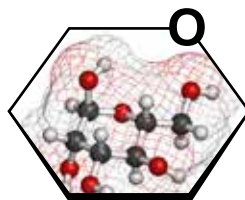


Glycobiotechnology in Berlin-Brandenburg

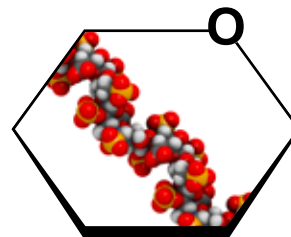
Potentials – Profiles – Perspectives

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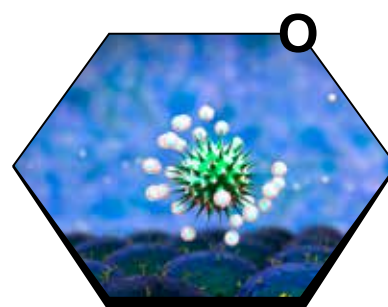
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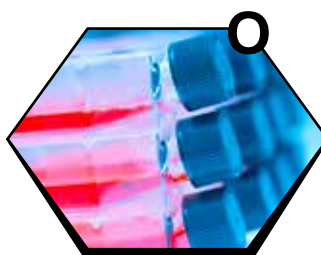
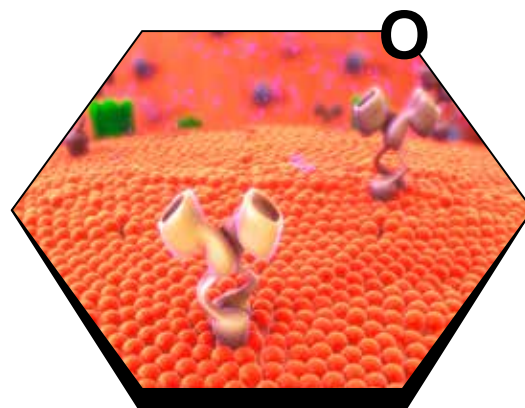
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Glycobiotechnology in Berlin-Brandenburg – The Best Conditions to Yield Sweet Fruit

Molecules with sugar components – carbohydrates – are the most abundant biomolecules on Earth. They are ubiquitous in all living organisms and have innumerable functions. Simple carbohydrates are essential for energy metabolism and complex carbohydrates, or glycans, serve as energy storage, build structural component in cells, and are substantially involved in any kind of cell-cell interactions. They are, for instance, essential for the interaction between egg and sperm during fertilization. Being a constitutive part of receptors, ion channels, antibodies and antigens, carbohydrates significantly influence physiology, developmental processes and play an important role in disease development and immunology. An impressive example of the impact of glycans can be seen in the heterogeneity of the Influenza A virus. Several subtypes of the flu virus exist and differences in glycosylation patterns can lead to different pathogenicity. Agents causing avian influenza such as H5N1 or H7N9 became a matter of global concern because of its transmission to humans.

Impact of carbohydrates in nature

- Essential source of energy
e.g. glucose, fructose, lactose
- Essential energy storage
e.g. starch and glycogen
- Essential element to build structures
e.g. cellulose, lignin, chitin
- Essential element for recognition processes
e.g. enzymes, receptors, antibodies, glycoproteins, glycolipids

Glycoscience (derived from Greek *glykòs* for sweet) is dedicated to unraveling the complexity of carbohydrates and their functions. After genomics, proteomics, and metabolomics, glycomics has been established as a distinct discipline that focusses on the glycome – the entirety of sugars in living organisms.

According to a recent European White Paper on glycobiology, the glycomics R&D market includes enzymes, monoclonal antibodies, instruments, kits and reagents, and is expected to rise to \$930 billion by 2019. Berlin-Brandenburg is well positioned to significantly participate in this rapidly growing market.

Berlin and Brandenburg recognized the potential of glycosciences early on, and undoubtedly, Werner Reutter, one of the global pioneers exploring this field of science, was a key driver who was consistently committed throughout his life to advancements in glycosciences. From 1979 when he became Head of the Institute of Biochemistry and Molecular Biology at the Freie Universität Berlin until his death in 2016, he infected numerous scientists with his enthusiasm for the exciting field of glycosciences. Political driven activities to enhance this research area in the region reach back to the beginning of the 2000s. Already in 2004,



“Berlin-Brandenburg – like very few other regions world-wide – has a network of academic scientific institutions and research-based companies that cover the whole range of glycosciences and glycobiotechnologies: from basic research to applied sciences – from industrial development to applications in medicine and biotechnologies. This competence can give the Berlin-Brandenburg region a leading edge in this highly competitive field of biotechnologies and biomedicine in the international context. New technologies developed in the region like automated synthesis of carbohydrates are now paving avenues for the biomedical application of glycosciences in diagnostics, therapy and preventive medicine. Glyco-engineered biopharmaceuticals and novel diagnostic tools contribute to important advances in the treatment of various diseases in medicine.”

Rudolf Tauber

Charité – Universitätsmedizin Berlin, Head Institute of Laboratory Medicine,
Clinical Chemistry and Pathobiochemistry

BioTOP introduced the glycobiotechnology assets of the region in a special issue of its BioTOPics publication. In the same year an “Innovation Forum Glycans” was initiated. Many other congresses, workshops and events followed and continue up to the present – including seven “Glycan Forums” and the “Treffpunkt Bioinformatik und Glykobiotechnologie”. The strong local commitment was also demonstrated by the establishment of the glycobiotechnology network “Glykostrukturfabrik” (Glycostructure Facility). The goal of this platform, which existed from 2005 to 2008, was to combine excellence in basic glycobiological research and the development of related products and services, while building a glycobiological center of excellence in the Berlin-Brandenburg region. The ongoing strong political commitment is also reflected in the joint innovation strategy of Berlin and Brandenburg (Masterplan Gesundheitsregion Berlin-Brandenburg). It incorporates future support for the development of glycobiology as an enabling technology for the biotech sector.

One more recent result of regional activities was the establishment of the glyconet Berlin-Brandenburg in 2016. The network supports research and development in glycobiotechnology, synthetic chemistry, analytical sciences and medical chemistry. The main goal is to strengthen and visualize regional skills and education in these areas of the life sciences. Soon after its kick-off in December 2016 glyconet BB set up a “Winter School” for junior scientists to provide insights on the theory and practice of glycosciences.

Glyconet BB is also organizing its first international congress in September 2017. The congress on “New and Emerging Technologies” will provide a platform for international scientific exchange and showcase the newest developments in glycobiotechnology, biochemistry, molecular biology, biofunctional materials, antibody technologies, synthetic biology and related areas.

The capital region is also attractive for other event organizers active in the glycosciences field. In early 2017, Berlin was the venue of the first “GlycoBioTec”, an international symposium hosted by the Max Planck Institute of Complex Technical Systems Magdeburg. Since October 2009, Berlin and Potsdam have been venues for five international “Beilstein Glyco-Bioinformatics Symposia”, organized by the Frankfurt-based foundation Beilstein Institut, and with Peter Seeberger, Director of the Max Planck Institute of Colloids and Interfaces as member of the scientific committee.

