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Tuning specificity and topology of lectins through synthetic biology

Simona Notova and Anne Imberty

Abstract

Lectins are non-immunoglobulin and non-catalytic glycan binding proteins that are able to decipher the structure and function of complex glycans. They are widely used as biomarkers for following alteration of glycosylation state in many diseases and have application in therapeutics. Controlling and extending lectin specificity and topology is the key for obtaining better tools. Furthermore, lectins and other glycan binding proteins can be combined with additional domains, providing novel functionalities. We provide a view on the current strategy with a focus on synthetic biology approaches yielding to novel specificity, but other novel architectures with novel application in biotechnology or therapy.

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Keywords

Synthetic biology, Lectins, Glycans, Multivalency.

Introduction

Glycans are present in the form of a complex array on the surface of all living cells, and a significant number of genes are involved in the biosynthesis of these biomolecules [1]. Through the variety of monosaccharides and linkages, the occurrence of branching structures and their dynamic behavior, the resulting “glycome” represents a maximal possibility of conveying signals. The resulting concept of “glycode” [2] is accompanied by the necessity of receptors able to decipher this code. Lectins and other glycan-binding proteins such as carbohydrate-binding modules, antibodies and variable lymphocyte receptors play a crucial role in the social life of cells by deciphering the code. Lectins are therefore of

high interest for studying the role of complex glycans on cells, but they are also tools in research, diagnostics, biotechnology and other [3]. Numerous lectins have been discovered in nature and many of those found their applications. However, there are certain drawbacks, such as not sufficient selectivity or affinity, that might be disadvantageous for lectins. Therefore, in the last decades, lectins became a target of protein engineering with the clear vision to create suitable candidates with the potential in biotechnology and biomedicine applications.

Synthetic biology is a rapidly growing, multidisciplinary field that comprises the design and engineering of biomolecules, biological machines, and even the creation of novel biological systems and life forms, i.e., artificial cells and organisms [4]. Synthetic glycobiology uses the same approaches as synthetic biology with different areas such as the engineering of glycosylation for the production of novel glycoconjugates [5,6], the alteration of glycocalyx [7] or the engineering of carbohydrate-active enzymes [8] and glycan-binding proteins [9]. In this chapter, we focus on lectin engineering with a particular interest in introducing additional specificity and multivalency.

Engineering of lectin specificity

Engineering the lectin specificity is of strong interest, either for obtaining tools for yet unlabeled glycans or for narrowing too broad specificity. Unfortunately, lectin binding sites do not present the same adaptability as classical antibodies or variable lymphocyte receptors (VLRs) from Lamprey that can be selected towards glycans of interest [10]. Tuning the specificity of lectins *via* mutagenesis and directed evolution is challenging [9,11,12].

Since the pioneering work of Drickamer [13], who switched the specificity of an animal C-type lectin from mannose to galactose, classical methods based on site-directed mutagenesis, usually from structure-derived information, were applied. Narrowing the specificity of a galactose-specific galectin from the fungus *Agrocybe cylindracea* could be performed through a single amino acid modification, resulting in the α GalNAc specificity [14]. Similarly, increasing the selectivity of subtilase cytotoxin (SubB) of *Escherichia coli* towards N-glycolyl

neuraminic acid (NeuGc) towards its more common N-acetyl analog (NeuAc) could be obtained by shortening a peptide loop in the active site [15]. The importance of structural information combined with molecular simulations is illustrated in recent success in the engineering of a mutated form of E-selectin with specificity towards 6'-sulfo-sialyl Lewis X instead of not sulfated natural ligand [16].

Directed evolution is largely used in protein engineering and allowed to increase the specificity of fungal *Aleuria aurantia* lectin towards α 1-6 linked fucose over other linkages [17]. Random mutagenesis and expression screening using ribosome display resulted in sialic acid specificity from the β -trefoil earth worm lectin EW29Ch [18]. This approach obtained the rare 6-sulfo-galactose specificity from the same scaffold [19]. However, in both cases, some residual binding to galactose is maintained, illustrating the difficulties of altering carbohydrate binding sites.

An original approach, allowing for more discrete modifications of the binding site, is incorporating non-canonical amino acids (ncAAs). Such lectin "variants" were first obtained through peptide solid phase synthesis, incorporating fluoro-phenylalanine and naphthylalanine in a hevein-like peptide [20]. More recent approaches use engineered *E. coli* strains auxotrophic for tryptophan (Trp) that could be supplemented with indol compounds serving as tryptophan-analogue precursors [21]. The Trp-rich lectin RSL from *Ralstonia solanacearum* was modified with different fluoro analogs [22]. The resulting variants demonstrated an altered preference for blood group oligosaccharides. Indeed, fluorination affects the hydrogen bonds pattern but also kills the aromaticity of the Trp indol ring, resulting in the loss of stacking interaction with sugar.

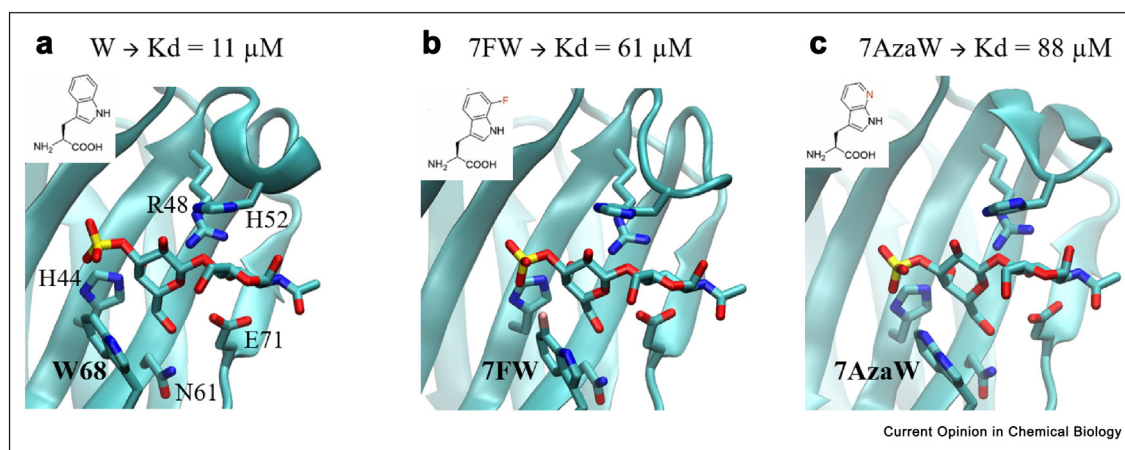
Subsequently, human Galectin 1 (Gal-1), which contains only one Trp residue located in the binding site, was engineered with the same approach, using 12 different Trp non-canonical variants [23]. Two variants with 7-azatryptophan and 7-fluorotryptophan showed significantly reduced affinity for 3'-sulfated glycans while maintaining binding to 6-sulfated glycans (Figure 1). The approach is therefore, promising for finely tuning of specificity, although efforts are needed to rationalize the effects of the inclusion of ncAA on functionality.

