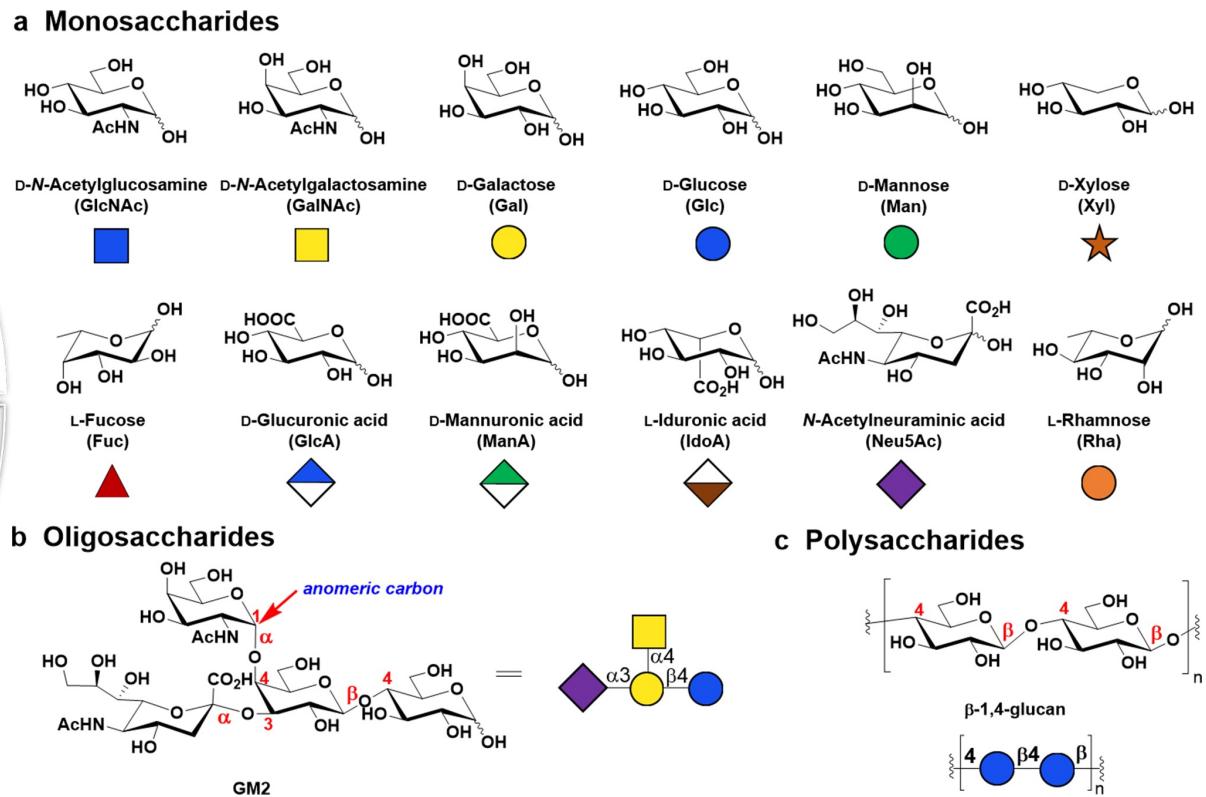
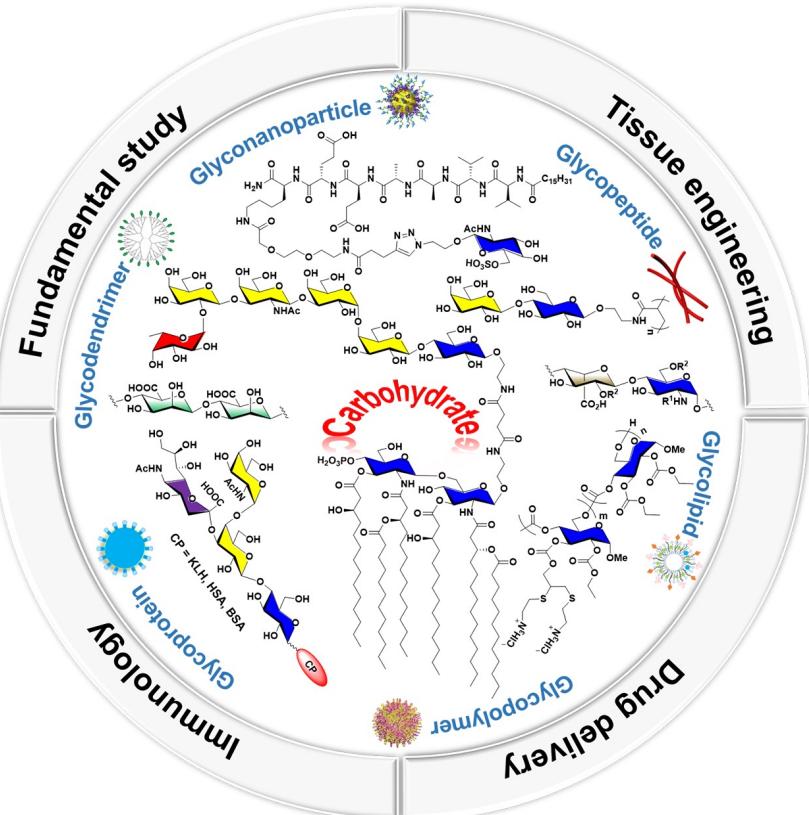


## Chapter 2

# Polysaccharide-Based Theranostics

# Carbohydrate-Based Macromolecular Biomaterials

- **Carbohydrate-based macromolecular biomaterials** with multiscale structure–function relationship in fundamental study, delivery system, tissue engineering, and immunology.

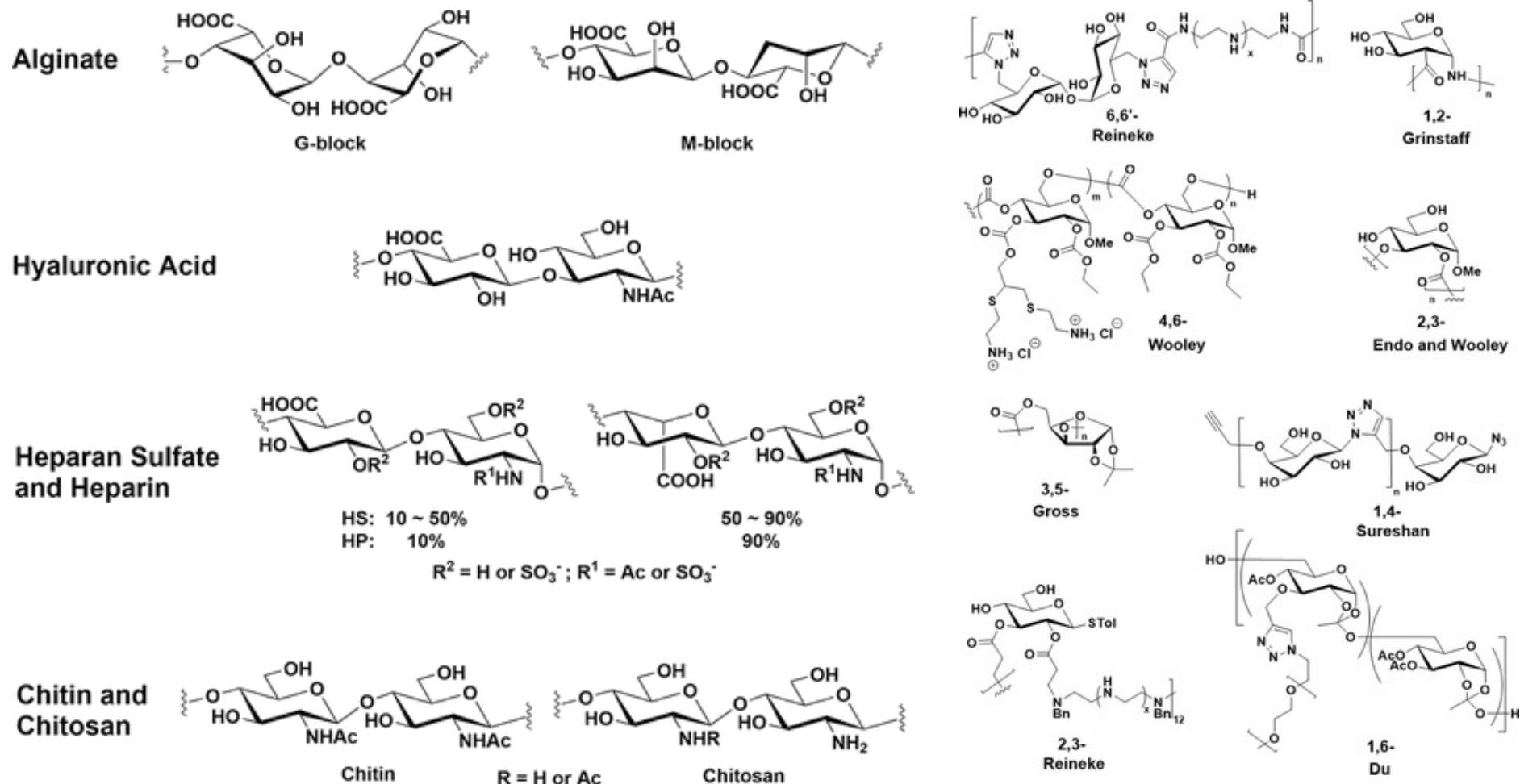


The common monosaccharides (a), oligosaccharides (b), and polysaccharides (c) with their anomeric linkage labeled in red.

**Carbohydrate-based biomaterials** are categorized into five basic species (1) naturally occurring polysaccharides, (2) naturally derived synthetic polysaccharides, (3) glycopolymers and glycodendrimers, (4) supramolecular glycopolymers, and (5) synthetic glycolipids and glycoproteins.

