AIMMS Annual Meeting
April 18th 2019

Amsterdam Institute for Molecules, Medicines and Systems

www.aimms.vu.nl
info@aimms.vu.nl

VU
VRIJE UNIVERSITEIT AMSTERDAM
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Word from the Director

Dear participants of the 2019 AIMMS Annual Meeting,

Welcome to the 9th annual meeting of AIMMS, our institute that will soon be the Amsterdam Institute of Molecular and Life Sciences. Yes, this will be our new name, but we will keep the well-known acronym. This change is necessary to reflect the broadened scope of our institute, which also includes environmental studies and science relevant for sustainability. Note that we will formally roll out the name later in 2019 to prevent using two names at the same time.

Last year we have streamlined the Training and Supervision Plans, set up a Metabolism Core Facility, revived the PhD council and developed an introductory course for new AIMMS PhD students. We have also seen a change in the management team, where Iwan de Esch made place for Matthias Bickelhaupt. We thank Iwan for his efforts and wish him lots of fun with more science again! This year we will work together with a new Science Advisory Board on communication, value proposition and strategic collaborations, within AIMMS and together with partners.

This annual day is an opportunity to meet each other, to learn about the areas of expertise and techniques within AIMMS, and to set up new collaborations. We highlight research topics that we work on within AIMMS, through oral presentations by AIMMS PIs and selected PhD students, and through many posters. This year, the meeting was organised not by the AIMMS management, but by a committee of Edith Houben, Henry Vischer and Tim Jonkers. I want to thank them for putting this great program together, and Joyce Braam, Jurgen Haanstra and the MCB secretaries for their help in organising this event.

This year our speakers are Marjorie van Duursen, new professor in Environmental Health and Toxicology, Alex Speer who is a postdoc at Molecular Microbiology, and Maikel Wijtmans, assistant professor from Medicinal Chemistry. We also have an external speaker, Rob Wolthuis, who is professor in Oncogenetics at the Cancer Centre Amsterdam of the AUMC, location VUmc.

On behalf of the AIMMS management team (Jurgen Haanstra, Jacob de Boer, Matthias Bickelhaupt, Peter van Hoorn, and Holger Lill) I wish you a pleasant and inspiring annual day!

Bas Teusink
Scientific Director
Programme

Programme 9th Annual Meeting AIMMS 18 April 2019
Auditorium, O|2 Lab Building, VU Amsterdam

Welcome (Main hall and Auditorium, O|2 Lab Building)
08.45  Registration and coffee
09.15  Opening of Annual Meeting – Bas Teusink (director AIMMS)

AIMMS PIs showcase our scientific diversity and strengths (Auditorium, O|2 Lab Building)
   Chair: Henry Vischer (Molecular Pharmacology)
09.30  Majorie van Duursen (Environmental Health and Toxicology)
   Towards better test methods for female reproductive toxicity of endocrine disruptors
10.00  Alex Speer (Medical Microbiology, VUmc)
   ESX-5 as drug target: compounds that block ESX-5 secretion reduce mycobacterial growth in vivo

Poster competition (Main hall, O|2 Lab Building)
10.30  Coffee break and poster presentations part 1 (even numbers)

AIMMS PIs showcase our scientific diversity and strengths (Auditorium, O|2 Lab Building)
   Chair: Edith Houben (Molecular Microbiology)
11.30  Maikel Wijtmans (Chemistry and Pharmaceutical Sciences)
   Photopharmacology for GPCR receptor proteins: 1st and 2nd generation chemical biology tools

Lunch break (Pop-up room O|2 Lab Building – next to the Auditorium)
12.00  Lunch and informal poster viewing/networking
**PhD oral presentation competition (Auditorium, O|2 Lab Building)**

**Chair:** by Tim Jonkers (Environmental Chemistry & Toxicology)

13.30 **Valeriia Kuzyk** (BioAnalytical Chemistry)
*Abundant protein glycosylation as potential biomarker signature for colorectal cancer*

13.45 **Philipp Schmidt** (Systems Bioinformatics)
*When and how to switch? – Studying gene expression at single cell level in Baker’s yeast*

14.00 **Ewa Skoczynska** (Environmental Chemistry & Toxicology)
*Heterocyclic PAHs in rubber granulates*

14.15 **Sandra Ortega Ugalde** (Molecular and Computational Toxicology)
*Suicide inhibitors for Cytochrome P450 enzymes from Mycobacterium tuberculosis*

14.30 **Juami van Gils** (Bioinformatics)
*To fold or not to fold*

14.45 **Maarten Bebelman** (Target and Systems Biochemistry)
*Exosomal release of the human cytomegalovirus-encoded chemokine receptor US28*

15.00 **E-voting chaired by Maikel Wijtmans and Danny Scholten**

**Poster competition (Main hall, O|2 Lab Building)**

15.05 **Tea break and poster presentations part 2 (odd numbers)**

**Keynote and Awards ceremony (Auditorium, O|2 Lab Building)**

**Chair:** Bas Teusink (Scientific Director)

16.00 **Rob Wolthuis** (Cancer Center Amsterdam, theme leader Tumor Biology and the head of the Section of Oncogenetics)
*Using Next-Gen CRISPR Screening to Connect Genes to Your Molecules, Medicines and Systems*

16.45 **Bas Teusink** (Scientific Director)
*Awards ceremony*

**Afterparty (Eat, Meet, Work, O|2 Lab Building)**

17.15 **Drinks**

18.00 **Buffet dinner**
AIMMS PIs abstracts

**AIMMS PIs showcase our scientific diversity and strengths, 09.30h**

**Prof. Majorie van Duursen**

Professor Environmental Health and Toxicology, AIMMS, VU Amsterdam, The Netherlands

Towards better test methods for female reproductive toxicity of endocrine disruptors

It is becoming increasingly clear among scientists, regulators and society that many chemicals in our food chain and environment can disturb endocrine processes, threatening the reproductive health of humans, critical food livestock and wildlife globally. Increasingly, women experience problems with getting pregnant or carrying pregnancy to term. A woman’s fertility is already largely established early in her life, during embryonic and fetal development and puberty. Evidence shows that exposure to endocrine disrupting chemicals (EDCs) during these specific times in her life can negatively affect a woman’s fertility. Strikingly, there is surprisingly limited knowledge on the mechanisms by which EDCs can impair female reproduction. As part of an EU-funded project, our laboratory investigates human-specific biomarkers of female reproductive health and develop test methods to address this in a regulatory context. This presentation will address the issue of endocrine disruptors, present challenges and future perspectives for better assessment of EDC properties and related health effects.

**AIMMS PIs showcase our scientific diversity and strengths, 10.00h**

**Dr Alexander Speer**

Post-doc, AIMMS, VU Amsterdam, The Netherlands

ESX-5 as drug target: compounds that block ESX-5 secretion reduce mycobacterial growth in vivo

An important part of the antibiotic persistence problem of Tuberculosis is that Mycobacterium tuberculosis is protected from harmful compounds by an unusual and highly impermeable cell envelope; however, this layer must also be functionalized. To secrete proteins across this cell envelope, mycobacteria use specialized secretion systems known as type VII secretion (T7S). The T7S system called ESX-5 is only found in virulent mycobacteria. It is essential for survival by modulating the immune response and facilitating nutrient uptake; therefore, ESX-5 is an interesting target for drug development. Performing a whole cell-based high throughput screening campaign, we succeeded in identifying a new class of small molecules that efficiently inhibit the secretion of ESX-5 substrates, while the T7S-independent extracellular proteins continue to be produced. We synthesized an optimized version of the initial hit compound with increased activity (nM range) and lower toxicity. Importantly, these compounds, comparable to some 1st line antibiotics, significantly reduced the bacterial burden in zebrafish and macrophages infected with M. marinum and M. tuberculosis, respectively. In line with our hypothesis, ESX-5 inhibitors work in a very limited host range and are not active against other Gram+, Gram-, or fast-growing mycobacteria. Interestingly, these inhibitors also seem to affect secretion of a second ESX system (ESX-1). Blocking two important targets simultaneously is known to significantly reduce development of antibiotic resistance.
Photopharmacology for GPCR receptor proteins: 1st and 2nd generation chemical biology tools

Photopharmacology is a discipline that uses photoswitchable ligands as pharmacological tool compounds to yield spatiotemporal control of protein activity with light. However, photopharmacology in the G protein-coupled receptor (GPCR) field is still in its infancy. In this presentation, our general approach towards GPCR photopharmacology will be discussed as well as recent contributions from our laboratory including several series of photoisomerisable azobenzene-based GPCR ligands. First-generation series involve photoswitchable antagonist or agonist ligands that shift affinity/potency for an aminergic GPCR. In a second-generation series, we incorporated a change in the ligand efficacy for a peptidergic GPCR upon illumination, thus establishing a photoswitch from antagonism to agonism. Our contributions deliver a toolbox of compounds capable of photomodulating GPCR signaling in complementary ways.
Valeriia Kuzyk - Chemistry and Pharmaceutical Sciences – BioAnalytical Chemistry

**Abundant protein glycosylation as potential biomarker signature for colorectal cancer**

Valeriia Kuzyk1,2, Guinevere S.M. Lageveen-Kammeijer2, Rob Haselberg1, Manfred Wuhrer2, Govert W. Somsen1

1Vrije Universiteit, Amsterdam,
2Leiden University Medical Centre, Leiden

Email: v.kuzyk@vu.nl

Protein glycosylation is altered in malignant cells and their microenvironment. Carcinoembryonic antigen (CEA) is a large glycoprotein, that is expressed by colorectal cells upon malignant transformation and is secreted into blood circulation. It's glycosylation is abundant and, therefore, may harbour glycosignatures of malignant phenotype. This study aims to link the CEA glycoprofile heterogeneity to the characteristics of the tumor cells producing it. We have evaluated the CEA production in 14 colorectal cancer (CRC) cell lines in both quantitative and qualitative manner. This demonstrated significant variation in amount of protein secreted, it's degree of glycosylation and/or presence of protein isoforms. To access glycosylation microheterogeneity, CEA was isolated from CRC cell extracts and subjected to combined proteolytic digestion. The samples were analysed with CE-ESI-MS/MS and annotated manually. We have compared the resulting glycoprofiles in attempt to specify the traits correlating to the CRC cell origin, tumor stage, degree of differentiation and phenotype.
When and how to switch? – Studying gene expression at single cell level in Baker’s yeast

Phillipp Schmidt¹, Johan van Heerden¹, Bas Teusink¹ and Frank Bruggeman¹

¹Systems Bioinformatics, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

E-mail: p.schmidt@vu.nl, j.van.heerden@vu.nl, b.teusink@vu.nl, f.j.bruggeman@vu.nl

When *S. cerevisiae* grows in batch-cultures on glucose it produces ethanol. After glucose depletion, cells start to use the ethanol as carbon source. This phenomenon of observing two growth phases is called diauxie. We want to study how this shift is regulated on the gene expression level and which external signal is determinative. In particular, it is not clear if it is a hard-wired response to glucose depletion or if it also requires a certain concentration of ethanol. We use fluorescent reporters and a flow cytometer to study gene expression at the single cell level. We performed batch growth experiments and use multiple genes as readout for the initiation of gluconeogenesis. The analysis of mean fluorescence over time showed that cells clearly induced gene expression shortly before or around the point of glucose depletion. At the single cell level, we detected varying subpopulations of cells that show induction. The observed behaviour can be explained by anticipation of glucose depletion/ethanol consumption. While we don’t understand the mechanism, we conclude that a higher ethanol concentration leads to a higher fraction of cells that anticipate the shift and can switch immediately.
Rubber granulates originating from recycled car tiers are widely used in artificial football pitches, playgrounds, sport schools and even in domestic gardens. The recycled tire rubber contains polycyclic aromatic hydrocarbons (PAHs), phthalates, vulcanisation additives, heavy metals and other hazardous substances.

