Master’s programme
Biomolecular Sciences

Course Manual
2006/2007
# Content

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>4</td>
</tr>
<tr>
<td>Programme</td>
<td>5</td>
</tr>
<tr>
<td>Course Descriptions</td>
<td>10</td>
</tr>
<tr>
<td>Master Course Biomolecular Sciences</td>
<td>11</td>
</tr>
<tr>
<td>Master Classes</td>
<td>14</td>
</tr>
<tr>
<td>Advanced Lipid and Protein Analysis</td>
<td>15</td>
</tr>
<tr>
<td>Advanced Mass Spectrometry in Proteomics and Structural Biology</td>
<td>17</td>
</tr>
<tr>
<td>Advanced Protein Crystallography</td>
<td>18</td>
</tr>
<tr>
<td>Biomolecular Mass Spectrometry</td>
<td>19</td>
</tr>
<tr>
<td>Biomolecular NMR</td>
<td>20</td>
</tr>
<tr>
<td>Biomolecular Synthesis</td>
<td>21</td>
</tr>
<tr>
<td>Biotechnology</td>
<td>22</td>
</tr>
<tr>
<td>Essentials of Glycochemistry and Glycobiology</td>
<td>23</td>
</tr>
<tr>
<td>Functional Analysis of the Genome</td>
<td>24</td>
</tr>
<tr>
<td>Integrative Physiology</td>
<td>25</td>
</tr>
<tr>
<td>Profiles research groups</td>
<td>28</td>
</tr>
<tr>
<td>Biochemical Physiology</td>
<td>29</td>
</tr>
<tr>
<td>Biochemistry and Cell Biology (Vet. Medicine)</td>
<td>31</td>
</tr>
<tr>
<td>Biochemistry of Lipids</td>
<td>35</td>
</tr>
<tr>
<td>Biochemistry of Membranes</td>
<td>37</td>
</tr>
<tr>
<td>Biomolecular Mass Spectrometry</td>
<td>39</td>
</tr>
<tr>
<td>Biomolecular NMR</td>
<td>40</td>
</tr>
<tr>
<td>Cell Biology (Biology)</td>
<td>42</td>
</tr>
<tr>
<td>Cell Biology (UMC-Medicine)</td>
<td>44</td>
</tr>
<tr>
<td>Glycoscience &amp; Biocatalysis</td>
<td>45</td>
</tr>
<tr>
<td>Infectious Disease and Immunology (Vet. Medicine)</td>
<td>48</td>
</tr>
<tr>
<td>Medicinal Chemistry</td>
<td>49</td>
</tr>
<tr>
<td>Membrane Enzymology</td>
<td>50</td>
</tr>
<tr>
<td>Molecular Biophysics</td>
<td>52</td>
</tr>
<tr>
<td>Molecular Genetics</td>
<td>53</td>
</tr>
<tr>
<td>Molecular Microbiology</td>
<td>55</td>
</tr>
<tr>
<td>Protein Crystallography</td>
<td>57</td>
</tr>
<tr>
<td>Protein Folding</td>
<td>60</td>
</tr>
<tr>
<td>Thrombosis and Haemostasis Lab (UMC-Medicine)</td>
<td>62</td>
</tr>
<tr>
<td>Names and Addresses</td>
<td>66</td>
</tr>
</tbody>
</table>
Introduction

Utrecht Prestige Master’s Programme

Biomolecular Sciences is a rapidly developing discipline in the modern life sciences, at the crossroads of chemical, biological, physical and computational sciences and focusing on the molecular understanding of how cells function.

Utrecht University has gained an outstanding reputation in this field for advanced structural analysis of biomolecules in the context of cellular systems. Research groups in the Faculties/Departments of Biology, Chemistry, Physics, Pharmacy, Medicine and Veterinary Medicine are involved in this interdisciplinary programme, which is directly linked to the PhD programmes of the Bijvoet Centre for Biomolecular Research and the Institute of Biomembranes. In 2004 the Utrecht MSc programme in Biomolecular Sciences was awarded the distinction of ‘Utrecht Prestige Master’s Programme’ by the University Board, based on its excellent research programme.

The MSc programme in Biomolecular Sciences aims to transfer this unique combination of interdisciplinary research to the next generation of students. The programme is research-oriented and has a broad character. Students are trained in state-of-the-art techniques, ranging from advanced methods in genetics and cell biology to structural biology. Participants will acquire extensive knowledge in the fields of:

- Molecular Recognition and Regulation
- Cell Signalling
- Membrane Biogenesis and Sorting
- Chemical Approaches to Biology
- Genomics and Proteomics

On completing their MSc programme, students will be well trained in the field of Biomolecular Sciences and will have developed the communication skills necessary for a scientific career. The majority of graduates will move on to a career in a university (becoming a PhD student) or research institution, or in the pharmaceutical or biotechnology sectors. A growing number of students are also choosing to take up opportunities in management positions in industry and governmental organisations, consulting, science journalism, technology transfer and patent law.

The master’s programme Biomolecular Sciences is officially a part of the Utrecht Graduate School of Life Sciences.
1. Programme

The MSc in Biomolecular Sciences offers a challenging programme with a high degree of freedom to choose from a number of outstanding research groups and several master classes. Students can select the order of course components, depending on their educational background and ambition. The programme committee will advise students on career development and help them set out an individual tailor-made programme. The duration of the MSc programme is 2 years (120 ECTS).

Overview of the MSc programme

<table>
<thead>
<tr>
<th>Major Research Project (36 weeks, approx. 9 months) incl. presentation and report</th>
<th>Master’s Course Biomolecular Sciences</th>
</tr>
</thead>
<tbody>
<tr>
<td>51 ECTS</td>
<td>10 ECTS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Minor Research Project (24 weeks, approx. 6 months) incl. presentation and report</th>
<th>Master Classes</th>
<th>Optional Courses</th>
<th>MSc-thesis</th>
<th>ABC seminars</th>
</tr>
</thead>
<tbody>
<tr>
<td>33 ECTS</td>
<td>6 ECTS</td>
<td>9 ECTS</td>
<td>9 ECTS</td>
<td>2 ECTS</td>
</tr>
</tbody>
</table>

*one week is considered to be 40 hours and is equal to approx. 1.5 ECTS

Major Research Training (51 ECTS)

A 36-week research project with one of the participating research groups. This includes giving a presentation and writing a report. All the research groups belong to one of the research institutes: (i) Bijvoet Centre for Biomolecular Research or (ii) the Institute of Biomembranes. For a comprehensive overview of all the participating departments and related research projects, see section 3, Department Profiles. If approved by the programme committee, it is also possible to conduct the Major Research Project outside Utrecht University under the supervision of one of the research groups.
**Minor Research Project (33 ECTS)**

A 24-week research project in a field of interest related to the Biomolecular Sciences, performed either at Utrecht University or abroad, in academia or industry. This includes writing a report. All external projects are subject to the approval of the programme committee and have to be supervised by a principal investigator (PI) of Utrecht University.

Courses or traineeships in the fields of ‘Science and Business’ or ‘Science and Education’ can be substituted for this minor research project (see below: profiles). Students who have a BSc degree from a Dutch higher education vocational college (HBO) or laboratory training college (HLO) can exchange parts of their research training for courses to fill in gaps in their education (upon approval).

**Master’s Course Biomolecular Sciences (10 ECTS)**

The annual Master’s Course in Biomolecular Sciences is compulsory for all MSc students. The aim of this course is to introduce a broad range of literature in the field of biomolecular research. Students will be trained to:

- read research articles critically
- present a research paper
- analyse the research goal
- analyse and discuss the approach (methodology/techniques)
- discuss the essential steps in research design
- discuss the outcome of research articles in the group
- design new lines of research based on the articles

Teachers will introduce the field of research (context) and act as a moderator during the discussions. The course consists of four parts, with weekly sessions held over a period of one year:

- **Part 1** Molecular Cell Biology
- **Part 2** Molecular Recognition and Regulation
- **Part 3** Membrane Biogenesis, Protein Folding and Sorting
- **Part 4** Genomics, Proteomics and Bioinformatics

Each part is worth 3 credits, so you only have to do 3 out of 4. You can choose the parts you like the most or the ones that best suits your expertise. Of course it is allowed to follow all parts. For participating in the introduction week, you will receive the final credit.
Master Classes (6 ECTS)

The MSc programme offers a variety of advanced practical and theoretical courses, ranging from genetics, biotechnology, biomolecular synthesis, methods in cell biology, and structural biology.

- Functional Analysis of the Genome
- Biotechnology
- Advanced Protein Crystallography
- Biomolecular NMR
- Integrative Physiology
- Biomolecular Mass Spectrometry
- Advanced Mass Spectrometry in Proteomics and Structural Biology
- Essentials of Glycochemistry and Glycobiology
- Advanced Lipid and Protein Analysis
- Biomolecular Synthesis

Optional Courses (9 ECTS)

There are three types of options possible (6 weeks’ duration)

- Various MSc or PhD courses.

  For a comprehensive overview of Biomedical courses, see:  
  [www.bms.uu.nl](http://www.bms.uu.nl) → Masters → Courses  
  For Chemistry and Physics courses, see:  
  [www.chem.uu.nl](http://www.chem.uu.nl) → Onderwijs → Master

  For Biology courses, see:  
  [www.bio.uu.nl/AnimalBiology/classesandcourses/](http://www.bio.uu.nl/AnimalBiology/classesandcourses/)  
  [www.bio.uu.nl/plantbiology/courses/](http://www.bio.uu.nl/plantbiology/courses/)  
  [www-binf.bio.uu.nl/master/](http://www-binf.bio.uu.nl/master/)

  For PhD courses  
  [www.biomembranes.nl](http://www.biomembranes.nl)  
  [www.bijvoet-center.nl](http://www.bijvoet-center.nl)

- Extension of the major/minor research project (by 6 weeks). upon approval
- Courses from BSc programmes to fill in gaps in a student’s education, upon approval
MSc thesis (9 ECTS)

There are two options for the MSc thesis:

(i) Students write a thesis on a subject of their own choice, supervised by a member of staff from one of the research groups participating in the MSc programme.

(ii) Students write a research proposal based on the outcome of either their major or minor research project.

The choice of subject can be determined by personal interest and will often be related to future career plans, e.g. possibilities for a PhD project or specific areas of research in connection with industrial job opportunities. Duration: approximately 6 weeks.

Please note the requirements for the MSc thesis may vary depending on the Education and Examination Regulations of each faculty or department (e.g. for Biomedical Sciences students only option (i) is applicable).

ABC Seminars (2 ECTS)

A series of monthly seminars organised by the ABC’s MSc programmes. Students are expected to write a short essay on each seminar, which will be evaluated by the programme co-ordinator. See also www.abc.uu.nl.
Different profiles within the MSc programme

There are various profiles to choose from within the MSc programme. The 6-month minor research project (see above) can be replaced by theoretical courses or traineeships in the field of ‘Management and Business’. Students will be awarded an MSc degree in ‘Chemical, Biological or Biomedical Sciences’, specifying the chosen profile (profile achievements are listed separately with grades). There are three different profiles:

‘Research’ profile. The majority of students in Biomolecular Sciences follow the research profile. This profile is eminently suited as preparation for a career in scientific research but it also provides a strong basis for other career perspectives. Students undertake two research projects of 6 and 9 months (see programme overview above).

