Foreword

The first brochure on the Bijvoet Center for Biomolecular Research described the mission of the Bijvoet Center as “The quest for structures”. These four words provide still the shortest and most to the point statement on the continuing activities of the Bijvoet Center. The architecture of biomolecules and biomolecular aggregates is based by nature on a limited array of relatively simple building blocks. These building blocks are combined in an endless variety to yield complex three-dimensional structures. The structures thus created are continually changing, thereby creating a dynamic and diverse molecular world. This forms the basis for the interaction of molecules with their environment and their ability to recognise and to be recognized. The dynamic interplay of architecture and function is of vital importance for an optimal quality of life, hence the elucidation of molecular structure is essential to gain further understanding of the molecular basis of life.

The Netherlands Foundation for Chemical Research and Utrecht University founded the Bijvoet Center for Biomolecular Research in 1988, thereby linking major research groups in Utrecht to which they give considerable support. In the past ten years many biomolecular systems have been studied, with the help of advanced instrumental techniques and computational methods, in order to obtain insight into the mechanisms of biomolecular recognition and interaction. This area is an important aspect in structural biology, because the resulting fundamental knowledge can be used to develop a rationale for the treatment of human diseases.

The search for biomolecular structures and their function in tuning living systems has been the quest of the Bijvoet Center and will continue to be so in the foreseeable future. This booklet looks back on some of the more important achievements obtained by the individual research teams in the Bijvoet Center. Though these research highlights are grouped with the individual research groups, this does not mean that they have been obtained by the teams operating in isolation. The strength of the Bijvoet Center is the integrated approach towards the structural and dynamic aspects of biomolecular interaction, and this integrated approach has been an aspect of all examples being presented.

Prof.Dr. Hans Vliegenthart
Research Director

Introduction

The Netherlands Foundation for Chemical Research and Utrecht University founded the Bijvoet Center for Biomolecular Research as a para-university research institute on March 25th, 1988. The goal of this collaboration was to create a well equipped, with respect to staff and advanced instrumental and computational infrastructure, research and expertise center in the area of structural biology with a national and international reputation for high quality research. In the Bijvoet Center major research groups at Utrecht University, for which the research is focussed on the mechanisms of molecular recognition and interaction, were brought together to pursue their common goal: the elucidation of molecular structures and dynamics to gain further insight into the molecular basis of life.

The study of the relation between the structure and dynamic behaviour at atomic resolution of a biomolecule or biomolecular aggregate on the one hand and the properties and function of these systems on the other has an important role in structural biology research. It is of importance for several areas, such as the development of new drugs and vaccines, the development and optimization of biotechnological processes and procedures, the development of improved foods and food supplements (functional foods) and materials, and for the development of advanced diagnostic procedures. Establishing the structure and dynamics of a biomolecule is one of the last steps in a process that leads to establishing a direct relationship between the structure and dynamic behaviour of a biomolecule and its function and properties. In many cases the research activities of the Bijvoet Center will thus be concentrated on the final steps in fundamental research programmes that are aimed at the elucidation of cellular processes at the molecular level. In view of the advanced and exceedingly costly infrastructure and the highly specialised expertise needed for obtaining the necessary structural and dynamic information, this type of research will in general be concentrated in a few specialised institutes. The Bijvoet Center was created to be one of these institutes in the Netherlands. On the other hand, once the necessary expertise and infrastructure are in place, such a research institute can deal with and participate in a broad range of research programmes in collaboration with research partners from other institutes both nationally and internationally.
The complex structure of bioactive molecules and the fine tuning of molecular processes brought about by subtle changes in those structures, can only be studied through a multi-disciplinary approach. In the Bijvoet Center the studies on the role of the three-dimensional structure, and dynamics, in the mutual interactions of biomolecules and into the interactions between biomolecules and biomolecular complexes are focussed on carbohydrates, unsaturated fatty acids, nucleic acids, peptides, proteins and biomembranes. The research is based on the use of three complementary spectroscopic techniques: Nuclear Magnetic Resonance Spectroscopy, X-Ray Diffraction and Mass Spectrometry, which are integrated with computational science. In-vivo NMR spectroscopic studies link the high-resolution NMR studies of the Bijvoet Center with the whole-body NMR imaging and NMR Spectroscopic studies of the medical sciences. This integrated approach, using advanced instrumental and computational methods for the study of a limited number of biomolecular systems, should contribute to the fundamental understanding of the molecular basis of life and provide a foothold for strategic and applied research, oriented towards designing compounds for the treatment of health disorders and improving the quality of human life.

The mission of the Bijvoet Center has thus been formulated as follows:

The Bijvoet Center will develop, use, and keep up to date, the necessary expertise, methods, and infrastructure for obtaining insight into the relation between the structure and the function/activity at the molecular level for biomolecules that are involved in recognition, regulation and interaction processes in the area of structural biology. The Bijvoet Center will make its expertise, methods, and infrastructure available to the scientific community as a whole. The Bijvoet Center strives to retain and improve its international top position in this area.

Within the context of this mission the Bijvoet Center constitutes an important aspect of the biomedical research at Utrecht University. The Bijvoet Center collaborates furthermore closely with industry and with organizations concerned with specific diseases, and formal links exist with a multitude of international research groups, often in the form of formal research networks funded by the European Union.

The initiative for creating the Bijvoet Center has proven to be a successful endeavour. Important results in an organisational context are:

- the integration of the national X-ray crystallographic participation research project of the Netherlands Foundation for Chemical Research (SON), and Utrecht University (UU) into the Bijvoet Center;
- the creation at the Bijvoet Center of a national facility for in-vivo NMR spectroscopy in collaboration with several research councils of the Netherlands Organisation for Scientific Research (NWO), the Ministry of Education, Culture and Science (OCW), and Utrecht University (UU);
- the creation at the Bijvoet Center of a facility for 750 MHz NMR spectroscopy, in a collaboration between the Ministry of OCW, SON/NWO and UU;
- the recognition of the SON NMR large scale facility for biomolecular NMR (SONNMRLSF) as a European Union supported large scale facility in its Human Capital and Mobility, and Training and Mobility of Researchers programmes, thus enabling access to the biomolecular NMR facilities of the Bijvoet Center to European researchers;
- the conclusion of a research alliance between the Bijvoet Center and the Nijmegen SON Research Center (BNRA), which involves the boards of Utrecht University, the University of Nijmegen and SON;
- the conclusion of a research agreement between the BNRA and the European Molecular Biology Laboratory at Heidelberg (EMBL);
- the accreditation of the Bijvoet Graduate School for Biomolecular Chemistry, for which the Bijvoet Center is the central part, by the Royal Netherlands Academy of Arts and Sciences (KNAW) in 1992 and 1997.

The Bijvoet Center has proven to be an interesting partner in European Union supported research networks, both in its Human Capital and Mobility, and Training and Mobility of Researchers programmes. It is also a sought after institute for Marie Curie fellows. The partners in the Bijvoet Center participated and/or are currently participating in European Union supported research contracts with a net value to the partners in the Bijvoet Center of over six million ECU. At the national level the Bijvoet Center is among others supported with programme grants for starting up a protein crystallography project, and for a research project on the biopolymer cellulose.