This study aims at determining the presence of heterocyclic- and methyl-PAHs in recycled tire rubber granulates of synthetic turf football pitches and rubber tiles. Heterocyclic PAHs, like benzothiazole and its derivatives, are commercially used and together with methyl-PAHs pose serious risk to human health and environment although they are still not regularly included in the analysis of priority polyaromatic compounds (PACs).

The analytical approach initially includes non-target screening to investigate the presence of heterocyclic PAHs and other toxic substances. The non-targeted analysis involves two-dimensional gas chromatography (GCxGC) coupled with a time-of-flight-mass spectrometry detector (ToF-MS) measurements of raw extracts and is followed by the development of targeted analysis of the identified heterocyclic PAHs. The sample pre-treatment is optimized and the identification and quantification of target compounds is done on GC-MS and GCxGC coupled with atmospheric pressure chemical ionization-mass spectrometry (APCI-MS).

The non-target screening allowed to tentatively identify 22 heterocyclic PAHs that subsequently were identified and quantified together with 16 EPA PAHs. The \( \Sigma \)PAHs varied from 27 to 5000 mg/kg and the concentrations of the heterocyclic-PAHs varied from 0,1 to 150 mg/kg.

The analytical method is still under the development.
Suicide inhibitors for Cytochrome P450 enzymes from Mycobacterium tuberculosis

Ortega Ugalde S., Wallraven K., Bitter W., Jennings P., Grossmann T., Commandeur JNM.

1AIMMS Division of Molecular Toxicology, VU University Amsterdam, The Netherlands
2Department of Chemistry and Pharmaceutical Sciences, VU University Amsterdam, The Netherlands
3Division of Molecular Microbiology, Faculty of Sciences, VU University Amsterdam, The Netherlands

E-mail: s.ortegaulde@vu.nl

Tuberculosis (TB), caused by the human pathogen Mycobacterium tuberculosis (Mtb), was responsible for 1.3 million deaths in 2017, thereby becoming again the deadliest infectious disease known to humankind. The emergence of resistant Mtb strains has prompted the development of new drugs for TB. Some Cytochrome P450 enzymes (CYPs) are considered essential for the viability of Mtb in vitro and therefore are considered attractive therapeutic targets. Fragment-based approaches applied to several CYPs have yielded a subset of compounds with high potencies but low antimycobacterial activity (MIC, ≥50 μM). Their mechanism of action is direct inhibition which may be limited due to the reversibility of the CYP inhibition. Alternatively, suicide inhibition, resulting from the activation of a drug by the CYP into highly reactive metabolites, may lead to a longer lasting inactivation of the CYP which can only be restored with de novo protein synthesis.

The major goal of this project was to synthesize tentative suicide inhibitors for a Mtb CYP based on structural modification of its natural substrate. This novel approach could provide the starting point as a novel strategy to fight against this burden disease.

A) Direct inhibition

B) Suicide inhibition

References

Juami van Gils - Bioinformatics

To fold or not to fold

Juami van Gils1, Maryam Hashemi Shabestari2, Halima Mouhib3, Peter van Ulsen4, Wouter Roos2, Gijs Wuite2 and Sanne Abeln1

1Dept. Bioinformatics, Vrije Universiteit, Amsterdam, The Netherlands
2Dept. Physics of Living Systems, Vrije Universiteit, Amsterdam, The Netherlands
3Dept. Theoretical Chemistry, Université Paris-Est Marne-la-Vallée, Paris, France
4Dept. Molecular Microbiology, Vrije Universiteit, Amsterdam, The Netherlands

E-mail: j.h.m.van.gils@student.vu.nl, s.abeln@vu.nl

While it is currently possible to study protein structures in their native state, it is difficult to probe misfolded proteins, due to the ensemble of different conformations. Hemoglobin protease is an autotransporter secreted by E. coli. The protein only folds into its native state in vitro if the correct machinery is in place, otherwise it reaches a misfolded state. We studied the unfolding and refolding ability of a truncate of the passenger domain using atomic force microscopy (AFM) and MD simulations (1). The results show that the construct unfolds from N- to C-terminus and is able to refold in the AFM setup. However, when the protein is denatured with urea, we obtain very distinct force-extension profiles, with high force at lower extension lengths. Simulation results show similar unfolding patterns as the AFM, both for native and misfolded proteins. Hence, we are able to discriminate folded from misfolded states in the AFM setup. The combination of simulations with single-molecule measurements provides a new method to probe misfolded configurational ensembles of proteins.

Figure 1. Force-extension of natively folded Hbp in an MD simulation. Top: Force-extension curve of Hbp. The raw data is shown in blue, the smoothed data in red, unfolding events are indicated with black dots and worm-like-chain model fits are shown with black lines. Bottom: LGA analysis of the unfolding process. White indicates that at a certain elongation (x-axis) a given residue (y-axis) is still folded, while an orange/red color indicates that a residue is unfolded.

References

Maarten Bebelman - (Target and Systems Biochemistry)

Exosomal release of the human cytomegalovirus-encoded chemokine receptor US28

M.P. Bebelman1,2, J. van Senten1, L.van de Ven1, R.J.P Musters3, G. van Niel4, D.M. Pegtel2, M.J. Smit1

1 Division of Medicinal Chemistry, Amsterdam Institute for Molecules Medicines and Systems, VU University Amsterdam, de Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands.
2 Exosome Research Group, Dept. Pathology, Cancer Center Amsterdam, VU University Medical Center, de Boelelaan 1118, 1081 HV Amsterdam, The Netherlands.
3 Departement of Physiology and Advanced Optical Microscopy Core in O|2, VU University Medical Center, de Boelelaan 1118, 1081 HV Amsterdam, The Netherlands.
4 Centre de Psychiatrie et Neurosciences Paris, France.

Email: m.p.bebelman@vu.nl

Introduction: Exosomes are nanosized extracellular vesicles (EVs) that originate from the fusion of multivesicular bodies (MVBs) with the plasma membrane. These vesicles mediate intercellular communication via the transfer of proteins and RNA. In the last decade, it has become clear that many viruses, including herpesviruses, modulate exosomal communication to create an optimal environment for viral persistence and dissemination. In this study, we show that the human cytomegalovirus (HCMV)-encoded chemokine receptor US28 is released via exosomes. This viral receptor is expressed in both latent and lytic stages of HCMV infection and constitutively activates proliferative and pro-angiogenic signaling pathways. We hypothesize that exosomal release of US28 might contribute to HCMV pathology.

Methods: We developed an optical reporter based on US28 and a pH-sensitive GFP (pHluorin) that enables live cell imaging of the fusion of US28-containing MVBs with the plasma membrane. Furthermore, we generated a HCMV strain containing US28-pHluorin to study exosomal release of US28 in HCMV-infected cells.

Results: Live cell TIRF microscopy on HCMV-infected cells revealed that US28-pHluorin-containing MVBs fuse with the plasma membrane. In line with this, EVs isolated from the culture supernatant of infected cells contain US28. Moreover, analysis of the EV-fraction by super-resolution STED and electron microscopy confirmed the presence of US28-pHluorin-positive EVs with a diameter of 50-100 nm, corresponding to the size of exosomes.

Summary/conclusion: Together, these results suggest that HCMV-infected cells release US28 via exosomes. In future studies, the US28-pHluorin system can be used to study the functional consequences of US28 exosome release and to identify potential strategies to block exosomal communication by HCMV.
Keynote lecture - biosketch & abstract

Keynote lecture, 16.00h

Dr Rob Wolthuis

Using Next-Gen CRISPR Screening to Connect Genes to Your Molecules, Medicines and Systems

Rob Wolthuis studied molecular biology and biochemistry at Utrecht University and did a PhD on the topic of growth factor receptor-tyrosine kinase signal transduction pathways under supervision of prof. Hans Bos in Utrecht. He then worked as a KWF postdoc at the Gurdon Institute, University of Cambridge (UK), where he developed live cell imaging technologies to follow ubiquitin-mediated proteolysis in relation to cell division. Next, as a Junior Group Leader at the Netherlands Cancer Institute, he started a research group to study cell proliferation and genomic stability using molecular cell biology, advanced fluorescence microscopy and genomics. There, he first started to employ screening approaches to interrogate drug-gene interactions.

Currently, he is a Cancer Biology theme leader at the Cancer Center Amsterdam (CCA) and an associate professor at the AUMC Department of Clinical Genetics, leading the Oncogenetics section. He is also a co-manager of the CCA CRISPR facility. Very recently, his lab developed and implemented novel approaches for genome-wide screening using CRISPR-Cas9.

Currently, his lab offers a comprehensive CRISPR-screening pipeline to identify all the human genes that determine cellular responses to individual drugs and therapeutics. Furthermore, tools are under construction to create interaction maps that predict gene function and druggability of gene networks on the basis of CRISPR screens. These topics will also be part of the presentation.

