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Structural Studies on Different Ligand Binding Ability of Sialoadhesin Using Molecular Modeling Techniques

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ABSTRACT

Siglecs are the major homologous subfamily of I-type lectins with an ability to recognize sialylated glycans. Siglecs are attractive therapeutic targets because of their endocytic properties, ability to modulate receptor signaling and cell-type specific expression pattern. Sialoadhesin (Sn/Siglec-1/CD169), a member of the Siglec family expressed on subsets of resident and inflammatory macrophages and involves in modulation of inflammation and immunity. In this work, 3-D structure of human Siglec-1 (hSiglec-1) was predicted based on X-ray crystallo-graphically determined structure of mouse Siglec-1[mSiglec-1(PDB ID: 1QFP)] using molecular modeling techniques. The structure of complexes in solution of hSiglec-1 with ligands, glycopeptide and 3′-sialyllactose were predicted using a novel docking technique comprising of repeated cycles of molecular dynamics and energy minimization. Calculation of the free energies of binding of complexes suggested that glycopeptide can form stable complex with dissociation constant value of 3.31 μM whereas complex formation of 3′-sialyllactose with the protein in aqueous medium is thermodynamically unfavorable. The structural analysis of theses complexes represent the functional recognition interactions of this protein with the bound sugar molecule and as such provide detailed information about functional roles of such sugar binding protein.

KEYWORDS

Glycopeptide, 3′-Sialyllactose, Docking, Sialic acid, Sialoadhesin.

INTRODUCTION

A specialised subgroup of the immunoglobulin (Ig) superfamily, which shares a considerable sequence similarities and can identify sialylated glycoconjugates, called as Siglecs [1]. Sialic acid (Neu5Ac) is an acidic monosaccharide having nine carbons and found at the end of secreted O-glycans, *N*-glycans and glycolipids, which are located on the cell surface (*i.e.* glycocalyx present on the cell surface). For recognition, various pathogens utilise different derivatives of sialic acid; multicellular organisms employ the conjugates of sialic acid for mediating protein-protein interactions, cell adhesion and protein trafficking through receptors that recognize sialic acid. Conjugates play a crucial role in non-specific electrostatic repulsion among different cells [2-10]. Animal lectins or mammalian carbohydrate-binding proteins are categorized into different groups based on their structural features and on the type of the recognized carbohydrate ligands [11]. Siglecs are the membrane proteins of type 1, which comprise a N-terminal V-set Ig domain that binds to sialic acid and identifies sialy-

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lated glycoconjugates, different numbers of C2-set Ig-like domains, a cytoplasmic tail and a transmembrane domain [12,13]. Siglecs are classified into two subgroups on the basis of the similarity in the sequence of intracellular and extracellular regions. The first subgroup is composed of sialoadhesin (Siglec-1), CD22 (Siglec-2), MAG and Siglec-15; this subgroup shares the sequence identity of 25-30% in the extracellular region and has varying cytoplasmic tails. The second subgroup is com-posed of CD33 (Siglec-3) related Siglecs [14]. The proteins of this subgroup share a sequence similarity of 50-80% and have two substantially conserved motifs based on tyrosine in cytoplasmic tails. Mainly in the immune and hematopoietic systems in humans, all human Siglecs are expressed in the cell type-specific manner, which indicates involvement in wide-ranged discrete functions including activating B cell (CD22), controlling CD33 and myeloid cell interactions (sialo-adhesin), maintaining myelination in nervous systems (MAG), and regulating neuronal cell growth [15-20].

Siglec-1 controls the immune response of infections, especially the infection of human immunodeficiency virus (HIV), through the recognition of viral membrane ganglioside and is highly promising in inflammatory disease treamtemts [21-23]. In present study, the 3-D structures of hSiglec-1 along with specific ligands, glycopeptide and 3′-Sialyllactose were predicted. The structural analyses of the predicted complexes and the theoretical dissociation constant values were also calculated for the complexes, which facilitated to compare the relative binding affinity.