‘Management’ or M-profile. Skills in Chemical, Biological and Biomedical Sciences will be combined with courses in Management and Business Economics (30 ECTS). The ‘Fundamentals of Business and Economics’ (FBE) course consists of 8 intensive modules and 4 workshops. This course can be taken instead of the 6-month research project.

‘Communication and Education’ (C&E) profile. This course (30 ECTS) offers a theoretical and practical introduction to science communication and education. This profile is an excellent preparation for a career as a science journalist or communications adviser, in profit and non-profit organisations.
2. Course descriptions
# 2. A Master’s Course Biomolecular Sciences, 
aangepast

**Coordinator**
- Molecular Cell Biology: Dr. C. Rabouille, Tel. 250 9280, e-mail C.Rabouille@lab.azu.nl
- Molecular Recognition and Regulation: Prof. Dr. M. Egmond, Tel. 253 3526, e-mail m.r.egmond@chem.uu.nl
- Membrane Biogenesis, Protein Folding and Sorting: Prof. Dr. A. Killian, tel. 253 3442, e-mail j.a.killian@chem.uu.nl
- Genomics, Proteomics and Bioinformatics: Dr. A.F.J.M. van den Ackerveken tel 253 3013 e-mail: a.f.j.m.vandenackerveken@bio.uu.nl

**Lecturers**
See www.bio.uu.nl/biomolecularsciences

**Discipline group**
-----

**Work load**
Each exam is worth 3 ECTS, the introduction week is worth 1 ECTS

**Semester**
- Sep-Nov 2006 (Mol Cell Biology)
- Nov-Jan 2007 (Molecular Recognition and Regulation)
- Feb-Apr 2007 (Membrane Biogenesis, Protein Folding and Sorting)
- Apr-Jun 2007 (Gen, Prot and Bioinf)

**Enrolment**
[http://www.uu.nl/osirisstudent](http://www.uu.nl/osirisstudent)

**Work form**
Lectures, presentations, discussions

**Materials**
Lecture notes

**Evaluation**
Written test

**Level**
M (master)

**Entry requirements**
-----

## Course aims

This course aims to introduce students to a broad range of literature on biological processes at the molecular scale. The students are trained to critically read and discuss research articles.

## Course content

In the Master Course of the Biomolecular Sciences programme, the students discuss a wide range of seminal papers (from high impact journals) in Biomolecular Sciences that have been selected by the teachers. Each student prepares and presents once a research article. Teachers will introduce the field of research (context) and act as a moderator during the discussions. Each meeting will be finished by a group discussion with the teacher functioning as chairman. Students will be trained to:

- read research articles critically
- present a research paper
- analyse the research goal
- analyse and discuss the approach (methodology/techniques)
- discuss the essential steps in research design
- discuss the outcome of research articles in the group
- design new lines of research based on the articles

The course consists of four parts, with weekly sessions held over a period of one year:

Part 1 Molecular Cell Biology (Dr. Catherine Rabouille)
Part 2 Molecular Recognition and Regulation (Prof. Dr. Maarten Egmond)
Part 3 Membrane Biogenesis, Protein Folding and Sorting (Prof. Dr. Antoinette Killian)
Part 4 Genomics, Proteomics and Bioinformatics (Dr. Guido van den Ackerveken)
2.B Master Classes
Advanced Lipid and Protein Analysis, aangepast

Coordinator  Dr. J. Holthuis (030-2536630), j.c.holthuis@chem.uu.nl
Lecturers  Dr. E. Breukink, Mw. Prof. Dr. A. Killian, Mw. Prof. I. Braakman, Prof. Dr. H. Tabak, Dr. S. Rüdiger, Dr. A. v/d Zand, Prof. Dr. G. van Meer, Dr. J. Holthuis, Ing. J. van den Dikkenberg

Discipline group  Bijvoet Center and Institute of Biomembranes

Work load  3.0 ECTS
Enrolment  http://www.uu.nl/osirisstudent

Work form  Practical work and data analysis.
Materials  Written instructions from the participating groups.
Evaluation  Active participation in the course
Level  M (master)
Capacity  12 students
Entry requirements  Basic knowledge of Biochemistry

Course content and aims

The duration of this course will be 2 weeks and will be organized by 3 groups in the Bijvoet Center and Institute of Biomembranes: Biochemistry of Membranes (BvM), Cellular Protein Chemistry (CPC) and Membrane Enzymology (ME)

Biochemistry of Membranes (E. Breukink, R. Sijbrandi and A. Killian)

Theme: Characterization of the techoic acid biosynthesis pathway of Gram-positive bacteria.

Techniques: Techoic acids are involved in the virulence of pathogenic bacteria and may thus be interesting targets for antibiotic development. The students will develop methods to characterize the different steps in the bacterial wall techoic acid synthesis pathway. This synthesis pathway makes use of the undecaprenylphosphate carrier. Using the water-soluble substrate GlcNAc-pyrophosphorylgeraniol and membrane preparations that carry the necessary enzymes the students will attempt to build a water soluble version of the basic building block of wall teichoic acid. Techniques as HPLC, TLC and Mass spectroscopy will be used to determine the success of the approaches taken.

Cellular Protein Chemistry (I. Braakman, H. Tabak, S. Rüdiger, A. v/d Zand)

Theme and techniques:

The students will apply fluorescence microscopy to study the formation of new peroxisomes (subcellular organelles) in living cells of Saccharomyces cerevisiae (baker’s yeast). One of the key proteins involved in this process will be purified applying modern strategies that allow the purification of proteins without the help of tags, which potentially may alter protein structure and activity. The students will be trained to make full use of the automatisation potential of modern chromatography equipment.
Membrane Enzymology (J.C.M. Holthuis, J. van den Dikkenberg, G. van Meer)

**Theme:** Characterization of sphingomyelin synthase, a key enzyme of mammalian sphingolipid synthesis.

**Techniques:** The students will develop and carry out discrete steps of an experimental approach aimed at the identification of a key enzyme of sphingolipid synthesis. Candidate genes will be selected from the database and after heterologous expression in baker’s yeast subjected to a detailed functional analysis. The students will gain experience in database mining, functional cloning strategies, membrane protein expression and reconstitution techniques, and in the application of fluorescent lipid probes (lipid extraction and 2D-TLC analysis).
Advanced Mass Spectrometry in Proteomics and Structural Biology, aangepast

Coordinator
Dr. R.H.H. van den Heuvel, contact: C.C. van Dijk (030-2535871), sec.biomass@pharm.uu.nl

Lecturers

Discipline group
Biomolecular Mass Spectrometry and Proteomics

Work load
1.5 ECTS

Semester
First week of June and first week of December, provided that there is a group of 4-10 students

Enrolment
http://www.uu.nl/osirisstudent or sec.biomass@pharm.uu.nl

Work form
Literature discussions, tutorials and presentations

Materials
Lecture notes, literature

Evaluation
Active participation in the course and written and oral presentation

Level
M (master)

Entry requirements
Structural Biology or Biomolecular Mass Spectrometry course

Course aims

The main aim of this course is to come to a detailed understanding of feature articles in proteomics and biomolecular mass spectrometry. Students will be supervised in critical reading scientific papers and presenting highlights and novelties of such work.

Course content

Mass spectrometry is a method of choice in both Proteomics and Structural Biology. Mass spectrometry is used successfully to identify unknown proteins and protein networks, to monitor and quantify differences in protein expression levels between normal and deviant cells, to analyze protein post-translational modifications, receptor-ligand binding, protein folding and non-covalent protein interactions.

In this course the emphasis is on extending the understanding of the scope and limitations of modern mass spectrometric techniques in Proteomics and Structural Biology. Students will receive four research articles, either on new applications of mass spectrometry or on new mass spectrometry-based methods. The group discusses each publication with one of the lecturers. Each session will take approximately 1.15 hours. In addition, students will write and present a (short) research project proposal using the mass spectrometry methods discussed in the course.
Advanced Protein Crystallography, aangepast

Coordinator  Prof. Dr. P.Gros, p.gros@chem.uu.nl
Lecturers  Prof. Dr. P. Gros, Dr. L.M.J. Kroon-Batenburg
Discipline group  Protein Crystallography
Work load  3.0 ECTS
Semester  2nd Semester (February/March 2007)
Enrolment  http://www.uu.nl/osirisstudent
Work form  Lectures
Materials  Lecture notes and recommended literature
Evaluation  Written test
Level  M (master)
Capacity  "unlimited"
Entry requirements  It is assumed students are acquainted with basic aspects of (protein) crystallography as discussed in "Introduction to Protein Crystallography" as part of the course "Structural Biology". A background in Mathematics (at least 2nd year level of Chemistry Bachelor) is recommended.

Course aims

This course aims to introduce the student to the theoretical background of the various methods and software packages used in modern-day protein crystallography.

Course content

In this lecture course we will deal with the theoretical background of protein crystallography. Topics discussed include diffraction, structure-factor algebra and statistics, phasing methods and maximum-likelihood optimization.
Biomolecular Mass Spectrometry

Coordinator
Prof.dr. A.J.R. Heck, contact: C.C. van Dijk (030-2535871), sec.biomass@pharm.uu.nl

Lecturers

Discipline group
Biomolecular Mass Spectrometry

Work load
1.5 ECTS following course only, 3.0 ECTS when exam is passed

Semester
25 September-29 September 2006

Enrolment
http://www.uu.nl/osirisstudent

Work form
Lectures

Materials
Lecture notes, course manual (€ 50,-)

Evaluation
Written examination

Level
M (master)

Capacity
28 students

Entry requirements
Bachelor chemistry, biomedical sciences or biology with course structural analysis recommended

Course aims

After following this course the student will have a very good understanding of basic concepts in mass spectrometry as applied to research in proteomics and structural biology

Course content

In recent years, the field of biomolecular mass spectrometry has grown rapidly through the introduction of various new methods. The course aims to give an overview of the present state-of-the-art in Biomolecular Mass Spectrometry, highlighting several recent applications in chemistry, pharmacy, biology and medicine. Basic fundamentals and instrumentation will not be overlooked.

The course is meant for those who wish to learn about these new developments in mass spectrometry and how they may contribute to their own area of research. Additionally, the course is also meant for active mass spectrometrists who want to update or broaden their knowledge in the area of biomedical applications.

