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Synthesis of MUC1 Tn/Sialyl-Tn Glycopeptides and Combinatorial Libraries for Lectin Binding Studies

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Introduction

Mucin 1 (MUC1) is a heavily glycosylated transmembrane protein, altered in both expression and glycosylation pattern in human carcinomas of the epithelium. The incomplete or truncated glycan structures, often capped by sialic acid are overexpressed in premalignant and malignant tissue.[1,2]

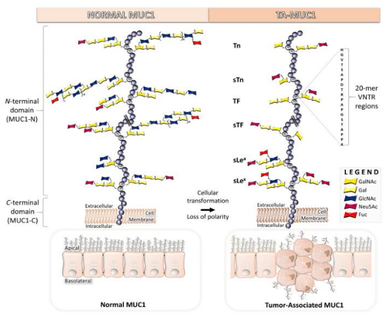


Fig 1: Structural differences between normal and tumor- associated MUC1.

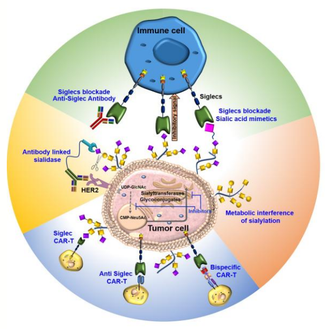


Fig 2: Strategies to target Siglec-sialylated MUC1 immune axis in cancer.

Interactions between the tumor-associated carbohydrate antigens (TACAs) of MUC1 and the carbohydrate-binding proteins, lectins, often lead to the creation of a pro-tumor microenvironment favoring tumor initiation, progression, metastasis, and immune evasion.

Macrophage galactose binding lectin (MGL) is a C-type lectin receptor found on antigen-presenting cells that facilitates the uptake of carbohydrate antigens for antigen presentation, modulating the immune response homeostasis, autoimmunity, and cancer.

The specific aim of this research is to synthesize mono and multiple glycosylated MUC1 glycopeptide models carrying the Tn or sTn (sialyl Tn), that allow for the control of the complexity of the chemical space of the multivalent ligands.[3,5] In addition, we use Positional Scanning Synthetic Glycopeptide Combinatorial Libraries (PS-SGCLs) as a tool to model the diversity in the type and positions of *O*-glycans on MUC1.[4] This focused combinatorial library with defined structural complexity allow us to evaluate the effect of neighboring residue glycosylation, glycan density, and/or the presence of unique patterns of *O*-glycan clusters on binding to lectins, thus helping us understand the multivalent carbohydrate–lectin recognition processes at the molecular level.

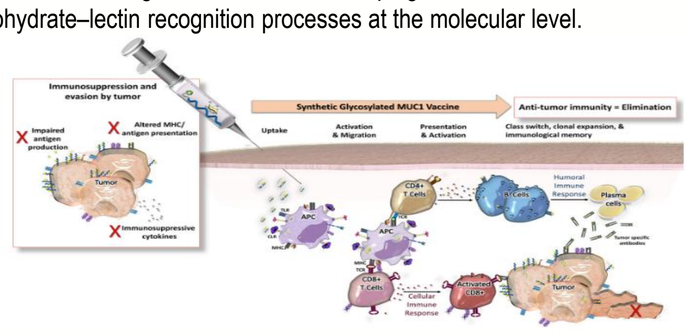
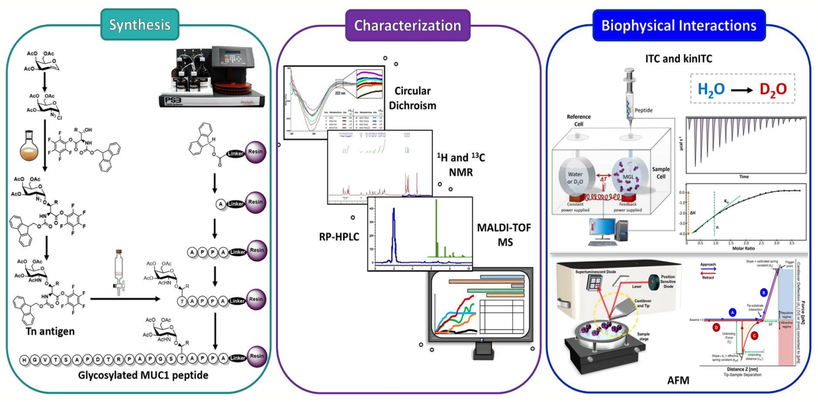