EXPERIMENTAL

Through homology modelling based on knowledge, the initial structure of human Siglec-1 (hSiglec-1) was predicted using in-house softwares of MODELYN and ANALYN [24]. For homology modelling, the initial scaffold was the structure of Mus Musculus (PDB ID: 1QFP) determined using X-ray crystallography. The starting structure of a Siglec-ligand complex was acquired by superposing the experimental Mus Musculus (PDB ID: 1QFO) structure on the structure of modeled hSiglec-1 and then, by optimizing this structure through the repeated simulations of dynamics and energy minimization. The InsightII 2005 of Accelrys (San Diego, USA) provided with the molecular dynamics and energy minimisation module called DISCOVER was used to refine the structure. The structure was optimised through energy minimisation (100 steps of each conjugate gradient and steepest descent methods) by using cff91 force field and then dynamics simulations. Typically, a dynamics run comprised 1000 equilibration steps using a conformational sampling strategy of 1 in 100 steps at 300K followed by 1,00,000 steps of 1 femto-second. By using the InsightII ANALYSIS module, the conformation having the lowest potential energy was selected for the subsequent refinement cycle at the end of dynamics simulations. This combination of dynamics and minimisation was iterated to obtain suitable conformational parameters.

By using the assembly/soak option of InsightII, the water molecule spheres of 18 Å radius were added roughly at the centre of ligand molecules so that water molecules were completely surrounded to explore the effect of water on ligand binding. In the aqueous environment and presence and absence of protein molecules, the ligand structure was optimized through molecular dynamics and energy minimization simulations. The method of linear interaction energy approximation reported by Aqvist *et al*. [25] was employed to absolute binding energies by using the free energy values of ligand complex formation obtained in water and water-protein environments. The relation used is $\Delta G_{bind} = \alpha \Delta < V^{el}_{l\text{-}s} > + \beta \Delta < V^{vdw}_{l\text{-}s} >$, where ΔG_{bind} denotes the absolute energy of binding and Δ is the difference between the van der Waals ($V^{vdw}_{l\text{-}s}$) and electrical ($V^{el}_{l\text{-}s}$) components of the free energies of ligand solvent (l-s) systems, *i.e.* in protein containing and pure water environments. According to the study of Åqvist *et al*. [25] and other studies, the factors of the contributions of van der Waals and electric components were considered 0.16 (β) and 0.5 (α), respectively [26,27]. The thermodynamic relation $\Delta G_{bind} = -RT\ln K_a$ was used to calculate the association constant (K_a), using where T is the absolute temperature and R is the ideal gas constant; the inverse of K_a was calculated as the dissociation constant (K_d).

MODELYN was run on both a FUEL workstation of Silicon Graphics, Inc. in the IRIX environment and IBM-compatible personal computer in windows. In the same environment, InsightII was run on the Altrix 350 server and FUEL workstation of Silicon Graphics, Inc. MOLMOL was employed to analyze the electrostatic potential surface of proteins [28], while the PROCHECK software was used to investigate the structural parameters [29]. In the UNIX operating system, both MOLMOL and PROCHECK were executed on FUEL. In the UNIX system, InsightII was executed on the FUEL workstation of Silicon Graphics, Inc. The all-atom contact analysis was performed using MOLPROBITY [30] for clashscores (number of atoms with atom pair overlaps ≥ 0.4 Å present in 1000 atoms) and for rotamer outlier calculations. The InsightII DOCKING module was employed to determine the binding affinities of Siglec-ligand complexes.

The BUILDER module of InsightII was employed to produce ligand structures followed by optimization using iterated molecular dynamics and energy minimisation simulations. To acquire homologous sequences, protein BLAST [31] was employed.

RESULTS AND DISCUSSION

The combinations ANALYN and MODELYN were used to predict the initial three-dimensional structure of the target protein. Molecular dynamics and energy minimization were conducted to regularize the most influenced segments during insertion, loop grafting and deletion. The common structural characteristics of the predicted model were verified by measuring all bond angles and distances and calculating the deviations in these parameters from their standard values. The backbone conformation quality was verified by drawing Ramachandran's plots for the structure and calculating psi and phi dihedral angles. Table-1 presents the root mean square deviation (RMSD) of the bond angles and lengths of the predicted structure and the percentages of the Phi–Psi angles of backbones observed in the different areas of Ramachandran's plots obtained after 3D structure prediction.

