



Leibniz Institut
für Naturstoff-Forschung und Infektionsbiologie
Hans-Knöll-Institut



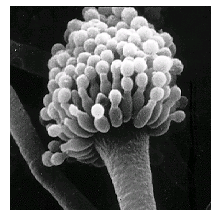
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Proteome analysis for pathogenicity and new diagnostic markers

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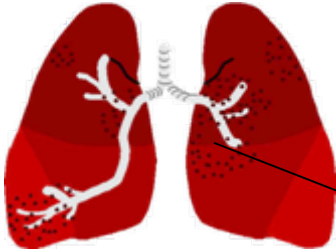
Friedrich Schiller University, Department of Microbiology/Molecular Biology, Jena



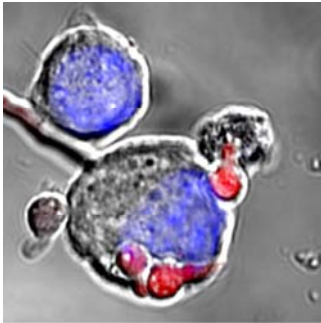
Outline

- I. Introduction
- II. Proteome map of *A. fumigatus*
- III. Pathogenomics: Stress response of *A. fumigatus* to ROI
- IV. Proteomics and diagnosis of IA

Stress and host defence during infection

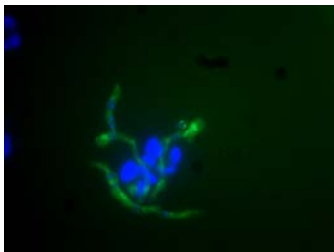


Temperature
Nutrients, trace elements like iron



(Behnsen et al. 2007)

1. Alveolar macrophages → ROI and low pH

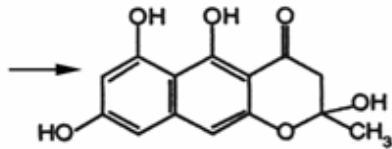


(Lessing and Löffler)

2. Neutrophilic granulocytes → ROI and defensins

Proteomic approach to study virulence

Malonyl-CoA
Acetyl-CoA



PksP

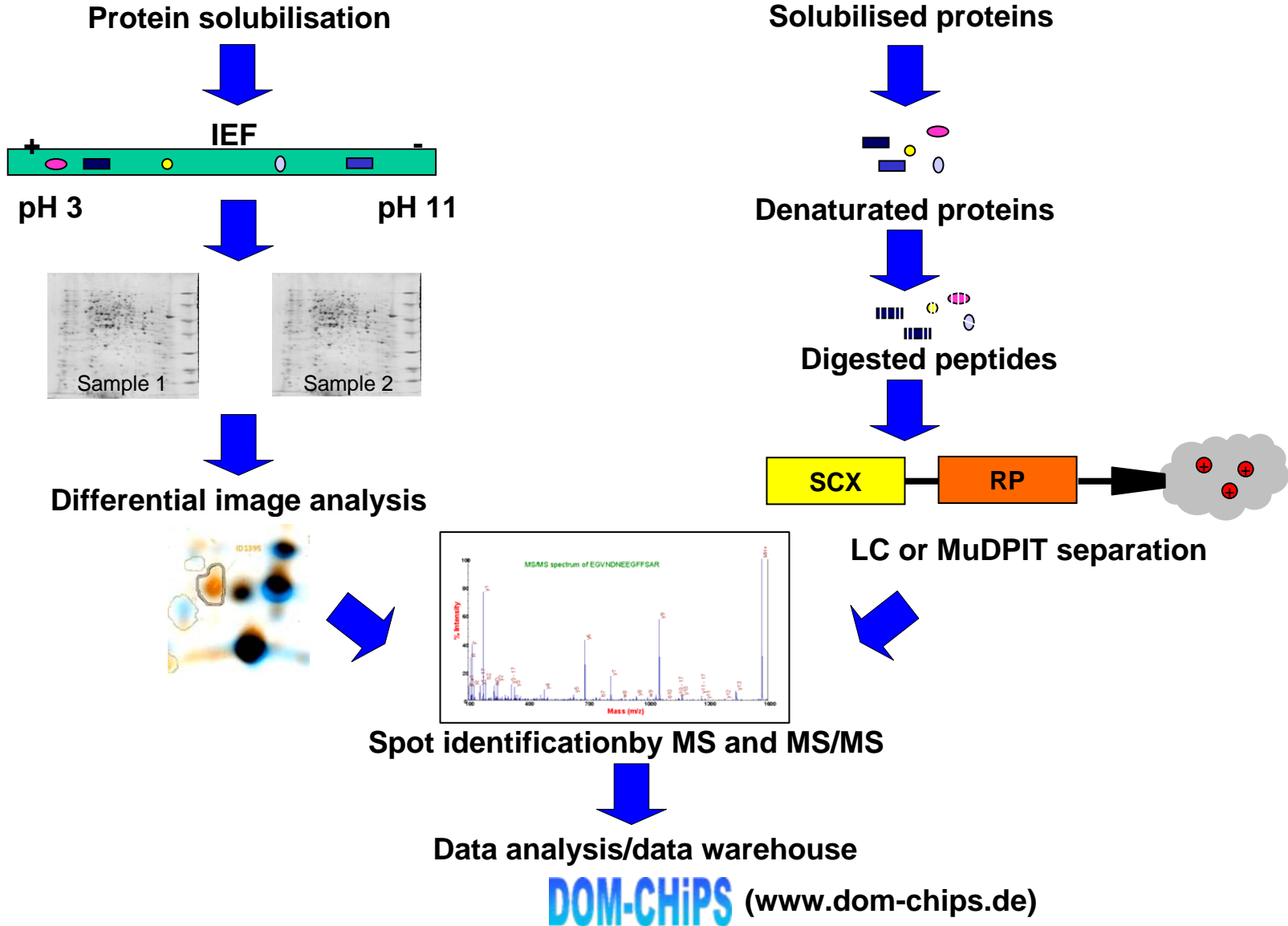
YWA1

(Langfelder 1998)

Some virulence determinants were found by genetic approaches, but the pathogenicity of *A. fumigatus* is a multifactorial process

Goal: Identification of regulatory networks and proteins important for stress tolerance and virulence.