Awards ceremony, 16.45h

Prof. Bas Teusink
Scientific director AIMMS

Announcement of Prize winners
- Poster prize
- PhD competition
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Poster 1  Anticoagulant fingerprint profiling of Black Spitting Cobra (Naja nigricollis) venom

Arif Arrahman¹,², Taline Kazandjian³, Kristina B.M. Still¹, Ben Bruyneel¹, Freek Vonk⁴, Govert W. Somsen¹, Nicholas R. Casewell³, Jeroen Kool¹

¹Amsterdam Institute for Molecules, Medicine, and Systems, Division of Bioanalytical Chemistry, Department of Chemistry and Pharmaceutical Sciences, Faculty of Sciences, Vrije Universiteit Amsterdam, De Boelelaan 1085, 1081HV Amsterdam, The Netherlands,
²Faculty of Pharmacy, Universitas Indonesia, Kampus Baru UI Depok 16424, West Java, Indonesia,
³Alistair Reid Venom Research Unit Herpetarium, Parasitology Department, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L35QA, United Kingdom,
⁴Naturalis Biodiversity Center, 2333CR Leiden, The Netherlands

E-mail: a.arrahman@vu.nl | j.kool@vu.nl

Many Cobra venoms contain anticoagulant toxins which prevent blood clotting and importantly help neurotoxins spread and reach their targets. This study aims at the application of nanofractionation analytics for unraveling the Black Spitting Cobra (Naja nigricollis) venom toxin responsible for venom-induced coagulopathic toxicities after envenoming. An integrated analytical approach was used in which venoms were analyzed by liquid chromatography coupled with mass spectrometry (MS) followed by nanofractionation on a 384-well plate for bioassaying coagulopathic properties of eluting venom constituents. After centrifuging the fraction on the 384-well plates, a spectrophotometric bioassay kinetically measured coagulation for 1.5 h after rapid automated pipetting of 20 µL of CaCl₂ (20 mM) followed by 20 µL of citrated bovine plasma to initiate coagulation. Data processing involved plotting the coagulation potency measured for each well against the fraction time to create bioactivity chromatograms. In the wells showing anticoagulation activity, MS analysis revealed that Naja nigricollis venom was found to consist mostly of acidic phospholipase A2 (PLA2) proteins and many three-finger toxins were identified. It was found that the venom also contains metalloproteinases as minor components. An inhibition assay was performed against PLA2s and metalloproteinases using a specific inhibitor. We conclude that PLA2s, three-finger toxins, and metalloproteinases are the most likely candidates responsible for the observed anticoagulant properties of Naja nigricollis venom.
**Poster 2**  
*Exosomal release of the human cytomegalovirus-encoded chemokine receptor US28*

M.P. Bebelman¹², J. van Senten¹, L.van de Ven¹, R.J.P Musters³, G. van Niel⁴, D.M. Pegtel², M.J. Smit¹

¹Division of Medicinal Chemistry, Amsterdam Institute for Molecules Medicines and Systems, VU University Amsterdam, de Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands.
²Exosome Research Group, Dept. Pathology, Cancer Center Amsterdam, VU University Medical Center, de Boelelaan 1118, 1081 HV Amsterdam, The Netherlands.
³Departement of Physiology and Advanced Optical Microscopy Core in O|2, VU University Medical Center, de Boelelaan 1118, 1081 HV Amsterdam, The Netherlands.
⁴Centre de Psychiatrie et Neurosciences Paris, France.

E-mail: m.p.bebelman@vu.nl

**Introduction:** Exosomes are nanosized extracellular vesicles (EVs) that originate from the fusion of multivesicular bodies (MVBs) with the plasma membrane. These vesicles mediate intercellular communication via the transfer of proteins and RNA. In the last decade, it has become clear that many viruses, including herpesviruses, modulate exosomal communication to create an optimal environment for viral persistence and dissemination. In this study, we show that the human cytomegalovirus (HCMV)-encoded chemokine receptor US28 is released via exosomes. This viral receptor is expressed in both latent and lytic stages of HCMV infection and constitutively activates proliferative and pro-angiogenic signaling pathways. We hypothesize that exosomal release of US28 might contribute to HCMV pathology.

**Methods:** We developed an optical reporter based on US28 and a pH-sensitive GFP (pHluorin) that enables live cell imaging of the fusion of US28-containing MVBs with the plasma membrane. Furthermore, we generated a HCMV strain containing US28-pHluorin to study exosomal release of US28 in HCMV-infected cells.

**Results:** Live cell TIRF microscopy on HCMV-infected cells revealed that US28-pHluorin-containing MVBs fuse with the plasma membrane. In line with this, EVs isolated from the culture supernatant of infected cells contain US28. Moreover, analysis of the EV-fraction by super-resolution STED and electron microscopy confirmed the presence of US28-pHluorin-positive EVs with a diameter of 50-100 nm, corresponding to the size of exosomes.

**Summary/conclusion:** Together, these results suggest that HCMV-infected cells release US28 via exosomes. In future studies, the US28-pHluorin system can be used to study the functional consequences of US28 exosome release and to identify potential strategies to block exosomal communication by HCMV.
Poster 3  Synthesis of radiolabelled $[^{11}C]$formamides; a new carbon-11 labelled building block to access novel PET tracers

Coralie M. Bonnemaire¹, Jurriën W. Collet², Tom R. Roose², Eelco Ruijter², Romano V. A. Orru², Albert D. Windhorst¹, Danielle J. Vugts¹

¹Amsterdam UMC, VU University, Radiology and Nuclear medicine, Radionuclide Center, De Boelelaan 1085c, Amsterdam, The Netherlands
²Department of Chemistry & Pharmaceutical Sciences, Amsterdam Institute for Molecules, Medicines & Systems, Vrije Universiteit Amsterdam, HZ, Amsterdam, The Netherlands

E-mail: c.bonnemaire@vumc.nl

Formamides are important building blocks in medicinal chemistry¹. As such they are also interesting for PET tracer synthesis upon labelling with carbon-11 ($t_{1/2}=20.4$ min). However to date the synthesis of radiolabelled formamides has not been reported. The aim of our research is to synthesize carbon-11 labelled formamides and to apply them in the synthesis of novel PET tracers. As a first example, N-[^11]C benzylformamide was synthesized in 2 steps starting from[^11]C CO₂. First,[^11]CCO₂ was reduced to[^11]Cformic acid using LiEt₃BH. The reaction mixture was transferred to a second vessel where[^11]Cformic acid was reacted with benzylamine using BOP/pyridine resulting in N-[^11]C benzylformamide in less than 25 min with a radiochemical yield of 90±6 % ($n=2$) and a radiochemical purity higher than 95% (based on HPLC analysis, decay corrected to end of beam). Results with other carbon-11 labelled formamides are depicted in scheme 1, all in a radiochemical purity comparable to N[^11]C benzylformamide.

A fast and easy method for the synthesis of radiolabelled N[^11]C benzylformamide has been developed. This method was applied in the synthesis of a small series of radiolabelled formamides. Further optimization is ongoing as well as application of these new carbon-11 labelled building blocks in the synthesis of novel PET tracers.

References
Poster 4  Ranking E. coli’s ammonium transport and assimilation networks by how they fit diverse experiments

Kazuhiro Maeda¹, Hans V. Westerhoff²,³,⁴, Hiroyuki Kurata¹, Fred C. Boogerd⁵

¹Kyushu Institute of Technology, Japan ²VU University Amsterdam ³The University of Manchester, United Kingdom ⁴University of Amsterdam

E-mail: kmaeda@bio.kyutech.ac.jp; Hans.Westerhoff@manchester.ac.uk; kurata@bio.kyutech.ac.jp; f.c.boogerd@vu.nl

1. Introduction. E. coli is capable of growing in media with ammonium present in the low µM range because of the transporter AmtB. The energetics of the transport remains a matter of debate. A quantitative model is necessary for a decision on the controversy. Given the heterogeneity of the data sets in terms of quality, relevance, and completeness, how can modelers still develop models that are sufficiently realistic to test hypotheses about the ammonium transport?

2. Approach. We developed a novel constrained optimization-based parameter estimation technique that allows the integration of heterogeneous experimental data sets. The technique enables us to objectively quantify the plausibility of models. We implemented the technique on a supercomputer with a fast genetic algorithm.

3. Results. We developed two kinetic models based on either the active or the passive transporter hypothesis. As training datasets, we employed diverse experimental data from three different expert laboratories. For both models, we found solution parameter sets, indicating that both models could fit the training data. However, the model plausibility of the active transporter model was 130 times greater than that of the passive transporter model.


E. coli’s ammonium transport and assimilation network
Poster 5  Cell type specific modulation of Air-Liquid Interface cultures, a new way to measure airway remodeling in vitro

Jelle van den Bor¹, Martine Smit¹

¹Amsterdam Institute for Molecules Medicines and Systems, Division of Medicinal Chemistry, Faculty of Science, Vrije Universiteit Amsterdam, The Netherlands.

E-mail: j.vanden.bor@vu.nl

Chronic Obstructive Pulmonary Disease (COPD) has a prevalence of 251 million cases (2016) and 3.17 million deaths (2015) worldwide (WHO). The primary cause of COPD is tobacco smoke which constantly injures the lung epithelium leading to characteristic pathological changes of the epithelium including goblet cell hyperplasia and basal cell (BC) squamous metaplasia (Araya et al., 2007). The progenitor function of the BC in the human airway epithelium has been linked to these pathological changes, wherein the number of BC progenitors positively correlates with the functional spirometrical parameters of the lung (Ghosh et al., 2017). Additionally, a decreased BC progenitor population was observed in COPD patients. Understanding how the progenitor function of the BCs is regulated could therefore potentially result in a way to improve lung function and subsequently cure COPD.

To study the outcome of the BC progenitor function, BCs are differentiated in air-liquid interface cultures to generate airway epithelia in vitro (Figure 1). In our study, we investigate new ways to kinetically track cell populations in the ALI culture by using fluorescent proteins under the control of cell type specific promotors. Subsequently, these techniques are used to study BC progenitor function.

References


Poster 6  Deciphering the complex distribution of cellulose ethers

Tijmen S. Bos¹, Rob Haselberg¹, Peter J. Schoenmakers², Govert W. Somsen¹

¹ Biomolecular Analysis, Amsterdam Institute for Molecules, Medicines and Systems, Vrije Universiteit Amsterdam, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands
² Van ’t Hoff Institute for Molecular Science (HIMS), University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands

E-mail: t.s.bos@vu.nl

Within the UNMATCHED project, universities and companies are working together to develop new analytical methods for the characterization of complex (bio)polymers, such as cellulose ethers. The ultimate goal is to achieve polymer products with enhanced properties by a better understanding of molecular structures through improved separation and detection. Chemically substituted celluloses find a broad range of application. For example, in the pharmaceutical industry, they are used as excipients for controlled drug release, but they are also widely applied as essential thickening agents in e.g. paint and food.

Cellulose ethers are obtained from (hydroxyl)alkylation of cellulose, which results in a highly complex chain structure and heterogeneity. The distribution at the molecular level determines polymer properties, such as solubility, viscosity, and biodegradability. In order to reveal these molecular structures, new methods for characterization are required with a focus on sequence distributions of the various substituents. In this projects we will explore the potential of two-dimensional LC, capillary electrophoresis and field-flow fractionation in hyphenation with mass spectrometry and light scattering detection modes. Advanced chemometric methods will be developed to interpret and connect the obtained data in order to obtain new insights into the functional structure of cellulose ethers.
Poster 7  Ligand binding kinetics for the H1 receptor- properties of long binders

R. Bosma¹, J. van den Bor¹, L. Inkoom¹, S. Kuhne¹, A.J. Kooistra¹, Z. Wang¹, C. de Graaf¹, H.F. Vischer¹, I.J.P. de Esch¹, R. Leurs¹.