# Natural / Synthetic Polysaccharides

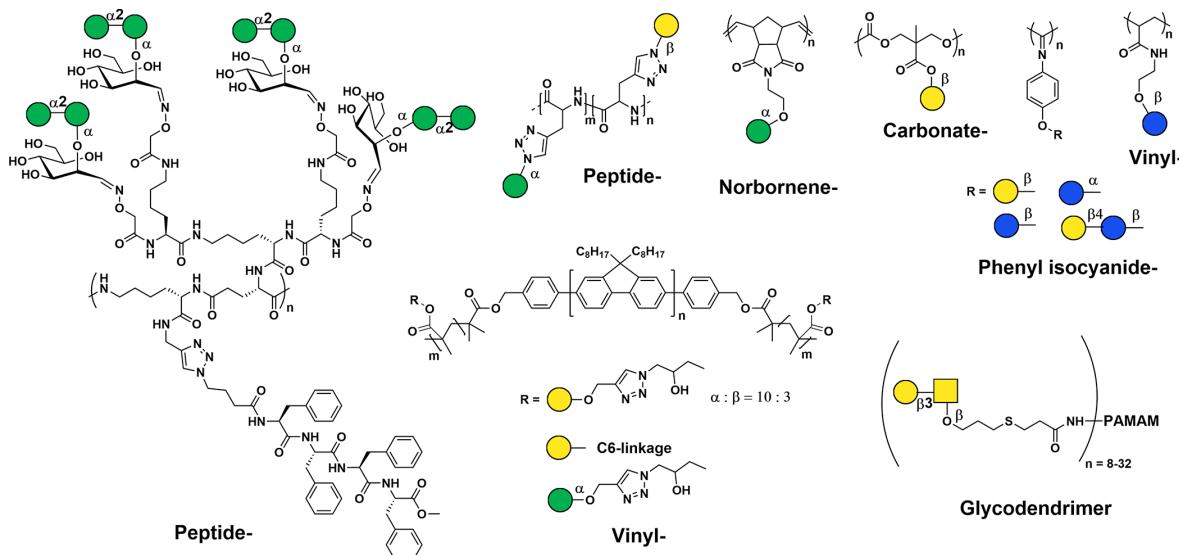
- The only way to construct polysaccharides via a **chemical or chemoenzymatic method** is step-by-step synthesis.



**Naturally occurring polysaccharides:**  
 1) Alginate, 2) Hyaluronic Acid, 3)  
Heparin/Heparan Sulfate, 4) Chitin and Chitosan.

**Synthetic polysaccharides:** 1) Poly(saccharide carbonate)s, 2) Poly-Amido-Saccharides. A variety of synthetic polysaccharides with ortho-ether, ester, amide, carbonate, triazolyl, or a combination linkages.

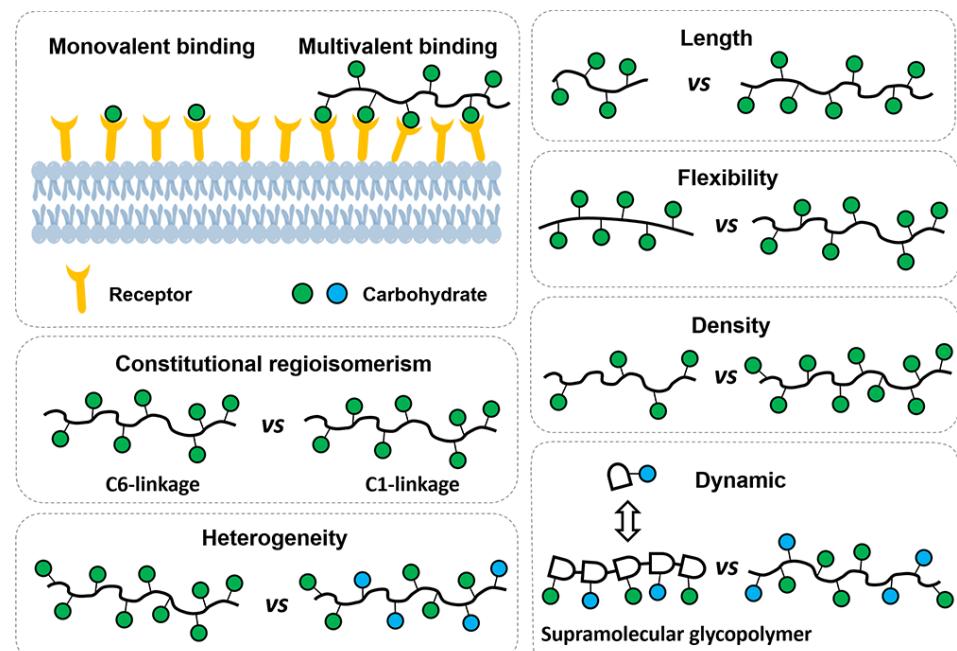
# Glycopolymers and Glycodendrimers



Examples of glycopolymers and glycodendrimers.

- ✓ **Glycan-binding proteins (GBPs)** are found in all living organisms and fall into lectins and sulfated GAG-binding proteins.
- ✓ The **monovalent interactions** of the carbohydrate and its **carbohydrate-recognition domains (CRDs)** are relatively weak (i.e.,  $K_a = 10^3\text{--}10^4 \text{ M}^{-1}$ ).
- ✓ **Multivalent interactions** are employed throughout biological processes, such as cell differentiation, intercellular signal transduction, inflammation, and the immune response, which can not only enhance the functional affinity of cell surface CPIs, but also improve CPIs specificity.

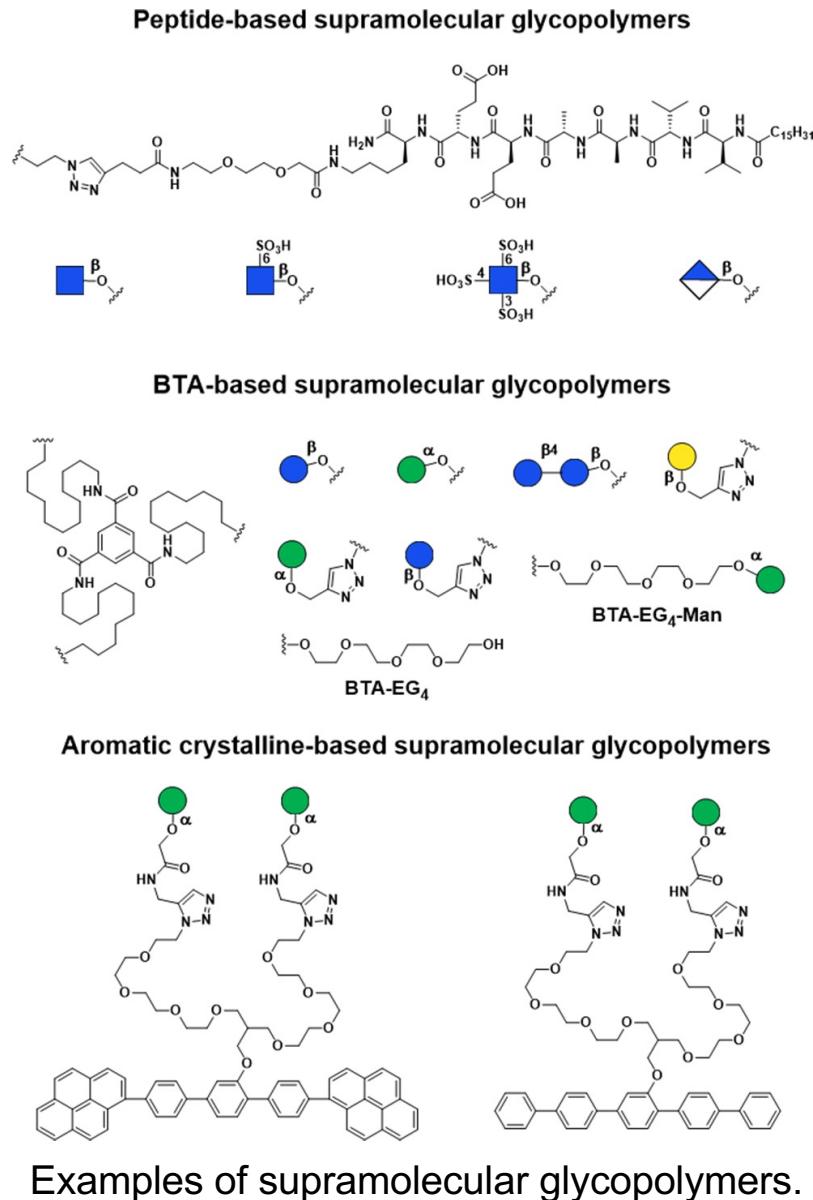
**Long glycopolymers tend to show higher binding affinities** than short ones owing to the enthalpy gain with multiple binding.  
**Long glycopolymers provide more opportunities to induce supramolecular polymerization/organization** of the receptors anchored on the supported lipid bilayer or cell membrane, to increase the local concentration of CRDs, which could also contribute to a higher binding affinity.



**Multivalency effect on CPIs** with some influencing factors.

# Supramolecular Glycopolymers

- **Supramolecular glycopolymers**, that is well-defined nanofiber structure



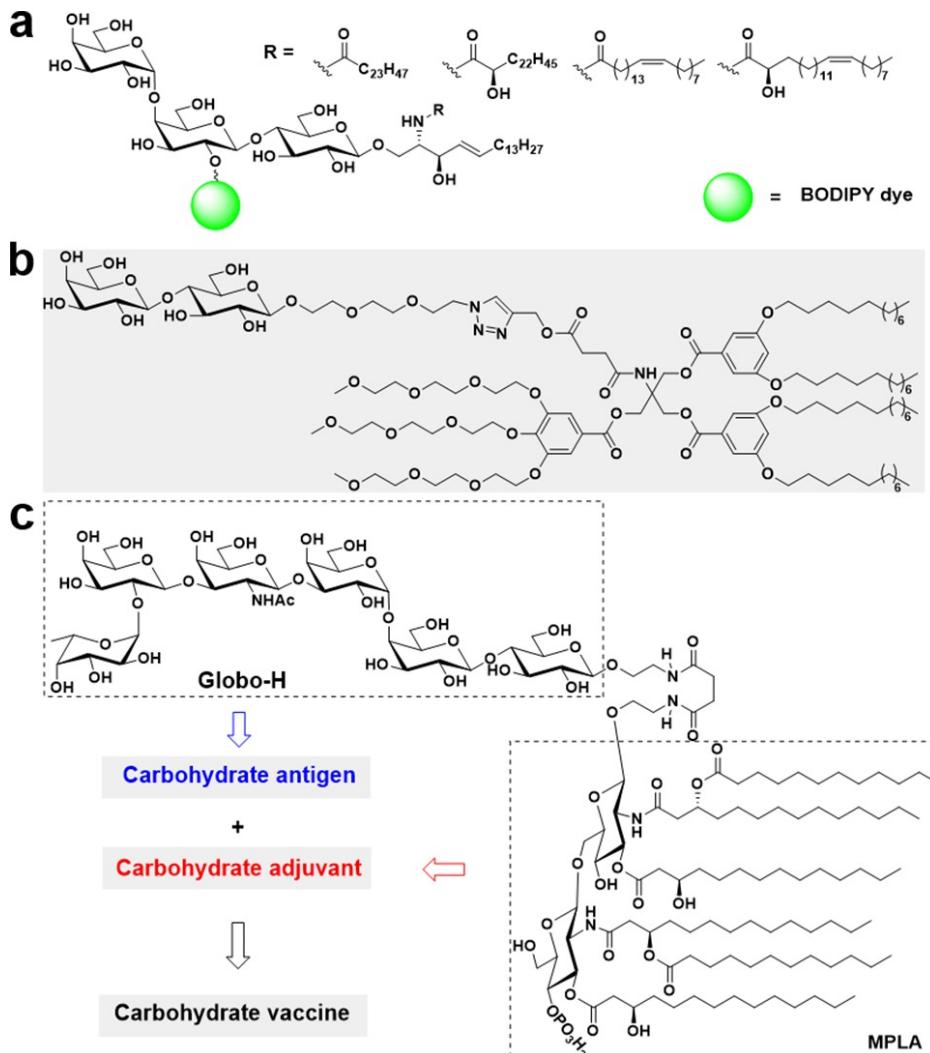
**Supramolecular glycopolymers** include three categories according to the hydrophobic scaffold, namely, 1) peptide, 2) C3-benzene-1,3,5-tricarboxamide (BTA), and 3) aromatic crystalline.