Potential of carbohydrates in man-made applications

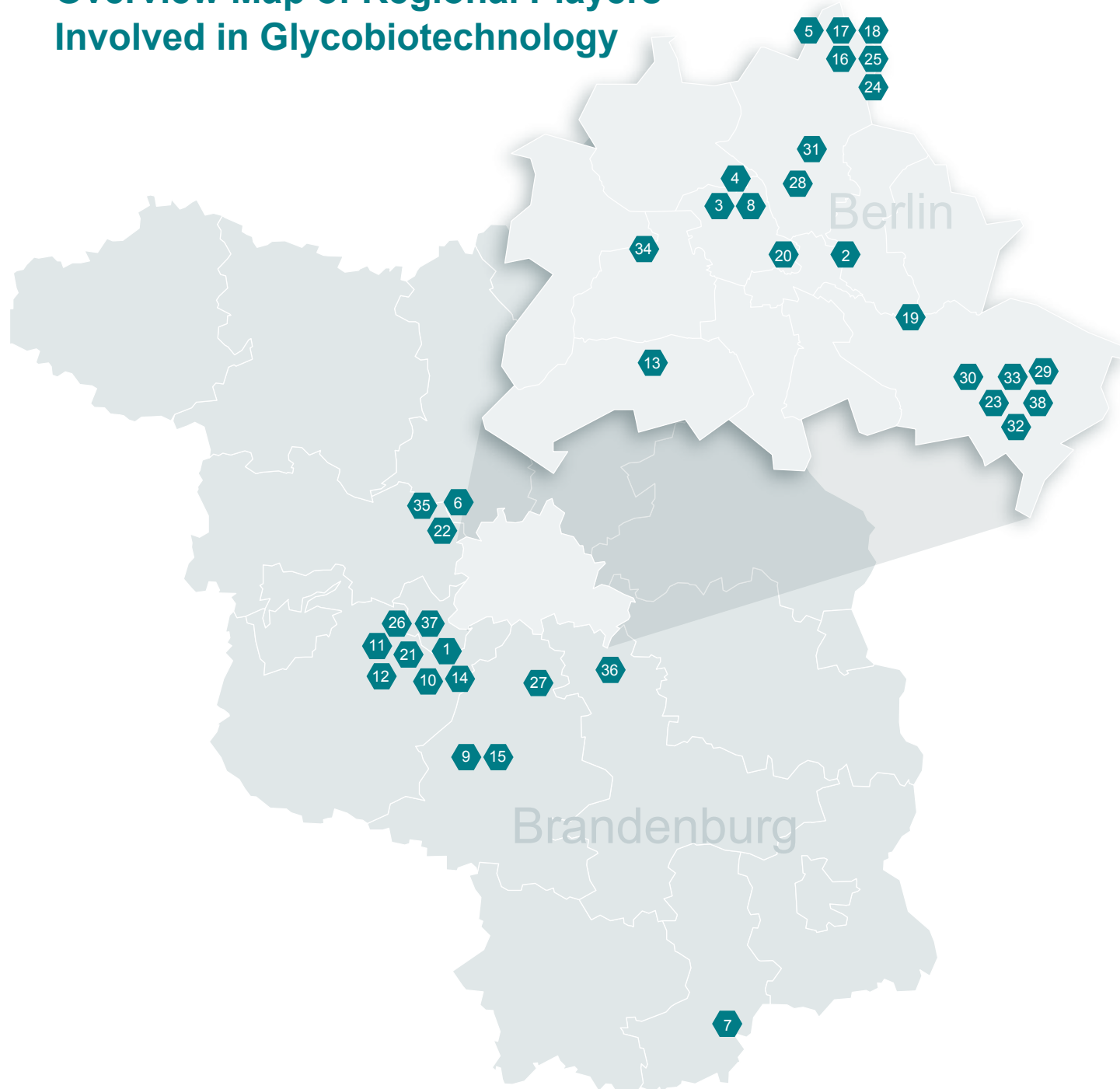
- Novel diagnostics
- Novel drugs
- Optimized drugs
- Novel vaccines
- New methods of drug delivery
- Novel biomaterials
- Food optimization
- Applications for agriculture

The key players which make the Berlin-Brandenburg region one of the leading centers of glycosciences and glycobiotechnology in Europe are the universities, the research institutes and last but not least an increasing number of companies. About 50 players in science and industry are committed to exploring the secrets of carbohydrates and focusing their research activities on the development of new approaches and applications which lead to improvements in healthcare. Key players in the academic environment are the Max-Planck-Institute for Colloids and Interfaces with Peter Seeberger as a globally renowned pioneer of glycosciences, and the Charité – Universitätsmedizin Berlin (Véronique Blanchard and Rudolf Tauber working group). Among other institutions the Beuth University of Applied Sciences Berlin (Stephan Hinderlich working group), Fraunhofer Institute for Cell Therapy and Immunology (Stefan Kubick working group) as well as the Fraunhofer Institute for Applied Polymer Research (Alexander Böker working group) are strongly engaged in glycosciences. Major industry actors include Glycotope GmbH and ProBioGen AG in the glycoengineering area, GlycoUniverse GmbH & CO KGaA, providing automated carbohydrates synthesizers, and Scienion AG, active in the development of glycan-based microarrays. These and other players use their distinct glycosciences expertise – often in collaborative efforts – for comprehensive glycoanalytics and to develop novel diagnostics and therapeutic agents, biosimilars and biobetters, new vaccines and innovative materials. Examples will be presented on the following pages.

Pioneering sugar technologies for centuries

Berlin has been a pioneer of sugar technologies for more than 200 years. The chemist Andreas Sigismund Marggraf (1709–1782) discovered the high sugar content of beets and laid the foundation for the industrial process for extracting sugar from sugar beet – a breakthrough, since this technology allowed producers to achieve independence from expensive cane sugar.

Overview Map of Regional Players Involved in Glycobiotechnology



- | | | |
|--|--|---|
| 1 Analyticon Discovery GmbH | 12 Fraunhofer Institute for Cell Therapy and Immunology - Potsdam-Golm location IZI-BB | 25 Max Delbrück Center for Molecular Medicine Berlin-Buch |
| 2 BDW Berliner Diagnostik Werke GmbH | 13 Freie Universität Berlin | 26 Max Planck Institute for Colloids and Interfaces |
| 3 Berlin-Brandenburg Center for Regenerative Therapies | 14 German Institute of Human Nutrition | 27 Medipan GmbH |
| 4 Beuth University of Applied Sciences Berlin | 15 Glycon Biochemicals GmbH | 28 MicroDiscovery GmbH |
| 5 Biosyntan GmbH | 16 Glycotope GmbH | 29 Octapharma Biopharmaceuticals GmbH |
| 6 biotechrabbit GmbH | 17 GlycoUniverse GmbH | 30 PlasmaChem GmbH |
| 7 Brandenburg University of Technology | 18 HealthTwist GmbH | 31 ProBioGen AG |
| 8 Charité - Universitätsmedizin Berlin | 19 htw Berlin – University of Applied Sciences | 32 Proteome Factory AG |
| 9 Chiracon GmbH | 20 Humboldt-Universität zu Berlin | 33 Scienion AG |
| 10 evocx technologies GmbH | 21 Hybrotec GmbH | 34 Technische Universität Berlin |
| 11 Fraunhofer Institute for Applied Polymer Research | 22 InVivo BioTech Services GmbH | 35 UGA Biopharma GmbH |
| | 23 JPT Peptide Technologies GmbH | 36 University of Applied Sciences Wildau |
| | 24 Leibniz-Forschungsinstitut für Molekulare Pharmakologie | 37 University of Potsdam |
| | | 38 Vaxxilon GmbH |

Key Areas of Research and Development – Advancements in Healthcare Will Be Sweet

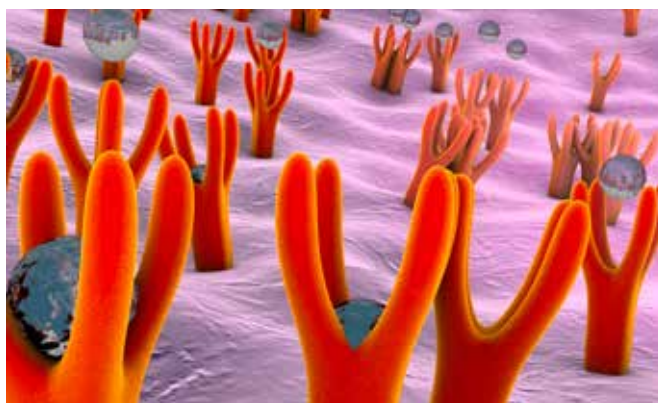
Berlin and Brandenburg have built a strong expertise in glycosciences and glycobiotechnology and the results of research efforts enjoy worldwide recognition in the scientific community. At the same time, an increasing trend is visible in which research results are being transferred to applications and practice. Universities, research institutes and companies cover the whole value chain from basic and applied research to developing and commercializing products.

