

Structural Medicine: The Importance of Glycomics for Health and Disease

European Science Foundation Policy Briefing

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Foreword

Biomedical research has entered the post-genomic phase following the completion of the Human Genome project and other related sequencing efforts. The functional analysis of gene products (functional genomics) gave rise to the 'omics' disciplines including proteomics, defined as the identification and characterisation of proteins and their interactions as well as of protein expression. However, proteomics does not describe post-translational modifications of proteins that are in many instances necessary for biological activity. While recent years have witnessed an unprecedented growth of knowledge in proteomics, the time for generating broad insights into post-translational modifications has now arrived, especially in the field of *glycomics* (glycoscience), *lipidomics*¹, phosphorylation 'omics' and even Structural Medicine as such.

Glycomics describes and analyzes the structure of sugar molecules in glycoconjugates. Carbohydrates present in glycoconjugates such as glycolipids and glycoproteins contribute decisively to their functionality. Certain glycosylation patterns give proteins specific physical, biochemical and biological characteristics and cause their structural and functional variance. Currently, some far-reaching biomedical consequences of changes in the structure and metabolism of glycans are already known: these are indicators of diseases such as congenital disorder of glycosylation, inclusion body disease, tumors, inflammation, diabetes mellitus, intoxications (alcohol, drugs) and microbial infections. Changes in glycan structure themselves play a causative role in the development of inflammation, arteriosclerosis, immune defects and autoimmunity, infections (e.g. influenza, virus hepatitis, meningitis and HIV) and the invasion of cancer cells.

Expanded knowledge on disease-related changes in glycosylation patterns and its integration in genome and proteome data provides new basic biomedical insights and thus, far-reaching possibilities for diagnostic application (prevention, first diagnosis, treatment follow-up, and prognostic assessment) as well as for the development of new therapeutic approaches.

The European Medical Research Councils (EMRC) Committee of the European Science Foundation (ESF) established a task force comprised of leading European scientists in the field of glycoscience with the goal to develop a science policy briefing (SPB) addressed to national and European research organisations. The scientific experts had been nominated by the ESF Member Organisations and through a wide consultation within the European Scientific community. The task force met on several occasions to address scientific issues and to formulate a draft policy briefing. This SPB was presented to the EMRC Standing Committee, which reviewed the recommendations in the wider context of medical research priorities.

The EMRC of ESF sees a need for an acceleration of research in Structural Medicine and recommends a strong and coordinated interdisciplinary research effort on a European scale to overcome the fragmentation currently observed and to regain strength in this previously very prominent European research domain. The European Science Foundation calls upon national and European research organisations, as well as the Structural Medicine research community, to give serious consideration to the recommendations in this Science Policy Briefing.

Bertil Andersson
ESF Chief Executive

1. A second ESF Science Policy Briefing entitled "Structural Medicine: The Importance of Lipidomics for Health and Disease" is planned.

Introduction

Structural medicine is an area of biomedical research where fundamental knowledge of the structures and interactions of molecules is acquired. This information may in subsequent steps be applied to medical treatments and health care. Consequently, both basic and applied research is needed in this field to achieve meaningful progress.

Life – DNA – Structural Medicine

The discovery of the DNA structure as a double helix in 1953 is an important milestone for structural medicine. In more recent years, it has been recognized that knowledge about the structure of genes, i.e. the sequence of nucleotides, is not sufficient to decipher the functional role of biological systems. Additional information is required on the dynamic expression of relevant proteins and, in particular, their potential post-translational modifications such as glycosylation.

The theme “Structural Medicine” with a focus on glycans and glycoscience aims at improving human health by enhancing our insights into the structure of glycoconjugates (glycoproteins, glycolipids, proteoglycans, glycosylphosphatidylinositol anchors) and microbial polysaccharides, and by eventually translating it into reliable diagnostics and treatment strategies.

Based on a great history of carbohydrate research, European Research groups have developed and led to maturity the areas of Structural Biology related to glycoscience, especially glycochemistry and glycoanalysis, and initiated glycobiology and glycomedicine. However, in the area of combining the underlying molecular characteristics with function, Europe has been losing ground. The USA and Japan are currently ahead in glycobiology and glycomedicine, and, heavily supported by national grants, glycoscience consortia have been created, i.e. “the USA Consortium for Functional Glycomics” and “the Japan Consortium for Glycobiology and Glycotechnology”. It is recommended to fund European initiatives that aim at reinforcing the historic strengths in glycoscience, and to make the information available through cross-linked databases and expert systems. Furthermore, a solid maintenance base for such infrastructure should be established. To let Structural Medicine with a focus on glycoscience flourish, young scientists must be attracted, and they should be trained in this highly relevant area of research that crosses the barriers between various disciplines.

