A quest for structure and function

Visions behind the Bijvoet Center

20 years

BIJVOET CENTER FOR BIOMOLECULAR RESEARCH

Welcome by the scientific director

The Bijvoet Center for Biomolecular Research (Research Institute and Graduate School) celebrates its 20th anniversary in 2008.
This is, I believe, testimony to the fact that, building on the foundations led by the pioneering scientific director Hans Vliegenthart and the preceding Scientific Director Rob Kaptein, we have established an institute of lasting value. As the Bijvoet Center we have been able to preserve quality in the changing scientific climate. The Center is adaptable to new developments in science and scientific-funding. In comparison to other scientific institutions, 20 years perhaps seems not that old, but in the current age with ever growing demands for administrative restructuring of universities, their faculties and departments, and the seemingly constant introduction of new fashionable names, an institute that celebrates its 20th birthday is quite exceptional.

The Bijvoet Center in its first 20 years has established itself as a stronghold for biomolecular research with a special emphasis on structural biology. The initial strengths of the Bijvoet Center were in the main its developments in protein NMR, X-ray structure elucidation and carbohydrate biochemistry. However, significant extensions and changes in direction have been part of the Bijvoet Center's development over the years. Small molecule X-ray crystallography has evolved, and been extended, to include serious efforts in protein crystallography with now first-rate international reputation. Although the stronghold in carbohydrate chemistry has been reduced in recent years, related efforts in the Medicinal Chemistry group have surfaced to balance this. The Mass spectrometry department has seen a huge expansion in infrastructure and expertise and is now a world-leading research group, both in proteomics as well as in macromolecular mass spectrometry. An entirely new research line that has been incorporated in recent years is that of protein folding, both *in vivo* and *in vitro* as performed in the Cellular Protein Chemistry group. There is strong cross-fertilisation between this group and the structure technology oriented groups in protein NMR, crystallography and mass spectrometry. The Faculty of Science has always had strong representation in biomembrane related research, both within the Bijvoet Center and the Center for Lipid and Membrane Enzymology. The recent merger of these two institutes has been a natural process and has further extended the cross-fertilisation of research in all groups of these two centres. With these changes, the Bijvoet Center has now emerged from its teenage years and has matured into an important centre for molecular structural biology in Europe.
All this has only been possible by continuous support from our university, the science faculty, and Dutch funding agencies. Most of the plaudits and thanks however, should go to the young researchers from the Netherlands and abroad, who find the Bijvoet Center a fertile ground for the initial part of their careers either as undergraduate and graduate students or as postdoctoral fellows.

In the years ahead we will have to anticipate the ever expanding possibilities of new technologies and the new opportunities they provide to study the detailed biology of cells at the molecular level. We will increasingly understand the intricate play of the biomolecules present in the cell which is required for biological function. This expansion of our knowledge of protein networks and protein-small molecule interactions allow for new areas such as molecular system biology and chemical biology to come within reach. Unique for the Bijvoet Center, with only a few comparable initiatives in Europe, is the powerful combination of the structural biology expert facilities in crystallography, microscopy, mass spectrometry and NMR, next to the functional molecular biology expertise groups. Holistically, we are optimally geared-up for the comprehensive analysis of cellular biology at the molecular level starting from molecular structures, via protein-networks and protein-protein/protein-DNA and protein/membrane interactions, through to sub-cellular localisation and cellular function.

Concomitantly, we seek to constantly rejuvenate our Bijvoet School activities in order to further enhance the education of our talented students and introduce further tools to increase cross-fertilisation between the Bijvoet Center's embedded groups. Naturally we also ensure that our students not only experience the best that locally based research has to offer, but provide opportunities for international exposure. We have expanded our core base of quality-teaching and research by inviting internationally renowned researchers to Utrecht and we also encourage students to visit international research groups and conferences. Utrecht University's educational structure is dynamic and the position of institutes such as the Bijvoet School is, therefore, often subject to change. The Bijvoet School has shown a ready ability to adapt to these requirements for change by holding to the single premise of maintaining excellent standards of research and education throughout these shifts.

In this book, published to celebrate our anniversary, you will find interviews with some of the current and former Bijvoet Center participants. We hope this will provide you with a flavour of our activities and will give you a snapshot of the Bijvoet Center as experienced by the people responsible for the research and education. Many of the interviewed researchers have addressed questions on their personal motivation, and their scientific dreams and ambitions. I sincerely hope you enjoy reading it at the time of our anniversary or in the years ahead, to see how we have lived up to our dreams.

Sincerely yours,

Albert J. R. Heck | Scientific Director Bijvoet Center

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Illustrations used in this book: Chemistry Research Day 19 April 2007 'Imaging and Imagination'

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interviews

Group leaders

Piet Gros (45) is Head of the Crystal and Structural Chemistry section at the Bijvoet Center. Their research involves unravelling molecular structures of a wide range of molecules, from small pharmaceuticals and organic compounds to large protein complexes. These structures show the chemistry that occurs when, for example, proteins work together in immune response and wound healing.

Crystallography: a big *Aha-Erlebnis*

‘A crystal structure gives us very valuable insights. It gives an overall 3D picture of the molecules we are interested in. Essentially, a protein molecule is a very mechanical, architectural thing in most cases. The function of a protein depends on its shape. Knowing the shape, if you will the structure, provides a framework for understanding the protein’s biochemical data. Once you are able to see the structure it is often an ‘*Aha-Erlebnis*’, one is able to put the biochemical insights together in one big picture and then start to understand how the molecule works.

Protein crystallography involves many disciplines. Initially, it requires a lot of molecular biology and biochemistry preparation (making proteins and testing their functions) and then you arrive at the unusual step: growing a crystal. The next steps are all physics and mathematics. We use X-rays to shine on the crystal and collect the diffracted X-ray beams. From the X-ray diffraction data we can then compute the information back to our model. All this work results into a 3D map of the protein molecule that demonstrates how the thousands of atoms make up the complete architecture of the molecule. Seeing the structure appearing is always a great moment, a breakthrough in the research.’

The complement system

‘Most of the projects in protein crystallography are medically related topics, such as our research on the complement system. The complement system is the part of the immune system in our blood that protects against invading bacteria. The system consists of many proteins working together, which evolved long before antibodies. Antibodies form an adaptive immune system, whereas the complement system is a primordial, innate immunity system that recognises foreign cells. Flies, for example, do not have antibodies, but they have similar proteins to the complement system in order to defend themselves.

The complement system was discovered in 1902 and consists of thirty to forty proteins that are able to recognise and kill bacteria. These proteins are generally very large molecules. We want to know how they work together to discriminate between foreign cells and our own cells, and how this system is activated.

The process starts with recognition, which is basically the binding of molecules. The next step is called “activation”. Understanding the molecular processes of activation is a particularly structural question. In many cases activation, and regulation, means there is a complex formation of different proteins and these proteins actually change shape as they become activated. It is like a dormant system, which must be activated suddenly when there is a bacterium in your blood.

We began this project five years ago and have managed to unravel the heart of this system. The complement system is a proteolytic cascade of proteins. Most of the proteins were discovered in the 1970s when proteins did not get nice fancy names, so they have simple names like C1, C2 all the way up to C9. Protein C3 is at the core of the system. This is the one that has to be attached to the bacterium to give the signal that it is foreign and has to be removed. C3 is the dormant state of the molecule. It becomes activated when it is cleaved into two parts, C3a

Structural characterization of proteins by X-ray

Harma Brondijk
Crystal and Structural Chemistry

Crystals of the extracelullar domain of a receptor protein involved in the prevention of auto-immune reactions.

and C3b. The smaller part, C3a, is important for invoking inflammatory responses. The bigger part, C3b, is crucial to the complement system. It is the C3b protein that will actually attach to the bacterium. If many C3b molecules attach to a particle, it will be cleared by phagocytosis. C3b provides a very strong biological signal.
The fundamental question now is what makes the difference between the dormant state C3 and the activated form C3b. C3 binds to almost nothing but when it becomes C3b it binds to the surface and it binds series of different proteins.
We succeeded in resolving the crystal structure of the dormant C3 and the activated C3b; and thus far this is our most prominent result. After C3b attaches, basically there are three possible responses. The stimulation of B-cells and phagocytosis are the cellular responses and in addition to this there is a protein response. This is called the terminal pathway, the proteins response is to make a hole in the bacterium that then leads to lysis. Last summer we published our results on this structure that essentially shows how the hole is being made.'

Medical interest

'Over activation of the complement system leads to tissue damage. This happens in transplanted organs for example, which leads to an excessive complement reaction. The complement system is also involved in autoimmune problems and the defence against MRSA. There is clearly a great deal of medical interest in this field. Our research forms a starting point for new drug design.
Another big success is our Haemostasis project. Haemostasis is the arrest of bleeding at the site of an injured blood vessel. Also in this case as a starting point we began by studying the regulatory process. An important protein in this process is the Von Willebrand factor which is a huge protein. It binds both a receptor on blood platelets and collagen that is exposed when the vessel wall is damaged. This way it forms a bridge between platelets and the wound, leading to platelets forming a plug to fill the hole in the vessel. Normally the dormant proteins and the platelets swim around in our blood but do not bind. We showed how the Von Willebrand factor binds to its platelet receptor and indicated how shear stress may play a crucial role in activating the Von Willebrand factor. Mutations in either the Von Willebrand factor or the platelet receptor disturb the balance between the dormant and active state, which may cause bleeding diseases. Therefore, understanding the regulation and activation of this protein is crucial for finding a cure for this type of condition.'

Future challenges

'In our field we face big challenges in the future. Proteins do not work alone, they form networks. We want to use crystallography to see how these proteins change their shape during these interactions. How do the individual interactions form a biological response? What is the chemistry that makes biology happen? Our recent work on the complement system shows that we can address these issues, our results gave a breakthrough in the understanding of the C3 molecule central to this. Now we need to understand better the initiation process and

the regulation processes that protect our own cells from harm. It is the interplay between thirty to forty different proteins that is responsible for a balanced biological response.
The same type of questions holds for many different regulatory pathways and molecular machineries. Understanding the biological complexity at a chemical level requires detailed insights into the various interactions and the molecular effects.
In the past century, I consider the discovery of DNA to be the most important scientific breakthrough. In 1900, we did not even know it was a molecule, now we know everything. The next century will be about the molecular working of the brain: what is memory? How do we store information? In the end it must be chemistry. Our thoughts are made out of molecules. I am not a neurobiologist, yet I would predict that at the end of this century we will have a basic molecular understanding of memory and thinking. There is a lot of progress in neurobiology, in understanding the wiring and chemistry of the brain. We are already down to the cellular level; the next step is the molecular level. I also think that the complement system, that we study, is involved in the shaping of the brain because the complement system is so very good at getting rid of cells. This process is exactly what happens after a child is born. Initially, there is a lot of wiring between the different parts of the child's brain which later needs to be pruned. Cells have to be cleared again, and it is likely that the complement system plays a major role in that process.'

interviews

Group leaders

Marc Baldus is specialised in Solid-state NMR. He has worked for eight years at the Max Planck Institute for Biophysical Chemistry in Göttingen. He will become Professor of the NMR Spectroscopy section at the Bijvoet Center this year

The right mix of techniques

‘I have been a group leader here at Göttingen for eight years now, before that I had already spent some time in the Netherlands. I worked for a while as a PhD student in Nijmegen and I have also been employed as assistant professor at Leiden University. During my study in physics, I became especially interested in biophysics. I have a background in NMR methods and I am interested in cellular processes such as the folding of proteins and membranes. Most NMR researchers study protein folding and complex structures in solution. I, however, wanted to look at these structures in a cellular context, and for that you need to do solid state NMR. That way you can look at the cellular membranes directly or, for example, study the function of membrane ion channel activation or inactivation. My work is closely linked to medicinal chemistry and chemical biology, areas that Rob Liskamp and Antoinette Killian work in.

If you want to learn something about membranes you really need a mix of techniques, and, importantly, the right application of these different methods. Therefore, I believe it was visionary to organise institutes like the Bijvoet twenty years ago. They combine the different disciplines in one learning institute and have room for basic fundamental science but also for applied research.’

Quantum mechanics

‘I remember a particular striking moment from the time I was studying quantum mechanics to predict how the atoms are structured in biomolecules. I looked at a small molecule and conducted a solid-state NMR spectroscopy to determine distances between atoms. At that time that was very difficult to achieve. Utilising quantum mechanics, I succeeded in designing an experiment that could be used to determine the structure of the molecule. I found this to be very exciting as it demonstrated that quantum mechanics is actually useful!

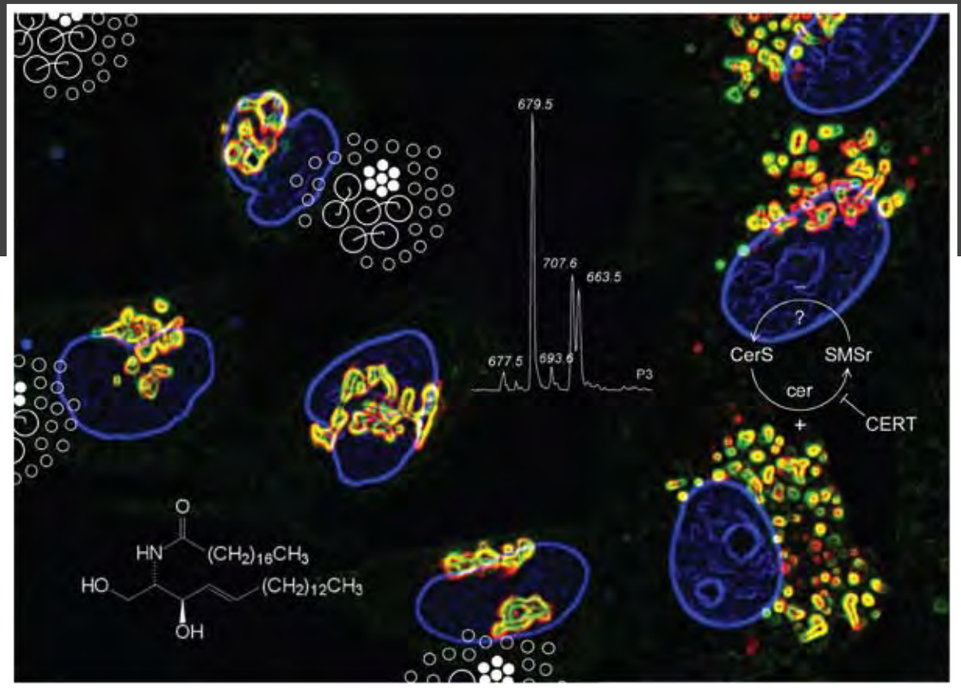
At the moment, I am studying red blood cells. In order to collect the blood for these experiments we had to enter the clinic, with real live patients providing the test tubes full of their blood. Entering a clinic really is a different world for a physicist. People do not always understand the way we do our experiments. I once studied a toxin secreted by scorpions. Of course I did not use real scorpions, I used a toxin produced by bacteria. When I published a paper about how this toxin binds to ion channels in membranes, I received phone calls from people actually wanting to see the scorpions on my desk, which luckily was not the case!

NMR combined with other tools will make it possible to study complex molecular systems. Teaming up with other biophysical methods will increase the size of systems that we can handle. The role of computational biology and molecular dynamics will also be very important in this process. The prediction of molecular structure by computational biology is already speeding up our work dramatically. In ten years it will be normal to combine computer power and NMR, or other methods, in structural biology. In twenty years, I think we will really be able to understand why something is not working in the human body in cases like Parkinson’s disease. That is the ultimate goal for me, to make a contribution to something that improves people’s health.’

Sensing lipids with a bad reputation

Ana Vacaru and Fikadu Geta Tafesse
Membrane Enzymology

We discovered a membrane protein that functions as a sensor for ceramide, a lipid with a bad reputation that can drive cells to commit suicide if they contain too much of it. Removal of this sensor causes a rise in cellular ceramide levels and a collapse of the Golgi complex, a ribbon like structure (stained red and green) that is normally juxtaposed to the cell nucleus (stained blue; note that the three cells on the right lack the sensor). Our current work focuses on understanding how this lipid sensor works and how it influences cell organization and fate.



Fruit fly experts

'I am very much looking forward to working at the Bijvoet Center. Each of the groups in the Bijvoet Center is a leading group in their field. Together these groups create a unique research opportunity. It is becoming more and more important to work together across disciplines and not use one single method only. Of course, this research requires a great deal of funding but that alone is not enough. It is extremely important to be able to talk to people from different fields. The contact between people will promote new discoveries. That is what I really like about the Bijvoet Center, this spirit of a teaching school. You have to know a little about biology, but also about other fields, other methods. If I look at my own career, I started out studying superconducting materials, which is not even close to where I am now.
It is important for students nowadays to learn to look at a problem from different angles. Of course, in science you will always have to look in-depth at one specific problem, but you need to use different approaches in order to gain a more complete understanding.
Communication can often be a problem. Scientists from different fields often use nomenclature that excludes others, for instance fruit fly experts and laser experts might find it difficult to understand each other. People are often easily bedazzled by this specific language. There are so many different acronyms used, so many genes, so many protein families. A biologist will of course see this as standard knowledge, but we physicists can easily get lost! To solve this, you need to take a step back. Then you are able to communicate with and better understand colleagues. And then maybe somebody will say: "That change in molecular structure is the same thing I see when I look at chaperone activity" and the meaning will become clear to both.
At my institute in Göttingen we solved this communication problem by organizing a PhD seminar series, which all groups attend. Each PhD student is expected to present his or her work, but has a host from a completely different field. For instance, I would be partnered with a researcher involved in fruit flies, and they would present the talk to me first. In this way you can ensure that people outside of your own field are able to understand your message and you in turn will receive new ideas. Science will always be about details. But nowadays you have to look around; you cannot keep playing in your own sandpit any more.'

interviews

Group leaders

Ineke Braakman

is Head of the Cellular Protein Chemistry section at the Bijvoet Center. She investigates protein folding using different model proteins, *in vitro* and *in vivo*.

Understanding protein folding

‘The four model proteins which we use are the influenza virus hemagglutinin, HIV Envelope glycoprotein, the LDL receptor, and CFTR. The latter is involved in the transport of chloride ions across the cell’s surface membrane. When it does not function properly it causes cystic fibrosis (CF).

We carry out our folding assays under physiological conditions. We conduct *in vivo* studies using intact cells, but also are engaged in *in vitro* studies, where we take cell membranes and synthesise the protein in the test tube. We aim to bridge the gap between folding studies in the intact cell and the true *in vitro* folding studies of a purified protein. With the various assays we can distinguish different stages of protein folding: *in vitro* at the atomic level (by NMR, for instance, in collaboration with Rolf Boelens), and *in vivo*, at the functional level, using antigenic epitopes and limited proteolysis.

It takes many steps to complete one experiment including, of course, the use of many controls. The experiments are therefore exhaustive and very hard work. Some of my chemistry colleagues have remarked that they chose not to specialise in biochemistry because of this, but by doing so they miss the thrill of a difficult experiment coming together and giving a beautiful result. My sense of accomplishment is much greater when more work and skill went into accomplishing it.

Fortunately, not all the steps in an experiment are a black box until you see the result. The trained lab members are in control of their actions. If I ask people at the end of an experiment: ”Before you see the results, do you think it has worked or not?”, they usually reply: “Yes, it did,” or “Well, there was this one step where I knew I lost concentration or where I may have switched something”. With these experiments of some hundred steps, everybody is allowed to make mistakes. Where work is being done, mistakes are made, that is normal. I mistrust anyone who never reports failures. It only starts to become worrying when the same mistake is made two or three times.’

