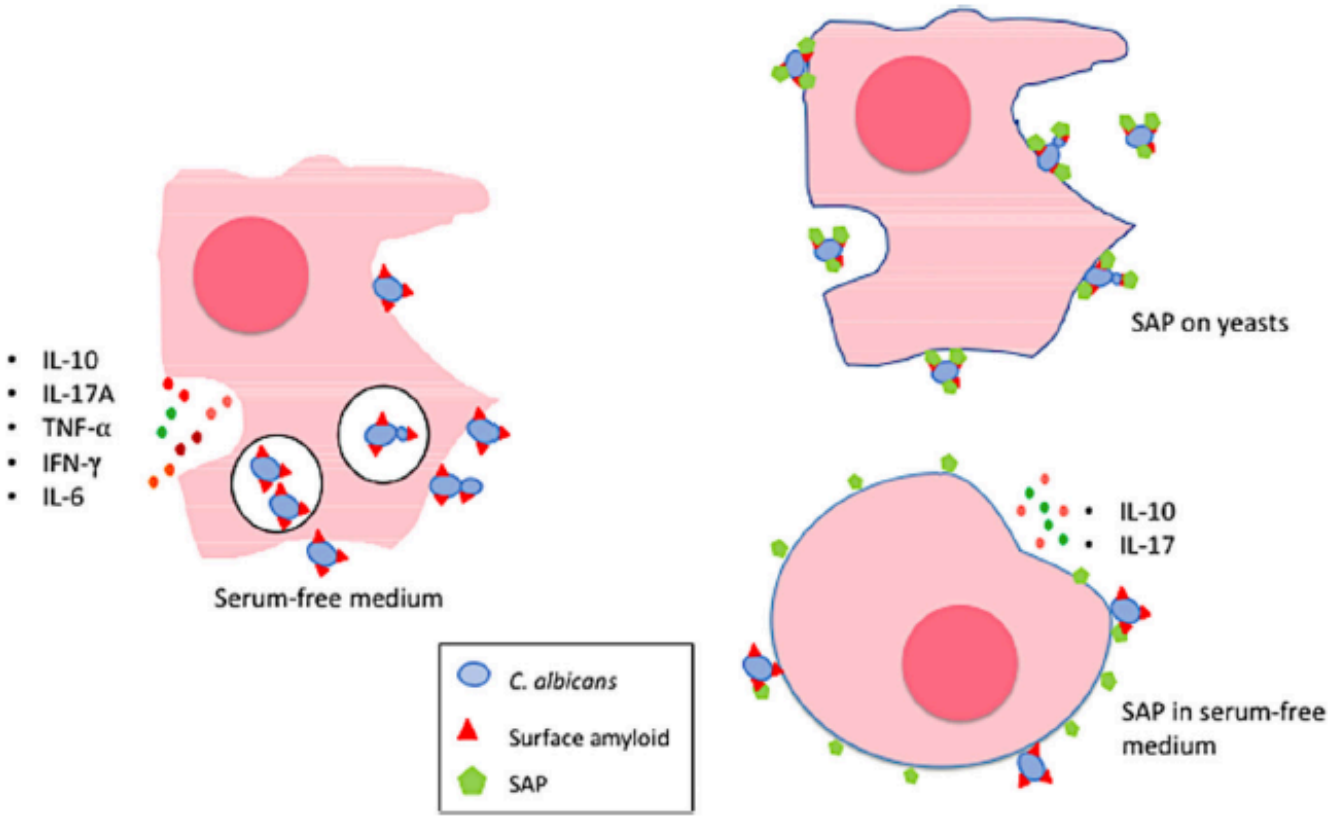