The Post-Genomic Era

Biology and medicine have been revolutionized by the dramatic developments in genomics and proteomics. The emergence of new technologies boosted these developments, in particular the high-throughput sequencing of the genome and mass spectrometry of the proteome. The burst of techniques associated with functional analysis of genes has given rise to huge amounts of data obtained and classified by experts in genomics, transcriptomics and proteomics. The newly available data were stored in databases and made available to the scientific community. They have allowed enormous progress in the identification of the molecular bases of disease and have greatly contributed to exploring the identification, cure and prevention of a number of life-threatening diseases.

DNAs, RNAs and proteins are directly related to genes. The situation becomes more complex when extended to systems biology, taking into consideration metabolic pathways including the establishment of concentration in time and space of all cellular metabolites, cell signalling, gene regulation, etc. Since this concerns a wide diversity of chemical structures, with tremendous variations in concentration, the development of suitable technology and the handling of the huge load of data is a great challenge for bio-informatics. Further sources of precious information are the 3D-structures of biomolecules. In particular X-ray crystallography and NMR spectroscopy have demonstrated their potential in this area. Infrastructures and methodologies have been improved both in terms of capacity and quality.

Significant efforts have been directed towards the structural investigation of proteins, whereas often equally important glycans/glycoconjugates have almost been neglected, although they are essential in many recognition, signalling and regulation processes. In the case of glycans, however, real high-throughput analysis has not yet been achieved, as the assignment of structural features is a much more complex process than the linear sequencing of DNA. The analysis of glycan structures is indeed challenging due to their heterogeneity.

To counter the relative deficiency of information of this functionally important class of biomolecules, a multidisciplinary research initiative focussing on glycoscience and glycomics is required in Europe.

Glycoscience and Glycomics

Glycomics can be defined as the full characterization of the entire repertoire of glycans in a living organism. Presently, this inventory is far from being complete as new glycan structures (glycoconjugates and polysaccharides) are constantly being elucidated. It is also important to study the expression of proteins involved in carbohydrate metabolism, transport and function as well as the regulation of their genes.

In the context of cells and organisms, glycoconjugates contribute to the dynamics of biological membranes, to energy storage and expenditure, and to conveying messages at all cellular levels, including the nucleus. Consequently, multidisciplinary approaches are required to further the insight into in-vivo functions of glycoconjugates both in natural and pathological states. The same holds for the microbial glycans that are of importance in health and disease.

The Value of 'Structure'

The knowledge of cellular and molecular structures lies at the basis of modern medicine. Translating structural information to medicine is applying structural information to health. Specific interaction and/or recognition at the molecular level is the basis for all signalling, regulation and control processes in and between cells of all living organisms. The importance of subtle molecular details in relation to health may be best demonstrated by the immune system which protects against a wide range of intruders in human bodies through i) its remarkable power to distinguish between self and non-self (or non-dangerous and dangerous) and ii) its memory potential which enables it to be better prepared when the same molecular entity is recognized again. In this context, glycan structures play an important but largely – at the present time – undefined role.

One of the most characteristic features for correct immune surveillance is the continuous recirculation of cells from blood to lymph. In this process carbohydrates play a vital role. P- and E-selectins are localized on endothelial cells and, when activated in e.g. trauma situations, interact with carbohydrate structures displayed on leukocytes. A multi-step process occurs that involves slowing down lymphocyte movement through dynamic biomolecular interactions and cell rolling, tethering, firm adhesion and transmigration. Glycan-recognizing proteins, selectins, on lymphocytes (L-selectin) and on the vascular endothelium (P-selectin) are involved in this process which represents an important step in leukocyte trafficking to inflammatory sites. Leukocyte integrins (which are glycoproteins) are also important: they are activated by the rolling and then bind to their counter-receptors on endothelial cells, leading to the adhesion of the leukocytes.

In general, a disease state can be associated with a deviating biomolecular balance, either as the cause or as the result of the disease. Analysis of the biomolecular imbalance or the occurrence of specific carbohydrate epitopes may be the key to disease diagnostics in the hospital laboratory. Likewise, detailed insight on the structure and action of relevant carbohydrates needs to be obtained in order to improve the development of innovative and more effective medicines.