Life and death

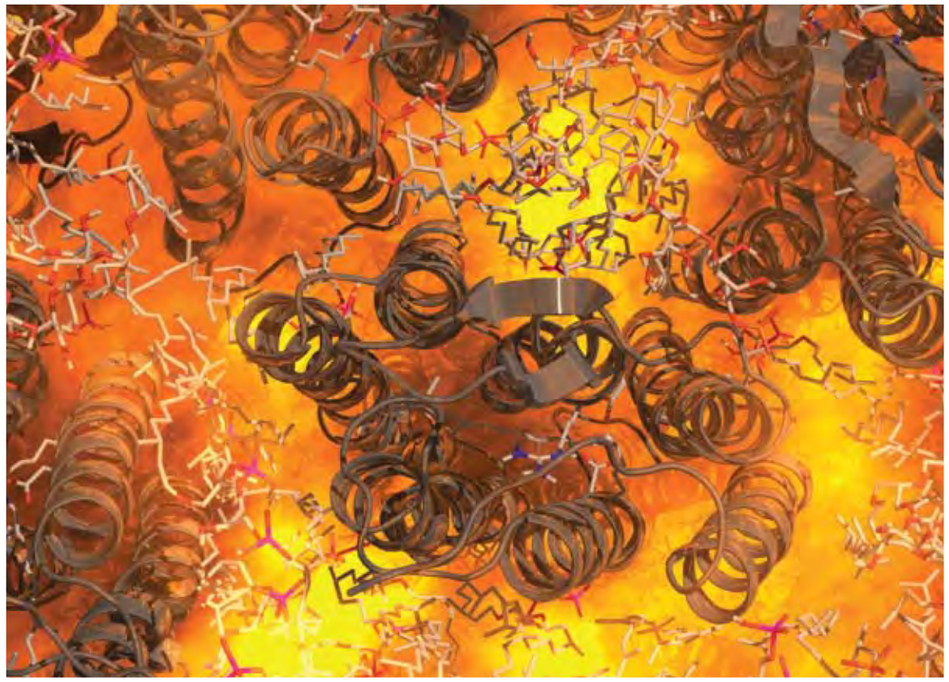
‘The CFTR work is aligned relatively closely with its application. I am a member of an international consortium of nine scientists working on the folding and trafficking of the CFTR protein. Some of the research is financed by the American Cystic Fibrosis Foundation. The foundation itself is very hands on and has a habit of looking over our shoulders at the work we do, whilst we do it. Many of their personnel have family members with CF and so this approach is understandable. Imagine your sister or your child has CF; what would you want? They stimulate all of us to work faster and to stay focused. It is clear that these nine scientists and their groups will accomplish more working together than as individuals. This in turn means that we all must share data. Some of the team are more open than others, but it generally works out.

The majority of patients have the disease because of a single mutation. This leads to a protein that is temperature sensitive for folding. In CF it is 7 degrees that make the difference between life and death. If we could live at 30 degrees Celsius these people would not

Salt, saltier, saltiest’

Tsjerk Wassenaar
NMR Spectroscopy

Bacteriohodopsin is a light-harvesting protein found in archaea which thrive best at salt concentrations of 3.3M. This image shows a volumetric density rendering of the distribution of sodium around bacteriorhdopsin and associated lipids during a 25ns molecular dynamics simulation.



be patients. The implication is that the mutant CFTR protein is not far from being properly folded. At the moment, all hope for therapy is on drugs that can help the protein to fold and that stabilise it. In the cell this works, but to bring a compound from cell to patient takes a long time and many compounds get lost or stranded somewhere along the way.
We contribute by finding out how certain promising compounds influence CFTR folding. In the near future, I expect to see how our results have helped select compounds that can actually work as drugs. In the end, as a scientist it is not curing a disease that I am interested in, I want to understand how proteins fold, the real fundamental science behind it. I would be very unsatisfied if we were to develop a successful treatment for CF without understanding what is really happening. This in particular because it would not give us clues for improvement.'

Eureka moment

'The most striking moment in my career was not a singular experiment, but rather the coincidence of several things coming together at the right time in an area where I already had an interest. I still consider it somewhat of a miracle how I ended up in the folding field.
I started out doing pharmacokinetics during my PhD, where I learned that I really wanted to go for explanations, for mechanisms and to really understand our observations. This meant that I had to dive into the cell, therefore I decided to do a post-doctorate in cell biology.
I already had a broad interest in this. As a PHD student I had gotten into the habit of scanning titles in Current Contents for relevant articles and requested papers about sharks, protein folding, anything really that could be even loosely related to my interest.
For my postdoc I ended up in Ari Helenius' lab at Yale University. From all the literature I had collected during my PhD work, I took a number of reviews on protein folding with me, even though I was due to work on viruses. When I arrived at Yale, a technician had done an assay that showed some unexpected results, this turned out to be the start of a folding assay. I am not superstitious at all, but I still feel that this was my Eureka moment. That I had collected, read and travelled with reviews on protein folding just at a moment when folding studies in the cell became possible and then presented themselves by coincidence as my future project.
Students often talk about career planning, but I am not sure you can or should plan every step for your future. Perhaps at some point you need to do some planning, but the best career move is to do what you enjoy most. The things we do well are usually the things we love.
If you were to ask my advice for any young scientist it would be: do not fear anything, fear is a bad adviser. Just go for it, go for a postdoc in a lab that has high ambitions for instance. What practical advice I can give is to only go to places where your supervisor will be a good mentor. This may not be as obvious as it appears. Your academic career is your own responsibility, but a good mentor during your PhD and postdoc periods will teach and guide you, and should be generous in giving you credit for your work. How do you know who will be a good mentor? A difficult question, but be sure to talk to the people in the lab before it is too late.'

Future plans

'I hope that in a few years we can connect our *in vitro* and *in vivo* studies. For instance, one of our other model proteins, the LDL receptor, contains seven repeats of approximately forty amino acids that are very similar in sequence and structure. Despite this similarity, we found to our surprise that they all show very different folding behaviour *in vivo*. We do not know yet whether this will be the same *in vitro*. If this is the case then we can study their folding and start to understand why. If *in vitro* they behave in the same manner, we can conclude that it is the cell that determined the difference. To discover the mechanism behind this is likely to take more than five years experimenting and work.

In 2008, I am taking a sabbatical. I still need to make detailed plans, but the invitations are there. I am welcome in many places but I am definitely going to visit Phil Thomas in Dallas and Art Johnson at Texas A&M University. Over the past thirty years, Art has developed a technique to incorporate into any particular site in a protein an amino acid modified with a fluorescent probe or an activatable cross-linker. Art Johnson is really marvellous at this technique. I am not planning on setting up the same technique here, but I do want to collaborate with them. A related collaborative project we are starting is with Sheena Radford from Leeds University. When we incorporate fluorescent probes into our LDL receptor repeats using Art's technique, Sheena can study folding of single molecules of these repeats.'

interviews

Group leaders

Gerrit van Meer (54) heads the Membrane Enzymology section at the Bijvoet Center. He did his PhD with L.L.M. van Deenen in Utrecht in 1981 and returned to the same department as Professor of Biochemistry in 2001. In between he spent five years at EMBL Heidelberg, and fifteen years in total with the cell biology departments of the academic hospitals UMC Utrecht and AMC Amsterdam.

The accidental lipid guy

‘I have worked in the lipid field for thirty years now, but actually I got into lipids by accident. When I started as a student I initially chose what I really liked: molecular cell biology. I did experiments for some time, but it turned out there were many problems within the cell biology department. I had decided that my last topic should be at a top laboratory because I wanted to go on and study for a PhD. The lipid department on the sixth floor was already a famous international department directed by Van Deenen, and because of this, that is where I went for the last part of my practical work. Therefore, as a result of the coincidence of internal and political problems in my original department, I became a lipid guy. That is the way things work sometimes, but for me it was a positive choice. I chose for the best, internationally renowned department available to me at the time and I have never since had a reason to leave the lipid field.’

Lipid transporter

‘I have always been fascinated by the question why eukaryotic cells synthesise such a variety of membrane lipids. They make more than a thousand different lipids. My goal is to unravel the network of how these lipids behave in cells and how cells use these lipids for their physiological functions.

We have three main approaches. We study how and where the various lipids are synthesised and degraded, and how the respective enzymes are regulated. We also look at the transportation of lipids within and between cellular membranes. Our third project is the function of lipids in the sorting, transport and activity of membrane proteins.

So far, our research has brought us to the discovery of lipid rafts as platforms of protein sorting and signalling, to the role of multidrug transporters in moving lipids across membrane bilayers, and to the crucial role of specific lipids in pigmentation.

Cells have all sorts of membranes, not just the cell membrane, but all organelles are surrounded by membranes. The lipids in these membranes are arranged in asymmetrical double layers. This asymmetry between the inner and outer leaflet of a membrane was discovered in Cambridge and here in Utrecht in 1972 and 1973.

The question of how cells are able to arrange this asymmetry is answered by their use of flippases. These are enzymes in the membrane that pump phospholipid molecules between the two leaflets that compose a cell’s membrane. Part of our group, under Joost Holthuis, works on this flippase system.

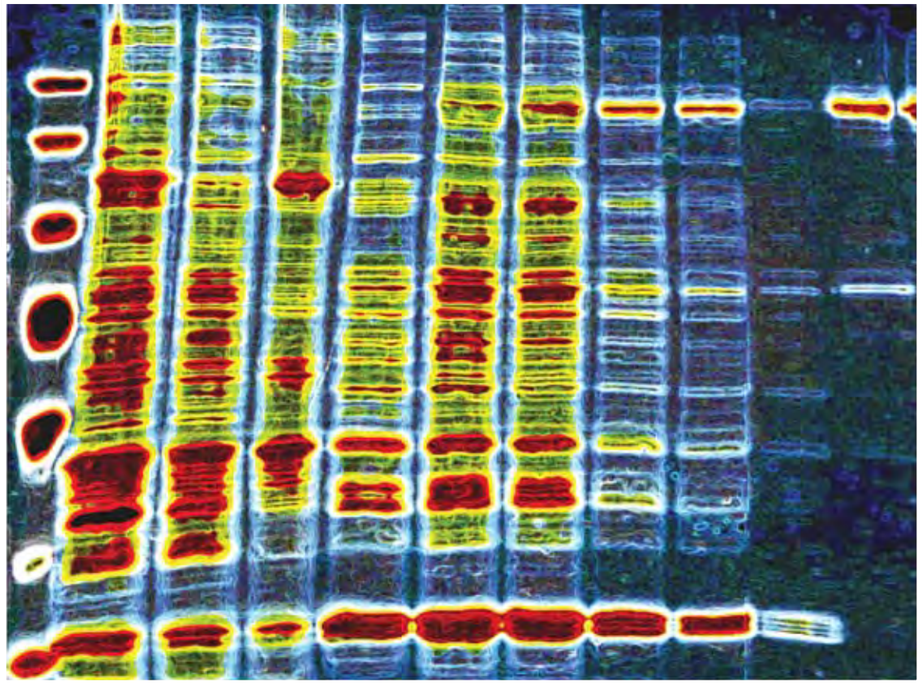
These flippases are extremely interesting. Before I came to the Bijvoet Center, I worked for fifteen years as a medical scientist at the UMC and AMC hospitals. Our major discovery was that lipids can be moved across a lipid bilayer by a pump that was already known by the name of multidrug resistance protein no.1.

These multidrug transporters are a problem in cancer. When cancer cells are treated with medicine, at some stage they can become resistant: they start to pump out the drugs again. They only remove drugs that are slightly lipid soluble. What we found out is that the protein responsible for this, is actually a lipid exporter. Suddenly one of the most famous proteins turns out to be the pump that we were searching for! This was one of the first examples of

The art of membrane protein purification

Guillaume Lenoir
Membrane Enzymology

I work on a calcium pump-related class of lipid transporters implicated in diabetes, obesity and a severe human liver disease. These so-called flippases consist of multiple subunits but nobody knows how they function. To study their inner workings, I purify these activities from bath-tubs full of yeast. The challenge is to prevent the flippase from falling apart during the purification process. The image shows a silver-stained protein gel loaded with fractions obtained during the purification process, from crude extract (ultra-left) to the purified flippase complex (ultra-right).



this class of proteins that is a lipid exporter. This MDR paper was a very famous paper; I think it is my most cited paper.'

Pigmentation

'A couple of years ago we touched on pigmentation disease by accident. We were interested in the function of a specific lipid, and Japanese colleagues had made a mutant cell line that could no longer make this lipid. My PhD student Hein Sprong cultured the mutant cells and the original cells from which the mutant was derived.
I can remember his phone call, he said: "I did not know glycolipids were black". That was fantastic! You could actually see black and white cells in the centrifuge tubes. The Japanese never saw this, either because they are chemists and never looked at their cells properly, or because by some accident or variation under their conditions, the difference in colour was not so apparent.
So what makes these cells black? The cells come from a melanoma cell line, skin cancer cells. Melanoma cells make black pigment, yet apparently cells without those specific glycolipids cannot make this pigment. This discovery then brought us to another completely different topic: how are lipids connected to pigment formation? Making pigment is a chemical reaction facilitated by several enzymes. As it turned out, the enzyme is still there, but not in the right location. It should be in the pigment bodies but it got stuck in the Golgi apparatus. This meant that there was a transport defect.
So we began to study how enzymes (proteins) move in cells. This happens in small membrane vesicles. The enzyme has to enter the lipid membrane of a vesicle, and there something goes wrong. In a paper that we are currently writing, we find that the information for this behaviour is determined by a specific part of the protein that sticks into the vesicle.
It has been known for a long time that the inside of vesicles has a special property: it is acidic. This low pH is probably relevant for the movement of these enzymes. What we have observed is that it is these glycolipids that make the inside more acidic. The lipids stimulate the pump that pumps protons into the vesicle. This lipid is there from the earliest stage of eukaryotes, and low pH is present in all living beings. This means we are working on extremely basic principles and I think this is very original work. So in a series of papers, which started in 2001, we have been unravelling how lipid metabolism is linked via defined steps to a very central physiological parameter in living cells.'

Lipid rafts

'The most important discovery in my career I made in Heidelberg in 1988, where I studied how lipids on one side of the epithelium are different from those on the other side. I used fluorescent lipids and found that these analogues behaved exactly as predicted from the natural lipid composition. Therefore I thought: if we understand how these analogues are sorted between the one side and the other side, and how the analogues are preferentially transported, we will understand the mechanism. My idea was that within one membrane these lipids must aggregate spontaneously. In the literature I found some indication

that this could happen. My idea has now became a famous hypothesis: the lipid raft hypothesis. It is still famous, or infamous, as it is still contested, and there are now thousands of papers on this idea. Everybody thinks it must be true in some way, but how? I think that it is a basic principle of how cells work. Lipid rafts act as platforms of protein sorting and signalling.
That then was my big success. One does of course sometimes miss opportunities. For instance, some time ago a friend called me from the US and told me he had been treating a set of membranes with detergents and that the membranes seemed resistant, they did not dissolve. He asked me to look at the lipid composition because he himself is a protein chemist. He sent me the details across and I looked at the lipids. It was a big mess because they were full of soap. At the time I felt that this was not very important and told my friend I could not do the study.

Two years later, a dramatic paper was published that stated that these lipid rafts, which we had proposed, were resistant to detergents. I had missed my chance to find this because I had not been careful enough in my analysis of the samples. The study went on to become a fantastic, basic paper for this whole field. I bet that it has been quoted at least five times more then my original paper!
I was not angry though. In those days we did not have mass spectrometry as we do now. I had too few samples and it would, therefore, have been difficult for me to proceed in any case. But I was so very close! Still, other researchers have also had near misses with some of my findings, you win some, you lose some.

Twenty years from now

'Twenty years from now I think we will understand how membrane proteins recognise different environments in membranes and how they are regulated by lipids. When does a cell decide that an organelle is big enough? How does it sense what is going on? That is highly exciting; there must be sensors of some physical property which decide: "now it's enough, stop making new lipids".
I also believe we will have solved lipid storage diseases, where cells cannot degrade a certain lipid and then start to accumulate these lipids. Good examples are Gaucher's Disease or NPC (Niemann-Pick disease), but there are many others. We know some of these are transport diseases, and some people think that NPC is a cholesterol transport disease. However, we are pretty sure that it is something else and that cholesterol just follows.
We will also have understood the molecular mechanism of flippases and how they are recognised. This means we will understand what regulates storage of fat; what makes a person obese. Obesity is now one of the biggest health problems facing western societies. One theory is that obese persons are just more efficient in extracting calories. In our team we think it is a regulation problem, although it is not just biochemical regulation. It has to do with psychology and hormone regulation.
Another field we will have explored is the involvement of lipids in cancer. People now use mass spectrometry analysis to see patterns of lipids. These lipid patterns become like a fingerprint or a biomarker for a certain type of cancer. These questions and challenges will all hopefully be unravelled within the next twenty years.'

interviews
Group leaders

Alexandre Bonvin (43) is Associate Professor at the NMR Spectroscopy section. Bonvin is specialised in biomolecular modelling and computational structural biology. He was awarded a VICI grant by NWO, the Netherlands Organisation for Scientific Research.

Solving 3D puzzles

‘I studied chemistry in Switzerland but did my PhD here with the NMR group at the Bijvoet Center. I actually never did NMR experiments myself during this time, I worked solely with computers, I think I did my last ‘real’ experiment in Switzerland. We study biomolecular interactions in our group. Sometimes, NMR only gives you an idea about a binding site, but not the full 3D structure. Then, we can try to make a computer model. It is like solving a 3D puzzle with all kinds of molecules, trying to see how they might fit together and where they bind. It is modelling of protein interactions. Our computational models are not only based on NMR data; they can be built from other information sources and used by other scientists too. For instance, I could use the mass spectrometry interactions that Albert Heck studies, and try to make a 3D model based on the known components of a complex. The first step is always to understand how the system works. If you have a general idea about that, you can then try to change the system or introduce a fix when it is broken. It is similar to a lock and a key. Once you know the structure of the lock, you can develop a key that will fit. Applications for this kind of modelling are all biological, for instance in the field of HIV, DNA repair or cancer related proteins.’

Critical experimentalists

‘I was very happy when I was offered a position here at the Bijvoet Center. After my PhD, I did postdocs at Yale in the United States (Piet Gros and Ineke Braakman were both there at the time), and in Switzerland at the ETHZ, but it is here that I have been most productive. So I was very glad to have the opportunity to come back. Here at the Bijvoet Center I am doing computational work in an experimental setting, and that is the ideal way of doing this kind of work. The experimentalists do not believe you easily, they are very critical. I have to sell my work to people who are doing the real experiments and that is the hard part, but it is also the strength of it. Without these critical eyes you will start to believe in your own reality, so you need to check things carefully.
When is a model correct? The model should not be an end; it should just be a starting point for further experiments. With a good model of a complex you can design experiments to test it. You can mutate the residues: for instance if the model is telling me a certain residue is really important, you can check that by changing it experimentally and see what happens. If you can predict what will happen, it is a good proof that your model makes sense. My models do not show the atomic structure, like NMR or crystallography, but they are models that biologists can use to gain understanding and design new experiments. A model is very useful, but it has to be validated. From time to time it turns out your model is wrong, but that is life. When you are developing methods, you learn the most from your mistakes. If things work out easily you do not think about what can go wrong.’

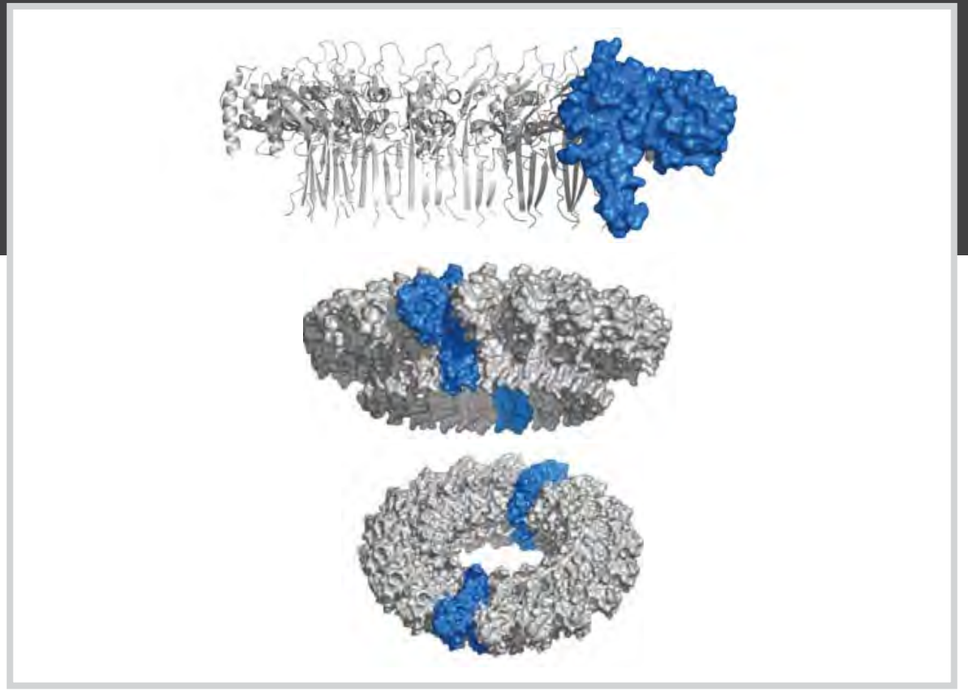
Blind competition

‘We also test our methods in a blind competition on an international level. When a piece of structure of a complex has been solved by a research group somewhere

Return of the MAC

Michael Hadders
Crystal and Structural Chemistry

Model of the Membrane Attack Complex pore based on the structure of a MACPF domain. These pores form holes in pathogenic bacteria, resulting in their death.



in the world, they make it available to a computational community called CAPRI, before they publish it. If you are registered, you can participate. It is obviously very confidential, as these structures are not yet published and usually very hot. All members of CAPRI then have three weeks to predict the 3D structure of the complex. So you have no information and only three weeks to do your best and try to find the solution. It is fun, but also challenging. They do not call it a competition, but it surely is. We are competing with other computational groups in the world. It is a very good way of testing your methods. Since you do not know the system and do not have any information, you cannot make *a priori* choices; you simply have to do your best with what you have. Afterwards, sometimes things look right and wonderful, but equally sometimes things go wrong. Then you try to find out what went wrong. You learn a lot in this way. It is very useful in generating new ideas and new models.
After these three weeks, you get an evaluation. Once the real structure is published you can really go back to your predictions and look at what went right and what went wrong. We get it right about 65% of the time. That puts us on top in the worldwide CAPRI community. Yet we are seen as the outsiders to this group, we are the new kids on the block. Coming from an experimental setting, we try to base our models on real experimental data when available. When we first joined CAPRI, we were seen as arriving out of the blue and moving directly to the top of the pile. Some people said we were cheating, but we are just using better information for our models. It would be nice to be able to predict a structure purely on physics, but if you have experimental information you are just more likely to get the correct answer. It is a different approach that is becoming big business; we now belong to this community and are recognised for what we are doing. So they are now understanding that they have to check their systems the same way we do.'