Engineering of lectin multivalency

Lectins are multivalent, and the avidity generated by the presence of several binding sites compensates for the somewhat weak affinity of protein-carbohydrate interaction. Increasing the number of binding sites while controlling their resulting spatial position and orientation is a topic of intense interest [24].

Several lectin domains or carbohydrate binding modules (CBMs) can be covalently linked together by incorporating a flexible linker at the gene level. Since markers for sialic acid (NeuAc) are of high interest, divalent NeuAc specific lectins were obtained by assembling tandem repeats CBMs: CBM40 associated with sialidase NanH from *Vibrio cholerae* [25] and NanI from *Clostridium perfringens* [26] resulting in neo-lectins with a strong affinity for sialylated oligosaccharides. The same approach was used with CBM77_{Rf} from the bacterium *Ruminococcus flavefaciens* resulting in the divalent neo-lectin diCBM77_{Rf} specific to homogalacturonic acid with a possible application as a pectin probe (Figure 2a) [27]. Both examples support the idea that CBMs are suitable candidates for engineering lectin by creating multimeric modules with higher affinity. The same

Figure 1



Effect of incorporation of ncAAs on affinity. Snapshot from molecular dynamics simulation of the complex between 3'-sulfated disaccharide and lectin Gal-1 wt (a) and its two variants with ncAA 7-fluorotryptophan (b) 7-azatryptophan (c). Experimentally derived values of the dissociation constant are indicated above each complex.

linker strategy was used for assembling algal lectin tandem repeats [28]. Dimerization of high mannose-specific actinohivin from actinomycete improved its anti-HIV activity [29]. The effect of the peptide linker's nature and length on homodimers binding properties was thoroughly examined for the galectins assembly [30–32]. In the case of Gal-1 [31], varying the size of the linker and the number of domains did not affect the affinity for disaccharide nor the specificity of the glycan array but generated differences in the histochemical staining profiles.

Dimerization can also be obtained by conjugating “handles” inserted by incorporating ncAA with bio-orthogonal reactivities. Human Gal-1 was produced in a methionine auxotrophic *E. coli* host to introduce L-azidohomoalanine (Aha) or L-homopropargylglycine (Hpg) at the only methionine residue at the N-terminal position, and dimerization was obtained through cyclo-addition reaction [21].

Avidine-based non-covalent tetramerization, associated with site-specific biotinylation, may be preferred for better mimicking of multivalent presentation of carbohydrate recognition domains (CRDs) [34]. Fusion of SigLec-7 and murine analogs of DC-SIGN with a peptide substrate of biotinylation enzyme ByrA, resulted in tetrameric assemblies that were used to characterize B-cell subpopulations [35]. Similarly, a biotin-mimic tag was added to *Pseudomonas fluorescens* agglutinin (PFA) specific for oligomannose. It tetramerized through streptavidin and was immobilized on beads for efficient binding to HIV gp120 [36]. TETRALEC, an artificial tetrameric assembly of DC-SIGNR, was obtained by adding oligo-glycine at the N-terminus followed by the activity of sortase A, SrtA, which anchored a biotinylated peptide (Figure 2b) [37]. The neutravidin-assembled TETRALEC displays glycan selectivity comparable to DC-SIGNR. However, the spatial organization of binding sites differs and might affect some interactions. The same streptavidin strategy was used for assembling two biotinylated analogs of cholera toxins into a cross-linking decavalent architecture able to induce hemifusion and fusion of glycolipid-presenting vesicles [38].

Non-covalent oligomerization can also be achieved by genetic fusion with a self-assembling peptide domain. Fc-fragment from IgG has been widely used for C-type lectins [39,40] and SigLecs [41]. Further engineering made possible the creation of a library of multivalent SigLecs used for mapping ligands on healthy and cancer tissues [42]. The Fc dimerization strategy was also applied to Gal-1 to create homodimers [43]. Even greater stability and, in turn enhanced biological activity of Gal-1 could be obtained by fusion with a cysteine-terminated variant of the FBJ osteosarcoma viral oncogene homolog (FOS) leucine zipper, which forms disulfide-linked dimers [44]. The dimerizing *de novo*