Proteomics Workflows

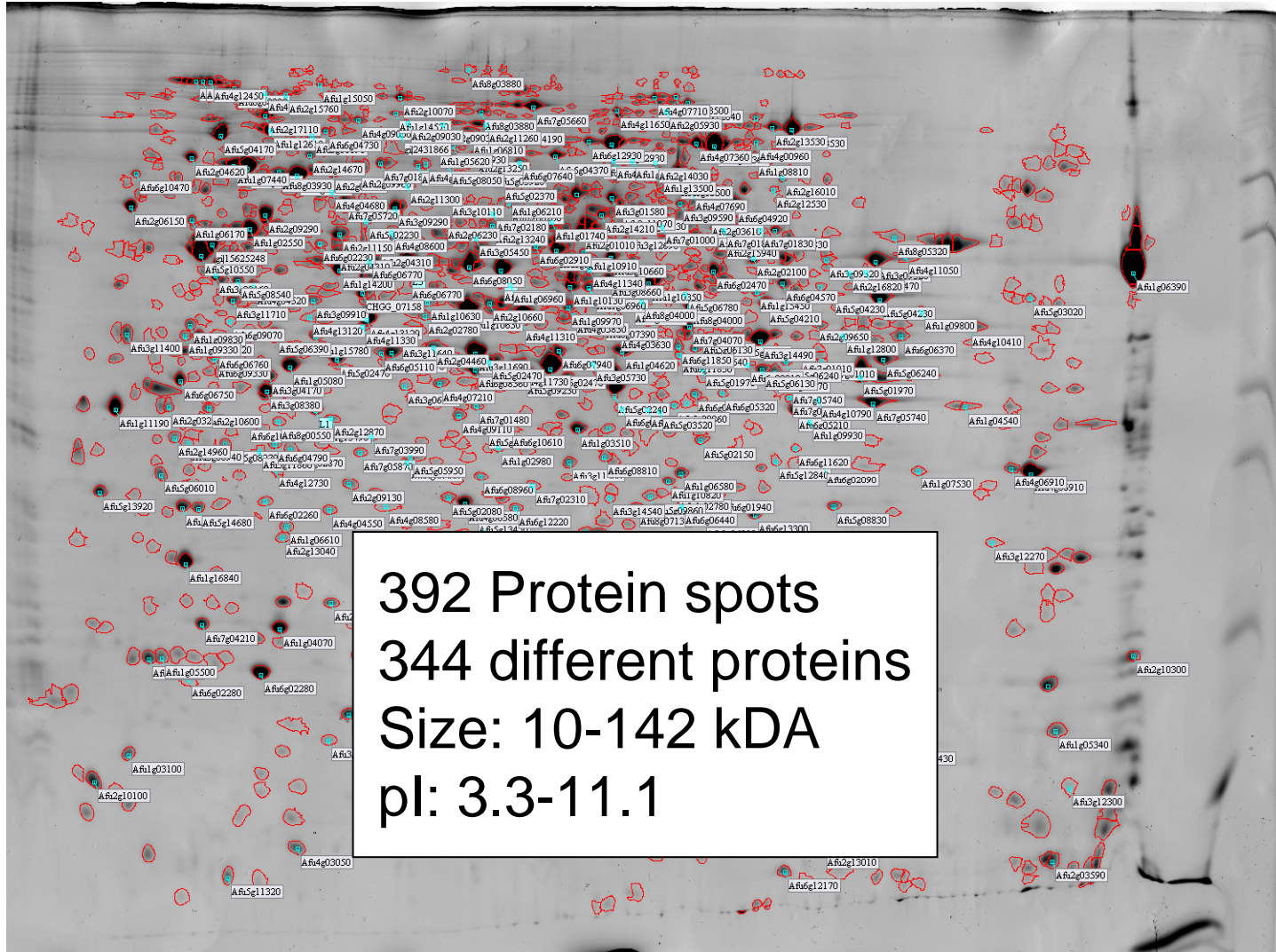


Which proteins do we find in 2D-gels?

Problem: High dynamic range of protein
concentrations of around 10^5

Proteome map of *A. fumigatus*

pH 3-11 NL

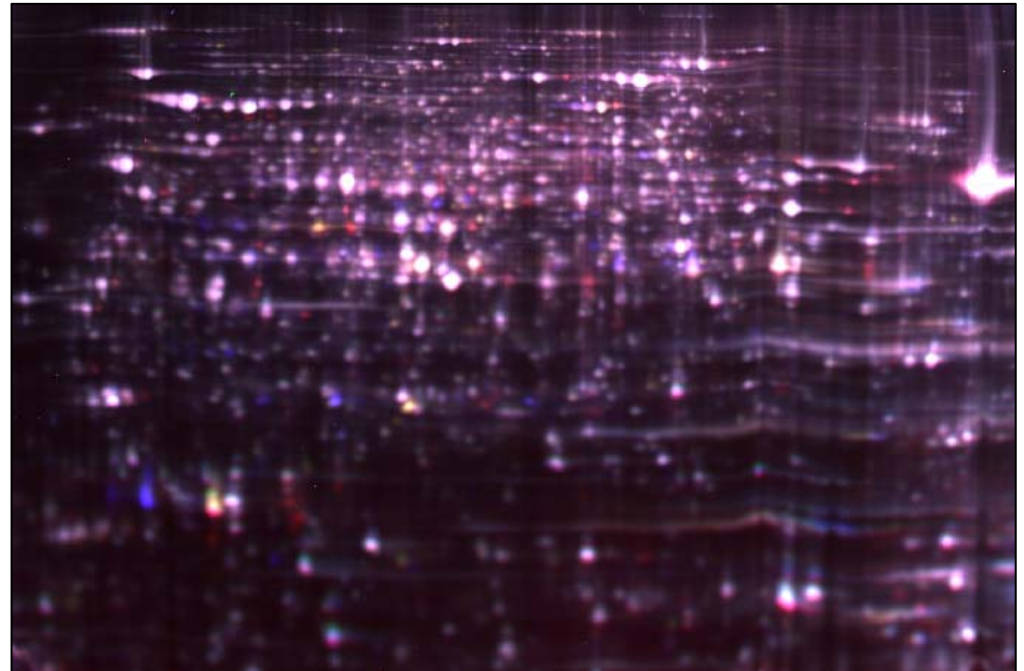
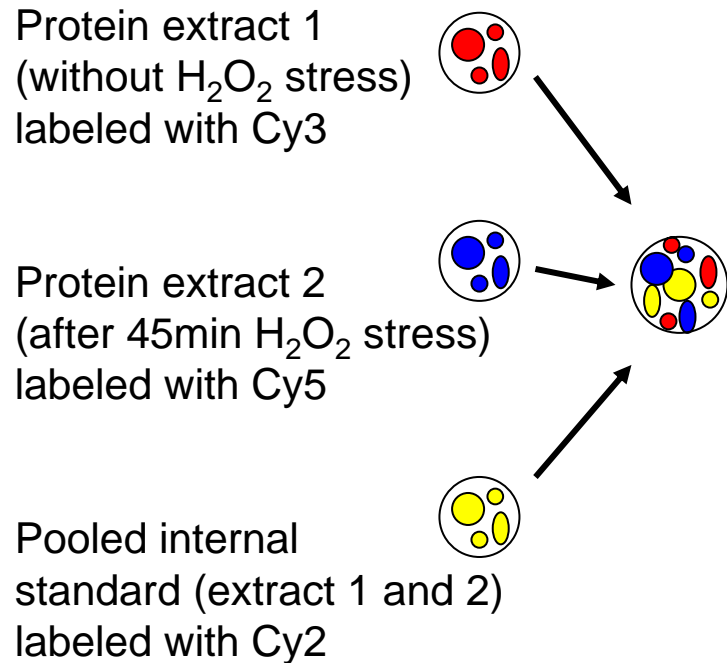