Unravelling the interactome

'I think in the future computational modelling will be a common tool in the toolbox of biologists. Simulations will meet with experiments. My dream is to be able to make an *in silico* prediction of the human interactome at atomic resolution. The interactome is the map of all the interactions between human proteins. The connection between the dots. We might be able to make a movie out of it. Some people already talk about a Google.cell, just like Google.earth. You combine different techniques on different levels, and can zoom in from a complete human down to an organ, a cell, and then in the cell to the organelles and finally to the bio-molecules, to see what is happening. The Google.cell will thus be able to look at specific interactions and reactions.
We have in the Bijvoet Center many complementary methods such as for example NMR, crystallography, electron microscopy and mass spectrometry. If we are able to put everything together you can really get a complete picture of what is happening at different levels.
That is the dream of every biological scientist, to be able to look inside the cell, and I think that computational modelling will contribute to that, by combining all this information.'

interviews

Group leaders

Stefan Rüdiger (39) joined the Bijvoet Center in 2004 as Assistant Professor in Cellular Protein Chemistry. In 2005 he received a Marie-Curie Excellence Grant of the European Union, the same year he also got a VIDI grant of the Netherlands Organisation for Scientific Research, NWO. In 2006 he became High Potential of Utrecht University.

Unboiling an egg

‘My reasons for coming to the Bijvoet Center were that they had the biophysical equipment that I needed and, due to the presence of Ineke Braakman’s chaperone group, the scientific context was excellent for me to establish a research team that works on protein folding. The protein folding field is broad and interdisciplinary. It requires high-tech machines and cutting edge biophysics, but at the same time you have to be able to relate your *in vitro* test tube results to the situation in the living cell *in vivo*. For the *in vitro* experiments I need high level NMR in particular, and here we have the biggest machine that money can buy at the moment. Rolf Boelens’s NMR group is an excellent team to collaborate with, the same holds true for Ineke’s group for *in vivo* protein folding experiments.

Evidently I did not come to Utrecht because I liked mountain climbing in the Utrechtse Heuvelrug, but because at the Bijvoet Center I have a really good environment for my science!

I have my own scientifically independent group, and I arrived with my own research projects. This was a conditio sine qua non for me. Almost all of my group have their salaries paid from my own individual grants. If you ask important questions and look for the best place to answer them, you are in a good position to attract funding.’

Chaperones

‘I want to understand how proteins fold. We all know that if you boil an egg it tastes different then an unboiled egg. That is because by boiling you unfold the proteins in the egg white. Boiling an egg is easy; anybody can do that. Reversing this process is much more complicated. Starting with an unfolded egg white and managing to get folded active proteins from that.

Most proteins require a specific three dimensional conformation to be functionally active. Folding and unfolding processes in the cell are controlled by a special class of proteins called chaperones. Studying chaperone complexes allows biochemical access to this otherwise mysterious process.

Hsp90 chaperones constitute a particular exciting chaperone class. They are evolutionary conserved, appear in most compartments and most substrates are oncogenes. Hsp90 is the most abundant chaperone in the cytosol, it makes up one percent of the total protein mass, which is quite a lot.

There are roughly twenty thousand proteins in a human cell. From those there are now 130 that are supposed to be substrates of Hsp90. Every protein needs chaperones to promote folding. So why does this Hsp90, the most abundant one of them, take only about 130 proteins for substrate. The fundamental question is how Hsp90 recognises this small subset and what kind of proteins this subset consists of. We already know that around 60 proteins of this subset are kinases, but other substrates for Hsp90 are transcription factors, which are completely different in structure from kinases.’

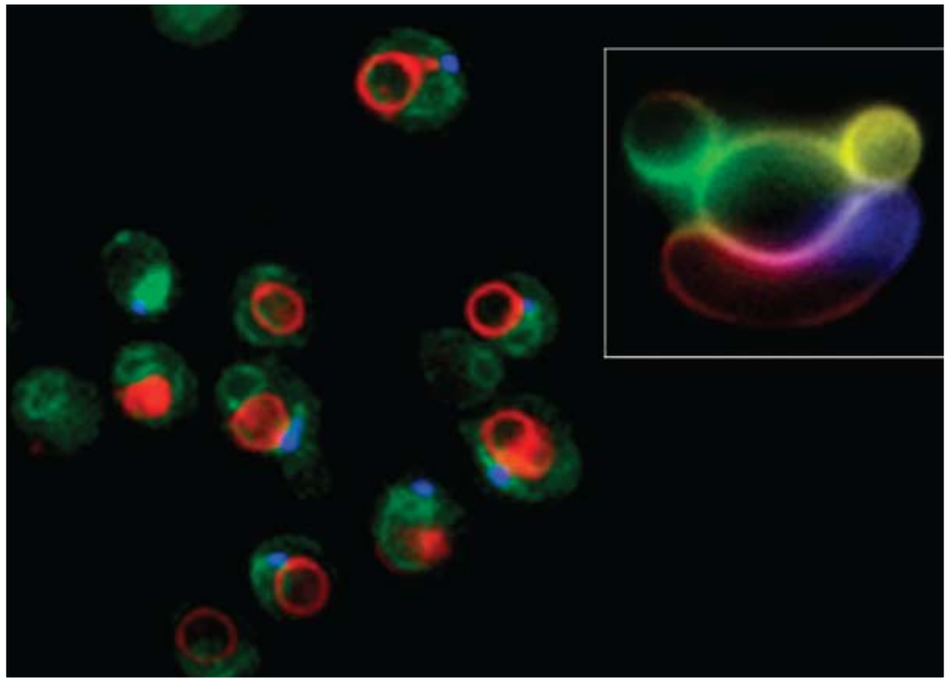
Ideology

‘A lot of the substrates of Hsp90 are interesting for cancer research. For instance, sometimes kinases get

The birth of a peroxisome / Happy Yeast

Adabella van der Zand
Cellular Protein Chemistry

To capture ‘nascent’ peroxisomes, genetically modified cells were studied using fluorescence microscopy. New peroxisomes are shown in blue. To demonstrate where in the cell new peroxisomes are formed, two additional organelles are shown; the endoplasmic reticulum (green) and vacuoles (red).
(inset) ‘Happy Yeast’: Sometimes you get lucky and acquire an image that captures the imagination. Here vacuoles are shown with an uncanny resemblance to a clown-face. In fact these are un-manipulated cells, but for fun ‘false’ colors were used to illustrate the vacuoles.



oncogenetic mutations and become a risk for the body. These kinases need Hsp90, otherwise they are unstable. Due to this, researchers actively look for inhibitors of Hsp90. Some of those inhibitors are now being tested in clinical trials for tumour therapy. Another example of a cancer related substrate is p53. This is an important transcription factor that regulates the cell cycle and functions as a tumour suppressor.

Due to this 'cancer drugging' effect there is a lot of money in the field, but also a lot of ideology. It makes it easier to apply for and gain grants, but it does not necessarily improve the science behind it.

The protein folding field is an extremely interdisciplinary field, which is what makes it so exciting. Colleagues coming from theoretical biophysics, study protein folding *in vitro* or just *in silico*. Others come from genetics and have very different paradigms.

I did some initial experiments in my postdoc time with p53, and I found it is unfolded when it binds to Hsp90. It is hard to imagine that all proteins that are substrate for Hsp90 are totally unfolded until they are activated. That would actually mean it is a bit of a mess in the cell. Perhaps that really is the case, at the moment we just do not know!

A lot of people think it has to be more specific. They assume that most substrate proteins for Hsp90 are already in a late folding stage so they already have some structure. This might be true but there is no real proof supporting that theory. There is only one paper that concluded, in a circumstantial way, that Hsp90 binds to an almost folded late folding stage.

However, this was really the answer the field was already looking for. What you see happening now is that the first review citing this paper has already appeared. Further reviews then just cite this in a sort of rolling stone effect, and before you know it such an opinion spreads without a good experimental basis to support the claim. This is what I referred to as an ideology.'

Open approach

'I prefer an open approach, I think it is important to design experiments with an open mind. We like to take kinases, make a complex with Hsp90 and just look with NMR at what happens to the structure of these kinases. Are they unfolded, or do they have a certain structure? Once we have tested all possibilities we will hopefully know for sure.

From an NMR point of view, structured substrates would be much more useful because you can do further experiments and investigate those. On the other hand, if you find that they are completely unfolded that would be spectacular! It would suggest that all these proteins would live unfolded in the cell and adopt structure only upon activation.

The most important piece of information for us to discover is how chaperones actually contribute to this folding of their substrates. To know what is really going on before a protein gets functional you have to quench its early folding intermediates. The problem is that it is so difficult to analyse because folding intermediates tend to aggregate as egg white.

For our study we need several biophysical methods, but above all it is crucial to have a high level NMR, because these protein complexes are huge: we are talking about 200 kDa whereas normally 25 kDa is what people put into the magnet. It is true that NMR loses sensitivity when you use bigger proteins, but there are some recent developments that still make this possible to use. It is not easy to make the samples, but it is the only method that gives structural information of proteins that are not fully folded.

The different groups in the Bijvoet Center work on quite diverse projects. What unifies us is that we can carry out almost all important techniques at the highest level. That gives us the opportunity to always find a collaborator from a different group. This is a huge advantage. If, for example, I purify a protein for the first time, I would be stupid not to try and set up crystallisation trials as well, but in normal circumstances one would only do so if it were easy. Due to the Bijvoet Center's structure, here it is very easy. In terms of the physical techniques that I need to answer my question, there is nothing that is missing. I think that is the strength of the institute, but one that provides everything our researchers need.'

interviews

Group leaders

Antoinette Killian works as a Professor at the Biochemistry of Membranes section. **Roland Pieters** works as an Associate Professor at the Medicinal Chemistry and Chemical Biology section and just received a VICI grant. What their research has in common, is that they both work on medical related issues, especially antibiotics and infectious diseases.

Fundamental science for medical applications

Antoinette Killian's main research interest is the field of membrane protein structure and protein-lipid interactions. 'I want to know how lipids influence the structure of membrane proteins and their dynamics, how they affect insertion of proteins in the membrane, and what their role is in assembling multimeric membrane proteins'.

Killian's section has several research subjects. Her own main research deals with trying to understand complex membrane proteins by using very simple models of lipids and transmembrane peptides that are easy to manipulate. 'With these models we study, for instance, the role of tryptophans in membrane proteins, which is a very conserved motif. We synthesise an alpha helix that has this motif and then we change it, to see how this modulates the behaviour of the peptide. This line of research is closely linked to Roland Pieters' department, where members synthesise these transmembrane helical peptides, and put in any amino acids at will as well as labels so we can use different biophysical approaches to find out how the peptides behave.'

Peptide antibiotics

An important discovery from Antoinette's section originating in 2004, was a new model of action for antibiotics. For this project her colleague Eefjan Breukink collaborated with the NMR section in the Bijvoet Center to find out how nisin recognises Lipid II. 'Nisin is produced by bacteria to kill other bacteria. It shows substantial specificity for Lipid II, which is an essential precursor for the synthesis of bacterial cell walls. Together, Lipid II and nisin can very efficiently form pores in the membrane and thereby kill the bacteria.' Because of this property and the fact that Lipid II occurs in all bacteria, nisin is an attractive candidate for use in the pharmaceutical industry to prevent or fight infections. The results of these nisin studies can be used to design a new class of antibiotics. Another line of research is the role of amyloids, insoluble fibrous protein aggregations sharing specific structural traits. Abnormal accumulation of amyloid in organs may lead to amyloidosis, and may play a role in various other diseases. 'Amyloid deposits are a deposition of proteinaceous mass, like the plaques in the brain of people with Alzheimer's. Amyloid deposits are now also found to play a role in Diabetes II.'

These studies gave new insights in the role of membranes in amyloid fibril formation and in causing cell death of insulin producing cells in the pancreas of patients with type II diabetes. The membranes interact with the proteins and catalyse fibril formation, whereby some lipids are taken up in the growing amyloid fibres. The interaction also leads to membrane leakage, which could be responsible for cell death.

Killian did a 6-month sabbatical period at the University of Umeå in Sweden in 1993, where she studied peptides in bilayers and micelles by diffusion NMR techniques. 'It is good to know about different techniques. When focussing on studying membrane proteins with NMR, we plan to collaborate more intensively with the NMR department. Actually, one of my PhD students will probably work in the NMR section for a while, to learn more about solid state NMR on membrane peptides and

proteins. The Bijvoet Center really is a good place to meet other people, from outside your own field.'

Synthetic molecules

The Medical Chemistry and Chemical Biology section focuses on design, chemo-synthesis or biosynthesis of compounds with biological or potential medicinal applications, and interactions with their receptors. Insights thus obtained could lead to new bioactive compounds, new approaches for the treatment of still incurable diseases and new approaches to unsatisfactory treatments.

Roland Pieters' own research interests are the synthesis and biomolecular recognition of carbohydrate and peptide derivatives and glycomics. The research is aimed at the design and synthesis of compounds to be used in ligand receptor interaction studies. These are used to better understand the molecular part of bio recognition in disease-related processes and to interfere with them. Modified peptides and carbohydrates in particular are used.

'Medicinal chemistry is basically synthetic chemistry. We synthesise molecules, for instance peptides, with the goal of affecting biological activity. The nice thing is that we can introduce unnatural amino acids, which we cannot do with biological means.'

Pieters comes from the pharmaceutical sciences department so the focus of his research is on diseases. 'Blocking bacterial adhesion is one of our goals, while bacterial toxins and certain cancer proteins are some of our other targets. We also work on new antibiotics, using synthetic molecules.'

Multivalency

Pieters studies carbohydrate recognition. These interactions are weak, so he is looking for methods to make them bind more strongly. A powerful approach to this increase in binding affinity is multivalency. Multivalency is when the naturally occurring target proteins involved in disease have more than two binding sites, which the research team attempt to block simultaneously.

'Scientists in my field used to prefer only small drug molecules, but often one does not get any further with affinity, especially with sugars. More and more now, we try to design better and stronger binders for multivalent targets. If you attach molecules to a dendrimer (a sort of hand shaped molecule with several attachment sites) you can, in some cases, really see much stronger binding. This then opens new doors to us.'

The technique is not suitable for all targets; the delivery is different so you have to choose the targets carefully. 'A good target for instance is the bacterial toxin of cholera. It is in the intestinal tract and therefore easily accessible to large molecules. We have made a molecule that binds 300,000 times better to the toxin than the natural sugar does. I think this is a very interesting direction. In twenty years I hope several of these compounds, based on this multiple approach, will make it to therapeutic and diagnostic applications.'

The antibacterial area of research has a variety of activities. 'The problem with antibiotics is resistance; you need new ways to kill them or to block the path towards disease. One approach is to block the attachment of

Signaling networks in stem cells

Javier Muñoz, Dennis van Hoof, Jeroen Krijgsveld
Biomolecular Mass Spectrometry and Proteomics

We study the mechanism involved in the loss pluripotency of stem cells when they are induced to differentiate to more specialized cells. We are particularly interested how phosphorylation levels change during this process, since this indicates the signaling cascades that are activated. We approach this by quantitative proteomics using stable-isotope labeling of cells, and enrichment of phosphopeptides from induced and uninduced stem cells. This allows us to monitor the dynamics of phosphorylation of thousands of peptides over time.

bacteria to cell surfaces; this is why we use multivalent sugars. Another way is to puncture the membrane of the bacterium. Natural peptides are available that do this, but we make them more potent. The compounds make pores in the bacterium, this causes the bacteria to be killed because the cell contents leak out.'

Future

When asked his opinion on the future of the Bijvoet Center, Pieters says: 'I think we will increasingly study more complex systems and uncover new biological mechanisms, and simultaneously increase the atomic resolution of biomolecular interactions.'
To study these complex questions, the heterogeneity encouraged at the Bijvoet Center is a big advantage. Pieters explains: 'There is a lot of information exchange between the sections. PhD students from different sections organise seminars, we have tutorial symposia, all these activities are very valuable for us. Although we do not work in the area of proteins, we do need to know a lot about them. Essentially, our goal is interfering with them, so evidently we have to know our enemies.'
In his own field, Pieters expects a lot from the fact that chemists are getting better and better in synthetically preparing larger and larger molecules, with very interesting biological activities. 'This line of work will yield drugs possessing novel working mechanisms, alongside more traditional small molecules. Furthermore, technological developments and miniaturisation will allow amazingly accurate detection of specific markers in complex samples, without big machines.'

interviews

Group leaders

Rolf Boelens (56) is Head of the NMR Spectroscopy section. Their research is focused on understanding the molecular basis of protein-DNA recognition and gene regulation.

Magnetised by protein-DNA interactions

‘I am an NMR spectroscopist. As a postdoc I worked with Rob Kaptein on the Lac repressor for a long time. The Lac repressor is a DNA regulatory switch that binds very specifically to a stretch of DNA, called the operator. We wanted to know how the protein recognises this sequence. A crucial step towards this was the structure of the Lac headpiece, the DNA binding domain of the Lac repressor in 1985, and the model of DNA binding in 1987. So our group started working on DNA binding proteins, but over the years extended to also studying human transcription factors and proteins involved in DNA repair. Defects in these proteins are often associated with severe diseases, such as cancer.

We are specialised in high resolution NMR spectroscopy, and have a long tradition in biomolecular computing. A big advantage of NMR is that we can study proteins in solution, replicating conditions in the cell. Down in the large basement of the specially designed NMR building, eight NMR machines are busy measuring and computing day and night. They are very expensive. The biggest one, the 900 MHz NMR costs €5,000,000. We are a national NMR facility, but we also provide access to research groups from across Europe. It is obvious that we must make these instruments available to others. At this moment we have about forty European users running projects here.’

Large structures

‘Most biomolecular complexes involved in regulation of DNA expression are large and dynamic. This in itself presents us with a big challenge. Almost each nucleus gives an NMR signal. So with a big molecule the spectrum gets very crowded. In a big molecule the signals also become broader and less intense, and because of that at some point you cannot distinguish the various lines any more. The problems are significant but ones for which we have developed solutions.

There are several technical tricks to get the data we need. First of all, the size of the magnets helps to spread out the signals. It is also not always necessary to study the full system, so we can express a small part of the protein that we want to study.

By 13C and 15N isotope labelling in combination with 3D NMR we can highly reduce the overlap in the NMR spectra. The line broadening can also be reduced by deuterating our proteins.

In the past, the NMR structures were always based on finding NOE cross-peaks between all nuclei. In a big protein, therefore, there would be many NOEs to identify. But in recent years new tricks, such as residual dipolar couplings and use of specifically attached spin-labels, have become available that make it much easier to analyse large complexes.

Common perception is that we cannot go higher than 50 kDa, but this has changed because of the so called TROSY effect in deuterated proteins. We now study a complete dimeric Lac repressor with DNA, a complex of 90 kDa, and the changes that occur when we add the inducer to the Lac repressor. Really a first step towards understanding the genetic switch. At the moment, people even conduct NMR studies on proteins and complexes as large as 900 kDa. In a system that big it is not possible

Hot curiosity in a cold exactness’

Tiago Rito & Elif Karagöz
Cellular Protein Chemistry

In the upper image we see a scientist exposing a protein gel to an autoradiography film. Overlayed is the temperature display of a freezer. In the lower image we depicted two curious researchers analyzing a DNA gel using a new fluorescent dye excited with blue light. Overlayed is the temperature display of an incubator used for bacterial growth.

to analyse all the NMR signals, but that is not always necessary either.'

