



NUI MAYNOOTH

Ollscoil na hÉireann Má Nuad

Gliotoxin Reductase in *Aspergillus fumigatus*?

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Why are we interested in the ubiquitous filamentous fungus, *Aspergillus fumigatus*?

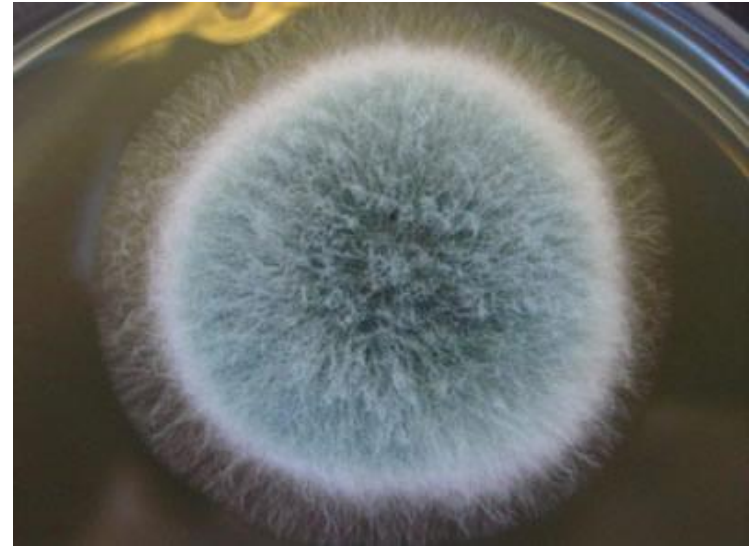
1. Human Pathogen- immunocompromised patients.

Invasive aspergillosis.
High mortality rate.

2. Limited antifungal drug repertoire.

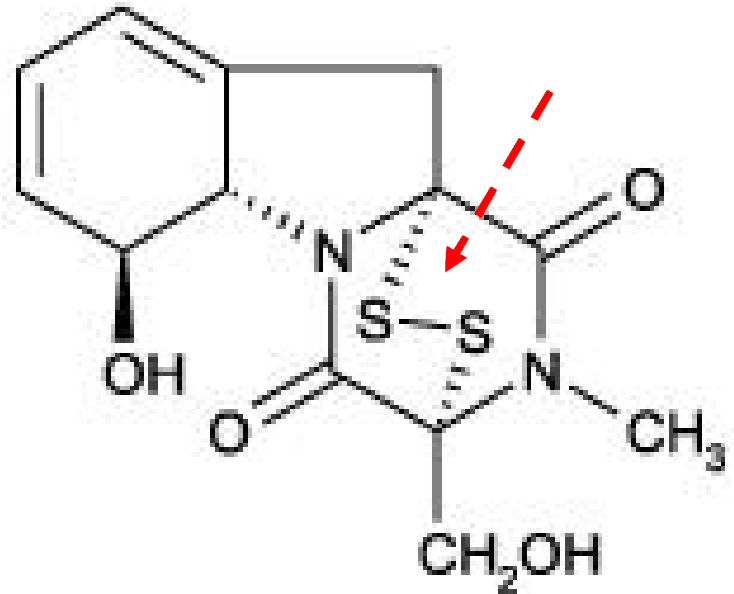
Voriconazole, AmpB and
Echinocandins

3. Filamentous fungi - reservoir of biologicals.



Gliotoxin (GT).

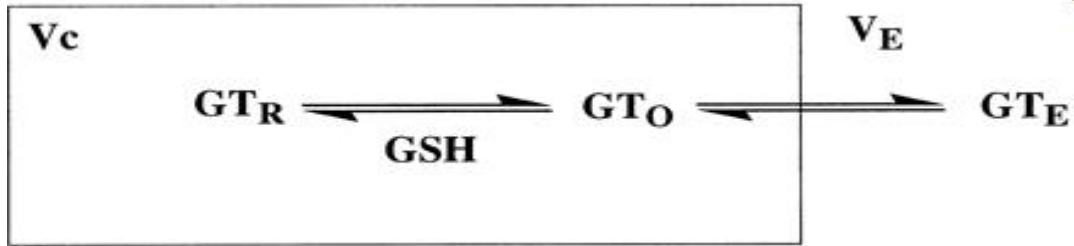
- Epipolythiodioxopiperazine (ETP) type toxin.
- 326 Da
- At least 14 known ETPs.
- Essential disulphide bridge.
- GT toxicity in mammalian cells:
 - Direct inactivation of essential protein thiols.
 - Inhibits NADPH oxidase complex formation in neutrophils.
 - Redox cycling leading to H_2O_2 formation.....or dismutation.



Many contradictions in the gliotoxin story.....

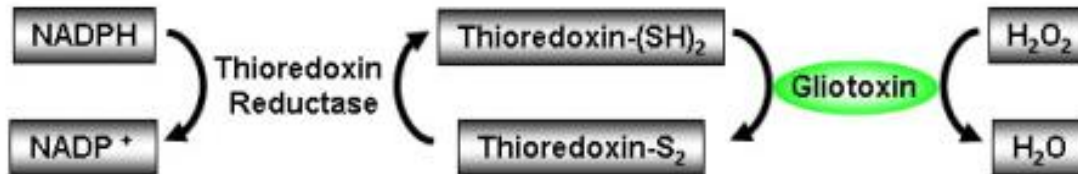
Primary role of Gliotoxin?

Apoptotic Effect in mammalian cells



Bernardo et al. (2003) J. Biol. Chem.

