LectomeXplore, an update of UniLectin for the discovery of carbohydrate-binding proteins based on a new lectin classification

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ABSTRACT

Lectins are non-covalent glycan-binding proteins mediating cellular interactions but their annotation in newly sequenced organisms is lacking. The limited size of functional domains and the low level of sequence similarity challenge usual bioinformatics tools. The identification of lectin domains in proteomes requires the manual curation of sequence alignments based on structural folds. A new lectin classification is proposed. It is built on three levels: (i) 35 lectin domain folds, (ii) 109 classes of lectins sharing at least 20% sequence similarity and (iii) 350 families of lectins sharing at least 70% sequence similarity. This information is compiled in the UniLectin platform that includes the previously described UniLectin3D database of curated lectin 3D structures. Since its first release, UniLectin3D has been updated with 485 additional 3D structures. The database is now complemented by two additional modules: PropLec containing predicted propeller lectins and LectomeXplore including predicted lectins from sequences of the NBCI-nr and UniProt for every curated lectin class. UniLectin is accessible at https://www.unilectin.eu/

INTRODUCTION

Glycans, present as part of glycoconjugates at the surface of all living cells, are involved in cellular communication and a range of biological processes. Carbohydrates are therefore considered as ‘the third alphabet of life’, with information encoded in their composition, their complex structure and their dynamics (1). Lectins, are proteins able to decipher the so-called glycome, they are defined by their functions since they contain one or more non-catalytic domains able to specifically bind mono or oligosaccharides (2). Glycan–lectin interactions play a crucial role in many biological processes, and are considered as hot-spots for therapeutic strategies (3). Lectins are also used for biotechnology and diagnostic: they are able to decipher fine differences in glyco-phenotypes of tissues, giving therefore crucial information for personalized medicine (4). Lectins occur in all branches of the living kingdoms, and the variety of their origins and folds challenges their classification. Previous attempts to classify lectins upon their origin or specificity do not reflect the subtle functional differences and versatility of these proteins (2). Among the recent suggestions, a grouping into 48 families based on fold and Pfam families (5) was detailed (6).

Glycobioinformatics databases and tools are increasingly being developed as described in recent reviews (7,8). Yet, only a limited number are devoted to support the study of lectins and their interactions with glycoconjugates. SugarBindDB describes adhesins and glyco-targeted toxins of microbial pathogens (9). The Lectin Frontier Database (LfDB) contains affinity data based on frontal affinity chromatography (10). The lectin pages in GlyCosmos (11) list protein entries annotated as lectins in UniProt. Information on lectins are also available from databases with a broader view on protein-carbohydrate interactions, such as ProCarbDB, a database of carbohydrate-binding proteins (12), the Database of Anti-Glycan Reagents (DAGR) that covers glycan-targeted antibodies and lectins as reagents (13), and the carbohydrate binding modules (CBM) pages in CAZy (14). The complete list of resources is listed in Supplementary Table S1.

The UniLectin portal was created in 2018, with the UniLectin3D module dedicated to lectin 3D structures and lectin/glycan complexes (15,16) and providing 1740 struc-
tutes covering 428 different lectins and 765 references (as of 29 August 2018). UniLectin3D was confirmed as the main source of information on 3D structures of lectins and their interactions with ligands, as pointed in recent glycomics reviews (7,17). It has also been recognized as a resource complementing new tools for determining the specificity of proteins (12,18), as well as for analyzing glycosylation patterns in personalized glycomedicine (4,19,20).

In order to extend UniLectin, the first focus was on lectins containing tandem repeats and resulted in launching the PropLec module, for the identification of β-propeller lectin candidates accurate on every blade composing the propeller. In PropLec, candidate lectins were identified from conserved motifs distinguishing six β-propeller lectin classes. The November 2019 UniProt release was predicted to contain 4877 β-propeller lectins. The prediction was validated by the identification and structural characterization of a novel type of propeller assembled by dimerisation of 3-blade repeat (21).

