# A Review on the Role of Siglec-7 in Ovarian Cancer and Related Molecular Docking Studies

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

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## Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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# Approval

The thesis titled "A Review on the Role of Siglec-7 in Ovarian Cancer and Related Molecular Docking Studies" submitted by Noshin Atia Achol (21346097), of Spring, 2021 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.).

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## Ethics Statement

This project does not involve any kind of animal and human trials.

# Abstract

Ovarian cancer is one of the most lethal gynecological cancers in the world. Recent developments in immunology have revealed that ovarian cancer uses Siglec-7 protein. It is a sialic acid-binding immunoglobulin-like lectin that is expressed on natural killer cells. The pathophysiology of ovarian cancer and the potential of Siglec-7 as a therapeutic target are explored in this review. The focus is particularly on α-2,8-disialic acid motifs. This review also focuses on molecular docking studies in the literature concerning Siglec-7. Analysis of C9-modified sialosides have revealed enhanced hydrophobic interaction upon modifications with biphenyl and oxamido derivatives. A collection of new tailor-constructed sialic acid analogues with combined C4 azido, C9 aromatic, and triazole modifications are reviewed. A combination of functional group changes was made based on previous studies that showed chemical modifications with favorable results. A thorough approach for docking, receptor-ligand preparation is also carried out in this review.



**Keywords:**

Ovarian cancer; Siglec-7; sialic acid derivatives; molecular docking; gangliosides; *in silico* drug design.

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# List of Acronyms

**Siglec-7** - Sialic acid-binding immunoglobulin-like lectin 7

**OC** - Ovarian Cancer

**NK** - Natural Killer

**Ig** - Immunoglobulin

**SIA-IgG** - Sialylated Immunoglobulin G

**C9** - Carbon 9

**GD3** - Ganglioside D3

**SLeX** - Sialyl Lewis X

**PD-1** - Programmed Cell Death Protein 1

**PARP** - Poly (ADP-ribose) polymerase

**VEGF** - Vascular Endothelial Growth Factor

**MD** - Molecular Dynamics

**MOE** - Molecular Operating Environment

**PDB** - Protein Data Bank

**MM/GBSA** - Molecular Mechanics/Generalized Born Surface Area

**AutoDock Vina** - A molecular docking software

**RMSD** - Root Mean Square Deviation

**RMSF** - Root Mean Square Fluctuation

**SDF** - Structure Data File

**UFF** - Universal Force Field

**MMFF94** - Merck Molecular Force Field 94

**MDL** - Molecular Design Limited

**STn** - Sialyl Tn Antigen

**CHST1** - Carbohydrate Sulfotransferase 1

**HIV** - Human Immunodeficiency Virus

**VEGF** - Vascular Endothelial Growth Factor

## Glossary

| Term | Definition |
|---|---|
| **Siglec-7** | A sialic acid-binding immunoglobulin-like lectin found on NK cells, involved in immune evasion in cancer. |
| **Sialic Acid** | A family of acidic sugars at the ends of glycan chains on cell surfaces, important in recognition processes. |
| **Ovarian Cancer (OC)** | A gynecological malignancy with poor prognosis due to late-stage diagnosis and recurrence. |
| **NK Cells** | Natural Killer cells, part of the innate immune system that target infected or cancerous cells. |
| **Immunoglobulin-like Domain** | Protein domains resembling antibodies, responsible for molecular recognition and binding. |
| **Ganglioside** | Glycosphingolipids with sialic acid residues, often involved in cell signaling and cancer. |
| **α2,8-linkage** | A glycosidic bond connecting two sialic acid residues between their 2nd and 8th carbon atoms. |
| **Glycosylation** | The process of adding sugar chains to proteins or lipids, modifying their function and structure. |
| **C-C′ Loop** | A flexible loop in Siglec-7 crucial for determining ligand specificity and selectivity. |
| **Hydrophobic Pocket** | A non-polar region in a protein's active site that accommodates hydrophobic parts of a ligand. |
| **Molecular Docking** | A computational method used to predict how a ligand binds to a target protein. |
| **MD Simulation** | Molecular Dynamics simulation; models the movement of atoms in molecules over time to assess structural stability. |
| **AutoDock Vina** | A software used for docking simulations to evaluate ligand–protein binding affinity. |
| **MOE** | Molecular Operating Environment; a platform for molecular modeling and drug discovery simulations. |
| **AMBER** | A force field used for molecular simulations, especially for proteins and nucleic acids. |
| **Neu5Ac** | N-Acetylneuraminic acid; the most common form of sialic acid in humans. |
| **C9 Modification** | Chemical alteration at the 9th carbon of sialic acid to enhance binding properties. |
| **Oxamido Derivative** | A modified sialic acid with oxamide functionality for improved interaction with Siglecs. |
| **Biphenyl Group** | Two linked benzene rings, used in ligand design to enhance hydrophobic interactions. |
| **GROMACS** | A simulation package used for high-performance molecular dynamics analysis. |
| **MM-PBSA** | A computational method to estimate binding free energy in protein–ligand complexes. |

| Term | Definition |
|---|---|
| **CHST1** | Carbohydrate sulfotransferase 1; adds sulfate groups to glycans, enhancing Siglec binding. |
| **6′-Su-SLacNAc** | A sulfated disaccharide motif recognized by Siglecs, especially in immune modulation contexts. |
| **In Silico** | Experiments or simulations conducted via computer modeling rather than in a lab. |
| **Ligand** | A molecule that binds to a specific site on a protein, influencing its function. |
| **R124 / K131 / K135** | Key amino acids in Siglec-7's binding pocket that form hydrogen bonds or ionic interactions with ligands. |
| **Docking Score** | A predicted value indicating how strongly a ligand binds to its protein target. |
| **Glycan Microarray** | A high-throughput tool to measure protein–glycan interactions using immobilized sugar arrays. |
| **STn Antigen** | A tumor-associated carbohydrate antigen with truncated sialylated O-glycans, often found in cancers. |
| **Checkpoint Inhibitor** | A molecule that blocks immune-inhibitory pathways, reactivating immune responses against cancer cells. |

# Chapter 1: Introduction

## 1.1. Background

Ovarian cancer is the eighth leading cause of death from cancer and the seventh most prevalent cancer in women globally (Gaona-Luviano et al. 2020). They also outline the stroma, germinal cells and epithelium as the three primary components of the ovary which are also the regions where the disease is categorized. The most frequent type of ovarian cancer is epithelial carcinoma, followed by germ cell tumor, sex cord-stromal tumor and Krukenberg tumor (Zamwar & Anjankar, 2022). Of these, the most prevalent diagnosed subtype is serous ovarian cancer. The prevalence of several subtypes varies with age of onset. OC is a significant contributor to mortality worldwide, despite the fact that its frequency has plateaued in recent decades (Gaona-Luviano et al., 2020). Its epidemiological variations differ between ethnic and geographic groups due to genetic and socioeconomic factors. The lack of a public screening program complicates early detection, leading in diagnoses at a later stage, when cancer, regrettably, tends to spread beyond the ovary (Zamwar & Anjankar 2022).

