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Glycopolymer Code: Programming Synthetic Macromolecules for Biological Targeting

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Targeting in a cellular level is still one of the major challenges in biomedical treatments. However, new synthetic and analytical techniques now allow the development of precisely prepared macromolecules. Thus, glycopolymer chains are reported to be prepared with controlled length, monomer sequences, as well as chain-folded structures. A high level of complexity in synthetic macromolecules also allows increased selectivity in targeting, which is a key factor in biomedical applications.

Over the last two decades, an abundance of synthetic glycopolymer–based materials have been developed for the elucidation of multivalent protein–carbohydrate recognition, which is crucially essential for a variety of biological processes, including cell growth regulation, adhesion, cancer cell metastasis, inflammation by bacteria and viruses, and immune responses. Besides their utilization as building blocks and energy production in our bodies, carbohydrates have a specific code/message to the sugar-specific receptors (lectins) and this coding system works similaraly to a key and lock mechanism. This is not surprising as many types of cells in Nature are covered by a sugar shell on their surface that could reach 100 nm thickness at the apical border of some epithelial cell.[1] The complexity of glycans from their highly branched nature is still very challenging to be mimicked completely by synthetic chemists due to the insufficient and inefficient synthesis techniques. Even though the first synthetic glycopolymers were very simple due to their linear and short-chain length, and homo structures, a huge number of different well-defined and complexed oligo- and polysaccharides have been proposed so far to investigate their biological interactions. All these glycopolymers with different properties, such as saccharide density, backbone flexibility, glycopolymer, and linker length, presented that each property has a big influence on binding ability and cannot be ignored.[2]

Recently developed precise control synthesis approaches allow to design new polymeric architectures in high precision with improved binding affinity due to their enhanced glycocluster effect.[3] These studies are very important to understand and modulate the involvement of lectin–glycopolymer interaction in terms of different biological and medical functions. In particular, the calcium-dependent carbohydrate-binding lectins (C-type lectins) on immune cells providing very high carbohydrate binding affinity can recognize different types of pathogens and viruses selectively by their cognate carbohydrate ligands to isolate infectious events, and trigger adaptive immunity.[4] Hence, the importance of recent advancements in glycopolymer synthesis to manipulate lectin–carbohydrate recognition has been linked directly to the discovery of new therapeutic targets.

Much uncertainty still exists about this specific and selective interaction between glycans and lectins, hence, understanding glycopolymer code will help to design more suitable and functional glycopolymers for better therapeutic approaches. Based on both biological and chemical perspectives, the synthesis of precision glycopolymers that can be used as a glycan in terms of the density, clustering, and contact sites of glycans and their specific biological interactions with counterparts is still challenging mainly due to lack of appropriate chemical synthesis techniques. Therefore, to find the ideal glycopolymer that perfectly binds to the targeted lectin is a challenging topic, but once the rapid developments and advancements in the polymer science over the past decades are considered, it does not seem far away to succeed it. It is so obvious that a multidisciplinary collaboration between polymer chemists, organic chemists, material scientist, biochemist, cell and microorganism biologists, and immunologists are so crucial to achieve this carbohydrate receptor-targeting paradigm for future avenues.

In the glycoscience field, synthesis of well-defined glycopolymers with a variety of multiple functionalities was a crucial first step and it has been achieved by synthesis of linear glycopolymers. Generally, methods in glycopolymer synthesis are carried out either by postmodification of preformed polymers or by direct polymerization of glycomonomers.[5] First glycopolymers were linear and simple structures that were used to evaluate carbohydrate–lectin binding properties. There has been a great deal of interest in this topic after the first optimistic results. At present, there are different routes in glycopolymer synthesis that allow to design more complex macromolecules with specific functionalities and properties in order to imitate complex biological systems.[6] In particular, an integration of single-chain technology to glycopolymer synthesis would have great potential to create 3D glycopolymers

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as reversible self-folding structures in terms of interfacial molecular recognition.

In recent years, a few futuristic and innovative examples have been reported by Bertozzi and her research group, one of the leading groups in the World in glycan chemistry. They have investigated bio-orthogonal chemistry, which allows to develop many enabling technologies within and beyond the field of glycobiology, for example, site-specific antibody–drug conjugation.[7] She is one of the cofounders of Palleon Pharmaceuticals Company and their novel discoveries in the field of glycoimmunology are being translated to commercial therapeutic platforms in this company by inhibiting the Siglec-Sialoglycan axis to activate both innate and adaptive antitumor immune responses and treat cancer patients who do not respond to T-cell checkpoint therapies.[8] Last year, a team led by Bertozzi has achieved to develop a quick and simple way to detect the bacteria that causes tuberculosis using chemically tweaking a sugar molecule known as trehalose.[9] The in vivo and imaging results are very promising for the possible clinical applications to fight against tuberculosis.

