Molecular interactions between pathogenic fungi, bacteria and their host

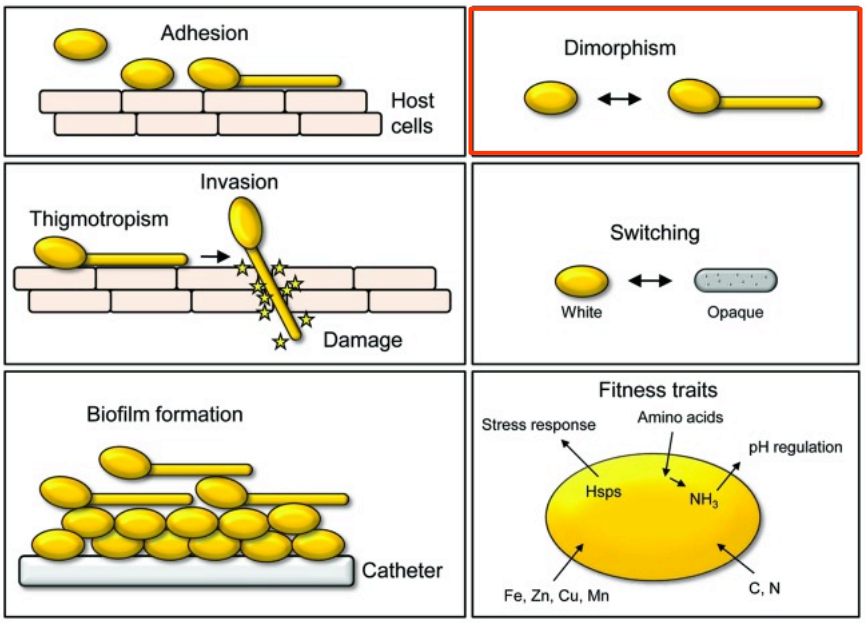
Patrick Van Dijck

VIB KU LEUVEN

Outline of the presentation

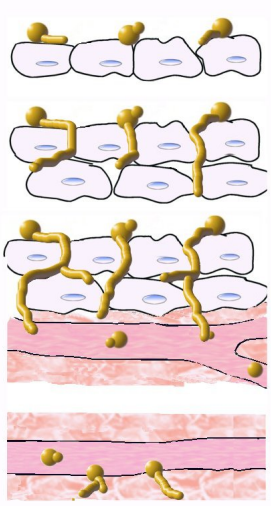
- Virulence factors of *Candida albicans*
- Role of adhesins in biofilm formation
- Role of adhesins for interspecies interaction
- In vivo mixed species infections
- Interaction between *C. albicans* and the host

Focus on *Candida albicans*



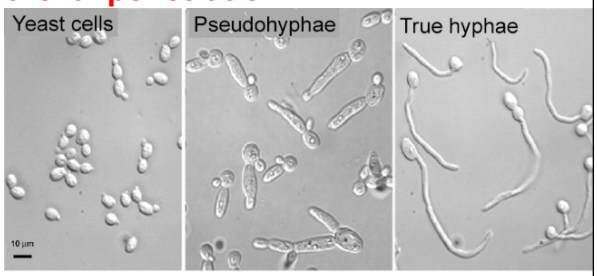
Candida albicans **is a pleiomorphic organism that can adapt its lifestyle to any host niche**

Many virulence factors



adhesion & colonisation

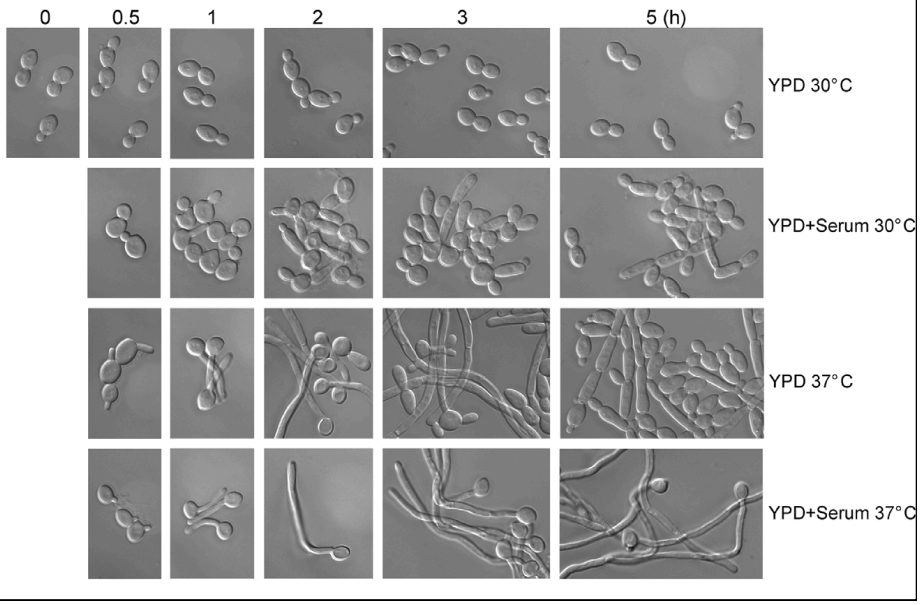
epithelial penetration



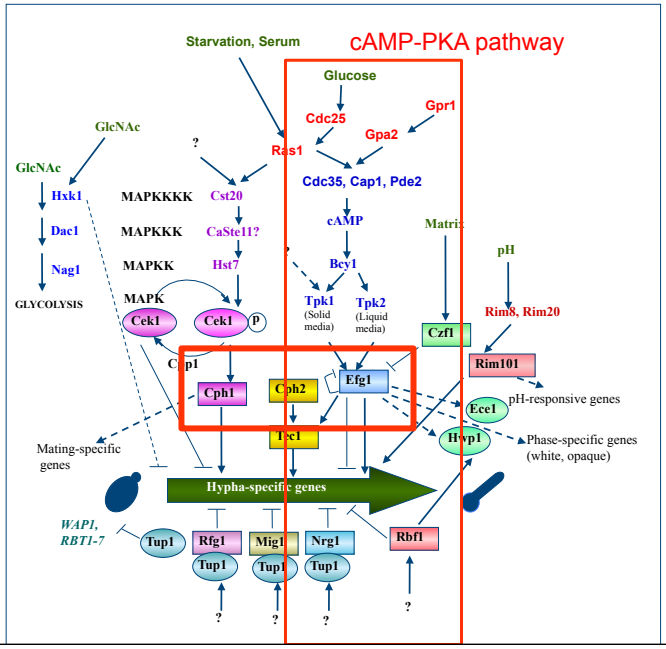
vascular dissemination

endothelial colonisation and penetration

Combined increase in temperature and addition of serum are strong inducers of filamentation



C. albicans pathways regulating morphogenesis

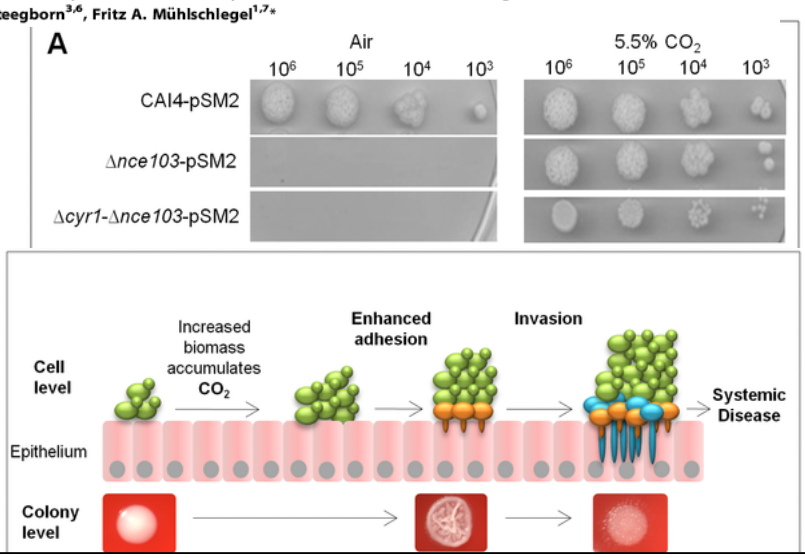


Biswas et al., 2007

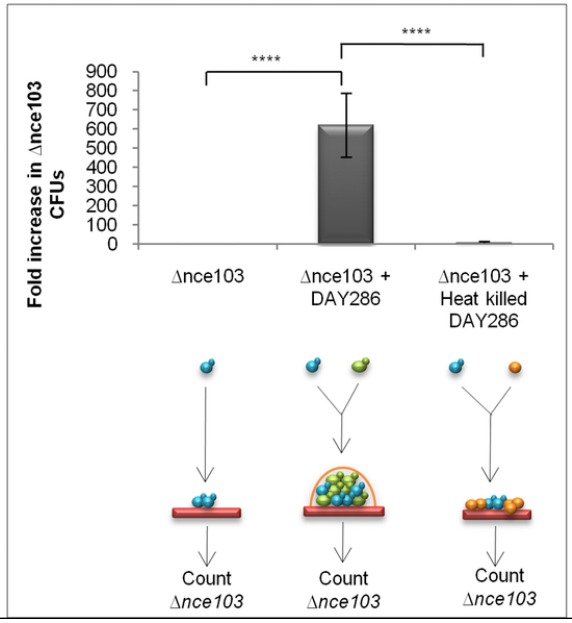
OPEN ACCESS Freely available online PLOS PATHOGENS 2010

CO_2 Acts as a Signalling Molecule in Populations of the Fungal Pathogen *Candida albicans*

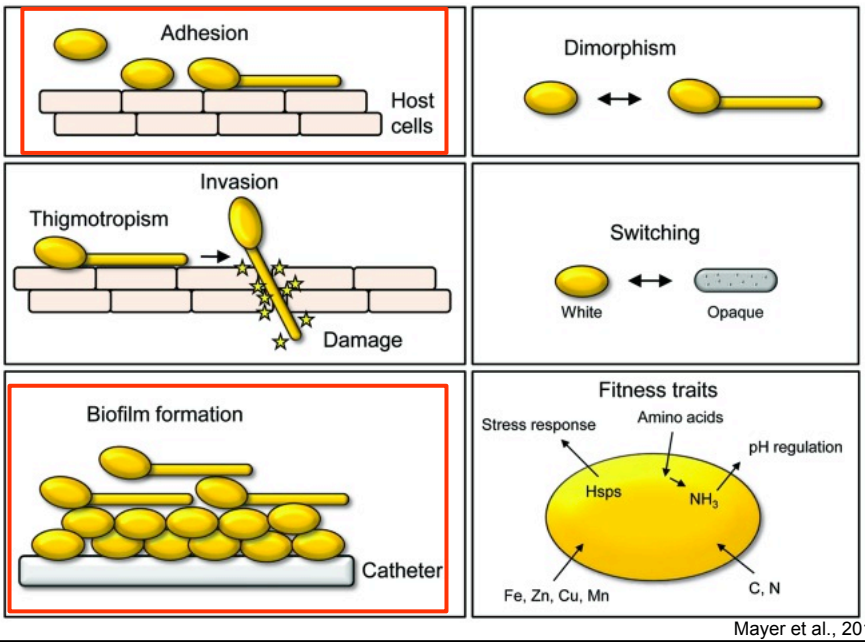
Rebecca A. Hall[1,2], Luisa De Sordi[1], Donna M. MacCallum[2], Hüsnü Topal[3], Rebecca Eaton[1], James W. Bloor[1], Gary K. Robinson[1], Lonny R. Levin[4], Jochen Buck[4], Yue Wang[5], Neil A. R. Gow[2], Clemens Steegborn[3,6], Fritz A. Mühlschlegel[1,7]*



Nce103 mutant can be used as biosensor for CO_2 and shows that within colonies CO_2 is produced and utilized