Protective Effect in mammalian cells

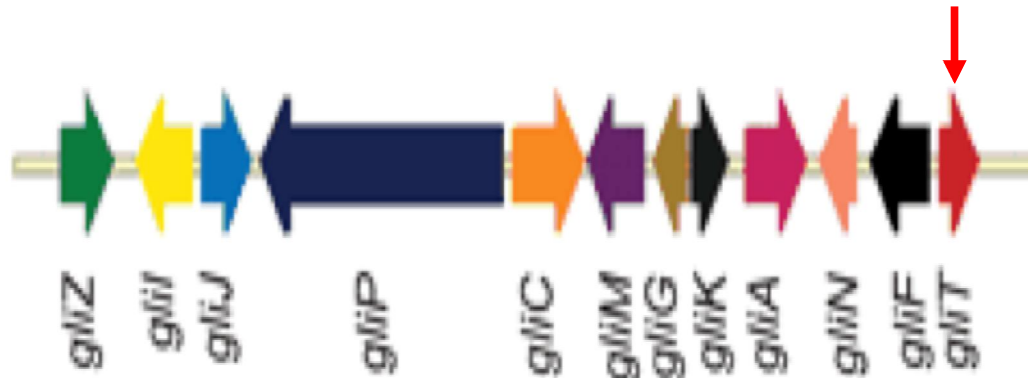


Choi et al. (2007) BBRC












Gliotoxin reduces H_2O_2 to H_2O by replacing the function of peroxiredoxin *in vitro* and decreases intracellular level of H_2O_2 in HeLa cells.

A. fumigatus gliotoxin gene cluster.

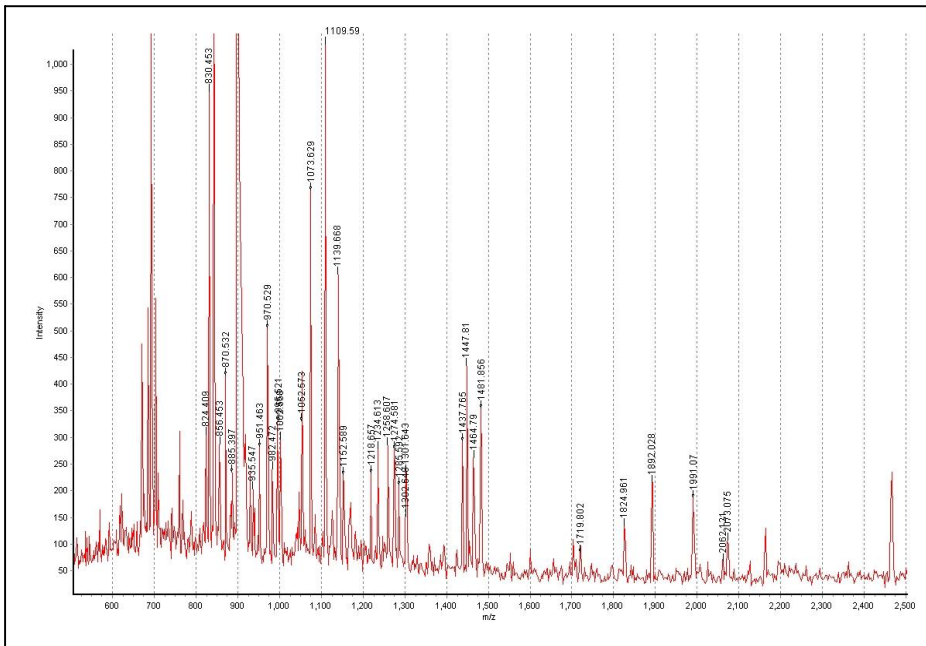
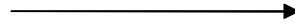
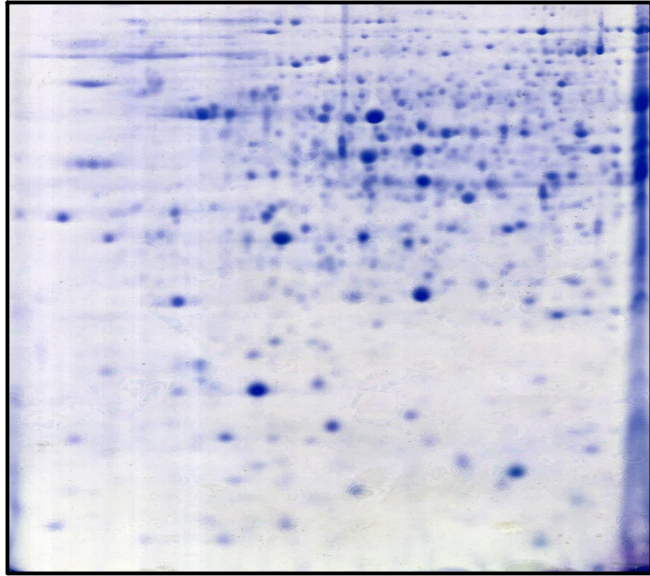
Aspergillus fumigatus putative gliotoxin gene cluster