The RMSD in the bond angles and lengths of approximately 3º and 0.02 Å, respectively, from their respective standard

TABLE-1
GENERAL AND BACKBONE STRUCTURAL PARAMETERS OF THE MODELED STRUCTURE OF THE TARGET SEQUENCE AS WELL AS THE X-RAY STRUCTURES OF THE SIGLEC

Siglecs	Accession No	% of AA Identity (positive score)			% of Phi-Psi pairs in the area			
			Bond (Å)	Angle (°)	Core	Allowed	Generously allowed	Dis-allowed
mSiglec-1	1QFP	100	0.018	2.33	77.2	19.8	2.0	1.0
mSiglec-1	1QFO	100	0.016	2.51	83.0	16.0	0.0	1.0
hSiglec-1	Q9BZZ2	77(89)	0.014	2.26	78.2	18.8	2.0	1.0

values showed that general structural parameters of the predicted structure were satisfactory. The good backbone conformation quality of the modeled structure was indicated by the overall values of > 95% of Phi–Psi pairs in the core and allowed areas of Ramachandran's plot.

PROCHECK was employed to determine the planarity of the side chains of planar groups in tyrosine, phenylalanine, histidine, tryptophan, glutamine, arginine, glutamic acid, asparagines and aspartic acid, in which the deviations from the standard planarity were identified through the calculation of the root mean square (RMS) of the distances of planar atoms from the most suitable plane. Residues with the RMS distances of > 0.03 and 0.02 Å for rings and other groups, respectively, were considered outliers [29] (Table-2). The rotamer and clash-scores outliers were calculated by employing MOLPROBITY to analyze the protein geometry of the modeled structure [30] (Table-2).

The Siglec-1, multi-domain–Ig-like receptor protein connected to the surface of macrophage membrane, is expressed in the highest level under non-inflammatory conditions in the secondary lymphoid and haemopoietic (that bind preferentially to a mature granulocyte) tissues [32,33]. In lymphoid tissues, Siglec-1 may behave as lymphocyte adhesion molecules. The selective expression of Siglec-1 on macrophages in a marginal zone of spleens indicates its role in antigen presentation to the B-cells [34,35]. In bone marrow, Siglec-1 is present at the location of contacts between developing granulocytes and macrophages. Under chronic inflammatory conditions, such as rheumatoid arthritis and atherosclerosis, high levels of Siglec-1 are expressed in active inflammatory macrophages [15,33]. Siglec-1 comprises an N-terminal V-set Ig-like domain (SnD1) and 16 C2-set Ig domains and is the largest known human Ig-like lectin. These 16 C2-set Ig domains ensure that the terminal V-set domain does not contact with the residues of sialic acid on macrophages and exhibits activity towards the sialic acid conjugate present on target cells [36]. The hSiglec-1 structure was modeled by using the crystal structure of the unliganded form of mSiglec-1(PDB ID: 1QFP) as the template. The hSiglec-1 (Accession No: Q9BZZ2) showed 89% and 77% approximately sequence similarity and identity, respectively, with query sequence. Moreover, Siglec-1 preferentially binds with α(2,3) Sia-linked ligands [37-39], while mSiglec-1 binds more strongly with glycopeptide ligands than with 3′-Sialyllactose [40]. Thus, the following ligands were selected to investigate the binding preferences of hSiglec-1.
Glycopeptide: Ala-Gly-His-Thr(Neu5Ac)-Trp-Gly-His-NH2 and; **3′-Sialyllactose:** NeuAcα2,3Galβ1,4Glc.