The latest module named LectomeXplore describes candidate lectins identified in available proteomes from all kingdoms and for all available lectin classes. These predictions are not provided in other lectin-related databases. Both PropLec and LectomeXplore are based on screening the UniProt (Swiss-Prot + TrEMBL) and NCBI-nr (non-redundant) protein databases to identify lectin candidates. The LectomeXplore module is based on a new lectin classification that was built from a collection of conserved motifs. This classification is composed of 109 lectin classes derived from 35 distinct folds detailed in (22). Each class is defined with a Hidden Markov Model (HMM) sequence profile generated from a manually curated protein alignment with HMMER-hmmbuild (23). Screening was performed with the HMMER-hmmsearch followed by post processing described further in this article and in (22). The new LectomeXplore module is introduced here in detail and illustrated with examples.

UPDATE OF THE UNILECTIN PORTAL AND NEW CLASSIFICATION OF LECTIN STRUCTURES

The UniLectin3D module now includes 2225 3D-structures from 535 different lectins, corresponding to an increase of more than 25% in the last 2 years. Searching was improved by simplifying the graphical interface and adding a 'glycan search' to query the database by specificity, i.e., by monosaccharides and oligosaccharides bound to the lectin structures. The protein topology provided by the PDBe (24) is now available. External links to the full glycan structures covering 428 different lectins and 765 references (as of 29 August 2018). UniLectin3D was confirmed as the main source of information on 3D structures of lectins and their interactions with ligands, as pointed in recent glycomics reviews (7,17). It has also been recognized as a resource complementing new tools for determining the specificity of proteins (12,18), as well as for analyzing glycosylation patterns in personalized glycomedicine (4,19,20).

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CONSERVED MOTIFS AND CONSTRUCTION OF THE LECTOMEEXPLORE MODULE

Within each class, the lectin sequences were aligned with the Muscle software (30). Sequence redundancy was automatically discarded. The conserved regions resulting from the multiple alignments were then fed to a Hidden Markov tool with the HMMER-hmmbuild tool (31) to generate characteristic profiles of each lectin class, using default parameters except a symfrac set at 0.8. An HMM sequence profile is a probabilistic model which encodes amino acid conserva-
Figure 1. Hierarchical tree of the UniLectin3D lectin classification based on both sequence and structure. The tree is expanded for the Shiga toxin, F-type and hevein-like lectin classes in order to exemplify the variety of classes and families.

Figure 2. Distribution of curated versus predicted lectin folds across the kingdoms of life. Red circles represent the presence of reference 3D structures, blue circles represent the predicted lectins. In white are represented the fold not yet discovered for the corresponding kingdom.

tion at each position in a defined region. A typical example is displayed in Supplementary Figure S2, representing the conserved motif of the F-type lectin class.

Using these 109 profiles, potential lectin sequences were searched in UniProtKB (UniProt April 2019) (32) and NCBI-nr (non-redundant July 2019) (33). Protein datasets were screened with all HMM profiles using HMMER-hmmsearch, with default parameters and a $P$-value below $10^{-2}$. The probabilities of substitution of each amino acid by any other are stored in the BLOSUM62 substitution score matrix that was used for the generation of the profiles (Supplementary Table S1: databases and tools). Further filtering was applied to the cases of very similar sequences (multiple strains of the same species, natural mutation, sequencing errors, ...). A post-processing step led to identify a single representative whenever proteins of the same
species have identical 100 consecutive amino acids. Domains shorter than 10 amino acids were also filtered out. An HMMER bit score is generated for each predicted lectin. Because each family profile is generated independently of one another, the score values are not comparable across motifs used in the prediction. As a result, it is impossible to use a single cut-off value for all lectin classes. For the sake of simplicity, LectomeXplore provides a score easy to understand (between 0 and 1). The LectomeXplore normalized prediction/similarity score reflects the similarity between a prediction and the matched profiles. It is generated to rank the results and select the top one, as previously described in (22).

OVERVIEW OF THE LECTOMEXPLORE MODULE

LectomeXplore supports multiple ways to explore the predicted lectins with entries based on the taxonomy, the lectin class, the protein name and other identified functional domains by Pfam. The LectomeXplore homepage provides an overview of the predicted lectins with a score over 0.5. Expanding the search with the use of lower score is possible via the advanced search page, which provides a broad set of criteria. A tutorial is available at https://unilectin.eu/predict/tutorial.