¹Amsterdam Institute for Molecules Medicines and Systems, Division of Medicinal Chemistry, Faculty of Science, Vrije Universiteit Amsterdam, The Netherlands

E-mail: r.bosma@vu.nl

The binding kinetics of a drug for its target receptor directly influences the efficacy of that drug in vivo. Understanding the molecular mechanisms that shape the binding kinetics between a drug and receptor is therefore of interest in the design of new drug molecules with desirable receptor binding kinetics. The histamine H1 receptor (H1R) is a prototypical class A GPCR for which many clinically used drugs have been described. The drug-receptor binding kinetics is explored here in the context of the H1R.

It is shown that ligands substituted with a carboxylic acid functional group have an increased target residence time ($k_{off}^{-1}$) at the H1R. Similarly, structural analogs with and without a tricyclic aromatic core were shown to have longer and shorter residence time at the H1R, respectively.

Olopatadine, a second-generation antihistamine, has both a tricyclic aromatic core scaffold as well as a carboxylic acid moiety. As was therefore expected: [³H]olopatadine was measured to have a long residence time at the H1R. Upon probing the H1R by mutagenesis, the long residence time of [³H]olopatadine was shown to be the consequence of distinct interactions in the binding pocket as well as by interactions along the egress pathway of olopatadine from the receptor.
**Poster 8**  
*In vivo characterisation of fluorescent proteins in budding yeast*

Dennis Botman¹, D.H. de Groot¹, Philipp Schmidt¹, Joachim Goedhart², Bas Teusink¹

¹Systems Bioinformatics/AIMMS, Vrije Universiteit Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands.  
²Section of Molecular Cytology, van Leeuwenhoek Centre for Advanced Microscopy, Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands.

E-mail: d.botman@vu.nl

**Introduction:** Fluorescent proteins (FPs) are widely used in many organisms, but are commonly characterised *in vitro*. However, the *in vitro* properties may poorly reflect *in vivo* performance. Therefore, we characterised 27 FPs *in vivo* using *Saccharomyces cerevisiae* as model organism.

**Methods:** We linked FPs via a T2A peptide to a control FP, producing equi-molar expression of the 2 FPs from 1 plasmid (Fig. 1). Using this strategy, we characterised the FPs for brightness, photostability, photochromicity and pH-sensitivity, achieving a comprehensive in vivo characterisation.

**Results:** Many FPs showed different in vivo properties compared to existing in vitro data. Additionally, various FPs were photochromic, which affects readouts due to complex bleaching kinetics. Finally, we codon optimized the best performing FPs for optimal expression in yeast, and found that codon-optimization alters FP characteristics.

**Discussion:** The created yeast FPs improve experimental signal readout, opening new experimental possibilities. Our results may guide future studies in yeast that employ fluorescent proteins.

![Figure 3. Example of practical brightness quantification using the T2A peptide linker. yoeCFP shows a low brightness compared to mCherry. In contrast, mTq2 shows a higher brightness than mCherry. Calibration bar indicates the ratio value when dividing the CFP by the RFP channel (i.e. the relative brightness to mCherry).](image-url)
Poster 9 Applying the APEX2 proximity biotinylation system to identify 14-3-3 interaction partners

Brink, H.J.1, Baginska, U.1, Bebelman, J.P.1, Siderius, M.1, Smit, M.1 and De Boer A.H.1

1Vrije Universiteit, Fac. Exact Sciences, Dept. Medicinal Chemistry, The Netherlands

Email: h.j.brink@vu.nl

The small molecule fusicoccin (FC) is able to stabilize protein-protein interactions (PPIs) between the regulatory proteins 14-3-3 and a subset of their client proteins. Stabilization occurs at the extreme C-terminus of the client protein which interacts with 14-3-3 based on a phosphorylated serine/threonine type III motif (xxx-pS/pT-(VLI)-COOH). In the past identification of mode-III FC stabilized 14-3-3 interaction partners has relied on coupling FC to magnetic hydrazide beads and performing pulldown experiments. Whilst these experiments have provided insights into 14-3-3’s interactome they were limited to analysis of complexes still intact in whole cell lysates. Here we show the implementation of the APEX2 proximity biotinylation system to identify 14-3-3 interaction partners in living cells. APEX2 is an engineered peroxidase that can be activated with H₂O₂ to use biotin-phenol to generate biotin-phenoxyl radicals that can rapidly biotinylate proteins within a 20 nm radius on tyrosine, cysteine, histidine and tryptophan residues. Unlike previous live cell biotinylation systems such as BirA* and BioID2 which required multiple hours to achieve sufficient labelling, APEX2 can achieve the same in minutes. In this study we generated APEX2-14-3-3η fusion proteins and validated the system by labelling the 14-3-3 mode-III interaction partner cancerous inhibitor of protein phosphatase 2A (CIP2A).
Poster 10  Human induced Pluripotent Stemcell (iPSC) derived renal proximal tubular epithelial like cells for the application of nephrotoxicity

Vidya Chandrasekaran¹, Anja Wilmes¹ and Paul Jennings

¹Division of Molecular and Computational Toxicology, Amsterdam Institute for Molecules, Medicines and Systems, Vrije Universiteit Amsterdam, The Netherlands

E-mail: v.chandrasekaran@vu.nl

Renal proximal tubular epithelial cells are constitutive transporters which transport substances to and from the lumen and thus play an essential role in whole body homeostasis. Xenobiotics are also transported here and can reach cytosolic concentrations not seen in other tissues, which may cause injury contributing to acute and chronic renal disease. The main objective of this study was to explore the possibility of differentiating induced human Pluripotent Stem cells into cells representing some of the phenotype of proximal tubule cells in vivo, especially with respect to aminoglycosides and nanoparticle uptake mechanisms. iPSC cells were differentiated by driving them towards an intermediate mesoderm using CHIR and TTNBP, and the towards a proximal tubulus using a cocktail of specific growth factors (undisclosed). PTL were characterised by assessing the expression of pluripotency markers, renal development markers and maturation markers via immunofluorescence and western blot analysis. The outcomes of the progress of these studies are presented.
Poster 11  A simple dye dilution method for studies of mycobacteria persister cells

Priyanka Chauhan¹, Dirk Bald², Frank J Bruggeman¹

¹Systems Bioinformatics & ²Structural biology section, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), Vrije Universiteit, Amsterdam, The Netherlands

E-mail: p.chauhan@vu.nl

Tuberculosis (TB) obliteration is dogged by multiple challenges. One of them is the presence of non-growing, or dormant, antibiotic tolerant cells during infections. These cells are more likely to persist antibiotic treatment than actively growing cells and, hence, cause treatment failure and recalcitrant infections.

Our understanding of the physiological state of persistent cells is hampered by lack of suitable tools to identify, isolate and characterize non- or slowly growing mycobacteria. Until recently, qualitative parameters, such as colony forming units, have been applied to study these cells, which, however, do not allow for precise quantification of the dormancy fraction. Though fluorescent reporters have enabled some quantitative techniques for study microbial subpopulations, but require tuning of expression and genetically engineered strains. Precise quantification of the persister faction is required for evaluation of new drugs, as minor changes in their fraction may considerably influence the results of drug dose and treatment.

In our study, we aim to develop a simple and quantitative dye-based detection and isolation technique for persisters, and apply it to study dynamics of persister formation as function of combinations of conditions which effect dormancy of mycobacterium. I will present and illustrate the working of the dye that allows us to quantify persister subpopulations of mycobacteria.
**Poster 12**  
A PE/PPE substrate pair determines the system specific secretion of EsxB\(_1\)/EsxA\(_1\) by the type VII secretion pathway in *Mycobacterium marinum*

Trang H. Phan\(^1\), Merel P. M. Damen\(^1\), Roy Ummels\(^2\), Wilbert Bitter\(^{1,2}\), Edith N. G. Houben\(^1\)

\(^1\)Section Molecular Microbiology, Amsterdam Institute of Molecules, Medicines & Systems, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands, \(^2\)Department of Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, The Netherlands.

E-mail: m.p.m.damen@vu.nl

Type VII secretion systems (T7SSs) secrete a range of proteins, which play important roles in the bacterial viability and host-pathogen interactions of mycobacteria. There are four classes of T7SS substrates; the Esx, PE, PPE and Esp proteins. Some of these substrates are secreted as heterodimers by just one of the five T7SSs (i.e. by ESX-1, ESX-3 or ESX-5). This raises the question how system specificity is determined. PE/PPE heterodimers specifically interact with their cognate EspG chaperones, which determines their destined secretion pathway. However, both structural and pulldown analysis suggest that EspG chaperones are unable to interact with Esx proteins. Therefore, the determinant factor for system specificity of these substrates remains unknown. Here, we investigated the secretion specificity of the ESX-1 substrate pair EsxB\(_1\)/EsxA\(_1\) in *M. marinum*. While this substrate pair was hardly secreted when heterologously expressed, secretion was observed when EsxB\(_1\)/EsxA\(_1\) were co-expressed with PE35/PPE68\(_1\), which are expressed from the same operon. Surprisingly, co-expressing EsxB\(_1\)/EsxA\(_1\) with PE35/PPE68\(_1\), carrying the EspG\(_5\) chaperone binding domain and redirecting this substrate pair to the ESX-5 system, also resulted in secretion of EsxB\(_1\)/EsxA\(_1\) via ESX-5. Our data suggest a secretion model in which PE35/PPE68\(_1\) are the determinant factor for the system specific secretion of EsxB\(_1\)/EsxA\(_1\).
**Poster 13**  
*Identification of trade-offs between growth rate and adaptation to new environments in Lactococcus lactis*

**Sieze Douwenga¹, Filipe Branco dos Santos², Bas Teusink¹, Herwig Bachmann¹**

¹Systems Bioinformatics, VU Amsterdam, The Netherlands  
²Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands  

E-mail: s.douwenga@vu.nl

The phenotypic properties of an organism are determined by cellular constraints that can be of physical/chemical nature, or by limitations resulting from the evolutionary history of an organism. Such constraints lead to trade-offs, which restrict organisms in optimizing multiple phenotypic properties simultaneously. In the case of environmental transitions, trade-offs can manifest themselves in an intermediate growth arrest.

An example is the intermediate lag phase observed during diauxic growth on different carbon sources. The underlying reasons for such a lag phase can be the time needed to change the cellular composition to grow on the new substrate, and/or that only a part of the population can resume growth on the second carbon source.