Glycopeptides and 3′-sialyllactose were docked at the binding sites of the modeled structure by superposing them onto X-ray structures, which were bound to glycopeptides (1URL) and 3′-sialyllactose (1QFO) with respect to the regions that were structurally conserved and then glycopeptides and 3′-sialyllactose were transferred to the binding sites. The complex structures were optimized by iterating energy minimization and molecular dynamics in the presence of water. The free energy of complex formation was calculated. Tables 3 and 4 present the binding energies and their H-bonding patterns, respectively. Linear interaction energy approximation was used to calculate the ΔG_{bind} values for modeled hSiglec-1 complexes with glycopeptides and 3′-sialyllactose (Table-3). The calculated ΔG_{bind}

TABLE-2
GENERAL AND BACKBONE STRUCTURAL PARAMETERS OF THE MODELED STRUCTURE OF THE TARGET SEQUENCE IN COMPARISON WITH THE X-RAY STRUCTURES OF THE SIGLEC

Siglecs	Accession No	All atom clashcore (per 1000 atom)	Rotamer outliers (%)	Planarity outliers (%)
mSiglec-1	1QFP	3.16	3.42	0.0
mSiglec-1	1QFO	3.26	4.81	0.0
hSiglec-1	Q9BZZ2	4.57	4.95	0.0

TABLE-3
EMPIRICAL FREE ENERGIES, THEIR DIFFERENCE IN WATER AND WATER-PROTEIN ENVIRONMENTS AND CORRESPONDING ΔG AND K_d VALUES FOR THE COMPLEX FORMATION BETWEEN THE hSiglec-1 AND ITS SPECIFIC LIGANDS IN THE AQUEOUS SOLUTION

Complex	Free energy (kcal/mol)			Difference		ΔGbind (kcal/mol)	K_d
	Vdw	Electrical	Total	Vdw	Electrical		
hSiglec-1- Glycopeptide in solution	-91.45	-430.58	-522.03	+8.09	-17.71	-7.57	3.31 μM
Glycopeptide*	-99.54	-412.87	-512.41				
hSiglec-1-3'-Sialyllactose in solution	-70.77	-106.58	-177.35	+0.53	+138.51	+69.34	
3'-Sialyllactose*	-71.30	-245.09	-316.39				

*Values corresponding to the interaction energies in presence of water molecules only.

TABLE-4
HYDROGEN-BOND NETWORK WITHIN THE BINDING SITE OF hSiglec-1 IN COMPLEX WITH GLYCOPEPTIDE. DISTANCES ARE MEASURED BETWEEN HYDROGEN AND ACCEPTOR OR DONOR ATOM

Ligand-protein hydrogen-bonds		
Atoms of glycopeptide	Atoms of hSiglec-1	Distance (Å)
Neu5Ac		
O1A	Arg-97:NH2	2.05
O1B	Arg-97:NH1	1.84
O1A	Arg-105:NH1	2.03
Hia		
ND1	Tyr-41: OH	1.66
N	Glu-99: OE1	1.90
Gly		
N	Glu-99: OE1	2.01
His		
ND1	Arg-105:NH1/Arg-105:NH2	2.00/1.83
Ligand-protein hydrogen-bonds mediated by water		
Atoms of glycopeptide	Atoms of hSiglec-1	*Distance (Å)
His NE2	Glu-102: OE2/OE1	4.91/4.83
Hia N	His-53: NE2	3.64
Intramolecular hydrogen-bonds		
Atoms of glycopeptide	Atoms of glycopeptide	Distance (Å)
Neu5Ac O8	Neu5Ac O1B	2.00
Neu5Ac O7	Neu5Ac O9	1.88