The intensive course will be given in one full week. A variety of lecturers will cover a diverse array of fundamentals and applications. The course will be informal with ample time for discussion and questions. Via lab-tours and interaction with the researchers, participants will get an opportunity to survey state-of-the-art instrumentation and several examples of biomolecular mass spectrometric analysis. To encourage group interactions, the number of participants is limited. A course manual will be provided.
### Biomolecular NMR

<table>
<thead>
<tr>
<th>Coordinator</th>
<th>Prof. dr. R. Boelens (030-2534035), <a href="mailto:r.boelens@chem.uu.nl">r.boelens@chem.uu.nl</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecturers</td>
<td>Prof. dr. R. Boelens; Dr. A. Bonvin</td>
</tr>
<tr>
<td>Discipline group</td>
<td>Biomolecular NMR, Biochemistry of Membranes</td>
</tr>
<tr>
<td>Work load</td>
<td>1,5 ECTS</td>
</tr>
<tr>
<td>Semester</td>
<td>2nd Semester</td>
</tr>
<tr>
<td>Enrolment</td>
<td><a href="http://www.uu.nl/osirisstudent">http://www.uu.nl/osirisstudent</a></td>
</tr>
<tr>
<td>Work form</td>
<td>Lectures, tutorials</td>
</tr>
<tr>
<td>Materials</td>
<td>Lecture notes</td>
</tr>
<tr>
<td>Evaluation</td>
<td>Written test</td>
</tr>
<tr>
<td>Level</td>
<td>M (master)</td>
</tr>
<tr>
<td>Capacity</td>
<td>20 students</td>
</tr>
<tr>
<td>Entry requirements</td>
<td>We assume that the students are familiar with the fundamental concepts of NMR described in the following two mandatory courses Structural Biology (Chemistry/Biology 3rd year) and 'Structuuranalyse' (Chemistry/Biology 2nd year).</td>
</tr>
</tbody>
</table>

**Course aims and content**

Nuclear magnetic resonance is a very powerful and versatile technique for investigating the structure and dynamics of biomolecules such as proteins and their complexes in solution and even in more solid-like environments, such as membranes. The importance of NMR in biomolecular sciences and in particular in structural biology has recently been recognized by the attribution of the 2002 Nobel price in chemistry to K. Wüthrich (ETH Zürich).

This course consists of a theoretical part of 1 week, which comprises lectures in the morning and assignments in the afternoon. It covers both high-resolution NMR methods to study water-soluble proteins and solid-state NMR methods to study membrane proteins and lipids. The course is open to a large number of students (max. 20). The students are expected to actively participate in all sessions and make one assignment at home each day.
Biomolecular Synthesis

Coordinator
Prof. dr. R.M.J. Liskamp (030-2537396. secr 030-2537307), r.m.j.liskamp@pharm.uu.nl

Lecturers
Prof. dr. R.M.J. Liskamp, Dr. R.J. Pieters, Dr. Ir. D.T.S. Rijkers, Dr. Ir. J.A.W. Kruijtzer, Dr. J.A.F. Joosten

Discipline group
Medicinal Chemistry

Work load
3 ECTS

Semester
Spring 2007

Enrolment
http://www.uu.nl/osirisstudent

Work form
Lectures, tutorials

Materials

Evaluation
Written test

Level
M (master)

Capacity
# students

Entry requirements

Course aims

After this course the student will be familiar with the chemical behavior of peptides and carbohydrates. In addition, he/she will be able to plan and understand synthesis of these biopolymers.

Course content
- principles amino acid and carbohydrate structure and chemical behavior
- functional group transformation and manipulation
- protection group strategies
- modular synthesis and coupling strategies
- (solid phase) peptide and carbohydrate synthesis

Demonstration of equipment: peptide synthesizer, robot synthesizer, solid phase, characterization and structural elucidation equipment (mass, NMR, nanoprobe)
Biotechnology, aangepast

Coordinator
Prof. dr. H. Wösten (030-2533448), h.a.b.wosten@bio.uu.nl

Lecturers
Prof. dr. H. Wösten, Prof. dr. M. Egmond, Prof. Dr. D. Bosch, Prof. dr. J. Tommassen, Dr. R.P. de Vries, Dr. J. Hanson, Dr. F. Meijboom, Dr. B. Defize, Dr. M. Koster

Discipline group
Molecular Microbiology

Work load
4.5 ECTS

Semester
Start 28th of May 2007, 2½ weeks

Enrolment
Prof. dr. H. Wösten (030-2533448), h.a.b.wosten@bio.uu.nl

Work form
Lectures and assignments

Materials
--

Evaluation
Assignments, participation in course

Level
M (master)

Capacity
24 students

Entry requirements
A bachelor course in genetics, molecular microbiology or a related course is highly recommended

Course aims
At the end of the course the student is expected to:

• have an overview of the different fields of biotechnology
• to be able to express its opinion on scientific and ethical aspects of biotechnology
• be able to critically read primary literature

Course content
This course is an introduction in Biotechnology. It will not only give an overview of the different fields (DNA, protein, carbohydrate and lipid technology; microbial biotechnology; plant biotechnology; animal biotechnology; vaccine development; stem cells; human genetics and forensics; environmental biotechnology;) but will also give in depth information of new developments. Moreover, workshops on patents, ethics, debating and setting up your own company are included. Attendance of lectures and workshops is compulsory.
Essentials of Glycochemistry and Glycobiology,
aangepast

Coordinator
Prof. dr. J.P. Kamerling (030-2533479), j.p.kamerling@chem.uu.nl

Lecturers
Prof. dr. J.P. Kamerling, Prof. Dr. D. Bosch

Discipline group
Bio-Organic Chemistry

Work load
3.0 ECTS

Semester
In the period May-June 2007

Enrolment
http://www.uu.nl/osirisstudent

Work form
Lectures

Materials
Lecture notes

Evaluation
Oral tentamen

Level
M (master)

Capacity
# students

Entry requirements
Bachelor level: Basic knowledge of Carbohydrate Chemistry (e.g. J. McMurry: Organic Chemistry sixth edition, chapter 25; P. Yurkanis Bruice: Organic Chemistry fourth edition, chapter 22), Basic knowledge of Mass Spectrometry and NMR Spectroscopy (Course Structural Analysis SK-BSTRUC, B-B3STRU03; Course Chemistry for Biologists). The course Structural Biology (SK-BSBIOL) is advised.

Course aims
At the end of the course the student is expected to have general insight into the area of glycoscience.

Course content
In the General introduction to Glycoscience (glycoproteins, glycolipids, proteoglycans, polysaccharides, glycosylphosphatidylinositol anchors), mono- and oligosaccharide structures, simple carbohydrate reactions, structural parameters, and nomenclature of carbohydrates will be discussed. In addition, biosynthetic pathways of glycoprotein glycans, glycolipids, bacterial polysaccharides, and glycosylphosphatidylinositol anchors will be presented. Detailed attention will be paid to the basics of primary structural analysis of polysaccharides and glycoconjugate glycans (microchemical and enzymatic protocols, liquid chromatography, lectins, mass spectrometry, NMR spectroscopy, high-throughput glycan profiling), and some insight will be given into conformational analysis of glycans. Furthermore, the course will shortly focus on: Glycan epitopes such as sialic acids, immunodeterminants; Unusual glycosylation events; The biological roles of glycans; Disorders in biosynthesis and degradation of glycans; Glycosylation changes in cancer; Acquired glycosylation changes in human diseases; Carbohydrate-based vaccines; Natural and synthetic inhibitors of glycosylation; Animal and plant lectins, Carbohydrate-protein and carbohydrate-carbohydrate interactions; Glycotechnology: recombinant glycoproteins expressed in mammalian cells, yeast cells, plant cells or transgenic animals; Basics of organic-chemical and enzymatic synthesis of carbohydrate chains and glycoconjugates.
Functional Analysis of the Genome

Coordinator Dr. G. van den Ackerveken (030-2533013), G.vandenackerveken@bio.uu.nl
Lecturers Dr. G. van den Ackerveken, Dr. F. Menke
Discipline group Molecular Genetics
Work load 1.5 ECTS
Semester March 2007
Enrolment Course coordinator
Work form Lectures, hands-on workshop, excursion, group discussions, short presentations, writing short proposal
Materials Lecture hand-outs, notes and recommended literature
Evaluation Participation and performance
Level M (master)
Capacity 20 students
Entry requirements Basic knowledge of Genetics and Molecular Biology

Course aims

This course aims to introduce student into (i) genomics technologies (HT sequencing, expression profiling, etc), (ii) DNA, protein, SNP, and expression databases, and (iii) the implementation of these technologies/databases in biological research. At the end of the course, student will be familiar with gene analysis and methods to understand gene-function.

Course content

The availability of the complete genome sequences of an ever-growing number of organisms, including human, is having an enormous impact on molecular biology and genetics. Students will exploit existing DNA, protein and SNP databases in bioinformatics workshops for a number of experimental problems/examples. An introduction into DNA microarray technology will be provided, and students will work on large gene-expression datasets obtained by expression profiling using DNA-chips. Clustering methods will be used to group genes. The last part of the course will deal with analysis of gene function. A broad spectrum of techniques will be presented that are used to study the role of selected genes in biological processes, e.g. gene knockouts, RNAi, site-directed mutagenesis, etc. The availability of a huge amount of biological resources, especially for model organisms, e.g. yeast, Drosophila, Arabidopsis, and the mouse, will be included in workshops.
Integrative Physiology, aangepast

Coordinator
Dr. Kees W. Rodenburg, 030-2539331; k.w.rodenburg@bio.uu.nl

Lecturers
Dr. Kees W. Rodenburg, guest lecture: to be announced

Discipline group
Biochemical Physiology, Department of Biology

Work load
1.5 ECTS

Semester
16-20 October 2006

Enrolment
Course coordinator

Deadline for enrolment
2 October 2006

Work form
Practical course (4-8 participants)

Materials
Experimental tools provided at the start of the course

Evaluation
Attendancy, degree of active participation, and presentation of the results in poster form

Level
M (master)

Capacity
8 students (2 x 4)

Entry requirements
B (Bachelor): Biology, Chemistry, Biomedical Sciences, or Pharmaceutical Sciences; Students of the Master Programmes Biomolecular Sciences and Animal Biology have priority over other students.

Course aims

After following the course the student will be able to understand how to integrate experimentally studied molecular aspects at the level of the animal metabolism as a whole.

Course content

Study of the physiological role of the insect LDL receptor, LpR, in lipid storage

The function of the fat body is analogous to that of both liver cells and adipocytes in mammals. In the insect organ, energy is stored as glycogen and lipid droplets. For lipid storage in the fat body, lipid is transported from the gut to the fat body cells in a lipoprotein. At the cell membrane, the lipoprotein is bound and endocytosed by the insect LDL receptor, LpR. This LpR is a unique member of the LDL receptor family, since after transfection in mammalian cells, endocytosed ligands are not directed to lysosomes for degradation, but are recycled, and most likely resecreted. In the insect, LpR is present on moments that the fat body is “empty” and requires to be loaded with lipids. According to the physiological situation of the insect, these moments of lipid loading may occur in several developmental stages, different between males and females, as well as after flight activity.

Practical approach: insect (locust or Drosophila) fat bodies at various developmental stages, after (mimicking of) flight activity, sex, stage, or starvation, will be analysed for their capacity to endocytically take up lipoprotein or other ligands. The uptake will be visualised by using immunofluorescence and fluorescence microscopy. Based on a introduction
into the relevant research status and theory the students, in groups of two, will be asked to design and plan experiments. The course aims at developing insight into analysing specific molecular process in the context of the animal as a whole.
3. Profiles research groups
The group is involved in two master programmes: Biomolecular Sciences and Animal Biology. MSc students in both master programmes are invited to take Biochemical Physiology as their major (or minor) research project.

The two master programmes have different scopes, from the functioning of the animal (Animal Biology) to understanding of cellular function at the molecular level (Biomolecular Sciences). Both scopes are reflected in the focus of the research programme of the group.