We aim to understand the relation between growth rate and adaptability in *Lactococcus lactis* by detailed characterization of the growth response to environmental transitions. One focus will be the types of adaptation strategies (bet-hedging, proteome adaptation, or generalist strategies) used by *L.lactis*. We will study the response of *L.lactis* to around 100 environmental transitions, including various carbon sources and stress conditions. Initially we will observe the growth characteristics throughout transitions by optical density measurements, which will be followed by single cell analysis using flow cytometry and/or time-laps microscopy. This should allow us to identify conditions where significant trade-offs occur between growth rate and adaptability, and to identify the adaptation strategies used by *L.lactis* during environmental transitions.
Poster 14  To fold or not to fold

Juami van Gils¹, Maryam Hashemi Shabestari², Halima Mouhib³, Peter van Ulsen⁴, Wouter Roos², Gijs Wuite² and Sanne Abeln¹

¹Dept. Bioinformatics, Vrije Universiteit, Amsterdam, The Netherlands
²Dept. Physics of Living Systems, Vrije Universiteit, Amsterdam, The Netherlands
³Dept. Theoretical Chemistry, Université Paris-Est Marne-la-Vallée, Paris, France
⁴Dept. Molecular Microbiology, Vrije Universiteit, Amsterdam, The Netherlands

E-mail: j.h.m.van.gils@student.vu.nl, s.abeln@vu.nl

While it is currently possible to study protein structures in their native state, it is difficult to probe misfolded proteins, due to the ensemble of different conformations. Hemoglobin protease is an autotransporter secreted by *E. coli*. The protein only folds into its native state in vitro if the correct machinery is in place, otherwise it reaches a misfolded state. We studied the unfolding and refolding ability of a truncate of the passenger domain using atomic force microscopy (AFM) and MD simulations (1). The results show that the construct unfolds from N- to C-terminus and is able to refold in the AFM setup. However, when the protein is denatured with urea, we obtain very distinct force-extension profiles, with high force at lower extension lengths. Simulation results show similar unfolding patterns as the AFM, both for native and misfolded proteins. Hence, we are able to discriminate folded from misfolded states in the AFM setup. The combination of simulations with single-molecule measurements provides a new method to probe misfolded configurational ensembles of proteins.

Figure 1. Force-extension of natively folded Hbp in an MD simulation. Top: Force-extension curve of Hbp. The raw data is shown in blue, the smoothed data in red, unfolding events are indicated with black dots and worm-like-chain model fits are shown with black lines. Bottom: LGA analysis of the unfolding process. White indicates that at a certain elongation (x-axis) a given residue (y-axis) is still folded, while an orange/red color indicates that a residue is unfolded.

References

**Poster 15  TooCOLD – Development of a Toolbox for studying the Chemistry Of Light-induced Degradation**

Iris Groeneveld¹, Mimi den Uijl², Govert Somsen¹, Freek Ariese¹, Peter Schoenmakers², Maarten van Bommel²,³

¹Vrije Universiteit Amsterdam,  
²Universiteit van Amsterdam,  
³Cultural Heritage Agency of The Netherlands

E-mail: i.groeneveld@vu.nl

Many organic compounds change under the influence of (UV) light. Sometimes this is beneficial, for example in water purification, but in many other cases this is undesirable, for example when cultural-heritage objects fade, affecting their esthetical value, or when healthy food ingredients (e.g. vitamins) degrade or when toxic components are formed. Studying photochemical conversion is challenging and can be very time consuming. Often it is difficult to establish a strong link between the degradation products and the starting materials which results in poor degradation-prediction models.

Therefore, we propose to develop an innovative, high-resolution and fully orthogonal system to study the degradation of a wide range of mixture components under the influence of light. The starting mixture will first be separated using liquid chromatography (LC). The isolated components will be characterized and trapped in an exposure cell. Next, these isolated components will be degraded by light using lasers or LEDs, a process which could be monitored using Raman Spectroscopy. This helps understanding the degradation mechanisms and will indicate whether the degradation is at a stage to proceed to the next separation step. The degradation products will then be separated using LC coupled with high-resolution MS.
Poster 16  **Nanobody-targeted photodynamic therapy selectively kills viral GPCR expressing glioblastoma cells**

*Timo W.M. De Groof¹, Vida Mashayekhi², Tian Shu Fan¹, Paul van Bergen en Henegouwen², Raimond Heukers¹³, Sabrina Oliveira²⁴, Martine J. Smit¹*

¹Division of Medicinal Chemistry, Amsterdam Institute for Molecules Medicines and Systems (AIMMS), Vrije Universiteit Amsterdam, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands
²Division of Cell Biology, Department of Biology, Utrecht University, Utrecht, The Netherlands
³QVQ B.V., Yalelaan 1, 3484 CL Utrecht, The Netherlands
⁴Pharmaceutics, Department of Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

E-mail: t.w.m.degroof@vu.nl

Photodynamic therapy (PDT) eradicates tumors by the local activation of a photosensitizer with near-infrared light. One of the aspects hampering the clinical use of PDT is the lack of selectivity of the photosensitizer. To improve this, a new approach was introduced for targeted PDT, conjugating photosensitizers to nanobodies. Diverse G protein-coupled receptors (GPCRs) show aberrant overexpression in tumors and are therefore interesting targets in cancer therapy. Here we show that GPCR-targeting nanobodies can be used in targeted photodynamic therapy. We have developed a nanobody specifically binding to the viral GPCR US28, which is detected in tumors like glioblastoma. The nanobody was site-directionally conjugated to the water-soluble photosensitizer IRDye700DX and selectively killed US28-expressing glioblastoma cells both in 2D and 3D cultures upon illumination with near-infrared light. This study is the first example of a GPCR as target for nanobody-directed photodynamic therapy. With the emerging role of GPCRs in cancer, this data provides a new angle for exploiting this large family of receptors for targeted therapies.
Poster 17  Factors Controlling the Diels–Alder Reactivity of Hetero-1,3-Butadienes

Song Yu¹, Hans M. de Bruijn¹, Trevor A. Hamlin¹, F. Matthias Bickelhaupt¹²

¹Department of Theoretical Chemistry, Amsterdam Center for Multiscale Modeling (ACMM), Vrije Universiteit Amsterdam, De Boelelaan 1083, 1081 HV Amsterdam (The Netherlands); ²Institute for Molecules and Materials (IMM), Radboud University, Heyendaalseweg 135, 6525 AJ Nijmegen (The Netherlands).
E-mail: t.a.hamlin@vu.nl, f.m.bickelhaupt@vu.nl

We have quantum chemically explored the Diels–Alder reactivities of a systematic series of hetero-1,3-butadienes with ethylene by using density functional theory at the BP86/TZ2P level. Activation strain analyses provided physical insight into the factors controlling the relative cycloaddition reactivity of aza- and oxa-1,3-butadienes. We find that dienes with a terminal heteroatom, such as 2-propen-1-imine (NCCC) or acrolein (OCCC), are less reactive than the archetypal 1,3-butadiene (CCCC), primarily owing to weaker orbital interactions between the more electronegative heteroatoms with ethylene. Thus, the addition of a second heteroatom at the other terminal position (NCCN and OCCO) further reduces the reactivity. However, the introduction of a nitrogen atom in the backbone (CNCC) leads to enhanced reactivity, owing to less Pauli repulsion resulting from polarization of the diene HOMO in CNCC towards the nitrogen atom and away from the terminal carbon atom. The Diels–Alder reactions of ethenyldiazene (NNCC) and 1,3-diaza-butadiene (NCNC), which contain heteroatoms at both the terminal and backbone positions, are much more reactive due to less activation strain compared to CCCC.
Poster 18  Identification of a 14-3-3 binding epitope in human aminopeptidase N

Kiehstaller, S.1, Hennig S.1

1Department of Chemistry and Pharmaceutical Sciences, VU Amsterdam, 1081HZ Amsterdam.
E-mail: s.kiehstaller@vu.nl; s.hennig@vu.nl

Aminopeptidase N (APN, CD13; figure 1A) is a zinc-dependent type II transmembrane ectopeptidase which is located on the surface of many cell types such as fibroblasts, epithelial and myeloid cells. It plays an important role in different cellular mechanisms including tumour cell invasion and angiogenesis. Elevated levels of APN were also found in several kinds of malignancies. One interaction partner of APN is the adapter protein 14-3-3. Previous research has shown, that extracellular binding of 14-3-3 to APN leads to an upregulation of MMP1 transcription within fibroblasts. Additionally, it has been observed that 14-3-3ε binding to the surface of chondrocytes seems to be dependent on APN expression. The family of 14-3-3 proteins are highly conserved eukaryotic proteins and 7 different homologues have been detected in mammals. 14-3-3 forms homo- and heterodimers with other homologues and serves as an adapter protein for more than 200 binding partners. The monomer consists of 9 α-helices that form an amphiphatic binding groove (figure 1B).

Although the cellular effects of the interaction have been shown, the binding mechanism and the activation of APN by 14-3-3 are still unknown. We therefore used an in silico approach to identify potential binding motifs in APN and analysed most promising epitopes in vitro. We could identify and characterize a best binding protein sequence and analysed its binding mode by X-ray crystallography.

Figure 1: Crystal structure of aminopeptidase N (A, pdb: 4FYQ) and 14-3-3ε (B, pdb: 3MHR).

References
Poster 19  

Lactobacillus crispatus growth on glycogen is dependent on its type 1 pullulanase gene variant.

Rosanne Y. Hertzberger¹,², Alicia Brandt ²,³, Charlotte vd Veer⁴, Jorne Swanenburg¹,⁵, Remco Kort¹,⁵

1 VU University Amsterdam, Netherlands,
2 REBLAB, Netherlands,
3 University of Groningen, Netherlands,
4 Public Health Service, Amsterdam, Netherlands,
5 TNO, Zeist, Netherlands

Email: r.y.hertzberger@vu.nl

Glycogen is an abundant carbohydrate in the vagina of reproductive-age women. Here we study whether Lactobacillus crispatus, a prominent member of the vaginal microbial community in reproductive age women, can utilize this carbon source colonization and acidification.

We studied growth, enzymatic activity and metabolite production of a group of 19 Lactobacillus crispatus strains that were isolated from reproductive age women. 13 out of these 19 isolates were able to grow on glycogen. Starch was used as a proxy to study glycogen breakdown and starch metabolism activity was found in both supernatant as well as in the washed pellets after growth on glycogen, but not after growth on glucose.

A survey of the genomes to track down any carbohydrate active enzymes showed the presence of a putative cell surface associated type 1 pullulanase. The gene shows high similarity to the extracellular cell wall attached pullulanase previously found in a human gut isolate of Lactobacillus acidophilus.

Although a copy of the pullulanase gene was found in the genomes of all 19 L. crispatus strains, the six non-glycogen consuming strains all carried one of three different mutations in the N-terminal signal peptide sequence, expected to disrupt transcription of at least part of the gene.

