

## Thomas Hankemeier: 'How can biological systems be described over time?' From target analysis to a holistic approach

**H**ow to measure all components in a complex mixture? How to know which components are relevant for certain properties of a material or an organism, or for the onset of a disease? These are the general denominators in Thomas Hankemeier's research. From October 1, the energetic and enthusiastic young analytical chemist is the LACDR's new Professor of Analytical Biosciences at Leiden University.

Thomas Hankemeier has been extremely lucky. His grandfather was an avid chemistry teacher. Together they bought laboratory equipment including a balance and a Bunsen burner. As a school kid of seven or eight years old Thomas performed chemistry experiments for his mother on top of the kitchen table. Later on, in collegial co-operation, grandfather and grandson made rockets.

'Here my love for chemistry started', smiles Hankemeier thirty years later. With a slightly sad undertone he adds: 'It's really a pity that my granddad is not able to share the pleasure of my professorship in our mutual research area.'

From October 1, 2004, the 37-year young, German-born chemist is the new Professor of Analytical Biosciences at the Leiden branch of the LACDR, in addition to Professor Jan van der Greef.

### Hard choice

After the gymnasium Thomas had to choose between chemistry, medicine and biochemistry. Although it was a hard choice, it became chemistry. His Masters-degree in analytical chemistry he got at the University of Ulm in Baden-Württemberg.

'It was a very pleasant, rather free study', he explains. 'What I really appreciated is that we learned – especially in the discussions with fellow students – how to see interconnections between the disciplines.' Hankemeier has a very strong opinion about this stimulating atmosphere he experienced in Ulm. 'The art of thinking has to return to the university. Stimulating creativity should be as important as learning hard facts.'

After having visited a large number of European universities in order to make the proper next step in his education, he chose for the Vrije Universiteit Amsterdam as the place to get his PhD degree, with Professor Udo Brinkman as his supervisor. 'I liked the open atmosphere, and the dynamic and inspiring research environment', he memorizes his stay in Amsterdam.

The preparation of a PhD thesis was interrupted after three and a half years, when he became a group leader at the Netherlands Organization for Applied Research TNO. His very successful research group rapidly grew from four to thirty. However, academia was never far away. He supervised PhD students and was a guest scientist at the University of Amsterdam. In 2000 Thomas Hankemeier himself ultimately got his PhD degree at the Vrije Universiteit.

### Common denominator

During his years at TNO, research subjects ranged from oligomers in packaging materials, the characterization of polymeric materials to biological systems. Despite this variety

of subjects and objects it has never been hard to find a common denominator in Hankemeier's research. He explains why: 'The general questions have always been and will also be for the next period: How can we measure (almost) all components in a complex mixture? How do we know which components are relevant for certain properties of a material or a biological system?'

He finds it a highly fascinating field of research. He gives an example. Plastics are very complex mixtures of shorter and longer polymers. During their lifetime they become older.

Which components are responsible for wearing and ultimately for breaking? Another example is the complexity of the metabolic routes in microorganisms. Which steps in the production routes are the bottle-

necks for a higher production of a certain product? Which pre-cursors are produced at a too low rate? And when talking about diseases: which compounds are good early diagnostic markers?

'Our research effort has to change from target analysis to holistic analytical methods to investigate complex systems and processes', Thomas Hankemeier explains. 'We no longer try to find substances, products or metabolites that we know in advance – the targets – but to determine all known and unknown substances, products or metabolites that are being formed over time and over varying circumstances. This ultimately gives us new insights in all relevant processes that take place in living organisms'.

### Great love

The new approach in which analytical chemistry, multivariate analysis (statistics) and biology closely cooperate has brought Hankemeier back to the great love of his childhood: biochemistry and medicine. 'Only now we are starting to be able to measure all the molecules that are

relevant for very complex biological processes. It is a great challenge to measure as many biological molecules as possible, locally – even in one single cell –, dynamically – during time –, and in small quantities.'

How can biological systems be described over time in the best possible way? How does the communication between cells, between organs and the brain take place? What causes the onset of a disease? How can we treat diseases as early as possible? 'We can only tackle these problems as a multidisciplinary team of bio-analytical chemists, biologists, biopharmaceutical scientists, biochemists and statisticians', states the newly appointed Professor of analytical Biosciences at the LACDR. 'The greatest challenge of the next years will be the integration of all genetic, proteomic and metabolic data using bio-informatics to obtain new insights. This is actually the aim of the concept of systems biology.' It appears that the highly energetic Hankemeier can't wait a second to reach his goal. Even before his start at the LACDR he has laid the first scientific contacts inside and outside Leiden for his new position.



Professor Thomas Hankemeier: 'In doing experiments together with my grandfather, my love for chemistry started.'

Contents	Page
* Thomas Hankemeier, Professor of Analytical Biosciences at Leiden University	1
* Ron de Kloet, Academy Professor 2004 of the KNAW	2
* Highlight Centre of Human Drug Research	2
* LACDR Fall symposium	3
* Highlight Division of Analytical Biosciences,	3
* Professor Fred Regnier as Nauta Professor 2004,	4
* Highlight Division of Toxicology,	4
* Professor Steven Lamberts as Marius Tausk Professor 2004	5
* Highlight Division of Medicinal Chemistry Amsterdam	5
* PhD theses completed	6
* New PhD students appointed	6

## People Awards Grants



**Maroesja van Nimwegen, PhD student**, division of Toxicology has won the Best Presentation Award during the Tumor Cell Biology Meeting, held on April 20-21, 2004 in Lunteren.



On March 24 **Professor Meindert Danhof**, Head of the Division of Pharmacology, received the Rawls Palmer Award at the Annual Meeting of the American Society for Clinical Pharmacology Therapeutics in Miami, Florida. The award was presented to him by Professor Gerhard Levy for his pioneering research on mechanism-based pharmacokinetic-pharmacodynamic modelling.



**Peter Keizers**, division of Molecular Toxicology, received the "best poster award" of the KNCV-section of Pharmacology at NWO-CW discussion group Pharmacology in Lunteren.



**Douwe Breimer** (former Director of Research), receives an honorary doctorate from the Montreal University, Canada, in September 2004 for his outstanding research in the area of the pharmaceutical sciences.