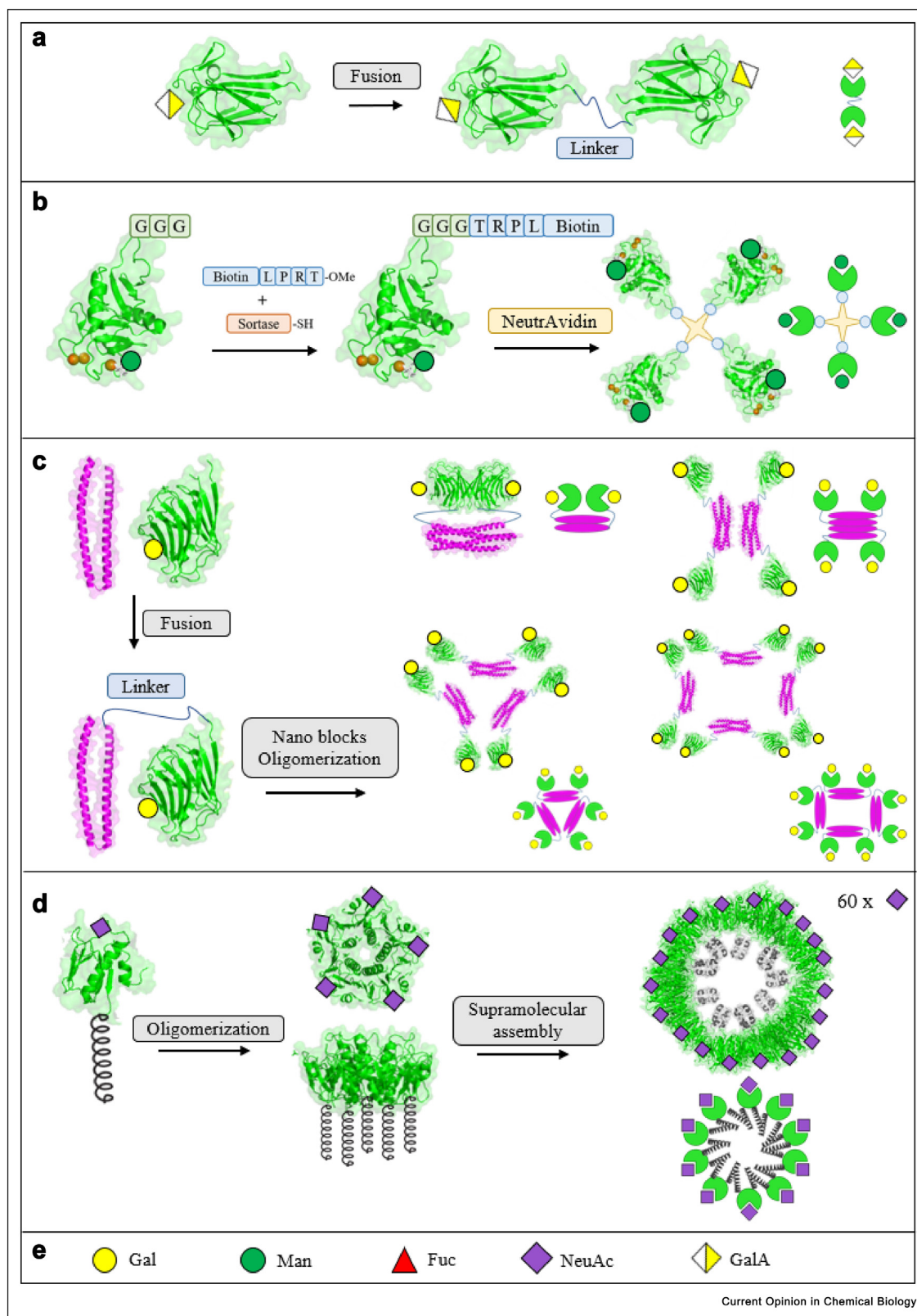
protein WA20 was used for building lectin nano-blocks by fusion with ACG (*Agroclybe cylindracea* galectin). The use of different linkers resulted in the self-assembling of di-, tetra-, hexa-, octa- and decameric structures that were structurally characterized by small-angle x-ray scattering (SAXS) (Figure 2c) [45]. More versatile architectures could be obtained with the fusion of coiled-coil domains that form helices based on heptad repeats of polar and hydrophobic residues. B-subunits of pentameric cholera toxin (CTB) were appended with α -helical coiled-coil, and structural studies demonstrated the formation of tubes composed of 12 pentamers where all CTB binding sites aligned at the external face of the tube (Figure 2d) [46]. Such strategy might be of interest for the formation of novel supramolecular structures. Large oligomers were also obtained by fusion of SigLec-8 with pentamerizing domain of cartilage oligomeric matrix protein (COMP), a spontaneously resulted in the identification of endogenous ligands in human airways [47]. Hexavalent sialic-acid binding proteins were obtained by engineering tandem repeat of CBMs from *V. cholerae* or *Streptococcus pneumoniae* linked to a trimerizing domain from *Pseudomonas aeruginosa* sialidase [48]. This construct masks sialic acid on airway epithelia and is therefore of interest as a protection against viruses such as influenza [48] and SARS-Cov2 [49].

Shuffling of carbohydrate-recognition domains: super lectins and Janus lectins

Multivalent lectins can also be assembled from different domains, resulting in heterodimers of more complex architectures. Connecting Gal-7 with Gal-1 or Gal-3 through several types of linkers resulted in the acquisition of altered properties [32]. The binding properties and biological activity of the resulting heterodimers depended on their composition, arrangement order, and nature of the linker. Gal-1 and Gal-3 were assembled either through a peptide linker or coiled-coil zipper [50], resulting in enhanced extracellular activity (Figure 3a).

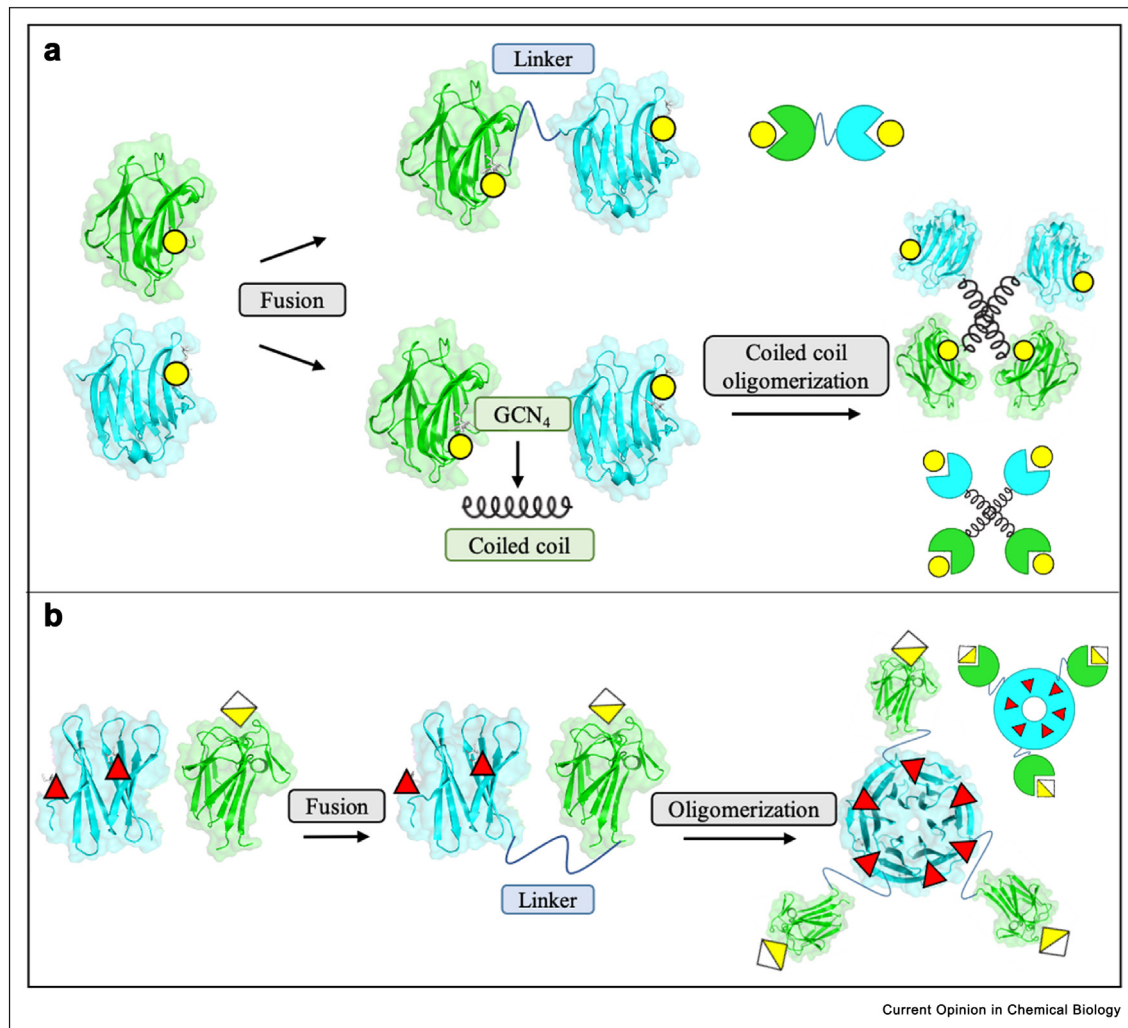
More complex architecture are the Janus lectins, built as a bispecific chimera with two faces specific for different glycans. Janus lectins have been first obtained by associating a trimeric β -propeller lectin (RSL from *R. solanacearum*) and CBMs from a different origin. RSL-CBM40 presents six binding sites for fucose on one side/face and three for sialic acid on the other [51], resulting in crosslinking of vesicles decorated with fucosylated and sialylated glycolipids, respectively. Since RSL-CBM40 Janus lectin can detect hypersialylation, a physiological defect occurring in numerous cancer types, it was used to crosslink fucose-decorated liposomes with sialic-rich H1299 cancer cells [52]. Strikingly, the intact liposome uptake into the cell-mediated by lectin was observed, suggesting that bispecific lectins could find their application in targeted drug delivery systems.