Stupp group: Peptide-based supramolecular glycopolymers are used to mimic glycosylated protein function. Glycopeptide supramolecular nanofibers displaying on the surface with a high density showed a rather good binding ability and bioactivity toward multiple proteins in vivo.

Meijer group: BTA system, revealing the structure-dynamic-property relationship on supramolecular (co)polymerization in water. BTA glycopolymers show higher dynamic properties yet allow for control over supramolecular copolymerization via hydrophobic or H-bonding.

**Aromatic crystalline-based glycopolymers** with the dynamic property of the hydrophobic core, allowing for strong  $\pi-\pi$  stacking and hydrophobic interaction, while the multiple OH groups of the carbohydrates allow for extensive H-bonding, to fabricate dynamic and self-healing materials.

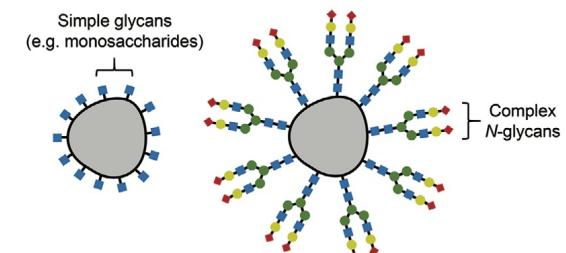
# Synthetic Glycolipids and Glycoproteins



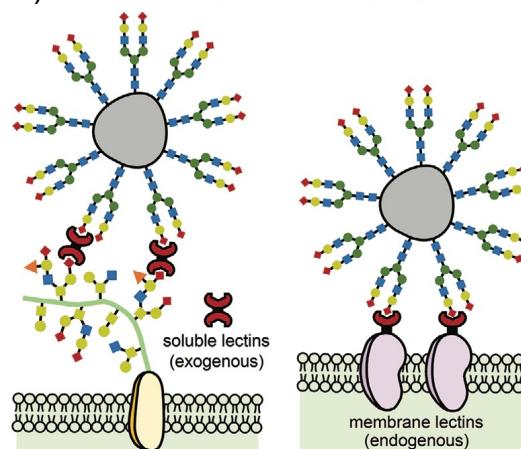
**Structures of glycolipids.** (a) Chemical structure of Gal-derived single-chain glycolipid. (b) Amphiphilic Janus glycodendrimer. (c) MPLA-Globo-H conjugate.

## a) Artificial glycoproteins

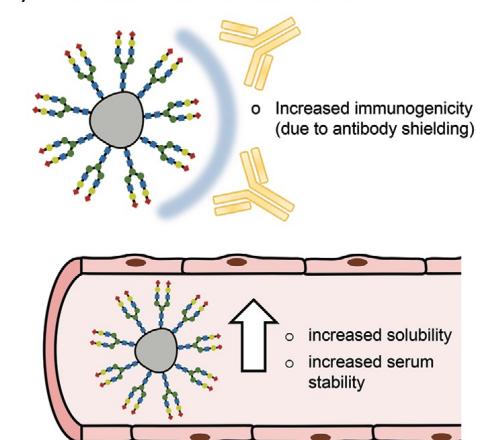
- o Proteins decorated with non-natural assembly of glycans
- o Prepared with either simple or complex glycans



## b) (Lectin-directed) glycan-based targeting



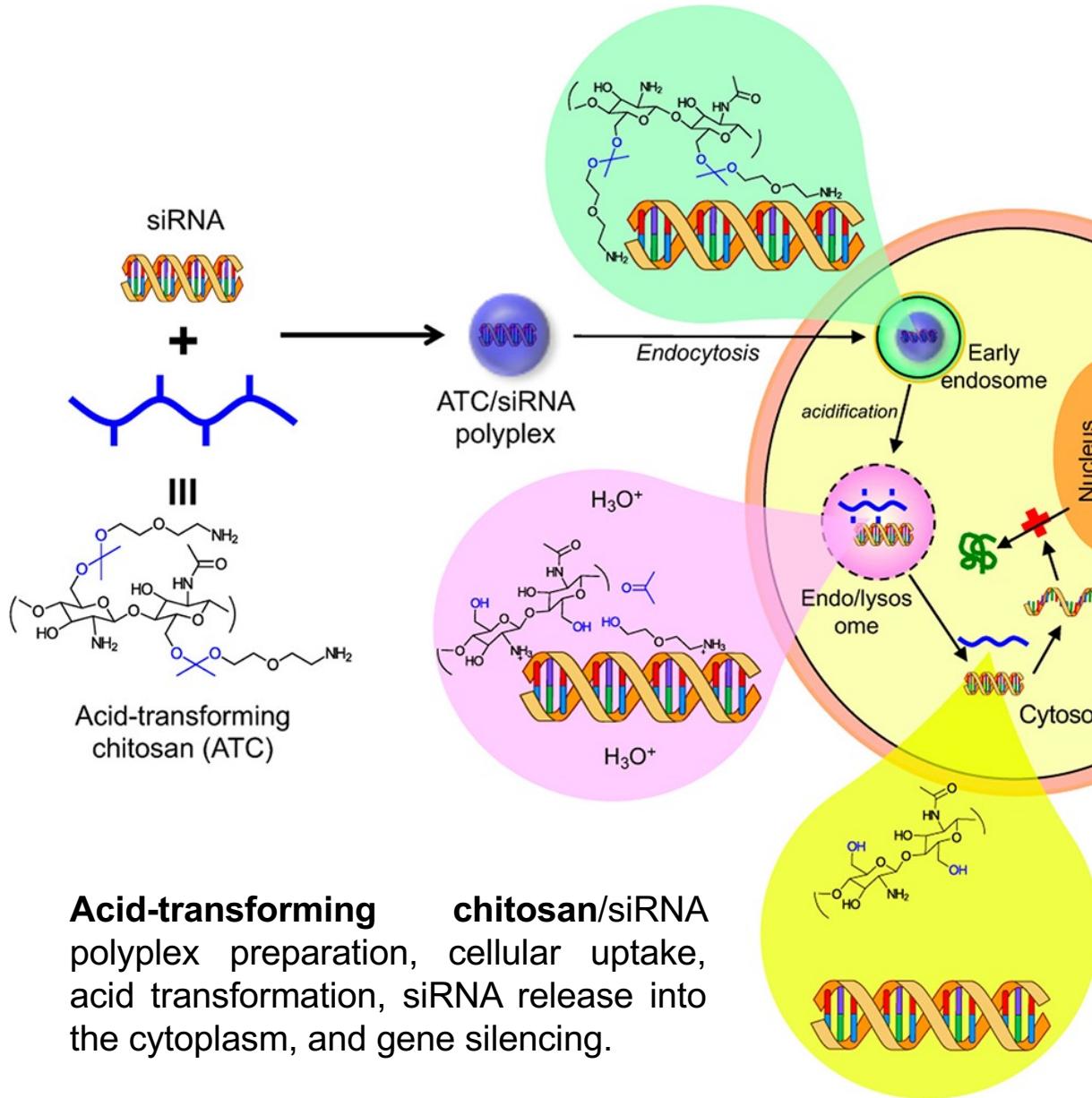
## C) Advantages of protein glycosylation



**a) Artificial glycoproteins** is based on constructing protein complexes that are decorated with a non-natural assembly of glycans (monosaccharides or complex glycans). **b) Lectin-based recognition of artificial glycoproteins** can be performed by lectins differentially expressed on cellular surfaces. **c) Protein glycosylation** is known to improve aspects related to immunogenicity, serum solubility, and other physical properties.

# Carbohydrate-Based Therapeutic Delivery Systems

- Delivery biomaterials with **carbohydrate-based macromolecules as the skeleton**



**Acid-transforming chitosan/siRNA** polyplex preparation, cellular uptake, acid transformation, siRNA release into the cytoplasm, and gene silencing.