**Henk Timmerman** (former Director of Research), was awarded a "Doctor Honoris Causa of Medicine" degree by the Medical University of Łódź, Poland, for his outstanding contributions to the fields of medicinal chemistry in September 2004.

continued on page 2

From page 1



**Yan Ping Zhang**, division of Medical Pharmacology, received the S.E. de Jongh Prize 2003 in pharmacological sciences of Leiden University and Leiden University Medical Centre.



**Paola Casarosa**, division of Medicinal Chemistry (Amsterdam), has won the first prize for her poster presentation within the biology section, in the Pfizer postgraduate symposium in Sandwich, UK. She also obtained one year support from the Board of the Vrije Universiteit, which has started a special program for talented students and graduates, which allows Paola to work for one year in the group in order to finish some exciting last experiments. Paola started in January 2004 as post-doc in the Division of Medicinal Chemistry (Amsterdam).



**Cathelijne Knibbe, PhD**, division of Pharmacology, has been awarded with the annual PhD Theses Award of the Dutch Society for Clinical Pharmacology and Biopharmacy.



**Miranda van Eck, PhD**, division of Biopharmaceutics, has won the Young Investigator Award for her poster presentation on "Macrophage ABCA1 overexpression inhibits atherosclerotic lesion progression at the XIIIth International Symposium on Atherosclerosis in Kyoto, Japan.



**Professor Henk J. de Jong**, has been chosen as 1st vice-president of the European Pharmaceutical Committee for the period 2004-2007.



**Gijs van Puyvelde**, division of Biopharmaceutics, won the best poster presentation award during the Fifth ST-Gerlach vascular biology workshop. He also won a prize for the best abstract of the 7th Dutch Atherosclerosis Society Symposium, held in Ermelo.

continued on page 3

Ron de Kloet: 'This *had* to be of utmost importance'

## Academic freedom leading to excellence

LACDR's medical pharmacologist Professor Ron de Kloet has been appointed Academy Professor 2004 by the Royal Netherlands Academy of Arts and Sciences 'for his excellent contributions to stress biology'. His research resulted in new concepts for the treatment of a disturbed stress response homeostasis. Academy Professors are exempt from administrative duties, in order to concentrate on doing innovative research and coaching talented young researchers. De Kloet gained five new years of academic freedom.

The screensaver of Ron de Kloet's computer constantly changes from one to another colorful image of seahorses. The marine fish with the pointed horse head is depicted everywhere in his office: a poster embellishes the wall, several smaller examples in brass and glazed ceramics lay distributed on his desk. De Kloet winds up a music box with a cute little example on top of it. A non-relevant jingle sounds. He grins below his graying mustache. 'Its tail is wrongly wrapped: backwards instead of forwards!'

LACDR Professor of Medical Pharmacology admits to have a collection of hundreds of seahorses, ranging from pillows to even a costume. Why this exuberant diversion for such a serious investigator? The ignorant interviewer has to be explained that 'hippocampus' is the Latin name of these strangely shaped fish. The name is derived from the Greek 'hippokampos': horse-like sea monster. The small seahorse-shaped brain organ with the same name is his research object of choice of the last thirty years.

De Kloet picks up a picture from the table in front of him, showing a gray oval with some sharp, bent black lines. With this almost abstract radiophotograph it all started in 1973. He explains that it shows the cross section of a rat brain. The black lines indicate the presence of stress hormone receptors in the rat's hippocampus. Radiolabeled stress hormones concentrate at one distinct area.

The leitmotiv in De Kloet's research has always been the question

how hormones coordinate an individual's physical and mental processes in the preparation for and the handling of stress and how these hormones help the individual to recover from stressful situations. The mission of the Leiden Division of Medical Pharmacology is to discover how stress hormones cause stress-related brain disorders such as major depressive illness, anxiety disorders, post-traumatic stress disorders and how they affect susceptibility to degenerative diseases like Alzheimer's disease.

### French stone

The hippocampus is De Kloet's Rosetta stone. It is the intermediate that realizes context: it gives the individual the spatial coordinates of memory as well as the emotional aspects that are involved. Ron de Kloet gives as an example the stone that you recover in a box in the garage, many years after you found that stone in France.

'The hippocampus brings you back at the spot in the French Alps where you collected it; it lets you memorize the weather at the moment you found the stone, and even how pleasant your vacation was. Without this tiny but extremely important organ you lose your way. You know the looks of the railway station, but you have forgotten how to reach the place.'

Stress hormones influence the function of the hippocampus, the organ that tells you how serious a stressful situation is and how to handle it. When you are distressed, the hormones help you recover. Moreover they make you prepared for stressful situations in the future.



The extremely useful brain organ, however, turns out to be a two-sided sword. De Kloet explains: 'The breakthrough in our hippocampus research came when we realized that this organ has two types of receptors for one single stress hormone cortisol. One receptor helps to *organize* the stress response that is *terminated* by the other. With this finding we established a firm foundation under our research. We found the goldmine.'

The brain is the grand regulator of all body processes, e.g. energy metabolism, immune and inflammatory reactions, physiological processes that have to be in equilibrium, in homeostasis. The hormone cortisol, an essential factor for survival, has a strategic function in this respect. If the cortisol system is disordered, nasty processes are switched on.

continued on page 3

## Clamping for stable ethanol levels

Centre for Human Drug Research

The Centre for Human Drug Research developed the first highly practical way to maintain stable pseudo-steady state levels of ethanol, requiring no specific equipment or expertise. The method is very suited for ethanol effect studies and for accurate drug-alcohol interaction studies.

Alcohol interaction studies are an important part of many drug development programs. Acute drug-alcohol interactions can be determined most reliably if ethanol levels are kept constant, while drug concentrations change after concomitant administration. However, it has been impossible to maintain stable alcohol levels, because of the large intersubject variability and Michaelis-Menten pharmacokinetics of ethanol.

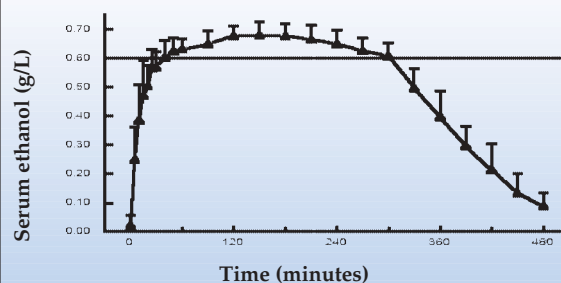
To overcome these problems, O'Conner et al designed a clamping method which starts with an oral alcohol loading dose, followed by an ethanol infusion that is constantly adapted to frequently measured breath alcohol (BrAC) levels [Alcohol Clin Exp Res 22(1), 202, 1998]. However, this method has never been widely used, probably

because of the experience needed to perform the clamp and the frequent adaptations of the infusion rates, and possibly also because of the fluctuations that occur after the oral loading phase.