Striking moments

'One of the striking moments in my career was the first international meeting I went to as a PhD student. It was the first time I visited the United States, so that in itself was already an exciting experience. The meeting was one of those incredibly big American events, with thousands of people attending. I had displayed my poster in this huge hall and Britton Chance, at that time a big shot in my research field, came to speak to me about my poster. He gave some good input and suggestions. As a PhD student I of course found this very impressive.
A less successful moment from my PhD period in Amsterdam was the time we had to evacuate the entire biochemistry lab because of me. I was doing experiments using nitrogen monoxide, a very dangerous, poisonous gas. Suddenly my gas bottle started leaking; I could not do anything about it. All this gas was escaping, with a huge brown cloud of the gas leaking around me. I had to give an alarm. Luckily, one of the security people came and basically grabbed the bottle and then dumped it in the Plantage Muidergracht. There was no real danger anymore, but the Slater Institute was a very big building and all the six floors had to be evacuated. Of course people then started asking who had caused this. I was the cause of this, just a PhD student from the first floor!
I was mortified.

Future

'If you were to look at the increasing strength of the magnetic field in NMR machines over the last twenty years, you should see a very nice linear graph. This development will continue. In twenty years or so we will be able to have a magnetic field of 1.5 or 2 GigaHerz. The increasing monetary price, however, will not present such a neat linear graph alas. This increases very quickly . These machines could conceivably cost 20 or 30 million Euro. They can only be financed on a European scale. Theoretically, these machines may only be available in one of the EU countries. Possibly France or Germany, but who knows, perhaps it will be here at the Bijvoet Center. We would prefer this latter option of course!
In the future I expect a lot more research that combines different techniques, like NMR in combination with crystallography or mass spectrometry. Other questions could also be solved using electron microscopy. Also, I see in the future that biomolecular modelling becomes increasingly powerful.
In the next twenty years it could well be that a whole new technique will be developed. Now with NMR we use RF coils but there are other possibilities to heighten the sensitivity to a level that one can see every atom, like with the atomic force microscope. We already have an NMR equivalent. The problem is that you can only study surfaces. Hopefully this might change in the coming twenty years.'

interviews

Group leaders

Joost Holthuis (43) is Associate Professor in Membrane Enzymology at the Bijvoet Center. His group studies the inner workings and cellular functions of a disease-related class of lipid pumps. In 2004 he received a VIDI grant from the Netherlands Organisation for Scientific Research and became High Potential of Utrecht University.

Synthetic dream

‘My scientific career took shape when I was a postdoc at the MRC-LMB in Cambridge (UK). I worked on syntaxins, a family of proteins that guide transport vesicles in cells to fuse with the correct target organelle. Removal of multiple syntaxins in bakers’ yeast allowed me to create cells that no longer had an endosomal system. By putting back each syntaxin individually, I could reconstruct the endosomal system step-by-step and draw-up a new road map for membrane traffic in cells.

At the MRC-LMB, I met Mark Bretscher and Sean Munro, two great minds responsible for some thought-provoking concepts in lipid cell biology. They sparked my enthusiasm to work on lipids as co-organisers of membrane traffic. The problem was that I did not know much about lipids. Then Gerrit van Meer, arguably the most lipophilic guy on the globe, was looking for an Assistant Professor. I decided to apply for the job.’

Synthases and sensors

‘Unless you wish to become the understudy of the chair of the department, it is vital to set out your own lines of research and secure your own funding. Supported by a Royal Academy fellowship and a liberal chair, I started my own group to study two topics that caught my interest. The first concerns the question of how cells exploit the organising potential of a specialised class of membrane lipids, the sphingolipids. The second investigates the origin and significance of membrane lipid asymmetry.

An important breakthrough in the first line of research was our discovery of a family of sphingomyelin synthases. These enzymes had been sought for many years and are believed to serve key functions in membrane traffic and cell growth regulation. Their identification is widely viewed as a landmark in the field, as it opened up important new avenues for studying sphingolipid function in mammals. Unexpectedly, our recent work brought to light that some members of this family function as lipid sensors rather than lipid synthases. This is an exciting development because little is known about how cells control the lipid composition of their membranes, even though this is crucial for a proper functioning of their organelles.’

Flippases and vesicle budding

‘A fascinating aspect of cellular membranes is that the different lipid species are often asymmetrically distributed across the bilayer. For example, in the plasma membrane you will find the aminophospholipids almost exclusively in the inner leaflet. In multicellular organisms this arrangement is critical for cell survival as the appearance of aminophospholipids on the cell surface causes cells to be eaten by macrophages. Even cells living in solitude, like yeast, invest energy to keep aminophospholipids inside. This suggests that lipid asymmetry serves a fundamental role.

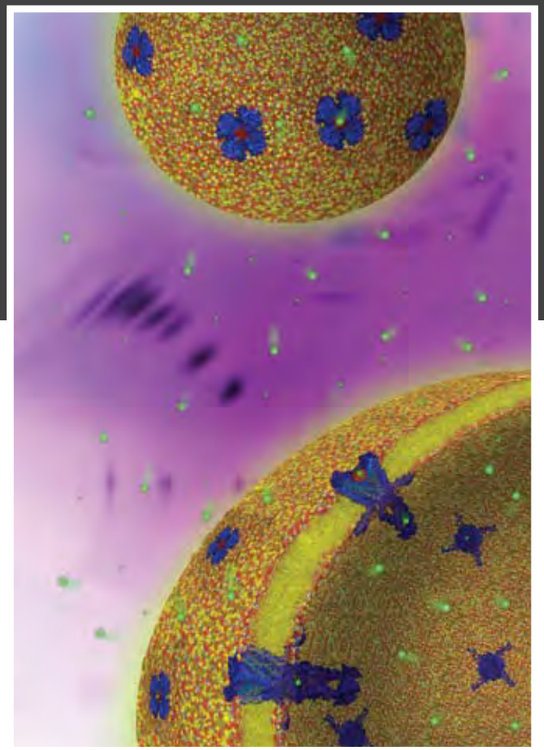
Using yeast as a model system, we identified two ATP-fuelled transporters that are required for flipping aminophospholipids from the outer to the inner leaflet of the plasma membrane. We also found that removal of these so-called P4 ATPases blocks the formation of endocytic vesicles at the plasma membrane.

This was a remarkable finding, given that a central dogma in cell biology maintains that vesicle formation is mainly driven by the assembly of coat proteins onto the cytosolic

How do lipids affect transmembrane proteins?

Jacques Doux
Chemical Biology

Our group studies effects of lipids on designed peptides and engineered proteins by biophysical methods such as solid state NMR. This allows us to get insight in the fundamentals of protein-lipid interactions. The picture represents KcsA potassium channels, reconstituted in liposomes, and potassium ions.



surface of the membrane. Due to their intrinsic curvature, these coat complexes are thought to deform the bilayer into a bud. Our studies indicate that this is not the whole story. By virtue of their ability to pump lipids inward, P4 ATPases are able to expand the inner leaflet of the bilayer at the expense of the outer leaflet. We postulate that this eventually leads to invagination and vesiculation of the plasma membrane, and that coat assembly mainly determines where vesicles bud. Formulating hypotheses is great. The real challenge is to prove them.'

Synthetic dream

'So far, the concept that flippases help drive vesicle formation is mainly based on genetic evidence. That is not good enough. P4 ATPases belong to a conserved super family of cation transporters that include calcium, potassium and heavy metal pumps. From a mechanistic point of view, it is a complete mystery how P4 ATPases acquired the ability to flip lipids.

Our recent work suggests that these ATPases do not act alone but in complex with another class of membrane proteins. Our current approach is to purify these protein complexes in an enzymatically active form and to reconstitute them into giant liposomes that can be observed under the light microscope. The idea is that addition of ATP would trigger flippase activity. This, in turn, can be monitored by shape changes of the giant liposome. Hence, our ambition is to define, for the first time, the molecular composition of a flippase and to unravel its role in vesicle formation.

This is a high risk/high impact undertaking that, in addition to a stroke of luck, requires input from a dedicated and strong-minded group of researchers.

I count myself lucky that I have been able to secure two prestigious grants to build up such a team. Reconstitution of flippases is not the end point, but rather the beginning of the project. The Bijvoet Center offers an ideal environment to try and obtain crystals of the purified protein complexes to solve their atomic structures and thereby get a glimpse of how flippases actually work.

Mutations in human P4 ATPases have been linked to diabetes, obesity and a life-threatening liver disease.

My group has teamed up with the group of Leo Klomp at the UMC to unravel the molecular basis of these diseases.'

High horses

'The current trend at Dutch universities is to increase management of science and to put all high horses together to create one giant horse. I have some mixed feelings about these developments. Giant horses tend to provide shelter for many little ponies. I also think that scientists in a research institute like the Bijvoet Center would be bored to death if they all had to work on a single topic, such as AIDS or ageing. Curiosity-driven research is a vital part of science, and by definition not manageable. As long as the criteria for hiring academic staff are firm, and based on quality and ambition, I believe that research institutes should nurture, or at least tolerate, a certain level of anarchy to ensure a healthy scientific future. In this respect, the future of the Bijvoet Center looks bright. It houses not only brilliant scientists but also some of the most idiosyncratic people I have ever met.'

interviews

Group leaders

Albert Heck (43) is Director of the Bijvoet Center since 2006 and Head of the Biomolecular Mass Spectrometry section since 1998. His section focuses on two main research areas. One is proteomics, studying how the cell changes at the protein level. The other main area is developing new technologies for structural biology.

Riding the back of a protein

‘An important overall aspect of all our research projects is the interaction between proteins in cells. We generally have two different ways to study these interactions using mass spectrometry. The standard method is by using a specific protein or antibody as bait and then ‘fishing out’ other proteins that interact with it. This is a good method to identify reasonably high-throughput networks of protein complexes.

The second method is more unique: we have developed a technology enabling the measurement by mass spectrometry of intact, very large protein complexes, such as the ribosome, the proteosome and viruses. These are somewhat unstable complexes because they are bound, not chemically but non-covalently, by a mixture of electrostatic and hydrophobic interactions. For this kind of research we use a unique mass spectrometer. It is a rather specialised and difficult technique to use; only a few groups in the world can do this at the moment.

We have recently published an article in Structure. The article describes an experiment using this technique to look at RNA polymerases. We transferred the whole 600 kDa polymerase into the vacuum of the mass spectrometer. In the ionisation process the complex loses all its bound water molecules but at the same time it picks up charges. This allows us to make an accurate mass analysis within 0.2% of the exact mass.

This way we determine the mass of the complex, and from the more standard proteomics experiments we determine the mass of the sub-units. The sum of all sub-units must add up to the intact complex. Solving such puzzles allows us to determine which sub-units are in the complex. By detecting polymerase sub-complexes, we also discovered that two sub-units were more loosely attached than the other sub-units. This means that it maybe the case that in the living cell, part of the polymerase complex is present in a slightly different form.’

New science

‘This is an example of novel technology that is interesting for other researchers in the Bijvoet Center as well. Structural biologists throughout the world study the same polymerase and its sub-units with NMR and X-ray crystallography.

If you have the crystal structure, you understand everything, including the atoms. That is really beautiful but unfortunately we cannot do that with mass spectrometry. However, what we can easily do is measure stoichiometries and impurities. We can count which pieces are really there, and whether there are two copies, by measuring the mass. In this case, we can show that there is a mixture of the two. That is something you cannot easily see with crystallography. So this part of mass spectrometry adds a tool for research in Structural Biology, complementary to crystallography and NMR, for looking at large protein complexes.

I am probably most proud of a new analytical method we developed a few years back in order to target protein phosphorylation. Developing the method took us several years. It is based on a material called titanium dioxide which we obtained from a Japanese company. It was a risk, as we had no idea whether it would work.

CFTR biogenesis: protein assisted folding’

Elena Ganusova
Cellular Protein Chemistry

The picture is my association with cell protein factory and it is devoted to the protein I’m working with. Several cell compartments are presented and all flowers are either chaperons (a lot of white daisy-like flowers) or other proteins in the cell. Caterpillars present proteasome, the upper part – is plasma membrane.

Fortunately, it worked out very well, so well in fact that nearly everyone who looks at protein phosphorylation by proteomics has adopted this method. We published our initial article in 2004, and last year there were a few hundred publications which used our method as a building block.

Our first report on such a method was published in a specialised analytical journal, which was surprisingly difficult to submit the paper to. It is very rewarding to see that a couple of years later, people who use this method in order to solve problems are publishing in Nature and Cell. Analytical work and method development do not get the credit that they should. Particularly from magazines like Nature, we often receive comments along the lines of 'nice work, but too methodological'. I would like to see this type of attitude turned on its head. If we do not develop new methods, there will not be any new science. Progress in science, is only made following the development of new methods. I think the scientists that developed the analytical methods that allowed us to sequence the Human Genome, should receive at least the same credit and plaudits as those who carried it out.

Holy Grail

'I started studying physical chemistry because I wanted to control my chemical systems; and that was certainly not possible in biology. Now I am a bit older and more relaxed, I know that I cannot control the whole cell, but I still want to understand how it works. It is still my dream to see how one entity in the cell is moving around and how it interacts.

The Holy Grail for me would be being able to make moving images of single proteins in cells. To be able to sit on the back of a protein and see what it does. To look around in the cell and then come back and write it all down. That would be sublime. I have already imagined that the title of this publication would be: "Riding the back of a protein inside the living cell".

The funny thing is that this dream appears to be closer and closer to reality. We can already make movies of proteins with a fluorescent probe, yet it would be nice to do that with the resolution of NMR or crystallography. Now all you see is a blurry blob that moves to the membrane for instance. That is alright, but we want to know what happens before it reaches the membrane, how it anchors, how it communicates with other biomolecules.

Twenty years from now, I think we will be there. A problem right now is that most of the structural biology is centred on *in vitro* systems. A bridge has to be made between these live cell imaging techniques and the high resolution structural data that structural biology offers. For instance, in organic and physical chemistry they are able to make so called quantum dots, very fine clusters of atoms that light up very sharply. Like the experiment in which atoms light up to form the letters IBM. People have now started to use these quantum dots to label a protein.

Probably then, this bridge will be made not by a biologist, but by a physicist or a chemist. Maybe an organic chemist who wants to become a structural biologist and then tries to use his old methods for new problems. That is how breakthroughs are made: by people in situations they are not used to. Like so many things in life, to move on, you need input from totally different directions.'

Future of the Bijvoet Center

'My hope for the Bijvoet Center is that we make a major contribution to this dream of mine. I believe that in order to realise this dream we probably have the right ingredients in place here.

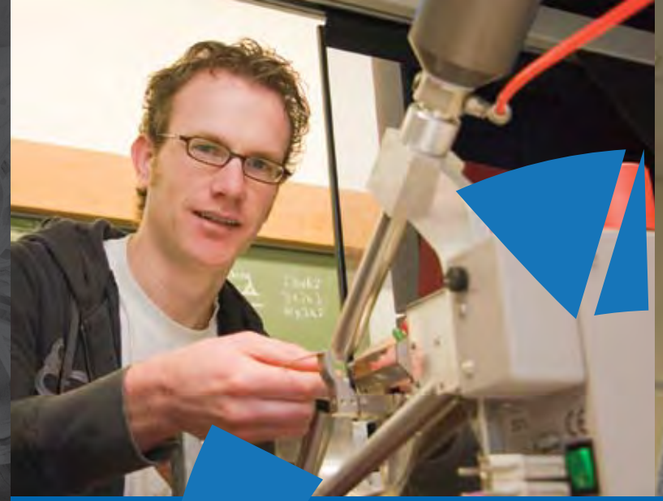
On a day to day basis, a more down to earth challenge is being able to define joint interests. There is a conflict because even though every scientist is partly working for the greater good, at the same time they all have their own projects, and are also working for their own egos. I hope, therefore, that in the future, even more than in the past, the sum of the groups will be more than the separate components.

I truly believe in such a multidisciplinary approach. That is something I miss when I look at graduate students sometimes. Too often I see that a graduate student in mass spectrometry decides to be a mass spectrometrist for the rest of his career. They should try to switch. You may not be able to use all your old knowledge in a new field, but you do get a lot of things in return. I still think my background as a physical chemist is an added value to the way I look at the problems that we have here.'

interviews

Junior Researchers

Michael Hadders & Kristina Lorenzen
Chris Arnusch & Klaartje Houben
Ewald van den Bremer



Michael Hadders (28) and **Kristina Lorenzen** (29) are both PhD students at the Bijvoet Center. Michael works at the Crystal and Structural Chemistry section, Kristina works at the Biomolecular Mass Spectrometry section.

Being a PhD student at the Bijvoet Center

What topics are you studying?

Michael: 'I work at the Crystal and Structural Chemistry section. We use a technique called X-ray diffraction to determine the 3D structure of proteins. This 3D structure can give a lot of information on how these proteins function. My particular project focuses on a part of the immune system, the complement system, which forms the first line of defence against invading bacteria. When this system is activated, a large protein complex is made that makes big holes in the bacteria causing them to lyse and die.'

Kristina: 'I am studying protein complexes by mass spectrometry. It allows the analysis of non-covalent protein complexes, even when these complexes are heterogeneous and the interactions weak, in an environment where the proteins are supposed to keep their quaternary conformation. A major advantage of macromolecular mass spectrometry is that it allows measurements of dynamics, stoichiometry and stability on the level of protein-ligand, protein-protein and protein complex interactions.'

Why did you choose to do your PhD at the Bijvoet Center?

Michael: 'I studied biology and was always fascinated by X-ray crystallography. I was lucky that, during my biology study, the undergraduate course in structural biology was also open to biologists. This course really opened my eyes. My first impression was: wow! There is so much you can do with structural biology! Also because of the vast array of different techniques you can learn at the different research groups.
That course made me realise that I really wanted to know how to solve a protein structure myself. It turned out it was possible to do an internship here at the Bijvoet Center, so that is what I did. I worked at the crystallography section and it was great, I really liked it. During that internship, my supervisor asked me if I might be interested in doing a PhD in their section. One of the reasons I decided to accept the offer is the fact that, although the Bijvoet Center is very technique-oriented, they work on biological questions. I can go to my neighbours here and use all their techniques on my biological problem. For instance, NMR is a difficult technique, but here I can learn to use it for my own research. That is not the case in more traditional institutes where researchers often all use the same kind of techniques.'

Kristina: 'The mass spectrometry laboratory I am working in is one of the best in the world. Of all mass spectrometry laboratories there are only a handful that have the equipment that is needed to look at big protein complexes. When I was looking for a PhD position the vacancy they had here was the one that captivated me. So here I am.'

Do you have much contact with other PhD students?

Michael: 'We have a PhD platform with representatives from every research section. This platform organises lectures by PhD students and young postdocs. I think it is one of the best things here, I like it very much. We regularly meet, have dinner, paid for by the Bijvoet Center, and then three of us will hold a lecture on his or her research. It broadens your horizon. Sometimes it is difficult to follow, but in most cases you can grasp the concepts and you can always ask questions. Also, if the lecture is very technical, the person giving the talk should realise that his audience is not on the same level as his peers and will, therefore, adjust his presentation to cater to the audience. That is a challenge too, to learn to do that.'

Kristina: 'I am lucky because I am already in a very big group and the contact between the PhD students is very good. At the Bijvoet Center, the PhDs organise their own evenings where we meet and give talks about our projects, without the supervisors or professors. This creates a very informal atmosphere which stimulates problem-orientated discussion. It also especially helps the new PhD students to find helpful contacts quickly. Additionally there is the social element of it, after the talks we usually go for dinner. I really enjoy these evenings.'

What was the most striking moment in your career?

Michael: 'My first first-author article, which was published in Science last September. I solved the crystal structure of one of the proteins from the complement immune system. After the 1980s the advance in the field of complement slowed down, in part due to a lack of high resolution crystal structures of the proteins involved. However, with the new technical advancements, so much more is becoming possible. We were the first to be able to solve the crystal structure with one of those complement proteins. The result was very interesting, because when we captured the structure it turned out that it looked a lot like bacterial toxins from gram positive bacteria, that cause disease in humans. This was very unexpected, because the genetic sequence is totally different. When we searched the literature for structures of all kinds of pore forming proteins, we found a picture in a review article that looked very similar to our structure. Yet this belonged to a bacterial toxin. So the toxin uses pore forming proteins to kill human cells, but the human immune system uses the same trick and makes pore forming proteins that can kill the bacteria. That was a fantastic discovery.'