Obviously, the scientific achievements of the Bijvoet Center for Biomolecular Research are the most essential achievements of the Center. Highlights from these achievements are contained in the following sections. At the end of this book some information is given on the composition of the Bijvoet Center, the recipients of the Bijvoet Medal, and selected personal achievements of members of the staff of the Bijvoet Center.

### Bio-organic Chemistry

Prof.Dr. J.F.G. Vliegenthart

Prof.Dr. J.P. Kamerling

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The main research efforts of the department were focused on:

- Structure and function of carbohydrates en glycoconjugates
- Biocatalysis

The research resulted in 42 PhD-theses and about 250 publications in scientific journals. Approximately a 100 lectures were presented upon invitation to highlight recent results.

**Structure and function of carbohydrates en glycoconjugates.**

Carbohydrates and glycoconjugates exert in nature a large variety of essential biological and physio-chemical functions [12]. Gaining insight in the functioning of such molecules requires as a first step the determination of the primary and three-dimensional structure. However, from a structural point of view, carbohydrates belong to the most complex type of bio-macromolecules. The unambiguous identification of a complex carbohydrate requires the establishment of many structural parameters like constituting monosaccharides, including absolute configuration, ring size and anomeric configuration, position of glycosidic linkages, type and position of non-carbohydrate substituents and molecular mass distribution. For this purpose advanced NMR spectroscopy and mass spectrometry are employed in conjunction with chemical and biochemical methods.
As a first example, the studies have led to the unraveling of the cell-surface carbohydrate chains that are responsible for the species specific cell adhesion of the sponge *Microciona prolifera* [2,3]. This is a perfect illustration of the direct involvement of cell-surface-bound carbohydrates for the social behavior of the living cell. It clearly illustrates the general role of cell-surface glycans in the regulation of contacts of the cell with the outer world, comprising physiological and pathological features.

From the same perspective, the structure of the HNK-1 carbohydrate epitope, as expressed on neural recognition molecules, has been established [4]. This epitope was first identified on human natural killer cells, is involved in cell interactions that control cell-type specific neurite outgrowth and regeneration. Furthermore it is a target for autoimmune IgM antibodies in de myelinating neuropathies of the peripheral nervous system in humans.

Schistosomiasis is a wide-spread disease. It is caused by the parasite blood fluke *Schistosoma mansoni*, residing in the portal and mesenteric veins of humans and various mammalian species. Although the parasite is exposed to a cellular and humoral immune response, it persists in the host for 3 tot 5 years at least. In elucidating the immunological and immunopathological interactions between *S. mansoni* and its host, the attention was focused on the antigens secreted by the parasite. These antigens circulate in the host and are glycoproteins. The O-linked glycans of these antigens represent the immunoreactive parts. In both cases the structures of these glycans are unique [5,6]. The strong immunity of CAA (Circulating Anodic Antigen) formed the starting point for an organic synthesis project aimed at the generation of new diagnostic reagents to screen for this parasitic infection.

The zona pellucida is the glycoprotein matrix surrounding the mammalian oocyte. It mediates a number of essential steps in the fertilization process, including species-specific sperm-oocyte binding, induction of the sperm acrosome reaction, prevention of polyspermy and physical protection of the growing embryo until implantation in the uterus. The primary structures of O-linked glycans were determined for the porcine zona pellucida glycoproteins [7,8]. Present studies are aimed at correlating the structure of the N- and O-linked carbohydrate chains to the biological activities.

Tamm-Horsfall glycoprotein is produced in the kidney as a phosphatidyl-inositol anchored glycoprotein. After cleavage from the anchor, it is excreted as the most abundant protein in the urine. It has been proposed that the carbohydrate chains are essential in many (patho)physiological features. For that reason the structures of the glycans were studied. It turned out that the glycosylation pattern of this glycoprotein is extremely complex. A large variety of N-linked glycans is present even in the urine of an individual donor. The branching varies from di- to tetraantennary. On the non-reducing termini several different acidic epitopes are found [9].

The repertoire of protein glycosylation was extended by the discovery of C-mannosylation of the indole ring of tryptophane in human ribonuclease U₅ [10-13].

Bacterial exopolysaccharides are widely applied in the food industry as gelling and thickening agents. Recently, a strong interest has arisen in exopolysaccharides produced by lactic acid bacteria, because these bacteria are food grade. In order to gain insight into the relation between structure and rheological properties, the primary structure has been determined of a number of such exopolysaccharides [14-19]. As a next step extended computational studies have been started for a repeating unit of the exopolysaccharide excreted by *Lactobacillus sake* 0-1. A helix-like structure could be deduced for this compound [20].

In recent years significant progress has been made with the determination of the three-dimensional structure of intact glycoproteins in solution. These studies have revealed new insights into the mutual interaction of glycans and protein in a glycoprotein. Also a new approach was developed in defining the mobility of the constituting monosaccharides in a glycan. Exciting results were obtained for pine-apple stem bromelain. In particular the comparison of the glycan in the intact glycoprotein with that in the glycopeptide showed the dramatic influence of the large polypeptide backbone [21,22]. For the a-subunit of human chorionic gonadotropin the solution structure of the protein and the glycans were derived. Interestingly, the glycans at the two N-glycosylation sites are significantly different with respect to the mobility near the asparagine residue involved [23-26].
Biocatalysis

The industrial utilisation of enzymes, in particular aimed at the production of fine chemicals like flavour compounds, may have several advantages over the use of nonenzymatic processes. Biocatalytic processes can be energy-efficient and show a high stereo selectivity. The latter is very relevant in the production of flavour compounds, pharmaceuticals and agrochemicals. Biocatalysis is growing as a scientific discipline, because the successful introduction of a suitable biocatalyst requires basic research on its structure and on the mechanism of the reaction. Lipoxygenase research in the Department of Bio-Organic chemistry has focussed on mechanistic aspects of the oxidative modification of natural polyunsaturated fatty acids, and on establishing the structures of reaction products. The primary products of the reaction are chiral, cis, trans-conjugated fatty acid hydroperoxides. Lipoxygenases, from both plant and mammalian sources contain iron as the only non-amino acid cofactor, which is essential for catalytic activity. During the catalytic cycle its valence shuttles between Fe(II) and Fe(III). At the beginning of the cycle the valence state must be Fe(III) in order to be able to react with linoleic acid. However, many native lipoxygenases contain Fe(II), which makes an activation step to the Fe(III) state necessary. Once this has occurred, the catalytic cycle is fully self-supporting. The activation causes complex kinetics at the start of the reaction. In addition to spectroscopic evidence from EPR, fluorescence and other optical techniques, a detailed kinetic investigation including the use of synthetically prepared diynoic inhibitors, has shown that the amount of iron present as Fe(III) fully determines the kinetic behaviour. EXAFS (Extended X-ray Absorption Fine Structure) measurements have indicated that the iron most probably is in a distorted octahedral environment with nitrogen and oxygen as its direct ligands. The EXAFS technique has also yielded direct evidence that the iron environment of the Fe(III) enzyme species is different from that of the Fe(II) species. The physiological functions of lipoxygenases still cannot be completely defined. In mammalian systems, lipoxygenases are the key enzymes in the production of leukotrienes, in particular in pathological conditions like asthma bronchiale. Reaction products may also be involved in apoptosis and signal transduction pathways. More recent developments comprise the possible involvement of lipoxygenase in the metabolism of anandamide, an endo-cannabinoid, and an excellent substrate for lipoxygenase in vitro. Recent work on the use of lipoxygenase as a biocatalyst owes much to the accumulated knowledge on the mechanism of the reaction and the structure of the products. In a typical project, a crude protein fraction from soybean meal was used as the source of lipoxygenase activity, while the substrate consisted of an aqueous suspension of hydrolysed safflower oil. Each vital parameter of the reaction, i.e. pH, oxygen partial pressure, stirring speed, was controlled and optimised to yield a pure product: a stereoselectively oxygenated unsaturated fatty acid, suitable to be subsequently used as the precursor of a natural flavour compound: (R or S)-13-decalactone. Current research activities focus on the formation of other flavour compounds, in particular small aldehydes and alcohols, as well as the enzymatic mechanisms of their formation.