Based on this original idea, CHDR has now developed a practical and reliable alcohol clamping method that allows alcohol levels to be kept very stable for hours on end, with minimal expertise. In the first 10 minutes of the clamp, ethanol 10% is infused with a fixed regimen. After this loading phase, the ethanol infusion is continued at a rate that is adapted to the results of BrAC-measurements using a simple spread-sheet program. This program calculates a regression equation between the infusion rates and the BrAC-changes over the preceding time interval. This equation is then used to predict the infusion rate that will lead to the desired increase or decrease of the BrAC-level.

The alcohol clamping procedure was performed in twelve healthy male and female volunteers, with a target alcohol level of 0.6 g/L, just above the legal driving limit in the Netherlands. Breath- and serum ethanol concentrations and a battery of central nervous system tests were obtained repeatedly.

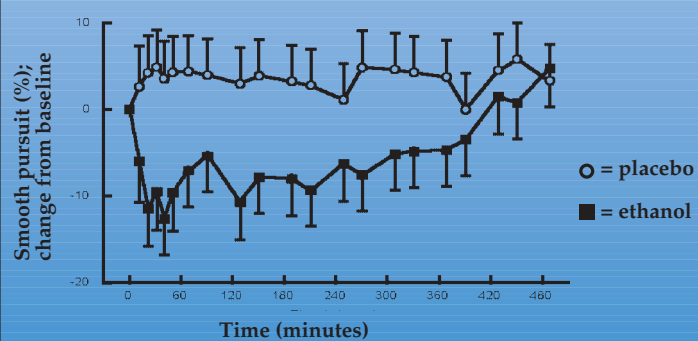
The results showed very stable ethanol levels, without overshoots and with very little inter-subject variation (Figure 1).



**Figure 1:** Very stable serum ethanol concentrations with very little inter-subject variation ( $M \pm SD$ ).

Among several pharmacodynamic measurements that were affected significantly by this low ethanol level, smooth pursuit eye movements responded most clearly (Figure 2).

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**Figure 2:** Smooth pursuit eye movements were affected most by low ethanol levels.

from page 2

This finding has brought De Kloet to the central question: how is it possible that this survival hormone can turn into a signal of death?

### Epigenetics

The solution of this question comes from research in laboratory animals as well as in tissue systems. The Leiden medical pharmacologists identify in animals the genes that are responsible for the stress response and study the emotional and cognitive expressions of behavior – e.g. in the interaction with other individuals.

From this research it has become quite clear that early-life experiences are of utmost importance. De Kloet: 'Stress response is genetically determined but can be programmed very early in life.' The cause of this programming effect appears to occur in part by 'epigenetics': the way the structure and function of chromatin is changed.

De Kloet's group for instance studied rats that were deprived temporarily from maternal care: animals that were taken away from their mothers shortly after birth for several hours. It was known that more maternal stimulation leads to a less reactive stress system. The deprived animals, however, were more sensitive to stress in mid-life. Later in life at senescence these deprived animals performed either good or bad, without any animals with an average performance as is commonly observed in old rats reared as pups normally by the dam.

'This was really an eye-opener for us. The brain mechanism programmed in early life appears to be permanent.'

The explanation of all of this is that for the development of the brain a stable climate of cortisol is essential. The presence or absence of this hormone together with the nature and extent of maternal care influences epigenetics, the way DNA is processed. Everything during brain formation is regulated in a matter of seconds. Stressful situa-

tions during embryo development and after birth in the newborn lead to permanent function changes because the 'wiring' of nerve circuits is restructured.

A large part of the research of LACDR's Division of Medical Pharmacology has a major clinical impact, and Ron de Kloet is proud of that. He gives an example. Psychotically depressed patients often are therapy resistant. The only quite successful way was electroshock treatment. This has changed recently. De Kloet: 'Now we know that anti-glucocorticoids act quite rapidly. By blocking one receptor, the activity of the other becomes more prominent. Our understanding of how this functions makes me very happy!'

### Academic freedom

Ron de Kloet, now sixty, really is a happy person. He was nicely surprised with his Academy Professorship. 'It made me very happy at this moment in my life', he says. 'In retrospect I must say that - although I had phases of success - every new day I have to make a head start. Every day my next paper can be torn into pieces. But, this type of activity works addictive. I never stop.'

He has one final message: the necessity of academic freedom. 'The choice of my research has always been very clear to me: this had to be of utmost importance. Even as a student I was already interested in the functioning of hormones and the brain. Hormones are the essential tools to let brain, body and soul cooperate.'

The fundamental message is that this kind of research was only possible because we had the freedom to study this ourselves. You have to figure out yourself what kind of research you do. Fundamental research takes a lot of time. In my case: this has been a matter of thirty years.'

One thing is clear: with his Academy Professorship Ron de Kloet gained what he always fought for: the ultimate academic freedom. He is a lucky guy: the next five years he is fully exempt from administrative duties.

# LACDR Fall Symposium

30 november 2004

Gorlaeus Laboratories, Lecture Hall 1,  
13.15 h – 18.00 h  
Einsteinweg 55, 2300 RA Leiden

## Pharmacogenomics and Drug Research

Invited speaker: **Dr. Marcel van Duin**  
(Head Department of Pharmacology, Organon N.V., Oss)  
"Genomics and drug development"

**Dr. Erno Vreugdenhil**  
(Division of Medical Pharmacology, LACDR)  
"How stress affects the genes in our brain:  
a pharmacogenomics approach"

**Dr. Johan Kuiper**  
(Division of Biopharmaceutics, LACDR)  
"In vivo identification and evaluation of genes  
involved in atherosclerosis"

**Dr. Bob van de Water**  
(Division of Toxicology, LACDR)  
"Toxicogenomics & Toxicoproteomics and  
mechanisms of cytotoxicity"

Invited speaker: **Prof. Dr. René Bernards**  
(Netherlands Cancer Institute, Amsterdam)  
"Novel tools for functional genomics"

Presentation **SE de Jongh Award** by  
Prof.dr. E. Ron de Kloet

If you wish to attend, please register at:  
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From page 2



**Arnaud Hauer**, division of Biopharmaceutics, has received the "Young Investigator Award" during the 7th European Atherosclerosis Society Congress in Sevilla, Spain



**Yanina Revsin**, division of Medical Pharmacology, has won the best poster prize during the 3rd Dutch Endo-Neuro-Psycho Meeting in Doortwerth.