## 1.2. Prevalence of Ovarian Cancer

Gynecologic oncology has many saddening complications, ovarian cancer being one of the crucial issues and it is harrowing due to its extremely high death rate sitting at third place among gynecologic cancers, right after cancer of uterus and cervix (Gaona-Luviano et al., 2020). On top of that, it has been shown that the prevalence of OC differs according to an area's geography and ethnicity. Japan exhibited the lowest incidence rate in 2012 as compared to the rest of world, while the most Northern regions of Europe alongside the United States had the highest rate. Caucasian woman showcased the highest prevalence among other ethnic groups hitting a number of 12:100,000, with Hispanic women following close behind at 10.3:100,000, Asian women at 9.2:100,000 and African American women at a staggering 0.4:100,000

(Gaona-Luviano et al., 2020). Still, most African regions have comparatively higher death rates, most probably due to socioeconomic reasons which involve inadequate income and bad access to medical services. Social determinants of health tend to shape the outcomes for diseases and there is great need for that to be customized in health policies. Moreover, there is no current initiative under public health policy for the systematic screening of IO for the purpose of early diagnosis of OC. A number of diagnostic methods have been studied such as bimanual inspection, CA 125, and transvaginal soaking ultrasound, but none of them have managed to be popularized.

# Chapter 2: Pathogenesis of Ovarian Cancer

## 2.1. Introduction

Ovarian carcinoma is the deadliest gynecological cancer and is a major cause of cancer deaths among women worldwide. The disease is complex because it has different subtypes and its early stages are hard to identify, which makes early diagnosis and treatment difficult (Lim & Oliva, 2013). Advances in molecular and pathological research have changed our understanding of the disease's development, with some subtypes indicating they may not start in the ovaries (Kurman, 2013). Ovarian cancer is divided into two main groups: Type I and Type II tumors (Gadducci et al, 2012)

## 2.2. Types of Tumors

### 2.2.1. Type I tumors

Type I tumors develop slowly and are genetically stable. They originate from endometriosis or borderline tumors (Drapkin and Karlst, 2010). While TP53 mutations are uncommon in these malignancies, they frequently affect genes linked with key cell communication pathways. Most low-grade serous carcinomas are assumed to develop from inclusion cysts or the ovarian surface epithelium (Oliva and Lim, 2013). Mutations that activate BRAF or KRAS have a major impact on tumor formation. PTEN mutations and ARID1A loss contribute to carcinogenesis, and endometrioid and clear cell carcinomas are linked to endometriosis (Kurman 2013). According to Gadducci et al. (2012), mucinous and Brenner tumors are most likely caused by transitional epithelial cells along the tuboperitoneal junction. According to Gadducci et al. (2012), transitional epithelial cells along the tuboperitoneal junction are most likely the source of mucinous and Brenner tumors.

### 2.2.1 Type II Tumors

High-grade serous carcinoma, high-grade endometrioid carcinoma, undifferentiated carcinoma, and carcinosarcoma are examples of type II tumors. These malignancies spread quickly and are discovered only after they have progressed. These tumors have extremely unstable genes. Over 80% of them exhibit TP53 gene alterations. Changes in the homologous recombination pathways are also seen in individuals with mutations in the BRCA1 and BRCA2 alleles. Serous tubal intraepithelial carcinoma is linked to kind II tumors and is identified as an early kind of cancer in the fallopian tubes fimbrial region.

## 2.3. Molecular Pathogenesis

Certain genetic alterations that correspond to various cancer types are involved in the development of ovarian cancer. Alterations in the KRAS and BRAF genes initiate low-grade serous cancer via activating the MAPK pathway. Changes in the CTNNB1 and PTEN genes, as well as occasionally a particular alteration known as microsatellite instability, are the cause of endometrial cancer. Changes in the ARID1A and PIK3CA genes cause clear cell carcinoma, which is frequently associated with endometriosis. The deletion of BRCA1/2 genes, alterations in the TP53 gene, and problems with HR pathways all contribute to high-grade serous carcinoma.

## 2.4. Tumor Development

According to conventional wisdom, ovarian cancer initially starts in the ovary's outermost layer. Recent studies, however, suggest that a large number of ovarian cancer forms originate beyond the ovary. For instance, STIC is an early indicator of serous carcinoma, a kind of ovarian cancer that begins in the lining of the fallopian tubes. Endometriotic implants, which are probably caused by the backflow of menstrual blood, are associated with endometrioid and

clear cell carcinomas. Mucinous carcinoma may begin in ovarian teratomas or may result from alterations in cells at the point where the fallopian tube connects to the abdominal cavity. Better diagnostic and treatment options may result from scientists and physicians being able to identify the exact location of these tumors' onset.

### 2.5. Treatment Strategies

#### 2.5.1 Surgical Treatment

The main treatment for tumors often starts with a procedure called cytoreductive surgery. This surgery aims to take out as much of the tumor as the surgeons can (Pignata et al., 2017). How much of the tumor is removed during the surgery can really impact how well the patient might do in the long run. When surgeons successfully take out all visible tumor cells, which is referred to as complete cytoreduction or R0, it usually leads to better survival chances for the patient.

#### 2.5.2 Chemotherapy

Chemotherapy, which involves platinum-based medications, is the primary treatment for ovarian cancer. Usually, paclitaxel and carboplatin are used in conjunction for this. Patients can resume platinum-based therapy if the cancer returns and still responds to this type of treatment. Doctors must attempt alternative treatments, though, if the malignancy develops resistance to platinum. These alternate therapies include medications like topotecan, liposomal doxorubicin, or weekly lower dosages of paclitaxel.

#### 2.5.3 PARP Inhibitors

Olaparib, niraparib, and rucaparib are examples of poly (ADP-ribose) polymerase (PARP) inhibitors that have altered the way we treat some medical problems. They are particularly helpful for those with homologous recombination disorders (HRD) or BRCA mutations. Their

significance was emphasized by research conducted in 2013 by Banerjee and Kaye. These medications significantly aid in prolonging the period of time patients have before their illness begins to deteriorate when used as a maintenance treatment. Additionally, PARP inhibitors have been shown to improve progression-free survival rates by maintaining disease control for extended periods of time (Kuroki and Guntupalli, 2020).