Candida albicans virulence factors



Mayer et al., 2013

ALS genes: common structure

Hoyer et al., Med Mycol. 2008, 46, 1-15

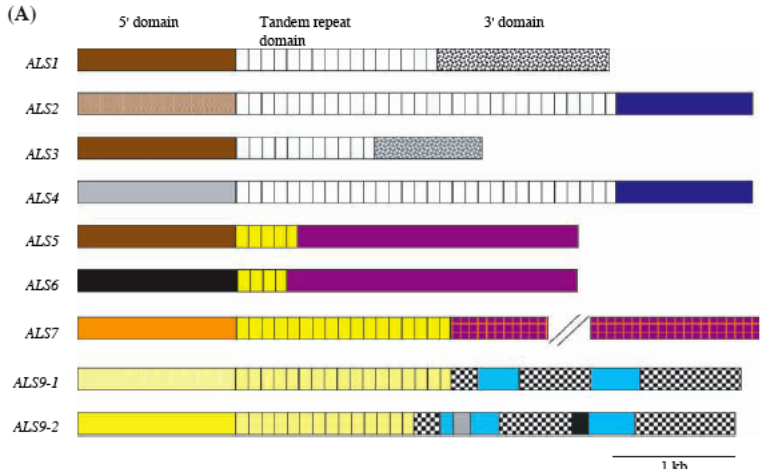


Fig. 1 (A) Line drawing showing selected features of the *Candida albicans* ALS genes. Color coding is used to show regions of similarity between genes and matches the color coding used in Fig. 1B. The length of the 5′ domain is similar in all ALS genes (approximately 1.3 kb) [1]. The tandem repeat domain is drawn as individual repeated units to emphasize the composition of this portion of the coding region. The number of tandemly repeated copies in each ALS gene varies by *C. albicans* strain, and often between alleles within the same strain. For all genes except *ALS9*, the tandem repeat copy number depicted here represents the most common allele or the mean of tandem repeat copy numbers observed from analysis of *C. albicans* strain collections (see text). For *ALS9*, the two alleles from strain SC5314 are shown [9,10]. Length of the 3′ domain is variable across the ALS family and is drawn to scale. There is a repeated domain in the 3′ end of *ALS7* that is of variable length. The location of the repeated region is marked with parallel, diagonal lines. (B) An anthropomorphic presentation of ALS gene relationships: the updated *C.*

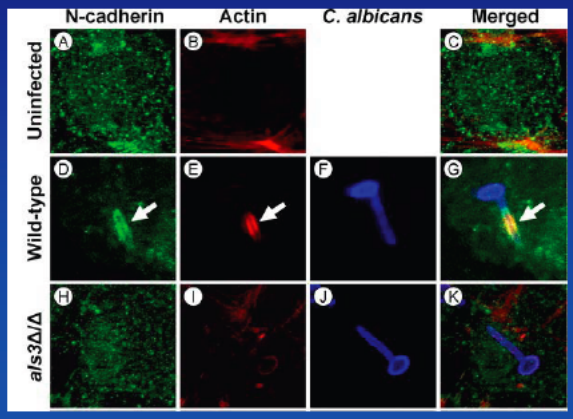
Als3 is important for induced endocytosis

Adhesion/Invasion

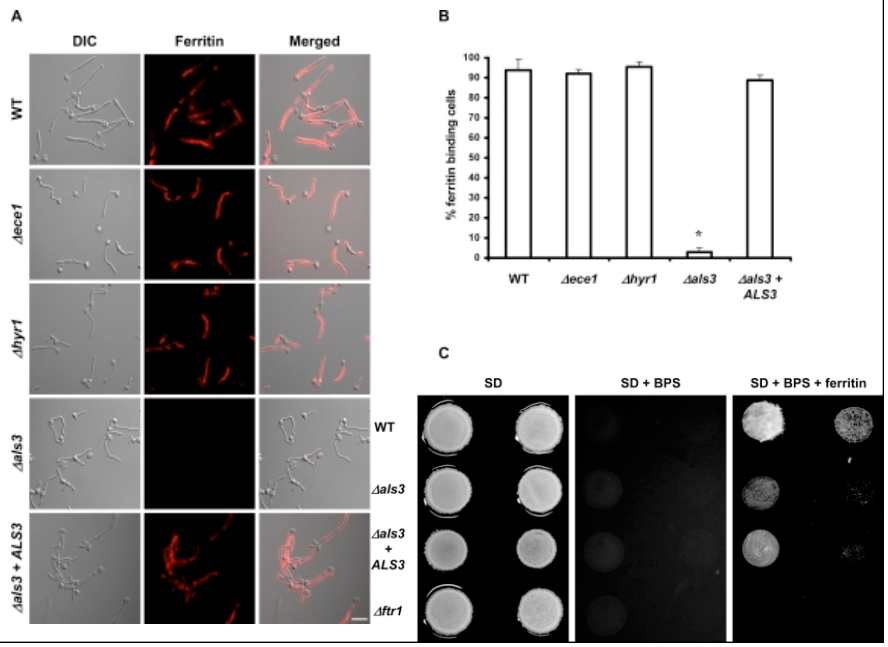
Invasion of endothelial cells

ALS3 promotes invasion of *C. albicans* into endothelial and epithelial cells

Phan, et al. (2007) *PLoS Biol.* 5:e64



Als3 is essential for ferritin binding



Als3 is important for adhesion

Als3 is important for invasion

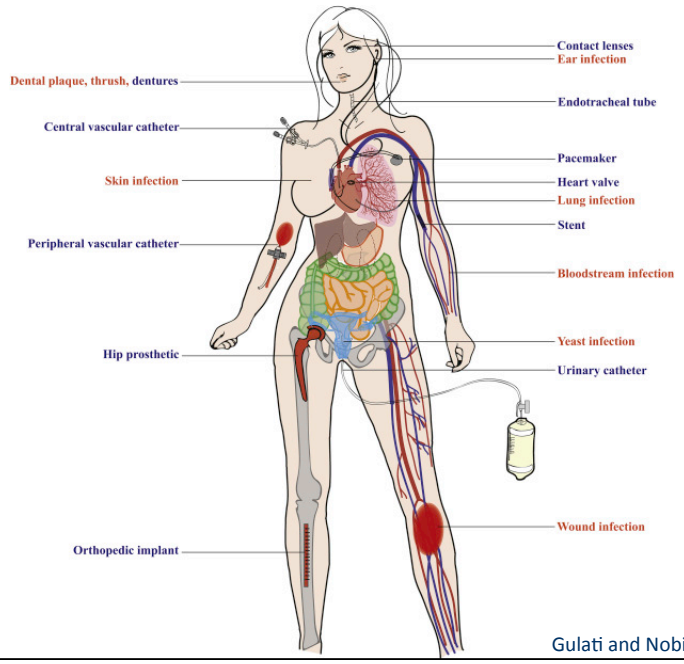
Als3 is important for iron acquisition

Als3 is important for biofilm formation

Biofilms cause major problems in hospitals

- Approximately 60 % of all hospital-associated infections = over 1 million cases per year in US
- Cells are up to 1000-fold more tolerant to antimicrobial drugs
- Use of indwelling devices will only increase
- Complex interaction between bacteria and fungi

Candida albicans biofilm-associated infections



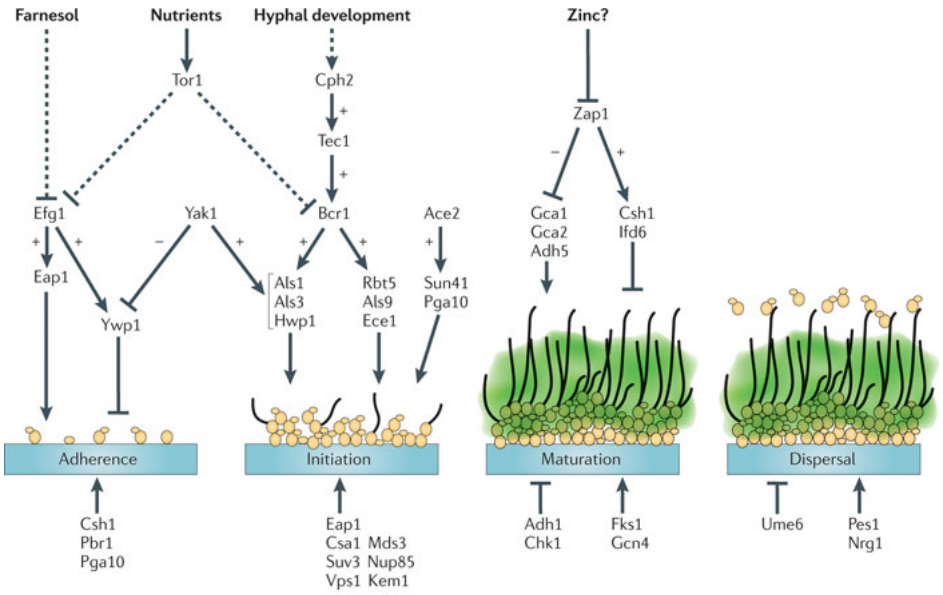
Gulati and Nobile, 2016

C. albicans biofilms on medical implants in the US

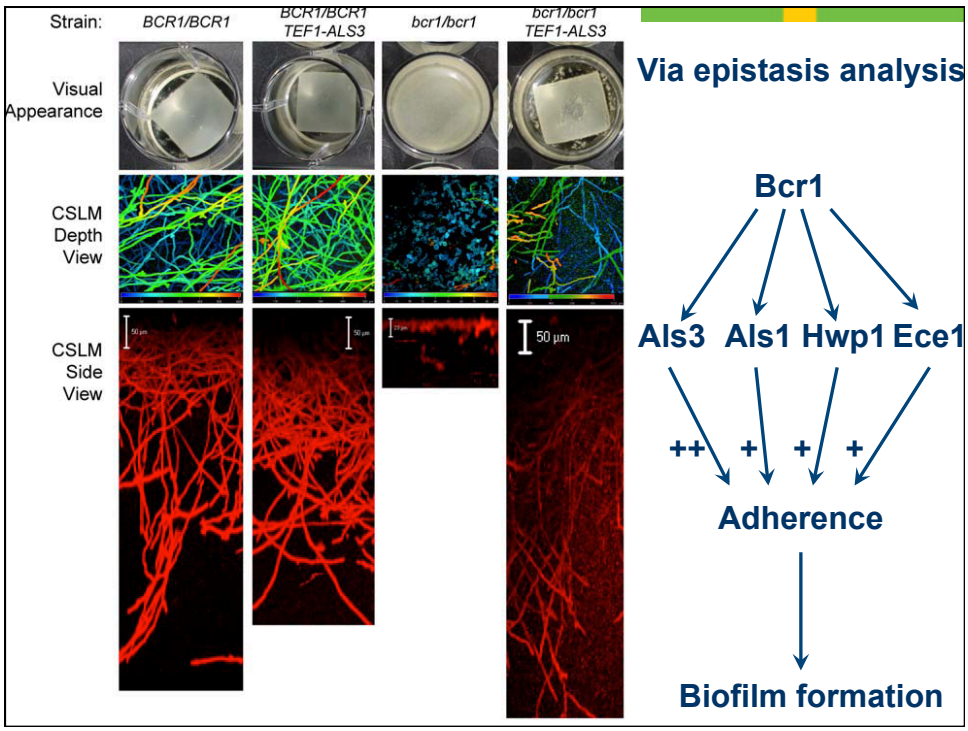
Device	Usage/year	Infection risk (%)	Main species
Central and peripheral venous catheters	5 million	3-8	*albicans, glabrata, parapsilosis*
Hemodialysis and peritoneal dialysis catheters	240 000	1-20	*albicans, parapsilosis*
Urinary catheters	Tens of millions	10-30	*albicans, glabrata*
Endotracheal tubes	Millions	10-25	*albicans*
Intracardiac prosthetic devices	400 000	1-3	*albicans, glabrata, parapsilosis, tropicalis*
Breast implants	130 000	1-2	*albicans*
Prosthetic joints	600 000	1-3	*parapsilosis, albicans, glabrata*
Dentures	> 1 million	5-10	*albicans, glabrata*
Voice prostheses	Thousands	50-100	*albicans, tropicalis*