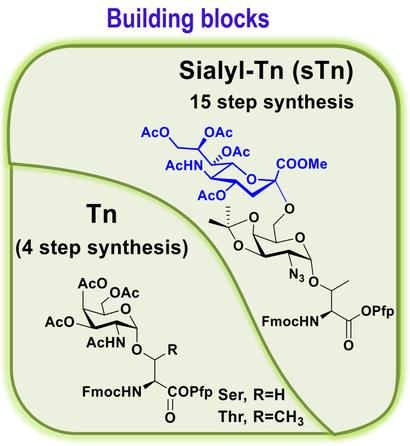
Fig3: Cancer vaccines either aim to prevent precancerous tumors from shifting into a microenvironment that favors regulatory and immunosuppressive mechanisms (preventive) or to restore immunosurveillance (therapeutic).

Considering the crucial role of tumor-associated forms of MUC1 with MGL in tumor immunology, a thorough understanding of this interaction is essential for it to be exploited for cancer vaccine strategies.

Methodology



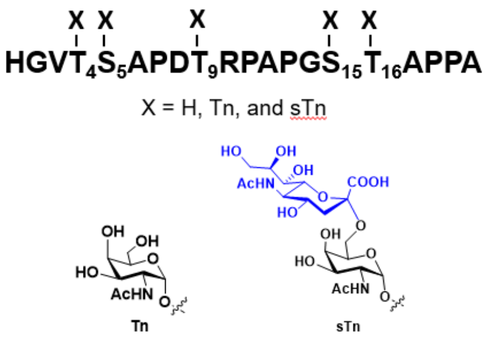
Building blocks



The Tn antigen building block was prepared via one-pot azidochlorination to generate the glycosyl donor, followed by a modified Koenigs–Knorr reaction to incorporate the Fmoc-protected Thr acceptor.[3] For the sTn analog, orthogonal protections enabled selective C6 glycosylation using a phenylthiosialoside donor activated by NIS/TMSOTf.[5]

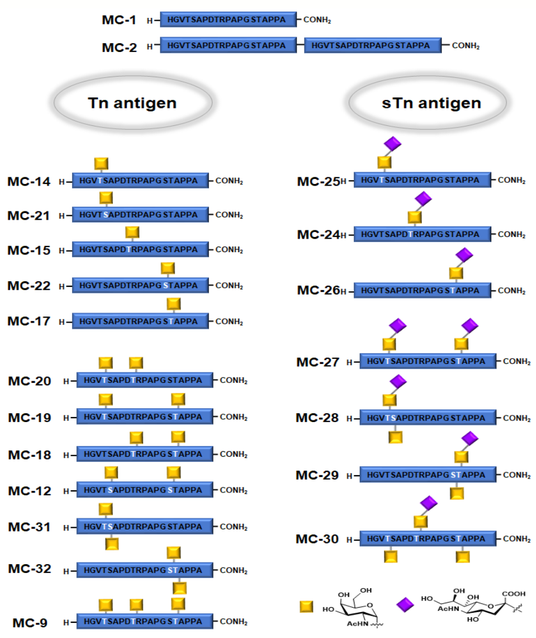
Positional Scanning Synthetic Glycopeptide Combinatorial Library (PS-SGCL)

A PS-SGCL was constructed using the "tea bag" method and standard Fmoc solid-phase peptide synthesis (SPPS). In each positional sublibrary one position on either Thr or Ser is fixed while the remaining glycosylation sites were incorporated as the isokinetic mixtures.