ROS response in *A. fumigatus*

Role for pathogenicity?

Lessing et al. (2007)

2D-gel electrophoresis with DIGE



Upregulated proteins in *A. fumigatus* under H₂O₂ stress

Protein	fold-change
Proteins with antioxidant properties	
Allergen Asp F3	10.3*
Antioxidant protein LsfA	3.7
Cu/Zn superoxide dismutase	2.7
Catalase I	1.3
Heat shock	
30 kDa heat shock protein	2.5
Pentose phosphate pathway	
Transketolase TktA	2.1*
Transaldolase	2.3*
Glycolysis and TCA cycle	
Glyceraldehyde-3-phosphate-DH	2.3*
Pyruvate-DH	3.2*
Polyamine pathway	
Spermidine synthase	2.9
Unclassified and proteins of unknown function	
GMC-oxidoreductase	6.2

Red = putative Yap1 binding site

Downregulated proteins in *A. fumigatus* under H₂O₂ stress

Protein	fold-change
Proteins with antioxidant properties	
Cytochrome C peroxidase	-2.0
Proteases	
Pitriysin family metalloprotease Cym1	-2.0
Fatty acid, alcohol and lipid metabolism	
Zn-dependent alcohol dehydrogenase	-2.1
Amino acid metabolism	
Glutamine synthetase	-2.0
Carbamoylphosphate synthase	-2.2
Cytoskeleton	
Actin interacting protein 2	-3.1
Beta-tubulin	-1.9
Topomyosin	-1.7
Trehalose, glycogen, beta-glucan metabolism	
UDP-glucose-pyrophosphorylase	-2.1*
Unclassified and proteins of unknown function	
NAD-(FAD)-dependent dehydrogenase	-2.1

Red = putative Yap1 binding site

Yap1 homologue in *A. fumigatus* (Afyap1)