1a J. Montreuil, J.F.G. Vliegenthart and H. Schachter (Eds)
1b J. Montreuil, J.F.G. Vliegenthart and H. Schachter (Eds)
Characterization of a novel pyrrolylated carbohydrate unit implicated in the cell aggregation of the marine sponge Microciona prolifera
Characterization of a novel sulfated carbohydrate unit implicated in the carbohydrate-carbohydrate-mediated cell aggregation of the marine sponge Microciona prolifera
4 H. Voshol, C.W.E.M. van Zuylen, G. Orberger, J.F.G. Vliegenthart and M. Schachner
Structure of the HNK-1 carbohydrate epitope on bovine peripheral myelin glycoprotein P0
The immunologically reactive O-linked polysaccharide chains derived from circulating cathodic antigen isolated from the human blood fluke Schistosoma mansoni have Lewis x as repeating unit
6 A.A. Bergwerff, G.J. van Dam, J.P. Rotmans, A.M. Deelder, J.P. Kamerling and J.F.G. Vliegenthart
The immunologically reactive part of immunopurified circulating anodic antigen from Schistosoma mansoni is a threonine-linked polysaccharide consisting of -6)-[beta-D-GlcpA-(1-3)]-beta-D-GalpNAc-(1-repeating unit
7 C.H. Hokke, J.B.L. Damm, J.P. Kamerling and J.F.G. Vliegenthart
Structure of three acidic O-linked carbohydrate chains of porcine zona pellucida glycoproteins
8 C.H. Hokke, J.B.L. Damm, B. Penninkhof, R.J. Aitken, J.P. Kamerling and J.F.G. Vliegenthart
Structure of the O-linked carbohydrate chains of porcine zona pellucida glycoproteins
9 K. Hård, G. van Zadelhoff, P. Moonen, J.P. Kamerling and J.F.G. Vliegenthart
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New type of linkage between a carbohydrate and a protein: C-glycosylation of a specific tryptophan residue in human RNase Us

11 T. de Beer, J.F.G. Vliegenthart, A. Löffler and J. Hofsteenge
The hesropyranosyl residue that is C-glycosidically linked to the side-chain of tryptophan-7 in human RNase Us is alpha-mannopyranose

12 J. Hofsteenge, A. Löffler, D.R. Müller, R.J. Richter, T. de Beer and J.F.G. Vliegenthart
Protein C-glycosylation

Spectroscopic and protein chemical analysis demonstrate the presence of C-mannosylated tryptophan in intact human RNase 2 and its isoforms

14 M. Gruter, B.R. Leeflang, J. Kuiper, J.P. Kamerling and J.F.G. Vliegenthart
Structure of the exopolysaccharide produced by Lactococcus lactis subspecies cremoris H414 grown in a defined medium or skimmed milk

15 M. Gruter, B.R. Leeflang, J. Kuiper, J.P. Kamerling and J.F.G. Vliegenthart
Structural characterisation of the exopolysaccharide produced by Lactobacillus delbrückii subspecies bulgaricus rr grown in skimmed milk

The structure of the exopolysaccharide produced by Lactobacillus helveticus 766

17 G.W. Robijn, D.J.C. van den Berg, H. Haas, J.P. Kamerling and J.F.G. Vliegenthart
Determination of the structure of the exopolysaccharide produced by Lactobacillus sake 0-1

Predicting helical structures of the exopolysaccharide produced by Lactobacillus sake 0-1

Conformations and internal mobility of a glycopeptide derived from bromelain using molecular dynamics simulations and NOESY analysis

Conformational analysis of the xylose-containing N-glycan of pineapple stem bromelain as part of the intact glycoprotein

Rapid and simple approach for the NMR resonance assignment of the carbohydrate chains of an intact glycoprotein. Application of gradient-enhanced natural abundance (H-1)-(C-13) HSQC and HSQC-TOCSY to the alpha-subunit of human chorionic gonadotropin

Site-specific and complete enzymatic deglycosylation of the native human chorionic gonadotropin alpha-subunit

NMR studies of the free a subunit of human chorionic gonadotropin; structural influences of N-glycosylation and the b subunit on the conformation of the a subunit

Glycosylation beyond the Asn78-linked GlcNAc residue has a significant enhancing effect on the stability of the a subunit of human chorionic gonadotropin
Biochemistry of Membranes

Prof. Dr. B. de Kruijff

Dr. A. Killian

Membrane research in the Bijvoet Center is geared to obtain molecular insight into the structure, function and dynamics of biological membranes in relation to the chemical and physical characteristics of the individual membrane components and their mutual interactions. This goal is achieved via a multi-disciplinary experimental approach involving both advanced spectroscopic as well as biochemical techniques using systems of decreasing complexity ranging from intact biomembranes to relatively simple model systems, the properties of which closely mimic that of the biological membrane.

Membrane insertion and translocation of proteins are essential processes for the normal growth of cells and organelles, for secretion and membrane biogenesis. But also in pathological situations, such as infection by viruses and bacteria, very often proteins have to negotiate the hydrophobic barrier of the membrane. A major research effort in our department is the elucidation of the underlying molecular mechanisms or the various membrane insertion and translocation processes in cells with special attention for the lipid-protein interactions involved.

A crucial and intriguing finding was made ten years ago in the bacterial protein export process [1]. By making use of lipid biosynthetic mutant strains of Escherichia coli, we could establish that the negatively charged lipid phosphatidylglycerol (PG) was essential for protein translocation across the cytoplasmic membrane. That observation led to the search for the components in the translocation reaction which required these type of lipids for activity. Two key observations were made: the signal sequence which is essential and sufficient to direct proteins into the secretory pathway was shown to insert into the lipid bilayer via electrostatic interactions between the positively charged N-terminus and PG, resulting in large changes in polypeptide and lipid structure. This conclusion was based on model experiments, using broad line and high resolution NMR techniques [2,3], as well as on translocation experiments using mutant signal sequences [4]. In the inserted state the signal sequence can initiate membrane translocation of the attached protein by undergoing a structural change from a looped to an extended transmembrane organization. Unfolding was mediated by the transmembrane potential which was shown to act both on the signal sequence and the core translocase complex consisting of the SecYEG subunits [5].

The other key observation was the finding that SecA, the motor protein which drives translocation via its ATPase activity, required PG for activity. This lipid-protein interaction was required to mediate deep membrane insertion of SecA, which was modulated by ATP binding and hydrolysis [6].