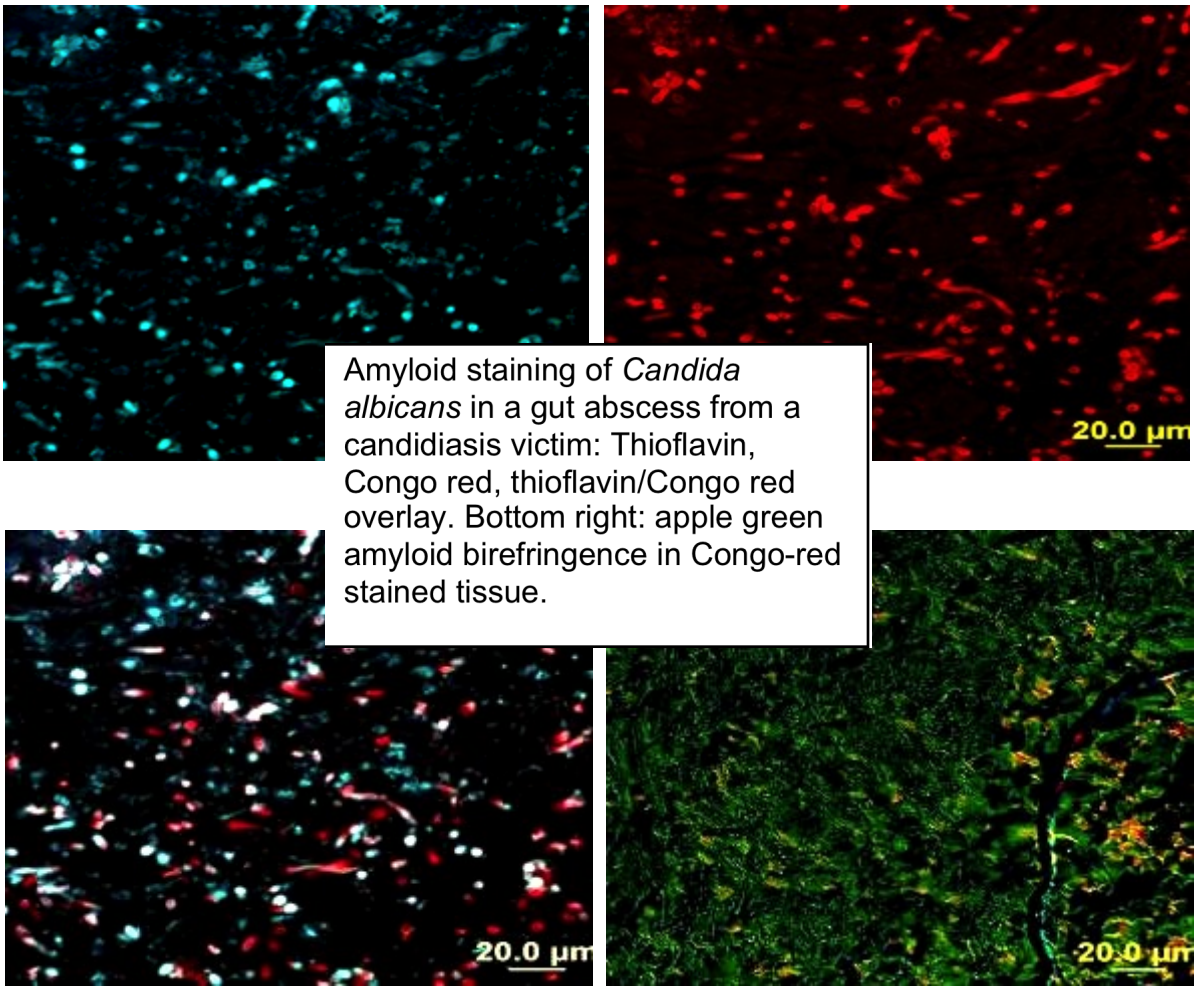