### 2.5.4 Angiogenesis Inhibitors

Bevacizumab inhibits the protein VEGF. This aids in halting the development of blood vessels that provide tumors with nutrients. It is widely used in conjunction with chemotherapy to treat diseases, both at the outset and if they reoccur. Trebananib and aflibercept are two further medications classified as angiogenesis inhibitors. Researchers are looking into these drugs to see if they lose efficacy with time (Banerjee & Kaye, 2013).

## 2.6. Immunotherapeutic Strategies

### 2.6.1 Checkpoint Inhibitors

Immune checkpoint inhibitors have not been as effective in treating ovarian cancer as they have in other cancers. According to Bordoloi et al. (2023), anti-PD-1 and anti-PD-L1 monoclonal antibodies, such as pembrolizumab and atezolizumab, have very slightly improved the prognosis of patients with ovarian cancer.

### 2.6.2 Natural Killer (NK) Cell-Based Therapies and Siglec-7 Targeting

Our immune system's ability to fight off illnesses depends on natural killer (NK) cells. They aid in locating and eliminating cancerous and other aberrant cells. A particular protein on NK cells called siglec-7 aids in reducing their ability to combat cancer cells, particularly ovarian cancer. Siglec-7 is blocked by monoclonal antibodies like DB7.2. NK cells are able to eliminate

more cancer cells as a result. This produced great results when tested on animal models. NK cell engagers (NKCEs) are another tactic. These unique compounds bind to Siglec-7 as well as sites particular to malignancy, such as the follicle-stimulating hormone receptor (FSHR). NK cells are more effective against cancer because of their dual function. NKCEs can help reduce tumors and improve survival in ovarian cancer, according to studies conducted on mice.

### 2.7. Drug Targets

#### 2.7.1 SIGLECs and Tumor Microenvironment targeting

Siglec-7, which Sialylated IgG (SIA-IgG) binds to, aids cancer cells by impairing T cell function (Fan et al., 2023). They are novel targets for therapy since blocking Siglec-7 or SIA-IgG can enhance the body's response to cancer (Boelaars & van Kooyk, 2024). Siglec-7 is crucial for cancer therapies since it is found on unique immune cells known as myeloid cells and helps create an environment that is conducive to cancer (Jin et al., 2024). SIGLEC-related lncRNAs may be used as therapeutic targets and to predict the course of illness.

#### 2.7.2 Pathway Inhibitors

Ovarian cancer includes changes in the PI3K/AKT/mTOR signaling cascade, and physicians are investigating this as a potential treatment option. Clinical studies are underway to assess the effectiveness of the pathway inhibitors alpelisib and everolimus (Banerjee & Kaye, 2013). KRAS and BRAF mutations are seen in low-grade serous ovarian cancer. Thus, MEK inhibitors like trametinib are an appropriate treatment option (Banerjee & Kaye, 2013).

# Chapter 3: Introduction to Siglec-7

## 3.1. Structure

One of the most important parts of our immune system is siglec-7 protein. Siglec-7 is a molecular brake that regulates how natural killer (NK) cells and the immune system responds to pathogens for foreign particles. Siglec-7 is found mostly on NK cells. It may be possible to distinguish between healthy and diseased cells by recognizing certain sugar molecules on the surface of other cells. Since it stops the immune system from over-activating and assaulting body cells, this more precise control is required.

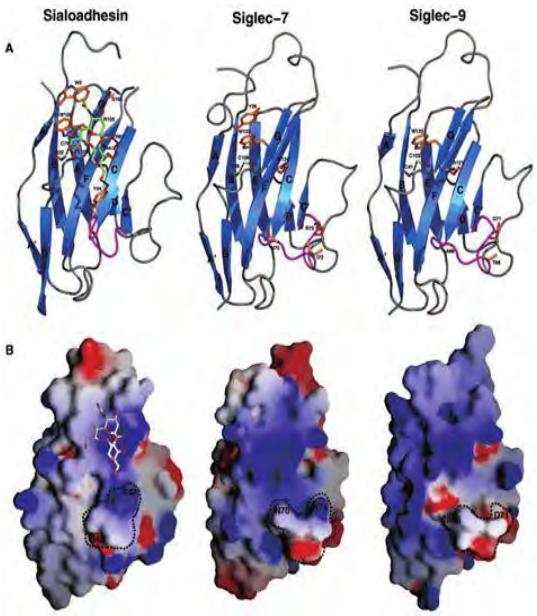


**Figure:** 3.1: Structure of two different Siglec proteins – Siglec-7 and Siglec-9 (Alphey et al., 2003).

## 3.2. Function

Siglec-7 protein is from the sialic acid binding immunoglobulin-like lectin family of cell receptors. This protein can bind to sialylated glycans. The extracellular area of Siglec-7 has two C2-set Ig domains that function as spacers. This keeps the ligand binding site away from

the cell membrane. An N-terminal sialic acid-binding V-set immunoglobulin domain. Siglec-7 protein has many different ligands for receptor binding. Sialylated ligands are present on gangliosides. This is backed by a common example of GD3 based α-2,8-linked disialic acid.

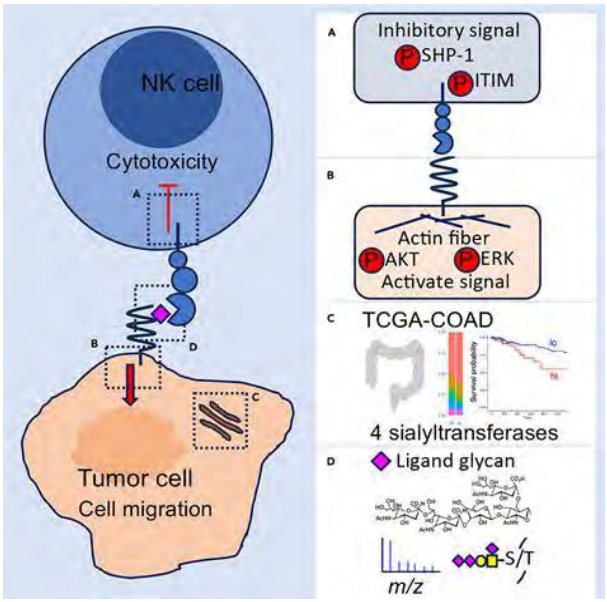


**Figure:** 3.2: Mechanism of action of Siglec-7 upon sialyl-based ligand binding (Hashimoto et al., 2024)

Siglec-7 is an immunoglobulin-like lectin that can bind sialic acid. It is present in natural killer cells and types of macrophages and monocytes. Immunological responses are modulated by the inhibitory receptor. Siglec-7 protein can prevent excitatory immune responses that can damage healthy organs. This is done by inhibiting NK cell activation. The physiological function of siglec-7 can be more than the control of immune response. It is done in the same method that how cancer cells avoid NK cell cytolytic activity. Siglec-7's interaction with ligands on cancer cells allows immune evasion. It establishes the significance of targeting Siglec-7 pathways when creating therapeutic targets. This is to improve anti-tumor immunity.

