



ELSEVIER

Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in
Structural Biology

Fungal lectins: structure, function and potential applications

Annabelle Varrot, Soorej M Basheer and Anne Imberty

Lectins are a widespread class of proteins implicated in many essential cellular and molecular recognition processes. They recognize carbohydrates in a non-catalytic, specific and reversible manner. Fungi, which include mushrooms, microfungi and yeasts, have attracted wide interest in recent years. They are indeed a promising source for novel lectins with unique specificity and potential for biomedical and biotechnological applications. Information on fungal lectins, particularly structural insight, is scarce compared to that on their plant and animal counterparts. This review therefore focuses on the structure, function, and exploitable properties of fungal lectins.

Addresses

CERMAV-CNRS (UPR5301, Affiliated with Université Grenoble Alpes and Belonging to ICMG), BP53, F8041 Grenoble cedex 9, France

Corresponding author: Imberty, Anne (imberty@cermav.cnrs.fr)

Current Opinion in Structural Biology 2013, 23:xx–yy

This review comes from a themed issue on **Protein–carbohydrate interactions**

Edited by Gideon Davies and Bernard Henrissat

0959-440X/\$ – see front matter, Published by Elsevier Ltd.

<http://dx.doi.org/10.1016/j.sbi.2013.07.007>

Introduction

Lectins are versatile and ubiquitous proteins of non-immune origin that bind reversibly and specifically to carbohydrates. They are often multivalent which give them the well-known ability to agglutinate cells. They mediate recognition events in many biological and pathological processes: cellular signaling, differentiation, host–pathogen interactions and tissue metastasis. Lectins are highly sought proteins since they present great therapeutic and biotechnological potentials. Fungi have become a rich source for new lectins with unique carbohydrate specificities. Numerous fungal lectins have indeed been reported with 82% arising from mushrooms, 15% from microfungi (molds) and 3% from yeasts [1]. Various physiological roles have been proposed for fungal lectins even if they remain elusive in many instances (Figure 1) [2–4]. They serve as storage proteins like in plants and intervene during fungal growth, development and morphogenesis. They mediate host recognition necessary to ectomycorrhizal symbiosis [5] and association with algae or cyanobacteria in lichens [6], or yeast flocculation [7]. Fungal lectins also take part in the

defense of fungi since some present toxic activities such as insecticidal, vermifugal or antiviral, and their interaction with host glycoconjugates can be involved in the infection process of pathogenic fungi as described below.

The localization, biochemical properties and biological activities of many fungal lectins have been determined [2–4]. In contrast, very few fungal lectins have been structurally characterized or produced in a recombinant form mainly because their primary sequence is generally unknown. Structural data started to become available only ten years ago and were reviewed once in 2007 [8]. Herein, we attempt to give an overview of recent structural and functional aspects of fungal lectins and on their possible applications.

Fold and quaternary association in fungal lectins

Today only 26 different fungal lectins have been structurally characterized resulting in approximately 100 crystal structures, 40% of them being in complex with carbohydrate ligands. This represents 8.5% of all lectin structures as gathered in the Lectin 3D database (<http://lectin3d.cermav.cnrs.fr>). Ten different folds have been identified, some of them being classical lectin or carbohydrate binding modules (CBM) folds, widely spread in all living kingdom, such as β -trefoil, β -sandwich (galectin, Ig and L-lectin types) or LysM domain.

Cyanovirin-type fold is shared with cyanobacteria while 6-blade β -propeller is observed in soil bacteria, illustrating the probable exchange of genetic material between fungi and soil inhabitants. The calcium-containing β -sandwich of flocculin has only been observed in yeasts. The integrin-like 7-blade β -propeller is unique to lectins from fungi, as well as the actinoporin-like fold, also called fungal fruit body lectin fold. Representatives of the major classes are described below.

Galectins constitute a conserved family of β -galactoside-binding proteins present in vertebrates, invertebrates and in some fungi [9]. Galectins from *Agrocybe aegerita* AAL [10] and *Coprinopsis cinerea* CGL2 [11] present the prototype galectin fold which consists of a β -sandwich formed by two parallel 6 stranded antiparallel β -sheets, one of them forming a concave surface specialized in the binding of β -galactosides (Figure 2a). While prototype lectins are usually dimeric, the *C. cinerea* galectins have the particularity to be tetrameric assembling as four-leafed clover [12].

Lectins from the fruiting bodies of several fungi such as *Boletus edulis* BEL [13], *Xerocomus chrysenteron* XCL [14],

2 Protein-carbohydrate interactions

Figure 1

ROLES	ORIGIN	RESEARCH AND BIOTECHNOLOGICAL APPLICATIONS
➤ Storage proteins  ➤ Growth and morphogenesis ➤ Parasitism/infections ➤ Host recognition  ➤ Adhesion ➤ Molecular recognition ➤ Mycorrhization ➤ Lichens ➤ Defense    ➤ Cell flocculation/Mating process	Mushrooms  Microfungi  Yeast 	➤ Glycoproteins and carbohydrates purification  ➤ Glycomics studies ➤ Biomarkers ➤ Cancer research ➤ Markers and diagnosis  ➤ Immunostimulating ➤ Antiproliferative/Antitumor ➤ Antiviral  ➤ Insecticide/Vermicide  ➤ Targeted drug delivery

Current Opinion in Structural Biology

Roles and potential applications of fungal lectins.