FIG 6 Figurative rendition of important findings of this investigation involving human macrophage-SAP-*Candida albicans* yeast interactions. (Left) The macrophage is a control macrophage (cultured in serum-free medium) and *C. albicans* yeasts added to it in serum-free medium. The cell secretes numerous cytokines and phagocytoses yeasts in great numbers. (Top right) The macrophage is cultured like the control macrophage, but the *C. albicans* yeasts are soaked in normal human serum or in purified SAP for an hour before addition to the macrophage, allowing SAP to bind to the fungal functional amyloid. Phagocytosis is reduced >80%. (Bottom right) The macrophage is cultured identically to the control macrophage except that it is incubated with SAP (30 μg/ml) for 1 h before *C. albicans* yeasts are added. Like the macrophage above it, there is a >80% reduction in phagocytosis of yeast cells, but only IL-10 and IL-17A are secreted in increased amounts. After SAP treatment, the macrophages were more rounded in their overall shape, a reflection of their quiescence.

Behrens, N.E., P.N. Lipke, D. Pilling, R.H. Gomer and S.A. Klotz. Serum Amyloid P Component Binds Fungal Surface Amyloid and Decreases Human Macrophage Phagocytosis and Secretion of Inflammatory Cytokines. 2019. mBio 10:e00218-19. https://doi.org/10.1128/mBio.00218-19. PMC6414697

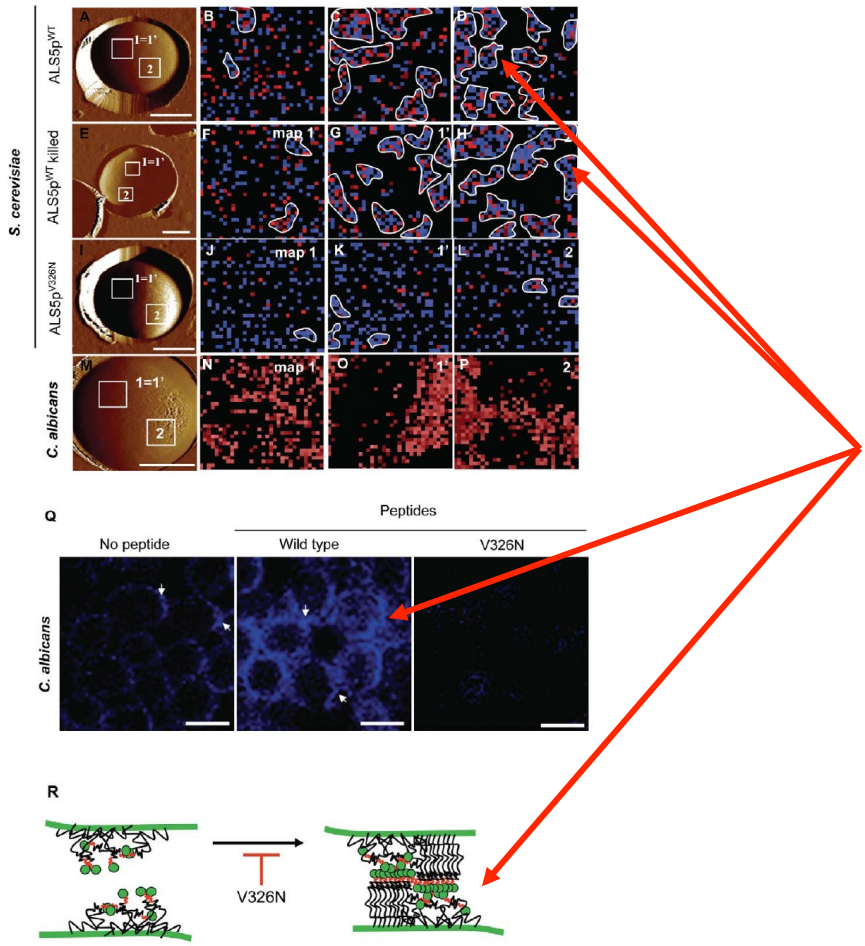
Yeast surface amyloids are detectable in tissue from abscesses, and appear to down-regulate inflammatory responses to fungal infections!



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.*

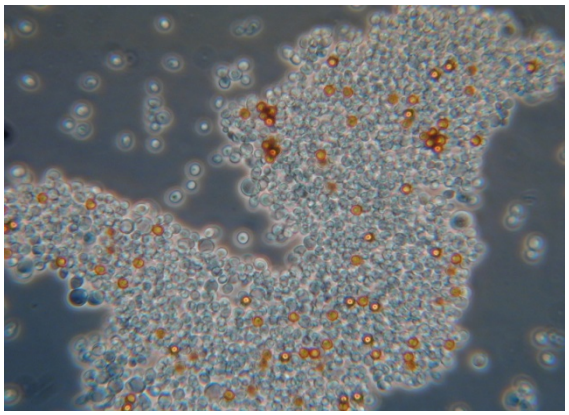
Lipke, P.N., M.C. Garcia, D. Alsteens, C.B. Ramsook, S.A. Klotz and Y.F. Dufrêne. 2012. Strengthening relationships: amyloids create adhesion nanodomains in yeasts. Trends Microbiol. 20: 59-65.

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.