Ramage et al., 2006

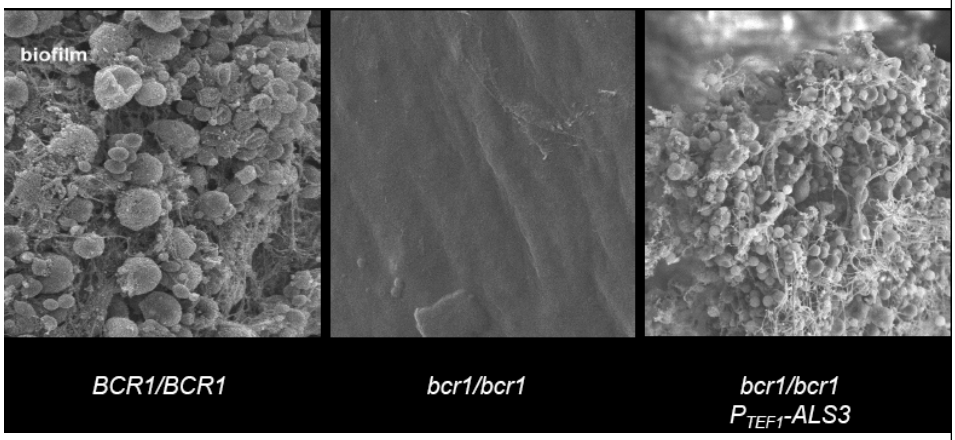
Regulation of biofilm development



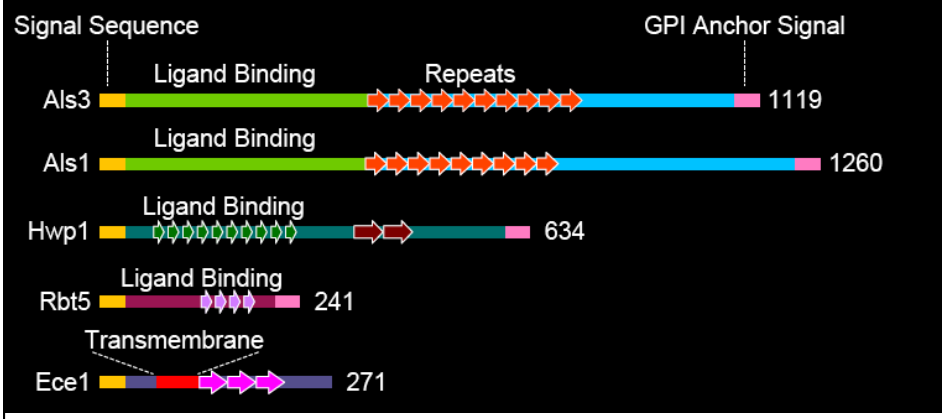
Finkel and Mitchell, 2011



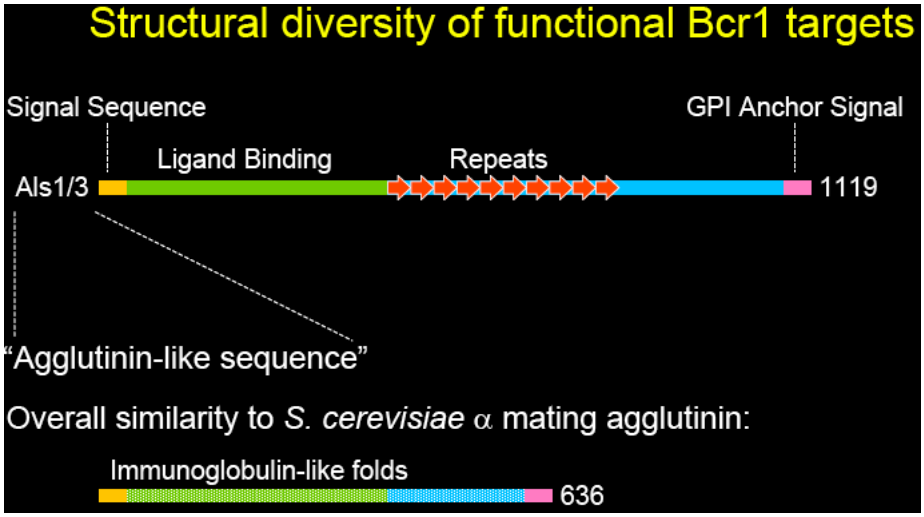
ALS3 OX rescues biofilm formation in a *bcr1* mutant

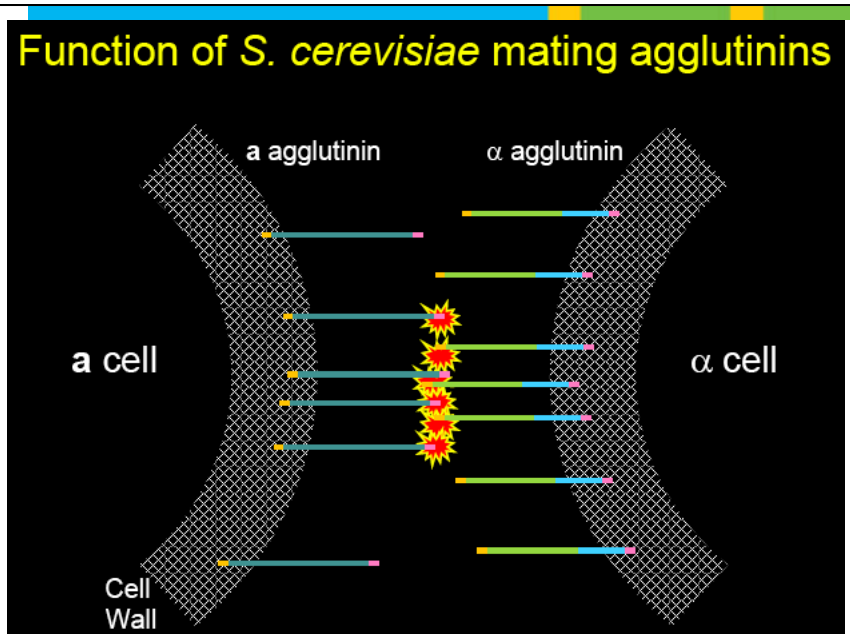


Mystery: Structural diversity of functional Bcr1 targets



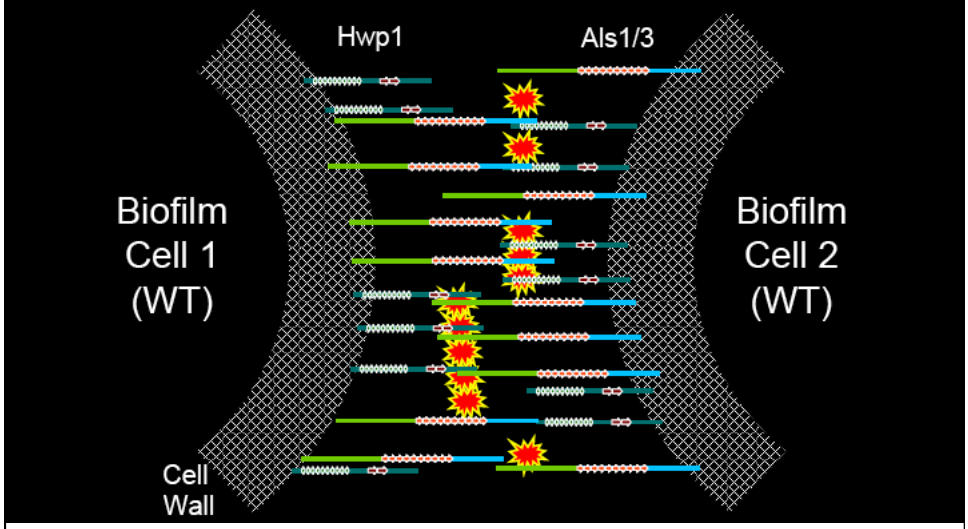
als1/als1 als3/als3: no biofilm formation *in vivo*
hwp1/hwp1: no biofilm formation *in vivo*



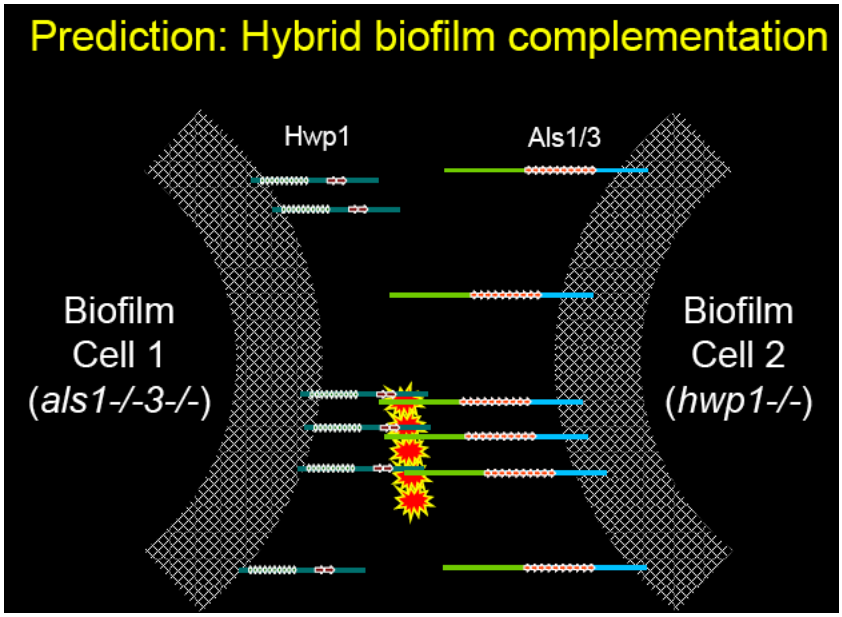


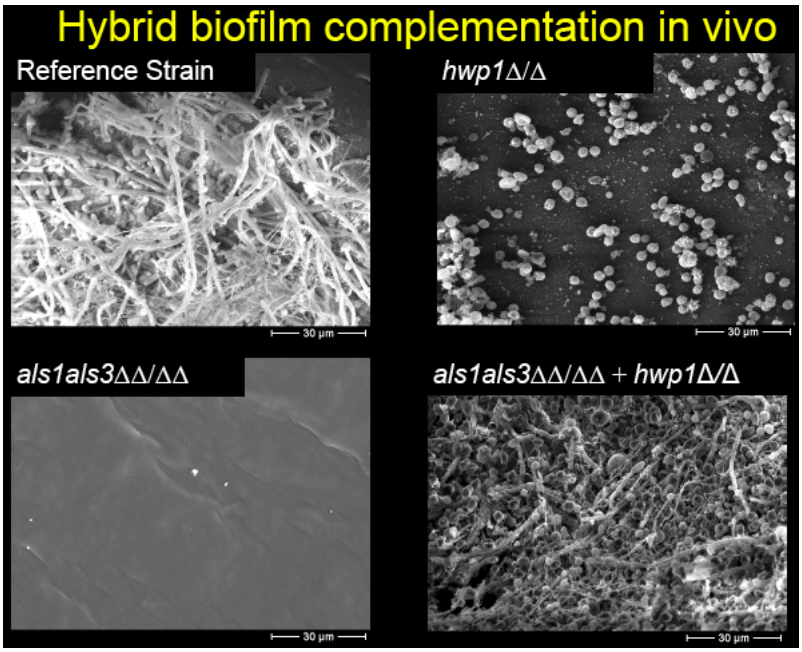
In *C. albicans*, Hwp1 maybe functions as a agglutinin (and Als3 as the alpha agglutinin

Mechanism: Complementary Als1/3-Hwp1 binding?

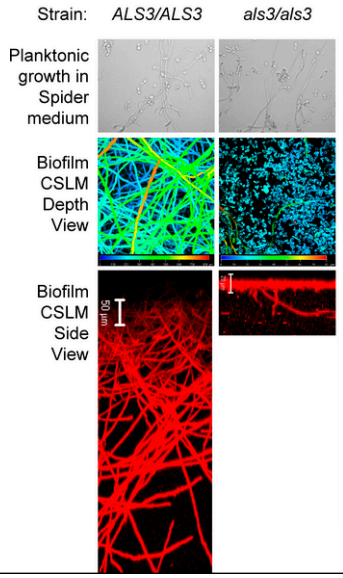


Prediction: Hybrid biofilm complementation



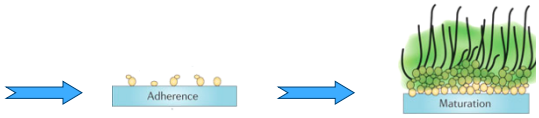


Inactivation of Als3 as a novel method to tackle the problem of biofilms

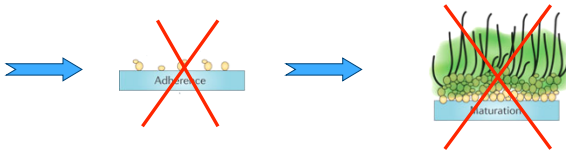


Nobile et al., 2006

Als3 as an example for a novel class of antifungals: aggregation-inducing peptides



Targeted aggregation of Als3 via short peptides



- Tango

```
                1        10        20        30        40        50        60
                |--------+---------+---------+---------+---------+---------|
Als3      GYHQRAPFTLRWTGYRNSDAGSNGIVIVATTRTVTDSTTAVTTLPFDPNRDKTKTIEILK
Als5      DYHQHAPFTLRWTGYKNSDAGSNGIVIVATTRTVTDSTTAVTTLPFNPSVDKTKTIEILQ
Als1      SRSQSKPFTLRWTGYKNSDAGSNGIVIVATTRTVTDSTTAVTTLPFNPSVDKTKTIEILQ
Consensus .gwQ.aPFTLRWTGYkNSDAGSNGIVIVATTRTVTDSTTAVTTLPF#PsvDKTKTIEILq
```

Als3 has two TANGO regions of which one with very high propensity

ALS3

NGIVIVATTR (peptide E1)
RKLLFNLGSRNGIVIVATTR (peptide F9)
RKLLFNLGSRNGIVIVPTTR (peptide 13_negat)

aggregation-booster sequence

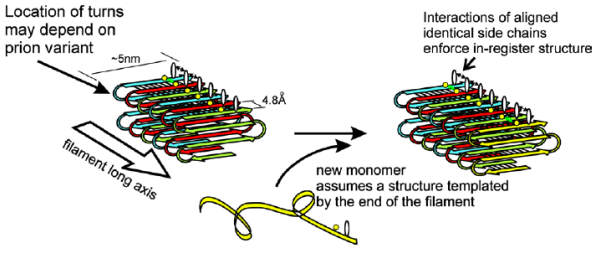
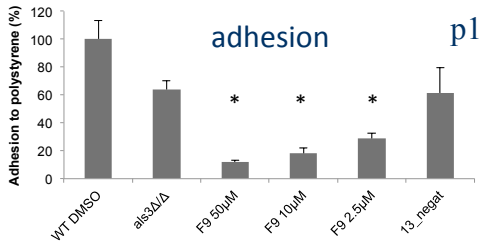
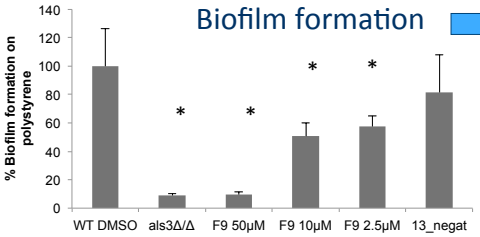


Figure 3. The in-register parallel beta sheet architecture with longitudinal folds of yeast prion amyloid filaments can explain transmission of conformational information to monomers joining the end of the filament. The same interactions among aligned identical amino acid side chains that hold the structure in-register also drive the monomer joining the end of the filament to have the same conformation as the molecules already in the filament (103,106).