Research projects

Regulation of metabolic key processes

The research program addresses animal metabolism, and is particularly focused on regulation of energy generation and lipid metabolism. Underlying questions lead to fundamental knowledge, whereas spin-off is applied:

- How can animal metabolism sustain exercise for longer periods of time (e.g. flight activity of insects)? Main topics are lipid storage, mobilization, transport and uptake.
- What is the molecular and structural basis of lipoprotein functioning in lipid transport? Insect lipoproteins are used as a model system for the structure and metabolism of human lipoproteins. Latter lipoproteins are involved in transport of lipids such as cholesterol, but also in diseases (from cardiovascular, diabetes, to Alzheimer's disease).
- How are evolutionary data on lipoprotein receptor functioning applicable for a better understanding of this process in human tissues? Many aspects of lipoprotein receptors in lower animals differ from those in man.
- Is defective recycling of a fatty acid transporter (CD36) in the heart involved in diabetes type-2, and does this transporter provide a target for treatment?

Methods & Techniques

- cell and tissue culturing
- immunofluorescence labeling and fluorescence microscopy
- PCR and cloning
- protein purification and Western- and immunoblotting, radiolabeling
- protein structure determination (electron microscopy, X-ray crystallography, modeling)
- density gradient ultracentrifugation
- hybrid gene construction
- 2-hybrid screening
- different enzyme assays to determine enzyme kinetics and cell physiology (signal transduction)
- different assays to determine protein-lipid interaction.

For more details contact:

Dr. Kees Rodenburg (Kruyt W208, 2539331, k.w.rodenburg@bio.uu.nl)

or consult our website: http://www.bio.uu.nl/physiology/
Biochemistry and Cell Biology

(Veterinary Medicine)

The Department of Biochemistry and Cell Biology (B&C) belongs to the faculty of Veterinary Medicine of Utrecht University. Its mission is an active involvement in teaching and strong scientific research. B&C contributes to the teaching of students in veterinary medicine as well as to students in biomedical sciences. B&C performs basic as well as clinically-oriented biochemical research in membrane dynamics and in cell biology.

*Biochemistry of Membrane Dynamics*

*Helms/Tielens/Gadella*

The biochemical research is focused to understand the molecular principles of membrane dynamics in animal cells. Our view of biological membranes has evolved dramatically over the last few decades. Initially a static model was proposed by Singer and Nicholson that describes a lipid bilayer with attached and embedded proteins. During the last two decades, this view has changed and we now envision membranes as highly dynamic structures. In the cell, membranes are continuously transported between organelles. This process includes protein and lipid sorting during transport to maintain the unique membrane composition of each organelle. In membranes, a select group of lipids and proteins can dynamically segregate in microdomains or so-called lipid rafts to regulate cellular processes.

Thus, biological membranes behave much more dynamic than previously anticipated. The dynamic properties of biological membranes appear to play a crucial role in many normal cellular functions and many diseases result from dysfunction of membrane dynamics. Several (dys)functions of membrane dynamics are studied in our department.

**Research Projects**

**Research line 1: Membrane dynamics and pathogenesis**

The Golgi apparatus is a unique organelle that traditionally has been regarded as the post office of a cell, modifying and sorting proteins. An emerging and unexpected role of the Golgi is its involvement in various signaling pathways controlling important cellular functions. The Golgi thus functions as a central checkpoint device, integrating information from various membrane trafficking and signaling pathways. These properties make this organelle an attractive target for pathogens to escape the immune system.
We investigate the role of the Golgi complex in host-pathogen interactions. This will allow the development of novel therapeutics and diagnostics.

**Research line 2: Membranes and bioenergetics of parasites**

This research group aims to discover and characterize special adaptations in the biochemistry and molecular biology of parasitic worms and protozoa. These parasite-specific adaptations are scientifically interesting as they often provide novel models to study general biological phenomena, and because they are unique targets for the development of new anti-parasitic drugs.

**Research line 3: Membrane dynamics during fertilization**

Human infertility is an increasingly important research area and optimized assisted reproductive technologies are economically increasingly relevant for veterinary breeding industries. However, molecules involved in the process of fertilization and signalling pathways involved in activation gametes in order to obtain competent fusion partners are essentially unknown. Central topics in this research line are the dynamics in adhesive and fusion properties, molecular composition and architecture of the two gamete’s membranes as well as membrane-derived signaling leading to embryo development.

**Research line 4: Membrane dynamics and lipid metabolism**

Mammalian cell membranes contain a great variety of lipids. The significance of this complex lipid mixture is illustrated by the fact that breakdown products of cellular phospholipids are biologically active and can interfere with cell physiology and pathology. Disturbance in the cellular homeostasis of phosphatidylcholine for example results in a dramatic increase in lipid droplets, indicating that these organelles are not just lipid storage containers but have an active role in lipid metabolism. Research in the mechanisms that regulate the synthesis and breakdown of lipids and their cellular homeostasis may have important implications for diseases such as obesitas (adipositas), atherosclerosis, and diabetes.

**Research line 5: Membrane dynamics during cartilage mineralisation**

Cartilage plays an essential role in bone growth and elongation. A first step in the transformation to bone is mineralization of cartilage. At the onset of mineralization, large amounts of matrix vesicles are produced by cells (chondrocytes) that are present in cartilage. Matrix vesicles are believed to play a crucial role in the initiation of mineralization by the formation of primary hydroxyapatite crystals, which function as nucleation cores for further mineralization. This research line aims to obtain more insight in the mineralisation process of cartilage by studying the formation of matrix vesicles. Defects in these processes may result in a delayed mineralization, retention and thickening of cartilage, which may the basis of several diseases such as osteochondrosis and rachitis.
**Methods and Techniques**

- Molecular biology (recombinant DNA techniques, yeast-two-hybrid system)
- Lipid chemistry (lipid separation and analysis, lipidomics by mass spectrometry analysis)
- Protein chemistry (recombinant protein synthesis and purification, HPLC/FPLC, Proteomics/2D-PAGE)
- Cell Biology (cell culture, FACS analysis, Confocal laser scanning microscopy, phase-contrast microscopy, fluorescence microscopy, electron microscope)
- Biochemistry (subcellular fractionation, membrane isolation, protein characterization, biochemical assays, both in vivo and in vitro)

**Cell Biology**

**Stoorvogel**

For more information on the research projects of Cell Biology please contact Prof.dr. Willem Stoorvogel (details below).

**For more details contact:**

**General information:**

Department of Biochemistry and Cell Biology  
Tel: +31-30-2535387  
email: BC@vet.uu.nl  
website: www.bc.vet.uu.nl

**Biochemistry of Membrane Dynamics:**

Prof.dr. J.B. Helms: j.b.helms@vet.uu.nl  
Prof.dr. A.G.M. Tielens: tielens@biochem.vet.uu.nl  
Dr. B.M. Gadella: b.gadella@vet.uu.nl

**Cell Biology:**  
Prof.dr. W. Stoorvogel: W. Stoorvogel@vet.uu.nl
Life requires death. Organisms eliminate damaged or superfluous cells by a stereotypical program of cell suicide, called apoptosis. Necrosis or ‘accidental cell death’ is the classical counterpart of apoptosis and occurs when cells are overwhelmed by toxic stimuli. Apoptosis is important during normal embryonic development and also contributes to the pathogenesis of a number of human diseases, including cancer, viral infections, autoimmune diseases, stroke, neurodegenerative diseases and AIDS. Apoptosis can be induced by many stimuli: UV-light, activation of the ‘death receptors’, oxidative stress by reactive oxygen species (ROS), etc.

There are two closely related research lines in our laboratory, which address the mechanisms of apoptosis.

**Research projects**

*Regulation of lipid signaling, cell proliferation and apoptosis by phosphatidylinositol transfer proteins (PI-TPs)*

Two isoforms of the phosphatidylinositol transfer protein, PI-TPα and β, have been identified in almost all mammalian tissues. Both proteins catalyze the transfer of PI between membranes in vitro. In addition, PI-TPβ demonstrates the ability to bind and transfer sphingomyelin (SM) in vitro. We have studied the effects of increased expression of PI-TPα and β in NIH3T3 mouse fibroblast cells. Overexpression of PI-TPα (SPIα cells) leads to an increased growth rate, a higher saturation density and increased intra- and extracellular levels of bioactive lipid metabolites. On the other hand, overexpression of PI-TPβ (SPIβ cells) decreases the growth rate as well as the saturation density and affects intracellular SM traffic. In addition to an increased rate of proliferation, SPIα cells also demonstrate, in contrast to wtNIH3T3 and SPIβ cells, a high survival upon UV-induced apoptosis. Medium conditioned by the SPIα cells was shown to be mitogenic for quiescent wtNIH3T3 and SPIβ cells and is also able to protect these cells against UV-induced apoptosis.

Our goal is to analyze the signaling pathways and to identify the bioactive lipids and proteins involved in the PI-TP-mediated stimulation and inhibition of apoptosis.

Apoptosis can be estimated by determination of the number of blebbing cells (A), or the number of cells with condensed DNA (B).
For more details contact:

Dr. Gerry Snoek (253 4668, g.t.snoek@chem.uu.nl) and Dr. Dmitri Sakharov (253 2852, d.sakharov@chem.uu.nl)
or consult our website: http://cble.chem.uu.nl/biolip/
Biochemistry of Membranes, aangepast

*De Kruijff/Killian/De Kroon/Breukink*

Biological membranes form the barriers separating in from out in biological systems. They play essential roles in numerous processes, ranging from transport of metabolites to communication with the environment. Membranes are dynamic structures consisting of a complex mixture of lipids and many different proteins. For the living cell to function properly it is crucial that newly synthesized membrane building blocks are correctly integrated and assembled into its membranes.

Research in the department is focussed on unravelling the molecular mechanisms of the insertion and assembly of newly synthesized membrane proteins, and the integration of new lipid molecules. Membranes are important targets for antibiotics and drugs. Other lines of research within the department include the mode of action of membrane active antibiotics, the role of membranes in amyloid formation in type II diabetes, and the development of a membrane-coated formulation of the anti-cancer drug cisplatin.

The methods and techniques used include a wide range of biochemical, molecular biological and cell biological techniques, lipid analysis, preparation of model membrane systems, fluorescence spectroscopy, fluorescence microscopy, circular dichroism, monomolecular layers, calorimetry (DSC, ITC), solid state and high resolution NMR, and atomic force microscopy (AFM).

**Current research projects**

- Integration and assembly of proteins in membranes. Basic questions to be answered are: how do newly synthesized proteins get into the membrane, what determines their conformation, how do the subunits of oligomeric membrane proteins like K⁺-channels find each other, how do lipids affect proteins and, vice versa, how do proteins affect lipids?
- Phosphatidylcholine (PC) in yeast: metabolism and transport. PC is an essential membrane building block in eukaryotic cells. Using the powerful molecular genetic tools provided by the model eukaryote *Saccharomyces cerevisiae*, the synthesis and turnover of PC, and the transport of PC to mitochondria are investigated. The specific functions of PC in mitochondria are addressed in a proteomics approach.
- Mode of action of the membrane active antibiotic nisin. After binding to the receptor lipid II, nisin kills target bacteria by forming pores in the membrane. Elucidation of nisin’s mode of action at the molecular level, provides a basis for developing a new class of antibiotics.
- The role of lipid II in bacterial cell wall synthesis. Lipid II transports subunits across the membrane to be coupled to the growing cell wall. Insight into this process may provide targets for new antibiotics.
- Cisplatin nanocapsules. Nanoprecipitates of the anti-cancer drug cisplatin coated with a lipid bilayer have an unprecedented cytotoxicity against tumor cells in vitro. Research is aimed at a new cancer therapy.
- IAPP-membrane interactions in type II diabetes. The IAPP peptide aggregates to form ‘amyloid fibers’ that destroy insulin producing cells. Which role do interactions with the cell membrane play in this process?