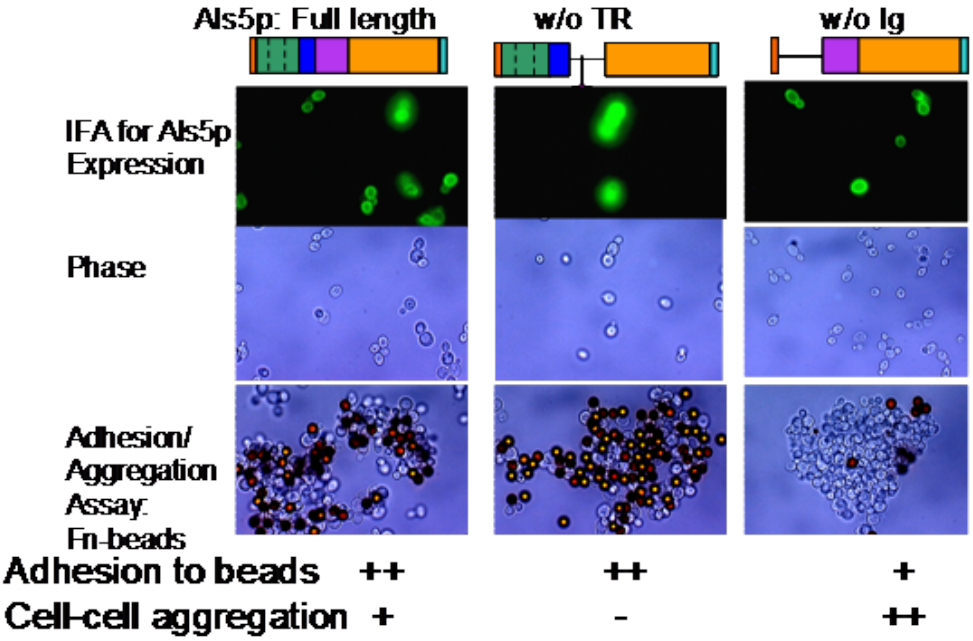


Rauceo, J., N.K. Gaur, S.A. Klotz, K.-G. Lee, and P.N. Lipke. 2004. Global Cell-surface Conformational Shift Mediated by a *Candida* Adhesin. *Infect. Immun.* 72: 4948-4955.

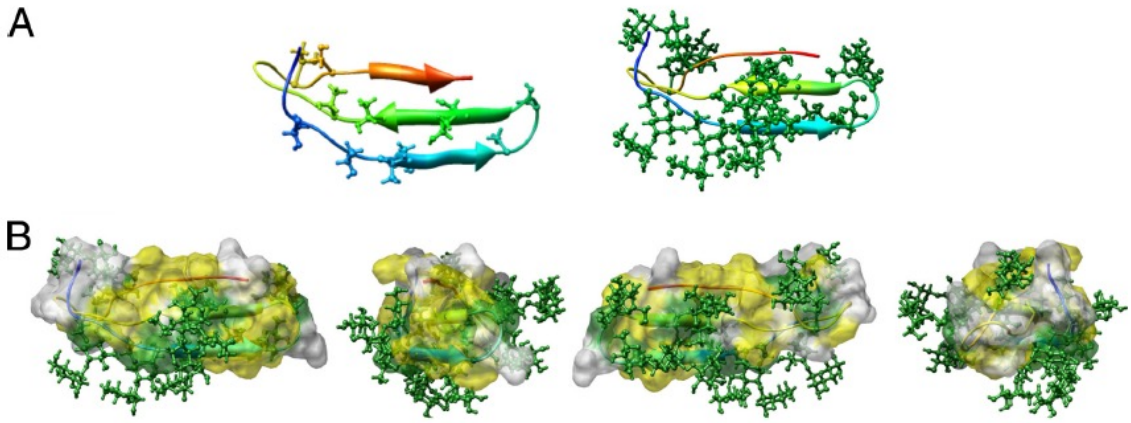
Gaur NK, Klotz SA, Henderson RL. Overexpression of the Candida albicans ALA1 gene in Saccharomyces cerevisiae results in aggregation following attachment of yeast cells to extracellular matrix proteins, adherence properties similar to those of Candida albicans. Infect Immun. 1999 Nov;67(11):6040-7.

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.



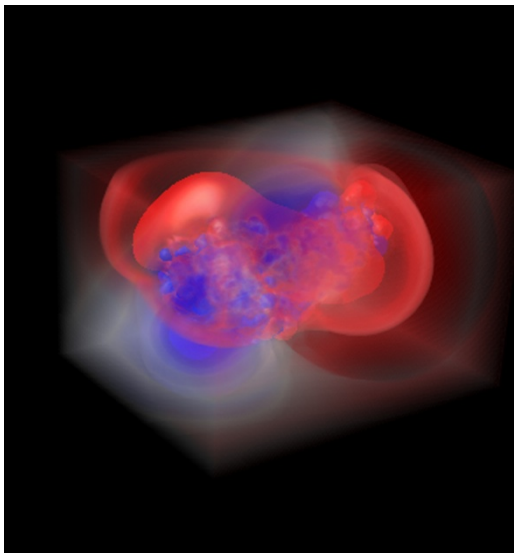
Rauceo JM, De Armond R, Otoo H, Kahn PC, Klotz SA, Gaur NK, Lipke PN. 2006 Threonine-rich repeats increase fibronectin binding in the Candida albicans adhesin Als5p. Eukaryot Cell. 5:1664-73.



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