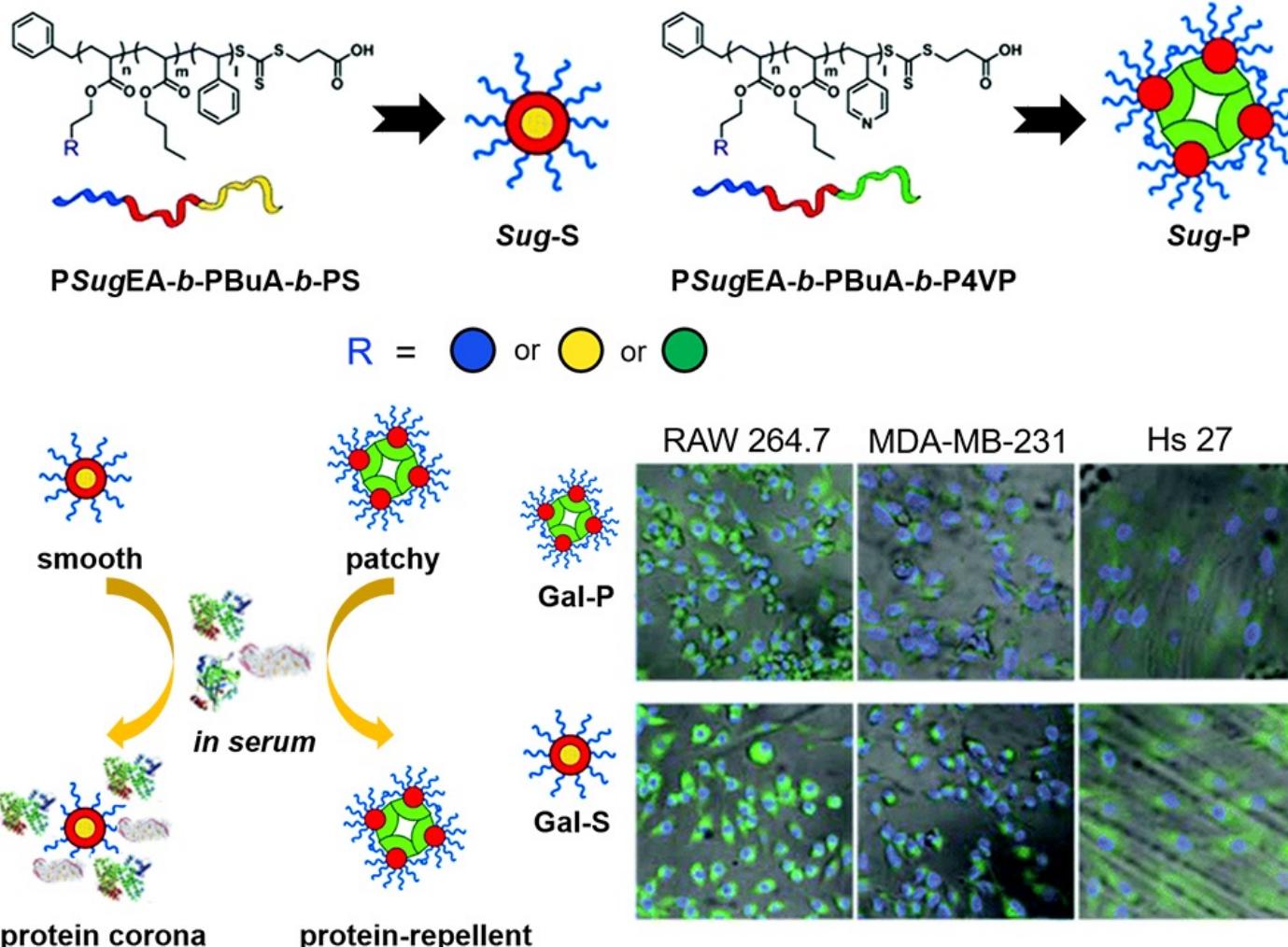
Apart from **tuning the molecular weight and acetylation degree**, functionalization could provide better transfection efficacy. Enhanced **stiffness and bulkiness of chitosan were induced by a high acetylation degree**.

An **acid-responsive siRNA delivery system** was achieved by conjugating flexible, aqueous-soluble aminoethoxy branches to the modified chitosan via **acid-cleavable ketal linkages**, showing greatly enhanced aqueous solubility and improved siRNA complexation. The mildly acidic endosome/lysosome environment could effectively trigger the hydrolysis of ketal linkages.

Cationic chitosan, modified with stearic acid and blood-brain barrier (BBB) crossing peptide (TGN) in drug delivery systems.

# Carbohydrate as the Hydrophilic Shell

- Delivery nanocarriers with carbohydrate-based macromolecules as the hydrophilic shell

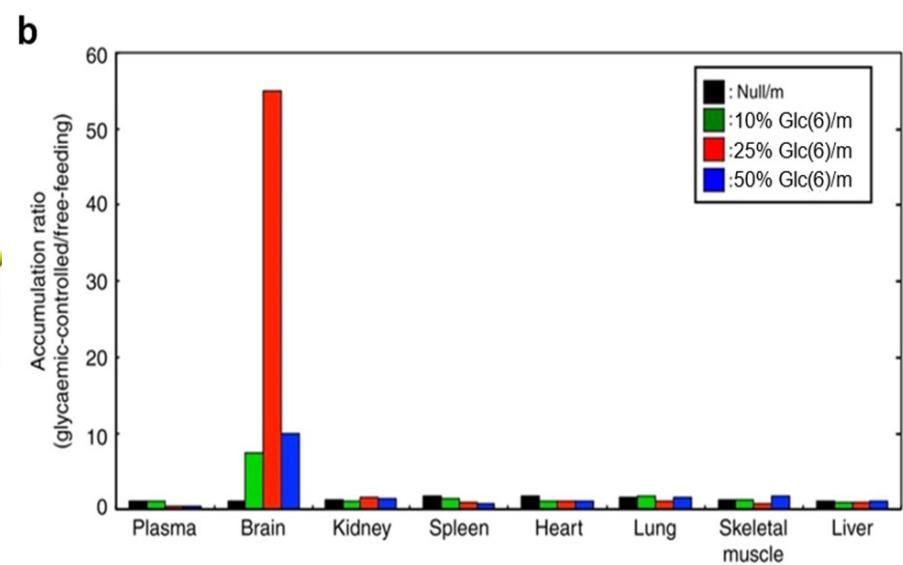
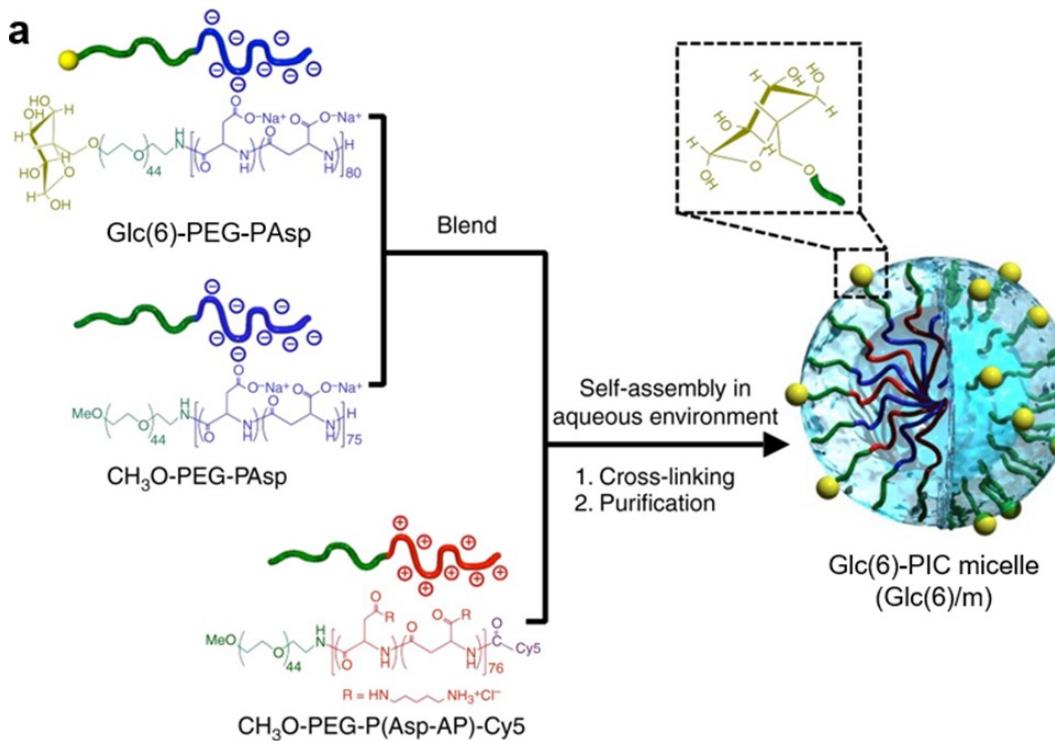


Compartmentalized spherical nanoparticles were prepared from a set of **linear ABC triblock glycopolymers with Glc, Man, or Gal patches**, allowing for combined evaluation of the surface topography and functionality. The patchy nanoparticles displayed significantly reduced serum protein absorption therefore lowered nonspecific uptake by different cell lines, including macrophages, breast cancer cells, and fibroblasts. Moreover, the carbohydrate type influenced the relative protein abundance on the corona of the smooth nanoparticles, with Man and Gal showing more profound absorption.

The formation of smooth and patchy nanocarriers (above) with representative confocal images showing internalization of Gal-based nanoparticles after 24 h of incubation (below).

# Carbohydrates as Targeting Agent

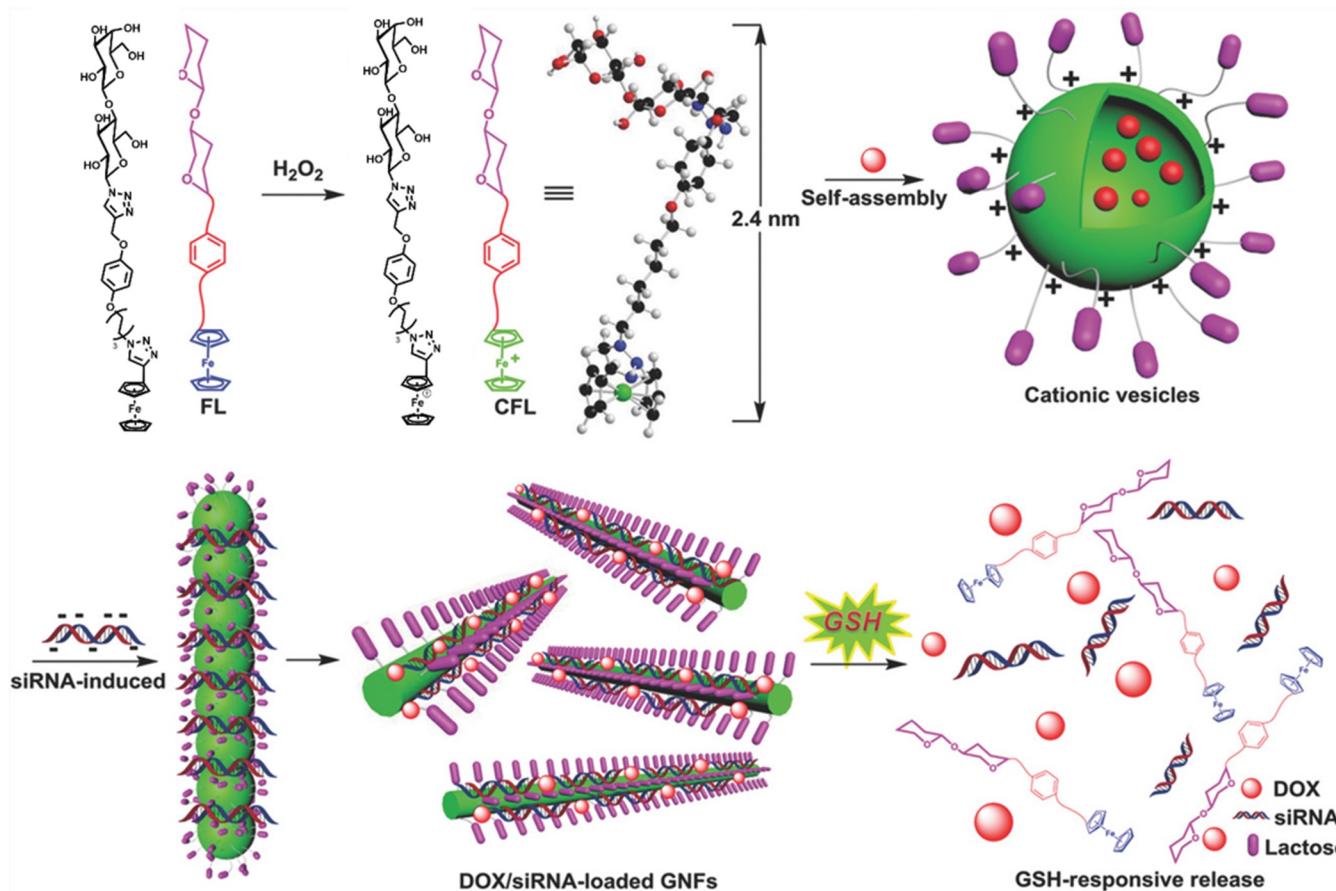
- **Glucose transporter-1 (GLUT1)**, highly expressed in brain capillary endothelial cells, could be potentially employed for enhancing the delivery of the carriers across the BBB to achieve accurate brain tumor diagnosis and satisfactory therapeutic effects.