*Distances between atoms linked through hydrogen-bonding *via* water molecule

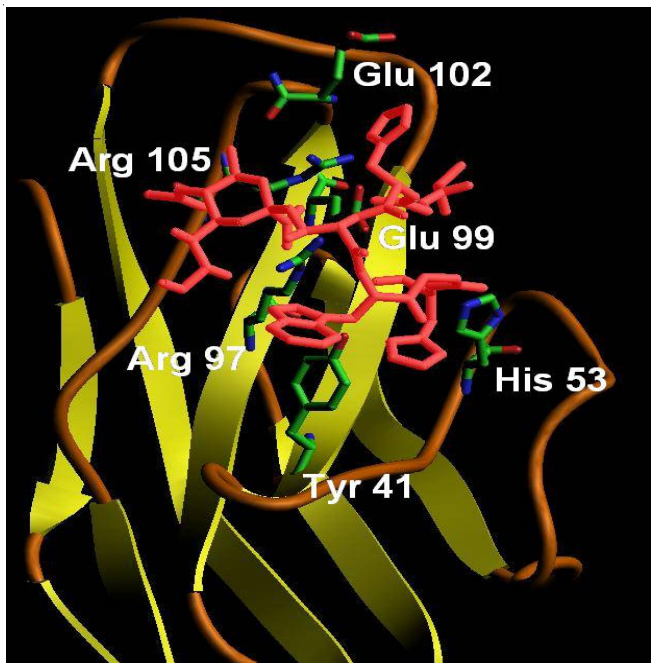

Fig. 1. Mode of ligand binding in hSiglec-1: Ligand binding environment is shown in the secondary structure environment of the modeled lectin. Beta sheets are shown in yellow with an arrow indicating the C-terminus and random coils as thin cylinder coloured in maroon. The residues of the protein involved in hydrogen bonding with the ligand are shown in stick representation, coloured as atoms (C=Green, O=Red and N=Indigo) and labeled as AA-ResidueID. The ligand, glycopeptide, is shown in stick representation in red colour

value for the glycopeptide complex with hSiglec-1 is negative; however, the calculated ΔG_{bind} value of 3′-sialyllactose complex with hSiglec-1 is positive, which indicated that in aqueous medium, the formation of 3′-sialyllactose complex with the protein is thermodynamically unfavourable. Therefore, the protein forms a stable complex with glycopeptides because compared with only water, in the protein-water environment, the complex exhibits lower free energy. The dissociation constant (K_d) of 3.31 μM corresponds to the ΔG_{bind} value for the glycopeptide-protein complex.

A large portion of electrical energy emerges from the hydrogen bonding, which plays a substantial role in the binding affinity. Vital interactions between the carboxyl group of sialic acid and Arg-97 are conserved in the structure [37,38,40]. Furthermore, the side chains of Tyr-41, Arg-105 and Glu-99 directly form hydrogen bonding with ligands, whereas those of His-53 and Glu-102 form water-mediated hydrogen bonding (Fig. 1). Two intramolecular hydrogen bonding stabilizes the bound conformations of ligands.

Conclusion

In this work, the 3D structure of human Siglec-1 was predicted and refined to obtain best backbone and sidechain conformation by executing repeated molecular dynamics and energy minimization and picking the most reliable structure. Although, the structural models do not cover the entire sequence of the biochemical lectin, which participate in many crucial phenomena of the mammalian life process, present predictions were limited only to the extent of the experimental structures available for proteins homologous to the hSiglec-1. None-theless, the structure encompassed the important segments known to participate in their biological activities. Structures of the complexes of the modeled hSiglec-1 with its specific ligands, glycopeptide and 3′-sialyllactose were predicted using a novel docking technique. The nature of interactions of the ligands with hSiglec-1 was examined in details in order to understand the origin of their specificity at the atomic levels. The involvement of the crucial amino acids, identified by experimental techniques, was confirmed from the modeled structure by exploring the involvement of evolutionary conserved amino acids. The chemical environment leading the stability of the bound ligands were analyzed in atomic details in presence of water molecules to simulate closely the aqueous environment. Dissociation constant (K_d) value of 3.31 μM for glycopeptide-protein complex reflects a very high binding affinity of glycopeptide. The absolute binding energy (ΔG_{bind}) value is positive for the complex of 3′-sialyllactose with hSiglec-1 indicating that the complex formation in the aqueous medium is thermodynamically unfavorable. Thus, present structural studies using predicted model of human Siglec-1 and its complexes with specific ligands have contributed significantly in understanding the interactions involving sialic acid containing bioactive molecules, which are implicated in many important biochemical phenomena. The knowledge of these modeled structures and particularly of their carbohydrate recognition domains may provide valuable information in developing potent therapeutics.