bZIP

```
      50      60      70      80      90      100
yap1p GEDSEQPKKKGSKTSKKQDLDPETKQKR TAQNRAAQRAFRRERKERKMKLEKQVQSLESI
      . . . . . : . . : : . . . . . : : : : : : : : : : : : : : : : : : :
A.f.  GKKRRE SDEKAAKPKGRKPLTSEP TSKRKAQNRAAQRAFRRERKEKHLKDLAKVEELQKA
Yap1  140    150    160    170    180    190

      110    120    130    140    150    160
yap1p QQQNEVEATFLRDQLITLVNELKKYRPE TRNDSKVLEYLARRDPLHFSKNNVHNSNSEP
      . . . . . : : : : : : : : : : . . . . . : : : : : : : : : : :
A.f.  SDNANQENGLLRAQVERLQLELKEYRKRLSWFGDL--PGSHLFTNTQTSKSNQNKAKDNP
Yap1  200    210    220    230    240    250

      170    180    190    200    210    220
yap1p IDTPNDDIQENVKQKMNFTFYPLDNDNDNDNSKNVKGQLPSPNDP-SHSAPMPINQTK
      : : : : : : : : : : . . . . . : : : : : : : : : : : : : : :
A.f.  TATPRSEAQ--VPGVLRNR-DLKI SSPNGLSNGPSPAKSTPSGQTPNSQTS TRP GSGTLN
Yap1  260    270    280    290    300    310

      230    240    250    260    270
yap1p KLSDATDSSSATL--DSLNSN-DVLNNTPN---STSMDWLDNVIYTNRFVSGDDGSNS
      : . . . . : : : : . . . . . : : : : . . . . . : : : :
A.f.  GAVDNNGAARGYQVNSSYSASTKQATHDTPSSDSPSSSDSHQS QLLSSNGTSPEP SLHS
Yap1  320    330    340    350    360    370

      280    290    300    310