Achieved **boosted BBB crossing and brain accumulation of Glc-modified nanocarriers** through rapid glycemic control. The size and Glc density of the nanocarriers were precisely controlled by multimolecular association of oppositely charged pairs of PEG-based block copolymers. The resulting nanocarrier Glc(6)/m was ca. 30 nm, and the Glc density was tuned as 0%, 10%, 25%, and 50%. In vivo study with glycemic control showed **a 56-fold higher brain accumulation of a 25% Glc(6)/m formulation** (up to 6% dose/g-brain) compared to the free-feeding mice, suggesting that BBB crossing was dependent on blood Glc concentration and promoted by GLUT1.

# Liver Cancer Targeting

- ASGPR, predominantly expressed at the surface of hepatic cells, is a hetero-oligomer consisting of two homologous transmembrane proteins with a  $\text{Ca}^{2+}$ -dependent CRD that interacts with Gal, GalNAc, and related galactosides.

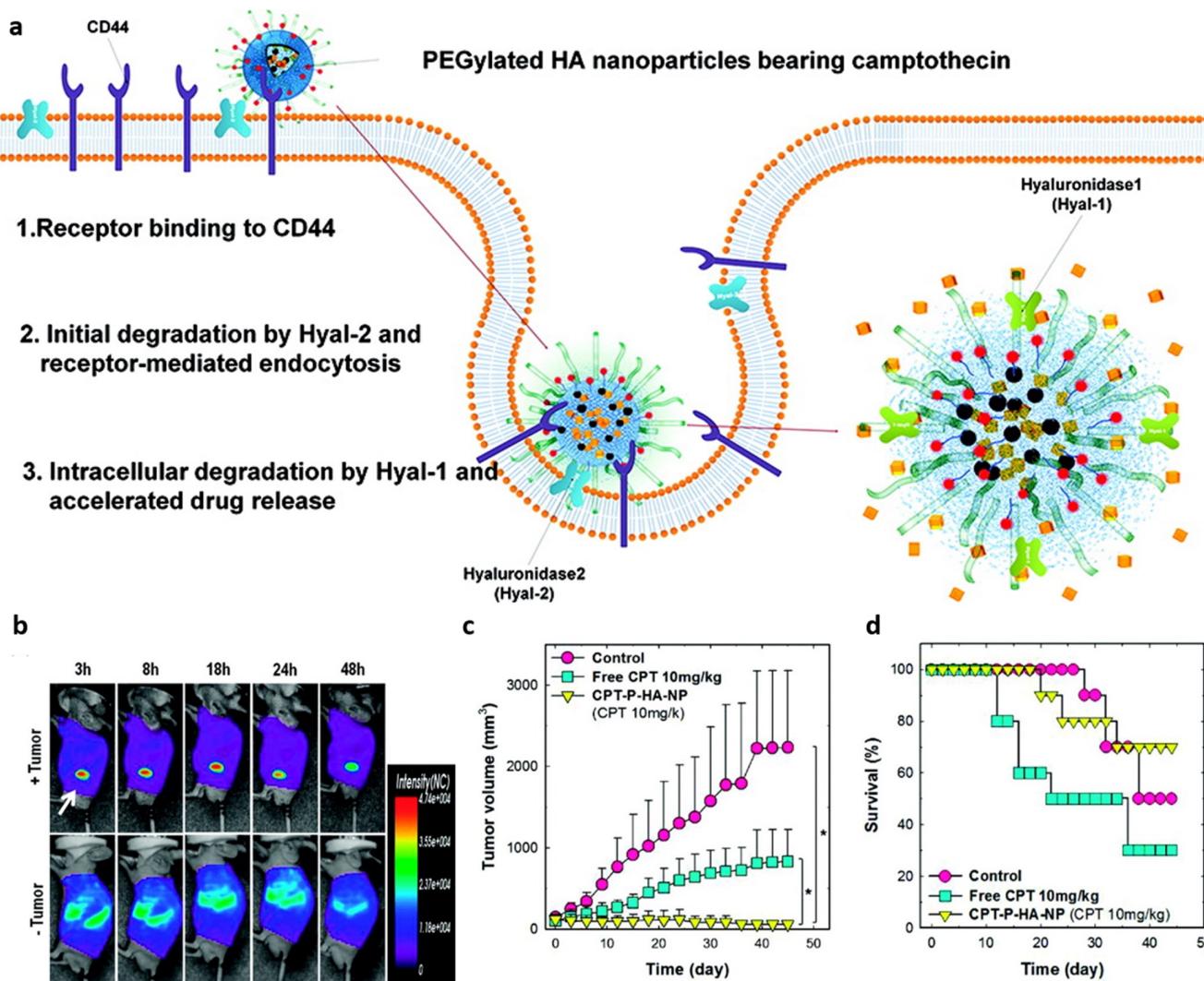


The molecular structure of CFL, formation of DOX/siRNA-loaded GNFs via self-assembly of CFL in the presence of DOX and siRNA induction, and disassembly of the GNFs upon GSH stimulus.

**Hepatic-targeted codelivery with controlled release of drug/siRNA** in vitro and in vivo. Amphiphilic cationic ferrocenium-modified lactose derivatives (CFLs) underwent supramolecular co-assembly with DOX, fabricating drug-loaded cationic vesicles, which further complexed with polyanionic siRNA and triggered the formation of nanofibers (GNFs). These GNFs displayed excellent biocompatibility, enhanced cell-penetrating ability, and hepatoma target ability. In vivo study in both HepG2 and HepG2/ADR subcutaneous tumor-bearing nude mice showed excellent tumor targeting delivery, enhanced therapeutic efficacy, and reduced systemic toxicity to organ.

# CD44-Mediated Targeting

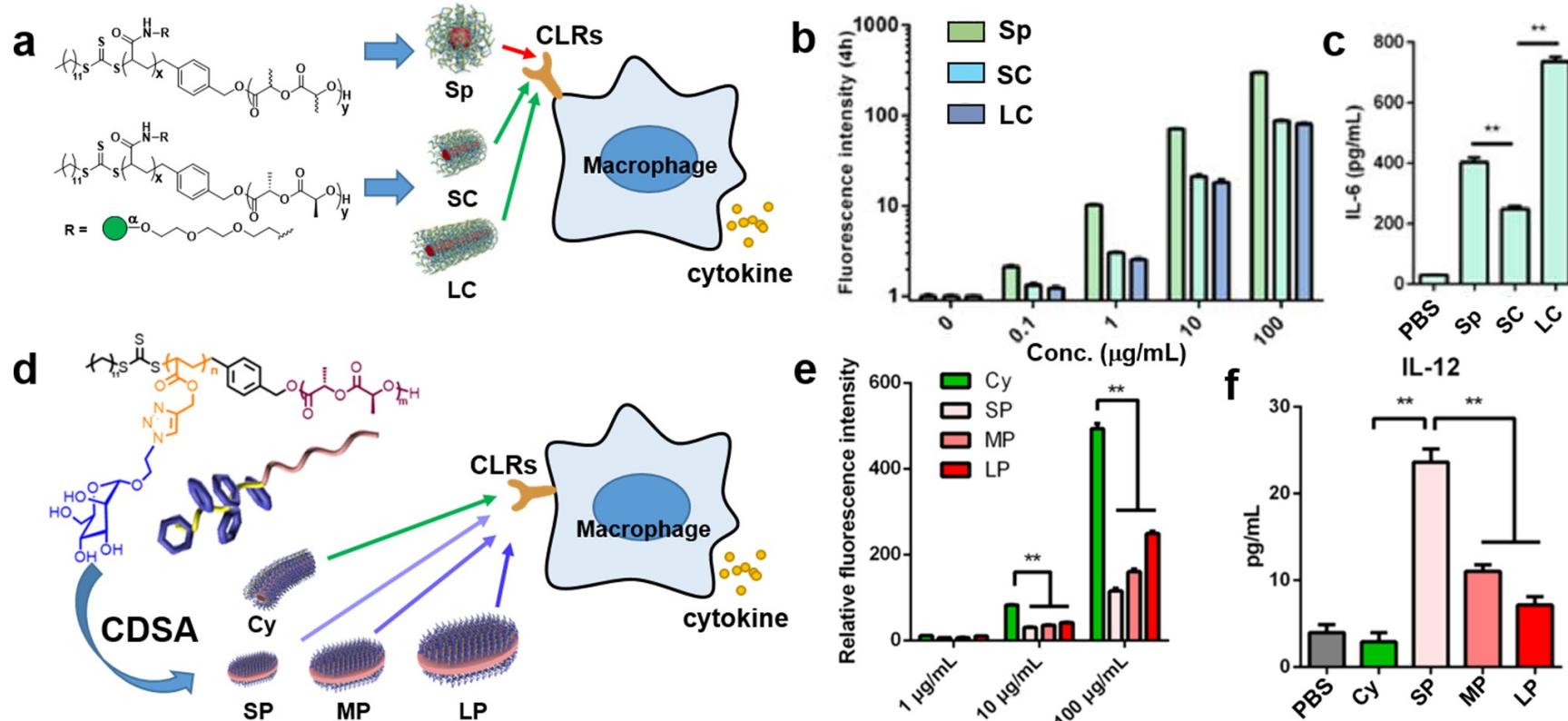
- CD44 is a cell-surface glycoprotein, which is strongly expressed on chondrocytes and some tumor cells (e.g., breast cancer cells)



- ✓ **Hyaluronidases-responsive nanocarriers** for cancer therapeutics consisting of amphiphilic PEGylated-HA conjugates (P-HA-NPs) and hydrophobic anticancer drug camptothecin (CPT).
- ✓ The P-HA-NPs could be internalized into cancer cells through receptor-mediated endocytosis but not by normal fibroblasts, **showing cancer cell-specific uptake**.
- ✓ In *vivo* noninvasive fluorescence images indicated the specific accumulation of CPT-P-HA-NPs in tumor tissue owing to the **prolonged circulation and HA-mediated CD44-targeting**.