Together with progress made in other laboratories, these results provided a molecular picture of the translocation process of which the initial phase is schematically illustrated in Figure 1. The significance of electrostatic interactions for anchoring positively charged protein domains at the membrane interface turned out to be of a much more general nature [7]. In fact, these lipids turned out to be crucial topological determinants for integral membrane proteins [8]. In membrane proteins very often positively charged amino acids are present close to the cytosolic side of transmembrane alpha-helical segments (positive inside rule). Modulating the charge distribution around the membrane spanning part of a protein and varying the level of anionic lipids by regulated expression of the corresponding biosynthetic enzymes revealed that the interaction of the positively charged amino acids and the anionic lipid in the E. coli inner membrane anchored these residues at the cytosolic side of the membrane and thereby controlled protein topology.

The potential of the use of lipid biosynthetic mutants for the search of the function of the various classes of membrane lipids became also very apparent in another area of membrane biology. Phosphatidylethanolamine (PE) is an abundant membrane lipid which belongs to the class of the so-called non-bilayer lipids. Non-bilayer lipids are membrane lipids which themselves do not prefer to self organize in the lipid bilayer but instead prefer to organize in inverted non-bilayer structures. The presence of such lipids provides a paradox, because the overall bilayer organization of biomembranes is undisputed. Why would nature bother to invest in the synthesis of such lipids while other membrane lipids are perfect bilayer formers. A crucial finding towards answering this paradox came from studies on a E. coli mutant which could not synthesize PE. The cells now had an absolute requirement for high concentrations of specific divalent cations for growth. Analysis revealed that the cells actively maintained a membrane environment. In the last year a dramatic number of new observations throughout the world were made to support this hypothesis, which was recently published [12]. The hypothesis is presented in Figure 2 and is further explained in the legend to that figure.

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Anionic phospholipids are determinants of membrane protein topology.


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De bijbehorende figuren en legenda staan op het schijfje: Killian

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**Crystal and Structural Chemistry**

Prof. Dr. J. Kroon
Dr. P. Gros
Dr. B.P. van Eyck

In the past ten years the department of Crystal and Structural Chemistry, working in the field of X-ray Crystallography and Computational Structural Chemistry, has published about 600 scientific articles. These papers cover methodological and experimental developments, structural studies ranging from small to macromolecular biomolecules and many crystal structure determinations by the National Facility of the SON/UU Scientific Service of X-ray Crystallography. Here we highlight appealing results obtained by our Protein Crystallography group (that is relatively new to the Bijvoet Center and was started in mid-1994 supported by a 5-years grant from SON/NWO) and by our long standing research on cellulose (in collaboration with the department of Bio-organic Chemistry and Akzo-Nobel Research, Arnhem, supported by the programmes IOP-k, EET, EU-FAIR and SON/STW).

**Protein crystallography**

The protein crystallography group is focussed on molecular recognition in biology. Regulatory mechanisms in living cells, ranging from gene transcription to molecular targeting, are studied by deriving detailed structural information (up to atomic resolution) of proteins and of protein complexes of physiological relevance.

This research performed in collaboration with several biochemical and biomedical research groups has already led to structures determined in the fields of immune response, haemostasis and gene transcription with more structures in the process of determination. The results yield a starting point for structure-based design of experimentally immunogenic peptides against bacterial meningitis; as well as a structural understanding of the adhesion process of multimeric plasma protein von Willebrand factor to collagen in haemostasis. In gene transcription we have studied a potent co-activator of transcription: human replication and transcription cofactor PC4.

Positive cofactors (PCs) enhance the transcription rate by functionally and physically mediating interactions between transcription activators and the basal transcription machinery\(^1\). The most potent positive cofactor known is PC4. Its role as mediator in gene-transcription activation is reflected by its potency to associate with different protein factors and different forms of DNA. We have studied the C-terminal domain of PC4 (residue 63-127), that is capable of binding single-stranded DNA (ssDNA) and partially melted duplex DNA, as in a heteroduplex with consecutive base pair mismatches\(^2\). In full-length unphosphorylated PC4 this activity is absent and appears "masked" by the N-terminal domain. Binding to intact double-stranded DNA (dsDNA) involves both the C-terminal and the N-terminal domains. The phosphorylation sites are located in the N-terminal domain, that is also responsible for association with other protein factors, such activators (e.g. VP16) and components of the basal machinery (TFIIA and TFIID).

The crystal structure of the C-terminal domain of PC4 (PC4\(_{CTD}\)) reveals a tetramer of dimers\(^3\). PC4\(_{CTD}\) forms tight dimers containing two anti-parallel grooves formed by curved b-sheets lined with basic and aromatic residues suited for binding ssDNA. The dimeric arrangement allows binding of two opposing strands of DNA over a length of 8 to 10 bases, which is in full agreement with observed optimal binding of heteroduplex DNA with 8 consecutive dT:dT mismatches. A tetramer of dimers is arranged in a right-handed spiral in the crystal structure. Arginines positioned on the b-ridge separating the ssDNA binding grooves point inwards forming a positively

http://www.nmr.chem.uu.nl/bijvoet_brochure/brochure.html#nmr
charged interior of the spiral. Charge, pitch and diameter of the spiral appear excellently suited for wrapping dsDNA.

These data present the first structural data from the class of positive cofactors. The data support a potential dual functional role for PC4 in transcription activation, where enhancement of transcription rate is achieved by assisting recruitment possibly by the dsDNA binding mode and establishing or maintaining melted DNA at promoters or origins of replication by the ssDNA binding mode.

Cellulose research

Cellulose is the most abundant macromolecules on earth and presents a huge source of renewable raw material. Our research on this polymer that forms highly-crystalline fibres, started with the investigation of a remarkable difference in mechanical properties between cellulose I and II, the two major polymorphs of cellulose. We were able to verify that this difference is caused by the formation of an additional intra-molecular hydrogen bond in cellulose I, providing it with a larger chain modulus. This larger chain modulus would have potential applications in high-strength polymeric materials, were it not that cellulose I appears to be the unstable polymorph. Any industrial treatment causes the transition of the natural cellulose I form into the regenerated/mercerized cellulose II form. To find the origin of this phenomenon several approaches were taken.

The transition of cellulose I to II always involves polar solvents. Our combined NMR and modelling studies on cellulose (models) have shown that the orientation of the hydroxy-methyl group is related to intra-molecular hydrogen bond formation and that the conformation typical for cellulose I (tg) is unstable in polar solvents. It seems that nature is capable of making the highest-strength form of cellulose due to biosynthesis by a protein complex that provides a hydrophobic environment, thus imposing this unfavourable tg conformation.

The behaviour of the polymer in solution is strongly dependent on the stiffness of the chain and on the occurrence of folds. These properties can be characterized by the persistence length. A procedure has been developed to calculate the persistence length of polymers like cellulose from MD simulations of small repeating units (e.g. cellulobiose) in solution. By our novel approach the solvent effect is included and a systematic study of the influence of solvent and temperature has become feasible. We have already found some remarkable substitution and solvent effects for cellulose. This is mainly caused by enhanced importance of folded conformations in some cases, which leads to a drastic drop in persistence length. A recently started project in the EET program continues research along this line.