The Als3 peptide F9 prevents adhesion and biofilm formation

F9 RKLLFNLGSR**NGIVIVATTR**
p13 RKLLFNLGSR**NGIVIV**P**TTR**

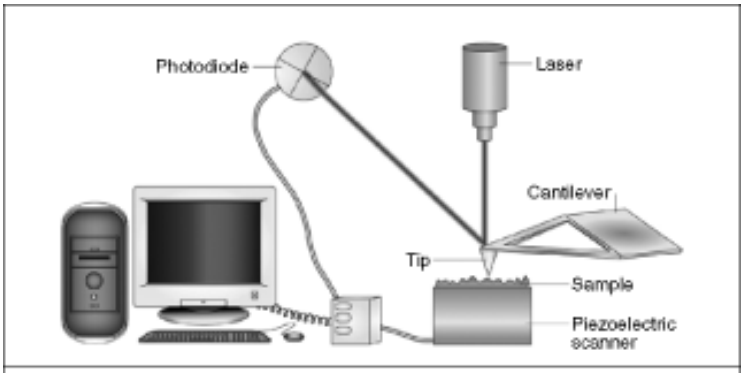




One amino acid change in the TANGO region affects the potency of the peptide

Kucharíková et al., in preparation

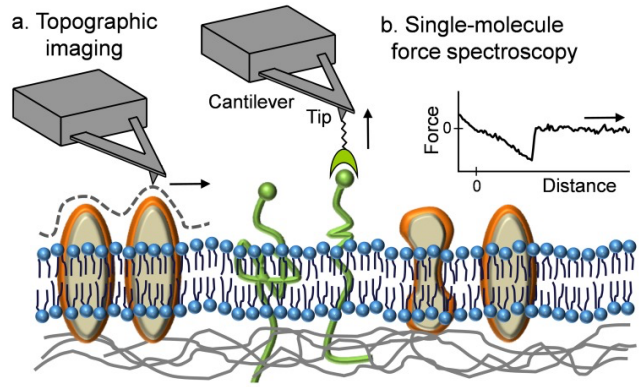
Atomic force microscopy to probe protein-cell or cell-cell interactions



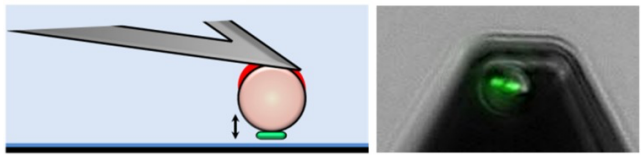
Imaging	Force spectroscopy
- nanoscale resolution	- pN sensitivity
- minimal sample preparation	- single molecule interactions
- in aqueous solution	- physical properties
- in real time	- lateral mapping

Dague et al., 2007

Atomic force microscopy to probe protein-cell or cell-cell interactions

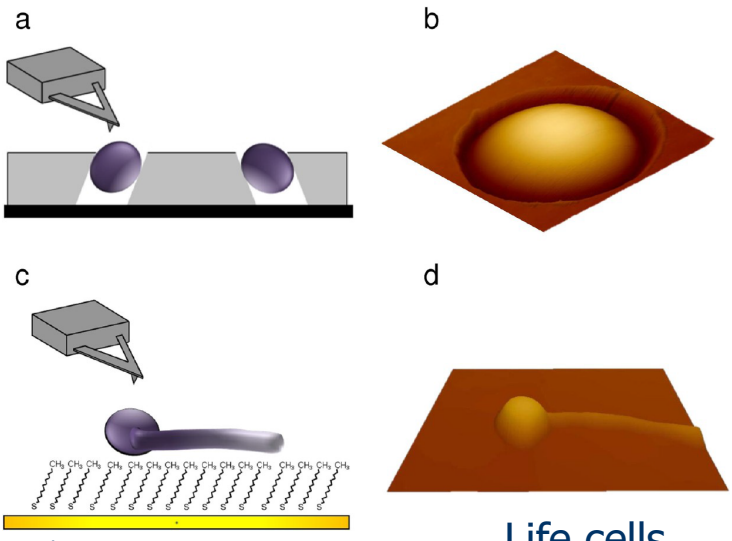


c. Single-cell force spectroscopy



Dufrêne, 2014

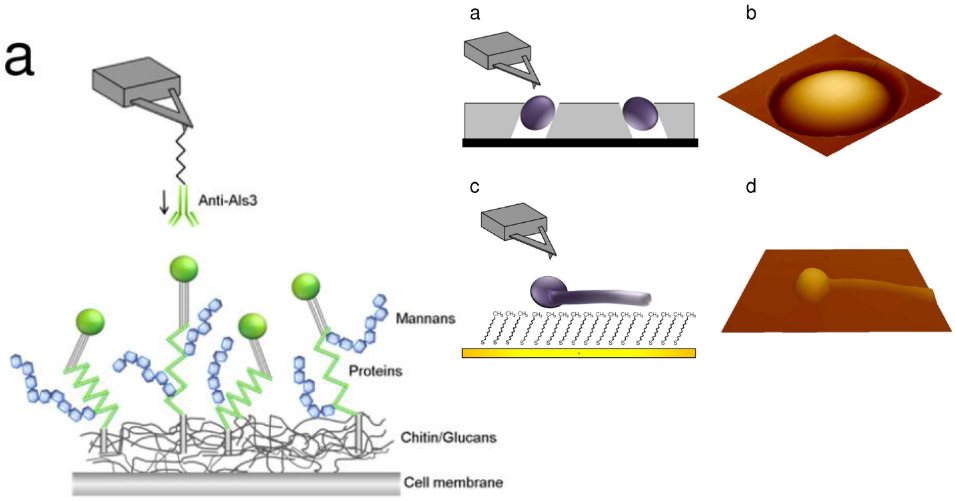
Atomic force microscopy for Als3 detection



Beaussart et al., ACS nano 2012

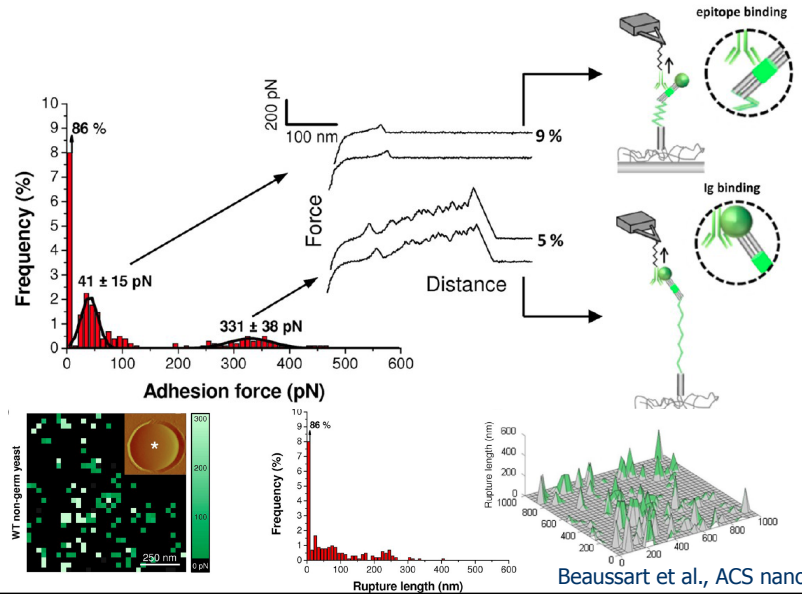
Life cells

Do we target Als3?
Atomic force microscopy for detection of Als3



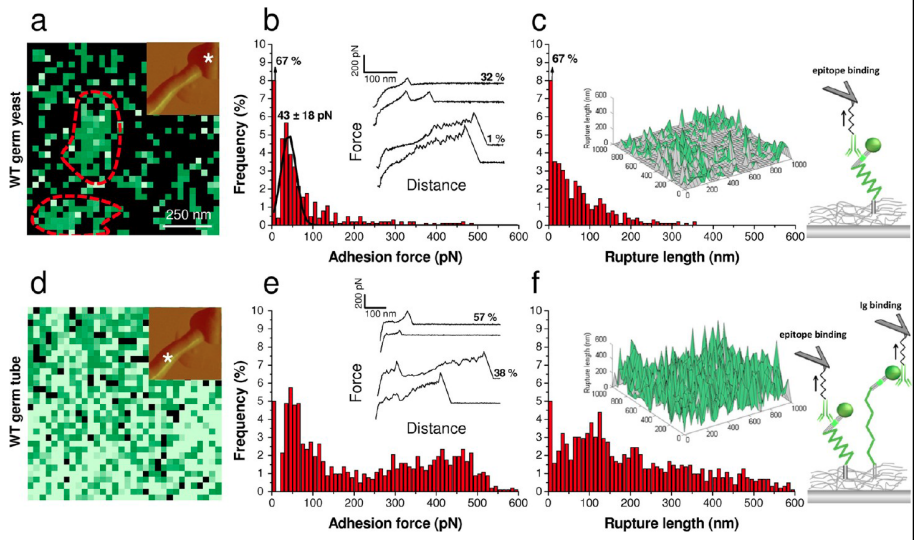
Beaussart et al., ACS nano 2012

AFM allows determination of distribution, adhesion, unfolding, and extension of Als adhesins



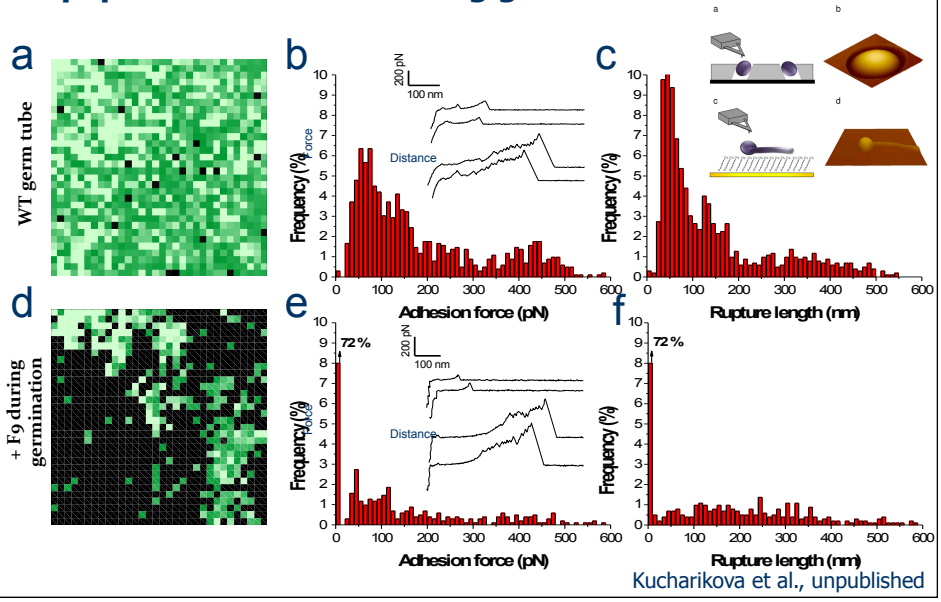
Beaussart et al., ACS nano 2012

Cellular morphogenesis leads to major increase in distribution and extension of Als3 proteins