Figure 2



Increase of lectin valency through engineering. a) Galacturonic-specific diCBM77_{Rf} resulting from assembling two CBM77_{Rf} (PDB code 5FU5) through a linker (blue). b) Mannose-specific artificial tetramer of DC-SIGNR TETRALEC obtained by sortase-mediated insertion of covalently linked biotin on modified DC-SIGNR (PDB code 1K9I) and assembly on neutravidin (c) Various self-assembling oligomers of nano blocks were obtained by fusion WA20

Figure 3



Hetero-chimeric lectins obtained through engineering. a) Galactose-specific lectins Gal-1 (green PDB code 1GZW) and Gal-3 (cyan PDB code 3ZSJ) associated by a peptide linker (blue) or a coiled-coil domain (black). b) Double-faced Janus lectin obtained by trimerization of RSL monomer (cyan PDB code 2BT9) fused to CBM77_{Rf} (green PDB code 5FU5).

Janus lectin is also the perfect candidate for building multilayered biomaterial. Fusion of fucose-specific RSL with CBM77_{Rf} from *R. flavefaciens* specific for galacturonic acid provided a “gluing protein” RSL-CBM77_{Rf} that was used for crosslinking pectin and hemicellulose and building supported artificial plant cell wall (Figure 3b) [27]. The concept has been extended to different lectin topologies and RSL-MOA associates fucose specificity with α Gal binding property from *Marasmius oreades* β -trefoil agglutinin [53]. The chemical “handles” inserted by incorporating nAA with bio-orthogonal reactivities could also be used for the

obtention of hetero-oligomers, although the oligomerization state appeared more challenging to control due to undesired side reactions [21].

Bringing new functionalities through fusion to additional domains

Some lectin domains are part of larger peptide sequences, and participate in the targeting of the proteins to glycosylated tissues. Such natural “chimerlectins” could include protease activity [54], toxin activity [55,56], and others. Therefore, it is possible to mimic

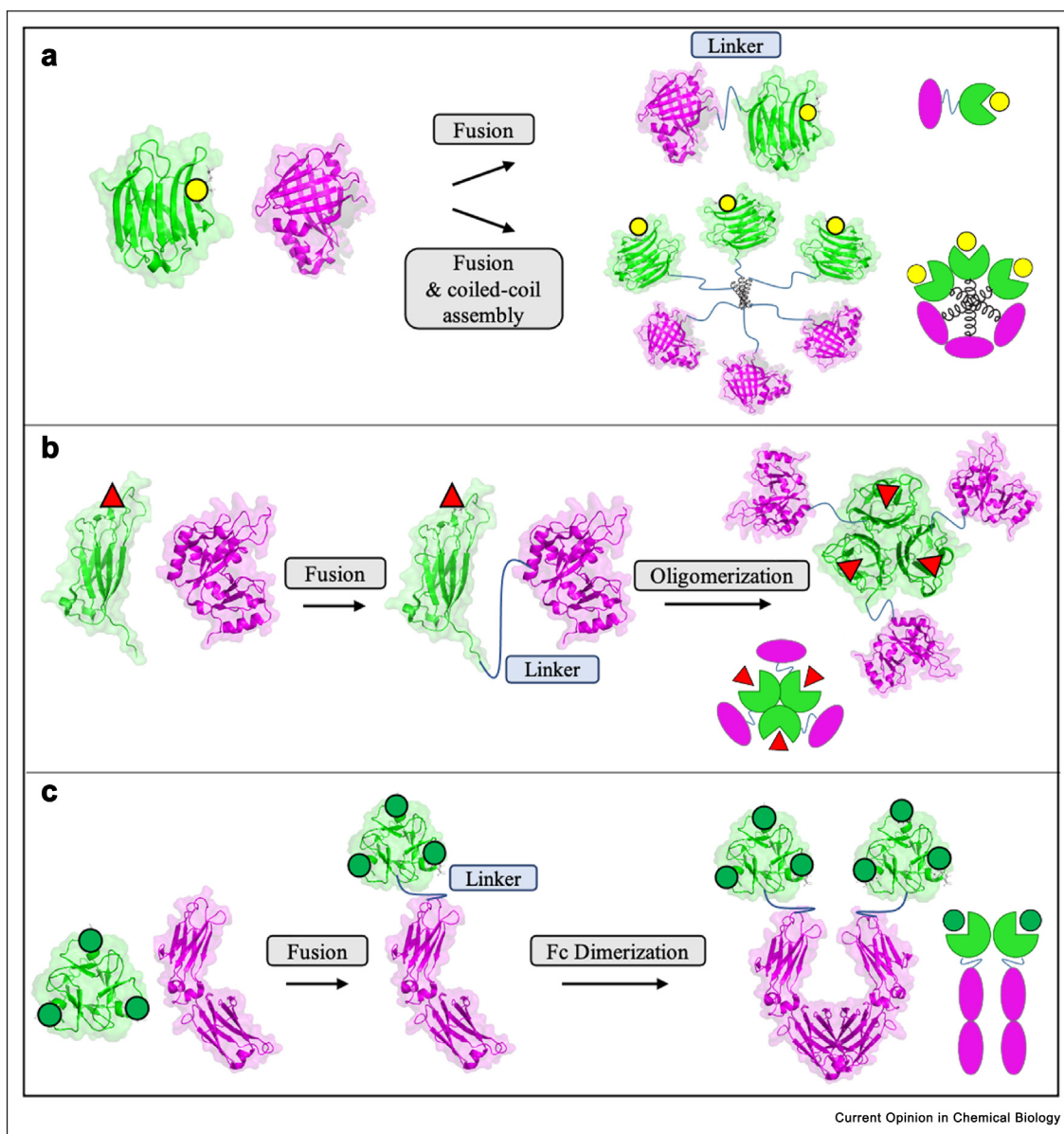
(magenta, PDB code 6KOS) and galactose-specific lectin ACG (PDB code 1WW4). D) Nanotube supramolecular assembly of lectin B-subunits of pentameric sialic acid binding cholera toxin (CTxB, PDB code 1PZJ) by fusion with coiled-coil domains (black) which resulted in an assembly where up to 60 binding sites for sialic acid can be found on the outer ring of such structures. Lectins domains are colored in green, and the representation of monosaccharides follows the SNFG convention represented in panel E [33].

such natural architecture and introduce new functions by fusion of an additional domain, with an application, especially in the biotechnology and medical fields. For example, galectins have been fused with luciferase (Figure 4a) [57] and green fluorescent protein using coiled-coil peptides allowing for mastering their oligomerization states [58].