For more details contact:

Dr. A. de Kroon (Kruyt Z610, 2533424, a.i.p.m.dekroon@chem.uu.nl)

or consult our website: http://cble.chem.uu.nl/biomem/
The research in the group of Heck is centred around the development and application of proteomics and biomolecular mass spectrometry technologies. The identification and characterisation of proteins, including their relevant post-translational modifications receives special emphasis. We also study functional protein complexes and protein higher-order structures. The group Biomolecular Mass spectrometry is embedded in both the Department of Chemistry and Pharmacy. Most of the research is multi-disciplinary and in collaboration with other groups in the ABC faculties and/or outside the university.

**Research Projects**

- Dynamic changes in protein expression levels (= the proteome), for instance to investigate which proteins determine the process of cell proliferation or embryonic development
- Analysis of functional multi-protein complexes, to study their role in important cellular processes
- Protein folding, protein-membrane interactions and protein-higher order structures

**Methods & Techniques**

2-D gel electrophoresis, mass spectrometry, image analysis, bio-informatics, phospho(proteomics), affinity-purification, bio-physical spectroscopy, investigation of interactions using surface plasmon resonance technology.

**For more details contact:**

Dr. R.H.H. van den Heuvel (Went W146, 2537500, (R.H.H. van den Heuvel@chem.uu.nl)

or consult our website: [http://www.chem.uu.nl/bioms/](http://www.chem.uu.nl/bioms/)
The research of the Utrecht NMR group deals with problems in biochemistry and molecular biology studied by the method of high-resolution nuclear magnetic resonance (NMR) spectroscopy. The group also contributes to the development of NMR methodology.

The infrastructure in the NMR department is excellent, with NMR spectrometers covering the range from 360 to 900 MHz, including a 500 MHz spectrometer equipped with a laser for the study of photoactive proteins and a 600 MHz spectrometer equipped with a cryoprobe for high sensitivity measurements, state of the art computers including a 36 processor Beowulf cluster and new laboratory facilities for cloning and protein expression and characterization of protein-protein and protein-DNA interactions through molecular biology techniques.

**Research projects**

*Regulation of gene expression*

A major research theme is that of the regulation of gene expression with aspects of both protein-nucleic acid interaction and protein-protein interaction. These interactions are studied in bacterial repressors, eukaryotic transcription factors and initiation factors involved in protein biosynthesis. The aim is to understand these biomolecular recognition processes in terms of detailed three-dimensional structures and dynamics.

*NMR methodology*

Novel NMR experiments and methods for structure refinement and for the quality assessment of protein NMR structures are being developed in the group. An important line of research deals with the development of rapid methods for the determination of protein structures within a structural genomics effort. This requires developments at all stages of the NMR structure determination process from protein expression and purification through NMR data acquisition and analysis to structure calculations and validation.

*Protein dynamics*

The dynamics of biomolecules is tightly associated with their function. A better understanding of their dynamic properties would thus lead to a better understanding of their function. NMR provide an unique tool for studying the dynamic properties of biomolecules since it is able to monitor motions at an
atomic level over time scales ranging from picoseconds thought micro- and milli-seconds to days. Research in this area covers both methodology developments in pulse sequences to extract new dynamic information and application to various systems under study in the laboratory.

Protein folding/unfolding

NMR can be used to monitor a residue level through, for example 1H-15N HSQC spectra, the folding or unfolding of proteins under various conditions such as higher temperatures or in the presence of co-solvents or denaturants. Next to NMR spectroscopy other biophysical methods such as circular dichroism spectroscopy and computational methods such as molecular dynamics are used as well. The information obtained in this way can be used for a better understanding of folding mechanisms and pathways or, for example, for protein engineering to improve the stability of industrial enzymes.

Biocomputations

Molecular modeling techniques are being used to study diverse aspects of biomolecules and of their interactions with other molecules, such as protein dynamics and folding, the effect of mutations on structure and stability and the prediction of the structure of biomolecular complexes. For the latter in particular, new methods combining bioinformatics with limited biophysical and/or biochemical information have been successfully developed. The computational work within the NMR department remains, when possible, tightly connected to experimental NMR data.

Methods & Techniques

- homo- and heteronuclear multidimensional NMR spectroscopy
- data processing
- structure determination
- bioinformatics
- biocomputing
- molecular modeling; molecular dynamics
- protein cloning, expression and purification
- protein characterization with molecular biology techniques

For more details contact:

Prof.Dr R. Boelens (Bloembergengebouw 1.14, 2532652, r.boelens@chem.uu.nl)
or consult our website: http://www.nmr.chem.uu.nl
Cell Biology (Department of Biology),
aangepast
Verkleij/Boonstra/Verrips/Van Bergen en Henegouwen/ Post/Humbel/Müller

The research program of the group Cell Biology of the Department of Biology is studying the dynamics of biological processes at the cellular level. Cell biological processes are characterized by the fact that they depend on spatio-temporal regulation. Understanding the principles of cell biological processes requires an integrated multidisciplinary approach, in which the spatio-temporal aspects are investigated by a combination of advanced microscopical, biochemical, biophysical and molecular biological methods. The research program contains five subprograms: Cell cycle & growth, Transport, Ageing, Antibody technology, and Imaging.

Cell cycle & growth (Boonstra)

In our research we aim to unravel the molecular machinery by which external factors regulate the molecular motors (the cyclin/CDK complexes) that cause progression of the cells through the cell cycle. In mammalian cells we study the activation of focal adhesion complexes during the transition from mitosis to the G1 phase in relation to cell attachment. Special attention will be given to the role of the actin microfilament system. In these studies special emphasis will be given to the cellular localization of the components of interest. Proliferation of yeast cells relies mainly on the presence of nutrients in the environment.

Transport (Van Bergen en Henegouwen, Müller)

The research of the transport group is directed towards the understanding at the molecular level of the regulation of intracellular transport. Essential for growth control is the downregulation of EGFR signaling which is mediated by endocytosis and degradation of in lysosomes of activated EGFRs. vBeH is investigating the role of raft lipids and protein mono-ubiquitination of proteins like Eps15 in this highly dynamic process. Muller is interested in the mechanism of secretion in fungi. Both endocytosis and secretion are subjects that are studied in collaboration with (inter)national research groups and
industries like Ablynx and DSM using various imaging methods and Llama antibodies, two other topics of the group Cell Biology.

**Ageing (Post, Verkleij, Verrips)**

Our ageing research focuses on the understanding of the ageing process at organ and cellular level, the definition of ageing markers and the development of anti-ageing intervention strategies. The major focus of our research is on ageing of the human vessel wall, in collaboration with (inter)national research groups and industries. The research combines genomics, proteomics, cell biology and mathematic modeling and studies vessel walls, cultured vessel wall cells and plasma. The spatio-temporal aspect of the vascular ageing process, with specific interest in the barrier function of the endothelium, is the heart of a recently started IOP project, combining many aspects of the research topics of Cell Biology.

**Antibody technology (Verrips)**

The Llama antibody technology is an enabling technology that fits excellent in the overall purpose of our group the study of cell architecture and dynamics. Antibodies are by far the best molecules to recognize other molecules, even when present in complexes. As the binding domain of Llama antibodies are quite small (only 21-15 kD) and can be modified (labelled) easily they are excellent suited to study molecular processes in the cell. At present we use this technology to study (age) related muscle diseases (together with LUMC), general ageing processes, cellular transport processes and the detection and removal of (age) proteins in blood (also with LUMC). Together with CNRS Marseille and Radbout University we studied the physical properties of these antibodies. On these research subjects we have collaborations with a number of companies like Ablynx, FEI and BAC/Unilever

**Imaging (Verkleij, Muller, Humbel)**

The group houses the central Electron Microscope Facility of the Department of Biology, which is used also by the Faculties/Departments of Chemistry, Geology, Physics and Medicine as well as by the Veterinary School, the Hubrecht Laboratory and the ‘Centraalbureau voor schimmelcultures’. The activities of the 3D-EM group are directed towards the development of methods for automated electron tomography using transmission electron microscopy. Our goal is to elucidate the cellular arrangement of cells and to localize individual macromolecules and structures in relative thick (100-500 nm) specimens (e.g. in small cells, in organelles) by means of gold-labeling and 3D pattern recognition.

**For more details contact:**

Prof. Dr. J. Boonstra (Kruyt W503, 2533189, J.Boonstra@bio.uu.nl)
or consult our website: [http://www.cellulararchitecture.nl](http://www.cellulararchitecture.nl)
The general research focus of this department: all eukaryotic cells critically depend on the compartmentalization of biochemical reactions in distinct intracellular compartments to provide optimal environments for enzymes with often distinct pH, redox and substrate requirements. Critical to the generation and maintenance of these compartments is their ability to communicate with each other via tubulo-vesicular transport carriers to exchange content such as substrates and co-factors. Genetic lesions in these processes are often lethal. Acquired perturbations due for instance to viral and microbial infections, or aging generally lead to more or less serious human diseases. Our research aim is to define general principles underlying organel maintenance and communication. Because this is a fundamental question that is approached with a wide variety of methods, its results can be applied to a wide variety of scientific problems.

Research Projects
- Ubiquitination and function of the growth hormone receptor (Strous)
- Molecular mechanisms of membrane transport (van der Sluij)
- Principles of organ inheritance during cell division (Van der Sluijs)
- The role of endosomes in neuron function (Klumperman)
- Golgi stack formation in Drosophila melanogaster (Rabouille)
- Dissection of the molecular mechanism of autophagy (Reggiori)
- Regulatory pathways in Wnt secretion and signal transduction (Maurice)

Methods & Techniques
- combination of generic molecular biology
- protein biochemistry
- applied bio-informatics
- fluorescence microscopy
- live cell imaging
- electron microscopy
- tomography
- image analysis
- drosophila and yeast genetics
- mouse models

For more details contact:
Prof. G. Strous (UMCU G02.525, 250 6476, strous@med.uu.nl) or consult our website: http://www.med.uu.nl/celbiol/celbiol.htm
Bio-organic Chemistry, aangepast

Kamerling

Glycoscience

In biomedical science, carbohydrate parts of glycoconjugates (glycoproteins, proteoglycans, glycolipids) and polysaccharides exert essential biological and physico-chemical functions in a great variety of organisms. In food technology, especially the polysaccharides of plant or bacterial origin play major roles. Typical keywords are: glycoscience, carbohydrate chemistry, glycobiology, cell-cell and cell-matrix recognition events, protein solubility, protease resistance, clearance from the circulation, activation/deactivation of host immunological systems, acting as immunodeterminants, correct folding and secretion, stabilization of the protein conformation, protein targeting, charge and waterbinding capacity.

Research projects Glycoscience

The multidisciplinary glycoscience program is focused on the integral approach of studying structure – function relationships of glycans.

(i) Gaining insight into the functioning of carbohydrates and glycoconjugate glycans requires as a first step the determination of the primary and three-dimensional structures. As protein glycosylation is a major post-translational event, glycoproteomics (glycomics) is of special importance.