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REFERENCES

1. P.R. Crocker, E.A. Clark, M. Filbin, S. Gordon, Y. Jones, J.H. Kehrl, S. Kelm, N. Le Douarin, L. Powell, J. Roder, R.L. Schnaar, D.C. Sgroi, K. Stamenkovic, R. Schauer, M. Schachner, T.K. van den Berg, P.A. van der Merwe, S.M. Watt and A. Varki, Siglecs: A Family of Sialic-Acid Binding Lectins, *Glycobiology*, **8**, v (1998); https://doi.org/10.1093/oxfordjournals.glycob.a018832
2. O. Blixt, B.E. Collins, I.M. van den Nieuwenhof, P.R. Crocker and J.C. Paulson, Sialoside Specificity of the Siglec Family Assessed using Novel Multivalent Probes-Identification of Potent Inhibitors of Myelin-Associated Glycoprotein, *J. Biol. Chem.*, **278**, 31007 (2003); https://doi.org/10.1074/jbc.M304331200
3. S. Kelm, R. Schauer, J.C. Manuguerra, H.J. Gross and P.R. Crocker, Modifications of Cell Surface Sialic Acids Modulate Cell Adhesion Mediated by Sialoadhesin and CD22, *Glycoconj. J.*, **11**, 576 (1994); https://doi.org/10.1007/BF00731309
4. L.D. Powell and A. Varki, The Oligosaccharide Binding Specificities of CD22 beta, A Sialic Acid-Specific Lectin of B Cells, *J. Biol. Chem.*, **269**, 10628 (1994).
5. E.C. Brinkman-Van der Linden and A. Varki, New Aspects of Siglec Binding Specificities, Including the Significance of Fucosylation and of the Sialyl-Tn Epitope, *J. Biol. Chem.*, **275**, 8625 (2000); https://doi.org/10.1074/jbc.275.12.8625
6. A.L. Cornish, S. Freeman, G. Forbes, J. Ni, M. Zhang, M. Cepeda, R. Gentz, M. Augustus, K.C. Carter and P.R. Crocker, Characterization of Siglec-5, A Novel Glycoprotein Expressed on Myeloid Cells Related to CD33, *Blood*, **92**, 2123 (1998); https://doi.org/10.1182/blood.V92.6.2123
7. G. Nicoll, J. Ni, D. Liu, P. Klenerman, J. Munday, S. Dubock, M.-G. Mattei and P.R. Crocker, Identification and Characterization of a Novel Siglec, Siglec-7, Expressed by Human Natural Killer Cells and Monocytes, *J. Biol. Chem.*, **274**, 34089 (1999); https://doi.org/10.1074/jbc.274.48.34089
8. A. Varki, Sialic acids as Ligands in Recognition Phenomena, *FASEB J.*, **11**, 248 (1997); https://doi.org/10.1096/fasebj.11.4.9068613
9. K.A. Karlsson, Meaning and Therapeutic Potential of Microbial Recognition of Host Glycoconjugates, *Mol. Microbiol.*, **29**, 1 (1998); https://doi.org/10.1046/j.1365-2958.1998.00854.x
10. P.R. Crocker and A. Varki, Siglecs, Sialic Acids and Innate Immunity, *Trends Immunol.*, **22**, 337 (2001); https://doi.org/10.1016/S1471-4906(01)01930-5
11. K. Drickamer, Increasing Diversity of Animal Lectin Structures, *Curr. Opin. Struct. Biol.*, **5**, 612 (1995); https://doi.org/10.1016/0959-440X(95)80052-2
12. K.F. Bornhöfft, T. Goldammer, A. Rebl and S.P. Galuska, Siglecs: A Journey through the Evolution of Sialic Acid-Binding Immunoglobulin-Type Lectins, *Dev. Comp. Immunol.*, **86**, 219 (2018); https://doi.org/10.1016/j.dci.2018.05.008
13. T. Angata, R. Hingorani, N.M. Varki and A. Varki, Cloning and Characterization of a Novel Mouse Siglec, mSiglec-F-Differential Evolution of the Mouse and Human (Cd33) Siglec-3-Related Gene Clusters, *J. Biol. Chem.*, **276**, 45128 (2001); https://doi.org/10.1074/jbc.M108573200
14. P.R. Crocker, Siglecs: Sialic-Acid-Binding Immunoglobulin-like Lectins in Cell-Cell Interactions and Signalling, *Curr. Opin. Struct. Biol.*, **12**, 609 (2002); https://doi.org/10.1016/S0959-440X(02)00375-5
15. P.R. Crocker, A. Hartnell, J. Munday and D. Nath, The Potential Role of Sialoadhesin as a Macrophage Recognition Molecule in Health and Disease, *Glycoconj. J.*, **14**, 601 (1997); https://doi.org/10.1023/A:1018588526788
16. S.D. Freeman, S. Kelm, E.K. Barber and P.R. Crocker, Characterization of CD33 as a New Member of the Sialoadhesin Family of Cellular Interaction Molecules, *Blood*, **85**, 2005 (1995); https://doi.org/10.1182/blood.V85.8.2005.bloodjournal8582005
17. J.G. Cyster and C.C. Goodnow, Tuning Antigen Receptor Signaling by CD22: Integrating Cues from Antigens and the Microenvironment, *Immunity*, **6**, 509 (1997); https://doi.org/10.1016/S1074-7613(00)80339-8