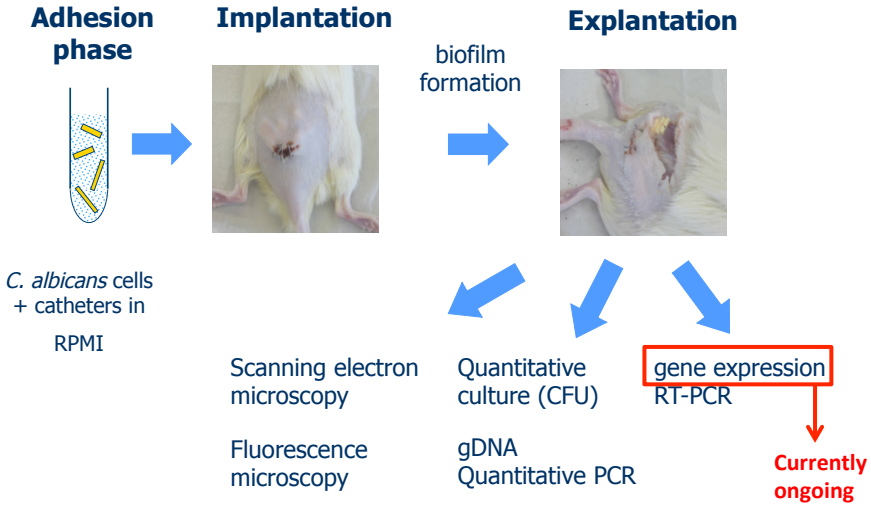
Beaussart et al., ACS nano 2012

Atomic force microscopy data support lower Als3 levels on the cell wall of hyphal cells but only when peptide F9 is added during germ tube formation



Kucharikova et al., unpublished

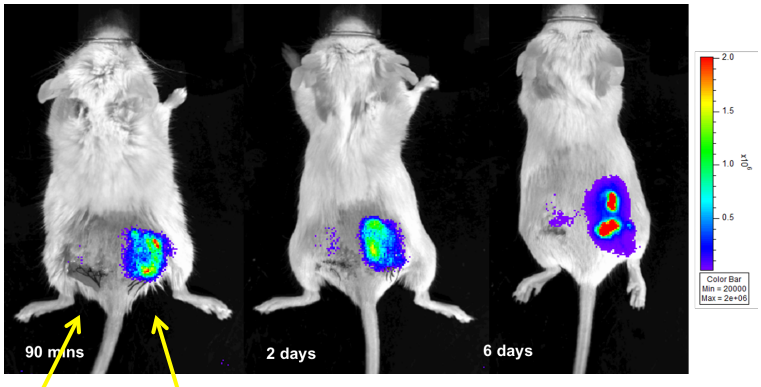
Development and use of a 'simple' *in vivo* model system



Kucharikova et al., Jove, 2015

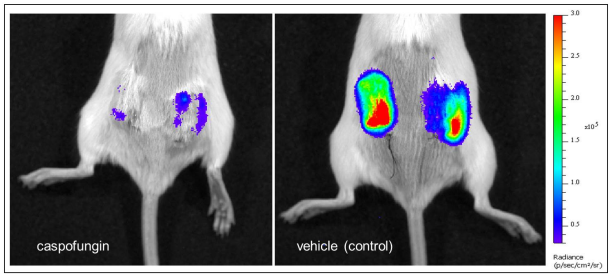
Řičicová et al., 2010

In vivo C. albicans biofilm formation visualized by bioluminescence imaging



Vande Velde, Kucharíková et al. 2014b

Regression of biofilms by treatment with caspofungin

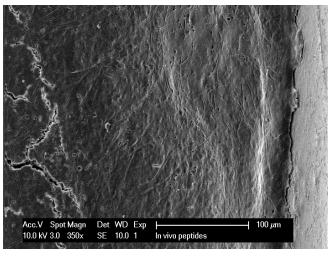


Longitudinal BLI of antifungal activity against biofilms under *in vivo* conditions. Typical *in vivo* BLI images from a mouse treated with caspofungin and a mouse treated with placebo, imaged 9 days after catheter implantation.

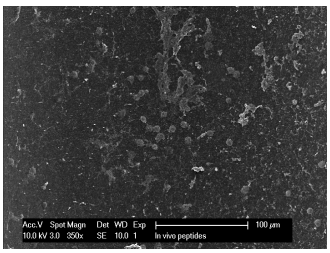
→ Ideal tool for screening novel antibiofilm compounds

Vande Velde et al., In preparation

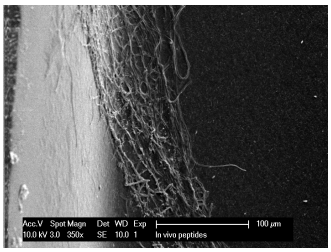
The effect of peptide on catheter-associated infections developed *in vivo*



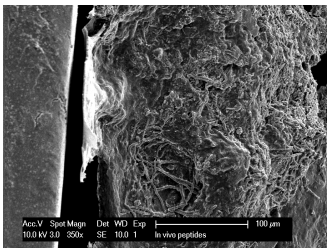
Control (no peptide)



als1Δ/Δ als3Δ/Δ



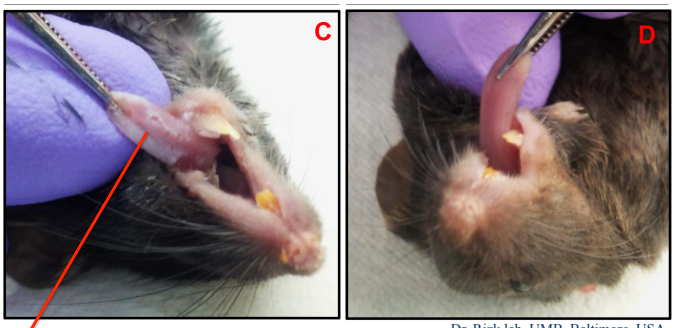
SC5314 + F9



SC5314 + 13_negat

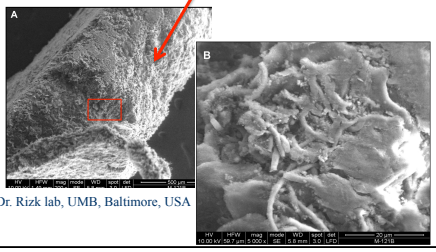
The peptide F9 works *in vivo* in an oral infection model system

Oral candidiasis



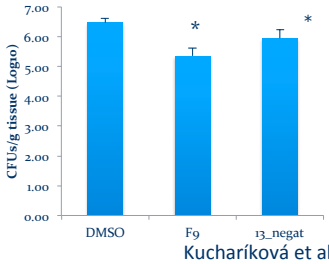
Dr. Rizk lab, UMB, Baltimore, USA

SEM



Dr. Rizk lab, UMB, Baltimore, USA

Ex vivo model of oral candidiasis



Kucharíková et al., in preparation

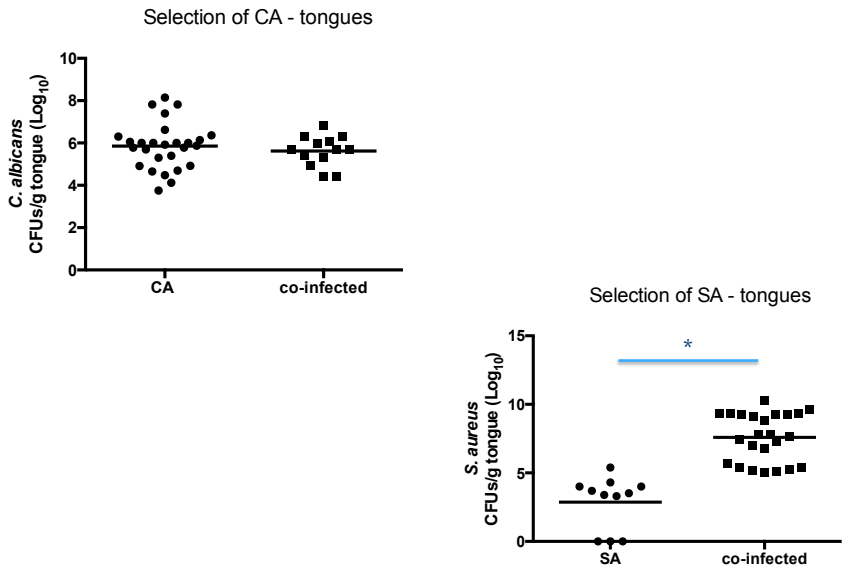
Dual-species biofilms:
closer to the real life situation

Development and use of an oral dual species biofilm model system in mice

(Kong, Kucharikova et al., 2015)

*

Candida and *S. aureus* recovery from tongues

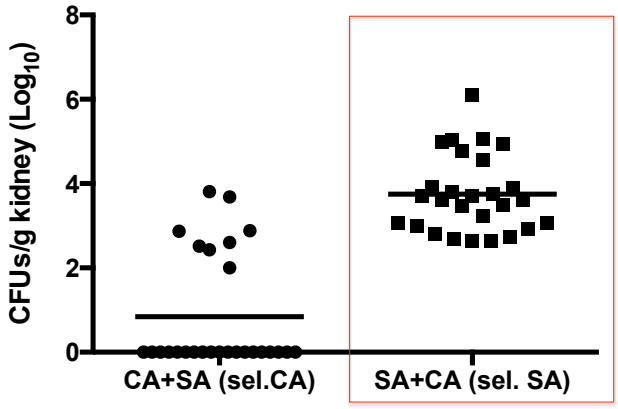


*$p<0.05$

Kong, Kucharíková *et al.*, 2015, Infection & Immunity

Candida and *S. aureus* recovery from kidneys

Candida alone or *Staph* alone do not disseminate but when co-infected....

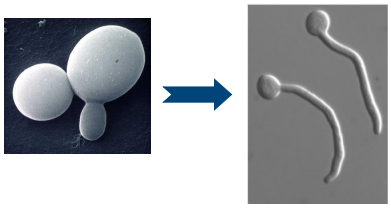


***Candida* helps *Staph* to disseminate and cause disease.**

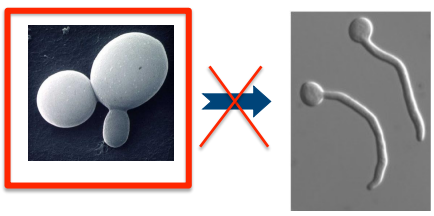
Kong, Kucharíková *et al.*, 2015, Infection & Immunity

The role of yeast to hyphae transition during development of clinical disease

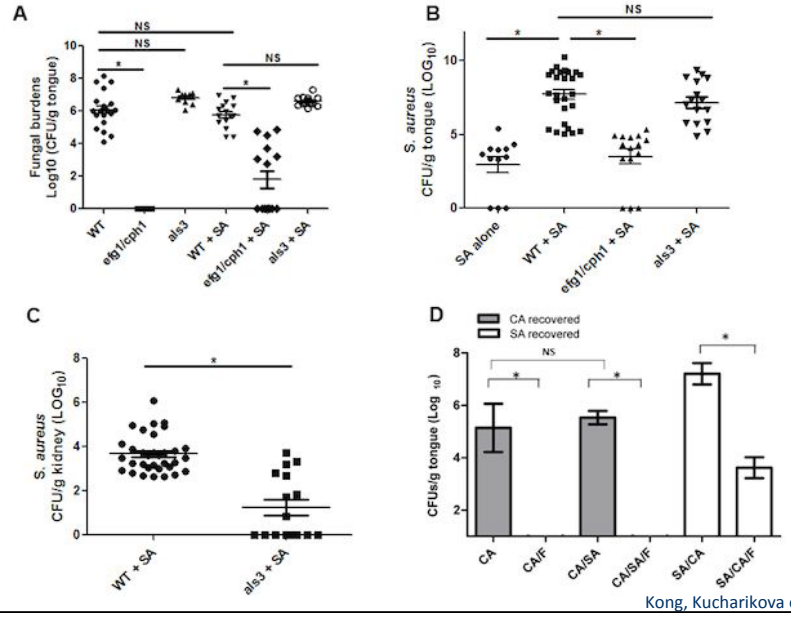
- *C. albicans* WT



C. albicans cph1/cph1 efg1/efg1

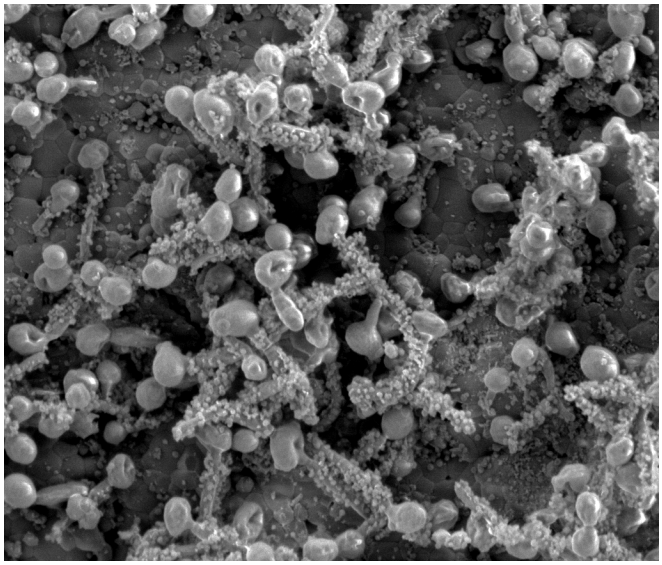


Tissue microbial recovery from mono and co-infected mice with and without fluconazole therapy



