



Title	Exploring the In situ Pairing of Human Galectins and -Dystroglycan O-Mannosylated Core M1 Glycopeptides [an abstract of dissertation and a summary of dissertation review]
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Abstract of Doctoral Dissertation

Degree requested Doctor of Life Science Applicant's name Larena L. Villones Jr.

Title of Doctoral Dissertation

Exploring the *In situ* Pairing of Human Galectins and α -Dystroglycan
O-Mannosylated Core M1 Glycopeptides
 (コア M1 型糖鎖を有する α -ジストログリカン糖ペプチドとガレクチンの *In-situ* ペア探索研究)

Dystroglycan (DG), which constitutes a part of the dystrophin–glycoprotein complex, connects the extracellular matrix to the cytoskeleton. DG glycans are presented by the extracellular α -DG, serving as a contact point beyond the well-studied interaction between matriglycan and laminin G-like domains, providing muscular and neural cell stability (Fig. 1). However, it remains unknown as to whether core M1 (GlcNAc β 1-2Man) structures can serve as ligands among the various *O*-Mannosylated (*O*-Man) glycans. On the other hand, galectin (Gal) is a family of carbohydrate-binding proteins (CBPs) that bind specifically to β -galactose-containing glycoconjugates modulating wide-range of (patho)physiological processes, such as cell growth/adhesion/differentiation, regulation of immune response, inflammatory function, and tumor development and progression. Therefore, based on the presence of *N*-acetyllactosamine (LacNAc) in this type of glycan following core extension, the binding interactions with adhesion/growth-regulatory galectins were explored.

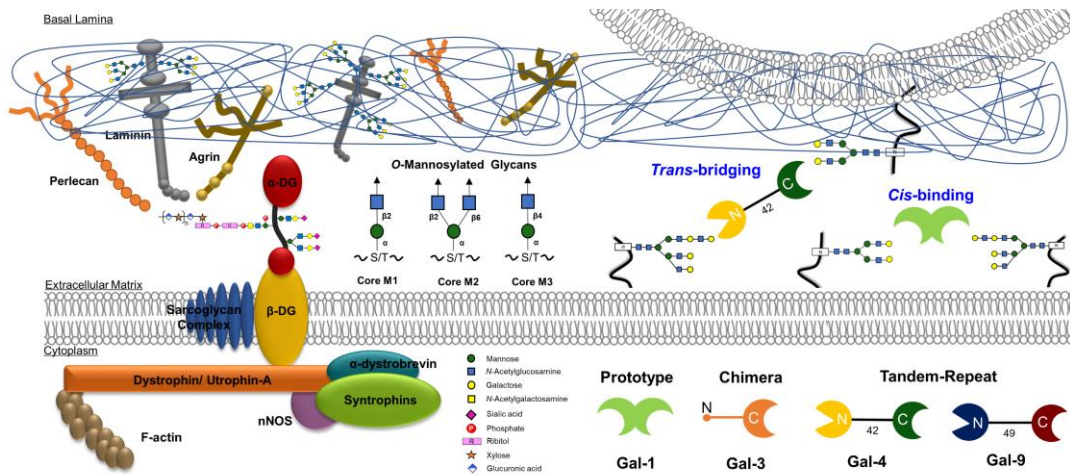


Figure 1. The dystrophin-glycoprotein complex (DCG) and types of human galectins. *O*-Man glycans identified in α -DG is also presented.

To elucidate this process, the interaction between the galectin (Gal)-1, -3, -4 and -9 with the core M1-based glycopeptide library of the α -DG fragment 372 TRGAIHQPTLGPIQPTRV 390 were profiled, using glycan microarray. The binding of the galectins was revealed irrespective of the type of modular architecture (Gal-1 >> Gal-4 > Gal-9, but very weak interaction with Gal-3), adding galectins to the list of possible binding partners of α -DG peptide and LacNAc presenting core M1 glycoconjugates by *cis*-binding via peptide- and carbohydrate-protein interactions, respectively. The binding of galectins was abrogated by α 2,3-sialylation of the LacNAc units. This molecular event was further verified by nuclear magnetic resonance studies, wherein the LacNAc-terminated α -DG glycopeptide was found to simultaneously interact with both the S- and F-faces

of Gal-1, thereby inducing oligomerization. Furthermore, the *trans-bridging* capabilities of Gal-1 with α -DG core M1 structures and laminins (-111, -121, -211, and -221, but little -511) were observed (Fig. 2), which proposed the possible mechanism by which Gal-1 prevents muscular dystrophies; however, this proposal warrants further investigation.