The distribution of the predicted lectins (with a score >0.5) spans 44186 proteins in Eukaryotes, 31 589 in Metazoa, 7780 in Viridiplantae, 4081 in Fungi, 8180 proteins in Viruses (including 4044 in Influenza) and 6943 proteins in Bacteria. In comparison, the NCBI-nr protein datasets contain 16 million proteins in Eukaryotes, 9 million in Metazoa, 8 million in Viridiplantae, 10 million in Fungi, 1 million proteins in Viruses and 116 million proteins in Bacteria. Some species, such as Escherichia coli, cover a large number of sequenced strains with similar proteins and after removal of the redundant protein sequences, 2076 predicted lectins remain in E. coli (based on 20922 strains of E. coli in the NCBI-nr dataset). Interestingly, even with a high score threshold most of the predicted lectins are annotated as Uncharacterized proteins (4403/59424 for a score of 0.5). Two classes of predicted lectins were very populated due to the high number of false positives, i.e. proteins wrongly identified as lectin candidates. TIM lectins, that are closely related to glycosylhydrolases, and Variable Lymphocyte Receptor (VLR), that are adaptive immune receptors from jawless fishes able to bind to a large number of antigens, were then excluded from whole proteome predictions. In other words, only 107 classes are used in all predictions.

LectomeXplore was assessed using the UniProt/SwissProt curated protein dataset. Among 622 reviewed proteins with ‘lectin’ in their name, 86 were not predicted, which is expected since the motifs cover only lectins with known 3D-structures. In contrast, only 50 Galactose-related enzymes
Figure 4. Ficolin-like candidate lectin as represented in LectomeXplore, from the species *Dendronephthya gigantea*. Top: Panel with the main information on the protein. Middle: Panel with the lectin domain aligned with the reference consensus sequence. Bottom: the amino acids involved in glycan-binding in the reference PDB structure.

EXAMPLE OF LECTOME EXPLORATION

The lectome of any organism with a complete proteome can be investigated and compared to other lectomes. Considering species with top numbers of predicted lectins (Supplementary Table S4), many aquatic organisms, fishes and molluscs, are listed and appear to produce at least 20 lectins. Among them, cnidarians, such as sea anemones and corals, have large lectomes that have been shown to be involved in innate immunity (34). Indeed, these immobilised marine animals filter large amounts of sea water and use lectins for aggregating bacteria. Interestingly, large panels of different sugars can be recognized by the lectome of these invertebrates, probably linked to the ability to bind a variety of different bacteria.

As an example, Figure 3 displays a selection of lectins among the 24 predicted to occur in the genome of the soft coral *Dendronephthya gigantea* described in Supplementary Table S5. The prediction also includes widely distributed lectins, such as calnexin, calreticulin and malectin, involved in the quality control during the biosynthesis of glycoproteins. Otherwise, lectins with high score and well conserved carbohydrate binding sites, spread over nine different classes, corresponding to proteins classical involved in innate immunity (Figure 3). It should be noted that only 50% of them are correctly annotated. Some of the identified classes are widely distributed in the animal kingdom, such as ficolins or C-type lectins. F-lectins consist of assembly of...
fucose-binding domains present in animals and in bacteria (26). On the other hand, some classes are specific to invertebrates, such as oyster lectins or sea anemone lectins and were identified only recently (35–37).

The LectomeXplore interface gives access to the identification of predicted lectins. For each predicted sequence, the alignment with the reference consensus sequence (generated by HMMER) of the class is displayed when clicking on the arrow in the top right corner of the summary box as shown in Figure 4. This view brings out the quality of the sequence similarity and highlights the amino acids involved in binding sugars below the alignment.

In the analysis of the *Dendronephthya gigantea* lectome, the ficolin-1 class is predicted with a strong score of 0.665. Several proteins are predicted to belong to this lectin class, some of them being annotated as ficolin-2, and others as ryncolin-1-like (Supplementary Table S5), due to their similarity to these snake venom proteins. M-Ficolins bind to acetylated sialic acid on pathogen-associated molecular patterns (PAMPs) (38). The very high conservation in the binding site in the coral ‘yncolin-1-like’ protein, in particular for basic amino acids, indicates that the sialic acid binding function is likely to be maintained in the coral protein. This example illustrates how LectomeXplore can reveal novel carbohydrate-binding activity in organisms.

