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Laminin G-like domains: dystroglycan-specific lectins

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Abstract

A unique *O*-mannose-linked glycan on the transmembrane protein dystroglycan binds a number of extracellular matrix proteins containing laminin G-like (LG) domains. The dystroglycan-matrix interaction is essential for muscle function: disrupted biosynthesis of the matrix-binding modification causes several forms of muscular dystrophy. The complete chemical structure of this modification has been deciphered in the past few years. We now know that LG domains bind to a glycosaminoglycan-like polysaccharide of [-3GlcAβ1,3Xylα1-] units, termed matriglycan, that is attached to a highly unusual heptasaccharide linker. X-ray crystallography has revealed the principles of Ca^{2+}-dependent matriglycan binding by LG domains. In this review, the new structural insights are applied to the growing number of LG domain-containing proteins that bind dystroglycan. It is proposed that LG domains be recognised as “D-type” lectins to indicate their conserved function in dystroglycan binding.

Introduction

The proper functioning of skeletal muscle depends on mechanical linkage of the cytoskeleton of muscle cells to the extracellular matrix surrounding the cells. Disruption of this linkage underlies a group of inherited diseases characterised by progressive weakening and degeneration of skeletal muscle, termed muscular dystrophies [1]. The most common form is Duchenne muscular dystrophy, which results from mutations in the cytoplasmic protein dystrophin. A series of seminal studies by Kevin Campbell’s laboratory in the early 1990s identified the dystrophin-glycoprotein complex, which spans the muscle plasma membrane and provides the necessary linkage to the extracellular matrix [2]. The matrix-binding component was revealed to be dystroglycan (DG), which consists of a heavily glycosylated extracellular α subunit (α-DG) and a membrane-spanning β subunit; the two subunits are derived from a single gene product by post-translational cleavage. An anionic *O*-linked glycan on α-DG appeared to be mediating the interaction with the matrix protein laminin, but all of the plausible candidates (glycosaminoglycans, sialylated glycans) were eventually ruled out. Deciphering the chemical structure and biosynthesis of the laminin-binding carbohydrate modification of α-DG took nearly 25 years and was full of surprises. The problem was eventually solved by a combination of human genetics and mass spectrometry, with a good helping of traditional biochemistry. As these discoveries are

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described in detail in a number of recent reviews [3–6], only a brief outline will be given here. My review instead focuses on the recent advances in understanding laminin binding to α-DG.

Biosynthesis of α-DG's laminin-binding modification

α-DG consists of two globular domains separated by a mucin-like region containing many serines and threonines. The N- and C-terminal halves of the mucin-like region are modified by *O*-Man and *O*-GalNAc cores, respectively, with a total of ~40 *O*-linked glycans identified in glycoproteomic studies [7–11]. Out of this number, only a few *O*-Man cores (Thr317, Thr319 and Thr379 in human α-DG) are extended to the laminin-binding modification (Figure 1A) [12,13]. *O*-mannosylation of α-DG is carried out by a complex of POMT1 and POMT2, which is embedded within the membrane of the endoplasmic reticulum [14]. Still within the endoplasmic reticulum, the *O*-Man cores destined to become the laminin-binding modification are extended by POMGNT2 and B3GALNT2 to form the so-called core M3 structure, GalNAcβ1-3GlcNAcβ1-4Man-*O*-Thr [13], which is then phosphorylated by POMK at position 6 of the mannose [15]. The remaining *O*-Man sites are elaborated to different structures, which are indirectly required for further synthesis of the laminin-binding modification [16,17•]. The Golgi-resident enzymes fukutin (FKTN) and fukutin-related protein (FKRP) now add a tandem ribitol-5-phosphate (RboP) moiety onto the phospho-core M3 [18••,19••,20••,21••,22•]. The presence of RboP in a vertebrate glycan was a major surprise, as this moiety previously was only known to exist in the teichoic acids of bacterial cell walls. TMEM5 next adds a xylose to the second RboP [20••,23••], and B4GAT1 adds a glucuronic acid to the xylose [24,25]. The resulting GlcAβ1-4Xyl terminal is a substrate for the bifunctional glycosyltransferase LARGE, which builds a polysaccharide of repeating [-3GlcAβ1,3Xylα1-] units [26]. It is this polysaccharide that confers laminin binding to α-DG. As we will see, other extracellular matrix proteins also bind to the LARGE-synthesised polysaccharide. Yoshida-Moriguchi and Campbell, therefore, coined the term matriglycan for the $[\text{-3GlcA}\beta\text{1,3Xyl}\alpha\text{1-}]_n$ polysaccharide on α-DG [3].