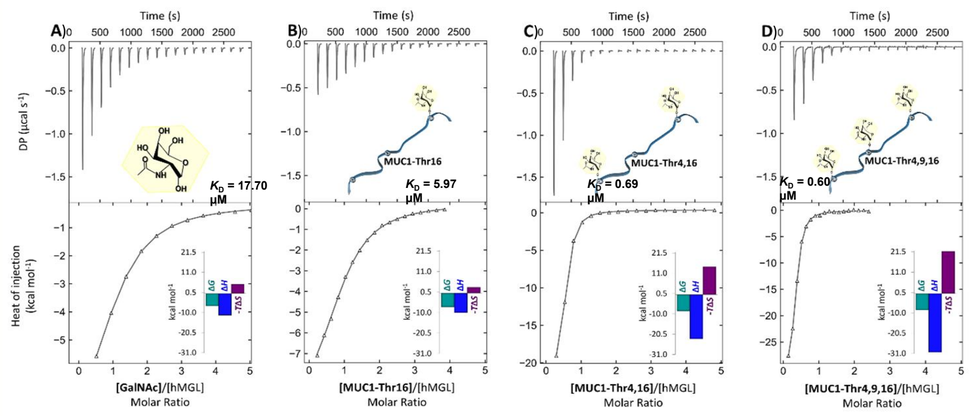


Results

MUC1 Glycopeptide models



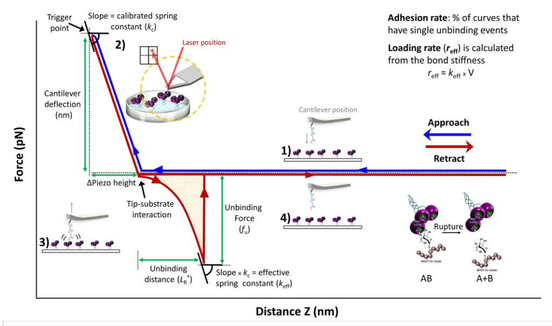
Thermodynamics of hMGL binding to GalNAc and MUC1 Glycopeptides



Selected ITC binding curves including thermographic profiles for interaction between (A) Gal*N*Ac (1.50 mM) with hMGL (29.6 μM), (B) MUC1-Thr^{16}(Tn) (0.50 mM) with hMGL (22 μM), (C) MUC1-Thr^{4,16}(Tn) (0.50 mM) with hMGL (20 μM), and (D) MUC1-Thr^{4,9,16}(Tn) (0.25 mM) with hMGL (20 μM) in buffered H_2O determined by ITC. Isotherms and thermograms were reproduced in GUSSI v.1.4.2 from data generated by MicroCal PEAQ-ITC software.

Kinetics of hMGL binding to GalNAc and MUC1 Glycopeptides

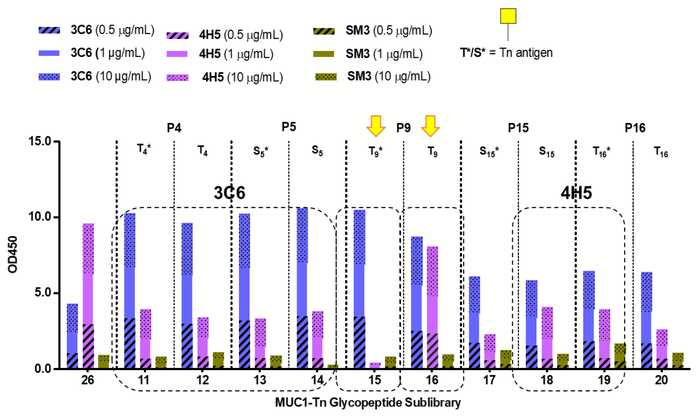
Force-Distance Rupture Curve



Parameters for the Energy Landscape of Immobilized Ligand: Receptor	MUC1-Thr^4(Tn): hMGL	MUC1-Thr^9(Tn): hMGL	MUC1-Thr^{16}(Tn): hMGL
Most Probable Force, F^* (pN)	54	34	33
Width of the energy barrier, x_β (nm)	0.353	0.807	0.733
Off-rate, k_{off} (s^{-1})	1.823	0.0789	1.159
Lifetime, τ_0 (s)	0.548	12.672	0.862