12 Genes

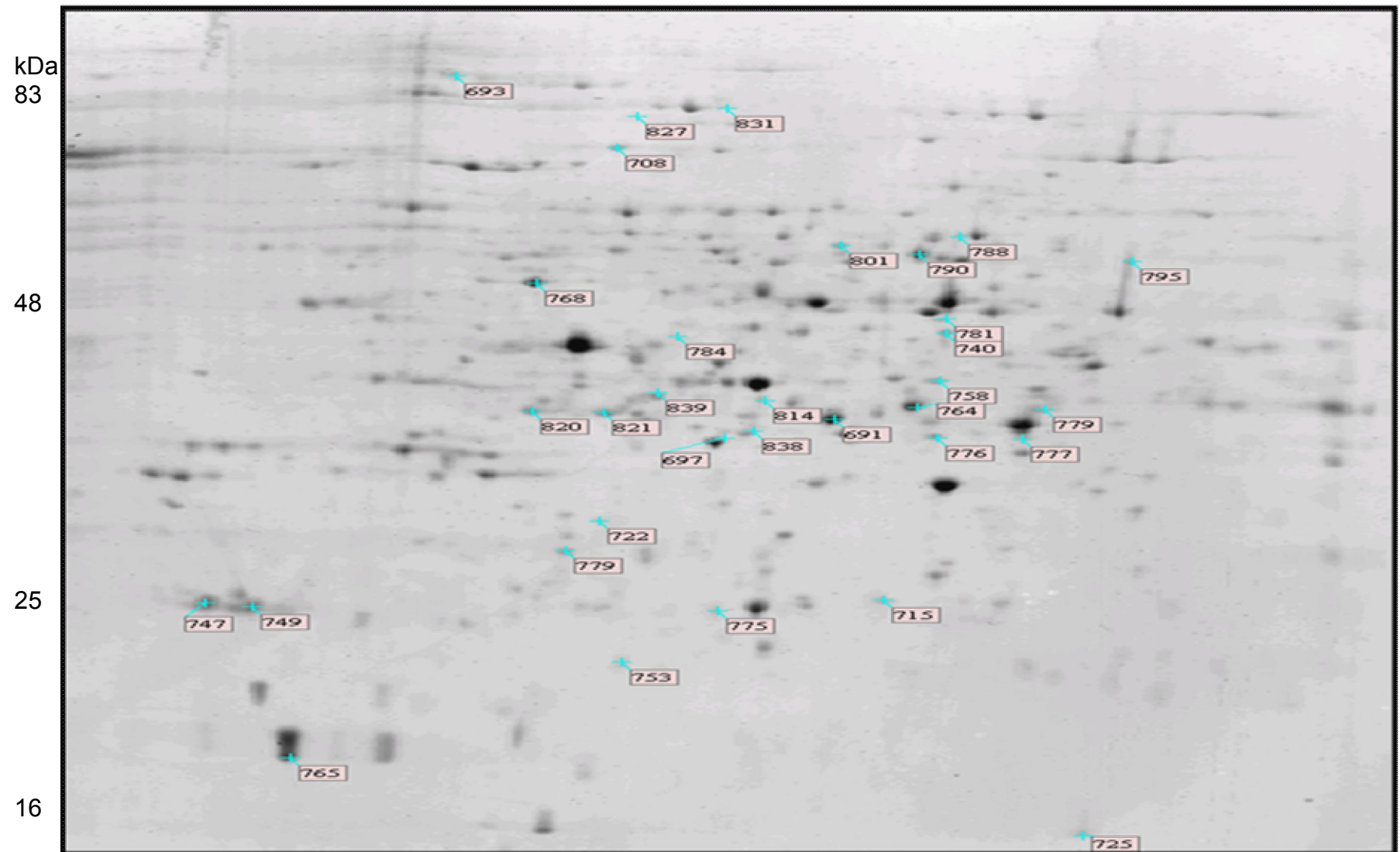
- | | | |
|---------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
|  Peptide synthetase |  O-methyl transferase |  Methyl transferase |
|  Thioredoxin reductase |  Glutathione S transferase |  Zinc Finger |
|  Cytochrome P450 monooxygenase |  Dipeptidase |  Transporter |
|  Aminocyclopropane carboxylate synthase |  Other | |

Classic proteomics workflow



pH 4

pH7



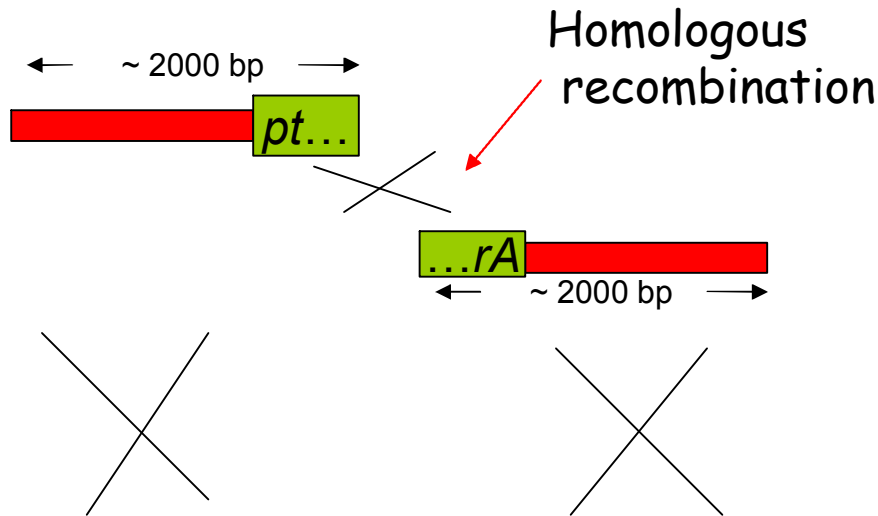
Reference 2D-PAGE of *A. fumigatus* challenged with exogenous gliotoxin ($40 \mu\text{M}$) resulted in the identification of **36 proteins** that were differentially regulated in response to the exogenous gliotoxin. Proteins were separated across the entire p.I. range of the pH 4-7 strip and were present from 16 - 83 kDa.

Exposure to Gliotoxin **Up-Regulates** the Expression of Selected Proteins in *A. fumigatus*.

Proposed/Actual function	Fold Increase	% Coverage	Mass (Da)	CADRE I.D.	Spot #
Cu,Zn Superoxide dismutase	(+)Unique	33.5	16361	Afu5g09240	725
Short chain dehydrogenase	(+)Unique	29.6	30928	Afu4g08710	779
Eukaryotic translation elongation factor beta 1 subunit	(+)Unique	25.9	30142	Afu1g11190	714
Adenine phosphoribosyltransferase	20.5	33.6	23621	Afu7g02310	715
Uridylate kinase	10.3	33.2	25560	Afu7g03990	722
Aminopeptidase	10.1	26	106227	Afu4g09030	693
vip1 protein	9.5	24.2	28265	Afu2g10030	777
vip1 protein	7.0	44.2	28265	Afu2g10030	776
Thiazole biosynthesis enzyme	7.0	27.7	35492	Afu6g08360	814
Allergen Asp F3	5.8	30.4	18453	Afu6g02280	765
GlIT → Pyridine nucleotide-disulphide oxidoreductase, putative	3.5	33	36004	Afu6g09740	821
Nitroreductase family protein	3.0	49.3	24314	Afu5g09910	753
* Unknown function protein	2.9	30.8	25439	Afu5g14680	749
Heat shock protein (Sti1), putative Z	2.9	17.6	65031	Afu7g01860	708
Allantoicase	2.7	21.3	46273	Afu3g12560	740
Mitochondrial processing peptidase beta subunit	2.7	31.5	53270	Afu1g14200	768
GlIT → Pyridine nucleotide-disulphide oxidoreductase, putative	2.5	33.2	36004	Afu6g09740	820
Glycerol dehydrogenase	2.5	25.8	36828	Afu4g11730	691
* Unknown function protein	2.1	37.9	25439	Afu5g14680	747
Thiamine biosynthesis protein Nmt1	1.9	33.9	38323	Afu5g02470	764
Phosphoglycerate kinase	1.9	34.2	44761	Afu1g10350	758

Elucidation of *GliT* function: A Functional Genomic/Proteomic Approach.