Altogether, the biosynthesis of the matrix-binding modification on α-DG requires ten glycosyltransferases, one kinase, at least six enzymes involved in the synthesis of sugar donors, and most likely one or several transporters [3–6,27]. Mutation of any of the genes coding for this set of proteins results in the absence of functional α-DG modification and, hence, muscular dystrophy. Because α-DG also has important functions in the nervous system, these so-called dystroglycanopathies are frequently accompanied by brain and eye abnormalities [1].

There are several intriguing parallels between α-DG's unique matrix-binding modification and the more widespread glycosaminoglycan (GAG) modifications of proteoglycans: in both cases, a linker is synthesised first, which is then extended by a bifunctional glycosyltransferase; phosphorylation of the linker is an obligatory step; and the anionic polysaccharides serve as a binding platform for a variety of extracellular matrix proteins [28–30]. For these reasons, it would make sense to consider matriglycan as a type of GAG, even though its disaccharide repeat does not contain an amino sugar. Interestingly, there may be overlap between the two types of modification: LARGE2 (the paralogue of LARGE) is

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able to attach matriglycan chains to proteoglycans, most likely by extending the GAG tetrasaccharide linkers [31•].

Structural basis of laminin binding to α-DG

Laminins are αβγ heterotrimers that polymerise on the extracellular face of the plasma membrane to form a cell-associated extracellular matrix, the basement membrane (BM) [32]. Mammalian genomes encode five laminin α chains, three β chains, and three γ chains, which assemble into 15 distinct laminin heterotrimers. The groundbreaking biochemical experiments on α-DG binding were all done using laminin-111 (α1β1γ1), which actually is not present in the BM of adult skeletal muscle; the predominant laminin isoforms in muscle contain the α2 chain [33]. Fortunately, the α-DG-binding properties of the laminin α1 and α2 chains have turned out to be quite similar.

The α-DG binding site(s) of laminins are located within the G (globular) region at the C-terminus of laminin α chains [34,35]. This region consists of five laminin G-like (LG) domains of ~200 residues each; in the laminin heterotrimer, LG1-3 forms a compact cloverleaf-shaped structure that is connected to LG4-5 through a flexible linker [36–38]. An important early observation was that laminin binding to α-DG strictly requires Ca^{2+} ions [39]. Intriguingly, the crystal structure of laminin α2 LG5 revealed a conserved Ca^{2+} binding site bound to one edge of the β-sandwich that makes up the LG fold [40]. Subsequent mutational studies established that the Ca^{2+} ion in LG4, and the region surrounding it, represents a major α-DG binding site in all laminin α chains [41,42]. An additional site is present in LG1-3 but has not been mapped to a single domain [35]. How LG domains recognise the carbohydrate modification of α-DG remained a mystery until recently.

The identification of LARGE as a bifunctional glucuronyl- and xylosyltransferase [26] made structural studies possible. When presented with only one type of sugar donor, LARGE will catalyse a single reaction, e.g. reacting GlcA-β-MU with UDP-Xyl will produce Xylα1-3GlcA-β-MU (MU denotes 4-methylumbelliferone). The reaction product can be purified and reacted with the other type of sugar donor, and so on. In this way, defined oligosaccharides can be obtained in sufficient quantity for X-ray crystallography and biophysical experiments. By soaking one such oligosaccharide into crystals of laminin α2 LG4-5, we were able to determine the structure of a laminin-carbohydrate complex at 1.4 Å resolution (Figure 1B) [43••].