Having evolved from a specialist's discipline, glycosciences and glycobiotechnology have become integral areas of research in various areas of life sciences such as biology, biochemistry, medicine, pharmacology as well as other science specialties like material sciences or energy production. While the tremendous impact of glycans in all living beings is undisputed today, deciphering its innumerable functions and unraveling its complex structures remains a gigantic task.

Glycoanalytics addresses both, analyzing the structure of carbohydrates as part of glycoconjugates and their function in health and disease. Altered glycosylation patterns can be associated with many diseases and these findings are leading to a new class of glycan-based biomarkers which can be used for novel diagnostics.

R&D activities utilizing glycoengineering technologies primarily aim at creating and improving glycoprotein drugs or carbohydrate-based vaccines by rational design.

Other regional research activities focus on research on rare hereditary diseases known as Congenital Disorders of Glycosylation (CDG) which are characterized by a variety of glycosylation anomalies. Uwe Kornak and his group at



the Institute for Medical Genetics and Human Genetics, Charité – Universitätsmedizin Berlin, investigate the Golgi compartment as the most important site for posttranslational protein modifications. Mutations in Golgi proteins have been detected in patients with CDG of variable severity. Uwe Kornak is a member of the E-Rare research project EURO-CDG-2 on molecular diagnostics, mechanisms and treatment of CDGs.

Further research involves multidisciplinary approaches and includes projects in bioinformatics, polymer sciences, nutrition and plant glycobiotechnology.



“Glycobiotechnology is a growing field in research on the role of glycans and development of applications utilizing sugar molecules in therapy, diagnostics and functional food. Many small and medium enterprises and academic institutions in the Berlin-Brandenburg area are focusing on the production of biosimilars of established glycopharmaceuticals as well as on the development of biobetters for improved medical application. This includes therapeutic antibodies, recombinant clotting factors, inflammation inhibitors and vaccines. New methods for glyco-based diagnostics are also important issues in the rapidly expanding field of glycobiotechnology.”

Stephan Hinderlich

Professor of Biochemistry,
Beuth University of Applied Sciences Berlin, Department Life Sciences and Technology

Systematic Glycoanalytics – Unveiling Complex Glycan Structures and Functions and Identifying New Biomarkers

Glycoanalytics build the basis for all glycobiotecnology applications. Several technologies are applied to increase knowledge about glycans, its constituents and structure as well as its innumerable functions in health and disease.

Beside their functions in energy metabolism, carbohydrates are constituents of glycoproteins, glycolipids, proteoglycans (glycosaminoglycans) and glycosphingolipids, and many diseases and disorders can be linked to alterations in glycosylation. To give one example of the great variety of glycans: linking three different monosaccharides can lead theoretically to over 20,000 different structures, which is incredible when one considers that linking three amino acids produces just 27 tripeptides.

Almost all regional academic institutions and several companies committed to the development of glycoengineered therapies or vaccines employ glycoanalytical techniques as part of their research efforts and to confirm their results. These include particularly the companies Glycotope GmbH, ProBioGen AG, Octapharma Biopharmaceuticals GmbH and UGA Biopharma GmbH. One of the major players is the Institute of Laboratory Medicine, Clinical Chemistry and Pathobiochemistry, Charité – Universitätsmedizin Berlin. The working group of Véronique Blanchard aims at the systematical analysis of the glycome in the context of physiological mechanisms in human health and disease. The group is able to analyze glycans from human sources, animal models and even plants. Several technical devices are used in the laboratory to integrate different technologies such as mass spectrometry (including Matrix-Assisted

Laser Desorption/Ionization Time-of-Flight Mass Spectrometry, MALDI-TOF MS), capillary electrophoresis and 2D gel electrophoresis, or other chromatographic techniques, namely high performance liquid chromatography (HPLC) and high-pH anion-exchange chromatography (HPAEC). The group uses these different techniques to unravel the whole glycome: N/O-glycans, glycosaminoglycans and glycosphingolipids.

Kevin Pagel from the Institute of Chemistry and Biochemistry, Department of Biology, Chemistry, Pharmacy, at the Freie Universität Berlin addresses a tricky challenge of glycoanalytics. Carbohydrates are often branched and have a complex regio- and stereochemistry. This in turn leads to a vast number of possible isomers of identical atomic composition and mass, which are difficult to distinguish using established analytical tools. To solve this problem, Kevin Pagel uses a combination of ion mobility-spectrometry and mass spectrometry (IM-MS), a very promising new tool in glycan analysis that has emerged in recent years. Additionally, he applies cold-ion spectroscopy, a method that provides highly diagnostic absorption features for oligosaccharides, resulting in a spectral fingerprint that is unique for each oligosaccharide. The unprecedented resolution of cold-ion spectroscopy coupled with tandem MS may render this the key technology to unravel complex glycomes.



“Protein glycosylation plays a crucial role in essential biological processes, e.g., immune response, cell differentiation and development, cell-cell interaction and signal transduction. Alterations in glycosylation are linked to vast numbers of diseases and disorders. Systematic knowledge of disease-related changes of glycosylation as well as their integration in genomic and proteomic data allows for a new understanding of biomedical mechanisms and thus opens up new possibilities for diagnostics, therapeutic approaches and prevention. Our working group, aiming at the systematical analysis of the glycome, is part of the excellent basic research environment based in the area Berlin-Brandenburg. Additionally, key players from the industry are also present in the region, meaning that paths for joint R & D projects are particularly short.”

Véronique Blanchard

Head Department Glycodesign and Glycoanalytics, Institute of Laboratory Medicine, Clinical Chemistry and Pathobiochemistry, Charité – Universitätsmedizin Berlin

Glycan-based biomarkers leverage a new dimension of diagnostics

Changes in glycosylation are associated with many diseases and have been documented for several disease conditions such as cardiovascular, metabolic, infectious and inflammatory diseases as well as for some hereditary disorders. Tremendous research efforts all over the world focus on exploring altered glycosylation in various malignancies as tumor-related glycosylation patterns can provide valuable diagnostic information on the type of cancer, staging, progression and the patient's prognosis.

The identification and evaluation of glycan-based biomarkers represents a prominent field of research in Blanchard's lab. One of the dedicated research projects – together with the Department of Gynecology of the Charité – focuses on serum glycome profiling to identify tumor markers for the diagnosis of epithelial ovarian cancer (EOC). Common tumor markers for this type of cancer show poor specificity; therefore, better biomarkers are needed. The group was able to develop a GLYCOV index combining upregulated structures that were fucosylated and sialylated, and a group of

downregulated structures that were of the high-mannose type. The research results indicate that serum glycome profiling could contribute to an improved diagnosis of ovarian cancer as GLYCOV could differentiate between early stage EOC and benign ovarian growths.