# Immunoregulation by Targeting Lectin Receptors

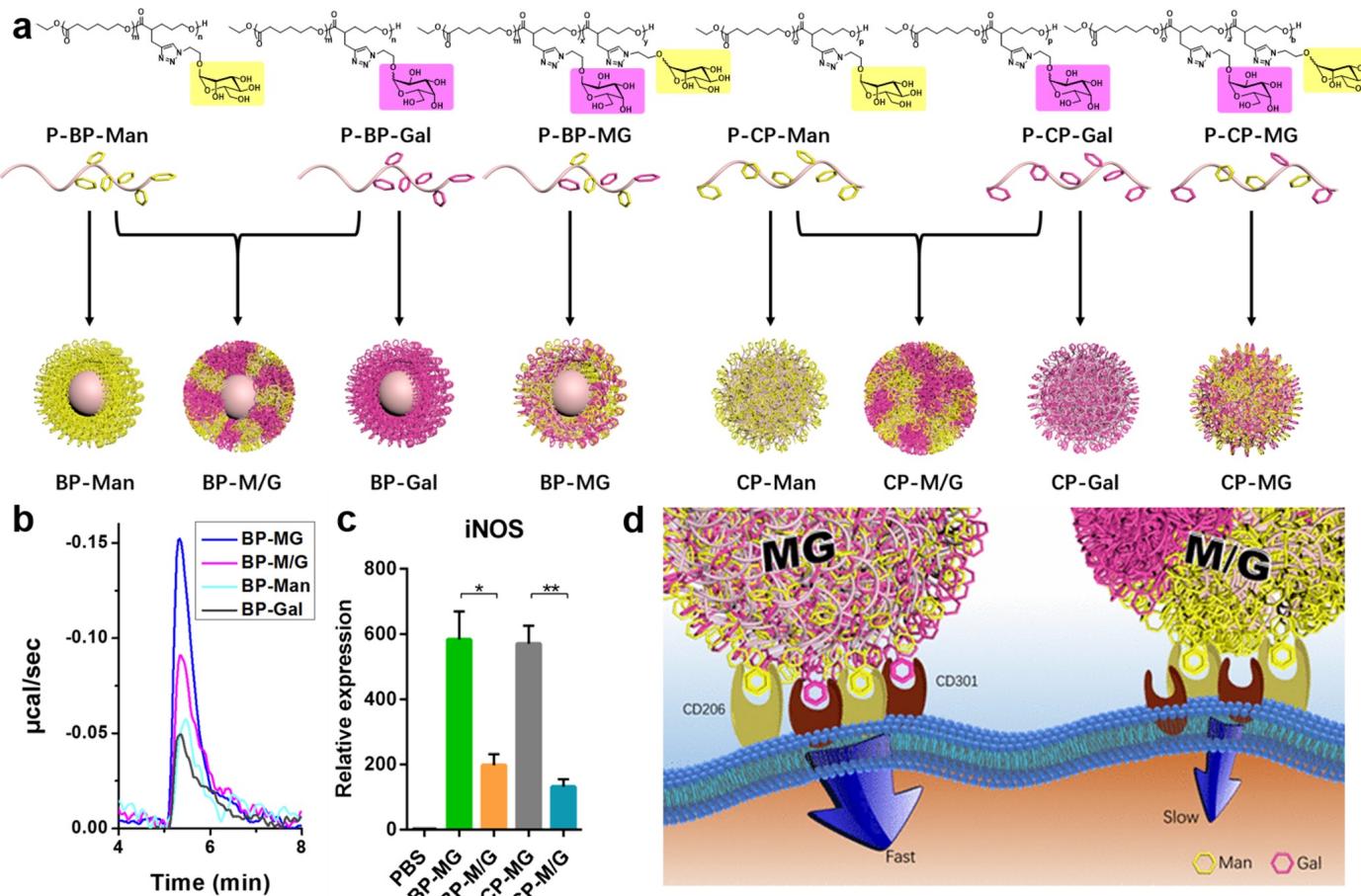
- C-Type lectin receptors (CLRs) are expressed by several immunologically relevant cell types, such as DCs and macrophages.



(a) **Glycomaterials with different shape and size to induce immune response.** (b) Dose dependence of the binding of GNPs with macrophages. (c) **GNPs promoted the IL-6 secretion of macrophages (inflammatory response)** after 24 h incubation measured by ELISA. (d) Immune response induced by GNPs with different shape by interaction with macrophages. (e) **Endocytosis of different fluorescent PLLA33-b-PMan12 GNPs by macrophages.** Cy, cylinder; SP, small platelet; MP, medium platelet; LP, large platelet. (f) **GNPs with different size/shape promoted IL-12 secretions of macrophages (immunostimulatory activities)** after 24 h incubation measured by ELISA.

# Immunoregulation by Targeting Lectin Receptors

- Besides the shape and size, the architectures of GNPs have a significant effect on cellular uptake and lectin binding abilities for immunoregulation.



- (a) GNPs with different shell architectures made by self-assembled glycopolymers. (b) Heat release of block copolymer (BP) series GNPs binding with lectins. (c) iNOS release under equal endocytosis amounts. (d) Interaction between two different saccharides and their receptors.

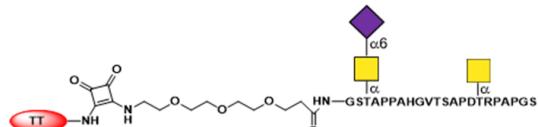
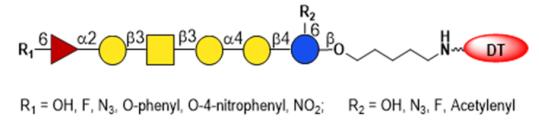
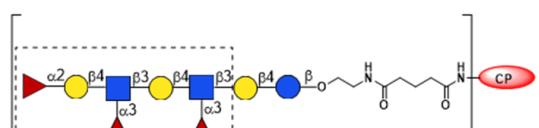
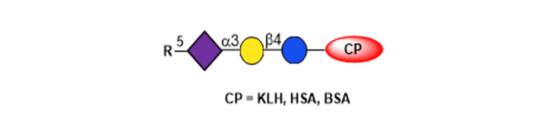
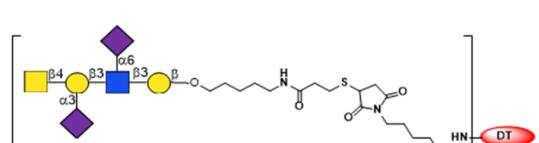
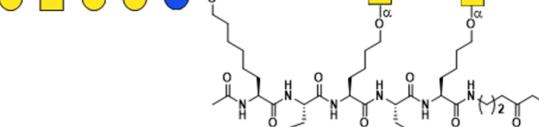
The endocytosis of these GNPs by macrophages showed that a **homogeneous mixture**, exhibited higher efficiency in cellular uptake and binding abilities.

Macrophages were also more efficiently activated by the homogeneous mixtures, than GNPs with blend-mixed coronas.

GNPs with a homogeneous corona allowed for simultaneous interactions between two different saccharides and their corresponding receptors.

# Cancer Vaccine

## ➤ Glycoprotein Vaccines

TACA	Carrier protein	Vaccine	Ref
MUC1 <sup>123,528-533</sup>			
Tn <sup>530,534</sup>	TT		529
TF <sup>533</sup>			
STn <sup>527,535-537</sup>			
Globo-H	DT		526
Lewis <sup>x</sup>	KLH		
Lewis <sup>y</sup>	HSA		538
KH-1			
GM3	KLH		524,525
RM2	DT		539
Globo-H			
GM2			
STn	KLH		540-542
TF			
Tn			



**Carbohydrate** = *Mucin1, Tn/TF/STn, Lewis<sup>x</sup> / Lewis<sup>y</sup>, GM2/GM3, Globo class*

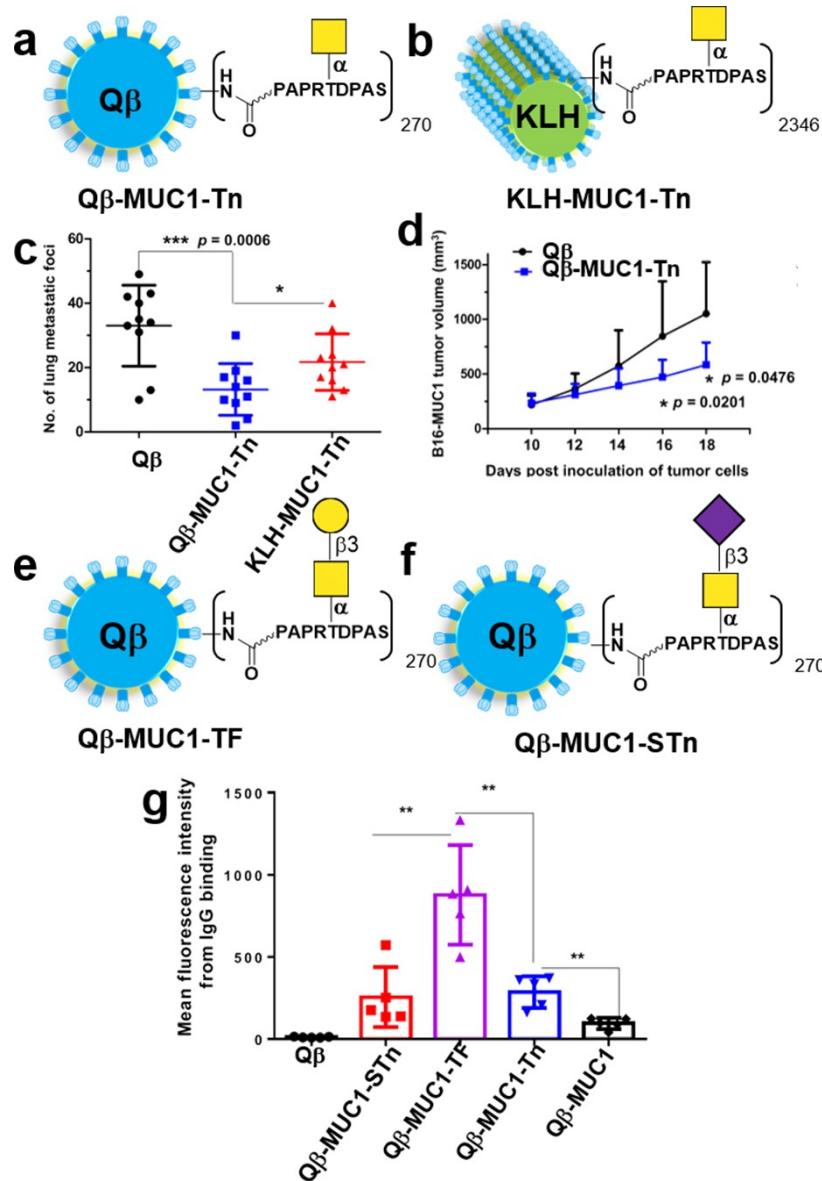
**Protein** = *KLH, HSA, CRM197, TT etc.*

## Structure of glycoprotein cancer vaccines.

To **improve the immunogenicity of carbohydrate antigens**, tumor-associated carbohydrate antigens (TACAs) are always conjugated to carrier proteins through various linkers. Carrier proteins not only provide T-cell epitopes to transform a T-cell-independent immune response to a T-cell-dependent type but also present carbohydrate antigens in multivalent form to **promote aggregation of IgM on the surface of B cells**, facilitating class switching of antibodies from IgM to IgG. The key points to design TACAs-based vaccines include (a) appropriate TACAs antigens, (b) proper carrier proteins, (c) suitable linkers between TACAs and carriers, and (d) the number and type of TACAs.