(ii) Subsequently, biologically relevant glycan chains and mimics thereof are selected and synthesized using organic chemical and enzymatic routes to generate potentially active components in such amounts that glyco-biological studies are feasible.

(iii) Finally, unravelling the recognition of glycans by studying the interactions on the molecular level between carbohydrates and complementary biomolecules contributes to understanding of (glyco)biological phenomena, and may generate leads to solve medical, pharmaceutical and rheological problems.

Primary structural analysis studies: recombinant glycoproteins (therapeutics), bacterial polysaccharides (pathogenic bacteria and GRAS bacteria).

Chemical and/or enzymatic synthesis: Preparation of oligosaccharide-protein conjugates as vaccine candidates against pneumoniae. Novel procedures for the synthesis of in vivo stable glycopeptides libraries, via combinatorial
chemistry, to develop cell- or tissue-specific drug targeting devices. Glycogoldnanoparticles for molecular interaction studies.

Interaction studies: Between carbohydrate and carbohydrate synthetic mimics (self-recognition in sponge cells), and between carbohydrate and antibodies (carbohydrate-based vaccines and diagnostics).

Methods & Techniques

- microchemical methods
- enzymatic methods
- chromatography (GLC, HPLC, FPLC, HPAEC)
- mass spectrometry (GLC--EI-MS, MALDI-TOF-MS, LC--ES-MS)
- surface plasmon resonance (SPR, Biacore)
- NMR spectroscopy
- electron microscopy

For more details contact:

Glycoscience:
Prof. Dr. J.P. Kamerling (Kruyt Z709, 2533479, j.p.kamerling@chem.uu.nl)

or consult our website: http://www.bijvoet-center.nl/boc2/
Infectious Diseases and Immunology
(Veterinary Medicine)

Rottier/De Groot

General aim of the research performed at the Division of Virology is the understanding of the infection process of viruses at the level of the cell, the organism and the population, and the development of strategies for the diagnosis of and protection against these infections. With respect to the viruses, we focus on enveloped plus-strand RNA viruses with particular attention to nidoviruses (i.e. coronaviruses, toroviruses and arteriviruses), important pathogens of humans and animals.

Research themes

Central themes in our research program are: the structure and assembly of nidoviruses; the targeting and cell entry of coronaviruses; the functions of structural and nonstructural proteins in the infection process and in the pathogenesis of nidoviruses; interactions of nidoviruses with host cells and with the host immune system; vaccine development.

Highlights:
- Fusion-inhibitors as potential antiviral drugs
- Genetically-modified life coronavirus vaccines
- Genetically-modified coronaviruses as anti-tumor agents
- Identification of host cell proteins involved in viral replication
- Structure-function analysis of receptor-binding proteins

Methods and Techniques

- Classical and state-of-the-art molecular virological techniques (including reverse genetics of (+) strand RNA and DNA viruses);
- standard molecular biological techniques (cloning, heterologous gene expression, sequence analysis, RIPA, Western-, Southern-, Northern-blotting, RT-PCR, quantitative Taqman (RT)PCR), bio-informatics, selected cell biological techniques (including RNAi), selected immunological techniques (including three-color flow cytometry)

For more details contact:
Dr. R.J. de Groot, Division of Virology
Androclus building
Room 508
Tel.: 2531463/2485
E-mail: R.J.deGroot@vet.uu.nl
Web: http://www.vet.uu.nl/site/viavet_english/faculty/departments/i_i/virology
Medicinal Chemistry

Liskamp

Chemical approaches are aimed at answering questions related to understanding as well as influencing peptide-protein, carbohydrate-protein and protein-protein interactions. There is a certain bias towards interactions that underlie infection and immunological disorders. However, many other research topics are studied in collaboration with groups in the ABC faculties. Especially modified (phospho-, glyco- and lipo-) peptides and peptidomimetics as well as carbohydrates are used in conjunction with defined receptors.

Research Projects

- Modulation of and interference with protein-protein interactions of signal transduction involving proteins containing SH2 domains by (phospho)peptides and peptidomimetic constructs
- Interference with protein-protein interactions leading to inhibition of protein-protein interaction and fibril formation of amyloid proteins in diabetes, Alzheimer etc.
- Protein-protein interactions in antibody-antigen interactions directed towards artificial antibodies and discontinuous epitopes
- Interference with carbohydrate-protein interactions by design and synthesis of high-affinity monovalent and multivalent carbohydrate ligands
- Development and evaluation of chemical probes for functional proteomics

Methods and Techniques

Modern Synthetic Organic Chemistry; Synthesis of Peptides, Peptidomimetics and Carbohydrates; Solid Phase Synthesis and Combinatorial Chemistry including employing peptide- and robot-synthesizers; GC, analytical and preparative HPLC; Assays for biological activity (SPR)/screening (fluorescence microscopy); NMR/conformational analysis; MAS-NMR with nanoprobe; MS (ESI and MALDI-TOF); Molecular modeling

For more details contact:

Prof. dr. Rob M.J. Liskamp (Went Z717, Z703 (secr), 2537396 or 2537307, r.m.j.liskamp@pharm.uu.nl or secrmedi@pharm.uu.nl)

or consult our website: wwwcmc.pharm.uu.nl/MedChemWEB/
Membrane Enzymology, aangepast
Bosch/Egmond/Holthuis/Spring/van Meer

Research Topics

- Specificity and response of lipid-protein interactions.
- Sphingolipid-protein interactions in the lateral organization and sorting of membrane proteins.
- Protein and lipid modifications in the secretory pathway.
- Key enzymes of sphingolipid metabolism, roles in cell growth regulation and homeostasis.
- Mechanism of transbilayer lipid transport in relation to membrane deformation and vesicular trafficking.
- Mechanism of lipid uptake and release by lipid transfer proteins
- Structure-function relations of lipolytic enzymes

Research Highlights

• We identified two P-type ATPases required for aminophospholipid transport across the yeast plasma membrane and uncovered a functional link between ATPase-dependent lipid pumping and endocytic vesicle formation (Pomorski et al., 2003). We found that the yeast Golgi contains two related ATPases with a critical role in secretory vesicle budding (Alder-Baerens et al., 2006). Our current work aims to unravel the mechanism of ATPase-dependent lipid transport in relation to membrane deformation and vesicular trafficking. A collaboration in the UU High Potential program of Joost Holthuis with Leo Klomp (UMCU) focuses on the 15 human orthologs of these ATPases, which have been implicated in severe liver disease, obesity and neurological disorders.

• Using a bioinformatics and functional cloning strategy in yeast we identified a family of sphingomyelin synthases (Huitema et al., 2004). These enzymes play a key role in cell growth regulation and are upregulated in tumor cells and Plasmodium, the parasite causing malaria. We developed a high throughput screen for sphingomyelin synthase inhibitors (patent pending) that may facilitate identification of a novel class of drugs against cancer and malaria.

• The simple glycosphingolipid glucosylceramide (GlcCer) is required for pigment synthesis in melanocytes and is needed for the sorting of melanosomal proteins (Sprong, 2001). We have now observed that GlcCer lowers the pH in secretory organelles (with Dave van den Heuvel and Hans Gerritsen, Debye Inst.), and specifically activates the proton-ATPase in isolated membranes. GlcCer is translocated from its cytosolic site of synthesis to the lumenal aspect of the Golgi, where it is used for complex
glycolipid synthesis. The underlying molecular mechanisms are being elucidated to check the possibility that ceramide, the central lipid in sphingolipid metabolism, regulates the pH in the secretory pathway through GlcCer.

• How lipids carry out specialized functions at the molecular level is our focus of interest. The functionality of each lipid is determined by its local concentration in time, as it arises from lipid turnover and transport (van Meer, 2005). Key in understanding (sphingo)lipid functionality in cells is knowledge about the ways in which the lipid composition in membranes is sensed and controlled. Lipid transfer proteins play an important part in such processes. Their structure-function relations are investigated in vitro and within the living cell.

• Proteins that are covalently linked to lipids tightly associate with membranes. We found that the morphogens Hedgehog and Wnt use their lipid-anchors to bind to lipoproteins. Further biochemical and genetic analysis revealed that lipoproteins are the major carrier of morphogens through tissues (Sprong et al., 2005). We recently discovered that parasites like schistosomes and trypanosomes transfer their lipid-linked surface proteins to host lipoproteins, most likely to deceive the host immune system. Together, these findings imply a widespread application of the interaction of lipid-linked proteins with lipoproteins (Eaton et al., 2005).

• Many of the important recombinant therapeutic proteins are glycoproteins. The quality of the glycans attached to these proteins is determined by the activity as well as localization of glycan modifying enzymes in the various secretory compartments. In order to develop plants as a production platform for therapeutic proteins, we have humanized their glycosylation machinery by introducing catalytic domains of human glycosyltransferases coupled to plant sub-Golgi anchor sequences. The data show that plants can be tailored to synthesize human glycoproteins.

• Lipid transfer proteins have recently been identified in playing regulatory functions in cellular metabolic processes of vital importance. While interactions between protein and single lipid molecules were known in molecular detail, little was known about the mode of action of the transfer proteins. We obtained evidence for lipid transfer proteins acting on lipid membranes like lipolytic enzymes such as phospholipases. This implies that lipid transfer is controlled by the molar fraction of the lipid to be transferred in the membrane rather than its surface concentration.

• The active site of serine hydrolases is a unique target for the introduction of new catalytic functions, e.g. using phosphonate derivatives as active site directed molecules. Thus a metallopincer molecule was introduced stereospecifically into a lipase yielding a new catalyst.
**Key Publications**


**Patents**


The department has expertise in the following areas:

- Molecular biology (cloning, site-directed mutagenesis, overexpression in bacteria and yeast, knock-out by RNA interference)
- Enzymology (substrate specificity, kinetics)
- Protein chemistry (purification of proteins, protein-ligand interactions, peptide synthesis, protein reconstitution)
- Lipid chemistry (purification, mass spec analysis, assays for transmembrane orientation)
- Combinatorial chemistry (peptide banks)
- Cell biology (immunofluorescence microscopy, subcellular fractionation, membrane isolation, protein sorting assays)

For more details contact:

Prof.dr G. van Meer (Kruyt N607, 2533427, g.vanmeer@chem.uu.nl or consult our website: http://cble.chem.uu.nl/epe/
Molecular Biophysics, aangepast

Gerritsen

Biophysics; the development and application of state-of-the-art fluorescence microscopy and spectroscopy techniques. Spectroscopic techniques are employed in combination with fluorescence microscopy to obtain more detailed and quantitative information at the microscopic level. For the applications the group collaborates with several of the ABC groups.

Research projects

Fluorescence lifetime and spectral imaging

Fluorescence lifetime imaging and spectral imaging are used in combination with confocal microscopy and multi-photon excitation microscopy for investigating co-localization and interactions between biomolecules. Examples include receptor ligand interaction studied by means of Fluorescence Resonance Energy Transfer.

Single Molecule Imaging

Here, the signal of a single fluorescent labeled molecule is followed using a microscope. This offer the opportunity to observe details that are hidden in conventional microscopy due to averaging effects. Effects that can be observed include enzymatic activities, conformational changes etc. At present we investigate the diffusion of single molecules in artificial membranes (bilayers). This work will be extended to include studies on processes such as dimerization and single molecule work on living cells.