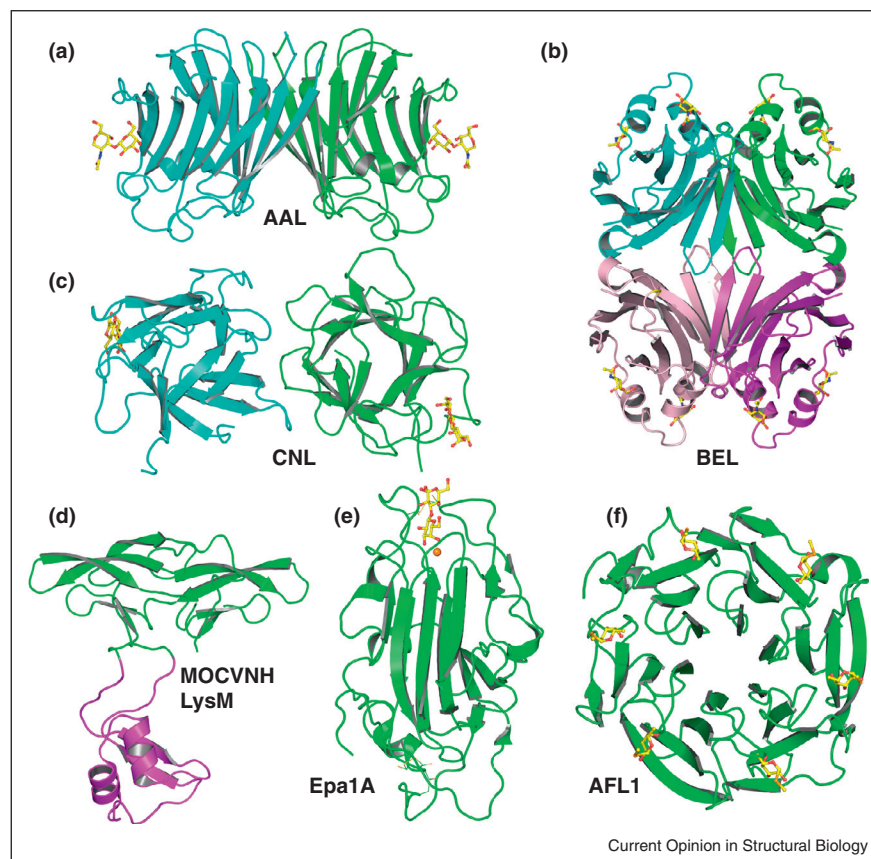
Agaricus bisporus ABL [15] and *Sclerotium rolfsii* SRL [16] present an actinoporin-like fold which consists of a β -sandwich made by two β -sheets composed of six and four β -strands respectively and connected by a helix-loop-helix motif (Figure 2b). Those lectins form dimers or tetramers with two distinct binding sites on each monomer found on each side of the helix-loop-helix motif. The primary site is specific for GalNAc while the secondary site is specific for GlcNAc.

The β -trefoil fold, or ricin-B fold, is very versatile and characterized by three subdomains α , β , and γ assembled around a pseudo-3-fold axis. A four-stranded β -sheet composes each subdomain that can present one carbohydrate-binding site (Figure 2c). The lectins from *Polyporus squamosus* PSL [17] and *Marasmius oreades* MOA [18] present an additional domain at the C-terminus involved in dimerization. The pore forming lectin from *Laetiporus sulphureus* LSL is hexameric and presents an elongated C-terminal domain belonging to the aerolysin family of β -pore-forming toxins [19]. The *Sclerotinia sclerotiorum* agglutinin SSA [20] and *Coprinopsis cinerea* lectin CCL2 [21**] are monomeric while the *Boletus edulis* lectin BEL β -trefoil [22], the *Clitocybe nebularis* lectin CNL [23] and the *Rhizoctonia solani* agglutinin RSA [24] present various dimeric associations. Presence and orientation of key residues define the functionality of the three potential carbohydrate binding sites. In the case of MOA and BEL β -trefoil, all binding sites are functional while only the site of subdomain α or γ is functional for CNL or SSA respectively.

Several fungal lectins are homologs of the cyanovirin-N (CVNH), a small viricidal lectin produced by the cyanobacterium *Nostoc ellipsosporum*. They are monomeric and mostly belong to type I CVNH with one copy of cyanovirin-N such as GzCVNH lectin from the wheat head blight fungus *Gibberella zeae* [25]. GzCVNH consists of two CVNH repeats related through a pseudo twofold symmetry referred to as domain A and B. Carbohydrate binding can occur in both domains but often only one binding site is functional in the fungal CVNHs. The structure of MOCNVH-LysM from *Magnaporthe oryzae* gave the first structural insights for type III CVNH where a LysM domain is inserted between the two CVNH repeats (Figure 2d) [26**]. The LysM domain recognizes chitin oligomers while the CVNH part binds oligomannosides in both A and B domains.

Yeast adhesins involve in flocculation like Flo5 from *Saccharomyces cerevisiae* [7] or in epithelial adhesion like Epa1p from the human pathogen *Candida glabrata* [27*,28**] present at their N-terminus a lectin domain with a β -sandwich topology that resembles the PA14 domain from the anthrax toxin protective antigen. A calcium-dependent carbohydrate-binding site is found opposite to the N and C-termini that are tethered by disulfide bridges (Figure 2e). The calcium ion is bound through a DcisD motif in a surface loop. Flo5 presents also a secondary binding site and a Flo5 subdomain, stabilized by two disulfide bridges and composed of five short β -strands that helps to discriminate oligomannosides. [7].