yap1p KTKNLD SNMF S NDFNFENQFDEQVSEFC SKMNQVCGTRQCP
      . . . . . : . . . . . : : : : : . . . . . : : :
A.f.  PAVKATESSTPHACTYTTINGEE--SFC AQLSMACGNINNP
Yap1  380    390    400    410

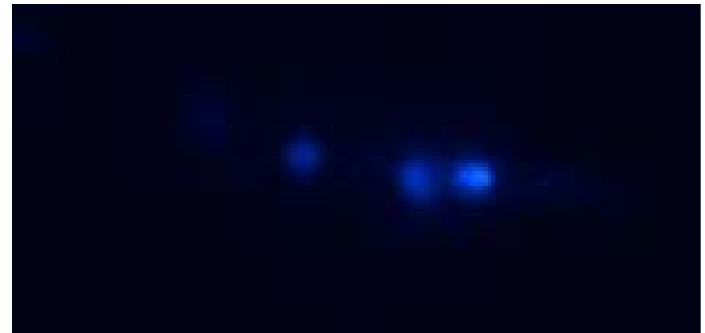
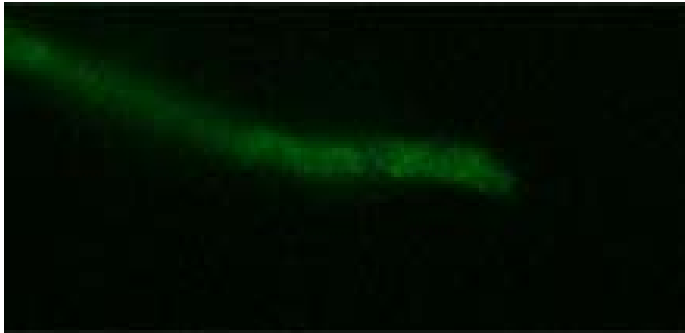
      590      600      610      620      630
yap1p DNDNDVVP SKEGS-LLRCEIWDRIITHPKY--SDIDVDGLCELMAKAKC SERGVVINA
      : : : : : : : : : : : : : : : . . . . . : : : : : : : : : :
A.f.  DEDEEVVPGEDKSQMLT CNKIWDRLQ SMEKFRNGEIDVDNLCELRTKARCEGSGVVVNQ
Yap1 510    520    530    540    550    560

      640
yap1p EDVQLALNK
      . . . . .
A.f.  KDVEDIMGR
Yap1 570
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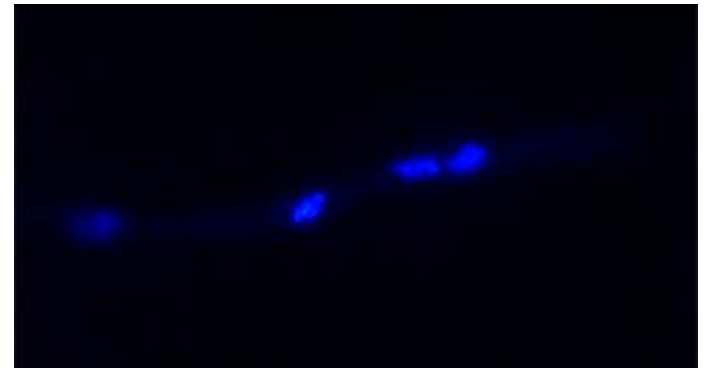
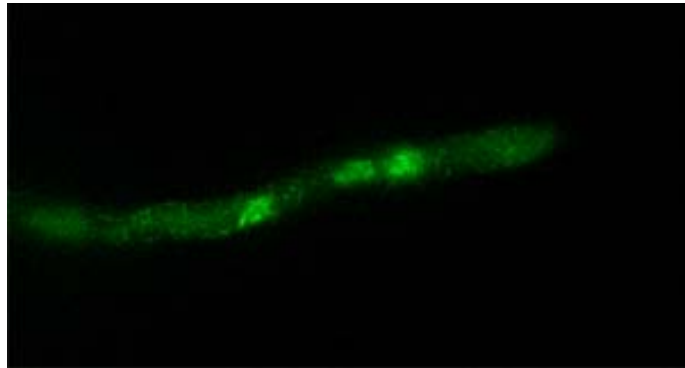
Cys rich domain

Nuclear accumulation of Afyap1-eGFP upon H₂O₂ stress

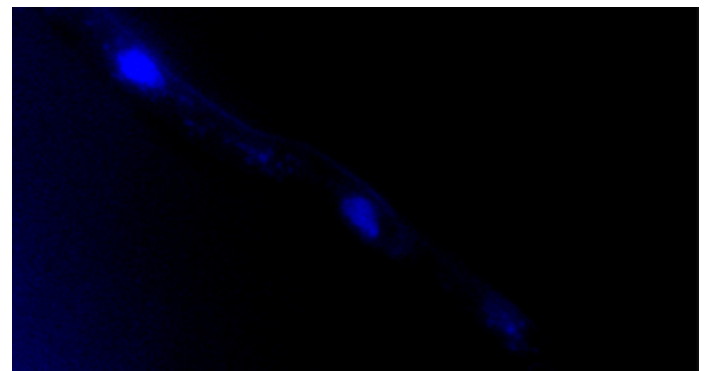
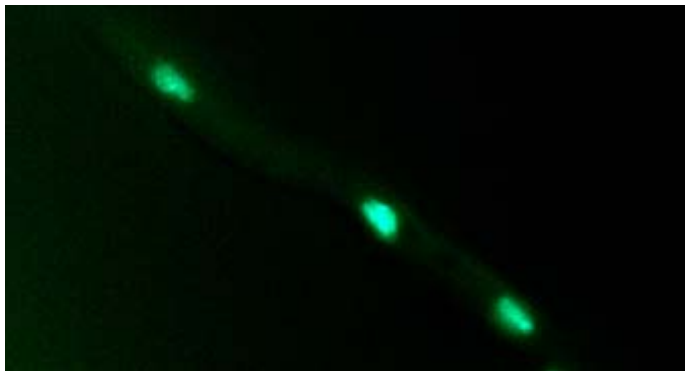
T₀



H₂O₂ T₂₀

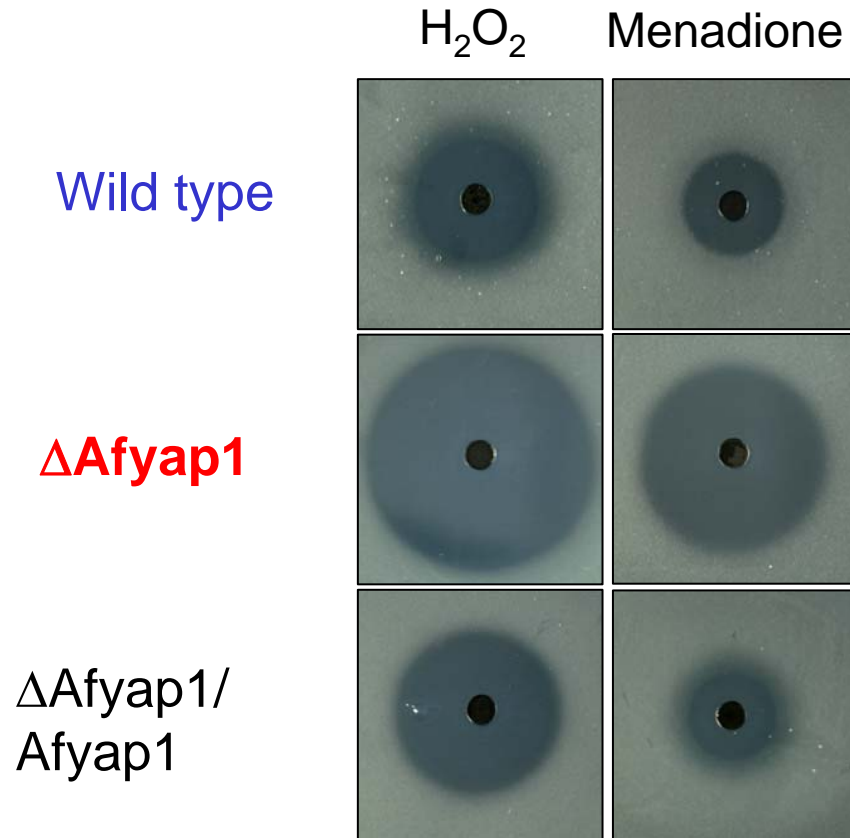


H₂O₂ T₄₅



Sensitivity of Δ Afyap1 against ROI

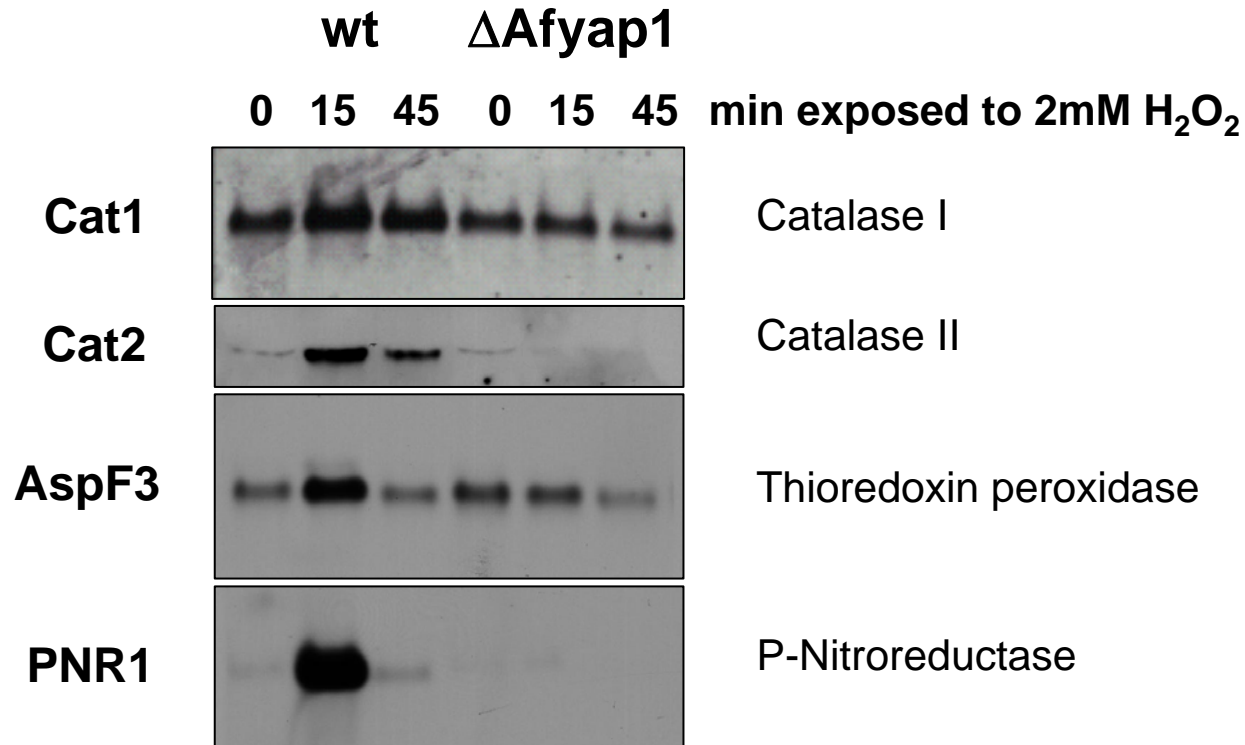
- Generation of an Afyap1 deletion strain
- Characterisation of the Δ Afyap1 strain:



No growth difference in the presence of diamide, high salt, SDS, CdCl₂

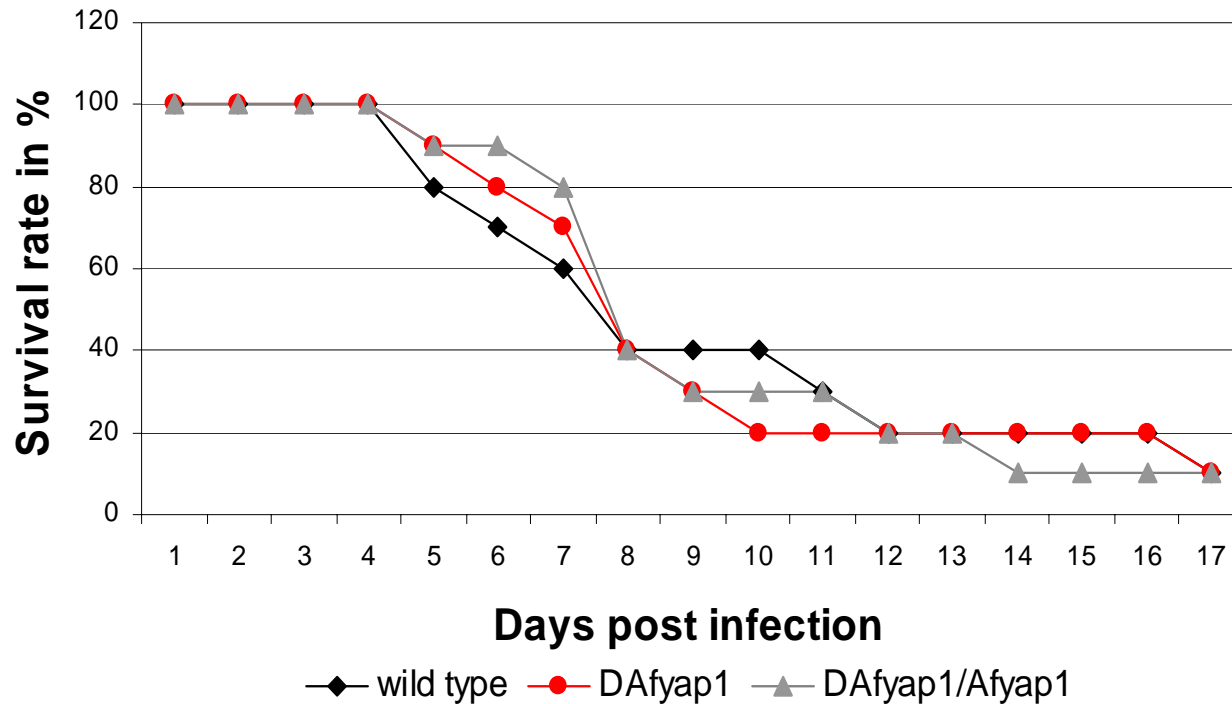