Blanchard's group is also working towards the next generation of stem cell (SC) markers. The scientists work with adult multipotent bone marrow SCs and embryonal SCs in collaboration with the Berlin-Brandenburg Center for Regenerative Therapies (BCRT). SCs have the potential to develop into several tissues and are ideal candidates for applications in regenerative medicine. The group has identified key glycan structures in SCs that are promising candidate markers to detect and distinguish SCs from their adipogenic progeny.

These and other projects reveal the potential of glycan-based biomarkers as determinants of physiological processes in health and disease. Furthermore, new glycan-based diagnostic methods enable more personalized therapy approaches and improved patient care.



Glycan Microarrays for Improved Diagnostics – Tracking the Affinity to Distinctive Carbohydrates

Carbohydrate microarrays have become an indispensable tool for functional studies of glycans. They offer versatile screening platforms to analyze interactions of oligosaccharides with proteins that control cell growth, cell differentiation, cell-cell-interactions, bacterial attachment to target cells and signaling events involving the extracellular matrix.

However, access to sufficient amounts of carbohydrates is an essential prerequisite to produce glycan arrays. The isolation of carbohydrates from natural sources is challenging if possible at all, and it results only in limited amounts of glycans. On the other hand, classical solution phase synthesis of oligosaccharides is often a time-consuming task. Peter Seeberger, Director at the Max Planck Institute of Colloids and Interfaces (MPICI) and Freie Universität Berlin, did groundbreaking work to overcome these limitations. He and his team successfully developed a technology that allows automated solid-supported oligosaccharide synthesis. In 2013, the spin-off GlycoUniverse GmbH & Co KGaA was founded to market the Glyconeer®, the first commercial, fully automated oligosaccharide synthesizer.

Scienion AG is another major player in the field of carbohydrate microarrays. The company is well positioned in the ultra-low volume liquid handling systems and microarray technology markets. Scienion has a longstanding collaboration with the Seeberger group at the MPICI and with the group of Véronique Blanchard, Charité – Universitätsmedizin Berlin, Institute of Laboratory Medicine, Clinical Chemistry and Pathobiochemistry. The three partners successfully pursued the joint project “GLYCARDIAG” (GLY-Can ARray DIAGnostics) which aimed at the development of a glycan-based microarray platform for In-Vitro Diagnostics.

The detection of Heparin-induced thrombocytopenia (HIT) served as a first application example. This reduction of platelets below normal range can occur as a side effect after administration of the anticoagulant heparin. HIT can lead to deep vein thrombosis and life-threatening conditions such as stroke, myocardial infection or pulmonary embolism. In this project glycans have been used as capture molecules for a completely new sugar-based molecular diagnostic for the first time. The project also included a final bioinformatic analysis of microarray data by MicroDiscovery GmbH, a leading provider of high quality software for biomolecular research.

More recently, Scienion and MicroDiscovery carried out a feasibility study to analyze technical requirements for establishing a glycan-based diagnostic platform. The specific focus was on quality-assured production of the arrays and its automatic quantitative analysis. The encouraging results clearly demonstrate the potential of glycan-based rapid tests for diagnostics. A further development of this platform opens up new perspectives for personalized medicine. Future fields of application range from specific pathogen detection up to complex multi-parameter assays, e.g. for differentiation between acute or latent infections or for detection of different types of antibody classes.



“Carbohydrate microarrays have become important tools for functional glycomics studies and SCIENION provides an ideal technology for handling the precious carbohydrate samples. High throughput analysis of carbohydrate interactions in a microarray format allows us to study a wide range of carbohydrate binding partners, including proteins, RNA, whole cells and viruses. Glycan-based arrays point the way towards a completely new kind of in-vitro diagnostics. Together with our regional partners we are strongly committed to accelerating this exciting development and transferring our joint research efforts into innovative IVD products.”

Holger Eickhoff
CEO SCIENION AG

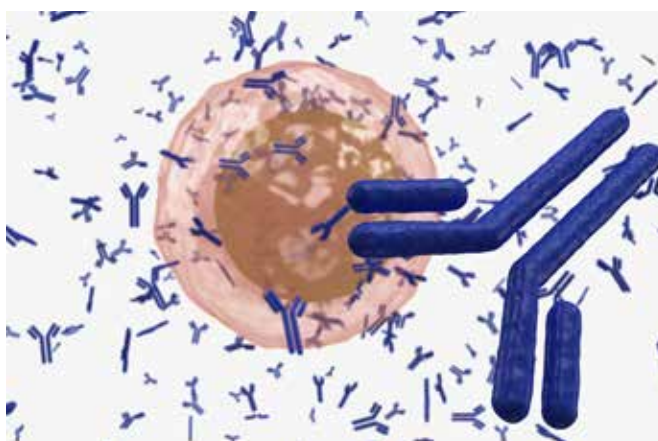
Rational Glycoengineering – Sugars Can Improve Drug Efficacy

The launch of the first recombinantly produced insulin in 1982 marked the start of a new era of biopharmaceuticals. Today, therapeutic antibodies account for the majority of approved biologics. About 400 recombinant protein drugs are available and another 1300 under development. Glycosylation is a posttranslational modification of proteins that has a crucial impact on the activity, stability and pharmacokinetics of biopharmaceuticals. Therefore, glycoengineering technologies have become increasingly important for the development of improved, glyco-optimized drugs.

With the expiration of numerous patents for blockbuster biopharmaceuticals, the efforts towards biosimilar production have been considerably increased. Due to the manufacturing process, biosimilars or follow-on biologics cannot be completely identical to the reference drug. Being a cell-system dependent process, it varies with expression systems and growth conditions. The term “biosimilar” was introduced to differentiate these products from “generics” of the small molecule type. Technological progress and especially advances in glycomics opened doors to not only replicate existing drugs but to create “biobetters” by means of a rational design process. Biobetters provide improved product properties resulting in better efficacy, increased serum half-life or improved pharmacokinetics.

Several universities, research institutes and companies in Berlin and Brandenburg apply highly sophisticated glyco-engineering techniques, while the latter translate scientific evidence into innovative products.

Glycotope GmbH is a clinical stage immuno-oncology company built on leading global glyco-biology expertise. Using its unique GET and GEX® technologies, Glycotope has developed a mature and growing pipeline of proprietary cancer



therapies, as well as products in infertility and blood coagulation. GET makes it possible to identify and target specific glyco-epitopes found on tumor cells while GEX® enables the glyco-optimization of fully human glycosylated biopharmaceuticals. With the combined advantages of optimized sialylation, galactosylation, fucosylation, branching and absence of non-human sugar structures, GEX® products may result in higher efficacy, fewer side effects and/or broader coverage of patients and indications. Three oncology products are currently in advanced clinical development.



“At Glycotope, we aim to play our part in the immuno-oncology revolution by using our world-leading platform technologies to develop new, better targeted and glyco-optimized immunotherapeutics in cancer and other areas of high unmet medical need. Our glyco-engineered biopharmaceuticals allow us to address tumor specific glyco-epitopes giving the potential for powerful anti-cancer activity and reduced side-effects. Furthermore, our human GlycoExpress® production platform is drawing increased interest at companies worldwide to be used as a new expression system for glyco-optimized biopharmaceuticals.”

Paul Higham
CEO Glycotope GmbH



ProBioGen AG is a specialist for the development and manufacturing of complex therapeutic glycoproteins and cell-based vaccines for clinical use. Combining both state-of-the-art development platforms together with intelligent product-specific technologies yields biologics with optimized properties.