18. C. Li, M.B. Tropak, R. Gerlai, S. Clapoff, W. Abramow-Newerly, B. Trapp, A. Peterson and J. Roder, Myelination in the Absence of Myelin-Associated Glycoprotein, *Nature*, **369**, 747 (1994); https://doi.org/10.1038/369747a0
19. D. Montag, K.P. Giese, U. Bartsch, R. Martini, Y. Lang, H. Blüthmann, J. Karthigasan, D.A. Kirschner, E.S. Wintergerst, K.-A. Nave, J. Zielasek, K.V. Toyka, H.-P. Lipp and M. Schachner, Mice Deficient for the Glycoprotein Show Subtle Abnormalities in Myelin, *Neuron*, **13**, 229 (1994); https://doi.org/10.1016/0896-6273(94)90472-3
20. T. Angata, Siglec-15: A Potential Regulator of Osteoporosis, Cancer, and Infectious Diseases, *J. Biomed. Sci.*, **27**, 10 (2020); https://doi.org/10.1186/s12929-019-0610-1
21. Y.-C. Liu, M.-M. Yu, Y.-F. Chai and S.-T. Shou, Sialic Acids in the Immune Response during Sepsis, *Front. Immunol.*, **8**, 1601 (2017); https://doi.org/10.3389/fimmu.2017.01601
22. S. Duan and J.C. Paulson, Siglecs as Immune Cell Checkpoints in Disease, *Annu. Rev. Immunol.*, **38**, 365 (2020); https://doi.org/10.1146/annurev-immunol-102419-035900
23. T. Angata, C.M. Nycholat and M.S. Macauley, Therapeutic Targeting of Siglecs using Antibody- and Glycan-Based Approaches, *Trends Pharmacol. Sci.*, **36**, 645 (2015); https://doi.org/10.1016/j.tips.2015.06.008
24. C. Mandal, MODELYN – A Molecular Modelling Program Version PC-1 0 Indian Copyright No 9/98 (1998).
25. J. Aqvist, C. Medina and J.-E. Samuelsson, A new Method for Predicting Binding Affinity in Computer-aided Drug Design, *Protein Eng.*, **7**, 385 (1994); https://doi.org/10.1093/protein/7.3.385
26. Johan and S.L. Mowbray, Sugar Recognition by a Glucose/Galactose Receptor-Evaluation of Binding Energetics from Molecular Dynamics Simulations, *J. Biol. Chem.*, **270**, 9978 (1995); https://doi.org/10.1074/jbc.270.17.9978
27. J. Hulte'n, N.M. Bonham, U. Nillroth, T. Hansson, G. Zuccarello, J. Åqvist, A. Bouzide, B. Classon, U.H. Danielson, A. Karlen, I. Kvarnstrom, B. Samuelsson and A. Hallberg, Cyclic HIV-1 Protease Inhibitors Derived from Mannitol: Synthesis, Inhibitory Potencies and Computational Predictions of Binding Affinities, *J. Med. Chem.*, **40**, 885 (1997); https://doi.org/10.1021/jm960728j
28. R. Koradi, M. Billeter and K. Wuthrich, MOLMOL: A Program for Display and Analysis of Macromolecular Structures, *J. Mol. Graph.*, **14**, 51 (1996); https://doi.org/10.1016/0263-7855(96)00009-4
29. R.A. Laskowski, M.W. MacArthur, D.S. Moss and J.M. Thornton, PROCHECK: A Program to Check the Stereochemical Quality of Protein Structures, *J. Appl. Cryst.*, **26**, 283 (1993); https://doi.org/10.1107/S0021889892009944
30. I.W. Davis, L.W. Murray, J.S. Richardson and D.C. Richardson, MOLPROBITY: Structure Validation and All-Atom Contact Analysis for Nucleic Acids and their Complexes, *Nucleic Acids Res.*, **32(Web Server)**, W615 (2004); https://doi.org/10.1093/nar/gkh398
31. S.F. Altschul, T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller and D.J. Lipman, Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs, *Nucleic Acids Res.*, **25**, 3389 (1997); https://doi.org/10.1093/nar/25.17.3389
32. P.R. Crocker, S. Freeman, S. Gordon and S. Kelm, Sialoadhesin Binds Preferentially to Cells of the Granulocytic Lineage, *J. Clin. Invest.*, **95**, 635 (1995); https://doi.org/10.1172/JCI117708
33. P.R. Crocker, S. Kelm, C. Dubois, B. Martin, A.S. McWilliam, D.M. Shotton, J.C. Paulson and S. Gordon, Sialoadhesin Binds Preferentially to Cells of the Granulocytic Lineage, Purification and Properties of Sialoadhesin, A Sialic Acid-Binding Receptor of Murine Tissue Macrophages, *EMBO J.*, **10**, 1661 (1991); https://doi.org/10.1002/j.1460-2075.1991.tb07689.x
34. T.K. van den Berg, J.J. Brevé, J.G. Damoiseaux, E.A. Döpp, S. Kelm, P.R. Crocker, C.D. Dijkstra and G. Kraal, Sialoadhesin on Macrophages: its Identification as a Lymphocyte Adhesion Molecule, *J. Exp. Med.*, **176**, 647 (1992); https://doi.org/10.1084/jem.176.3.647