Another approach to understand the cellulose I to II transition was to study the crystalline state in more detail. A systematic analysis of possible polymorphs of cellulose has been undertaken through MD simulations. In our group appreciable progress has been made in developing methods and improving force fields to predict carbohydrate crystal structures and polymorphy. Part of these developments could be used in the cellulose project. An important issue is whether cellulose molecules pack in the crystal in a parallel or antiparallel way. All previous evidence pointed to a parallel packing for cellulose I and an antiparallel packing for cellulose II. However, in view of the solid state transition of I to II in the mercerization process without loss of crystal morphology, the parallel to antiparallel conversion was not conceivable. We found that there may exist two forms of cellulose II, one parallel and one antiparallel, with almost indistinguishable cell dimensions, so that, depending on the kind of conversion process, either form could occur. Further evidence for this can be obtained from fibre diffraction studies. However, fibre diffraction techniques are in need of improvement. With support from SON/STW we are currently contributing in this field. With the advent of modern area detection systems and computational techniques a lot of progress can be made in this field.

Research on larger oligomers of cellulose and hemi-celluloses is currently undertaken in a project in the EU-FAIR program. It involves the study of the interactions between these oligomers and cellulose crystalline surfaces.

References


Legends to Figures:
Fig. 1. Crystal structure of the C-terminal domain of replication and transcription cofactor PC4; shown are Ca traces of the PC4<sub>CTD</sub> dimer (left) and the octameric spiral arrangement (right).

Fig. 2. Anti-parallel dimeric arrangement of the ssDNA binding grooves in PC4 allows binding of two opposing strands of ssDNA over a length of approx. 8 to 10 bases and, thus, may stabilize melted duplex DNA in transcription or replication. Shown is the proposed model of melted dsDNA bound to the PC4<sub>CTD</sub> dimer.

Fig. 3. Theoretical model of PC4<sub>CTD</sub> interactions with dsDNA (for clarity 3 out of 4 dimers are shown). The octamer yields a positively charged spiral positioning arginines ideally for interactions with the phosphate backbone of dsDNA.

Fig. 4. Hydrogen bonded sheets in the two major polymorphs of cellulose. In cellulose I (left) two intramolecular hydrogen bonds along the fibre direction occur, giving it a larger elastic modulus than cellulose II (right) where only one such hydrogen bond occurs.

Fig. 5. Five examples of cellulose molecules obtained from computer simulations of cellobiose in water. From complete sets of such conformations physical properties like the persistence length are calculated.
Fig. 6 MD model of parallel cellulose I and two MD models of cellulose II, antiparallel (top right) and parallel (bottom right). Transition from I to II goes along the top-arrow for the regeneration process, and could go along the bottom-arrow in the mercerization process.

Fig. 7 X-ray fibre diffraction pattern obtained from regenerated cellulose II fibres. Accurate intensity determination and data reduction from such patterns gives detailed structural information.

NB. De eerste drie figuren horen bij de protein crystallography en de laatste vier bij cellulose

NMR Spectroscopy

Prof.Dr. R. Kaptein
Prof.Dr. R. Boelens

The NMR research at the Bijvoet Center is focussed on obtaining insight at the molecular level in biological processes, through the study of the three dimensional molecular structures derived from the geometrical information contained in multi-dimensional NMR spectra. In the following three highlights are given from different research topics in the department.

The N- and C-terminal domains of HIV integrase.

Three essential proteins of the human immunodeficiency virus - HIV protease, reverse transcriptase and integrase - are important for the design of drugs against aids. For the first two a wealth of structural information is already available. In contrast, for the integrase protein only recently the structure of the catalytic core domain was solved by X-ray crystallography. In collaboration with the group of Prof. R.H.A. Plasterk (NCI, Amsterdam) the solution structures of the two remaining domains of HIV integrase was solved.

In the life cycle of the virus integration of a DNA copy of the viral RNA into the genome of the infected cell is an essential step. Reverse transcription of the viral single-stranded RNA, catalysed by the viral reverse transcriptase, results in a double-stranded blunt-ended DNA copy. This DNA copy is then integrated into the host genome by the viral integrase (IN) protein, which catalyses two distinct reactions: (i) site-specific cleavage of two nucleotides from both 3' ends of the viral DNA and (ii) integration of the recessed viral DNA into the target DNA.

Integrase consists of three functional domains: an N-terminal putative Zn-finger domain, a catalytic core domain and a C-terminal DNA-binding domain (DBD). The structure of the 51-residue DBD was solved using multidimensional NMR and is shown in Figure 1. It is a symmetrical dimer, which presents a challenge for the NMR
analysis, since the interpretation of NOE’s in terms of intra- or intersubunit contacts is not unambiguous. This problem could be solved by introducing asymmetry using 15N and 13C isotopically enriched protein in conjunction with isotope filtered NMR experiments.

A surprising result was that the basic fold of the domain is homologous to that of Src-homology 3 (SH3) domains. Since SH3 domains are often found in proteins involved in signal transduction where they contact other proteins, this raises the intriguing possibility that the integrase DBD is similarly involved in protein-protein interaction as well as interaction with DNA. As a Lys 264 mutant has been identified that abolishes DNA binding, the DNA contacting region could be established as the loop region in the protein opposite to the dimer interface.

Recently, the structure of the N-terminal domain (residues 1-55) was also solved by NMR. This domain is known to bind zinc and contains a conserved HHCC motif (two histidines and two cysteines) that is found in all retroviral integrases. The structure is shown in Figure 2. It consists of a three-helical core stabilized by the HHCC zinc-binding unit. The helical arrangement is structurally homologous to the DNA-binding domains of several proteins such as trp repressor, prd paired domain and TC3A transposase. The combination of a three-helical unit and zinc-binding motif has not been previously observed in protein structures.

The structure and dynamics of a protein-DNA complex.

Virtually all biological control mechanisms involve highly specific interactions between biomolecules. How do these molecules interact? In general terms this is through a surface complementarity involving hydrogen bonds, hydrophobic and charge-charge interactions. However, often this complementarity does not pre-exist, but is induced during binding. Thus, the binding mechanism is not of a simple “lock-and-key” type but rather an “induced-fit”. This requires that the partners adapt their conformations with respect to each other and must be flexible to some degree in order to accomplish this. NMR spectroscopy is uniquely suitable to study this flexibility in addition to defining the structure of biomolecular complexes in solution. In particular, for 15N and 13C labelled biomolecules the measurement of heteronuclear spin relaxation parameters (T1, T2 and NOE) provides information on local dynamic behaviour. For example, with uniform 15N labelling of a protein local mobility of the complete backbone can be sampled at the amide positions and the same is true for many of the side chains (arginine, lysine, asparagine, glutamine and histidine).

As an example in the area of protein-nucleic acid interaction we have made a combined study of the structure and dynamics of the lac repressor headpiece complexed with an 11 base pair lac operator. The headpiece of 56 amino acid residues is the DNA binding domain of the repressor. The structure of the complex is essentially based on NOEs (see Fig. 3) and gave an excellent account of the specificity of sequence recognition by the repressor. Many interactions between amino acid side chains and DNA could be delineated, that had been implicated previously by mutagenesis studies. In fact the structure of the complex provides a framework for interpretation of a vast body of biochemical and genetic data.