Blood clotting – Heparin – Optimised medication

Blood clotting is an essential response to damage inflicted upon tissue. However, under certain – controlled – conditions it can be an unwanted process that must be suppressed by medication. The blood-clotting cascade consists of a series of enzymes and a complex control mechanism for activation and deactivation. Heparin is a natural sulphated proteoglycan that reduces blood clotting by enhancing the action of antithrombin III. Structure-function analysis revealed that a specific pentasaccharide fragment of heparin is responsible for this activity. Several structural analogues of the pentasaccharide have been synthesised and tested for their activity. Based on these studies, an optimized therapy is available now for patients requiring anticoagulant treatment. These synthetic drugs have a high effect/dose ratio, which is largely due to their considerably longer half-lives in the body (e.g. 120 hours for a pentasaccharide variant; application once a week) than the natural compound (1 hour for heparin; application three times a day). The prolonged half-life provides great benefits and an increased quality of life to patients relying on these drugs.

The modes of action of enzymes depend on molecular details as well. Subtle modification of a protein can change its activity, and subsequently also change the occurrence of other proteins in a cascade. Such a change, caused by e.g. a single amino acid exchange (single DNA mutation), or a different post-translational modification of the protein, can have serious health implications. Essential biochemical pathways may be perturbed or even blocked.

The Value of 'Structure' for Diagnostics, Disease and Drug Development

The value of 'structure' in living systems, including humans, is evident. All specificity and selectivity in life processes relies on structural differences. From the perspective of medicine the structural characteristics are precious as well. Diagnostic tools can distinguish between pathogenic states on the basis of the spectrum of structures presented. The continuing developments in essential instrumental techniques such as high-resolution NMR spectroscopy and imaging, cryo-electron microscopy and mass spectrometry have resulted in valuable structure-tools and infrastructures for medicine.

The value of structure for medicine is further expressed in the understanding of disease-causing processes and the potential to develop preventive and therapeutic medication.

Disease can have a multitude of origins. Numerous diseases originate from some toxin or disease-causing organism penetrating our body, whereas others stem from a dysfunction of the complex biochemical production machinery or its regulation and control system.

Bacterial infection: Vaccines against *Haemophilus influenzae* type b

The commercially available capsular polysaccharide vaccines against pathogenic bacteria, like *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* type b, are poorly immunogenic in high risk groups, such as children under the age of two and old persons. Polysaccharides are thymus-independent type 2 antigens that induce a poor immunological memory. These problems can be overcome by conjugating the polysaccharides to proteins, thus initiating a T-cell dependent response. A clear demonstration of a multidisciplinary approach, including glycan analysis, organic synthesis, and immunology resulted in a complete synthetic oligosaccharide-protein conjugate vaccine. This vaccine is now used in the battle against bacterial infections.

Bacteria, viruses and parasites are the major agents leading to disease. Although a wide range of bacteria flourish in harmony with the human body, certainly not all do so. The disease-causing properties of many pathogenic bacteria comprise the excretion of bacterial toxins, of which many are lectins (i.e., carbohydrate recognising proteins). In addition to producing toxins that bind to cell surface glycans, various types of bacteria adhere to sugar-containing structures on host cell surfaces. Bacterial adhesion may be harmless as a normal mechanism of coexistence. It may, however, also be the first step of the infection process. It is well established that the infection by viruses and bacteria often starts by specific interactions with host-driven glycans. The next step involves the actual penetration of the cellular membrane. Both steps, the cellular adhesion and the internalisation, are of high interest for preventive medicine. Following infection the immune system plays an essential role in the defence, which may be directly utilized in human health care. Vaccination with capsular polysaccharides of encapsulated pathogenic bacteria representing key virulence determinants is a well-established tool to prepare the immune system against such specific pathogens. In some cases, however, it is powerless against the invasion. Viral infections (HIV) or some parasite infections are good examples thereof. Furthermore, some pathogenic organisms are capable of disguising themselves in such a way that the immune system considers them as being 'self'.

Viral infection: Influenza – medication

Several viral infections are initiated through binding to terminal sialic acid residues on cell surface glycoproteins and glycolipids. Haemagglutinin functions as biological glue in this process enabling invasion of the cell while neuraminidase (sialidase) acts as a biological scissor (required for an efficient release of newly formed virus particles). Thus, drug design targets both haemagglutinin and sialidase. Synthesis of potential drugs designed by computational chemistry and inhibition studies of several variants of sialidase-inhibitors resulted in a modified monosaccharide transition-state analogue as a highly potent drug that is now used to treat influenza infections in man.