Figure 2



Most common folds of fungal lectins. **(a)** Galectin fold of *Agrocybe aegerita* lectin AAL [10] (PDB 3AFK). **(b)** Actinoporin-like fold of *Boletus edulis* lectin BEL [13] (PDB 3QDV). **(c)** β -Trefoil fold of *Clitocybe nebularis* ricin B-like lectin CNL [23] (PDB 3NBD). **(d)** Cyanovirin-N like fold with LysM domain of MOCVNH-LysM from *Magnetophobus oryzae* [26] (PDB 2L9Y). **(e)** Yeast adhesin fold for the epithelial adhesin 1A domain (Epa1A) from *Candida glabrata* [27] (PDB 4AF9). **(f)** β -Propeller fold of the *Aspergillus fumigatus* lectin AFL1 [29] (PDB 4AGI). Coloring as per monomer and carbohydrates are represented in balls and sticks with yellow carbon atoms.

The only other lectin from a human fungal pathogen with an available crystal structure is AFL1 from *Aspergillus fumigatus* [29]. It adopts a 6-blade β -propeller fold similar to the one of *Aleuria aurantia* lectin AAL [30] (Figure 2f). Each blade consists of four antiparallel β -strands that arranges around a pseudo sixfold axis. The β -propeller is closed as a zipper through hydrogen bonds. AAL and AFL1 are both dimeric fucose binding lectins but the dimer contacts are different. The fucose binding sites, located at the interface between two blades, present only minor variations. All six potential sites are functional in AFL1 while there are only five in AAL.

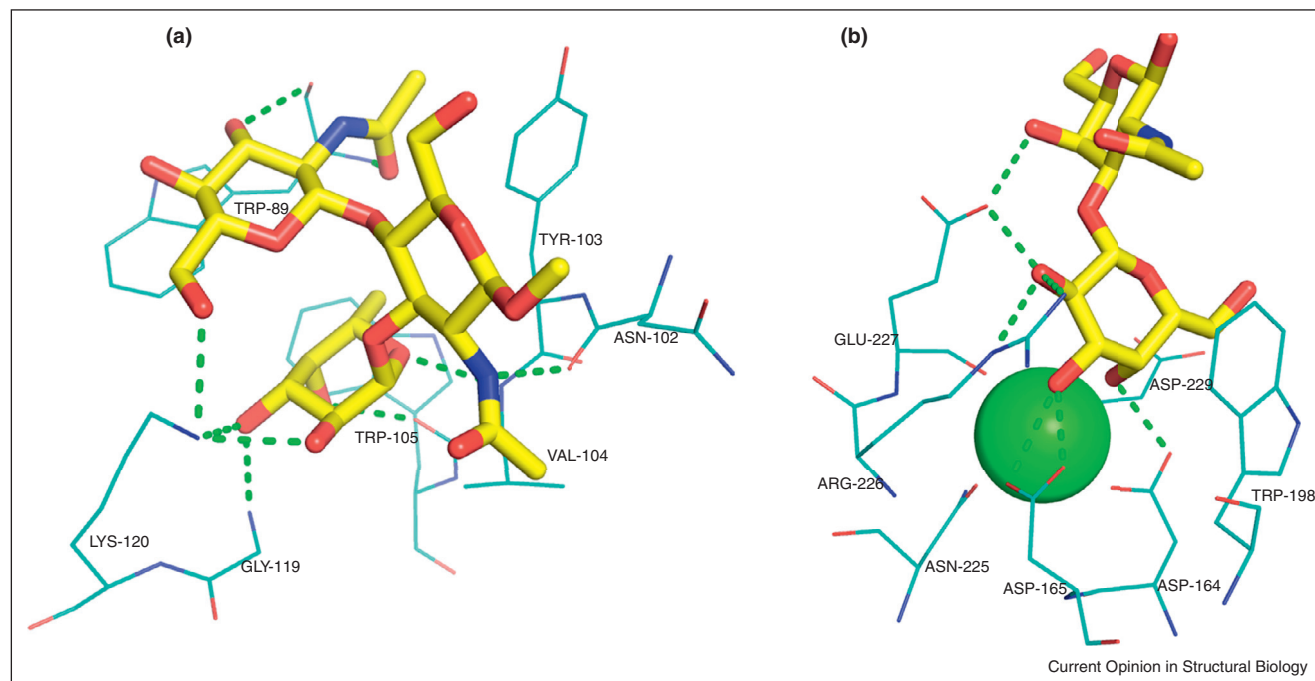
Mushroom lectins as defense proteins

Since fruiting bodies of mushroom contain a large number of toxic proteins involved in their defense against parasites and feeders, fungal lectins have been tested for their insecticidal and nematocidal properties. XCL, the actinoporin-like protein from *X. chrysenteron* is indeed toxic when incorporated in diet of *Drosophila melanogaster* [31]

and has been reported to have pest control application against the aphids *Acyrtosiphon pisum* [31] and *Myzus persicae* [32]. The β -trefoil lectins present in the sclerotes [33[•]] (compacted forms of mycelium) from *S. sclerotiorum* and *R. solani* are toxic towards the aphid *A. pisum* [34] and the cotton leafworm *Spodoptera littoralis* [33[•]], respectively. In both case, it was demonstrated that the lectin toxicity is related to binding to midgut epithelium. Toxicity may be dependent on the multivalency of the β -trefoil lectin, as demonstrated for *C. nebularis* lectin CNL that requires a dimeric state for its toxic activity towards *Caenorhabditis elegans* [23]. In the case of *M. oreades* lectin MOA, a chimerolectin with a β -trefoil domain and a cysteine-protease domain [35,36^{••}], the toxicity towards *C. elegans* necessitates both the binding to the worm glycolipids and the enzymatic activity [36^{••}]. The precise identification of the targeted glycoconjugates is very challenging, mostly because of the lack of knowledge about glycomes of invertebrates and other soil inhabitants. Very recently, the β -trefoil lectin CCL2 from

4 Protein-carbohydrate interactions

Figure 3



(a) Trisaccharide from nematode N-glycan in the binding site of *Coprinopsis cinerea* β -trefoil lectin CCL2 [21^{••}] (PDB code 2LIQ). (b) Human Gal β 1-3GalNAc mucin epitope in the binding site of *Candida glabrata* adhesin Epa1 [28^{••}] (PDB code 4ASL), with Dc1D bond between Asp164 and Asp165. Hydrogen bonds are represented as green dotted lines and calcium ion as green sphere.