Our results show for the first time that certain vaginal isolates of Lactobacillus crispatus are capable of metabolizing one of the most abundant vaginal carbohydrates. We identify a pullulanase that may be essential for this activity. These findings bring us further to understanding the basic mechanisms of Lactobacillus colonization and acidification of the human vagina.

This project is part of REBLAB, an Open Kitchen Science initiative. For more information see www.reblab.org.
Poster 20  London dispersion interactions without density change

Derk P. Kooi\textsuperscript{1}, Paola Gori-Giorgi\textsuperscript{1}

\textsuperscript{1} Department of Theoretical Chemistry and Amsterdam Center for Multiscale Modeling, Faculty of Science, Vrije Universiteit Amsterdam, 1081HV Amsterdam, The Netherlands

E-mail: d.p.kooi@vu.nl

London dispersion interactions play a crucial role in physical, chemical and biological processes. Despite their nature being elucidated already in the 1930s, calculating accurately the strength of these interactions remains a computational challenge and is one of the main challenges for Density Functional Theory (DFT) today. In our work we have introduced a class of variational wavefunctions that capture the long-range interaction between neutral systems (atoms and molecules) without changing the diagonal of the density matrix of each monomer. As the individual monomer densities are kept fixed, we can also unambiguously assess the effect of the density distortion on London dispersion interactions: for example, we obtain virtually exact dispersion coefficients between two hydrogen atoms up to C\textsubscript{10}, and relative errors below 0.2\% in other simple cases.

Poster 21  
**Optimization of a phenotypic hit, NPD-2975, for the African sleeping sickness**

Yang Zheng¹, An Matheeussen², Marco Siderius¹, Geert Jan Sterk¹, Louis Maes², Guy Caljon², Rob Leurs¹

¹Division of Medicinal Chemistry, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands;  
²Laboratory for Microbiology, Parasitology and Hygiene, University of Antwerp, Wilrijk, Belgium.

E-mail: r.leurs@vu.nl

In our consortium PDE4NPD, in the search for novel potential treatments of several Neglected Parasitic Diseases, we followed a two-pronged approach: SBDD based on parasitic phosphodiesterase inhibition and a phenotypical approach by screening on several parasites *in vitro*. During these efforts, we found, phenotypically, a class of pyrazolopyrimidinones with good efficacy against the parasite *Trypanosoma brucei*, the causative agents of the African sleeping sickness: pIC$_{50}$ *in vitro* between 10 and 100 nM. Optimization within this chemical class resulted in a lead compound, NPD-2975, with efficacy in several mouse models after oral application. In this poster we describe our efforts for optimization on potency, PK properties and tolerability together with the results in animal models.

![Chemical structures](image)

**Discovery and modification of NPD-2975**
Prediction of microbial cross feeding with genome-scale models

Julia Lischke1, Frank Bruggeman1, Bas Teusink1

1Systems Bioinformatics, Vrije Universiteit Amsterdam

E-mail: j.lischke@vu.nl

Microbial communities shape our environment, influence our health and revolutionize biotech engineering. Still, our understanding of the interactions in microbial communities is very limited. Though measurement and screening technologies improved in the last decade, it is a challenge to identify, quantify and characterize the members of communities and their influence on the whole community. To enhance the understanding of mutualistic growth and to guide the experimental efforts, we develop a mathematical modelling approach to predict cross feeding with genome scale models.

For developmental purposes, we use data from synthetic communities. First starting with artificially cross-feeding E.coli strains, generated from a common ancestor1. This data suggests that the mutant strains gain a growth benefit by division of labour. Therefore, resources can be reallocated and used more efficiently to achieve higher growth.

We use protein constrained genome scale models, to analyse and predict efficient growth modes and their resource allocation strategy in single strain cultures and communities2. This will lead to the prediction of the metabolites, which are most beneficial for the community to cross feed.

This research is embedded in the EraCoBiotech project YogurtDesign and will help to improve our understanding of yogurt fermentation and its engineering opportunities.

References


G protein-coupled receptors (GPCRs) play an essential role in the regulation of cell function by activating G protein- and/or β-arrestin-mediated intracellular signaling upon binding of their cognate ligands, and are consequently important targets for therapeutic intervention. The last couple of years it became apparent that GPCRs can interact with other non-canonical proteins that may modulate GPCR functions. These so-called GPCR interacting proteins (GIPs) are hypothesized to modulate or even initiate distinct biochemical signaling in a more cell type-specific manner. We recently identified novel GIPs that constitutively interact with H4R using a split-ubiquitin membrane yeast two-hybrid screen (SUMY2H) on a T lymphocyte cDNA library under basal conditions. Interestingly, stimulation of the H4R with histamine decreased this interaction. We validated these ligand-modulated interactions in HEK293T cells by using bioluminescence resonance energy transfer (BRET) and enzyme-fragment complementation (EFC)-based assays to dynamically monitor their proximity, and subsequently aim to determine the functional consequences of these H4R-GIP interactions.
**Poster 24**  
**A new antibiotic target in mycobacterial energy metabolism**

Ludovica Mascolo

Department of Molecular Cell Biology, Amsterdam Institute for Molecules, Medicines and Systems, Faculty of Earth and Life Sciences, Vrije Universiteit Amsterdam

E-mail: l.m.mascolo@vu.nl

Tuberculosis (TB) is a major global scourge firstly because of the emergence of drug-resistant strains and, also for the presence of an overtaken and uncomfortable therapy. For these reasons a new regimen is needed and new drugs targeting energy metabolism are highly promising to defeat this menace. In particular, the ATP synthase inhibitor bedaquiline (BDQ), was included in anti TB regimen in 2012.

Our goal in this project is to characterize small-molecule inhibitors active on other complexes of energy metabolism, in particular on the terminal oxidases. We aim to optimize these inhibitors in terms of target affinity and stimulation of companion drugs, combined with minimal toxicity.

The effect of drug candidates on whole cells oxygen consumption by *Mycobacterium tuberculosis* will be presented.
**Poster 25**  Photoswitchable ligands as tools for dynamic modulation of histamine receptors

Tamara A.M. Mocking¹, Niels. J. Hauwert¹, Albert J. Kooistra¹, Daniel da Costa Pereira¹, Yara Huppelschoten¹, Marek Staszewski², Iwan J.P. de Esch¹, Chris de Graaf¹, Krzysztof Walczyński², Maikel Wijtmans¹, Henry F. Vischer¹, Rob Leurs¹

¹Department of Medicinal Chemistry, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), Vrije Universiteit Amsterdam, Amsterdam, 1081HZ The Netherlands.
²Department of Synthesis and Technology of Drugs, Medical University of Łódź, ul. Muszyńskiego 1, 90-145 Łódź, Poland

E-mail: t.a.m.mocking@vu.nl

Histamine receptors are widely distributed throughout the body and respond (in a paracrine/autocrine manner) to local increases in histamine. Hence, it would be therapeutically advantageous to only locally influence histamine receptors rather than systemic targeting of the receptor to avoid unwanted side effects. Temporal and spatial control of drug effects can be induced utilizing photoswitchable ligands. Photoswitchable ligands can reversibly photo-isomerize from the trans to the cis isomer upon illumination with specific wavelengths. The resulting conformational change in the ligand may result in distinct changes in binding affinity and/or intrinsic activity of the isomer for the receptor. To this aid, we have developed and characterized a toolbox of photoswitchable ligands for the different histamine receptor subtypes that enables dynamic optical control of their activity with high temporal resolution. Moreover, the option to locally modulate the receptor activity with light opens opportunities to investigate the local role of histamine receptor signaling in vivo.
**Poster 26**  **Specific Flux Optimization in the Threonine Pathway**

**Riccardo Muolo¹,³, Robert Planqué², Frank J. Bruggeman¹, Bas Teusink¹,³**

1 Systems Bioinformatics, VU Amsterdam, the Netherlands;  
2 Department of Mathematics, VU Amsterdam, the Netherlands;  
3 Marie Curie ITN SynCrop, European Commission Horizon 2020.

E-mail: r.muolo@vu.nl

Inspired by recent observations in microbiology indicating that gene control might be optimized for growth rate maximization, we propose a method to design synthetic gene control systems that optimize specific product formation fluxes in biotechnological applications.

We reformulate an existing method, called “$q$ORAC”, for Specific Flux ($q$) Optimization by Robust Adaptive Control, to make it applicable to biotechnology. $q$ORAC is a way of modeling “blind sensing” by cells, i.e., a cell’s ability to adapt to changing environments without sensors for environmental cues. Through $q$ORAC, we can design a circuit that finds optimal metabolite and enzyme concentrations, given pathway stoichiometry and enzyme kinetics.

We illustrate the approach for an existing, experimentally validated, kinetic model of the metabolism of threonine biosynthesis in *Escherichia coli*. Given a demand flux for threonine, can we find a gene control circuit that would steer pathway enzyme synthesis to minimal levels, in the face of a dynamic environment? Such an implementation would then impose a minimal burden on the resources of the cell.

This framework offers biotechnologist and metabolic engineers theoretical guidelines for design of optimal gene regulation that maximizes the specific flux of a biotechnological product in dynamic environments.

**References**

Poster 27  The dynamic co-culture metabolic modeling of cheese starter cultures

Emrah Özcan1,2, Emrah Nikerel4, Tunahan Çakır3, Ebru Toksoy Öner2, Bas Teusink1

1Systems Bioinformatics, Amsterdam Institute for Molecules, Medicines and Systems, VU Amsterdam, The Netherlands
2IBSB, Department of Bioengineering, Marmara University, Istanbul, Turkey
3Department of Bioengineering, Gebze Technical University, Gebze, Kocaeli, Turkey
4Genetics and Bioengineering Department, Yeditepe University, Istanbul, Turkey

E-mail: e.o.ozcan@vu.nl

Lactic acid bacteria (LAB) commonly used in cheese starter cultures were grown in co-cultures in chemically defined medium under anaerobic conditions without pH maintenance. Co-cultures containing *L. lactis* and *L. mesenteroides* strains were accepted to represent mesophilic cheese starter cultures, while co-cultures containing *L. lactis* and *S. thermophilus* strains were accepted to represent thermophilic cheese starter cultures.

Genome-scale metabolic models of the LAB used in the experiments were then used to reconstruct the dynamic co-culture metabolic models (Fig. 1) to evaluate dynamic genome-scale metabolic patterns of co-cultures.

The glucose, organic acids, amino acids and individual biomass concentration profiles predicted by co-culture models were consistent with experimental data, which allowed us to estimate the potential metabolic interactions among the LAB and flavour compound production capacities of the co-cultures.