Many diseases, however, do not originate from the penetration of external pathogens. Instead, the regulation and control mechanisms within the particular organism may be disturbed by various internal causes. A DNA point mutation may result into a single amino-acid exchange in a protein which may completely change or obliterate the function of such a protein. This may happen in isolated cells of grown individuals, or it may be inherited, resulting in inborn errors, such as congenital disorders of glycosylation (CDGs), lysosomal storage diseases, and von Willebrand factor deficiency. Likewise, cancer or autoimmune diseases, such as rheumatism, are typical examples of fatal errors in the body's regulation and control system.

- Cancer is associated with changes in glycosylation of proteins exposed on the outer cell surface. Therefore, monitoring of temporal changes in glycosylation profiles has a potential as a diagnostic tool or as a prognostic indicator. Furthermore, it may also serve as a target for selective delivery of agents that can kill tumor cells.
- Complex carbohydrate epitopes play their roles in allergy and immune reactions against parasites. They are of great concern in xeno-transplantation and in the biotechnological production of recombinant therapeutic glycoproteins.
- Another field of high interest is termed metabolic oligosaccharide engineering, which leads to a biosynthetically changed cell-surface repertoire through the introduction of unnatural sugar residues into cellular glycans. Such engineering is of high value in investigations on e.g. cell-cell interactions.

Statements and Recommendations

To further develop diagnostic tools, preventive medicines (vaccines) and therapeutic drugs, a better understanding of the structural basis of glycosylated molecules is required. The recommendations listed below address the need to accelerate research in the field of Structural Medicine in Europe with special emphasis on glycomics.

Recommendation: Focus on Structure and Function of Glycans

Glycoconjugates play key roles in many cellular processes including signalling and molecular recognition in health and disease. Knowledge of the respective structures is a prerequisite for the elucidation of function(s) and of therapeutic applications².

Today, a wide panel of powerful analytical techniques for a detailed structural elucidation of glycans and glycoconjugates is available in European research institutions. These include high-resolution NMR spectroscopy, dichroism spectroscopy, mass spectrometry and X-ray crystallography. Furthermore, fundamental knowledge in carbohydrate chemistry and enzymology are prerequisites for the success in glycan structural analysis. These undoubtedly existing European expertises, however, are fragmented and need to be interlinked.

ESF recommends a strong coordinated interdisciplinary research effort on the occurrence and function of glycans and glycoconjugates in Europe to enhance our insights into the structure of these molecules in health and disease.

Recommendation: Development of Rapid Diagnostics

Changes in metabolite concentrations and profiles of biomolecules expressed at the surface of infected or diseased cells (e.g. cancer) have great potential to be exploited for rapid and accurate diagnostic tools. The application of powerful micro-array assays that have been developed for gene research should be adapted for the detection of a wide range of metabolites or glycans/glycoconjugates.

ESF recommends fostering the development of high-throughput-diagnostic tools for the rapid analysis of glycan profiles.

Recommendation: Protected and Targeted Delivery

Glycans often play a pivotal role in intercellular interaction and recognition processes without being recognised by the immune system. Consequently, these types of molecules have great potential for targeted delivery of active compounds to the appropriate location within the body. Since these active compounds may be toxic, targeted delivery through selective biomolecular interactions would be beneficial. Furthermore, glycans can protect the active compound itself, thus preventing an undesirably fast clearance in the body.

ESF recommends stimulating research that combines targeted delivery with protection of the active agent.

Recommendation: Glyco-Databases

The challenge for glycoscience is to make existing and emerging information available through databases and expert systems. As a first step, a corresponding design study, EUROCarbDB, has been launched. Further development of such databases and, in particular, a solid maintenance base for such infrastructures should be established. Techniques developed for genomics and proteomics need to be adopted and adapted to the specific requirements. The expert systems have to meet the inherent complexity of glycoscience and open up the knowledge for the larger life science community. Furthermore, the new databases should be cross-linked with genomics, proteomics and protein-structural databases.

ESF recommends supporting European initiatives that aim at cross-linking databases and expert systems exploiting recent advances in bioinformatics of the “omics”.

Recommendation: Investment in Human Capital

The opportunities of glycosciences within Structural Medicine can only be realized when sufficient resources in human capital are created through investment in adequate interdisciplinary educational and training programmes. A broad variety of disciplines including synthesis, molecular structure and interaction, functional assays, spectroscopy/spectrometry and bioinformatics in the context of glycochemistry, (systems) glycobiology and glycomedicine need to be taught in training programmes ranging from summer schools to specialized PhD and MD/PhD programmes with the aim to satisfy clinical, academic and industrial needs.

ESF recommends investing in European interdisciplinary educational programmes aimed at training scientists in glycoscience in the context of Structural Medicine.

² In the future, glycans may also play an important role in designing specific types of functional food, including baby and infant food.



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