As elucidated above, the design of sequence and architecturally controlled glycopolymers could enhance the selective binding activity on multivalent scaffolds in glycoscience due to more predictable biological properties. Very recently, Hartmann and co-workers have developed sequence-defined and monodisperse glyco(oligoamides) as side chains of soluble brush glycopolymers with a high level of control over the branched architecture.[10] These synthesized glycopolymers having variations of structural parameters are good example of glycan mimetics according to their binding results through different C-type lectins. Moreover, our research group has recently demonstrated a reversible single-chain folding of well-defined triblock glycopolymer structures into an α-shape using different density of sugar moieties.[11] These single-chain folded glycopolymer structures obtained in an aqueous solution under high dilution represented better binding affinity than unfolded single-chain glycopolymer, which proved that the unprecedented effect of secondary structures of glycopolymers on their biological activities and the integration of sequence-controlled glycopolymers to single-chain folding technology could bring a new point of view into the field of glycoscience. From both biology and polymer chemistry perspectives, this combination is essential to establish a full understanding on the functional roles of heterogeneity and multivalency in glycans.

Furthermore, self-assembled glyconanoparticles or glycopolymer-coated nanoparticles have paved the way in glycobiology due to their importance in achieving enhanced lectin-glycopolymer binding. In nature, these types of self-assembled nanostructures can be easily found on the scale of cells and organelles and the selectivity and specificity were observed via these nanostructures in Nature. Therefore, a huge number of glyconanoparticles with different morphologies and packing parameters have been developed to evaluate their interaction with different types of lectins in the last years. One of the most active research groups on the synthesis of glyconanoparticles is Stenzel group. In a joint project with Barner-Kowollik, Stenzel and co-workers have developed the light-induced collapse of single glycopolymer chains in water generating fluorescent single-chain glyconanoparticles (glycol-SCNPs) and their subsequent coating onto nanodiamonds.[12] In another recent work, Stenzel and co-workers have prepared glycomicelles with different concentrations and arrangement of fructose in the shell of the micelle and studied that how these properties affect the biological activity in living cell systems, which is one the most critical and missing point in lectin-glycopolymer binding studies.[13]

The fabrication and engineering of well-defined different types of glycoconjugates ranging from dendritic, brush, graft, hyperbranched, star-shaped, and cyclic polymers have provided an opportunity to understand better the pivotal role of carbohydrates in their interactions better at molecular level.[14] However, there are still some challenges that need to be achieved, such as the stability of nanoparticle materials under physiological conditions, poor permeability to various biological membranes and rapid renal elimination. Glycopolymers coated nanosized inorganic cores, such as gold, iron oxide

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and quantum dots, have attracted much attention in last decades due to their unique properties and their easy modification in size and composition.[15] pH-responsive drug-conjugated glycopolymer-coated gold nanoparticles were reported and used for cancer treatment through three different cancerous cell lines.[16] Additionally, metallic core based glyconanoparticles have been designed as a photoacoustic imaging agent and photothermal therapy agent of tumors, too.[17]

The use of generation 3 glycodendrimers targeting both DC-SIGN and Langerin lectins, which enhanced activation of gp100 specific CD8+ T cells in combination with TLR stimulation was reported by Duinkerken et al.[18] In vivo results showed that an intradermal glyco-vaccine simultaneously can activate antitumor immune responses via dual DC-SIGN and Langerin targeting. Star shaped glycopolymers that are another advanced glycostructures can bind to lectins with high affinity due to their multivalency. Four-arm star glycopolymers were prepared by using a core-first approach and used for lectin-binding studies.[19] It was noted that not only glycopolymer length and molecular weight, but also backbone rigidity has an important influence on binding affinity toward lectins. Furthermore, Mitchell et al. represented the possible use of star glycopolymers in immune response modulation by influencing cytokine expression.[20] We recently reported a synthesis of reversible single-chain folded glycopolymers containing adamantane and cyclodextrin at the rear ends of the polymer.[11] The host–guest interaction between adamantane and cyclodextrin allowed to obtain a single-chain folded structure under high dilution condition in water. The lectin binding results demonstrated that single-chain folded structures enhanced greatly the binding ability in comparison to the unfolded linear structures.

As a conclusion, synthetic difficulties to design complex and branched glycostructures as a naturally existing glycan mimicry are the main reasons why glycopolymers are lagging far behind other synthetic polymers in biological and therapeutic applications. Even though many research groups have contributed to biological research on the interactions between glycopolymers and lectins, the exploitation of carbohydrate-based systems in biological and medical applications has still not been realized because of low plasma half-life, low-affinity binding of carbohydrate-selective interactions, the stability of nanoparticle materials and insufficient target cell recognition. Considerable work still remains to be carried out to improve these properties for the biological and medical applications and it is believed that integration of glycopolymers into the precisely defined glycostructures will bring new features to these materials. Once a certain level of progress is achieved, glycopolymers will open unprecedented avenues in the development of immunotherapy, vaccination, and mapping patterns of glycan as functional markers. By providing specific coding/information to future smart glycopolymers, particularly immune cells and pathogens could be controlled and manipulated in terms of their interaction, trafficking, and signaling, which will provide us a powerful tool to prevent many diseases.

Conflict of Interest

The authors declare no conflict of interest.

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