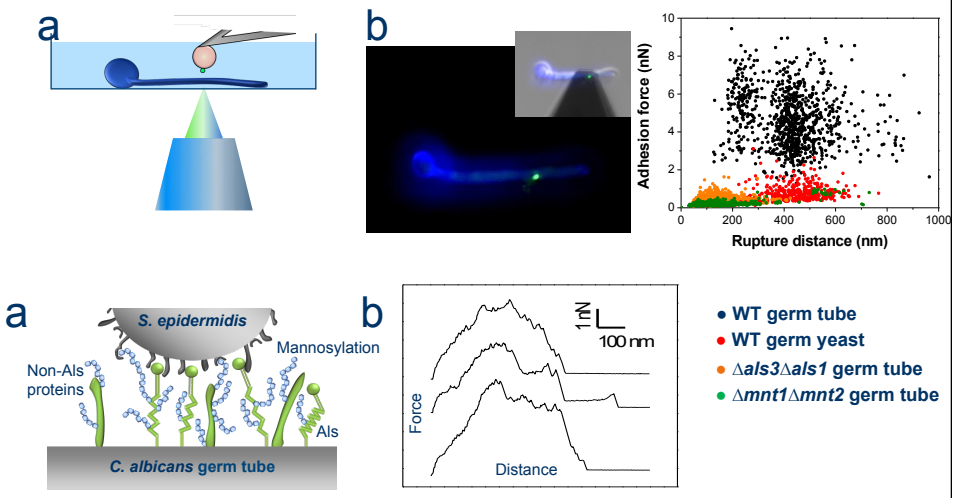
Kong, Kucharikova et al., 2015

Scanning electron microscopy of a mature biofilm of *C. albicans* and *S. aureus*



S. aureus only interacts with hyphae (Why?)

Probing *Staphylococcus epidermidis-Candida albicans* interaction using single-cell force spectroscopy



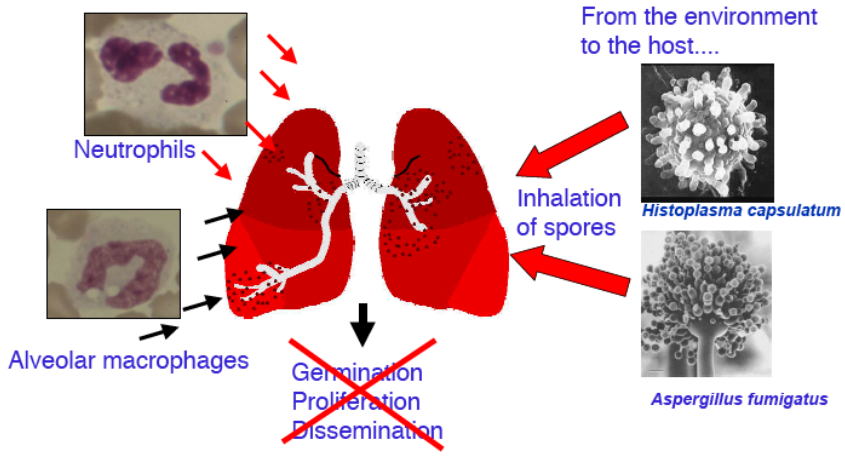
Glycosylation of mainly Als3-Als1 is required for the interaction

Beaussart et al., 2013

Host-pathogen interactions

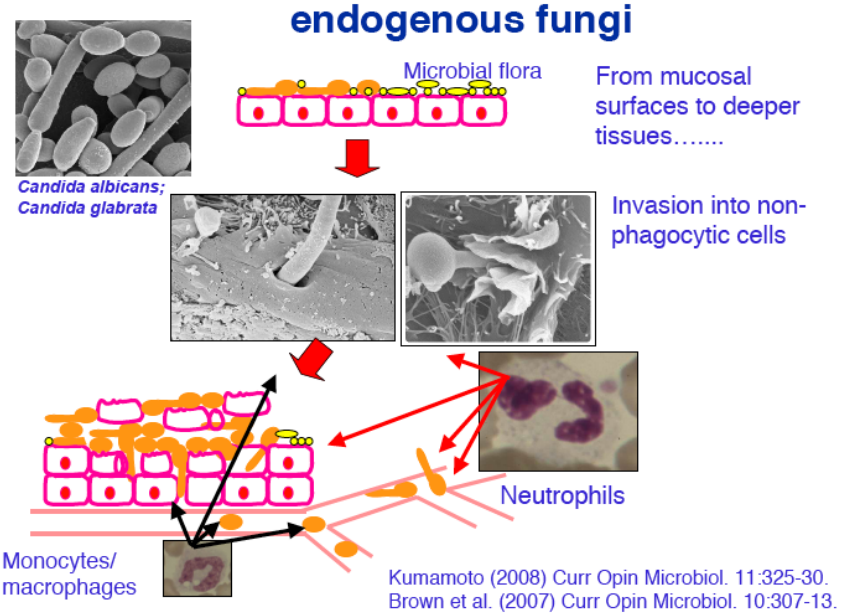
First contact between host and pathogen: innate immune system

The routes of fungal infections: environmental fungi



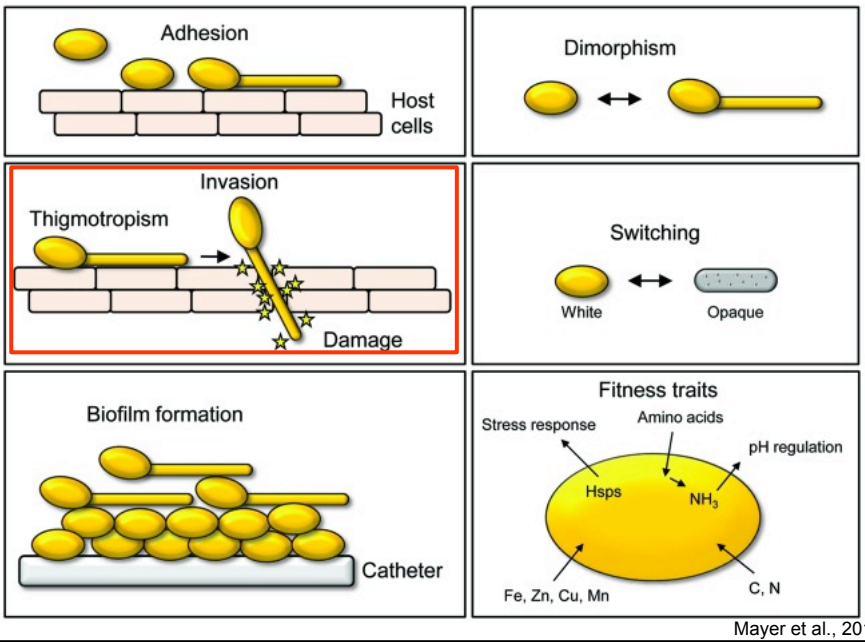
Askew (2008) Curr Opin Microbiol. 11:331-7.
Holbrook and Rappleye (2008) Curr Opin Microbiol. 11:318-24.
Klein and Tebbets (2007) Curr Opin Microbiol. 10:314-9.
Behnsen et al. (2007) PLoS Pathog. 3:e13.

The routes of fungal infections: endogenous fungi



Kumamoto (2008) Curr Opin Microbiol. 11:325-30.
Brown et al. (2007) Curr Opin Microbiol. 10:307-13.

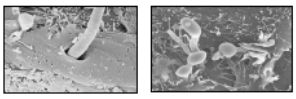
Candida albicans virulence factors



Mayer et al., 2013

How does the host defend pathogenic fungi?

Barriers!



Immediate action!

Recognition!

Innate immunity

Early detection of invaders!

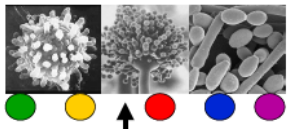
What type of invader?

Appropriate response!

Antimicrobial peptides, complement, iron binding molecules, reactive oxygen species, reactive nitrogen species, proteases

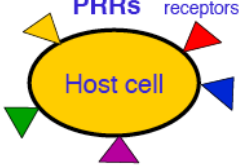
Pathogen associated molecular pattern

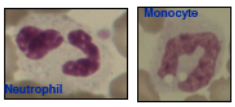
PAMPs



Pattern recognition receptors

PRRs

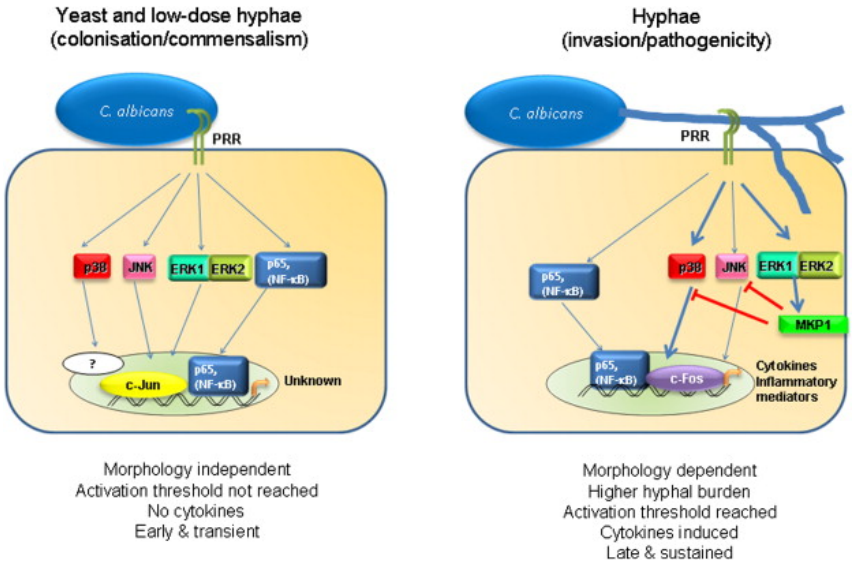




phagocytes

Netea et al. (2008) Nat Rev Microbiol. 6:67-78.

Candida albicans detection in epithelial cells and the host response



Morphology independent
Activation threshold not reached
No cytokines
Early & transient

Morphology dependent
Higher hyphal burden
Activation threshold reached
Cytokines induced
Late & sustained

Initial detection also results in the induced expression of antimicrobial peptides

A peptide originating from the Ece1 protein is important for induction of damage response pathway

a

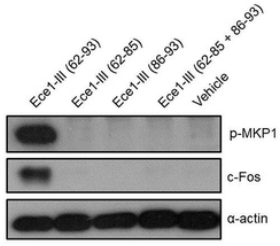
***Candida albicans* Ece1p amino acid sequence:**

MKFSKIACATVFALSSQAAIIHHAPEFNMKRDVAPAAPAAPADQAPTVPAPQEFNTAITKRSIIGIIMGILGNIPQVIQIIMSIVKA
FKGNKREDIDSVVAGIIADMPFVVRAVDTAMTSVASTKRDGANDDVANAVVRLPEIVARVATGVQQSIENAKRDGVPDVGLNLVANA
PRLISNVFDGVSETVQQAKRDGLEDFLDELLQRLPQLITRSAESALKDSQPVKRDAGSVALSNLIKKSIETVGIENAAQIVSERDIS
SLIEEYFGKA

b

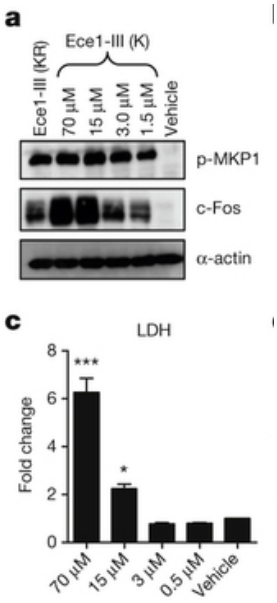
Ece1-I_{1-31} MKFSKIACATVFALSSQAAIIHHAPEFNMKR
Ece1-II_{32-61} DVAPAAPAAPADQAPTVPAPQEFNTAITKR
Ece1-III_{62-93} SIIGIIMGILGNIPQVIQIIMSIVKAFKGNKR
Ece1-IV_{94-126} EDIDSVVAGIIADMPFVVRAVDTAMTSVASTKR
Ece1-$V_{127-160}$ DGANDDVANAVVRLPEIVARVATGVQQSIENAKR
Ece1-$VI_{161-194}$ DGVPDVGLNLVANAPRLISNVFDGVSETVQQAKR
Ece1-$VII_{195-228}$ DGLEDFLDELLQRLPQLITRSAESALKDSQPVKR
Ece1-$VIII_{229-271}$ DAGSVALSNLIKKSIETVGIENAAQIVSERDISSLIEEYFGKA

f



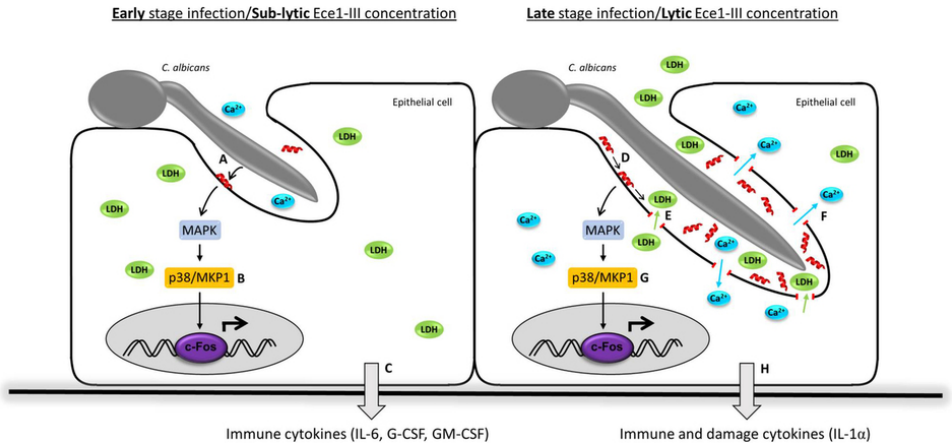
Moyes et al. 2016

A peptide originating from the Ece1 protein is important for induction of damage response pathway but only at high concentrations



Moyes et al. 2016

A peptide originating from the Ece1 protein is important for induction of damage response pathway but only at high concentrations



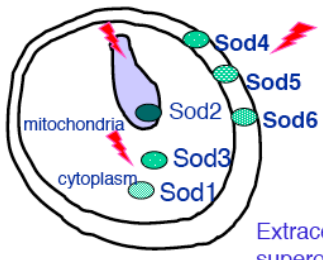
Moyes et al. 2016

Host-pathogen interaction:

Example of fungal host evasion

Fungal immune evasion strategies!