In the next paragraphs, we will focus on present efforts for adding a functional domain able to kill the targeted

cells. Associating a lectin to a toxin domain is an efficient strategy for targeting some subtype of cells. A successful example is the use of BC2LCN from *Burkholderia cenocepacia* with narrow and exclusive specificity for fucosylated H-type1/3 epitopes [59], that are present on human pluripotent stem cells (hPSCs). Fusion of BC2LCN with exotoxin A from *P. aeruginosa* resulted in an efficient tool for removing hPSCs, which are potential tumorigenic, from stem cell cultures (Figure 4b) [60]. Since enhanced fucosylation is a signature of several

Figure 4



Lectin functionalization by fusion with additional domains. a) Gal-3 covalently linked (green PDB code 3ZSJ) with luciferase (magenta PDB code 1LCI) resulting in a lectin/enzyme chimera, with either a peptide linker or a coiled-coil domain resulting in a trimeric association. b) Fucose-specific lectin BC2LCN (green PDB code 2WQ4) fused with a toxin domain of Exotoxin A (magenta PDB code 1IKQ) c) "Lectibody" from the combination of mannose-specific actinohivin variant (green PDB code 4DEN) and Fc fragment of human IgG antibody (magenta PDB code 1H3W).

cancer types, the same lectin-toxin construct was assayed against pancreatic [61], colon [62] and gastric cancer [63]. Tumor growth was inhibited, but only some cell lines were responsive to such treatment.

Lectin-mediated cytotoxicity is also obtained through “lectibody” – a chimeric protein composed of lectin and Fc fragment of IgG antibody. Once the lectin is bound, the Fc part activates the immune system *via* cell receptors or complement proteins. Such lectibody was prepared as a fusion of Fc fragment with actinohivin lectin variant (Avaren) specific for high-mannose-type glycans present on the envelope glycoproteins (gp120) of the HIV viruses (Figure 4C) [64]. The AvFc lectibody exhibited potent antiviral activity against several strains of HIV and Fc-mediated cell killing activity against HIV-1-infected cells. Since hyper-mannosylation is also a signature of several cancers, AvFc was tested on several cancerous cell lines and demonstrated strong binding and cytotoxic activity, confirming the potential of lectibody strategy for drug development and targeting [65]. The concept was extended through an *in vitro* approach by protein ligation, allowing for the production of lectibodies from libraries of lectins and Fc fragments. This approach resulted in a novel lectibody based on the high mannose-specific scytovirin from the cyanobacteria *Scytonema varium* [66]. Recently, an anti-CD3 single-chain antibody fragment was coupled through bio-orthogonal reactive nAA to the B-subunit of Shiga toxin to target Gb3+ cancer cells, resulting in very fine targeting of the toxicity [67].

Lectin chimera can also be part of cell therapy strategy. Lectin-based chimeric antigen receptors (CARs) have been developed as a fusion between an extracellular lectin domain, a transmembrane domain, and several effectors/signaling intracellular domains [68] and have been expressed in different types of lymphocytes. H84T-BanLec, a variant of BanL with antiviral properties but devoided of mitogenic effect [69], was introduced in a lectin-based CAR designed to direct NK cells to SARS-CoV-2 glycoproteins [70] specifically. H84T-BanLec CAR-NK cells are activated against SARS-CoV-2 and represent promising antiviral therapy. Tumor cells can also be targeted by lectin-based CAR T cells. Three different lectins (STx from *Shigella dysenteriae*, LecA from *P. aeruginosa*, and engineered Mitsuba), all specific for α Gal1-4Gal epitope present on cancer-related Gb3 glycosphingolipid, were selected and fused to a second-generation CAR. The resulting lectin-based CAR T cells demonstrated cytotoxicity against Burkitt’s lymphoma cell lines but also against cells from solid tumors [71].

Conclusion and perspective

Designing new lectins with enhanced properties in terms of specificity, multivalency and added function is

still a novel domain. The review focused on protein engineering of lectins and CBMs, but novel opportunities can be opened if using other modules such as variable lymphocyte receptors [10], single chain antibodies (nanobodies) [72], or novel scaffolds now being developed. Similarly, the frontier between protein engineering through molecular biology methods and protein conjugation through chemical ways is getting porous. Including non-canonical amino acids allows for creating novel linkers with bioorthogonal reactive groups [21]. After an exploration period, the field now enters into more thoroughly designed multimeric protein architectures, where the distance and orientation of the binding sites can be controlled. The length and flexibility of linker sequences are also parameters of interest: for antibodies, higher affinity was demonstrated to be reached using rigid linker that optimally spans the distance between sites [73] but this may be different when binding to flexible glycans presented on cell surface. Multidisciplinary studies will provide complete glycan binding protein in the future, probably away from the classical lectin scaffolds, allowing for more application for glycosciences in research and health or material sciences.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

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

- * of special interest
- ** of outstanding interest

1. Varki A: **Evolutionary forces shaping the Golgi glycosylation machinery: why cell surface glycans are universal to living cells.** *Cold Spring Harbor Perspect Biol* 2011, 3.
2. Gabius HJ: **Cell surface glycans: the why and how of their functionality as biochemical signals in lectin-mediated information transfer.** *Crit Rev Immunol* 2006, 26:43–79.
3. Lis H, Sharon N: **Lectins as molecules and as tools.** *Annu Rev Biochem* 1986, 1986:35–67.
4. Benner SA, Sismour AM: **Synthetic biology.** *Nat Rev Genet* 2005, 6:533–543.
5. Jarontomechai T, Taw MN, Li M, Aquino A, Agashe N, Chung S, Jewett MC, DeLisa MP: **Cell-free synthetic glyco-biology: designing and engineering glycomolecules outside of living cells.** *Front Chem* 2020, 8:645.