**DISCUSSION AND CONCLUSION**

The gradual increase of structural data in UniLectin3D was key to determining relevant criteria to classify these proteins. In turn, the resulting classification revealed an underlying order that played a critical role in profiling lectins and support their detection in very large amino acid sequence collections. We surmise that our broad screening strategy will lead not only to consolidate the definition of the chosen criteria but also to improve coverage with the potential creation of new classes or families. The latter could arise from the release of new lectin structures in the PDB. If this classification is adopted by the community, it can serve as a basis for a nomenclature. An option would be to make this information more accessible through an ontology and semantic web technologies (39). But first, we aim at contributing to the annotation of putative lectins in protein sequence databases.

The search tool of the LectomeXplore module is versatile. The occurrence of a given lectin class can be searched in all kingdoms. All lectins present in one species can be tile. The occurrence of a given lectin class can be searched in different databases. According to the annotation of putative lectins in protein sequence databases, the ficolin-1 class is predicted with a strong score of 0.665. Several proteins are predicted to belong to this lectin class, some of them being annotated as ficolin-2, and others as ryncolin-1-like (Supplementary Table S5), due to their similarity to these snake venom proteins. M-Ficolins bind to acetylated sialic acid on pathogen-associated molecular patterns (PAMPs) (38). The very high conservation in the binding site in the coral ‘yncolin-1-like’ protein, in particular for basic amino acids, indicates that the sialic acid binding function is likely to be maintained in the coral protein. This example illustrates how LectomeXplore can reveal novel carbohydrate-binding activity in organisms.

**SUPPLEMENTARY DATA**

Supplementary Data are available at NAR Online.

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**REFERENCES**

1. Kaltner, H., Abad-Rodríguez, J., Corfield, A.P., Kopitz, J. and Gabius, H.-J. (2019) The sugar code: letters and vocabulary, writers, editors and readers and biosignificance of functional glycane–lectin pairing. *Biochem. J.*, 476, 2623–2655.
2. Sharon, N. and Lis, H. (1989) Lectins as cell recognition molecules. *Science*, 246, 227–234.
3. Valverde, P., Ardá, A., Reichardt, N.-C., Jiménez-Barbero, J. and Gimeno, A. (2019) Glycans in drug discovery. *Med. Chem. Commun.*, 10, 1678–1691.
4. Gourdin, J.-P.F., Brush, M.H., Vasilievsky, N.A., Shefchek, K., Köhler, S., Matentzoglu, N., Munoz-Torres, M.C., McMurry, J.A., Zhang, X.A., Robinson, P.N. *et al.* (2019) Representing glycoconjugates: semantic unification of glycobiology resources for disease discovery. *Database*, 2019, baq114.
5. El-Gebali, S., Mistry, J., Bateman, A., Eddy, S.R., Luciani, A., Potter, S.C., Qureshi, M., Richardson, L.J., Salazar, G.A., Smart, A. *et al.* (2019) The Pfam protein families database in 2019. *Nucleic Acids Res.*, 47, D427–D432.
6. Fujimoto, Z., Tateno, H. and Hirabayashi, J. (2014) Lectin Structures: Classification Based on the 3-D Structures. In: Hirabayashi, J. (ed). *Lectins, Methods in Molecular Biology*. Springer, NY, Vol. 1200, pp. 579–606.
7. Abrahams, J. L., Taherzadeh, G., Jarvis, G., Guttmann, A., Zhou, Y. and Campbell, M. P. (2020) Recent advances in glycoinformatic platforms for glycomics and glycoproteomics. *Curr. Opin. Struct. Biol.*, 62, 56–69.

8. Aoki-Kinoshita, K. F., Lisacek, F., Mazumder, R., York, W. S. and Packer, N. H. (2020) The GlySpace Alliance: toward a collaborative global glycoinformatics community. *Glycobiology*, 30, 70–71.