In the structure of the complex, the carbohydrate ligand completes the octahedral coordination sphere of the Ca^{2+} ion in LG4. Specifically, a single GlcAβ1-3Xyl disaccharide unit chelates the Ca^{2+} ion, with coordination bonds formed by the carboxylate group of GlcA and the 4-OH group of Xyl. In addition, a total of four hydrogen bonds are formed between LG4 and the carbohydrate ligand, three with the Ca^{2+}-chelating GlcA-Xyl disaccharide and one with the following GlcA. Water molecules are notably scarce at the binding site. This binding mode is similar to that employed by C-type lectins [44,45], but there is one important difference: the Ca^{2+} ion in C-type lectins is coordinated by a single sugar (Figure 1C), whereas the Ca^{2+} site in the LG4 domain is coordinated by a

disaccharide. This difference likely accounts for the high specificity and affinity of the laminin-matriglycan interaction. Specificity for GlcA results from the two strong hydrogen bonds to the sugar's carboxylate group; specificity for Xyl results from close contacts between the LG domain and the C5 atom of Xyl, which discriminate against hexoses; and specificity for the β1-3 glycosidic linkage is dictated by the Ca^{2+} coordination geometry. NMR titration of a GlcA-Xyl-GlcA-Xyl-GlcA-MU pentasaccharide with laminin α2 LG4-5 gave a dissociation constant of 230 nM [43••]. By comparison, solid-phase binding assays with full-length laminin and native dystroglycan typically give dissociation constants of 1-10 nM [46]. The tighter binding likely results from the presence of two independent matriglycan binding sites in laminin (LG1-3 and LG4-5) and the many GlcA-Xyl repeats in native matriglycan, i.e. from an avidity effect.

Laminin LG domains also bind other anionic carbohydrate ligands, namely heparan sulphate/heparin and sulphated glycolipids [32]. Heparin inhibits α-DG binding to laminin-111, but not to laminin-211 [47]. Importantly, however, heparin binding does not require Ca^{2+} [48]. Results from site-directed mutagenesis suggest that basic residues in laminin α1 LG4-5 and α2 LG4-5 are important for both heparin and matriglycan binding [41,42]. In our crystal structure of laminin α2 LG4-5 with a matriglycan oligosaccharide only Arg2803 interacts directly with the ligand [43••]. The role of the other basic residues may be to reduce the electrostatic repulsion between laminin and the polyanionic matriglycan chain.

A couple of anomalies remain in our current understanding of the laminin-matriglycan. First, it is not clear why certain Ca^{2+}-binding LG domains do not seem to bind α-DG (e.g. α1 LG5 [35]). Second, α5 LG4 binds α-DG Ca^{2+}-dependently [49], yet neither of the two aspartic acid residues that coordinate the Ca^{2+} ion in α1 LG4 and α2 LG4 are conserved in α5 LG4. Clearly, further structure-function studies are needed.

Other α-DG-binding proteins with LG domains

Apart from laminins, LG domains are present in a number of large multidomain proteins that have been shown to bind to α-DG (Figure 2A). These include: agrin and perlecan, the two major BM proteoglycans; neurexin, a synaptic transmembrane protein; pikachurin, a matrix protein located in the photoreceptor ribbon synapse; and Slit2, a matrix protein important for axon guidance. In all these cases, Ca^{2+} is essential for α-DG binding and suitable Ca^{2+} ligands are present in at least one of the LG domains (Figure 2B). These Ca^{2+} sites are predicted to bind matriglycan in the same way as in laminin α2 LG4 [43••].