8 Glycobiology

6. Kightlinger W, Warfel KF, DeLisa MP, Jewett MC: **Synthetic glycobiology: parts, systems, and applications.** *ACS Synth Biol* 2020, **9**:1534–1562.
 7. Purcell SC, Godula K: **Synthetic glycoscapes: addressing the structural and functional complexity of the glycocalyx.** *Interface Focus* 2019, **9**, 20180080.
 8. Czlapinski JL, Bertozzi CR: **Synthetic glycobiology: exploits in the golgi compartment.** *Curr Opin Chem Biol* 2006, **10**: 645–651.
 9. Ward EM, Kizer ME, Imperiali B: **Strategies and tactics for the development of selective glycan-binding proteins.** *ACS Chem Biol* 2021, **16**:1795–1813.
- A review on successful engineering of lectins, CBMs, antibodies and variable lymphocyte receptor.
10. McKittrick TR, Bernard SM, Noll AJ, Collins BC, Goth CK, McQuillan AM, Heimburg-Molinaro J, Herrin BR, Wilson IA, Cooper MD, *et al.*: **Novel lamprey antibody recognizes terminal sulfated galactose epitopes on mammalian glycoproteins.** *Commun Biol* 2021, **4**:674.
 11. Hirabayashi J, Arai R: **Lectin engineering: the possible and the actual.** *Interface Focus* 2019, **9**, 20180068.
 12. Warkentin R, Kwan DH: **Resources and methods for engineering "designer" glycan-binding proteins.** *Molecules* 2021, **26**:380.
- A comprehensive overview of available methods for engineering all types of glycan-binding proteins.
13. Iobst ST, Drickamer K: **Binding of sugar ligands to Ca(2+)-dependent animal lectins. II. Generation of high-affinity galactose binding by site-directed mutagenesis.** *J Biol Chem* 1994, **269**:15512–15519.
 14. Hu D, Tateno H, Sato T, Narimatsu H, Hirabayashi J: **Tailoring GalNAcalpha1-3Galbeta-specific lectins from a multi-specific fungal galectin: dramatic change of carbohydrate specificity by a single amino-acid substitution.** *Biochem J* 2013, **453**: 261–270.
 15. Day CJ, Paton AW, Higgins MA, Shewell LK, Jen FE, Schulz BL, Herdman BP, Paton JC, Jennings MP: **Structure aided design of a Neu5Gc specific lectin.** *Sci Rep* 2017, **7**:1495.
 16. Wang X, Hanes MS, Cummings RD, Woods RJ: **Computationally guided conversion of the specificity of E-selectin to mimic that of Siglec-8.** *Proc Natl Acad Sci U S A* 2022, **119**, e2117743119.
- A successful strategy of rational design and molecular simulation that resulted in the highly sought specificity towards sulfated oligosaccharide.
17. 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.
 18. Yabe R, Suzuki R, Kuno A, Fujimoto Z, Jigami Y, Hirabayashi J: **Tailoring a novel sialic acid-binding lectin from a ricin-B chain-like galactose-binding protein by natural evolution-mimicry.** *J Biochem* 2007, **141**:389–399.
 19. Hu D, Tateno H, Kuno A, Yabe R, Hirabayashi J: **Directed evolution of lectins with sugar-binding specificity for 6-sulfo-galactose.** *J Biol Chem* 2012, **287**:20313–20320.
 20. Chavez MI, Andreu C, Vidal P, Aboitiz N, Freire F, Groves P, Asensio JL, Asensio G, Muraki M, Canada FJ, *et al.*: **On the importance of carbohydrate-aromatic interactions for the molecular recognition of oligosaccharides by proteins: NMR studies of the structure and binding affinity of AcAMP2-like peptides with non-natural naphthyl and fluoroaromatic residues.** *Chemistry* 2005, **11**:7060–7074.
 21. Tobola F, Sylvander E, Gafko C, Wiltschi B: **'Clickable lectins': bioorthogonal reactive handles facilitate the directed conjugation of lectins in a modular fashion.** *Interface Focus* 2019, **9**, 20180072.
 22. Tobola F, Lelimosin M, Varrot A, Gillon E, Darnhofer B, Blixt O, Birner-Gruenberger R, Imberty A, Wiltschi B: **Effect of non-canonical amino acids on protein-carbohydrate interactions: structure, dynamics and carbohydrate affinity of a lectin engineered with fluorinated tryptophan analogs.** *ACS Chem Biol* 2018, **13**:2211–2219.
 23. Tobola F, Lepsik M, Zia SR, Leffler H, Nilsson UJ, Blixt O, Imberty A, Wiltschi B: **Engineering the ligand specificity of the human galectin-1 by incorporation of tryptophan analogs.** *ChemBiochem* 2022, **23**, e202100593.
- The incorporation of non-canonical amino acid in the binding-site of galectin-1 proved to be a successful approach for fine-tuning the specificity.
24. Tobola F, Wiltschi B: **One, two, many: strategies to alter the number of carbohydrate binding sites of lectins.** *Biotechnol Adv* 2022, **60**, 108020.
- A recent review describing various approaches in lectin engineering with a special focus on protein valency and topology.
25. Connaris H, Crocker PR, Taylor GL: **Enhancing the receptor affinity of the sialic acid-binding domain of *Vibrio cholerae* sialidase through multivalency.** *J Biol Chem* 2009, **284**: 7339–7351.
 26. Ribeiro J, Pau W, Pifferi C, Renaudet O, Varrot A, Mahal LK, Imberty A: **Characterization of a high-affinity sialic acid specific CBM40 from *Clostridium perfringens* and engineering of divalent form.** *Biochem J* 2016, **473**:2109–2118.
 27. Notova S, Cannac N, Rabagliati L, Touzard M, Mante J, Navon Y, Coche-Guérente L, Lerouxel O, Heux L, Imberty A: **Building artificial plant cell wall on lipid bilayer by assembling polysaccharides and engineered proteins.** *ACS Synth Biol* 2022, **11**:3516–3528.
- Rational design of a janus lectin with "glue-like" application for surface assembling of biopolymers mimicking plant cell wall.
28. Hwang HJ, Han JW, Jeon H, Han JW: **Induction of recombinant lectin expression by an artificially constructed tandem repeat structure: a case study using *Bryopsis plumosa* mannose-binding lectin.** *Biomolecules* 2018, **8**.
 29. Takahashi A, Inokoshi J, Hachiya A, Oka S, Omura S, Tanaka H: **The high mannose-type glycan binding lectin actinohivin: dimerization greatly improves anti-HIV activity.** *J Antibiot (Tokyo)* 2011, **64**:551–557.
 30. Garcia Caballero G, Beckwith D, Shilova NV, Gabba A, Kutzner TJ, Ludwig AK, Manning JC, Kaltner H, Sinowatz F, Cudic M, *et al.*: **Influence of protein (human galectin-3) design on aspects of lectin activity.** *Histochem Cell Biol* 2020, **154**: 135–153.
 31. Kutzner TJ, Gabba A, FitzGerald FG, Shilova NV, Garcia Caballero G, Ludwig AK, Manning JC, Knospe C, Kaltner H, Sinowatz F, *et al.*: **How altering the modular architecture affects aspects of lectin activity: case study on human galectin-1.** *Glycobiology* 2019, **29**:593–607.
 32. Ludwig AK, Michalak M, Gabba A, Kutzner TJ, Beckwith DM, FitzGerald FG, Garcia Caballero G, Manning JC, Kriegsmann M, Kaltner H, *et al.*: **Imitating evolution's tinkering by protein engineering reveals extension of human galectin-7 activity.** *Histochem Cell Biol* 2021, **156**:253–272.
- Engineering homodimers of Gal-7 and heterodimers with other galectins demonstrated no alteration for binding, but different cellular responses when applied to array of cells
33. Varki A, Cummings RD, Aebi M, Packer NH, Seeberger PH, Esko JD, Stanley P, Hart G, Darvill A, Kinoshita T, *et al.*: **Symbol nomenclature for graphical representations of glycans.** *Glycobiology* 2015, **25**:1323–1324.
 34. Powlesland AS, Ward EM, Sadhu SK, Guo Y, Taylor ME, Drickamer K: **Widely divergent biochemical properties of the complete set of mouse DC-SIGN-related proteins.** *J Biol Chem* 2006, **281**:20440–20449.
 35. Gieseke F, Mang P, Viebahn S, Sonntag I, Kruchen A, Erbacher A, Pfeiffer M, Handgretinger R, Muller I: **Siglec-7**