C. cinerea was demonstrated to bind to the α 1,3-fucosylated N-glycan core structures of nematode N-glycans [21^{••}] and the crystal structure of the complex gave the atomic details of this novel binding site (Figure 3a). Similarly, the CGL2 galectin from the same fungus was crystallized with the Gal β 1,4Fuc α 1,6GlcNAc epitope which is another modification of *C. elegans* N-glycans core [11]. Therefore fungal lectins not only are excellent models systems for the study of self/non-self recognition but also have potential utility against helminthic infections or as pest control agents.

Fungal lectins and pathogenicity

Many fungi adopt a parasitic life style and are pathogens towards plants or animals. As observed in bacterial infections where lectins can be involved in specific recognition of host glycans [37], fungal lectins can also participate in the early stages of infection. As described above, several lectins from phytopathogenic fungi have been structurally characterized including β -trefoil lectins from *R. solani* [24] and *S. sclerotiorum* [20], actinoporin-like lectins from *S. rolfisii* [16] and *X. chrysenteron* [14] and cyanovirin-like lectin from *G. zeae* [25], but no relationship could be established with infectious process. Only a lectin of rice blast fungus *M. oryzae* with alternating CVNH and LysM domains could be linked to the early stages of plant infection [26^{••}].

In addition, several lectins from yeasts and microfungi have been shown to be directly involved in human pathogenesis. For example, paracoccin, a GlcNAc binding lectin from *Paracoccidioides brasiliensis*, binds to laminin, a component of extracellular matrix, during all stages of infection and stimulates the release of TNF- α and nitric oxide by macrophages, two compounds that are key mediators in paracoccidioidomycosis [38]. Likewise, lectins from *A. fumigatus* and *C. glabrata*, two fungi responsible for major hospital-acquired diseases, recently revealed specific binding to human oligosaccharides. AFL1 from *A. fumigatus* is localized on the surface of conidia and binds to fucosylated human blood group oligosaccharides. The lectin has a strong pro-inflammatory effect on human bronchial cells that could be inhibited by the addition of exogenous fucose [29]. *C. glabrata* pathogenic yeast contains a large number of Epa epithelial adhesins that are GPI-anchored cell wall proteins [27[•],28^{••}]. The fine differences in specificity for the N-terminal calcium dependent lectin domain of three *C. glabrata* adhesins were determined by use of glycan arrays [39]. Epa1, Epa3 and Epa6 all bind to terminal galactose with Epa1 displaying higher affinity for Gal β 1-3GalNAc ($K_d = 3.5 \mu\text{M}$) as determined by fluorescence spectroscopy. The different specificity or promiscuity for oligosaccharides could be rationalized through several complex structures of Epa1 and subtype-switched Epa

Table 1

Representative fungal lectins with known crystal structure and potential use in biotechnology. Color code as follows. Yellow disk: Gal, blue square: GlcNAc, yellow square: GalNAc pink diamond: NeuAc, red triangle: Fuc

Fungus	Lectin (fold)	Specificity	Affinity	Recomb.	Application
<i>Laetiporus sulphureus</i>	LSL (β -trefoil)		$K_d = 170 \mu\text{M}^a$ (lactose) [19]	LSL150 [44]	Used as protein tag for purification on agarose [45]
<i>Marasmius oreades</i>	MOA (β -trefoil)		$K_d < 100 \mu\text{M}^a$ (Gal α 1-3Gal) [46]	[47]	Immobilized on column for purifying xenoantigen [48]
<i>Polyporus squamosus</i>	PSL (β -trefoil)		$K_d = 1.3 \mu\text{M}^a$ (6'sialyllactose) [17]	[49]	Labeling of Neu5Ac α 2-6 on colon cancer [50]
<i>Clitocybe nebularis</i>	CBL (β -trefoil)			[51]	Antiproliferative activity [51]
<i>Sclerotium rolfsii</i>	SRL (actinoporin-like)			—	Inhibition of the growth of human colon cancer cells [52]
<i>Agaricus bisporus</i>	ABL (actinoporin-like)			—	Inhibition of cell proliferation of some ocular and cancer cell lines [53]. Increase pancreatic cell proliferation [54]
<i>Agrocybe aegerita</i>	AAA (galectin)		$K_d = 23 \mu\text{M}^b$ (3'sialyllactose) [55]	[56]	Engineering for higher selectivity for 3'sialyllactose [56]
<i>Aleuria aurantia</i>	AAL (β -propeller)		$K_d = 12 \mu\text{M}^c$ (2'fucosyllactose) [30]	[57]	Detection of fucosylated glycoconjugates/Engineering for higher selectivity for core fucose [43*]
<i>Psathyrella velutina</i>	PVL (β -propeller)		$K_d = 214 \mu\text{M}^a$ (GlcNAc) [58]	—	Detection of asialo IgG in rheumatoid arthritis [59]

^a Determined by isothermal titration calorimetry.