35. B. Steiniger, P. Barth, B. Herbst, A. Hartnell and P.R. Crocker, The Species-Specific Structure of Microanatomical Compartments in the Human Spleen: Strongly Sialoadhesin-positive Macrophages Occur in the Perifollicular Zone, But Not in the Marginal Zone, *Immunology*, **92**, 307 (1997); https://doi.org/10.1046/j.1365-2567.1997.00328.x
36. J. Munday, H. Floyd and P.R. Crocker, Sialic Acid Binding Receptors (Siglecs) Expressed by Macrophages, *J. Leukoc. Biol.*, **66**, 705 (1999); https://doi.org/10.1002/jlb.66.5.705
37. N.R. Zaccai, K. Maenaka, T. Maenaka, P.R. Crocker, R. Brossmer, S. Kelm and E.Y. Jones, Structure-Guided Design of Sialic Acid-Based Siglec Inhibitors and Crystallographic Analysis in Complex with Sialoadhesin, *Structure*, **11**, 557 (2003); https://doi.org/10.1016/S0969-2126(03)00073-X
38. A.P. May, R.C. Robinson, M. Vinson, P.R. Crocker and E.Y. Jones, Crystal Structure of the N-Terminal Domain of Sialoadhesin in Complex with 3′-Sialyllactose at 1.85 Å Resolution, *Mol. Cell*, **1**, 719 (1998); https://doi.org/10.1016/S1097-2765(00)80071-4
39. A. Varki and T. Angata, Siglecs-The Major Subfamily of I-Type Lectins, *Glycobiology*, **16**, 1R (2006); https://doi.org/10.1093/glycob/cwj008
40. J.T. Bukrinsky, P.M. St. Hilaire, M. Meldal, P.R. Crocker and A. Henriksen, Complex of Sialoadhesin with a Glycopeptide Ligand, *Biochim. Biophys. Acta*, **1702**, 173 (2004); https://doi.org/10.1016/j.bbapap.2004.08.015