9. Mariezhko, J., Khatib, K., Aloci, D., Campbell, M. P., Karlish, N. G., Packer, N. H., Mullen, E. H. and Lisacek, F. (2016) SugarBindDB, a resource of glycan-mediated host–pathogen interactions. *Nucleic Acids Res.*, 44, D1243–D1250.

10. Hirabayashi, J., Tateno, H., Shikanai, T., Aoki-Kinoshita, K. and Narimatsu, H. (2015) The Lectin Frontier Database (LiDB), and data generation based on frontal affinity chromatography. *Molecules*, 20, 951–973.

11. Yamada, I., Shiota, M., Shinmachi, D., Ono, T., Tsuchiya, S., Hosoda, M., Fujita, A., Aoki, N. P., Watanabe, Y., Fujita, N. et al. (2020) The GlyCosmos Portal: a unified and comprehensive web resource for the glycosciences. *Nat. Methods*, 17, 649–650.

12. Copotu, L., Torres, P. H. M., Ascher, D. B., Blundell, T. L. and Malhotra, S. (2020) ProCarDB: a database of carbohydrate-binding proteins. *Nucleic Acids Res.*, 48, D368–D375.

13. Sterner, E., Flanagan, N. and Gildersleeve, J. C. (2016) Perspectives on anti-glycan antibodies gleaned from development of a community resource database. *ACS Chem. Biol.*, 11, 1773–1783.

14. Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P. M. and Henrissat, B. (2014) The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res.*, 42, D490–D495.

15. Bonnardel, F., Mariezhko, J., Saltentin, S., Robin, X., Schroeder, M., Perez, S., Lisacek, F. and Imberty, A. (2019) UniLectin3D, a database of carbohydrate binding proteins with curated information on 3D structures and interacting ligands. *Nucleic Acids Res.*, 47, D1236–D1244.

16. Bonnardel, F., Perez, S., Lisacek, F. and Imberty, A. (2020) Structural database for lectins and the UniLectin web platform. In: Hirabayashi, J. (ed.) *Lectin Purification and Analysis. Methods in Molecular Biology*. Springer US, NY, Vol. 2132, pp. 1–14.

17. Cao, W.-Q., Liu, M.-Q., Kong, S.-Y., Wu, M.-X., Huang, Z.-Z. and Yang, P.-Y. (2020) Novel methods in glycemics: a 2019 update. *Expert Rev. Proteomics*, 17, 11–25.

18. Haab, B. B. and Klamer, Z. (2020) Advances in tools to determine the glycan-binding specificities of lectins and antibodies. *Mol. Cell Proteomics*, 19, 224–232.

19. Kunet, T. (2019) Rise of systems glyciobiology and personalized glycomedicine: why and how to integrate glycomics with multiomics science? *OMICS*, 23, 615–622.

20. Silva, M. L. S. (2019) Lectin biosensors in cancer glycan biomarker detection. In: *Advances in Clinical Chemistry*. Elsevier, Vol. 93, pp. 1–61.

21. Bonnardel, F., Kumar, A., Wimmerova, M., Lahmann, M., Perez, S., Varrot, A., Lisacek, F. and Imberty, A. (2019) Architecture and evolution of blade assembly in β-propeller lectins. *Structure*, 27, 764–775.e3.

22. Bonnardel, F., Haslam, S. M., Dell, A., Feizi, T., Liu, Y., Tajadura-Ortega, V., Akune, Y., Sykes, L., Bennett, P. R., MacIntyre, D. A. *et al.* (2020) Proteome-wide prediction of bacterial carbohydrate-binding proteins as a tool for understanding commensal and pathogen colonisation of the vaginal microbiome. bioRxiv doi: https://doi.org/10.1101/2020.09.10.291781, 18 September 2020, preprint: not peer reviewed.

23. Eddy, S. R. (2011) Accelerated profile HMM searches. *PLoS Comput. Biol.*, 7, e1002195.

24. Armstrong, D. R., Berrisford, I. M., Conroy, M. J., Gutmanas, A., Anyango, S., Choudhary, P., Clark, A. R., Dana, J. M., Deshpande, M., Dunlop, R. *et al.* (2020) PDBe: improved findability of macromolecular structure data in the PDB. *Nucleic Acids Res.*, 48, D335–D343.