**Are ROI detoxifying catalases in *A. fumigatus*
regulated by Afyap1?**

Northern Blot analysis



Afyap1 is a key regulator of *A. fumigatus* required for defence against ROI.

Mouse infection model



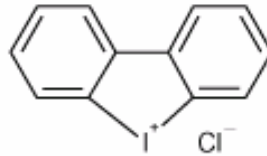
No attenuation in virulence of the $\Delta Afyap1$ strain in a leukopenic murine infection model

ROI production by granulocytes (PMNs)?

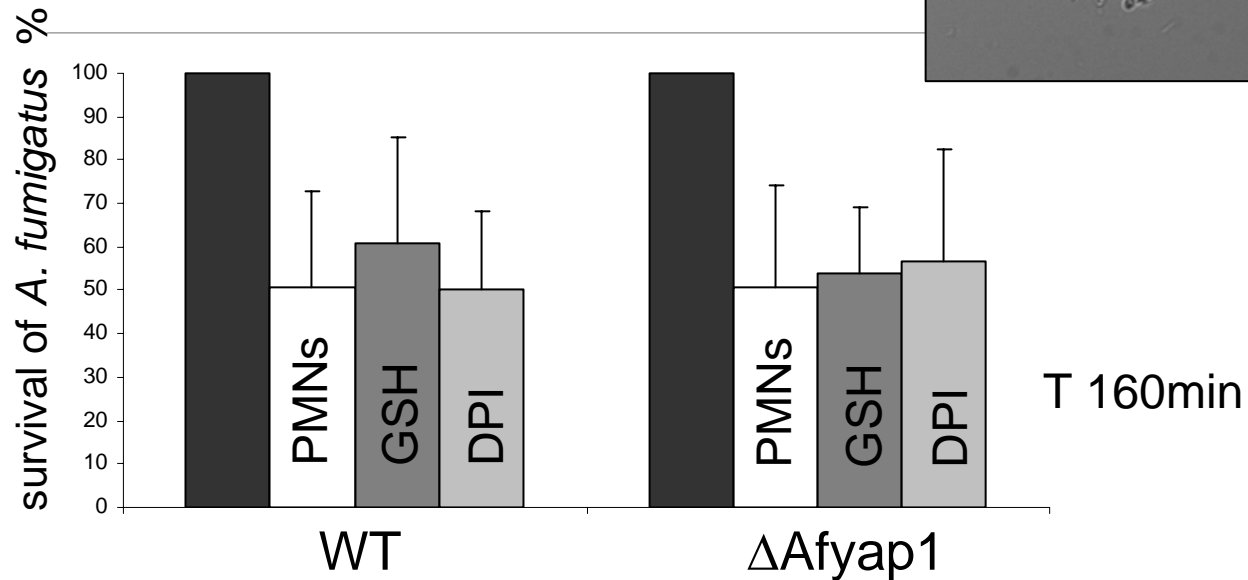
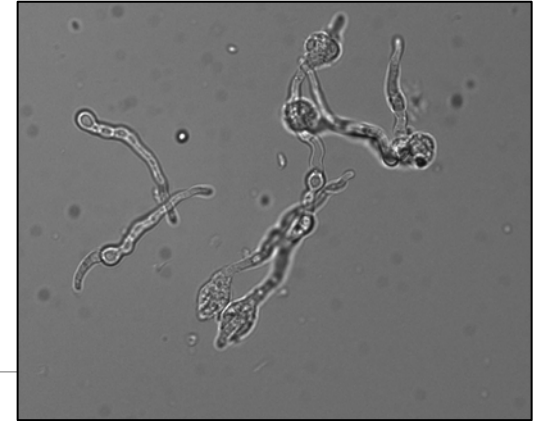
**Coincubation of granulocytes with hyphae
after addition of ROI inhibitors**

Killing of *A. fumigatus* hyphae by PMNs

Diphenyleneiodonium chloride



DPI



Addition of glutathione and DPI had no effect on survival of *A. fumigatus* WT and Δ Afyap1 coincubated with PMNs.

Summary

- Proteome map established
- Response to peroxide at proteome level:
 - (i) transient
 - (ii) antioxidant defence enzymes, heat shock proteins, the pentose phosphate shunt, protein translation and cytoskeleton
- A lot of the identified proteins are apparently regulated by the Yap1 homolog *Afyap1*. Nuclear localisation was shown.
- Deletion of *Afyap1* led to increased sensitivity against O_2^- and O_2^{2-} . **No** attenuation in virulence or reduced killing by neutrophil granulocytes. **Direct indirect role of ROI?**