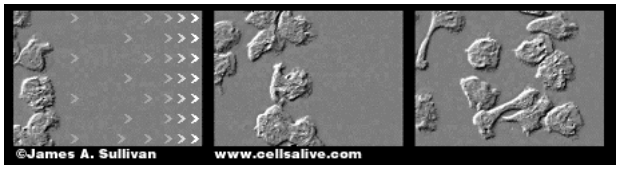
- Avoid detection
- Molecular mimicry
- Downregulate complement
- **Detoxification/Protection**



Frohner et al. (2009) Mol Microbiol. 71:240-52.
Liu et al. (2008) Cell. 135:174-88.
Ejzykowicz et al. (2009) Mol Microbiol. 72:155-69
Schmaler-Ripcke et al. (2009) Appl Environ Microbiol. 75:493-503.
Fradin et al (2005) Mol Microbiol. 56:397-415.

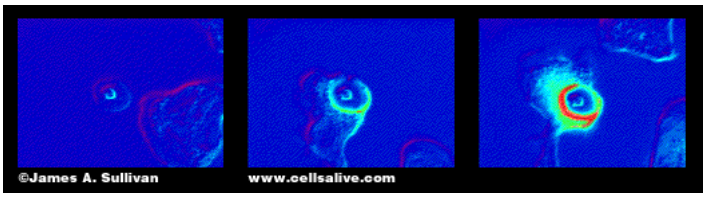
The reaction to pathogens is a three-step process

1. Chemotaxis

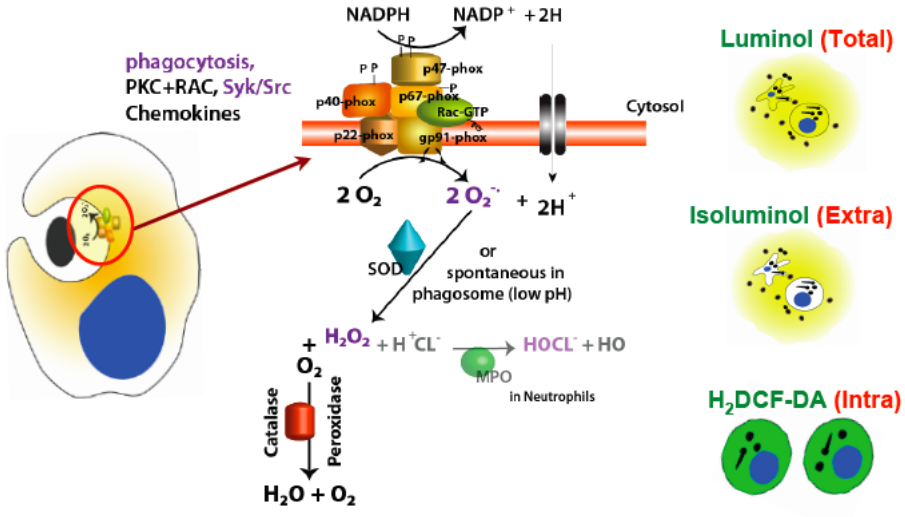


2. Phagocytosis

3. Oxidative burst

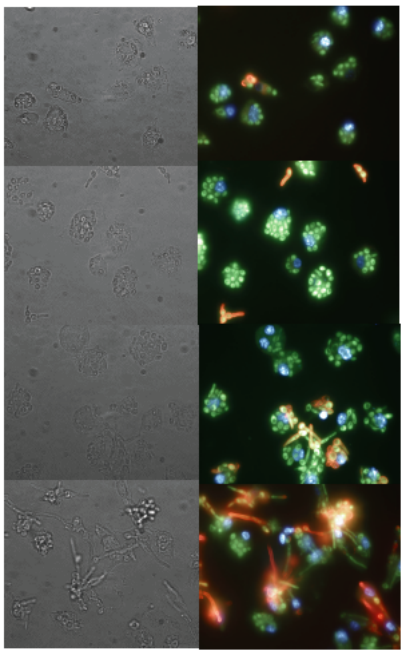


Host Generate ROS Cells During Phagocytosis



dichlorodihydrofluorescein diacetate

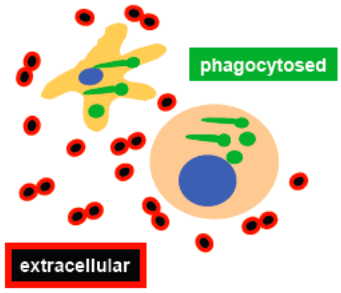
Why are *C. albicans* cells not killed by macrophages



20min

***Candida albicans* Infecting Primary Macrophages**

20+30min



20+90min

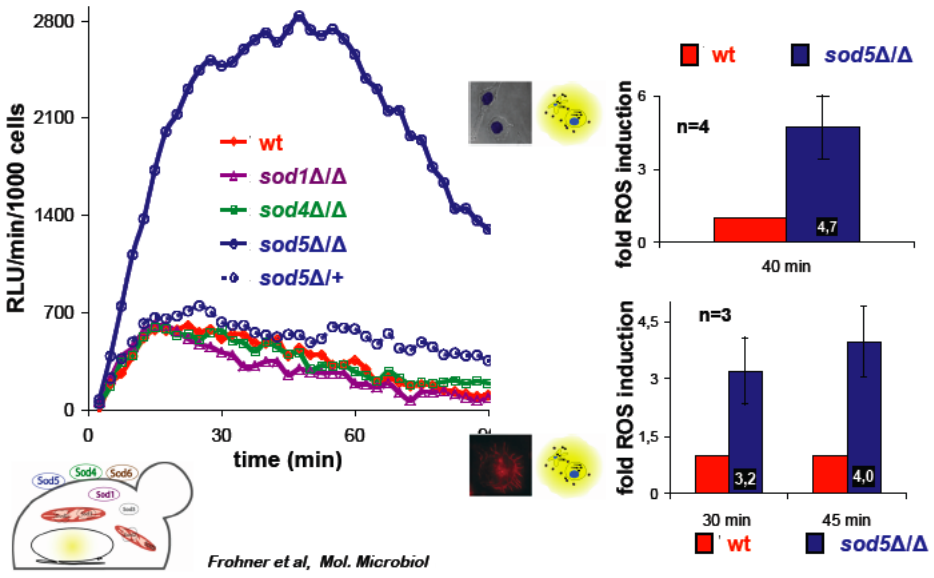
20+180min

Fungal Superoxide Dismutases & Host Interaction

- *Candida albicans* - Cytosolic CuZn-SOD - *SOD1*
  - *SOD1* - Important for survival in MpH, virulence factor
- *Candida albicans* - Cell Wall CuZn-SODs - *SOD4,5,6*
  - *SOD4* - up-regulated in the white → opaque switch
  - *SOD5* - virulence factor in mice; no protection from Mph, up-regulated upon contact with neutrophils, blood

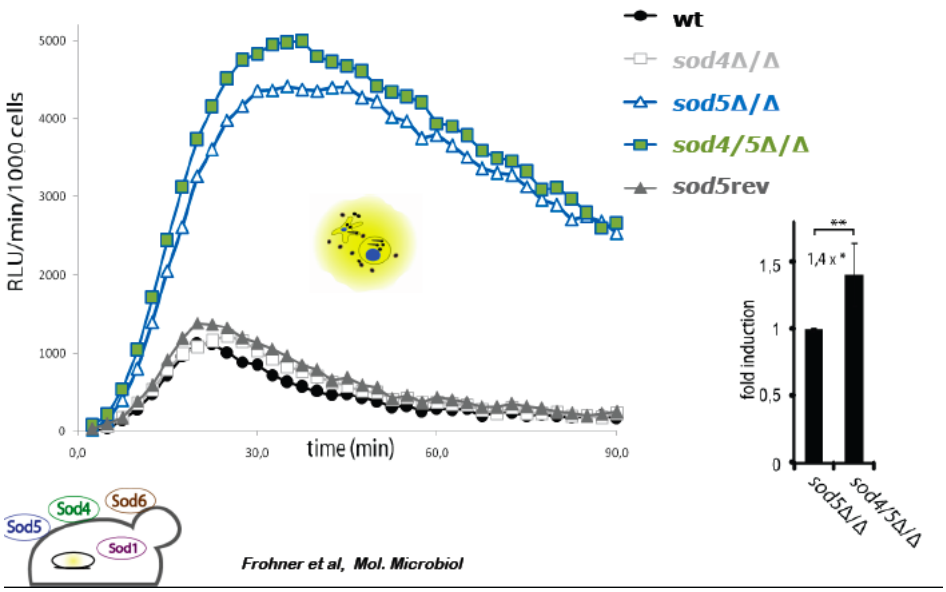
Are the Sod enzymes responsible for survival in Mph?