^b Determined by frontal affinity chromatography.

^c Determined by surface plasmon resonance.

variants engineered by mutations, demonstrating the major role of two inner loops in both calcium binding and ligand recognition [27*,28**].

Potential applications in diagnostic and biotechnology

Many fungal lectins bind with high specificity to epitopes present on human glycoconjugates. Some of these oligosaccharides are overexpressed in pathological situation, and several fungal lectins are therefore useful markers in inflammation and cancer (see Table 1). By competing with human lectins, they can also have direct effect on tumor cell proliferation, or can be used for targeted drug delivery [15]. Recently, AAL2, a GlcNAc specific lectin from *A. aegerita*, has shown antitumor activity on hepatoma cells and potential for cancer diagnosis [40]. Fungal lectins are routinely used in research laboratory for glycomics studies. They are now included in lectin microarrays that are emerging tools for high-sensitivity profiling of glycomes [41].

Examples of properties and application of fungal lectins, together with the references for production of recombinant form, are listed in Table 1. The most widely used

fungal lectin is AAL from *A. aurantia* that is able to bind all fucosylated human oligosaccharides [30]. The lectin from *Aspergillus oryzae* (AOL) is very similar in sequence and specificity but displays preference for core fucosylation, that is, the presence of a fucose α 1-6 linked to the first GlcNAc residue of N-glycans [42]. Core-fucosylation is of high interest for diagnosis of some hepatic carcinomas and this particular post-translational modification inhibits the antibody-dependent cellular cytotoxic activity of therapeutic antibodies. On the basis of structural knowledge of the binding site [30], it has been possible to engineer AAL in order to increase its selectivity for Fuc α 1-6GlcNAc motif [43*].

Conclusion

Fungal lectins present some similarity and a convergence of fold with bacterial lectins probably as a result of divergent evolution. With the latest advancements in genome sequencing and production of recombinant proteins, more fungal lectins are expected to become available. There is still a vast number of species to be analyzed with the possibility to increase the variety of structures and specificity, thus increasing the catalog of biomedical and biotechnological applications.