Kristina: 'I am not sure if there was one striking moment. There are various small moments where you finally succeed in an experiment that did not want to work for a long time, or when you get good results after spending a lot of time and effort on it.'

What was you biggest mistake?

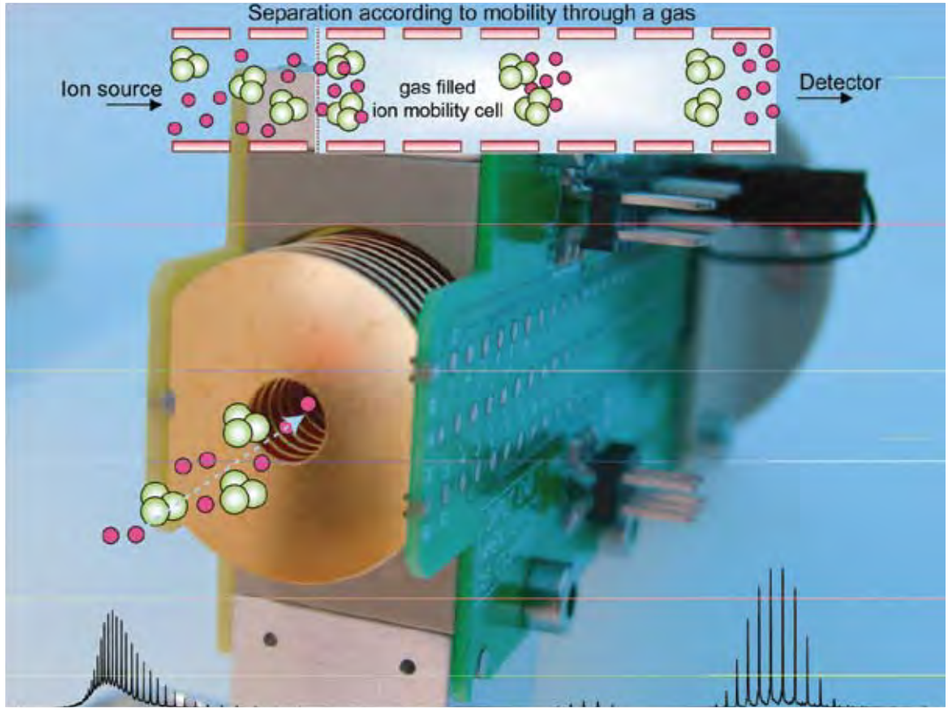
Michael: 'Nothing specific comes to my mind. However, I remember a couple of times when I was not paying enough attention during the purification of a protein. I did not notice it was flowing down the drain. This often means a lot of work for nothing. The feeling you get when you realise that is not a pleasant one.'

Kristina: 'Not backing up the data on my computer in a sufficient way resulting in its loss after the computer crashed and we were not able to retrieve it.'

Structural and functional characterization of protein

Esther van Duijn, Kristina Lorenzen, Charlotte Uetrecht
Biomolecular Mass Spectrometry and Proteomics

Ion mobility mass spectrometry separates ions based their size, shape and mass to charge ratio. This novel structural biology technique can be used to explore protein conformations in the gas-phase.



What would be the title of the ultimate research paper you wish to publish before you die?

Michael: 'At this moment: Crystal structure of the Membrane Attack Complex.'

Kristina: 'Science is about advancement in your field as well as in yourself. There are always new questions coming up along the way which you are trying to find answers for. When you discover these, you try to pass the information on by publishing them. Often the ideas and new questions come with the problem you are working on at that moment in time. For me it is a continuous process, I am not able to tell what question will captivate me ten years from now.'

Where does structural biology stand twenty years from now?

Michael: 'Hard to say. There are enough questions out there. I think, as methods advance, people will be tackling bigger, more complex questions. For instance we study the membrane attack complex, a very big complex. We have now solved the crystal 3D structure of one domain, of one protein. As a whole, the complex consists of five proteins, and one of those has fifteen copies in this structure. Therefore, I think in the future the questions will move to looking at the aspects of whole complexes of macromolecules. That would be fascinating and the technical advances will make it possible. It is important to know the whole complex. Now we often only look at parts, and we make assumptions on how the whole thing works, but we are not sure. Maybe the sum of it is more than its parts?'

Kristina: 'The trend is always moving towards a smaller and smaller scale. It might well be that we will be able to monitor reactions in the living cell down to the atom level on a visual basis.'

With which Bijvoet Center section would you choose to spend a sabbatical?

Michael: 'Probably with the NMR section. I would like to learn some new techniques that I could apply to my own research and with NMR you can do many exciting things.'

Kristina: 'The Bijvoet Center already gives me the opportunity to address every researcher when one needs it, as well as to go to their labs and establish collaborations. For me this possibility to interact within the Bijvoet Center is one of the great benefits of working here.'

What are your hopes for the Bijvoet Center?

Michael: 'I hope it can hold its international reputation of being an excellent Research Center. I have become rather used to it, but a foreign student made me realise how good a reputation we have internationally. So my hope for the centre is for it to stay at the top.'

Kristina: 'That it keeps the interaction and exchange between scientists, their knowledge and their instrumentational possibilities moving on a non-bureaucratic level and thus driving the science onwards.'

Which would be your favourite undergraduate course if you had the choice today?

Michael: 'Structural biology! Our group gives a part of that course. It is very broad, touching all aspects of structural biology, also from a practical point of view. It opened my eyes when I took the course as a biology student several years ago.'

Kristina: 'Looking back, a course starting from the gene to protein expression, purification and kinetics would have been very useful.'

What is your best advice for the young investigator?

Michael: 'Be critical and focused. Think about what you want to get out of your PhD and do it. Do not become the pipetting robot of your supervisor...
You have to bring in your own ideas. I have a lot of freedom. Of course sometimes your supervisor tells you to do something, and you have to do it, and that is all right, because it turns out he was right. I do not pretend to know everything, but the possibility to discuss the line of research with your supervisor is very important.'

Kristina: 'Always try to improve and never give up.'

interviews
Junior Researchers

Chris Arnusch (31) and Klaartje Houben (31) both work as postdocs at the Bijvoet Center. Arnusch works in the Chemical Biology and Organic Chemistry section and the Medicinal Chemistry and Chemical Biology section, Houben in both the NMR and Membrane Enzymology sections. A common interest they share are pores in membranes, although from a very different angle and with very different methods.

Life as a postdoc at the Bijvoet Center

What topics are you studying?

Chris: 'My focus is on antibiotics. I am interested in creating new antibiotics from the ones that have already been found in nature. Making new combinations and watching their mode of action and activity. I work with magainin, vancomycin and nisin, and study their interactions with the membrane and lipid II. This is a precursor building block, which is used in bacteria to form the cell wall(the protective coat). Nisin binds to lipid II and forms a pore in the cell membrane, killing the bacterium. Lipid II is also the target of vancomycin, but with a different mechanism. Magainin is an antimicrobial peptide that targets the membrane and was first isolated from frog skin.'

Why study these antibiotics, are they not just fine as they are?

Chris: 'Nisin is an antimicrobial peptide with an activity far greater than that of magainin, and we want to show that it is possible to increase the activity of magainin via multivalency. Ultimately, we want to make magainin as active as nisin or vancomycin.'

Klaartje: 'So what is the mode of action of vancomycin again? Is it not a pore former?'

Chris: 'No, vancomycin just targets the small tri-peptide on lipid II and it blocks cross-linking of the cell wall, so the cell wall cannot become strong. So nisin and vancomycin basically target the bacteria in the same place, but have different killing mechanisms.'

Why is nisin itself not good enough?

Chris: 'Well, it is good enough, since it is currently used as a food preservative; it is one of those "E" numbers you see in products. We want to take a weak pore forming antibiotic like magainin and make it more effective. Magainin needs to aggregate before it disrupts the membrane. We want to "pre-form the pore" by connecting the magainin peptides in a pore-like shape. Ultimately, we want one construct to make one pore, kind of like a bullet…'

Once you have a pore, the cell gets killed?

Chris: 'Hopefully, yes. If you manage to disrupt that membrane the cell might leak out ATP and other important things and just say "I've had it, I'll just die".'

But bacteria try to repair themselves, don't they?

Chris: 'That is true, and I think maybe that is how resistance can form, because it then brings out another mechanism to create the cell wall for example. But resistance when it comes to changing something as basic and necessary as a lipid membrane would be difficult. That is why these antimicrobial peptides that target the membrane are so important to study.'

Klaartje: 'I guess that is why nisin is so effective; you disrupt the cell membrane by making a pore and block cell wall synthesis as well, by targeting lipid II.'

What are the future applications?

Chris: 'It is just a matter of time to see what applications I can apply it to. I have shown now, with different types of model membranes, that you can leak the contents out by adding the multivalent magainins. The activity really increases with some of them, even as active as nisin! However, we have also tested for safety using a hemolysis test, and some of the compounds we have made would be very toxic if they would be in our bloodstream.

So now you know how to kill red blood cells, very convenient.

Chris: 'Yes, well, it is just another piece of evidence that this covalent oligomerisation perhaps does make a pre-formed pore, and is then able to be inserted into any membrane.

Klaartje: 'What is the most challenging part? Is it the organic synthesis of the compounds , or is it making them soluable, or designing them? What is the hardest part?'

Chris: 'Actually the synthesis was relatively straight forward with the smaller oligomers, because it was just peptide chemistry and then conjugation onto small dendrimers using "click" chemistry. So that was pretty easy. The most challenging were the larger oligomers, connecting four or even eight magainin peptides onto one dendrimer. The octomer had a MW of about 25 kDa – a little synthetic protein. So I had to use some biochemical techniques like SDS page to analyse them.'

What about you, Klaartje, what is your topic?

Klaartje: 'I just started in October. Before that I spent three years in Grenoble doing NMR research. When I started to write the application, I knew I wanted to continue working with NMR and I wanted to work with membrane proteins, because I think this is a challenging group of proteins. So that is why I contacted Gerrit van Meer's group, because they work with very interesting membrane proteins. The problem with NMR is that you cannot work on too large systems because for the moment this is rather challenging. You want your proposal to be challenging but also feasible.
So I decided to work on a family of small proteins, sixty residues or less. They exist in plants and yeast but also in several bacteria and they are all stress-related proteins. They are induced by cold stress or salt stress.
The idea is that these types of proteins depolarise the membrane, slowing down the influx of positive ions. It is a defence mechanism. Sodium ions are toxic for plants, so if there is a lot of salt in the soil, these ions should not get into the cell. The hypothesis is that these proteins make a pore….'

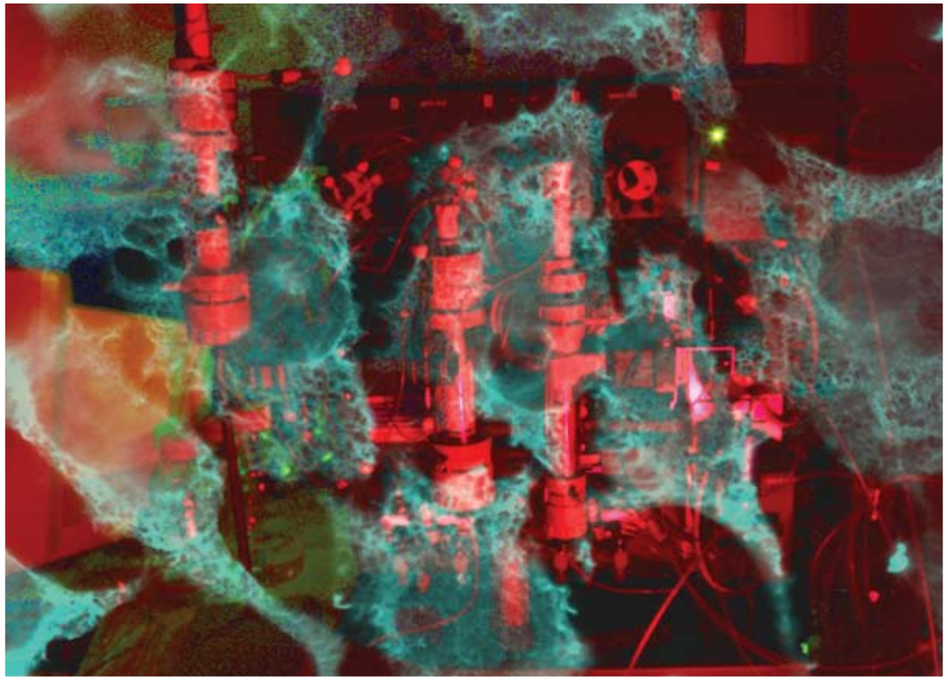
Chris: 'Hey, that is interesting.'

Klaartje: '…but these are very small pores that let only protons go into the cell which prevents other ions which are toxic, like sodium, to go into the cell. My goal is to understand the mode of action of these kinds of proteins by studying their 3-dimensional structure by NMR.
First I want to do a large-scale screening of the different proteins in this family because the problem with solution NMR and membrane proteins is that they are not soluble. Therefore, you have to search for the best behaving target-protein. The second problem is finding detergents that protect the hydrophobic part of the protein. I need to create a system for NMR that is feasible to study but also functional. You cannot have a

On the road to purity'

Elif Karagöz and Tiago Rito
Cellular Protein Chemistry

Overlay of a liquid chromatography system photo with immunofluorescence picture of cells expressing Hsp90, ubiquitous cytosolic chaperone. Our research aim is to purify Hsp90 to study the mechanism by which it helps the folding of its substrates.



complete membrane in the NMR sample because that becomes a huge system, too big for solution NMR. What you need, then, are micelle forming detergents that protect the hydrophobic part.
I am going to take all these different proteins from these different organisms and make three constructs of each protein with different fusion proteins. This way I hope to find a protein that expresses in large amounts and then I will put it into a micelle. Right now, I am still cloning to make the constructs; I have not started the expression experiments yet.'

Can you already mention a striking moment in your career, or is it too early for that?

Chris: 'When I started looking at the red blood cells and other types of cells under the microscope, I guess that was a striking moment. It really gave me a wake up call that we are quite physical. That we are just made up of all these little cells, but the sum of all those cells makes up something more than just a physical being, it makes us individuals. This sort of struck me. These cells are just so vulnerable and so simple, yet so very complex as well. And when they are all put together they really make up more than just a physical bucket of cells. It is really cool to see these little red blood cells.'

Klaartje: 'That is funny, that is because you are a chemist.'

Chris: 'Well, yes, as a chemist, you make white powders all the time. You are not busy thinking about biological processes, so when you move to the biological stuff, I guess you have the Van Leeuwenhoek experience. You see this droplet of blood and realise that it is "alive". That the blood I am looking at through a microscope is also in my body doing its job.'

Klaartje: 'I think that is the nice thing of the Bijvoet Center actually, we are mainly chemists, but we can use our chemistry background to ask questions about biology. This triangle of biology, chemistry and also physics is what I like about the Bijvoet Center.'

Do you feel there is a difference between technology-driven and biology-driven scientists?

Klaartje: 'Yes, the NMR people are often more technology-driven. I think that in one way this is a problem for the Bijvoet Center. Sometimes people do not speak the same language. People can be a bit negative, for instance saying that the NMR people are only thinking of their spectra. On the other hand, the NMR people say, "these biologists, they think we can solve everything, but it is not that easy".
That is what I like about my project. I am working together with Joost Holthuis, who is from a more biological field, and we are trying to understand each other. For example, Joost is worried about the fact that I am working in a model system, because I am going to work with micelles, and that it is not a natural biological environment. We try to find assays that we can do to show that the proteins are functional. Although the environment might not be biological, the protein structure that I will study most probably is the same in a micelle as it is in the lipid bilayer. For me it is already a challenge to work with a micelle system and Joost

understands this. I also agree with him that it is always better to answer the real biological question, but you do need this step in between.'

But you are all scientists, why is this so difficult to explain?

Klaartje: 'Yes, we are all scientists, but we are very specialised and we do not always use the same language. If you give a group lecture for your own group or for the Bijvoet Center as a whole, you have to adapt the talk because when I show my NMR spectra, not everybody knows what I am talking about. You have to make an effort to explain NMR to non-experts.'

And what about your most striking moment yet?

Klaartje: 'For now I would say getting the Veni Grant. I was really happy. It means they say: "We trust you; you have a good idea and should go for it". That is very supportive and encouraging.'

You both study pore forming. Have you considered a collaborative project?

Chris: 'Well, Klaartje is studying pore-forming proteins, and I am basically trying to make home-made pore-forming proteins. If Klaartje can get a good NMR spectrum of her protein, maybe I could give her my home-made proteins and she could see what she could make of them.'

Klaartje: 'Yes, it would interest me a lot to look at these kind of pores. I know the lipid II/nisin story a little. Danny Hsu, a former PhD student in my group, studied nisin and its interaction with lipid II using NMR. So there is already a connection.'

Chris: 'Yes, NMR played a very important role in showing where the nisin is binding to the lipid II.'

Klaartje: 'NMR is very useful, when you design something it is always nice to check if the molecule you designed is actually formed in the way you think it does.'

Why did you choose to do a postdoc at the Bijvoet Center?

Chris: 'I need to believe in my project. The subject and type of work motivates me and gives me vision. Going to work at Harvard just because it is a top institute on a project that does not really excite me is not my idea of being honest to myself. Is it right to say that I chose the Bijvoet Center? No, because of the high quality project, the Bijvoet Center chose me.'

Klaartje: 'After my postdoc in Grenoble I wanted to try to get a Veni-fellowship which gives you the possibility to develop your own research topic. I am very interested in the application and development of solution/solid-state NMR for the study of structure and dynamics of membrane proteins, so I searched for a host-institute where I could best focus on this research theme. In my opinion, the Bijvoet Center is the place for that. It offers a solution NMR group with impressive equipment and computational force, another group using solid-state NMR to look at transmembrane peptides, a Membrane Enzymology group studying many interesting membrane proteins, a crystallography group experienced with the structural biology of membrane proteins, and so on.'

How do you see the future?

Chris: 'Sometimes insights in the future can be found by looking at the past. About fifteen years ago, I had a vision of myself designing new drugs, especially antibiotics. Being in Europe and doing what I am doing now I could not even have imagined at that point. So I trust that in the future, the next step for me will come when the time is right, and get me closer to my original vision.'

Klaartje: 'Already some people in the world are using high-resolution solution NMR to study membrane proteins, but here in Utrecht nobody is working on it yet. I really would like to establish this technique. I would like to make the link with the Bijvoet Center and try to work with other interesting systems here. Like the system Chris told me about, or those of Joost or Gerrit van Meer. I would like to contribute to establishing interesting collaborations between these groups. By contributing to the development of solution NMR, increasingly adapted for the study of membrane proteins, I hope to convince researchers of the Bijvoet Center of the power of NMR for this field of research.

Several people in the Bijvoet Center are studying huge membrane proteins, and there are really a lot of specific questions one could ask and answer with NMR once the technique has been further developed and made sufficiently robust. Personally, the ultimate theme would be to work on a big protein like for example the V-type ATPase that has some 13 sub-units, and study it with NMR.

In the future I think that different groups in the Bijvoet Center will increasingly unite in order to cooperate and to use the force of all the powerful techniques - NMR, X-rays, MS, EM, etcetera. These should be combined by the Bijvoet Center to study the folding, assembly and activity of huge biomolecular complexes on a detailed atomic level.'

What are your hopes for the Bijvoet Center in the future?

Chris: 'If you see other people's work and apply your own background to that new work, then you could achieve something greater than if you work all by yourself. Perhaps the Bijvoet Center in the future could stimulate that even more. For example allow researchers to learn more techniques, do a short project and really learn the NMR and then return to your own project.'

Klaartje: 'Yes, that would be very good. I know the Bijvoet Center started as a sort of artificial construct in order to get money, and then became more integrated as a research school, educating PhD students. So now, at the end of your PhD you can say: "I am a PhD student of a Biomolecular Research Center, not just a PhD student of an NMR group."

interviews
Junior Researchers

Ewald van den Bremer (34) started his career in 1998

as an HLO technician at Numico in Wageningen. Two years later he moved on to do a PhD in Mass Spectrometry at the Bijvoet Center which he finished in 2004. He currently works in the field of monoclonal antibodies designed to treat cancer and autoimmune diseases at Genmab BV in Utrecht.

The opportunity of a lifetime

‘As a technician at Numico, I started working with mass spectrometry for the first time and I liked it very much. What I really wanted was to be able to design my own experiments because I had a lot of ideas that I wanted to look into. However, being a technician that is not always possible. I knew it was possible to do a PhD with an HLO background and in order to move forward I had to do this.