25. Aloci, D., Mariethoz, J., Gastaldello, A., Gasteiger, E., Karlish, N. G., Kolarich, D., Packer, N. H. and Lisacek, F. (2019) GlyConnect: glycoproteomics goes visual, interactive, and analytical. *J. Proteome Res.*, 18, 664–677.

26. Vasta, G. R., Amzel, L. M., Blanchet, M. A., Cammarata, M., Feng, C. and Saito, K. (2017) F-Type lectins: a highly diversified family of fucose-binding proteins with a unique sequence motif and structural fold, involved in self/non-self-recognition. *Front. Immunol.*, 8, 1648.

27. Sillio, L., Dawson, N., Lewis, T. E., Das, S., Lees, J. G., Ashford, P., Tolulope, A., Scholes, H. M., Senatorov, I., Buja, A. *et al.* (2019) CATH: expanding the horizons of structure-based functional annotations for genome sequences. *Nucleic Acids Res.*, 47, D280–D284.

28. Hirabayashi, J. and Ari, R. (2019) Lectin engineering: the possible and the actual. *Interface Focus*, 9, 20180068.

29. Chandaoni, J.-M., Fox, N. K. and Brenner, S. E. (2017) SCOPe: manual curation and artifact removal in the structural classification of proteins – extended database. *J. Mol. Biol.*, 429, 348–355.

30. Edgar, R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.*, 32, 1792–1797.

31. Potter, S. C., Luciani, A., Eddy, S. R., Park, V., Lopez, R. and Finn, R. D. (2018) HMMER web server: 2018 update. *Nucleic Acids Res.*, 46, W200–W204.

32. The UniProt Consortium (2019) UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res.*, 47, D100–D105.

33. O’Leary, N. A., Wright, M. W., Brister, J. R., Ciufol, S., Haddad, D., MeVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D. *et al.* (2016) Reference sequence (RefSeq) database at NCBI: current status, taxonomy expansion, and functional annotation. *Nucleic Acids Res.*, 44, D733–D745.

34. Vasta, G. R., Ahmed, H. and Odom, E. W. (2004) Structural and functional diversity of lectin repertoires in invertebrates, protocohetes and ectothermic vertebrates. *Curr. Opin. Struct. Biol.*, 14, 617–630.

35. Unno, H., Matsuyama, K., Tsuji, Y., Goda, S., Hiemori, K., Tateno, H., Hirabayashi, J. and Hatakeyama, T. (2016) Identification, characterization and X-ray crystallographic analysis of a novel type of mannose-specific lectin CGL1 from the Pacific Oyster Crassostrea gigas. *Sci. Rep.*, 6, 29135.

36. Unno, H., Nakamura, A., Mori, S., Goda, S., Yamaguchi, K., Hiemori, K., Tateno, H. and Hatakeyama, Y. (2018) Identification, characterization, and X-ray crystallographic analysis of a novel type of lectin AJLec from the sea anemone Anthopleura japonica. *Sci. Rep.*, 8, 11516.

37. Jiang, S., Wang, L., Huang, M., Jia, Z., Weinert, T., Warkentin, E., Liu, C., Song, X., Zhang, H., Witt, J. *et al.* (2017) DM9 domain containing protein functions as a pattern recognition receptor with broad microbial recognition spectrum. *Front. Immunol.*, 8, 1607.

38. Gout, E., Garlatti, V., Smith, D. F., Lacroix, M., Dumeust-Pétrard, C., Lunardi, T., Martin, L., Cebron, J.-Y., Arlaud, G. J., Gaboriaud, C. *et al.* (2010) Carbohydrate recognition properties of human ficolins: glysan array screening reveals the sialic acid binding specificity of M-ficolin. *J. Biol. Chem.*, 285, 6612–6622.

39. Gutierrez, F. (2017) Semantic Technologies and Bio-Ontologies. In: Huang, J., Borchert, G. M., Dou, D., Huan, J., Lan, W., Tan, M. and Wu, B. (eds) *Bioinformatics in MicroRNA Research*. *Methods in Molecular Biology*. Springer, NY, Vol. 1617, pp. 83–91.