In the field of glycobiotechnology, the company has developed the GlymaxX® technology to optimize cell lines producing therapeutic antibodies. Antibodies produced by GlymaxX®-engineered cells contain a reduced amount of core-fucose and show an increased ability to recruit natural killer cells, a type of cytotoxic lymphocytes, mediating an efficient antibody dependent cellular cytotoxicity response. In addition, ProBioGen is based on a variety of proprietary technologies, e.g. to modulate the degree of galactosylation and sialylation to calibrate the desired glycosylation pattern of biosimilars and new biological entities.

UGA Biopharma, a CRO in cell line development, offers ready-to-use biosimilar expressing cell lines and customized cell line development services for new biological entities (NBEs). Bioprocess optimization, downstream development and analytics are included.

The success is based on the choice of an appropriate host cell line, optimized expression vectors and use of a proprietary, high-performance cell culture medium (optimized for the CHO platform). Chinese Hamster Ovary (CHO) clones are the most commonly used cell lines for the production of recombinant proteins. With a very streamlined workflow in hand, UGA Biopharma is seeking new cooperation partners for out-licensing existing research cell lines from the portfolio or starting a new development project for a biosimilar/ NBE expressing cell line together with clients.

At the Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Christian Hackenberger and his group study the biological role of protein glycosylation and other post-translational modifications and decorate proteins with fluorescent moieties that permit their visualization. In collaboration with Stephan Hinderlich, Beuth University of Applied Sciences Berlin, the scientists generate a repertoire of unnaturally modified glycoproteins by metabolic oligosaccharide engineering. These novel biopolymers will be investigated for the development of new diagnostic and therapeutic glycoproteins.

Both groups also investigate the functional contribution of sialic acids to disease development. Research efforts focus on a specific enzyme, *N*-acetylmannosamine kinase, which plays a key role in the biosynthesis of sialic acids and glycosylation of proteins. Sialylated glycoconjugates affect a large number of biological processes, including immune modulation and cancer transformation. In searching for effective inhibitors of *N*-acetylmannosamine kinase the scientists apply high-throughput screening of drug-like small molecules.

The comprehensive competence in the glycoengineering area has been enhanced by pronounced collaborative efforts of regional players in industry and academia that also include Charité – Universitätsmedizin Berlin, HTW Berlin – University of Applied Sciences, Max Planck Institute for Colloids and Interfaces, Fraunhofer Institute for Cell Therapy and Immunology and others.

Carbohydrate Vaccines – A Promising Way to Prevent Infectious Diseases

Vaccines represent the most powerful tool to combat infectious diseases and are indispensable in modern medicine. The benefits of immunization have saved millions of lives and today more than 70 vaccines are available to prevent infections from about 30 pathogens. Despite all medical progress, infectious diseases still remain a challenge. Carbohydrate vaccines open up a new pathway for rational vaccine design.

Modern vaccination reaches back to the late 18th century when Edward Jenner first used a vaccine against smallpox. It was he who introduced the term “vaccine” since his inoculant derived from a virus affecting cows (Latin: vacca for cow). A hundred years later the Charité became renowned for this field of research. Robert Koch, Emil von Behring and Paul Ehrlich – all of them Nobel Prize Laureates in Physiology or Medicine in the first decade of the 20th century – received worldwide recognition for their breakthrough achievements in immunology.

With the advancements of glycomics in recent times, the potential of glyco-conjugate vaccines soon became apparent as carbohydrates have a huge impact on pathogen-host interactions. The surface of microbes is covered with carbohydrates and the sugar residues build unique glycan epitopes which are key for the development of protective antibodies. The Max Planck Institute of Colloids and Interfaces (MPICI) is among the pioneers exploiting this area of research. In Peter Seeberger’s group “Glycobiology and Vaccine Development” scientists synthesize pathogen surface carbohydrates and evaluate them for potential applications in the field of vaccine development. The group currently focuses on chemical synthesis and biological evaluation of carbohydrates present on the bacteria *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Yersinia pestis*, *Chla-*

mydia, *Haemophilus influenzae*, *Neisseria meningitidis* and *Clostridium difficile* as well as on surface carbohydrates of unicellular protozoa like *Leishmania* and *Plasmodium falciparum* which causes malaria. The most advanced project aims at developing a vaccine against *Streptococcus pneumoniae* a bacterium that causes lung infections. The Paul Ehrlich Institute already gave its go-ahead for a first clinical trial. Due to the promising results of research efforts, in 2015 the spin-off Vaxxilon AG was founded to drive forward the development and commercialization of carbohydrate vaccines. Peter Seeberger is also co-founder of BDW Berliner Diagnostik Werke GmbH, a company developing carbohydrate-based microarrays to determine general vaccination status as well as companion diagnostics for the vaccination status related to specific vaccines.

Other regional players committed to vaccine development are the Beuth University of Applied Sciences and the Technical University of Applied Sciences Wildau. Their joint “Ipo-Gly” project aimed at developing glycoengineered vaccines integrating antigen and adjuvant functions in one molecule. Improved vaccine efficacy was achieved by atypical glycosylation of a recombinant protein of the respiratory syncytial virus, a pathogen causing severe infections of the respiratory tract in infants and in the elderly.



“The German capital region is a global center of the glycosciences that span from basic research at universities and institutes to companies that translate those scientific breakthroughs into products. Access to defined glycans by automated chemical assembly has been the basis for the development of novel research tools, diagnostics and vaccines. Carbohydrate-conjugate vaccines can protect people from many bacteria including those that are responsible for resistant hospital acquired infections. The ability to protect millions of lives creates a multi-billion Euro market globally. Berlin provides a perfect stage for top scientists and entrepreneurs to pursue their dreams.”

Peter Seeberger

Director Max Planck Institute of Colloids and Interfaces / Co-Founder Vaxxilon AG

Creating Synergies – Combining Glycosciences with Other Disciplines Leads to Cross Innovation

Glycobiotechnology offers such a huge range of possible applications that other science disciplines are becoming increasingly excited to integrate their own expertise with the newest findings from glycosciences. The potential of glycobiotechnology reaches unprecedented levels when different disciplines are involved.

Bioinformatics tools are essential for glyco-biology research

Bioinformatics has already become an integral and indispensable part of all glycomics activities as specific software tools are needed to manage the enormous flood of experimental data generated. The development of sophisticated algorithms is the basis for a precise analysis and evaluation of glycobiology research. MicroDiscovery GmbH, a leading provider of high quality software for biomolecular research, offers custom-tailored software development. Managing Director Arif Malik is also co-founder of BDW Berliner Diagnostik Werke GmbH, a company developing carbohydrate-based microarrays. HealthTwiSt GmbH is another bioinformatics specialist supporting customers with data management and statistical analysis services.

Creating new functional materials by combining glycans and polymers

The Fraunhofer Institute for Applied Polymer Research tackles a completely new field of carbohydrate-based applications. The institute explores the potential benefits of combining glycans with polymers for a broad variety of applications. Ruben R. Rosencrantz and his team work on

glycan-based coatings, e.g. for contact lenses to achieve better tolerability. The group also conducts research on glycan-based polymers involving mucins. Mucins, glycosylated proteins, are a component of the mucus in the mouth or other mucous membranes. Beside other functions they serve as a protective barrier against microbes. Possible applications include coatings for handrails, hand grips for toilets in trains and other frequently touched objects that might transmit pathogens. Another project aims at developing an intelligent coating of cell culture plates which allows for a more gentle detachment of cells. The scientists are also engaged in diagnostic applications. They develop glycan-based sensors to detect bacteria or its toxins that bind to certain carbohydrate structures of human cells. Their aim is to develop point-of-care diagnostics for pathogens like *Clostridium*, *Helicobacter pylori*, *Pseudomonas* or enterohemorrhagic *E. coli* (EHEC).