Binding of agrin to α-DG was discovered independently by four laboratories in 1994 [50]. As for laminins, more than one LG domain of agrin is involved in α-DG binding, but additional complexity is introduced by alternative splicing of agrin's LG domains. A splice insert in LG2 is required for heparin binding by agrin, and a splice insert in LG3 is required for the acetylcholine receptor-clustering activity of neuronal agrin. Both of these inserts reduce the affinity for α-DG, in agreement with the location of the splice sites close to the conserved Ca^{2+} sites in LG2 and LG3 [51,52]. Binding of perlecan α-DG also seems to involve more than one LG domain, but no detailed studies have been carried out [35].

Binding of neurexin to α-DG was reported in 2001 [53], but the physiological significance of the interaction is still unclear. The α-DG binding sites in LG2 and LG6 of neurexin-1 have been probed by site-directed mutagenesis and map to the immediate surroundings of the conserved Ca^{2+} sites [54]. Interestingly, the Ca^{2+} coordination in LG2 involves only one protein side chain instead of the usual two [55]. As for agrin, alternative splicing of neurexin LG domains has a negative impact on α-DG binding [54].

The interaction of pikachurin with α-DG is required for the proper synaptic connection between retinal photoreceptor and bipolar cells [56]. Dissection of pikachurin's LG region revealed that no single LG domain is sufficient for α-DG binding and that the LG2-EGF-EGF-LG3 fragment fully retains the α-DG binding activity of pikachurin [57].

The interaction of Slit2 with α-DG was discovered in a genetic screen in mice [58], which revealed axon guidance defects resulting from mutations in *B3gnt1* (now known to code for the glucuronyltransferase B4GAT1 [24,25]), *ISPD* (now known to code for the enzyme that synthesises the CDP-ribitol used by FKTN and FKRP [18••,19••,20••,59]), and *dystroglycan* itself. These defects resembled those of *Slit/Robo* mutants, and biochemical experiments indeed established a direct interaction of Slit2 with α-DG [58]. Slit2 is unique among α-DG binders in that it contains only a single LG domain.

Considering these examples, I would argue that the presence of multiple LG domains in a secreted protein is sufficient evidence to warrant an examination of α-DG binding. An intriguing candidate in this regard is EYS, a large extracellular matrix protein located in the connecting cilium and outer segment of retinal photoreceptor cells [60,61]. EYS contains five LG domains, the last three of which are predicted to have functional Ca^{2+} binding sites (Figure 2B). Mutations in *EYS* are a common cause of autosomal recessive retinitis pigmentosa [62–64]. Over 100 unique missense variants have been detected, two of which affect a putative Ca^{2+} ligand in LG4 (Asp2746). Functional studies in vertebrates are complicated by the lack of a functional EYS gene in mice, but EYS function can be studied in zebrafish, which have a functional O-mannosyl/matriglycan system [60,61,65].

Cooperativity in α-DG binding

With the exception of Slit2 (and possibly the laminin α5 chain), proteins use multiple LG domains to bind α-DG. The length of the matriglycan chains *in vivo* is not known, but may exceed 100 disaccharide repeats [66]. By repeating the glycosidic angles observed in the oligosaccharide bound to laminin α2 LG4-5 [43••], the matriglycan chain can be modelled as a helix with a rotation of 107° and a translation of 7.8 Å per GlcA-Xyl disaccharide. This model does not take into account any flexibility, of course, but is nevertheless useful to appreciate the relative dimensions of protein and carbohydrate (Figure 3). A single LG domain will block access to no more than ten disaccharide units, allowing for multiple binding events on a matriglycan chain. The simultaneous binding of several extracellular matrix proteins to a single maytriglycan chain may be important for a compact BM structure [66].

Concluding remarks

The last few years have seen a remarkable confluence of genetic, biochemical and structural studies, which finally have demystified the matrix-binding modification of α-DG. Given the increasing number of LG domain-containing proteins that bind to this modification, it seems timely to award the LG domain its own class in the lectin family. The LG domain fulfils all of the necessary criteria: evolutionary relationship, conserved structure of the carbohydrate binding site, conserved function in different proteins, ligand specificity, and so on. I would like to propose the term “D-type lectins”: D for dystroglycan, of course, but also for Dave (Briggs), whose crystallographic skills were instrumental in unveiling their mode of carbohydrate binding.