Characterization and evaluation of fluorescent labels

This work includes the investigation of conventional dyes as well as novel labels such as quantum dots and fluorescent colloids. Properties that are investigated include the effects of the local environment (cellular, solvent) on the fluorescence decay time, emission spectrum and polarization.

Methods & Techniques

Fluorescence Lifetime Imaging (FLIM), Spectral Imaging (SPIM), Confocal Microscopy (CLSM), Two-photon Excitation Microscopy (TPEM), Fluorescence Resonance Energy Transfer (FRET)

For more details contact:
Prof.dr H. Gerritsen (Ornstein Lab. 060, 253 2824, h.c.gerritsen@phys.uu.nl) or consult our website: http://www1.phys.uu.nl/wwwmbf/
The research of the section Molecular Genetics of the Department of Biology is focused on the themes development, signal transduction and plant-microbe interactions. Within the Plant-Microbe Genetics Group we study the molecular processes that are crucial in establishing a successful plant-microbe infection. The focus is on molecular changes during the infection process, the communication between host and pathogen, and the identification and analysis of host genes required for pathogen infection. The plant *Arabidopsis thaliana* (a mustard weed) is our favourite model organism. It has important advantages for basic research in genetics and molecular biology, e.g. full genome sequence, large number of mutant lines and genomic resources, efficient transformation, etc.

**Research projects**

*Molecular genetics of plant disease susceptibility & resistance*

Several *DMR* genes have been cloned in our group that are involved in disease susceptibility, as mutations in those genes render the plant resistant to downy mildew infection. Current research is aimed at understanding the molecular role of the *DMR* genes in defense and pathogen infection. In collaboration with plant breeders we work on the transfer of *DMR* technology to crop plants.

*Downy mildew genomics*

The genome sequence of the Arabidopsis downy mildew pathogen *Hyaloperonospora parasitica* has recently been determined. In this genomics project we identify and functionally analyse secreted proteins of *H. parasitica*. Two major classes of secreted proteins have been identified; (i) the extracellular proteins with potential inhibitory or enzymatic functions that are required for the infection process (e.g. suppression of host defense proteins), and (ii) the translocated proteins, which travel into the host cell via an unknown mechanism, and interfere with host cell processes (e.g. with defense signal transduction). We study a subset of effector proteins and their mode of action in establishing disease.

*Signalling at the host-pathogen interface*

The pathogens we work with are extracellular organisms. Yet they modulate host processes and can grow efficiently at the cost of the host. To understand the signalling and transport processes at the plant plasma-membrane (PM) we study both host membrane proteins and secreted pathogen effector
proteins. To identify PM proteins with an important role during disease we use a combination of genomic and proteomic approaches, as well as GFP-fusion proteins to determine pathogen-associated localization.

Methods & Techniques

- molecular biology (DNA/RNA isolation, cloning, etc.)
- genetic analysis, mutagenesis, overexpression
- expression profiling (DNA microarrays)
- genome analysis, bioinformatics
- reporter gene analysis (GFP, GUS, LUC)
- microscopy (DIC, fluorescence, CSLM)

For more details contact:

Dr. Guido van den Ackerveken (Kruyt room N410, 2533013, G.vandenAckerveken@bio.uu.nl), or consult our website: http://www.bio.uu.nl/mg/
Eukaryotes Group

Filamentous fungi fulfil important roles in nature by degrading organic waste or by forming symbiotic or pathogenic relationships with other organisms. They grow by means of hyphae that extend at their apices while branching subapically. As a result, a mycelium is formed consisting of a network of hyphae. Within the mycelium the cytoplasm is more or less a continuum since hyphae are compartmentalized by porous septa only.

Fungal hyphae grow under variable conditions. They may colonize aqueous substrates, grow into the air to form reproductive structures, or grow on a hydrophobic surface of a host. To adapt to their environment fungi secrete a spectrum of proteins. Secreted enzymes degrade polymers in the substrate to acquire nutrients, whereas secreted structural proteins determine the surface characteristics of the hypha enabling for instance cell-cell contacts, attachment to surfaces, or efficient dispersal.

We are interested in the process of protein secretion and the role these secreted proteins fulfil in fungal growth and development. We study the saprofytic fungi *Schizophyllum commune* and *Aspergillus niger* and the pathogenic fungi *Ustilago maydis* and *Cryptococcus neoformans*. *S. commune* is the model organism for mushroom forming fungi, while *U. maydis* and *C. neoformans* are important plant and human pathogens, respectively. *A. niger* is used in the industry for large scale protein production. Thus, the research is not only interesting from a fundamental point of view, it is also of significance for the industry, agriculture and medicine.

Research Projects Eukaryotes Group

- Protein secretion within the fungal mycelium
- Protein secretion within a fungal hypha
- The role of secreted proteins in fungal growth
- The role of secreted proteins in reproduction and pathogenicity

Methods & Techniques

We use molecular techniques at DNA and protein level as well as microscopy (LM, CLSM, SEM, TEM).
**Prokaryotes Group**

The cell envelope of Gram-negative bacteria consists of two membranes, the inner membrane and the outer membrane, which are separated by the periplasm containing the peptidoglycan layer. The inner membrane is a phospholipid bilayer, whereas the outer membrane is an asymmetrical bilayer with phospholipids and lipopolysaccharides (LPS) in the inner and outer monolayer, respectively. The outer membrane functions as a protective barrier allowing the influx of nutrients by passive diffusion via pore-forming proteins, designated porins. Since the outer membrane is not energized, active transport processes usually require complex machineries and energy-coupling mechanisms. Our fundamental research programme focuses on the elucidation of transport processes in the Gram-negative bacterial cell envelope, including the biogenesis of the outer membrane, nutrient uptake and protein secretion. The knowledge generated is applied, amongst others, in vaccine development and biotechnology.

**Research projects Prokaryotes Group**

- Biogenesis of the outer membrane
- Mechanisms of protein secretion
- Vaccine development

**Methods & Techniques**

We use a myriad of techniques in the fields of molecular biology, biochemistry, microbiology, genetics, cell biology and immunology, including gene cloning and sequencing, site-directed mutagenesis, in vitro transcription-translation assays, in vitro protein translocation assays, pulse-chase experiments, immunoprecipitation, protein purification, enzyme assays, in vitro protein folding, circular dichroism, thin-layer chromatography, enzyme-linked immunosorbent assays, subcellular fractionation, sucrose density gradient centrifugation and immunofluorescence microscopy.

**For more details contact:**

*Eukaryotes*
Prof.dr. H.A.B. Wösten (Kruyt W401, 2533448, H.A.B.Wosten@bio.uu.nl)

*Prokaryotes*
Prof.dr. J.P.M. Tommassen (Kruyt W411, 253 2999, J.P.M.Tommassen@bio.uu.nl)

or consult our website: [http://www.bio.uu.nl/~microbio/](http://www.bio.uu.nl/~microbio/)
The research of the protein crystallography group focuses on bio-molecular recognition and regulation processes with emphasis on bio-medically important proteins. In many cases, the proteins involved are multi-domain proteins that function in large complexes. Our goal is to reveal by X-ray diffraction the precise atomic (re)arrangements which underlie highly specific recognition and tight regulation.

The main themes include haematology and immunology. In these projects we collaborate with biomedical and biological research groups of the Utrecht University. The projects provide a structural basis for host-pathogen interactions and for understanding molecular disorders in diseases, which is crucial for structure-based approaches to develop novel drugs and vaccines.

In addition, our department has a long tradition in crystallographic methodology with two exciting current research lines that focus on developing diffraction methods and on the principle phase problem in (protein-) crystallography.

**Research projects**

*Immune defence by the human complement system*

Protection against pathogenic bacteria critically depends on the complement system in blood plasma. This system of ~35 multi-domain proteins enables the host to specifically recognize and kill invading microbes. We study proteins involved in initiation and activation steps in the classical pathway of complement activation. This research involves structural biology of protein domains as well as intact multi-domain proteins and complexes thereof. The goal in this challenging area is to reveal the spatial mechanisms employed to strictly regulate i) initiation by the formation of immune complexes on the bacterial surface, ii) amplification in the protease cascade and iii) lysis of the pathogenic bacterium through the formation of the “membrane-attack complex”.

*Regulatory steps in bleeding arrest*

The protein von Willebrand factor (VWF) is a huge multimeric glycoprotein from blood plasma and is a typical example of a multi-domain protein with separate functions assigned to separate domains. Together with the dept. of Haematology (UMCU) we study the roles of these domains and their complexes in recognition and regulation. The primary tool is crystal-
structural analysis combined with mutagenesis and functional studies. These studies provide fundamental insights into the molecular cause of various (VWF-related) bleeding disorders. The structures serve as a starting point for designing potential anti-thrombotic peptides.

Membrane proteins and membrane-associated proteins

Membranes and membrane proteins are essential for a wide range of biological functions. Fortunately, significant advances in the field of structural biology of membrane proteins enable detailed studies of this exciting class of proteins. We study the outer-membrane proteins of pathogenic bacteria, in particularly those from Neisseria meningitidis that may cause life-threatening meningitis and sepsis (in coll. with dept. of Microbiology). The goal is twofold, i) to gain fundamental insights into outer-membrane functioning (translocation, transport, membrane biogenesis) and ii) to aid in development of vaccines and anti-microbial compounds against serotype B of N. meningitidis.

Cross-beta structure

Protein misfolding diseases, such as Alzheimer's disease, Parkinson's diseases and prion diseases are associated with the deposition of amyloid in specific tissue. The major component of amyloid consists of proteins or peptides forming the cross-beta structure. The toxicity of amyloid has recently been found to be related to soluble oligomers. Several proteins and antibodies were found that interact with amyloid, irrespective of the amyloid amino acid sequence. We study the structures of both of these multi-ligand receptors and the peptides forming amyloid by single crystal and fibre X-ray diffraction. The goal is to understand the specific requirements for the recognition of amyloid, and the role that certain proteins play in the regulation of amyloid in vivo.

Crystallographic methods development

Methods development focuses on two aspects of crystallographic computing. Using the in-house diffraction-data-processing package EVAL we develop new methods and software to address complex diffraction patterns (e.g. from fibrous material) and to improve data quality from weakly diffracting crystals (e.g. from huge protein complexes). Using the program CNS ("Crystallography and NMR System) we expand the refinement technology with the goal of circumventing the central phase problem in protein crystallography.

Methods & Techniques

- cloning and expression
- cell culture
- protein purification
- protein characterization with biophysical techniques
- protein crystallization
- single-crystal and fibre diffraction
- X-ray data processing
- crystallographic computing
- structure determination

For more details contact:

Dr E.G. Huizinga (Kruyt N-810, 2532866, e.g.huizinga@chem.uu.nl)
or consult our website: http://www.crystal.chem.uu.nl
Protein Folding (Bio-organic Chemistry 1)

Braakman/Rüdiger/Tabak

Most newly synthesized proteins in a cell fold in either the cytoplasm or the endoplasmic reticulum (ER). Molecular chaperones and folding enzymes in these compartments assist the folding process and check the quality of folding proteins. Young proteins in the ER undergo various modifications essential for proper folding, such as glycosylation of asparagine residues, cis-trans isomerization of prolines, and oxidation of cysteines to disulfide bonds. The quantity of ER in a cell (i.e. the amount of membrane as well as the amount of chaperones and folding enzymes) is adapted to the need, to the circumstances. If the ER fills up with aggregated misfolded proteins, or when a large amount of protein needs to be synthesized and folded, the compartment increases its size.