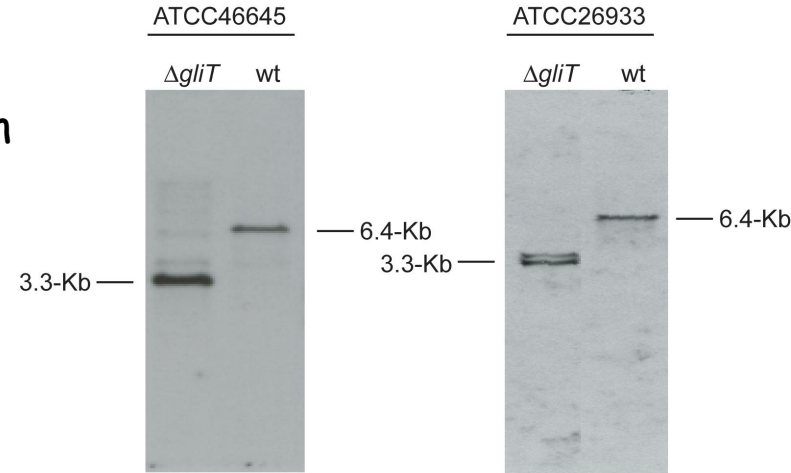
1. Gene Disruption



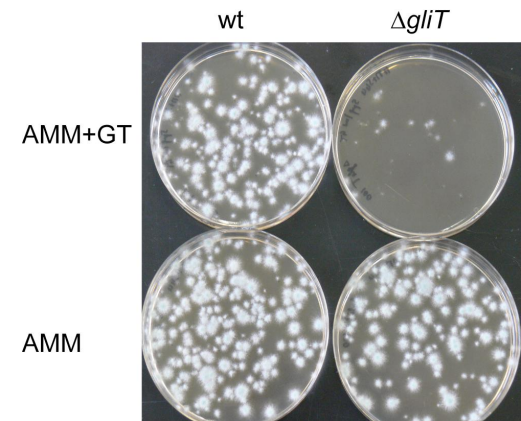
Gene Disruption/Replacement
with Selection marker