What remains to be done? An obvious question is whether the twenty-odd gene products required to make the matrix-binding modification of α-DG are also used to modify other proteins. Is Nature really so wasteful as to use them only for α-DG? It is also important to stress that the recent ground-breaking studies all used recombinant α-DG fragments. The complete structure of the matrix-binding modification on tissue-derived α-DG still needs to be confirmed and may well throw up another few surprises.

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Highlights

- The chemical structure of dystroglycan's laminin-binding modification is now known
- Laminin G-like domains bind to a polysaccharide of glucuronic acid-xylose units
- Crystal structure analysis has revealed the atomic details of this interaction

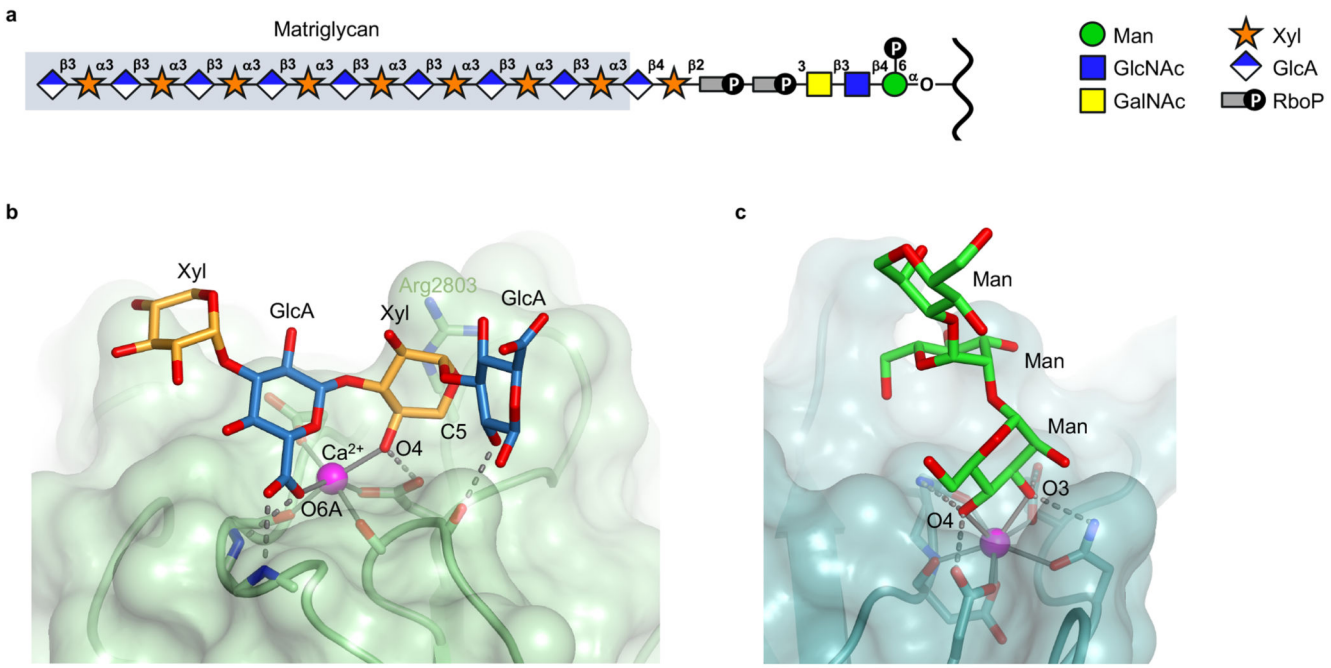

Figure 1.