Proteomics and its application to diagnosis

Diagnosis of invasive aspergillosis

I. Direct techniques

Microscopy and histology

II. Cultivation

III. Serological techniques

Antigenes: Galactomannan, (1,3)- β -D glucan

Antibodies directed towards *A. fumigatus*

Metabolites

PCR tests

IV. Radiological imaging

Serological testing for aspergillosis

Serological testing for acute invasive pulmonary aspergillosis:

- Mostly based on detection of antigens
- The relevance of antibody testing is under debate: Impaired antigen presentation in neutropenic patients is assumed (Reichenberger et al. 2002)
- Antibody production has been documented in some cases (e. g. Chan et al. 2002)
- Antibody assays may confer prognostic information (Hope et al. 2005)
- High titers of anti-*Aspergillus* antibodies in many patients before transplantation (Sarfati et al. 2006)

Known antigens and allergens

Around 20 allergens for *A. fumigatus* are known (ABPA):

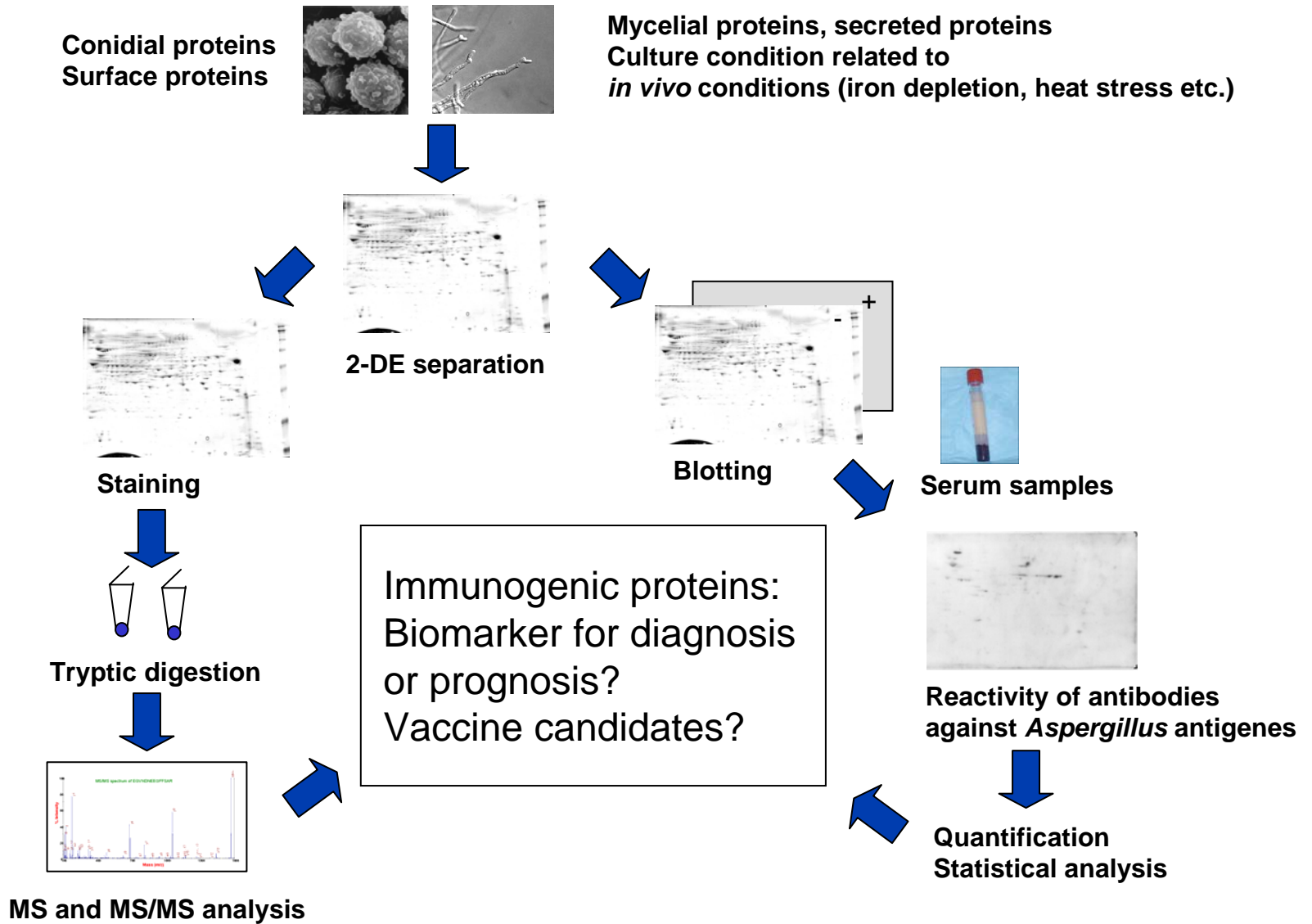
Asp f 1-Asp f 13, Asp f 17, Asp f 18, Asp f 22, Asp f 23,
Asp f GST, Asp o lipase

Antibody detection in Invasive Aspergillosis:

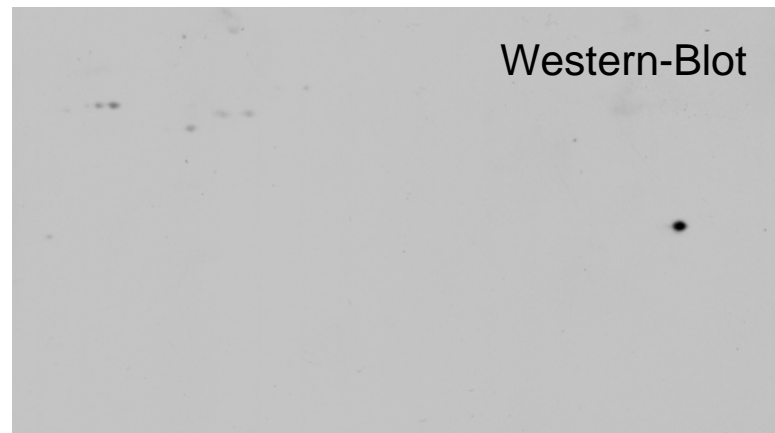
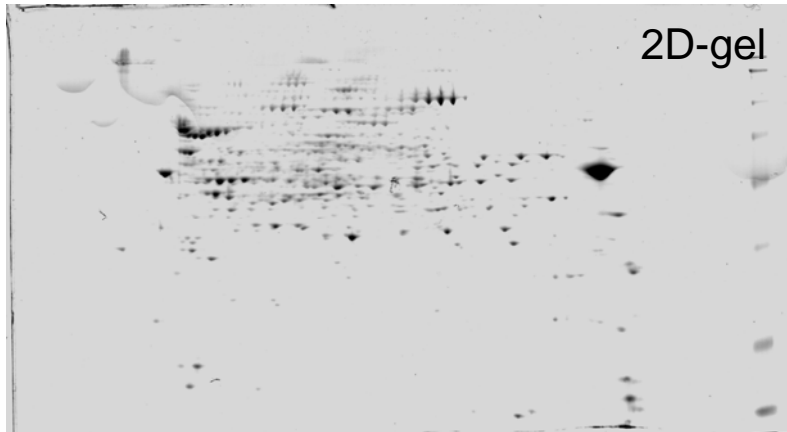