**Dr. Martine Smit**, division of Medicinal Chemistry Amsterdam was awarded a NWO-VIDI grant for her work on viral chemokine receptors. Her project proposal, entitled: "Cytomegalovirus-encoded receptors as modulators of cellular signalling networks" was one of the eight grants awarded by Chemische Wetenschappen and will allow the appointment of 2 Ph.D. students. She also obtained a grant from the Vrije Universiteit. The so-called "Talent-gelden" will pay for 50% of her salary for 5 years and allows the division of Medicinal Chemistry to appoint additional staff.



**Dr. Rob Leurs**, Division of Medicinal Chemistry (Amsterdam), has received a grant from the NIH to investigate the interactions of new ligands with the H<sub>1</sub> receptors. The four year project is led by Dr. Raymond Booth (University of North Carolina) and aims to design and develop novel phenylaminotetra- (PAT) analogs as functionally selective probes and drugs that target histamine H<sub>1</sub> G protein-coupled receptors (GPCRs) to modulate brain catecholamine neurotransmitter synthesis.



**Ing Atilla Akdemir** and his supervisor Dr. Iwan de Esch, division of Medicinal Chemistry Amsterdam were awarded a 4 year grant from NWO (Mozaik programma) to start a Ph.D. program on nicotine receptors. The project entitled, "Ligand-gated ion channels: from structure to drugs" was one of the 20 awarded grants from a total of more than 200 applications.



**Professor Joke Bouwstra** of the Division of Drug Delivery Technology, received a STW-project in

continued on page 4

## Unequivocal identification of metabolites in plasma

Division of Analytical Biosciences

The extremely high resolution of Fourier Transform Ion Cyclotron Resonance mass spectrometry (FT-MS) allows a detailed profiling of metabolites present in body fluids.

A unique feature is that accurate masses of the metabolites are generated on a chromatographic time scale.

Profiling the metabolome is probably the most challenging task as compared to the studies of the related "omics" fields (genomics, proteomics). The metabolome is a complex mixture of a very diverse group of compounds, different in size, polarity, chemical properties and concentration. Profiling such mixtures require state-of-the-art technology with highest available resolution and sensitivity.

In the past years, the Division of Analytical Biosciences has developed various methods for profiling the metabolite composition of body fluids. Mass spectrometry forms the basis of the profiling, either

coupled to liquid chromatography (LC-MS), capillary electrophoresis (CE-MS) or chip-based methods.

Such requirements are met by the latest generation Fourier Transform Ion Cyclotron Resonance mass spectrometers (FT-MS). The extremely high resolution of the FT-MS allows a detailed profiling of the metabolites present in body fluids. Furthermore the apparatus delivers accurate masses of the metabolites, a prerequisite for the unequivocal identification of the metabolites. A unique feature is that these data, including MS/MS data are generated on a chromatographic time scale. Based on FT-MS, methodology for metabolic profiling will be further improved and will be applied to clinical samples delivered by the various partners in the CMSB, which eventually may lead to a better therapy of the diseases under study.

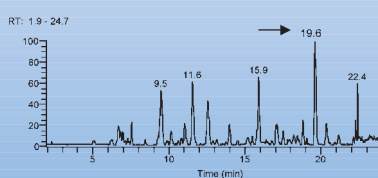
The Center for Medical Systems Biology (CMSB) is a recently established multidisciplinary collaboration between Leiden University, the Leiden University Medical Center, the Erasmus Medical Center Rotterdam, TNO and the Free University at Amsterdam. The joint research is focussed on the identification of novel drug targets and disease markers by using the Systems Biology approach.

Within this framework, the Division of Analytical Biosciences will further expand its expertise on the development of methodology and instrumentation for metabolic profiling.

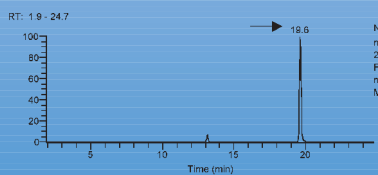
### Reference

Van der Greef J, van der Heijden R, Verheij ER: The Role of Mass Spectrometry in Systems Biology: Data Processing and Identification Strategies in Metabolomics. In: *Advances in Mass Spectrometry, Volume 16*. Edited by Ashcroft AE, Brenton G, Monaghan JJ. Amsterdam: Elsevier Science: 2004, pp. 145-164

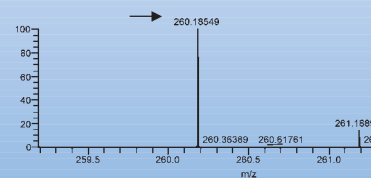
### Accurate mass determination



Typical LC-FT-MS profile (base peak vs retention time) of metabolites from human plasma. The compound eluting at 19.6 minutes is (butylated) glutamic acid.



Mass trace at m/z 260.1856 with a tolerance of 10 ppm (!) shows only butylated glutamic acid.



Accurate mass determination of butylated glutamic acid; the difference between the measured mass and the theoretical mass is only 0.56 ppm.

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From page 3

cooperation with Utrecht University (Prof. Wim Hennink) and Leiden University Medical Centre (dr. Maja Ponc). Title: "Novel strategies to improve barrier function and repair"

The Division of Drug Delivery Technology have received two grants of ICI. One grant is focussed on growing human skin. The other grant concerns the interaction between active ingredients and human skin *in vitro* and *in vivo*.



Dr. Bob van de Water, division of Toxicology, has been successful within a SENTER granted IOP Genomics project entitled: **Genomic analysis and in vivo imaging of stress in aging-related disease** with a contribution from the Division of Toxicology.