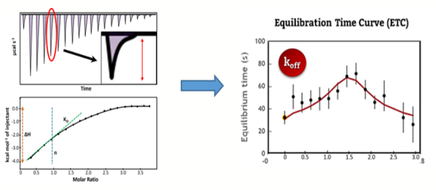
Screening and deconvolution of MUC1-Tn PS-SGCL by ELISA assay

Anti-MUC1 mAbs



- **Sublibrary 16** showed a strong preference for a non-glycosylated Thr^9 in the PDTR epitope of MUC1 for **4H5** anti-MUC1 mAb.

Kinetic parameters and lifetime calculated from experimental ITC data using AFFINImeter KinITC software

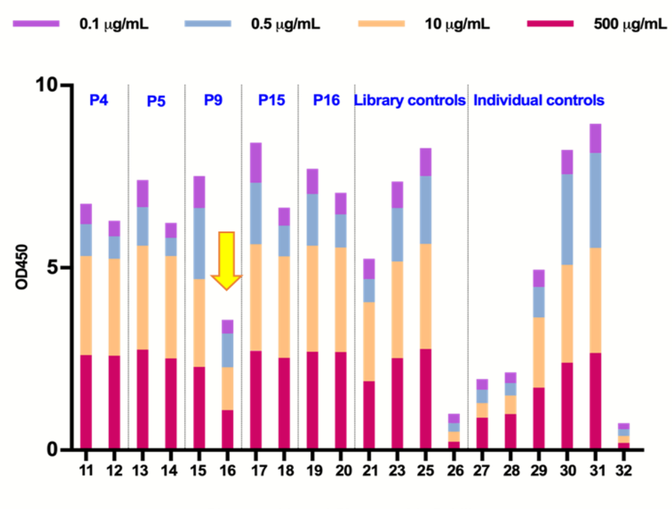


Ligands	k_{on} $x\ 10^6\ M^{-1}\ s^{-1}$	k_{off} s^{-1}	τ s
GalNAc	-	-	-
MUC1-Thr^4(Tn)	-	-	-
MUC1-Thr^9(Tn)	-	-	-
MUC1-Thr^{16}(Tn)	0.096	0.60	1.67
MUC1-Thr^{9,16}(Tn	0.032	0.049	20.41
MUC1-Thr^{4,16}(Tn)	0.058	0.051	19.61
MUC1-Thr^{4,9}(Tn)	0.016	0.060	16.67
MUC1-Thr^{4,9,16}(Tn)	0.026	0.019	52.63

- KinITC analysis revealed a prolonged lifetime of the lectin–glycan complex with increasing glycan valency.

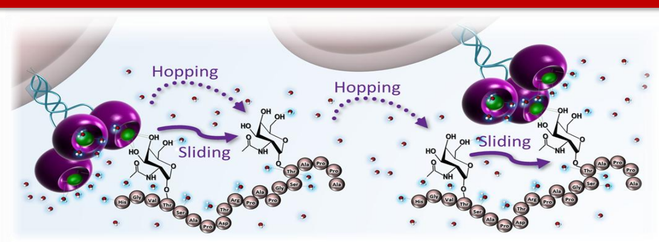
Screening and deconvolution of MUC1-Tn PS-SGCL by ELLA assay

Glycine max soybean agglutinin (SBA)



- **Sublibrary 16** showed a major loss in affinity when Thr^9 is non-glycosylated while other positions are mixtures.

Conclusion



- Glycan density, as well as the topology in the vicinity of the glycan site of attachment in the MUC1 epitope impact lectin affinity differently and can be used to manipulate thermodynamic and kinetic signatures of binding.
- "Bind and jump" mechanism is evident, from the additive –ΔH, non-additive -ΔS, and increasing lifetimes as the glycan density increases.
- The success of vaccine strategies is also dependent on a better understanding of the role of the tumor microenvironment (TME) in cancer progression.

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