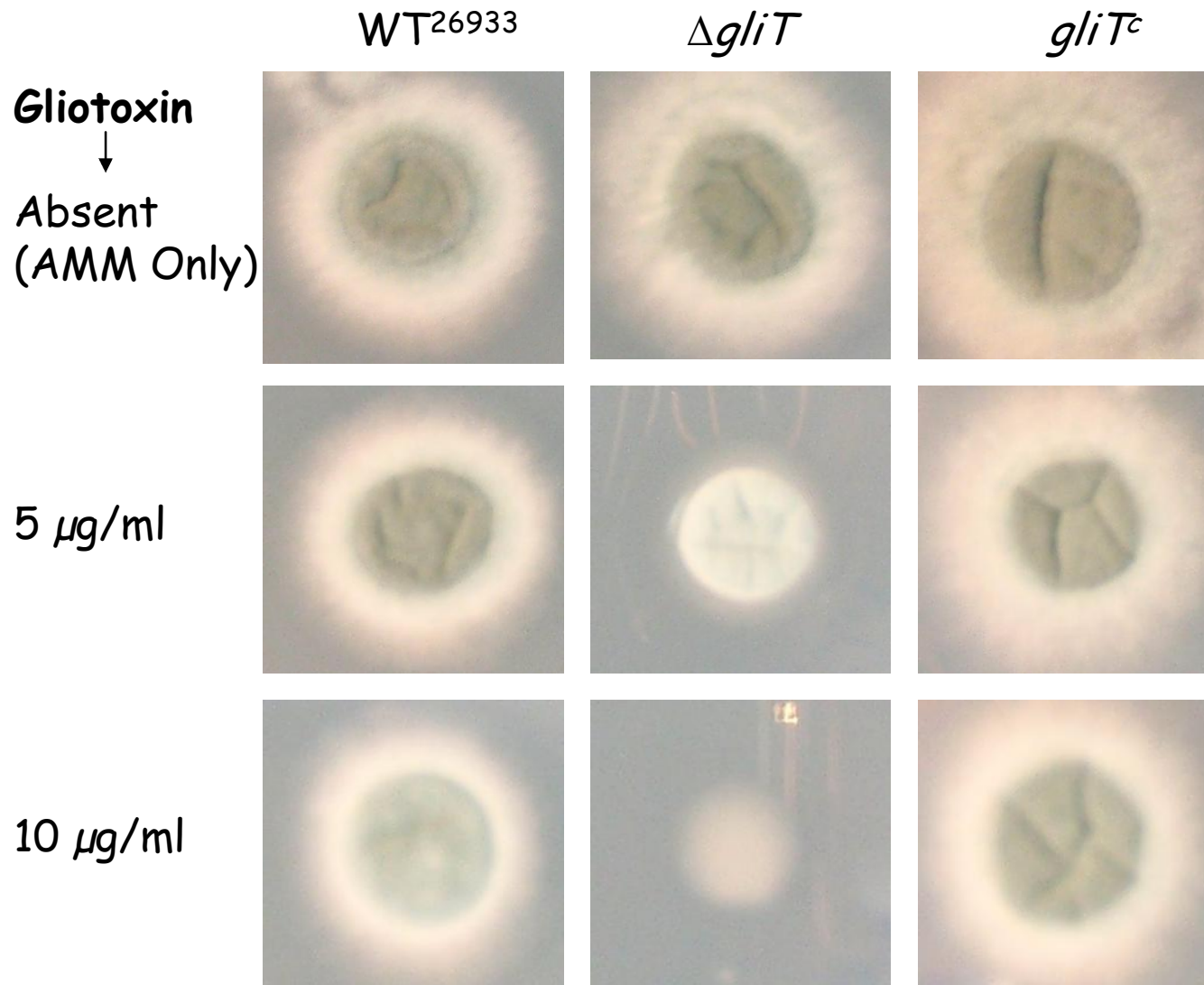
2. Analysis



3. Phenotype

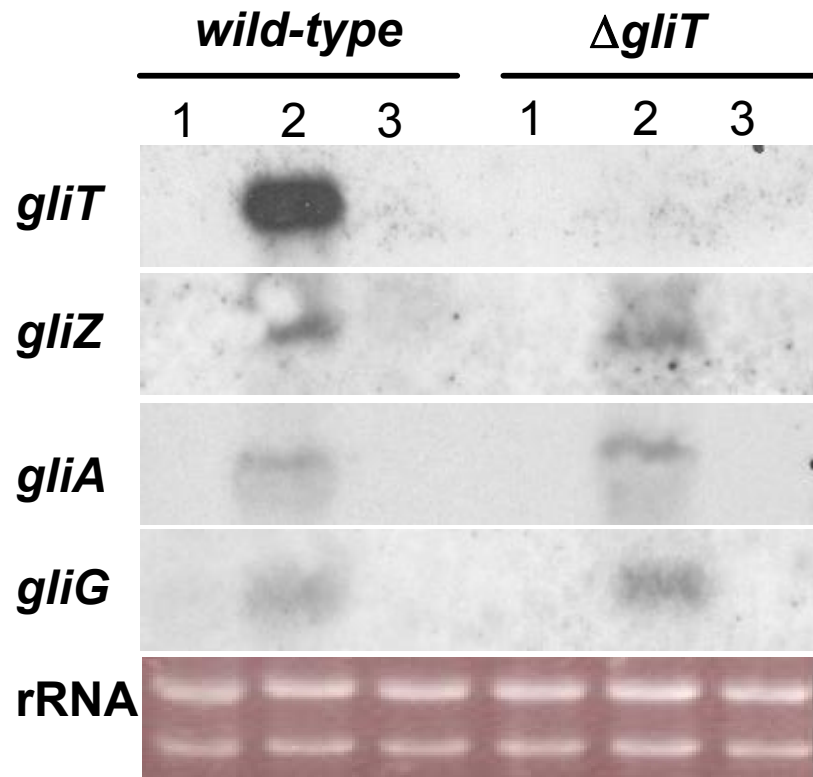


gliT Mediates Protection against Exogenous Gliotoxin

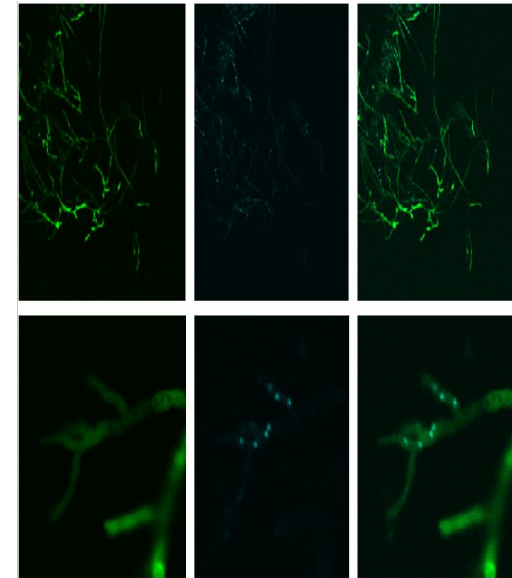


Incubation time: 40 h at 37 °C.