# Virus-Like Particles-Based Vaccines

- VLPs are highly organized structures which are self-assembled from subunit proteins, and VLPs have been widely used as carriers in vaccines.



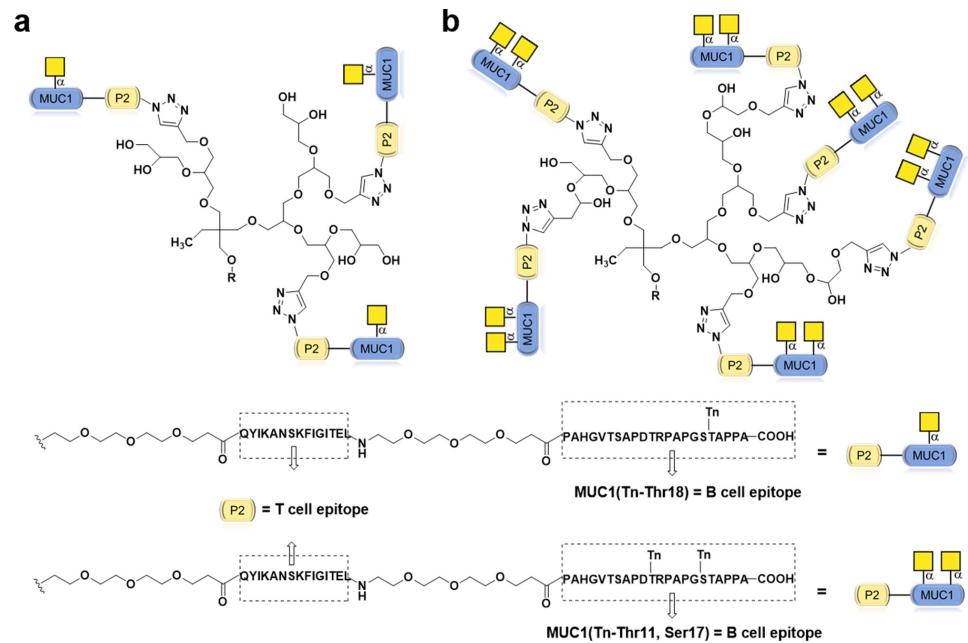
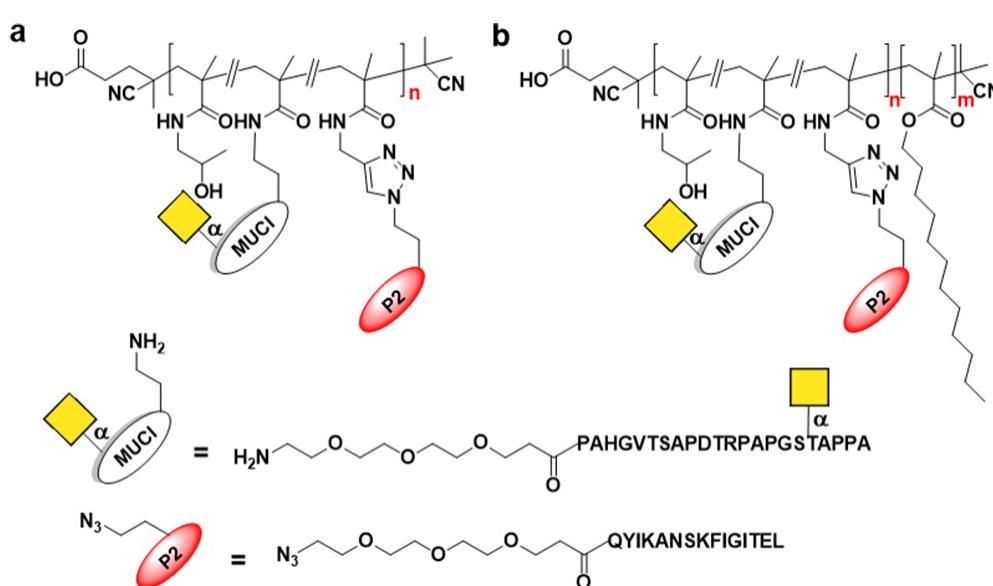
**VLPs harness high immunogenicity of viruses but are safe to humans and animals owing to the lack of viral genome. The size of VLPs ranges from 20 to 200 nm, which are **beneficial for being uptaken by APCs and trafficking to lymph nodes**.**

- ✓ The newly designed Q $\beta$ -MUC1 conjugate (**bearing monosaccharide TF**) provided significant tumor protection in both solid tumor and metastatic models.
- ✓ Q $\beta$ -MUC1-TF (**bearing disaccharide TF**) can largely reduce the number of tumor foci in a lung metastasis model, implying the translational potential of this structure as cancer vaccines.

Design of MUC1 (mucin family)-based vaccine for effective tumor protection in immunotolerant mice.

# Polymer-Based Vaccines

- The multivalent effects of pendant carbohydrates on the glycopolymer backbone can not only promote the binding of these carbohydrates to receptors but also facilitate the applications of glycopolymers in design of cancer vaccines.

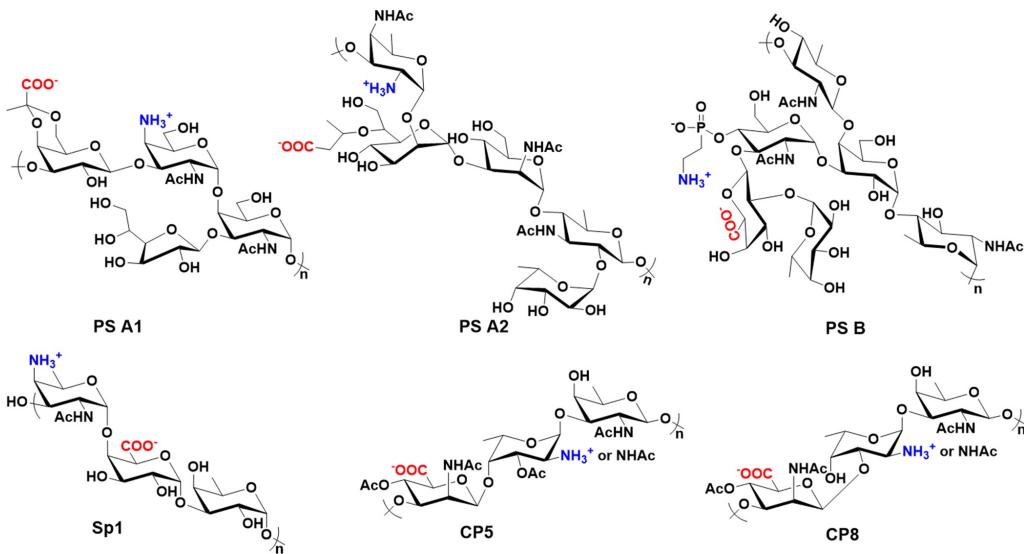


- ✓ Structure of **water-soluble polymers coupled with glycopeptide antigens and T-cell epitopes as potential antitumor vaccines**, including polymers without (a) or with (b) additional hydrophobic block.

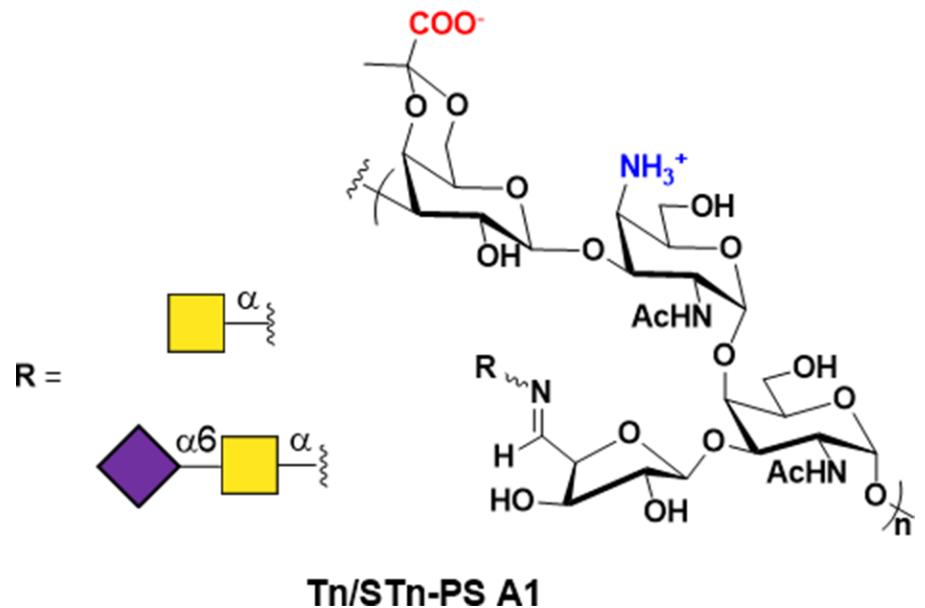
- ✓ **MUC1 glycopeptide cancer vaccines based on hyperbranched polymers.** (a) HPG-based cancer vaccine containing on average five MUC1 glycopeptides. (b) HPG-based cancer vaccine containing on average eight MUC1 glycopeptides. R can contain additional glycerol units with two MUC1 glycopeptides on average.