Lack of Sod5 Leads to ROS Accumulation



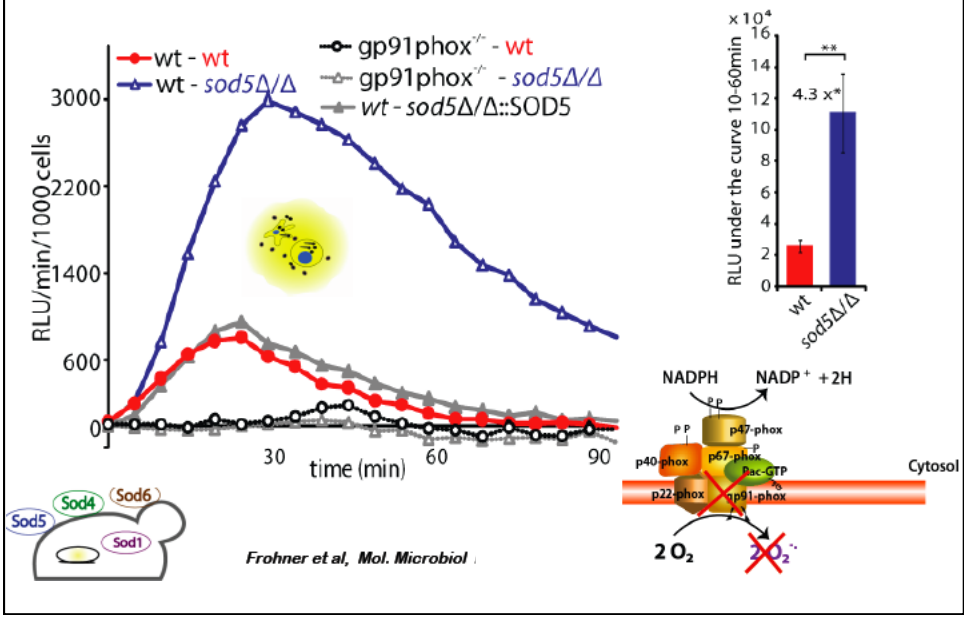
Frohner et al, Mol. Microbiol

Lack of both Sod4 & Sod5 boosts ROS levels



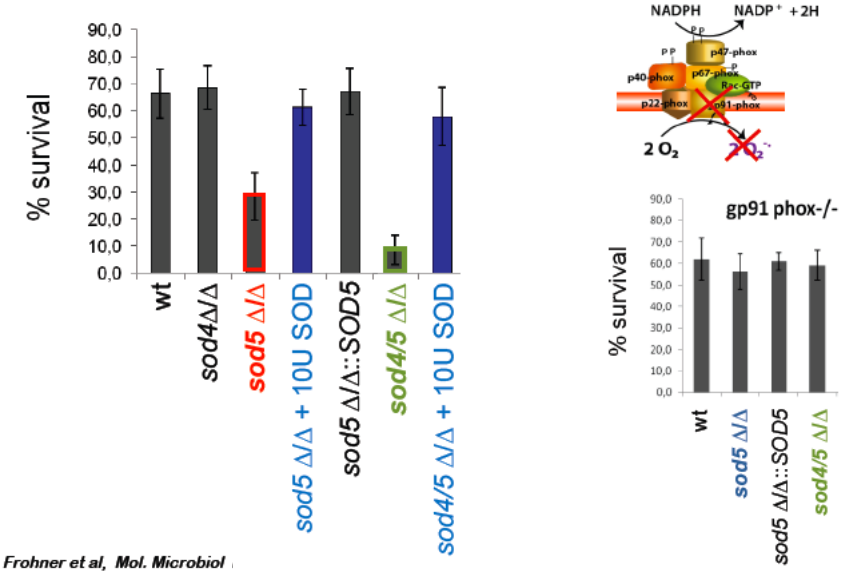
Frohner et al, Mol. Microbiol

Host ROS Accumulate when *sod5*Δ/Δ Infect mDCs



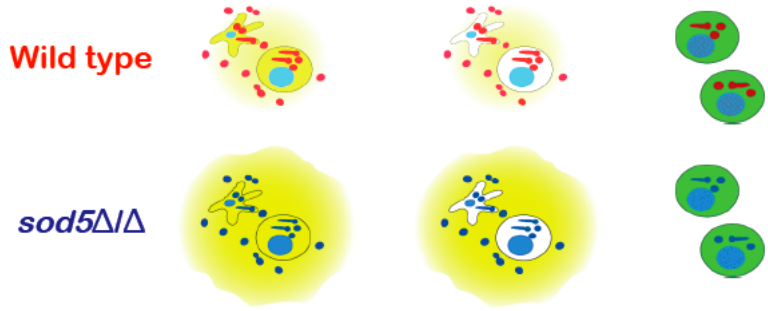
Frohner et al, Mol. Microbiol

Lack of Sod4 & Sod5 Increases ROS Sensitivity



Frohner et al, Mol. Microbiol

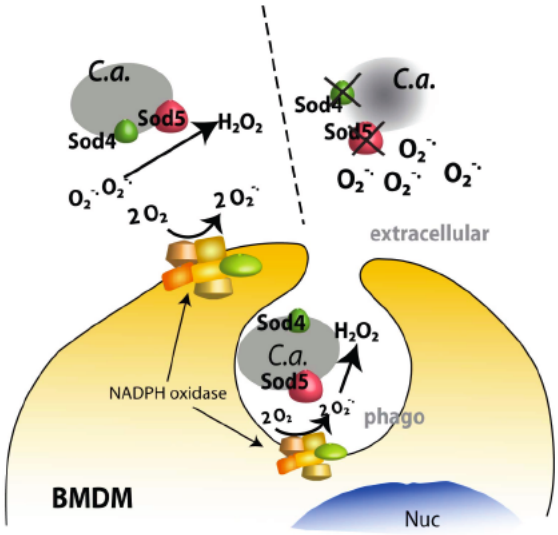
C. albicans Sod5/Sod4 Destroy Host-Derived ROS



C. albicans strategy to escape host-defense & facilitate host invasion

Counteracting oxidative burst of immune cells

Sod4/5 are Defense Genes Decomposing Host ROS



Microbiology and Molecular Biology Reviews

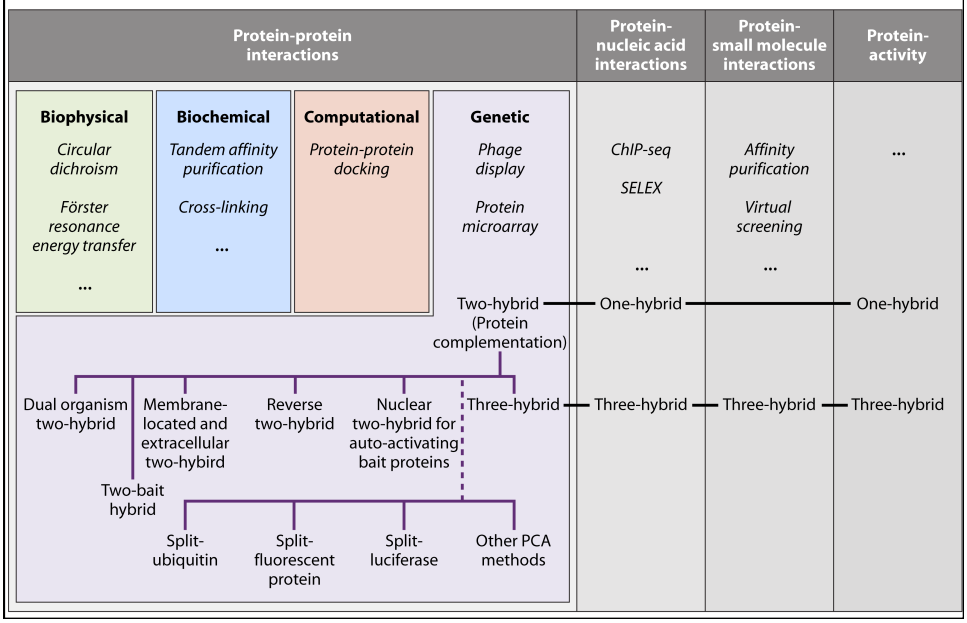
Diversity in Genetic *In Vivo* Methods for Protein-Protein Interaction Studies: from the Yeast Two-Hybrid System to the Mammalian Split-Luciferase System

Bram Stynen, Hélène Tournu, Jan Tavernier and Patrick Van Dijck

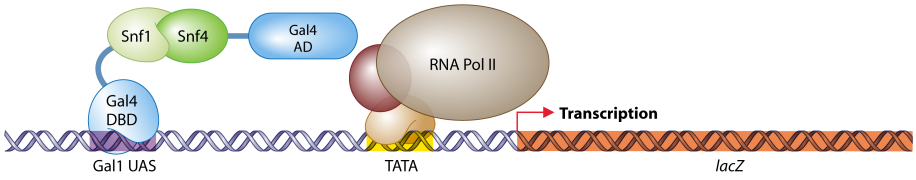
Microbiol. Mol. Biol. Rev. **2012, 76(2):331. DOI: 10.1128/MMBR.05021-11.**

Different protein-protein interaction systems

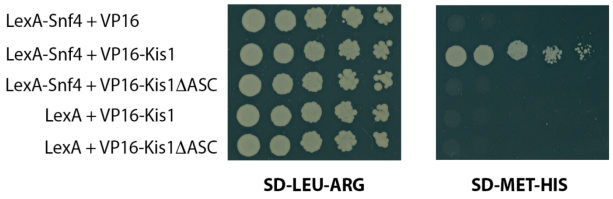
Stynen et al. MMBR 2012



The first two-hybrid system

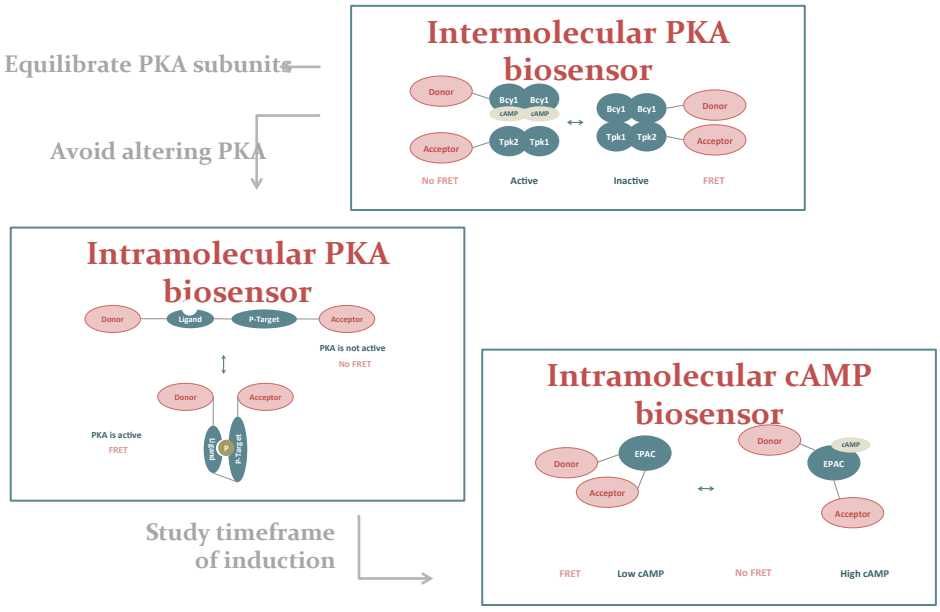


We adapted this for use in *C. albicans* (see presentation by Floris Schoeters)



We also develop BiFC and FRET biosensors for *C. albicans*

Integrative system for monitoring cAMP/PKA signaling



Troubleshooting optimization cAMP/PKA biosensors

Fluorophore intensity in *Candida albicans*

Certain fluorophores are not as stable or bright in *C. albicans* as they are in *Saccharomyces cerevisiae* and mammalian cells. It is therefor important to choose an appropriate FRET donor and acceptor.

→ CFP-YFP should be replaced by mTurquoise-Venus.

PKA activity correlates with autofluorescence

PKA positively regulates riboflavin synthesis through modification of iron metabolism. The spectral properties of riboflavin are the same as those of the FRET signal.

→ This can cause false positive results.

Aggregation of proteins in *C. albicans*

Certain parts of the biosensor aggregate under certain conditions. Biosensor accumulates in aggregates. Impossible to localize or quantify the FRET signal.

→ The cAMP EPAC biosensor was modified and nonessential domains were eliminated, this solved the issue of the aggregation.

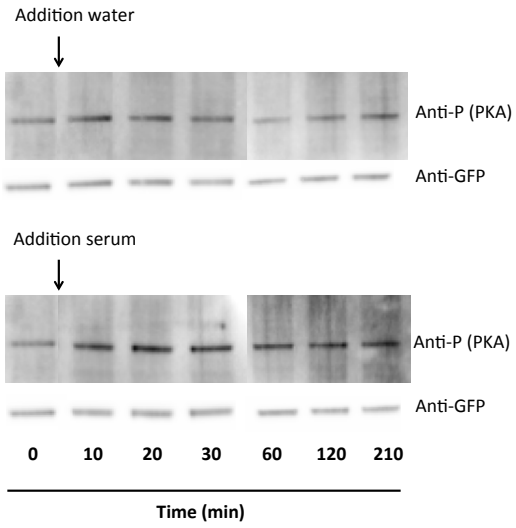
Immobilization of *C. albicans* cells

FRET measurements at second timescale requires immobile cells. Coating glass surface doesn't work adequately

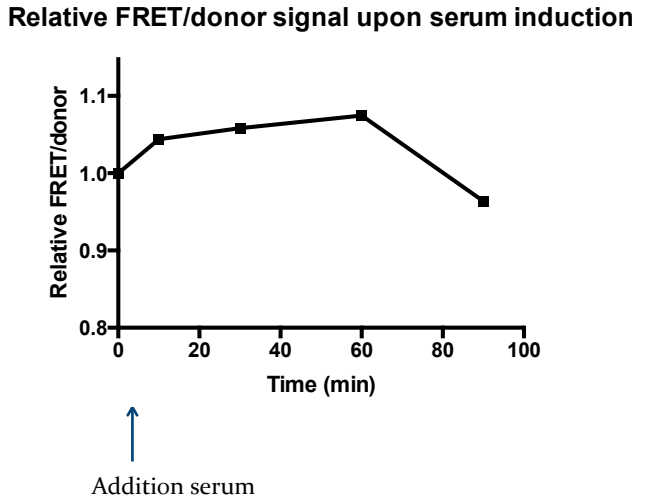
→ Microfluidics device avoids focus shift

Preliminary results AKAR – PKA activity biosensor

What is the timeframe we are looking at?



Preliminary results AKAR – PKA activity biosensor



Conclusions

Biofilms cause a major problem in hospitals

We developed a 'simple' *in vivo* model system

This system allows screening for drugs, testing mutants, performing expression analysis, study host-biofilm interaction

Als3 peptides are promising antibiofilm molecules

VIB KU LEUVEN

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Prof. Joost Schymkowitz

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UCL
Prof. Yves Dufrêne
Dr. Audrey Beaussart
Dr. David Alsteens

The MCB laboratory general lab meeting 2015