6 Protein-carbohydrate interactions

Acknowledgements

The authors would like to thank the French cystic fibrosis association 'Vaincre la mucoviscidose' and the Finovi foundation for financial support. The authors are thankful to CNRS and the COST actions CM1102 and BM1003 for support.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Singh RS, Bhari R, Kaur HP: **Characteristics of yeast lectins and their role in cell-cell interactions.** *Biotechnol Adv* 2011, **29**:726-731.
 2. Guillot J, Konska G: **Lectins in higher fungi.** *Biochem Syst Ecol* 1997, **25**:203-230.
 3. Khan F, Khan MI: **Fungal lectins: current molecular and biochemical perspectives.** *Int J Biol Chem* 2011, **5**:1-20.
 4. Singh RS, Bhari R, Kaur HP: **Mushroom lectins: current status and future perspectives.** *Crit Rev Biotechnol* 2010, **30**:99-126.
 5. Pujic P, Fournier P, Alloisio N, Hay AE, Marechal J, Anchisi S, Normand P: **Lectin genes in the *Frankia alni* genome.** *Arch Microbiol* 2012, **194**:47-56.
 6. Diaz EM, Vicente-Manzanares M, Sacristan M, Vicente C, Legaz ME: **Fungal lectin of *Peltigera canina* induces chemotropism of compatible Nostoc cells by constriction-relaxation pulses of cyanobiont cytoskeleton.** *Plant Signal Behav* 2011, **6**:1525-1536.
 7. Veelders M, Bruckner S, Ott D, Unverzagt C, Mosch HU, Essen LO: **Structural basis of flocculin-mediated social behavior in yeast.** *Proc Natl Acad Sci USA* 2010, **107**:22511-22516.
 8. Goldstein IJ, Winter HC: **Mushroom lectins.** In *Comprehensive Glycoscience*, vol 3. Edited by Kamerling JP. Elsevier; 2007:601-621.
 9. Vasta GR: **Galectins as pattern recognition receptors: structure, function, and evolution.** *Adv Exp Med Biol* 2012, **946**:21-36.
 10. Feng L, Sun H, Zhang Y, Li DF, Wang DC: **Structural insights into the recognition mechanism between an antitumor galectin AAL and the Thomsen-Friedenreich antigen.** *FASEB J* 2010, **24**:3861-3868.
 11. Butschi A, Titz A, Walti MA, Olieric V, Paschinger K, Nobauer K, Guo X, Seeberger PH, Wilson IB, Aeby M et al.: ***Caenorhabditis elegans* N-glycan core beta-galactoside confers sensitivity towards nematotoxic fungal galectin CGL2.** *PLoS Pathog* 2010, **6**:e1000717.
 12. Walti MA, Walser PJ, Thore S, Grunler A, Bednar M, Kunzler M, Aeby M: **Structural basis for chitotetraose coordination by CGL3, a novel galectin-related protein from *Coprinopsis cinerea*.** *J Mol Biol* 2008, **379**:146-159.
 13. Bovi M, Carrizo ME, Capaldi S, Perduca M, Chiarelli LR, Galliano M, Monaco HL: **Structure of a lectin with antitumoral properties in king bolete (*Boletus edulis*) mushrooms.** *Glycobiology* 2011, **21**:1000-1009.
 14. Birck C, Damian L, Marty-Detraves C, Lougarre A, Schulze-Briesse C, Koehl P, Fournier D, Paquereau L, Samama JP: **A new lectin family with structure similarity to actinoporins revealed by the crystal structure of *Xerocomus chrysenteron* lectin XCL.** *J Mol Biol* 2004, **344**:1409-1420.
 15. Carrizo ME, Capaldi S, Perduca M, Irazoqui FJ, Noreas GA, Monaco HL: **The antineoplastic lectin of the common edible mushroom (*Agaricus bisporus*) has two binding sites, each specific for a different configuration at a single epimeric hydroxyl.** *J Biol Chem* 2005, **280**:10614-10623.
 16. Leonidas DD, Swamy BM, Hatzopoulos GN, Gonchigar SJ, Chachadi VB, Inamdar SR, Zographos SE, Oikonomakos NG: **Structural basis for the carbohydrate recognition of the *Sclerotium rolfii* lectin.** *J Mol Biol* 2007, **368**:1145-1161.
 17. Kadirvelraj R, Grant OC, Goldstein IJ, Winter HC, Tateno H, Fadda E, Woods RJ: **Structure and binding analysis of *Polyporus squamosus* lectin in complex with the Neu5Ac[alpha]2-6Gal[beta]1-4GlcNAc human-type influenza receptor.** *Glycobiology* 2011, **21**:973-984.
 18. Grahn EM, Winter HC, Tateno H, Goldstein IJ, Krenzel U: **Structural characterization of a lectin from the mushroom *Marasmius oreades* in complex with the blood group B trisaccharide and calcium.** *J Mol Biol* 2009, **390**:457-466.
 19. Angulo I, Acebron I, de las Rivas B, Munoz R, Rodriguez-Crespo I, Menendez M, Garcia P, Tateno H, Goldstein IJ, Perez-Agote B et al.: **High-resolution structural insights on the sugar-recognition and fusion tag properties of a versatile beta-trefoil lectin domain from the mushroom *Laetiporus sulphureus*.** *Glycobiology* 2011, **21**:1349-1361.
 20. Sulzenbacher G, Roig-Zamboni V, Peumans WJ, Rouge P, Van Damme EJ, Bourne Y: **Crystal structure of the GalNAc/Gal-specific agglutinin from the phytopathogenic ascomycete *Sclerotinia sclerotiorum* reveals novel adaptation of a beta-trefoil domain.** *J Mol Biol* 2010, **400**:715-723.
 21. Schubert M, Bleuler-Martinez S, Butschi A, Walti MA, Egloff P, Stutz K, Yan S, Collot M, Mallet JM, Wilson IB et al.: **Plasticity of the beta-trefoil protein fold in the recognition and control of invertebrate predators and parasites by a fungal defence system.** *PLoS Pathog* 2012, **8**:e1002706.
- This study determines how a fungal lectin binds to a glycoepitope that is characteristic for invertebrates but absent from fungi. This oligo-saccharides, also present for example in bee venom, may be recognized as non-self by both fungal and mammalian innate immunity system.
22. Bovi M, Cenci L, Perduca M, Capaldi S, Carrizo ME, Civiero L, Chiarelli LR, Galliano M, Monaco HL: **BEL beta-trefoil. A novel lectin with antineoplastic properties in king bolete (*Boletus edulis*) mushrooms.** *Glycobiology* 2013, **23**:578-592.
 23. Pohleven J, Renko M, Magister S, Smith DF, Kunzler M, Strukelj B, Turk D, Kos J, Sabotic J: **Bivalent carbohydrate binding is required for biological activity of *Clitocybe nebularis* lectin (CNL), the N,N'-diacetyllactosidamine (GalNAc[beta]1-4GlcNAc, LacdiNAc)-specific lectin from basidiomycete *C. nebularis*.** *J Biol Chem* 2012, **287**:10602-10612.
 24. Skamnaki VT, Peumans WJ, Kantsadi AL, Cubeta MA, Plas K, Pakala S, Zographos SE, Smagghe G, Niernman WC, Van Damme EJ et al.: **Structural analysis of the *Rhizoctonia solani* agglutinin reveals a domain swapping dimeric assembly.** *FEBS J* 2013 <http://dx.doi.org/10.1111/febs.12190>.
 25. Matei E, Louis JM, Jee J, Gronenborn AM: **NMR solution structure of a cyanovirin homolog from wheat head blight fungus.** *Proteins* 2011, **79**:1538-1549.
 26. Koharudin LM, Viscomi AR, Montanini B, Kershaw MJ, Talbot NJ, Ottonello S, Gronenborn AM: **Structure-function analysis of a CVNH-LysM lectin expressed during plant infection by the rice blast fungus *Magnaporthe oryzae*.** *Structure* 2011, **19**:662-674.
- This paper presents how a LysM domain is inserted between individual repeats of a single CVNH domain resulting in a large super lectin. Distinct glycoepitopes are recognized by the LysM and CVNH domains. Over-expression in fungal cells specialized in host infection suggests role in pathogenesis.
27. Ielasi FS, Decanniere K, Willaert RG: **The epithelial adhesin 1 (Epa1p) from the human-pathogenic yeast *Candida glabrata*: structural and functional study of the carbohydrate-binding domain.** *Acta Crystallogr D Biol Crystallogr* 2012, **68**:210-217.
- The structural characterization of Epa1p has demonstrated similarity with *S. cerevisiae* flocculins. The higher affinity for carbohydrate ligands observed for this adhesin is explained as a result of differences in the binding site.
28. Maestre-Reyna M, Diderrich R, Veelders MS, Eulenburg G, Kalugin V, Bruckner S, Keller P, Rupp S, Mosch HU, Essen LO: **Structural basis for promiscuity and specificity during *Candida glabrata* invasion of host epithelia.** *Proc Natl Acad Sci USA* 2012, **109**:16864-16869.
- The Epa epithelial adhesin presents many variants with different specificities towards mucin O-glycans. The construction of chimeric Epa1

variants and their structure determination led to establish the role of several loops in glycan specificities.