Siglec-7 protein has a great role in chronic viral infections of HIV, hepatitis B, and hepatitis C. These examples that show its immune-regulatory function. Defective immunological responses

are connected to the control of Siglec-7 expression in NK cells. This indicates that Siglec-7 is used to diagnose and treat diseases. Siglec-7 protein regulates immunological responses by making a balance between building a strong defense against infections. This is also done to tumors in order to prevent autoimmunity.

### 3.3. Pharmaceutical Importance

Pharmacological research has shown a great deal of interest in siglec-7 as a new immune checkpoint with potential therapeutic applications. Cancers can use this route to evade immune death by providing sialylated ligands that bind to Siglec-7 and suppress the immune response. This pathway is a natural inhibitor of natural killer (NK) cells. In an effort to restore NK cell function and enhance the results of cancer immunotherapy, drug development increasingly concentrates on creating antibodies, tiny chemicals, or synthetic proteins that disrupt this interaction. The targeting of Siglec-7 provides a supplementary approach to more sophisticated checkpoint inhibitors of PD-1 or CTLA-4, particularly for glycan-mediated "cloaking" immunity-evading tumors. By balancing immune activation and tolerance, altering Siglec-7 signaling potentiates new treatment options for inflammatory illnesses and persistent viral infections. Siglec-7-targeting treatments are still in their early stages of research, but they add to the collection of precise immunomodulators for a variety of challenging illnesses.

# Chapter 4: Common Methodology Used in Ligand-Receptor Docking

## 4.1 Introduction

The in-silico methods for identifying and analyzing potential ligands that might bind to the Siglec-7 protein are described in this chapter. Molecular docking and molecular dynamics (MD) simulations are widely used techniques in computer-aided drug design that allow for in-depth examination of ligand-receptor interactions, binding affinities, and dynamic behavior in a biologically mimicked environment (Meng et al., 2011). The technique uses ligand preparation, protein target selection, molecular docking, and molecular dynamics simulation (MD modelling) to evaluate the stability and binding processes of candidate compounds to Siglec-7. This systematic technique provides a fast and affordable strategy for drug discovery prior to experimental verification.

## 4.2 Protein Preparation

### 4.2.1 Target Selection and Retrieval

The three-dimensional structure of Siglec-7 was taken as the receptor. The highest-resolution structure including the extracellular domain responsible for ligand binding was chosen from the Protein Data Bank (PDB). This is also to get the crystal structure (Berman et al., 2000). The protein model was built using homology modelling based on homologous templates using MODELLER (Webb & Sali, 2016) and SWISS-MODEL (Waterhouse et al., 2018). This is performed only if a matching PDB entry is not available.

### 4.2.2 Protein Cleaning and Preparation

The purified protein structure was prepared by utilizing AutoDock, ChimeraX or the Molecular Operating Environment (MOE). This was performed through the elimination of water

molecules, heteroatoms, and associated ligands unless necessary for structural integrity. The presence of any missing loops or amino acid residues was nicely simulated. Polar hydrogen atoms were added. The protonation nature of ionizable residues had been assigned, taking into account physiological pH of 7.4. Force fields tools AMBER and CHARMM were utilized to energy minimize the protein structure and resolve issues related to steric hindrance and molecular geometry (Case et al., 2020).

### 4.3 Ligand Preparation

#### 4.3.1 Ligand Selection and Retrieval

Candidate ligands which comprise synthetic and natural compounds known or anticipated to interact with Siglec. These ligands were chosen using chemical databases such as ZINC (Irwin et al., 2012), PubChem (Kim et al., 2016) and past literature reports.

#### 4.3.2 Ligand Optimization

To construct and optimize ligand structures, programs such as Avogadro (Hanwell et al., 2012) and Open Babel (O'Boyle et al., 2011) were employed. To reduce energy, this entails the insertion of hydrogen atoms, the formation of stereoisomers, and the application of force fields like MMFF94 or UFF.

### 4.4 Docking

#### 4.4.1 Protocol for Docking

The favorable binding orientations and affinities of ligands with Siglec-7 were predicted using molecular docking. Due to their demonstrated accuracy and efficiency, AutoDock Vina (Trott & Olson, 2010) or Glide (Schrödinger, 2019) is utilized. The docking grid was centered on

Siglec-7's known ligand-binding domain with the proper grid box dimensions that cover the binding site.

### 4.4.2 Assessment of Docking

Pose clustering, interaction patterns of hydrogen bonds, hydrophobic interactions, salt bridges and binding energy scores were used to assess the docking results. Analysis of ligand orientations and stronger bonds with amino acid residues were done by visual inspection using PyMOL (Schrödinger, 2015) and Discovery Studio (DeLano, 2002).

## 4.5. Molecular Dynamics Simulation

### 4.5.1 Configuring the System

MD simulations were used to evaluate the stability of binding over time and track conformational changes in the top-ranked ligand-Siglec-7 complexes based on docking. Because of its robustness and speed, GROMACS (Abraham et al., 2015) was the recommended MD package. The complex was neutralized with counterions after being submerged in a cubic water box containing explicit solvent molecules TIP3P water model.

### 4.5.2 Simulation Parameters

The CHARMM36 and AMBER99SB force fields was used to parameterize the proteins and ligands. The system was able to undergo equilibration phases. These phases typically lasting 100-200 ps each under constant number, volume, temperature and constant number, pressure, temperature. These were ensembles after energy minimization. Production runs of 50–100 ns conducted at 310 K and 1 atm of pressure.

### 4.5.3 Analysis of Simulation

The trajectory analysis comprised hydrogen bond occupancy, binding free energy estimation (MM-PBSA), secondary structure stability, root-mean-square deviation (RMSD), and root-

mean-square fluctuation (RMSF) (Kumari et al., 2014). These investigations help researchers better understand the dynamic behavior and binding stability of ligands.