Cell-free systems for synthesis, modification and analysis of glycoproteins

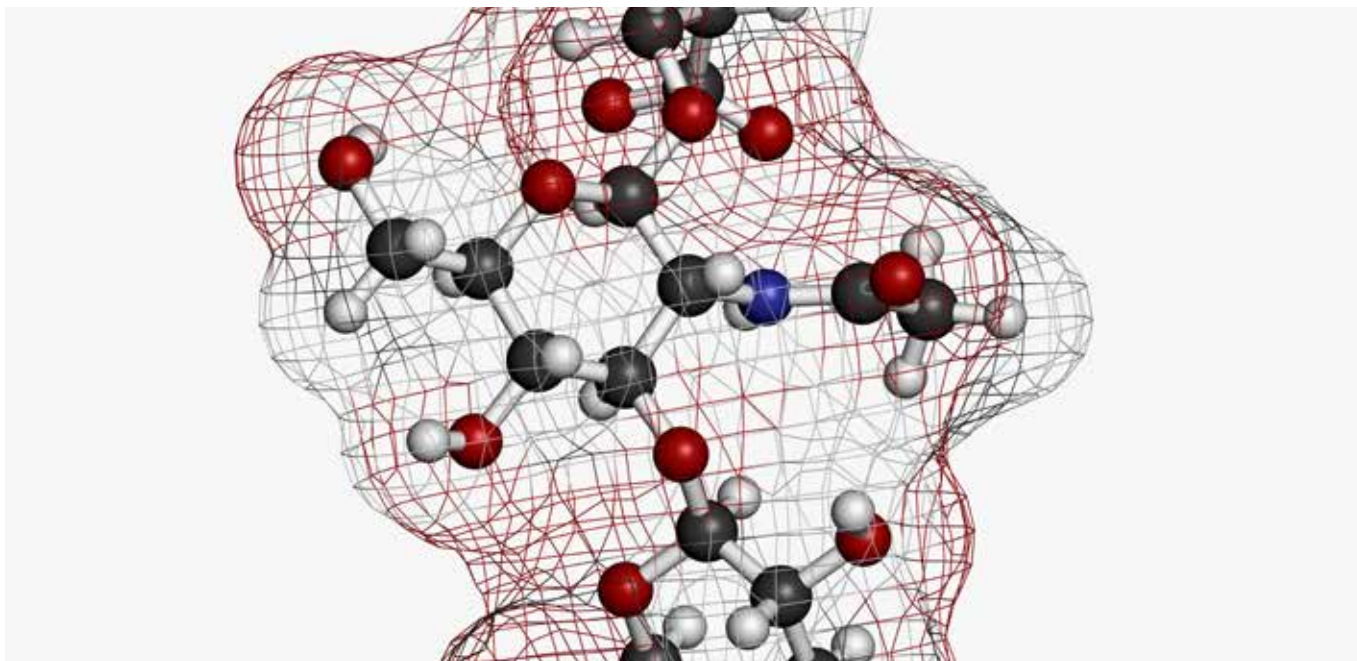
The need for enzymes, complex peptides and glycoproteins, or synthetic biomolecules in general is constantly increasing in food technology and the agricultural, cosmetics, detergent industries and, above all, in the health



"Glycans are biomolecules which exhibit one of the greatest variety of different functionalities in nature. Integrating glycans into polymers allows to create new tailored materials with a broad range of applications, such as fast diagnostics of pathogens, encapsulation for drug delivery, anti-fouling surfaces and functional coatings, to name just a few. By combining nature's diversity with the possibilities of modern programmable and functional materials, the Fraunhofer IAP wants to break new ground in polymer science."

Ruben R. Rosencrantz

Project leader at Fraunhofer Institute for Applied Polymer Research IAP, Functional Protein Systems / Biotechnology



sector. In this context, cell-free systems are gaining increased interest, e.g. for the production of tailor-made glycoproteins. Cell-free bioproduction only uses the sub-cellular components of the organisms that are required for the synthesis. Extensive work has been carried out at the Fraunhofer Institute for Cell Therapy and Immunology IZI, Branch Bioanalytics and Bioprocesses Potsdam-Golm (IZI-BB) to establish this technology and to develop it further.

Applied glycosciences in nutrition

The increasing knowledge on carbohydrates enables the generation of probiotics and nutritional products with distinct new properties. Regional players utilize glycosciences to develop innovative products that meet the needs of customers in the food and beverages industry.

AnalytiCon Discovery GmbH addresses glycosylated natural products by applying medicinal or natural product chemistry to develop innovative products with beneficial features for the pharma sector as well as for nutrition and cosmetics applications. The company is co-initiator of the DOLCE program which offers food companies support to develop and produce natural sweetening solutions in order to achieve sugar-and calorie reductions in diverse food and beverage categories.

Apart from its enzyme business, evocx technologies GmbH offers innovative carbohydrates produced by enzymatic bioconversion using simple raw materials like sucrose and fructose. The company has several carbohydrate products under development that address present trends and the demands of the global food industry like low calorie products, slowly digestible carbs or dietary fibers.



“At Fraunhofer IZI, our current research activities are particularly focused on the cell-free synthesis of glycoproteins and their functional characterization. The enormous time savings are a key advantage of the cell-free system. Glycoproteins can be synthesized in just 90 minutes, whereas cell-based protein synthesis takes over 24 hours. Cell-free systems also have an ‘open’ design, meaning that external components can be added to the system in order to have a targeted impact on the quality and quantity of the glycoprotein. One of the outstanding perspectives of cell-free protein synthesis consists in the production of glycoproteins containing amino acids with different types of modified side chains.”

Stefan Kubick

Head of Department Cell-free and Cell-based Bioproduction, Fraunhofer Institute for Cell Therapy and Immunology, (IZI), Branch Bioanalytics and Bioprocesses Potsdam-Golm (IZI-BB)

More Innovation through Cooperation – the glyconet Berlin Brandenburg

In the last 15 years, the Berlin-Brandenburg region has emerged as a global center of glyco-sciences and its multiple applications. In 2016, the glyconet Berlin Brandenburg was established as a platform to connect various players from academia and industry and to join forces in this fascinating and promising area of research and development.

Several companies, universities and research institutes in Berlin and Brandenburg are committed to glycobiotechnology research and its applications. The glyconet BB provides a forum to enable exchange on current R&D activities and to facilitate more intense collaboration and synergies.

With a growing number of members, glyconet BB is a network that supports research and development in the innovative fields of glycobiotechnology, synthetic chemistry, analytical sciences and medical chemistry for use in medicine and life sciences. The main goal is to strengthen and visualize regional skills and education in this area of life sciences. Glyconet BB members have a broad range of expertise, from glycoanalytics and glycoengineering, proteomics, cell biology, immunology, biopharmaceuticals and vaccine development, to bioinformatics and polymer sciences.

With respect to education, glyconet BB set up a “Winter School” which is planned as a regular annual event. The first of these one-week training courses for junior scientists

took place early 2017 and provided insights into the theory and practice of glycosciences technologies.

Another initiative targets the establishment of a new international congress format, also planned as a regular event. The first congress on “New and Emerging Technologies” is scheduled for September 2017 in Potsdam. As an interdisciplinary networking event, the conference provides a platform for international scientific exchange and showcases the newest developments in glycobiotechnology, biochemistry, molecular biology, biofunctional materials, antibody technologies, synthetic biology and related areas.