The matrix-binding modification of α-DG and its interaction with LG domain 4 of the laminin α2 chain. (**a**) Schematic structure of the O-linked glycan on α-DG [3–6]. The matriglycan polysaccharide is highlighted by grey shading. (**b**) Crystal structure of a matriglycan oligosaccharide bound to laminin α2 LG4-5 [43••]. A GlcAβ1,3Xyl disaccharide unit straddles the Ca^{2+} ion in LG4 (magenta sphere). Metal ion coordination bonds and hydrogen bonds are shown as solid and dashed lines, respectively. Selected atoms are labelled (see text). (**c**) Ca^{2+}-dependent mannose binding to mannose-binding protein, a member of the C-type lectin subfamily [44,45].

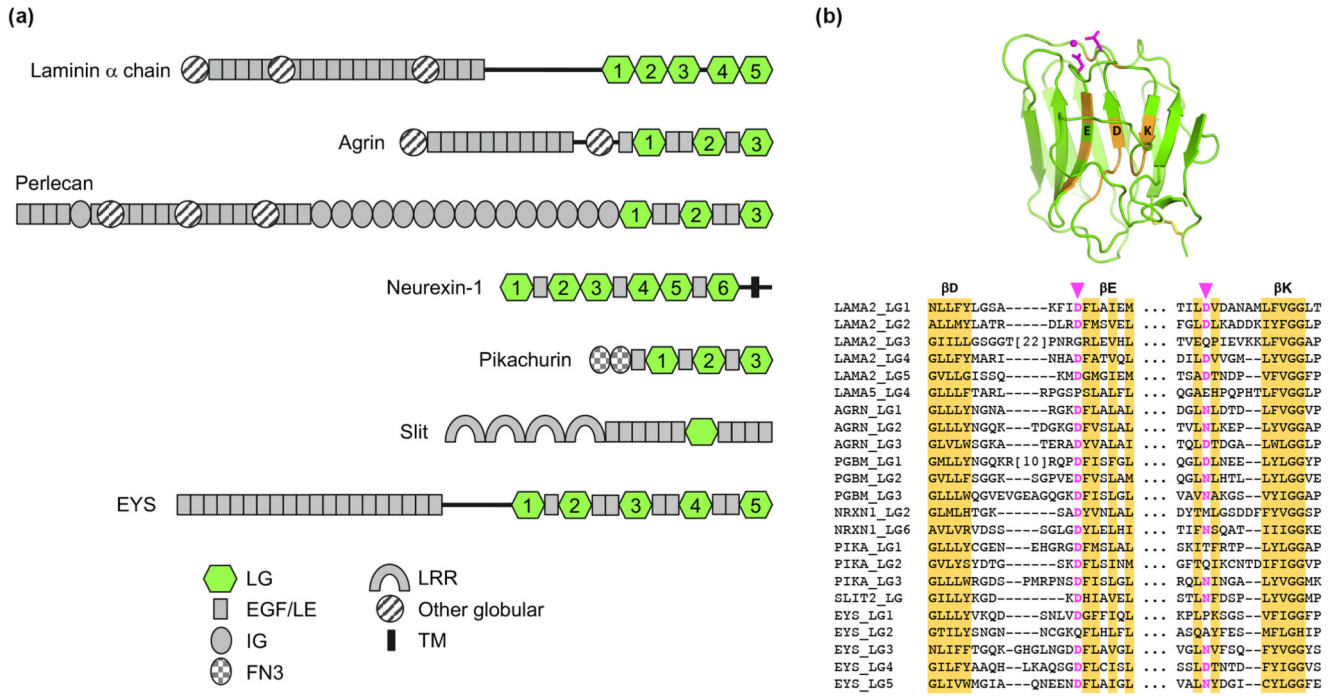

Figure 2.