The project is based on a strong research consortium (the **imAGING consortium**) between established research groups in the area of (cell) biology, genetics and bio-informatics of the department of Genetics of the Erasmus MC (Prof.dr. J.H.J. Hoijmakers/Dr. G.T.J. van der Horst), the Department of Toxicogenetics of the LUMC (Dr. H. Vrieling), the Division of Toxicology of the LACDR (Dr. B. van de Water), the Micro-Array Department of the University of Amsterdam (Dr. T. Breit) and the RIVM (Dr. H. van Steeg). Altogether 4 postdocs and 2 technician will be working on the project.



The Dutch 'technologiestichting' STW has awarded a grant to **dr. Erno Vreugdenhil** of the Division of Medical Pharmacology.

The aim of the research project is to use a novel gene-silencing technology, the so-called small interference (si) RNA for *in vivo* application in the brain.

## Proteomics expert Fred Regnier as Nauta Professor 2004

Prof. Dr. Fred Regnier (Purdue University, West-Lafayette, USA) was appointed as Nauta Professor 2004 at the Vrije Universiteit in Amsterdam. Prof. Regnier is seen as one of the 'godfathers' of proteomics and Systems Biology and has been the founder of Perceptive Biosystems.

**PURDUE**  
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Prof. Regnier visited the LACDR from June 14-18 and gave a 4-day workshop entitled *Proteomics: Yet another avenue to better living through chromatography and analytical chemistry*.

On Wednesday, June 16, he presented a plenary lecture at the auditorium of the Vrije Universiteit entitled *Proteomics as a Tool in the Search for Disease markers*.

The workshop, which attracted 44 participants, covered a wide range of bioanalytical topics, reflecting the experience and expertise of Prof. Regnier in this field.

In his lectures, Prof. Regnier addressed key issues of proteomics such as the separation and quantification of proteins, the miniaturization of analytical technologies (micro total analytical systems), and Systems Biology. The huge interest in his workshop nicely reflects the increasing interest within the LACDR to apply proteomic approaches in drug discovery research.



The Nauta-chair has been installed at the Vrije Universiteit in Amsterdam following the initiative of the Stichting "Prof. Dr. W. Th. Nautafonds".

The chair is to be occupied by an eminent scientist in the area of the Pharmaceutical Sciences. Previously, the Nauta-chair has been occupied by prof. Dr. Graeme Milligan (Glas-

gow, expert on G-protein coupled receptors) and prof. Dr. Gert Folkerts (ETH Zurich, expert on computational drug design).

Prof. Dr. Wybe Nauta was the founder of the Amsterdam branch of the LACDR and has been instrumental for the development of the field of Medicinal Chemistry in the Netherlands.

## Workshop on visualisation leads to new co-operations

LACDR has started a number of workshops intended to discuss new technologies that are of interest to many LACDR scientists. The focus of the first one, organized on July 1st by dr Fred Nagelkerke, was on state-of-the-art **methods for visualization**.

Professor Jan-Pieter Abrahams (Leiden Institute of Chemistry, Leiden University) introduced Cyttron, a consortium of a number of universities and companies that recently received a substantial Bsik grant from the Ministry of Economic Affairs. The mission of Cyttron is to integrate and improve visualization techniques from the (sub)molecular to the cellular scale and subsequently to distribute these techniques between all participating parties.

Professor Abrahams stipulated that this Programme would offer unique opportunities to the participating groups, including those from LACDR: the wide range of innovative technologies developed in collaboration with relevant industrial partners will allow unequalled research at the cellular level *in vitro* and *in vivo*.

Dr Clemens Löwik from the department of Endocrinology of the Leiden University Medical Center (LUMC) introduced whole body-imaging of intact living animals (mice). Cells that contain the luciferase protein are injected into mice and are visualized using luciferin. Similarly, transgenic mice that contain the luciferase gene show luciferase activity at selected specific sites. These mice can be used to visualize promoter activity at those selected sites.

Subsequently Fred Nagelkerke (LACDR) introduced new, high-through-put, laser scanning cytometry and Fluorescence Resonance Energy transfer to study protein-protein interactions.

The LACDR participants discussed the application of these technologies in relation with visualization requirements for their own projects.

**This resulted in a number of new co-operations, one of the purposes of these meetings.**

## Towards more efficient anti-cancer therapies

Division of Toxicology

For the migration, survival and invasion the metastasizing cells, extracellular signals are critical. Focal adhesion kinase (FAK) is a central protein in all these processes. New data of the Division of Toxicology show that (survival) signals mediated by FAK are important in the resistance of tumor cells against doxorubicin and necessary for metastasis formation. This finding may lead to new roads of anticancer therapy.

A major problem in the treatment of cancer is the increased resistance of tumor cells towards cytostatics. Most cancer deaths are not caused by the primary tumor but by distant, highly resistant metastases. For the migration, survival and invasion of the metastasizing cells,

extracellular signals are critical. Focal adhesion kinase (FAK) is a central protein in all these processes. Increased expression and activity of FAK is observed in several human tumors, especially in invasive and metastatic tumor tissue, which is associated with amplification of chromosome 8q and with poor disease prognosis.

We have investigated the role of FAK in the regulation of breast tumor cell susceptibility towards anticancer drugs as well as tumor cell metastasis behaviour. The cytostatic doxorubicin caused formation of well-defined focal adhesions in the mammary adenocarcinoma cell line MTLn3 as well as F-actin cytoskeleton stress fibers early after treatment. Later, focal adhesions and stress fibers were lost, which coincided with the onset of apoptosis and was associated with the downregulation of the activity of FAK. Overexpression of the dominant negative acting focal adhesion kinase-related non-kinase (FRNK) induced some apoptosis in MTLn3 cells. Moreover, overexpression of FRNK sensitized MTLn3 cells to apoptosis caused by doxorubicin.

To further evaluate the role of FAK under *in vivo* conditions we next generated MTLn3 cells with doxycycline-regulated conditional expression of HA-tagged FRNK. The expression of HA-FRNK had an inhibitory effect on growth rate, attachment and migration of MTLn3 cells *in vitro*.

Furthermore, expression of HA-FRNK also promoted doxorubicin-induced cell death. In the tumor-metastasis model, HA-FRNK expression reduced tumor volume of the primary tumor in the mammary fat pad. Strikingly, HA-FRNK almost completely prevented lung metastasis formation after tail vein injection of the breast tumor cells (see Fig). We expect that pharmacological inhibition of FAK may inhibit tumor metastasis and increase the efficacy of anticancer therapy in patients.