![Figure 1. The dynamic co-culture metabolic modeling approach.](image)
**Poster 28  Genome-scale Proteome Constrained model of L. lactis**

Eunice van Pelt-KleinJan¹, Yu Chen², Brett Olivier¹, Douwe Molenaar¹, Herwig Bachmann¹, Jens Nielsen², Bas Teusink¹

¹Systems Bioinformatics, Vrije Universiteit Amsterdam ²Department of Biology and Biological Engineering, Chalmers University of Technology, Göteborg, Sweden

E-mail: e.van.pelt-kleinjan@vu.nl

Microorganisms face constraints, e.g. limited protein resources, on their adaptability to new conditions. As a result trade-offs can occur: one trait can only be optimized at the cost of another. We study the constraints that are responsible for these trade-offs.

We do this with a PC (Proteome Constrained) model, which is a metabolic model extended with the reactions for protein and mRNA synthesis and degradation. Solving a PC-model gives a flux distribution that also takes into account the cost of enzyme synthesis and the limited cell volume. PC-models can therefore predict the impact of cellular constraints on functionality much more realistically than traditional genome-scale models. We started to construct such an integrated model of the cellular economy for L. lactis.

Visualization of the different levels in the PC-model would greatly help analyzing results. We therefore developed an automatic visualization pipeline to generate a structured map of the network. Advantages of our visualization are the interactivity and the possibility to visualize both metabolic fluxes and expression data of different conditions. This way of visualization is especially useful for modeling and data integration with next generation models such as PC-models and will improve the understanding of occurring trade-offs in L. lactis.
**Poster 29**  
*Enabling systematic comparison of protein-ligand binding free energies by combining LIE theory with TI for ligand solvation*

**Eko Aditya Rifai¹, Valerio Ferrario², Jürgen Pleiss², and Daan P. Geerke¹**

¹AIMMS Division of Molecular and Computational Toxicology, Department of Chemistry and Pharmaceutical Sciences, Vrije Universiteit Amsterdam, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands  
²Institute of Biochemistry and Technical Biochemistry, Universität Stuttgart, Allmandring 31, 70569 Stuttgart, Germany  

E-mail: e.a.rifai@vu.nl

Calculating binding free energies ($\Delta G_{\text{bind}}$) between ligands and target proteins is a major interest in drug discovery and safety, yet it is still attributed with several challenges and difficulties. Linear interaction energy (LIE) is one of the *in silico* end-point methods available to predict $\Delta G_{\text{bind}}$, and it is suitable for calculations of $\Delta G_{\text{bind}}$ for flexible proteins that can bind their ligands in multiple orientations¹⁻³. For 28 cytochrome P450 2A6 (CYP2A6) ligands, we demonstrated that coupling LIE with solvation free energies ($\Delta G_{\text{solv}}$) obtained from thermodynamic integration (TI) helps improving correlation with respect to reference binding data (Pearson’s $r = 0.77$).

![Graph showing correlation between experimental and TI-calculated binding free energies](image)

**References:**

(2) van Dijk et al. *J Chem Inf Model* (2017) 57:9  
Poster 30  When and how to switch? – Studying gene expression at single cell level in Baker’s yeast

Phillipp Schmidt¹, Johan van Heerden¹, Bas Teusink¹ and Frank Bruggeman¹

¹Systems Bioinformatics, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

E-mail: p.schmidt@vu.nl, j.van.heerden@vu.nl, b.teusink@vu.nl, f.j.bruggeman@vu.nl

When S. cerevisiae grows in batch-cultures on glucose it produces ethanol. After glucose depletion, cells start to use the ethanol as carbon source. This phenomenon of observing two growth phases is called diauxie. We want to study how this shift is regulated on the gene expression level and which external signal is determinative. In particular, it is not clear if it is a hard-wired response to glucose depletion or if it also requires a certain concentration of ethanol. We use fluorescent reporters and a flow cytometer to study gene expression at the single cell level. We performed batch growth experiments and use multiple genes as readout for the initiation of gluconeogenesis. The analysis of mean fluorescence over time showed that cells clearly induced gene expression shortly before or around the point of glucose depletion. At the single cell level, we detected varying subpopulations of cells that show induction. The observed behaviour can be explained by anticipation of glucose depletion/ethanol consumption. While we don’t understand the mechanism, we conclude that a higher ethanol concentration leads to a higher fraction of cells that anticipate the shift and can switch immediately.

Figure 4: Example experiment of CEN.PK21-C ICL1-ymNeongreen growing on 15mM glucose and different ethanol concentrations with the following measurements: cell concentration (A), ethanol concentration (B), glucose concentration (C) and mean fluorescence area (D). t=0h is set at the moment of glucose depletion. (E) Histograms for the fluorescence concentration (fluorescence divided by side scatter) over time for 20mM ethanol. The orange lines represent the mean and mean ± standard deviation. Two peaks are visible after glucose depletion. The first bimodal distribution is shown in red. (F) The fraction of uninduced cells over time (small peak in histogram). The red dot indicates the moment were the distribution becomes bimodal. The ethanol present during glucose depletion lowers the fraction. These data indicate two subpopulations; one that can induce gene expression immediately, while the other keeps inducing over the course of a few hours.
Characterisation of the signaling properties of the HCMV-encoded receptor UL78

Irfan M. Setiawan¹, Jelle van den Bor¹, Timo de Groof¹, Marco Siderius¹ and Martine J. Smit¹

¹VU University Amsterdam, Faculty of Sciences, Division of Medicinal Chemistry

E-mail: mj.smit@vu.nl; i.m.setiawan@vu.nl

Human Cytomegalovirus (HCMV) is a member of the β-herpesvirus family and widely spread in a large percentage of the population¹. Infection of HCMV does not cause serious disease in an immunocompetent individual, but it can be a dangerous pathogen in immunocompromised persons. The genome of HCMV encodes four viral G Protein-Coupled Receptors (GPCRs) namely US28, US27, UL33, and UL78. Among these GPCRs, US28 is the most characterized receptor. US28 is activated by various chemokines and activates various inflammatory and proliferative signaling pathways in a constitutively active manner². In contrast to US28, UL78 is considered an orphan receptor since no ligands were identified to bind this receptor yet. The UL78 like genes are present in all β-herpesvirus and are highly conserved in clinical isolates³, yet little is known about the UL78 signaling properties. The aim of this study is to determine the signaling properties of UL78, whether it is able to constitutively activate various signalling pathways. Insight into this is important to attribute a potential role of this receptor in HCMV associated diseases.

References
1. Cannon et al. 2010, Reviews in Medical Virology 20:202
**Poster 32**  
*Identification of coagulopatic snake venom peptides and enzymes using nanofractionation coagulation analytics*

**Julien Slagboom¹, Marija Mladić², Govert W. Somsen¹, Nicholas Casewell³**  
**Jeroen Kool¹**

¹Division of BioAnalytical Chemistry, Amsterdam Institute for Molecules Medicines and Systems, VU University Amsterdam, De Boelelaan 1083, 1081HV, Amsterdam, The Netherlands  
²Animal Sciences and Health, Institute of biology Leiden, University of Leiden, Sylviusweg 72, 2333BE Leiden, The Netherlands  
³Alistair Reid Venom Research Unit, Parasitology Department, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK

E-mail: j.slagboom@vu.nl

Snake bite is a neglected tropical disease and the majority of deaths resulting from snake bites are caused by venoms that act on the blood coagulation cascade. Current treatments do not suffice and therefore it is crucial to develop a generic and cheap alternative. This study describes the identification of snake venom compounds that act on the blood coagulation cascade by applying the direct combination of analytical and pharmacological methods called nanofractionation. Crude snake venoms which have previously shown to act on the blood coagulation cascade were separated using reversed-phase liquid chromatography. A post-column split allowed a small fraction to be analyzed with mass spectrometry and a larger fraction to be collected onto 384-well plates. The well plates containing the collected snake venom fractions were subjected to vacuum centrifuge drying and subsequently analyzed directly using the new plasma coagulation assay. When bioactivity was observed, the accurate masses of the bioactive compounds were determined by correlating the mass spectrometry data with the bioassay data. The wells from a duplicate nanofractionated 384-well plate, containing the same bioactive compounds, were subjected to tryptic digestion for further proteomics analysis by nanoLC-MS/MS and database searching. For this, the obtained data was run through the Mascot database for identification of bioactive compounds. This high-throughput approach can be used to identify toxins that are not neutralised by existing antivenoms and can serve as a powerful tool in guiding future antivenom design.
**Poster 33**  
*Picofractionation System enabling Parallel Coupling of NanoLC with Microarray Bioassays and Nano-ESI-MS for Screening of Aquatic Pollutants*

**Kristina B. M. Still**¹, **Timo Hamers**², **Peter H. Cenijn**², **Govert W. Somsen**¹, **Marja H. Lamoree**², and **Jeroen Kool**¹

¹Division of BioAnalytical Chemistry, Amsterdam Institute of Molecules, Medicines and Systems, Vrije Universiteit Amsterdam, de Boelelaan 1085, 1081 HV Amsterdam, The Netherlands;  
²Institute for Environmental Studies, Amsterdam Institute of Molecules, Medicines and Systems, Vrije Universiteit Amsterdam, de Boelelaan 1085, 1081 HV Amsterdam, The Netherlands;  

Email: k.b.m.still@vu.nl

Effect directed analysis (EDA) combines chemical and biological methods in an effort to screen the aquatic environment for compounds with bioactive properties of concern. Platforms applied in common water screening laboratories are mostly EU regulated and facilitate (semi)automated analytical instrumentation for targeted and untargeted analysis of these compounds. EDA approaches are often laborious and insufficiently result in identification of bioactives. Therefore, new high-throughput (HT) screening platforms for EDA are developed. For that, liquid chromatography (LC) with parallel online high-resolution fractionation and mass spectrometric (MS) data collection are combined. This helps to enhance throughput and increase sensitivity and selectivity for improved bio-assaying and identification of (unknown) bioactive compounds.

In order to further increase MS sensitivity and allow analysis of limited-volume samples, a new ‘picofractionation’ screening platform was developed. The new system combines nanoLC with microarray bioassays and nano-electrospray ionization (nanoESI) MS in parallel. For this, nanoliter-volume fractions of the nanoLC effluent were discretely deposited on a glass slide and subsequently interrogated by a microarray bioassay of choice. This was performed by first drying the nanoliter fractions and then sequentially deposit nL-volumes of reagents on top of the spots. Unwanted evaporation of bioassay liquids was circumvented by employing mineral oil droplets. A fluorescence microscope was used for assay readout in a kinetic mode. Parallel identification of bioactives was achieved by implementation of online nanoESI-TOF-MS(/MS) detection after nanoLC, or by offline MALDI-TOF-MS(/MS) after deposition on MALDI target plates.