LG domain-containing proteins that bind α-DG. (**a**) Domain organisation in α-DG-binding proteins. The LG domains are highlighted in green. Note that α-DG binding by EYS has not been demonstrated; the protein is included as a likely candidate (see text). (**b**) Conservation of the Ca^{2+}-binding site in many LG domains. The structure of laminin α2 LG4 is shown at the top (PDB 5IK5; magenta, Ca^{2+} ligands; orange, conserved residues). Below, a partial sequence alignment of the conserved regions is shown. Putative Ca^{2+}-binding residues are highlighted in magenta. LAMA2, human laminin α2 chain; AGRN, human agrin; PGBM, human perlecan; NRXN1, human neurexin 1; PIKA, human pikachurin; SLIT2, human Slit2; EYS, human EYS.

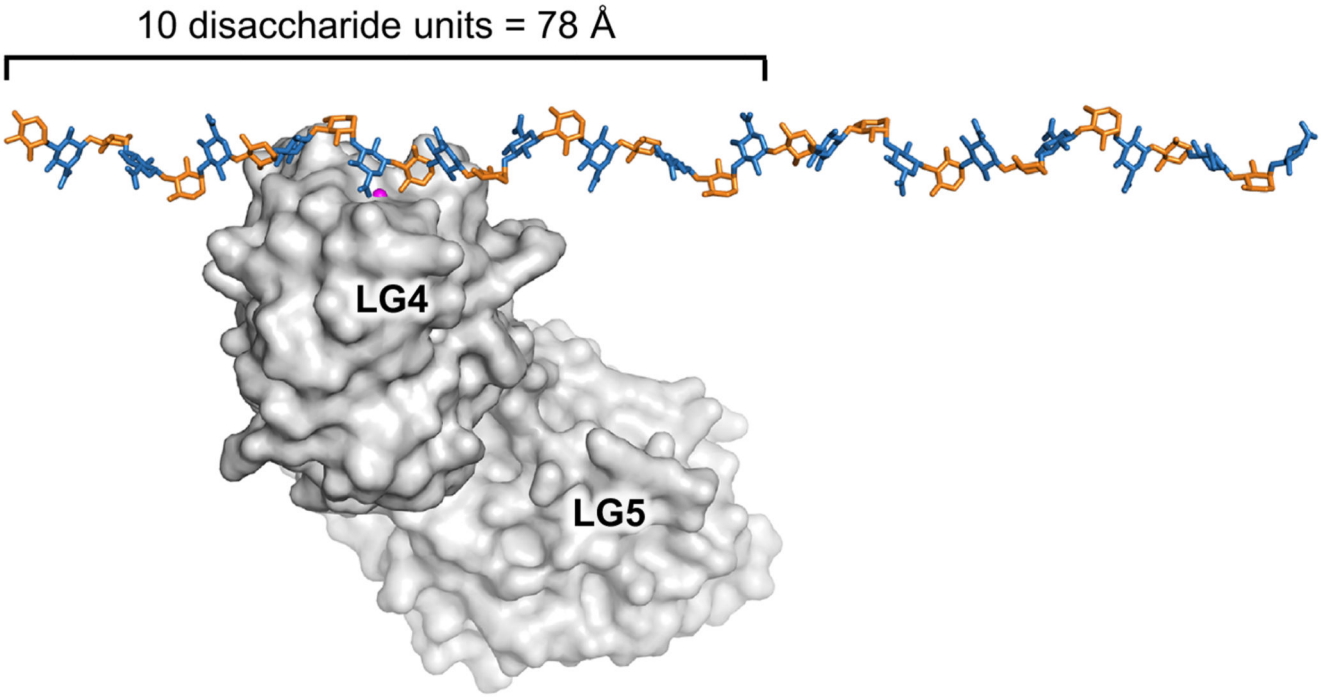

Figure 3.

Relative dimensions of LG domains and the matriglycan polysaccharide. The matriglycan chain was modelled by repeating the tetrasaccharide observed in the crystal structure with laminin α2 LG4-5 [43••]. This procedure gives a helix with a rotation of 107° and a translation of 7.8 Å per GlcA-Xyl disaccharide.