AUTOMATED GLYCAN ASSEMBLY ENABLES THE GLYCOSCIENCES

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My View of the Present State of Research on Exploring the Chemistry–Biology Interface

Three major classes of repeating biopolymers are at the heart of all biological processes: the genetic material DNA encodes via RNA proteins that in turn produce polysaccharides. Access to pure oligomers and polymers is key to understanding these complex biomolecules. Since isolation from biological sources is often not possible, chemical synthesis has been key to understanding the molecular processes in all of biology. Heroic total synthesis efforts were required to assemble even short oligomers until solid-phase peptide synthesis invented in 1962 by B. Merrifield laid the basis for automated assembly. Automated solid-phase synthesis of DNA (1981) and RNA (1997) invented by M.H. Caruthers enabled molecular biology breakthroughs by access to oligonucleotide primers for cloning and second-generation sequencing applications. The chemical synthesis of polysaccharides was a chemically much more challenging task since carbohydrates, unlike peptides and DNA, are branched and each new glycosidic linkage is a new stereogenic center that needs to be controlled.

My recent research contributions to Exploring the Chemistry–Biology Interface

Our laboratory solved the chemical challenges and reduced oligosaccharide synthesis to a task that can be carried out by instruments providing access to glycans as long as 100 units that can in turn be coupled to access even larger polysaccharides. The chemical methods for rapid access to defined carbohydrates created important tools that are fueling biological and medical research as well as material science.

Automated glycan assembly provides now access to defined glycans that used to take years can now accomplished in a few hours. Initially, we developed the chemical foundations [1] that we systematically expanded into a general synthesis of the largest class of biopolymers that resulted in the first commercial carbohydrate synthesizer [2], now used in more than 30 laboratories worldwide to prepare polysaccharides [3], and the challenge of installing multiple cis-glycosidic linkages was solved [4].

Synthetic glycans were the basis for creating glycan microarrays, glycoconjugate vaccines, monoclonal antibodies against glycans and carbohydrate materials (see Fig. 1).

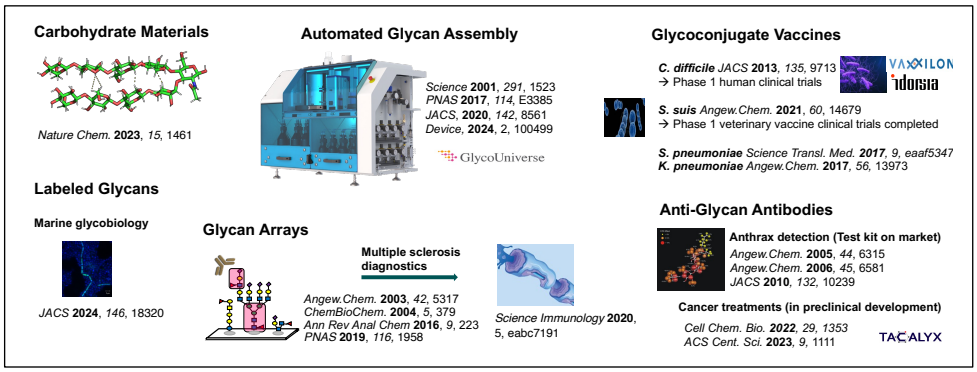

Fig. 1. Molecular glycobiology enabled by Automated Glycan Assembly.

The synthetic glycan tools were used in collaborations to address long standing problems in the carbohydrate field. With Prof. Pagel we employed ion mobility mass spectrometry to sequence glycans [5] and ultra-cold IR spectroscopy to determine the structure of a glycosyl cations that had never been observed experimentally before [6]. Single glycans can be imaged using a combination of soft-landing electrospray ion beam deposition and low-temperature scanning tunneling microscopy [7] to gain fundamental insights into the very basis of carbohydrate folding [8]. These insights subsequently resulted in the first designed oligosaccharide hairpin as an entry into the creation of glycan foldamers [9].

Synthetic glycans have been used to define protein-carbohydrate interactions in many biological systems including chemokine-heparan sulfate-mediated T cell recruitment [10], heparan sulfate proteoglycan presentation of PCSK9 to the LDL receptor [11], and glycan-dependent two-step cell adhesion mechanism of Tc toxins [12]. Defined molecules helped to understand the alteration of cell wall antigen glycosylation to subvert the protective host defense in *Staphylococcus aureus* [13]. Glycan arrays are now a ubiquitous tool that enabled the identification of glycan epitopes in multiple sclerosis [14] among many other applications. Synthetic oligosaccharides were recently used in fundamental studies of marine glycobiology [15].

Rapid access to synthetic sugars has fundamentally changed the approach to glycoconjugate vaccine development: departing from isolated polysaccharides to a medicinal chemistry approach based on defined oligosaccharides. Carbohydrate vaccine candidates against a host of bacteria such as *Streptococcus pneumoniae* [16] and *Klebsiella pneumoniae* [17] are advancing towards clinical testing much like the *C. difficile* glycoconjugate vaccine that successfully completed Phase 1 human clinical trials [18]. Rapid and reliable synthesis of oligosaccharides resembling glycans found on the

surface of bacteria, parasites and human cancers helped to identify diagnostic markers. An antibody against *Bacillus anthracis* [19] resulted eventually in a commercial diagnostic kit used to detect this bioweapon. Antibodies and nanobodies against tumor-associated carbohydrate antigens are currently in pre-clinical development [20].

Outlook to future developments of research on the Chemistry–Biology Interface

Automated Glycan Assembly provides rapid chemical access to defined glycans and as such is the basis for molecular glycobiology. Itis now used by laboratories around the world. All areas of biology involving carbohydrates can now be studied on the molecular level from immunology to marine biology, plants and fungi. In addition to basic research, the method is employed by companies interested in developing synthetic glycoconjugate vaccines against a host of infectious diseases vaused by bacteria, fungi and parasites. Inducing the production of monoclonal antibodies against glycans on the surface of cancer cells is the basis for diagnostic and therapeutic approaches. Much like for nucleic acids and proteins, chemistry enables fundamental insights and subsequently applications to address medical needs in the field of glycobiology.

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