The new picofractionation system is being developed and evaluated using a standard EDA method as starting point. Its applicability will be tested using the transthyretin (TTR) displacement assay as toxic biochemical readout. The TTR displacement assay, an endocrine disruptor assay, screens for compounds competing with thyroid hormones by binding to TTR, a thyroid hormone transport protein. Environmental polluting compounds with these properties can be found plentiful in the aquatic environment and have possible risks to human health.
**Poster 34**  
*A systematic assessment of current genome-scale metabolic reconstruction tools*

**Bas Teusink**, Sebastián N. Mendoza, Brett G. Olivier, Douwe Molenaar  

1 Systems Bioinformatics, AIMMS, VU Amsterdam, The Netherlands  

E-mail: b.teusink@vu.nl

Several genome-scale metabolic reconstruction software platforms have been developed and continuously updated during the last fifteen years. These tools have been widely applied to reconstruct metabolic models for hundreds of microorganisms ranging from important human pathogens to species of industrial relevance. So far, these platforms have not been systematically evaluated with respect to software quality, best potential uses and intrinsic capacity to generate high-quality genome-scale metabolic models. In consequence, this lack of assessment keeps users away from selecting the tool that best fits the purpose of their research. In this work, we reviewed the current genome-scale reconstruction software platforms and we performed a systematic assessment of the most promising tools. We defined a list of features for assessing software quality related to genome-scale reconstruction which we hope to be useful for potential users. In addition, we compared the output networks generated using each of these tools with the high-quality manually curated models of *Lactobacillus plantarum* and *Bordetella pertussis*, representatives of gram-positive and gram-negative bacteria, respectively. We showed that none of the tools outperform the others in all the studied features and that users should carefully choose one or another, or even combining them, depending on the particularities of their projects.
Poster 35  Resolving protein conformational states with hybrid trapped ion mobility spectrometry-high resolution mass spectrometry

Robert L.C. Voeten¹,², Rob Haselberg¹, Govert W. Somsen¹

¹ Biomolecular Analysis, Amsterdam Institute of Molecules, Medicines and Systems, Vrije Universiteit Amsterdam, de Boelelaan 1085, 1081 HV Amsterdam, The Netherlands,  
² TI-COAST, Science Park 904, 1098 XH Amsterdam, The Netherlands

E-mail: r.l.c.voeten@vu.nl

Like most macromolecules, proteins exhibit complex structural dynamics. Activity, binding and other biological properties of proteins heavily depend on their conformational state. Information on (changes in) protein conformation is essential to understand biological function. In this work, we exploited the feasibility of gas-phase trapped ion-mobility spectrometry (TIMS) in combination with high-resolution time-of-flight mass spectrometry (TOFMS) for the detection and resolution of protein conformational states.

Bovine carbonic anhydrase II (BCA) was dissolved (8 μM) in ammonium acetate or formate solutions containing small amounts of organic solvents. A trimodal charge state distribution (CSD) marked the transition point of pH-induced unfolding. Ammonium formate and increasing ionic strength appeared to stabilize the holo-state of BCA. Introducing TIMS prior to TOFMS allowed ion-mobility analysis of single charge states of BCA throughout the CSDs obtained under the different conditions (Figure 5). Mobilograms of single charge states of bovine carbonic anhydrase (BCA) showed the coexistence of different conformational states of BCA in solutions of varying pH. Conformational transitions reflecting protein unfolding in solution were revealed. Native-like conformations at increased charge states were observed by comparison of holo and apo state protein populations. TIMS shows to be an effective and fast tool for studying protein conformational changes induced by solution composition.

Figure 5: Left: ESI mass spectra for BCA II in ammonium acetate of decreasing pH showing shifts to higher charge states. Right: ion mobilograms for the 17+ ions showing coexisting BCA species of different mobility and indicating pH-induced conformational changes (unfolding).
**Poster 36**  
A new approach for constraining peptide secondary structures through hydrocarbon crosslinks

K. Wallraven¹, P.M. Cromm², A. Fürsnter³, T.N. Grossmann¹

¹VU University, Amsterdam, The Netherlands;  
²Max-Planck-Institute of Molecular Physiology, Dortmund, Germany;  
³Max-Planck-Institute for Coal Research, Mülheim/Ruhr, Germany

E-mail: k.wallraven@vu.nl

Stapled peptides are well studied probes to elucidate biological systems. They comprise an \( \alpha \)-methylated, hydrocarbon crosslink that stabilizes \( \alpha \)-helical secondary structures. Such macrocyclized peptides are known to provide higher target affinities compared to their unmodified counterparts by reducing conformational flexibility. These peptides adopt the bioactive conformation already in their unbound state thereby reducing entropic penalty upon target engagement. Also, stapled peptides reveal increased proteolytic stability. Due to their excellent surface recognition properties, such modified peptides are expected to fill the gap between small molecule drugs and biologicals. Therefore, they are gaining increased interest as inhibitors of protein–protein interactions (PPIs).\(^1\) However, peptide stapling is designed to stabilize \( \alpha \)-helical structures only and thereby leaving a variety of secondary structures unaddressed. Although the crosslink aims for conformational restriction it is relatively flexible itself and remains unfunctionalized. Here we present a novel approach of ring-closing alkyne metathesis (RCAM) to constrain an irregular peptide secondary structure through an alkyne-containing hydrocarbon crosslink.\(^2\) The alkyne moiety provides new steric and conformational features and serves as a platform for post-crosslinking functionalization.

![Image](image-url)

**References**

**Poster 37**  Ligand rigidification has a pronounced effect on the residence time of histamine H1 receptor antagonists

Zhiyong Wang¹, Reggie Bosma¹, Sebastiaan Kuhne¹, Jelle Van den Bor¹, Henry F. Vischer¹, Chris de Graaf¹, Maikel Wijtmans¹, Rob Leurs¹, Iwan J. P. de Esch¹

1 Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), Division of Medicinal Chemistry, Faculty of Science, Vrije Universiteit Amsterdam, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands.

E-mail: w.z.y.wang@vu.nl, i.de.esch@vu.nl

The histamine H₁ receptor (H₁R) mediates important effects of histamine in allergic conditions, including urticaria, allergic rhinitis, and pollenosis. Well-known tricyclic H₁R antagonists such as doxepin and desloratadine have a much longer drug-target residence time (RT) when compared to other H₁R antagonists such as tripipridine and mepyramine¹, which may be the consequence of different rigidification of the structures.² We therefore explored the restraining of the conformational freedom of the aromatic head group as a potential strategy to modulate the RT. The role of a rigid tricyclic head group was first explored by determining the binding kinetics, including RT at the H₁R of a diverse set of typical H₁R clinically used drugs. A subsequent set of tailored synthesized derivatives allowed the direct comparison of ligands with non-fused aromatic ring systems with ligands in which the rings were fused by an ethane or ethylene bridge. A similar comparison was executed for the moiety connecting the aromatic part to the heterocycle. Similar trends for residence time were observed for each series of comparisons, leading to well-defined structure-kinetics-relationships that describe the role of rigidification on the ligand binding kinetics.

![Chemical structures](image)

X = C, N

**References**


Poster 38  Enhancing Protease Stability of Stapled Peptides for Targeting Protein–Protein Interactions

Mathias Wendt, Philipp M. Cromm, Jochen Spiegel, Philipp Küchler, Laura Dietrich, Julia Kriegesmann, Roger S. Goody, Herbert Waldmann, Tom N. Grossmann

1 Department of Chemical Biology, Max-Planck-Institute of Molecular Physiology, Otto-Hahn-Strasse 11, D-44227 Dortmund, Germany
2 Technische Universität Dortmund, Fakultät für Chemie und Chemische Biologie, Otto-Hahn-Strasse 6, D-44227 Dortmund, Germany
3 Chemical Genomics Centre of the Max Planck Society, Otto-Hahn-Strasse 15, D-44227 Dortmund, Germany
4 VU University Amsterdam, Department of Chemistry & Pharmaceutical Sciences, De Boelelaan 1108, 1081 HV, Amsterdam, The Netherlands
5 Structural Biochemistry, Max-Planck-Institute of Molecular Physiology, Otto-Hahn-Strasse 11, D-44227 Dortmund, Germany

E-mail: m.wendt@vu.nl / t.n.grossmann@vu.nl

StRIP3 is a hydrocarbon stapled peptide inhibitor of the protein–protein interaction between the small GTPase Rab8a and an effector protein. It is derived from the binding interface between the Rab6-interacting protein1 and Rab6a. The bioactive conformation is hereby retained by the introduction of two \( \alpha \)-methyl, \( \alpha \)-alkenyl amino acids. Using ring closing metathesis a macrocyclic peptide can be formed, which locks the peptide in a helical conformation. Stapled peptides are widely recognized to have a higher proteolytic stability than their corresponding wild-type sequences. However our model peptide StRIP3 does not show a high increase in proteolytic stability. With our approach we were able to increase proteolytic stability significantly by the incorporation of \( \alpha \)-methyl amino acids. Nonetheless the variations had a negative influence on the target affinity. Therefore, introduction of a 2nd cross-link into the peptide sequence, was a new approach, that resulted in high proteolytic stability and retained target affinity. Finally the overall negative charge of the modified peptides were reduced leading to both a highly protease resistant peptide with good cellular uptake. The described role case shows a strategy to implement proteolytic stability while retaining affinity and increasing cellular uptake of a conformational constrained peptide.

References


**Poster 39**  
*Effect of varespladib on neutralizing the coagulopathic and phospholipase A2 activities of haemotoxic snake venoms*

**Chunfang Xie, Kristina B. M. Still, Laura-Oana Albulescu, Freek Vonk, Govert W. Somsen, Nicholas R. Casewell, Jeroen Kool**

Amsterdam Institute for Molecules Medicines and Systems, Division of BioAnalytical Chemistry, Department of Chemistry and Pharmaceutical Sciences, Faculty of Sciences, Vrije Universiteit Amsterdam, De Boelelaan 1083, 1081HV Amsterdam, The Netherlands.

E-mail: c.xie@vu.nl

Phospholipase A₂ (PLA₂) enzymes are important players for controlling cellular signaling and are abundant in the mammalian pancreas. Venom PLA₂s show a variety of toxicities, including those related to haemolysis and/or anticoagulation. Varespladib is a broad-spectrum inhibitor of PLA₂s which has demonstrated highly potent efficacy in suppressing venom-induced PLA₂ activity. This study applied chromatography combining mass spectrometry and bioassays to investigate the effect of varespladib on the coagulopathic toxicities and enzymatic PLA₂ activities induced by individual venom toxins of several medically important snake venoms. For this, snake venoms were separated by liquid chromatography followed by nanofractionation for bioassaying, and parallel mass spectrometry and proteomics analysis for identification of the venom toxins. The results show that varespladib is not only capable of neutralizing different PLA₂ activities in haemolytic venoms known to be rich in PLA₂s, but also capable of efficiently neutralizing coagulopathic toxicities (most profoundly anticoagulation) induced by venom components. This further indicates the potential clinical utility of varespladib for mitigating the toxic effects of certain snakebites.