Research projects

Characterizing the folding pathway of our model proteins in the ER

These model proteins are the Influenza virus hemagglutinin (HA), HIV-1 Envelope glycoprotein gp160, the low density lipoprotein receptor (LDL-receptor), and CFTR. The latter two proteins contain mutations in patients with FH (familial hypercholesterolemia) and cystic fibrosis, respectively.

Determine the role cellular factors play in protein folding and quality control

These factors include ATP, calcium, redox-milieu, but also molecular chaperones and folding enzymes. We characterize the known folding factors and identify and study new ones.

Molecular mechanisms of substrate recognition of molecular chaperones

Hsp90 chaperones select specifically for kinases and transcription factors. The molecular mechanisms of this selection are unclear, and it is not known how the chaperone actually folds its substrates. We study these questions by biochemical and biophysical methods. The aim of this research line is to gain insights into the structural properties of unfolded proteins in the cell.

Peroxisome

This research line does not focus on the ER but on another cellular organelle, the peroxisome. We study the role of cytosolic chaperones and associated
proteins in the maturation and import of proteins into the peroxisome. Since we found peroxisomal membranes to originate from the ER, we plan to examine an intermediate compartment between these two organelles.

**Methods & Techniques**

- mammalian and yeast cell culture
- (recombinant) virus infection
- radioactive pulse-chase
- in vivo and vitro folding assays
- in vitro translations
- (co-)immunoprecipitation
- SDS-PAGE
- Immunofluorescence
- DNA cloning
- yeast genetics
- proteomics
- 2D-gel electrophoresis
- protein expression and purification
- Fluorescence spectroscopy
- NMR spectroscopy

**For more details contact:**

The Department’s secretary office (Kruyt O706, T: 030 - 253 2184, E: folding@chem.uu.nl)

or consult our website: http://www.bijvoet-center.nl/boc1/
Thrombosis is the major cause of death in the western society. Arterial thrombosis may lead to myocardial infarction and stroke, venous thrombosis to pulmonary embolism. Thrombosis is the pathological counterpart of hemostasis, which is the mechanism that following vessel wall injury prevents excessive blood loss. Aim of the laboratory’s research efforts is to better understand the control of hemostasis thereby findings means to improve therapeutic intervention of arterial and venous thrombotic disease.

Research projects

Platelet-vessel wall interaction
Platelet adhesion to the injured vessel wall is the first step in the pathogenesis of thrombosis. This process is studied in a perfusion chamber to better mimic physiological conditions. Studies are focussed on the role of von Willebrand factor (vWF), an adhesive protein that binds to the platelet receptor glycoprotein Ib thereby coupling platelets to the site of injury. Current projects are (i) studies on glycoprotein Ib binding to vWF at the atomic level and, (ii) real-time analysis of platelet deposition on collagen. Von Willebrand Factor is not only involved in the adhesion of platelets, but also provides an adhesive surface for leukocytes (neutrophils, monocytes). The molecular basis of this interaction is studied in perfusion chambers, employing recombinant proteins and various leukocytic cells.

Von Willebrand Factor clearance

Von Willebrand’s disease (VWD) is the most common inherited bleeding disorder affecting up to 1% of the population. In our laboratory, we have discovered that increased clearance of VWF contributes to the pathology of this disease. Further in vitro and in vivo studies are being performed to understand the molecular basis of how increased clearance contributes to the low levels of vWF in patients with VWD.

Antiphospholipid antibodies

The anti-phospholipid syndrome is defined by recurrent venous and arterial thrombosis associated with the presence of antiphospholipid antibodies. These antibodies are directed against β2-glycoprotein I bound to anionic phospholipids of blood platelets. Formation of bivalent antibody-β2-glycoprotein I complexes are considered a major cause of the pathology of the syndrome. We have constructed a dimer of β2-glycoprotein I molecule
which is used as a substitute for anti-ß2-glycoprotein I antibodies to study the mechanism by which antiphospholipid antibodies induce thrombosis.

**Platelet activation and signal transduction**

Blood platelets are small, anucleated cells that are essential elements in the cessation of bleeding and the development of thrombosis. Platelets are formed from megakaryocytes in the bone marrow, circulate during about 10 days and in part sequester in the spleen. Under normal conditions the cells maintain a resting, disc shaped conformation but once activated, platelets change shape, form pseudopods, stick to adhesive surfaces and couple to other activated platelets thus forming an aggregate. In the meantime activated platelets facilitate the coagulation process and secrete substances that induce vasoconstruction.

The research of the platelet signal transduction group is focussed on the mechanisms that regulate the activation and inhibition of platelet functions. Specifically, the concept of “stimulus-response coupling” is characterized in detail including receptor identification, signal generation by activated receptors, signaltransduction pathways in the cytosol and the mechanisms that execute the different platelet functions. Part of these studies make use of cultures of human megakaryocytes to assess the biogenesis of activating and inhibiting pathways of the platelet.

Recent studies have indicated that sphingolipid- and cholesterol-rich microdomains (rafts) exist in the platelet plasma membrane. These membrane domains are highly dynamic and play a crucial role in signal transduction. Upon surface activation, cholesterol-rich domains cluster in the tips of platelet filopodia, together with Src-kinases and co-stimulatory molecules (CD63). Our studies are focused on the characterization of raft-dependent signaling pathways during platelet adhesion to collagen via glycoprotein VI leading to aggregation and to von Willebrand factor vail glycoprotein Ib leading to the formation of spread platelets that fail to aggregate.

**LRP**

The research line is related to the interaction between pro- and anti-coagulant proteins and surface-exposed receptors. In this regard, part of the studies is directed to the identification and characterisation of the molecular mechanisms that contribute to receptor-mediated endocytosis of these haemostatic proteins. In addition, the potential of ligand/receptor complexes to influence processes such as gene expression and cell proliferation is studied.

**Nitric Oxide**

Nitric oxide (NO) is an important mediator in the inhibition of thrombotic and pro-atherogenic events such as platelet aggregation and monocyte adhesion
to the endothelium. Production of NO is regulated by endothelial nitric-oxide synthase (eNOS), or via induction of the inducible NOS isoform (iNOS). Recent studies in several cell types have shown that NO–mediated protein modification (i.e. protein tyrosine nitration, S-nitrosylation of cysteine residues) plays a crucial role in cell signaling pathways. For example, iNOS induction and NO formation is important for megakaryocyte development and platelet formation. NO also regulates exocytosis by S-nitrosylation of N-ethylmaleimide-sensitive factor. The project focuses on (i) determination of \textit{in situ} end products of NO and oxidative stress (peroxynitrite-induced protein nitration) and, (ii) the role of NO-mediated S-nitrosylation and tyrosine nitration in thrombosis and atherosclerosis.

\textit{Interplay between coagulation and platelet functioning}

The formation of both a platelet plug and a fibrin clot are required for the generation of a stable hemostatic plug. Platelet plug formation and fibrin generation have in the past been investigated separately, but it has become clear that both processes influence each other in several ways. We are currently investigating the role of fibrin in platelet aggregate formation. Inhibitors of fibrin-mediated platelet plug formation may be a relevant new targets in treatment of thrombosis.

\textit{Protein misfolding and hemostasis}

Each protein has a unique 3-dimensional conformation, which determines its specific function. Proteins can lose their structure. This can occur spontaneously, but can also be caused by aging or disease. Unfolded proteins can aggregate and fibrillize. Such fibrillar aggregates can accumulate in various tissues and are associated with degenerative diseases. The term “amyloid” describes these fibrillar deposits (or plaques). Diseases characterized by amyloid are referred to as amyloidosis and include Alzheimer’s disease, type II diabetes, and Creutzfeld-Jacob Disease (the human variant of mad cow disease). Thus, avoidance of amyloid formation and clearance of misfolded proteins is of critical importance for cell viability. We recently discovered that two proteases that control hemostasis recognize misfolded proteins. Together with other evidence this implicates that the hemostatic system is involved in the clearance and/or toxicity of protein aggregates and suggests that the hemostatic system has evolved to protect against toxic aggregates of misfolded proteins. The recent finding that protein misfolding and formation of toxic aggregates is an inherent property of proteins raises the possibility that aggregates of any protein might occur spontaneously and account for systemic and neurological disorders not yet associated with amyloid formation. The focus of our research group is to clarify the role of the hemostatic system in conformational diseases.

\textit{Methods & Techniques}

- protein cloning
- expression and purification
- site-directed mutagenesis
- protein crystallization (in collaboration with Bijvoet Center)
- analysis of receptors and second messengers
- flow models with whole blood
- protein-protein interaction (Biacore)
- confocal and electron microscopy

For more details contact:

Prof. Dr. J.W.N. Akkerman (030-2506512; j.w.n.akkerman@lab.azu.nl) or consult our website: www.thrombosis.umcutrecht.nl
Names and Addresses

Addresses programme

**Programme Director:** Prof. Dr. P. Gros  
Protein Crystallography  
Kruyt building, room N806  
Padualaan 8  
3584 CH Utrecht  
Tel.: +31 (0)30-2533127/3502  
Fax: +31 (0)30-2533940  
E-mail: p.gros@chem.uu.nl

**Programme Coordinator:** Dr. V.J. Winter  
Kruyt building, room W502  
Padualaan 8  
3584 CH Utrecht  
Tel.: +31 (0)30-2532598  
E-mail: V.J.Winter@bio.uu.nl

**Board of Studies Biomolecular Sciences**

Prof. Dr. P. Gros  
Prof. Dr. A. Killian  
Prof. Dr. H. Wösten  
Prof. Dr. G. Strous  
Dr. P. van Bergen en Henegouwen  
Dr. V.J. Winter

**Admissions Committee Biomolecular Sciences**

Prof. Dr. R. Boelens  
Dr. M. Slijper  
Dr. P. van Bergen en Henegouwen  
Dr. V.J. Winter

**Student administration**  
(Questions regarding ECTS, Osiris etc.)

*Student Administration Chemistry:*  
Went building, room Z.003  
Sorbonnelaan 16  
3584 CA Utrecht  
e-mail: boi@chem.uu.nl  
Office hours: Mon, Tue, Thu, Fri from 11:00-12:30 and from 13:00-15:00 (closed on Wed)
Student Administration Biology:
Went building, room Z.005
Sorbonnelaan 16
3584 CA Utrecht
e-mail: onderwijszaken@bio.uu.nl
Office hours: From Mon to Thu, from 8:30-13:30, Fri from 8:30-12:00

Student Administration Medical Biology:
Stratenum, lobby
Universiteitweg 100
3584 CG Utrecht
e-mail: studentenbalie@med.uu.nl
Office hours: From Mon to Fri, from 11:00-14:00
Graduate School of Life Sciences

Department of Chemistry, Utrecht Science Faculty
Department of Biology, Utrecht Science Faculty
Biomedical Sciences, Faculty of Medicine

Copyright
University of Utrecht
Department of Chemistry
School of Chemical Sciences
Sorbonnelaan 16
3584 CA Utrecht

August 2006

Editors
Drs. M.J. Thurnim
Dr. V.J. Winter