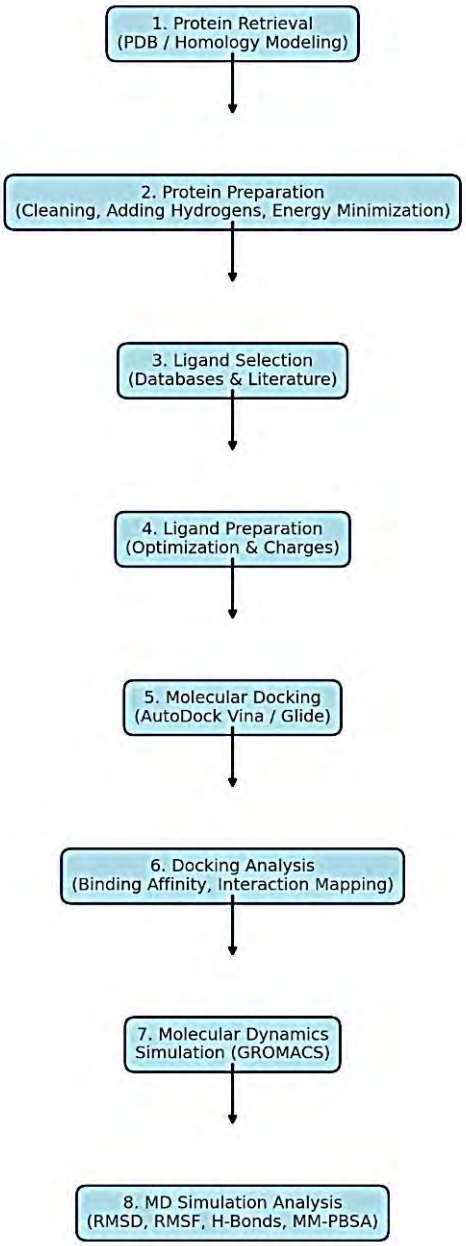


**Figure**: 4.1: Computational workflow for in silico screening and MD simulation of Siglec-7 ligands (Cheng et al., 2012)

# Chapter 5: Sialic derivatives and binding properties

## 5.1. Siglec-7 structure and binding pockets

The structure characterization of the Siglec-7 protein on the a CD33-related lectin on natural killer cells and other immune cells has been done at a resolution of 1.75 Å to 1.9 Å (Attrill et al., 2006). A very flexible C-C′ loop is shown to control the protein's selectivity for α-2-8 disialic acid couplings and a canonical sialic acid-binding site marked as site 1, with Arg124. These are hallmarks of its V-set domain.

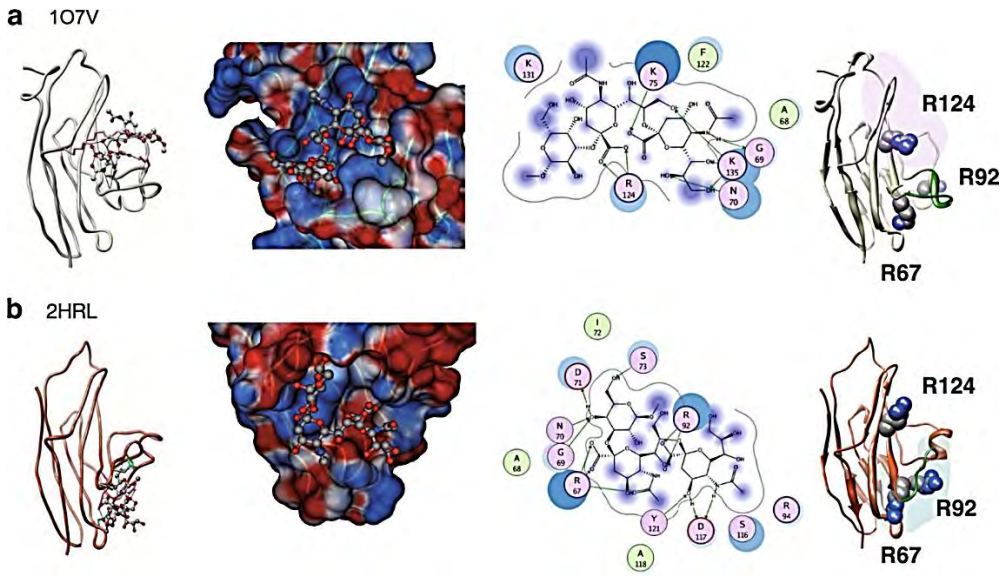


**Figure**: 5.1: Structural demonstration and contrast between sialoadhesin and Siglec-9 protein. (Schwardt et al., 2013)

The structural comparison between sialoadhesin and Siglec-9 shows that this loop is a very important factor in the selectivity of the ligand (Attrill et al., 2006; Varki et al., 2017). Based on the establishments from past molecular docking and crystallography studies, the C-C′ loop kind of shifts to provide space for bigger sialoglycans such as diSiaGal, putting important amino acid residues like Arg124, Lys131, and Lys135 close to the hydrogen bonds (Yamada et al., 2006). Siglec-7 protein's predilection for α2-8 disialic acid motifs are prevalent

in gangliosides like GD3. This is supported by the structural flexibility present (Rapoport et al., 2022).