A comparison of the protein free in solution and complexed to DNA showed that the basic three-helical fold of the headpiece remains intact, but the loop between the second and third helix changes its conformation drastically upon binding (see Fig. 4). This is an example of adaptation of the protein to allow a number of side chain interactions with DNA to be made Interestingly, 15N relaxation data showed that this loop region has a greater mobility than the core of the protein in the free state. This mobility is frozen, however, in the complex. At the same time the relaxation data for the protein side chains suggest that there is a substantial residual flexibility even for residues in the protein-DNA interface. Thus, from this and other studies the conclusion emerges that biomolecular complexes are not so rigid as hitherto thought but that they retain a lot of flexibility. NMR with its ability to provide information on dynamics as well as on structure is eminently suitable to provide a detailed picture of biomolecular interactions.

Photoactive Yellow Protein and its photocycle.

Photoactive Yellow Protein (PYP) is a photosensory protein of Ectothiorhodospira halophila. It protects the bacterium from harmful blue and UV light by inducing a negative phototactic response. PYP is a water-soluble 14 kD protein, which makes it a good model system for NMR studies of its ground state (pG) and intermediates in addition to defining the structure of biomolecular complexes in solution. For example, with uniform 15N labelling of a protein local mobility of the complete backbone can be sampled at the amide positions and the same is true for many of the side chains (arginine, lysine, asparagine, glutamine and histidine).

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We have generated pB by Argon laser irradiation in the NMR probe and studied its stucture in solution. We find that this photo-intermediate is largely unfolded and consists of an ensemble of rapidly interconverting conformers. Figure 6 shows regions of the protein (in red) where the largest conformational changes occur. Surprisingly, this result contradicts that of a recent time-resolved Laue diffraction study, which indicated a much more limited conformational change. We have also followed the refolding to the ground state of PYP by a series of 15N HSQC spectra taken at various delays after light irradiation. Refolding rates were different for different regions of the protein. A picture emerges for the refolding of the protein, in which the β-sheet and part of the helical regions refold first. This promotes then the cis to trans isomerization of the chromophore after which the protein can fully refold into its pG state.

Legends to the figures:

Fig. 1 Structure of the C-terminal DNA-binding domain of HIV integrase
Fig. 2 Structure of the N-terminal (zinc-finger) domain of HIV integrase

Fig. 3 The complex of lac repressor headpiece with DNA as solved by NMR spectroscopy. Hydrogen bonds are indicated.

Fig. 4 Backbone of the lac repressor headpiece (thick line) in complex with DNA. The DNA is depicted on the left side. The backbone of the headpiece free in solution is shown too, overlapping with the bound structure, in a thin line.

Fig. 5 Photocycle and Photoactive Yellow Protein. pG: ground state; pR: red shifted intermediate; pB: blue-shifted intermediate.

Fig. 6 Structure of PYP with α-strands indicated I-VI and β-helices A-D. The extent in which pB deviates from pG is colour coded (red: large deviations, blue: small deviations).

Mass spectrometry
The developments in mass spectrometric techniques and methods in the last decade have opened up numerous possibilities for advanced mass spectrometric studies of biomolecular systems. In the following a few of the recent highlights are presented. The first concerns a mass spectrometric approach to the study of the molecular-recognition processes involved in host-symbiont interactions.

The soil enriching properties of leguminous plants have been recognised for more than two millennia, and is exploited widely in systems of sustainable agriculture, such as crop rotation and intercropping. The soil-improving ability of leguminous plants is due to the ability of legumes alone among higher plants to establish a symbiotic relationship with soil bacteria of the genus *Rhizobium*. This symbiosis results in the development of root organelles, the nodules, in which the bacteria are able to convert atmospheric nitrogen into ammonia that is available to the plant and the surrounding environment as combined, metabolizable nitrogen.

The legume-rhizobium symbiosis is very species-specific, with only certain species of *Rhizobia* able to form root nodules on only certain species of host legume. This species-specificity is mediated by two sets of molecular signals. The plant roots secrete a specific cocktail of flavonoids that are recognized by the bacterium, and in the symbiotic act to induce the biosynthesis of the bacterium's own signal molecules, a novel type of glycolipid known as lipo-chitin oligosaccharides (LCOs). The LCOs act on the roots of specifically the host legume to initiate nodule formation. LCOs consist of an oligosaccharide backbone having between two and six N-acetylgalactosamine residues in 1-4 linkage, to the non-reducing terminal residue of which a fatty acyl chain is amide-linked to the glucosamine nitrogen.

The detailed chemical structure of the LCOs encodes the species-specificity, by means of chemical 'decoration' of, generally, the termini of the chitin backbone. Examples include the attachment of acetyl, methyl, carbamoyl, or sulphate groups, or of an additional monosaccharide residue. In order to understand the molecular recognition processes that underlie the host-symbiont interaction, with the ultimate hope of being able to exploit this understanding for the purposes of optimising the efficiency with which leguminous plants can be grown and used, a systematic study of the chemical structures of LCOs produced by a wide variety of wild-type and mutant or recombinant *Rhizobia* has been undertaken as part of an interdisciplinary research collaboration with Professor Herman Spanik, of the Institute of Molecular Plant Sciences, University of Leiden.

Since LCOs are active, and therefore produced, at very low concentrations (typically 10⁻⁹ M) and frequently represent a heterogeneous mixture of chemically-related structures, their structural analysis presents a significant challenge. Mass spectrometry, with its attributes of high sensitivity and suitability for analysing mixtures, is the obvious method of choice for application to this problem. Over the last six years, we have applied mass spectrometry to the study of this intriguing glycobiological system. Our collaborative successes include demonstrating that simply exchanging a C18:4 fatty acid chain for its C20:4 analogue accounts for the difference in host specificity between two very closely-related species of *Rhizobium*, and that this difference is the result of only a few amino acid differences in the highly conserved central domain of one gene, nodE. In addition we have demonstrated that unrelated species of *Rhizobium* having different hosts make identical LCOs, and that the difference in species-specificity derives from the difference in legume-specific flavonoid signals. Mass spectrometry has also been used to help study the biosynthesis of LCOs and has led us to identify two novel bacterial glycosyl transferases that are the analogues of mammalian enzymes, in addition to leading us to the gene encoding one of them. The search for the gene for the second enzyme is now the subject of urgent research efforts.

The future for the application of mass spectrometry to such studies in glycobiology is very bright, with the advent of a new generation of extra sensitive mass spectrometers that are currently being acquired by the Mass Spectrometry Department of the Bijvoet Center. The exceptional sensitivity these new instruments offer makes it feasible to scale back our bacterial cultures and biological starting material by at least one thousand fold, allowing us to design and attempt ever more ambitious and intriguing experiments. Our most urgent aim is to exploit this enormous sensitivity enhancement to obtain chemical evidence to support the results of radio labelling experiments that suggest that chitin-like signal molecules act also as developmental regulators in developing fish embryos.

The second example concerns the structural analysis of peptoids using high-energy CID tandem mass spectra.

Peptoids are synthetic oligomers based on N-substituted glycines. Their structures differ from those of peptides in that the amino acid-specific side chains are located on the nitrogen atom rather than on the α-carbon atom (Scheme 1). The interest in these compounds centers around their therapeutic value that is based on their biological
activity and proven enzymatic stability. The absence of the chiral $\alpha$-$C$ atom in peptoids can be considered to be an advantage because spatial restrictions that are present in peptides do not exist when dealing with peptoids. Structural analysis of isomers such as peptides and peptoids requires their MS characteristics to be distinguishable and preferably that recognizable sequence ions are produced.