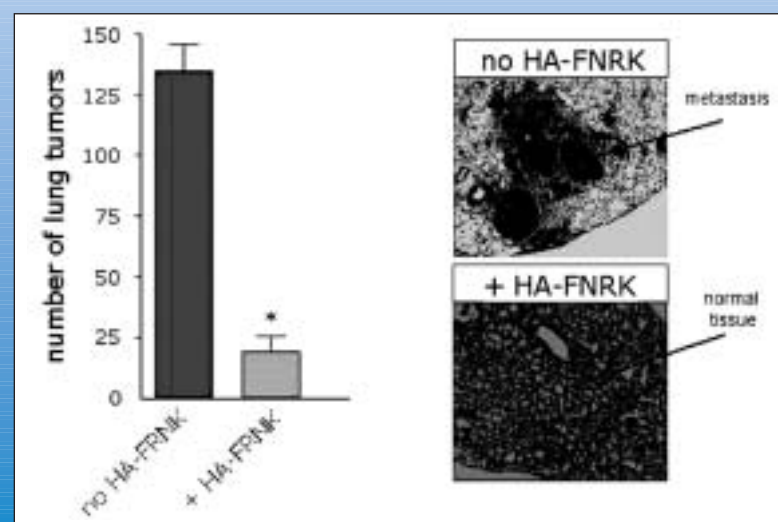
This work was sponsored by the Netherlands Cancer Foundation (KWF Kankerbestrijding) project 2001-2477.

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### FAK controls breast tumor lung metastasis formation.

**Left panel**  
Lung metastasis formation of tumor cells with conditional expression of dominant negative acting FAK (FRNK) were injected in the tail vein of mice. At different days after injection the FRNK expression was upregulated and after 28 days the number of surface metastasis was counted.

**Right panel**  
When FRNK is expressed no lung metastasis were present in lung at the histological level.



## Leiden University installed Finnigan LTQ-FT LC-MS/MS

In January 2004 a LTQ-FT mass spectrometer of Thermo Electron was installed by the Division of Analytical Biosciences (Prof. J. van der Greef, c.s.) This mass spectrometer will be used in the Centre for Medical Systems biology (CMSB), which is a multidisciplinary joint venture in the field of systems biology within the NWO-Genomics programme.

recognition techniques and bioinformatics can be compared with control population. The contribution of the Division of Analytical Biosciences in this project consists of further developments of LC-MS methods for metabolite profiling. The high resolution of this LTQ-FT mass spectrometer guarantees an extremely detailed characterization of the metabolites existing e.g. in sam-



Several research groups including the Leiden University, Leiden University Medical Centre, Erasmus Medical Centre Rotterdam, TNO and the Vrije Universiteit of Amsterdam are involved in the CMSB. The research is focussed on the identification of new drug targets and disease markers, as a result of modern systems biology approaches. The basis for this approach is the integral analysis of the transcriptome, the proteome and the metabolome of patient populations, which by the use of pattern

ples of plasma and urine. For further identification of individual metabolites, accurate mass-determinations and MS/MS spectra, as provided by this machine, are required.

The required facilities are provided by the LTQ-FT of Thermo Electron. Furthermore the start-up and the data-application take place via a well-known and user-friendly Xcalibur software programme. The LTQ-FT will be of great importance for the proteomic applications with-

## Steven Lamberts Marius Tausk professor 2004

Dr Steven Lamberts, professor in Medicine, and currently Rector Magnificus of the Erasmus University, Rotterdam, was appointed to the 'Marius Tausk' Chair 2004 at Leiden University.

Steven Lamberts has published over 500 scientific articles about various aspects of fundamental and clinical endocrinology and cancer research. He has made hallmark contributions to the clinical application of somatostatin analogs.

Another recent highlight was the discovery and functional analysis of glucocorticoid receptor single nucleotide polymorphisms (SNP's). One particular polymorphism predisposed for metabolic disease and cognitive decline during aging, while another one would possibly qualify as longevity gene. This interest in glucocorticoid receptors shares dr Lamberts with his host, professor Ron de Kloet.

One of the highlights of dr Lamberts appointment is the workshop to be held on Monday December 6 in the Conference hotel 'Kasteel Oud-Poelgeest', entitled: *Genetics of glucocorticoid resistance in stress and aging*.

The objective of this workshop is to identify strategies aimed towards integration of basic and clinical studies to explore the functional significance of glucocorticoid receptor SNP's for health and Disease. The keynote lecture at the annual Endo-Neuro-Psycho meeting in Doorwerth, June 1, entitled *Genetic Endocrinology of Aging* underscores the success of having dr. Lamberts as Marius Tausk professor 2004.



The Marius Tausk Chair, accommodated at LACDR, was established in 1998 at the Leiden University Medical Center (LUMC) by N.V. Organon and the Netherlands Endocrine Society.

From 1926-1967, Professor Tausk has been, as Director, important for the success and growth of Orga-

non. In honour to Marius Tausk the Chair is designated each year to a scientist who has made an extraordinary contribution to research in the area of endocrinology.

## Highly Cited researchers

ISI Highly Cited Researchers has identified the 250 most cited authors worldwide over the past two decades (ISIhighlycited.com).

Four LACDR researchers are amongst them:

- Professor of Pharmacology Douwe. D. Breimer, in the area of Pharmacology
- Professor of Molecular Toxicology N.P.E. Vermeulen, in the area of Pharmacology

- Professor of Pharmacology M. Danhof, in the area of Pharmacology
- Professor of Medical Pharmacology E.R. de Kloet, in the area of Neurosciences

## Highly selective H<sub>3</sub> receptor agonist

Division of Medicinal Chemistry

A recent discovery that is expected to have a major impact on histamine receptor research is the development of a selective histamine H<sub>3</sub> receptor ligand.

Over the years the Division of Medicinal Chemistry at the Vrije Universiteit Amsterdam has developed various ligands that are now widely being used in academia and industry to characterise the four histamine receptor subtypes.