One day in 1999, I read an interview with Albert Heck in Chemisch Weekblad. Heck was newly appointed at the Bijvoet Center, very enthusiastic and just 35 years old. I decided to write an open job application for a PhD project. I just had to; otherwise I would have wondered about this opportunity for the rest of my life. After a few interviews, Heck hired me as a PhD student. During my HLO studies I did a one-year traineeship at the Portland State University in the United States. I think this experience was an advantage when I applied for a PhD. Being a technician, I felt I was rather skilled in the lab but lacked the knowledge that comes from reading lots of literature. I needed to work on that, especially during my first year, but it worked out well. Starting my PhD was no doubt the most striking moment in my career.’

Colicins

‘I studied four different proteins from a class of proteins called colicins. These are toxins made by bacteria under stressed conditions like lack of nutrients. Under such conditions bacteria excrete these toxins to kill other bacteria in order to survive.

My PhD had mainly two goals. One was learning more about this protein family itself. The fact that these proteins are able to kill bacteria makes them medically relevant, because a lot of bacteria, like MRSA, get resistant to drugs that are presently available. So knowing more about the colicins might be important for the development of new treatments for bacterial infections. The other goal was a more technical one but perhaps even more important. That was whether mass spectrometry is suitable to study protein folding and conformational states. My experiments showed that colicins exist in differently folded populations. These different populations are a very interesting phenomenon that you are not able to see when you look at the proteins with more conventional techniques. For instance, if you take fluorescence, you can only measure the ensemble, to sum of everything; you cannot really see the different populations in solution.

Using mass spectrometry, I studied the relation between charge state distributions and the conformational state of a protein. When a protein is compact, it has a different charge state compared to a more unfolded protein. This is because the basic amino acid residues buried in the core of the protein are not able to adopt a charge. Hence, a compact protein will adopt less charges when compared to the identical protein when it is more unfolded loosely packed. This way you can discriminate between folded and unfolded states of a protein.

What I noticed, was that at room temperature and neutral pH, the colicin DNAse domains are in a dynamic conformational equilibrium between two quite

distinct forms. This observation was quite surprising. Furthermore, even more surprisingly, the dynamic equilibrium disappeared when the protein adopted a divalent metal ion, leaving one compact conformational state. This was the first time we could see this for this class of proteins. The different forms are probably necessary to execute two important tasks. Firstly, to enter the bacterium and secondly, to actually activate the reactions that lead to killing the bacterium.'

Validating mass spectrometry

'Because we saw these different protein populations with mass spec first, we had to prove it was not an artefact of our method. Mass spectrometry (ESI-MS) is a gas-phase based technique and life occurs in solution. So we had to check whether this was also really happening in the solution phase. I needed to verify my mass spec results using more accepted methods.

At the beginning I did not have access to the equipment I needed to validate my mass spec findings. I wanted to do fluorescence measurements and differential scanning calorimetry. I took my problem to other people, inside and outside of the Bijvoet Center, and explained what I needed. That is how I got to know Wim Jiskoot from the UIPS pharmaceutical research center. He put me in contact with people in Wageningen and at Organon. They got enthusiastic as well and were willing to help me out. Later on Jiskoot became my co-promotor.

Having access to other equipment, I started to perform intrinsic tryptophan quenching studies, to see if we could monitor differences in tryptophan accessibility as function of divalent metal ion binding as I would have expected. The results of these studies were fantastic.

Also by differential scanning calorimetry measurements we found that between the two conformations - folded and unfolded - there was a big difference in melting temperature. That also correlated well with my mass spec findings. I later extended the study to three other protein family members, which were believed to share similar folding behaviours. However, my mass spec results showed that each had its own unique folding properties and this also correlated well with other solution phase measurements.

I think I was quite fortunate seeing that all these pieces fell together. It resulted in four articles on this family of proteins. A different protein might have been a bigger challenge. They still use my findings in Albert Heck's lab to study a lot of other things, for example in ion mobility mass spectrometry.'

After the Bijvoet years

'After my graduation I left the Bijvoet Center for a job in industry. That decision was always clear to me and after four years of academic research I missed working on more applied sciences.

What I have learned during the years at the Bijvoet Center is that mass spec (ESI-MS) is a multifaceted tool that allows simultaneous monitoring - differentiating by mass - or even conformation - differentiation by charge - of multi-component species present in solution. It allows the investigation of dynamic features, such as assembly or disassembly, and equilibriums, and therefore opens up the

Conformational Complexity of Complement Component C3b'

Bert Janssen
Crystal and Structural Chemistry

A surface representation of human C3b with its twelve domains colored differently.

possibility to study particular problems that cannot easily be solved by alternative methods.
The application of ESI-MS in structural biology is still in its infancy, but I believe that in the near future it will grow extensively in the direction of more fundamental research towards more pharmaceutical applications.
As an alumni member of the Bijvoet Center I know were to find the people that could provide me with answers to interdisciplinary questions ranging from mass spectrometry to crystallography and NMR. Unfortunately, I think that for others outside the academic world, the Bijvoet Center is not always clearly visible. That is a pity because I think both worlds, academia and industry, can learn from each other and, in general, there are many interfaces for interesting collaborations on both sides.

At my work at Genmab, for instance, I have recently demonstrated the use of ESI-MS in structural biology and applied science. It was great to see how a more academic mass spec application provided clear answers for us at Genmab BV to questions that we could not otherwise directly have solved in such a short time.
At Genmab BV we conduct research in the field of fully human monoclonal antibodies. These are designed to treat cancer and autoimmune diseases. In general, the use of monoclonal antibodies is a very exciting field, which is rapidly expanding and is an open-ended endeavour. I think that within twenty years most cancers and many other diseases can be effectively treated by the use of antibodies or antibody-like molecules in targeted therapies. Also it will be standard practice that patients are being screened before they receive treatment in order to prevent needless medication and undesired side-effects.’

interviews

Advisors

Ad Bax
Emmo Meijer
Ivo Ridder
Bas Leeflang
Hans Vliegenthart

interviews
Advisors

Ad Bax (51) is member of the Advisory Board of the Bijvoet Center. He received a PhD in Applied Physics in Delft. He now heads the Section on Biophysical NMR Spectroscopy at the Laboratory of Chemical Physics, National Institutes of Health, Bethesda.

Sharing the same coffee machine

I never worked at the Bijvoet Center myself, but I have many friends at the Center, in particular Rob Kaptein. I received my PhD in 1981 from the Delft Technical University for work done on the development of new NMR methods, and Kaptein was a member of my thesis committee. He was still in Groningen at that time and clearly had great foresight; realising early on the importance of two-dimensional NMR spectroscopy. Utrecht University indeed made a very clever move by luring him to the Bijvoet Center.

In Delft I was a member of a team that built a novel type, highly versatile NMR spectrometer, that was immediately capable of conducting two-dimensional NMR experiments after they were first proposed in the literature. Kaptein asked us to record such spectra to study hen egg-white lysozyme, a small protein extensively studied by various biophysical methods in those days.

He sent us a sample but, unfortunately, both for him and for us, I was not a biologist, I was trained as a physicist, not a biochemist. So, when the sample arrived as a solid white aggregated substance, instead of the clear solution it was supposed to be, I had no inkling that anything was amiss and simply reported back "very sorry, no signal". If only I had realised that the sample was disintegrated! Rob would have beaten Kurt Wüthrich towards getting high quality two-dimensional NMR spectra of proteins, work that was a cornerstone in earning Wüthrich his 2002 Nobel Prize for determining the three-dimensional structure of biological macromolecules in solution.'

Team efforts

'I visit the Bijvoet Center whenever I go back to the Netherlands. Why is the Bijvoet Center special? It has the perfect set-up: a number of complementary groups that are much stronger together than they would be separately. Communication between different people is the key. For example, it is important to have close interactions with colleagues that are experts in the wide array of modern biophysical techniques, or that can help to generate sufficient quantities of a protein in pure and stable forms. It is also very important to have a common interest, so you are not working "in a vacuum". The Bijvoet Center is stellar in this respect, with outstanding groups not only in protein NMR, but also in protein crystallography, mass spectrometry, protein expression, as well as membrane and glycoproteins. This gives the students unprecedented opportunities to get trained in a vast array of experimental approaches.

In the US we see this much less, and groups tend to be organised in a more individualistic manner. Internationally they remain at the forefront, primarily because of a limited number of very smart people, mostly at the top universities, and usually only capable of addressing a smaller, well-defined question. However, for addressing larger problems, the Bijvoet Center may well be in a better situation than any of the top US groups individually.

I do not believe that Dutch people are necessarily better at collaborating, but it may be the difference in the funding system that stimulates working together. It was apparently mandated by the Department of Education

that only "Centres of Excellence" would get access to the shrinking pool of research funds. So they forced such structures to develop. Although, to many, this transition may have been an unpleasant experience to undergo, I believe it has been a good decision in the long term because it enhances the level of science attainable.
At least in the biological sciences, the future is moving away from smaller, individual efforts to more collaborative work. It is not like it was in the eighties any more. The field is evolving and I expect many of the major future discoveries to result from team efforts.
In that respect, the restructuring seen in the Netherlands appears to be setting the pace.'

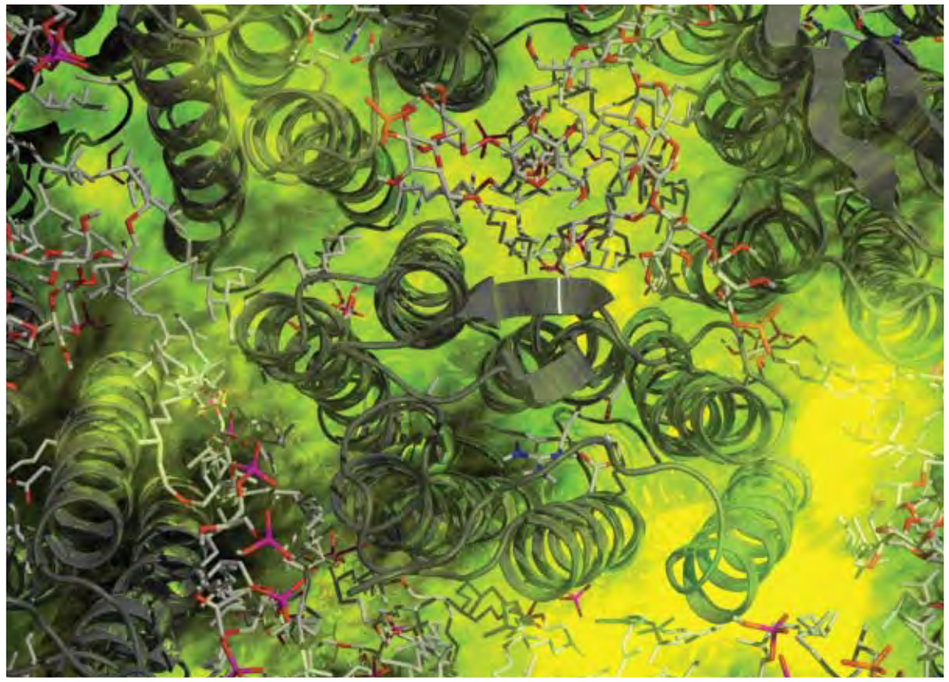
Sharing the same coffee machine

'More and more you see a shift in focus towards solving really major problems. There are about half a dozen major problems, maybe ten, which scream for biophysical input. For instance, signal transduction in cancer, transcription regulation, the role of small RNAs, or protein folding and its failure which underlies diseases ranging from Parkinson's to Alzheimers. The Bijvoet Center could make breakthrough contributions solving some of these puzzles.
In the past we saw a lot of individual technical research contributions. However, with some exceptions, I expect that in the future the recognition will increasingly emphasise what can be solved with technological advances. Developing novel techniques alone, unless they address completely new territory, will not do it. The visibility will largely depend on what you have done with these novel techniques. So I hope the Bijvoet Center, with the ideal position that it has, will be able to do just that. The Bijvoet Center provides a perfect setting for making a major breakthrough that might even lead to a Nobel Prize some day. Why should it not be them? They have got the brains and the set-up.
A theme that joins the individual expertise would be good. It does not necessarily have to be a disease, but you have to consider visibility for funding too. In this light, focus on a disease would be good. On the other hand, the Bijvoet Center is also known for its strength in developing new technology, but that is perhaps harder to get funding for.
Choosing to work on a major theme should come naturally, you cannot dictate that from above. Not because it is not a nice thing to do, but because it will not work. A natural convergence, for whatever logical reason, would be good. A natural close collaboration on a shared problem may be the best way to keep the Bijvoet Center's star shining in the future. They have strong people in a wide range of different areas, and it is hard to choose one topic, but the Center might work on a shared problem. Let's say Parkinson's disease, or viral entry. Study of the whole mechanism, would require membrane experts, crystallography, NMR and mass spec, glycobiology, complemented by a range of other advanced biophysical tools.
In any case, it is critical that different groups talk intensively. The new NMR building does have its down side. It is great architecturally but the NMR group is perhaps a bit isolated. Different buildings always tend to

Salt, saltier, saltiest'

Tsjerk Wassenaar
NMR Spectroscopy

Bacteriohodopsin is a light-harvesting protein found in archaea which thrive best at salt concentrations of 3.3M. This image shows a volumetric density rendering of the distribution of sodium around bacteriorhdopsin and associated lipids during a 25ns molecular dynamics simulation.



be a problem; you might as well put people in different cities. When people are in the same building they share the same coffee machine. That is what makes the difference in the end, the talks with colleagues at the coffee machine, that is where the real science takes place. Here at the NIH we have a yearly retreat. We are forced to go, and every year I hate it, because there is never enough time for taking care of my regular research. Afterwards, though, it always turns out to be helpful. Sometimes it may not be so bad to be forced to do something.'

The next great question

'Structural biology so far has mostly been about detecting structures. The next great question is: how does it work? Interactions between proteins are key in this process, requiring proteomics and mass spectrometry. Understanding how such biology works at the molecular level will require structural input, where in particular NMR spectroscopy is likely to play a major role. In terms of pure structure determination, hopefully either NMR or crystallography will conquer the study of GPCRs. G-protein-coupled receptors are hugely important signalling systems and drug targets. They control virtually everything and there is only a rudimentary model for this class of proteins. I predict that quest will be successful in the next ten years, maybe less.

I can also connect to Albert Heck's vision of a 3D movie of everything that happens in the cell at a molecular level. On the other hand, you have to walk a fine line between imagination and fact finding. So, the task is to find the data that allow us to make such a realistic 3D movie, better than you can currently find on the Internet.

As a Bijvoet Center adviser, once a year I get to see what people have done. Our input is really minimal, I am convinced of that, but we do have some distance, making it easier to develop perspective. I am sure I learn more from being on the board than they get out of me, I get to look into their kitchen, but I do not feel guilty about that at all.

For the Bijvoet Center it is important to get some independent input on how their work is viewed and also how they treat students. For instance, it is quite useful for a student to have a broad training. Sometimes the faculty wants students on a single narrow track, because it takes less time. Particularly at the Bijvoet Center, students have the opportunity to look around and broaden their skills.

Asked about what advice to give young investigators, that is difficult. It is a delicate balance.

As a young investigator you have to trust your gut instincts, be adventurous, but you have also got to be willing to listen to advice. If you are a bright student and you know what you want, go for it, but do not waste four years on something impossible.'

interviews
Advisors

Emmo Meijer (1951) has been Chair of the Scientific Advisory Board of the Bijvoet Center from 2003 until 2007. He studied chemistry at the VU in Amsterdam and got his PhD in 1979. He went on to work for DSM finally in the role of CTO and is now senior vice president of Unilever Foods Research & Development.

PhD students and postdocs are the real workforce

‘My background is industrial but my role as adviser to the Bijvoet Center was to help develop it as a fundamental research institute. I did it just because I am interested in fundamental research and that is why I am still a part time professor. I do not think it is always a good idea, or necessary, to try and relate fundamental research directly to industrial research. Let them just do excellent research. For the industry we also have the TTIs (The Top Institutes) in place.
The Bijvoet Center has a technological infrastructure that a single industry could never afford. We will never buy the same technology. In addition it is not necessary to do the same work. The Bijvoet Center, in that way, can be seen as a support centre for the industry, next to their scientific ambitions.’

NWO Research Schools

‘Apart from my time as Chairman of the Advisory Board, I have also been involved in NWO in different capacities. NWO played an important role in establishing centres of excellence like the Bijvoet Center, organised around expensive infrastructure. I believe that this was a visionary approach, creating a strong infrastructure for fundamental sciences at carefully selected places in the Netherlands. Many research groups are benefiting from it inside and outside the Netherlands. In the case of the Bijvoet Center it was the rapid developments in NMR and its impact on life sciences that triggered NWO to support the establishment of this Center.

Of course, it is not just a matter of infrastructure. People play an even more important role. If I was allowed to mention one name that built the reputation of the Bijvoet Center then it would be Rob Kaptein. He was instrumental in shaping the Center into a European Centre of Excellence.
I believe it is now one of the few centres in the Netherlands which has worldwide recognition. With the NMR that they have now, the high resolution is amazing! In the old days we only had X-ray crystallography. With NMR it is now possible to look at processes in living cells in pretty amazing detail. NMR opened new avenues for many subjects in life sciences. Studying proteins in action is now within reach.’

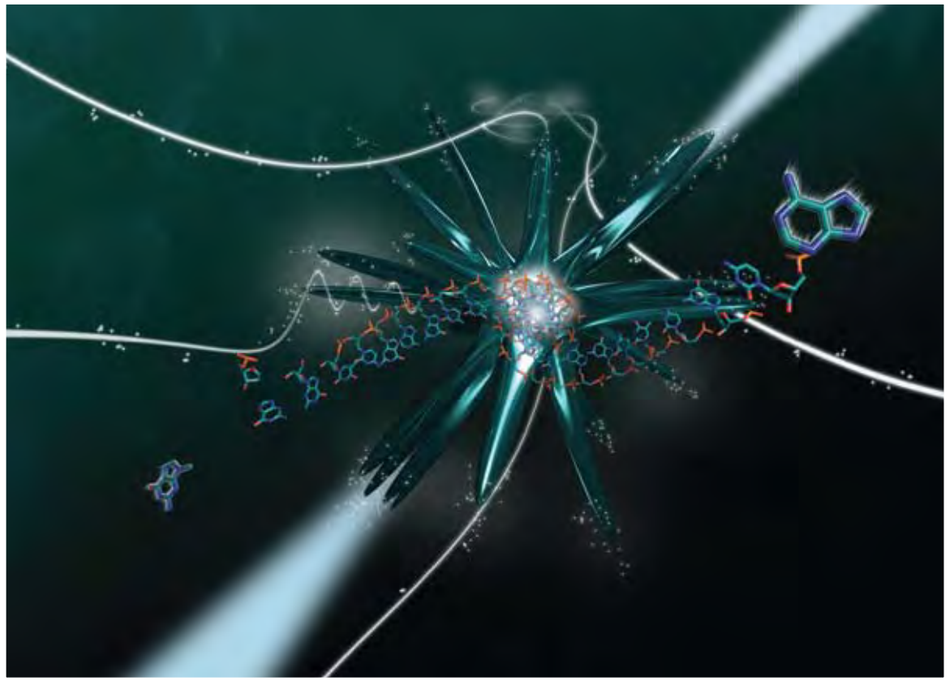
The real workforce

‘The Advisory Board has two main functions. Firstly, to look at the scientific work and to advise the Director about important new subjects in science. The second important activity is meeting the PhD students at the Bijvoet Institute in order to talk about their work. They are only just starting their careers, but do not forget most of the actual research is done by PhD students; and the postdocs of course. The professors influence the choice of subjects, but the PhD students are the real workforce. That is why it is very important to talk to the PhD students. They are brilliant people. They are selected very carefully. What they need is room for their own ideas, but also guidance from senior scientists, to talk to them and to challenge them. That is one of the reasons to create a

DNA: (un)ravelling the double strand

Mark van Dijk
NMR spectroscopy

An artistic impression of DNA double strand formation by the DNA replication machinery.



school around professors who have 'impact'.
In the United States the situation is very different. They have tenure track which is much more focused on individuals. I, however, think it is in the genes of Dutch people to work together. In the Netherlands there is a lot more teamwork than elsewhere. In industrial science you can see a similar pattern. Dutch people like to team up. Teamwork becomes increasingly important because nobody can master all the disciplines and techniques for a certain programme. Nevertheless, it is important to educate your students in a multidisciplinary manner these days. Younger generations are much quicker and much more open-minded than we were in the old days. In my time, as a PhD student, you were not stimulated to work at other departments and to pick up new approaches developed in other disciplines. Generally, professors were the kings and there was a strict hierarchy. This has changed completely.
I think young researchers should be able to do the things they are really excited about. It is so good to have a period in your life to really follow your interest. In those years you make new discoveries, you sharpen your mind, you learn how to write concise papers. These years are the most wonderful period in your life. This period is a tremendous start for many different careers. I went on to work in the industry; I was more interested in management of innovations, but I still build upon what I learned during those four years working on my thesis.'