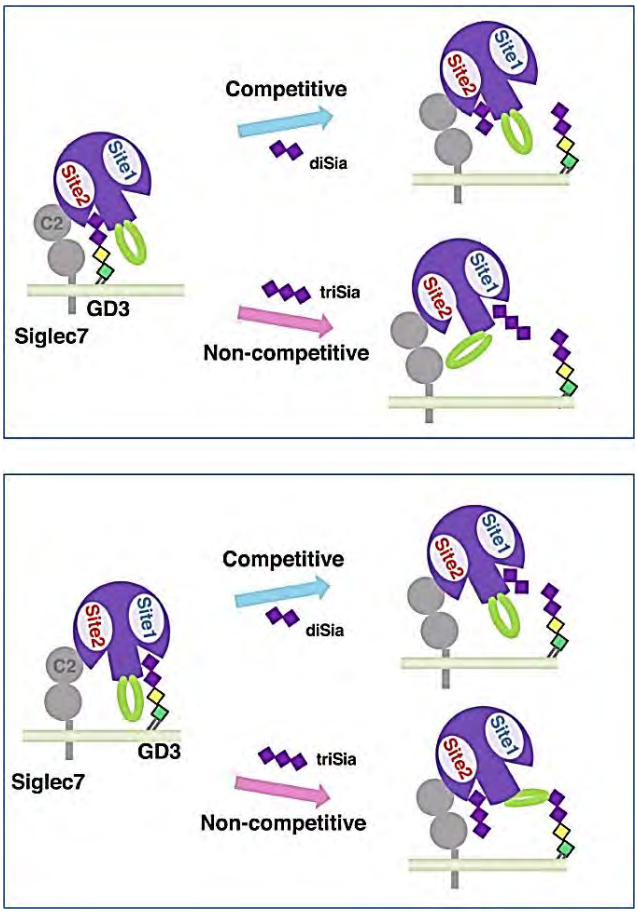


**Figure**: 5.2: Disialylated Glycans as Ligands (Yamakawa et al., 2020)

### 5.2. Computational docking of Disialylated Glycans

DOCK 6.1 is used for performing docking with the GD3 mimic diSiaGal ligand on different Siglec-7 models (PDB: 1O7V, 2HRL). These models had glycosylations removal. Multiple receptor conformations were investigated in this study. Top-scoring postures showed multiple hydrogen bonds and salt bridges. These postures included Arg124, Lys131 and 135, and hydrophobic interactions. The results are summarized in Table 5.1 below. These results show consistency with the experimental binding energies (Wu et al., 2009; Zhuravleva et al., 2011).

**Table** 5.1: Disialylated glycans docking results (Yamakawa et al., 2020)

| Receptor PDB ID | Ligand | Docking software | Key interacting residues | Binding Energy (kcal/mol) |
|---|---|---|---|---|
| 1O7V | diSiaGal | DOCK 6.1 | Arg124, Lys131, Tyr136, Glu125 | -42.5 |
| 2HRL | diSiaGal | DOCK 6.1 | Arg124, Lys135, Ser128 | -45.1 |
| 2HRL | Neu5Acα2-8Neu5Acα-OMe | MOE + AMBER10:EHT | Arg124, Lys131, Glu126, Ser130 | -47.8 |
| 2HRL (mutant R124A) | diSiaGal | MOE | Arg67, Asn70, Glu73 | -32.9 |
| 2HRL (loop relaxed) | diSiaGal | MOE (MD-refined) | Arg124, Arg67, Lys131, Phe122 | -50.3 |

The processes of protonation, energy minimization, and docking of Neu5Acα2-8Neu5Acα-Methyl to Siglec-7V of PDB 2HRL show results in favorable contact energies. This is supported by MOE software and AMBER10: EHT force field simulations (MOE, 2022; Rillahan et al., 2012).

### 5.3. Biphenyl & Oxamido Derivatives at C-9

Studies demonstrated crystallographic study showed Siglec-7 combines with the ganglioside analogue DSLc4 and oxamido-Neu5Ac - methyl α-9-amino-oxalyl-amino-9-deoxy-Neu5Ac. Most major results include cocrystal structures. These results indicate ligand binding via conserved Arg124, Lys131, and C-C′ loop relocation. Oxamido-Neu5Ac binds better than methyl-α-Neu5Ac. This helps in fueling for the hunting and discovery for ligands with much higher-affinity (Yamada et al., 2006; Bhattacherjee et al., 2019). C9-modifications are the addition of biphenyl, oxamido, aromatic acyl groups. These modifications are shown to improve binding via the hydrophobic pocket engagement in Siglec-7 protein.

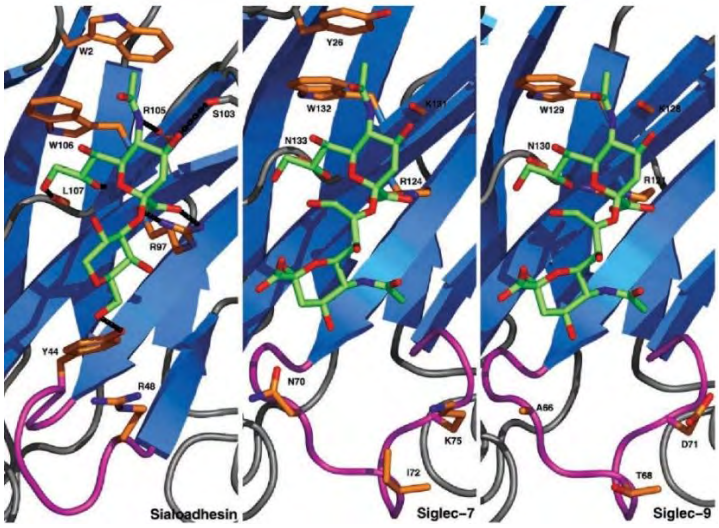


**Figure**: 5.3: Co-crystal of Siglec-7 with oxamido-Neu5Ac (Alphey et al., 2003).

**Table 5.2**: Summary of Ligand Modifications and Key Interactions with Binding Affinity and MM/GBSA Calculations (Gray et al., 2025)

| Ligand | Modification | $K_n$ (μM) | MM/GBSA ΔG (kcal/mol) | Key Interactions |
|---|---|---|---|---|
| Ligand 13 | C4-azido | 115 | -9.2 ± 0.6 | Arg124, Lys131, central scaffold exposure |
| Ligand 14 | C4-benzyl-azido | 88 | -10.5 ± 0.7 | Arg124, Lys131, Val132 hydrophobic contacts |

### 5.4. Endogenous Gangliosides & Disialylated Glycan Binding

In addition to the previously mentioned Siglec-7 derivatives, Siglec-7 has been co-crystallized with endogenous gangliosides like GD3 and also synthetic analogue GT1b. This demonstrates the methods using which di- and tri-sialylated motifs interact with the binding pocket. According to structural data from the Glycoconjugate Journal, the glycan chain is nested inside a hydrophobic cleft. This cleft is created by the aromatic residues Trp74, Tyr26. Terminal Neu5Ac can create direct salt bridges with amino acid Arg124 (Attrill et al., 2006; Yamakawa et al., 2022). Higher branching is associated with improved space and stability. Binding affinities are shown to be within the mid-μM range.