29. Houser J, Komarek J, Kostlanova N, Cioci G, Imberty A, Wimmerova M: **Protein oligomerization in *Aleuria aurantia* lectin family – importance and difficulties.** *Mater Struct Chem Biol Phys Technol* 2012, **12**:20-21.
30. Wimmerova M, Mitchell E, Sanchez JF, Gautier C, Imberty A: **Crystal structure of fungal lectin: six-bladed b-propeller fold and novel recognition mode for *Aleuria aurantia* lectin.** *J Biol Chem* 2003, **278**:27059-27067.
31. Trigueros V, Lougarre A, Ali-Ahmed D, Rahbe Y, Guillot J, Chavant L, Fournier D, Paquereau L: ***Xerocomus chrysenteron* lectin: identification of a new pesticidal protein.** *Biochim Biophys Acta* 2003, **1621**:292-298.
32. Jaber K, Cuartero Diaz G, Haubruge E, Francis F: **Investigation of carbohydrate binding property of a fungal lectin from *Xerocomus chrysenteron* and potential use on *Myzus persicae* aphid.** *Commun Agric Appl Biol Sci* 2008, **73**:629-638.
33. Hamshou M, Van Damme EJ, Caccia S, Cappelle K, Vandenborre G, Ghesquiere B, Gevaert K, Smaghe G: **High entomotoxicity and mechanism of the fungal GalNAc/Gal-specific *Rhizoctonia solani* lectin in pest insects.** *J Insect Physiol* 2013, **59**:295-305.
- This paper shows that the toxicity of RSA towards insects is correlated to strong binding to midgut tissues. A caspase-dependent apoptotic mechanism is proposed from *in vitro* assays using insect midgut CF-203 cells.
34. Hamshou M, Smaghe G, Shahidi-Noghabi S, De Geyter E, Lannoo N, Van Damme EJ: **Insecticidal properties of *Sclerotinia sclerotiorum* agglutinin and its interaction with insect tissues and cells.** *Insect Biochem Mol Biol* 2010, **40**:883-890.
35. Cordara G, Egge-Jacobsen W, Johansen HT, Winter HC, Goldstein IJ, Sandvig K, Krengel U: ***Marasmius oreades* agglutinin (MOA) is a chimerolectin with proteolytic activity.** *Biochem Biophys Res Commun* 2011, **408**:405-410.
36. Wohlschlagel T, Butschli A, Zurluh K, Vonesch SC, auf dem Keller U, Gehrig P, Bleuler-Martinez S, Hengartner MO, Aebi M, Kunzler M: **Nematotoxicity of *Marasmius oreades* agglutinin (MOA) depends on glycolipid binding and cysteine protease activity.** *J Biol Chem* 2011, **286**:30337-30343.
- The fairy ring mushroom lectin consists of an N-terminal ricin B-type lectin domain and a C-terminal dimerization domain that is demonstrated to have sequence and function similarity to proteases and both domains are required for defense against predators.
37. Imberty A, Varrot A: **Microbial recognition of human cell surface glycoconjugates.** *Curr Opin Struct Biol* 2008, **18**:567-576.
38. Coltri KC, Casabona-Fortunato AS, Gennari-Cardoso ML, Pinzan CF, Ruas LP, Mariano VS, Martinez R, Rosa JC, Panunto-Castelo A, Roque-Barreira MC: **Paracoccin, a GlcNAc-binding lectin from *Paracoccidioides brasiliensis*, binds to laminin and induces TNF-alpha production by macrophages.** *Microbes Infect* 2006, **8**:704-713.
39. Zupancic ML, Frieman M, Smith D, Alvarez RA, Cummings RD, Cormack BP: **Glycan microarray analysis of *Candida glabrata* adhesin ligand specificity.** *Mol Microbiol* 2008, **68**:547-559.
40. Jiang S, Chen Y, Wang M, Yin Y, Pan Y, Gu B, Yu G, Li Y, Wong BH, Liang Y et al.: **A novel lectin from *Agrocybe aegerita* shows high binding selectivity for terminal N-acetylglucosamine.** *Biochem J* 2012, **443**:369-378.
41. Hirabayashi J, Yamada M, Kuno A, Tateno H: **Lectin microarrays: concept, principle and applications.** *Chem Soc Rev* 2013, **21**:4443-4458.
42. Matsumura K, Higashida K, Ishida H, Hata Y, Yamamoto K, Shigeta M, Mizuno-Horikawa Y, Wang X, Miyoshi E, Gu J et al.: **Carbohydrate binding specificity of a fucose-specific lectin from *Aspergillus oryzae*: a novel probe for core fucose.** *J Biol Chem* 2007, **282**:15700-15708.
43. Romano PR, Mackay A, Vong M, DeSa J, Lamontagne A, Comunale MA, Hafner J, Block T, Lec R, Mehta A: **Development of recombinant *Aleuria aurantia* lectins with altered binding specificities to fucosylated glycans.** *Biochem Biophys Res Commun* 2011, **414**:84-89.
- A directed mutagenesis approach resulted in a set of recombinant AAL proteins that displayed altered binding affinities. One mutant revealed higher specificity for 6-linked core fucosylation.
44. Tateno H, Goldstein IJ: **Molecular cloning, expression, and characterization of novel hemolytic lectins from the mushroom *Laetiporus sulphureus*, which show homology to bacterial toxins.** *J Biol Chem* 2003, **278**:40455-40463.