Heretofore known H<sub>3</sub> receptor agonists show only a limited selectivity (27-55 fold) for the H<sub>3</sub> receptor over the related H<sub>4</sub> receptor. Our efforts, in collaboration with UCB Pharma (Belgium), resulted in the discovery of immethridine as a potent and selective H<sub>3</sub> receptor ag-

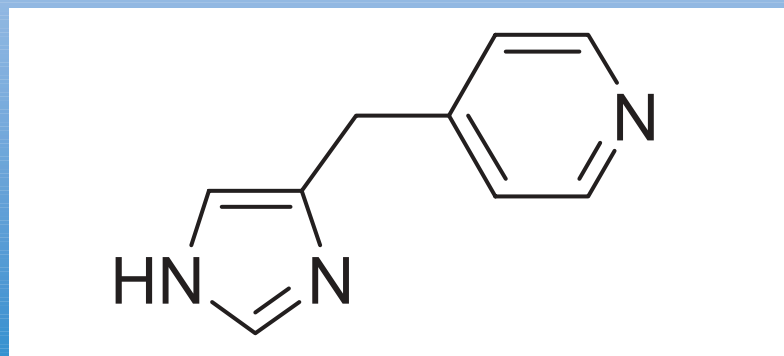
onist, exhibiting a 300-fold selectivity for the H<sub>3</sub> receptor over the related H<sub>4</sub> receptor and negligible H<sub>1</sub> receptor and H<sub>2</sub> receptor affinity.

It is anticipated that immethridine will play a major role in elucidating the role and function of the H<sub>3</sub> receptor in vivo, thereby facilitating the development of H<sub>3</sub> receptor-related drugs.

More information: far@few.vu.nl, +31 20 444 76 00

### Reference

R. Kitbunnadaj, O.P. Zuiderveld, B. Christophe, S. Hulscher, W.M.P.B. Menge, E. Gelens, E. Snip, R.A. Bakker, S. Celanire, M. Gillard, P. Talaga, H. Timmerman, R. Leurs (2004) Identification of 4-(1H-Imidazol-4(5)-ylmethyl) pyridine (Immethridine) as a Novel, Potent and Highly Selective Histamine H<sub>3</sub> Receptor Agonist. J Med. Chem. 47, 2414-2417.



Immethridine, a potent and selective H<sub>3</sub> receptor agonist

## Abstract books SPRING SYMPOSIUM available

Within the LACDR a wide range of projects in the field of medicines research is ongoing.

Once every year almost all of these are discussed at the Spring Symposium, in a poster session.

If you want to know what is going on at the LACDR, and if you want to talk with our PhD students: this is the opportunity!

For information on the next Spring Symposium on April 19, 2005: please contact

Ms Martha van der Ham (m.ham@lacdr.leidenuniv.nl) who also has still a limited number of the abstract books of the 2004 symposium available for those interested.

# LACDR Agenda

- 13 - 16 October **Course on Pharmacokinetics-Pharmacodynamics**  
Holiday Inn Hotel, Leiden  
Course leaders are Prof.dr. M. Danhof and Prof.dr. B. Jusko
- 26 - 29 October **13th School on Medicinal Chemistry**  
NH Conference Hotel De Leeuwenhorst, Noordwijkerhout  
Course directors: Prof.dr. A.P. IJzerman, Prof.dr. H. Timmerman
- 12 November **Inaugural lecture of Prof dr J. Bouwstra at Leiden University**
- 30 November LACDR Fall symposium "**Pharmacogenomics and Drug Research**"  
Gorlaeus Laboratories, Leiden
- 24 - 26 November **Farmacokinetiek** (Dutch spoken)  
City Hotel, Oss  
Course director: Prof.dr. M. Danhof
- 6 December **Symposium Marius Tausk**  
Genetics of stress and aging
- 2005**
- 12 - 14 January **Farmacokinetiek** (Dutch spoken)  
City Hotel, Oss  
Course director: Prof.dr. M. Danhof
- 19 April **LACDR Spring Symposium**  
VU - Amsterdam

6

## Training Course on Quality Management in Pharma and Biotech

The courses teaches:

Essentials of quality management

and is addressed to:

Professionals in pharma and biotech industry

Professionals in institutions and contract research

organisations

Hospital pharmacists

Postgraduate students planning an industrial career

Information on: [www.lacdr.nl](http://www.lacdr.nl) (courses and symposia)

## Courses and Symposia

LACDR is an active organiser of Courses and Symposia on topics that represent the specific expertise available within the Center.

### Information

For information on future events:

LACDR-Secretariat,

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email: [m.ham@lacdr.leidenuniv.nl](mailto:m.ham@lacdr.leidenuniv.nl).

See also our website: [www.LACDR.nl](http://www.LACDR.nl)

## PhD theses completed since October 2003

**Rianne de Ligt** (20 March 2003)  
Division of Medicinal Chemistry (Leiden)  
Promoter: Prof.dr. A.P. IJzerman  
Inverse agonism on adenosine receptors. Constitutive activity, inverse agonism, and allosteric modulation

**P.H.M. van der Kuy**  
Division of Pharmaceutical Technology and Biopharmaceutics  
Promoter: Prof.dr. F.W.H.M. Merkus  
Studies on intranasal drug delivery in migraine

**Adriaan M. Karszen**  
Division of Medical Pharmacology  
Promoter: Prof.dr. E.R. de Kloet;  
Co-promoter: Dr. O.C. Meijer  
Glucocorticoid access to the brain; involvement of efflux transporter P-glycoprotein.

**Marloes Schaddelee**  
Division of Pharmacology  
Promoters: Prof.dr. M. Danhof, Prof.dr. A.P. IJzerman;  
Co-promoter: Dr. A.G. de Boer  
Adenosine A<sub>1</sub> receptor agonists. Blood/Brain Barrier Transport and PK/PD Correlations in Neuropathic Pain

**Denise Jacobs**  
Former Division of Pharmacognosy  
Promoter: Prof.dr. R. Verpoorte  
Proteome analysis of the medicinal plant *Catharanthus roseus*

**Daniël M. Jonker**  
Division of Pharmacology  
Promoter: Prof.dr. M. Danhof;  
Co-promoter: Dr. R.A. Voskuyl  
Pharmacodynamic analysis of antiepileptic drug-drug interactions *in vivo*.