- Mitogillin (Weig et al 2001; Schwienbacher et al. 2005)
- Chitosanase chiB1 (Schwienbacher et al. 2005)
- Mannoproteins Afmp1p and Afmp2p (Woo et al. 2002; Ken et al. 2004)
- Cu,Zn superoxide dismutase (Holdom et al. 2000)
- Ribonuclease, catalase and dipeptidylpeptidase V (Sarfati et al. 2006)

→ **New more *A. fumigatus* antigens for diagnosis of IA, ABPA, aspergilloma?**

Proteomic approaches I – 2D gel electrophoresis

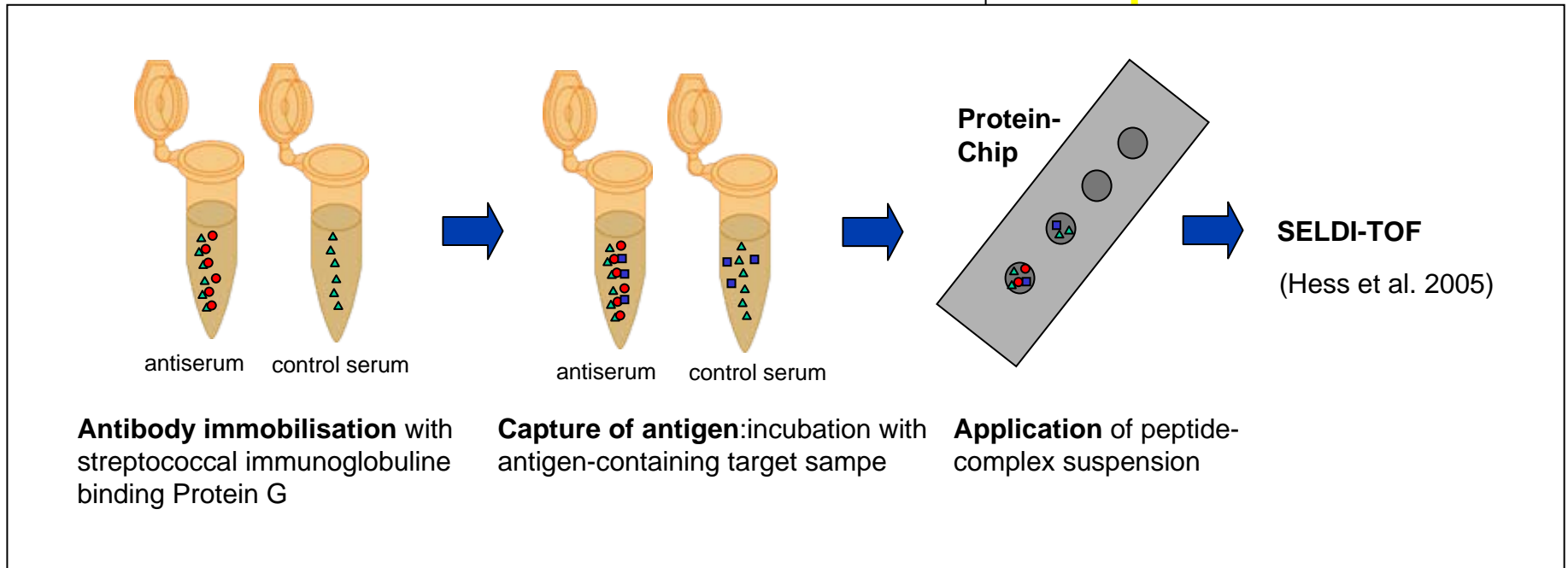


2D-gel electrophoresis



Detection with anti human IgG antibodies

Proteomic approaches II – SELDI-TOF



SELDI-ProteinChip

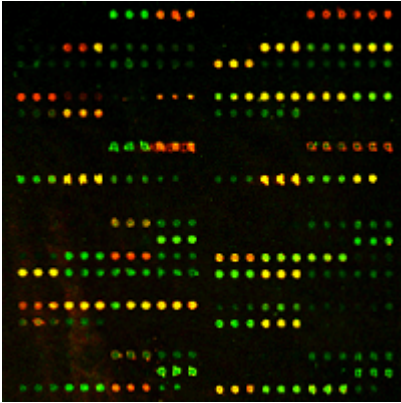
Chemical, biochemical or biological capture surface,
e. g. IMAC, ion exchance, hydrophobicity, affinity etc.

Positive

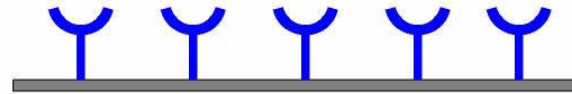
Negative

SELDI-TOF = Surface-enhanced laser desorption/ionisation time-of-flight

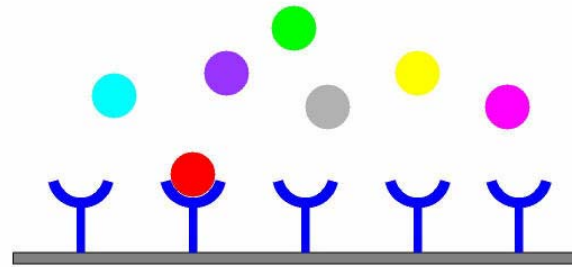
Protein arrays



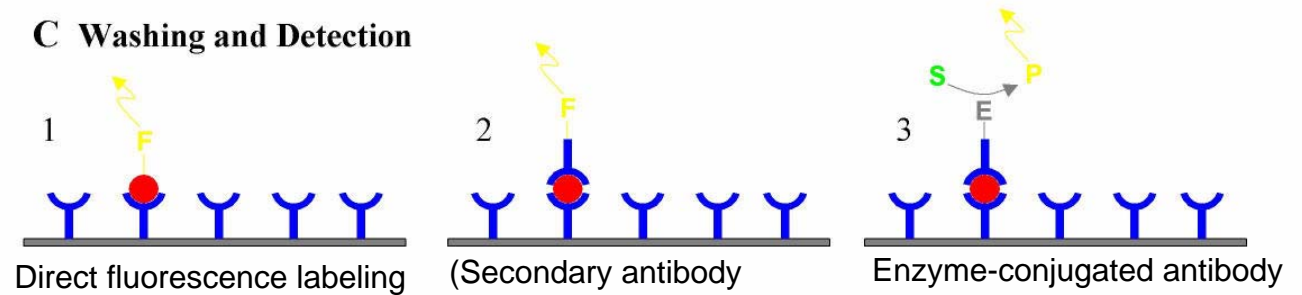
A Immobilization of Capture Agents



B Sample Incubation



C Washing and Detection



Proteome Science 2003 1:3

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Steinbach



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