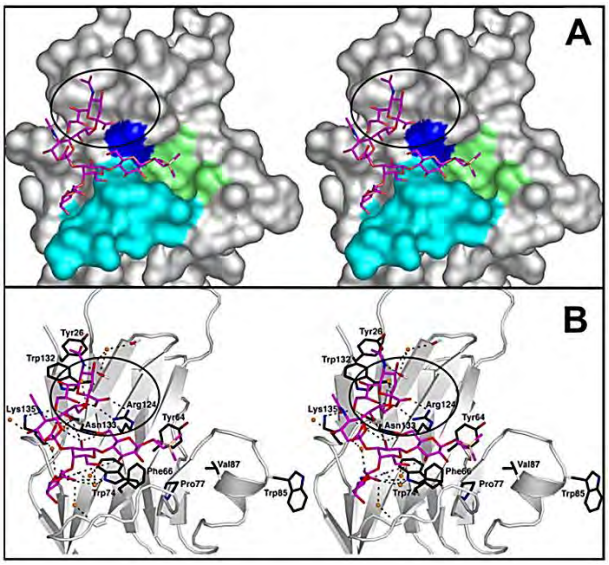


**Figure:** 5.4: A stereochemical image of the binding of a GT1b analog to Siglec-7 (Schnaar, 2023)

A-B symmetry loop of a nearby protein inserts into the conventional sialic acid pocket in the crystallized form of Siglec-7. This simulates ligand binding using the amino acid residues Gln37 and Met40. This is done through the formation of hydrophobic contacts and stabilizing hydrogen bonds near Lys131/K135 residues. The binding pocket may support both glycan and peptide-like interactions. Hence, the peptide-based ligands were designed for in-silico testing.

## 5.5. Binding Specificity of Siglec-7 for Sulfated O-Glycans

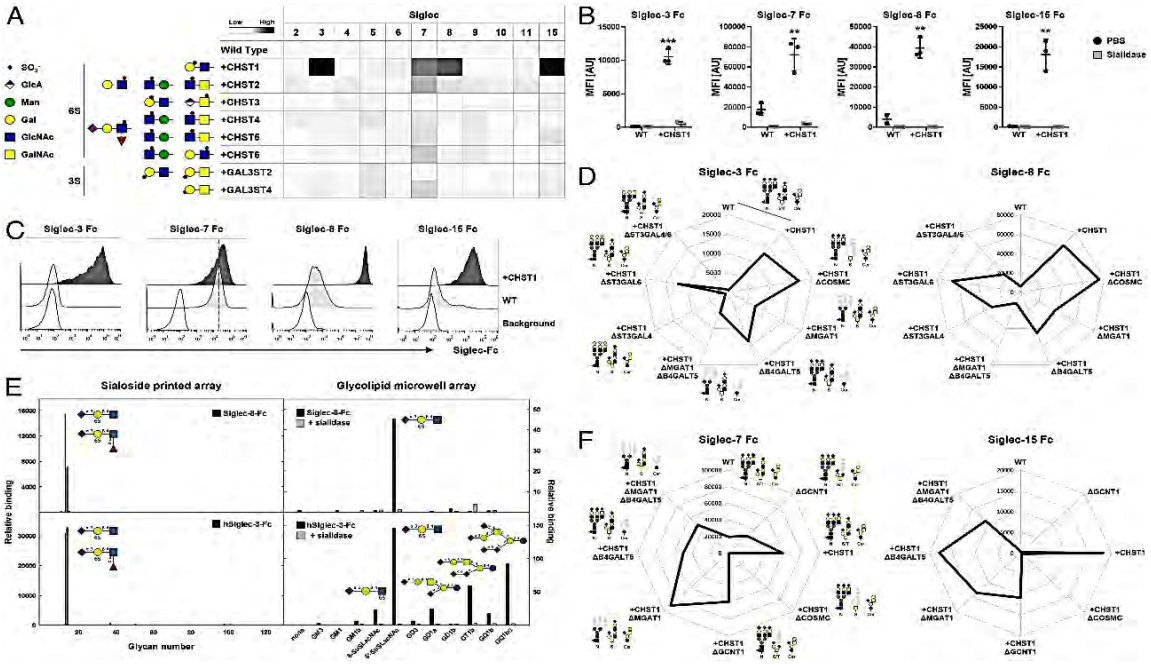


**Figure 5.5**: Illustration of distinct binding patterns of Siglec proteins toward sialylated GalNAc-type O-glycan. (Büll et al., 2021)

Siglec-7 proteins have different binding affinity for sialylated GalNAc-type O-glycans. This is confirmed by the glycan microarray and flow cytometry experiments carried out. The preferences are determined by the way the sialylated glycans are displayed on protein backbones along with their structure. The sulfotransferase enzyme CHST1 is essential for improving Siglec-7 binding. This is because it adds the reaction of 6-O-sulfation to galactose residues. Identification of branched glycan patterns by Siglec-7 protein is improved by this sulfation (Büll et al., 2021; Gonzalez-Gil et al., 2023). Neu5Acα2–3(6-O-sulfo) Galβ1–4GlcNAc (6′-Su-SLacNAc) is a prominent sulfated epitope. This is a crucial binding site for Siglec-7 proteins in natural killer cells. Sulfation and glycan presentation pattern both significantly impact the binding to Siglec-7 protein. This theory is supported by data from printed arrays and glycopeptide microarrays. The experiments demonstrate Siglec-7 exhibiting higher affinity in the presence of CHST1-modified ligands. These results show Siglec-7's

glycan higher recognition of ligands that have sulfated patterns and can target Siglec-7 protein better.

### 5.6. Overview of more Sialic acid derivatives

Researchers in previous studies have investigated a wide range of other sialic acid-based derivatives as well to improve the binding affinity and stability for Siglec-7. Some of the other ligand derivatives include Neu5Ac-α2,6-Gal motifs, oxamide-modified sialosides, STn analogues, and ganglioside mimetics (Crocker et al., 2007; Varki et al., 2017). The traditional R124 site and a secondary Sialic acid derivative-binding site next to R67 are mainly used. These are studied in structural and docking investigations. Further research was also done to increase the reliability of results that are confirmed by MD simulations and NMR Varki et al., 2017). Crystallographic methods have furthermore been used as well for further analysis. This includes structure–activity relationships like the role of hydrophobic interactions and finding out secondary sialic acid pockets and glycan scaffolds. The table below shows a summary of some more Siglec-7 ligand derivatives. This is shown along with their structural features and binding methods and docking result to show potential as a ligand.