**Edith Gelens**  
Division of Medicinal Chemistry (Amsterdam)  
Promoters: Prof.dr. H. Timmerman, Prof.dr. H.C.J. Ottenheijm  
The Solid-Phase synthesis of substituted imidazoles via a scaffold approach

**Jian Guo**  
Division of Biopharmaceutics  
Promoter: Prof.dr. Th.J.C. van Berkel;  
Co-promoter: Dr. P.H.E. Groot  
The role of bone marrow progenitor cell derived cc-chemokine receptor 2 and cathepsin k. in atherosclerosis

**David Mallet**  
Division of Analytical Biosciences  
Promoter: Prof.dr. J. van der Greef  
High throughput bioanalysis of pharmaceuticals by high performance liquid chromatography

**Remko Bakker**  
Division of Medicinal Chemistry (Amsterdam)  
Promoters: Prof.dr. H. Timmerman, Prof.dr. R. Leurs  
Modulation of histamine H<sub>1</sub> receptor activity

**Mathias Schmidt**  
Division of Medical Pharmacology  
Promoter: Prof.dr. E.R. de Kloet,  
Stress System Development: Molecular Mechanism of Inhibition and Activation

**Tim Schenk**  
Division of Analytical Biosciences  
Promoters: Prof.dr. J. van der Greef, Prof.dr. H. Irth; Co-promoter: Dr. U.R. Tjaden  
Detection and identification of bioactive compounds in complex mixtures using liquid chromatography-biochemical detection

**Loan Honeywell**  
Division of Drug Delivery Technology  
Promoters: Prof.dr. J.A. Bouwstra, Prof.dr. H.E. Junginger  
Surfactant-Based elastic vesicles and skin delivery vehicles

**Eleonora L. Swart**  
Division of Pharmacology  
Promoters: Prof.dr. L.G. Thijs, Prof.dr. M. Danhof  
Lorazepam *versus* midazolam for long-term sedation in critically ill intensive care patients

**Sittiruk Roytrakul**  
Former Division of Pharmacognosy  
Promoter: Prof.dr. R. Verpoorte  
Transports of alkaloids and its precursors through the vascular membrane of *Catharanthus roseus*

**Joost Rothbarth**  
Division of Toxicology  
Promoters: Prof.dr. G.J. Mulder; Prof.dr. C.J.H. vd Velde  
Treatment of liver metastases by isolated hepatic perfusion

**T.J. Raharjo**  
Former Division of Pharmacognosy  
Promoter: Prof.dr. R. Verpoorte  
Studies of Cannabinoids Biosynthesis in *Canabis Sativa*

**M. El Sayed**  
Former Division of Pharmacognosy  
Promoter: Prof.dr. R. Verpoorte  
Regulation of alkaloid pathways after strictocidine glucosidase in *Catharanthus roseus*

These costs € 20,- per copy and can be ordered at the LACDR secretariat

## New PhD students appointed

April 2003: **Yanina Revsin**, Division of Medical Pharmacology  
Promoter: Prof.dr. E.Ron de Kloet;  
co-promoter: Dr. Melly Oitzl  
Stress hormones and cognitive performance: study on animal models for comorbidity of depression and diabetes

October 2003: **Siem van der Laan**, Division of Medical Pharmacology  
Promoter: Prof.dr. E. Ron de Kloet;  
co-promoter: Dr. Onno Meijer  
Project: Coregulators and Corticosteroid Signalling in the brain

January 2004: **Thomas van Es**, Division of Biopharmaceutics  
Promoter: Prof.dr. Theo van Berkel,  
co-promoter: Dr. Johan Kuiper  
Project: Immunomodulation of the inflammatory response within the atherosclerotic plaque

February 2004, **Gijs Santen**, Division of Pharmacology  
Promoter: Prof.dr. Meindert Danhof; co-promoter: Dr. Oscar Della Pasqua  
Project: Integration of disease progression, compliance and placebo effect in the characterisation of the therapeutic response to anti-depressant therapy

February 2004, **Rogier Smits**, Division of Medicinal Chemistry (Amsterdam)  
Promoter: Prof.dr. Rob Leurs, co-promoter: Dr. Iwan de Esch  
Project: Synthesis, SAR and molecular modelling of histamine H<sub>4</sub> ligands

February 2004, **Regina Appiah-Opong**, Division of Molecular Toxicology  
Promoter: Prof.dr. Nico E.P. Vermeulen; co-promoter: Dr. Jan Commandeur

Project: Drug-drug interactions by plant-derived food components

March 2004: **Bob Meijboom**, Division of Biopharmaceutics  
Promoter: Prof.dr. Theo J.C. van Berkel; co-promoter: Dr. Eric Biessen  
Project: Modulation of gene expression as therapy for arteriosclerosis

April 2004: **Thomas Dijkmans**, Division of Medical Pharmacology  
Promoter: Prof.dr. E.R. de Kloet; co-promoter: Dr. Erno Vreugdenhil  
Gene silencing of corticosteroid-responsive genes in the brain: application of small interference RNA

April 2004: **Saskia de Jager**, Division of Biopharmaceutics  
Promoter: Prof.dr. Theo van Berkel; co-promoter: Dr. Eric Biessen  
Project: Leukocyte homeostasis in the advanced plaque: a key to understanding rupture-related syndromes

May 2004: **Martine van Miltenburg**, Division of Toxicology  
Promoter: Prof.dr. Gerard J. Mulder; co-promoter: Dr. Bob vd Water  
Project: Anticancer drug resistance and DNA-damage repair

July 2004: **Robert Rissmann**: Division of Drug Delivery Technology  
Promoter: Prof.dr. Joke A. Bouwstra  
Project: Novel strategies to improve skin barrier function and repair

July 2004: **Kamonchanok Sansuk**: Division of Medicinal Chemistry (Amsterdam)  
Promoter: Prof.dr. Rob Leurs; co-promoter: Dr. Remko Bakker  
Project: Purification and biophysical characterisation of histamine receptors

August 2004: **Lenneke van Hooijdonk**: Division of Medical Pharmacology  
Promoter: Prof.dr. E. Ron de Kloet; co-promoter: Dr. Erno Vreugdenhil  
Project: Anti-glucocorticoids action mechanism

September 2004: **Martine Bot**, Division of Biopharmaceutics  
Promoter: Prof.dr. Theo van Berkel; co-promoter: Dr. Eric Biessen  
Project: Modulation of lysophosphatidate homeostasis in the atheroma: a new strategy to prevent thrombotic complications after plaque rupture

**LACDR NEWSLETTER**  
is a publication of the Leiden/  
Amsterdam Center for Drug Research (LACDR)

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