- tetramers characterize B-cell subpopulations and leukemic blasts. *Eur J Immunol* 2012, **42**:2176–2186.
36. Lim B, Kydd L, Jaworski J: **A peptide-lectin fusion strategy for developing a glycan probe for use in various assay formats.** *Chemosensors* 2019, **7**.
37. Achilli S, Monteiro JT, Serna S, Mayer-Lambertz S, Thepaut M, Le Roy A, Ebel C, Reichardt NC, Lepenies B, Fieschi F, *et al.*: **TETRALEC, artificial tetrameric lectins: a tool to screen ligand and pathogen interactions.** *Int J Mol Sci* 2020:21.
38. Wehrum S, Siukstaite L, Williamson DJ, Branson TR, Sych T, Madl J, Wildsmith GC, Dai W, Kempmann E, Ross JF, *et al.*: **Membrane fusion mediated by non-covalent binding of re-engineered cholera toxin assemblies to glycolipids.** *ACS Synth Biol* 2022, **11**:3929–3938.
39. Lindenwald DL, Monteiro JT, Rautenschlein S, Meens J, Jung K, Becker SC, Lepenies B: **Ovine C-type lectin receptor hFc-fusion protein library - a novel platform to screen for host-pathogen interactions.** *Vet Immunol Immunopathol* 2020, **224**, 110047.
- A library of sheep C-type lectin-hFc fusion chimeras allow for identification of receptors for the ruminant pathogen *Mycoplasma mycoides*.
40. Willment JA: **Fc-conjugated C-type lectin receptors: tools for understanding host-pathogen interactions.** *Mol Microbiol* 2022, **117**:632–660.
41. Chang LY, Low PY, Sridharan D, Gerlovin K, Angata T: **Preparation of recombinant Siglecs and identification of their ligands.** *Methods Mol Biol* 2020, **2132**:85–98.
42. Rodrigues E, Jung J, Park H, Loo C, Soukhtehzari S, Kitova EN, Mozaneh F, Daskhan G, Schmidt EN, Aghanya V, *et al.*: **A versatile soluble siglec scaffold for sensitive and quantitative detection of glycan ligands.** *Nat Commun* 2020, **11**:5091.
- The Fc dimerization strategy was used for Siglecs multimerization allowing for glycan identification on healthy and cancerous tissues
43. Cedeno-Laurent F, Barthel SR, Opperman MJ, Lee DM, Clark RA, Dimitroff CJ: **Development of a nascent galectin-1 chimeric molecule for studying the role of leukocyte galectin-1 ligands and immune disease modulation.** *J Immunol* 2010, **185**:4659–4672.
44. van der Leij J, van den Berg A, Harms G, Eschbach H, Vos H, Zwiers P, van Weeghel R, Groen H, Poppema S, Visser L: **Strongly enhanced IL-10 production using stable galectin-1 homodimers.** *Mol Immunol* 2007, **44**:506–513.
45. Irumagawa S, Hiemori K, Saito S, Tateno H, Arai R: **Self-assembling lectin nano-block oligomers enhance binding avidity to glycans.** *Int J Mol Sci* 2022, **23**.
- An original approach to increase lectin avidity through combination with self-assembling peptides.
46. Ross JF, Wildsmith GC, Johnson M, Hurdiss DL, Hollingsworth K, Thompson RF, Mosayebi M, Trinh CH, Paci E, Pearson AR, *et al.*: **Directed assembly of homopentameric Cholera toxin B-subunit proteins into higher-order structures using coiled-coil appendages.** *J Am Chem Soc* 2019, **141**:5211–5219.
47. Gonzalez-Gil A, Porell RN, Fernandes SM, Wei Y, Yu H, Carroll DJ, McBride R, Paulson JC, Tiemeyer M, Aoki K, *et al.*: **Sialylated keratan sulfate proteoglycans are Siglec-8 ligands in human airways.** *Glycobiology* 2018, **28**:786–801.
48. Connaris H, Govorkova EA, Ligertwood Y, Dutia BM, Yang L, Tauber S, Taylor MA, Alias N, Hagan R, Nash AA, *et al.*: **Prevention of influenza by targeting host receptors using engineered proteins.** *Proc Natl Acad Sci U S A* 2014, **111**:6401–6406.
49. Fell R, Potter JA, Yuille S, Salguero FJ, Watson R, Ngabo D, Gooch K, Hewson R, Howat D, Dowall S: **Activity of a carbohydrate-binding module therapy, neumifil, against SARS-CoV-2 disease in a hamster model of infection.** *Viruses* 2022:14.
50. Fettes MM, Farhadi SA, Hudalla GA: **A chimeric, multivalent assembly of galectin-1 and galectin-3 with enhanced extracellular activity.** *Biomater Sci* 2019, **7**:1852–1862.
51. Ribeiro JP, Villringer S, Goyard D, Coche-Guerente L, Höferlin M, Renaudet O, Römer W, Imberty A: **Tailor-made Janus lectin with dual avidity assembles glycoconjugate multilayers and crosslinks protocells.** *Chem Sci* 2018, **9**:7634–7641.
52. Siukstaite L, Rosato F, Mitrovic A, Müller PF, Kraus K, Notova S, Imberty A, Römer W: **The two sweet sides of Janus lectin drive crosslinking of liposomes to cancer cells and material uptake.** *Toxins* 2021, **13**:792.
- Janus lectin with double specificity towards fucose and sialic acid induces the cellular uptake and burst of liposomes suggesting potential application for targeted drug delivery.
53. Notova S, Siukstaite L, Rosato F, Vena F, Audfray A, Bovin N, Landemarre L, Römer W, Imberty A: **Extending Janus lectins architecture: characterization and application to protocells.** *bioRxiv* 2022.
54. Cordara G, Egge-Jacobsen W, Johansen HT, Winter HC, Goldstein IJ, Sandvig K, Krenzel U: **Marasmius oreades agglutinin (MOA) is a chimerolectin with proteolytic activity.** *Biochem Biophys Res Commun* 2011, **408**:405–410.
55. Mancheno JM, Tateno H, Goldstein IJ, Martinez-Ripoll M, Hermoso JA: **Structural analysis of the Laetiporus sulphureus hemolytic pore-forming lectin in complex with sugars.** *J Biol Chem* 2005, **280**:17251–17259.
56. Notova S, Bonnardel F, Rosato F, Siukstaite L, Schwaiger J, Bovin N, Varrot A, Römer W, Lisacek F, Imberty A: **The choanoflagellate pore-forming lectin SaroL-1 punches holes in cancer cells by targeting the tumor-related glycosphingolipid Gb3.** *Comms. Biol.* 2022, **5**:954.
57. Farhadi SA, Bracho-Sanchez E, Fettes MM, Seroski DT, Freeman SL, Restuccia A, Keselowsky BG, Hudalla GA: **Locally anchoring enzymes to tissues via extracellular glycan recognition.** *Nat Commun* 2018, **9**:4943.
- Galectin-3 fusion with green fluorescent protein through coiled-coil peptides resulted in synthetic constructs with tunable valency.
58. Farhadi SA, Liu R, Becker MW, Phelps EA, Hudalla GA: **Physical tuning of galectin-3 signaling.** *Proc Natl Acad Sci U S A* 2021:118.
59. Šulák O, Cioci G, Delia M, Lahmann M, Varrot A, Imberty A, Wimmerová M: **A TNF-like trimeric lectin domain from *Burkholderia cenocepacia* with specificity for fucosylated human histo-blood group antigens.** *Structure* 2010, **18**:59–72.
60. Tateno H, Saito S: **Engineering of a potent recombinant lectin-toxin fusion protein to eliminate human pluripotent stem cells.** *Molecules* 2017, **22**.
61. Shimomura O, Oda T, Tateno H, Ozawa Y, Kimura S, Sakashita S, Noguchi M, Hirabayashi Y, Asashima M, Ohkohchi N: **A novel therapeutic strategy for pancreatic cancer: targeting cell surface glycan using rBC2LC-N lectin-drug conjugate (LDC).** *Mol Cancer Therapeut* 2018, **17**:183–195.
62. Kitaguchi D, Oda T, Enomoto T, Ohara Y, Owada Y, Akashi Y, Furuta T, Yu Y, Kimura S, Kuroda Y, *et al.*: **Lectin drug conjugate therapy for colorectal cancer.** *Cancer Sci* 2020, **111**:4548–4557.
- Fusion of fucose-specific lectin BC2LCN with a bacterial toxin results in the creation of lectin drug conjugate which significantly suppresses tumor growth
63. Yang Y, Akashi Y, Shimomura O, Tateno H, Saito S, Hiemori K, Miyazaki Y, Furuta T, Kitaguchi D, Kuroda Y, *et al.*: **Glycan expression profile of signet ring cell gastric cancer cells and potential applicability of rBC2LCN-targeted lectin drug conjugate therapy.** *Gastric Cancer* 2022, **25**:896–905.
64. Hamorsky KT, Kouokam JC, Dent MW, Grooms TN, Husk AS, Hume SD, Rogers KA, Villinger F, Morris MK, Hanson CV, *et al.*: **Engineering of a lectibody targeting high-mannose-type glycans of the HIV envelope.** *Mol Ther* 2019, **27**:2038–2052.
65. Oh YJ, Dent MW, Freels AR, Zhou Q, Lebrilla CB, Merchant ML, Matoba N: **Antitumor activity of a lectibody targeting cancer-associated high-mannose glycans.** *Mol Ther* 2022, **30**:1523–1535.
66. Jaakkonen A, Volkmann G, Iwai H: **An off-the-shelf approach for the production of Fc fusion proteins by protein trans-splicing towards generating a lectibody in vitro.** *Int J Mol Sci* 2020:21.