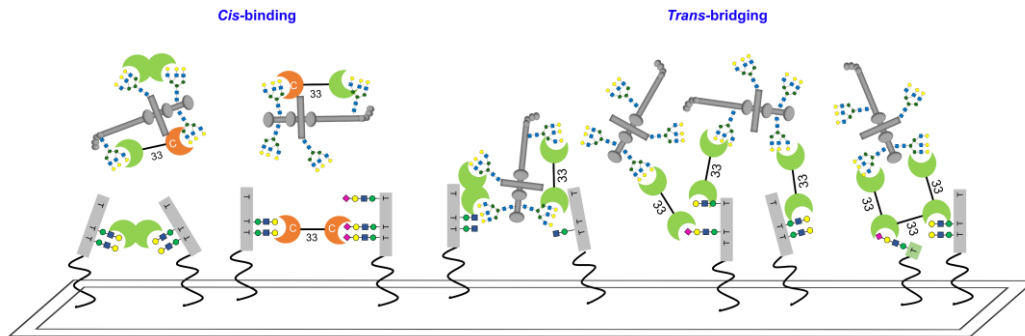


Figure 2. The *cis*-binding and *trans*-bridging activities of Gal-1 and its variants with α -DG core M1 glycopeptides and laminin *in situ*.

The multifunctionality of galectins regulating a broad range of fundamental cellular processes via *cis*- and *trans*-activities has achieved wide attention in exploring beyond the importance of natural specificity/selectivity to the glycoconjugate receptors, i.e., its modular architecture to present the carbohydrate recognition domain (CRD) variously. Combining Gal-1, -3, -4, and -9 variant test panels and synthetic α -DG *O*-Man core M1 glycopeptide microarray library, a detailed comparative analysis is possible in delineating design-functionality relationships within this lectin family towards its affinity to the prepared glycoconjugates of α -DG. Enhancement of affinity towards the prepared ligands was observed in linker-connected di- and tetramer Gal-1 variants, while converting this to a Gal-3-like protein decreased binding. Presenting Gal-3 as a prototype markedly increased the susceptibility to the test compounds. While inserting a peptide linker between Gal-3 CRDs to form a tandem-repeat type diminished the binding. The galectin-4 variants have shown that the natural linker is detrimental to its interaction with α -dystroglycan glycoconjugates. On the other hand, Gal-9 variants revealed the importance of the C-terminal CRD on the binding affinity. Compared to Gal-1 wild-type, Gal-1 variants demonstrated higher *trans*-bridging capabilities between LacNAc- and sialyl-LacNAc-terminated *O*-Man core M1 α -dystroglycan glycopeptides and laminins (-111, -121, -211, -221, and -511) *in situ* (Fig. 2). This suggests possible higher translational applications of these galectin variants in the treatment of some forms of α -dystroglycanopathy.

Overall, our experimental setup revealed that *O*-Man core M1 glycopeptides of α -DG could serve as ligands for galectins *in situ* via *cis*-binding. In addition, the prototype Gal-1 can *trans*-bridge *O*-Man core M1 glycopeptides of α -DG and laminins in microarray. We also demonstrated that the alteration of the galectin structures can give additional insights into the preferential modular architecture and binding behavior of this lectin family towards specific ligands. Furthermore, rational protein engineering is indeed a useful tool in redesigning lectins with possibly higher therapeutic potentials than their wild-type counterpart. Here, Gal-1 and its variants has proof-of-principle character in *trans*-bridging *O*-Man core M1 glycopeptides of α -DG and laminins *in situ*.