The positive ion mass spectra of peptoids resemble those of the corresponding peptides.

High-energy collision-induced dissociation (CID) spectra of [M+H]$^+$ ions reveal that peptoids also fragment at the amide bonds to yield isomeric B- and Y"-type ions (see Scheme 1).

The CID spectra of the [M+H]$^+$ ions of acetylated Leu-enkephalin amide (Ac-YGGFL-NH$_2$) and its corresponding peptoid (Ac-$\alpha$YGGFuL-NH$_2$) are given in Fig. 1a and b. CID spectra of [M+H]$^+$ ions of corresponding peptides and peptoids exhibit many sequence ions at identical m/z-values. The pronounced differences in the relative intensities of these sequence ions, however, can be related to structural characteristics of the compounds. B-type ions appear to be of comparable abundance in both compounds, however, more abundant Y"-type ions from peptoids are observed, most probably caused by the enhanced proton affinity of the secondary amine group in peptoids as compared to the primary amine function in peptides (compare Y" in Fig. 1a and b). Identification of the nature of the monomers can be obtained from high-energy CID spectra of selected immonium ions showing a preferential loss of an imine molecule (CH$_2$-NH) from $N$-substituted amino acids, not observed from the common amino acid immonium ions (see Fig. 1c and d).

High-energy CID spectra of [M+H]$^+$ ions show loss of amino acid-specific side chains, which occurs as relatively weak radical loss from peptides but as more pronounced molecular loss from peptoids. Facile elimination of a C$_{4}$H$_{6}$O molecule from all NTyr-containing ions (Fig. 1b) reveals the presence of this residue at the N-terminal position in the peptoid and corroborates sequence information obtained from backbone fragmentation. The presence of series of B- and Y"-type ions allows complete sequence analysis of these synthetic oligomers.

1) Peptoids were synthesized at the Utrecht Institute for Pharmaceutical Sciences, Faculty of Pharmacy.

Dept. of Medicinal Chemistry, University of Utrecht.

In vivo NMR spectroscopy

Dr. K. Nicolay

The effect of molecular structures and their function in tuning living systems can be fully revealed only by studying intact living systems rather than cells in culture, isolated tissues, organs etc. The department in vivo NMR spectroscopy was initiated in 1990 as a national center of expertise for non-invasive NMR studies of laboratory animals and intact plant systems. The in vivo NMR group on the one hand develops new techniques and on the other hand uses these to study fundamental biochemical and biological phenomena as well as animal models of human disease. The latter is done with several departments at the Utrecht University Hospital and other medical research institutions. Being part of the SON NMR large scale facility for biomolecular NMR, groups from other EU countries come to do experiments at the in-vivo center. The ever increasing number of groups that use the facilities, are an indication that there is a great need for this type of non-invasive technology. This development in the Netherlands is also seen internationally and underscores the growing impact of in vivo NMR in the biomedical sciences.

The technique development focusses on improving the speed of in vivo NMR studies, increasing the specificity of the information obtained and making experiment set-up more easy. An example of this is our specialization on the use of adiabatic radio-frequency pulses. Such amplitude- and phase-modulated pulses only require a one-time power calibration and because of their immunity to inhomogeneities in the strength of the B$_{0}$-field, they can be used in combination with a surface coil for optimal detection sensitivity. Figure 1 shows a series of T$_{2}$-weighted H-1 NMR images that were taken through the brain of a rat and measured with a single surface coil for excitation and reception of the NMR signal. The upper row that was acquired with adiabatic pulses shows a clean signal decay due to T$_{2}$ relaxation. By contrast, the bottom row that was measured with classical pulses exhibits strong ghosting artifacts.

Adiabatic pulses are very versatile and we have used them for many application studies on animal models of human disease. Figure 2 shows an example of the use of adiabatic pulses for metabolite editing, in this case for the measurement of the distribution of lactate in tumour-bearing rat brain with H-1 Spectroscopic Imaging. Aerobic glycolysis that is so typical for tumour metabolism leads to a high level of lactate in the malign tissue that can only be accurately sampled and discriminated from lipid signal when the lactate is edited with gradient techniques. Such techniques enable the non-invasive, longitudinal evaluation of tumour growth and regression.

The major part of the application studies are devoted to animal models of cerebrovascular diseases, including stroke, malignant hypertension, hydrocephalus and birth asphyxia. In the very early phase of stroke, part of the brain becomes ischemic due to an inadequate supply of oxygen and glucose. The depletion of high-energy phosphates that is the result of this, causes osmotic swelling of cells in the brain due to failure of the ATP-dependent ion pumps. We have shown that this swelling can be accurately monitored with diffusion-weighted NMR techniques: cell swelling leads to a retardation of water diffusion that generates contrast in NMR images that are
The Bijvoet Medal

The Bijvoet Medal was established in 1989 to recognize outstanding contributions to Biomolecular Research, which further the interests of individual research groups of the Bijvoet Center, or of the Center as a whole. The medal has so far been awarded to:

Prof.Dr. Jack D. Dunitz, ETH, Zürich, Switzerland
In recognition of his outstanding contributions to "Chemical Crystallography"

Prof.Dr. Brian R. Reid, University of Washington, Seattle, USA
In recognition of his outstanding contributions to "NMR spectroscopy of biomolecules"

Prof.Dr. Nathan Sharon, Weizmann Institute of Science, Rehovot, Israel
In recognition of his outstanding contributions to "The chemistry of carbohydrates and glycoconjugates"

Prof.Dr. Hartmut Michel, Max Planck Institut für Biophysik, Frankfurt, Germany
In recognition of his outstanding contributions to "Molecular membrane biology"

Prof.Dr. Isabelle L. Karle, Naval Research Laboratory, Washington DC, USA
In recognition of her outstanding contributions to "X-ray Crystallography"

Prof.Dr. Binne Zwanenburg, Nijmegen University, the Netherlands
In recognition of his outstanding contributions to "The foundation of the Bijvoet Center"

Prof.Dr. Joachim Seelig, Bio-Center, Basel, Switzerland
In recognition of his outstanding contributions to "Macroscopic in-vivo NMR spectroscopy"

The Bijvoet Family, The Netherlands

In recognition of "The eminent scientific contributions of Prof.Dr. J.M. Bijvoet and the lasting bond this has created of the family with the Bijvoet Center"

Dr. Adriaan Bax, National Institutes of Health, Bethesda MD, USA
In recognition of his outstanding contributions to "The development of Nuclear Magnetic Resonance"

Prof.Dr. Hans Paulsen, Universität Hamburg, Hamburg, Germany
In recognition of his outstanding contributions to "Carbohydrate Chemistry"

Prof.Dr. h.c. mult. Fred McLafferty, Cornell University, Ithaca, USA
In recognition of his outstanding contributions to "Mass Spectrometry"

Prof.Dr. Ivano Bertini, University of Florence, Florence, Italy
In recognition of his outstanding contributions to "NMR of paramagnetic molecules and to furthering European cooperation in biomolecular NMR"

http://www.nmr.chem.uu.nl/bijvoet_brochure/brochure.html#nmr

Legends to the Figures

Figure 1. H-1 NMR images measured from rat brain with a surface coil. From left to right the images are increasingly weighted for T2 relaxation, by repetitive refocusing of the spin-echo signal. The upper series was measured using adiabatic refocusing pulses while the lower series was collected with classical pulses. Note the strong ghosting in the latter case, that makes meaningful T2 evaluation impossible.