Holy Grails

'In the next twenty years the equipment will become even more advanced. There will be major breakthroughs there. I am sure the Bijvoet Center will position itself even better in that respect. This may also lead to a regrouping of activities in the same location following the dynamics in the field. It is important that researchers meet each other frequently in an informal way. Brilliant ideas mostly pop up at the coffee machine!
With respect to the direction of the research itself I do not think it is a good idea to focus on one major common problem, like a certain disease. It is true that right now it is a trend to focus on relevance for society and of course it would be good if an important disease would be cured in a couple of decades. However, I think it is important not to overemphasise it. There are many hypes, but they are short-lived. We have to be careful with that; to not focus on a dead end. I know that everybody working at the Bijvoet Center in the back of their minds have ideas how their research could impact society. That is enough.
There are still many holy grails, for example related to the functioning of proteins in membranes. I believe the Bijvoet Center can play an important role here. It has been some time ago since we had a Dutch winner of the Nobel Prize, but I think there is a good chance for it within the next twenty years in areas like this. Do not ask me to mention a name, but I do think it will happen.'

interviews
Advisors

Ivo Ridder

(39) works for the Chemical Sciences division of the Netherlands Organisation for Scientific Research (NWO). He studied chemistry at Utrecht University and received his PhD in 1999 at Groningen University.

A Center of Excellence '*avant la lettre*'

'I am involved with the Bijvoet Center in two ways. First of all I have spent some time here when I was a graduate student. Secondly, at the moment I am involved at a different level because of my work for the Netherlands Organisation for Scientific Research (NWO). As a graduate student I carried out research at the Bijvoet Center for a year and half; I graduated in 1993 under Professor Jan Kroon.

During that period I was also a student member of the chemistry group research board. An important issue at that time was the decision to expand our research field. Up until then, we mainly studied organic compounds, but we considered starting a new research line studying protein structures. It was a much discussed topic, not everybody was convinced this was necessarily the best strategy for the future. Finally it was decided to appoint Piet Gros as Professor of Crystallography.

I think that was a crucial decision, otherwise the whole group might not have survived to this day. Other universities that did not make this step do not have an independent crystallography research group any more. They only use crystallography as an analytical method.

I received my PhD at Groningen University. At that time it was the only place to go if you wanted to do protein crystallography. Nowadays there are four or five institutes that have a viable crystallography group. The Bijvoet Center has become very good at it.

At the time the Bijvoet Center was founded in collaboration with SON, the Dutch Foundation of Chemical Research, a part of NWO. The Ministry of Education, Culture and Science provided extra research money for establishing research centres. So by reorganising into such a research centre it was possible to make a claim for this extra money. That is one of the reasons the Bijvoet Center was founded twenty years ago. They did manage to get a lot of extra money. The fact that they managed to get an excellent NMR scientist, Rob Kaptein, to move from Groningen University to Utrecht also contributed to this. That really was a spectacular transfer in the scientific community, comparable to current transfers in soccer.'

The Bijvoet Center in international context

'I have been working for the successor of SON – now called the Chemical Sciences division of the Netherlands Organisation for Scientific Research - for eight years now. Through my work I have come to know Albert Heck and his colleagues quite well. If he needs something or has a question he calls us directly, just like his other chemistry colleagues. In the chemistry field, especially, there is close contact between NWO and the chemistry professors. Of course we cannot always indulge every wish or request, but we do try to help as much as we can.

Although these research centres were founded partly for political and financial reasons, this concept has certainly proven to be very successful. These institutes have an advantage when applying for grants, because they have the right infrastructure. Several institutes need very expensive equipment, but the Bijvoet Center has generally been more successful in actually getting the funding. For example, the NMR equipment has partially been paid for with money from NWO investment programmes. This is the outcome of a competition with other institutes of course, but it helps that the Bijvoet Center has an excellent infrastructure because of its combination of top scientists and already well-equipped laboratories. With regard to structural biology, the Bijvoet Center is unique, they are very complete, no other institute can compare to this.

So the Bijvoet Center has been very successful so far. In the future I think the general trend will be to have scientists working together on the same overall question, tackling it from their own disciplines. I do not know whether that will happen at the Bijvoet Center as well, but it is certainly a sensible approach. You just cannot do it on your own. Nobody can master every technique available. If your Institute has the right equipment it will be attractive for visiting scientists and other faculty members who are working on interesting subjects. That gives room for collaboration projects. Collaboration is a must nowadays; almost all good publications are joint efforts from different groups.

This trend to collaborate increasingly occurs at an international level. I would guess that all scientists, one way or another, collaborate at a European level. The same holds true for NWO itself, we also collaborate with partner organisations from other member states, although this does not yet have a major impact. Apart from that, the European Union also has its own research programmes, and recently the European Research Council has been founded. They have started with a talent programme comparable to the Dutch NWO Vernieuwingsimpuls and they are also setting up a special programme for professors. In terms of funding, we are talking about millions of Euros, but of course this is meant for all member states of the European Union. In the first round of the programme, for young investigators, they have made available three hundred million Euros. This may seem a lot of money, but there are about 9,000 applications. So there is a lot of competition. Due to this, I think young chemical researchers in the Netherlands have a better chance for funding by applying for a so-called NWO Vernieuwings grant.

Also, applying for European grants is not easy. There are a lot of conditions for collaboration with other member states. For instance, you have to prove that international collaboration has an added value. That is a problem when you are one of the best European institutes in a specific field. With what other group would collaboration add something to the proposal?

At the Bijvoet Center, the NMR group is a very successful European Large Scale Facility. They have many visiting scientists that come here to do their experiments. That is possible because they have not only the right equipment, but also the scientists to push this equipment to the limits. Also, the Netherlands Proteomics Centre is something to be very proud of and do not forget that Ton Spek and Piet Gros are both in the top 50 of most cited chemists worldwide.'

Science policy

'In the Netherlands, a student can study chemistry at ten different universities, but until recently, the number of

chemistry students was getting smaller every year. That is not an ideal situation, neither for education nor for research. It makes it difficult to maintain the best research groups.

The so-called 'Focus and Mass policy' of Utrecht University means that we want to concentrate on a large enough number of top scientists, equipment and support to be able to work on important topics, and at the same time to be able to give students a good education.

Another issue in the Netherlands is the strong coupling of research and education. As a result, excellent research groups may have to close down because their faculties do not have enough new students. At the same time, all universities decide individually which new research lines they want to start, which research lines they want to keep and which ones they want to end. From a national point of view this is an undesirable situation. Universities should communicate about these kinds of decisions, but they do not always do so because it is not in their own direct interests. To solve this problem two parties involved, the Regiegroep Chemie and the various university boards, have made a national proposal to strengthen the knowledge infrastructure. This proposal fits very well within the NWO chemistry strategy and all parties involved are trying hard to get the proposal funded. At this moment the plan for the chemistry field identifies around twelve different research areas that universities should focus on. For instance, structural biology, but also research into catalysis, theoretical chemistry and industrial biotechnology. Universities have to choose together which of these subjects they want to continue doing research on, and then will receive support to further develop that area of research. Within a research topic you then create enough mass, meaning you must be big enough in terms of people and infrastructure, to be able to make a real difference in the field. I think the founders of the Bijvoet Center realised this already twenty years ago and I expect they will stay at the forefront of science in the years to come.'

Bijvoet Medal Awards

Dr. Jack D. Dunitz [1]	October 27, 1989	Chemical Crystallography
Dr. Brian R. Reid [2]	October 27, 1989	NMR Spectroscopy of Biomolecules
Dr. Nathan Sharon [3]	October 27, 1989	Chemistry of Carbohydrates and Glycoconjugates
Dr. Hartmut Michel [4]	October 27, 1989	Molecular Membrane Biology
Dr. Isabelle L. Karle	October 27, 1989	X-ray Crystallography
Dr. Binne Zwanenburg	February 21, 1991	Founding of the Bijvoet Center
Dr. Joachim H. Seelig	September 23, 1991	Macroscopic *in vivo* NMR Spectroscopy
Bijvoet Family [5]	March 2, 1992	100th Birthday of Dr. J.M. Bijvoet
Dr. Ad Bax	January 6, 1993	Development of Nuclear Magnetic Resonance
Dr. Hans Paulsen	November 10, 1993	Carbohydrate Chemistry
Dr. Fred McLafferty	March 6, 1997	Mass Spectrometry
Dr. Ivano Bertini	March 25, 1998	NMR of Paramagnetic Proteins
Dr. Brian T. Chait	March 21, 2000	Biomolecular Mass Spectrometry
Dr. Hans F.G. Vliegenthart	March 21, 2000	Founding of and dedication to "Bijvoet"
Dr. Nicolaas Bloembergen	November 2, 2001	Nuclear Magnetic Resonance
Dr. Chris Dobson	March 28, 2002	NMR Spectroscopy and Protein Folding
Dr. Rod MacKinnon	April 16, 2004	Structural Studies of Ion Channels
Dr. Rob Kaptein	June 14, 2006	NMR Spectroscopy and dedication to "Bijvoet"
Dr. Rien de Bie	February 22, 2007	Founding and maturation of the Bijvoet Center
Dr. Wolfgang Baumeister	April 7-8, 2008	High Resolution Electron Microscopy
Dr. Bernd Bukau	April 7-8, 2008	Molecular Chaperones
Dr. Patrick Cramer	April 7-8, 2008	Structural Basis of Gene Transcription
Dr. Alan Fersht	April 7-8, 2008	NMR Spectroscopy and Protein Folding
Dr. Tony Kouzarides	April 7-8, 2008	Epigenetic Regulation of DNA Transcription
Dr. Matthias Mann	April 7-8, 2008	Proteomics and Signal Transduction
Dr. James C. Paulson	April 7-8, 2008	Functional Glycomics and Chemical Glycobiology
Dr. Kurt Wüthrich	April 7-8, 2008	NMR Spectroscopy of Large Biopolymers

interviews
Advisors

Bas Leeflang (44) is Managing Director of the Bijvoet Center and responsible for getting funding.

The burden of paperwork

'As a student I did crystallography experiments, my PhD was on carbohydrate NMR research. I have always been interested in the relation between 3D structures and how they bring about certain properties and functionalities. For instance, why is a cellulose fibre as strong as it is, or why is wood both strong and bendable.
After my PhD, I wanted to continue in science. When you really want to have a career in science it is an unwritten law that you should spend some time abroad. Due to personal reasons I could not do this, which more or less cut off my opportunities of becoming a group leader.
I still a had strong interest in what was going on in science, and wanted to stay involved. That is how I started to work with Rien de Bie, who was Managing Director at that time, and became an expert in European research funding. I remember many long car journeys with Rien. He was always "flying low" with his car, very fast and for long stretches. Rien and I made a sport out of reviewing all funding possibilities. We would see what would fit in with the Bijvoet Center, consider the deadlines for the calls and see whether we could find external partners to form a team to write a proposal with.
Looking for funding always involves a lot of paper work. As Managing Director it is basically my task to take away this burden from the group leaders so they can focus on their research.'

Reading between the lines

'To get the money you have to understand what funding agencies really want. It is not enough to just read the information on their website; you have to be able to read between the lines. All funding agencies have their own agendas. You should realise what the true objectives are of a particular funding agency to give away its money.
For instance: just writing good scientific proposals works with NWO calls, which are really focused on qualitative research only. It requires a different approach when applying to SenterNovem, for funds from Economic Affairs, or to the European Framework Programmes. In general, their agenda is that the research should be good for the economic situation in Europe or in some way beneficial to European citizens. That means health related proposals have a good chance of succeeding. Especially if you can demonstrate that, in the end, European companies will also benefit because of the possibility to sell tests for techniques used in the hospital to diagnose certain diseases.
Of course, research is always a challenge and we cannot guarantee what the outcome will be. However, we do know what we are good at, and we can make an estimate of what we think might be possible in due time.
The European Commission itself also publishes calls for research projects. Sometimes these calls are very specific, other times they are quite open. Method development for large scale analysis of biological relevant questions is an example of this, anyone can sign in for this. However, if they have a call for some special synchrotron project, of course it is only worthwhile if you actually have a synchrotron available.
Comparisons between Europe and Japan or the United States are always a good argument for funding,

because Europe has the goal to be the most competitive community in the world. That is the Lisbon agenda. For infrastructure (research facilities), the link to economy is further away. The European goal is to provide access to the best research facilities for European researchers. So in this aspect the key question is whether you are a top facility and whether providing access to this facility will help solve key research questions. That is one of the tasks that you have to be able to put on paper convincingly.'

Raw data sharing

'Apart from my funding work, I am scientifically involved in two major projects. One is the FP6 Marie Curie Research Training Network GlycoGold in which the potential application of gold glyco nano particles is being investigated. These particles are just like Lego building bricks, you can create them in any size or shape you want. They can be used for vaccines, or as inhibitors for bacterial adhesion to prevent infection. I am managing this scientific research project and help people conduct the NMR experiments.

The other project is a design study for a research infrastructure we started in 2005. That is a project I am really proud of. It is called EUROCarbDB and focuses on preserving and making available the raw experimental data, that now often gets lost, for the scientific community after publication. This provides an efficient use of resources and scientific transparency.

In carbohydrate research, HPLC, Mass spectrometry and NMR are key experimental techniques for the analysis of the primary and 3D structure, dynamics, and interactions of glycans. Only the results, which are the end product of all the analyses, get published, along with some nice pictures to show that you really did some NMR or mass spectrometry. Nevertheless, a few years later you might want to return to the original data, for instance because somebody has challenged your research. Possibly also because a new analysing method has been developed, and you would like to use this new method on your old data to see if you can get more information out of it.

On the hand, this database is a framework with tools for later analysis and data storage, which you can do locally at your own laboratory. But after your research has been published and there are no hot secrets any more, you can also team up with the European effort and make the data more broadly available by depositing the available data, locally or in a central database. That way someone from the outside can have a closer look at the original experimental data.

Raw data-sharing between scientists does not have to be a problem. In the end, people want to share data. Not when it is 'hot', of course, but at some stage, either when it is being submitted for patents or publication, it becomes clear that it is your invention or your interpretation of this sample. Once this is done often there is no longer a strong need to protect the underlying data.

For this project, implementation is the key issue right now. In 2006, we had a meeting at NIH, with Japanese, European and American representatives. There is a need for standardisation and common exchange formats so communication between databases becomes possible, whatever the structure of the local storage database.

Future

'At the Bijvoet Center in the future, I would like to see even closer interaction between the biological groups and the instrumental groups in defining and trying to answer research questions. Obviously there is always a clear joint element that binds us: the question of how, on a molecular level, things work in the cell or between cells. The topics are very broad indeed, and that is okay, but with more coordination the Bijvoet Center groups could benefit more from each other.

How to make that happen? I would not favour a model with an omnipotent director who tells all scientists what subject they should work on. When we hire group leaders we aim for scientific excellence. They are responsible for doing top research in their own discipline. I am convinced that enabling frequent scientific and informal interactions, and the notion of being part of a community, which the Bijvoet Center is, will result in the optimal coherence.'

interviews
Advisors

Hans Vliegenthart (71) is Honorary Professor of Bio-Organical Chemistry. He was Dean of the Faculty of Chemistry from 1985 until 1989, Scientific Director of the Bijvoet Center from 1988 to 2000 and played a crucial role in the foundation and realisation of the Bijvoet Center.

The vision behind a successful institute

I have always been interested in the structure of proteins and glycoproteins. For the latter class, my interest lies especially in their carbohydrate chains. During my time at the Bijvoet Center there were no methods available to gain unambiguous answers. In those days it was quite a challenge to determine the structure of carbohydrates! Due to my interest in NMR spectroscopy, I have exerted myself to make sure that we had the best and technically most advanced equipment available at the Center. We have probably had all generations of NMR machines at our lab at some time, with the aim of always trying to get ahead. Finally, with these advances, the resolution was good enough to be able to get some meaningful results on these carbohydrate structures. Importantly, it is not just a question of having the right equipment, no way! You have to have the right mind set to achieve this. You have to be convinced that it is possible, even when everybody else says it will never happen. In 1976, a very talented PhD student from the Faculty held a presentation at the FEBS conference in Copenhagen. The chairman commented on the presentation: 'This is impossible!'. A year later he had to admit we had been right all along, and you have to bear in mind that he was an NMR spectroscopist himself. Our paper showing that it was possible to use NMR to solve these carbohydrate structures was my biggest scientific breakthrough. Not only did it create a huge breakthrough in understanding the structure itself, it also showed people how to deduce the biosynthesis of carbohydrate chains. That was undoubtedly the most striking moment in my scientific career.'

Cutbacks

'I became Dean of the Chemistry Faculty in 1985. To be honest, that was not a very nice period. This was primarily because of huge financial cutbacks on scientific research at the time which meant that we had to selectively redistribute the funding and organise a new allocation of tasks. Those were hard days for the Chemistry Faculty. Some people were even forced into early retirement. The only advantage was that, at that time, the social arrangements were not as bad as they are today, but I recall it as a disastrous time. My predecessor had started this process and it was incumbent on me to finish it.
The question then was how to deal with what was left? We had to make a new start. It had always been clear to me that structural biology was going to be an important future research field. I went to the Board of the University and told them: 'If you want to do something to make up for all these cutbacks, then make sure that all of the analytical chemical methods that we are already good at, are organised into a strong scientific research infrastructure'. My argument was to focus on fundamental techniques, not for the sake of the technique itself, but aimed at advanced applications, focused on biomolecules. I wanted X-ray analysis, mass spectrometry and especially NMR at the highest level.
The President of the Board agreed with investing in NMR on one condition: I had to get the best NMR spectroscopist from the Netherlands to come to Utrecht. The next day I went to Groningen to talk to Rob Kaptein. At first he tried to use our offer to improve his position at Groningen University, but when they would not budge

Kaptein finally moved to Utrecht. That was an enormous success for me and the Faculty.'

The Bijvoet Center

'One day the Board of the University released a plan to organise the research in so-called 'Zwaartepunten' (Centres of Excellence). They understood they had to concentrate scientific research within special infrastructures. That provided me with a great opportunity. It meant that I could realise my earlier plans, to join the scientists involved in structural biology together into one research centre. So this is what I suggested to the Board .

Immediately we faced our first problem. When you want to start a group that needs that much heavy equipment, this is nearly impossible to finance for an individual university. We needed extra money! In those days, institutes that were not embedded within a university but carried out scientific research, could get extra funding from the Ministry of Education. They had a special programme for these so-called 'para university institutes'. SON (part of NWO, the Netherlands Organisation for Scientific Research) stepped forward with a solution to this problem. They proposed the possibility of entering into an agreement with the universities to found special institutes, that would be jointly funded by SON/NWO and the universities themselves. The Minister of Education agreed with this construction, and that is how the Bijvoet Center came into being.

SON, of course, had its own demands. They knew I wanted mass spectrometry, but again in order to get it, the proviso was that I had to lure the best Dutch professor in mass spectrometry to Utrecht. I discussed this with the then Amsterdam-based professor and we agreed terms for his move. However, he cancelled the move at the last moment because he felt too attached to the city of Amsterdam and did not want to leave.

I had to bring this bad news to SON. Their position was that without this particular professor we should propose a different field of research. Our first suggestion was *in vivo* NMR, and fortunately SON encouraged the idea and we were able to start. The final missing piece of the puzzle was a name for the new institute. The chairman of SON, Zwanenburg, suggested the name Bijvoet Center. We immediately liked the name, and so did the Board of the University. Bijvoet had been one of the most successful chemistry professors in Utrecht. Bijvoet is a well-known name and therefore easy to remember, which is very important. When we called the Ministry, just mentioning 'Bijvoet' was enough for them to know who we were. Scientifically the Bijvoet Center had everything going for it. It was very successful in all fields.

Initially, the only part still missing was mass spectrometry, but people just did not think it was necessary. I kept nagging on and on about it, but all I got was half a chair which was not enough. Then things changed, because the Pharmacy Faculty also wanted to spend money on half a chair. We decided to combine forces and put out a job advertisement in all of the major scientific journals. We got many reactions, but none of them were any good. It seemed that either people were using the negotiations to improve their position at their own employer's, or they

Bijvoet-centrum voor moleculair onderzoek

Samen met de stichting 'Scheikundig onderzoek in Nederland' (SON) wil de Utrechtse universiteit een para-universitair instituut voor moleculair structuuronderzoek oprichten. Dit 'Bijvoet-centrum' zal diensten verlenen aan instellingen met verwant onderzoek. De samenwerkingsovereenkomst zou op 1 april 1988 in werking moeten treden.