Further activities focus on joint applications and implementation of resulting research projects. To improve the network’s reach glyconet BB permanently seeks out for co-operation partners on regional, national and international level.



Sweet Perspectives – Paving the Way for Translating the Latest Glycosciences into Targeted Medicines and Other Applications

Compared to genomics or proteomics, glycomics is still in an early stage. This is mainly due to the lack of tools to explore the complex structures of glycans and their features. Players in Berlin and Brandenburg are committed to increasing the knowledge in glycosciences and to accelerating the development of its numerous applications. The region is well-positioned to shape future markets.

Automated solid-supported oligosaccharide synthesis, as developed at the Max Planck Institute of Colloids and Interfaces and marketed by its spin-off GlycoUniverse GmbH & Co KGaA, has proven to be a most valuable tool and will advance glycobiotecnology in virtually all fields of applications – from research to the development of diagnostics, biopharmaceuticals or vaccines.

The analysis of the complex structures of glycans still remains a challenge. Players in Berlin and Brandenburg use and will further integrate state-of-the-art technologies to advance improved glycoanalytics.

Berlin-Brandenburg has definite strengths in the development of biologics, biosimilars and vaccines. The latest glycoengineering technologies will be applied to generate glyco-optimized drugs and vaccines by rational design. Biosimilars are currently subject to a highly competitive market. Therefore, biobetter versions might be useful to outpace competitors as well as the reference product. A biobetter may be characterized by optimized glycosylation but it could also be reflected by a different formulation, e.g. the development of a subcutaneously administered drug instead of an intravenous one. While presently recombinantly produced antibodies represent the majority of biopharmaceuticals, future progress will also include the development

of other glycosylated proteins as substitutes for disease-related deficiencies. Another trend features enzyme replacement therapy which replaces an enzyme that is deficient or absent in patients.

Glycan-based in vitro-diagnostics represent another future market for glycobiotecnology applications. So far, carbohydrate-based microarrays are widely used for research purposes. However, no in vitro-diagnostics of this kind are yet available. Regional players like Scienion AG, together with MicroDiscovery GmbH, Charité – Universitätsmedizin Berlin and Max Planck Institute of Colloids and Interfaces, are well-positioned to conquer this market and to enable improved diagnostics and personalized medicine approaches.

The potential of glycobiotecnology applications goes far beyond: glycosciences is entering numerous other disciplines to exploit the unique advantages of combined knowledge. Application examples include polymer sciences, nutrition, agriculture, material sciences, renewable energies and many more. The Berlin-Brandenburg region with its density of universities, research institutions and companies with a life science focus provides an optimal environment to transform glycosciences from a field dominated by specialists towards an integrated discipline.



“Berlin and Brandenburg recognized the potential of glycosciences and its various applications very early and have, since the early 2000s, continuously supported the regional expansion of this promising field to become a leading European glycobiotecnology center of excellence. Regional scientists have gained international recognition for their groundbreaking work and meanwhile research results have been transferred to successful applications. With respect to a well-filled product pipeline, I am sure that we will see an increasing number of innovations made in Berlin and Brandenburg. Definitely, the future will be sweet!”

Günter Peine

Cluster Healthcare Industries Berlin-Brandenburg HealthCapital, Section Manager Biotech I Pharma

At a Glance – Glycobiotechnology Expertise of Regional Players

Institution / Company	Glycoanalytics & Biomarker	Assay Development	Glyco-engineering	Vaccines	Cross-cutting Issues
Analyticon Discovery GmbH	●	●			●
BDW Berliner Diagnostik Werke GmbH		●			
Berlin-Brandenburg Center for Regenerative Therapies	●				
Beuth University of Applied Sciences Berlin	●		●	●	
Biosyntan GmbH			●		
biotechrabbit GmbH		●	●		●
Brandenburg University of Technology					●
Charité - Universitätsmedizin Berlin	●	●	●		●
Chiracon GmbH					●
evovx technologies GmbH					●
Fraunhofer Institute for Applied Polymer Research	●	●			●
Fraunhofer Institute for Cell Therapy and Immunology - Potsdam-Golm location IZI-BB	●		●		●
Freie Universität Berlin	●		●		
German Institute of Human Nutrition	●				●
Glycon Biochemicals GmbH			●		●
Glycotope GmbH	●	●	●		●
GlycoUniverse GmbH				●	●
HealthTwist GmbH					●
htw Berlin – University of Applied Sciences	●		●		
Humboldt-Universität zu Berlin	●				
Hybrotec GmbH		●			●
InVivo BioTech Services GmbH		●			
JPT Peptide Technologies GmbH			●		
Leibniz-Forschungsinstitut für Molekulare Pharmakologie	●	●	●		
Max Delbrück Center for Molecular Medicine Berlin-Buch	●	●	●		
Max Planck Institute for Colloids and Interfaces	●	●	●	●	●
Medipan GmbH		●			●
MicroDiscovery GmbH					●
Octapharma Biopharmaceuticals GmbH	●	●	●		
PlasmaChem GmbH					●
ProBioGen AG	●	●	●	●	●
Proteome Factory AG	●				●
Scienion AG	●	●			●
Technische Universität Berlin	●				
UGA Biopharma GmbH			●		●
University of Applied Sciences Wildau				●	●
University of Potsdam	●				●
Vaxxilon GmbH				●	●

Directory of Regional Players Involved in Glycobiotechnology

Biotech Companies	
Analyticon Discovery GmbH	
AnalytiCon is a biotechnology company focusing on the development of compound libraries consisting of natural product (NP) and synthetic small molecules. As the global market leader in NP small molecule screening compounds, AnalytiCon is the only company which is able to provide vast collections with completely disclosed structural information. As a pure compound provider the company is offering its products and services to the pharmac., chemical, biotech, cosmeceutical and nutraceutical industries.	ac-discovery.com
BDW Berliner Diagnostik Werke GmbH	
BDW Berliner Diagnostik Werke GmbH was founded in 2017 to develop, manufacture and commercialize new diagnostic technologies that leverage the potential of carbohydrate-science for sensitive and specific multiplex assay solutions. First product developments focus on diagnostic assays for determining the general vaccination status as well as companion diagnostics for vaccination status related to specific vaccines. Other applications include the analysis of specific allergies, autoimmune and infectious diseases. Business areas include the development of own products and customized diagnostics for the healthcare industry.	
Biosyntan GmbH	
BIOSYNTAN GmbH - Custom Synthesis of Peptides BIOSYNTAN GmbH was founded in 1997 as a scientific service company, focused on custom synthesis of peptides. Rising interest among our customers for modified peptides has prompted us to develop new methods for the manufacturing of such compounds. As one of the leading specialists in the synthesis of linear and cyclic peptides with unusual residues and a multitude of modifications we can offer you an extensive range of services. Our core area is the manufacturing of fluorescently labeled peptides for assay development and the synthesis of post-translationally modified peptides.	biosyntan.de
biotechrabbit GmbH	
biotechrabbit GmbH is determined to offer the newest and best life science products and innovative solutions to scientists leading the fight against disease and fueling our lives with innovations. We supply ultra pure enzymes and high-quality antibody services for diagnostics and a life science product portfolio (including reagents for PCR and nucleic acid purification) for molecular biology and proteomics. The biotechrabbit advantage is our combination of passion for excellent research with the agility of true entrepreneurship.	biotechrabbit.com
Chiracon GmbH	
chiracon is a qualified partner to develop chiral intermediates at a constant high quality for innovative drugs regarding economic, environmentally acceptable and scalable aspects. We offer support through expert knowledge and key technology to meet your increasing needs. Our core competence is chirality. In addition we have a broad variety of chiral building blocks as an inhouse raw material source for high-level API production and development.	chiracon.de
evoxx technologies GmbH	
The core competencies lie in the targeted conversion of simple carbohydrate containing substrates (e.g. agricultural by-products, waste streams, inexpensive sugars) to high-value carbohydrate based products, their characterization, processing and testing. Marketable products include novel /optimized enzymes, expression systems, specialized biotransformation and down-stream processes, customized oligomers and polymers for food and non-food uses.	evoxx.com
Glycon Biochemicals GmbH	
Glycon Biochemicals is a specialist in the field of carbohydrate chemistry, ionic and non ionic carbohydrate detergents, first of all anomeric pure n-Dodecyl β -maltoside and building units used for the synthesis of new pharmaceutical compounds and diagnostics.	glycon.de
Glycotope GmbH	
Glycotope is a leading company in glycomics and immunotherapeutics. It has developed novel therapeutic antibodies and the unique GEX® platform technology for production and glyco-optimization of biological entities.	glycotope.com
GlycoUniverse GmbH	
GlycoUniverse GmbH & Co KGaA is a technology spin-off of the Max Planck Institute of Colloids and Interfaces. Registered on October 10th 2013, the company deals with the automated development and synthetic production of complex sugars and is seeking customers and collaboration with leading scientific institutes in Europe and world-wide.	glycouniverse.de