Figure 2. H-1 Spectroscopic Imaging (SI) of lactate distribution in rat brain. The images in (a) and (b) are taken from the same rat which has a C6 glioma tumour. In (a) a standard SI sequence was used to measure the distribution of protonated metabolites in the image plane. The iso-intensity contour lines show that the signal is dominated by lipids from outside the brain while intra cerebral metabolites are barely visible. In (b) the SI signal was filtered to only have the signal from the methyl protons of lactate remain. This was done with a gradient-enhanced multiple-quantum filtering technique. As a result, the lipid signal is completely suppressed, and lactate becomes visible and is confined to the tumour region.

Figure 3. H-1 NMR imaging and spectroscopy on a rat with an ischemic stroke in the right hemisphere of the brain. The contrast in the image is dominated by regional differences in the diffusion coefficient of tissue water: water diffusion is slowed down by cell swelling in the ischemic part, explaining the higher signal in most of the right hemisphere. H-1 Spectroscopic Imaging (SI) was next done to measure the distribution of lactate, a hallmark of brain ischemia. The lactate-edited SI data set consisted of 16x16 volume elements, as indicated by the grid overlayed on the image. Typical spectra (only containing a peak from the lactate methyl group at 1.3 ppm) from a voxel in the border region of the infarct and one from the center are depicted to the right. The spectrum from the border voxel has two satellites because it has been partly formed from 1-13C-glucose that was infused intravenously.

sensitized for diffusion effects. Figure 3 was measured from the brain of a rat, circa 10 minutes after a stroke was induced in the right hemisphere. The higher signal in the region to the right is the result of the slower rate of water diffusion. The diffusion technique enables the visualization of the ischemic territory within minutes after the infarct and has therefore rapidly become an important tool in the diagnosis and treatment of stroke. In the same experiment we have measured the distribution of lactate, using the above lactate editing technique. Two spectra that were taken from a large data set covering the entire brain, are plotted: one from the center of the infarct (showing a single peak from the lactate methyl group) and the other from the border region (displaying a strong center peak, with two smaller satellite peaks). In this experiment, the rat was infused with 13C-enriched glucose from the onset of stroke induction. The data indicate that lactate in the infarct core was formed from endogenous, 13C-glucose and that lactate in the periphery of the infarct is partly formed from 13C-glucose. Such labelling studies give unique information on lactate turnover and the spatial distribution thereof, and play an increasing role in our research on cerebrovascular disease models.
Composition of the Bijvoet Center

The Bijvoet Center comprises the following research groups:

Bio-organic chemistry
- Bio-organic chemistry of Glycoconjugates and carbohydrates. (Prof.Dr. J.F.G. Vliegenthart and Prof.Dr. J.P. Kamerling)
- Biocatalysis by lipoygenase and related enzymes. (Prof.Dr. J.F.G. Vliegenthart and Prof.Dr. G.A. Veldink)

NMR spectroscopy
- NMR in Molecular Biology and NMR Methodology. (Prof.Dr. R. Kaptein, and Prof.Dr. R. Boelens)

X-ray crystallography
- 3-D structures of small, intermediate and macromolecules. (Prof.Dr. J. Kroon, dr. P. Gros, and dr. B.P. van Eijck)

Mass spectrometry
- Mass spectrometry of biomolecules. (Prof.Dr. J. Haverkamp, dr. W. Heerma and dr. J. Thomas-Oates)

Biochemistry of membranes
- Structure-function relationships in biological and model membrane systems. (Prof.Dr. B. de Kruijff and dr. A.L. Spek)

In-vivo NMR spectroscopy (working group)
- In-vivo NMR spectroscopy and imaging. (Dr. K. Nicolay)

The Bijvoet Center is the central part of a research institute and of a KNAW accredited graduate school. Next to the above mentioned groups from the Bijvoet Center, the department of Organic Chemistry of Leiden University (Prof.Dr. J.H. van Boom), the department of Medical Chemistry of the Free University at Amsterdam (Prof.Dr. D.H. van den Eijnden), and the departments of Physiological Chemistry (Prof.Dr. P.C. van der Vliet) and Medicinal Chemistry (Prof.Dr. R.M.J. Liskamp) of Utrecht University are participants in the school.

Three national centers are embedded in the Bijvoet Center:

The 750 MHz NMR Laboratory
(Prof.Dr. R. Kaptein and dr. M. Czisch)

The in-vivo NMR Laboratory
(Dr. K. Nicolay)

The SON/UU X-ray crystallographic participation project
(Prof.Dr. J. Kroon and dr. A.L. Spek)

The permanent staff of the Bijvoet Center comprises 4 full professors and one part-time professor (ordinary chairs) next to three professors at personal chairs. The scientific staff comprises furthermore 13 senior scientists, of which 3 are directly involved with the national centers, and 3 junior scientists. 59 Temporary scientific staff complement the permanent staff. The technical support staff comprises 19 persons, of which 3 are directly involved with the national centers. 14 Temporary staff positions are funded by the university, and 17 temporary staff members are funded by the SON, while 9 post-doc positions are funded by the European Union. The other temporary staff members are funded by IOI's, industry, STW, other NWO foundations, and other sources.

The Bijvoet Center is managed by:

Prof.Dr. J.F.G. Vliegenthart, Director of Research
Dr. M.J.A. de Bie, Managing Director

The general board of the Bijvoet Center consists of:

Prof.Dr. R.A. van Santen (Chairman of the Netherlands Foundation for Chemical Research)
Dr. B.E. van Yucht Tijsen (Member of the Executive Board of Utrecht University)
Prof.Dr. G. van Koten (Dean of the Faculty of Chemistry of Utrecht University)

Personal recognitions (selected)

Dr. B.P. van Eijck
- Visiting Professor, Rudjer Boskovic Institute, Zagreb, May 1993
- Visiting professor University of Milan, November 1996

Prof.Dr. J.P. Kamerling
- Roy L. Whistler Award
- Bronze Medal of the Faculty of Sciences of the Lajos Kossuth University, Hungary
Foreword

Prof. Dr. R. Kaptein
Member of the Royal Netherlands Academy of Arts and Sciences

Dr. J.A. Killian
Doctor Honoris Causa, University of Umeå

Prof. Dr. B. de Kruijff
Member of the Royal Netherlands Academy of Arts and Sciences

Prof. Dr. G.A. Veldink
Visiting professor at Würzburg University (Germany)

Prof. Dr. J.F.G. Vliegenthart
Foreign Member of the Royal Swedish Academy of Sciences
Honorary Member of the American Society for Biochemistry/Molecular Biology
Member of the Royal Netherlands Academy of Arts and Sciences

Doctor Honoris Causa, L. Kossuth University, Hungary
Doctor Honoris Causa, Université des Sciences et Techniques de Lille, France
Doctor Honoris Causa, University of Stockholm, Sweden
Louis Pasteur Medal of the Université des Sciences et Techniques de Lille, France
Claude Hudson Award in Carbohydrate Chemistry from the ACS

Title:
A ten year "Quest for Structures"

Bijvoet Center for Biomolecular Research
1988 - 1998