Eén van de belangrijkste redenen is dat de stichting SON dan bij het ministerie van Onderwijs en Wetenschappen voor de Utrechtse onderzoekgroepen een bijdrage kan vragen uit het Intentioneel Apparatuurschema. Voor de periode 1989-1992 zal het centrum rond 8,5 miljoen gulden aan apparatuur claimen, waarvan 4,1 miljoen in 1989.

De plannen van de faculteit Scheikunde voor dit centrum dateren van 1986. De stichting SON geeft al jaren aanzienlijke bijdragen voor het internationaal gezien uitstekende onderzoek van een cluster samenwerkende groepen. Zij houden zich bezig met de moleculaire structuur en reactiviteit van chemisch en biologisch belangrijke verbindingen en systemen, en zoeken naar nieuwe methoden om die te bepalen. De leiding van dit onderzoek hebben de hoogleraren dr. R. Kaptein, dr. J. Kroon, dr. B. de Kruijff en dr. J.F.G. Vliegenthart. Laatstgenoemde kwam onlangs in opspraak om de manier waarop hij zijn taak als decaan waarneemt.

De grondslag voor het onderzoek legde tussen 1939 en 1962 prof.dr. J.M. Bijvoet, die wereldfaam verwierf met het gebruik van röntgenstralen om kristalstructuren te bestuderen.

Verwacht wordt dat de stimulering van dit onderzoek zal leiden tot essentieel nieuwe vindingen, die van invloed kunnen zijn op de levenswetenschappen en op maatschappelijk belangrijke terreinen, zoals de fijnche mie, de farmacochemie en de biotech nologie. b.k

Het laboratorium voor kristalchemi aan de Catharijnesingel, dat voor d nieuwbouw van Hoog-Catharijn werd afgebroken. Prof.dr. J.M. Bij voet woonde naast en werkte in di 'Kristalpaleis'.
Foto J.W. Hamminga, rond 1968

<< NRC Handelsblad – 1988

<< Rien de Bie

just were not suited for the job. We had already promised the Board that we would get someone very good to fill the chair.
Then out of the blue I received a call from Albert Heck. He told me he had received his PhD in Amsterdam, done postdoctorate work in the United States and was currently holding a position in the UK. He was excited about the challenge of the position but was worried that his youth might prevent him from being considered for the role.
I did not hesitate for a moment and told him he should apply immediately. He visited me for an interview and won me over instantly. Of course that did not mean the rest of the committee felt the same way! In the end, and after some persuasion on my part, we managed to agree to hire Albert. He has gone on to become a great success. The mass spectrometry section is really doing a fantastic job, and I could not imagine a better choice.'

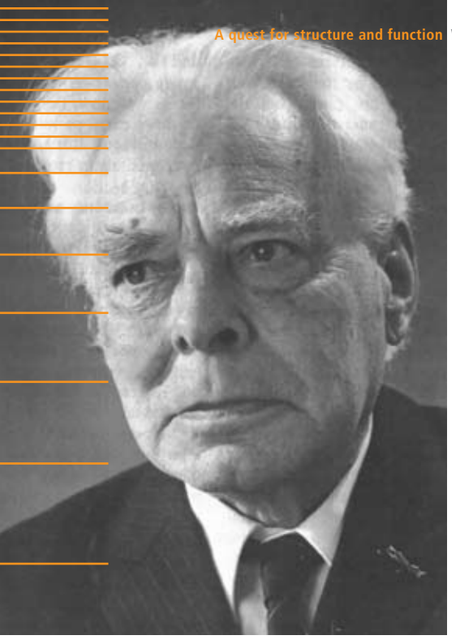
Prima donnas

'Nearly all successful scientists at one time or another develop some prima donna tendencies. This often means they will try to mark out their territory and are not always willing to cooperate for the greater good. People want the benefits of all facilities available, but they also have a posture of: "Don't meddle with me, and don't tell me what to do or whom to cooperate with".
When you have an institute with strong personalities it brings disadvantages along with the benefits. The 'personalities' are not always willing to put energy towards the institute as a whole. I would not say they are only in it for the benefits, that would be exaggerating, but it does play a role sometimes. I suspect that it is only human and certainly in some cases that is just the way it is. Being a Director, however, means that this is a factor that needs careful consideration and it demands some delicate manoeuvres.
In general I can say we handled that very well. The recognition for our early success should particularly go to my Managing Director at the time, Rien de Bie, who sadly passed away much too early and is missed very much. I could never have done it without him. He was an extremely obliging and very intelligent man, with a fine understanding of the organisation as well as the people. In addition to all that, he was also fantastic at writing funding proposals. I came up with the ideas, but Rien took care of the implementation. The Bijvoet Center would have been founded anyway, but thanks to Rien it became the flourishing institute it is today. He was the one that got us funding from the European Union. He made sure that, despite the opposition from some group leaders, we got in touch with the people in Brussels. At first nobody believed that it would do us any good. Commonly the advice we received was along the lines of: "Don't go there, it will never work, it's one big bureaucracy, you'll never be able to get through all the red tape." Rien proved these people wrong and today his worst sceptics are amongst the biggest winners from our approach for European facilities.
A large amount of the Bijvoet Center is funded by European money. When we wanted a 900 MHz NMR machine, the Ministry of Education told us not to expect them to pay the entire bill. We managed to purchase the machine with extra support from the EU. You have to think across the border on an international level in order to get these kinds of things done.'

<< Signing the memorandum of association of the Bijvoet Center on 25 March 1988. Left to right: Binne Zwanenburg (president of SON), Jan Veldhuis (president of CvB, UU).

Management philosophies

'At some point the Minister wanted to organise all excellent research into special 'Onderzoeksscholen' (research schools), that had to be approved by the Royal Netherlands Academy of Arts and Sciences. This was a change that was right down our alley. We were used to writing good proposals which connected different research fields. The Bijvoet Center was one of the first to be recognised as research school. That was a piece of cake for us but a great success. I am really in favour of research schools. This is because of the unique combination of the right people and the right equipment. It means that you can tackle the most challenging problems. Therefore, I think it is a pity that the Board of the University now wants to combine research schools to form one big graduate school. It is an unfavourable development that will lead to loss of identity. Things are going very well as they are and I do not think that there is an obvious win with regard to the scientific research. It is of course nice to profile yourself to the outside world like this, but when you look at the content and its true coherence, it is a rather disappointing development. The university tends to change their management philosophy every ten years. First centralisation, then decentralizations and then vice versa again. Now is the time for extreme centralisation. Everything that feels like a boundary has to be broken down. The Chemistry Faculty is gone, the Subfaculty is gone. It is a department now. This integration has gone on much too far. As a result, people in all sorts of positions are becoming increasingly unhappy. One of the attractions of working at the Chemistry Faculty was the feeling of a shared community, but the changes that have been implemented stifle this. It has all transformed into one big 'Bèta Science' Faculty.
I would like to make a plea to maintain the research school and the Bijvoet Center as separate entities. Especially because they are very well equipped to address the challenges in science for the next ten years. It is all so very coherently organised; I cannot see how integrating it with other units will yield any benefits. All the useful connections are already there. Albert Heck, for instance, not only cooperates within Utrecht University, but throughout the whole country. The NMR spectroscopy functions as a European facility. Where is the profit in removing the boundaries between research schools? I am surprised that the Board have continued this process of centralisation, while already our society as a whole is developing in a completely different direction. I, however, do hope for the best and wish that the Bijvoet Center will continue to play a major role in biomolecular research for years to come.'



Bijvoet, a name to be proud of!

The Netherlands Foundation for Chemical Research (SON) and Utrecht University founded a joint research institute on 25 March 1988. The objective of this collaboration was to create a research and expertise centre in the area of structural biology with an internationally acknowledged staff and access to an advanced instrumental and computational infrastructure.

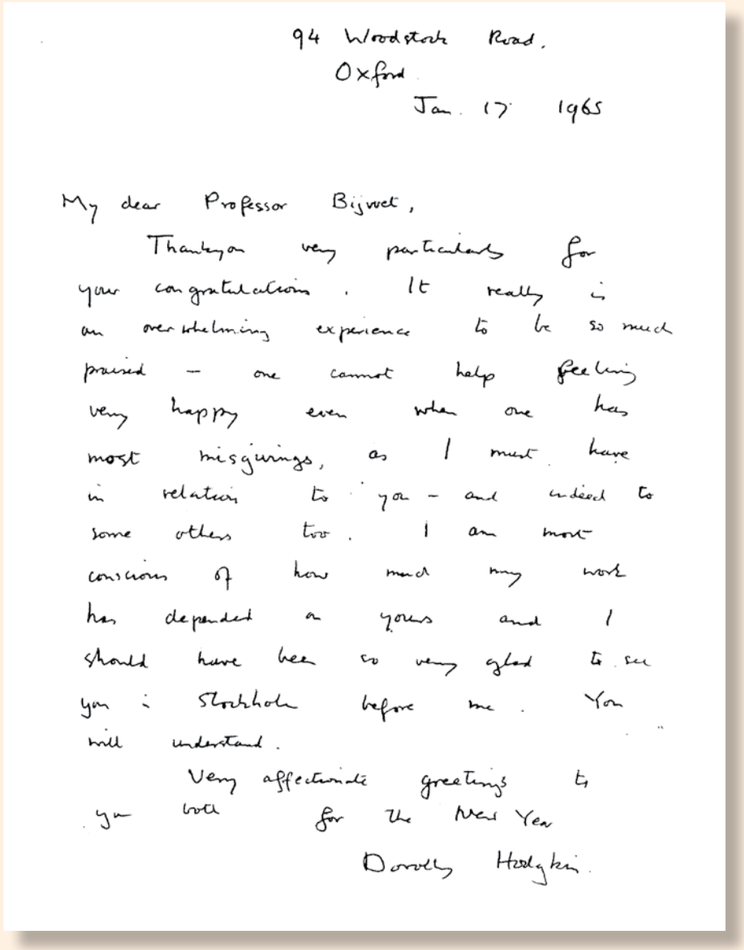
Johannes Martin Bijvoet (1892-1980) is the name-giver of the Bijvoet Center for Biomolecular Research. The choice for the 'grand master' in crystallography, eight years after his passing away, is a tribute to one of the most reputable scientists in his field.

Professor Bijvoet

In those days of classical hierarchical professors, Bijvoet was no exception. Many, however, remember his inspirational approach to research. His international network, broad background - from quantum mechanics and thermodynamics to crystallography - and perseverance made him a great motivator of his staff and student researchers. British Nobel laureate Dorothy Hodgkin (1964, Chemistry) declared in a personal letter to Bijvoet that she would not have received this ultimate acknowledgment without his results and would have wanted Bijvoet to receive the Nobel Prize before her.

In 1952, his laboratory was established at the Faculty of Chemistry in the so-called 'Kristalpaleis' (Crystal Palace) at the Catharijnesingel in Utrecht. Unfortunately, a lack of funding prevented him from setting it up to his standards. To partly overcome this, Bijvoet bought the back-rooms of the house and moved in with his family. In the fifties, Bijvoet acquired Utrecht University's first computer, the 'ZEBRA'. This meant a great break-through for the research in crystallography. Researchers from Mathematics and Physics were also keen to use this modern instrument.

Many remember his teaching as very intriguing, but completely incomprehensible. His research students were writing frantically in the lecture-room while later, in the labs trying to understand what the professor had said. As a high-class professor, Bijvoet wanted the best for his research. He was always very critical and controlling. This created several quarrels with fellow professors. However, people nowadays remember him as a very kind man, an



94 Woodstock Road,
Oxford
Jan. 17 1965

My dear Professor Bijvoet,

Thank you very particularly for your congratulations. It really is an overwhelming experience to be so much praised – one cannot help feeling very happy even when one has most misgivings, as I must have in relation to you – and indeed to some others too. I am most conscious of how much my work has depended on yours and I should have been so very glad to see you in Stockholm before me. You will understand.

Very affectionate greetings to you both for the New Year

Dorothy Hodgkin

<< Letter of Professor Dorothy Hodgkin to Professor Bijvoet on occasion of receiving her Nobel Prize in Chemistry 1964 for her determinations by X-ray techniques of the structures of important biochemical substances.

absent-minded and quirky professor: as a music lover he preferred staff that could play musical instruments. Music, preferably Mozart's, was always being played at his office and lab, and staff members held quartet evenings. Even at the end of his active career, he remained very critical. Potential PhD students were queuing up to work in his lab. Although he did not himself carry out any experiments in the lab anymore, his staff still needed to explain every step they took in their investigations. The lab, therefore, had taken over his critical attitude.

After his active years in the 'Kristalpaleis', Bijvoet still kept in touch with the latest studies. Once every month, he would come in, and the students that had their office in Bijvoet's former bedroom, had to clear their belongings and leave immediately, to make room for the professor. No changes were allowed in the laboratory, no instrument or library moved without his consent. He dedicated the years after his retirement to putting all his data in historical overviews, which are still very useful today. Bijvoet will always be remembered as one of the most famous scientists both in Utrecht and the Netherlands.

With many thanks to Professor Ton Spek and Dr. Loes Kroon-Batenburg.

The present Bijvoet Center

Research in the Bijvoet Institute focuses on the relation between the molecular structure and function of biomolecules involved in recognition, interaction, and regulatory processes. The Bijvoet Institute integrates technology-driven innovations with biochemical/biomedical relevant research themes. This approach requires the close collaboration of scientists with backgrounds in chemistry, biology, pharmaceutical sciences and medicine. With our cross-discipline research we wish to contribute to a better understanding of life processes at the molecular level, enabling contributions to public health and disease therapies.

Utrecht University

Utrecht University was founded in 1636 as a continuation of a medieval convent school. Located in the heart of the Netherlands, it is firmly founded on tradition. The university, which recently celebrated its 370th anniversary, has developed into one of Europe's largest institutes of research and education. Utrecht University offers a wider range of subjects than any other university in the Netherlands. The life sciences are well represented in Utrecht, and successful collaborations between the life sciences at the different faculties are abundant. Focal areas of research and education in which members of the Bijvoet Center participate are the Academic Biomedical Centre (ABC), the Centre for Biomedical Genetics (CBG), and the Netherlands Proteomics Centre (NPC).

NWO-CW

The Council of Chemical Sciences of the Netherlands Organisation for Scientific Research, (NWO-CW, formerly SON), promotes scientific research in the chemical sciences at Dutch universities by, amongst other thing, furthering national research institutes and facilities. The Bijvoet Center was founded with support from NWO-CW and continues to receive support for its activities. The support consists of NWO-CW contributions to two national facilities housed within the Bijvoet Center. The NMR section hosts the European SON NMR Large Scale Facility (SON NMR LSF), which provides access to state of the art NMR equipment and expertise both for national and international collaborators. The Crystallography section provides access to crystal structure elucidation for national research teams through the X-ray Participation Project. Approximately 3 researchers within the Bijvoet Center benefit from NWO grants. NWO has also contributed to the acquisition of costly state of the art equipment, like NMR and Mass spectrometers, X-ray diffractometers and Surface Plasmon Resonance equipment.

<< The interior of the 'Kristalpaleis' at the Catharijnesingel in Utrecht.

Quest for structure and function

At its 5th and 10th anniversaries, the Bijvoet Center published an overview of its activities under the title "The Quest for Structures". At the 20th anniversary of the Bijvoet Center for Biomolecular Research this quest for structures is still going strong. Over the years, the challenge of understanding structure and function relations has become increasingly achievable. In the mean time public interest in the structure and function of biomolecules has grown considerably. Genomes of human and other species have been established. With all human genes mapped, and more and more genome sequences from other organisms being produced, the existence of a great many proteins can be predicted. However, knowledge of the amino-acid sequences of proteins is still insufficient to properly understand their function. For an assessment of function, the three-dimensional structure, chemical modifications like glycosylation and phosphorylation, the localisation of a protein (cytosolic, membrane-associated), and the interactions with other biomolecules provide important leads as well. With the availability of complete genome sequences, we have entered the era of functional and structural genomics. The key topics now relate to the unravelling of interacting protein networks. The research

<< **First Bijvoet Symposium, 27 October 1989. First row, left to right: Prof. Jan Kroon, Mrs. Bijvoet and children of Prof. Bijvoet. Prof. Vliegenthart chairs this meeting.**

approach of the Bijvoet Center for Biomolecular Research has traditionally combined structural and functional studies. An advanced and integrated infrastructure with respect to staff and equipment renders the Bijvoet Center particularly well equipped to address the challenges of this new era. Within the Bijvoet Center, several research teams aim their efforts at the elucidation of biomolecular structures. For structural studies, the Bijvoet Center has access to in-house X-ray diffractometers and to a beam line at the European Synchrotron in Grenoble. Advances in Crystallography will permit the real-time observation of chemical reactions at protein surfaces. Mass Spectrometry is employed for structural characterisation of biomolecules in relation to their biological function. High-resolution NMR spectrometers with proton NMR frequencies ranging from 360-900 MHz allow structure determination of small to medium size proteins and other biomolecules. NMR spectroscopy and molecular dynamics studies allow for the assessment of dynamic functional interactions between biomolecules.

Other research teams employ a wide variety of biochemical and molecular biological techniques in addition to structural studies to gain further insight into the various functions of and interactions between biomolecules.

Research themes at the Bijvoet Center

Biomolecular recognition, interaction and regulation processes:

- The regulation of gene expression
- The role of post-translational modifications
- *In vivo* and *in vitro* protein folding; the role of molecular chaperones and folding enzymes
- Protein ligand binding and interactions at receptors
- Biomembrane organisation and dynamics
- Structural and functional glycomics / lipidomics

Development and application of methods for structural and biochemical characterisation:

- NMR spectroscopy
- X-ray diffraction
- Mass spectrometry / Proteomics

Directors of Research

Hans Vliegenthart	(1988 - 2000)
Rob Kaptein	(2000 - 2006)
Albert Heck	(2006 - present)

Managing Directors

Rien de Bie	(1988 - 2005)
Janneke Schepers	(2005)
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Keith Wilson, University of York, United Kingdom

Biography of Johannes Martin Bijvoet

1892	Born in Amsterdam on 23 January
1908	Graduated from HBS (secondary school)
1910	Started Chemistry studies at the University of Amsterdam
1914	Graduated 'with distinction'
1914-1918	Served during WW I, at the same time studying the work of Gibbs thermodynamics and static mechanics
1919	Passed doctoral exams 'with distinction'
	Started working at the laboratory of Dr. A. Smits, where he researched X-ray analytics with his friend Albert Karssen
1923	Concluded his doctoral degree studies with a thesis entitled; "X-ray investigation of the crystal structure of Lithium and Lithiumhydride"
	Worked with colleague and friend Albert Karssen at the laboratory of William Bragg in Manchester (UK)
1928	Became Professor at the University of Amsterdam
1929	Appointment as lector
1930	Married Maria Elisabeth Hardenberg. They got four children, three sons and one daughter.
1938	Publication of his famous book: "X-ray Analysis of Crystals"
	Established himself in the Van 't Hoff laboratory, after his appointment as Professor of General and Inorganic Chemistry at Utrecht University
1946-1951	Published many articles on new methods of X-ray analysis of crystal structures, on isomorphic change of heavy atoms and on anomic diffraction; herewith he gained international fame
1951	Selected as President of the International Union of Crystallography
1958	Awarded with the 'Companion of the Order of Orange-Nassau' distinction
1967-1971	Honorary doctorates at the Universities of Delft (1967), Zurich (1970) and Bristol (1971)
1962	Retired, but remained an active scientist still for many years
1980	Bijvoet passed away on 4 March in Winterswijk

The Utrecht Department of Chemistry congratulates the Bijvoet Center on their 20th anniversary!

Universiteit Utrecht

With her wide range of excellenct research, the Department of Chemistry at the Utrecht Faculty of Sceince plays an important role as interconnection between the Life Sciences and the Natural Sciences. It is here, that research on nanomaterials finds it's biomolecular partner. This symbiosis offers, through the outstanding research infrastructure, a fertile playground for all future chemists who want to expand their capabilities and work at the forefronts of science.

In the Bijvoet Institute at the Department of Chemistry biomolecular research integrates technology-driven innovations with biochemical/biomedical relevant research themes. This approach requires the close collaboration of scientists with backgrounds in chemistry, biology, pharmaceutical sciences and medicine. The aim of this cross-discipline research is to increase the understanding of life processes at the molecular level, enabling contributions to human health and disease therapies.

Discover the Utrecht Chemistry research and visit:
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