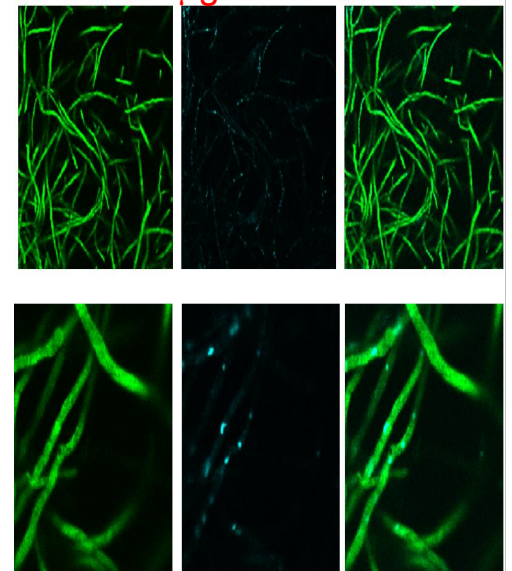
Gliotoxin induces GliT Expression



GT: 0 μ g/ml



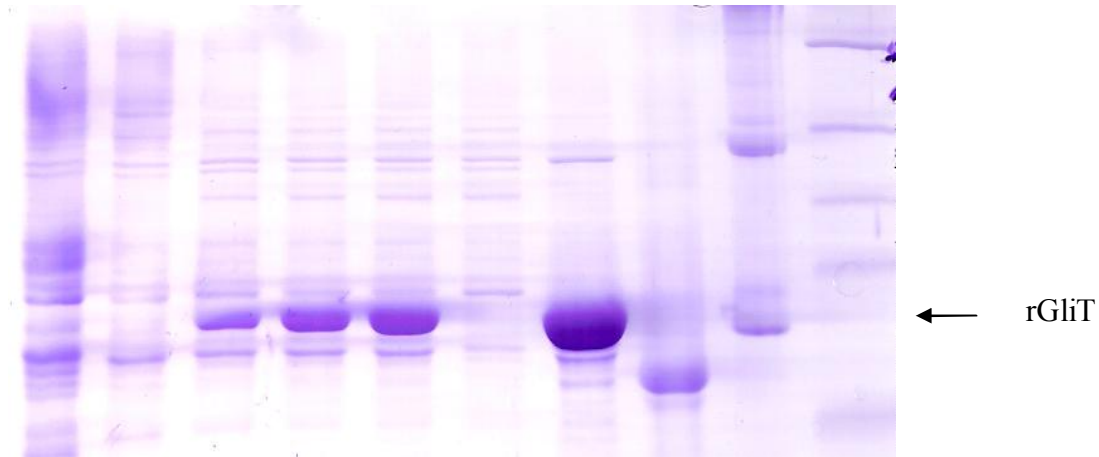
GT: 10 μ g/ml



- Induction of *gliT* upon treatment with exogenous gliotoxin
- No *gliT* expression in Δ *gliT*.
- Up-regulation of regulator, transporter and biosynthetic gene in WT and Δ *gliT*.

Recombinant Expression of GliT

SDS-PAGE

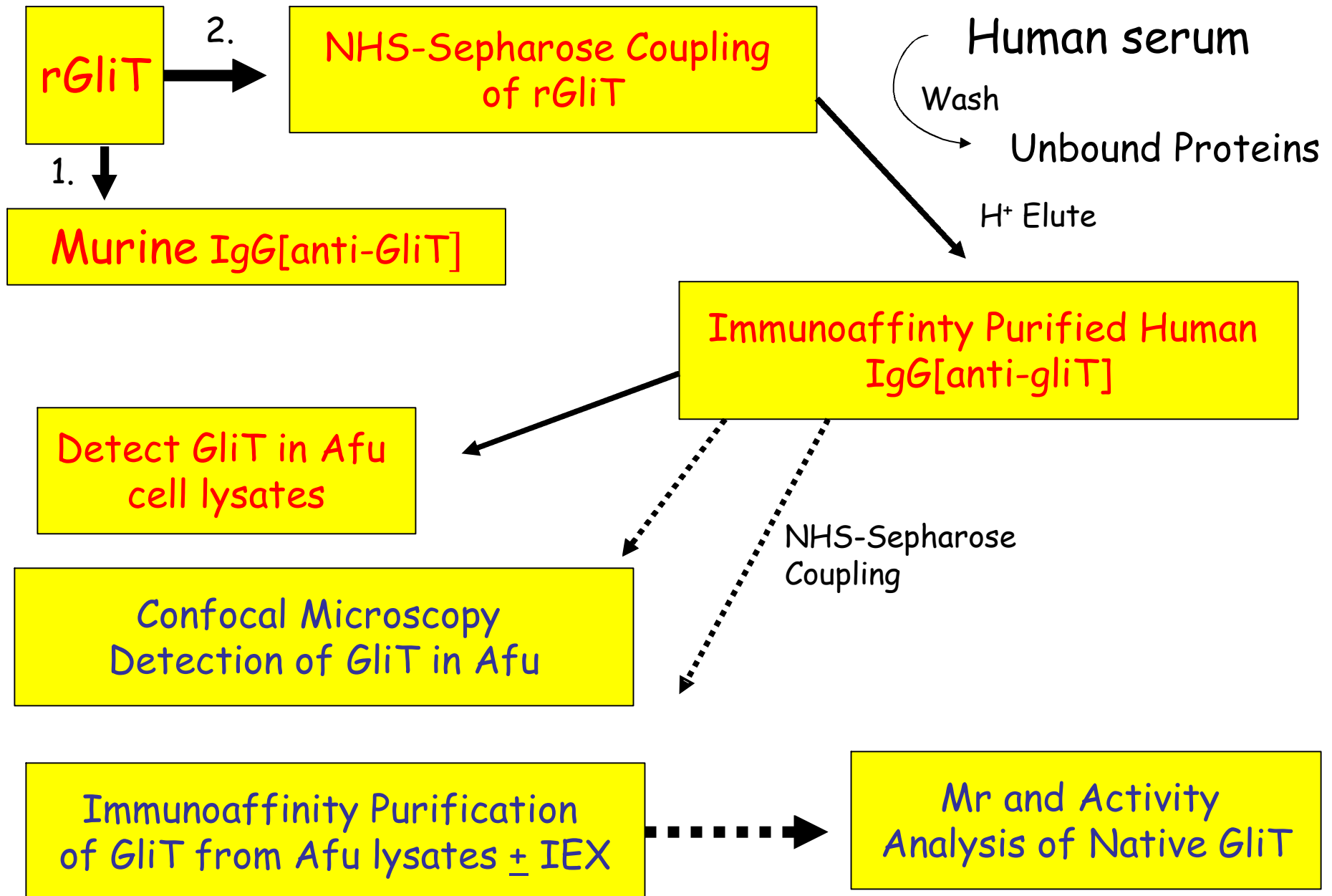


Western blot

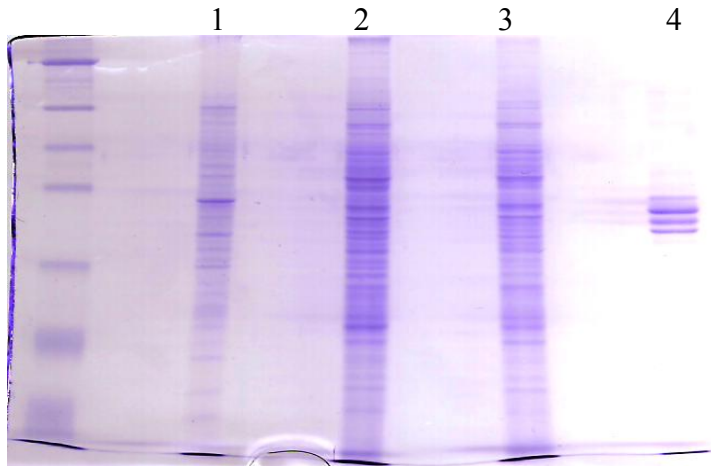


Recombinant GliT- Enzymatically Inactive.