# Polysaccharides-Based Vaccines

## Zwitterionic polysaccharides



## Entirely carbohydrate antigen conjugates



**Zwitterionic polysaccharides (ZPSs)** are polysaccharides comprised of both negative and positive charges. They are isolated from the capsule of commensal anaerobic bacteria and have the ability to elicit MHCII-mediated, T-cell-dependent immune responses and invoke production of IgG and IgM. Their effects are **comparable to carrier proteins**.

**An entirely carbohydrate vaccine candidate.** The Tn antigen was introduced by oxime formation with oxidized ZPS PS A1. High titers of immunoglobulins were generated in the absence of adjuvant. Specific IgG3 antibodies were also elicited, implying a T-cell-dependent immune response. Furthermore, STn has also been conjugated to PS A1 by the same protocol.

# Antimicrobial Vaccines

## ➤ Development of glycoconjugate antimicrobial vaccines

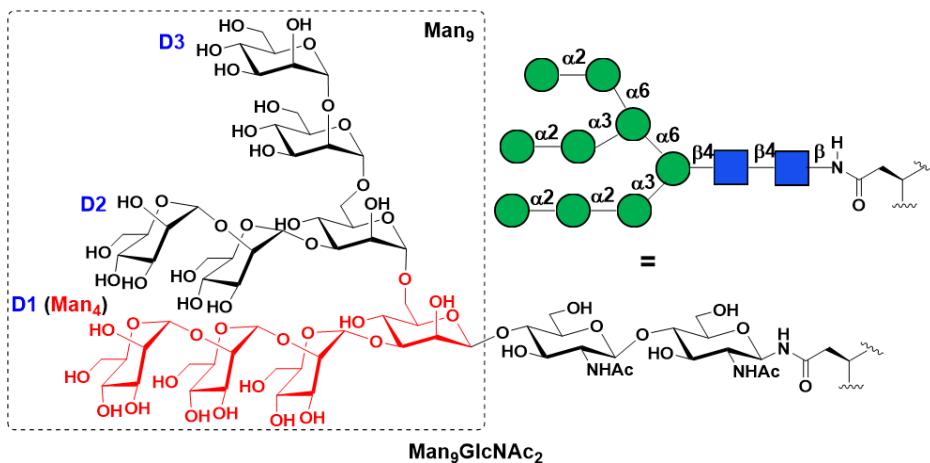
Carbohydrate		Carrier protein	Vaccine	Ref
Type	Source			
Teichoic acid	<i>E. faecium</i> U0317	KLH/HSA		619
Capsular polysaccharides	<i>Streptococcus pneumoniae</i> type 3			620
CPS	<i>Neisseria meningitidis</i> serogroup C			621
Cell wall polysaccharide	Group A streptococcus			622
CPS	<i>Staphylococcus aureus</i>			623
Cell wall polysaccharide	Fungal ( <i>Candida albicans</i> )			624
Cell wall polysaccharide	Fungal ( <i>Candida albicans</i> )	HSA/KLH		625

The structure of the carbohydrate antigen, the presence or absence of some functional groups, the nature of the carrier protein, the spacer and conjugation pattern, as well as the ratio of carbohydrate to protein, and their **influences on immune efficacy**.

# Antiviral Vaccines

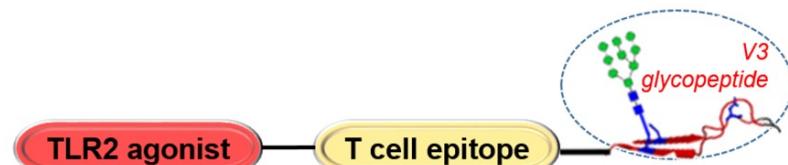
## ➤ Antiviral Vaccines (Anti-HIV Vaccines)

A high-mannose patch, **Man<sub>9</sub>GlcNAc<sub>2</sub>**, is mostly targeted by bnAbs, which has been used to mimic these epitopes.

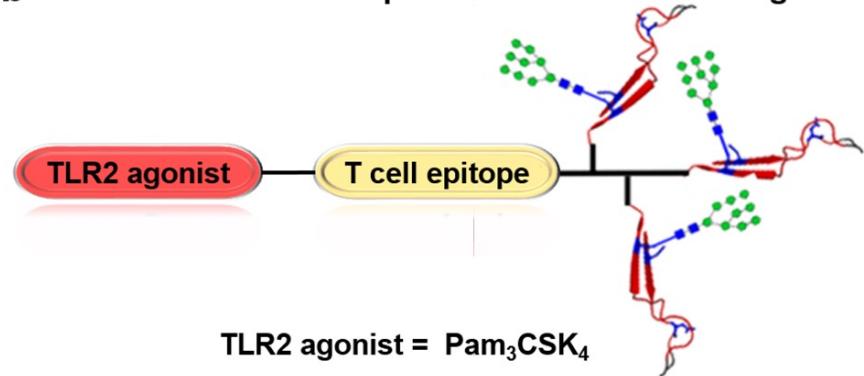


(a) monovalent and (b) trivalent three-component glycopeptide immunogens.

### a Monovalent three-component HIV-1 V3 immunogen



### b Trivalent three-component HIV-1 V3 immunogen



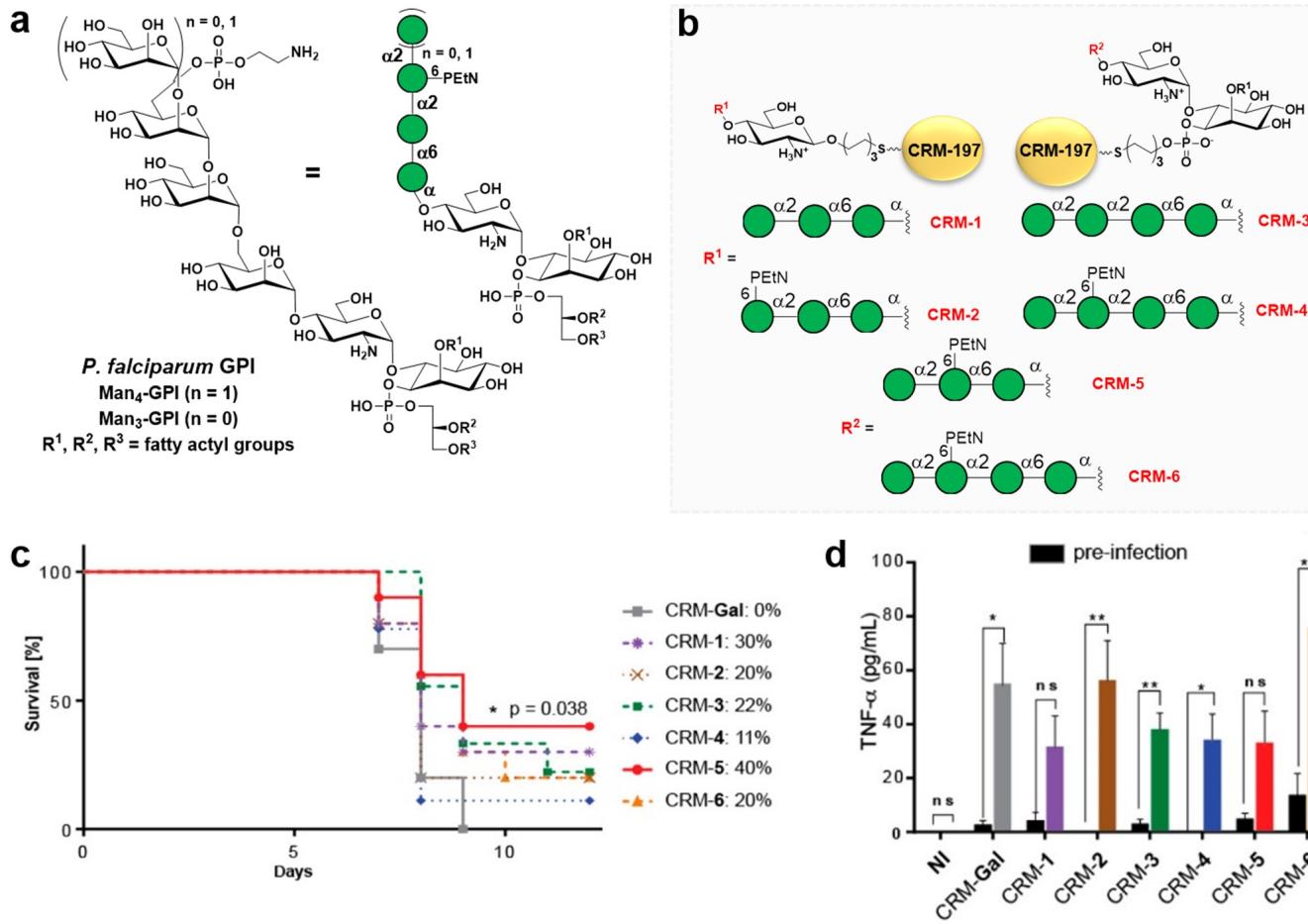
TLR2 agonist = Pam<sub>3</sub>CSK<sub>4</sub>

T cell epitope = ELHSASVKPVRLWFSVTFNNF

- ✓ The glycosylation type and site of the third variable (V3) domain of the HIV-1 gp120 envelope glycoprotein were crucial to high-affinity binding to bnAbs.
- ✓ Glycopeptide immunogen together with a TLR agonist Pam3CSK4 and a Th epitope, were coupled to form a **three-component glycopeptide vaccine**. The self-adjuvant synthetic glycopeptides elicited substantial glycan-dependent antibodies with broad recognition to several gp120s in rabbit immunization.
- ✓ Three-component trivalent HIV-1 V3 glycopeptides were constructed. The **immunogenicity of the V3 glycopeptide was substantially enhanced by the multivalency effect**. Glycopeptide-specific antibodies were induced, and the antisera induced by the trivalent glycopeptide displayed stronger binding to gp120.

# Antiparasitic Vaccines

## ➤ Carbohydrate-based Malaria vaccine



- (a) Natural glycosylphosphatidylinositol (GPI) structures of *P. falciparum*.
- (b) Synthetic GPI glycoconjugates as vaccine candidates against malaria.
- (c) Mice immunized with GPI conjugates exhibited an increased survival.
- (d) Serum levels of pro-inflammatory cytokines TNF-α.

Various kinds of GPI fragments were synthesized to evaluate their structure–immunogenicity relationship.

These GPI glycans were then coupled to authorized carrier protein CRM197; the resulting glycoconjugates were used to evaluate the **production of anti-GPI antibodies, T-cell activation, and protection of mice from experimental cerebral malaria**.

The best survival occurred in mice immunized with glycoconjugates CRM-5 containing the GPI glycan core with the PEtN at a non-natural position, by reducing activation of CD4+ and CD8+ T cells as well as release of pro-inflammatory cytokines IFN-γ and TNF-α.