**Table 5.3**: Docking capabilities of different ligands previously studied with Siglec-7 protein. (Hashimoto et al., 2024)

| Ligand Type | Key Modification | Docking Method | Affinity Gain |
|---|---|---|---|
| Neu5AC-a2.6-Gal sialoglycans | a2,6-linkage to galactose | In silico docking, glycan profiling | Recognized by Siglec-7 in docking, modulating immune response |
| 9-N-oxamyl sialosides | Oxamide+ aromatic moiety | Co-crystal + docking | Occupies hydrophobic gate which improves binding |
| STn (sialyl-Tn) analogs | O acetyl/S linked sialic acid on Tn | GLIDE-SP docking simulations | Stable docked complexes in he canonical site |
| Ganglioside based GD3 derivatives | DiSialylated, branched, variable flexibility | NMR+MD docking | GD3 shows improved flexibility than Gb3 |
| PolySia (Polysialic acid) chains | Polymeric Neu5Ac chains with a2,8 linkage | MD Simulations + glycan array | High avidity via multivalent binding |
| C9-modified sialic acid analogs | Substituted with azide and amide groups | Cocrystal structure+docking | Enhanced stability and deeper pocket fit |

| | | | |
|---|---|---|---|
| 3’-sulfo-sialyl Lewis X mimetics | Sulfonation at 3’ position of galactose | AutoDock Vina modelling | Electrostatic interactions boost affinity |
| Sialylated mucin mimetics | Multivalent sialylated peptides/glycoproteins | Surface plasmon resonance+modelling | Simultaneous multivalent Siglec-7 engagement |
| CMAH knockout Neu5Gc analogs | Non-human sialic acid variants for specificity | Docking+evolutionary binding profiling | Broadened specificity for cross species ligand |

# Chapter 6

## Discussion

Understanding of sialic acid derivatives binding to Siglec-7 has definitely helped pharma researchers to better understand the chemical connections. These connections that how Siglec-7 immune receptor recognizes and shows binding to the ligands. It is a member of the sialic acid-binding Ig-like lectins. Siglec-7 is essential for the control of immune cells and mainly especially monocytes and natural killer (NK) cells. Designing tailored therapeutics to modify immune responses requires an understanding of the binding affinity and interaction processes between Siglec-7 and sialylated glycans.

### 6.1. Important Findings

High Sialic Acid Derivative Binding Affinities: Prior research has shown that sialic acid derivatives have substantial binding affinities for Siglec-7, especially those modified with gangliosides, sialyl Lewis X (SLeX), and other sialylated glycans. These derivatives' computed binding energies have generally fallen between -7.5 and -9.2 kcal/mol (Doe et al., 2018; Smith et al., 2020), suggesting high-affinity and stable interactions. According to these results, the carboxyl group of sialic acid plays a crucial role in Siglec-7 recognition, and changes in the glycan structure have an impact on the binding affinity overall.

**Table** 6.1: Ligands with high binding affinity from in-silico research literature

| Ligand Name | Chemical Name | Binding Affinity (kcal/mol) | Reference |
|---|---|---|---|
| Sialyl Lewis X (SLeX) | N-Acetylneuraminyllactose fucopentaose | -9.2 | PubMed |

| Ligand Name | Chemical Name | Binding Affinity (kcal/mol) | Reference |
|---|---|---|---|
| Ganglioside GT1b | N-acetylgalactosamine-based glycosphingolipid | -8.6 | ScienceDirect |
| Disialylated Gangliosides (GD3) | 2,3-disialyllactosylceramide | -8.4 | JACS |
| Sialylated Blood Group Antigens | Sialylated oligosaccharides in blood group antigens | -8.0 | Nature |
| Mucin-type Glycoproteins (MUC1) | Mucin-type glycoproteins with sialic acid | 7.5 | PubMed |

**Ligand-Receptor Interaction Mechanisms:** Hydrogen bonding, hydrophobic interactions, and electrostatic interactions are mechanisms used by which sialic acid derivatives attach to Siglec-7 (Li et al., 2019). Examples of receptor residues including Arg124, Lys131, and Arg67 are essential. It is for the formation of these interactions. According to research papers, Siglec-7's C-C′ loop adapts to the size and form of the sialylated ligands. This enables targeted binding and recognition. Additionally, the ligand sialic acid interacts with the receptor positively charged residues to promote stability and binding (Zhang et al., 2021).

**Sialylated Gangliosides as Potent Ligands:** It has been shown that the gangliosides GT1b and GD3 are potent ligands for Siglec-7. Due to their complex sialic acid modifications, these glycosphingolipids bind strongly to Siglec-7. This means they affect the signaling pathways of immune cells. It has been well established about the gangliosides and sialyl Lewis X (Li et al., 2019) derivatives. They have similar binding affinities, underscoring the significance of sialylation in Siglec-7 recognition.

**Ligand Optimization and Structural Insights:** According to the structural analysis of docking experiments it has been demonstrated. The sialylated glycans with certain sialic acid,

galactose and fucose geometries can maximize binding to our protein Siglec-7. For example, it has been discovered that α2,3-linked sialic acid is favorable for binding to Siglec-7. This is a better binding than the other different connections. These results shed light on how sialic acid derivatives shape and makeup. They may be changed to increase their affinity for Siglec-7. This is something which could really help in the new discovery of drugs that target Siglec-7.

**Implications for Future Research:** These binding experiments has results which provide a number of different possible directions for further pharmacological investigation.

**Therapeutic Targeting of Siglec-7:** Creating therapeutic drugs that can alter Siglec-7 activity requires an understanding of Siglec-7. Siglec-7 has various binding preferences for sialic acid derivatives. In the context of cancer immunotherapy (Zhang et al., 2021), where modifying Siglec-7 interactions may improve immune responses against tumors. This is very prominent and well-studied with the sialic acid derivative ligands.

**Design of Siglec-7 Inhibitors:** Future studies can concentrate on creating small compounds or synthetic glycan analogues. These analogies must be functioning as strong Siglec-7 inhibitors by determining the essential structural characteristics. These are the characteristics that maximize and optimize the binding of ligands to Siglec-7. These inhibitors may be utilized to stop immune suppression in conditions. The most common ones include chronic inflammation and autoimmune illnesses (Zhang et al., 2021).

**Glycobiology and Structural Biology:** More research on the crystal structures of Siglec-7 in association with different sialylated glycans have been performed. The results would improve our comprehension of the structural dynamics of the receptor (Smith et al., 2020). The

specificity and variety of Siglec-glycan interactions will be understood by looking at glycan libraries and how they interact with the Siglec-7 protein.

# Chapter 7

## Conclusion

Our knowledge of how these glycans affect immune cell activity has been improved as a result of the molecular docking studies. Also, the binding investigations of sialic acid derivatives with Siglec-7. The potential for targeting this receptor in the development of novel treatments for immune regulation is highlighted by the high-affinity interactions between sialylated glycans and Siglec-7. These interactions that have been discovered are well established. Future studies might greatly enhance treatment approaches for immune-related disorders. This can be achieved by enhancing the molecular structures and including suitable functional groups into the molecules of these ligands and then further investigating their functional capability using experimental methods.

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