GliT ImmunoProteomics

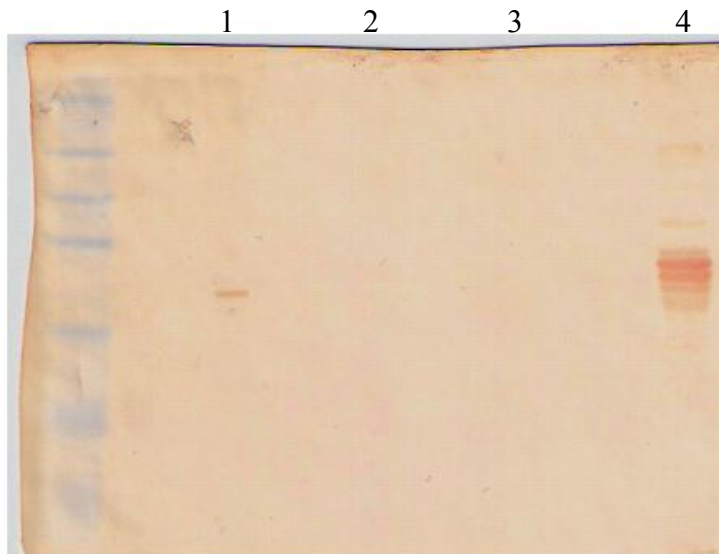


Confirmation of GliT Absence in *A. fumigatus* $\Delta gliT$



SDS-PAGE

1. *A. fumigatus* 26933 lysate (72 h);
2. *A. fumigatus* 46645 lysate (24 h);
3. *A. fumigatus* 46645 $\Delta gliT$ lysate (24 h);
4. Recombinant GliT (2 μ g).



DAB

Gliotoxin added during culture.

1^o Antibody: Immunoaffinity purified human IgG [anti-GliT].



ECL

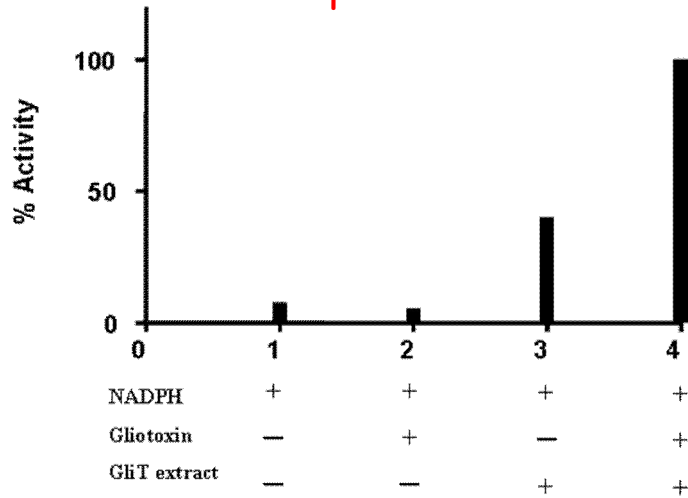
GliT IgG presence in human sera-Allergen?

GliT is a Gliotoxin Reductase.



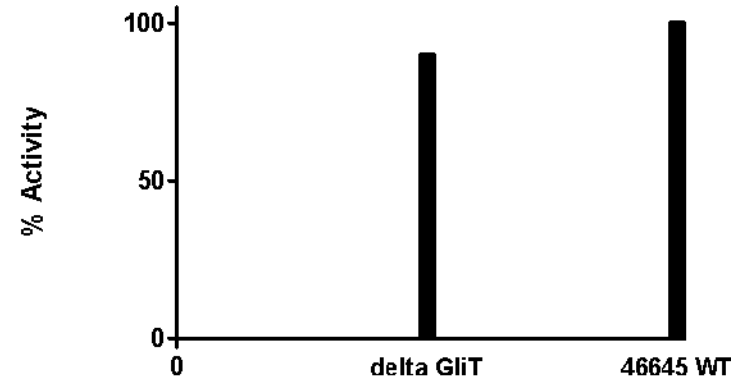
A.

Gliotoxin presence essential



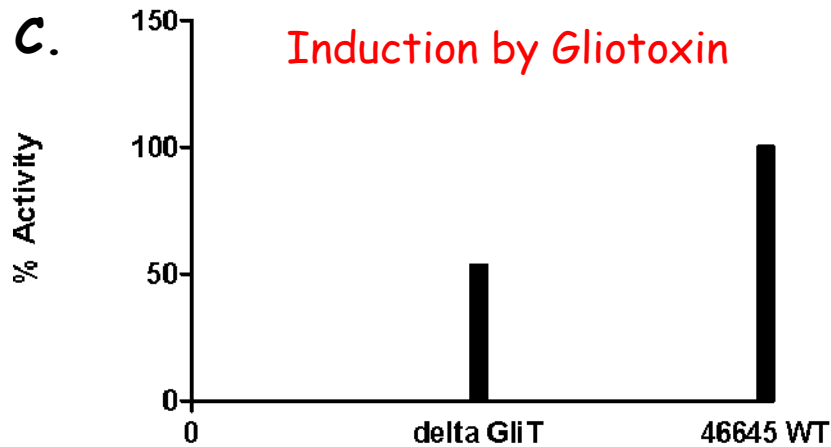
B.