45. Lopez-Gallego F, Acebron I, Mancheno JM, Raja S, Lillo MP, Guisan Seijas JM: **Directed, strong, and reversible immobilization of proteins tagged with a beta-trefoil lectin domain: a simple method to immobilize biomolecules on plain agarose matrixes.** *Bioconjug Chem* 2012, **23**:565-573.
46. Winter HC, Mostafapour K, Goldstein IJ: **The mushroom *Marasmius oreades* lectin is a blood group type B agglutinin that recognizes the Gal alpha 1,3Gal and Galalpha 1,3Galbeta 1,4GlcNAc porcine xenotransplantation epitopes with high affinity.** *J Biol Chem* 2002, **277**:14996-15001.
47. Kruger RP, Winter HC, Simonson-Leff N, Stuckey JA, Goldstein IJ, Dixon JE: **Cloning, expression, and characterization of the Gal alpha 1,3Gal high affinity lectin from the mushroom *Marasmius oreades*.** *J Biol Chem* 2002, **277**:15002-15005.
48. Loganathan D, Winter HC, Judd WJ, Petryniak J, Goldstein IJ: **Immobilized *Marasmius oreades* agglutinin: use for binding and isolation of glycoproteins containing the xenotransplantation or human type B epitopes.** *Glycobiology* 2003, **13**:955-960.
49. Tateno H, Winter HC, Goldstein IJ: **Cloning, expression in *Escherichia coli* and characterization of the recombinant Neu5Acalpha2,6Galbeta1,4GlcNAc-specific high-affinity lectin and its mutants from the mushroom *Polyporus squamosus*.** *Biochem J* 2004, **382**:667-675.
50. Toma V, Zuber C, Winter HC, Goldstein IJ, Roth J: **Application of a lectin from the mushroom *Polyporus squamosus* for the histochemical detection of the NeuAcalpha2,6Galbeta1,4GlcNAc sequence of N-linked oligosaccharides: a comparison with the *Sambucus nigra* lectin.** *Histochem Cell Biol* 2001, **116**:183-193.
51. Pohleven J, Obermajer N, Sabotic J, Anzlovar S, Sepcic K, Kos J, Kralj B, Strukelj B, Brzin J: **Purification, characterization and cloning of a ricin B-like lectin from mushroom *Clitocybe nebularis* with antiproliferative activity against human leukemic T cells.** *Biochim Biophys Acta* 2009, **1790**:173-181.
52. Inamdar SR, Savanur MA, Eligar SM, Chachadi VB, Nagre NN, Chen C, Barclays M, Ingle A, Mahajan P, Borges A et al.: **The TF-antigen binding lectin from *Sclerotium rolsii* inhibits growth of human colon cancer cells by inducing apoptosis *in vitro* and suppresses tumor growth *in vivo*.** *Glycobiology* 2012, **22**:1227-1235.
53. Yu LG, Fernig DG, White MR, Spiller DG, Appleton P, Evans RC, Grierson I, Smith JA, Davies H, Gerasimenko OV et al.: **Edible mushroom (*Agaricus bisporus*) lectin, which reversibly inhibits epithelial cell proliferation, blocks nuclear localization sequence-dependent nuclear protein import.** *J Biol Chem* 1999, **274**:4890-4899.
54. Wang Y, Liu Y, Wang H, Li C, Qi P, Bao J: ***Agaricus bisporus* lectins mediates islet beta-cell proliferation through regulation of cell cycle proteins.** *Exp Biol Med* 2012, **237**:287-296.
55. Hirabayashi J, Hashidate T, Arata Y, Nishi N, Nakamura T, Hirashima M, Urashima T, Oka T, Futai M, Muller WE et al.: **Oligosaccharide specificity of galectins: a search by frontal affinity chromatography.** *Biochim Biophys Acta* 2002, **1572**:232-254.
56. Imamura K, Takeuchi H, Yabe R, Tateno H, Hirabayashi J: **Engineering of the glycan-binding specificity of *Agrocybe cylindracea* galectin towards alpha(2,3)-linked sialic acid by saturation mutagenesis.** *J Biochem* 2011, **150**:545-552.
- Saturation mutagenesis on one position permitted the identification of a mutant that presented strong specificity for 3' sialylactose with loss of the galectin classical affinity for galactose-terminating glycoconjugates.

8 Protein-carbohydrate interactions

57. Fukumori F, Takeuchi N, Hagiwara T, Ito K, Kochibe N, Kobata A, Nagata Y: **Cloning and expression of a functional fucose-specific lectin from an orange peel mushroom *Aleuria aurantia*. *FEBS Lett* 1989, **250**:153-156.**
58. Cioci G, Mitchell EP, Chazalet V, Debray H, Oscarson S, Lahmann M, Gautier C, Breton C, Pérez S, Imberty A: **b-Propeller crystal structure of *Psathyrella velutina* lectin: an integrin-like fungal protein interacting with monosaccharides and calcium. *J Mol Biol* 2006, **357**:1575-1591.**
59. Liljeblad M, Lundblad A, Pahlsson P: **Analysis of agalacto-IgG in rheumatoid arthritis using surface plasmon resonance. *Glycoconj J* 2000, **17**:323-329.**