67. Rosato F, Pasupuleti R, Tomisch J, Melendez AV, Kolanovic D, Makshakova ON, Wiltshi B, Romer W: **A bispecific, cross-linking lectibody activates cytotoxic T cells and induces cancer cell death.** *J Transl Med* 2022, **20**:578.
 ** Combination of Gb3 binding shiga toxin and anti-CD3 single-chain antibody resulted in the selective redirection of T cell cytotoxicity, resulting in nearly complete tumor cell lysis
68. Raglow Z, McKenna MK, Bonifant CL, Wang W, Pasca di Magliano M, Stadlmann J, Penninger JM, Cummings RD, Brenner MK, Markovitz DM: **Targeting glycans for CAR therapy: the advent of sweet CARs.** *Mol Ther* 2022, **30**:2881–2890.
 ** Associating glycan binding protein to chimeric antigen receptors (CARs) appears as promising tools for cancer and antiviral therapy
69. Swanson MD, Boudreaux DM, Salmon L, Chugh J, Winter HC, Meagher JL, Andre S, Murphy PV, Oscarson S, Roy R, *et al.*: **Engineering a therapeutic lectin by uncoupling mitogenicity from antiviral activity.** *Cell* 2015, **163**:746–758.
70. Christodoulou I, Rahnama R, Ravich JW, Seo J, Zolov SN, Marple AN, Markovitz DM, Bonifant CL: **Glycoprotein Targeted CAR-NK Cells for the Treatment of SARS-CoV-2 Infection.** *Front Immunol* 2021, **12**:763460.
71. Melendez AV, Velasco Cardenas RM, Lagies S, Strietz J, Siukstaite L, Thomas OS, Tomisch J, Weber W, Kammerer B, Romer W, *et al.*: **Novel lectin-based chimeric antigen receptors target Gb3-positive tumour cells.** *Cell Mol Life Sci* 2022, **79**:513.
 ** First constructions of lectin-CAR T demonstrated their cytotoxic activity towards solid tumors and Burkitt's lymphoma cells
72. Khilji SK, Goerdeler F, Frensemeier K, Warschkau D, Lühle J, Fandi Z, Schirmeister F, Chen ZA, Turak O, Mallagaray A, *et al.*: **Generation of glycan-specific nanobodies.** *Cell Chem Biol* 2022, **29**:1353–1361 e1356.
73. Einav T, Yazdi S, Coey A, Bjorkman PJ, Phillips R: **Harnessing avidity: quantifying the entropic and energetic effects of linker length and rigidity for multivalent binding of antibodies to HIV-1.** *Cell Syst* 2019, **9**:466–474 e467.