No Induction



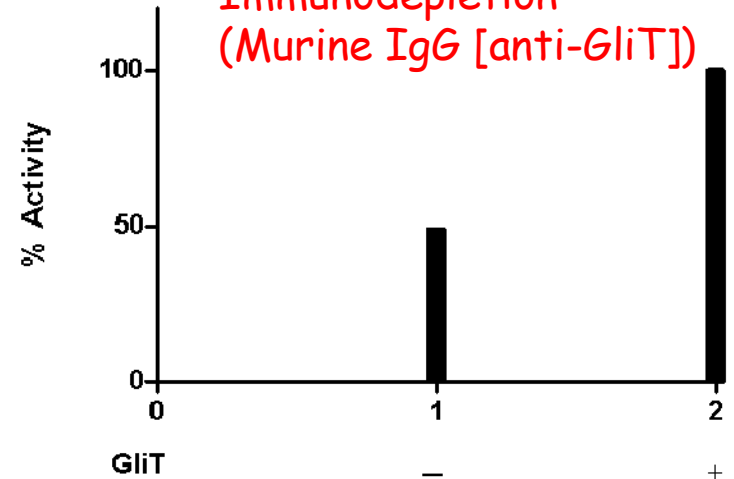
C.

Induction by Gliotoxin

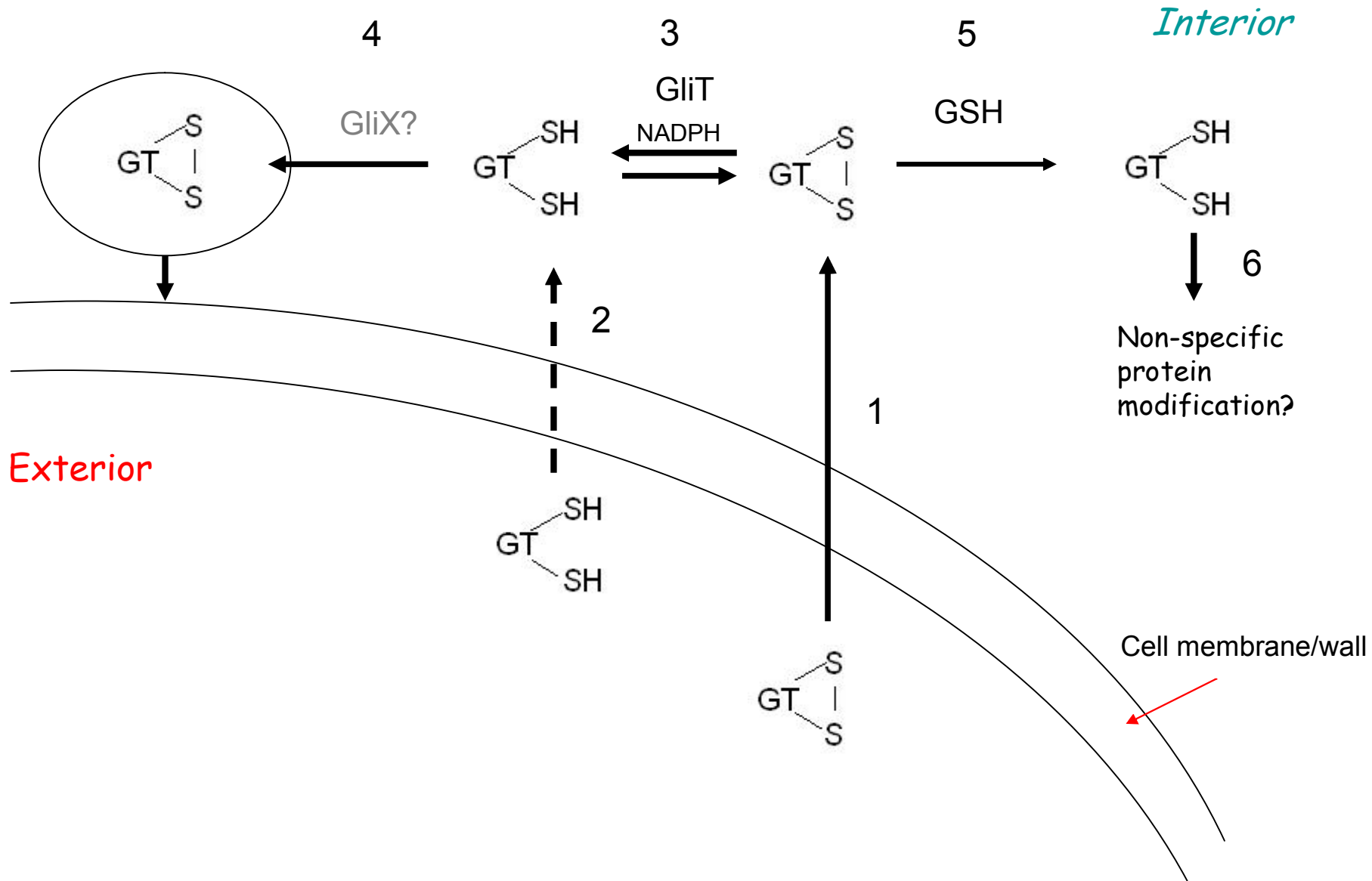


D.

Immunodepletion (Murine IgG [anti-GliT])



Our Proposed Model for GliT Role in *A. fumigatus*



Conclusions

1. Exogenous gliotoxin alters *A. fumigatus* proteome. GliT is differentially expressed relative to other *gli* cluster genes.
2. Absence of *gliT* sensitizes *A. fumigatus* to exogenous gliotoxin and confirms *gliT* as key component of an unknown autoprotective system. Selection marker potential.
3. GliT does not appear to be involved in gliotoxin biosynthesis (i.e., disulphide bridge closure) and gliotoxin is produced by $\Delta gliT$.
4. GliT is Gliotoxin Reductase. Previously unknown activity in fungi.
5. Gliotoxin may be a component of the intracellular redox control system, and not really a 'toxin'?

Prof. Sean Doyle

Dr. Kevin Kavanagh

Dr Markus Schrettl

Aine Nolan

Professor Hubertus Haas- Innsbruck

Professor Nancy Keller - U. Wisconsin

HEA

Higher Education Authority
An tÚdarás um Ard-Oideachas

HEA-PRTLII, Cycles 3, 4
and Equipment Grant.

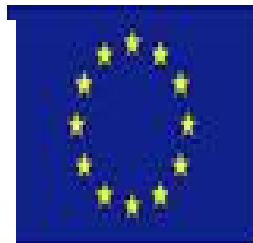


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