HealthTwist GmbH HealthTwist is a service provider for “Genetic Contract Research” in the fields of Functional Genomics, Epigenetics, Proteomics or Functional Food / Nutraceuticals. In particular, HealthTwist is offering access to extensively characterized human subjects and samples as well as prospective studies in twins, together with know-how in the analysis of complex genetic traits. Furthermore, database development, data management and statistical analyses for clinical projects are provided.	healthtwist.de
Hybrotec GmbH Hybrotec GmbH is specialized in the development of monoclonal antibodies and immunoassays (preferably homogenous assays) as a service to our customers. Our main focus lies on antibodies binding low molecular weight substances (e.g. toxins, fluorophores, hormones) as well as antibodies against novel proteins.	hybrotec.com
InVivo BioTech Services GmbH InVivo is a contract manufacturing organization (CMO) dedicated to the development and production of monoclonal antibodies and the expression of recombinant proteins for research, diagnostic and pre-clinical use. In the last 19 years more than 3000 monoclonal antibodies were produced on demand for customers of the IVD or pharma industry in any scale. Regarding immunoassay development InVivo covers every aspect of the whole value chain. From antigen production via transient gene transfection in HEK cells to CE approval of the final test, Invivo manufactures on demand and provides advice and support.	invivo.de
JPT Peptide Technologies GmbH JPT Peptide Technologies GmbH is the leading provider of innovative peptide-based services and products, as well as a partner for R&D projects in Immunotherapy, Proteomics and Drug Discovery.	jpt.com
Medipan GmbH Development, manufacturing and international distribution of radioactive (125J-IRMA) and non-radioactive (Elisa) in vitro-diagnostic tests, covering the field of Autoimmunity, mainly in thyroid and type 1 diabetes and automated interpretation of cell-based immunofluorescence tests by the AKLIDES system.	medipan.de
MicroDiscovery GmbH MicroDiscovery GmbH is a leading provider of high quality software for biomolecular research, innovative diagnostics and personalised medicine. The company is ISO 9001 certified and has a strong track record in creating software systems in accordance with relevant regulatory directives for the life science and IVD industry. Customised services ranging from biostatistical data analysis to bioinformatical design and support of complex research projects complete the product line of MicroDiscovery.	microdiscovery.de
Octapharma Biopharmaceuticals GmbH The department “Molecular Biochemistry” in Berlin investigates the influence of the manufacturing process steps on the molecular structure or biological activity of recombinant and plasma derived proteins by using state-of-the-art analytical tools. The results are important for process optimization as well as assurance of the native form of the proteins which is crucial for their physiological activity.	octapharma-biopharmaceuticals.com
PlasmaChem GmbH PlasmaChem GmbH is an innovative R&D and manufacturing company specializing in nanomaterials, plasma- and ultra-thin film technologies. The company manufactures nano-coated medical implants (stents), nanomaterials, additives for metal plating (applied e.g. by Siemens AG), new additives to engine oils on the base of nanodiamonds and other industrial nanoproducts.	plasmachem.com
ProBioGen AG ProBioGen is a Contract Development and Manufacturing Organization (CDMO) and Technology Provider, with extensive expertise in cell line engineering, process development (upstream- and downstream process) and GMP manufacturing of biopharmaceuticals. Offering state-of-the-art contract development and manufacturing services as well as access to proprietary cell lines (AGE1.cell lines®) for the manufacturing of biopharmaceuticals and vaccines. The company possesses the manufacturing authorization from the competent authority and meets the quality standards required by the EMA and the FDA guidelines.	probiogen.de
Proteome Factory AG Professional Proteomics and Protein Analysis. High resolution mass spectrometry is employed for epitope mapping of antibodies and identification, quantification and characterization of proteins and peptides including determination of phosphorylation and glycosylation sites and optionally their structure.	proteome-factory.com
Scienion AG Scienion is a leading solution provider in the markets of precision dispensing and diagnostics solutions. The product portfolio comprises hardware, consumables and services. Scienion's leading product is the sciFLEXARRAYER non-contact dispensing system, available in different versions for R&D and manufacturing purposes. It represents the perfect tool for automated ultra-low volume liquid handling of various types of samples. The company has a wide-ranging experience in developing microarrays for numerous applications, including glycan-based assays, e.g. for the detection of Heparin-induced thrombocytopenia, a life-threatening prothrombotic condition.	scienion.de

UGA Biopharma GmbH	
UGA Biopharma, a CRO in cell line development, offers ready-to-use biosimilar-expressing cell lines and customized cell line development services for NBE. Bioprocess optimization, downstream development and analytics are included. Its success is based on choice of an appropriate host cell line, optimized expression vectors and use of a proprietary, high-performance cell culture medium.	ugabiopharma.com
Vaxxilon GmbH	
Vaxxilon is a startup established by the Max Planck Society and Actelion Ltd to discover and develop novel vaccines against major infectious diseases. The innovative technologies are based on the scientific insights and the research of professors Peter Seeberger (the Max Planck Institute of Colloids and Interfaces) and Gennaro Libero (University Hospital of Basel). Vaxxilon is advancing several vaccine candidates using homogeneous, synthetically produced carbohydrate antigens.	vaxxilon.com

Universities, Research Institutions

Berlin-Brandenburg Center for Regenerative Therapies	b-crt.de
Humboldt-Universität zu Berlin	hu-berlin.de
Max Delbrück Center for Molecular Medicine Berlin-Buch	mdc-berlin.de
Technische Universität Berlin	tu-berlin.de
Brandenburg University of Technology	tu-cottbus.de
Charité - Universitätsmedizin Berlin	charite.de
Fraunhofer Institute for Applied Polymer Research	iap.fraunhofer.de
Fraunhofer Institute for Cell Therapy and Immunology - Potsdam-Golm location IZI-BB	izi.fraunhofer.de
Freie Universität Berlin	fu-berlin.de
German Institute of Human Nutrition	dife.de
htw Berlin – University of Applied Sciences	htw-berlin.de
Leibniz-Forschungsinstitut für Molekulare Pharmakologie	fmp-berlin.de
Max Planck Institute for Colloids and Interfaces	mpikg.mpg.de
Beuth University of Applied Sciences Berlin	beuth-hochschule.de
University of Applied Sciences Wildau	tfh-wildau.de
University of Potsdam	uni-potsdam.de

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